Investigation into the occurrence of the dinoflagellate, *Ceratium hirundinella* in source waters and the impact thereof on drinking water purification

N. VAN DER WALT

12775754

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Supervisor: **Prof. S. Barnard**

Co-supervisor: **Ms. A. Swanepoel**

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ABSTRACT

The *Ceratium* species occurring in the Vaal River since 2000, was identified as *Ceratium hirundinella* (O.F. Müller) Dujardin as proposed by Van Ginkel *et al* (2001). *Ceratium hirundinella* is known to cause problems in drinking water purification and has been penetrating into the final drinking water of Rand Water since 2006. *Ceratium hirundinella* is associated with many other water purification problems such as disrupting of the coagulation and flocculation processes, blocking of sand filters and algal penetration into the drinking water. *Ceratium hirundinella* also produce fishy taste and odorous compounds and causes discolouration of the water.

The aims of this study were to determine the main environmental factors which are associated with the bloom formation of *C. hirundinella* in the source water and to investigate the influence of *C. hirundinella* on the production of potable water. In order to optimise treatment processes and resolve problems associated with high *C. hirundinella* concentrations during the production of potable water, jar testing and chlorine exposure experiments were performed.

Multivariate statistical analyses were performed to determine the main environmental variables behind *C. hirundinella* blooms. Ten years data (2000 – 2009) from the sampling point C-VRB5T in the Vaal River, (5 km upstream from the Barrage weir) were used for this investigation, because *C. hirundinella* occurred there frequently during the ten year period. In this study, it was found that *C. hirundinella* was favoured by high pH, Chemical Oxygen Demand (COD), orthophoshapte (PO_4), and silica concentrations, as well as low turbidity and low dissolved inorganic nitrogen (DIN) concentrations. No correlation was found between *C. hirundinella* and temperature, suggesting that this alga does not occur during periods of extreme warm or extreme cold conditions, but most probably during autumn and spring. The results of the multivariate statistical analysis performed with historical data from Vaalkop dam, indicate that the dinoflagellate *C. hirundinella* seems to be favoured by low temperature and turbidity, and high DIN, Fe, Methyl-orange alkalinity, Cd, PO_4, Conductivity, pH, hardness and SO_4 concentrations.

In order to optimise treatment processes such as coagulation, flocculation and sedimentation, jar testing experiments were performed to investigate different coagulant chemicals namely: cationic poly-electrolyte only, cationic poly-electrolyte in combination with slaked lime (CaO) and CaO in combination with activated silica. Water from four different sampling localities were chosen to perform the different jar testing experiments: 1) sampling point M-FOREBAY (in the Forebay, connecting the canal to the Zuikerbosch Purification plant) near Vereeniging due to its proximity to the Zuikerbosch treatment plant, 2) M-CANAL_VD (upstream from the inflow of the recovered water from Panfontein) to determine the influence of (if any) the recovered water from

Panfontein on Forebay source water, 3) source water from Vaalkop Dam (M-RAW_VAALKOP) and 4) source water from Rietvlei Dam (water from both Vaalkop and Rietvlei Dams contained high concentrations of *C. hirundinella* at that time of sampling) to determine which coagulant chemical is the most effective in removing high concentrations of *C. hirundinella* cells during the production of drinking water.

The jar testing experiments with Vaalkop Dam and Rietvlei Dam source water (rich with *C. hirundinella*) indicated that using cationic poly-electrolyte alone did not remove high concentrations of *C. hirundinella* efficiently. However, when CaO (in combination with cationic poly-electrolyte or activated silica) were dosed to Vaalkop Dam source water a significant decrease of *C. hirundinella* concentration was observed. This indicates that the *C. hirundinella* cells were "shocked or stressed" when exposed to the high pH of the CaO, rendering it immobile and thereby enhancing the coagulation and flocculation process. However, when 10 mg/L CaO in combination with poly-electrolyte was dosed to Rietvlei Dam source water the turbidity and chlorophyll-665 results indicated that this coagulant chemical procedure was ineffective in removing algal material from the source water.

The jar testing experiments using the cationic poly-electrolyte alone or cationic poly-electrolyte in combination with CaO on M-FOREBAY and M-CANAL_VD source water, showed a decrease in turbidity, chlorophyll-665 concentration, and total algal biomass, with an increase of coagulant chemical. When CaO in combination with activated silica was dosed, the inherent turbidity of the lime increased the turbidity of the Vaalkop Dam, M-FOREBAY and M-CANAL_VD source water to such an extent that it affected coagulation negatively, resulting in high turbidity values in the supernatant. Regardless of the turbidity values, the chlorophyll-665 concentration and total algal biomass (*C. hirundinella* specifically in Vaalkop Dam source water) decreased significantly when CaO was dosed in combination with activated silica. Therefore it was concluded that a cationic poly-electrolyte alone is a good coagulant chemical for the removal of turbidity, but when high algal biomass occur in the source water it is essential to add CaO to "stress" or "shock" the algae for the effective removal thereof. However, when CaO in combination with activated silica was dosed to Rietvlei Dam source water a decrease in turbidity and chlorophyll-665 concentration was found with an increasing coagulant chemical concentration. These results confirm the fact that coagulant chemicals may perform differently during different periods of the year when water chemistry changes and that certain coagulant chemicals may never be suitable to use for certain source waters.

For the effective removal of algae during water purification, it is recommended that cationic poly-electrolyte in combination with CaO are used as coagulant chemical at the Zuikerbosch Water Purification Plant. Turbidity is not a good indication of algal removal efficiency during jar testing experiments. If problems with high algal concentrations in the source water are experienced it is advisable to also determine the chlorophyll-665 concentrations of the

supernatant water during the regular jar testing experiments, since it will give a better indication of algal removal.

Chlorine exposure experiments were performed on water from Vaalkop Dam (M-RAW_VAALKOP) and Rietvlei Dam source water, to determine the possibility of implementing pre- or intermediate chlorination with the aim to render the cells immobile for more effective coagulation. The chlorine exposure experiments with Vaalkop Dam and Rietvlei Dam source water showed similar results. The chlorine concentration to be dosed as part of pre- or intermediate chlorination will differ for each type of source water as the chemical and biological composition of each water body are unique. When the effect of chlorine on the freshwater dinoflagellate *C. hirundinella* was investigated, it was found that the effective chlorine concentration where 50 % of *Ceratium* cells were rendered immobile (EC_{50}) was approximately 1.16 mg/L for Vaalkop Dam source water. For the source water sampled from Rietvlei Dam, it was found that the EC_{50} was at approximately 0.87 mg/L. Results of analyses to determine the organic compounds in the water after chlorination revealed that an increase in chlorine concentration resulted in increase in total organic carbon concentration (TOC), as well as a slight increase in MIB and trihalomethanes ($CHCl_3$). Pre- or intermediate chlorination seem to be an effective treatment option for the dinoflagellate *C. hirundinella* to be rendered immobile and thereby assisting in its coagulation process. The use of pre- or intermediate chlorination to effectively treat source waters containing high concentrations of *C. hirundinella* is a viable option to consider. However, the organic compounds in the water should be monitored and the EC_{50} value for each source water composition should be determined carefully as to restrict cell lysis and subsequent release of organic compounds into the water.

Keywords: *Ceratium hirundinella*, coagulation, flocculation, sedimentation, drinking water purification, jar testing, chlorine exposure, Vaalkop Dam, Rietvlei Dam, Vaal River, coagulant chemicals, poly-electrolyte, slaked lime, activated silica

OPSOMMING

Die *Ceratium* spesie wat vanaf 2000 in die Vaalrivier voorkom, is geïdentifiseer as *Ceratium hirundinella* (O.F. Müller) Dujardin, soos voorgestel deur Van Ginkel *et al* (2001). *Ceratium hirundinella* is bekend daarvoor om probleme in die drinkwatersuiweringsaanleg te veroorsaak en dit dring deur tot in die finale drinkwater wat geproduseer word deur Rand Water reeds vanaf 2006. *Ceratium hirundinella* word geassosieer met vele ander watersuiweringsprobleme soos versteuring van die koagulasie en flokkulasie prosesse, die blokkasie van die sandfilters en die deurdringing van alge tot in die finale drinkwater. *Ceratium hirundinella* produseer ook verbindings met ‘n visserige reuk en smaak wat verkleuring van die bronwater veroorsaak.

Die doelwitte van hierdie studie was om die omgewingsfaktore wat geassosieer word met *Ceratium hirundinella*–opbloeie in die bronwater te bepaal sowel as die invloed van *C. hirundinella* tydens die produksie van drinkwater. Om watersuiweringsprosesse te verbeter en probleme te verhoed wanneer hoë konsentrasies *C. hirundinella* teenwoordig is tydens die produksie van drinkwater, is roertoetse en chloor-blootstellings-eksperimente uitgevoer.

Meervoudige veranderlike statistiese analises is uitgevoer om die hoof omgewingveranderlikes te identifiseer wat voorkom tydens tydens ‘n *C. hirundinella* opbloei. Tien jaar se data (2000 – 2009) van die monsterpunt C-VRB5T in die Vaal River, (5 km stroom-op van die Barragekeerwal) is vir hierdie doeleinde gebruik omdat *C. hirundinella* gereeld by hierdie punt voorgekom het tydens die tien jaar van ondersoek. Hierdie studie het gevind dat *C. hirundinella* voorkom tydens omgewingstoestande gekenmerk deur ‘n hoë pH, chemiese suurstofbehoefte (COD), ortofosfaat (PO_4) en silika konsentrasies, asook lae troebelheid en lae opgeloste anorganiese stikstof (DIN) konsentrasies. Geen korrelasie is gevind tussen *C. hirundinella* en temperatuur nie, wat daarop dui dat hierdie alg nie voorkom tydens uiterste hoë of uiterste lae temperature nie, maar bes moontlik tydens herfs en lente. Die resultate van die meervoudige veranderlike statistiese analises wat op die historiese data van Vaalkopdam uitgevoer is, het getoon dat die dinoflagelaat *C. hirundinella* voorkom tydens lae temperature en lae troebelheid, asook hoë opgeloste anorganiese stikstof (DIN), yster (Fe), Metieloranje-alkaliniteit, Kadmium (Cd), PO_4, geleiding, pH, hardheid en SO_4 konsentrasies.

Om die watersuiweringsprosesse soos koagulering, flokkulering en sedimentering te verbeter, is roertoetse uitgevoer om die effek van verskillende koagulant chemikalieë te ondersoek: slegs kationiese poli-elektroliet alleen, kationiese poli-elektroliet in kombinasie met gebluste kalk (CaO) en laastens, CaO in kombinasie met geaktiveerde silika. Bronwater van vier verskillende monsterpunte is gekies om die roertoetse op uit te voer 1) monsterpunt M-FOREBAY (in die voorgaarbak, wat die kanaal verbind met die Zuikerbosch-watersuiweringsaanleg in

Vereeniging), 2) M-CANAL_VD (stroom-op van die invloei van die herwinde water vanaf Panfontein slik-aanleg) om vas te stel of die herwinde water ‘n invloed (indien enige) het op die bronwater in die voorgaarbak, 3) die bronwater van Vaalkopdam (M-RAW_VAALKOP) en 4) die bronwater van Rietvleidam (hoë konsentrasies van *C. hirundinella* het in die bronwater van beide Vaalkop- en Rietvleidamme voorgekom tydens die monsterneming). Dié water is gebruik om vas te stel watter koagulante meer effektief is om hoë konsentrasies *C. hirundinella* selle tydens die drinkwaterproses te verwyder.

Roertoetse met kationiese poli-elektroliet alleen was nie effektief in die verwydering van hoë konsentrasies *C. hirundinella* in die bronwater van Vaalkop- en Rietvleidamme nie. Daarenteen, wanneer CaO (in kombinasie met kationiese poli-elektroliet of geaktiveerde silika) gedoseer is in Vaalkopdam bronwater, was daar ‘n duidelike afname van *C. hirundinella* konsentrasies na sedimentering. Dit dui aan dat *C. hirundinella* selle “geskok” is tydens blootgestelling aan die hoë pH van CaO, hierdie effek maak die selle onbeweeglik en verbeter so dus die koagulering- en flokkuleringsprosesse. In teenstelling hiermee dui troebelheid en chlorofil-665 resultate wanneer 10 mg/L CaO in kombinasie met poli-elektroliet in Rietvleidam bronwater gedoseer is daarop dat hierdie koagulant oneffektief was aangesien dit nie die algmateriaal uit die bronwater verwyder het nie.

Die roertoetse waar kationiese poli-elektroliet alleen of kationiese poli-elektroliet in kombinasie met gebluste kalk (CaO) op M-FOREBAY en M-CANAL_VD bronwater gebruik is, het dit ‘n afname in troebelheid, chlorofil-665 konsentrasie en totale algbiomassa met toenemende koagulantkonsentrasie tot gevolg gehad. Wanneer CaO in kombinasie met geaktiveerde silika gedoseer is, het die inherente troebelheid van die kalk, die troebelheid van Vaalkopdam, M-FOREBAY en M-CANAL_VD bronwater verhoog, en sodoende die koagulasieproses negatief beïnvloed, asook hoë troebelheidswaardes in die bovloeistof van die roerbeker tot gevolg gehad. Ten spyte van van die troebelheidswaardes, het die chlorofil-665 konsentrasie en totale algbiomassa (*C. hirundinella* spesifiek in Vaalkopdam bronwater) noemenswaardig afgeneem wanneer CaO in kombinasie met geaktiveerde silika gedoseer is. Die gevolgtrekking kan dus gemaak word dat ‘n kationiese poli-elektroliet alleen ‘n goeie koagulant is vir die verwydering van troebelheid in die bronwater, maar wanneer hoë algbiomassa in die bronwater voorkom, is moet CaO bygevoeg word om sodoende die alge te “skok” vir die effektiewe verwydering daarvan. ‘n Afname in troebelheid asook die chlorofil-665 konsentrasie is gevind met ‘n toename in koagulantkonsentrasie wanneer CaO in kombinasie met geaktiveerde silika gedoseer is in Rietvleidam bronwater. Die chlorofil-665 resultate het aangedui dat wanneer CaO in kombinasie met geaktiveerde silika gedoseer is die algmateriaal voldoende verwyder is uit die bronwater.

Vir die effektiewe verwydering van alge tydens watersuiwering, word voorgestel dat kationiese poli-elektroliet in kombinasie met gebluste kalk (CaO) gebruik word as koagulant by die

Zuikerbosch watersuiweringsaanleg. Troebelheid is nie altyd ‘n aanduiding van effektiewe alg-verwydering gedurende roertoetse nie. Indien probleme met hoë algkonsentrasies in die bronwater ondervind word, word voorgestel dat chlorofil-665 konsentrasie van die bo-vloeistof tydens die roetine roertoetse ook bepaal word, wat ‘n beter aanduiding kan gee van algverwydering.

Chloor is toegevoeg tot die bronwater van Vaalkop- en Rietvleidamme om te bepaal of pre- of intermediêre chlorineringstappe kan bydra tot die immobilisering van *C. hirundinella*-selle wat kan lei tot meer effektiewe koagulering.

Die chloor-blootstellings-eksperimente met Vaalkopdam en Rietvleidambronwater het soortgelyke resultate getoon. Die chloorkonsentrasie wat gedoseer moet word as deel van pre- of intermediêre-chlorineringstappe sal verskil vir elke tipe bronwater, aangesien die chemiese en biologiese samestelling van elke waterbron uniek is. Die effek van chloor op die varswater dinoflagelaat *C. hirundinella* is ondersoek en dit is bevind dat die effektiewe chloorkonsentrasie waar 50 % van die *Ceratium* selle onbeweeglik gelaat is (EC_{50}), ongeveer 1.16 mgL is vir Vaalkopdambronwater was. Vir Rietvleidambronwater was die EC_{50}, ongeveer 0.87 mg/L. Met ‘n toename in chloorkonsentrasie was daar ‘n toename in die totale organiese koolstof (TOC) konsentrasie, asook ‘n geringe toename in metielisoborneol (MIB) en trihalometaan ($CHCl_3$). Wanneer die blootstellings-chloorkonsentrasie verhoog is, het die konsentrasie onbeweeglike selle ook toegeneem. Dit wil voorkom asof pre- of intermediêre chlorinering ‘n effektiewe behandeling kan wees wat die dinoflagelaat *C. hirundinella* immobiliseer en dus die koagulasie-proses ondersteun. Die toepassing van pre- of intermediêre chlorinering kan effektief aangewend word om bronwater te behandel wat hoë konsentrasies *C. hirundinella* bevat. Die konsentrasie organiese komponente moet egter noukeurig gemonitor word en die EC_{50}-waarde vir elke tipe bronwaarde bepaal word om die opbreek van selle te verhoed waartydens organiese verbindings in die water vrygestel sal word.

Sleutelwoorde: *Ceratium hirundinella*, koagulasie, flokkulasie, sedimentasie, drinkwatersuiwering, roertoetse, chloor-blootstelling, Vaalkopdam, Rietvleidam, Vaalrivier, koagulant chemikalieë, poli-elektroliet, gebluste kalk, geaktiveerde silika

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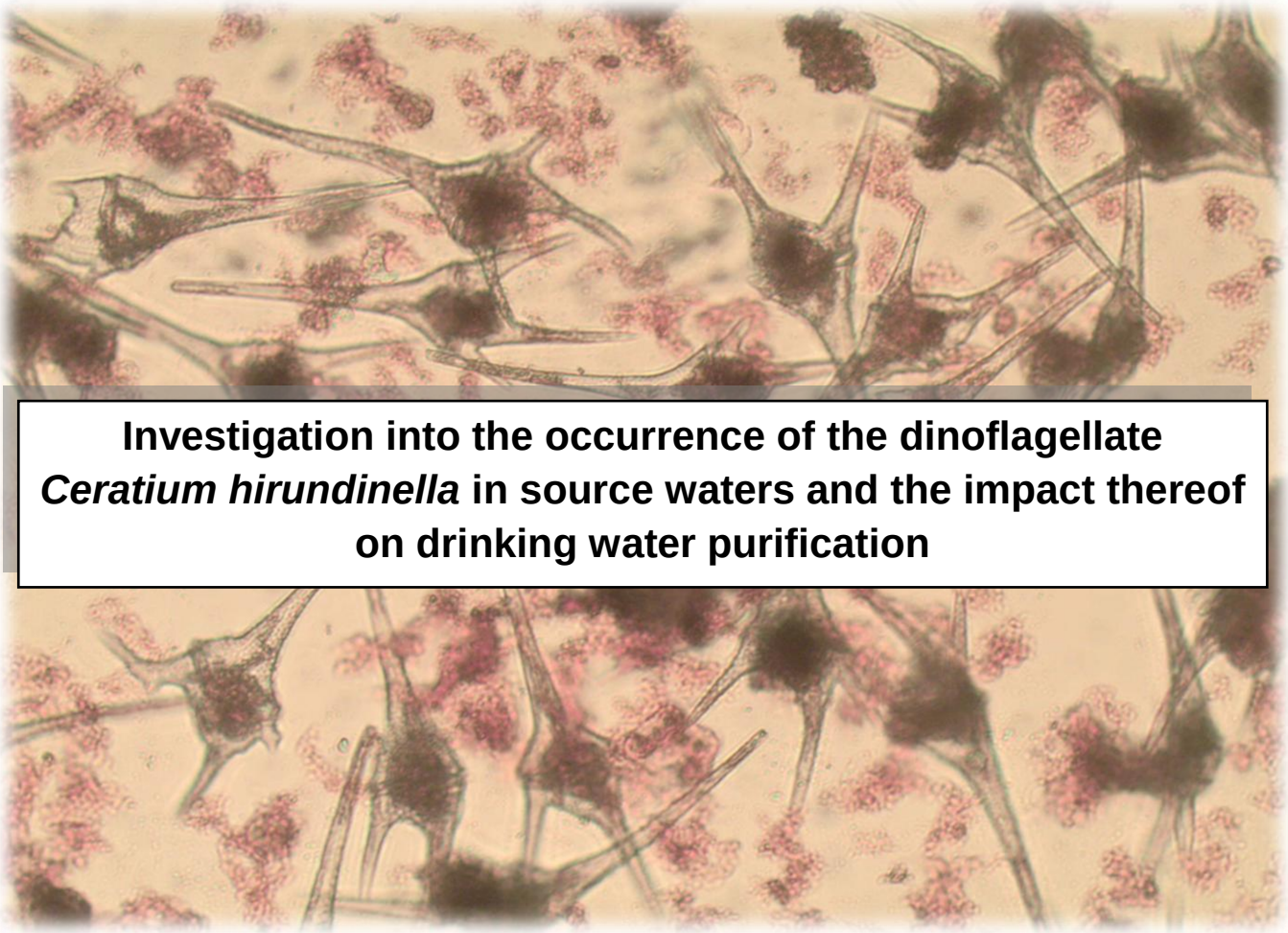
LIST OF ABBREVIATIONS

CA	Correspondence Analysis
CCA	Canonical Correspondence
COD	Chemical Oxygen Demand
DBP	Disinfection By-Products
DIN	Dissolved Inorganic Nitrogen
DIP	Dissolved Inorganic Phosphates
DOC	Dissolved Organic Carbon
HEA	Hybrid Evolutionary Algorithm
LHWP	Lesotho Highlands Water Project
MIB	Methylisoborneol
PCA	Principle Component Analysis
SS	Suspended solids
TDS	Total Dissolved Solids
THM	Trihalomethanes
TKN	Total Kjeldahl-Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphates



Investigation into the occurrence of the dinoflagellate *Ceratium hirundinella* in source waters and the impact thereof on drinking water purification

CHAPTER 1

INTRODUCTION

Aquatic algae are cosmopolitan and can be found in different types of waters such as fresh, marine or brackish water. Various environmental factors (e.g. temperature, light intensity, water currents and wind) influence the distribution, growth and diversity of algae in a water body (Harris *et al*, 1979; Heaney and Talling, 1980; Heaney and Furnass, 1980; Clegg *et al*, 2003). Nutrients such as phosphates and nitrates are essential to algal growth and can enter the aquatic ecosystem from sewage outfalls, agricultural or urban run-off or industrial effluent discharges. These nutrients can be considered to be pollutants since the excessive accumulation of it can cause excessive growth of nuisance plants and algae resulting in eutrophication (Strydom and King, 2009). Algal blooms can form as a result of increased nutrients (in addition to the appropriate light and temperature conditions) which can cause the water to change colour and are usually associated with obnoxious odours and tastes (Cantonati *et al*, 2003). During blooms of cyanobacteria and certain dinoflagellates toxic substances may be produced that can harm the aquatic life and even humans that use the water for recreation or consumption.

Eutrophication or nutrient enrichment has become a critical problem in many freshwater systems in South Africa including the Vaal River, one of the largest rivers and important water sources in the country (Pieterse and Janse van Vuuren, 1997). The Vaal River experience high levels of eutrophication due to exposure to untreated effluents from wastewater treatment plants and industries (Basson, 2000; Janse van Vuuren and Pieterse, 2005a). It is a well known fact that South African rivers (especially the Vaal River) are increasing in salinity at an alarming rate and total dissolved solids (TDS) concentration doubles every 10 years (Pieterse and Janse van Vuuren, 1997). According to Janse van Vuuren and Pieterse (2005a) heavy metal pollution is also becoming a particular problem in the Middle Vaal region and changes in the abundance of different phytoplankton groups become more apparent due to increasing concentrations of nutrients and salinity. It is of concern that this increase in salinity of the freshwater systems in South Africa may create environmental conditions that are favourable for the growth of specific salinity-tolerant algal species for example, dinoflagellates.

Algal-related water purification problems are very commonly found as the quality of South Africa's water sources are decreasing at a rapid rate (Basson, 2000). Algae (e.g. diatoms such as *Asterionella* sp. and *Fragilaria* sp. and dinoflagellates such as *Ceratium hirundinella*) have the potential to cause physical problems in the purification plant such as physical destabilisation of the flocs formed during flocculation and clogging of sand filters. Such problems increase the water purification and plant maintenance costs (Palmer, 1980). When algae (as part of the suspended particles in the water) cause a destabilisation of flocs and as a result thereof, are not

effectively removed by sedimentation, it results in higher algal loading onto the sand filters. This increased algal loading, results in reduced filter running times and increased backwashing (Palmer, 1980). It has been found that algae can avoid the purification process resulting in drinking water of aesthetically unacceptable quality (colouring, unpleasant tastes and odours) or drinking water that contain toxic substances that are detrimental to the health of consumers (Chow *et al*, 1999).

Ceratium hirundinella is the best known freshwater dinoflagellate and although it was identified in South Africa before 1999 (Van Ginkel *et al*, 2001; Hart and Wragg, 2009). *Ceratium hirundinella* (O.F. Müller) Dujardin has been identified in Rand Water's catchment area (refer to Appendix A, the species will be referred to as *Ceratium hirundinella* throughout the entire report), in the Vaal Dam and the upper Vaal River (twenty kilometres upstream from the Barrage weir) for the first time on the 1st of November 2000 (Swanepoel *et al*, 2008a). However, since this record the frequency of occurrence and concentration of *C. hirundinella* during spring and autumn has increased significantly in the Vaal River catchment (Swanepoel *et al*, 2008a). Unlike other algae, *C. hirundinella* is relatively large in cell size with robust cell coverings (theca plates) (Figure 1.1), which is the cause of many associated water purification problems such as 1) the disruption of the flocculation process, 2) clogging of sand filters, 3) production of fishy taste and odour substances and 4) penetration into the final drinking water. During 2008 to 2010, *C. hirundinella* was frequently found in the final drinking water as it was not removed by the different phases of purification in Rand Water's Zuikerbosch treatment plant (Ewerts, 2010).

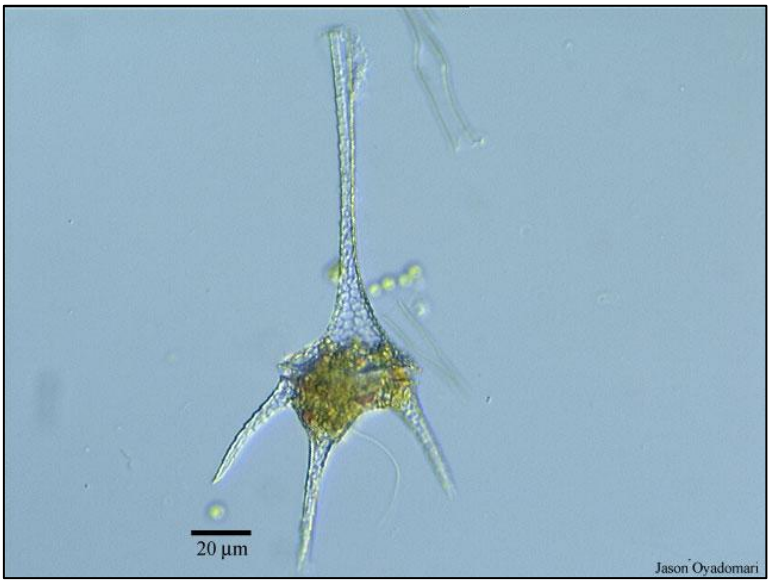


Figure 1.1: Light micrograph of the freshwater dinoflagellate *Ceratium hirundinella*, Phylum Myzozoa, Class Dinophyceae (Oyadomari J.K., 2008).

The objectives of this study were to investigate 1) the ecological reasons for the increase in *C. hirundinella* concentrations in the source water and 2) to determine the influence of high *C. hirundinella* concentrations on the water treatment processes specifically the coagulation, flocculation and sedimentation. These objectives were achieved by:

- Performing multivariate statistical analyses on physical, chemical and biological variables in order to identify the main associations behind the occurrence and increase of *C. hirundinella* in the source water at sampling point C-VRB5T (Vaal River Barrage) and Vaalkop Dam where *C. hirundinella* often occurred and

- Identifying and resolving problems associated with high *C. hirundinella* concentrations during the production of potable water. This was achieved by laboratory-scale experiments such as jar testing with different coagulant chemicals, as well as additional chlorine exposure experiments using source waters from the Forebay (before entering Rand Water's Zuikerbosch treatment plant), Vaalkop Dam and Rietvlei Dam.

CHAPTER 2

LITERATURE REVIEW

2.1. Ecology, morphology and physiological characteristics of the dinoflagellate *Ceratium hirundinella*

Ceratium hirundinella is a very common large, slow growing single-celled freshwater dinoflagellate. The cells are broad or narrow spindle-shaped and dorsiventrally flattened that can be up to 28 - 55 µm wide and 40 – 450 µm long (John *et al*, 2002). The cells are robust and have one anterior spine (on the epitheca) and 1 – 4 posterior horn-like spines (on the hypotheca) that may diverge widely from the cingulum (Figure 2.1). The size and number of horns, chemical composition of the plates of *C. hirundinella*, change according to season (Dodge and Crawford, 1970; Wetzel, 2001; van Ginkel *et al*, 2001; Gligora *et al*, 2003; Pizay *et al*, 2009). C*eratium hirundinella* is known for its seasonal polymorphism that can be recognised by the lengthening of the spines as the temperature increases from spring to summer, thereby also decreasing the sinking rate within the water-column (Sarjeant *et al*, 1987; Wetzel, 2001). It has been observed that during spring and autumn the cells may have less than three posterior horns which may be shorter than the apical horn.

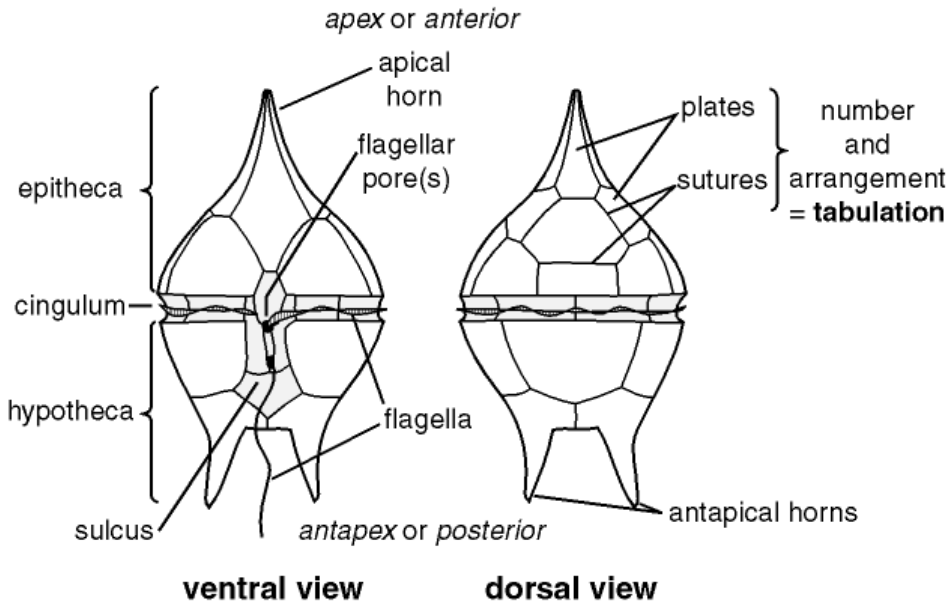


Figure 2.1: Morphological structure and anatomy of dinoflagellates (EWR, 2010).

Ceratium hirundinella has a cell covering (amphiesma) that consists of outer and inner continuous membranes (UCMP, 2006). Between these membranes flattened vesicles (alveoli) are found forming cellulose plates that overlap (Harland, 1988; NWE, 2008). These plates portray various shapes and arrangements that are used to distinguish between species. The thecal plate arrangements are smooth but coarsening on the horns (Figure 2.1). The plate

formula for *C. hirundinella* is Po, 4', 5'', 5c, ? [1]s, 6''', 2'''', fourth apical plate not reaching the apex of the cell (John *et al*, 2002). Dinoflagellates can be divided into two different taxa according to the structure of the amphiesmal vesicles, which may contain cellulose plates (armoured) or not (unarmoured or naked). *Ceratium hirundinella* is an example of armoured cells since it consists of a multi-layered cell wall of thick cellulose thecal plates that join to form an apical epitheca and posterior hypotheca, which is then divided by a groove called the cingulum (IWS, unknown). An example of unarmoured taxa such as *Gymnodinium* sp., known to cause red tides in the marine environment has thinner cell walls and no thecal plates.

The dinoflagellate is motile by means of two dissimilar flagellae, one transverse flagellum and the second longitudinal flagellum that extends from the sulcus on the rear (Canter-Lund and Lund, 1995; Fenchel, 2001). The flagellum's structure has been intensely studied by Sato *et al* (2004). Sato *et al* (2004) found that the longitudinal flagellum consists of three structures containing nanofilaments (2 - 4 nm), and the axoneme. This includes the paraflagellar rod, a thin striated fibre and the R-fibre which is a large bundle of fibre only found in the longitudinal flagellum of *C. hirundinella* (Sato *et al*, 2004). According to Heaney (1976), the flagellums allow for the cell movement within the water-column. The transverse flagellum is mainly providing the force necessary to drive the cell in a certain direction, and the longitudinal flagellum mainly steers the cell (Olney, 2002; NWE, 2008). According to studies done by Fenchel (2001), dinoflagellates have a unique swimming pattern. These studies indicated that the cell steer itself by changing the rhythm of one or both of the flagellums or by changing direction of the trailing flagellum. It was also found that most dinoflagellates swim in a clockwise helical path and that the dorsal side of the cell will always face the axis. Fenchel (2001) found that *C. hirundinella* swims slowly and rotates very slowly around its longitudinal axis and is able to reverse the swimming direction and move backwards.

The cells contain large oval chloroplasts that may vary in colour (yellow, brown or green) and are mostly bound by three membranes (Dodge and Crawford, 1970; Canter-Lund and Lund, 1995; John *et al*, 2002). Pigments such as chlorophyll's *a* and *c* and either peridinin or fucoxanthin and xanthophylls are found (Faust and Gulledge, 2002; NWE, 2008). Other organelles include a large nucleus (dinokaryon) in a central position containing condensed chromosomes and a nucleolus, food vacuoles, not a true eyespot but a reddish "corpuscular" body may be present in the mid-region of the cell. Dodge and Crawford's (1970) studies indicated the presence of other organelles such as mitochondria, lipid globules, food vacuoles, dictyosomes, trichocysts on the edges of the cell as well as Golgi bodies (Olney, 2002) (Figure 2.2). Olney (2002) also found that the horns are filled with cytoplasm and may also contain chloroplasts and even trichocysts.

[1] Taxonomists are uncertain about the number of theca plates in the sulcus (vertical groove) (Janse van Vuuren, S., 2010 [E-mail], *Ceratium hirundinella* taxonomy (Personal Communication , 8 February 2010).

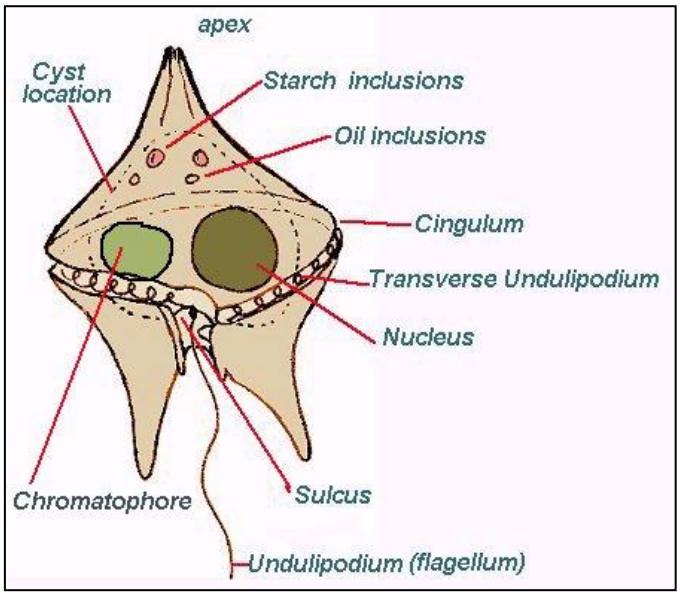


Figure 2.2: Diagram illustrating the morphology of dinoflagellates (Cavanihac, J., 2001).

The life cycle of dinoflagellates may be (Canter-Lund and Lund, 1995; Wetzel 2001):

- Haplontic: vegetative cells are haploid and the zygote is the only diploid cell or,
- Diplontic: vegetative cells are diploid and gametes are the only haploid cells or,
- Diplohaplontic: the alternation of diploid and haploid vegetative generations.

Dinoflagellates primarily reproduce asexually through binary fission, however, sexual reproduction is also found within *C. hirundinella*. (Sarjeant *et al,* 1987; Canter-Lund and Lund, 1995; Faust and Gulledge, 2002). Their investigation showed that during binary fusion the cell divides and each half has one or more of the mother cell's horns. The cell will produce more horns as it grows and thus become identical to the mother cell. Sexual reproduction occurs by fusion of two individuals to form a zygote that will remain motile or may form a resting dinocyst that will undergo meiosis to produce new haploid cells. Heller (1977) investigated the phased division of *C. hirundinella* and found that dividing alga cells in a population on a given day can indicate the population growth rate. According to Entz (1931) as cited by Heller (1977) the division of *C. hirundinella* occur during the night (20h00 - 22h00) and early morning.

Some dinoflagellates have the ability to form cysts when environmental conditions are unfavourable where after it can germinate to form a bloom (Wetzel, 2001). Sarjeant *et al* (1987) noted that *C. hirundinella* can encyst at any stage of its life cycles. In unfavourable conditions (e.g. insufficient nutrients or light and during cold weather) the life cycle changes, two vegetative cells fuse to form a planozygote and enter into a hibernation stage, then called a hypnozygote (Sarjeant *et al*, 1987). The organism then increase in size because of excess food intake and its shell gets harder and spikes can also form. When conditions become favourable again, it breaks out of its shell and enter a temporary stage (planomeiocyte), where they reform their individual thecae and return to the vegetative state (Canter-Lund and Lund, 1995; NWE, 2008).

Rengefors and Anderson (1998), Clegg *et al* (2003) and Collins (2006) mentioned various factors that contribute to cyst germination such as dissolved oxygen in the sediments, internal maturation processes, water temperature, light intensity and day length. Sarjeant *et al*, (1987) indicated that low light intensity, anoxia and low temperatures could delay or suppress cyst germination. Wetzel (2001) mentioned that *C. hirundinella* can form asexual resting stages or hibernating cysts. Cysts form inside the cell wall and will break open to release it. The cyst is surrounded by a thick cellulose membrane and contains food reserves such as lipids (Sarjeant *et al*, 1987). Sarjeant *et al* (1987) also found that the cyst wall is thick enough to resist fungal and bacterial attacks. *Ceratium hirundinella* cysts are triangular and short horns can be observed at each angle of the cell (Figure 2.3) (IWS, unknown). The cyst will sink to the bottom and remain there until environmental conditions become favourable, e.g. water temperature or light intensity increases (Sarjeant *et al*, 1987; Harland, 1988; Rengefors and Anderson, 1998). Cysts can survive in the lake sediment for several years (Sarjeant *et al*, 1987, e.g. cysts up to 6 ½ years old were found in Lake Zürich, Switzerland). Rengefors and Anderson (1998) have done studies in Lake Erken, Sweden, and found that a close relationship exists between the amount of cysts found in the sediment and the amount of vegetative dinoflagellate cells in the pelagic zone. Cysts may become entangled in the gills or other body parts of fish or other organisms and in this way be transported to new areas (Sarjeant *et al*, 1987; Collins, 2006).

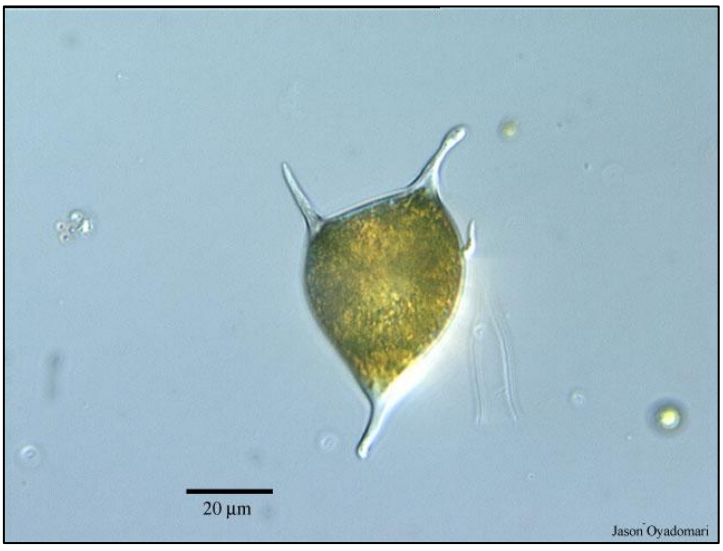


Figure 2.3: Light micrograph of *Ceratium hirundinella* cysts (Oyadomari J.K., 2008).

Dinoflagellates can be classified as photosynthetic autotrophs, heterotrophs or mixotrophs, thus playing an important role in the aquatic food-chain. According to Elgavish *et al* (1980) and Van Ginkel *et al* (2001) *C. hirundinella* can assimilate organic and inorganic phosphorus. It has also been observed that *C. hirundinella* is well adapted to obtain particulate food (Dodge and Crawford, 1970). Furthermore, it was found that *C. hirundinella* feeds on different types of food including bacteria, cyanobacteria and diatom frustules. Some *Ceratium* species can even

function as predators (by means of phagocytosis), endosymbionts or parasites (Harland, 1988; Faust and Gulledge, 2002; NWE, 2008). According to Baek *et al* (2009) potential grazers of *C. hirundinella* includes copepods and large ciliates.

Most species of dinoflagellates are cosmopolitan, and can occur in a variety of habitats such as pelagic, benthic, tropical seas, estuaries and freshwater (Harland, 1988; Canter-Lund and Lund, 1995; Faust and Gulledge, 2002). Dinoflagellate species are found to be common in deeper ponds and lakes that are oligotrophic to eutrophic (Rengefors and Anderson, 1998; John *et al*, 2002). According to Canter-Lund and Lund (1995) some species are commonly found in water bodies rich in phosphates and nitrates and high abundance of cyanobacteria. *Ceratium hirundinella* is known to grow in a warm stable epilimnion with low nutrient concentrations (Pfiester, 1971; Heaney and Talling, 1980; Whittington *et al*, 2000; Baek *et al*, 2008a, 2008b; 2009), however, Van Ginkel *et al* (2001), found *C. hirundinella* to bloom in the Hartbeespoort Dam, which has hyper-eutrophic conditions. Dinoflagellates are sensitive to changes in temperature, salinity and nutrient levels (Pfiester, 1971; Heaney and Talling, 1980; Frempong, 1984; Padisák, 1985; Whittington *et al*, 2000; Wetzel, 2001; Teubner *et al*, 2003; UCMP, 2006). For example, the development of certain environmental conditions such as an increase of salinity or nutrients may favour the growth of dinoflagellates and form blooms. *Ceratium hirundinella* can occur during all seasons, however, most occurrences have been reported in the warmer months (Wetzel, 2001; Tomec *et al*, 2002, as cited by Gligora *et al*; 2003; John *et al*, 2002; Baek *et al*, 2008a and Swanepoel *et al*, 2008a).

2.2. Environmental drivers behind the growth and occurrences of *Ceratium hirundinella* in freshwaters

Phytoplankton assemblages found in freshwater can serve as indicators of the water quality and level of pollution (Palmer, 1980). According to Khuantrairong and Traichaiyaporn (2008) a high diversity of phytoplankton communities illustrates a healthy ecosystem. The density and diversity of phytoplankton in particular can demonstrate various characteristics of the freshwater body (Khuantrairong and Traichaiyaporn, 2008). Environmental factors that have a large influence on phytoplankton succession include light intensity, salinity, availability of nutrients and temperature (Gligora *et al*, 2003; Clegg *et al*, 2003; NWE, 2008). Depending on these factors certain phytoplankton species may form blooms and grow extensively. Many theories have been formed relating to algal bloom formation and various studies have been done to investigate the reasons why certain phytoplankton species dominate with certain environmental conditions (Cantonati *et al*, 2003). However, few explanations have been found. For a bloom to be able to develop certain physical and chemical conditions for growth stimulation needs to be fulfilled (Reid, 1997).

The genus *Ceratium* is exceptionally species rich and can be found in different types of water, for example, in polar, marine and freshwater systems (Okolodkov and Dodge, 1996; Tunin-Ley *et al*, 2007; Santos-Wisniewski *et al*, 2007). Some species are known to cause red tide blooms (Reid, 1997; Nakano *et al*, 1999; Tunin-Ley *et al*, 2007). Tunin-Ley *et al* (2007) stated that little is known about the factors influencing its biodiversity and temporal patterns, however, numerous studies have been done on the growth rate, cell division, trophic relationships and taxonomy of this genus. The ecology and physiology of the alga *C. hirundinella* in freshwater bodies has been studied over the years in several countries especially its occurrence in Northern hemisphere lakes, however studies on its occurrences in the Southern hemisphere is rather limited (Heaney, 1976; Cranwell, 1976; Heller, 1977; Harris *et al*,1979; Nicholls *et al*, 1980; Heaney and Talling, 1980; Frempong, 1984; Padisák, 1985; Whittington *et al*, 2000; Buck and Zurek, 1994; Nakano *et al*,1999; van Ginkel, 2001; Grigorszky *et al*, 2003b; Tunin-Ley *et al*, 2007; Hart and Wragg, 2009).

According to van Ginkel *et al* (2001, 2007), the freshwater dinoflagellate *C. hirundinella* has been found to be dominant in many man-made impoundments with different trophic statuses in South Africa since the 1990s. Blooms appeared in 17 of 57 South African reservoirs during all seasons, examples of such impoundments include the Boskop Dam, Bronkhorstspruit Dam, Koster River Dam and Klipvoor Dam (DWA, 2001; van Ginkel *et al*, 2007 (van Ginkel and Silberbauer, 2006, as cited by Hart and Wragg, 2009). In the spring of 1999, *C. hirundinella* occurred with extreme bloom formations in the Hartbeespoort Dam, in hyper-eutrophic conditions. This contradicts the previous perception (Palmer, 1980; Padisak, 1985) that *Ceratium* sp. is an indicator species of clean water. *C. hirundinella* blooms also occurred in oligotrophic and mesotrophic waters (van Ginkel *et al*, 2001; Grigorszky *et al*, 2003b), e.g. Lake Biwa in Japan (other examples were studied by Padisák, 1985; Buck and Zurek, 1994; Rengefors and Anderson, 1998; John *et al*, 2002).

According to Granéli *et al* (1989) and Anderson (2009) algal blooms are usually found to be monospecific or dominated by a few species of the same taxonomic group. For an algal group or species to form a bloom it must have the ability to outcompete other species. When a water body is impacted by some type of pollution (e.g. sewage treatment plants that discharge effluents directly into the waterbody) it results in an accumulation of algal nutrients in water and in this way stimulate the growth of certain algae where some may form blooms. When algal blooms are formed the water quality is affected and reduced. Theories include that bloom formations can be linked to various environmental and anthropological factors such as pollution or discharges into the freshwater body, eutrophication, light intensity, nutrients, chelators and climatic changes (Granéli *et al*, 1989). For example, it has been found that there may be a correlation with iron-containing compounds that are discharged into the freshwater that can stimulate dinoflagellate blooms (Ingle and Martin, 1971, as cited by Granéli *et al*, 1989). It has

also been found that dinoflagellate growth can be stimulated by humic substances that may act as chelators or grazing pressure (Granéli *et al*, 1989). Previous studies done by Granéli *et al* (1985, 1989) indicated that dinoflagellates are more stimulated by humic substances than other phytoplankton groups such as diatoms.

It is known that certain algal blooms may have dramatic effects that may result in fish kills as a result of oxygen depletion (e.g. Palmer, 1980; Granéli *et al*, 1989; Reid, 1997; Recknagel *et al*, 1997; Collins, 2006; Anderson, 2009; Hart and Wragg, 2009). Fish kills occur when masses of algal material (during or after a bloom) is decomposed by aerobic bacteria – depleting the oxygen in the source water. During the summer of 1976, a *C. hirundinella* bloom in Heart Lake, Ontario Canada, caused a depletion of dissolved oxygen and resulted in a massive fish-kill in the lake (Nicholls *et al*, 1980). *Ceratium hirundinella* flourished because it was too large in cell size to be grazed on by smaller algae or other zooplankton. A study has shown that high concentrations of *C. hirundinella* can be found due to no competition for nutrients or organic matter in the lake sediment (Nicholls *et al*, 1980). According to Nicholls *et al* (1980) the lake underwent artificial mixing at that time by means of supplying compressed air to the bottom of the lake. Nicholls *et al* (1980) also found that there might be a relationship between the density of cyanobacteria and dinoflagellates, for instance the decline in *C. hirundinella* cells were observed when a high concentration of cyanobacteria was present. The study has indicated that the *C. hirundinella* bloom possibly collapsed because of a lack of inorganic nitrogen and the artificial destratification process. Other *Ceratium* sp. blooms such as those that occurred in Thailand and Japan; these blooms were also responsible for fish mortality due to oxygen depletion (Santos-Wisniewski *et al*, 2007). Heaney (1976) indicated that it is possible that large concentrations of *C. hirundinella* cells can increase the pH from 7 to 10 in the epilimnion due to photosynthesis (Kangro *et al*, 2005).

The *Ceratium* genus that occurs in the marine environment can release harmful substances during bloom formations which may be toxic to living organisms. Dinoflagellate blooms that occurred in 1980 and 1981 on the Swedish West Coast, Kattegat and Skagerrak, correlated with various environmental variables such as unusually high precipitation and high inorganic nutrient concentrations (Edler *et al*,1982; Nielsen and Aertebjerg,1984 as cited by Granéli *et al*,1989). According to Edler (1984) as cited by Granéli *et al* (1989), oxygen deficiency was observed in the bottom-near waters at the Kattegat and Skagerrak coast and may thus be considered a potential environmental factor that can contribute to bloom formation. The food-chain in the water body is very important - maintaining the balance between different consumers. Van Ginkel *et al* (2001) mentioned that it might be possible that bloom conditions can be triggered if the numbers of predators are less than it should be, e.g. Hartbeespoort Dam bloom that occurred in 1999 (van Ginkel *et al*, 2001). Reid (1997) indicated the important relationship between the occurrence of diatoms and dinoflagellates. Diatoms are known to be

found in excess during colder months and silica is an important constituent of its cell wall. Reid (1997) stated that when waters are low in silica, it would favour dinoflagellates that can migrate to regions below the pycnocline (density gradient in water).

2.2.1. Migration Pattern and Water Column Stability

According to Heaney (1976), *C. hirundinella* has a very distinct vertical migration pattern. The alga swims towards the water surface during the morning and early afternoon. In the late afternoon and at night the alga will redistribute to the region above the hypolimnion – avoiding the deoxygenated hypolimnion (Heaney, 1976; Harris *et al*, 1979; Heaney and Talling, 1980; Nicholls *et al*, 1980; Frempong, 1984; Whittington *et al*, 2000; van Ginkel, 2001; Baek *et al*, 2009). It was found that the *C. hirundinella* concentration increased in the metalimnion during the late afternoon (Heaney, 1976). The growth rates and distribution of phytoplankton are influenced by the nutrient availability in the water column (Baek *et al*, 2008b). The vertical migration of *C. hirundinella* allows the utilization of nutrients found in the epilimnion - indicating high cell concentrations in the upper 3 m layer of the lake (Teubner *et al*, 2003). However, Heaney (1976) has found that the alga may avoid migrating to the surface because of too high light intensities. From various studies it was found that the distribution pattern of *C. hirundinella* are influenced by a variety of growth-controlling factors such as algal migration, wind effects, light intensity, nutrient availability (especially DIN), Chemical Oxygen Demand (COD), optimum growth temperature, density, oxygen (tolerant to low oxygen levels) and water currents and mixing turbulence or stability of the water column (Pfiester, 1971; Weaver, 1979; Harris *et al*, 1979; Heaney and Talling, 1980; Heaney and Furnass, 1980; Nicholls *et al*, 1980; Padisák, 1985; Nakano *et al*, 1999; Whittington *et al,* 2000; John *et al*, 2002; Grigorszky *et al*, 2003a; 2003b; Gligora *et al*, 2003; Tunin-Ley *et al*, 2007; Baek *et al*, 2008a, 2008b; 2009).

The mobility of this dinoflagellate offers itself many advantages over other algae. For example, the alga may temporary move into the hypolimnion to utilize nutrients but return to the epilimnion for photosynthesis (Nakano, 1999; Whittington *et al*, 2000). During night-time when the alga redistribute to deeper levels of the lake it has access to large reserves of nutrients in the hypolimnion (Heaney, 1976; Frempong, 1984). According to Baek *et al* (2009) the diel vertical migration pattern (in a 24-hour period) and cell division timing might play a role as control factors in bloom formation. Because of the relatively large size of *C. hirundinella* together with its ability to swim, it is frequently found to interfere with the coagulation and flocculation processes during drinking water purification (Swanepoel *et al,* 2008a).

Padisák (1985) studied the population dynamics (from 1933 – 1982) of *C. hirundinella* in Lake Balaton in Hungary, Central Europe. His studies indicated the highest *Ceratium* cell abundance

occurred in 1978 with 48 500 cells/L distributed at the first two meter depths of the surface waters. In 1976 Harris *et al* (1979) investigated the alga's depth distributions, irradiance regimes and photosynthetic behaviour and found that certain environmental conditions were unfavourable for this alga, such as higher irradiances, wind (which create water currents) and low concentrations of inorganic nitrogen interfered with the vertical migration pattern of *C. hirundinella*. It was found that deep-mixed conditions in the epilimnion increased the biomass of the alga evenly throughout the water column – allowing optimal photosynthesis (Harris *et al*, 1979). Various studies have found that *C. hirundinella* populations tend to accumulate in the thermally stable region of the epilimnion where there is a sufficient amount of light, nutrients and dissolved oxygen (Pfiester, 1971; Harris *et al*, 1979; Heaney and Talling, 1980; Frempong, 1984; Padisák, 1985; Whittington *et al*, 2000; Teubner *et al*, 2003). According to Baek *et al* (2008b) a stable water column would provide a greater diversity of dinoflagellates and increase cell biomass.

Heaney and Talling (1980) observed the maximum cell density on a 3 – 4 m depth during low wind stress conditions, and they found a change in behaviour during the stationary phase where the alga concentrated near the surface during daytime. It has been found that strong wind conditions (>4 $m.s^{-1}$) decrease or eliminate population densities of *C. hirundinella* in the epilimnion. Ingleton *et al* (2008) indicated that the intensity and time of exposure of turbulent mixing can have an impact on the growth and life cycle of phytoplankton. Low cell densities (<1 cell/mL) were found during the winter, when the accumulation of starch in the cells and depletion of inorganic nitrogen and reactive phosphate occurred (Heaney and Talling, 1980). Various studies found that *C. hirundinella* avoids areas of strong water currents (Padisák,1985; Reid, 1997; van Ginkel *et al*, 2001).

2.2.2. Light Conditions

Padisák (1985) emphasized that a major factor influencing the diel vertical distribution of *C. hirundinella* is light intensity. Heaney and Furnass (1980) indicated that *Ceratium* cells migrate into the surface layers during low irradiances at <550 $\mu E.m^{-2}.s^{-1}$ while the cells moved downward avoiding the surface waters during irradiances higher than 1300 $\mu E.m^{-2}.s^{-1}$. Frempong (1984) also found that the alga migrated towards the surface during the day, and at high irradiance or during night-time the alga moved deeper into water but remained above the oxycline. Frempong (1984) emphasized that the migration pattern of the algae did not always correspond with the light-dark cycle. Grigorszky *et al* (2003a) found that light intensities between 60 – 180 $\mu mol.m^{-2}.s^{-1}$ photosynthetic active radiation is an optimum range for *C. hirundinella* during daytime. However, it is important to notice the relationship between

surface irradiance and temperature (e.g. complete avoidance at a surface irradiance of 1600 $\mu E.m^{-2}.s^{-1}$ at 14h00) was found by Heaney and Talling (1980).

Baek *et al* (2008a, 2009) stated that *C. hirundinella* avoids high irradiances to refrain themselves from light-induced cell damages (e.g. shrinkage and bleaching of chloroplasts). Harris *et al* (1979) also found that *C. hirundinella* tended to aggregate at a certain depth in response to population density. Baek *et al* (2008a, 2009) articulates that this specific ability can be seen as enhanced survival or growth under suboptimal light intensities. Studies done by Harris *et al* (1979) indicated that a *C. hirundinella* population was at its highest concentrations at depths with an irradiance level of 140 $\mu E.m^{-2}.s^{-1}$ - the highest photosynthesis net rate at that level. Péres-Martínez and Sánchez-Castillo's (2002) studied *C. hirundinella* during the autumn/winter mixing period in a southern Spanish reservoir indicating that light intensity and temperature might not influence growth during winter mixing in this particular water body. This reservoir has an irradiance of 150 $\mu E.m^{-2}.s^{-1}$ in the epilimnion throughout the year. However, it should be noted that the light intensity in the southern hemisphere is much higher in winter than in the northern hemisphere. Environmental conditions in the Southern hemisphere (light intensity and temperature) allow the growth of *C. hirundinella* throughout the year. Studies done by Baek *et al* (2009) indicated that the dinoflagellates *Ceratium furca* and *Ceratium fusus* preferred optimum temperatures from 18 to 28 ˚C and irradiances from 216 to 796 $\mu mol.m^{-2}.s^{-1}$.

According to Whittington *et al* (2000) *C. hirundinella* is able to migrate towards the surface from suboptimal depths at a velocity of 1.6 – 2.7 x 10^{-4} $m.s^{-1}$. Whittington *et al* (2000) found that this alga is able to migrate to the surface under different turbulent intensities, however it was found that at a turbulence of >5 x 10^{-3} $m.s^{-1}$ the accumulation of *C. hirundinella* to the surface was considerably reduced. The irradiance at the subsurface varied between 212 and 552 $\mu mol.m^{2}.s^{-1}$ photosynthetic active radiation, the accumulation of cells occurred at those depths rather than those found by Harris *et al* (1979) and Heaney and Furnass (1980). Whittington *et al* (2000) indicated that this might be due to higher light intensities in the Southern hemisphere.

2.2.3. Temperature and Seasonality

Physiological activities and cellular behaviour (e.g. distribution and swimming speed) in dinoflagellates are known to be closely related to temperature, light intensity and nutrient availability (Clegg *et al*, 2003; Baek *et al*, 2008a, 2008b; 2009). With an increase in temperature, the rate of photosynthesis and growth also increased. Various studies indicated that *C. hirundinella* has a variable seasonal pattern and that its occurrence may be correlated with water temperature (Padisák, 1985; Nakano *et al*, 1999; Tunin-Ley *et al*, 2007; Hart and Wragg, 2009). However, it has been found that *C. hirundinella* may occur as the dominant

species in winter and summer, e.g. Lake Vrana, Croatia (Tomec *et al*, 2002, as cited by Gligora *et al*, 2003). According to Nakano *et al* (1999) growth of the vegetative cell is found at approximately 10 ˚C and that optimal growth is at 20 ˚C in a stable water column. Nakano *et al* (1999) also found that temperature is an important factor with excystment of *C. hirundinella*, and that it occurred at a temperature between 15 ˚C and 30 ˚C. Temperatures higher than 30 ˚C suppressed excystment (Nakano *et al*, 1999). Studies have indicated that excystment may be an important inception of blooms (Heaney *et al*, 1983). According to van Ginkel *et al* (2001), *C. hirundinella* occurs mainly in the warmer months (Dottne-Lindgren and Ekbohm, 1975; Heaney, 1976; Gligora *et al*, 2003), however, they have also found *C. hirundinella* during winter in South Africa. Péres-Martínez and Sánchez-Castillo (2002) reports the presence of *C. hirundinella* in various reservoirs (e.g. Amadorio and El Gergal reservoir) during all seasons in Spain. Pfiester (1971) reported the presence of *C. hirundinella* during autumn and spring in a small pond in Calloway County, Kentucky, USA. Péres-Martínez and Sánchez-Castillo (2001, 2002) found winter dominance of *C. hirundinella* in a reservoir in the southern north-temperate Spain.

In South Africa, a *C. hirundinella* bloom occurred in the Hartbeespoort Dam bloom in 1999 and was the cause of fish kills. The water had a brown colour as a result of the large concentrations of *C. hirundinella* (DWAF, 2001; Faust and Gulledge, 2002; Collins, 2006). It started in the last month of winter and reached its peak cell concentrations in October that continued until December 1999. A distinct increase of chlorophyll-*a* concentrations were observed with the increase of the *C. hirundinella* population. A peak of 4243 mg/L chlorophyll-*a* was found on 12 October 1999 at the surface in comparison with a *Microcystis aeruginosa* bloom earlier the same year with the highest chlorophyll-*a* peak of approximately 100 mg/L during the month of April (van Ginkel *et al*, 2001). At a depth of 10 m a chlorophyll-*a* concentration of 100 mg/L were found showing that *C. hirundinella* was distributed throughout the water column. The ability of *C*. *hirundinella* to change its position in the water column benefits this organism over other species (van Ginkel *et al*, 2001). Van Ginkel *et al* (2001) also found average chlorophyll-*a* levels of up to 600 µg/L in the epilimnion and found cell densities of approximately 13 500 cells/mL. The bloom started at a temperature of 15 ˚C and during peak times the temperatures varied between 20 ˚C and 30 ˚C (van Ginkel *et al*, 2001; 2007). It has also been found that during the bloom the dissolved oxygen concentrations were higher in the upper layers of the impoundment than the previous year when *C. hirundinella* did not bloom.

A *C. hirundinella* bloom was found in the Albert Falls Dam (Kwa-Zulu Natal) in October 2004. In the following year a large increase in *C. hirundinella* concentrations were found in the early winter, and a subsequent increase in late spring/early summer (Hart and Wragg, 2009). In October 2006, a *Ceratium* sp. bloom caused rapid de-oxygenation below 5 m, and complete anoxia below 8 m was found in November, indicating the decomposition of algal matter after the

bloom. The lake was recovered and re-oxygenated by January 2007. In the same year (2007), there was another *C. hirundinella* increase during early spring. Hart and Wragg (2009) stated that *C. hirundinella* can be associated with temperatures between 15 ˚C and 20 ˚C. The Albert Falls Dam is a mesotrophic reservoir and the onset of this bloom was an indication of reduced water quality and ecological change (Hart and Wragg, 2009). In this particular bloom the *C. hirundinella* density increased to more than 5000 cells/mL and a consistent increase of the chlorophyll-*a* with depth was also found in October and January. The study also indicated a negative correlation of chlorophyll-*a* with temperature and dissolved oxygen saturation levels.

2.2.4. Nutrients and Other Chemicals

According to van Ginkel *et al* (2001) it is suspected that the *C. hirundinella* bloom that occurred in the Hartbeespoort Dam could be related to the change in nutrients within the impoundment, since positive correlations were found between chlorophyll-*a* and total nitrogen, dissolved inorganic nitrogen (DIN) and orthophoshorus concentrations. During the bloom it was observed that certain environmental variables such as DIN (nitrates, nitrites and ammonium) and phosphorus were present in high concentrations. Cantonati *et al* (2003) has found that phosphorus is an important nutrient for the metabolism of *C. hirundinella* and is the main nutrient necessary for bloom formation. According to the study done by van Ginkel *et al* (2001) it was found that the availability of dissolved inorganic nitrogen (DIN) and release of high concentrations of phosphorus from the sediments seemed to favour the growth of *C. hirundinella*. They suspected that phosphorus may be one of the driving forces behind bloom formation since *C. hirundinella* has the ability to assimilate both organic and inorganic phosphorus. No cyanobacteria were present during the bloom, thus contradicting the statement made by Nicholls *et al* (1980) that there might be a direct relationship between the density of cyanobacteria and dinoflagellates. The studies also showed a positive correlation between total nitrogen and chlorophyll-*a*. The TN:TP ratio increased from 21:8 (with the presence of a *Microcystis* sp. bloom) to 42:7 during the *C. hirundinella* bloom (van Ginkel *et al*, 2001). Van Ginkel *et al* (2001) and Hart and Wragg (2009) found that phosphates may act as an important factor leading to bloom formations of *C. hirundinella*. Péres-Martínez and Sánchez-Castillo (2001, 2002) found high values of alkalinity, conductivity, major ionic compounds: SO_4^{2-}, $CO_3^{2-,}$ Ca^{2+} and Mg^{2+} and a constant DIN concentration (>100 µg/L) during the autumn/winter period when *C. hirundinella* was dominant.

Ceratium hirundinella has increased in the Vaal River over the last decade. However, not many studies have been done to determine the possible environmental drivers that stimulate the growth of this dinoflagellate. Therefore, one of the objectives of this study was to investigate

the ecological reasons for the occurrence and increase of *C. hirundinella* in the Vaal River catchment.

2.3. Problems associated with *Ceratium hirundinella* during water treatment

The removal of algae and micro-organisms are one of the many challenges of every water treatment plant. Algae are known to cause many problems during water treatment such as:

- Increase in coagulant demand,
- Interference with the coagulation/flocculation processes,
- Filter clogging,
- Shortening of filter run-times and increased backwash regime,
- Production of nuisance odours and toxins,
- Increase in disinfection by-products (e.g. THM's),
- Formation of scum,
- Increase in chlorine demand and
- Increase in biofilm growth in the distribution network (Palmer, 1980; Recknagel *et al,* 1997; Basson, 2000; van Ginkel *et al*, 2001; Chen and Yeh, 2005; Swanepoel *et al,* 2008a; Hart and Wragg, 2009).

When producing potable water it is of critical concern to produce water free from revolting odours and tastes. Generally when algae are present in the source water taste and odour problems can occur (Palmer, 1980). Different odours are produced by different algae, for example a grassy odour is produced by green algae but seldom by diatoms or pigmented flagellates. A musty odour is mostly produced by cyanobacteria. Algae by itself are not always necessarily responsible for taste and odour problems. Sources that may cause taste problems can originate from inorganic compounds whereas organic compounds are mostly responsible for odour problems (Robison, 2007). According to Palmer (1980), the dinoflagellate *Ceratium* sp. produces a fishy odour when the community is in a moderate size and a stale odour when the community is large and abundant. The morphological and physiological characteristics of algae play an important role in their behaviour in water treatment (Basson, 2000; Chen and Yeh, 2005). Some of these morphological characteristics are responsible for poor floc formation and ineffective removal, such as:

- The shape and size of algal cells and presence of spines;
- Having a low specific density and negative surface charge;
- Presence of flagella and

- Mechanically resistant cell coverings (e.g. pellicle plates and bands, frustules, thecal plates).

Ceratium hirundinella has unique characteristics that prevent its removal by conventional water treatment processes. The morphological structure of this alga protects the cell from coagulant chemicals and rendering it less susceptible to charge neutralisation (Basson, 2000; Swanepoel *et al,* 2008a). This large alga is covered with theca plates and has a distinctive spiny structure that tends to avoid stable floc formation. Another morphological advantage of this alga is having flagellae and the ability to migrate in the water column (Heaney, 1976; Baek *et al*, 2009). It is speculated that C*. hirundinella* can swim out of flocs and thereby break up the already formed flocs during flocculation and therefore increasing the load on the sand filters. High concentrations of *C. hirundinella* can cause clogging of sand filters. This result in inadequate filtration, i.e. more algae, invertebrates and other small debris will be able to penetrate the final drinking water (Swanepoel *et al,* 2008a; Hart and Wragg, 2009). *Ceratium hirundinella* is large in size and contains more chlorophyll and other pigments than algal cells such as *Anabaena* sp. According to Sigee (2005) the biovolume of a single *C. hirundinella* cell estimated from linear measurements is 40 000 μm^3, in comparison with *Anabaena flos-aquae* that has a single cell biovolume of 2165 μm^3. Thus, the breakthrough of *C. hirundinella* together with other algae will result in high chlorophyll values in the final drinking water causing it to be aesthetically unacceptable to customers.

At Rand Water the presence of this dinoflagellate in the drinking water increase was noted in 2006, during the routine invertebrate analyses, where 5 m^3 of drinking water is filtered through a 50 µm mesh filter to entrap invertebrates in drinking water for identification and enumeration (Swanepoel *et al*, 2008a). Entrapment is done to identify and enumerate the invertebrates that penetrate into the final drinking water. It was during the summer of 2006 and 2007 that Swanepoel *et al* (2008a) found large amounts of brown residue that was identified as *C. hirundinella* cells in these invertebrate samples. It has also been observed that since 2006, the frequency and occurrence of *C. hirundinella* has increased significantly in the Vaal River catchment especially during spring and autumn. The study indicated that the Rand Water purification plant that receives water via the 20 km open canal, had the most algal breakthroughs. This algal breakthrough could possibly be because of additional algal growth since the water in the canal is still exposed to sunlight and the algae can photosynthesize and grow, whereas the other Rand Water purification plant receives water through closed pipelines which do not facilitate algal growth and therefore algal breakthrough is found to be less.

Swanepoel *et al* (2008a) found that the phytoplankton biomass were high in the source water, and decreased after coagulation, flocculation and sedimentation. However, an increase of phytoplankton biomass was found after rapid sand filtration. Swanepoel *et al* (2008a) mentioned that this might have occurred because of algal growth in the sand filters or the use of

poly-electrolyte as coagulant together with the re-circulation of all the filter backwash water from the treatment plant to the head of the works.

2.4. Management strategies for conventional treatment processes during dinoflagellate blooms

2.4.1. Predicting dinoflagellate blooms by developing and using a rule-based hybrid evolutionary algorithm (HEA) model

The development and investigation of ecological models to predict and control algal blooms have become more popular and can potentially also be implemented at water treatment facilities such as Rand Water. These predictive models will enable the water treatment plant operators to be prepared and be forewarned when an algal bloom occurs so that additional treatment options (such as pre-chlorination) can be implemented.

Van Ginkel *et al* (2007) developed a rule-based model based on the studies done by Recknagel *et al (*1997) to predict high concentrations (blooms) of dinoflagellates as biomass volume. This rule-based developed hybrid evolutionary algorithm (HEA) model relies on different input data such as TP (Total Phosphates), DIP (Dissolved Inorganic Phosphates), secchi disk depth, pH, TN (Total Nitrogen), DIN (Dissolved Inorganic Nitrogen), SO_4, chlorophyll-*a* (the most important factor) and dinoflagellate biomass as input drivers. The model was tested by real time data and allowed the model to generate future predictions of possible blooms based on the input data. It was found that the model predicted the maximum dinoflagellate biomass relatively accurately, however, under- and over-predictions were found, but even so the maximum biomass peaks were predicted correctly (van Ginkel *et al*, 2007). The designing of the model took many environmental factors into account, such as water temperature, chlorophyll-*a* concentrations and availability of nutrients. The model indicated the importance of the temperature range (5 – 17 ˚C) and DIP concentrations (< 82 µg/L) during low dinoflagellate biomass (van Ginkel *et al*, 2007). Van Ginkel *et al* (2007) also found that TN is the determining factor for the extent of the dinoflagellate bloom.

Van Ginkel's model was tested on the Albert Falls Dam in KZN (Hart and Wragg, 2009). However, with this particular case study the model was only casually tested without any training beforehand. The authors found that the observed and predicted values were different. The values were normalised and a broad sequential concordance was found when *C. hirundinella* was present. An inaccurate prediction of the continuing presence of the alga was found. However, the observed and predicted values correlated overall. There was also a correlation in the period where analyses were restricted when *C. hirundinella* was present. As the model uses chlorophyll values as the major input driver it has been found that when *C. hirundinella*

was absent and did not contribute to the chlorophyll values the model still predicted the alga's existence (Hart and Wragg, 2009). Hart and Wragg (2009) indicated that the model cannot distinguish the taxonomic origin of the chlorophyll level input driver.

This model has the potential to be developed and trained further as to provide more accurate results. A similar model may greatly assist Rand Water to predict dinoflagellate blooms, and thereby enabling plant managers to anticipate problems before they occur.

2.4.2. Optimisation of treatment processes

The goal of a water treatment plant is to produce water that is safe for consumption and fit for domestic, industrial, agricultural and commercial use. One of the challenges found today is the ability (or lack thereof) to abstract source water of a good quality, since most of the water sources are impacted by eutrophication or some type of pollution. Therefore, it becomes more and more difficult to produce water that is free from harmful synthetic organic substances and pathogens, when only conventional water treatment options are available.

Water treatment consists of various stages or processes to achieve the desired water quality. Rand Water uses conventional water treatment that is a series of processes to remove suspended and colloidal particles from the water, stabilising the water chemically and finally disinfecting it before it enters the reticulation network. The unit processes found and coagulants to be used within such a plant greatly depend on the source water quality.

Ways to optimise conventional treatment processes (Basson, 2000; Swanepoel *et al*, 2008a; MECC, 2009a):

- Optimise coagulant dosing by performing regular jar tests;
- Optimise treatment plant by adding process units e.g. pre-chlorination or intermediate chlorination; and
- Optimise filter performance (including back-wash optimisation and disinfection of sand filters).

2.4.2.1. Optimise coagulant dosing by performing regular jar testing experiments

Jar testing is a small scale laboratory procedure that demonstrates the physical conditions (coagulation, flocculation and sedimentation) found in a conventional water treatment plant (Figure 2.5). In each of the jar testing beakers, a different concentration of coagulant chemical is tested and the results compared to find the optimum or appropriate coagulant concentration that is needed for successful water purification. Water purification plants laboratory determines the lowest coagulant dosage concentration that is removing sufficient (not necessarily optimum) amounts of particulate matter, the term "appropriate dosage" (to be used by the plant) is used in

the present study. This takes into account that increasing dosage of coagulant chemicals, may most probably remove more particulate matter, but the financial implications will be such that it is not worth using the “optimum” concentration of the coagulant chemicals. It is imperative that this test resembles the conditions found on the water treatment plant. Thus, the stirring speed and duration should be determined and be similar to the operations of the plant (Hach Chemical Company, unknown). As water quality mostly differs on a daily basis, the operator must regularly perform this procedure and adjust the coagulant dosing (MECC, 2009a). The purpose of jar testing procedures includes (Poland and Pagano, 1998; Satterfield, 2005):

- The determination of the appropriate coagulant concentration, thereby predicting the dosage of a large scale treatment plant,
- The reduction of consumption of chemicals and costs of the water treatment process,
- It also allows one to control the pH, mixing speed and testing different coagulants and coagulant aids.

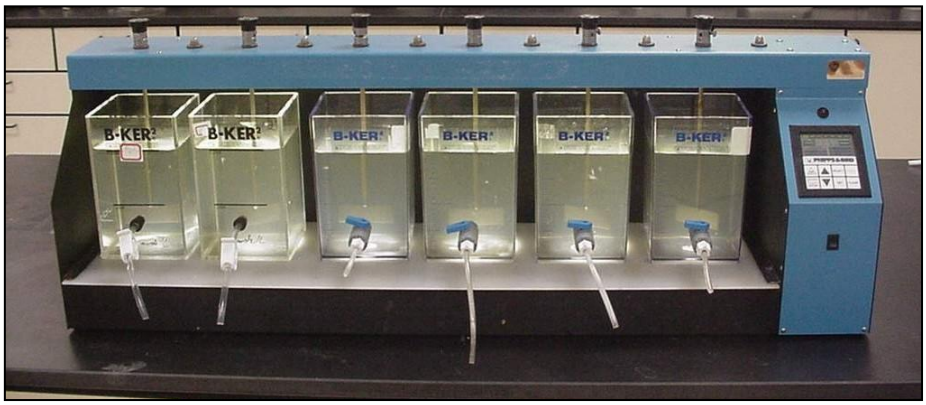


Figure 2.4: Example of a common jar testing apparatus.

2.4.2.1.1. The jar testing process:

First stage: Coagulant addition & rapid mixing

The jar testing apparatus (Figure 2.4) simulates the water purification process of the first stage that involves violent and rapid mixing of the coagulant with the untreated or source water for approximately two minutes. Rapid mixing is an essential process that greatly affects the flocculation process, and therefore it is suspected that the rate of coagulant adsorption also depends on the mixing conditions (Bolto and Gregory, 2007).

Different coagulant chemicals are used depending on the source water quality and budget of the water treatment company. The most commonly used chemicals are aluminium sulphate, lime, poly-electrolytes and iron salts which include ferric chloride and ferric sulphate. These

coagulant chemicals are either inorganic (e.g. aluminium sulphate) or organic compounds (e.g. poly-electrolytes). Cationic poly-electrolytes can be dosed to many different types of source water. These poly-electrolytes have the ability to destabilise and form bridges between molecules simultaneously (Leopold and Freese, 2009).

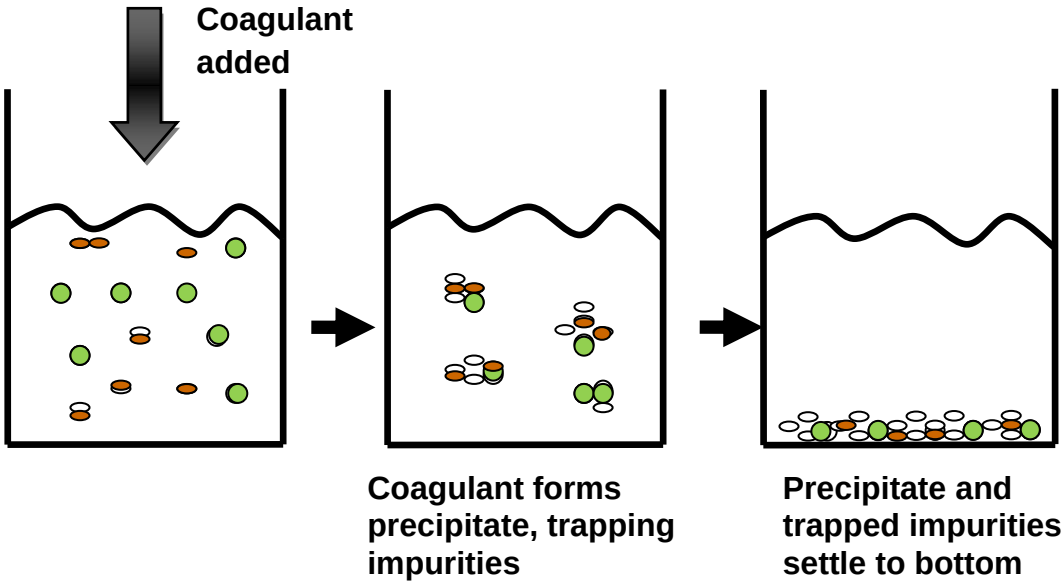


Figure 2.5: Jar testing procedure illustrating coagulation, flocculation and settling of flocs (MECC, 2009b).

Coagulant aids are used to improve coagulation and assist in the removal of turbidity. Ions will attach to the negatively charged colloidal particles and help with floc formation. Examples of coagulant aids include activated silica, weighting agents, lime, poly-electrolytes and iron salts such as ferric chloride and ferric sulphate. Underdosing of the coagulant chemical causes the final water to have high turbidity and increased colour levels, whereas overdosing results in resuspension of impurities and coagulant carryover to the filters, this may result in increasing backwash frequency (Bolto and Gregory, 2007). It has also been found that dosing poly-electrolyte as coagulant on the long-term, may increase the risk of mudball formation in the filters (Bolto and Gregory, 2007). Overdosing of a coagulant may also induce algal cell lyses and thereby releasing organic compounds (Henderson *et al*, 2008).

The optimum dosage is where coagulants cause particulate matter to combine large flocs that can settle during sedimentation. It should be noted however, that the “optimum” dosage will not necessarily be the “appropriate” dosage used in the plant. Although the optimum dosage would remove more colloidal material the lowest concentration that removes sufficient colloidal material (not necessarily the optimum) would be dosed due to financial implications. The response of the cell to the coagulant chemical has been found to be species specific

(Henderson *et al*, 2008). No literature has been found to indicate the response of the different coagulant chemicals used in this study specifically on *C. hirundinella.*

Coagulant chemicals are added and rapidly mixed into the source water to neutralise the electrical charges of particles in the water. These particles are very small and usually carry a negative charge and require a coagulant chemical with a positive charge to stabilize the water. Water with a higher turbidity is easier to treat since the particles are close to each other and can easily collide with each other. The primary coagulant is responsible for coagulation and neutralisation of the electrical charges. Coagulant aids assist in floc formation by increasing its stability and density (MECC, 2009b). As illustrated in Figure 2.6, particles in water carry a negative charge which will be neutralised by the positively charged coagulant. The zeta potential of particles should be considered. Zeta potential measures the amount of an electrical charge that surrounds the surface of the non-settleable or colloidal particle (Colloidal Dynamics, 1999). Thus, the amount of coagulant that should be added will be determined by the zeta potential. The higher the zeta potential, the more coagulant is needed (MECC, 2009b).

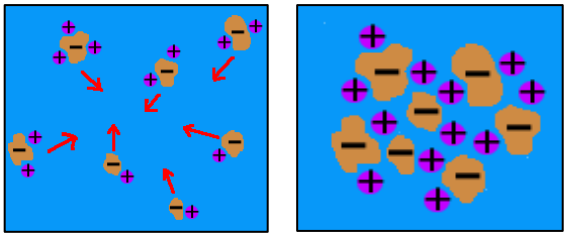


Figure 2.6: Negatively charged colloidal particles will bind with positively charged coagulant and neutrally charged particles will attract each other as a result of van der Waals forces (left) and will then aggregate to form larger flocs (right).

Second stage: Flocculation

Flocculation is a process where water is mixed slowly and gently to assist in the building up of floc particles. During the flocculation stage, the stirring speed is reduced and water is slowly mixed for a pre-determined time (also to simulate the mixing rates in the plant). The rate at which chemicals are mixed is determined by the rate at which the particles collide. If the mixing is slow, the flocs will grow slowly. The reduced mixing rate will promote floc formation by increasing particle collisions and flocs will increase in size. The slow mixing rate will also prevent sheering of the flocs. The flow of water into the flocculation chamber is controlled by baffles. Most flocculators are designed for taper flocculation. Taper flocculation causes the reduction of velocity as water passes through (Schutte, 2006).

Final stage: Sedimentation

The final stage of jar testing is the settling period where the stirring is stopped and the flocs are allowed to settle. According to MECC (2009b), the best floc size is 0.1 to 3 mm, flocs smaller or larger than that would not settle at all and may remain in the supernatant. After a certain time samples are removed by withdrawing the clear supernatant, turbidity can be measured to determine the effectiveness of the coagulation, flocculation and sedimentation processes.

2.4.2.1.2. Some of the coagulants and coagulant aids Rand Water use

Poly-electrolytes

Poly-electrolyte coagulants are high-molecular-weight molecules and consist of a very large structure containing monomer units that are repeated in a pattern. Poly-electrolytes are water-soluble and do not affect the pH of the water. It can have both positive and negative charged sites on the polymer chain or are classified as cationic polymers (overall positively charged) or anionic polymers (overall negatively charged) (Freese *et al*, 2004, Leopold and Freese, 2009). Cationic poly-electrolytes are commonly used during water treatment and have some benefits over other coagulants. Rand Water currently uses different cationic polymers, examples include *Südfloc 3850* and *Zetafloc 650*. Occasionally quicklime (CaO) is also used as a coagulant aid in combination with a polymer.

There are different flocculation mechanisms of particles when using poly-electrolytes as coagulant, which include polymer bridging, charge neutralisation and depletion flocculation. Poly-electrolyte adsorption between oppositely charged ionic groups is known to be strong and to occur in a flat equilibrium configuration with no loops and tails (Bolto and Gregory, 2007). According to Bolto and Gregory (2007) polymer bridging can form much stronger flocs in comparison with the other flocculation mechanisms charge neutralisation and depletion flocculation, this may be due to the flexibility of the links that permit it to stretch before flocs break up. According to Bolto and Gregory (2007) ageing of polymer solutions can influence the flocculation performance, also resulting in a reduction of viscosity and molecular weight. The optimum poly-electrolyte dosage will produce stable flocs, i.e. the coagulant neutralise the opposite charged particles or achieve a zeta potential close to zero.

Advantages of poly-electrolytes

The dosing of poly-electrolytes save costs since it can be dosed in lower concentrations (due to their high charge density) and reduce the dosage of other chemical coagulants (Freese *et al*, 2004). Other advantages include the formation of large and dense stable flocs that settle easily as well as the formation of less sludge and clearer supernatants (Bolto and

Gregory, 2007). Poly-electrolytes work very well in different types of source water. Studies done by Basson (2000) indicated that increasing dosage or using poly-electrolyte as coagulant will remove algae effectively. The effectiveness of the poly-electrolyte depends on its molecular weight together with its charge density (Freese *et al*, 2004).

Disadvantages of poly-electrolytes

According to Hamilton *et al* (1994), the higher charged a polymer, the more soluble it is and consequently more bioavailable to aquatic organisms such as algae. According to Bolto and Gregory (2007) cationic polymers are more toxic to aquatic organisms than anionic or non-ionic polymers. It has been found that fish are very sensitive to cationic polymers - it causes suffocation by gill blockage. Another disadvantage of using poly-electrolytes is that it may contain contaminants derived from its production process that may be harmful to human health (Freese *et al*, 2004). Cationic polymers can produce disinfection by-products (DBP's) such as trihalomethanes (THM's) when exposed to chlorine as part of pre-chlorination, while little DBP's are produced when polymers and chlorine are dosed in normal levels during post-chlorination (Bolto and Gregory, 2007).

Rand Water has found that the dosing of poly-electrolyte for extended periods of time encourages the growth of benthic green algae (mainly *Cladophora* sp.) in the flumes and sedimentation tanks on the plant[2]. A critical problem found in sand filters is the presence of mud balls which, in many cases, is caused by the overdosing of poly-electrolytes as coagulant chemical and exacerbated by the presence of mucus producing algae in the sand filter. This increased algal loading can result in reduced filter running times and increased backwashing (Palmer, 1980; Bolto and Gregory, 2007). The increased number of mud balls is known to reduce the effectivity of the sand filter to retain particulate matter including algae and invertebrates and therefore causing their penetration into the drinking water. The reduction in pennate diatoms are important as pennates are also known to produce mucilage, which together with the dosing of polyelectrolyte as coagulant chemical (Arendze and Geldenhuys, 2009) most probably can increase the formation of mud balls in the sand filters.

It is known that poly-electrolytes do not influence the pH of water, posing a major disadvantage when removing high concentrations of algae, invertebrates and microorganisms from the source water (Bolto and Gregory, 2007) as algae are relatively sensitive to pH changes. It seems to aid in the coagulation, flocculation and sedimentation of specifically algae if the pH is raised (e.g. dosing slaked lime) during water purification (Knappe *et al*, 2004). According to Steynberg *et al* (1994) the use of poly-electrolyte should be limited since high dosages are needed for high

[2] Swanepoel, A., 2010. [Conversation], Connection of Poly-electrolyte and algae in Rand Water's treatment plant. (Personal Communication: 10 May 2010).

turbidity water and that water produced by poly-electrolyte treatment is more corrosive and of lesser quality to that produced when using lime and activated silica.

Activated silica

Activated silica is an anionic inorganic coagulant aid used to improve coagulation, widen the pH range, and decrease the amount of coagulant dosage needed (Sidorkiewicz, Unknown). This flocculant is received as sodium silicate (Na_2SiO_3) and has to be activated by adding an acid (e.g. hypochlorous acid) to reduce the pH.

Advantages of activated silica

Activated silica forms strong stable flocs that settle quickly, enhances colour removal and allow good floc formation at low temperatures (AWWA, 2003; Leopold and Freese, 2009).

Disadvantages of activated silica

Sodium silicate solution can gel if the preparation method is not carefully followed. It has been found that when dosing too much silica it will affect the floc formation and cause filter clogging (AWWA, 2003).

Lime

Lime is used to stabilise corrosive water, increase alkalinity and adjust the pH. Rand Water uses lime as a coagulant chemical because it is inexpensive and easily accessible. It is usually received as limestone ($CaCO_3$). The $CaCO_3$ stones are crushed and heated in kilns to produce quicklime and carbon dioxide, the following reaction takes place during this process:

$$CaCO_3 + Heat\ (1200°C) \rightarrow CaO + CO_2$$

The quicklime are then mixed with the source water to react and produce slaked lime also known as hydrated lime ($Ca(OH)_2$), the following reaction takes place:

$$CaO + H_2O \rightarrow Ca(OH)_2$$

The reaction is exothermic and the heat released during this process causes temperatures to range from 70 – 80 ˚C. At Rand Water, lime dosage is normally around 60 mg/L as quicklime (CaO). If lime is not available, ferric chloride (4 mg/L) is used at Rand Water. After the addition of lime, pH increases to values between 10 and 11.

Advantages of lime

At a pH between 10 and 11, most of the heavy metals, bacteria, viruses and some organic matter are effectively removed. Chemical precipitation processes also occur due to the presence of cationic ions such as calcium and magnesium, and for example, magnesium is precipitated as magnesium hydroxide ($Mg(OH)_2$). Due to the high pH, an environment is created where algal growth is limited and as a result most algae are killed and form part of the flocs which are removed during the sedimentation stage. One of the benefits when using lime as coagulant aid is the removal of iron that also aids in the clarification of water.

Disadvantages of lime

Due to the increased pH, with the use of lime, the pH needs to be lowered again before the final treated water enters the distribution network, to prevent scaling of the pipes if the pH is not lowered. At Rand Water, the pH is generally lowered by means of CO_2 gas and if CO_2 gas is not available lime cannot be dosed as coagulant chemical.

2.4.2.2. Optimise filter performance

The increase of *C. hirundinella* and other algae (especially cyanobacteria) in the source water greatly impacts the treatment of drinking water, causing problems such as filter clogging. Swanepoel *et al* (2008a) suggested disinfection with chlorine (HTH) of all the sand filters at Rand Water purification plant during summer months when cyanobacteria are most abundant which can eleveate the problem. They also recommended that the normal cycle of two minute air scouring and five minute backwash should be replaced with a two minute air scouring and two minute backwash followed by another two minute air scouring and three minute backwash cycle. In this way more organic material will be removed and the filter performance will improve in the long run. Swanepoel *et al* (2008a) also suggested that the re-use of filter backwash water at Rand Water purification plants should be avoided during times of high concentrations of cyanobacteria. Since not all the organic substances can be removed during purification and will then be re-introduced into the plant.

2.4.2.3. Disinfection and additional treatment processes such as pre-chlorination

Disinfection is a process where disinfectants or oxidants are added to destroy or inactivate most pathogens or disease-causing microorganisms in order to ensure a safe water supply. Chlorine damages or destroys the cell membrane, DNA and enzymes, affecting the physiological cellular

processes (Chen and Yeh, 2005; Leopold and Freese, 2009; Van der Walt *et al*, 2009). According to Sukenik *et al* (1987) as cited by Chen and Yeh (2005), dosing chlorine at different concentrations have a distinctive effect on the cell structure of algae, causing the cells to rupture (lyse) and to release cellular organic compounds. It is desirable to remove or inactivating algae without cell rupture. Commonly used disinfectants are chlorine (Cl_2), monochloramine, chlorine dioxide (ClO_2), ozone (O_3), calcium hypochlorite/sodium hypochlorite and ultraviolet light. The selection of disinfectant is based on various factors, e.g. the water quality of the source water, the presence of harmful microorganisms or algae, the length of distribution network, and the residual chlorine needed.

Rand water uses chlorine gas as the primary disinfectant. It has the ability to react with natural occurring organic compounds and produce disinfectant by-products (DBP's) such as trihalomethanes (THM's) in the presence of organic material. Chlorine reacts with water to form hypochlorous acid and hydrochloric acid as shown in this reaction:

$$Cl_2 + H_2O \rightarrow HOCl + HCl \text{ (hydrolysis)}$$

Hypochlorous acid can dissociate or ionize to form hydrogen ions and hypochlorite ions ($HOCl \rightarrow H^+ + OCl^-$). Disinfection steps can be added at different stages during the water treatment process, e.g. at the beginning (pre-oxidation), midway (intermediate oxidation) or at the end of the process (post-oxidation). This is done to control microbial and algal growth as well as the concentrations of organic contaminants throughout the plant (Leopold and Freese, 2009). Rand Water uses chlorination at the end of the treatment process. During the disinfection stage there should be excess chlorine added to ensure that free chlorine remains in the water. The free chlorine assists in the elimination of remaining pathogens along the pipeline to the booster station.

In the secondary disinfection process, a less powerful disinfectant is added which will remain active for a certain period to protect the water until it reaches the consumer. At Rand Water, chlorine and ammonia are dosed at the booster pump stations to form monochloramine (Rand Water, 2011b):

$$NH_3 + Cl_2 \rightarrow NH_2Cl + HCl$$

Although monochloroamine is less reactive, it has the ability to prevent bacterial re-growth for up to 8 days (Rand Water, 2011b). This dosing rate of chlorine/ammonia is maintained when water enters the municipal distribution system. Chlorination is dependent on different factors influencing its effectivity, namely pH, temperature, chlorine concentration and contact time.

Pre-chlorination involves the addition of chlorine at the head of works, before the water enters the coagulation/flocculation stage, i.e. before any coagulant is added.

Advantages of pre-chlorination

This is done to disinfect the water, to control tastes and odours, and to enhance the flocculation of algae. Pre-chlorination is commonly used when there are high concentrations of algae in the source water. According to Leopold and Freese (2009) dead algal cells flocculate much better and therefore settles much easier than live algal cells. Chlorine is not only effective in killing algae but it will also bleach or oxidise the chlorophyll present, breaking the double bonds in the chlorophyll molecule (Van der Walt *et al*, 2009). Pre-chlorination is also used to remove iron and manganese in source waters (Leopold and Freese, 2009).

Other advantages of pre-chlorination are (BCWWA, 2010):

- Improved filter operation - a reduction of bacterial and algal load; and
- Improved coagulation.

Disadvantages of pre-chlorination

According to Basson (2000) it has been found that pre-chlorination could be inadequate in removing algal cells at high pH levels. Pre-chlorination has some other disadvantages such as (Basson, 2000; Leopold and Freese, 2009):

- Increased chlorine demand for disinfection;
- Impairing of flocculation caused by a release of algal organic matter thereby increasing coagulant demand;
- Formation of lower molecular weight organic material which are difficult to remove;
- Increase of THM concentrations in the final water;
- Release of algal toxins; and
- Increase of taste and odour problems caused by the rupture of cyanobacteria cells.

Basson (2000) found that if pre-chlorination is excluded from the treatment process an increase of the coagulant pH will result in a more effective removal of chlorophyll-*a*. Basson (2000) also found that intermediate chlorination might be more effective than pre-chlorination, especially if it is desired to remove more organic substances and reduce THM formation.

Chlorination inhibits physiological processes within the algae and renders it less mobile or totally immobile. Including additional chlorination may not necessarily remove more algae especially from waters with high diatom biomass. Diatoms have strong silica cell walls that increase their resistance to chlorine (Wetzel, 2001). Basson (2000) found that the algal removal rates were almost similar for intermediate and pre-chlorination. The studies indicated that pre-chlorination removed more green algae, although results were similar than those found with intermediate chlorination (Basson, 2000).

Basson (2000) did not specifically look into the effect of pre-chlorination on *Ceratium hirundinella.* Due to the problems experienced with coagulation and flocculation when *C. hirundinella* is present in the source water of Rand Water, this study will investigate the effect of chlorine on the mobility of *C. hirundinella*. Swanepoel *et al* (2008a) suggested that pre-chlorination might be the only treatment that could effectively aid in removing *C. hirundinella* during sedimentation.

CHAPTER 3

SAMPLING SITES, MATERIALS AND METHODS

3.1. Sampling sites

3.1.1. C-VRB5T, 5 km upstream from the Barrage weir

The Vaal River Barrage Reservoir was built by Rand Water in 1923. The reservoir has a storage capacity of 63 million litres and a surface area of 168 km^2 (Rand Water, 2011a). Rand Water does not abstract water from the Vaal River Barrage any longer since the water quality of it has significantly deteriorated over the last few years due to biological and chemical pollution. Rivers such as the Klip, Rietspruit, Waterval, Suikerbosrand, Taaibosspruit feed the Vaal River Barrage and flow through industrial and mining polluted areas such as Johannesburg and Sasolburg. The Vaal River Barrage is currently used for recreational purposes such as boating, skiing, swimming and fishing. The sampling point from which data was used to perform multivariate statistical analyses, was C-VRB5T (a Rand Water catchment sample in the Vaal River at 5 km upstream from the Barrage weir, close to the Vaalview Aquatic Club) (Figures 3.1, 3.2 and 3.3). This site was chosen because it had the most available environmental data where *Ceratium hirundinella* occurred frequently.

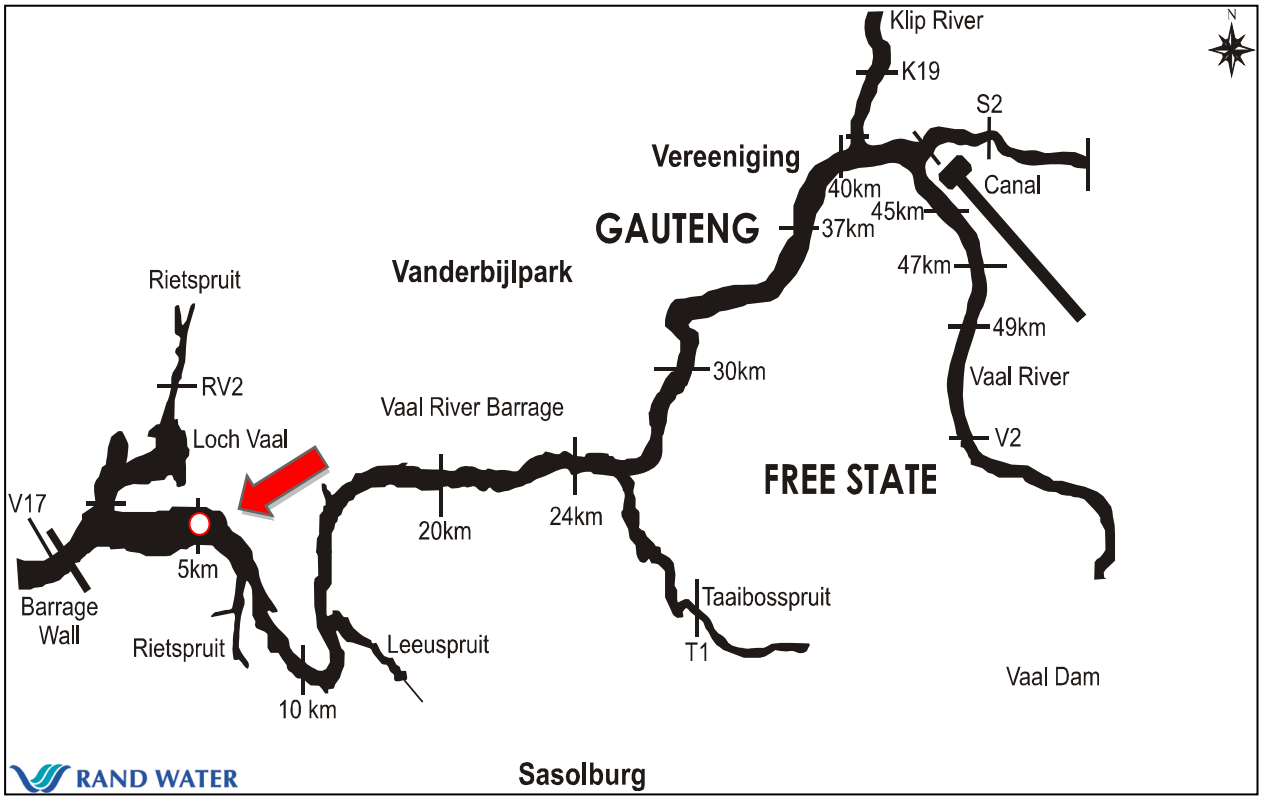


Figure 3.1: Map, indicating in white, the sampling point (C-VRB5T) in the Vaal River at 5 km upstream from the Barrage weir.

Figure 3.2: Picture of the sampling point (C-VRB5T) in the Vaal River at 5 km upstream from the Barrage weir.

Figure 3.3: Picture of developments and recreational activities found in the surrounding area of sampling point C-VRB5T.

3.1.2. M-FOREBAY, Zuikerbosch purification plant

The Vaal Dam is Rand Water's major supply of source water and a 20 km open canal (sampling point: M-CANAL) supplies water from the Vaal Dam into the Forebay (sampling point: M-FOREBAY) that has a capacity of 500 ML. Water from the Forebay enters the Zuikerbosch purification plant (Figure 3.4 and 3.5, point A). The sampling point M-FOREBAY was chosen due to its proximity to the Zuikerbosch plant and sampling was done twice a month from April to October 2010 by Rand Water samplers. This site was chosen because *Ceratium* has been known to break through into the final drinking water at the purification plant (Ewerts, 2010). Approximately 50 L of water was sampled to perform the different jar testing analyses, as well as extensive chemical and biological analyses of the water.

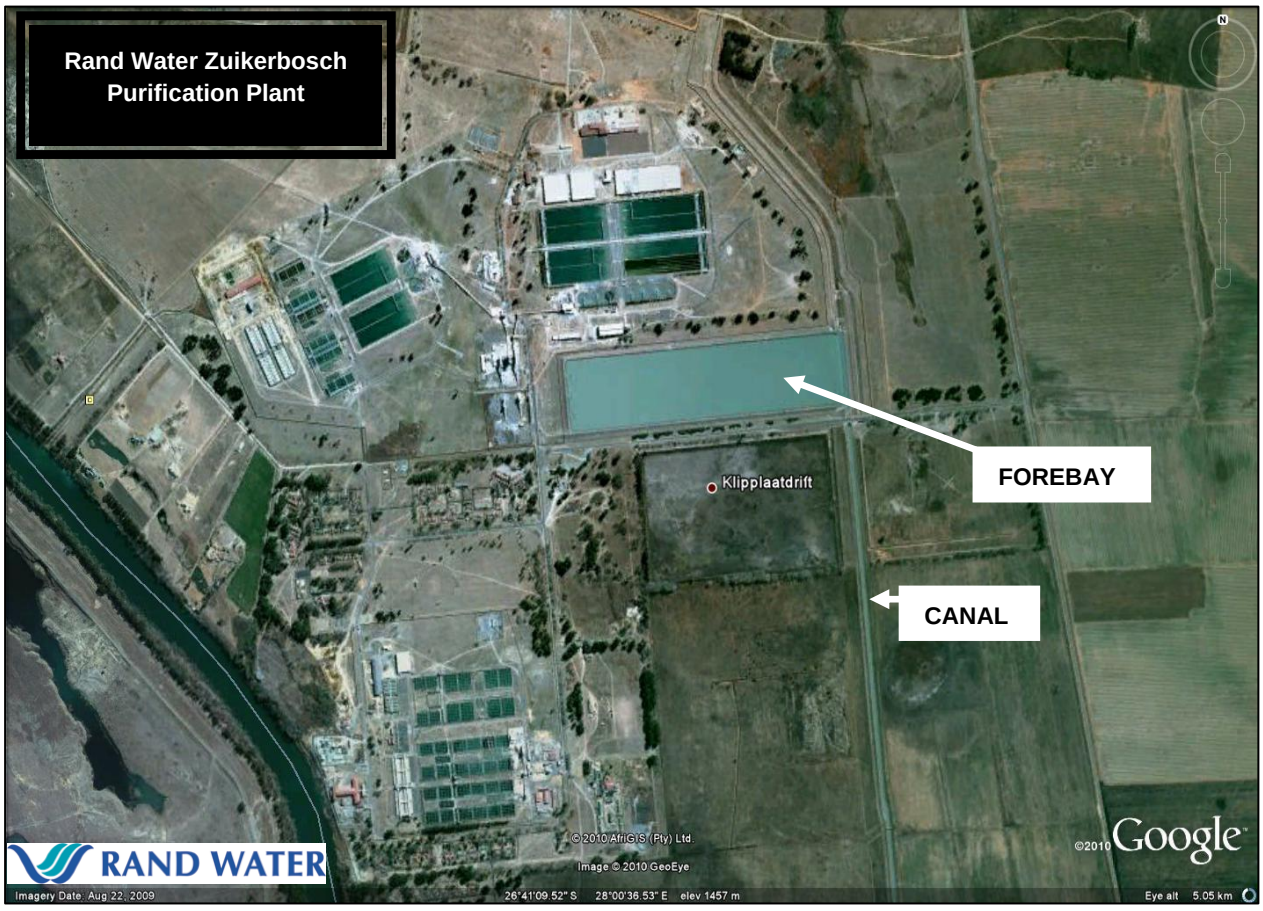


Figure 3.4: Satellite image showing the open canal (CANAL) flowing into the Forebay before water enters the Zuikerbosch purification plant (Google Inc., 2010).

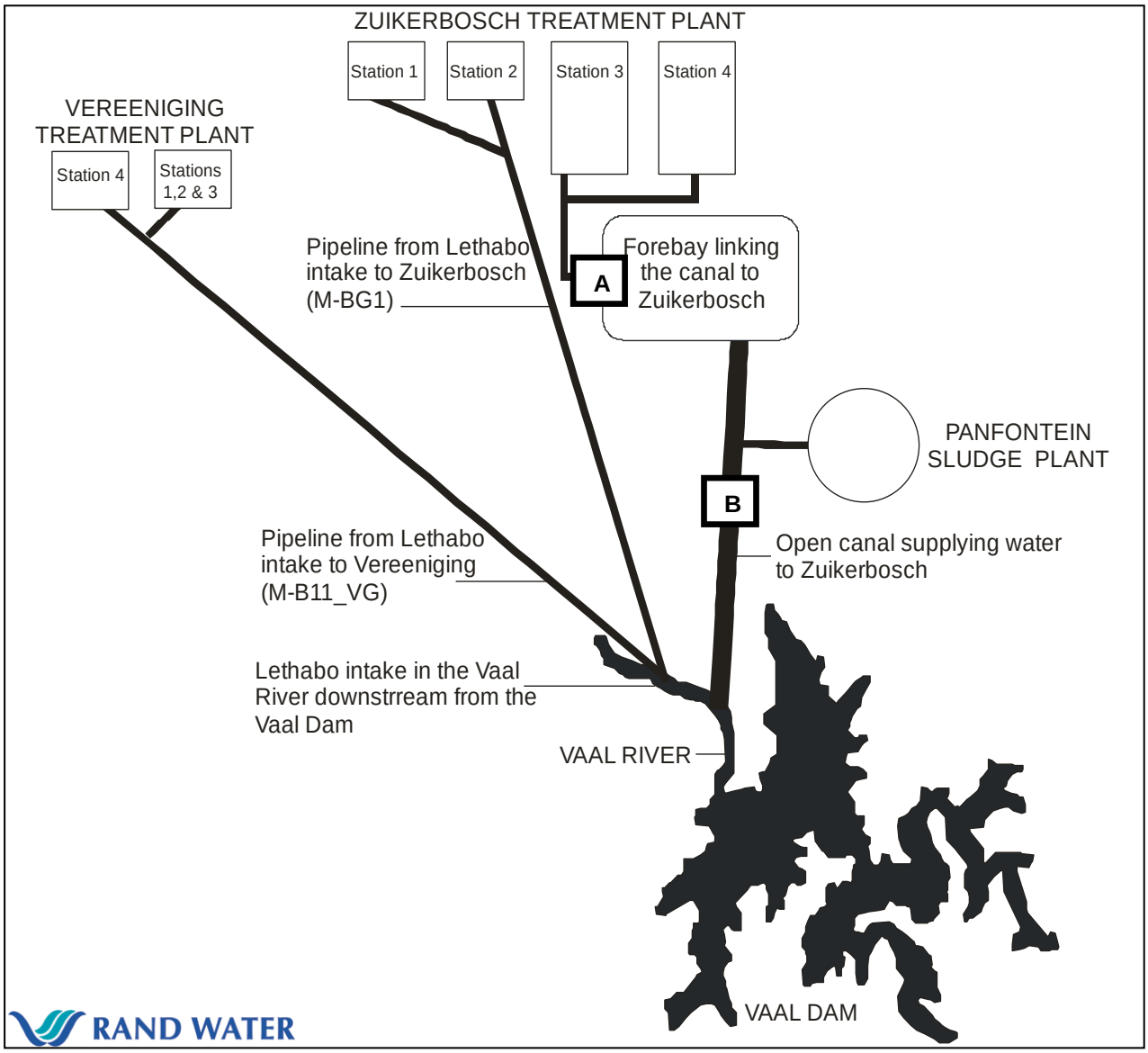


Figure 3.5: Water supply chain to Rand Water's purification plants from the Vaal Dam, indicating A, the sampling point M-FOREBAY and B, sampling point M-CANAL_VD, before recovered water from Panfontein is mixed with water from the Vaal Dam.

3.1.3. M-CANAL_VD, upstream from M-FOREBAY

After performing numerous jar testing experiments with source water from M-Forebay, weak floc formation and settling were constantly observed when using activated silica in combination with CaO. Due to these results it was decided to perform jar tests with source water from the sampling point, M-CANAL_VD. The sampling point M-CANAL_VD is located before the entrance of the recovered water from Panfontein (Rand Water's Sludge Disposal Site) (Figure 3.5, point B). Approximately 50 L of water was sampled on the 05/01/2011, to perform the different jar testing analyses, as well as chemical and biological analyses of the water.

3.1.4. M-RAW_VAALKOP, Vaalkop Dam

Vaalkop Dam is situated in the North West Province, between Brits and Pilanesberg (Figure 3.6). The dam lies within a nature reserve on the Elands River and has a surface area of 1110.5 Ha at full supply level (Rustenburg Local Municipality, 2007). Vaalkop Dam supplies source water to Magalies Water's Vaalkop Treatment Plant that provides potable water to Rustenburg and the surrounding areas. The dam is shallow with numerous islands (Figure 3.7a). It is an attractive site for angling, boating and bird watching. However, commercial farming is practised in the vicinity of Vaalkop Dam, the use of fertilizers causes an increase of nutrients in the dam (via runoff during rainstorms) and may contribute to eutrophication of the dam and the formation of algal blooms (Rossouw *et al*, 2008).

The Vaalkop Dam form part of Rand Water's monitoring programme, since Rand Water also supplies water to Rustenburg and water from Magalies Water and Rand Water are mixed for supply to Rustenburg. Sampling is done every second week by Rand Water samplers. The sampling point, M-RAW_VAALKOP was chosen due to a *C. hirundinella* bloom that occurred in the beginning of November 2010. On two occasions (03/11/2010 and 23/11/2010), approximately 75 L of water was sampled to perform two jar testing analyses and chlorine exposure experiments, as well as an extensive chemical analysis on the water. Data from October and November 2010, were also extracted from the Rand Water's LIMS Labware database, and organised in an Excel spreadsheet.

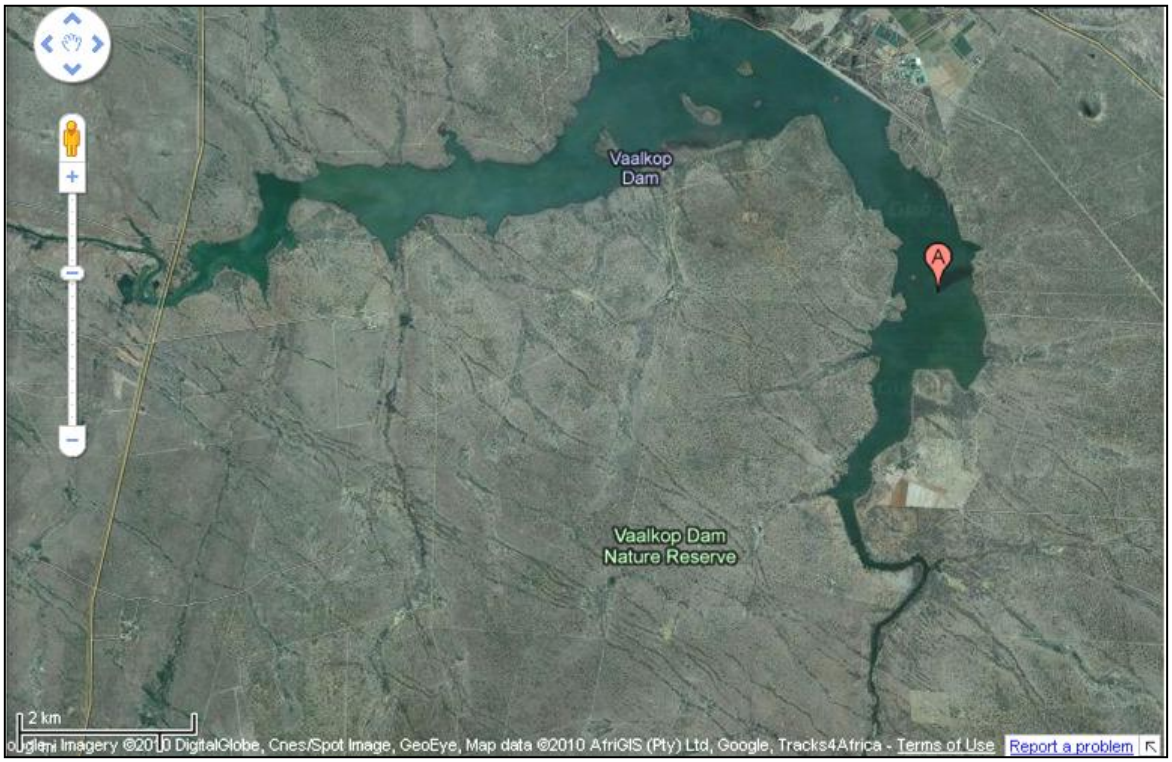


Figure 3.6: Satellite image of Vaalkop Dam located in the Vaalkop Dam Nature Reserve, 54 km North of Brits (Google Inc., 2010).

(a)

(b)

Figures 3.7 (a) and (b): Pictures of Vaalkop Dam showing (a) the islands and (b) the dam wall.

3.1.5. Rietvlei Dam

Rietvlei Dam is located on the south east side of the City of Tshwane. The Rietvlei Dam catchment is rather small (492 km^2). The Rietvlei water treatment works is located on the eastern side of the Rietvlei Dam. According to Coetzee *et al* (2010), the dam has a surface area of 204.13 Ha and a net capacity of 12 185 x 106 m^3. Agricultural activities are mostly found in this catchment area. This dam is of eutrophic nature and contains high concentrations of organic matter (especially phosphorus). Regular algal blooms occur as this dam also receives treated sewage water from Hartbeespoort waste water treatment works (Coetzee *et* al, 2010). This can contribute to taste and odour problems. Source water from Rietvlei Dam is one of the six water resources used (mixed with treated water from Rand Water) to provide Pretoria with drinking water (Coetzee *et al*, 2010). In July 2008, SolarBees have been installed in this dam as a lake management tool. SolarBees are solar powered water circulator positioned in the epilimnion layer to improve water quality – i.e. by habitat disturbance of the cyanobacteria. According to Coetzee *et al* (2010) SolarBees have numerous benefits such as a reduction in cyanobacterial blooms, a reduction in taste and odour and an increase of dissolved oxygen

levels in the water. During the summer of 2008 a significant *C. hirundinella* bloom occurred which reached a chlorophyll-*a* concentration of 681 µg/L (Coetzee *et al*, 2010). Another *C. hirundinella* bloom occurred during March/April 2009, with lower chlorophyll-*a* concentrations (200 µg/L) (Coetzee *et al*, 2010). The sampling point, Rietvlei Dam was chosen due to a *C. hirundinella* bloom that occurred in mid-February 2011. On two occasions (15/02/2011 and 21/02/2011), approximately 75 L of water was sampled to perform two jar test analyses and chlorine exposure experiments, as well as an extensive chemical analysis on the water.

Figure 3.8: Picture of Rietvlei Dam.

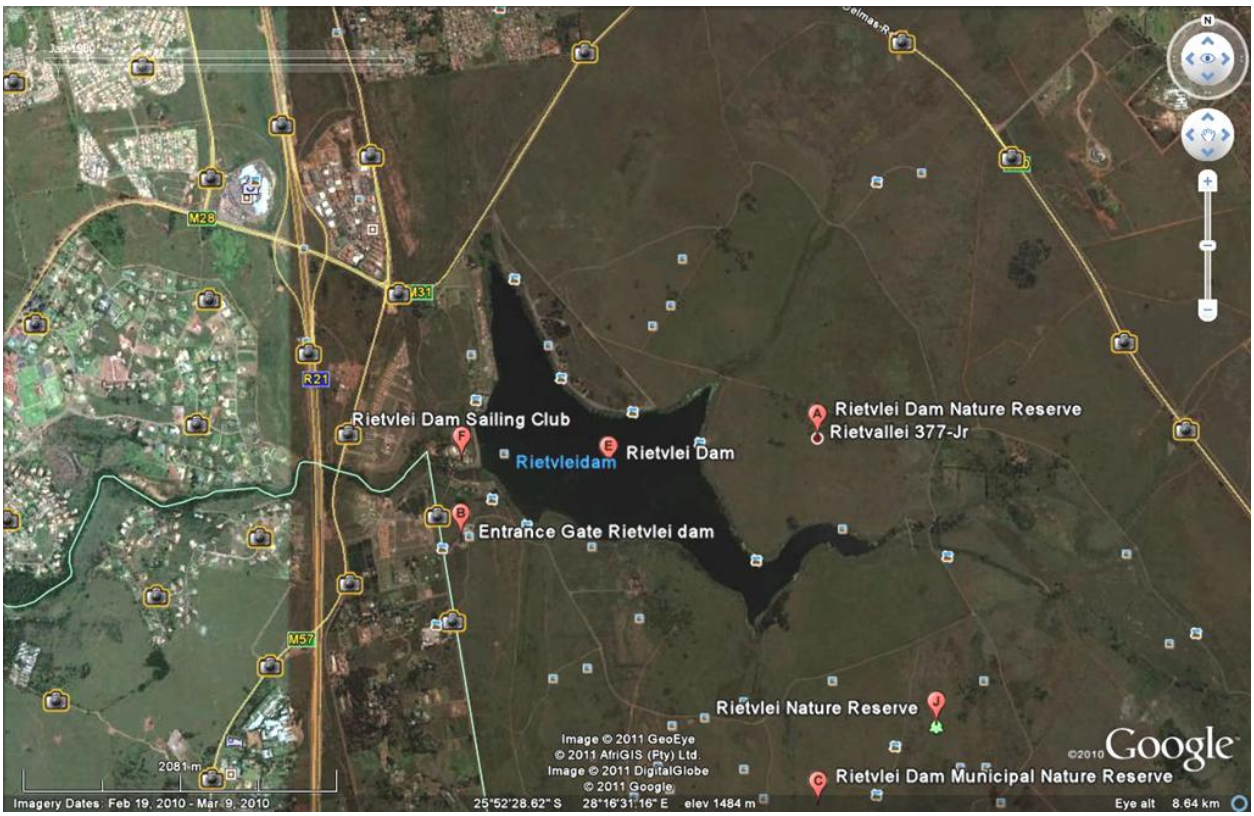


Figure 3.9: Satellite image of Rietvlei Dam located in the Rietvlei Dam Nature Reserve, 25 km from the Pretoria CBD (Google Inc., 2011).

3.2. Assessment of historical data of sampling points C-VRB5T and M-RAW_VAALKOP

To be able to determine the main environmental variables associated with *Ceratium hirundinella* blooms, ten years (2000 to 2009) for sampling point C-VRB5T and nearly eight years (2004 to February 2011) for sampling point M-RAW_VAALKOP, of physical, chemical and biological data were used. Data were extracted from the Rand Water's LIMS Labware database, organised and the algal species data were log-transformed in an Excel spreadsheet. Data reflected that samples were taken on a weekly or bi-monthly basis (C-VRB5T and M-RAW_VAALKOP respectively).

A Principle Component Analysis (PCA) was performed on all environmental data to determine the main environmental variables (principle components) at C-VRB5T and M-RAW_VAALKOP. A Correspondence Analysis (CA) and Canonical Correspondence Analysis (CCA) were also performed on algal and environmental variables to determine which environmental variables are responsible for the occurrences of *C. hirundinella* in the Vaal River and Vaalkop Dam. With regards to the algal species, it was decided to use only the species that occurred more than twenty times in the case of C-VRB5T, and more than ten times in the case of M-RAW_VAALKOP during the study period, since algae that occurred sporadic and in low

concentrations may cause a distorted image in the representation of the multivariate analyses (Ter Braak and Prentice, 1988; Legendre and Gallagher, 2001).

3.3. Assessment of treatment coagulants on source waters

In order to optimise treatment processes such as coagulation and flocculation, jar testing experiments were performed to investigate different coagulant chemicals (poly-electrolyte, poly-electrolyte in combination with slaked lime (CaO) and CaO in combination with activated silica). It is important to note that all samples were taken at the same depth which is at 30 cm below the surface for all the source waters assessed.

To determine the effectiveness of different coagulant chemicals during jar testing experiments, a series of replicates were needed to be able to statistically verify the trends found. In this study, fifteen jar testing experiments were performed with source water from sampling point M-FOREBAY, two jar tests with source water from Vaalkop and Rietvlei Dams (sampling points: M-RAW_VAALKOP and RIETVLEI DAM), and one jar test with source water from sampling point M-CANAL_VD.

The following source waters were selected:

- **M-CANAL_VD** – located in the canal, just before the inflow of the overflow water from the Rand Water's sludge disposal site at Panfontein, where the recovered water are diverted back into the open canal and thereby recycling it to Stations 3 and 4 at Zuikerbosch treatment plant for reprocessing together with the source water from the Vaal Dam. It was important to investigate the influence of the recovered water released from Panfontein on the source water that needs to be treated;
- **M-FOREBAY** – the water supplying Stations 3 and 4 at Zuikerbosch Treatment Plant downstream from the excess Panfontein Sludge Plant overflow water. M-FOREBAY was chosen due to its proximity to the Zuikerbosch plant and thereby investigating the immediate coagulant dosing action necessary when there is high concentrations of *C. hirundinella* cells present in the incoming source water;
- **M-RAW_VAALKOP** – water from Vaalkop Dam close to Brits that at the time when high concentrations of *C. hirundinella* occurred. This sampling point was chosen to determine which coagulant chemical is the most effective in removing high concentrations of *C. hirundinella* cells during the production of drinking water; and
- **RIETVLEI DAM** – water from Rietvlei Dam had high concentrations of *C. hirundinella* cells at the time of sampling. Jar testing experiments and chlorine exposure experiments were performed to determine the most effective coagulant chemical Magalies Water can

use on this type of source water, as well as evaluate the influence of different chlorine concentrations on *C. hirundinella* cells as part of a possible pre-chlorination step.

The results obtained from the jar testing experiments were recorded and organised into an Excel spreadsheet. PCA's were performed on all the jar testing results obtained with each of the source waters sampled (Forebay, Vaalkop Dam and Rietvlei Dam) for every treatment (cationic poly-electrolyte only, cationic poly-electrolyte in combination with slaked lime (CaO) and CaO in combination with activated silica). Another PCA was performed on the appropriate dosages specifically on Forebay source water of each jar test. This was done to investigate which treatment removed the most algae, total chlorophyll pigments content as Chlorophyll-665 (µg/L) and turbidity (NTU) (for the purpose of this dissertation, it should be noted that "cationic poly-electrolyte" will be referred to as "poly-electrolyte"). Regression analyses were performed for every treatment to determine which was the most effective to remove algae, total chlorophyll pigments (µg/L) and turbidity (NTU). The following criteria were selected to determine whether the process was effective: y-value = negative value (downward slope); or ineffective: y-value = positive value (upward slope). An R^2-value of > 0.7 with a negative slope is indicative of good removal.

3.4. Analytical methods

3.4.1. Physical, chemical and biological analyses

The chemical, physical and biological analyses of the samples for sampling points C-VRB5T, M-CANAL_VD, M-FOREBAY, M-RAW_VAALKOP and RIETVLEI DAM, were analysed at Rand Water's Chemistry and Biology Sections of Analytical Services (SANAS accredited laboratories) with methods based on standard methods (APHA, 2001).

The following physical and chemical variables were determined: Temperature (˚C), Turbidity (NTU), Secchi disk depth (cm), Suspended Solids (mg/L), pH, Dissolved Oxygen (mg/L), Conductivity (mS/m), Alkalinity (mg/L $CaCO_3$), Total Dissolved Solids (mg/L), Hardness (mg/L $CaCO_3$), Total Kjeldahl-Nitrogen (mg/L), Chemical Oxygen Demand (mg/L), Dissolved Organic Carbon (mg/L), Total Organic Carbon (mg/L), Geosmin (ng/L), Methylisorboneol (MIB, ng/L), Chloroform ($CHCl_3$, µg/L), Bromoform ($CHBr_3$, µg/L), Bromodichloromethane ($CHBrCl_2$, µg/L), Bromochloromethane (CHBrCl, µg/L), Total trihalomethanes (TTHM, µg/L), Phenols (µg/L), colour (mg/L), Arsenic (As, µg/L), Mercury (Hg, µg/L), Antimony (Sb, µg/L), Selenium (Se, µg/L), Strontium (Sr, µg/L), Uranium (U, µg/L), Li (µg/L), Barium (Ba, µg/L), Fluorine (F, mg/L), Bromide (Br, mg/L), Sulphur (S, mg/L), Chlorine (Cl, mg/L), Calcium (Ca, mg/L), Magnesium (Mg, mg/L), Sodium (Na, mg/L), Potassium (K, mg/L), Cadmium (Cd, mg/L), Chrome (Cr, mg/L), Cobalt (Co, mg/L), Copper (Cu, mg/L), Iron

(Fe, mg/L), Manganese (Mn, mg/L), Lead (Pb, mg/L), Zinc (Zn, mg/L), Nickle (Ni, mg/L), Aluminium (Al, mg/L), Boron (B, mg/L), Vanadium (V, mg/L), Molybdenum (Mo, mg/L), Total Silica (mg/L), Phosphorus (P, mg/L), Silicon (Si, mg/L), Ammonium (NH_4, mg/L), Nitrite (NO_2, mg/L), Nitrate (NO_3, mg/L), Orthophosphate (PO_4, mg/L) and Silicon dioxide (SiO_2, mg/L).

3.4.2. Turbidity determination

Turbidity in water is caused by suspended matter which includes clay, silt, organic and inorganic matter, plankton and other microscopic organisms. It is expressed in nephelometric turbidity unit (NTU) and was measured by using the bench top instrument HACH turbidity meter. Samples were taken from the sampling tap on the jar testing beaker, after the 15 minutes settling period during the jar testing procedure.

3.4.3. Chlorophyll-665 analyses

Algae contain chlorophyll that enables photosynthesis. Chlorophyll-665 represents the total photosynthetic pigments found in algal cells, e.g. chlorophyll-*a*, phaeophytin-*a* and other pigments. The pigments were extracted with methanol and read at an absorbance of 665 nm (Steynberg, 1986; Rand Water, 2009c, Swanepoel *et al*, 2008b). Samples were taken from the sampling tap on the jar testing beaker, after the 15 minutes settling period during the jar testing procedure. This sample represents the total algae biomass that will not settle out in the sedimentation tank and be carried over to the filter. Samples were analysed by the Hydrobiology Section of Analytical Services (a SANAS accredited laboratory) at Rand Water according to the accredited chlorophyll-665 method (Swanepoel *et al*, 2008b, Rand Water, 2009c: Method 1.1.2.02.1).

3.4.4. Phytoplankton identification and enumeration

Algae were identified and enumerated in the source water (control) samples as well as in the jar testing samples. Samples were taken from the supernatant of the jar, by means of a sampling tap on the jar testing beaker, after the 15 minutes settling stage during the jar testing procedure. This sample represents the algae that will not settle out in the sedimentation tank and be carried over to the filters. The results indicate which coagulant and concentration will be the most effective in algal removal.

The phytoplankton samples were preserved with Lugol's solution. Five milliliter of the fixed sub-samples was put into a counting chamber and centrifuged for 10 minutes at 3500 RPM. The algae were identified and enumerated on an inverted light microscope according to the

accredited phytoplankton enumeration method (Swanepoel *et al*, 2008b; Rand Water, 2009b: Method 1.1.2.03.1), based on the Utermöhl (1931, 1958) technique and modified by Lund *et al* (1958).

3.4.5. Jar testing procedures

For the purpose of this study a Phipps & Bird PB-700 series six-paddle standard jar tester was used. This instrument has six stainless steel paddles, spaced six inches apart that can be regulated to stir simultaneously at speeds of 1 – 300 RPM (Figure 3.10).

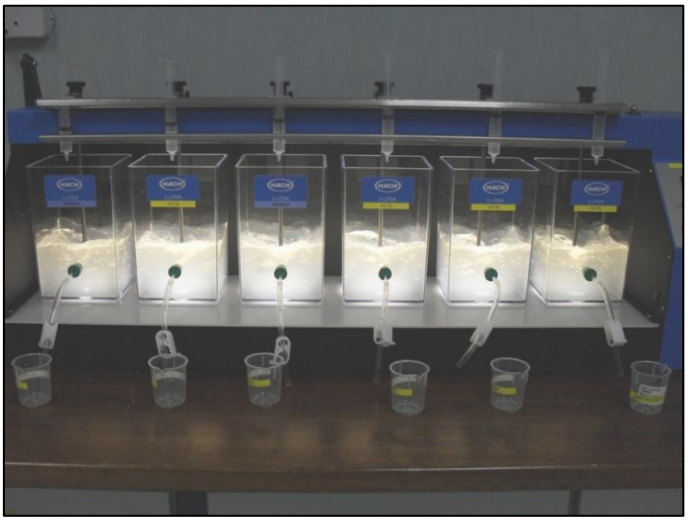


Figure 3.10: Jar testing with source water from the sampling point M-FOREBAY.

Preparation of coagulants:

The following section describes the preparation of the slaked lime (CaO), liquid polymeric coagulants (poly-electrolytes) and the activated silica.

Preparation of slaked lime (Rand Water, 2009a):

Step 1: A sample of quicklime with a known CaO content was collected from Rand Water Vereeniging Station Laboratory.

Step 2: To make up a 10 000 mg/L CaO stock solution, the mass was calculated (purity of quicklime known e.g. 85.7 %): 100/85.7 x 10 000/1 = 11668.6 mg quicklime will contain 10 000 mg/l CaO. Therefore, 11.67 g of quicklime was made up to 1000 mL to produce a 10 000 mg/L CaO.

Step 3: The calculated mass were weighed off and placed into a glass beaker.

Step 4: A few drops of cold water were added to the lime while stirring vigorously until all lime was in suspension. More tap water was added.

Step 5: The solution was brought to boiling point on a hotplate.

Step 6: After the mixture cooled down it was transferred to a 1 L measuring cylinder and diluted to mark with tap water, using some water to rinse all remaining CaO from the beaker. The contents were transferred to a 1 L plastic bottle for long term storage and use.

Preparation of liquid polymeric coagulants (Rand Water, 2009a):

Step1: A sample of the *Zetafloc* 650 was collected from Zuikerbosch Station Laboratory.

Step 2: 1.000 g of the liquid polymer was weighed into a weighing boat.

Step 3: The content of the weighing boat was rinsed into a 1 L volumetric flask and made up to the mark with distilled water giving a 100 mg/L stock solution.

Step 4: A fresh solution was prepared with each jar test.

Preparation of activated sodium silicate (Rand Water, 2009a):

Step 1: A concentrated sample was collected from the Rand Water Vereeniging Station Silica Plant (28.7 % m/v as SiO_2) and prepared to a 0.1 % m/v as SiO_2 solution.

Step 2: The mass of sodium silicate needed to make up a 0.1 % m/v (1000 mg/L SiO_2 stock solution from a 28.7 % m/v solution) were calculated as follows:

$$\frac{0.1\ \%}{28.7\ \%} \times \frac{1000}{1}$$

= 3.48 mL will contain 1000 mg of SiO_2.

Step 3: A solution that contains 1000 mg/L of SiO_2 was then prepared by diluting 3.48 mL (or 4.9 g) of 27.8 % m/v solution to 1000 mL.

Step 4: The pH of the sodium silicate sample was determined and activated if the pH was higher than 8.3.

Step 5: The pH was measured while slowly (drop wise) adding 2.5M HCl solution until the pH reached 8.3.

Step 6: The solution was matured for at least two hours before usage.

Step 7: A fresh solution was prepared with each jar test.

Jar testing methods performed with different coagulants:

Step 1: One litre of source water was poured into each jar.

Step 2: A constant amount of the different concentrations of the coagulant chemicals were added:

a. Poly-electrolyte procedure: Poly-electrolyte was added in its varying concentrations (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 mg/L).
b. Poly-electrolyte/slaked lime procedure: A constant amount of CaO was first added (10 mg/L) and 15 seconds later the poly-electrolyte in its varying concentrations (concentration 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 mg/L).
c. For slaked lime/activated silica procedure: activated silica was first added (2.5 mg/L) and 15 seconds later the slaked lime in its varying concentrations (30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 mg/L).

Step 3: The water was stirred for 2 minutes at 245 RPM, while coagulants (either 2a or 2b or 2c – depending on the coagulant being tested) were added.

Step 4: The speed was reduced to 30 RPM and stirred for another 8 minutes.

Step 5: The stirrers were switched off and flocs were allowed to settle for 15 minutes.

Step 6: The suitable volume of the supernatant was extracted through the sampling tap on the jars to determine the turbidity (NTU), chlorophyll-665 (µg/L) and phytoplankton identity and biomass (cells/mL).

3.4.6. Chlorine exposure investigation

This investigation was carried out to determine the effect of chlorine on the freshwater dinoflagellate *C. hirundinella*. It was necessary to determine which concentration would render the cell motionless, either by killing it or causing the flagellums to detach from the cell. This investigation was also carried out to assess the potential of incorporating pre-chlorination into Rand Water's purification process when high concentrations of *C. hirundinella* in the source water, is experienced. A commercial sodium hypochlorite (NaOCl) was used as a chlorine source for this investigation. The chlorine exposure experiments were conducted using Vaalkop and Rietvlei Dam source water, because of high concentrations of *C. hirundinella* (>1500 and >300 cells/mL respectively) occurring in the water at the time of sampling.

Ceratium hirundinella were exposed to various concentrations of NaOCl and the immobile or dead *Ceratium* cells were observed and counted.

Chlorine exposure method

Step 1: A known volume of source water rich with *Ceratium* cells was dosed with a pre-determined concentration of NaOCl. An example of this calculation is represented by the following:

For a 100 mL source water sample, it was needed to calculate the volume of NaOCl to dose to represent a 3 mg/L NaOCl concentration dosage, the NaOCl stock solution used had a known concentration of 12 500 mg/L from which 10 mL was made up to one liter with distilled water (reverse osmosis and by means of an Elgastat instrument):

$C_1V_1 = C_2V_2$

12 500 mg/L . V_1 = 3 mg/L . 100 mL

V_1 = 0.024 mg/L or 24 µg/L

The different chlorine concentrations were measured by means of a Hach colorimeter to be 0.825, 0.925, 1.025, 1.125, 1.225, 1.325, 1.825 and 2.325 mg/L NaOCl.

Step 2: The chlorinated sample was gently stirred for approximately 30 seconds and then five milliliter of the sample was poured into a counting chamber and a further 5 minutes contact time was allowed,

Step 3: The immobile or dead *Ceratium* cells were counted, using an inverted light microscope according to the accredited phytoplankton enumeration method (Swanepoel *et al*, 2008b; Rand Water, 2009b: Method 1.1.2.03.1), based on the Utermöhl (1931, 1958) technique and modified by Lund *et al* (1958).

3.5. Computer software packages

Data on algal counts and environmental variables was entered, organised and certain variables log-transformed into Microsoft® Excel 2007, spreadsheets. Multivariate analysis was carried out on physical, chemical and biological variables using the computer package CANOCO for Windows version 4.5 (Ter Braak and Šmilauer, 2002). The ordinations were graphically illustrated in CanoDraw for Windows Version 4.1. Box and whisker plots were created by using GraphPad Prism Version 4. Regression analysis was carried out in Curve Expert Version 1.3.

CHAPTER 4

RESULTS AND DISCUSSION

4. Introduction on Rand Water - its water resources and water treatment plants

Rand Water was established in 1903 to provide potable water to mines, industries and homes in the previous Transvaal province. Since then Rand Water's infrastructure has been expanded to meet the growth of demand in its service. Currently Rand Water is supplying to various customers such as municipalities, mines and other direct consumers. Rand Water supplies approximately 3500 ML water a day to about 10 million people (Rand Water, 2007).

Rand Water has two water purification plants namely Zuikerbosch and Vereeniging stations. The Vereeniging purification and pumping station is located 32 km from the Vaal River Barrage. Zuikerbosch Purification station is one of the largest conventional drinking water purification plants in the Southern hemisphere and is located on the banks of the Vaal River about 30 km downstream of the Vaal Dam (Rand Water, 2009e). Rand Water abstracts most of its source water from the Vaal Dam which is fed by the Vaal and Wilge Rivers. Source water from the Vaal Dam is of relatively good quality as the Vaal Dam is supplemented with source water from the Lesotho Highlands Water Project (LHWP) which is less polluted than most South African dams and rivers (Earle *et al*, 2005).

For this study water from different sources was sampled, refer to Chapter 3 for detail on the different sampling localities. The results of the historical data assessment and experiments performed will be discussed in the following section.

4.1. Assessment of historical data of sampling point C-VRB5T

In order to investigate the environmental conditions where *Ceratium hirundinella* frequently occur Principle Component Analyses (PCA) were performed to demonstrate the relationship between the different environmental variables (represented by arrows) (Figure 4.1). When interpreting the ordination diagrams the following should be taken into account: Two environmental variables are positively correlated if their arrows subtend a small angle to each other. They are uncorrelated if their arrows are at 90˚ from each other, and they are negatively correlated if their arrows are in opposite directions. Placement of environmental variables on the ordination diagram follows the reasoning that variables that are closer together on the ordination, have more in common than variables that are apart from one another. Environmental variables with the longest arrow relative to an axis have the greatest influence on that axis.

The first PCA on all environmental components (Table 4.1) was performed to reflect the conditions found at sampling point C-VRB5T from 2000 to 2009 (Figure 4.1).

Table 4.1: List of environmental variables included in the PCA ordination at sampling point C-VRB5T from 2000 to 2009

Environmental variable	***Abbreviation***	***Unit***
Temperature	*Temp*	*˚C*
Turbidity	*Turb*	*NTU*
Secchi Disc	*Secchi*	*cm*
pH	*pH*	*-*
Suspended solids	*SS*	*mg/L*
Dissolved Oxygen	*DO*	*mg/L*
Conductivity	*Cond*	*mS/m*
Chemical Oxygen Demand	*COD*	*mg/L*
Total Dissolved Solids	*TDS*	*mg/L*
Total Kjeldahl-Nitrogen	*TKN*	*mg/L*
Chrome	*Cr*	*mg/L*
Fluorine	*F*	*mg/L*
Bromide	*Br*	*mg/L*
Iron	*Fe*	*mg/L*
Sulphur	*S*	*mg/L*
Chlorine	*Cl*	*mg/L*
Nitrogen	*N*	*mg/L*
Calcium	*Ca*	*mg/L*
Magnesium	*Mg*	*mg/L*
Sodium	*Na*	*mg/L*
Potassium	*K*	*mg/L*
Cadmium	*Cd*	*mg/L*
Silica	*Si*	*mg/L*
Cobalt	*Co*	*mg/L*
Copper	*Cu*	*mg/L*
Nickel	*Ni*	*mg/L*
Manganese	*Mn*	*mg/L*
Aluminium	*Al*	*mg/L*
Boron	*B*	*mg/L*
Vanadium	*V*	*mg/L*
Molybdenum	*Mo*	*mg/L*
Total Silica	*Total Si*	*mg/L*
Phosphorus	*P*	*mg/L*
Silicon	*Si*	*mg/L*
Lead	*Pb*	*mg/L*
Zinc	*Zn*	*mg/L*
Sulphate	SO_4	*mg/L*
Dissolved Inorganic Nitrogen	*DIN*	*mg/L*
Orthophosphate	PO_4	*mg/L*
Hardness	*Hard*	*mg/L* $CaCO_3$
Alkalinity	*Alk*	*mg/L* $CaCO_3$
Chlorophyll-a	*Chl a*	*µg/L*

Although chlorophyll-*a* cannot be regarded as an environmental variable, it was included to determine the position of chlorophyll-*a* on the ordination diagram in order to illustrate the effect

of all the environmental variables on the total algal biomass. By including chlorophyll-*a* it will also allow explanations for the influence of certain environmental variables on the chlorophyll-*a* concentration in the water.

In the first PCA ordination (Figure 4.1), 40.7 % of the variance in environmental components could be explained on the first two canonical axes (Table 4.2). From Figure 4.1 it is evident that temperature correlated with turbidity, SS, pH, chlorophyll-*a*, DO, COD, TKN and different chemical elements such as Cd, Co, Br, Mo, V, F, Pb, Cr, Al and Fe. Turbidity correlated with suspended solids and showed a negative correlation was found with conductivity, hardness, Secchi disk depth and various other elements such as P, Cl, K, Na, Mg, S, Ca, B, and Ni.

Table 4.2: Results from the PCA analysis on all environmental variables at C-VRB5T from 2000 to 2009

Axes	1	2	3	4	Total variance
Eigenvalues	0.329	0.078	0.051	0.046	1.000
Cumulative percentage variance of species data	32.9	40.7	45.8	50.4	
Sum of all					1.000

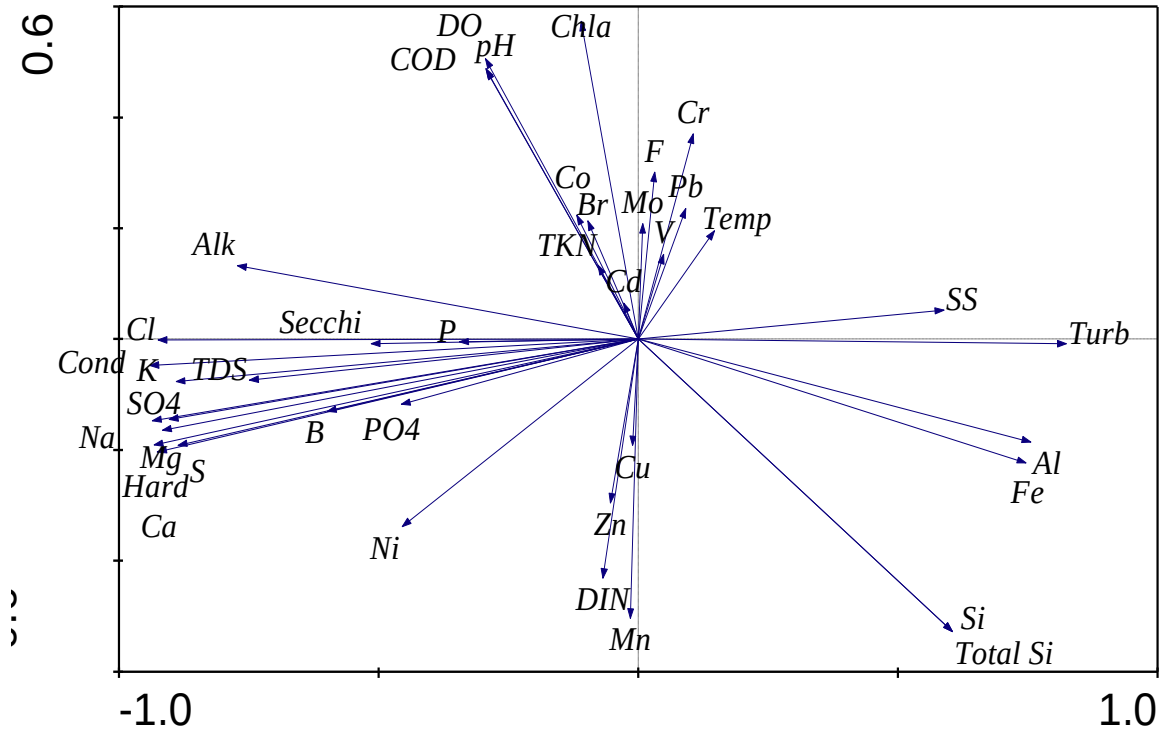


Figure 4.1: Bi-plot PCA ordination diagram showing all environmental variables measured at C-VRB5T for 2000 to 2009.

From the first PCA (Figure 4.1), principle components were identified and the rest omitted - variables not directly related to algal growth and physiology (such as Pb, V, Mo, F etc.). The variables important to algal growth and physiology were further used in the multivariate

analyses. The following principle components have been selected: Conductivity, Hardness, Silica (Si), Orthophosphate (PO_4), Turbidity, Dissolved Inorganic Nitrogen (DIN), Iron (Fe), Total Kjeldahl-Nitrogen (TKN), Chrome (Cr), Chlorophyll-a (Chl-a), Temperature (Temp), Alkalinity (Alk), Nickle (Ni), pH, Dissolved Oxygen (DO) and Chemical Oxygen Demand (COD).

A second PCA was done on the principle environmental components only to reflect the relationships between these variables found at sampling point C-VRB5T from 2000 to 2009 (Figure 4.2). Along the first two axes in the PCA ordination, a total 44.5 % of the variance in environmental data could be explained (Table 4.3).

Along the first axis (x-axis) in the PCA ordination, 31 % of the variance in environmental data could be explained (Table 4.3). Figure 4.2 indicated a negative correlation between turbidity and conductivity and between turbidity and PO_4. Turbidity indicates the clarity of water (the higher the turbidity the lower the clarity), and is mainly influenced by different concentrations of particulate material such as silt or algae in the water. During summer, the rainy season contributes to an increase of particulate material or runoff into the river and turbidity increases. High turbidity values will often suppress algal growth by limiting underwater light availability (Pieterse and Janse van Vuuren, 1997). The average turbidity at sampling point C-VRB5T was 13 NTU with a maximum of 106 NTU for the study period.

From Figure 4.2, it was found that the phosphates (PO_4) and nitrates (DIN) did not correlate with turbidity or temperature. There are residential developments found along the riverbank; however, high standards and regulations are set with regards to the sanitations systems of on-site septic tanks and wastewater works. The Free State Rietspruit, (a tributary to the Vaal River, 1 km upstream of sampling point C-VRB5T) (Figures 4.3 and 4.4) and Leeuspruit (another tributary 7 km upstream of C-VRB5T) are not likely to contribute to sewage inputs at this sampling point, since wastewater works in that area (e.g. Sasolburg) discard sewage via a pipeline downstream after the Barrage wall[3] (Figure 3.1). Little agricultural farming is practised upstream in the surrounding area of the Taaibosspruit. It should be emphasised that the Klip River upstream from C-VRB5T (more than 40 km) is responsible for high inputs of sewage effluents into the Vaal River. This most probably contributes most to the increasingly high concentration of nitrates and phosphates found at this sampling point (which is not necessarily linked to season). Wastewater works such as from Johannesburg, Emfuleni, Meyerton and Leeukuil wastewater works discharge sewage effluents into the Klip River. Mining activities are also found in the surrounding area of the Klip River, which contributes to an increase in salinity (TDS) in the Vaal River[3].

[3] Hoge, P. 2010. [Conversation], Pollution sources upstream of sampling point C-VRB5T. (Personal Communication, 21 May 2010).

Table 4.3: Results from the PCA analysis on the environmental data containing only the principle components at C-VRB5T from 2000 to 2009

Axes	1	2	3	4	Total variance
Eigenvalues	0.311	0.134	0.091	0.080	1.000
Cumulative percentage variance of species data	31.1	44.5	53.6	61.6	
Sum of all					1.000

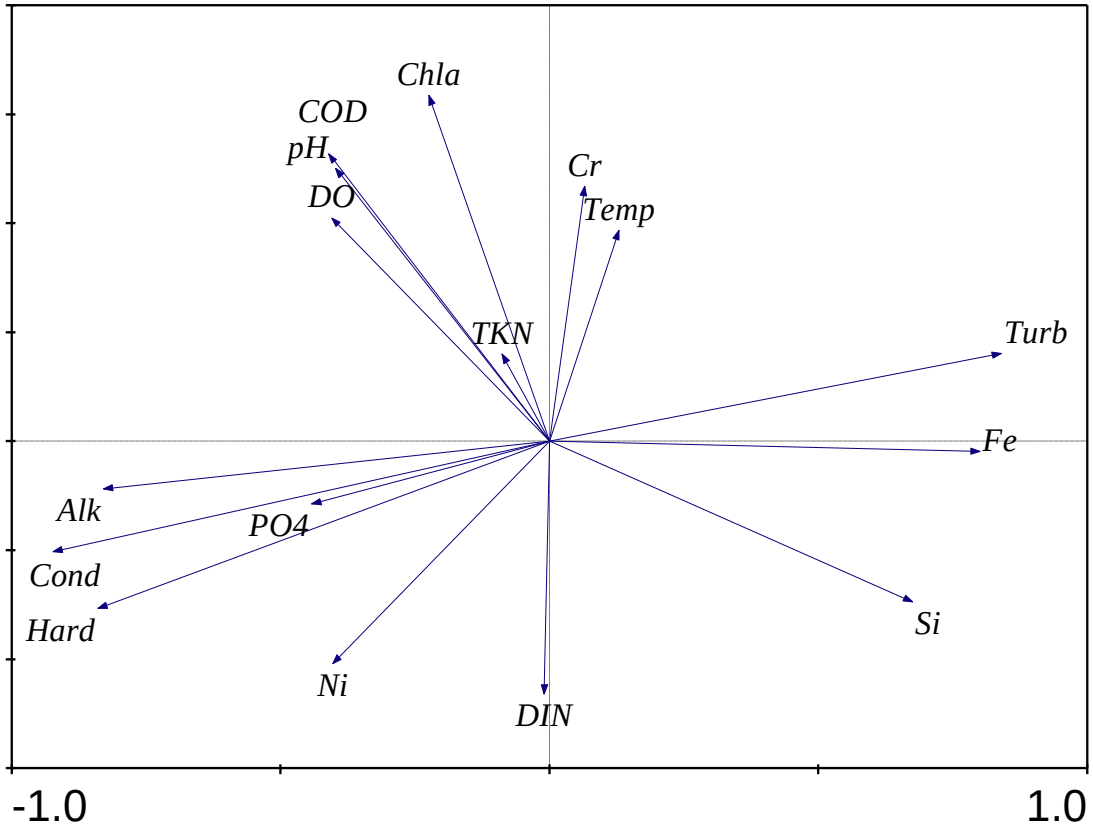
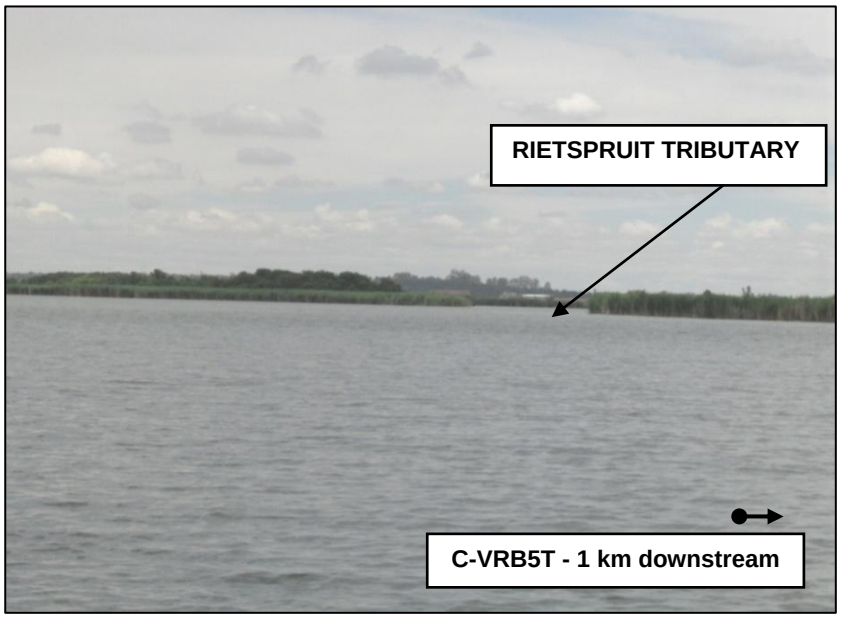


Figure 4.2: Bi-plot PCA ordination diagram showing only the main environmental variables (principle components) measured at C-VRB5T for 2000 to 2009 (LEGEND: Temp = Temperature; Turb = Turbidity; DIN = Dissolved Inorganic Nitrogen; Hard = Hardness; Cond = Conductivity; Alk = Alkalinity; TKN = Total Kjeldahl-Nitrogen; DO = Dissolved Oxygen; COD = Chemical Oxygen Demand; Chla = Chlorophyll-a; Cr = Chrome; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate).

Furthermore, 13.4 % of the variance in environmental data could be explained on the second axis (*y*-axis) of the PCA ordination (Table 4.3). The PCA (Figure 4.2) indicated that during periods of high temperature, the chlorophyll concentration is high. Thereby, indicating that the algae reached higher biomass during the summer. When chlorophyll concentration is high, photosynthesis is high where oxygen is released into and carbon dioxide removed from the water. Therefore, a positive correlation is found between chlorophyll-*a*, pH and oxygen provided it was sampled in high daylight. With high bio-activity (periods of high chlorophyll-*a)* the demand for oxygen increase (by aerobic decomposing bacteria) and a positive correlation with COD is

observed. The pH also reached its maxima during the warm summer periods and could most probably be related to the periods of increased algal biomass.



(a)

(b)

Figures 4.3 (a) and (b): Pictures of Rietspruit tributary 1 km upstream of sampling point C-VRB5T.

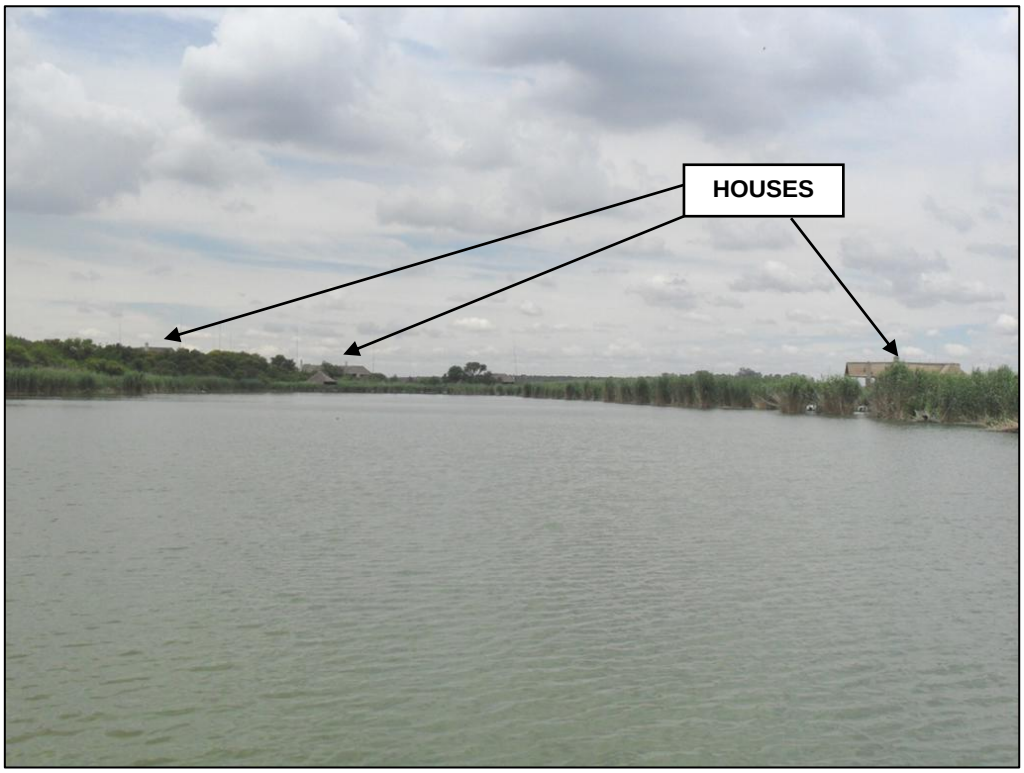


Figure 4.4: Picture showing developments found in the vicinity of Rietspruit.

A Correspondence Analyses (CA) was performed on the major algal taxa and principle environmental variables. It was found that the CA (Figure 4.5) and CCA ordination diagram (Figure 4.6) corresponds relatively well and appears to be mirror images. Therefore, it can be concluded that the environmental variables chosen for further multivariate analyses were the correct ones to explain the variation in the algal species data.

The Canonical Correspondence Analyses (CCA) was performed to reflect the relationship between the major algal taxa and environmental variables found at sampling point C-VRB5T from 2000 to 2009. This multivariate analysis method was used, because of the great variation found in the algal species and taxa data (Ter Braak and Prentice, 1988). The following main environmental components were included in the CCA ordination: Turbidity (Turb, NTU), Temperature (Temp, ˚C), pH, Conductivity (Cond, mS/m), Alkalinity (Alk, mg/L $CaCO_3$), Dissolved Oxygen (DO, mg/L), Total Kjeldahl-Nitrogen (TKN, mg/L), Chromium (Cr, mg/L), Iron (Fe, mg/L), Silica (Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Nickel (Ni, mg/L), Hardness (Hard, mg/L $CaCO_3$), Ortophosphate (PO_4, mg/L), Chemical Oxygen Demand (COD, mg/L) and Chlorophyll-*a* (Chl*a*, µg/L).

The seven major algal taxa included in the ordination diagram are Cyanophyceae (Cyano, cells/mL), Bacillariophyceae (Bacill, cells/mL), Chlorophyceae (Chloro, cells/mL), Cryptophyceae (Crypto, cells/mL), Dinophyceae (Dino, cells/mL), Chrysophyceae (Chryso, cells/mL) and Euglenophyceae (Eugleno, cells/mL).

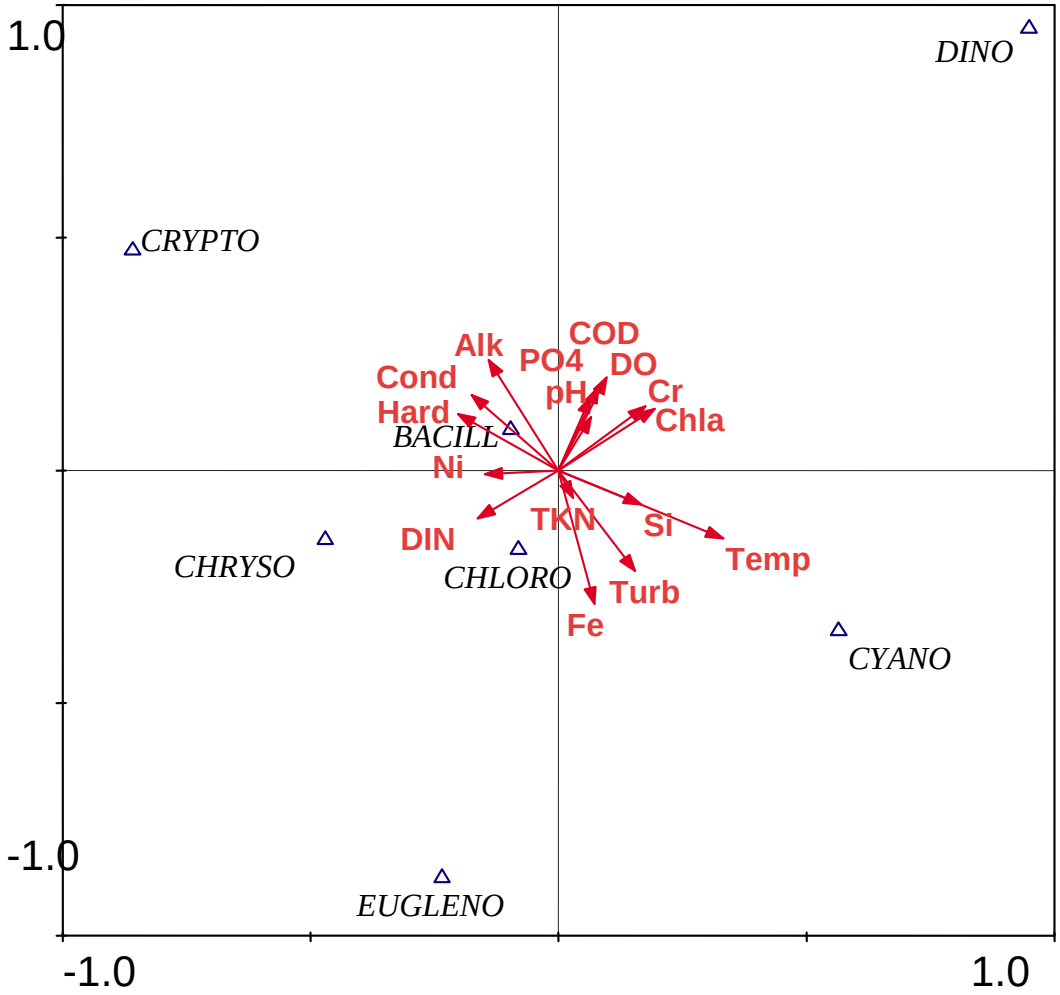


Figure 4.5: CA ordination diagram showing principle environmental variables and major algal taxa measured at C-VRB5T for 2000 to 2009 (LEGEND: Temp = Temperature; Turb = Turbidity; TKN = Total Kjeldahl-Nitrogen; DIN = Dissolved Inorganic Nitrogen; Hard = Hardness; Cond = Conductivity; Alk = Alkalinity; DO, Dissolved Oxygen; COD = Chemical Oxygen Demand; Chla = Chlorophyll-a; Cr = Chrome; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate; Cyano = Cyanophyceae; Bacill = Bacillariophyceae; Chloro = Chlorophyceae; Crypto = Cryptophyceae; Dino = Dinophyceae; Chryso = Chrysophyceae and Eugleno = Euglenophyceae).

From Table 4.4 it is evident that although the first two canonical axes (represented in Figure 4.6) explained only a total of 13 % of the variance within the algal data itself, it explained 75 % of the variance in the algal-environment relationship.

Table 4.4: Results from the CCA analysis on the environmental variables and major algal taxa at C-VRB5T from 2000 to 2009

Axes	1	2	3	4	Total inertia
Eigenvalues	0.067	0.043	0.019	0.008	0.847
Species-environment correlations	0.564	0.500	0.340	0.244	
Cumulative percentage variance of species data	7.9	12.9	15.2	16.0	
Cumulative percentage variance of species-environment relation	46.0	75.4	88.7	93.9	
Sum of all eigenvalues					0.847
Sum of all canonical eigenvalues					0.145

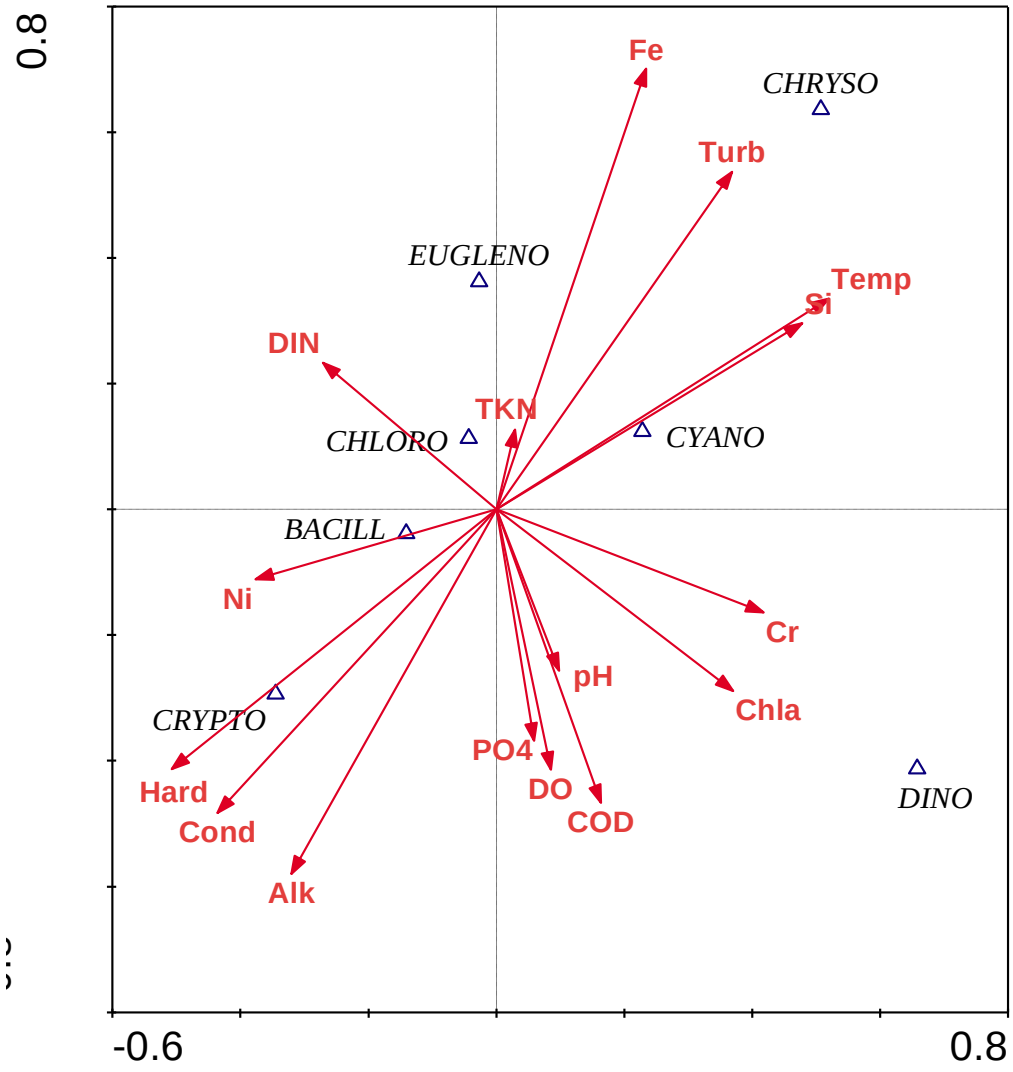


Figure 4.6: CCA ordination diagram showing principle environmental components and major algal taxa measured at C-VRB5T for 2000 to 2009 (LEGEND: Temp = Temperature; Turb = Turbidity; TKN = Total Kjeldahl-Nitrogen; DIN = Dissolved Inorganic Nitrogen; Hard = Hardness; Cond = Conductivity; Alk = Alkalinity; DO = Dissolved Oxygen; COD = Chemical Oxygen Demand; Chla = Chlorophyll-a; Cr = Chrome; Fe = Iron; Si = Silica; Ni = Nickel; PO4 = Orthophosphate; Cyano = Cyanophyceae; Bacill = Bacillariophyceae; Chloro = Chlorophyceae; Crypto = Cryptophyceae; Dino = Dinophyceae; Chryso = Chrysophyceae and Eugleno = Euglenophyceae).

The most important environmental variables influencing the growth of phytoplankton are light, temperature and nutrient availability (Janse van Vuuren and Pieterse, 2005a). A positive correlation was found between temperature and two algal classes namely Cyanophyceae and Chrysophyceae indicating that these organisms were favoured by warm water conditions. It is known from many studies (e.g. Palmer, 1980, Janse van Vuuren and Pieterse, 2005a; Du Preez and Van Baalen, 2006) that Cyanophyceae usually form blooms in the summer. According to Janse van Vuuren and Pieterse (2005a) Cyanophyceae tend to separate from other algal classes by preferring high water temperatures (higher than 20 ˚C) (Harding and Paxton, 2001). The Cryptophyceae and Bacillariophyceae (diatoms), on the other hand, tend to reach higher concentrations in water with lower temperatures (less than 15 ˚C) (Pieterse and Janse van Vuuren, 1997), as they correlate negatively with temperature (Figure 4.6). Similar results were found by Palmer (1980) and Løvstad and Bjørndalen (1990).

The negative relationship found between diatoms and silica may be the result of utilization and depletion of this essential nutrient during peaks in diatom biomass (Pieterse and Janse van Vuuren, 1997). Dinophyceae (*C. hirundinella*) were usually present during high pH, high COD, high PO_4 and low DIN concentrations. A correlation was also found between Dinophyceae and chlorophyll-a, which might be due to the cells containing high concentrations of chlorophyll. There was no correlation between with Dinophyceae and temperature. It seems that the Dinophyceae did not occur during extreme hot and severe cold conditions. The non-correlation indicates that the Dinophyceae may occur during periods of moderate temperatures such as experienced during spring and autumn. From Table 4.5 it is clear that the CCA analysis (for the first canonical axis as well as for all four axes) was statistically significant ($P < 0.05$) as judged on the basis of the Monte Carlo Permutation test (499 random permutations).

Table 4.5: Results of the Monte Carlo test from the CCA analysis on the principle environmental components and major algal taxa at C-VRB5T from 2000 to 2009

Test of significance of first canonical axis	
Eigenvalue	0.067
F-ratio	22.359
P-value	0.0020
Test of significance of all canonical axes	
Trace	0.145
F-ratio	3.374
P-value	0.0020

The second Canonical Correspondence Analyses (CCA) (Figure 4.7) was performed to reflect the relationship between the algal species and environmental data found at sampling point C-VRB5T from 2000 to 2009. The principle environmental variables included in the CCA ordination were: Turbidity (Turb, NTU), Temperature (Temp, ˚C), pH, Conductivity (Cond, mS/m), Alkalinity (Alk, mg/L $CaCO_3$), Dissolved Oxygen (DO, mg/L), Total Kjeldahl-

Nitrogen (TKN, mg/L), Chromium (Cr, mg/L), Iron (Fe, mg/L), Silica (Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Nickel (Ni, mg/L), Hardness (Hard, mg/L $CaCO_3$), and Orthophosphate (PO_4-P, mg/L), Chemical Oxygen Demand (COD, mg/L) and Chlorophyll-*a* (Chl*a*, µg/L).

The algal species (cells/mL) included are the cyanobacteria (Cyanophyceae): *Anabaena* sp., *Microcystis* sp. and *Oscillatoria* sp.; the green algae (Chlorophyceae): *Chlamydomonas* sp., *Pandorina morum*, *Pediastrum duplex*, *Oocystis* sp., *Coelastrum* sp., *Scenedesmus* sp., *Tetraedron* sp., *Monoraphidium* sp. and *Coccomonas* sp.; the Cryptophyceae: *Cryptomonas* sp.; the Dinophyceae: *Ceratium hirundinella*; the Euglenophyceae*: Euglena* sp.; *Trachelomonas* sp. and the Bacillariophyceae: *Aulacoseira* sp., pennate and centric diatoms.

Table 4.6 shows that although the first two canonical axes (as represented in Figure 4.7) explained only a total of 6.5 % of the variance within the algal data itself, it explained 48.3 % of the variance in the algal-environment relationship.

Table 4.6: Results from the CCA analysis on the principle environmental variables and algal species at C-VRB5T from 2000 to 2009

Axes	1	2	3	4	Total inertia
Eigenvalues	0.092	0.060	0.048	0.031	2.334
Species-environment correlations	0.579	0.561	0.525	0.457	
Cumulative percentage variance of species data	3.9	6.5	8.6	9.9	
Cumulative percentage variance of species-environment relation	29.2	48.3	63.4	73.3	
Sum of all eigenvalues					2.334
Sum of all canonical eigenvalues					0.315

From Figure 4.7, a positive correlation was found between temperature and the cyanobacteria *Microcystis* sp., *Anabaena* sp. and *Oscillatoria* sp. and green algae *Pediastrum duplex* and *Oocystis* sp. However, the diatoms (both centrics and pennates), *Cryptomonas* sp. and *Trachelomonas* sp. tend to reach higher concentrations during lower temperatures. A positive correlation was found between *C. hirundinella* and chlorophyll-*a* as did van Ginkel *et al* (2001) and Hart and Wragg (2009). It was found that the dinoflagellate *C. hirundinella* seem to be favoured by high pH, high COD, high chromium (also representing other cations namely, Cd, Mo, V, Co, Br, F and Pb), high silica as well as low turbidity, low iron and low nitrogen concentrations. Since both iron and nitrogen are essential for growth, it might have been utilized by the *Ceratium* sp. cells, decreasing the concentrations thereof. According to Janse van Vuuren and Pieterse (2005a), Dinophyceae are favoured during periods of low turbidity and high pH.

No correlation was found between *C. hirundinella* and temperature as did Hart and Wragg (2009). This non-correlation of *C. hirundinella* with extreme high or extreme low temperatures may indicate that *C. hirundinella* most probably occur during moderate temperature conditions, e.g. during spring and autumn, as was found during this study. The *C. hirundinella* bloom that occurred at the Albert Falls (KZN) reached a peak biomass in early winter and increased in the late spring/early summer (Hart and Wragg, 2009). Various studies have found the occurrence of *C. hirundinella* in different temperature ranges and seasons (Pfiester, 1971; Heaney, 1976; van Ginkel *et al*, 2001; Péres-Martínez and Sánchez-Castillo, 2002; Tomec *et al*, 2002, as cited by Gligora *et al*, 2003; Hart and Wragg, 2009).

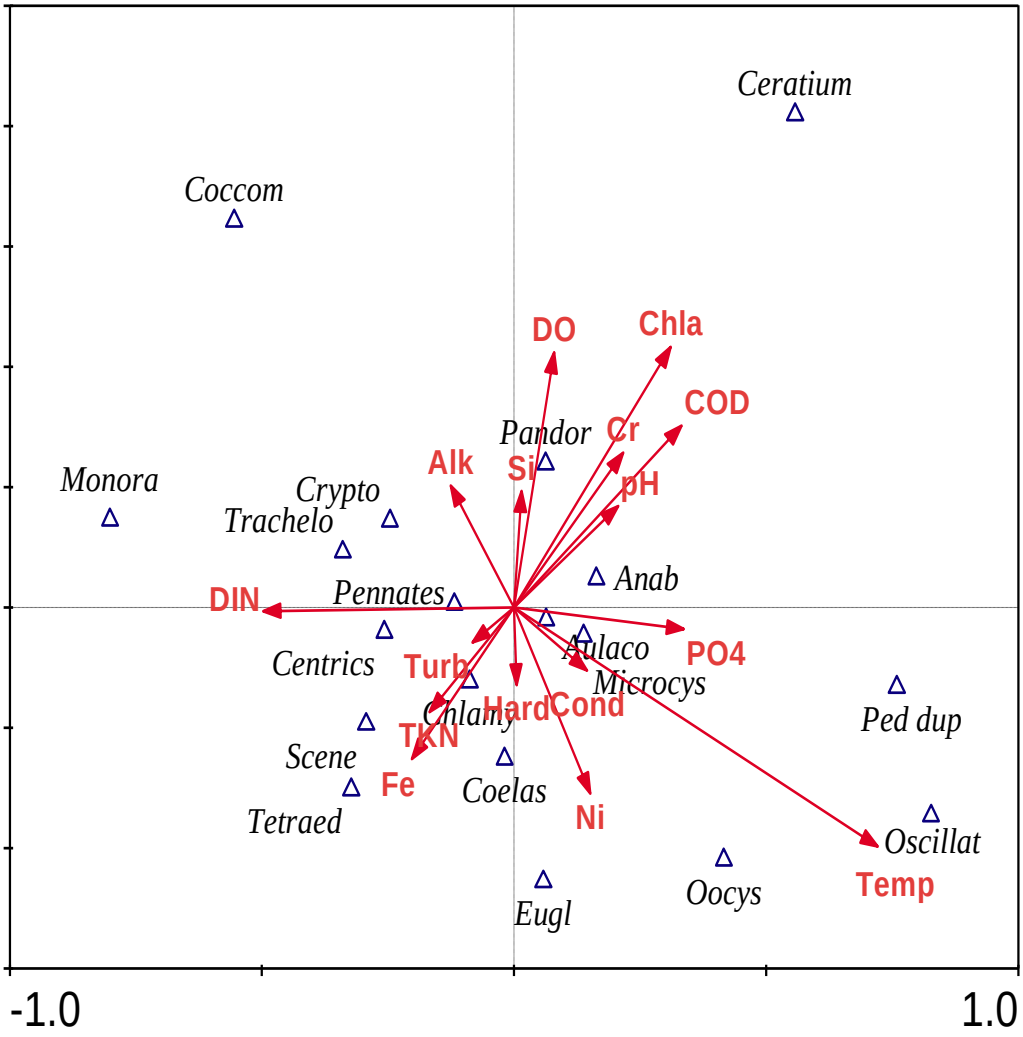


Figure 4.7: CCA ordination diagram showing principle environmental components and algal species measured at C-VRB5T for 2000 to 2009 (LEGEND: Temp = Temperature; Turb = Turbidity; TKN = Total Kjeldahl-Nitrogen; DIN = Dissolved Inorganic Nitrogen; Hard = Hardness; Cond = Conductivity; Alk = Alkalinity; DO = Dissolved Oxygen; COD = Chemical Oxygen Demand; Chla = Chlorophyll-a; Cr = Chrome; Fe = Iron; Si = Silica; Ni = Nickel; PO4 = Orthophosphate; *Ceratium* = *Ceratium hirundinella*; *Pandor* = *Pandorina morum; Anab* = *Anabaena* sp.; *Aulaco* = *Aulacoseira* sp.; *Microcys* = *Microcystis* sp.; *Ped dup* = *Pediastrum duplex; Oscillat* = *Oscillatoria* sp.; *Oocys* = *Oocystis* sp.; *Eugl* = *Euglena* sp.; *Coelas* = *Coelastrum* sp.; *Chlamy* = *Chlamydomonas* sp.; Centrics = Centric diatoms; *Scene* = *Scenedesmus* sp.; *Tetraed* = *Tetraedron* sp.; Pennates = Pennate diatoms;

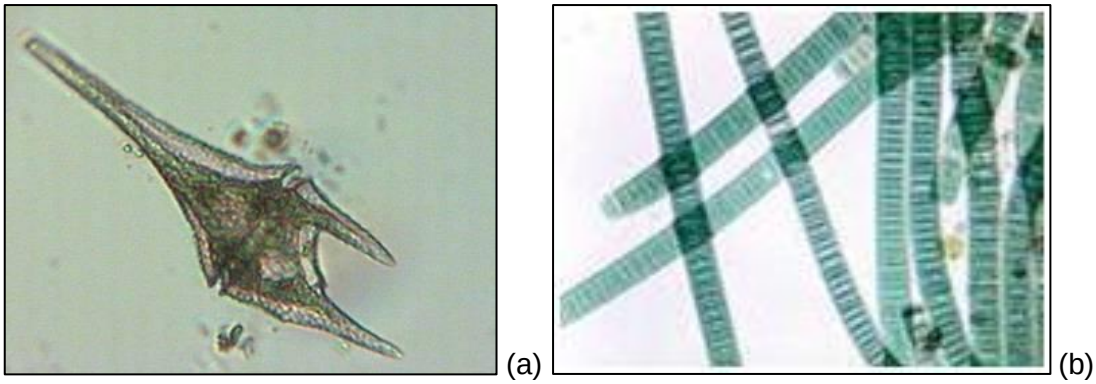
Trachelo = *Trachelomonas* sp.; *Monora* = *Monoraphidium* sp.; *Coccom* = *Coccomonas* sp.; and *Crypto* = *Cryptomonas* sp.).

Table 4.7 shows that this CCA analysis (Figure 4.7) was statistically significant ($P < 0.05$) as judged on the basis of the Monte Carlo Permutation test (499 random permutations). Thus, there is a statistically significant relationship between the environmental components and the algal species (represented in Figure 4.7).

Table 4.7: Results of the Monte Carlo Permutation test from the CCA analysis on the environmental data and algal species at C-VRB5T from 2000 to 2009

Test of significance of first canonical axis	
Eigenvalue	0.092
F-ratio	10.721
P-value	0.0020
Test of significance of all canonical axes	
Trace	0.315
F-ratio	2.547
P-value	0.0020

From Figure 4.10, an increase of *C. hirundinella* (Figure 4.8a) occurrences and concentration can be observed during the study period (2000 to 2009). The peaks in cell biomass coincided with periods showing maximum chlorophyll-*a* concentrations, especially from 2007 onwards. This is an indication that *C. hirundinella*, also of their large cell size, contribute most to the chlorophyll-*a* concentration when they are present in the source water.



(a) (b)

Figure 4.8: Light micrographs of a) *Ceratium hirundinella* and b) *Oscillatoria* sp.

Figure 4.9 explains the results obtained from the regression analysis performed between chlorophyll-*a* and *C. hirundinella* concentrations for the period 2007 to 2009. The regression analysis revealed a positive correlation ($R^2 = 0.72$), between higher *C. hirundinella* concentrations and higher chlorophyll-*a* concentrations, indicating that the species responsible for high chlorophyll-*a* concentrations, was probably *C. hirundinella*.

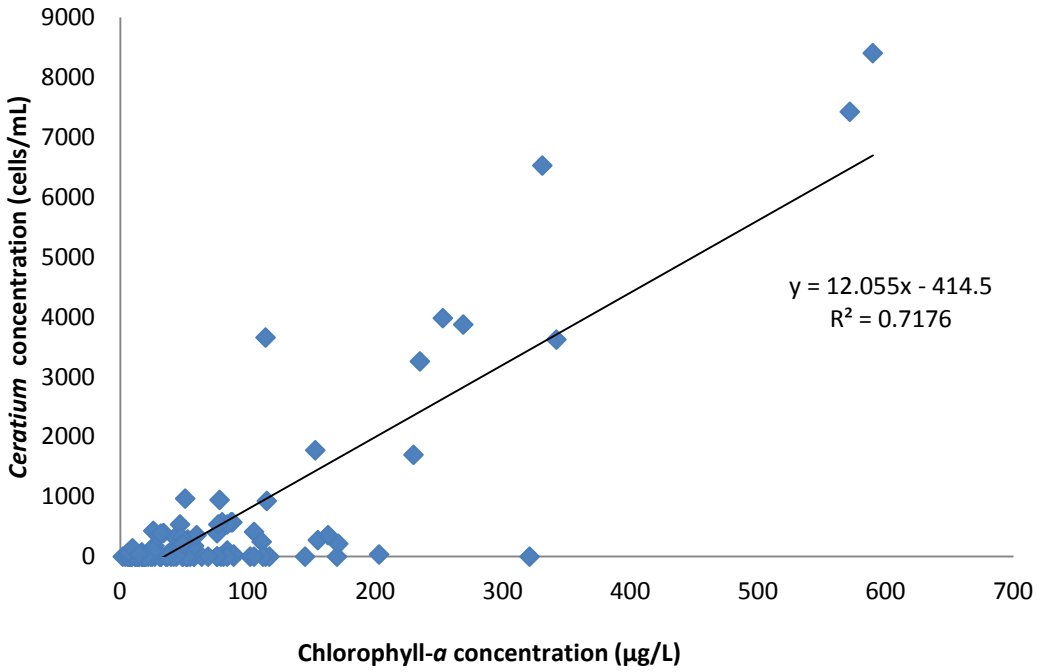


Figure 4.9: Linear regression between chlorophyll-*a* and *Ceratium hirundinella* from 2007 to 2009.

At sampling point C-VRB5T, the highest chlorophyll-*a* concentration was observed during 2008 (590 µg/L), and the average chlorophyll-*a* concentration from 2000 to 2009 is 48 µg/L. Van Ginkel *et al* (2001) found average chlorophyll-*a* levels of up to 600 µg/L and cell densities of approximately 13 500 cells/mL in the Hartbeespoort Dam during a *C. hirundinella* bloom in 1999. The maximum cell biomass of *C. hirundinella* was 8 400 cells/mL during October 2008 at C-VRB5T, with an average cell biomass of 630 cells/mL from 2000 to 2009. It is evident that the *Ceratium* concentration increase started around 2007 (Figure 4.10). The bloom detected during 2003, was due to the occurrence of the blue-green alga *Oscillatoria* sp. (Figure 4.8b), that was present in concentrations of > 65,000 cells/mL.

As the water quality of the freshwater systems in South Africa is decreasing as a result of pollution, conditions might become favourable to certain algal species. *Ceratium hirundinella* was identified in South Africa but it has only been found to occur in extreme bloom-forming proportions since 1999 (Hart and Wragg, 2009) and has been increasing in the Vaal River since 2007 (Figure 4.10). *Ceratium hirundinella* has unique characteristics as already mentioned in Chapter 2, which can cause problems during conventional water treatment. Two management strategies have been investigated in this study namely optimisation of coagulant dosing by performing jar testing experiments and chlorine exposure experiments (pre-chlorination) with source water containing high concentrations of *C. hirundinella*.

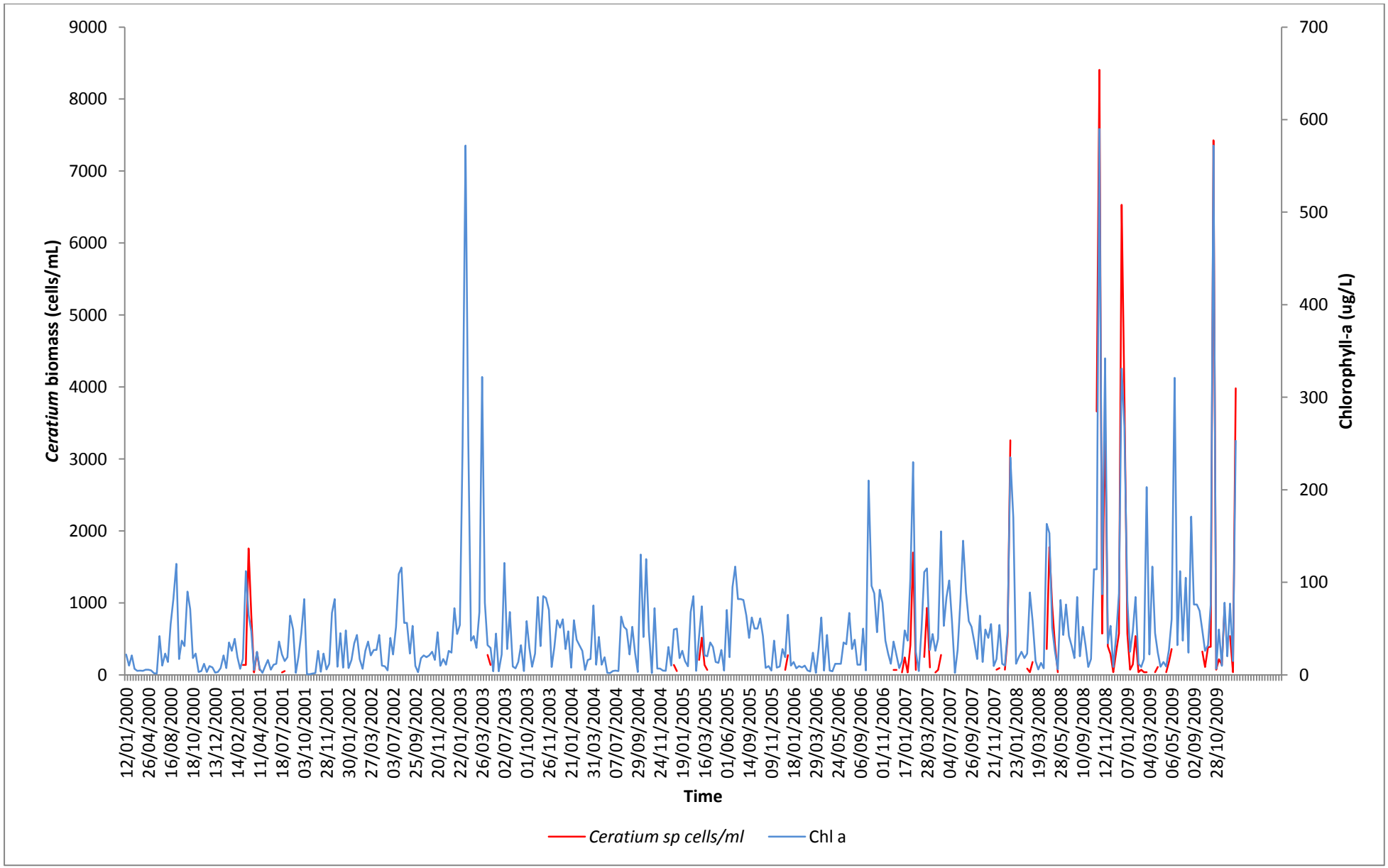


Figure 4.10: Weekly results for chlorophyll-*a* and *Ceratium hirundinella* concentrations at C-VRB5T from 2000 – 2009.

In Section 4.2., the results obtained when jar testing experiments were performed with three different coagulant chemicals (that Rand Water is currently using) on four different types of source waters, will be discussed accordingly.

4.2. Assessment of the effectivity of treatment coagulants on source waters

4.2.1. Assessment of water from sampling point M-FOREBAY during April to October 2010

Over a period of seven months (April to October 2010), a series of 15 jar tests were performed on the source water from sampling point M-FOREBAY, to determine which coagulant chemical is the most effective in removing high concentrations of *C. hirundinella* (Figure 4.8a) cells during the production of drinking water. However, during the study period there were only three occasions when *C. hirundinella* cells were detected in this source water (at less than 21 cells/mL).

4.2.1.1. Assessment of Forebay source water

In Figure 4.11, the concentration of the different algae present in the source water that was used for the jar testing experiments, are displayed.

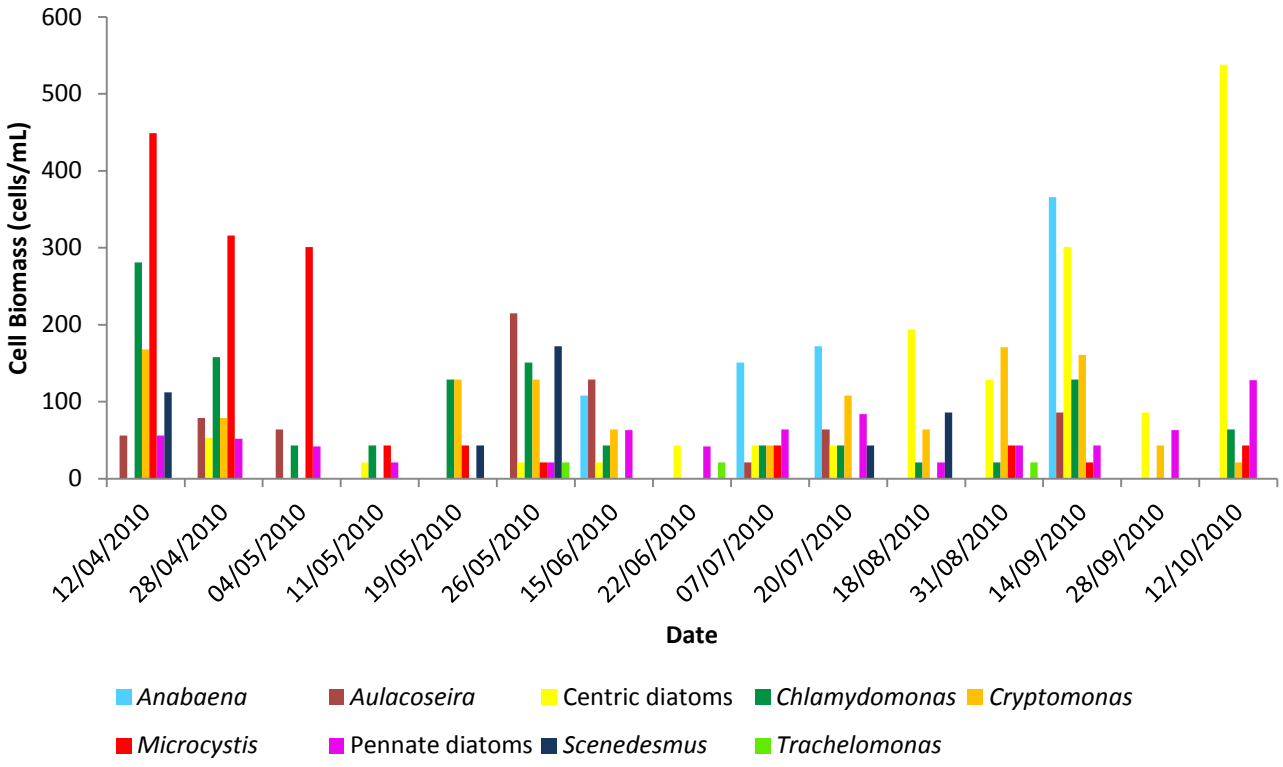


Figure 4.11: Histogram showing the algal genera and responding concentrations that occurred in the source water at the sampling point M-FOREBAY from April to October 2010.

The dominant algal genera found in the Forebay source water sample during April and May was *Microcystis* sp. (Figure 4.12b) and *Chlamydomonas* sp. (Figure 4.12c) that gradually decreased over the study period. During winter and spring there was an increase in diatoms (*Aulacoseira* sp. (Figure 4.12d), centric and pennate diatoms (Figures 4.12e and f)) at this sampling point, reaching dominant proportions during colder months. The occurrence of diatoms during low temperatures is well known (Løvstad and Bjørndalen, 1990; Pieterse and Janse van Vuuren, 1997), and in this study the same observation was found to occur at sampling point C-VRB5T (Section 4.1).

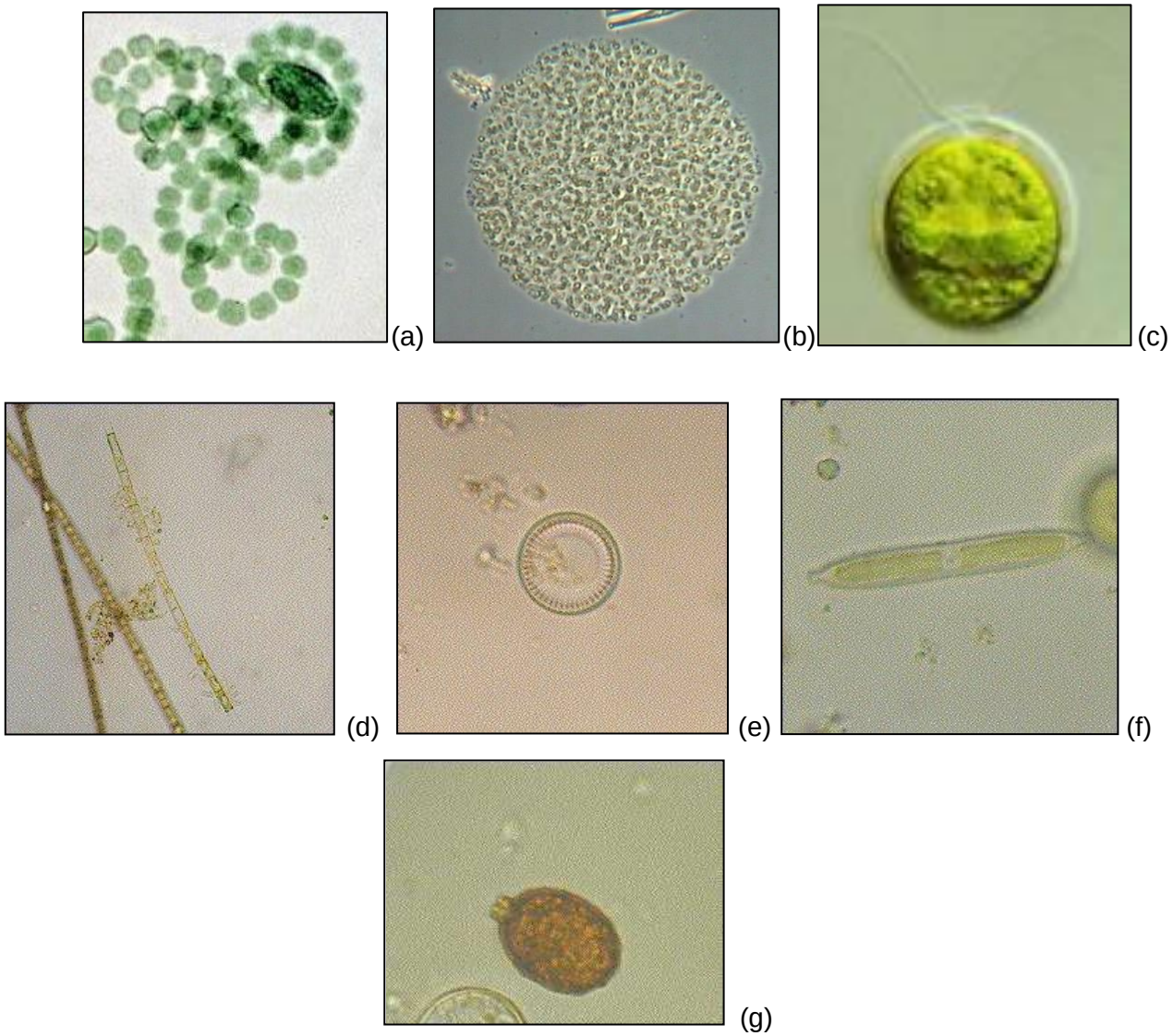


Figure 4.12: Light micrographs of (a) *Anabaena* sp., (b) *Microcystis* sp., (c) *Chlamydomonas* sp., (d) *Aulacoseira* sp., (e) centric diatoms, (f) pennate diatoms and (g) *Trachelomonas* sp.

Figure 4.11 shows an increase of the blue-green alga *Anabaena* sp. (Figure 4.12a) after winter. This was expected since this species is frequently found at this sampling point in large numbers.

This species is known to occur in high concentrations during warmer temperatures (Harding and Paxton, 2001; Janse van Vuuren and Pieterse, 2005a; Du Preez and Van Baalen, 2006).

The first Principle Component Analyses (PCA) on all environmental components was performed to reflect the conditions found at sampling point M-FOREBAY from April to October 2010 (Figure 4.13). The following environmental variables were included in the PCA ordination: Temperature (Temp, ˚C), Turbidity (Turb, NTU), pH, Suspended solids (SS, mg/L), Dissolved Organic Carbon (DOC, mg/L), Conductivity (Cond, mS/m), Chemical Oxygen Demand (COD, mg/L), Total Dissolved Solids (TDS, mg/L), Total Kjeldahl-Nitrogen (TKN, mg/L), Chrome (Cr, mg/L), Fluorine (F, mg/L), Bromide (Br, mg/L), Iron (Fe, mg/L), Sulphur (S, mg/L), Chlorine (Cl, mg/L), Nitrogen (N, mg/L), Calcium (Ca, mg/L), Magnesium (Mg, mg/L), Sodium (Na, mg/L), Potassium (K, mg/L), Cadmium (Cd, mg/L), Silica (Si, mg/L), Cobalt (Co, mg/L), Copper (Cu, mg/L), Nickel (Ni, mg/L), Manganese (Mn, mg/L), Aluminium (Al, mg/L), Boron (B, mg/L), Vanadium (V, mg/L), Molybdenum (Mo, mg/L), Total Silica (mg/L), Phosphorus (P, mg/L), Silicon (Si, mg/L), Lead (Pb, mg/L), Zinc (Zn, mg/L), Sulphate (SO_4; mg/L); Dissolved Inorganic Nitrogen (DIN, mg/L), Orthophosphate (PO_4, mg/L), Hardness (Hard, mg/L $CaCO_3$), Alkalinity (Alk, mg/L $CaCO_3$), and Chlorophyll-665 (Chl-665, µg/L).

In the first PCA ordination (Figure 4.13), 33.4 % of the variance in environmental variables could be explained on the first two canonical axes (Table 4.8). Figure 4.13 indicated that temperature correlated with turbidity, DOC, chlorophyll-665, TKN, alkalinity and different chemical elements such as Zn, F, Co, Mn, Mo, K and Cu. Turbidity correlated with DOC and alkalinity and a negative correlation was found with conductivity, hardness, SS, DIN and various other elements such as PO_4, Si, Fe, Cl, P and Al.

Table 4.8: Results from the PCA analysis on all environmental variables at M-FOREBAY from April to October 2010

Axes	1	2	3	4	Total variance
Eigenvalues	0.185	0.150	0.108	0.078	1.000
Cumulative percentage variance of species data	18.5	33.4	44.2	51.9	
Sum of all					1.000

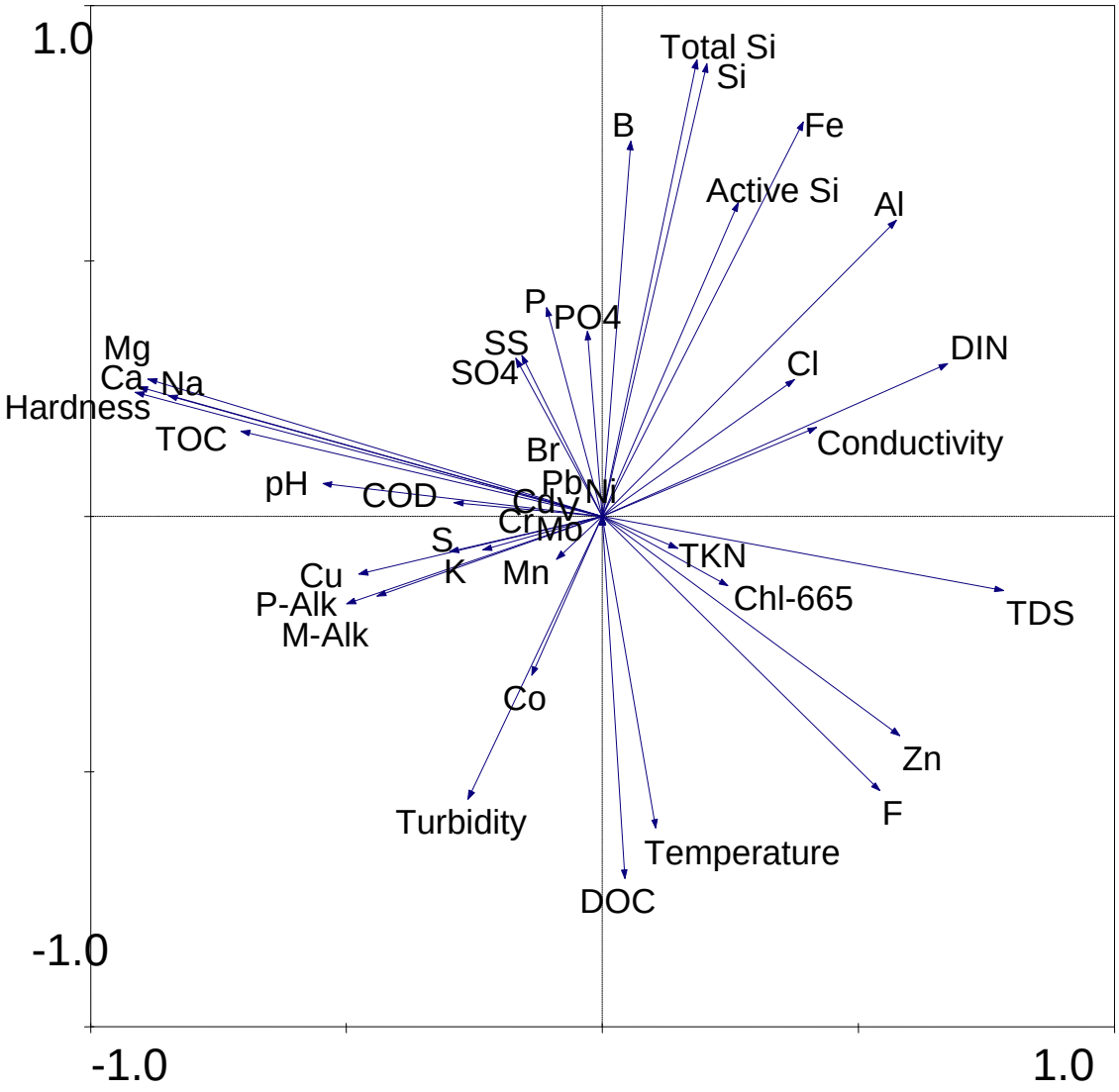


Figure 4.13: Bi-plot PCA ordination diagram showing all environmental variables measured at M-FOREBAY from April to October 2010 (LEGEND: Temperature; Turbidity; pH; Suspended solids = SS; Dissolved Organic Carbon = DOC; Conductivity; Chemical Oxygen Demand = COD; Total Dissolved Solids = TDS; Total Kjeldahl-Nitrogen = TKN; Chrome = Cr; Fluorine = F; Bromide = Br; Iron = Fe; Sulphur = S; Chlorine = Cl; Nitrogen = N; Calcium = Ca; Magnesium = Mg; Sodium = Na; Potassium = K; Cadmium = Cd; Silica = Si; Cobalt = Co; Copper = Cu; Nickel = Ni; Manganese = Mn; Aluminium = Al; Boron = B; Vanadium = V; Molybdenum = Mo; Total Silica = Total Si; Phosphorus = P; Silicon = Si; Lead = Pb; Zinc = Zn; Sulphate = SO_4; Dissolved Inorganic Nitrogen = DIN; Orthophosphate = PO_4; Hardness, Phenolphthalein Alkalinity = P-Alk; Methyl-Orange Alkalinity = M-Alk; and Chlorophyll-665 = Chl-665).

From the first PCA (Figure 4.13), the principle components were identified. Variables not regarded as principle components and those not directly related to algal growth and physiology (e.g. Pb, V, Mo, F etc.) were omitted. The variables also important in water treatment or indicators of point or non-point pollution sources were further used in the multivariate analyses.

The following variables have been selected as principle components:

- Active Silica (Active Si),
- Conductivity,
- Chlorophyll-665 (Chl-665),
- Dissolved inorganic nitrogen (DIN),
- Dissolved organic carbon (DOC),
- Turbidity,
- Alkalinity (M-Alk),
- pH,
- Hardness,
- Suspended solids (SS),
- Iron (Fe), Aluminium (Al), Manganese (Mn) and Orthophosphate (PO_4).

A second PCA was done on the principle environmental components only to reflect the relationships between these variables found at sampling point M-FOREBAY from April to October 2010 (Figure 4.14). Along the first axis (x-axis) in the PCA ordination, 28 % of the variance in environmental data could be explained and 20 % could be explained along the second axis (y-axis) (Table 4.9).

From Figure 4.14, it is evident that there is a negative correlation between turbidity and conductivity, DIN and chlorophyll-665. According to Swanepoel (1999) high flow rates can cause increasing turbidity due to an increase of suspended solids in the water. Higher conductivity results in lower turbidity due to increased coagulation and sedimentation in natural systems (Swanepoel, 1999). A positive correlation was found between turbidity and hardness as well as alkalinity. Minerals such as magnesium and calcium contribute to the hardness and alkalinity of the water. It was expected that alkalinity and conductivity would show a positive correlation because these minerals combine to form bicarbonates which may cause an increase in conductivity (Swanepoel, 1999), yet a negative correlation was found between alkalinity and conductivity in the present study. The PCA (Figure 4.14) indicated that during periods of high temperature, the chlorophyll-665 concentration is high, indicating that a higher algal biomass in the Forebay was observed during the summer. Nutrients such as phosphates (PO_4), silica (Si) and dissolved inorganic nitrogen (DIN) did not correlate with turbidity or temperature. A negative correlation was found between temperature and active silica. These previously mentioned environmental variables normally correlate positively (e.g. Janse van Vuuren and Pieterse, 2005a and 2005b) when high concentrations of diatoms are present in the water, and a positive correlation is due to the utilisation of silica by the diatoms, therefore it can be assumed that there were low concentrations of diatoms in the Forebay during

the study period. This was indeed the case as the maximum concentration of diatoms present in the Forebay was 709 cells/mL (8 mg/L Si). On one occasion (8/18/2010) the diatom concentration was 215 cells/mL and the silica concentration was 13 mg/L, indicating that the assumption made earlier was indeed acceptable as this study found the occurrence of higher concentration diatoms in the source water (709 cells/mL) when the silica concentration is much lower, therefore silica was indeed utilised by the diatoms. An average of 200 cells/mL (7.5 mg/L Si) was found during the study period.

Table 4.9: Results from the PCA analysis on the environmental data containing only the principle components at M-FOREBAY from April to October 2010

Axes	1	2	3	4	Total variance
Eigenvalues	0.28	0.197	0.14	0.105	1.000
Cumulative percentage variance of species data	28.0	47.7	61.7	72.2	
Sum of all					1.000

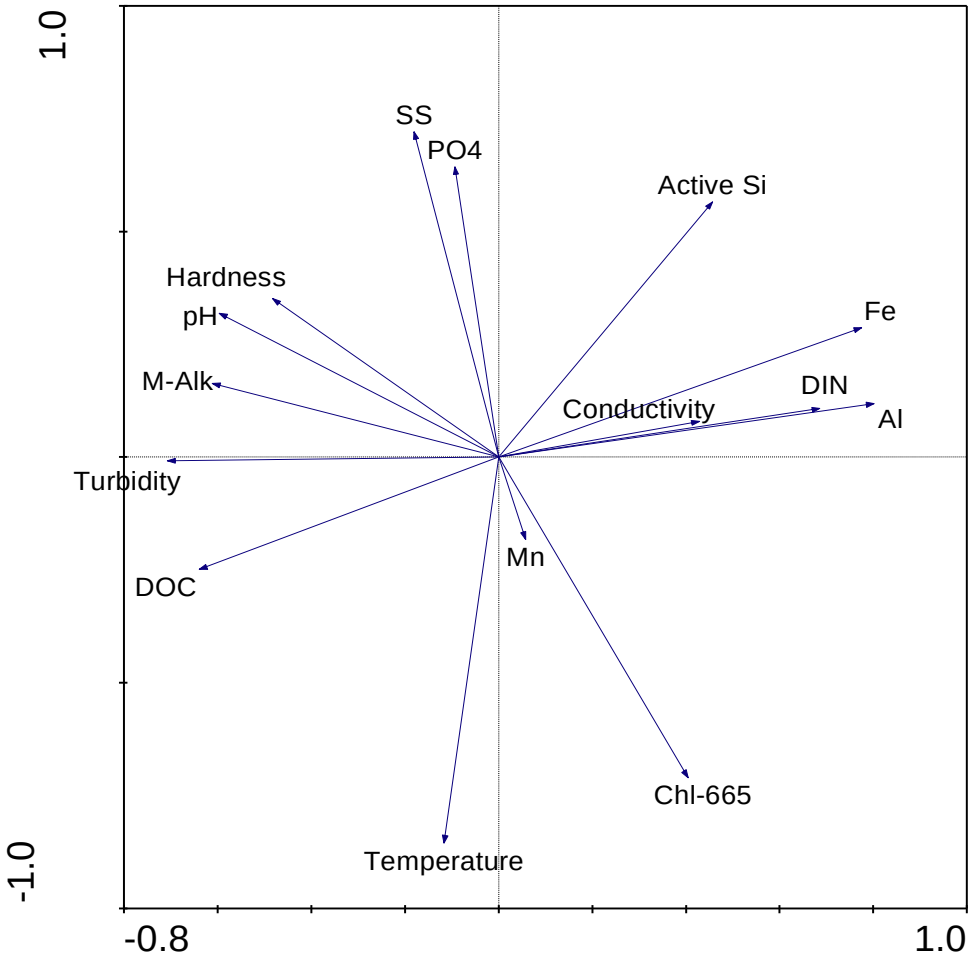


Figure 4.14: Bi-plot PCA ordination diagram showing only the main environmental variables (principle components) measured at M-FOREBAY from April to October 2010 (LEGEND:

Temperature; Turbidity; pH; Suspended solids = SS; Dissolved Organic Carbon = DOC; Conductivity; Iron = Fe; Manganese = Mn; Aluminium = Al; Active silica = Active Si; Dissolved Inorganic Nitrogen = DIN; Orthophosphate = PO_4; Hardness, Methyl Orange Alkalinity = M-Alk; and Chlorophyll-665 = Chl-665).

A Correspondence Analysis (CA) was performed on the major algal taxa and principle environmental variables. It was found that the CA and CCA ordination diagram (Figure 4.15) corresponded very well and appeared to be almost identical. Therefore, it can be concluded that the environmental variables chosen for further multivariate analyses were the correct ones to explain the variation in the algal species data. The Canonical Correspondence Analysis (CCA) was performed to reflect the relationship between the major algal taxa and environmental variables found at sampling point M-FOREBAY from April to October 2010. The following main environmental variables were included in the CCA ordination: Turbidity (Turb, NTU), Temperature (Temp, ˚C), pH, Conductivity (Cond, mS/m), Alkalinity (Alk, mg/L $CaCO_3$), Iron (Fe, mg/L), Active Silica (Active Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Hardness (Hard, mg/L $CaCO_3$), and Orthophosphate (PO_4, mg/L) and Chlorophyll-665 (Chl-665, µg/L). The seven major algal taxa included in the ordination diagram are Cyanophyceae (Cyano, cells/mL), Bacillariophyceae (Bacillario, cells/mL), Chlorophyceae (Chloro, cells/mL), Cryptophyceae (Crypto, cells/mL), Dinophyceae (Dino, cells/mL), Chrysophyceae (Chryso, cells/mL) and Euglenophyceae (Eugleno, cells/mL).

The first two canonical axes (represented in Figure 4.15) explained a total of 77.5 % of the variance within the algal data itself and variance in the algal-environment relationship (Table 4.10).

Table 4.10: Results from the CCA analysis on the environmental variables and major algal taxa at M-FOREBAY from April to October 2010

Axes	1	2	3	4	Total inertia
Eigenvalues	0.18	0.067	0.054	0.018	0.319
Species-environment correlations	1	1	1	1	
Cumulative percentage variance of species data	56.4	77.5	94.5	100.0	
Cumulative percentage variance of species-environment relation	56.4	77.5	94.5	100.0	
Sum of all eigenvalues					0.319
Sum of all canonical eigenvalues					0.319

A positive relationship was found between temperature and two algal classes, namely Cyanophyceae and Chlorophyceae, indicating that organisms in these two classes mostly occur during summer times. These two algal classes also seem to contribute to primary production more than others, since they occur during times of high pH (high photosynthesis rate – converting CO_2 to

O_2) and subsequently increasing the DOC. A positive correlation between Chlorophyceae and high pH was also found by Janse van Vuuren and Pieterse (2005a). Cyanophyceae and Chlorophyceae were also negatively correlated with conductivity and DIN. According to Janse van Vuuren and Pieterse (2005a) Cyanophyceae are generally favoured by high temperatures and low DIN concentrations. It could also be that high concentrations of these algae remove DIN from the water and therefore it seems as if it is favoured by low DIN concentrations, when in fact high Cyanophyceae and high Chlorophyceae concentrations reduce DIN concentrations. In contrast, the Bacillariophyceae and Cryptophyceae reached higher concentrations in water with lower temperatures, low pH and high DIN concentrations. These groups also showed positive correlation with silica, since it is most probably utilised by the diatoms as it is an essential nutrient (Janse van Vuuren and Pieterse, 2005a and 2005b). Representatives of the Euglenophyceae and Dinophyceae were generally very scarce or not present. Euglenophyceae seemed to be favoured by high temperatures, low pH, low DIN concentrations. These observations were also made by Janse van Vuuren and Pieterse (2005b).

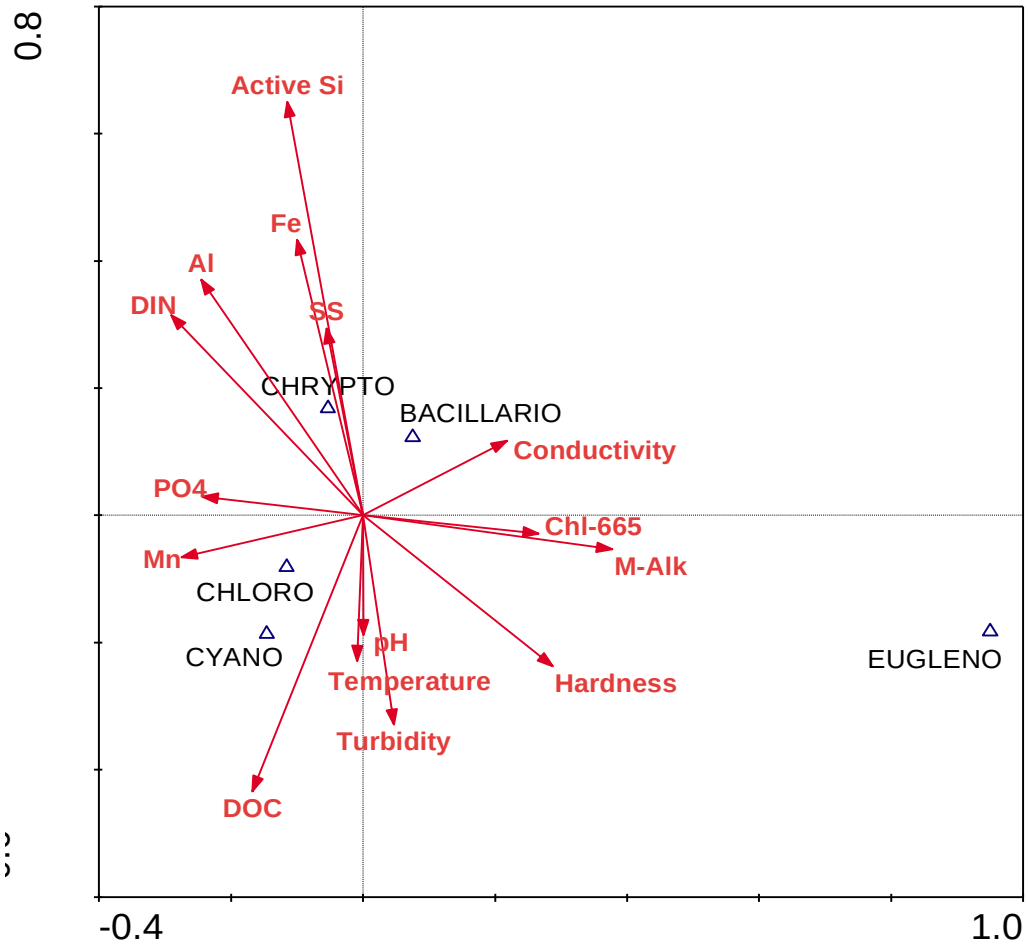


Figure 4.15: CCA ordination diagram showing principle environmental components and major algal taxa measured at M-FOREBAY from April to October 2010 (LEGEND: Temperature; Turbidity;

pH; Suspended solids = SS; Active silica = Active Si; Dissolved Organic Carbon = DOC; Conductivity; Iron = Fe; Manganese = Mn; Aluminium = Al; Dissolved Inorganic Nitrogen = DIN; Orthophosphate = PO_4; Hardness, Methyl Orange Alkalinity = M-Alk; Chlorophyll-665 = Chl-665 and the major algal taxa included in the ordination diagram are Cyanophyceae = Cyano; Bacillariophyceae = Bacillario; Chlorophyceae = Chloro; Cryptophyceae = Crypto and Euglenophyceae = Eugleno).

Table 4.11 shows that the CCA analysis (for the first canonical axis as well as for all four axes) was statistically insignificant (P = 1) as judged on the basis of the Monte Carlo Permutation test (499 random permutations).

Table 4.11: Results of the Monte Carlo Permutation test from the CCA analysis on the principle environmental components and major algal taxa at M-FOREBAY from April to October 2010

Test of significance of first canonical axis	
Eigenvalue	0.18
F-ratio	0
P-value	1
Test of significance of all canonical axes	
Trace	0.319
F-ratio	0
P-value	1

The second CCA (Figure 4.16) was performed to reflect the relationship between the algal species and environmental variables found at sampling point M-FOREBAY from April to October 2010.

The principle environmental variables included in the CCA ordination were: Turbidity (NTU), Temperature (˚C), pH, Conductivity (mS/m), Suspended Solids (SS, mg/L), Alkalinity (mg/L $CaCO_3$), Iron (Fe, mg/L), Active Silica (Active Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Hardness (mg/L $CaCO_3$), Dissolved organic carbon (DOC, mg/L), Orthophosphate (PO_4, mg/L), Mn (mg/L), Al (mg/L) and Chlorophyll-665 (Chl-665, µg/L). The algal species (cells/mL) included are the cyanobacteria (Cyanophyceae): *Anabaena* sp. and *Microcystis* sp.; the green algae (Chlorophyceae): *Chlamydomonas* sp. and *Scenedesmus* sp.; the Cryptomonads (Cryptophyceae): *Cryptomonas* sp.; *Trachelomonas* sp (Euglenophyceae); and the diatoms (Bacillariophyceae): *Aulacoseira* sp., pennate and centric diatoms.

Table 4.12 indicates that although the first two canonical axes (as represented in Figure 4.16 explained a total of 58 % of the variance within the algal data itself, and the variance in the algal-environment relationship.

Table 4.12: Results from the CCA analysis on the principle environmental variables and algal species at M-FOREBAY from April to October 2010

Axes	1	2	3	4	Total inertia
Eigenvalues	0.21	0.18	0.13	0.09	0.667
Species-environment correlations	1	1	1	1	
Cumulative percentage variance of species data	31.2	57.8	77.0	90.4	
Cumulative percentage variance of species-environment relation	31.2	57.8	77.0	90.4	
Sum of all eigenvalues					0.667
Sum of all canonical eigenvalues					0.667

According to Graham *et al* (1998) the positions of algae on ordination diagrams can give very valuable information about the species, e.g. algae found close together are more alike in response to certain environmental variables than those placed further apart. The authors also indicated that the species found furthest from the origin of the ordination have the best response to that particular variable explained by the ordination (e.g. *Anabaena* sp. (Figure 4.12a) and PO_4: (Figure 4.16)).

From Figure 4.16, a positive correlation was found between temperature and the cyanobacterium *Microcystis* sp. (Figure 4.12a); green algae *Scenedesmus* sp. (Figure 4.81c) and *Chlamydomonas* sp. (Figure 4.12c); and the Cryptophyte *Cryptomonas* sp. (Figure 4.49a). These species also seemed to be responsible for the highest primary production (indicated by high pH, high COD and high alkalinity, when photosynthesis is occurring at a high rate). The above mentioned species are also favoured at low conductivity, low nutrient concentrations (DIN, PO_4, and Si) possibly as a result of utilization for growth. Janse van Vuuren and Pieterse (2005b) found that green algae are associated with high levels of conductivity which is in contrast with what was found in this study.

Figure 4.11 indicates that there was an increase of cyanobacteria as temperatures increased (e.g. *Anabaena* sp., with a maximum total algal biomass of 366 cells/mL and *Microcystis* sp. 449 cells/mL, during the study period). However, it was found that *Anabaena* sp. tend to reach higher concentrations during lower water temperature, low pH and high PO_4. Diatoms (both centric and pennates) reached high concentrations during lower water temperature, low pH, low DOC, high conductivity and high DIN. Manganese was included in this ordination diagram for the reason that it is a pollutant resulting from mining activities. A positive correlation was found between *Trachelomonas* sp. (Figure 4.12g) and manganese, it is known that species from the algal taxa Euglenophyceae has loricas as cell coverings and can impregnate manganese and iron into it (Janse van Vuuren and Pieterse, 2005a).

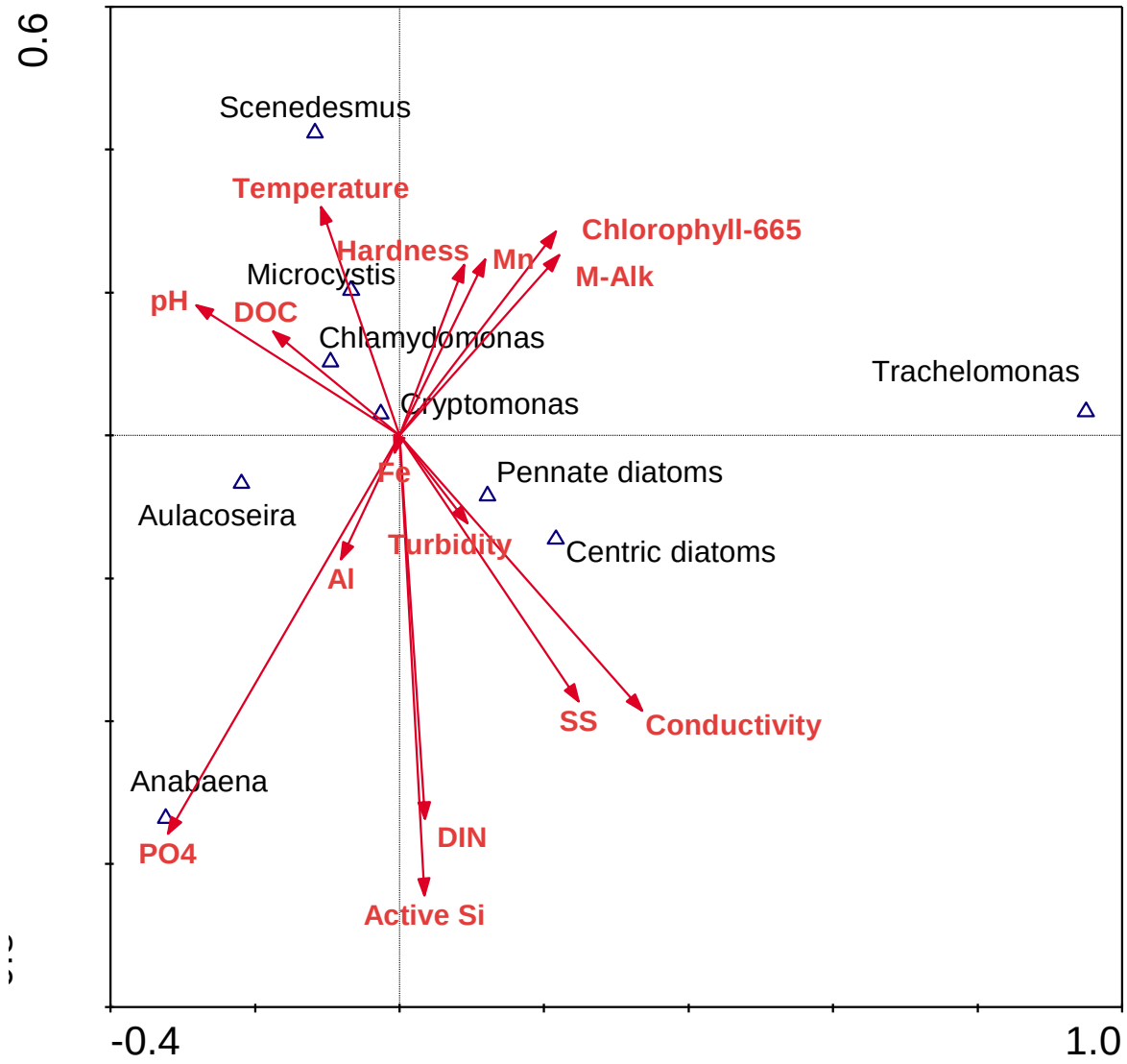


Figure 4.16: CCA ordination diagram showing principle environmental components and algal species measured at M-FOREBAY from April to October 2010 (LEGEND: Temperature; Turbidity; pH; Suspended solids = SS; Dissolved Organic Carbon = DOC; Conductivity; Iron = Fe; Manganese = Mn; Aluminium = Al; Active Silica, Active Si; Dissolved Inorganic Nitrogen = DIN; Orthophosphate = PO_4; Hardness, Methyl Orange Alkalinity = M-Alk; Chlorophyll-665 = Chl-665 and the algal species *Anabaena* sp.; *Microcystis* sp.; *Chlamydomonas* sp.; *Scenedesmus* sp.; *Cryptomonas* sp.; *Trachelomonas* sp.; *Aulacoseira* sp., pennate and centric diatoms).

From Figure 4.16, a positive correlation was found between nutrients and turbidity (e.g. DIN and Si). According to Janse van Vuuren and Pieterse (2005b) nutrients can adsorb onto silt particles under turbid conditions and become unavailable to phytoplankton. No correlation was found between chlorophyll-665 concentration and nutrients (e.g. DIN). A similar finding was made by Janse van Vuuren and Pieterse (2005b). According to Janse van Vuuren (2001) turbidity may be the most important variable to consider (as well as nutrients, physical and chemical variables) when investigating the changes in phytoplankton assemblages in the Vaal River. The Forebay source

water had an average turbidity value of 76 NTU and suspended solids concentration of 80 mg/L (for April – October 2010) in comparison with an average turbidity value of 13 NTU and suspended solids concentration of 17 mg/L (for 2000 – 2009) at sampling point C-VRB5T. According to Janse van Vuuren and Pieterse (2005b) high turbidity levels can be advantageous since it impacts environmental variables such as light penetration and temperature that will influence algal growth. This high turbidity found in the Forebay source water may be ascribed to high concentrations of particulate matter. One can be speculated that events such as runoff from the surroundings, effluent discharge (e.g. recovered water released from Panfontein upstream from the Forebay) or the confluence of the tributaries upstream of the Vaal Dam may contribute to the high turbidity levels found in the Forebay source water.

Janse van Vuuren and Pieterse (2005b) found that species such as *Anabaena* sp., *Microcystis* sp., *Oscillatoria* sp., *Trachelomonas* sp., and *Aulacoseira* sp. (Figure 4.12d) are able to grow under conditions of moderate to high turbidity levels (50 to 100 NTU); as seen in Figure 4.11, some of these species were constantly present in the Forebay source water during the study period. From Table 4.13 it is evident that this CCA analysis (Figure 4.16) was statistically insignificant (P = 1) as judged on the basis of the Monte Carlo Permutation test (499 random permutations). Thus, there was no statistically significant relation between the environmental components and algal species (Figure 4.16).

Table 4.13: Results of the Monte Carlo Permutation test from the CCA analysis on the environmental data and algal species at M-FOREBAY from April to October 2010

Test of significance of first canonical axis	
Eigenvalue	0.21
F-ratio	0
P-value	1
Test of significance of all canonical axes	
Trace	0.67
F-ratio	0
P-value	1

4.2.1.2. Assessment of jar testing experiments

Appropriate coagulant dosage

Turbidity is determined by the transparency or clarity of water. Turbidity is affected by the concentration of suspended particulate material in the source water. During the first stage of water treatment, positively charged coagulants are dosed into the source water that will bind to the negatively charged particulate material (silt, sand and algae) to form flocs. During the sedimentation stage most of the flocs will settle, and the clear effluent or supernatant will continue to the next treatment stage. Jar testing experiments simulate these processes, where the best coagulant and concentration to dose can be determined. After performing jar testing experiments the remaining particles (including the algae) in the supernatant were determined by 1) measuring turbidity, 2) measuring the chlorophyll-665 concentration and calculating 3) total algal biomass by enumeration. Turbidity readings indicate the suspended particulate matter as well as algal concentration, whereas chlorophyll-665 and total algal biomass indicate only algal biomass. Turbidity is commonly used to as an indicator to determine the optimum or appropriate dosage or to evaluate the coagulant chemical efficiency (Jun *et al*, 2001; Ma and Liu, 2002b; Ebeling *et al*, 2005; Cheng *et al*, 2008). However, Basson (2000) suggested investigating other criteria. Traditionally, choosing the most effective coagulant chemical has been highly dependent on the operator's experience based on jar testing experiments (Cheng *et al*, 2008). It must be emphasised that little research has been done to determine the most effective coagulant chemical based on flocculation conditions and floc formation (Cheng *et al*, 2008).

A turbidity value lower than 3 NTU is desired when choosing the appropriate coagulant dosing option. This value would apply to the turbidity of the supernatant that would be carried over to the filtration stage after coagulation/flocculation occurred and flocs are allowed to settle in the sedimentation tank.

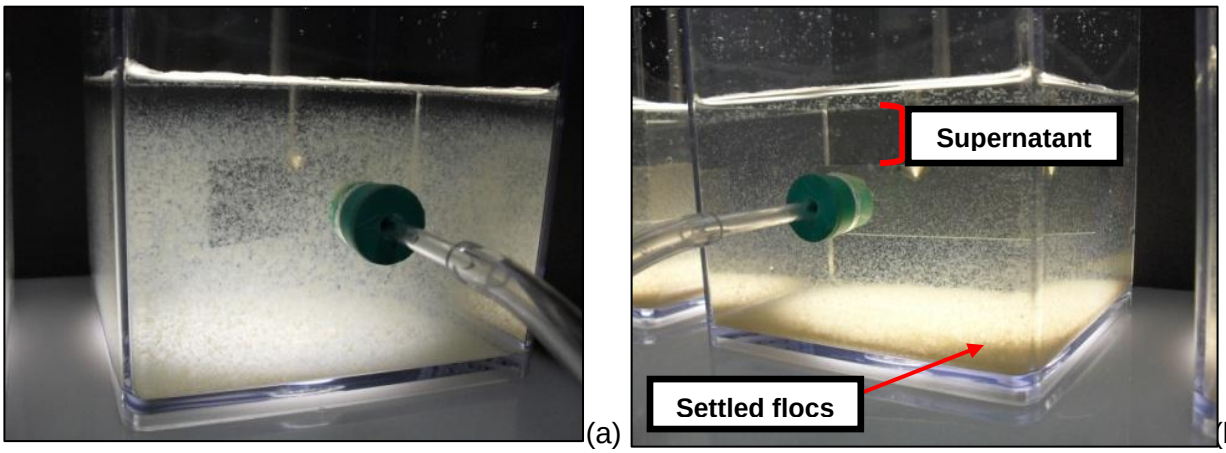
The plant laboratory uses turbidity only, to determine the "appropriate dosage" and not chlorophyll-665 or total algae biomass. When the appropriate dosage as determined by the chlorophyll-665 or total algae biomass is referred to, it will clearly be stated in the text. The appropriate coagulant dosage as determined by the turbidity, chlorophyll-665 and the total algae biomass is usually different, this can be due to a number of reasons: The first reason is that algae occur in filaments or colonies and therefore may form clumps (that is not necessarily picked up by the turbidity reading); the second reason is that the uncertainty involved in the determination of the turbidity value (Rand Water, 2009d), is much less than the uncertainty in the determination of the chlorophyll-665 (Rand Water, 2009c) or total algae biomass values (Rand Water, 2009b) and

therefore the turbidity seems to be the most appropriate way (the most stable and predictable value) to determine the appropriate dosage. The third reason is that measuring turbidity is quick while chlorophyll-665 and total algae biomass analysis requires expertise and expensive equipment.

Very few literature references explain the relationship between turbidity, chlorophyll-665 concentration and total algal biomass and the particular coagulant chemicals used in this study. However, similar findings with the same or different coagulant chemicals will be clearly stated and referred to.

4.2.1.2.1. Jar testing with poly-electrolyte as only coagulant chemical on source water from M-FOREBAY

In Figure 4.17, the fifteen minute settling period of the jar testing process is illustrated. As the flocs settle the clear supernatant becomes more apparent. The supernatant was drawn off for different analyses which include turbidity (NTU), chlorophyll-665 (µg/L, total pigments – representing the algal biomass) and phytoplankton identification and enumeration (cells/mL).



(a) (b)

Figure 4.17 (a) and (b): Jar testing with source water from sampling point M-FOREBAY, using only poly-electrolyte (13 mg/L) as coagulant.

Fifteen jar testing experiments were performed with source water from sampling point M-FOREBAY, the average values obtained for the different analyses are shown in Figures 4.19, 4.21 and 4.23, when poly-electrolyte only was used.

From Table 4.14, the appropriate dosages are shown for each jar testing experiment done during the study period - when poly-electrolyte was used as only coagulant chemical. It is important to

note that coagulant chemical concentrations to be dosed will differ every day as the source water undergo continuous changes in the physical, chemical and biological parameters.

Table 4.14: The turbidity value (NTU) obtained at the "appropriate" dosage concentration (mg/L) of the coagulant chemical used, in this case poly-electrolyte only, for each the jar testing experiments performed during the study period

Date	Appropriate dosage concentration* (mg/L)	Source water turbidity (NTU)	Turbidity (NTU)
12/04/2010	8	70.2	2.7
28/04/2010	8	73.6	2.67
04/05/2010	7	75.4	2.53
11/05/2010	10	82.6	2.82
19/05/2010	8	76.2	2.52
26/05/2010	12	80.5	2.92
15/06/2010	13	80.8	2.6
22/06/2010	11	82.7	3.06
07/07/2010	11	66.7	3.3
20/07/2010	14	73.5	2.81
18/08/2010	15	77.4	2.1
31/08/2010	16	76.3	2.85
14/09/2010	11	78.6	2.63
28/09/2010	9	75.5	2.93
12/10/2010	14	80.7	2.51

*The lowest concentration of coagulant chemical used for sufficient removal of colloidal material from the source water.

Figure 4.18 shows that the turbidity value decreased from a maximum value of 83 NTU in the source water to an average turbidity value of 7 NTU with initial coagulant chemical dosage that further decreased to an average of 3 NTU with an increase of coagulant chemical, in this case of using only poly-electrolyte. The solid line in the box represents the average value for that specific coagulant concentration over the study period.

The regression analysis revealed a strong negative correlation ($R^2 = 0.92$) between turbidity and the coagulant chemical poly-electrolyte (Figure 4.19). A study done by Ho and Newcombe (2005) showed that an increase of aluminium sulphate as coagulant chemical also resulted in a decrease of turbidity. Turbidity decreased from a turbidity value of 80 NTU in the source water to 2 NTU as the aluminium sulphate dosage increased (Ho and Newcombe, 2005). Numerous studies have

been done where cationic polymers were used as coagulant aids or secondary coagulants to improve flocs and turbidity removal (Jun *et al*, 2001; Aguilar *et al*, 2002; Ma and Liu, 2002a; Freese *et al*, 2004; Ebeling *et al*, 2005; Lee and Westerhoff, 2006). For example, Lee and Westerhoff (2006) found that dosing aluminium sulphate in combination with a cationic polymer improved the dissolved organic nitrogen removal. However, they also found that when dosing very high concentrations of aluminium sulphate, the cationic polymer had no effect in removing organics from the water. According to Bolto and Gregory (2007), very few examples exist in literature where polymer is used as only coagulant since it is commonly used in combination with a metal salt. However, using poly-electrolyte has been shown to be very effective, for example, Cornwall *et al* (2001) found a 50 % decrease of turbidity with a minimum polymer addition of 0.1 mg/L to a side-stream sedimentation plant.

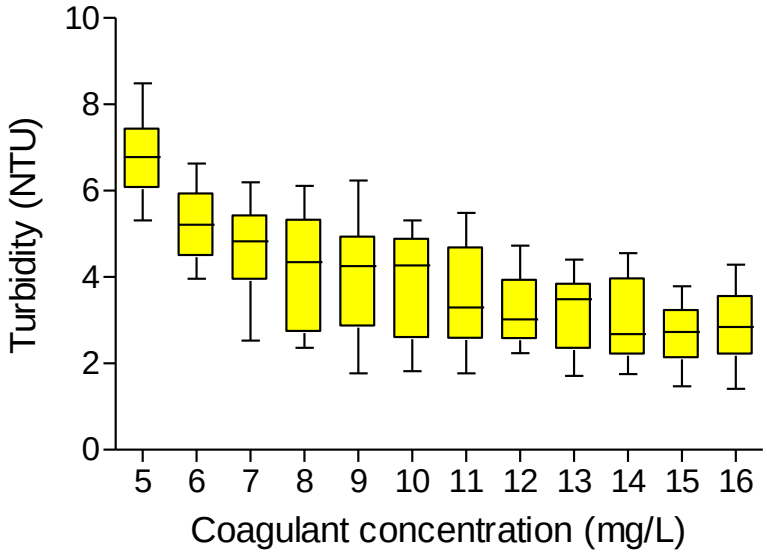


Figure 4.18: Box plot illustrating the changes in turbidity after jar testing over increasing concentrations of coagulant chemical when using only poly-electrolyte on Forebay source water for the study period of April to October 2010.

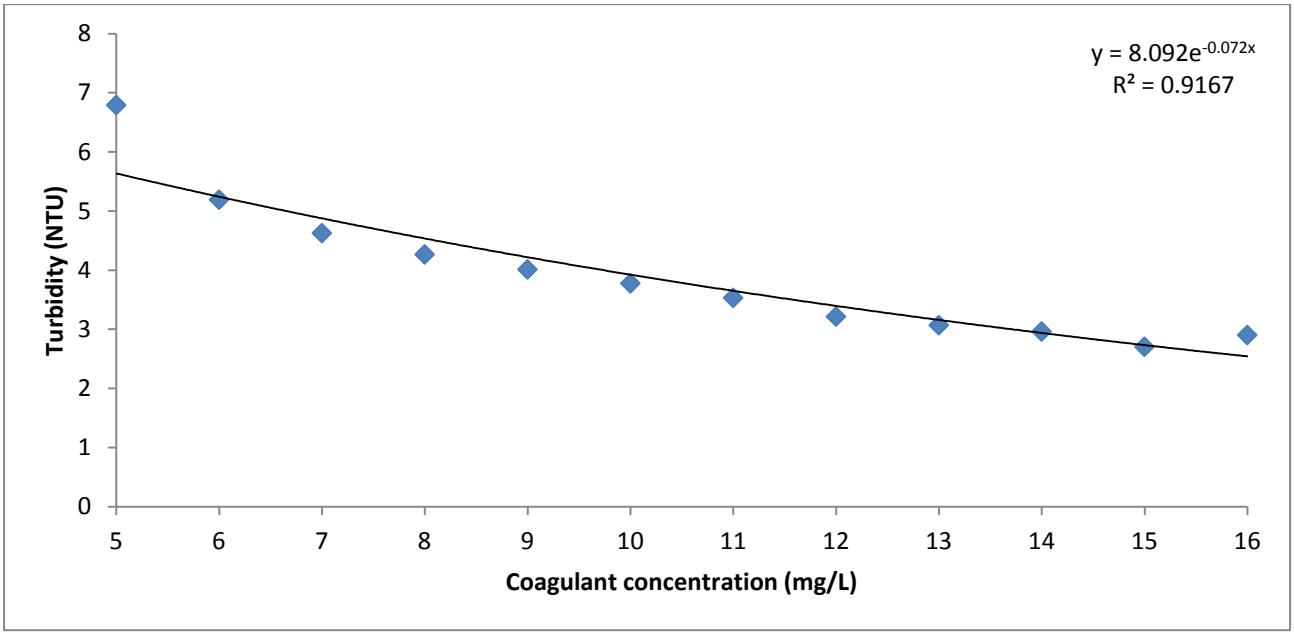


Figure 4.19: Regression analysis between average turbidity and poly-electrolyte dosage at concentrations of 5 – 16 mg/L when using only poly-electrolyte on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

Limited publications exist where chlorophyll-665 concentrations are considered to determine the appropriate dosage or evaluate the efficiency of coagulant chemicals. However, studies have been done to assess the effect of coagulant chemicals on cultured algae (e.g. Ma and Liu, 2002a) to determine the percentage algal removal.

The biological variable chlorophyll-665 represents the total photosynthetic pigment found in algae. Chlorophyll-665 concentrations in the source water varied between 1.7 and 4.7 µg/L during the study period. Figure 4.20 indicates that a decrease of chlorophyll-665 was found with an increase in the coagulant chemical poly-electrolyte. Basson (2000) also found that chlorophyll-*a* removal increased by increased coagulant chemical concentrations when using poly-electrolyte as secondary coagulant.

Figure 4.20 shows that the chlorophyll-665 concentration decreased from a maximum concentration of 4.7 µg/L in the source water to a value between 1 and 1.5 µg/L with initial coagulant chemical dosage that further decreased to values lower than 0.5 µg/L with an increase of coagulant chemical, in this case using only poly-electrolyte.

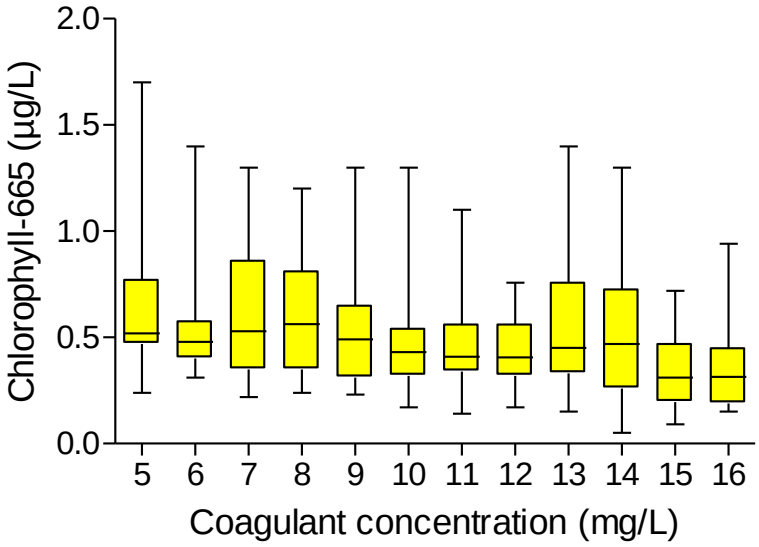


Figure 4.20: Box plot illustrating the change in chlorophyll-665 over increasing concentrations of coagulant chemical when using only poly-electrolytes on Forebay source water for the study period of April to October 2010.

The regression analysis between chlorophyll-665 and the coagulant chemical poly-electrolyte revealed a strong negative correlation (R^2 = 0.81) (Figure 4.21).

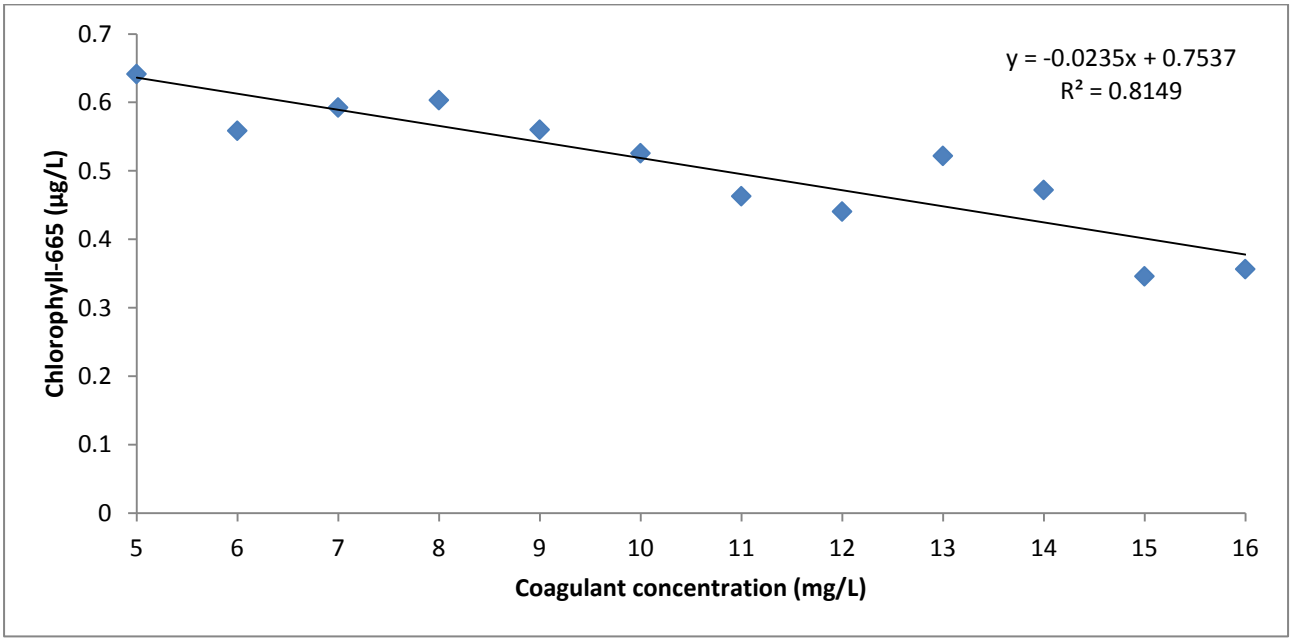


Figure 4.21: Regression analysis between average chlorophyll-665 and poly-electrolyte dosage at concentrations of 5 – 16 mg/L when using only poly-electrolyte on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

Some algae are not removed during the jar testing process (i.e. coagulation, flocculation and sedimentation) and remain in the supernatant, which are represented as the total algal biomass (in cells/mL) in Figure 4.22. It is important to remove algae by coagulation and sedimentation, to prevent filter clogging problems (Steynberg *et al*, 1994; Jun *et al*, 2001; Takaara *et al*, 2007) but sometimes algae are difficult to remove because of their morphology, size and low specific gravity (Ma and Liu, 2002a; Chen and Yeh, 2005). From Figure 4.22, it is evident that as the coagulant chemical concentration increased a decrease in algal concentration was found. The series of jar tests revealed that the total algal biomass decreased from a maximum concentration of 1234 cells/mL in the source water to a concentration of 925 cells/mL with initial coagulant chemical dosage and decreased further to levels below the detection limit, as the coagulant chemical concentration increased (Figure 4.22).

Various studies also made the same observation, for example, Ma and Liu (2002a) found increased algal removal with increasing coagulant chemical (potassium ferrate). Jun *et al* (2001) found that using a cationic polymer with alum removed more algae, resulting in larger stable flocs. Ma and Liu (2002a) also found that pre-treatment with potassium permanganate followed by coagulation with coagulants ferric sulphate and cationic polymer, improved particle and algal removal which are usually achieved in the direct filtration stage. According to Tenney *et al* (1969) cationic polymers can reduce the negative charge found on algae and enhance algal removal and coagulation, however, poly-electrolyte may not effectively remove algae with bristles or spines (such as *Scenedesmus* sp. or *Ceratium* sp.). Tenney *et al* (1969) also stated that various parameters such as pH, algal concentration, algal growth phase, algal size, algal surface area and temperature influences effective algal flocculation and the coagulant chemical concentration needed to dose.

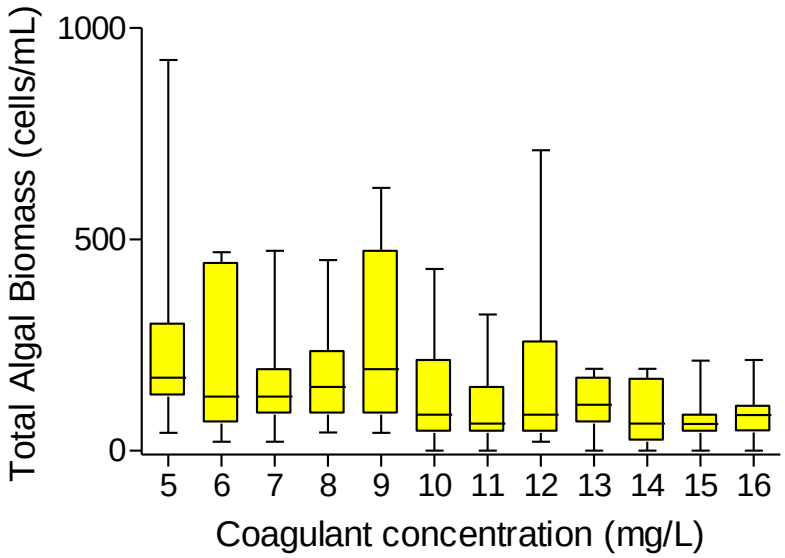


Figure 4.22: Box plot illustrating the change in total algal biomass over increasing concentrations of coagulant chemical when using only poly-electrolytes on Forebay source water for the study period of April to October 2010.

The regression analysis between total algal biomass and poly-electrolyte revealed a negative correlation (R^2 = 0.72) (Figure 4.23).

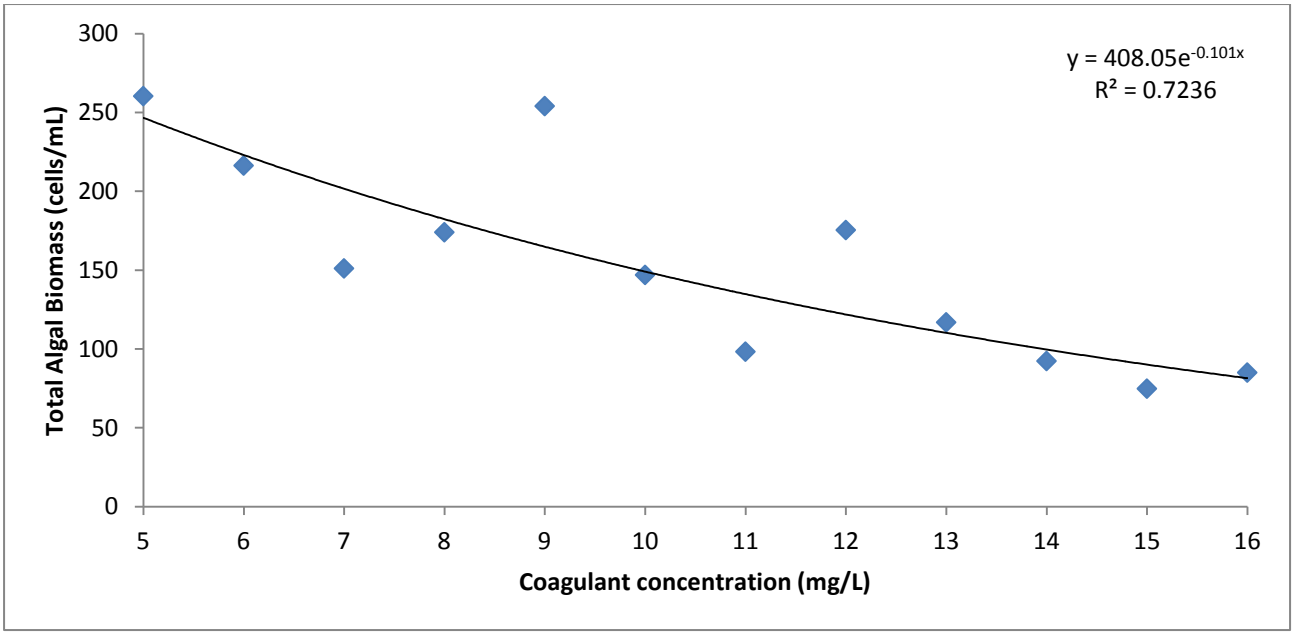


Figure 4.23: Regression analysis between average total algal biomass and poly-electrolyte dosage at concentrations of 5 – 16 mg/L when using only poly-electrolyte on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

The dominant algal species that remained in the supernatant after jar testing experiments were mostly *Anabaena* sp., *Microcystis* sp., *Cryptomonas* sp., *Aulacoseira* sp., *Chlamydomonas* sp. and low concentrations of diatoms. According to Steynberg *et al* (1994) some algae are able to escape the flocculation and sedimentation process due to their motility such as *Cryptomonas* sp. and *Chlamydomonas* sp. The cyanobacterium *Anabaena* sp. reached high concentrations in the source water during May, August and September and was also detected in the supernatant after jar testing, with varying concentrations between 172 and 753 cells/mL that were not removed by the coagulant chemical. It should however be noted that *Anabaena* sp. and *Microcystis* sp. (Figures 4.12a and b) are cyanobacteria that form gas vacuoles increasing their buoyancy and therefore sedimentation is avoided.

The use of "turbidity" as the indicator for the appropriate dosage when dosing poly-electrolyte alone seem to be a sound decision, since the regression analysis revealed an R^2 of turbidity vs. dosage concentration to be 0.92, which is higher than that of chlorophyll-665 vs. dosage concentration (R^2 = 0.81) and total algae biomass vs. dosage concentration (R^2 = 0.72) (Figures 4.19, 4.21 and 4.23). Turbidity is also an easy method to execute (colorimetric technique), unlike the method for determining chlorophyll-665 and total algal biomass, which is more involved and require very specific laboratory expertise. It must be noted that using a coagulant chemical does not remove all the turbidity found in source water, but does remove most of the turbidity during the first stage of treatment. This together with the other processes gives satisfactory results when the water reaches the customer.

4.2.1.2.2. Jar testing with Poly-electrolyte in combination with 10 mg/L slaked lime as coagulant chemicals on source water from M-FOREBAY

Very few literature references explain the use of slaked lime (CaO) as main coagulant chemical or coagulant aid with cationic polymers. According to Steynberg *et al* (1994) Rand Water dose between 55 and 70 mg/L CaO. They also emphasized that the use of poly-electrolyte should be limited since it has some disadvantages such as high dosages needed for high turbidity water and that water produced by poly-electrolyte treatment is more corrosive and of lesser quality to that produced when using lime and activated silica. Basson (2000) found that the addition of lime resulted in increased THM formation.

From Table 4.15, the appropriate dosages are indicated for each jar testing experiment done during the study period - when poly-electrolyte in combination with CaO was used. It is evident that there was also an increase of coagulant chemical concentration, just as in the case of using poly-

electrolyte alone. Thus, the composition of source water and environmental changes possibly explain the need to dose higher concentrations coagulant chemicals.

Table 4.15: The turbidity value (NTU) obtained at the "appropriate" dosage concentration (mg/L) of the coagulant chemical used, in this case poly-electrolyte in combination with 10 mg/L CaO, for each the jar testing experiments performed during the study period

Date	Appropriate dosage concentration* (mg/L)	Source water turbidity (NTU)	Turbidity (NTU)
12/04/2010	9	70.2	2.71
28/04/2010	8	73.6	2.71
04/05/2010	8	75.4	2.57
11/05/2010	7	82.6	2.81
19/05/2010	15	76.2	2.82
26/05/2010	9	80.5	2.74
15/06/2010	14	80.8	3.16
22/06/2010	14	82.7	2.76
07/07/2010	12	66.7	3.15
20/07/2010	15	73.5	3.12
18/08/2010	15	77.4	3.61
31/08/2010	10	76.3	3.16
14/09/2010	8	78.6	3
28/09/2010	12	75.5	2.79
12/10/2010	13	80.7	2.56

* The lowest concentration of coagulant chemical used for sufficient removal of colloidal material from the source water.

Figure 4.24 shows that turbidity decreased with increasing coagulant chemical concentrations (in this case using poly-electrolyte in combination with 10 mg/L slaked lime).

After performing the series of jar tests, the turbidity decreased from a maximum concentration of 85 NTU in the source water to a value between 6 and 9 NTU with initial coagulant chemical dosage and decreased further to a value between 1 and 3 NTU as the coagulant chemical concentration increased (Figure 4.24). The regression analysis revealed a strong negative correlation ($R^2 = 0.87$) between turbidity and the coagulant chemical poly-electrolyte with CaO (Figure 4.25).

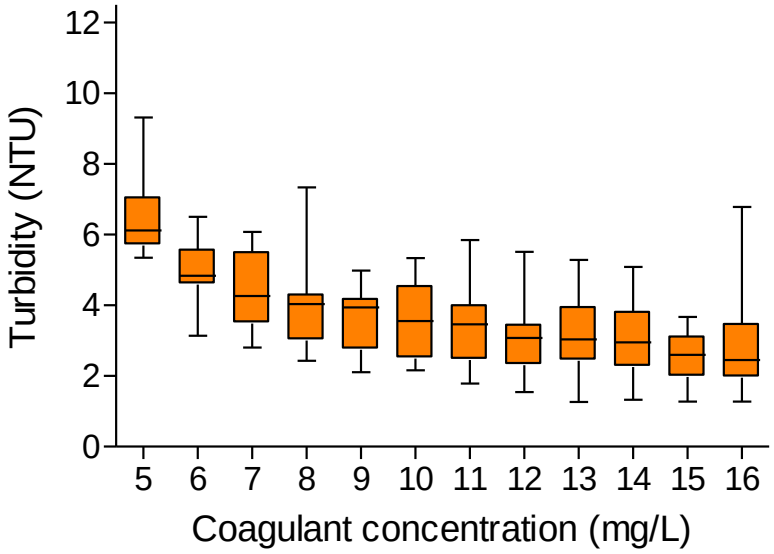


Figure 4.24: Box plot illustrating the changes in turbidity over increasing concentrations of coagulant chemical when using poly-electrolyte in combination with slaked lime on Forebay source water for the study period of April to October 2010.

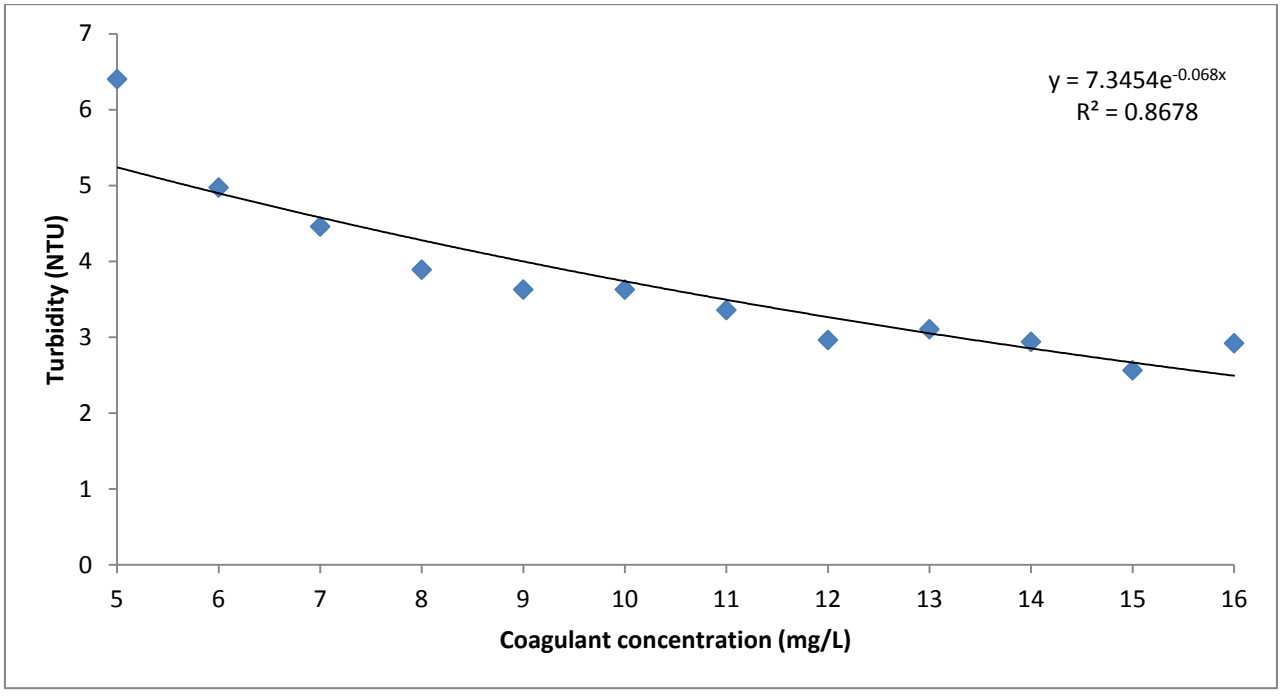


Figure 4.25: Regression analysis between average turbidity and poly-electrolyte dosage at concentrations of 5 – 16 mg/L with 10 mg/L CaO on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

Figure 4.26 displays the chlorophyll-665 concentration in the supernatant with increasing coagulant chemical concentration. A decrease in chlorophyll-665 concentration was found with increasing poly-electrolyte with CaO concentrations. After performing the series of jar tests it was clear that chlorophyll-665 decreased from a maximum concentration of 4.7 µg/L in the source water to a value between 1 and 2 µg/L with initial coagulant chemical dosage and decreased further to values below 0.5 µg/L, as the coagulant chemical concentration increased (Figure 4.26).

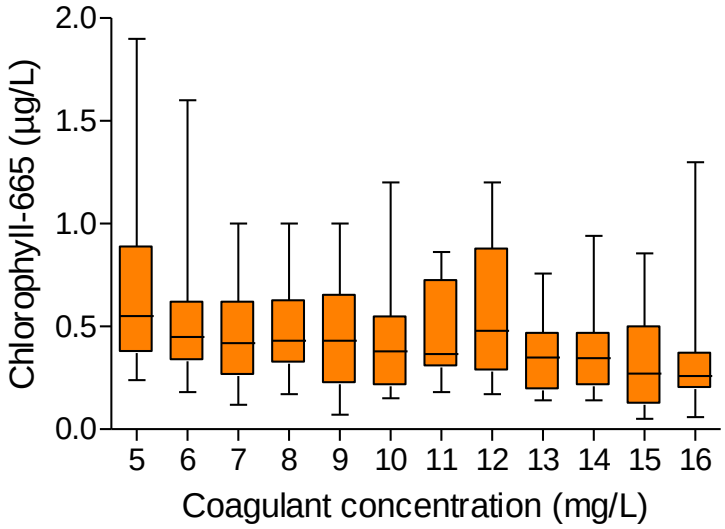


Figure 4.26: Box plot illustrating the chlorophyll-665 concentration with increasing concentrations of coagulant chemical when using poly-electrolyte in combination with slaked lime on Forebay source water for the study period of April to October 2010.

The regression analysis between chlorophyll-665 and the coagulant chemical poly-electrolyte with slaked lime revealed a negative correlation (R^2 = 0.65) (Figure 4.27).

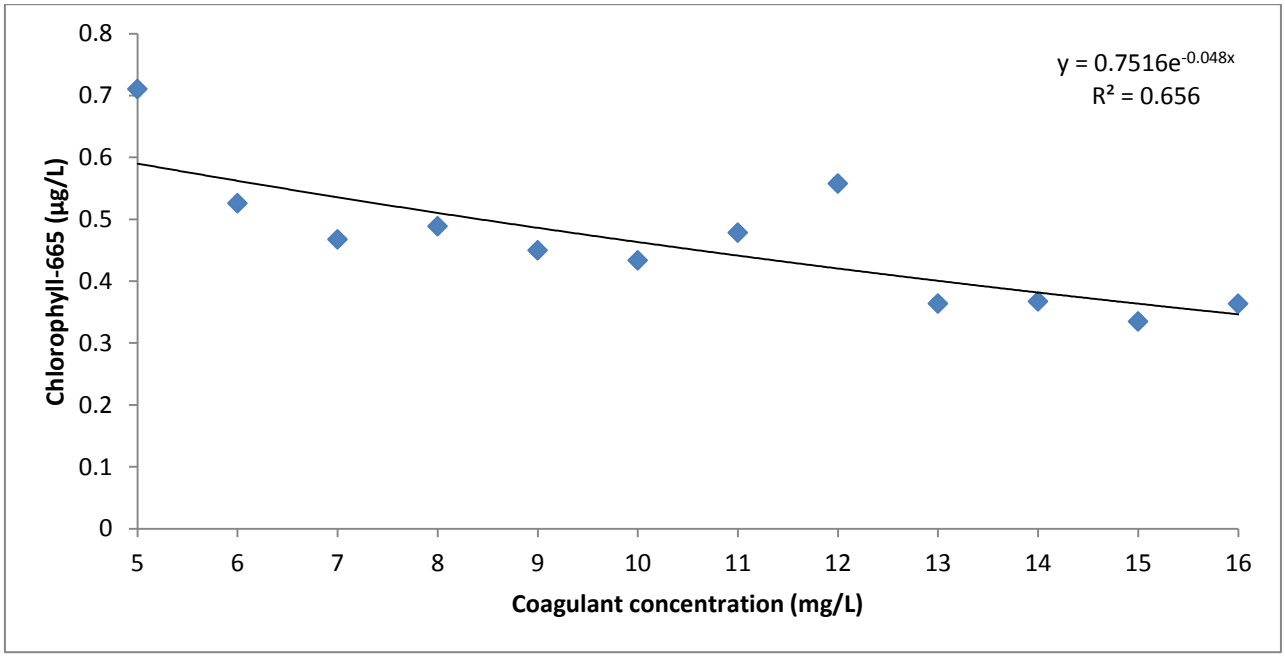


Figure 4.27: Regression analysis between average chlorophyll-665 and poly-electrolyte dosage at concentrations of 5 – 16 mg/L with 10 mg/L CaO on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

According to Steynberg *et al* (1994) the dosing of lime can aid in the reduction of algal concentrations in the water before entering the filtration stage. A decrease in algal concentration was found with increasing coagulant chemical concentrations (Figure 4.28). This same observation was made by Ma and Liu (2002a) that used potassium ferrate as coagulant chemical and increased coagulant concentrations resulted in increased algal removal, they also found that a dosage as little as 1 mg/L had an noticeable effect on algal concentrations.

After performing the series of jar tests, the total algal biomass decreased from a value of 2 195 cells/mL to ± 50 cells/mL, after coagulation/flocculation, as the coagulant chemical concentration increased (Figure 4.28). The regression analysis between total algal biomass and poly-electrolyte with slaked lime revealed a negative correlation ($R^2 = 0.5$) (Figure 4.29). The dominant algal species that remained in the supernatant during most of the jar tests were *Anabaena* sp., *Microcystis* sp., *Cryptomonas* sp., *Aulacoseira* sp. and *Chlamydomonas* sp. The cyanobacterium *Anabaena* sp. was detected in high concentrations in the supernatant with a concentration of 2195 cells/mL that were not removed by 11 mg/L poly-electrolyte with 10 mg/L CaO. However, it should be noted that cyanobacteria such as *Anabaena* sp. produce gas vacuoles that increase this alga's buoyancy, rendering it hard to remove by sedimentation.

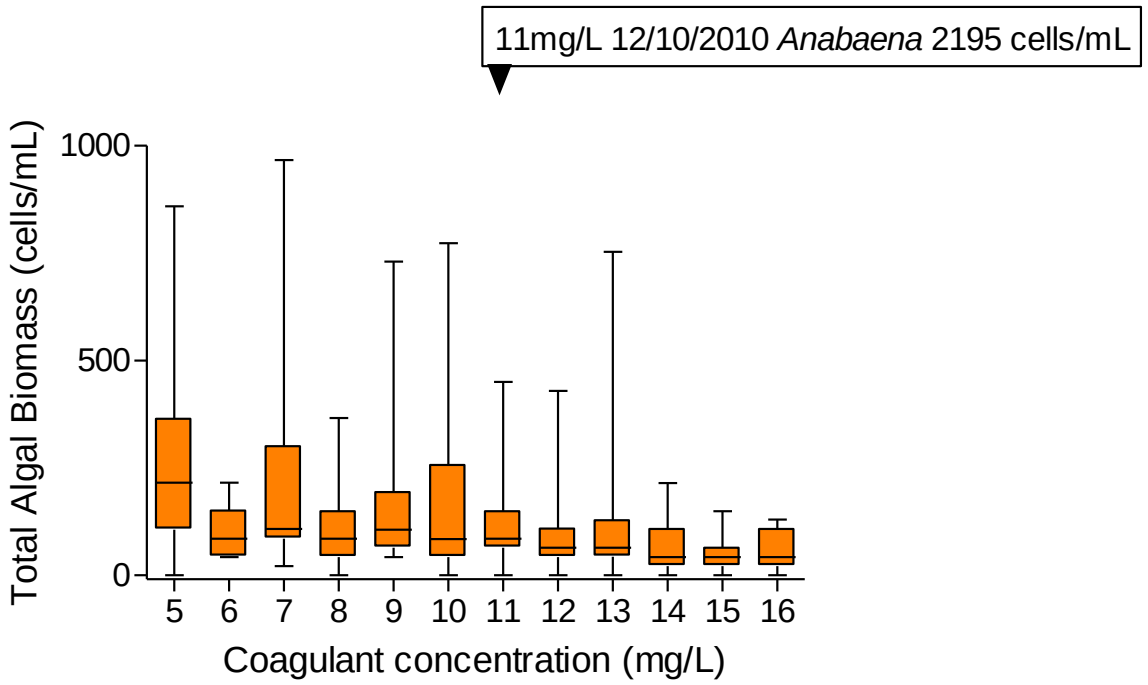


Figure 4.28: Box plot illustrating the change in total algal biomass over increasing concentrations of coagulant chemical when using poly-electrolyte in combination with slaked lime on Forebay source water for the study period of April to October 2010.

The use of "turbidity" as the indicator for the appropriate dosage seem to be a sound decision, since the regression analysis revealed an R^2 of 0.87 for turbidity vs. dosage concentration, which is higher than that of chlorophyll-665 vs. dosage concentration (R^2 = 0.65) and total algae biomass vs. dosage concentration (R^2 = 0.5) (Figures 4.25, 4.27 and 4.29).

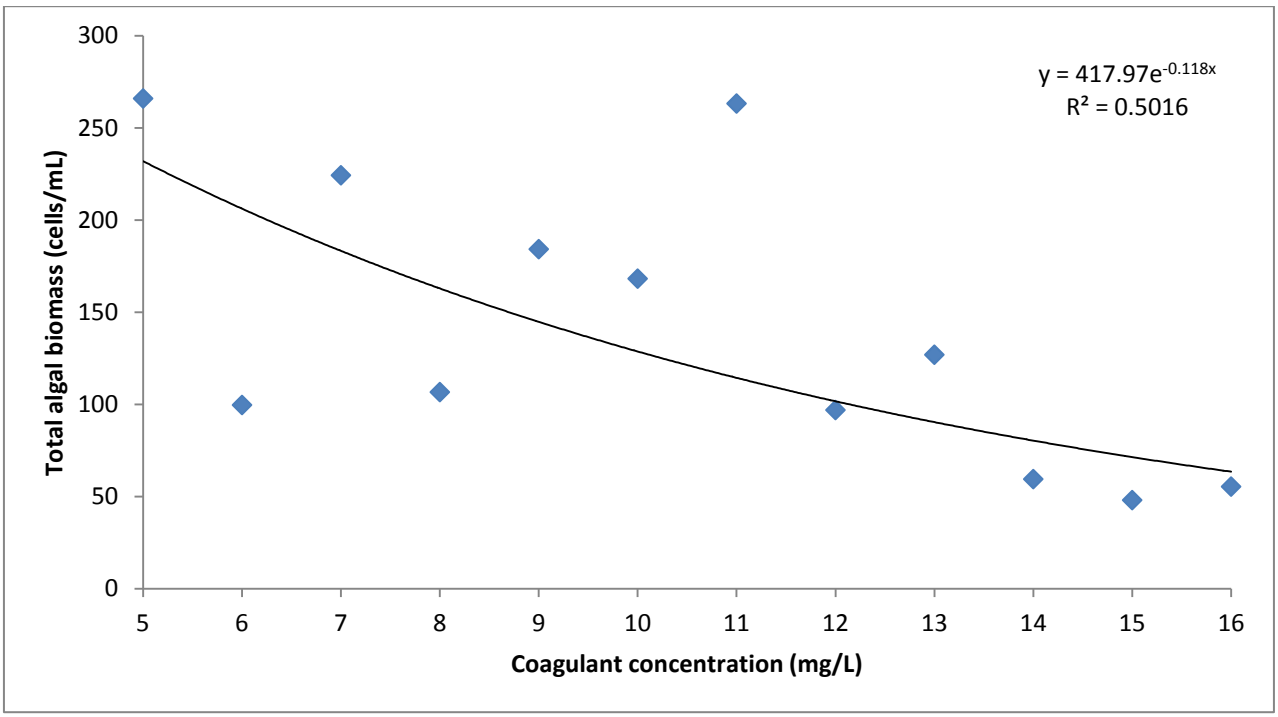


Figure 4.29: Regression analysis between average total algal biomass and poly-electrolyte dosage at concentrations of 5 – 16 mg/L with 10 mg/L CaO on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

4.2.1.2.3. Jar testing with slaked lime in combination with activated silica as coagulant chemical on source water from M-FOREBAY

Little information exist that describe the use of slaked lime (CaO) as main coagulant with activated silica as coagulant aid. It should be emphasised that the use of CaO and activated silica for destabilisation and flocculation is unique to source water from the Vaal Dam (Geldenhuys *et al*, 2000). Geldenhuys *et* al (2000) indicated that this is due to very little information that is known about the nature and origin of the organic material in the relatively unpolluted Vaal Dam. After jar testing, high turbidity values were obtained in the supernatant when using activated silica as chemical coagulant. According to Marais[4] (2010) it is difficult to simulate plant-like conditions when performing jar testing experiments using CaO and activated silica on Forebay source water. However, it should be noted that when CaO and activated silica are dosed on the plant, very good results are obtained.

Rand Water uses CaO (between 55 and 70 mg/L) for coagulation and flocculation and activated silica (between 1 and 3 mg/L) as flocculant aid (Steynberg *et al*, 1994; Geldenhuys *et al*, 2000). Lime improves the coagulation/flocculation process by limiting the growth of algae (due to a high

[4] Marais, E., 2010. [Conversation], Jar Testing experiments performed in Rand Water's Zuikerbosch water treatment plant. (Personal Communication: 5 April 2010).

pH 10 – 11), and also remove heavy metals, organic matter, bacteria and viruses (Steynberg *et al*, 1994; Geldenhuys *et al*, 2000; Basson, 2000). Activated silica is used as an inorganic coagulant aid as it improves turbidity removal and form larger flocs (Aguilar *et al*, 2002; Cheng *et al*, 2006).

From Figure 4.30, it is evident that the flocs formed when using the coagulant chemical CaO in combination with activated silica are very different from the flocs found when dosing poly-electrolyte only (Figure 4.17). The flocs were very small and did not seem to settle well. It is suspected that the coagulant chemical may not be suitable for this type of source water. It is important to note that the recovered water from Panfontein might have influenced the results obtained, for example an increase of pH and the re-introduction of organic compounds into the system. Therefore, it was decided to perform a jar test with source water from sampling point M-Canal_VD, where the recovered water from Panfontein has not yet been mixed with incoming water from the Vaal Dam (Figure 3.6), which will be discussed in section 4.2.2.

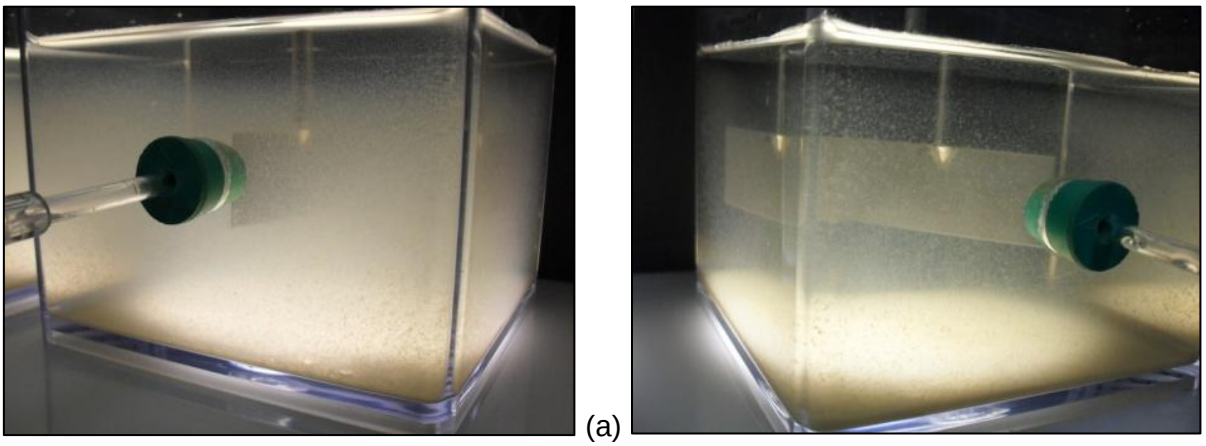

(a) (b)

Figure 4.30 (a) and (b): Picture of the jar tests performed with source water from sampling point M-FOREBAY, using CaO in combination with activated silica as chemical coagulant.

From Table 4.16, the appropriate dosages are indicated for each jar testing experiment done during the study period, when CaO in combination with activated silica was used. Figure 4.31 shows that higher turbidity values (after coagulation, flocculation and sedimentation) were found in comparison with the previous procedures where poly-electrolyte and poly-electrolyte in combination with CaO were used. However, the turbidity values still decreased as the CaO dosage concentration increased (although not with the same magnitude as the other procedures explained in Sections 4.2.1.2.1. and 4.2.1.2.2.).

Table 4.16: The turbidity value (NTU) obtained at the "appropriate" dosage concentration (mg/L) of the coagulant chemical used, in this case CaO in combination with 2.5 mg/L activated silica, for each the jar testing experiments performed during the study period

Date	Appropriate CaO dosage concentration* (mg/L)	Source water turbidity (NTU)	Turbidity (NTU)
11/05/2010	55	82.6	11.9
19/05/2010	75	76.2	15.1
26/05/2010	80	80.5	11.3
15/06/2010	50	80.8	5.63
22/06/2010	40	82.7	19.3
07/07/2010	60	66.7	3.08
20/07/2010	70	73.5	11.4
18/08/2010	60	77.4	15.6
31/08/2010	30	76.3	16
14/09/2010	85	78.6	12.5
28/09/2010	80	75.5	9.78
12/10/2010	85	80.7	15.3

* The lowest concentration of coagulant chemical used for sufficient removal of colloidal material from the source water.

From Figure 4.31, the overall turbidity values ranged between 3 and 60 NTU. The turbidity value decreased from a maximum concentration of 85 NTU in the source water to an average turbidity mostly ranged between 15 and 20 NTU. Geldenhuys *et al* (2000) also found an increase in macro particles with increased activated silica dosages. They found that high concentrations of CaO did not affect the formation of flocs (e.g. floc settling rate) and remove most of the turbidity found in the water. According to Geldenhuys *et al* (2000) dosages higher than 65 mg/L interfered with the formation of flocs. Freese *et al* (2004) found that when lime is dosed in higher concentrations that what the water supply requires, the supernatant will have high turbidity values because of residual lime particles.

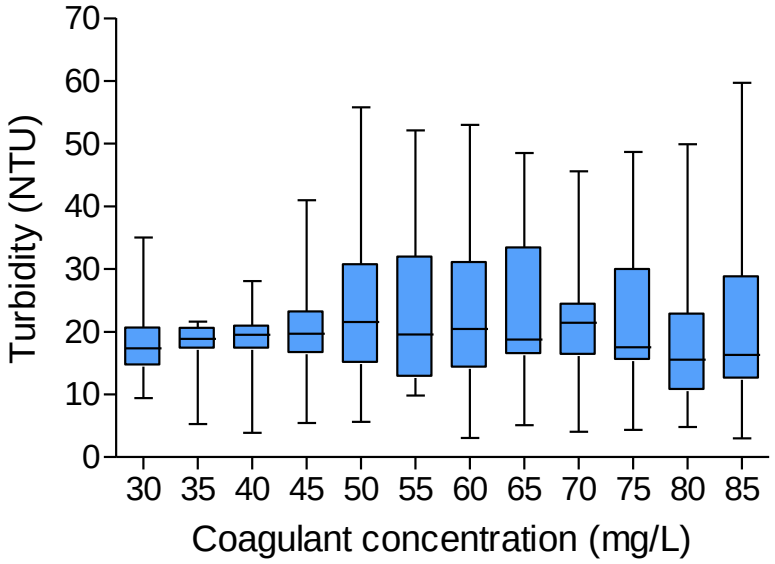


Figure 4.31: Box plot illustrating the changes in turbidity with increasing concentrations of coagulant chemical (varying concentrations of CaO ranging from 30 – 85 mg/L with 2.5 mg/L activated silica) on Forebay source water for the study period of April to October 2010.

The regression analysis between turbidity and the coagulant chemical CaO with activated silica did not reveal a good correlation (R^2 = 0.2) (Figure 4.32).

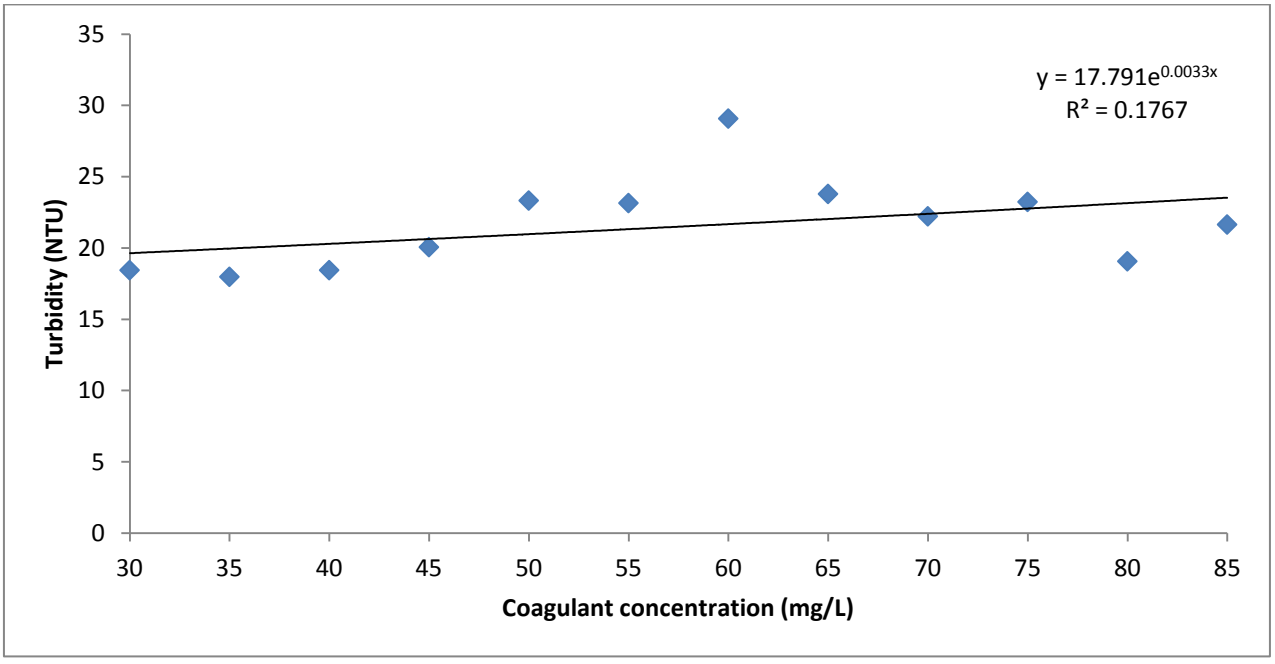


Figure 4.32: Regression analysis between average turbidity and coagulant dosage (dosing varying concentrations of CaO ranging from 30 – 85 mg/L with 2.5 mg/L activated silica) on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

From Figure 4.32 there seems to be an increase in turbidity with increasing coagulant dosage, suggesting that the correct range of concentrations were either below or above those tested and that the inherent increased turbidity of the coagulant chemical contributed significantly to the turbidity in the supernatant.

Figure 4.33 shows a decrease of chlorophyll-665 was found with increasing coagulant chemical concentration (CaO in combination with activated silica). Similar observations were made with poly-electrolyte and poly-electrolyte in combination with CaO as coagulant chemicals in the sections 4.2.1.2.1 and 4.2.1.2.2. It seems that the algae is relatively sensitive to pH changes and when increasing CaO concentrations algae will most probably die or be rendered immobile and therefore be removed effectively (Steynberg *et al*, 1994). The particulate matter penetrating to the sand filter will therefore consist of more suspended solids than algae when dosing CaO in combination with activated silica. Geldenhuys *et al* (2000) found that oxidants used with activated silica and CaO as coagulants did enhance the removal of chlorophyll containing cells.

After performing the series of jar tests it is clear that chlorophyll-665 concentration decreased from a maximum concentration of 4.7 µg/L in the source water to an average of 2 µg/L with initial coagulant chemical dosage that further decreased to values lower to an average of 0.6 µg/L as the coagulant chemical concentration increased (Figure 4.33). The regression analysis between chlorophyll-665 and the coagulant chemical activated silica with CaO revealed a strong negative correlation ($R^2 = 0.91$) (Figure 4.34).

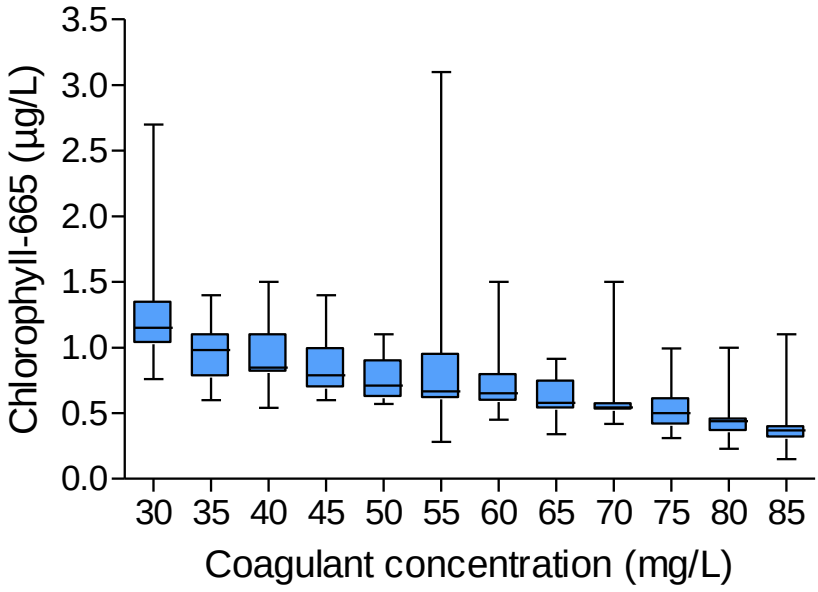


Figure 4.33: Box plot illustrating the changes in chlorophyll-665 over increasing concentrations of coagulant chemical when using varying concentrations of CaO ranging from 30 - 85 mg/L in combination with 2.5 mg/L activated silica with on Forebay source water for the study period of April to October 2010.

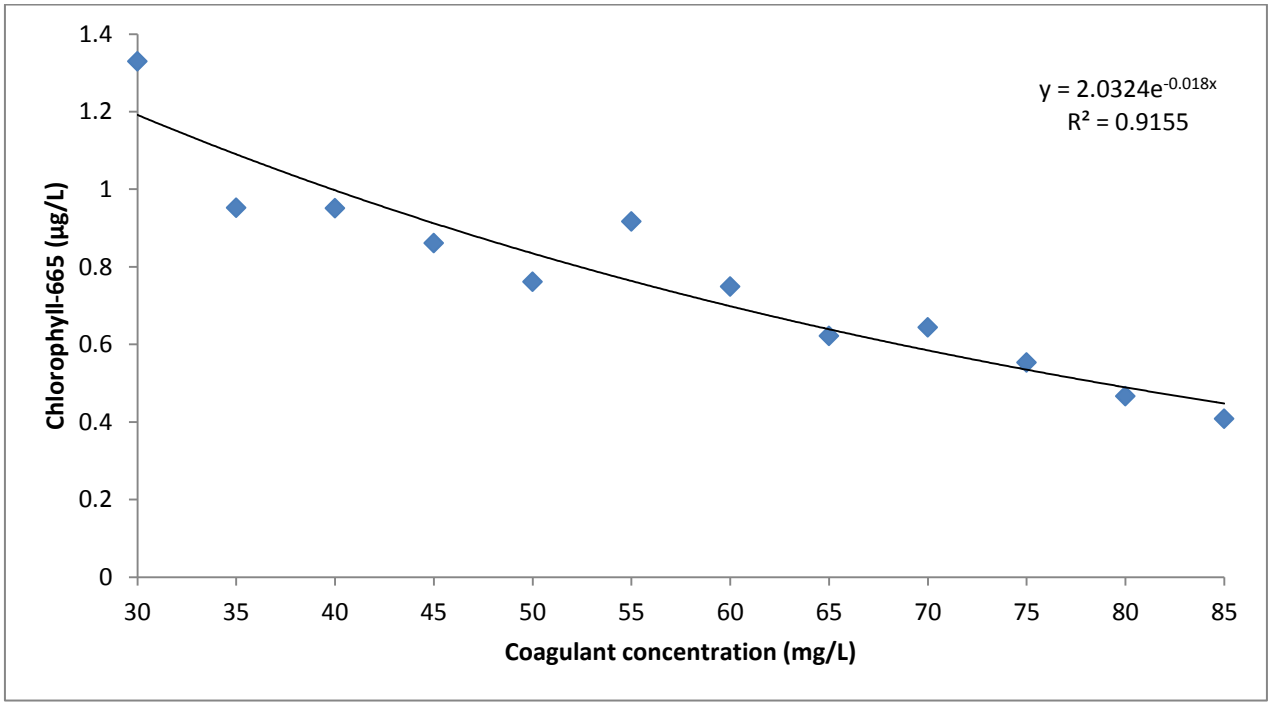


Figure 4.34: Regression analysis between average chlorophyll-665 and coagulant dosage concentrations of CaO ranging from 30 – 85 mg/L in combination with 2.5 mg/L activated silica is dosed with on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

A decrease in algal concentrations were found as the concentration of coagulant chemical increased (Figure 4.35). Similar observations were made with poly-electrolyte and poly-electrolyte in combination with CaO as coagulant chemicals in the sections 4.2.1.2.1 and 4.2.1.2.2. After performing the series of jar tests it is clear that the total algal biomass decreased from a maximum concentration of 1234 cells/mL in the source water to an average of 280 cells/mL with initial coagulant chemical dosage and decreased further to an average of 100 cells/mL as the coagulant chemical concentration increased (Figure 4.35). The regression analysis between total algal biomass and CaO with activated silica revealed an R^2 of 0.8 (Figure 4.36).

The dominant algal species that remained in the supernatant after the jar testing experiments were *Anabaena* sp., *Microcystis* sp., *Cryptomonas* sp., *Aulacoseira* sp., *Scenedesmus* sp., *Chlamydomonas* sp. and diatoms. The cyanobacterium *Anabaena* sp. was detected in high concentrations in the supernatant with a concentration of 987 cells/mL that were not removed by 35 mg/L CaO with 2.5 mg/L activated silica. During July 2010, high concentrations of *Oscillatoria* sp. (Figure 4.8b) were present in Forebay source water, and were also present (1076 cells/mL) in the supernatant of some samples after performing jar testing during that month.

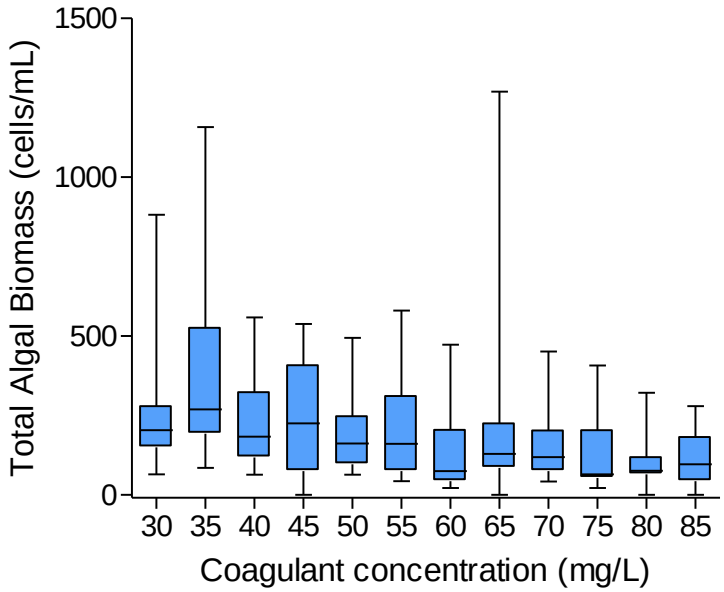


Figure 4.35: Box plot illustrating the changes in total algal biomass over increasing concentrations of coagulant chemical when using different concentrations of CaO ranging from 30 – 85 mg/L with 2.5 mg/L activated silica with on Forebay source water for the study period of April to October 2010.

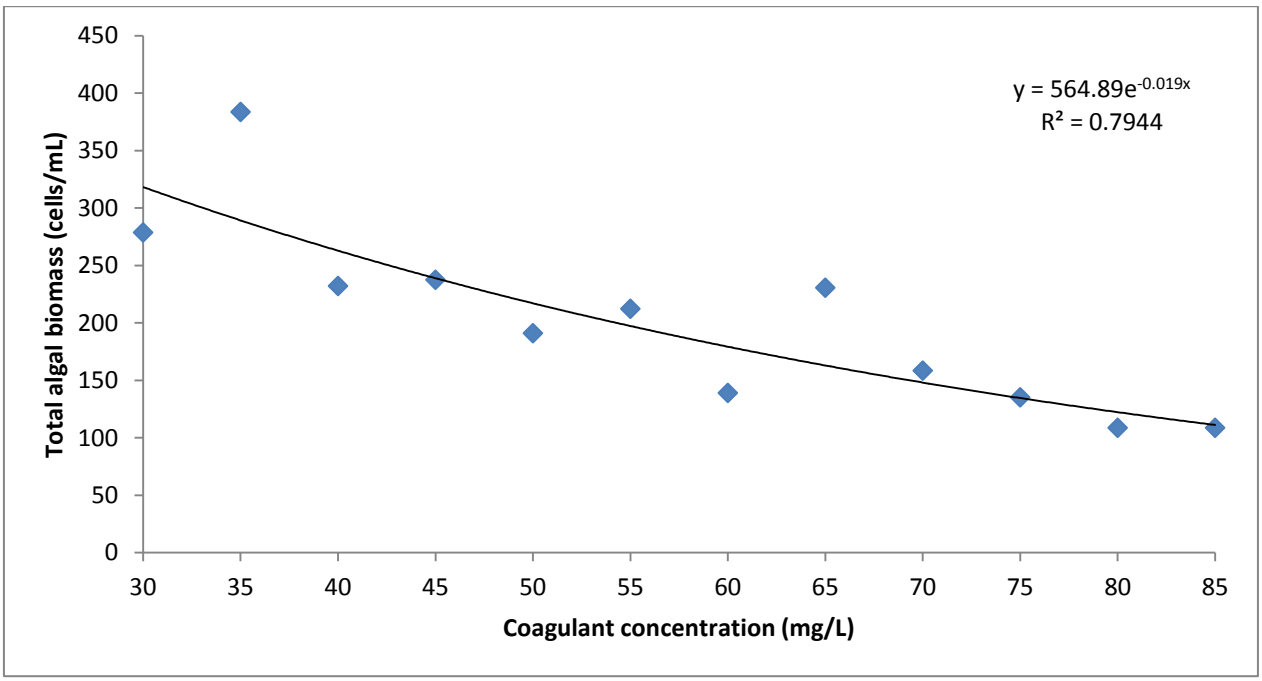


Figure 4.36: Regression analysis between average total algal biomass and coagulant dosage when using different concentrations of CaO ranging from 30 – 85 mg/L with 2.5 mg/L activated silica on Forebay source water. The average values were determined during all jar test experiments during the study period (April to October 2010).

Using different concentrations of CaO with activated silica, the turbidity of the supernatant after jar testing, seems to indicate that the coagulation and flocculation unit processes was ineffective when using this particular coagulant (R^2 = 0.2). However, the chlorophyll-665 results indicate that CaO in combination with activated silica is a very effective coagulant in removing specifically algal material from the source water (Figure 4.34: R^2 = 0.91), thereby suggesting that plant laboratories should not only use turbidity as indicator for the effectiveness of the simulated coagulation/flocculation unit processes, but also chlorophyll-665 (Figures 4.32, 4.34 and 4.36). The regression analysis also revealed an R^2 of 0.8 between total algae biomass and the coagulant chemical, showing that this particular analysis may also assist in determining the appropriate coagulant chemical and concentration.

The results of this specific jar test, indicates the negative effect that the pH of CaO in combination of activated silica has on the physiology of the algae (causing the death of algae and/or rendering it immobile for more effective removal) (Steynberg *et al*, 1994; Basson, 2000). The high pH seems to aid in the coagulation, flocculation and sedimentation of specifically algae during water purification (Knappe *et al*, 2004), indicating that CaO in combination with activated silica would most probably be the best coagulant to use during times of high chlorophyll concentrations in the source water.

4.2.1.3. Comparison of chemical coagulant treatments and coagulant concentrations on results from jar testing experiments on M-FOREBAY

Figure 4.37 indicates the relationship between the results of the remaining turbidity, chlorophyll-665 and total algal biomass concentrations in the supernatant, after jar testing with the different coagulant chemicals: poly-electrolyte (represented by a red circle (Poly)), a combination of poly-electrolyte and CaO (represented by a blue square (PolyCa)) and activated silica in combination with CaO (represented by a green diamond (SiCa)).

In the PCA ordination (Figure 4.37), 78 % of the variance in environmental data could be explained on the first two axes (Table 4.17). This ordination diagram includes all the concentrations of the different coagulant chemicals. Figure 4.37 indicates that turbidity and chlorophyll-665 (total photosynthetic pigments) were removed when dosing poly-electrolyte and poly-electrolyte in combination of CaO. However, when CaO in combination with activated silica was dosed, the turbidity and chlorophyll-665 were not removed effectively.

Table 4.17: Results from the PCA analysis on all jar testing procedures measured at M-FOREBAY from April to October 2010, with turbidity, chlorophyll-665 and total algal biomass for the different coagulant chemical treatment

Axes	1	2	3	4	Total variance
Eigenvalues	0.481	0.301	0.219	0	1
Cumulative percentage variance of species data	48.1	78.1	100	0	
Sum of all					1

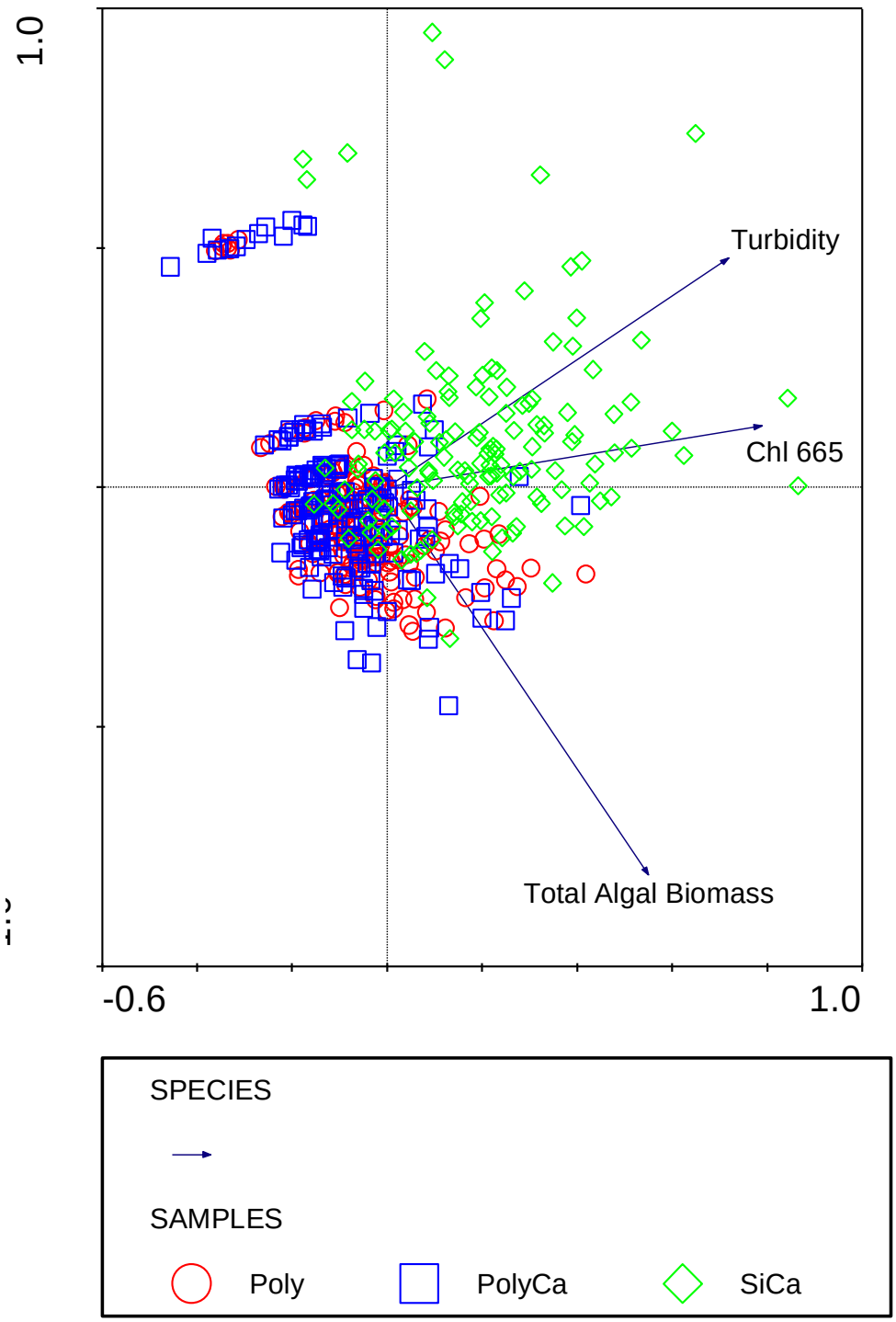


Figure 4.37: Bi-plot PCA ordination diagram showing the correlation of all jar testing experiments executed at M-FOREBAY from April to October 2010, with turbidity, chlorophyll-665 and total algal biomass for the different coagulant chemical treatments indicated by the different coloured symbols (LEGEND: poly-electrolyte (represented by a red circle (Poly)), a combination of poly-electrolyte and CaO (represented by a blue square (PolyCa)) and activated silica in combination with CaO (represented by a green diamond (SiCa)).

From all the results obtained for the different coagulant chemicals for the study period, the appropriate coagulant dosage were selected and multivariate analysis were performed to determine the relationship between the coagulant chemical with turbidity, chlorophyll-665 and total algal biomass.

Due to the number of replicates as well as the inclusion of all (appropriate and non-appropriate) dosage concentrations in Figure 4.37, definite trends and relationships could not be distinguished due to overcrowding of the diagram. It was therefore decided to select only the results of the appropriate dosage concentrations and perform an additional PCA analysis on such data. The results from this PCA are displayed in Figure 4.38 and Table 4.18.

In the PCA ordination (Figure 4.38), 80.4 % of the variance in the data could be explained on the first two axes (Table 4.18). Figure 4.38 indicates that when CaO in combination with activated silica was dosed, turbidity was not removed efficiently but instead CaO contributed to higher turbidity in the water. Similar findings were made by Freese *et al* (2004). When poly-electrolyte alone as well as poly-electrolyte in combination with CaO was dosed, chlorophyll-665 and turbidity were removed effectively. Algal biomass tended not to correlate with chlorophyll-665 in the Forebay since *Anabaena* sp. and *Microcystis* sp. were the dominant algal species. These cyanobacteria are very small algal cells that do not contribute significantly to the chlorophyll-665 concentration in the water unless in exceptionally high concentrations.

Table 4.18: Results from the PCA analysis showing the correlation of the "appropriate" concentration of coagulant chemical that was dosed with turbidity, chlorophyll-665 and total algal biomass for the different coagulant chemical treatment for jar tests done with source water at sampling point M-FOREBAY from April to October 2010

Axes	1	2	3	4	Total variance
Eigenvalues	0.538	0.266	0.196	0	1
Cumulative percentage variance of species data	53.8	80.4	100.0	0	
Sum of all					1

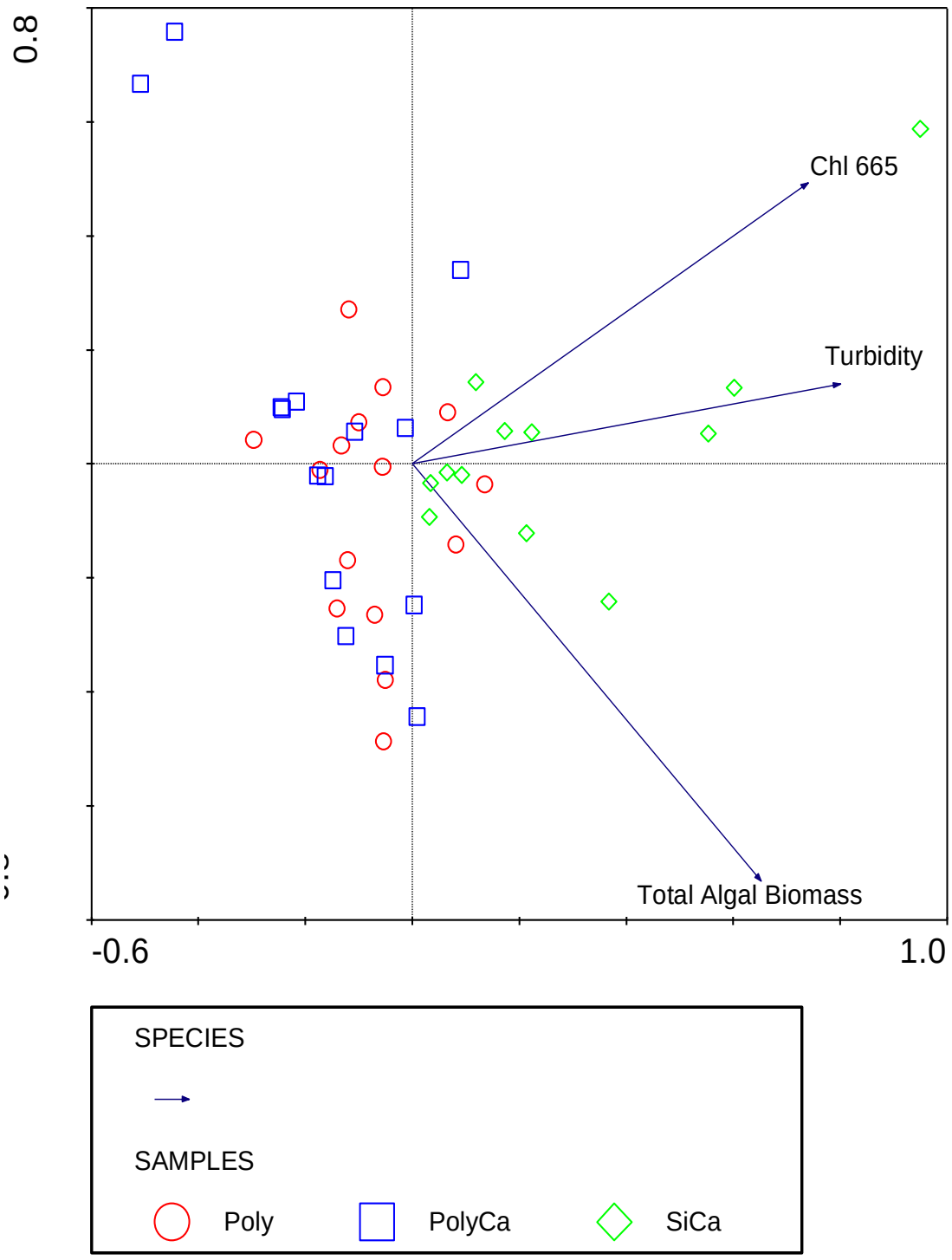


Figure 4.38: Bi-plot PCA ordination diagram showing the correlation with turbidity, chlorophyll-665 and total algal biomass where the "appropriate" concentration of coagulant chemical was dosed for jar tests done with source water at sampling point M-FOREBAY from April to October 2010, for the different coagulant chemical treatments (LEGEND: poly-electrolyte (represented by a red circle (Poly)), a combination of poly-electrolyte and CaO (represented by a blue square (PolyCa)) and activated silica in combination with CaO (represented by a green diamond (SiCa)).

4.2.2. Assessment of jar testing experiments from sampling point M-CANAL_VD

Due to the unusual results found during the jar testing experiment with CaO in combination with activated silica, it was decided to investigate whether the reason for the unexpected results were due to the influence of the recovered Panfontein water. Therefore an additional jar testing experiment was performed on source water from sampling point M-CANAL_VD, a site before the inflow from the recovered water from Panfontein.

4.2.2.1. Assessment of M-CANAL_VD source water

In Table 4.19, a summary of the physical, chemical and certain biological variables in M-CANAL_VD for 05/01/2011, during which period sampling took place for jar testing experiment, are displayed. There were not any major differences between the chemical and physical variables found at M-FOREBAY and M-CANAL_VD. However, silica concentrations were slightly lower than what was found at M-FOREBAY (Total silica: average concentration of 16 mg/L). This could be due to the presence of diatoms in the source water that utilises the silica. Conductivity, various minerals (e.g. Chloride), and nutrients such as PO_4, NO_3 and NO_2 were also found in lower concentrations than in the case of M-FOREBAY.

Table 4.19: Physical, chemical and biological parameters for sampling point M-CANAL_VD on 05/01/2011

Turbidity (NTU)	Conductivity (mS/m)	pH	Alkalinity (mg/L $CaCO_3$)	F (mg/L)	Br (mg/L)	SO4 (mg/L)	NH4 (mg/L)	PO4 (mg/L)	Cl (mg/L)
81	19	7.62	59	0.12	0.25*	14	0.243*	0.036*	5

TDS (mg/L)	Suspended solids (mg/L)	TKN (mg/L)	COD (mg/L)	Hardness (mg/L $CaCO_3$)	Ca (mg/L)	Mg (mg/L)	K (mg/L)	TOC (mg/L)	DOC (mg/L)	Na (mg/L)
185	86	2.1	13	50	11	5.5	2.2	6.2	6.2	6.6

Cd (mg/L)	Cr (mg/L)	Co (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Pb (mg/L)	Ni (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Zn (mg/L)
0.0025*	0.010*	0.015*	0.010*	0.44	0.003	0.008*	0.015*	0.43	0.02	0.008*

Al (mg/L)	B (mg/L)	V (mg/L)	Mo (mg/L)	Total Silica (mg/L)	P (mg/L)	S (mg/L)	Chlorophyll-665 (µg/L)	Si (mg/L)
0.76	0.05	0.03*	0.010*	7.9	0.041*	4.8	9.43	3.7

* Method Reporting limit

In Figure 4.39, the concentration of the different algae present in the source water on 05/01/2011 that was used for the jar testing experiments, are displayed.

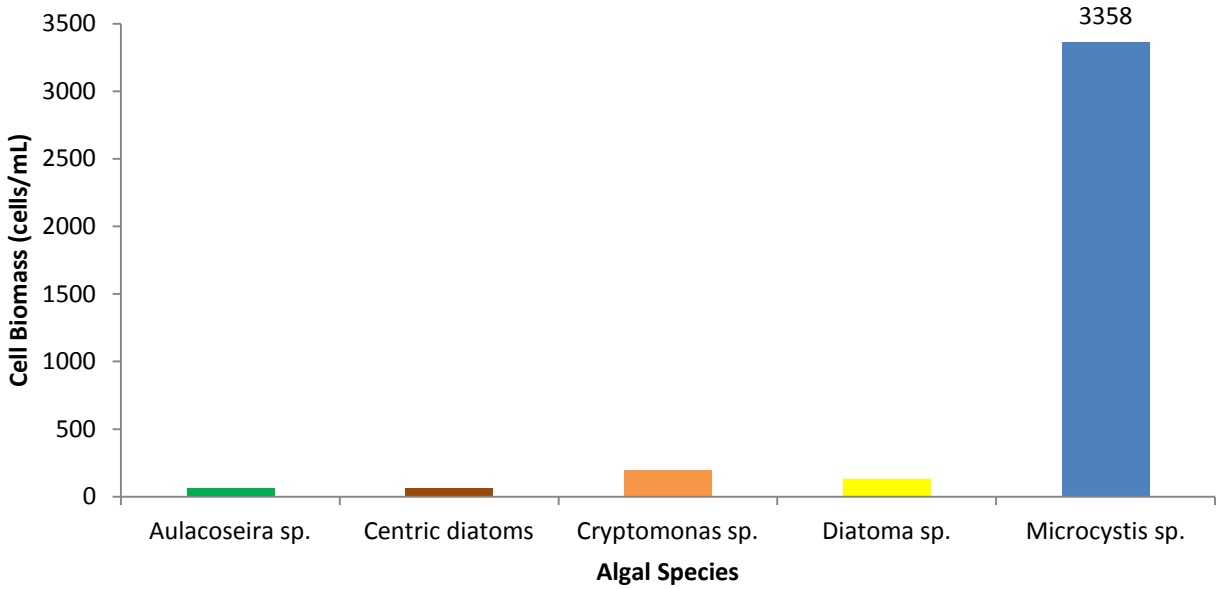


Figure 4.39: Histogram showing the algal species and concentration that occurred in the source water (control sample) at M-Canal_VD for 05/01/2011.

The dominant algal species found at sampling point M-Canal_VD was *Microcystis* sp., which occur most commonly during warmer temperatures (Harding and Paxton, 2001).

4.2.2.2. Assessment of jar testing experiments

The turbidity, chlorophyll-665 concentrations and algal composition results for the jar testing experiments are displayed in Figures 4.40, 4.43, 4.44, 4.45, and 4.48.

4.2.2.2.1. Jar testing with poly-electrolyte as only coagulant chemical

The results of the turbidity (NTU), chlorophyll-665 (µg/L) and algal composition (cells/mL) from the jar testing experiment, when using poly-electrolyte as coagulant chemical, are displayed in Figures 4.40, 4.43 and 4.44.

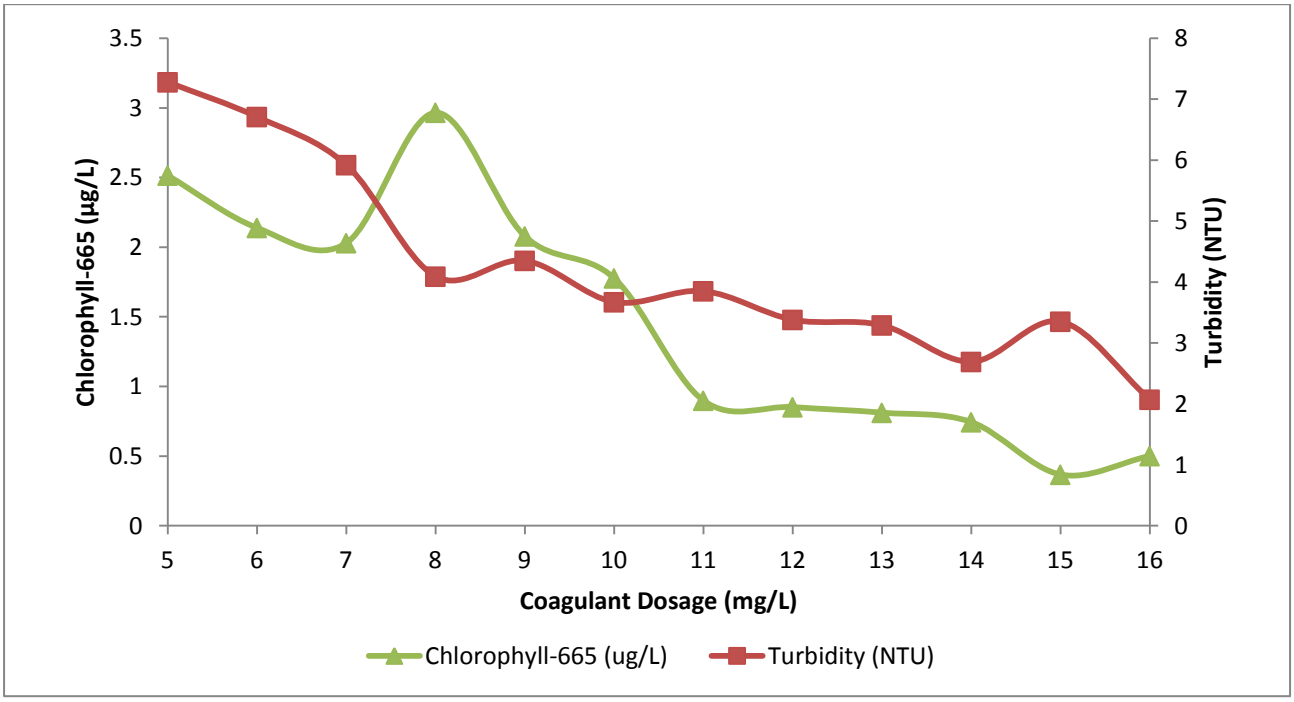


Figure 4.40: The comparison between turbidity and chlorophyll-665 when only poly-electrolyte is dosed at concentrations of 5 – 16 mg/L for sampling point M-CANAL_VD.

The turbidity values and chlorophyll-665 concentrations decreased as the coagulant dosage increased as was found with source water from the Forebay. This was expected since the source water quality of M-FOREBAY does not differ significantly from M_CANAL_VD source water. Other studies also indicated a decrease of turbidity and algal concentration with an increase of coagulant chemical (Basson, 2000; Geldenhuys *et al*, 2000; Ma and Liu, 2002a; Ho and Newcombe, 2005).

The regression analysis between turbidity vs. dosage concentration revealed a strong negative correlation (Figure 4.41: $R^2 = 0.88$), as well as between chlorophyll-665 vs. dosage concentration (Figure 4.42: $R^2 = 0.86$).

The jar testing experiment indicated that when using only poly-electrolyte as chemical coagulant, cyanobacteria such as *Microcystis* sp. and *Anabaena* sp. were still present in relatively high concentrations (*Microcystis* sp., maximum of 4365 cells/mL) (Figure 4.43). *Ceratium hirundinella* was not detected in the source water on this particular sampling date.

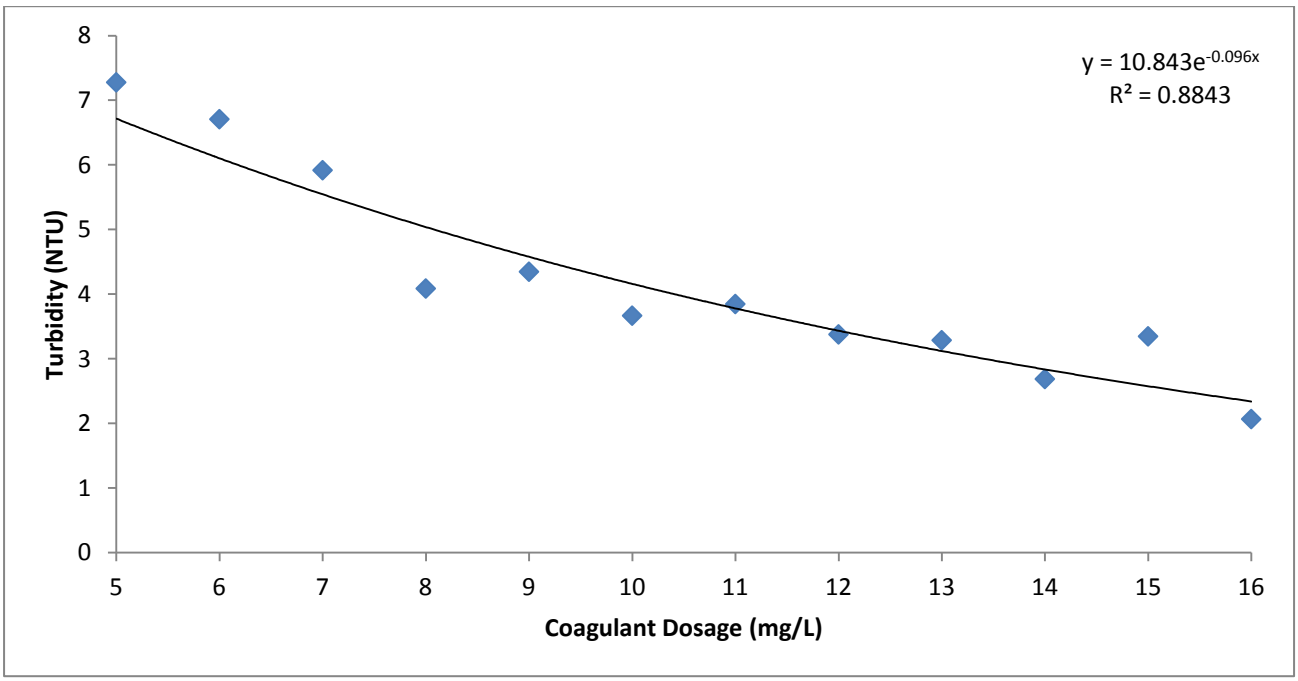


Figure 4.41: Regression analysis between turbidity and poly-electrolyte dosage at concentrations of 5 – 16 mg/L for sampling point M-CANAL_VD.

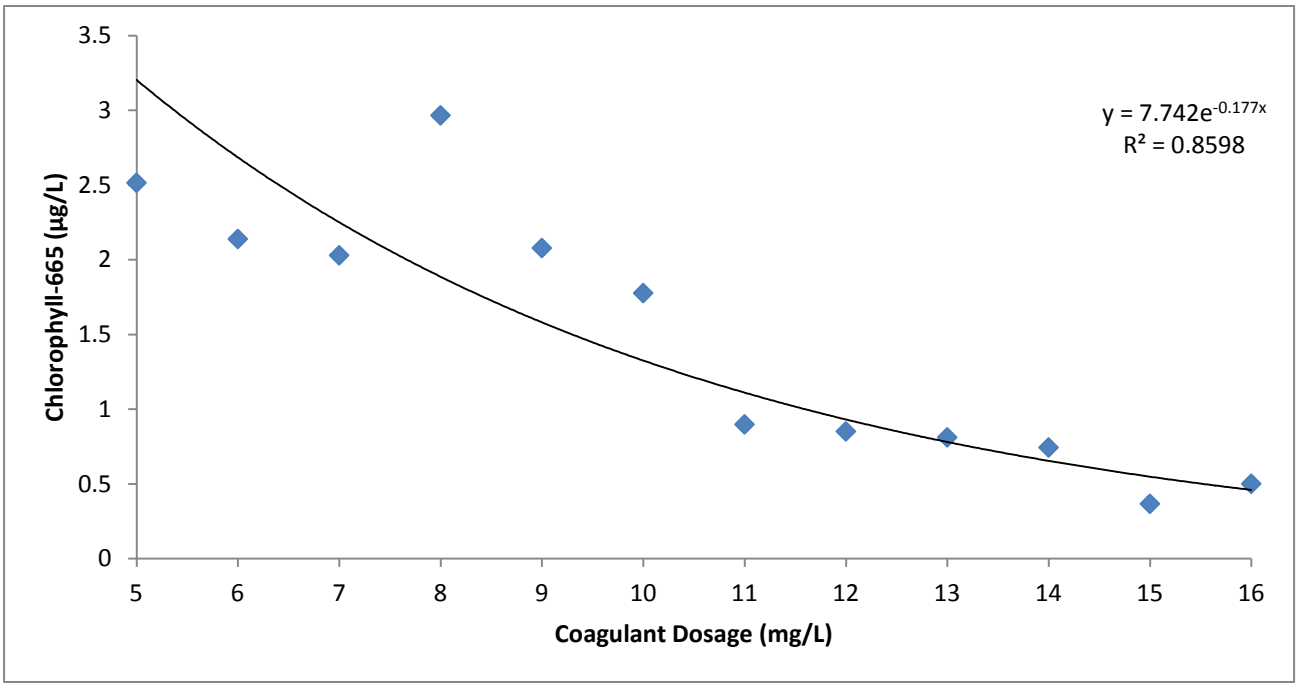


Figure 4.42: Regression analysis between chlorophyll-665 and poly-electrolyte dosage at concentrations of 5 – 16 mg/L for sampling point M-CANAL_VD.

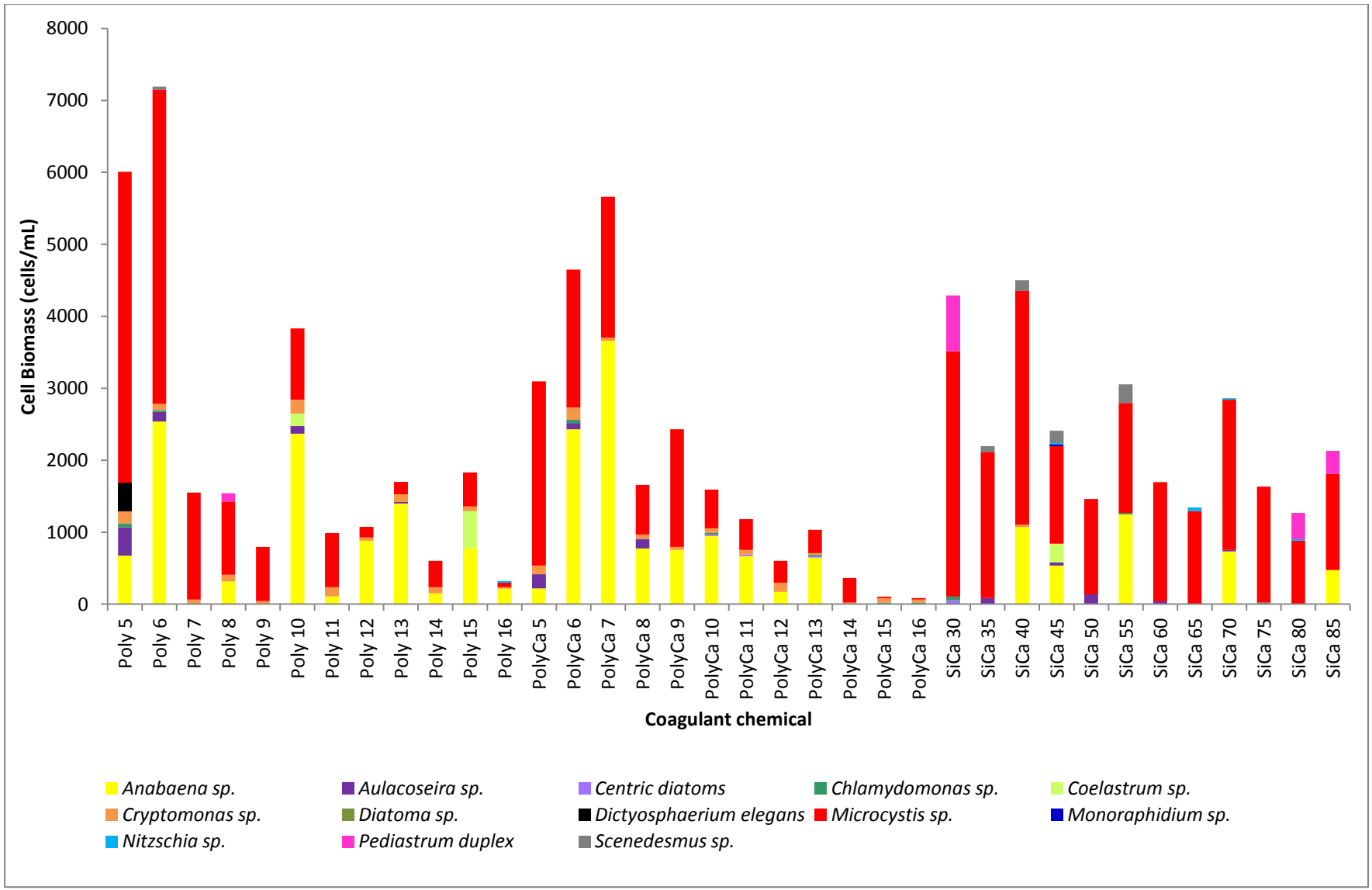


Figure 4.43: Histogram showing the algal species that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on source water from sampling point M-Canal_VD on 05/01/2011.

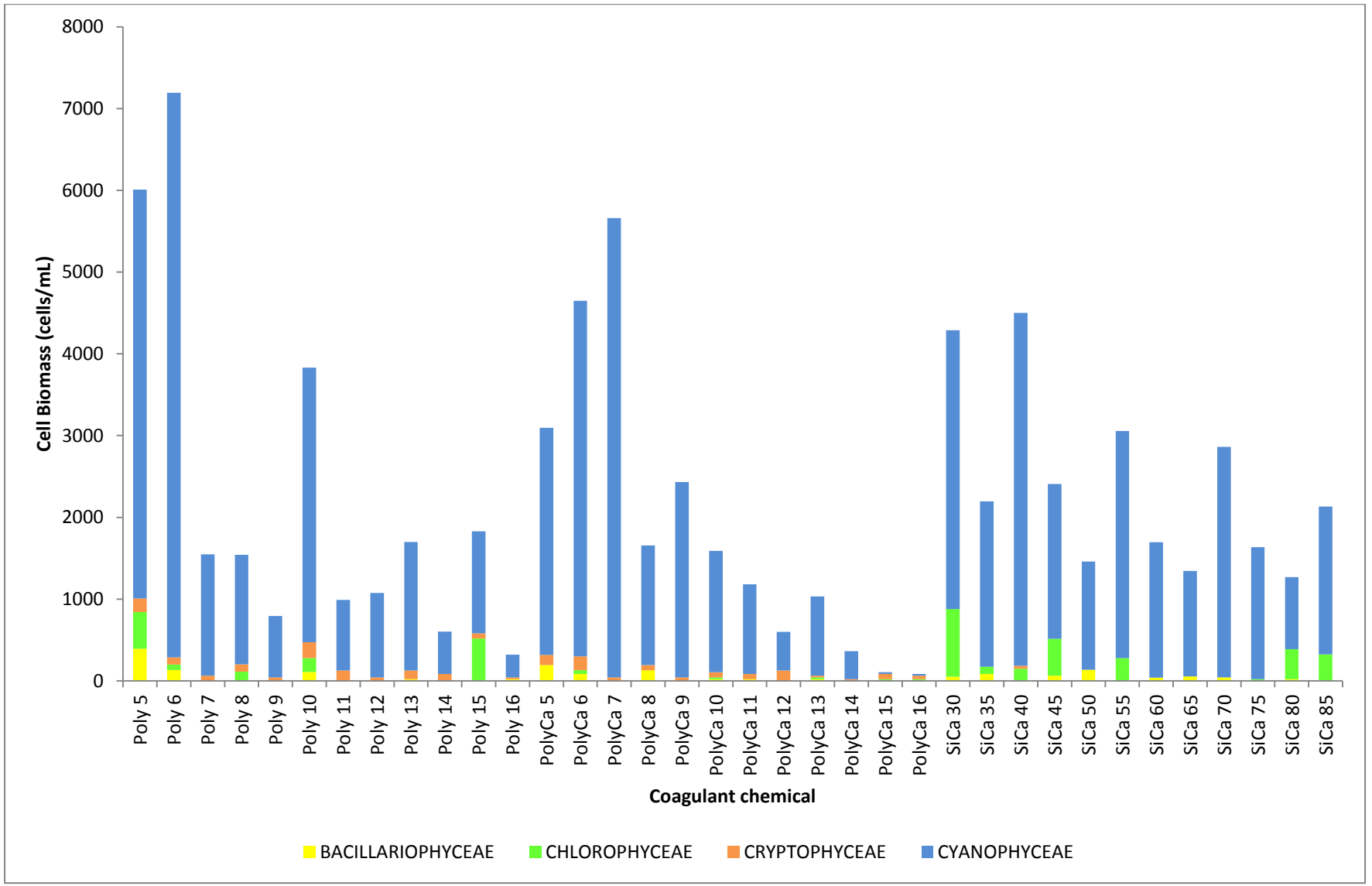


Figure 4.44: Histogram showing the major algal taxa that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on source water from sampling point M-Canal_VD on 05/01/2011.

4.2.2.2.2. Jar testing with poly-electrolyte in combination with CaO as coagulant chemical

The results of the turbidity and chlorophyll-665 concentrations and algal composition (cells/mL) from the jar testing experiment, when using poly-electrolyte with 10 mg/L CaO as coagulant chemical, are displayed in Figures 4.45, 4.43 and 4.44.

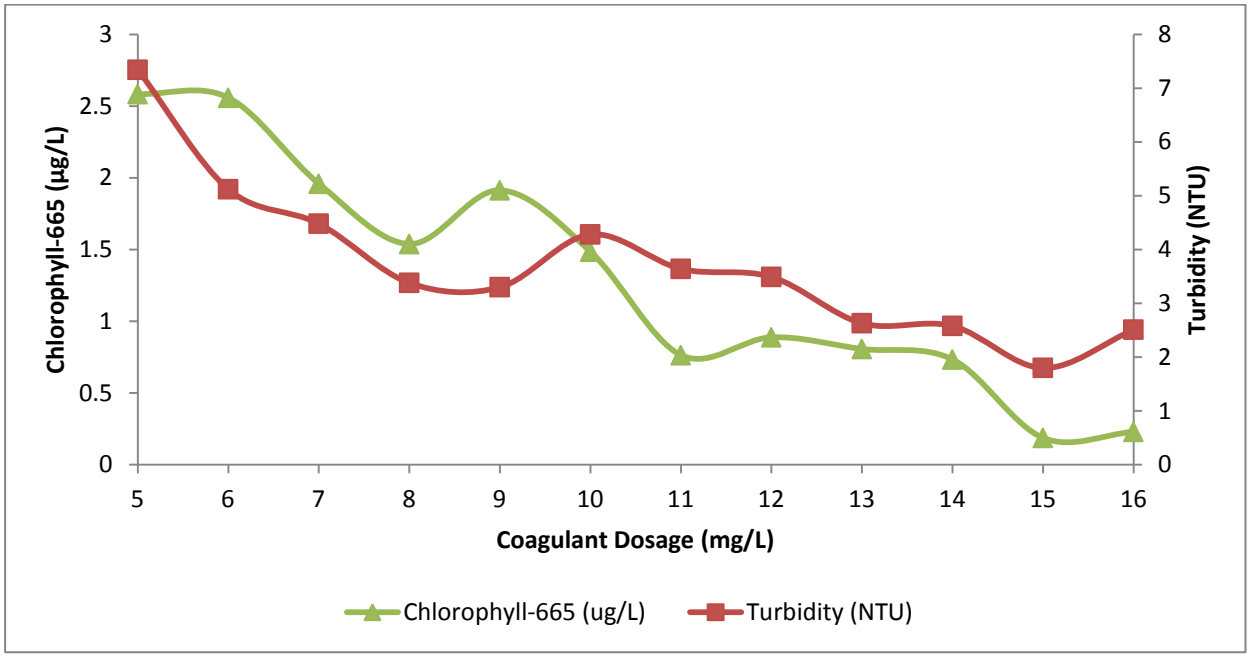


Figure 4.45: The comparison between turbidity and chlorophyll-665 when poly-electrolyte is dosed at concentrations of 5 – 16 mg/L with 10 mg/L CaO for sampling point M-CANAL_VD.

As previously found with Forebay source water, the turbidity values and chlorophyll-665 concentrations decreased as the coagulant dosage increased. It is known that when dosing lime a decrease in algal concentration will be found (Steynberg *et al*, 1994). Similar observations were made by Geldenhuys *et al* (2000). The regression analysis between turbidity vs. dosage concentration revealed a negative correlation (Figure 4.46: $R^2 = 0.79$). However, a stronger negative correlation was found between chlorophyll-665 vs. dosage concentration (Figure 4.47: $R^2 = 0.85$).

The jar testing experiment indicated that when using poly-electrolyte in combination with slaked lime as chemical coagulant, some cyanobacteria were still present in relatively high concentrations (Figure 4.43 and 4.44). A decrease in algal concentration were found with increasing coagulant chemical concentrations.

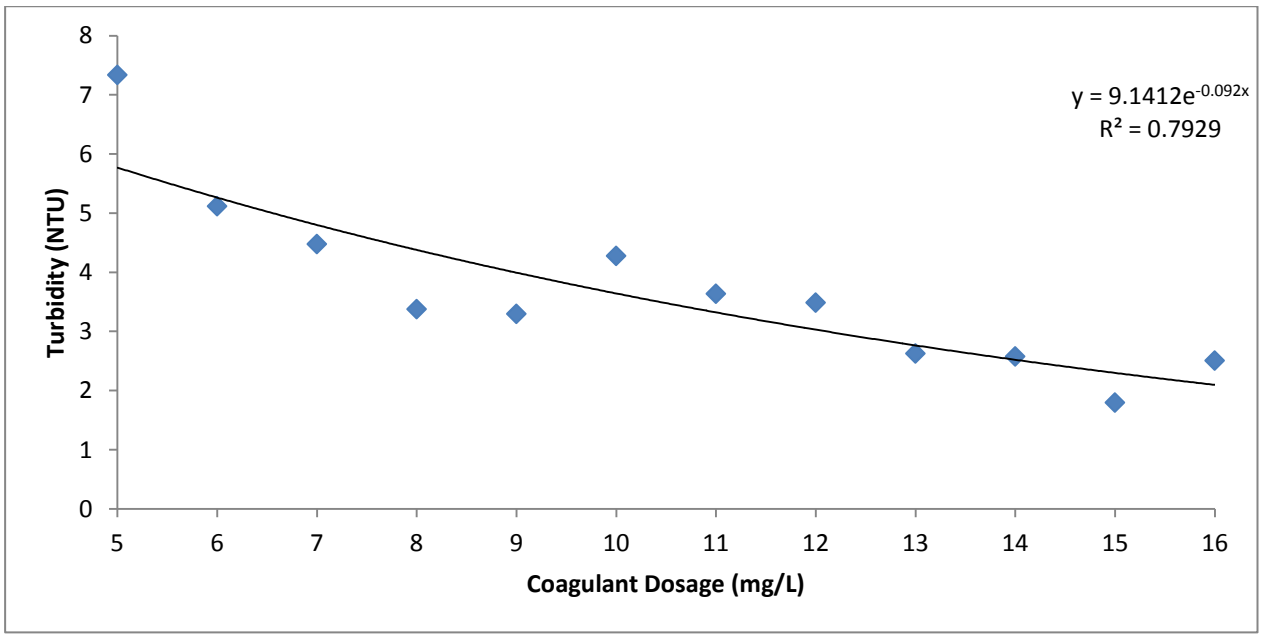


Figure 4.46: Regression analysis between turbidity and poly-electrolyte dosage at concentrations of 5 – 16 mg/L with 10 mg/L CaO for sampling point M-CANAL_VD.

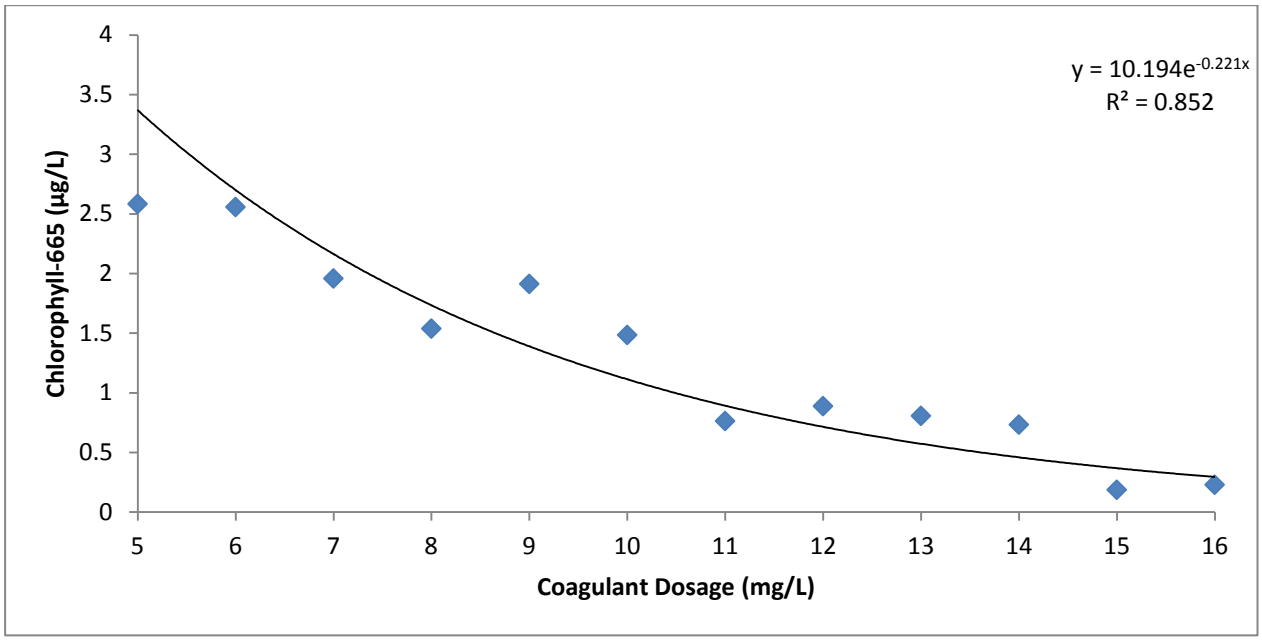


Figure 4.47: Regression analysis between chlorophyll-665 and poly-electrolyte dosage at concentrations of 5 – 16 mg/L with 10 mg/L CaO for sampling point M-CANAL_VD.

4.2.2.2.3. Jar testing with CaO in combination of 2.5 mg/L activated silica as coagulant chemical

The results of the turbidity and chlorophyll-665 concentrations and algal composition (cells/mL) from the jar testing experiment, when using CaO in combination with activated silica as coagulant chemical, are displayed in Figures 4.48, 4.43 and 4.44.

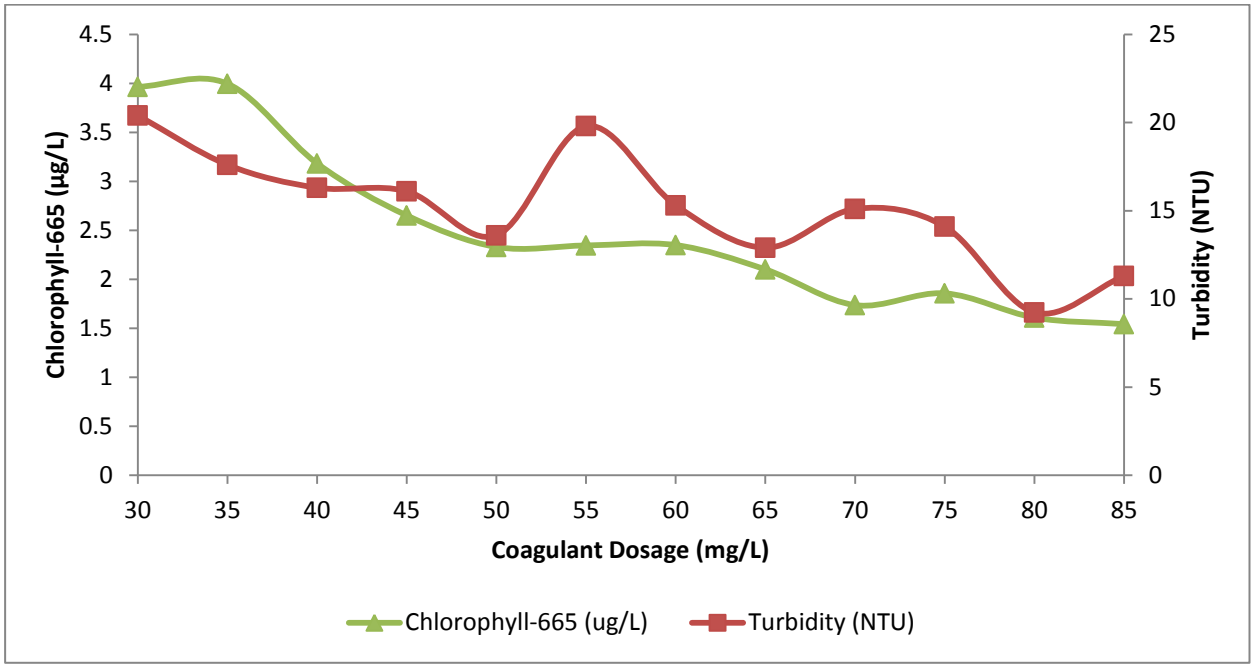


Figure 4.48: The comparison between turbidity and chlorophyll-665 when CaO in combination with 2.5 mg/L activated silica are dosed for sampling point M-CANAL_VD.

Figure 4.48 indicates that the turbidity values were higher than those found when using poly-electrolyte and poly-electrolyte in combination with CaO was used (similar to the findings with source water from M-FOREBAY). However, the turbidity values still decreased as the CaO dosage concentration increased. Chlorophyll-665 concentrations also decreased as the coagulant dosage increased. The regression analysis between turbidity vs. dosage concentration revealed a negative correlation (Figure 4.49: $R^2 = 0.62$). However, a stronger negative correlation was found between chlorophyll-665 vs. dosage concentration (Figure 4.50: $R^2 = 0.94$).

The jar testing experiment indicated that when using CaO in combination with 2.5 mg/L activated silica as chemical coagulant, cyanobacteria (*Microcystis* sp and *Anabaena* sp.) and the green algae *Pediastrum duplex,* were still not removed due to its spines or size.

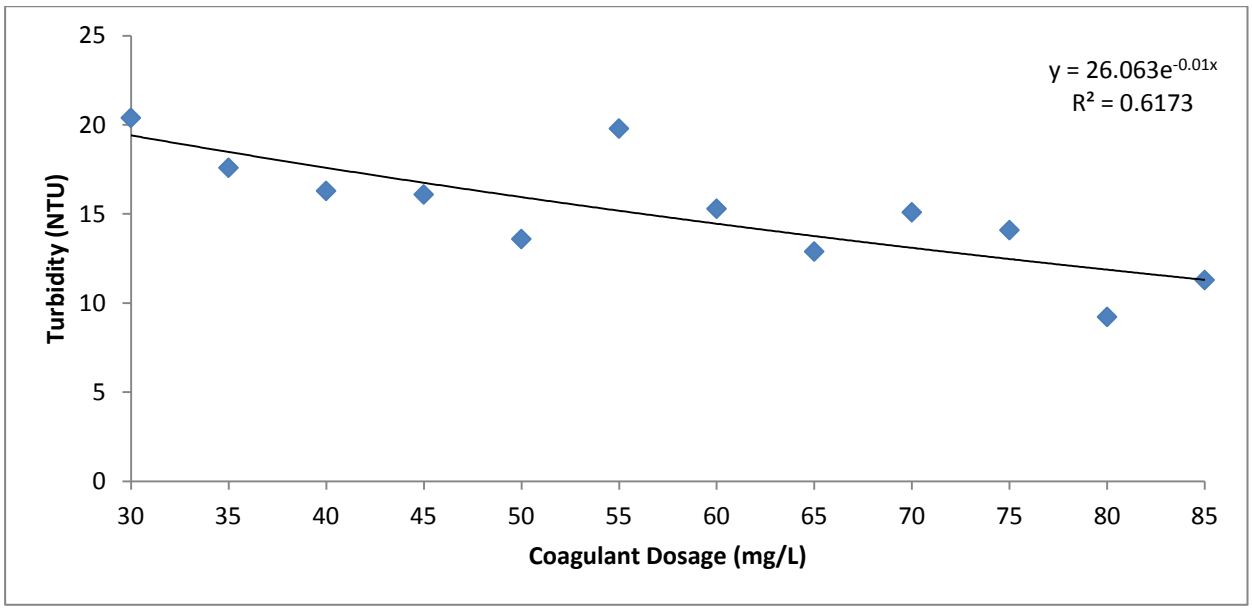


Figure 4.49: Regression analysis between turbidity and coagulant dosage when CaO in combination with 2.5 mg/L activated silica are dosed for sampling point M-CANAL_VD.

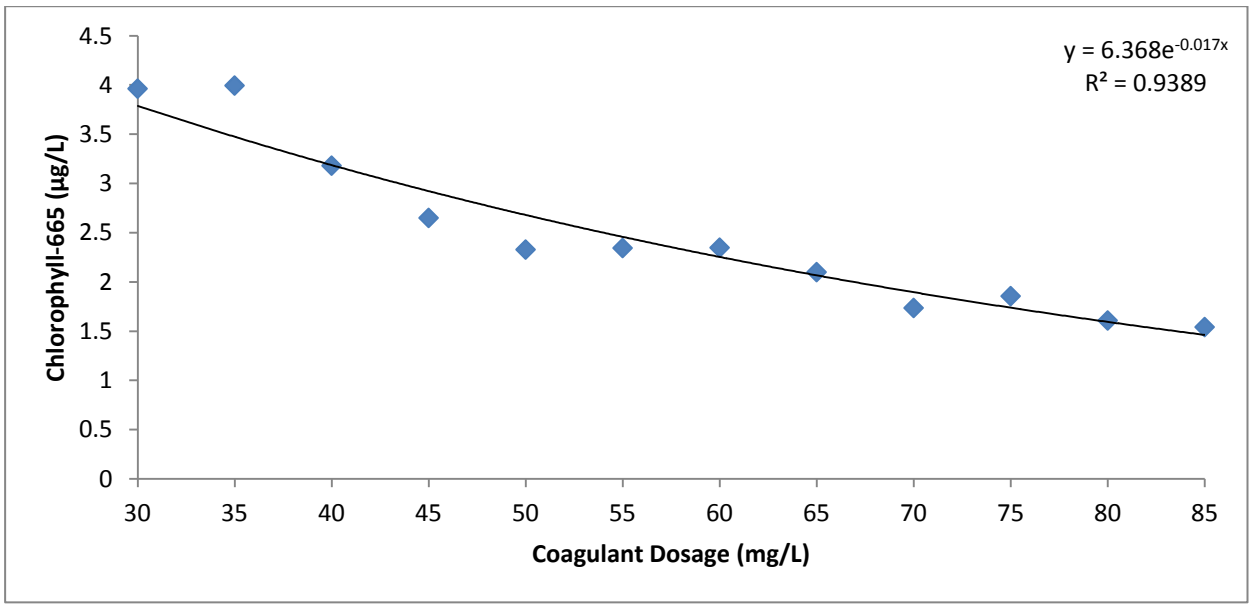


Figure 4.50: Regression analysis between chlorophyll-665 and coagulant dosage when CaO in combination with 2.5 mg/L activated silica are dosed for sampling point M-CANAL_VD.

Jar testing experiments with source water from sampling point M-CANAL_VD indicated that more algae (chlorophyll-665 concentration) were removed when poly-electrolyte in combination with CaO was dosed rather than poly-electrolyte on its own or CaO in combination with activated silica.

When CaO in combination with activated silica were dosed, the flocs formed were very small and did not settle well, as in the case of using Forebay source water (M-FOREBAY); hence the high turbidity values. Therefore, it seems that the recovered water from Panfontein did not have an influence on the results of the jar testing experiments with this particular coagulant chemical,

as was previously suspected, since the results were practically identical when compared to the results from Forebay source water.

4.3. Conclusions on Chapter 4

The multivariate analyses of ten years of environmental data for the sampling point C-VRB5T showed that the freshwater dinoflagellate *C. hirundinella* seem to be favoured by high pH, high COD, high PO_4, and high silica concentrations, as well as low turbidity and low nitrogen (DIN) concentrations. No correlation was found between *C. hirundinella* and temperature. It seems that *C. hirundinella* did not occur during extreme hot and severe cold conditions. The non-correlation indicates that the *C. hirundinella* may occur during periods of moderate temperatures such as experienced during spring and autumn. However, to gain more clarity on the relationship between different environmental variables and *C. hirundinella*, other sampling points in the Vaal River should also be included in future studies.

The results obtained in this study where three different coagulant chemical treatments (poly-electrolyte, poly-electrolyte with CaO and CaO in combination with activated silica) were used on Forebay source water that contained high concentrations of the cyanobacteria *Microcystis* sp. and *Anabaena* sp., the green alga *Chlamydomonas* sp., and diatoms, but very low (or insignificant) concentrations of *C. hirundinella* during the study period. It is evident that using the poly-electrolyte investigated alone turbidity, chlorophyll-665 concentration and total algal concentrations were reduced, however, it was found that some algae such as the cyanobacteria, did not coagulate well and were still present in the supernatant. It should be noted that poly-electrolytes do not influence the pH of water, however, when raising the pH with CaO, it “shocks” the algae and cause them to die or be rendered immobile (Steynberg *et al*, 1994; Bolto and Gregory, 2007). It was found that when poly-electrolyte was dosed in combination with CaO, there was a decrease in turbidity, chlorophyll-665 and total algal biomass. However, algae were still present in the supernatant. When CaO in combination with activated silica was dosed, it was found that the inherent turbidity of the lime increased the turbidity of the Forebay source water to such an extent that it affected coagulation negatively, resulting in high turbidity values in the supernatant. According to Freese *et al* (2004) this could be due to overdosing and therefore an increase in residual lime particles. However, regardless of the turbidity values, the chlorophyll-665 and total algal concentration decreased significantly.

The chemical composition of the Forebay source water was found to be very similar to source water from sampling point M-CANAL_VD (upstream from the Forebay). The source water had a high turbidity and low conductivity and TDS, low concentrations of nutrients, and low concentrations of specifically chlorides and sulphates in comparison with source water at

sampling point C-VRB5T. During the study period the source water from sampling point M-FOREBAY and M-CANAL_VD contained very low concentrations of *C. hirundinella*.

Thus, the jar testing experiments using the poly-electrolyte alone or poly-electrolyte in combination with CaO on M-FOREBAY and M-CANAL_VD source water, showed a decrease of turbidity, chlorophyll-665 concentration, and total algal biomass, with an increase in coagulant chemical concentration. When CaO in combination with activated silica was used on M-FOREBAY and M-CANAL_VD source water, it was found that the residual turbidity of the lime increased the turbidity of the water to such an extent that it affected coagulation negatively, resulting in high turbidity values in the supernatant. However, regardless of the turbidity values, the chlorophyll-665 and total algal concentration decreased significantly. After jar testing with source water from M-CANAL_VD, it seems that the recovered water released from Panfontein did not have an influence on the results of jar testing experiment with Forebay source water when CaO in combination with activated silica was used, as was previously suspected, since the results were practically identical when compared to the results from Forebay source water.

CHAPTER 5

RESULTS AND DISCUSSION

5. Case Studies

5.1. Introduction on case studies: Vaalkop and Rietvlei Dams

Due to the low concentrations of *C. hirundinella* in Rand Water's catchment during the study period, two case studies were investigated where *C. hirundinella* occurred in high concentrations, namely Vaalkop and Rietvlei Dams. Refer to the Chapter three (Sections 3.1.4 and 3.1.5) for more detail on the location of the dams and the sampling procedure.

The occurrence of *C. hirundinella* in relation to different environmental variables was investigated in Vaalkop and Rietvlei Dam. The chemical, physical and biological composition of the source water of these dams differ greatly (Compare Tables 5.7, 5.8 and 5.12). For example conductivity, hardness and the concentrations of sulphate, chloride, sodium and sulphur is much higher in Vaalkop Dam than in Rietvlei Dam. It should be noted that turbidity in Forebay source water was much higher than observed at Vaalkop and Rietvlei Dams. This indicates that *C. hirundinella* might prefer source water with a low turbidity and high conductivity as it was observed in Vaalkop and Rietvlei Dam where *C. hirundinella* occur frequently. Furthermore, *C. hirundinella* also occurred frequently in the Vaal River Barrage (C-VRB5T) which had an average turbidity of 13 NTU during the study period (2000 – 2009). As the salinity in the Vaal River System increase, a decrease in turbidity will be found, which favour the growth of dinoflagellates such as *C. hirundinella.* Almost eight years of historical data of Vaalkop Dam has been analysed to find a correlation between the occurrence of *C. hirundinella* and certain environmental variables, which will be discussed in the next section (section 5.2).

In order to optimise treatment processes such as coagulation and flocculation, jar testing experiments were performed to investigate different coagulant chemicals (poly-electrolyte, poly-electrolyte in combination with slaked lime (CaO) and CaO in combination with activated silica), thus to evaluate the effect of different coagulant chemicals and concentrations on the removal of *C. hirundinella.* Due to the problems experienced with coagulation and flocculation when *C. hirundinella* is present in the source water of Rand Water, this study will also investigate the effect of chlorine on the mobility of *C. hirundinella*. Chlorine exposure experiments were performed on these *Ceratium*-rich source waters to investigate the possibility of pre-chlorination during drinking water treatment, for the removal of *C. hirundinella* from source waters. Refer to Chapter 3, sections 3.1.4. and 3.1.5. for information about sampling sites and methods as well as section 3.4.6. for more detail on the chlorine exposure method.

5.2. Assessment of sampling point M-RAW_VAALKOP during January 2004 to February 2011

5.2.1. Assessment of historical data of sampling point M-RAW_VAALKOP

In the first PCA ordination (Figure 5.1), 31.9 % of the variance in environmental components could be explained on the first two canonical axes (Table 5.1). Figure 5.1 indicates that temperature correlated with turbidity, DIN, MIB, PO4, Geosmin and different chemical elements such as Mn, Al and F. A negative correlation was found between turbidity and variables such as pH, Chlorophyll-665, and M-Alkalinity.

Table 5.1: Results from the PCA analysis on all environmental variables measured at M-RAW_VAALKOP for 2004 to February 2011

Axes	1	2	3	4	Total variance
Eigenvalues	0.233	0.086	0.081	0.060	1.000
Cumulative percentage variance of species data	23.3	31.9	40.0	46.0	
Sum of all					1.000

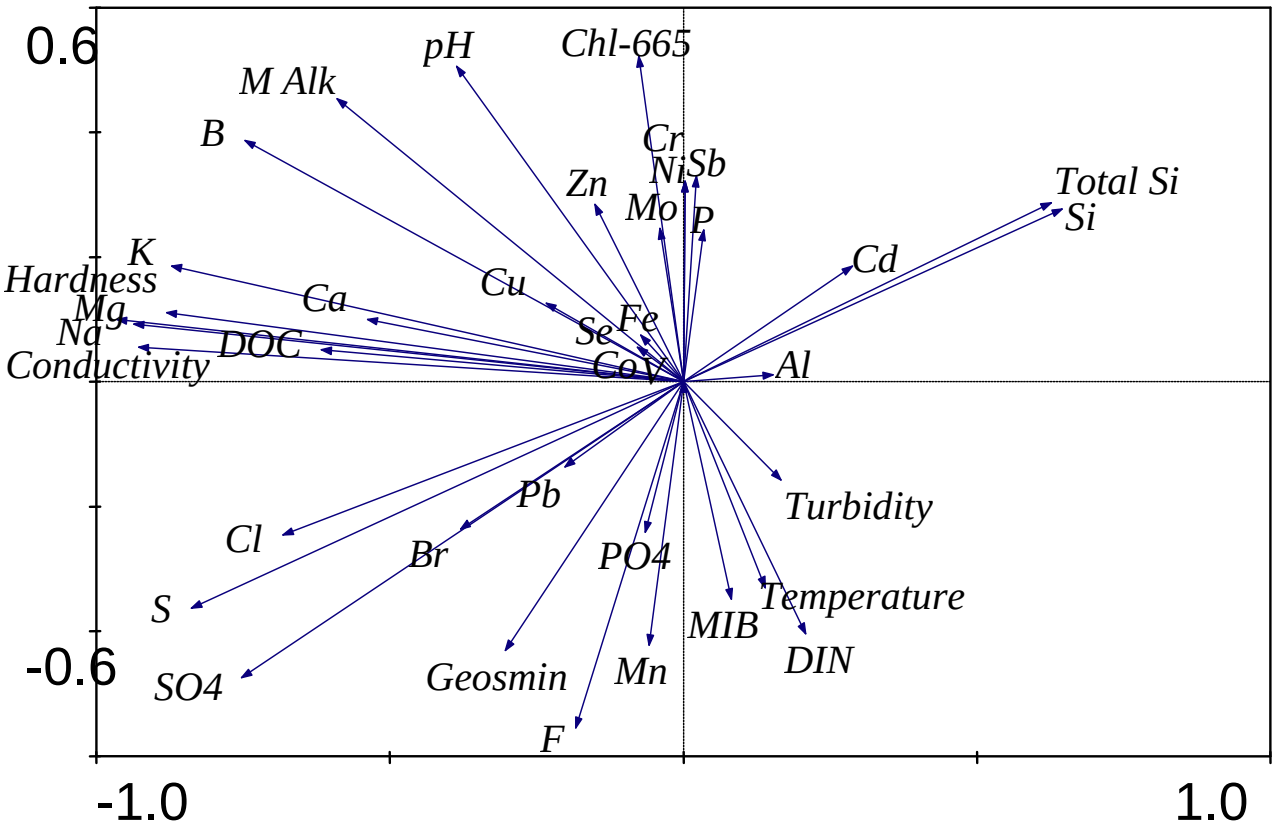


Figure 5.1: Bi-plot PCA ordination diagram showing all environmental variables measured at M-RAW_VAALKOP for 2004 to February 2011 (LEGEND: Dissolved Organic Carbon = DOC; Chemical Oxygen Demand = COD; Chrome = Cr; Bromide = Br; Iron = Fe; Sulphur = S; Chlorine = Cl; Calcium = Ca; Magnesium = Mg; Sodium = Na; Potassium = K; Cadmium = Cd; Silica = Si; Cobalt = Co; Copper = Cu; Nickel = Ni; Manganese = Mn; Aluminium = Al; Boron = B; Vanadium = V; Molybdenum = Mo; Total Silica = Total Si; Phosphorus = P;

Silicon = Si; Lead = Pb; Zinc = Zn; Selenium = Se; Antimony = Sb; Sulphate = SO_4; Dissolved Inorganic Nitrogen = DIN; Orthophosphate = PO_4; Hardness, Methyl-Orange Alkalinity = M-Alk; and Chlorophyll-665 = Chl-665; 2-Methylisorboneol = MIB).

From the first PCA (Figure 5.1), principal components were identified and the rest omitted that is of less importance to this study. The following principle components have been selected: Conductivity, Hardness, M-Alkalinity, pH, Fe, Chlorophyll-665, Si, PO_4, SO_4, Geosmin, Temperature, MIB, DIN, Turbidity and Cd were identified and used in further multivariate analysis.

A second PCA was done on the principal environmental components to reflect the relationships between these variables found at sampling point M-RAW_VAALKOP from January 2004 to February 2011 (Figure 5.2). Along the first two axes in the PCA ordination, a total of 40 % of the variance in environmental data could be explained (Table 5.2).

Table 5.2: Results from the PCA analysis on main environmental variables measured at M-RAW_VAALKOP for 2004 to February 2011

Axes	1	2	3	4	Total variance
Eigenvalues	0.245	0.153	0.091	0.081	1.000
Cumulative percentage variance of species data	24.5	39.8	48.9	57.0	
Sum of all					1.000

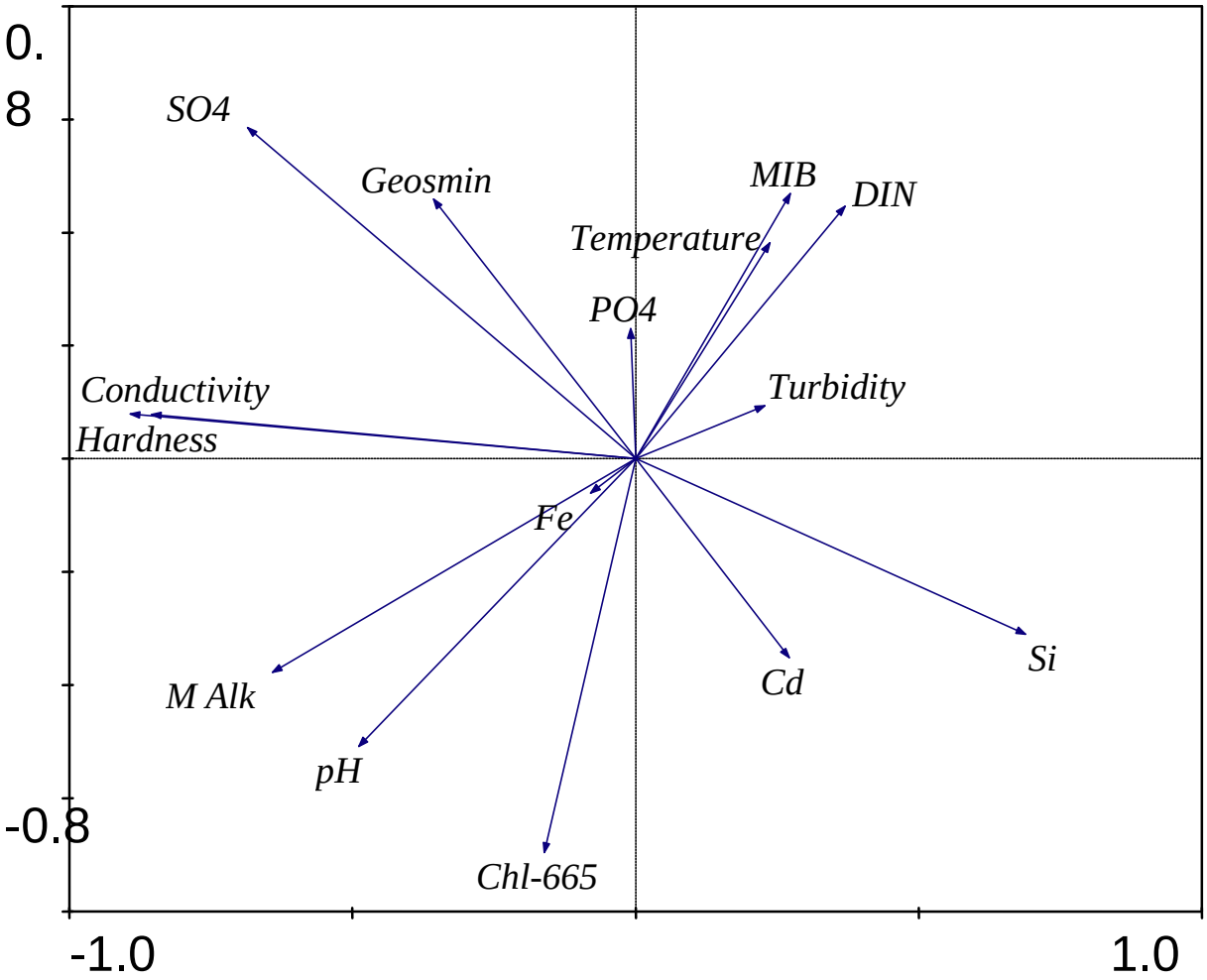


Figure 5.2: Bi-plot PCA ordination diagram showing only the main environmental variables measured at M-RAW_VAALKOP for 2004 to February 2011 (LEGEND: DIN = Dissolved Inorganic Nitrogen; M-Alk = Methyl-Orange Alkalinity; Chl-665 = Total Chlorophyll pigment; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate; Sulphate = SO_4; MIB, = 2-methylisorboneol).

Along the first axis in the PCA ordination, 24.5 % of the variance in environmental data could be explained (Table 5.2). Figure 5.2 indicates a negative correlation between turbidity and conductivity. Turbidity is mainly influenced by particulate material such as silt or algae suspended in the water. Turbidity increases during the rainy seasons due to an increase in particulate material or runoff into the river. The average turbidity at sampling point M-RAW_VAALKOP was 8.85 NTU for the study period.

Furthermore, 15.3 % of the variance in environmental data could be explained on the second axis of the PCA ordination (Table 5.2). The PCA (Figure 5.2) indicated that temperature, DIN, MIB, Geosmin and turbidity were positively correlated with one another, but showed a negative correlation with Chlorophyll-665 (total pigment analysis, Swanepoel *et al* (2008b)), pH, and M-Alkalinity. Although Chlorophyll-665 cannot be regarded as an environmental variable, it

allows for interpretation regarding the influence of some environmental variables on phytoplankton.

A Correspondence Analyses (CA) was performed on the major algal taxa and species respectively with the principle environmental variables. It was found that the CA and CCA ordinations diagram corresponds relatively well and appears to be mirror images. Therefore, it can be concluded that the algal species chosen were the correct ones to explain the variation in the algal data.

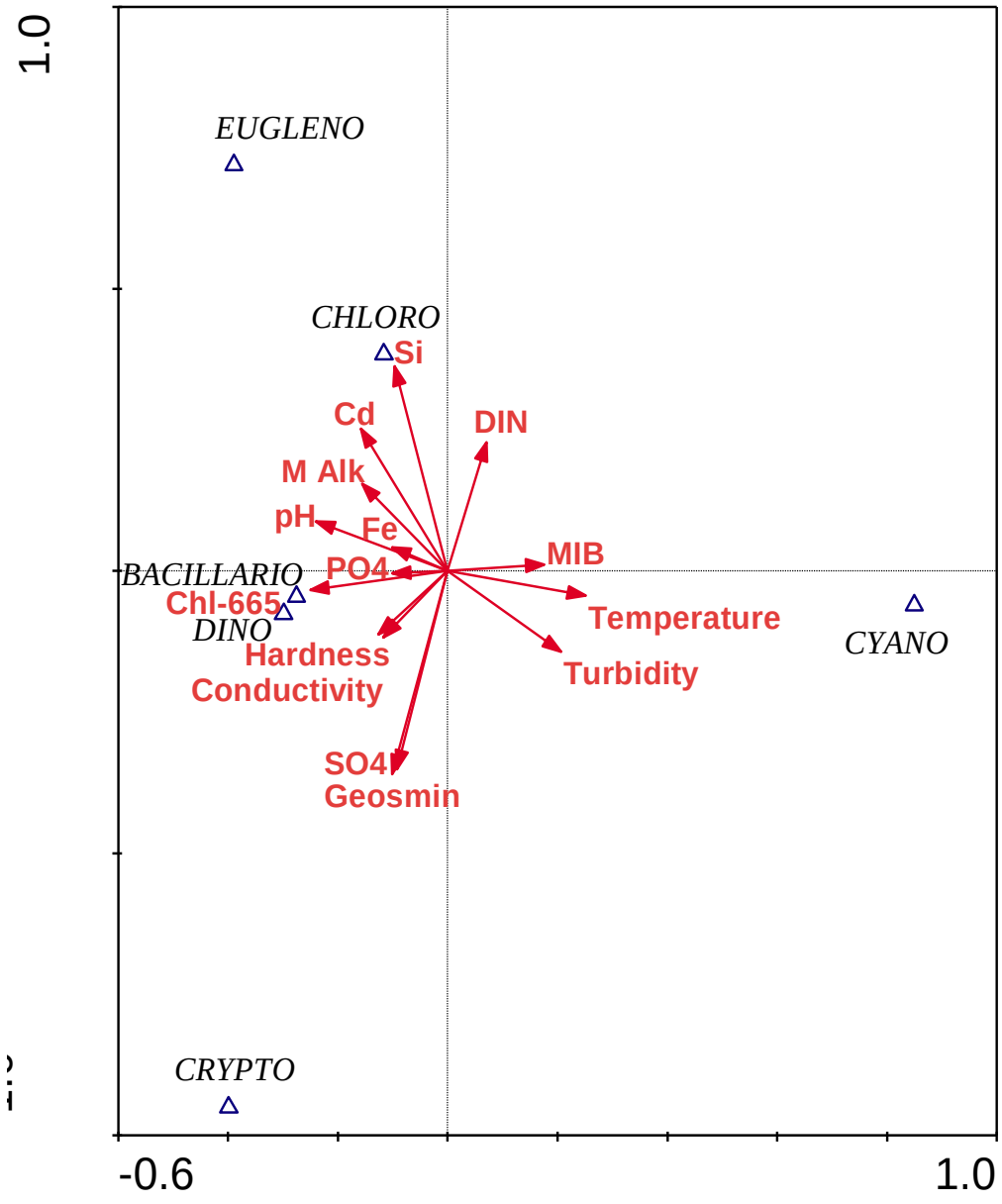


Figure 5.3: CA ordination diagram showing principle environmental variables and major algal taxa measured at M-RAW_VAALKOP for 2004 to February 2011 (LEGEND: DIN = Dissolved Inorganic Nitrogen; M-Alk = Methyl-Orange Alkalinity; Chl-665 = Total Chlorophyll pigment; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate; Sulphate = SO_4; MIB = 2-methylisorboneol; Cyano = Cyanophyceae; Bacill = Bacillariophyceae; Chloro = Chlorophyceae; Crypto = Cryptophyceae; Dino = Dinophyceae; and Eugleno = Euglenophyceae).

The Canonical Correspondence Analyses (CCA) was performed to reflect the relationship between the major algal taxa and environmental variables found at sampling point M-RAW_VAALKOP for 2004 to February 2011. The following main environmental components were included in the CCA ordination: Turbidity (NTU), Temperature (˚C), pH, Conductivity (mS/m), M-Alkalinity (M Alk, mg/L $CaCO_3$), Cadmium (Cd, mg/L), Iron (Fe, mg/L), Silica (Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Hardness (mg/L $CaCO_3$), Ortophosphate (PO_4, mg/L), Sulphate (SO_4, mg/L), 2-Methylisoborneol (MIB, ng/L), Geosmin (ng/L) and Chlorophyll-665 (Chl-665, µg/L).

The seven major algal taxa included in the ordination diagram are Cyanophyceae (Cyano, cells/mL), Bacillariophyceae (Bacillario, cells/mL), Chlorophyceae (Chloro, cells/mL), Cryptophyceae (Crypto, cells/mL), Dinophyceae (Dino, cells/mL), Chrysophyceae (Chryso, cells/mL) and Euglenophyceae (Eugleno, cells/mL).

The first two canonical axes (Figure 5.4 and Table 5.3) explained a total of only 23 % of the variance within the algal data itself; however, it explained 72 % of the variance in the algal-environmental relationship.

Table 5.3: Results from the CCA analysis on the environmental variables and major algal taxa at M-RAW_VAALKOP for 2004 to February 2011

Axes	1	2	3	4	Total inertia
Eigenvalues	0.146	0.074	0.05	0.018	0.973
Species-environment correlations	0.620	0.595	0.608	0.437	
Cumulative percentage variance of species data	15.0	22.6	27.7	29.6	
Cumulative percentage variance of species-environment relation	47.9	72.2	88.7	94.8	
Sum of all eigenvalues					0.973
Sum of all canonical eigenvalues					0.304

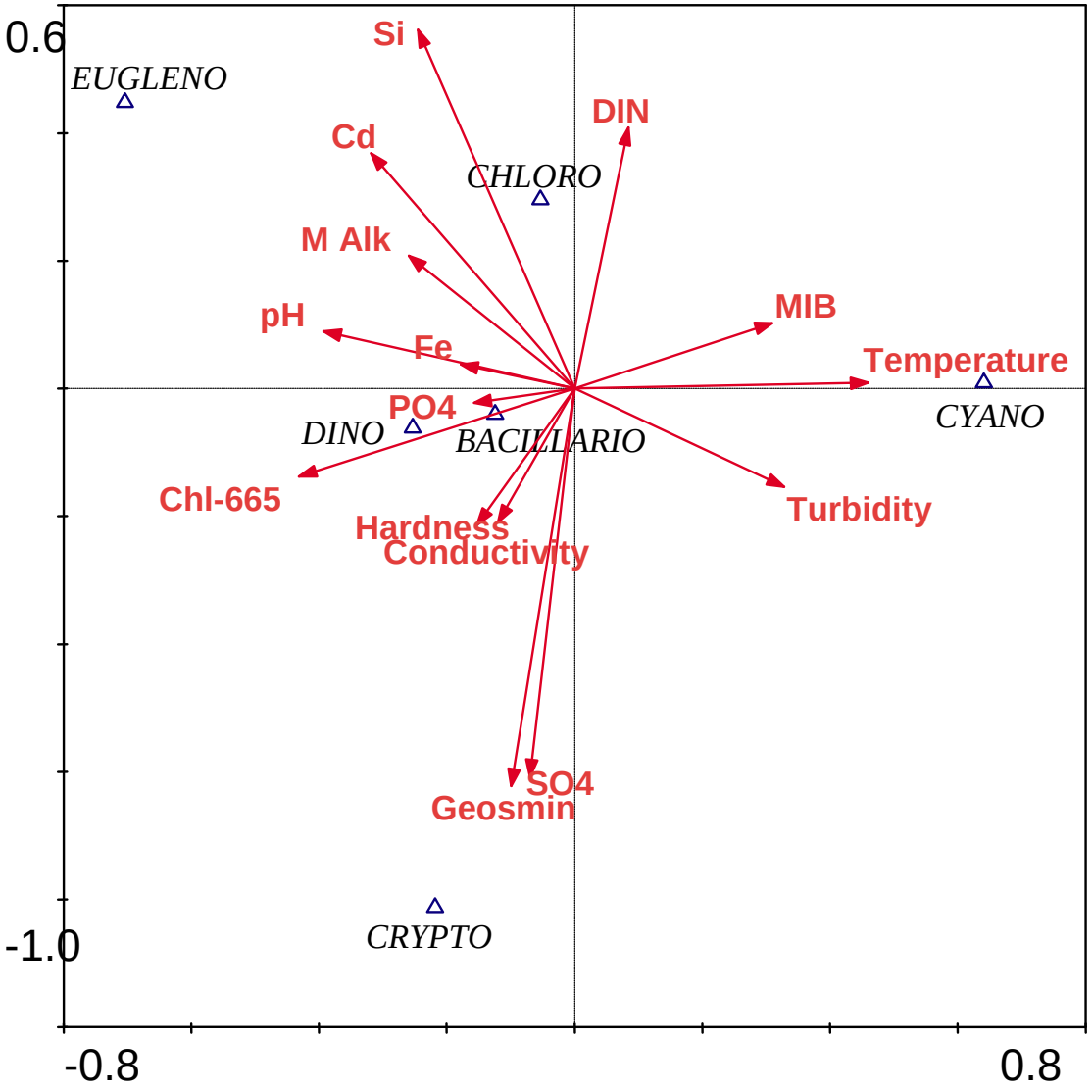


Figure 5.4: CCA ordination diagram showing principle environmental components and major algal taxa measured at M-RAW_VAALKOP for 2004 to February 2011 (LEGEND: DIN = Dissolved Inorganic Nitrogen; M-Alk = Methyl-Orange Alkalinity; Chl-665 = Total Chlorophyll pigment; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate; Sulphate = SO_4; MIB = 2-methylisorboneol; Cyano = Cyanophyceae; Bacill = Bacillariophyceae; Chloro = Chlorophyceae; Crypto = Cryptophyceae; Dino = Dinophyceae; and Eugleno = Euglenophyceae).

From Figure 5.4, a positive relationship is evident between temperature and the cyanobacteria (Cyanophyceae), indicating that these algae are favoured by warm water conditions (Janse van Vuuren and Pieterse, 2005a; Du Preez and Van Baalen, 2006). A negative correlation was found between temperature and two algal classes namely the diatoms (Bacillariophyceae) and Dinophyceae. Therefore, indicating that these organisms reached higher concentrations in water with lower temperatures (Pieterse and Janse van Vuuren, 1997). A negative correlation was also found between Bacillariophyceae and the nutrient silica, which might be as a result of utilization (Pieterse and Janse van Vuuren, 1997; Janse van Vuuren and Pieterse, 2005a). Dinophyceae were present during high conductivity, high alkalinity, high chlorophyll-665 concentrations, high PO_4 concentrations, low turbidity and low DIN concentrations. Supported

by results found earlier by Janse van Vuuren and Pieterse (2005a) similar results were obtained in this study with sampling point C-VRB5T (Section 4.1). The fact that Dinophyceae correlated positively with chlorophyll-665 is an indication that Dinophyceae (if present in the water) contributes significantly to the photosynthetic pigment concentration.

Table 5.4 shows that the CCA analysis (for the first and all canonical axes) was statistically insignificant ($P > 0.05$) as judged on the basis of the Monte Carlo Permutation test (499 random permutations).

Table 5.4: Results of the Monte Carlo test from the CCA analysis on the principle environmental components and major algal taxa at M-RAW_VAALKOP for 2004 to February 2011

Test of significance of first canonical axis	
Eigenvalue	0.146
F-ratio	8.984
P-value	0.07
Test of significance of all canonical axes	
Trace	0.304
F-ratio	1.546
P-value	0.016

The second Canonical Correspondence Analyses (CCA) (Figure 5.5) was performed to reflect the relationship between the algal species and environmental data found at sampling point M-RAW_VAALKOP for 2004 to February 2011. The following main environmental components were included in the CCA ordination: Turbidity (NTU), Temperature (˚C), pH, Conductivity (mS/m), M-Alkalinity (M Alk, mg/L $CaCO_3$), Cadmium (Cd, mg/L), Iron (Fe, mg/L), Silica (Si, mg/L), Dissolved Inorganic Nitrogen (DIN, mg/L), Hardness (mg/L $CaCO_3$), Ortophosphate (PO_4, mg/L), Sulphate (SO_4, mg/L), 2-Methylisoborneol (MIB, ng/L), Geosmin (ng/L) and Chlorophyll-665 (Chl-665, µg/L).

The algal species (cells/mL) included are the cyanobacteria (Cyanophyceae): *Anabaena* sp., *Microcystis* sp. and *Oscillatoria* sp.; *Cylindrospermopsis* sp.; the green algae (Chlorophyceae): *Chlamydomonas* sp., *Pediastrum duplex*, *Oocystis* sp., and *Scenedesmus* sp; Cryptophyceae: *Cryptomonas* sp.; Dinophyceae: *Ceratium hirundinella*; Euglenophyceae*: Trachelomonas* sp. and Bacillariophyceae: *Aulacoseira* sp., pennate and centric diatoms. With regards to the algal species, it was decided to use only the species that occurred more than ten times during the study period, since algae that occurred sporadic and in low concentrations may cause a distorted image in the representation of the multivariate analyses (Ter Braak and Prentice, 1988; Legendre and Gallagher, 2001).

From Table 5.5, the first two canonical axes (as represented in Figure 5.5) explained only a total of 20 % of the variance within the algal data itself, and 58 % of the variance in the algal-environment relationship.

Table 5.5: Results from the CCA analysis on the environmental variables and algal species at M-RAW_VAALKOP for 2004 to February 2011

Axes	1	2	3	4	Total inertia
Eigenvalues	0.372	0.146	0.093	0.075	2.575
Species-environment correlations	0.794	0.733	0.689	0.572	
Cumulative percentage variance of species data	14.4	20.1	23.7	26.6	
Cumulative percentage variance of species-environment relation	41.9	58.3	68.7	77.2	
Sum of all eigenvalues					2.575
Sum of all canonical eigenvalues					0.888

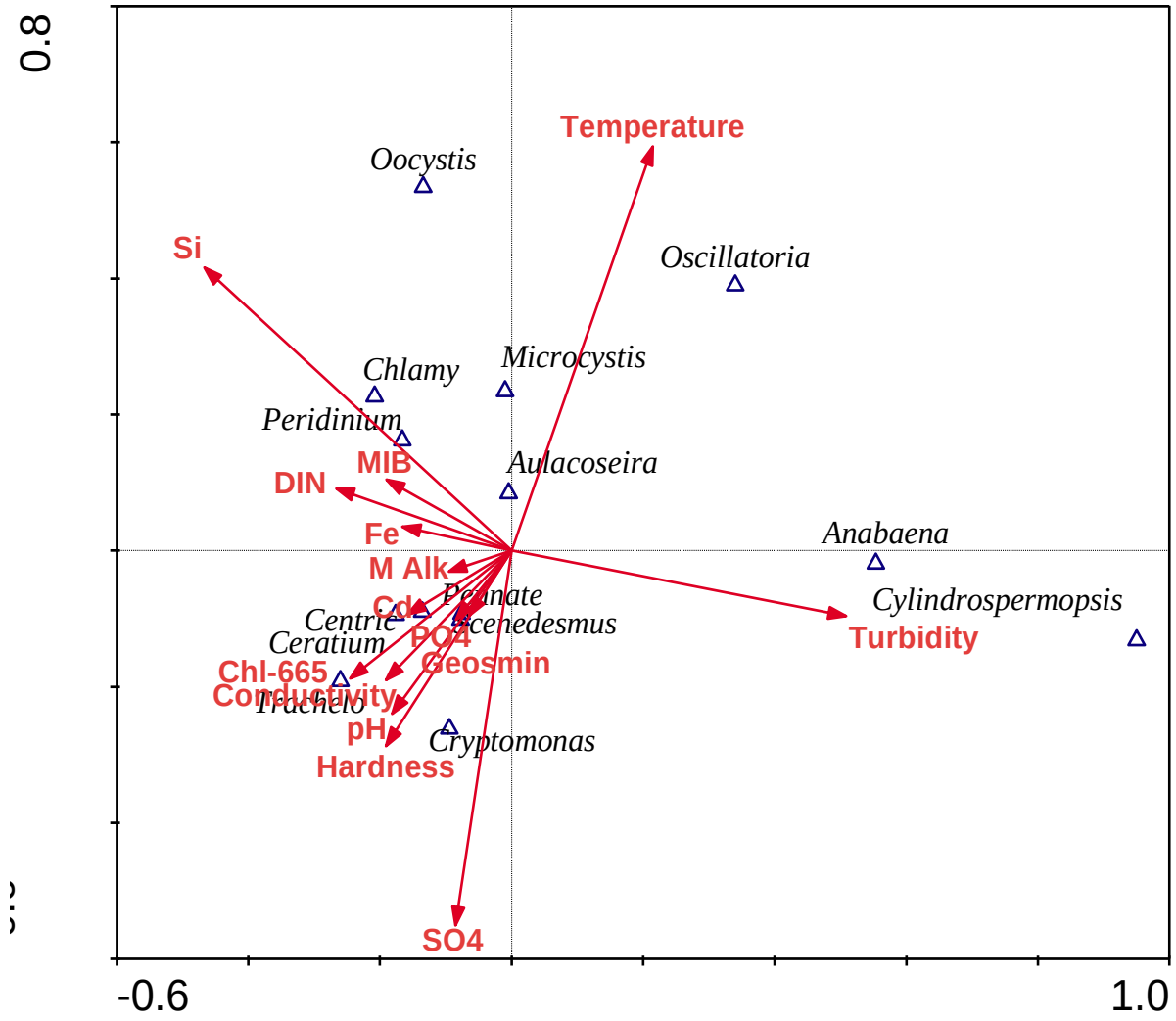


Figure 5.5: CCA ordination diagram showing principle environmental components and algal species measured at M-RAW_VAALKOP for 2004 to February 2011 (LEGEND: DIN = Dissolved Inorganic Nitrogen; M-Alk = Methyl-Orange Alkalinity; Chl-665 = Total Chlorophyll pigment; Fe = Iron; Si = Silica; Ni = Nickel; PO_4 = Orthophosphate; Sulphate = SO_4;

MIB = 2-methylisorboneol; and the algal species *Anabaena* sp.; *Microcystis* sp.; *Oscillatoria* sp.*; Cylindrospermopsis* sp.*; Chlamy* = *Chlamydomonas* sp.; *Scenedesmus* sp.; *Cryptomonas* sp.; *Trachelomonas* sp.; *Aulacoseira* sp., *Ceratium* = *Ceratium hirundinella, Peridinium* sp.*, Oocystis* sp., pennate and centric diatoms).

From Figure 5.5, a positive correlation was found between temperature and the cyanobacteria *Microcystis* sp. and *Oscillatoria* sp. and the diatom *Aulacoseira* sp. Other diatoms (centrics and pennates), as well as *Cryptomonas* sp. and *Trachelomonas* sp., *Scenedesmus* sp. and *Ceratium hirundinella* tend to reach higher concentrations during lower temperatures. The winter temperatures were very similar for the sampling points C-VRB5T ranged between 9 and 17 °C and M-RAW_VAALKOP between 12 and 19 °C, indicating the temperature range favoured by *C. hirundinella* at these two locations. The cyanobacteria *Anabaena* sp. and *Cylindrospermopsis* sp. correlated positively with turbidity. A positive correlation was found between *C. hirundinella* and chlorophyll-665 as did van Ginkel *et al* (2001) and Hart and Wragg (2009); as well as the results obtained in this study at sampling point C-VRB5T (Section 4.1), indicating that *C. hirundinella* contributes significantly to the total photosynthetic pigments. It was found that the dinoflagellate *C. hirundinella* seem to be favoured by low turbidity, and high DIN, Fe, M-alkalinity, Cd, PO_4, Conductivity, pH, hardness and SO_4 concentrations.

Table 5.6 showed that this CCA analysis (Figure 5.5) was statistically significant relationship between environmental variables and algal genera ($P < 0.05$) as judged on the basis of the Monte Carlo Permutation test (499 random permutations).

Table 5.6: Results of the Monte Carlo test from the CCA analysis on the principle environmental components and algal species at M-RAW_VAALKOP for 2004 to February 2011

Test of significance of first canonical axis	
Eigenvalue	0.372
F-ratio	8.434
P-value	0.002
Test of significance of all canonical axes	
Trace	0.888
F-ratio	1.755
P-value	0.002

The peaks in *C. hirundinella* cell biomass coincided to some extent with periods showing high chlorophyll-665 concentrations (Figure 5.8). It is also important to note that the concentration of *C. hirundinella* has decreased in Vaalkop Dam over the study period from January 2004 to the beginning of 2011. *Ceratium hirundinella* also occurred in Vaalkop Dam throughout all the seasons up until 2007. Its occurrence became sporadic after 2007, with peak concentrations in autumn and spring. During the study period, *C. hirundinella* reached an average concentration of 308 cells/mL and a maximum concentration of 3377 cells/mL in April 2004. Numerous cyanobacteria blooms occurred during the study period, especially *Cylindrospermopsis* sp.

(Figure 5.6) and *Oscillatoria* sp. For example, *Cylindrospermopsis* sp. blooms occurred during the study period: May 2007, maximum of 460 843 cells/mL; March 2009, maximum of 203 424 cells/mL and January 2010, maximum of 184 696 cells/mL.

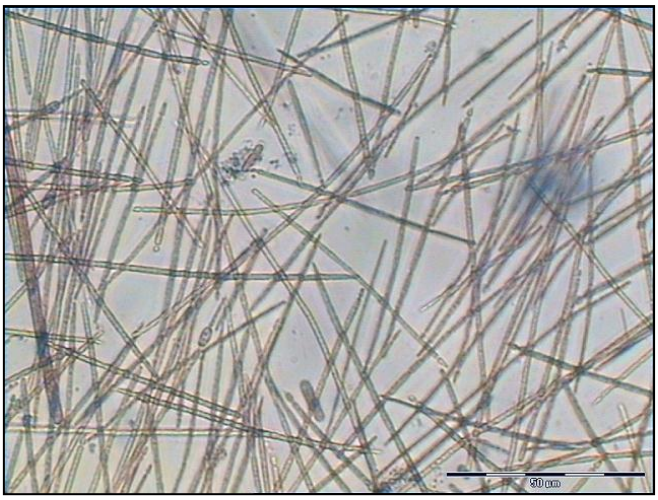


Figure 5.6: Light micrograph of *Cylindrospermopsis* sp.

Figure 5.7 shows the results obtained from the regression analysis performed between chlorophyll-665 and *C. hirundinella* concentrations for the period 2004 to February 2011. The regression analysis revealed a weak positive correlation (R^2 = 0.19) indicating that *C. hirundinella* was only one of a few phytoplankton species that contributed to the photosynthetic pigments at M-RAW_VAALKOP. At this sampling point, the highest chlorophyll-665 concentration was found during November 2004 (110 µg/L). The average chlorophyll-665 concentration from January 2004 to February 2011 is 43 µg/L.

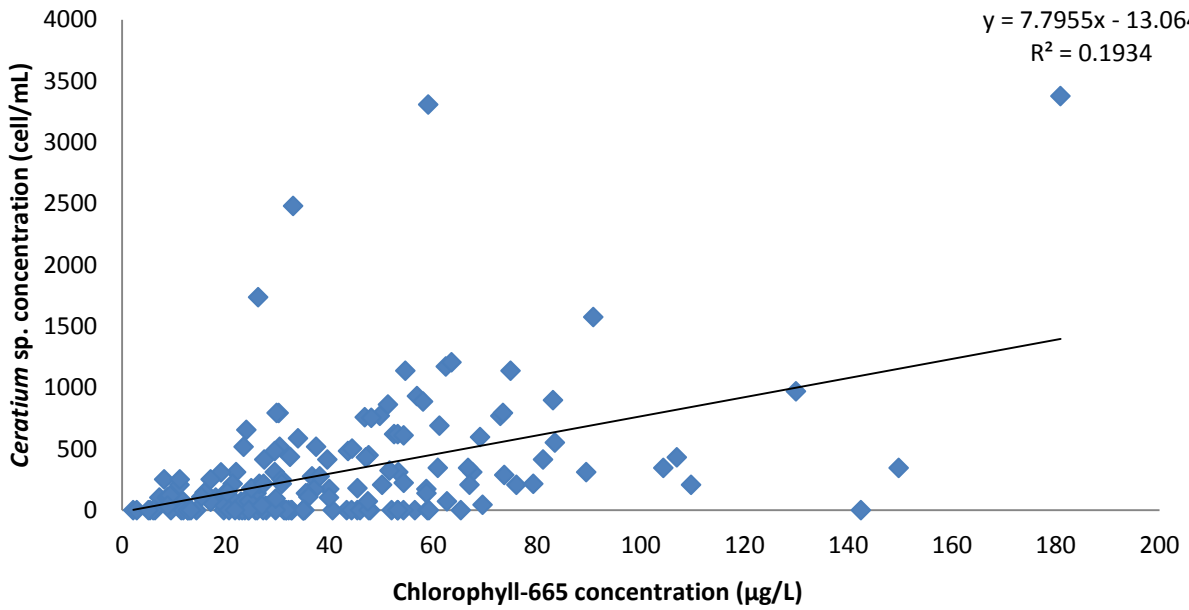


Figure 5.7: Linear regression between Chlorophyll-665 and *Ceratium hirundinella* from January 2004 to February 2011.

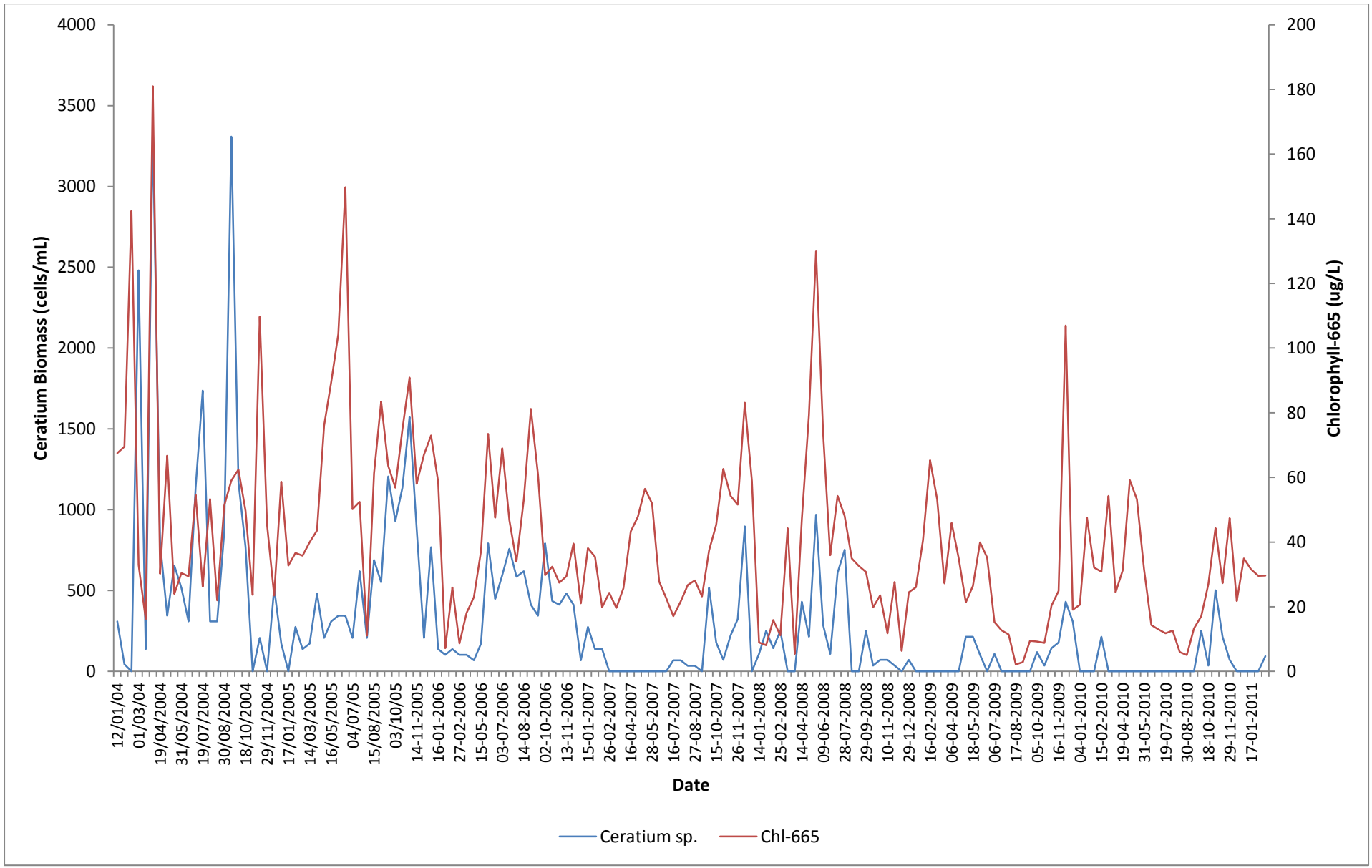


Figure 5.8: Bi-monthly results for chlorophyll-665 and *Ceratium hirundinella* concentrations at M-RAW_VAALKOP for the period January 2004 to February 2011.

5.2.2. Assessment of Vaalkop Dam source water during the presence of a *C. hirundinella* bloom during October and November 2010

In Table 5.7, a summary of the physical, chemical and certain biological variables measured in the Vaalkop Dam during October and November 2010, when sampling took place for jar testing experiments, are displayed. In comparison with Forebay source water that was used for some of the other jar testing experiments (section 4.2.1.2.) the following significant differences could be observed. From Table 5.7, it is evident that the turbidity in Vaalkop Dam was relatively low (Average 4.4 NTU) in comparison with what is found in Forebay source water (Average 76.4 NTU). According to Janse van Vuuren and Pieterse (2005a) the algal taxa Dinophyceae are favoured by conditions of low turbidity, high pH and high conductivity. The conductivity (average 68 mS/m) and total dissolved solids (average 419 mg/L) in Vaalkop Dam were significantly higher than in Forebay source water, which had an average conductivity of 18 mS/m and an average TDS concentration of 186 mg/L during the study period (April to October). The concentrations of sulphate, chloride, and sodium were also significantly higher in comparison with the water in Forebay (average SO_4, 0.24 mg/L; average Cl, 6.1 mg/L and average Na: 9.2 mg/L). According to Swanepoel (1999) an increase in conductivity results in a decrease in turbidity. These high concentrations of minerals might be as a result of human activities found in and around the catchment area, such as mining and farming, which caused an increase of these minerals in the source water.

From Table 5.7, it is evident that nutrients such as nitrates (Average of 0.11 mg/L) and orthophosphates (Average of 0.03 mg/L) were present in low concentrations during high concentrations of *C. hirundinella* (502 cells/mL); a similar observation was made by Grigorszky *et al* (2003a). Higher chlorophyll-665 concentration (average 33 µg/L) were also found in Vaalkop Dam (compared to the Forebay, average 3 µg/L), due to high concentrations of the dinoflagellate *C. hirundinella* present during October and November. *Ceratium* cells are large and contain high concentrations of chlorophyll in their large chloroplasts. *Ceratium hirundinella* reached a maximum concentration of 251 cells/mL near the end of October and increased to 502 cells/mL in the beginning of November in Vaalkop Dam. Van Ginkel *et al* (2001) found average chlorophyll-*a* levels of up to 600 µg/L and cell densities of approximately 13 500 cells/mL during a *C. hirundinella* bloom in the Hartbeespoort Dam during October to December 1999.

Other dominant algal species found in Vaalkop Dam during October and November was the cyanobacteria *Aphanocapsa* sp. (574 cells/mL; Figure 5.10c), *Merismopedia minima* (574 cells/mL; Figure 5.10d) and *Microcystis* sp. (610 cells/mL; Figure 5.10b); and the green-alga *Coccomonas* sp. (Figure 5.10e). According to Janse van Vuuren and Pieterse (2005b), the

cyanobacterium such as *Microcystis* sp. occur in high concentrations during periods of low conductivity (< 50mS/m) which is in contrast with what was found in this study.

In Figure 5.9, the concentrations of the different algae present in the source water (Vaalkop Dam, sampling point M-RAW_VAALKOP) during the month of October and November 2010, are displayed.

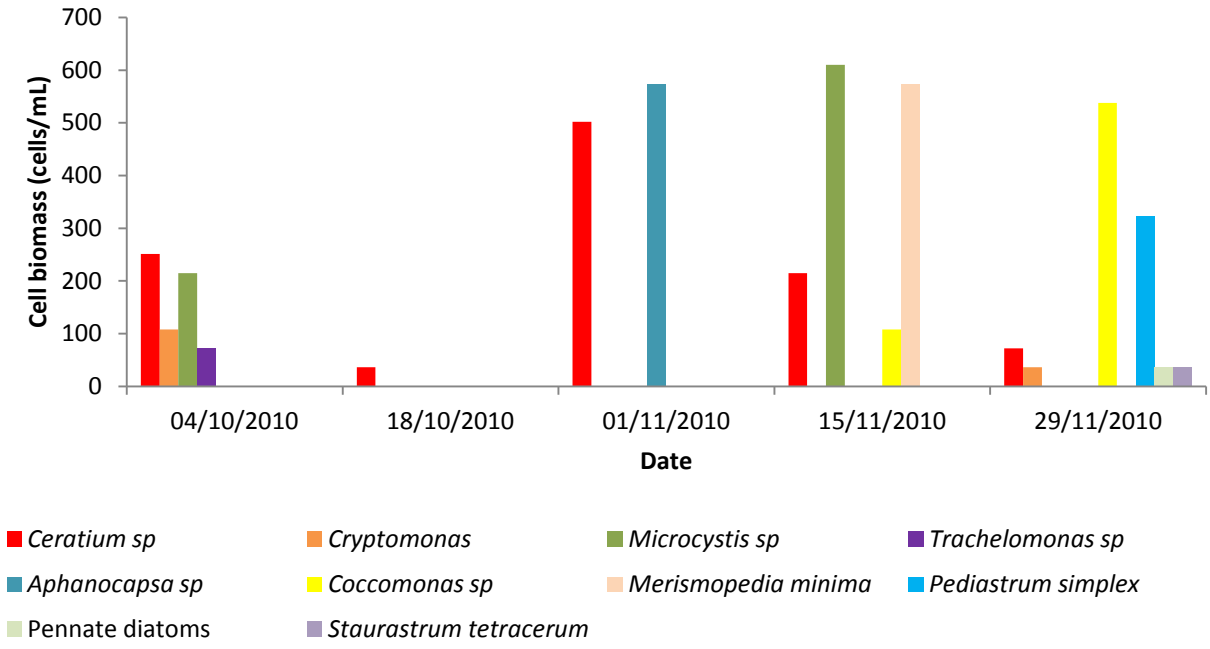


Figure 5.9: Histogram showing the algal species and concentration that occurred in the source water at the sampling point M-RAW_VAALKOP during October and November 2010.

Table 5.7: Minimum, maximum, average and standard deviation values for physical and chemical data for sampling point M-RAW_VAALKOP during October and November 2010

	Turbidity (NTU)	Conductivity (mS/m)	pH	Alkalinity (mg/L $CaCO_3$)	Fluoride (mg/L)	Bromide (mg/L)	Sulphate (mg/L)	Ammonia (mg/L)	Ortho Phosphate (mg/L)	Chloride (mg/L)
Minimum	1.43	65.7	6.92	100	0.43	0.22	85.5	0.032	0.009	72
Maximum	8.3	71.3	8.38	108	0.98	0.71	96.3	0.2	0.04	94.5
Average	4.4	68	7.71	104	0.60	0.34	92	0.10	0.03	88.4
Standard Deviation	2.5	2.13	0.62	3.83	0.22	0.20	4.15	0.07	0.01	9.46

	Total Dissolved Solids (mg/L)	Chemical oxygen demand (mg/L)	Hardness (mg/L $CaCO_3$)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Total Organic Carbon (mg/L)	Dissolved Organic Carbon (mg/L)	Sodium (mg/L)	Iron (mg/L)	Manganese (mg/L)
Minimum	362	17	148.5	27	20	5.38	4.21	2.14	41.4	0.006	0.003
Maximum	459	23	201	38.24	26	7.85	6	6.22	47.4	0.036	0.054
Average	419	19.6	176	33	23	7	4.9	4.95	45.14	0.012	0.021
Standard Deviation	38.45	2.61	19	4.15	2.15	0.94	0.7	1.60	3	0.013	0.023

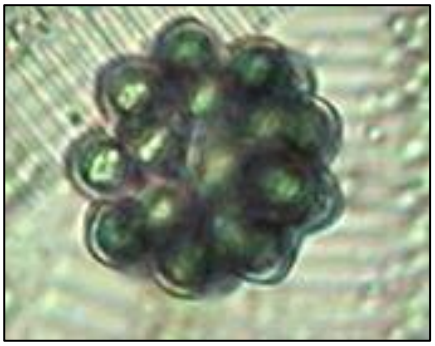
	Nickel (mg/L)	Zinc (mg/L)	Arsenic (µg/L)	Barium (µg/L)	Aluminium (mg/L)	Mercury (µg/L)	Antimony (µg/L)	Selenium (µg/L)	Silicon (mg/L)	Total Silica (mg/L)	Boron (mg/L)
Minimum	0.0003	0.003	0.447	35.3	0.004	0.022	0.11	0.13	0.3	0.65	0.01
Maximum	0.015	0.012	1.51	73.4	0.018	0.21	0.56	1	1.17	2.51	0.033
Average	0.009	0.007	0.91	50.39	0.01	0.077	0.3	0.57	0.82	1.75	0.022
Standard Deviation	0.007	0.003	0.41	16.3	0.005	0.076	0.17	0.31	0.32	0.68	0.009

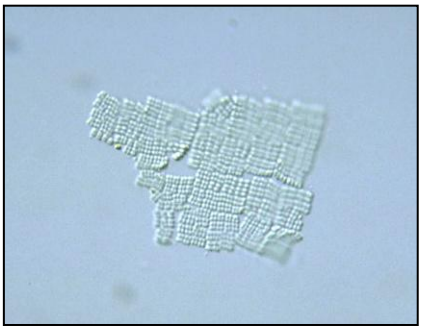
	Lithium (µg/L)	Colour (mg/L)	Sulphur (mg/L)	Nitrate (mg/L)	Strontium (µg/L)	Uranium (µg/L)	Chlorophyll-665 (µg/L)	Temperature (˚C)	Nitrite (mg/L)	Geosmin (ng/L)	Phenol (µg/L)
Minimum	0.83	7	23	0.074	169.6	0.745	17.08	20.9	0.02	5.72	3.45
Maximum	1.67	16	27	0.14	350	1.4	47.41	25.2	0.09	11.62	4.26
Average	1.08	10.8	25.4	0.11	237	1.05	33	23	0.05	8.3	3.94
Standard Deviation	0.39	3.56	1.53	0.03	80	0.32	12.8	1.77	0.035	2.95	0.43

A *C. hirundinella* bloom occurred during the first week of November 2010 in the Vaalkop Dam. Source water was sampled on two occasions (03/11/2010 and 23/11/2010) and was used for jar testing experiments, chlorophyll-665 and total algal biomass determination. The concentrations of the different algae present in the source water are shown in Figure 5.11. The source water for 03/11/2010 had a turbidity of 3.9 NTU and increased slighty to 4.6 NTU over a three week period. The chlorophyll-665 pigment concentration was 71 µg/L (on 03/11/2010) and decreased to 27 µg/L (23/11/2010) (Table 5.8), indicating a decrease of *C. hirundinella* concentrations (from 1263 cells/mL to 1011 cells/mL). These high concentrations of chlorophyll-665 can most probably be ascribed to *C. hirundinella* since these algae contain large concentrations of chlorophyll.

The dominant algal species found in the control sample (source water) on 03/11/2010 was *C. hirundinella* (1263 cells/mL), *Cryptomonas* sp. (735 cells/mL) (Figure 5.10a), *Coelastrum* sp. (316 cells/mL) (Figure 5.10b) and *Chlamydomonas* sp. (147 cells/mL) (Figure 4.12c). On the 23/11/2010, a decrease of *C. hirundinella* was found (1011 cells/mL), and high concentrations of cyanobacteria were present, namely *Oscillatoria* sp. (1565 cells/mL) (Figure 4.8b) and *Microcystis* sp. (833 cells/mL). The biomass of other algae such as *Cryptomonas* sp. (889 cells/mL) and *Chlamydomonas* sp. (838 cells/mL) also increased.

Nicholls *et al* (1980) found a decrease of *C. hirundinella* when high concentrations of cyanobacteria were present; however, it is known that cyanobacteria are favoured by warm temperatures (Janse van Vuuren and Pieterse, 2005a). In this study *C. hirundinella* occurred during periods of moderate temperature, as found during spring and autumn. These occurrences of *C. hirundinella* coincided with the periods of increasing (spring) and decreasing (autumn) cyanobacteria concentrations, therefore no obvious relationship between *C. hirundinella* and cyanobacteria could be established during this study. Van Ginkel *et al* (2001) found that *C. hirundinella* mainly occurs during the warmer months; however, they also found *C. hirundinella* during winter in South Africa.


(a)

(b)

(c)

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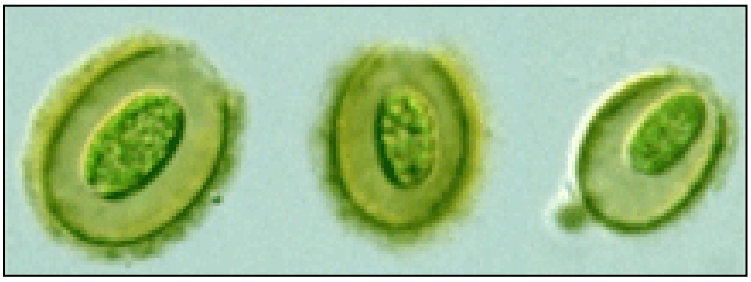

(e)

Figure 5.10: Light micrographs of (a) *Cryptomonas* sp., (b) *Coelastrum* sp., (c) *Aphanocapsa* sp., (d) *Merismopedia minima* and (e) *Coccomonas* sp.

Table 5.8: Physical and chemical parameters for sampling point M-RAW_VAALKOP on 03/11/2010 and 23/11/2010

Date	Turbidity (NTU)	Conductivity (mS/m)	pH	Alkalinity (mg/L $CaCO_3$)	Fluoride (mg/L)	Bromide (mg/L)	Sulphate (mg/L)	Ammonia (mg/L)	Ortho Phosphate (mg/L)	Chloride (mg/L)
03/11/2010	3.9	63	7.3	130	0.6	0.29	105	0.243*	0.036*	98
23/11/2010	4.6	67	8.23	97	0.34	0.25*	95	0.243*	0.036*	96

Date	Total Dissolved Solids (mg/L)	Suspended solids (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Chemical oxygen demand (mg/L)	Hardness (mg/L $CaCO_3$)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Total Organic Carbon (mg/L)	Dissolved Organic Carbon (mg/L)	Sodium (mg/L)
03/11/2010	475	10*	1.8	24	180	34	24	6.3	5.9	6	45
23/11/2010	395	10*	1.4	19	160	29	22	6.2	5.7	5.8	41

Date	Cadmium (mg/L)	Chrome (mg/L)	Cobalt (mg/L)	Copper (mg/L)	Iron (mg/L)	Manganese (mg/L)	Lead (mg/L)	Nickel (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Zinc (mg/L)
03/11/2010	0.0025*	0.01*	0.015*	0.01*	0.006*	0.003*	0.008*	0.015*	0.1	0.02	0.008*
23/11/2010	0.0025*	0.01*	0.015*	0.01*	0.006*	0.01	0.008*	0.015*	0.1	0.02	0.008*

Date	Aluminium (mg/L)	Boron (mg/L)	Vanadium (mg/L)	Molybdenum (mg/L)	Total Silica (mg/L)	Phosphorus (mg/L)	Sulphur (mg/L)	Chlorophyll-665 (µg/L)	Silicon (mg/L)
03/11/2010	0.11	0.02	0.03*	0.01*	1.9	0.041*	28	70.94	0.88
23/11/2010	0.01*	0.03	0.03*	0.01*	0.73	0.041*	25	26.64	0.34

* Method Reporting limit

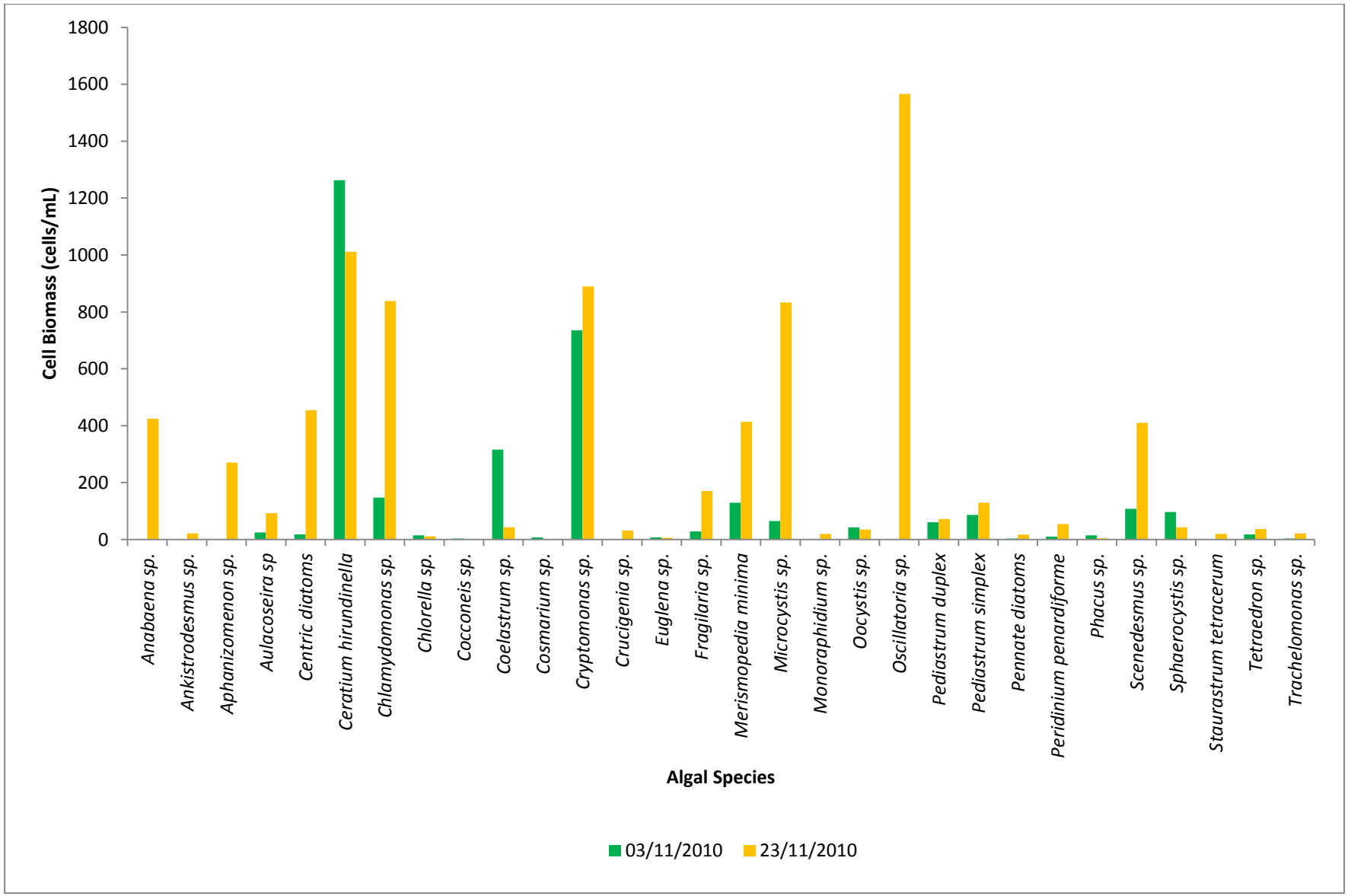


Figure 5.11: Histogram showing the algal species and concentration that occurred in the source water (control sample) at sampling point M-RAW_VAALKOP for 03/11/2010 and 23/11/2010.

5.2.3. Assessment of jar testing experiments

All analyses of Vaalkop and Rietvlei Dams described in Sections 5.2.3 and 5.3.1 were executed in exactly the same manner as for Forebay and M-Canal_VD described in Chapter 4. To avoid repetition the graphs of this section are captured in Appendix B.

The different coagulant chemicals were investigated to determine which removed high concentrations of *C. hirundinella*, and which analyses (turbidity, chlorophyll-665 or total algal biomass) gave a good or better indication of the appropriate dosage to do so. It should be emphasized that no literature were found where jar testing experiments were performed on source water containing high concentrations of *C. hirundinella*, to evaluate the appropriate coagulant chemicals and concentrations.

5.2.3.1. Jar testing with poly-electrolyte as only coagulant chemical on source water from Vaalkop Dam

No clear pattern was found from the results of turbidity and total algal biomass determinations. The turbidity varied between 3 and 5 NTU, and did not decrease significantly with an increase in coagulant chemical concentration. High turbidity with increasing coagulant chemical concentrations has however been found in other studies (e.g. Ma and Liu, 2002b and Jun *et al*, 2001). According to Jun *et al* (2001) reasons to explain poor turbidity removal includes restabilisation, as well as charge reversal or changes in floc size and density. However, an increase in turbidity at higher dosages of cationic poly-electrolytes is commonly found (Ebeling *et al*, 2005).

Table 5.9: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on sampling dates 03/11/2010 and 23/11/2010

Variable	Date	y-value	R^2-value
Turbidity	03/11/2010	-0.0428x + 5.106	0.0617
	23/11/2010	-0.0579x + 4.5851	0.36
Chlorophyll-665	03/11/2010	-1.9852x + 60.665	0.7156
	23/11/2010	-1.3659x + 29.971	0.4305
Total Algal Biomass	03/11/2010	-51.143x + 1656.9	0.1334
	23/11/2010	29.636x + 1024	0.0242

The jar testing experiment done on the 03/11/2010, indicated when using poly-electrolyte only, as coagulant chemical, the coagulation and flocculation unit processes were not effective

(Table 5.9: R^2 = 0.062). The appropriate dosage was 15 mg/L, as turbidity measured 3 NTU. The Chlorophyll-665 concentration in this sample was found to be 24.3 µg/L at the appropriate dosage of 15 mg/L. A decrease of chlorophyll-665 concentration was found as the coagulant dosage increased. The chlorophyll-665 results indicated that using poly-electrolyte only removed some algal material from the source water (Table 5.9: R^2 = 0.715). The chlorophyll-665 concentration in the supernatant remained higher than 20 µg/L indicating poor algal removal. The algal taxa Dinophyceae were not sufficiently removed as high concentrations were still present even as the coagulant chemical concentration increased (Figure 5.12). *Ceratium hirundinella* reached a maximum concentration of 1414 cells/mL that was not removed (Figure 5.13). Coagulation of algae is very intricate because of the large variability in morphology (e.g. spines and appendages and cell size) that reduces their settling velocity (Jun *et al*, 2001). It is also evident that the taxa Chlorophyceae (in particular *Chlamydomonas* sp. and *Coelastrum* sp.) and Cyanophyceae (specifically *Microcystis* sp.) were present in high concentrations, but decreased with increasing coagulant chemical concentration (Figures 5.12 and 5.13). Certain algae such as *Microcystis* sp. and *Coelastrum* sp. occur in colonies that contain mucilage that may also render it less susceptible to sedimentation.

The jar testing experiment done on the 23/11/2010, indicated when using poly-electrolyte only, that some of the turbidity was removed (less than 1 NTU difference between the lowest and highest dosage concentration (Table 5.9: R^2 = 0.36), and the chlorophyll-665 results indicated that this coagulant option removed some algae (Table 5.9: R^2 = 0.43). The appropriate dosage was 15 mg/L, and the chlorophyll-665 concentration in this sample was 7 µg/L. The total algal biomass concentrations remained relatively high since it was suspended in the supernatant and caused high turbidity and chlorophyll-665 values. According to Tenney *et al* (1969) synthetic cationic poly-electrolytes reduce the negative charge on algal surfaces and enhance coagulation and that anionic and non-ionic polymers alone do not coagulate algae sufficiently. However, poor turbidity and chlorophyll-665 removal was found when using a cationic poly-electrolyte, these results are most probably because restabilisation occurred and thereby influenced the algal coagulation (Jun *et al*, 2001), as was found in the present study. There was an insufficient removal of the algal taxa Chlorophyceae, Cyanophyceae and Dinophyceae (Figure 5.14) as in the previous jar testing experiment performed on the 03/11/2010. The biomass of the *Ceratium* cells in the source water decreased after the first sampling occasion (03/11/2010), indicating that unfavourable environmental conditions specific to *C. hirundinella* may have developed, which may include changes of chemical or physical parameters which may have resulted in the dominance of other algal groups such as Cyanophyceae. A maximum concentration of 904 cells/mL of *C. hirundinella* was observed in the supernatant (Figure 5.15). There were high concentrations of Cyanophyceae

present in the supernatant even when the coagulant chemical increased (*Microcystis* sp.: 258 cells/mL and *Oscillatoria* sp.: 2023 cells/mL) (Figures 5.14 and 5.15). The total algal biomass in the supernatant indicated that the coagulation and flocculation unit processes were ineffective for the first sampling date but even more ineffective for the second sampling date (Table 5.9: R^2 = 0.1334 for 03/11/2010 and R^2 = 0.0242 for 23/11/2010). When using poly-electrolyte alone, no clear trend was found between the biomass of *C. hirundinella* and the increasing concentrations of the coagulant chemical.

5.2.3.2. Jar testing with poly-electrolyte in combination with CaO as coagulant chemicals on source water from Vaalkop Dam

A decrease in turbidity, chlorophyll-665 and total algal biomass was found with an increase in coagulant chemical concentration for both sampling dates, as was found with Forebay source water (Section 4.2.1.2.2.).

Table 5.10: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when poly-electrolyte in combination with 10 mg/L CaO were dosed at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on sampling dates 03/11/2010 and 23/11/2010

Variable	Date	y-value	R^2-value
Turbidity	03/11/2010	-0.5528x + 14.663	0.4438
	23/11/2010	-0.3094x + 8.8117	0.4556
Chlorophyll-665	03/11/2010	$155.63e^{-0.229x}$	0.9325
	23/11/2010	$56.589e^{-0.273x}$	0.8786
Total Algal Biomass	03/11/2010	-88.51x + 1659.9	0.3305
	23/11/2010	-80.937x + 1332	0.7683

The jar testing experiment done on 03/11/2010, indicated that the appropriate dosage of 12 mg/L was found when using poly-electrolyte with 10 mg/L CaO. However, it should be noted that the turbidity value did not meet the requirements (i.e. < 3 NTU), therefore indicating that this coagulant chemical may not be suitable for this type of source water or an addition of a secondary coagulant should be considered. According to Steynberg *et al* (1994) the dosing of lime can aid in the reduction of algal concentrations, and this was found when CaO was dosed in combination with poly-electrolyte. Chlorophyll-665 in this sample was found to be 14.1 µg/L at 12 mg/L poly-electrolyte in combination with 10 mg/L CaO. Although the appropriate dosage was 12 mg/L (when considering turbidity only) the chlorophyll-665 concentration and total algal biomass (1015 cells/mL) were still high. The turbidity of the supernatant after jar testing, the coagulation and flocculation

unit processes were not effective when using this particular coagulant option (Table 5.10: R^2 = 0.44) because the turbidity values remained higher than 6 NTU irrespective of the coagulant chemical concentration. The chlorophyll-665 results indicated that using poly-electrolyte with CaO was also ineffective in removing algal material from the source water (Table 5.10: R^2 = 0.93). Most of the algal taxa were removed (Figure 5.12), however Dinophyceae were still present in relatively high concentrations (average concentration of 647 cells/mL). The dinoflagellate *C. hirundinella* decreased from a maximum concentration of 1808 cells/mL to 43 cells/mL as the coagulant chemical dosage increased from 5 to 16 mg/L (Figure 5.13). The Chlorophyceae were present in low concentrations (maximum of 209 cells/mL) (Figure 5.12). This decrease in algal concentration illustrates the negative effect of CaO on the algae, (specifically on *C. hirundinella*) creating unfavourable conditions (in this case, high pH) which most probably causes the cells to die or shed their flagellae and therefore can be removed more effectively (Steynberg *et al*, 1994; Geldenhuys *et al*, 2000; Basson, 2000).

The jar testing experiment done on 23/11/2010, show that the appropriate dosage was 14 mg/L, and the chlorophyll-665 concentration in this sample was 2 µg/L. The turbidity of the supernatant after the jar testing indicated that when poly-electrolyte in combination with CaO is used, the coagulation and flocculation unit processes were somewhat effective in removing silt and sand particles. (Table 5.10: R^2 = 0.45). However, the chlorophyll-665 results indicated that using poly-electrolyte with CaO is ineffective in removing algal material from the source water (Table 5.10: R^2 = 0.8786). From Figure 5.14, it is clear that most algal taxa (Bacillariophyceae, Chlorophyceae, Cyanophyceae and Dinophyceae) were removed when using poly-electrolyte in combination with CaO. The dinoflagellate *C. hirundinella* decreased from a maximum of 775 cells/mL to 43 cells/mL as the coagulant chemical dosage increased. There were high concentrations of Cyanophyceae present in the source water on this particular sampling date (Figure 5.11) and decreased significantly with increased dosage concentrations of poly-electrolyte in combination with CaO (Figure 5.15).

The total algal biomass results of the supernatant indicated that the coagulation and flocculation unit processes were somewhat effective for both sampling dates (Table 5.10: R^2 = 0.3305 for 03/11/2010 and R^2 = 0.7683 for 23/11/2010) when using poly-electrolyte in combination with CaO as coagulant chemical. The comparison between the removal of turbidity, chlorophyll-665 and total algal biomass showed that more particulate matter was removed with increased coagulant concentration. However, when poly-electrolyte in combination with 10 mg/L of CaO was used, the *Ceratium* biomass decreased significantly. Therefore, illustrating the effect of CaO on algae, creating an unfavourable environment (high pH 10 - 11), that renders the cells immobile or cause the cells to die (Steynberg *et al,* 1994; Geldenhuys *et al*, 2000; Ma and Liu, 2002a).

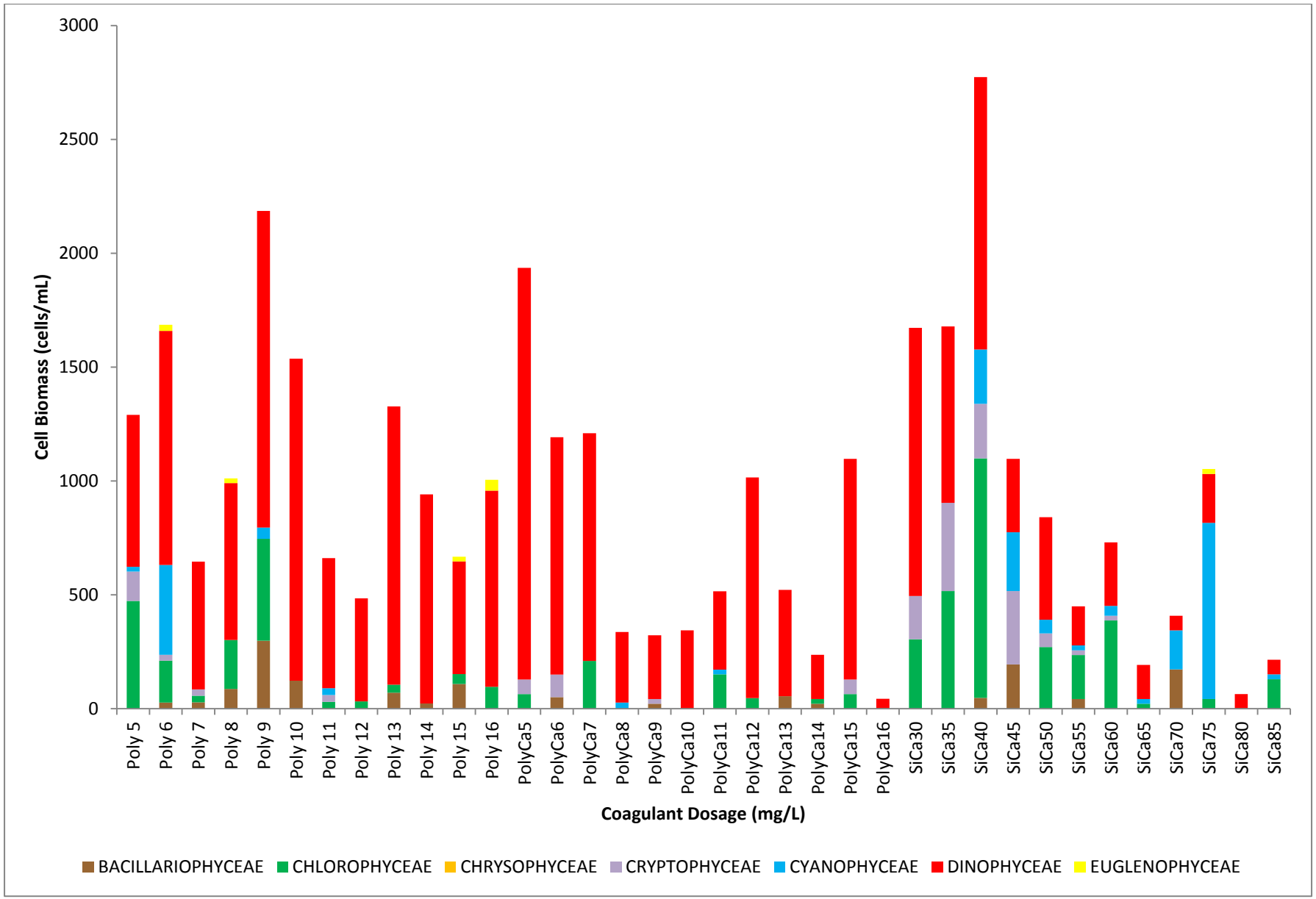


Figure 5.12: Histogram showing the major algal taxa that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on source water from sampling point M-RAW_VAALKOP on **03/11/2010**.

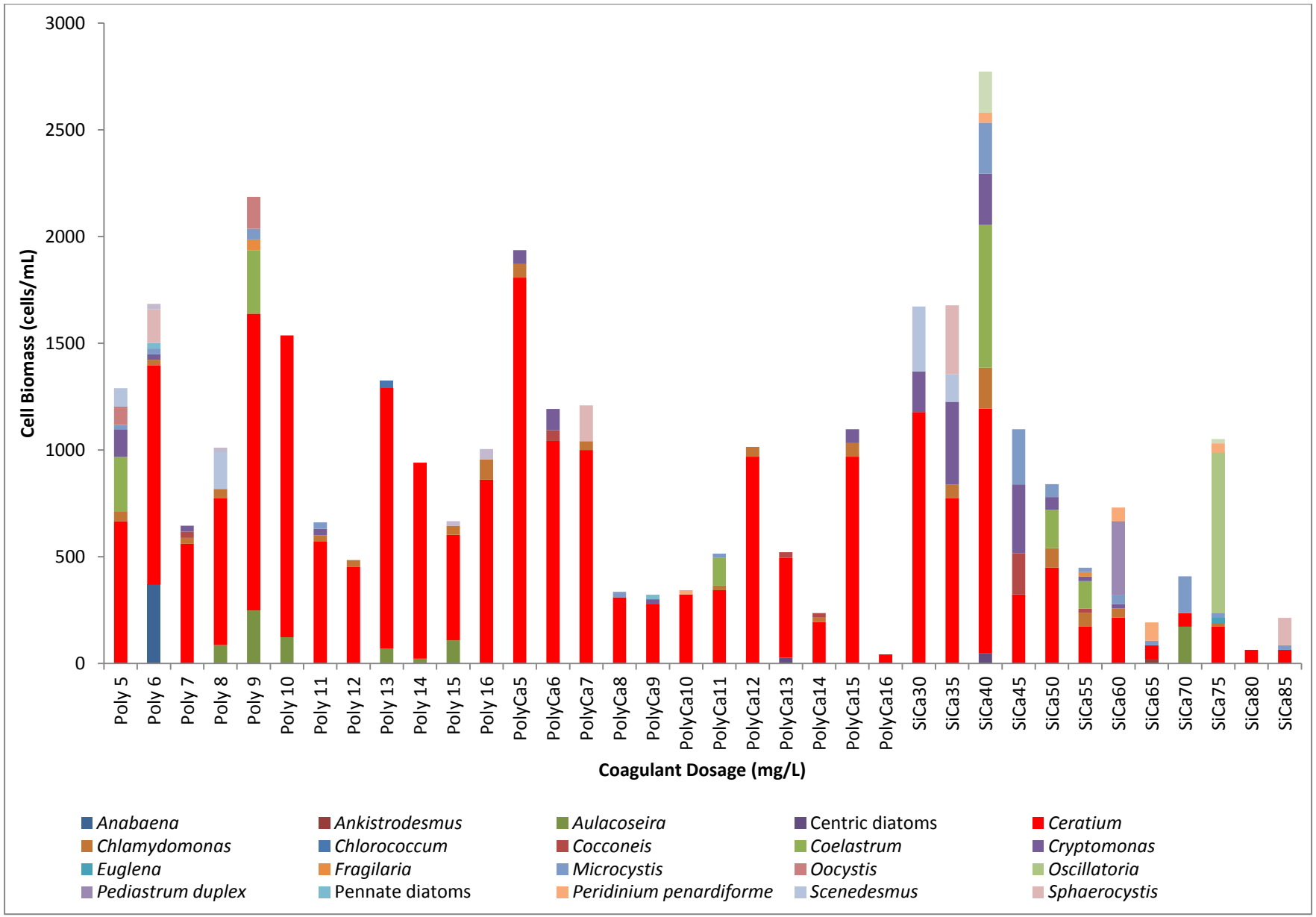


Figure 5.13: Histogram showing the algal species that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on source water from sampling point M-RAW_VAALKOP on **03/11/2010**.

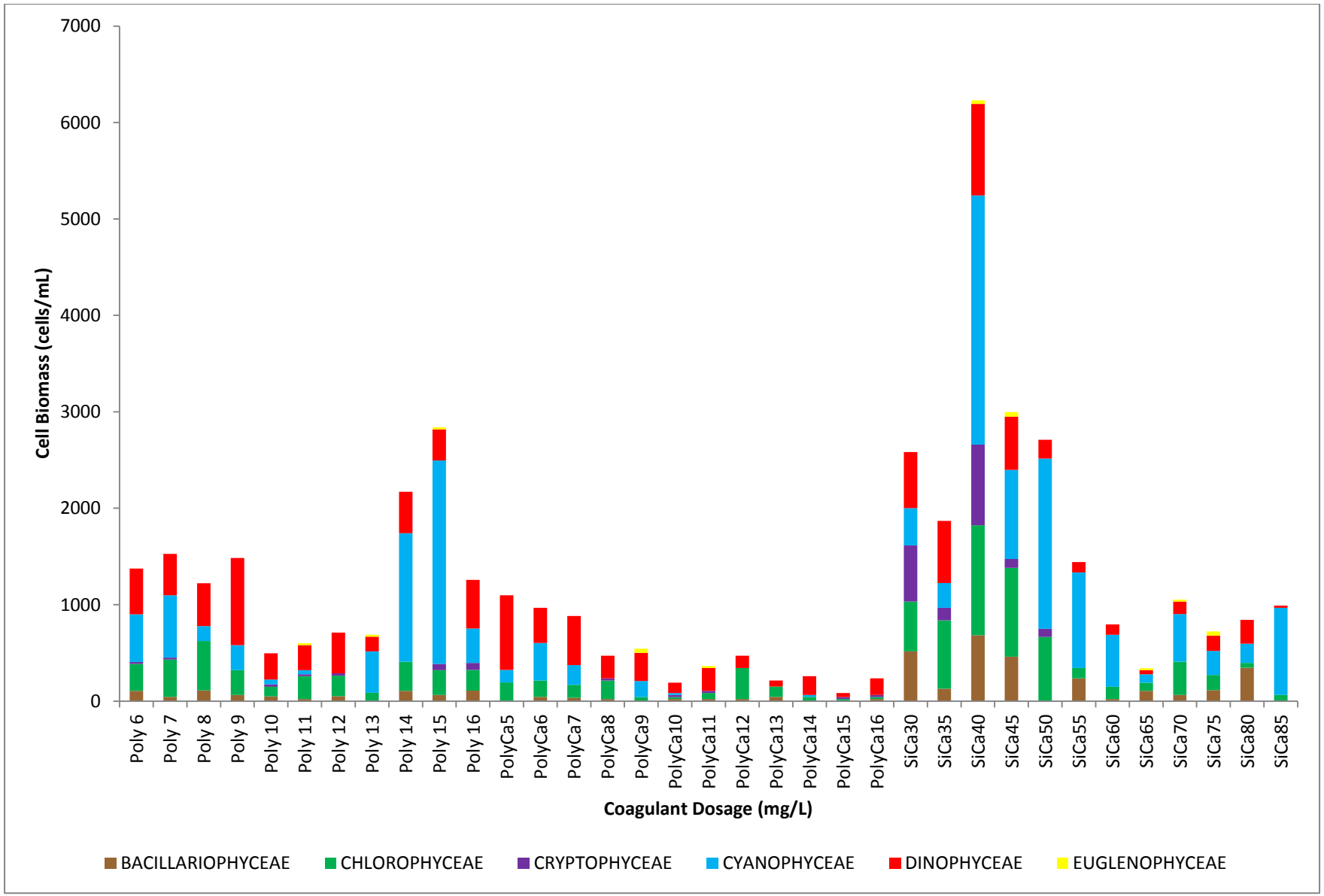


Figure 5.14: Histogram showing the major algal taxa that were not removed by the ranging concentrations of different coagulant chemicals for each jar testing procedure on source water from sampling point M-RAW_VAALKOP on **23/11/2010**.

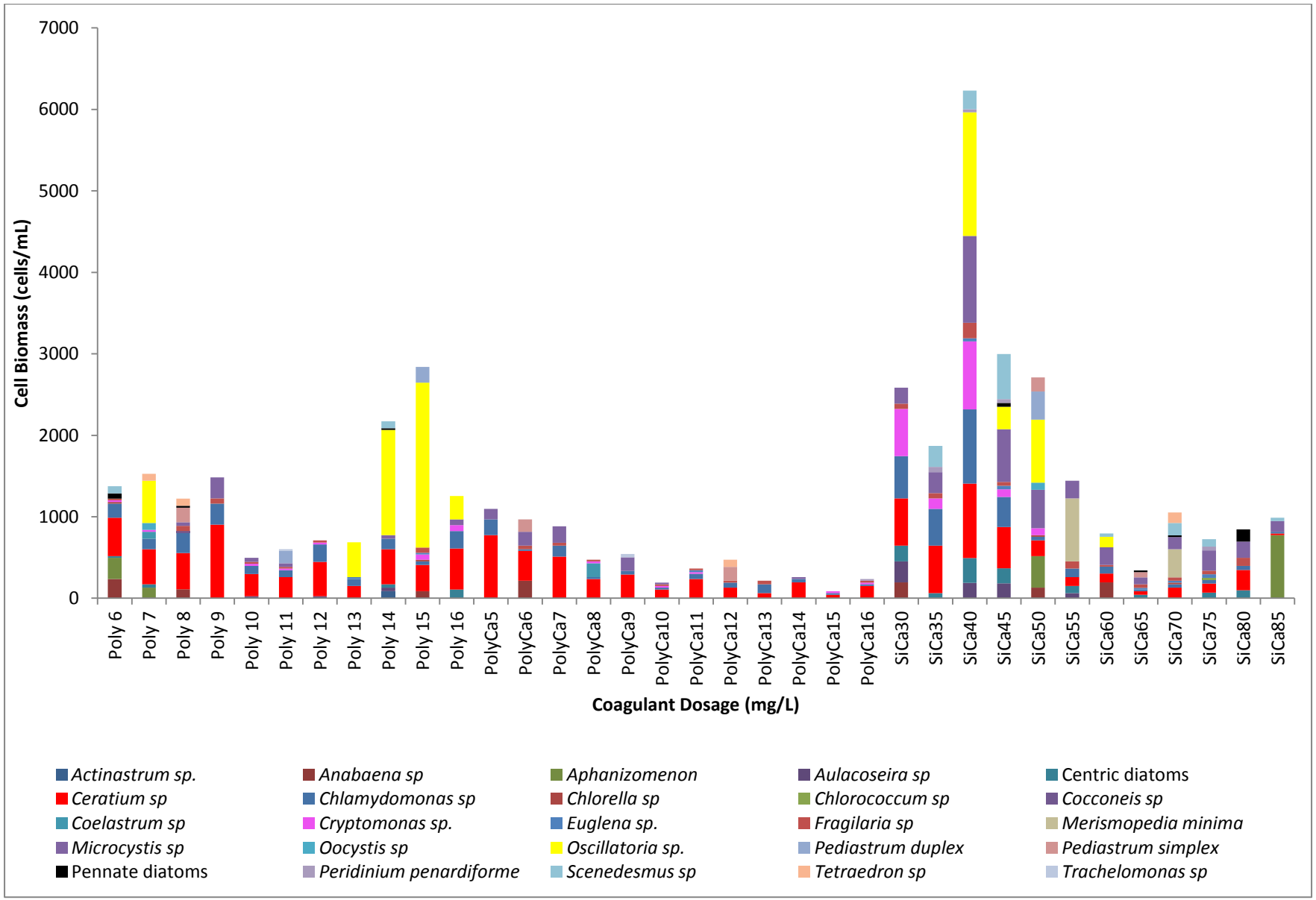


Figure 5.15: Histogram showing the algal species that were not removed by the ranging concentrations of different coagulant chemicals for each jar testing procedure on source water from sampling point M-RAW_VAALKOP on **23/11/2010**.

5.2.3.3. Jar testing with varying concentrations of CaO in combination with 2.5 mg/L activated silica as coagulant chemicals on source water from Vaalkop Dam

A decrease in turbidity (for only 03/11/2010), chlorophyll-665 and total algal biomass was found with an increasing coagulant chemical concentration for both sampling dates. From Table 5.8, it is evident that the Vaalkop Dam source water had a low turbidity (Table 5.8: 3.9 and 4.6 NTU for sampling dates 03/11/2010 and 23/11/2010 respectively). According to Cheng *et al* (2006) coagulant aids such as activated silica can be used in low turbidity waters to increase the turbidity and thereby enhancing coagulation.

Table 5.11: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when CaO in combination with 2.5 mg/L activated silica were dosed at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on sampling dates 03/11/2010 and 23/11/2010

Variable	Date	y-value	R^2-value
Turbidity	03/11/2010	-0.2464x + 36.258	0.7257
	23/11/2010	0.4026x + 15.41	0.8447
Chlorophyll-665	03/11/2010	-1.0208x + 81.622	0.9412
	23/11/2010	-0.3782x + 30.61	0.9255
Total Algal Biomass	03/11/2010	-33.37x + 2849.5	0.5762
	23/11/2010	-57.887x + 5210.1	0.4134

The jar testing experiment done on 03/11/2010, indicated that the appropriate dosage was 85 mg/L CaO when using CaO in combination with 2.5 mg/L activated silica. However, it should be noted that the lowest turbidity value was chosen as the appropriate dosage as the turbidity value did not meet the requirements (i.e. < 3 NTU); therefore indicating that CaO in combination with activated silica may not be suitable for this type of source water or that an addition of a secondary coagulant should be considered. The high turbidity value could be due to suspended residual lime particles in the supernatant (Freese *et al*, 2004). Chlorophyll-665 in the supernatant after the jar testing experiment was found to be 2.13 µg/L at this concentration (which is lower than what was found when using poly-electrolyte). A significant decrease in chlorophyll-665 concentration and total algal biomass in the samples were found as the coagulant dosage increased, indicating the effect of CaO on algae (as was found when using poly-electrolyte in combination with CaO as coagulant chemicals) (Steynberg *et al*, 1994). The coagulation and flocculation unit processes were effective (Table 5.11: $R^2 = 0.7257$). However, the chlorophyll-665 results indicated that using activated silica with CaO was effective in removing algal material from the source water (Table 5.11: $R^2 = 0.9412$).

The results from the jar testing experiment done on 03/11/2010 indicated that when using CaO in combination with activated silica as coagulant chemical that the algal taxa Dinophyceae, Chlorophyceae and Cyanophyceae were present in high concentrations (Figure 5.12). The dinoflagellate *C. hirundinella* decreased from a maximum concentration of 1178 cells/mL to 64 cells/mL as the coagulant chemical increased (Figure 5.13). The dominant green-algae include *Coelastrum* sp. (670 cells/mL), *Pediastrum duplex* (344 cells/mL) (Figure 5.16a), *Sphaerocystis* sp. (323 cells/mL) (Figure 5.16c) and *Scenedesmus* sp. (304 cells/mL) (Figure 5.16b); the cryptophyte *Cryptomonas* sp. (387 cells/mL); and high concentrations of cyanobacteria were also present in the supernatant, namely *Oscillatoria* sp. (753 cells/mL), and *Microcystis* sp. (258 cells/mL) (Figure 5.13). However, it should be noted that some of these algae mentioned form colonies or filaments and that may interfere with the coagulation, flocculation and sedimentation unit processes.

For the jar testing experiment done on 23/11/2010, it was found that when using CaO in combination with activated silica, the coagulation and flocculation unit processes were not effective when considering turbidity only (Table 5.11: $R^2 = 0.8447$). The appropriate dosage was 30 mg/L CaO (when considering turbidity results only). Turbidity increased with an increase of coagulant chemical, this therefore indicates that activated silica might not be a suitable main coagulant chemical. Despite this increase in turbidity, the chlorophyll-665 results indicated that using CaO in combination with activated silica was effective in removing algal material from the source water (Table 5.11: $R^2 = 0.9255$). Most of the algal taxa (Bacillariophyceae, Chlorophyceae, Cyanophyceae and Dinophyceae) remained in the supernatant (Figure 5.15). However, the algal concentrations decreased with an increase in coagulant chemical concentration. A maximum concentration of 912 cells/mL of *C. hirundinella* that were not removed from the supernatant was observed when 40 mg/L of the coagulant was dosed (Figure 5.15). There was a relatively high concentration of Cyanophyceae present in the supernatant and even with increasing coagulant chemical concentration that was not removed from the water (Figure 5.15) (*Microcystis* sp.: 1064 cells/mL, *Oscillatoria* sp.:1519 cells/mL and *Aphanizomenon* sp.: 775 cells/mL (Figure 5.16d)).

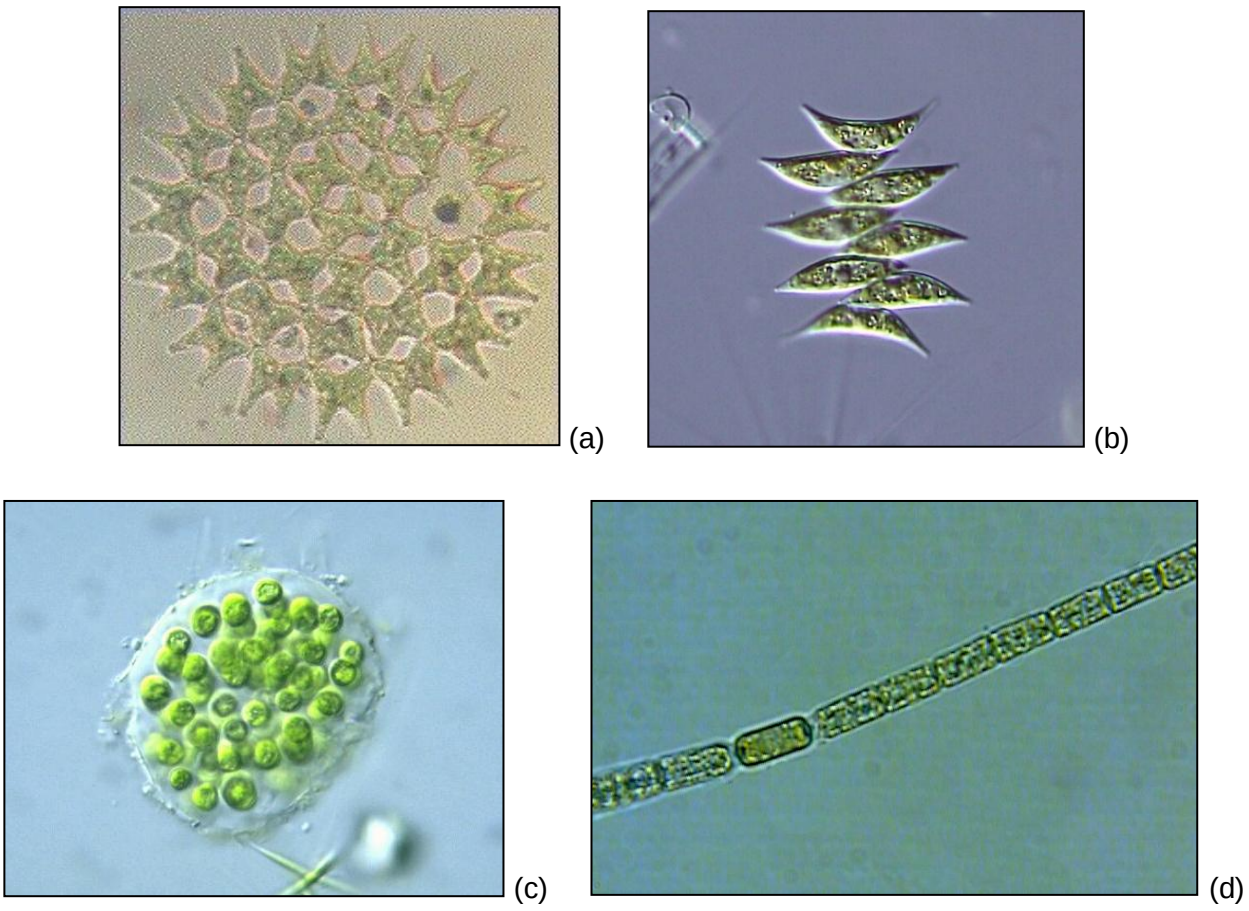


Figure 5.16: Light micrographs of (a) *Pediastrum duplex*, (b) *Scenedesmus* sp., (c) *Sphaerocystis* sp. and (d) *Aphanizomenon* sp.

The total algal biomass results of the supernatant indicated that the coagulation and flocculation unit processes were effective for both sampling dates (Table 5.11: R^2 = 0.5762 for 03/11/2010 and R^2 = 0.4134 for 23/11/2010) when using CaO in combination with 2.5 mg/L activated silica as chemical coagulants. The correlation between the removal of turbidity, chlorophyll-665 and total algal biomass showed that more particulate matter was removed with increased coagulant concentration. When CaO in combination with activated silica with was used, the *Ceratium* biomass decreased significantly with CaO concentrations higher than 45 mg/L. Thus, CaO as coagulant chemical greatly enhance algal removal, specifically *C. hirundinella*, as in this case.

The jar testing experiments for both sampling dates when using poly-electrolyte only, the desired turbidity was reached, but very little algae were removed with this coagulant chemical. In particular, *C. hirundinella* was still present at high concentrations (495 cells/mL at a concentration when dosing 15 mg/L coagulant chemical). The chlorophyll-665 analysis for the jar testing experiment done on 03/11/2010 indicated that more algae were removed when dosing poly-electrolyte in combination with CaO (14.1 µg/L) rather than poly-electrolyte alone (24.33 µg/L). The jar testing

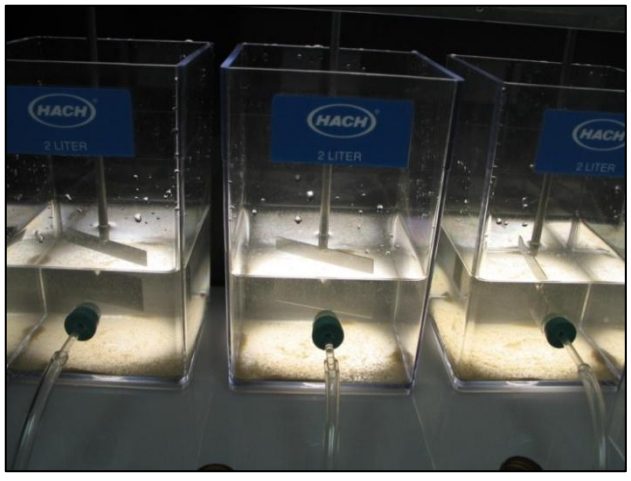
experiment for the 23/11/2010 illustrated the same trend as what was found on 03/11/2010, when poly-electrolyte was dosed alone 15 mg/L was needed to reach the desired turbidity. However, when 10 mg/L CaO was dosed in combination with poly-electrolyte an appropriate turbidity value was reached at 14 mg/L. Although dosing 15 mg/L poly-electrolyte only seems to be more attractive option financially, it is important to consider the results from chlorophyll-665 analysis indicating that algae is removed more effectively when poly-electrolyte is dosed in combination with CaO. In this particular case, the chlorophyll-665 analysis indicated that more algae were removed when dosing poly-electrolyte in combination with CaO (2 µg/L) rather than on its own (7 µg/L). When poly-electrolyte or poly-electrolyte in combination with CaO was used, a small number of flocs formed which did not seem to settle well. This explains the high turbidity values of the supernatant, which was higher than the turbidity of the control or source waters.

When using CaO in combination with activated silica, higher turbidity values (after coagulation, flocculation and sedimentation) were found in comparison with the procedures where poly-electrolyte and poly-electrolyte in combination with CaO were used. However, the chlorophyll-665 concentration and total algal biomass (in particular *C. hirundinella*) in the supernatant decreased significantly. Therefore, when considering turbidity alone, the coagulant chemical CaO in combination with activated silica might not be the suitable coagulant chemical for source waters containing high concentrations of *C. hirundinella*, or a secondary coagulant aid should be considered.

During this particular jar testing experiment, large and stable flocs formed when CaO was used in combination with activated silica as coagulant chemical (Figure 5.17), in comparison with the other coagulant chemicals (poly-electrolyte or poly-electrolyte with CaO). This was due to the reaction between the coagulant chemical and the type of source water (Cheng *et al*, 2006; Bolto and Gregory, 2007). Therefore, indicating that CaO in combination with activated silica work source waters with low turbidity values (Cheng *et al*, 2006). It is known that poly-electrolytes perform well on soft coloured source waters (Bolto and Gregory, 2007). Although the chlorophyll-665 concentrations and total algal biomass decreased significantly, a high turbidity was found due to residual lime particles in the supernatant (Freese *et al*, 2004). Therefore, adding a secondary coagulant should be investigated to aid in decreasing the turbidity.


(a)


(b)



(c)

Figure 5.17: Pictures of the jar testing process (a) during the two minute flash mixing period with activated silica in combination with CaO as coagulant chemical; and (b) during the fifteen minute settling period and (c) picture showing the clear supernatant after the fifteen minute settling period.

5.2.3.4. Comparison of the appropriate dosages of all coagulant chemicals for the jar testing experiments performed on Vaalkop Dam source water

The jar testing experiment done for both sampling dates (03/11/2010 and 23/11/2010) indicated that when using poly-electrolyte only as coagulant chemical, a high algal biomass was found to break through with the appropriate dosage at 15 mg/L in both cases (Figures 5.18 and 5.19). This particular coagulant option (for jar testing experiment on 03/11/2010) had high chlorophyll-665 concentrations and high algal biomass remaining in the supernatant, although a lower turbidity was found. It is well known that algae are difficult to remove because of their unique morphological characteristics and motility (Basson, 2000; Geldenhuys *et al*, 2000; Jun *et al*, 2001; Ma and Liu, 2002a). The jar testing experiment performed on 03/11/2010, poly-electrolyte in combination with 10 mg/L CaO was used. The appropriate dosage was 12 mg/L and had high concentrations of algal biomass remaining in the supernatant. This may be due to algae in the form of colonies or filaments that interfere with the coagulation, flocculation and sedimentation unit processes. The chlorophyll-665 concentration also decreased more significantly when compared to dosing poly-electrolyte only, confirming the negative effect of CaO on algae as found by Steynberg *et al* (1994). This was also found with the jar testing experiment performed on 23/11/2010, the chlorophyll-665 concentration and total algal biomass decreased when 14 mg/L poly-electrolyte was dosed in combination with 10 mg/L CaO.

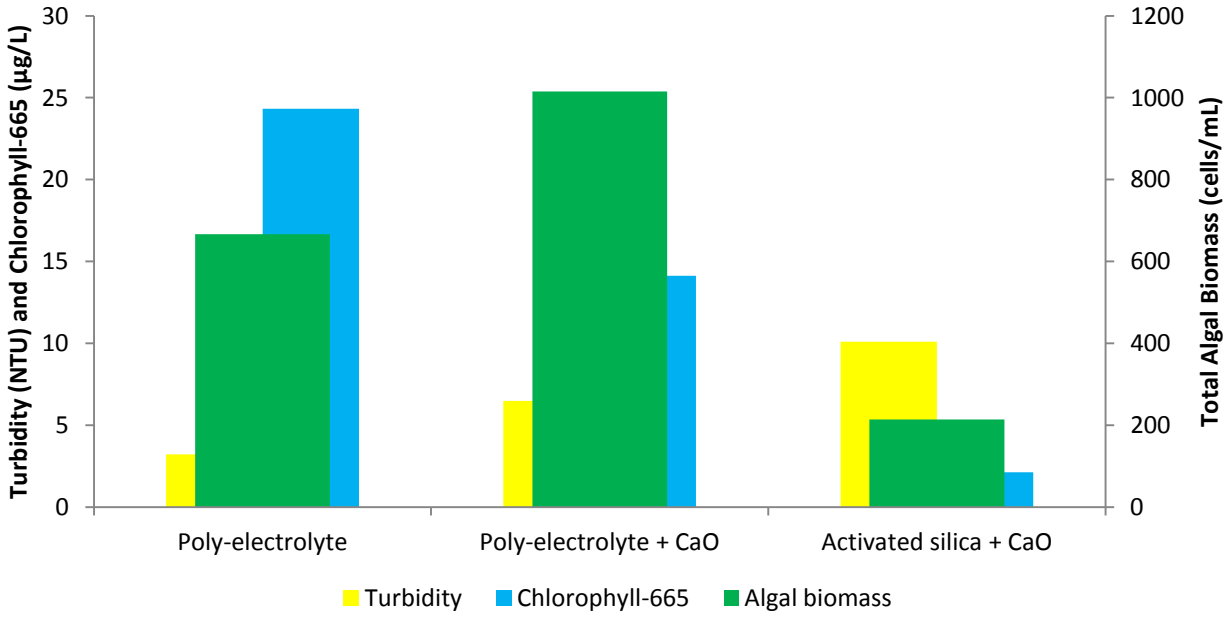


Figure 5.18: Comparison between turbidity, chlorophyll-665 and total algae biomass found in the samples where the "appropriate" concentration of coagulant was dosed for sampling point M-RAW_VAALKOP on **03/11/2010**.

For the jar testing experiment performed on 03/11/2010, the appropriate dosage was found to be 85 mg/L CaO, when CaO in combination with 2.5 mg/L activated silica was used. Turbidity was high although most of the chlorophyll-665 and total algal biomass were removed. This is most probably due to the presence of residual lime particles in the supernatant (Freese *et al*, 2004). For the jar testing experiment on 23/11/2010, an appropriate dosage was found at 30 mg/L CaO. However, it should be noted that the lowest turbidity value was chosen as no turbidity values smaller than 3 NTU were obtained. There were high concentrations of chlorophyll-665 and algae that remained in the supernatant at this appropriate dosage.

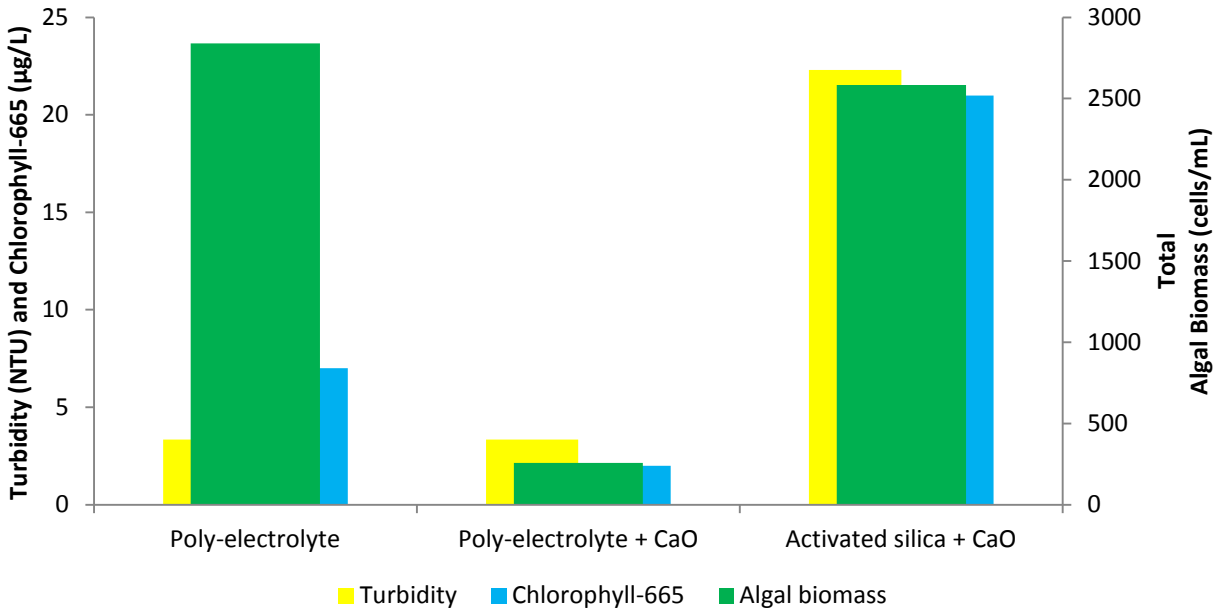


Figure 5.19: Turbidity, chlorophyll-665 and total algae biomass found in the samples where the "appropriate" concentration of coagulant was dosed for sampling point M-RAW_VAALKOP on **23/11/2010**.

Thus, using CaO as coagulant chemical causes a decrease in chlorophyll-665, illustrating the negative impact of CaO on algae. Thereby, suggesting that the plant laboratories should include chlorophyll-665 analysis as indicator of the effectiveness of the simulated coagulation/flocculation process (Basson, 2000), rather than only using turbidity, especially during times of high algal biomass in the source water and high algal related impacts on the water purification process.

5.2.3.5. Multivariate analyses for jar testing experiments performed with Vaalkop Dam source water

In the PCA ordination (Figure 5.20), 78 % of the variance in environmental data could be explained on the first two axes (Table 5.12).

Table 5.12: Results from the PCA analysis showing the correlation of the different concentrations of coagulant chemicals with turbidity, chlorophyll-665 and total algal biomass for jar tests done with source water at sampling point M-RAW_VAALKOP for 03/11/2010 and 23/11/2010

Axes	1	2	3	4	Total variance
Eigenvalues	0.412	0.367	0.221	0	1
Cumulative percentage variance of species data	41.2	77.9	100.0	0	
Sum of all					1

Figure 5.20 indicates that when poly-electrolyte (alone) and poly-electrolyte in combination with CaO are dosed, a negative correlation was found with turbidity, indicating that coagulation, flocculation and sedimentation removed most of the particulate matter or turbidity, however, there was an insufficient removal of chlorophyll-665 and algae present in the source water when only polyelectrolyte and poly-electrolyte in combination with CaO were dosed. The CaO in combination with activated silica showed a negative correlation with chlorophyll-665 and total algal biomass, indicating that this procedure removed most of the algal material although turbidity remained high. The CaO in combination with activated silica contributed to the turbidity because of the presence of residual lime in the supernatant after coagulation and flocculation (Freese *et al*, 2004).

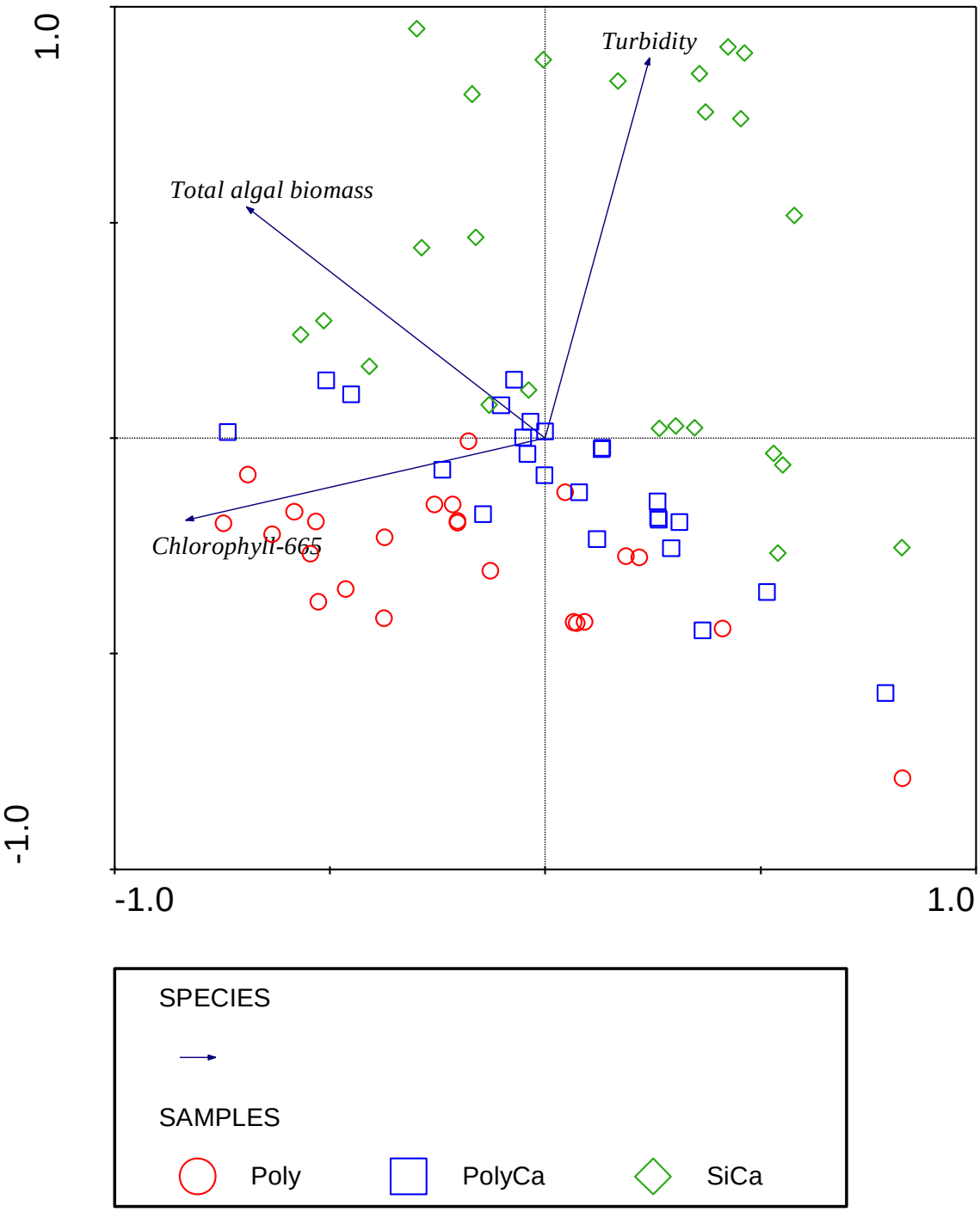


Figure 5.20: Bi-plot PCA ordination diagram showing the correlation of all jar test treatments with turbidity, chlorophyll-665 and total algal biomass for the different coagulant chemical treatments measured at M-RAW_VAALKOP for 03/11/2010 and 23/11/2010 (LEGEND: poly-electrolyte (represented by a red circle (Poly)), a combination of poly-electrolyte and CaO (represented by a blue square (PolyCa)) and activated silica in combination with CaO (represented by a green diamond (SiCa)).

5.2.4. Chlorine exposure experiments

Since *C. hirundinella* has unique characteristics that prevent its removal by conventional water treatment processes, it was imperative to investigate the effect of chlorine on high concentrations of this motile dinoflagellate in the source water, for the purpose of pre- or intermediate chlorination with the aim to render the cells immobile for more effective coagulation. Steynberg *et al* (1994), found an improvement (85 – 95 % removal) of the sedimentation and filtration stage when motile algae such as *Chlamydomonas* sp. or *Euglena* sp. were first rendered immobile by pre-oxidation. The oxidant attaches to the algal cell, increasing the weight and thereby enhances settling (Ma and Liu, 2002a). It should be noted that no literature were found that illustrated the effect of high concentrations of chlorine specifically on *C. hirundinella.* It is important to determine the exact chlorine concentration to inactivate *Ceratium* cells and not cause them or other algal cells to lyse and release toxins or other organic compounds into the potable water. It is well known that chlorination can change the algal surface properties and inhibit the physiological activities and thereby cause it to become immobile and be more susceptible to removal by the sedimentation stage (Bernhardt and Clasen, 1991; Steynberg *et al*, 1994; Basson, 2000; Ma and Liu, 2002a). However, chlorination can also cause the release of intracellular algal material which contributes to the formation of THM's after chlorination (Steynberg *et al*, 1994; Henderson *et al,* 2008).

Source water rich with the dinoflagellate *C. hirundinella* was sampled on two occasions (03/11/2010 and 23/11/2010) from Vaalkop Dam. On the first sampling date (03/11/2010), the average *Ceratium* concentration (cells/mL) in the Vaalkop Dam source water was 1263 cells/mL. The *Ceratium* cells were exposed to different concentrations of sodium hypochlorite (NaOCl) as a chlorine source and the immobile cells were recorded (Table 5.14). As the chlorine concentration increased the concentration of cells rendered immobile also increased, revealing a strong correlation (Figure 5.21: R = 0.95) between the NaOCl concentration and immobile *Ceratium* cells. Steynberg *et al* (1994) made the same observation where increased concentrations of chlorine enhanced algae removal. Steynberg *et al* (1994) also found that increased exposure time increased the mortality of algae. One should however be advised to adjust the pre-chlorination dose as the algal composition in the source water change, since algal species differ in their sensitivity to chlorine (Sarkiskova and Skripnik, 1988). This also emphasizes the need to determine the exact concentration necessary to render *C. hirundinella* cells immobile.

At chlorine concentrations lower than 1.125 mg/L there was less than 50 % immobile *C. hirundinella* cells, indicating ineffective NaOCl dosage (Table 5.15). The 50 % effective concentration (EC_{50}) of chlorine where 50 % of *C. hirundinella* were rendered immobile was

calculated according to the equation in Table 5.13. It was found that at a chlorine concentration of 1.17 mg/L, 50 % of the *Ceratium* cells were rendered immobile. The response of *Ceratium* cells to the different chlorine concentrations are displayed in Figure 5.21, also indicating the EC_{50} value.

Table 5.13: Calculation of EC_{50} value for sampling point M-VAALKOP_RAW for 03/11/2010 and 23/11/2010

Equation	03/11/2010	23/11/2010
$y = \frac{a}{(1 + be^{-cx})}$	a = 1530.2135; b = 3073219.2; c = 12.766837 and y = 770	a = 754.53534; b = 274.59196; c = 4.9007479 and y = 369
EC_{50}	**1.17 mg/L**	**1.14 mg/L**

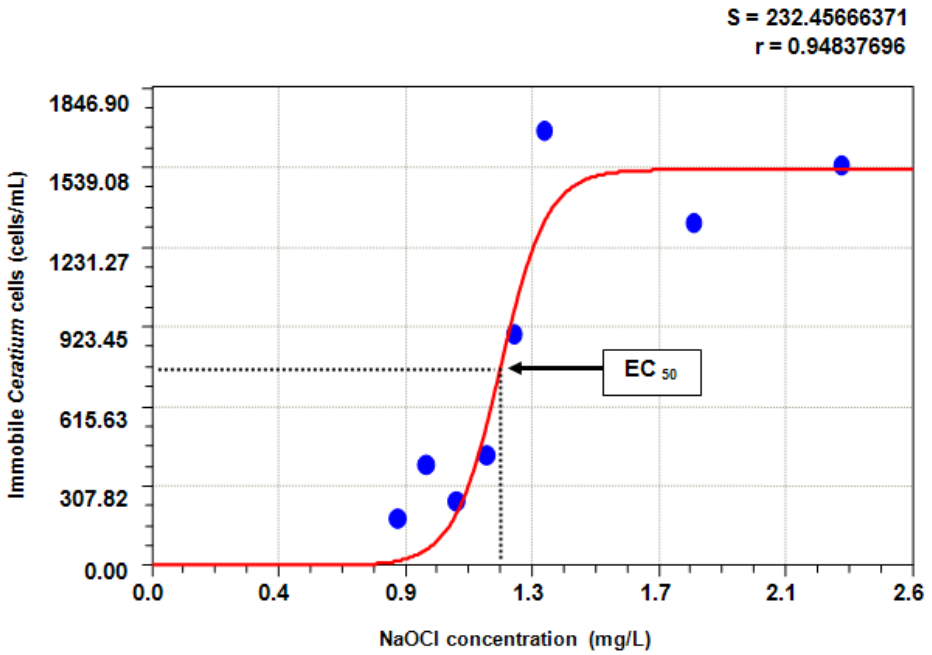
Steynberg *et al* (1994) found a strong relationship between residual chlorine, exposure time and inactivation of algae. They also found that the shorter the exposure time, the higher the residual chlorine concentration needed to reduce algae.

On the sampling date 23/11/2010, the average *Ceratium* cell counts in the Vaalkop Dam source water was 1011 cells/mL. As the chlorine concentration increased the percentage cell removal also increased (Table 5.15). At chlorine concentrations lower than 1.225 mg/L there was less than 50 % removal of *C. hirundinella*, indicating ineffective NaOCl dosage (Table 5.15). The response of *Ceratium* cells to the different concentrations of chlorine are displayed in Figure 5.22. A strong exponential correlation was found between the percentage *Ceratium* cells removed and the NaOCl concentration (Figure 5.22: R = 0.99).

For the source water sampled on two occasions from Vaalkop Dam, it was found that the effective concentration where 50 % of *Ceratium* cells were rendered immobile was at 1.17 mg/L and 1.14 mg/L respectively which may be due to a lower initial *Ceratium* concentration in the second experiment. However, it is very important to note that the over-all chlorine demand of a particular body of water (with unique water chemistry and water biology) will differ even from week to week. It is therefore of utmost importance to repeat this kind of experiments with the water that is being treated at the time of an incident as to determine the EC_{50} value of that particular water, as it will most definitely vary depending on the chlorine-demand in the water.

Table 5.14: Chlorine exposure results for sampling point M-VAALKOP_RAW for 03/11/2010

NaOCl concentration (mg/L)	Immobile *Ceratium* cells (cells/mL)
Control (source water)	**1263**
0.825	183
0.925	392
1.025	247
1.125	423
1.225	893
1.325	1679
1.825	1323
2.325	1549

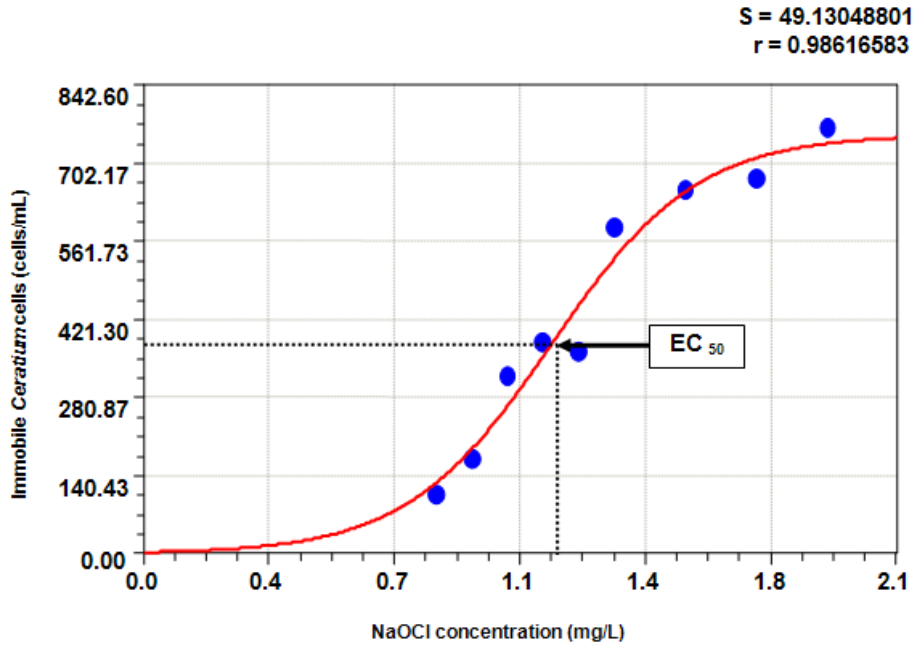


*Note: The indicated r-value is equal to the "correlation coefficient"

Figure 5.21: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for 03/11/2010.

Table 5.15: Chlorine exposure results for sampling point M-VAALKOP_RAW for 23/11/2010

NaOCl concentration (mg/L)	Immobile *Ceratium* cells (cells/mL)
Control (source water)	**1011**
0.825	107
0.925	172
1.025	321
1.125	380
1.225	362
1.325	588
1.525	654
1.725	675
1.925	766



*Note: The indicated r-value is equal to the "correlation coefficient"

Figure 5.22: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for 23/11/2010.

5.3. Assessment of Rietvlei Dam source water

Coetzee *et al* (2010), performed a redundancy analysis on historical data from 1989 to 2009, and found close correlations of *C. hirundinella* with environmental variables such as chlorophyll-*a* and PO_4. Coetzee *et al* (2010) also found that *C. hirundinella* seemed to be favoured by low pH and temperature, which is similar to what was found with multivariate analysis of Vaalkop Dam historical data.

In comparison with Vaalkop Dam (Table 5.8), many similarities favouring *C. hirundinella* can be found in Rietvlei Dam, such as low turbidity, high conductivity (sodium and chlorides especially), high sulphate concentrations and pH range between 7 and 8. These conditions seem to be common during the occurrence of *C. hirundinella* as was also found by Janse van Vuuren and Pieterse (2005a). According to Coetzee *et al* (2010), agricultural activities and livestock farmers are mostly found in the Rietvlei Dam catchment area. Table 5.16 shows the nutrients (PO_4, nitrate and nitrite) were present in low concentrations during the presence of *C. hirundinella* in the source water. Similar water quality results were found in Vaalkop Dam (Section 5.2), and also in studies done by Grigorszky *et al* (2003a). The effluent from Hartbeespoort waste water treatment works flows into the Rietvlei Dam and contributes to most of the nutrients found in this impoundment (Coetzee *et al*, 2010). *Ceratium hirundinella* reached a concentration of 297 cells/mL in the source water on 15/02/2011 and decreased to 151 cells/mL on the second sampling date (21/02/2011). Other dominant algal species found in Rietvlei Dam during the sampling period was the cyanobacteria *Microcystis* sp. and *Merismopedia minima* (Figure 5.23).

In Table 5.16, a summary of the physical, chemical and certain biological variables in the Rietvlei Dam for the sampling dates 15/02/2011 and 21/02/2011 are displayed. The source water contained high concentrations of *C. hirundinella*. Jar testing, chlorine exposure experiments, chlorophyll-665 and total algal biomass analyses were performed, as was discussed in Chapter 3.

In Figure 5.23, the concentrations of the different algae present in Rietvlei Dam during the sampling period are displayed.

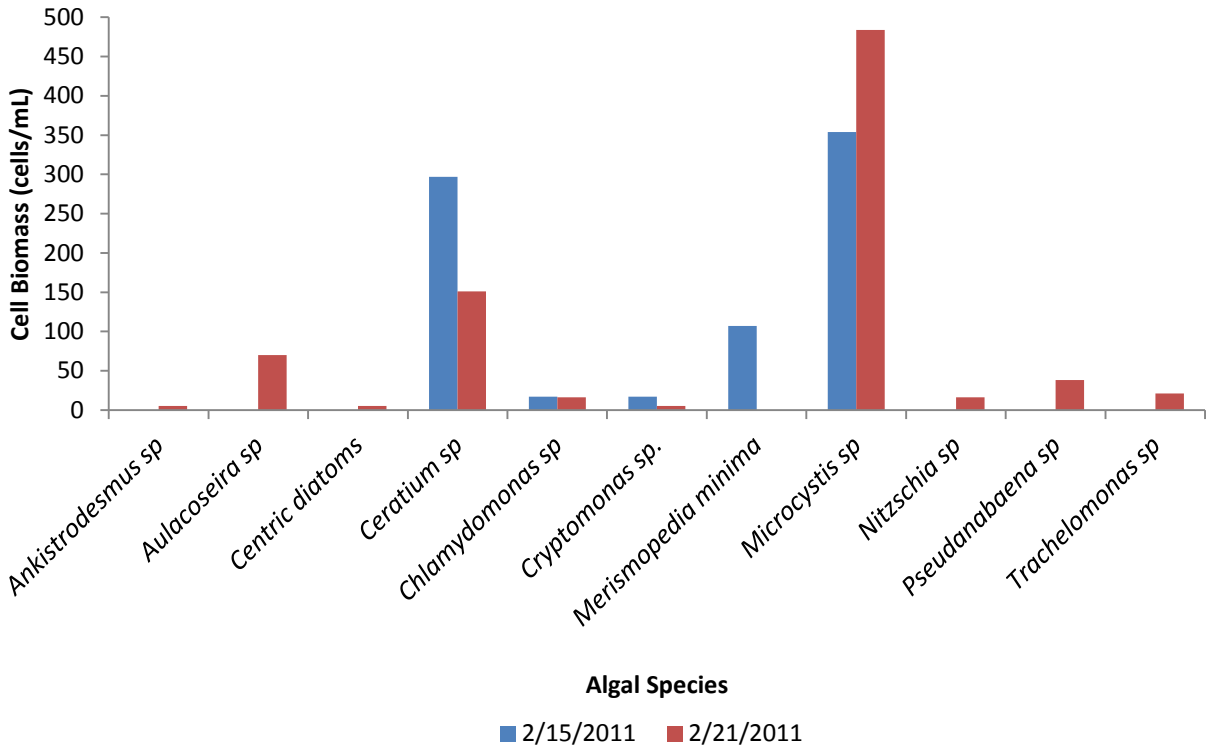


Figure 5.23: Histogram showing the algal species and concentration that occurred in the source water in Rietvlei Dam during 15th and 21st of February 2011.

Table 5.16: Physical and chemical parameters for source water sampled from Rietvlei Dam on 15/02/2011 and 21/02/2011

Date	Turbidity (NTU)	Conductivity (mS/m)	pH	Alkalinity (mg/L $CaCO_3$)	Fluoride (mg/L)	Bromide (mg/L)	Sulphate (mg/L)	Ammonia (mg/L)	Ortho Phosphate (mg/L)	Chloride (mg/L)
15/02/2011	2.9	35	8.09	105	0.2	0.25*	32	0.243*	0.2	18
21/02/2011	1.1	37	7.53	105	0.58	0.25*	32	0.34	0.24	20

Date	Total Dissolved Solids (mg/L)	Suspended solids (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Chemical oxygen demand (mg/L)	Hardness (mg/L $CaCO_3$)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Total Organic Carbon (mg/L)	Dissolved Organic Carbon (mg/L)	Sodium (mg/L)
15/02/2011	195	10*	1.5	22	100	23	10	4	7.8	8.5	21
21/02/2011	215	10*	1.8	18	115	27	12	4.8	8.7	8.3	24

Date	Cadmium (mg/L)	Chrome (mg/L)	Cobalt (mg/L)	Copper (mg/L)	Iron (mg/L)	Manganese (mg/L)	Lead (mg/L)	Nickel (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Zinc (mg/L)
15/02/2011	0.0025*	0.01*	0.015*	0.01*	0.01	0.003*	0.008*	0.015*	0.1	0.23	0.008*
21/02/2011	0.0025*	0.01*	0.015*	0.01*	0.02	0.04	0.008*	0.015*	0.22	0.1	0.008*

Date	Aluminium (mg/L)	Boron (mg/L)	Vanadium (mg/L)	Molybdenum (mg/L)	Total Silica (mg/L)	Phosphorus (mg/L)	Sulphur (mg/L)	Chlorophyll-665 (µg/L)	Silicon (mg/L)
15/02/2011	0.02	0.03	0.03*	0.01*	12	0.041*	8.8	4.77	5.7
21/02/2011	0.01*	0.05	0.03*	0.01*	14	0.17	9.2	5.6	6.4

* Method Reporting limit

5.3.1. Assessment of jar testing experiments

See sections 3.1.4. and 3.1.5 for information about sampling sites and methods. All analyses of Rietvlei Dams described in Section 5.3.1 were executed in exactly the same manner as for Forebay and M-Canal_VD described in Chapter 4. To avoid repetition the graphs of this section are captured in Appendix B.

Source water from Rietvlei Dam was sampled on the 15th of February 2011 to perform chlorine exposure experiments. *Ceratium hirundinella* was still alive in the sampled source water on the next day (16/02/2011) to perform more experiments. More source water was sampled on the 21st of February that was used to perform jar testing experiments. Another set of jar testing experiments were performed on the following day (22/02/2011) as *C. hirundinella* was still alive. The different coagulant chemicals were investigated with Rietvlei Dam source water to determine which removed high concentrations of *C. hirundinella*, and which analyses gave an indication of the appropriate dosage. The results of the jar testing experiments will be discussed in the following sections.

5.3.1.1. Jar testing with Poly-electrolyte as only coagulant chemical on source water from Rietvlei Dam

The jar testing experiment performed on the 21/02/2011, indicated that when using poly-electrolyte as only coagulant chemical, the appropriate dosage was 13 mg/L, as turbidity measured lower than 3 NTU. The turbidity varied between 2 and 4 NTU, and did not decrease significantly with an increase in coagulant chemical concentration (Table 5.17: R^2 = 0.0475). However, the chlorophyll-665 concentration initially decreased slightly and then a sudden increase was seen as the coagulant chemical concentration increased. The chlorophyll-665 concentration in this sample was found to be 4.55 µg/L at the appropriate dosage of 15 mg/L.

Table 5.17: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for Rietvlei Dam on sampling dates 21/02/2011 and 22/02/2011

Variable	Date	y-value	R^2-value
Turbidity	21/02/2011	$2.9616e^{0.0064x}$	0.0475
	22/02/2011	$2.9701e^{0.0055x}$	0.0431
Chlorophyll-665	21/02/2011	$4.8163e^{-0.004x}$	0.0062
	22/02/2011	$5.1946e^{-0.004x}$	0.0059
Total Algal Biomass	21/02/2011	-34.622x + 802.87	0.1975
	22/02/2011	-7.3392x + 418.64	0.0734

For the jar testing experiment performed on the 21/02/2011, it was found that when using poly-electrolyte, as only coagulant chemical, the coagulation and flocculation were ineffective (Table 5.17: R^2 = 0.0475). The chlorophyll-665 results indicated that using poly-electrolyte as only coagulant chemical did not remove algal material from the source water (Table 5.17: R^2 = 0.0062). The total algal biomass decreased with an increase in coagulant chemical concentration. With the second experiment there was no clear decrease in total algal biomass. The chlorophyll-665 concentration was found to fluctuate with an increase of coagulant chemical, and similar observations were found with the second experiment.

The jar testing experiment done on the 22/02/2011 indicated that the appropriate dosage was also 13 mg/L, and the chlorophyll-665 concentration in this sample was 5.24 µg/L. The results of these two experiments were very similar. The turbidity was not removed significantly (Table 5.17: R^2 = 0.0431), and the chlorophyll-665 results indicated that this coagulant option removed very little algae (Table 5.17: R^2 = 0.0059).

The jar testing experiments done on both sampling dates indicated that when using poly-electrolyte as only coagulant chemical, there was no clear trend in the removal of Dinophyceae. However, the concentration of Dinophyceae decreased overall as the coagulant chemical concentration increased (Figure 5.24). The other taxa Bacillariophyceae, Cryptophyceae and Cyanophyceae, in particular *Microcystis* sp. decreased as the coagulant chemical concentration increased (Figures 5.24 to 5.27). *Ceratium hirundinella* (taxon Dinophyceae) reached a maximum concentration of 295 cells/mL and 215 cells/mL on the respective sampling dates. The jar test experiment done on 22/02/2011 indicated that when using poly-electrolyte as only coagulant chemical, there was no clear trend in the removal of the algal taxa Chlorophyceae, Cyanophyceae and Bacillariophyceae (Figure 5.26). The biomass of the *Ceratium* cells in the source water decreased after the first sampling occasion (21/02/2011), indicating that unfavourable environmental conditions specific to *C. hirundinella* developed (an increase of TDS, hardness, Ca, Mn and P, see Table 5.16). The total algal biomass results of the supernatant indicated that the coagulation and flocculation unit processes were to some extent effective for both sampling dates (R^2 = 0.1975 for 21/02/2011 and R^2 = 0.0734 for 22/02/2011) when using poly-electrolyte as only coagulant chemical (Table 5.17).

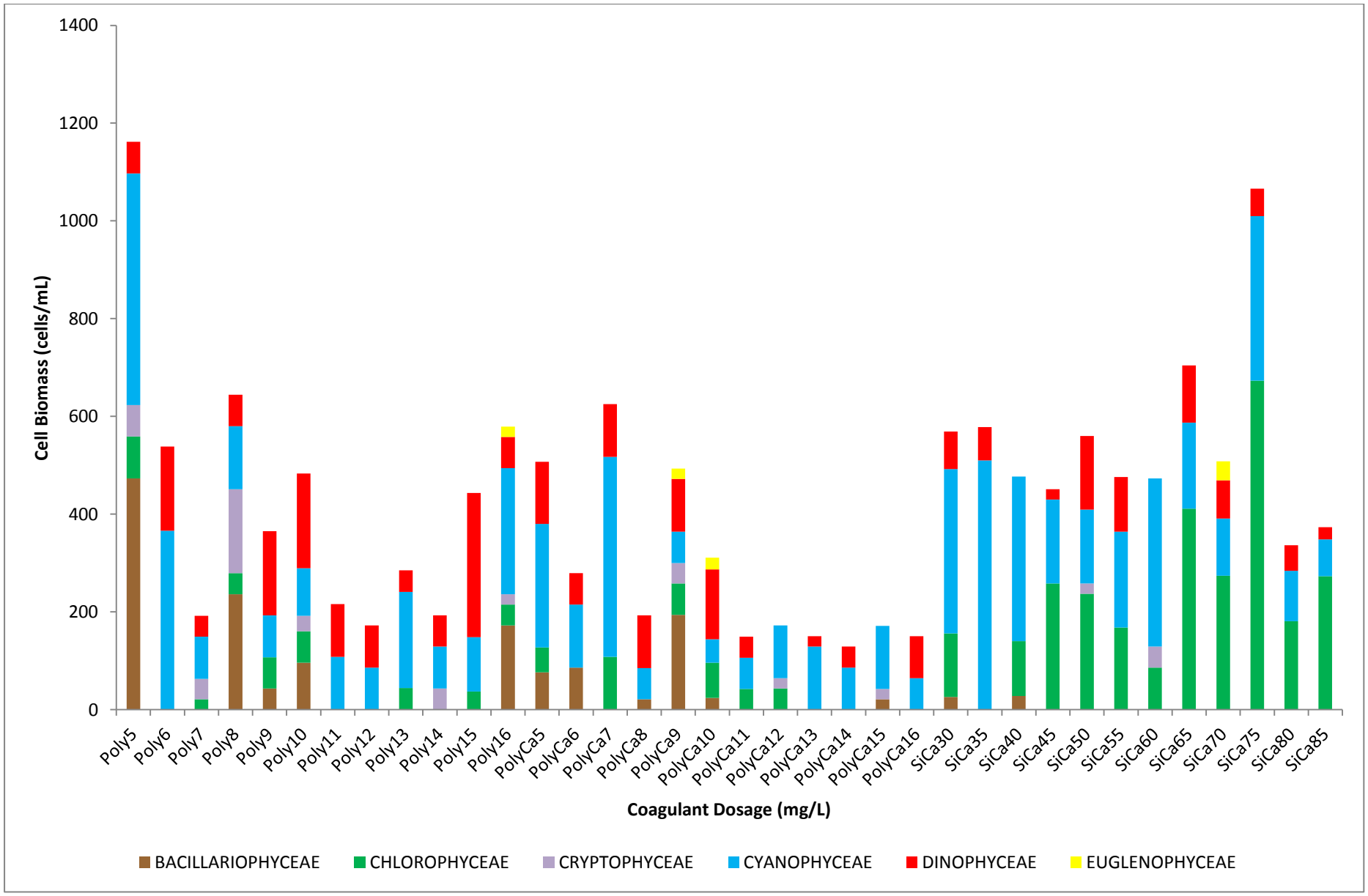


Figure 5.24: Histogram showing the major algal taxa that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on Rietvlei Dam source water for **21/02/2011**.

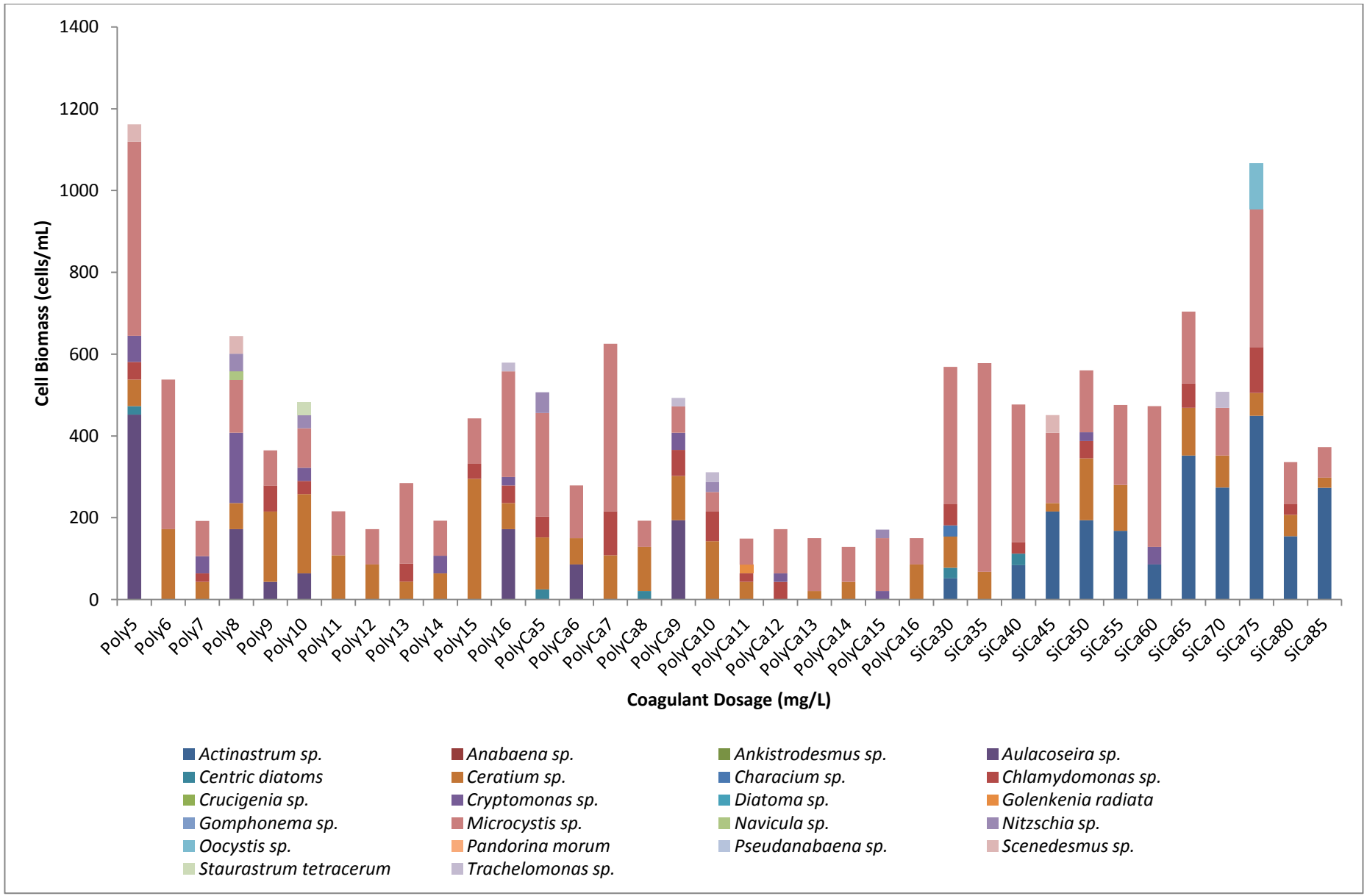


Figure 5.25: Histogram showing the algal species that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on Rietvlei Dam source water for **21/02/2011**.

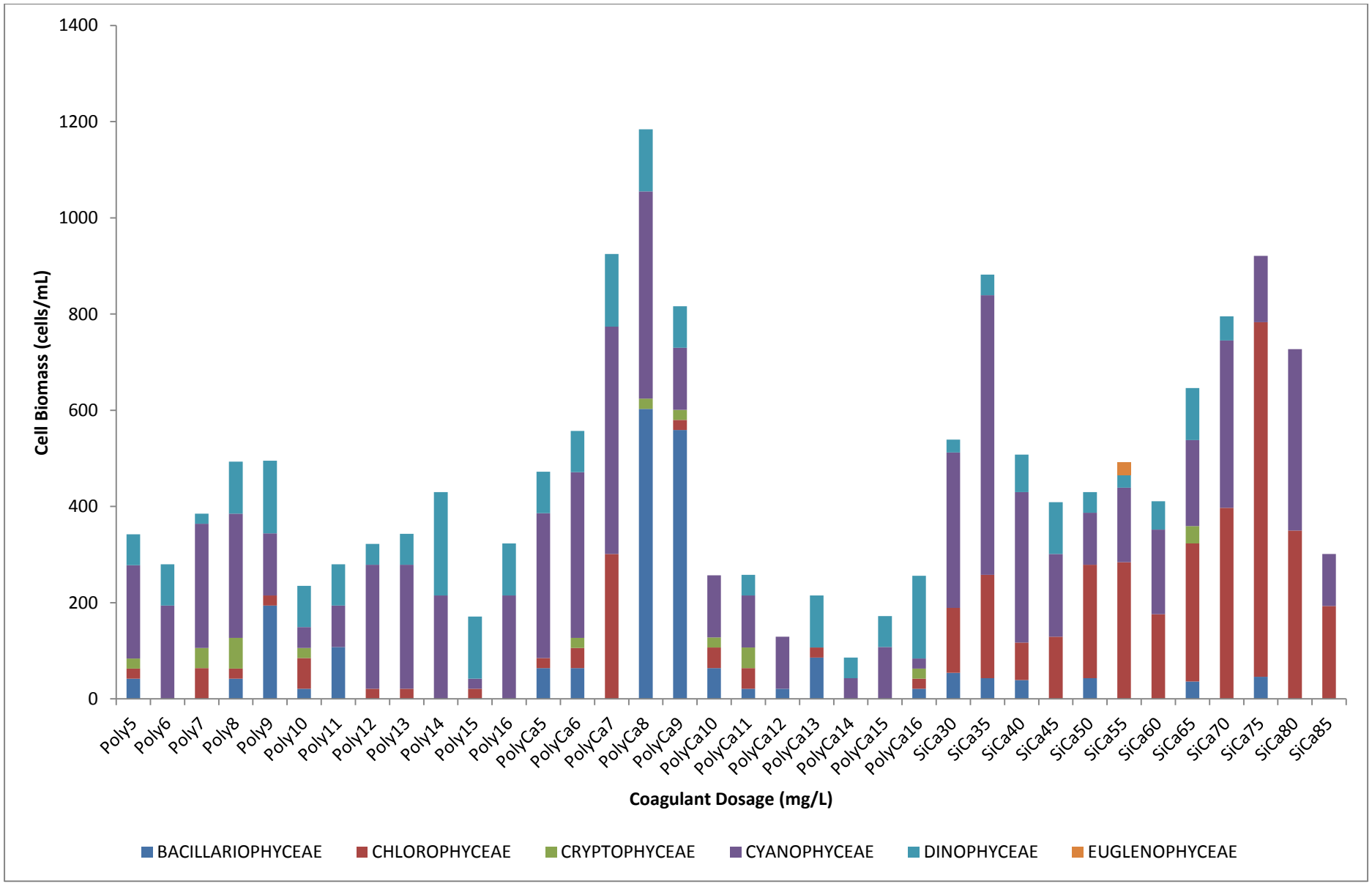


Figure 5.26: Histogram showing the major algal taxa that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on Rietvlei Dam source water for **22/02/2011**.

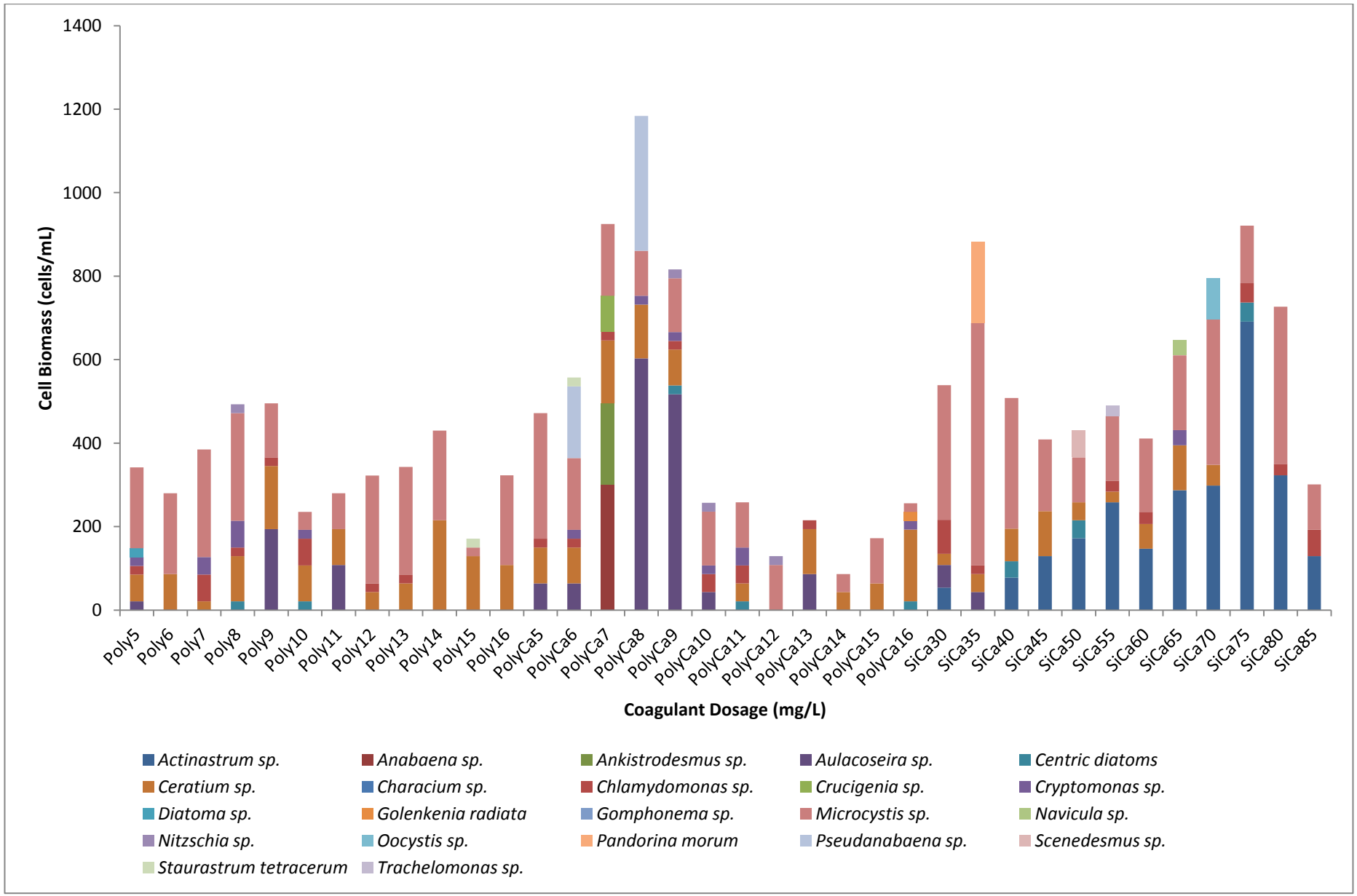


Figure 5.27: Histogram showing the algal species that were not removed by the ranging concentrations of different coagulant chemicals for each jar test procedure on Rietvlei Dam source water for **22/02/2011**.

5.3.1.2. Jar testing with poly-electrolyte in combination with CaO as coagulant chemicals on source water from Rietvlei Dam

The jar testing experiment performed on the 21/02/2011, indicated that the appropriate dosage of 15 mg/L (when considering turbidity only) was found when using poly-electrolyte in combination with 10 mg/L CaO. However, it should be noted that the turbidity value did not meet the requirements (i.e. < 3 NTU). The chlorophyll-665 concentration decreased and then a sudden increase in both chlorophyll-665 and turbidity were observed as the coagulant chemical concentration increased to the maximum dosage. The chlorophyll-665 concentration in this sample was found to be 2.56 µg/L at the appropriate dosage of 15 mg/L. The turbidity of the supernatant after jar testing, showed that the coagulation and flocculation unit processes was ineffective when using this particular coagulant option (Table 5.18: $R^2 = 0.65$). However, the chlorophyll-665 results indicated that when using poly-electrolyte in combination with CaO removed very little algal material from the source water but was still ineffective (Table 5.18: $R^2 = 0.15$).

Table 5.18: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when poly-electrolyte in combination with 10 mg/L CaO were dosed at concentrations of 5 - 16 mg/L for Rietvlei Dam on sampling dates 21/02/2011 and 22/02/2011

Variable	Date	y-value	R^2-value
Turbidity	21/02/2011	$9.0045e^{-0.049x}$	0.6504
	22/02/2011	$9.5247e^{-0.057x}$	0.6673
Chlorophyll-665	21/02/2011	$4.9697e^{-0.044x}$	0.1472
	22/02/2011	$5.3724e^{-0.05x}$	0.187
Total Algal Biomass	21/02/2011	-33.955x + 633.94	0.5125
	22/02/2011	-65.101x + 1127.5	0.4361

The jar testing experiment done on the 22/02/2011 indicated that the appropriate dosage was also 15 mg/L, and the chlorophyll-665 concentration in this sample was 2.9 µg/L. The turbidity of the supernatant after performing the jar test, indicated that the coagulation and flocculation unit processes were ineffective (Table 5.18: $R^2 = 0.67$). The chlorophyll-665 results indicated that using poly-electrolyte with CaO is ineffective in removing algal material from the source water (Table 5.18: $R^2 = 0.2$).

The jar testing experiments done on both sampling dates indicated that when using poly-electrolyte in combination with CaO as coagulant chemical, most of the algal taxa were removed (Figures 5.24 and 5.26). This decrease in algal concentration illustrates the negative effect of CaO on the algae,

creating unfavourable conditions that causes the cells to be removed sufficiently (Steynberg *et al*, 1994; Geldenhuys *et al*, 2000; Basson, 2000). The algal taxa Dinophyceae was still present as the coagulant chemical dosage increased. For example, the dinoflagellate *C. hirundinella* decreased from a maximum concentration of 143 cells/mL to less than 21 cells/mL (on the first sampling date) as the coagulant chemical dosage increased from 5 to 16 mg/L (Figure 5.25). It is evident that more particulate matter was removed with increased coagulant chemical concentrations. The total algal biomass results of the supernatant indicated that the coagulation and flocculation unit processes removed some algal material (R^2 = 0.51 for 21/02/2011 and R^2 = 0.44 for 22/02/2011) when using poly-electrolyte with CaO as chemical coagulant (Table 5.18).

5.3.1.3. Jar testing with varying concentrations of CaO in combination with 2.5 mg/L activated silica as coagulant chemicals on source water from Rietvlei Dam

A decrease in turbidity and chlorophyll-665 concentration was found with an increasing coagulant chemical concentration for both sampling dates. The jar testing experiment done on the 21/02/2011, indicated that the appropriate dosage was 80 mg/L CaO when using CaO in combination with 2.5 mg/L activated silica. The lowest turbidity value was chosen (3.41 NTU) as the appropriate dosage as the turbidity value did not fully meet the requirements (< 3 NTU). Chlorophyll-665 concentration in the supernatant after jar testing was found to be 0.56 µg/L at this concentration (which is the lowest value when compared to the results where only poly-electrolyte or poly-electrolyte in combination with CaO was used).

Table 5.19: Results of the regression analysis between turbidity, chlorophyll-665 and total algal biomass when CaO in combination with 2.5 mg/L activated silica were dosed at concentrations of 5 - 16 mg/L for Rietvlei Dam on sampling dates 21/02/2011 and 22/02/2011

Variable	Date	y-value	R^2-value
Turbidity	21/02/2011	$12.579e^{-0.014x}$	0.4187
	22/02/2011	$13.528e^{-0.015x}$	0.5143
Chlorophyll-665	21/02/2011	$7.1838e^{-0.03x}$	0.8364
	22/02/2011	$7.459e^{-0.027x}$	0.7888
Total Algal Biomass	21/02/2011	0.3517x + 527.36	0.0011
	22/02/2011	0.9622x + 533	0.0073

The jar testing experiment done on the 21/02/2011, indicated that when using CaO in combination with activated silica, the coagulation and flocculation unit processes were ineffective (Table 5.19: R^2 = 0.42). However, the chlorophyll-665 results indicated that using activated silica

with CaO (refer to Fig B.57 in Appendix B) removed some algal material with increased dosage concentration. Although it is still regarded as ineffective (Table 5.19: R^2 = 0.84), it seems to be more effective than polyelctrolyte or polyelectrolyte in combination with CaO. The jar testing experiment performed on 21/02/2011 also indicated that *Ceratium hirundinella* decreased from a maximum concentration of 151 cells/mL to less than 21 cells/mL. The dominant green algae include *Actinastrum* sp. (449 cells/mL), and the dominant cyanobacterium present in the supernatant was *Microcystis* sp. (510 cells/mL) (Figure 5.25).

For the jar testing experiment performed on the 22/02/2011, the appropriate dosage was 80 mg/L CaO when using CaO in combination with activated silica. The chlorophyll-665 concentration in the supernatant after jar testing was found to be 0.64 µg/L at this concentration. However, the turbidity of the supernatant after performing the jar test, indicated the coagulation and flocculation unit processes were ineffective (Table 5.19: R^2 = 0.51). The turbidity decreased with an increase of coagulant chemical, which is in contrast with what was found with this coagulant chemical on Vaalkop Dam source water (Section 5.2.3.3). However, the chlorophyll-665 results indicated that using CaO in combination with activated silica (refer to Fig B.59 in Appendix B) removed some algal material with increased dosage concentration. Although it is still regarded as ineffective (Table 5.19: R^2 = 0.79), it seems to be more effective than polyelctrolyte or polyelectrolyte in combination with CaO. *Ceratium hirundinella* was still present in the supernatant (a maximum of 108 cells/mL) (Figure 5.27).

The total algal biomass fluctuated with the different coagulant chemical concentrations for both sampling dates. The total algal biomass that remained in the supernatant after jar testing indicated that the coagulation and flocculation unit processes were ineffective for both sampling dates (Table 5.19: R^2 = 0.0011 for 21/02/2011 and R^2 = 0.0073 for 22/02/2011) when using CaO in combination with 2.5 mg/L activated silica as coagulant chemicals. It should be noted that the occurrence of high concentrations of small algal cells (containing little chlorophyll), in contrast to a small concentration of large algal cells (containing higher concentrations of chlorophyll) may give a scewed representation of algae removal (Figure B.61 in Appendix B), when compared to results from chlorophyll-665 analyses (Figure B.59 in Appendix B). The results from both the jar testing experiments performed indicated that when using CaO in combination with activated silica as coagulant chemical, the algal taxa Dinophyceae, Chlorophyceae and Dinophyceae were present in high concentrations in the supernatant (Figures 5.24 and 5.27).

5.3.1.2. Comparison of the appropriate dosages of all coagulant chemicals for the jar testing experiments performed on Rietvlei Dam source water

The jar testing experiments performed for both sampling dates (21/02/2011 and 22/02/2011) indicated that when using poly-electrolyte as only coagulant chemical, turbidity values were equal or less than 3 NTU with the appropriate dosages of 6 and 8 mg/L respectively (Figures 5.28 and 5.29). However, a high algal biomass and chlorophyll-665 was found to break through with the appropriate dosages on both sampling dates.

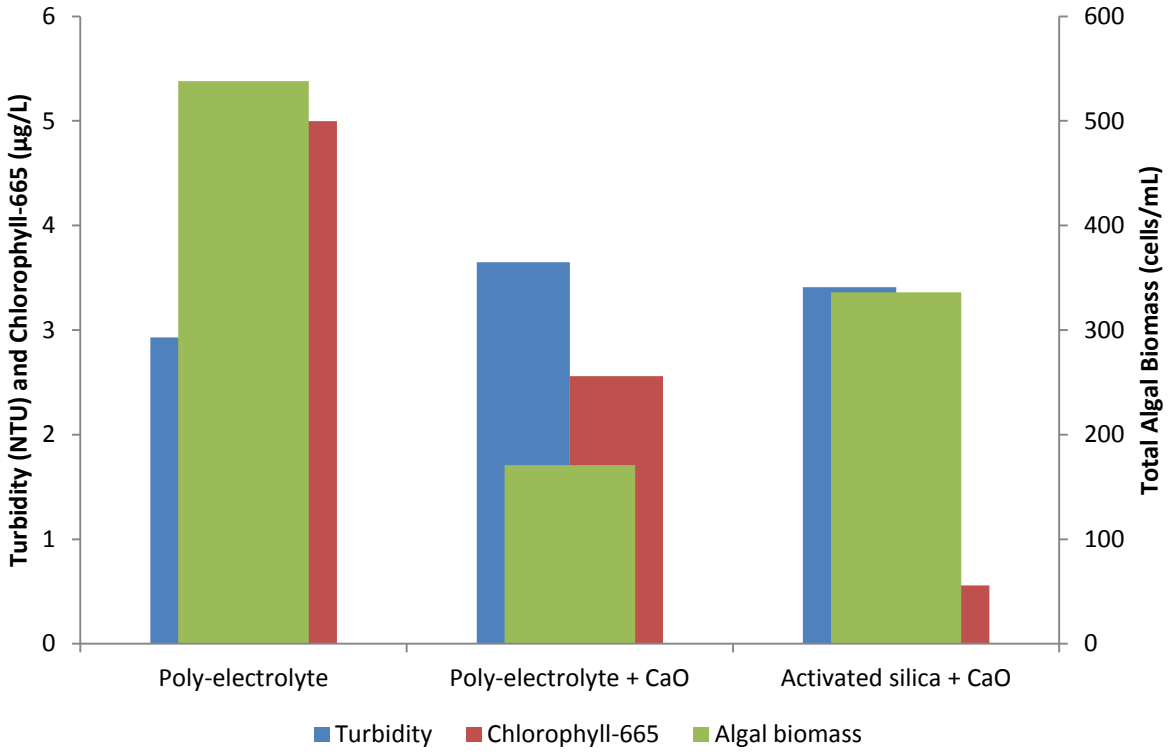


Figure 5.28: Comparison between turbidity, chlorophyll-665 and total algae biomass found in the samples where the "appropriate" concentration of coagulant was dosed in source water from Rietvlei Dam on 21/02/2011.

The chlorophyll-665 concentration decreased significantly when poly-electrolyte in combination with 10 mg/L CaO was used with the appropriate dosages of 15 mg/L in both cases (Figures 5.28 and 5.29). This was observed for both sampling dates, confirming the negative effect of CaO on algae as found by Steynberg *et al* (1994). The algal biomass also decreased significantly when CaO was used in combination with poly-electrolyte.

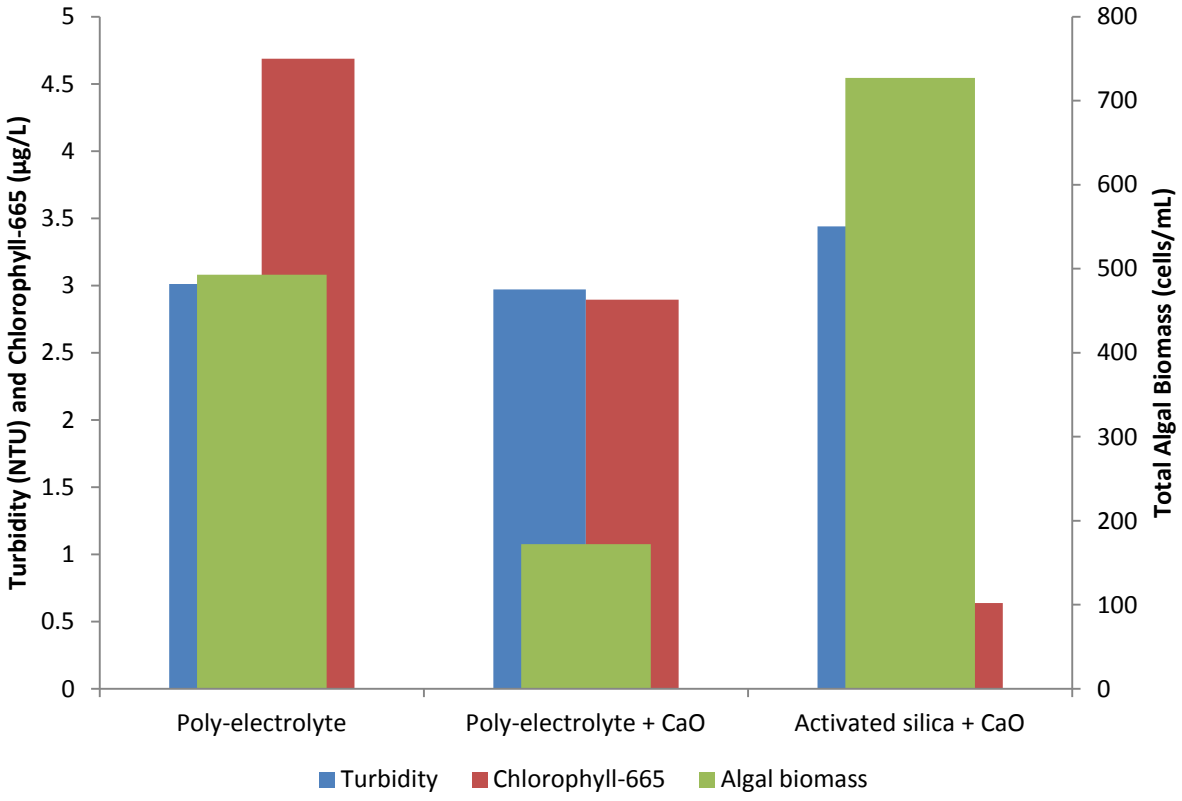


Figure 5.29: Comparison between turbidity, chlorophyll-665 and total algae biomass found in the samples where the "appropriate" concentration of coagulant was dosed in source water from Rietvlei Dam on 22/02/2011.

For the jar testing experiments performed on both sampling dates the appropriate dosage was found to be 80 mg/L CaO, when CaO in combination with 2.5 mg/L activated silica was used. Turbidity was slightly higher although there was a significant removal of chlorophyll-665 (similar to the findings with Vaalkop Dam source water). However, there were high concentrations of algae that remained in the supernatant at this appropriate dosage.

Therefore, the importance of dosing CaO as coagulant chemical aid is once again emphasized as it causes a decrease in chlorophyll-665 concentration. There were similarities with the previous jar testing experiments performed with source water from the Forebay and Vaalkop Dam. However, it should be noted that total algal biomass can give a skewed picture of algal removal, since many small cells may penetrate into the final drinking water, which will not be reflected by the total pigments (chlorophyll-665 concentration).

5.3.1.3. Multivariate analyses for jar testing experiments performed with Rietvlei Dam source water

In the PCA ordination (Figure 5.30), 75 % of the variance in environmental data could be explained on the first two axes (Table 5.20).

Table 5.20: Results from the PCA analysis showing the correlation of the different concentrations of coagulant chemicals with turbidity, chlorophyll-665 and total algal biomass for jar tests done with Rietvlei Dam source water on 21/02/2011 and 22/02/2011

Axes	1	2	3	4	Total variance
Eigenvalues	0.455	0.3	0.246	0	1
Cumulative percentage variance of species data	45.5	75.4	100	0	
Sum of all					1

Figure 5.30 indicates that when poly-electrolyte was dosed, a positive correlation was found with chlorophyll-665 concentration, indicating that this particular jar test procedure did not remove the chlorophyll-665. However, a negative correlation was found with turbidity and total algal biomass, therefore this procedure did remove the desired amount of particulate matter and algae.

When CaO was used in combination with activated silica, a positive correlation was found with turbidity, indicating that these procedures did not remove most of the particulate matter or turbidity, however, it did remove high concentrations of chlorophyll-665. Total algae biomass does not seem to correlate with any of the treatments, indicating that total algae biomass is not a good indication of algae removal. Algal biomass tended not to correlate with chlorophyll-665, since *Microcystis* sp. was one of the dominant algal species (Figure 5.23). This cyanobacterium forms a colony consisting of very small algal cells that does not contribute significantly to the chlorophyll-665 concentration in the water unless in exceptionally high concentrations. When poly-electrolyte was used in combination with CaO, a positive correlation was found with total algal biomass, meaning that this procedure did not remove the algal biomass.

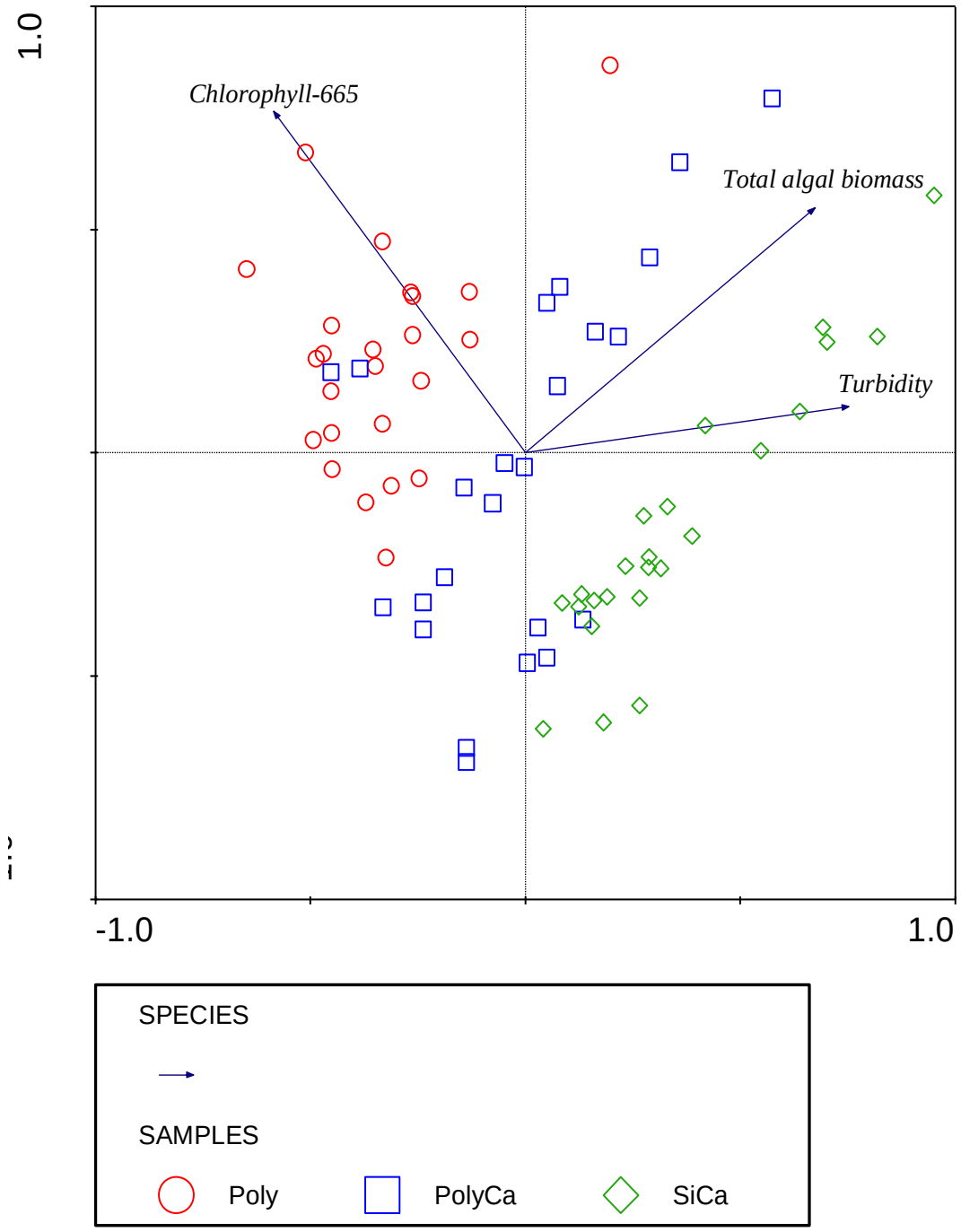


Figure 5.30: Bi-plot PCA ordination diagram showing the correlation of all jar test treatments with turbidity, chlorophyll-665 and total algal biomass for the different coagulant chemical treatments measured at Rietvlei Dam on 21/02/2011 and 22/02/2011.

5.3.2. Chlorine exposure experiments

Source water containing high concentrations of *C. hirundinella* was sampled on 15/02/2011 from Rietvlei Dam. Four replicas chlorine exposure experiments were performed to obtain an average value representing the concentration of NaOCl that could have been dosed. The average *Ceratium* concentration (cells/mL) in the Rietvlei Dam source water was 297 cells/mL (Figure 5.31). From Figure 5.31, the dominant algal species found in Rietvlei Dam during the time of sampling are illustrated. Tthe cyanobacterium *Microcystis* sp. also seems to occur in relative high concentrations on this particular sampling date. According to Janse van Vuuren and Pieterse (2005b), cyanobacteria (such as *Microcystis* sp.) and algae from the taxa Dinophyceae (such as *C. hirundinella*) occur in high concentrations during periods of low conductivity (< 50 mS/m), which was also found in this particular case study (Table 5.16).

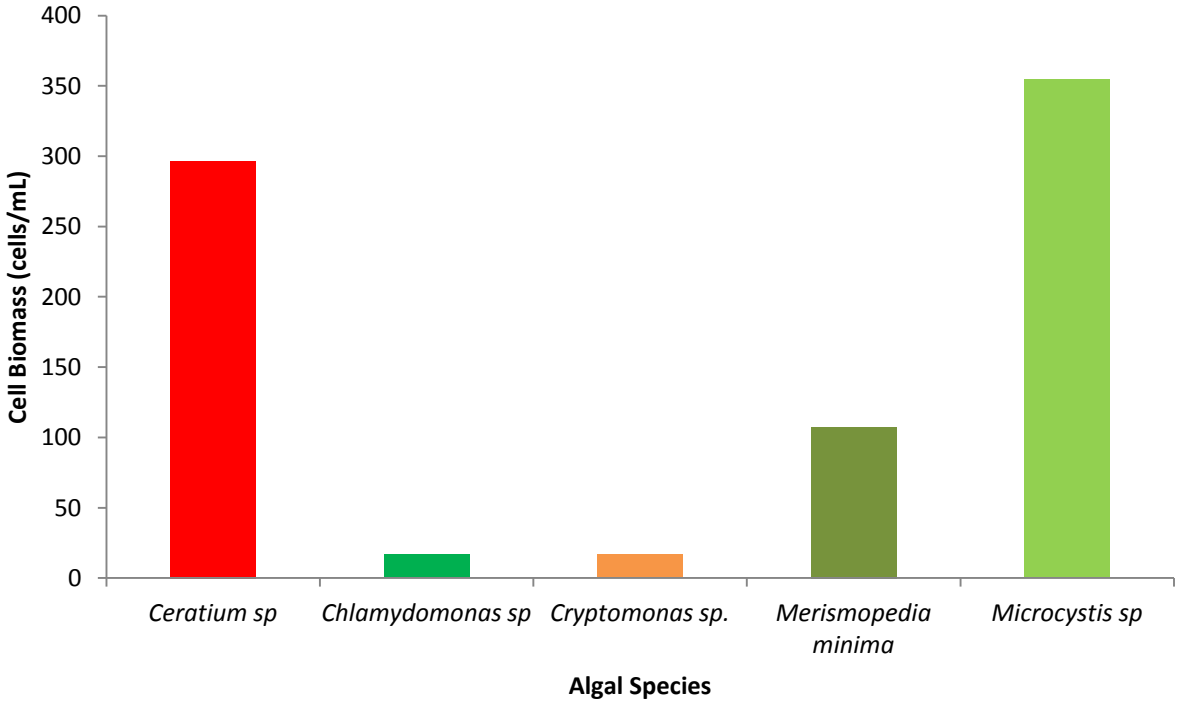


Figure 5.31: Histogram showing the algal genera and responding concentrations that occurred in Rietvlei Dam source water on 15/02/2011.

The *Ceratium* cells were exposed to different concentrations of NaOCl and the immobile cells were recorded (Table 5.22). The 50 % effective concentration (EC_{50}) of chlorine (where 50 % of *Ceratium* cells were rendered immobile) was calculated (Table 5.21) for each of the four experiments and an average value was obtained which would be the concentration NaOCl dosed to this type of source water during that particular sampling period.

The results for the four chlorine exposure experiments are shown in Table 5.22. After the source water was chlorinated the number of immobile *Ceratium* cells was recorded and chemical analyses were performed. These analyses were performed to determine the release of MIB, Geosmin (by the cyanobacteria present in the source water sample) and trihalomethanes with an increase of chlorine concentration.

Figures 5.32 to 5.35 indicate the chlorine concentration increased the concentration of cells rendered immobile also increased, revealing strong positive correlations for all four chlorine exposure experiments. From Table 5.22, an increase in chlorine concentration, the total organic carbon concentration (TOC) and MIB increased for all four experiments. There was also a slight increase of trihalomethanes ($CHCl_3$) released as the chlorine concentration increased (Table 5.22). The response of *Ceratium* cells to the different chlorine concentrations are displayed in Figures 5.32 to 5.35, also indicating the EC_{50} value.

Table 5.21: Calculation of EC_{50} value for the four chlorine exposure experiments performed on Rietvlei Dam containing high concentrations of *Ceratium* cells on the 15/02/2011

Equation	Experiment 1	Experiment 2	Experiment 3	Experiment 4

$y = \frac{a}{(1 + be^{-cx})}$	a = 317.66895;
	b = 63.336913;
	c = 4.6787647;
	y = 156
		a = 219.8252;
		b = 10.85943;
		c = 2.7281454;
		y = 102.2
			a = 281.37941;
			b = 29.961249;
			c = 3.2537276;
			y = 127.375
				a = 244.91471;
				b = 24.654299;
				c = 3.862638;
				y = 119.235

EC_{50}	**0.88 mg/L**	**0.822 mg/L**	**0.985 mg/L**	**0.815 mg/L**

The four replicates performed produced similar results: an increase of immobile *Ceratium* cells, TOC concentration, and a small release of MIB (due to relative high concentrations cyanobacteria in the source water, Figure 5.31) and $CHCl_3$ as the chlorine concentration increased. This is most probably due to chlorine oxidising the cyanobacteria cells causing them to lyse (rupture) and release the intracellular organic compounds into the water. For the source water sampled from Rietvlei Dam, it was found that the effective concentration where 50 % of *Ceratium* cells were rendered immobile (EC_{50}) was at 0.88 mg/L, 0.822 mg/L, 0.985 mg/L and 0.815 mg/L respectively. An average value was determined and it was found that pre- or intermediate chlorination at approximately 0.87 mg/L would be an effective treatment for the dinoflagellate *C. hirundinella* to be rendered immobile and thereby assisting in its coagulation process.

Table 5.22: Chlorine exposure experiment results with source water from Rietvlei Dam on 15/02/2011

NaOCl Concentration	Immobile *Ceratium* cells	MIB	Geosmin	TOC	$CHCl_3$	$CHBr_3$	$CHBrCl_2$	$CHBr_2Cl$	TTHM
mg/L	cells/mL	ng/L	ng/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Experiment 1									
0.4	75.5	<15	7.8	7.9	<0.328	<0.543	<0.558	<1.168	<1.168
0.6	97	<15	<6	7.6	<0.328	<0.543	<0.558	<1.168	<1.168
0.8	86	<15	<6	7.8	<0.328	<0.543	<0.558	<1.168	<1.168
1	150.5	15	<6	8	<0.328	<0.543	<0.558	<1.168	<1.168
1.2	331.5	18	<6	8	<0.328	<0.543	<0.558	<1.168	<1.168
1.4	340	<15	<6	8.2	<0.328	<0.543	<0.558	<1.168	<1.168
1.6	237	<15	<6	8.2	0.63	<0.543	<0.558	<1.168	<1.168
Experiment 2									
0.4	43	19	<6	7.7	0.42	<0.543	<0.58	<1.168	<1.168
0.6	118.5	<15	<6	7.7	<0.328	<0.543	<0.558	<1.168	<1.168
0.8	64.5	16	<6	7.8	<0.328	<0.543	<0.558	<1.168	<1.168
1	118.5	<15	<6	7.9	<0.328	<0.543	<0.558	<1.168	<1.168
1.2	161.5	18	<6	7.9	<0.328	<0.543	<0.558	<1.168	<1.168
1.4	194	<15	<6	8	<0.328	<0.543	<0.558	<1.168	<1.168
1.6	183	<15	<6	8	0.87	<0.543	<0.558	<1.168	<1.168
Experiment 3									
0.4	53.5	<15	<6	7.7	<0.328	<0.543	<0.558	<1.168	<1.168
0.6	53.5	<15	<6	7.9	<0.328	<0.543	<0.558	<1.168	<1.168
0.8	75	<15	<6	8	<0.328	<0.543	<0.558	<1.168	<1.168
1	118.5	<15	7.4	8	<0.328	<0.543	<0.558	<1.168	<1.168
1.2	193.5	19	<6	8.3	<0.328	<0.543	<0.558	<1.168	<1.168
1.4	214.5	<15	<6	8.4	0.48	<0.543	<0.558	<1.168	<1.168
1.6	236.5	19	<6	8.4	0.38	<0.543	<0.558	<1.168	<1.168
Experiment 4									
0.4	32	<15	7.2	7.5	<0.328	<0.543	<0.558	<1.168	<1.168
0.6	97	17	<6	8.1	<0.328	<0.543	<0.558	<1.168	<1.168
0.8	107.5	<15	<6	8.1	<0.328	<0.543	<0.558	<1.168	<1.168
1	129.5	<15	<6	8.1	<0.328	<0.543	<0.558	<1.168	<1.168
1.2	236.5	<15	<6	8.4	<0.328	<0.543	<0.558	<1.168	<1.168
1.4	214.5	<15	<6	8.4	<0.328	<0.543	<0.558	<1.168	<1.168
1.6	225.5	16	<6	8.5	0.72	<0.543	<0.558	<1.168	<1.168

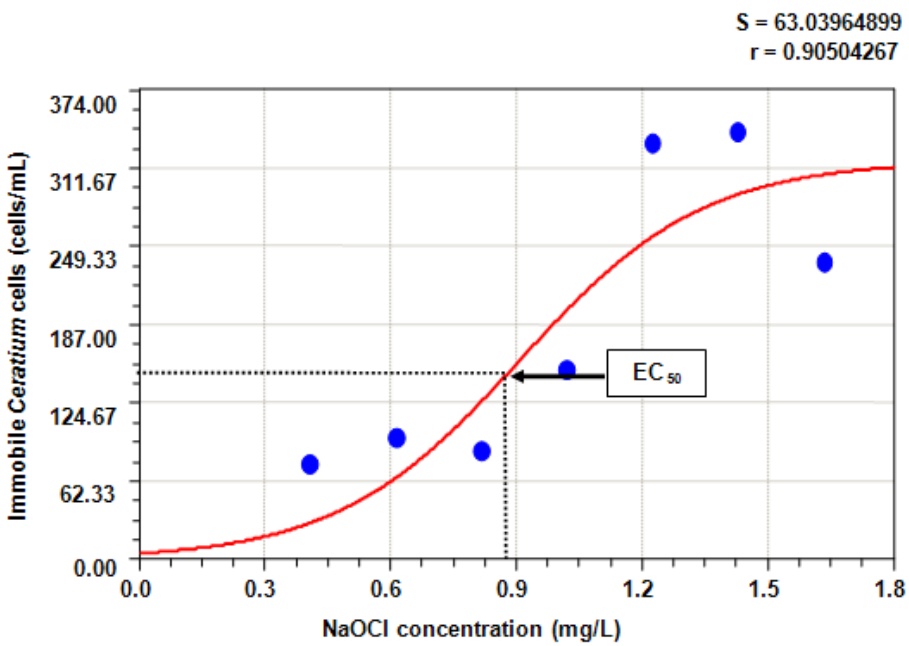


Figure 5.32: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for experiment 1 with source water from Rietvlei Dam on 15/02/2011

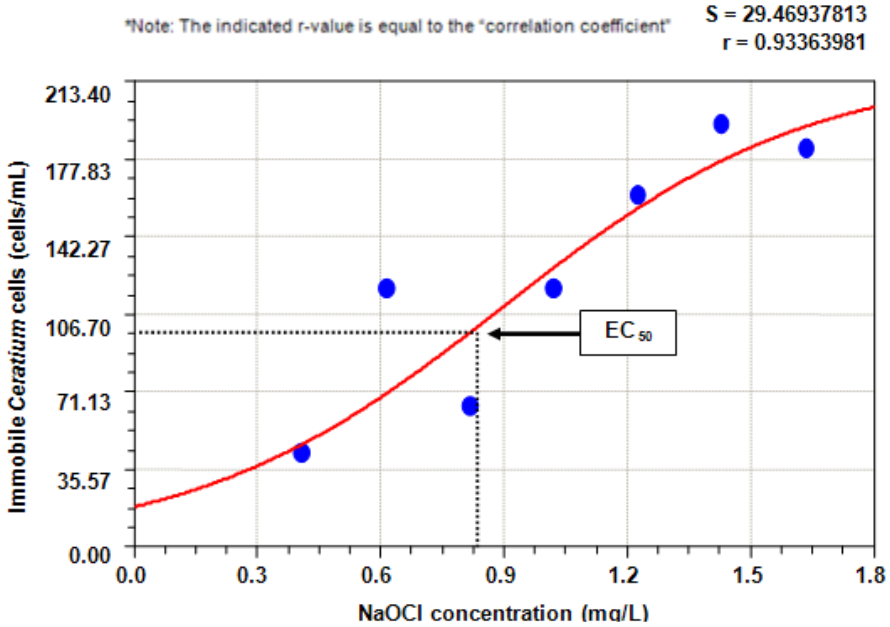


Figure 5.33: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for experiment 2 with source water from Rietvlei Dam on 15/02/2011

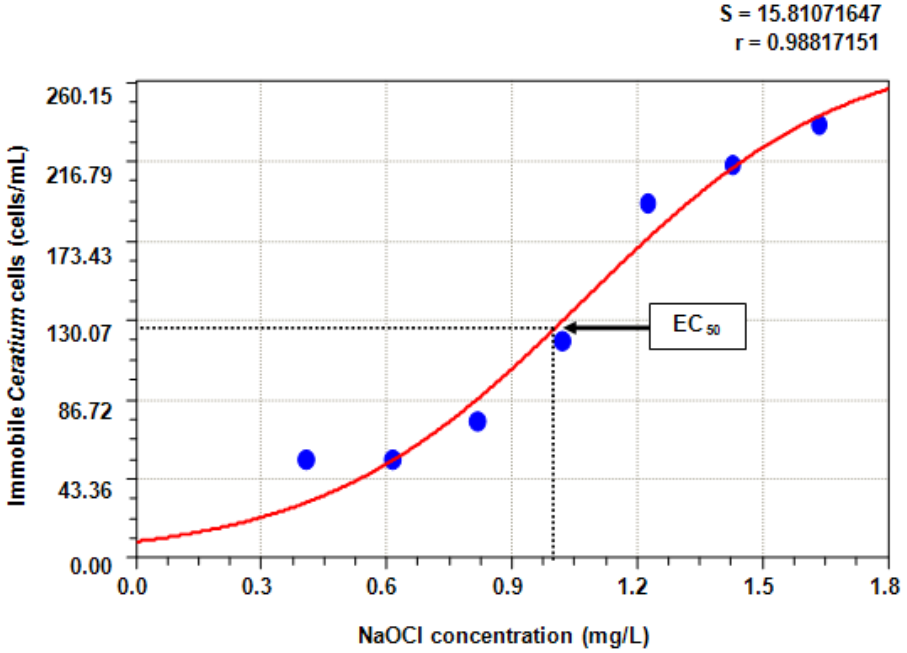


Figure 5.34: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for experiment 3 with source water from Rietvlei Dam on 15/02/2011

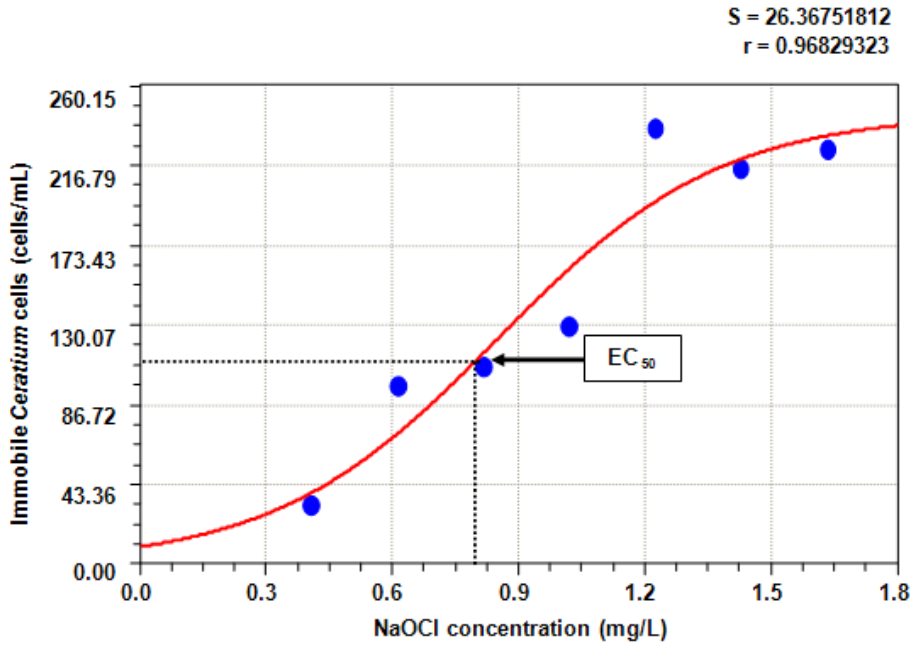


Figure 5.35: Sigmoidal correlation indicating the relationship between the NaOCl concentrations and immobile *Ceratium* cells for experiment 4 with source water from Rietvlei Dam on 15/02/2011

5.4. Conclusions on Chapter 5

The chemical, physical and biological composition of the source water of Rietvlei and Vaalkop dams differ greatly. The conductivity, hardness and the concentrations of sulphate, chloride, sodium and sulphur is much higher in Vaalkop Dam than in Rietvlei Dam. The turbidity in Forebay source water was much higher than observed at Vaalkop and Rietvlei Dams. This indicates that *C. hirundinella* might prefer source water with a low turbidity and high conductivity as it was observed in Vaalkop and Rietvlei Dam where *C. hirundinella* occur frequently. As the salinity in the Vaal River System increase, a decrease in turbidity will be found, which favour the growth of dinoflagellates such as *C. hirundinella.*

From multivariate statistical analysis with historical data of Vaalkop dam, it was found that the dinoflagellate *C. hirundinella* seem to be favoured by lower temperature and turbidity, and high DIN, Fe, M-alkalinity, Cd, PO_4, Conductivity, pH, hardness and SO_4 concentrations. The biomass of *C. hirundinella* (and cyanobacteria) has decreased in Rietvlei Dam over the study period from 2004 to beginning 2011 (Coetzee *et al*, 2010), possibly because of the installation of the SolarBees that disrupts the aquatic environment for algae to grow optimally. Coetzee *et al* (2010) performed multivariate statistical analysis with historical data of Rietvlei dam and found a close correlation of *C. hirundinella* with environmental variables such as chlorophyll-*a* and PO_4. Coetzee *et al* (2010) also found that *C. hirundinella* seemed to be favoured by low pH and temperature, which is similar to what was found with multivariate analysis of Vaalkop Dam historical data. Nutrients such as nitrates and orthophosphates were present in low concentrations during high concentrations of *C. hirundinella* in Vaalkop Dam. In this study *C. hirundinella* occurred during periods of moderate temperature, as found during spring and autumn. These occurrences of *C. hirundinella* coincided with the periods of increasing (spring) and decreasing (autumn) cyanobacteria concentrations, therefore no obvious relationship between *C. hirundinella* and cyanobacteria could be established during this study.

Jar testing experiments with Vaalkop Dam source water indicated that using the poly-electrolyte as only coagulant chemical investigated alone did not reduce the chlorophyll-665 concentrations or remove *C. hirundinella* efficiently. Poly-electrolytes are commonly used because it forms large stable flocs, and reduces turbidity to a great extent. However, poly-electrolytes do not influence the pH of water, posing a major disadvantage when removing high concentrations of algae from the source water (Bolto and Gregory, 2007). When CaO (in combination with poly-electrolyte or activated silica) was added to the Vaalkop Dam source water which revealed a significant decrease of chlorophyll-665 concentration and specifically *C. hirundinella*. However, when CaO in combination with activated silica was dosed, the residual lime in the supernatant (Freese *et al*, 2004) increased the turbidity of the Vaalkop Dam source water to such an extent that it affected coagulation negatively. Thus, high turbidity values in the supernatant were

observed. However, regardless of the turbidity values, the chlorophyll-665 concentration decreased significantly (to a greater extent than poly-electrolyte in combination with CaO) and it is recommended that the plant use chlorophyll-665 concentration as an indicator of the effectiveness of the simulated coagulation/flocculation process (Basson, 2000). Therefore, it can be concluded that poly-electrolyte in combination with CaO were the best coagulant combination to use on Vaalkop Dam source water.

Jar testing experiments with Rietvlei Dam source water indicated that when using the poly-electrolyte alone did not reduce turbidity and chlorophyll-665 concentration significantly with an increase of coagulant chemical concentration. However, the concentration of Dinophyceae cell biomass decreased overall as the coagulant chemical concentration increased. However, when 10 mg/L CaO was dosed in combination with poly-electrolyte the turbidity and chlorophyll-665 results indicated that this coagulant chemical procedure was ineffective in removing algal material from the source water. The turbidity and chlorophyll-665 concentration decreased and a sudden increase was seen as the coagulant chemical concentration increased to the maximum dosage. Most of the algal taxa were removed and illustrates the negative effect of CaO on the algae. The algal taxon Dinophyceae were still present as the coagulant chemical dosage increased and the total algal biomass remaining in the supernatant indicated that the coagulation and flocculation unit processes were still ineffective in removing the algae. When CaO in combination with activated silica was dosed a decrease in turbidity and chlorophyll-665 concentration was found with an increasing coagulant chemical concentration. The chlorophyll-665 results indicated that the use of activated silica with CaO was relatively effective in removing algal material from the source water. However, the algal taxa Dinophyceae, Chlorophyceae and Dinophyceae were still present in high concentrations. It is evident that turbidity decreased with an increase of coagulant chemical, which is in contrast with what was found with this coagulant chemical on Vaalkop Dam source water.

The varying results found in different source waters (e.g. Vaalkop, Rietvlei and Forebay) indicate that coagulant chemicals inevitably will have different results as water chemistry changes. It is therefore important to note that drinking water treatment plants should investigate the best coagulant chemical(s) for their particular source water.

The chlorine exposure experiments with Vaalkop Dam and Rietvlei Dam source water showed similar results. As the chlorine concentration increased the concentration of cells rendered immobile also increased, revealing a strong positive correlation between the NaOCl concentration and immobile *Ceratium* cells. However, it should be noted that the chlorine concentration to be dosed as part of pre- or intermediate chlorination will differ for each type of source water as the chemical and biological composition of each water body are unique and will differ with different chlorine demands. For the source water sampled on two occasions from

Vaalkop Dam, it was found that the effective concentration where 50 % of *Ceratium* cells were rendered immobile was at approximately 1.16 mg/L would therefore seem to be an effective treatment for the dinoflagellate *C. hirundinella* to be rendered immobile and thereby assisting in its coagulation process. For the source water sampled from Rietvlei Dam, it was found that the effective concentration where 50 % of *Ceratium* cells were rendered immobile was at approximately 0.87 mg/L would seem to be an effective pre- or intermediate chlorination treatment. It was evident that with an increase in chlorine concentration, the total organic carbon concentration (TOC) and MIB increased for all four chlorine exposure experiments performed on Rietvlei Dam source water. There was also a slight increase of trihalomethanes ($CHCl_3$) released as the chlorine concentration increased.

CHAPTER 6

OVERALL CONCLUSIONS

During this study environmental factors which are associated with the bloom formation of the freshwater dinoflagellate *Ceratium hirundinella* in the source water and the associated problems with high *C. hirundinella* concentrations during the production of potable water were investigated in this study. Algal-related water purification problems are very commonly found as the quality of South Africa's water sources are decreasing at a rapid rate. Algae have the potential to cause physical problems in the purification plant, e.g. physical destabilisation of the flocs formed during flocculation and clogging of sand filters. Such problems increase the water purification and plant maintenance costs. *Ceratium hirundinella* has been detected in Rand Water's catchment area since 2000, in the Vaal Dam and the upper Vaal River and this alga has been penetrating the final drinking water of Rand Water since 2006. During 2008 to 2010, *C. hirundinella* was frequently found in the final drinking water as it was not removed by the different phases of purification in Rand Water's Zuikerbosch treatment plant (Ewerts, 2010). The frequency of occurrence and concentration of *C. hirundinella* has increased significantly in the Vaal River catchment.

It was firstly determined that the *Ceratium* species in the Vaal River was indeed *Ceratium hirundinella* as proposed by Van Ginkel *et al* (2001) and therefore a source water sample was send for taxonomic analysis. The *Ceratium* species found in Rand Water's catchment was identified as *Ceratium hirundinella* (O.F. Müller) Dujardin (Class Dinophyceae, Order Peridiniales), by Phycologist, Sanet Janse van Vuuren (Ph.D) of the North-West University (refer to Appendix A). It was stipulated that *C. hirundinella* cells are normally 80 – 400 µm in length, with three straight and pointed antapical horns with closed tips; and the apical horns diverge slightly from each other and is long and narrow with a blunt tip. The cells are strongly compressed dorsiventrally. *Ceratium hirundinella* have a wide salinity tolerance and are commonly found in enriched eutrophied lakes and ponds. *Ceratium hirundinella* also produce a brown discoloration of the water during bloom formation.

Ceratium hirundinella is relatively large in cell size with theca plates and has a distinctive spiny structure that tends to avoid stable floc formation. Another morphological advantage of this alga has is the presence of flagella that gives it the ability to migrate in the water column and it is speculated that it causes a disruption of the coagulation, flocculation and sedimentation unit processes by swimming out of flocs and breaking up the already formed flocs. *Ceratium hirundinella* is also associated with many other water purification problems such as blocking of sand filters that causes reduced filter running times and algal penetration into the drinking water. *Ceratium hirundinella* can produce fishy taste and odorous compounds and contain

more chlorophyll than other smaller algae causing the drinking water to be aesthetically unacceptable to consumers.

Ten years of environmental data for the sampling point C-VRB5T was assessed, it was found that the freshwater dinoflagellate *C. hirundinella* seem to be favoured by high pH ranges, high COD, high PO_4, and high silica concentrations, as well as low turbidity and low nitrogen (DIN) concentrations. To accurately predict the occurrence of *C. hirundinella* in the Vaal River environmental data from other sampling points within the catchment should also be used.

Multivariate statistical analysis was also performed with nearly eight years of historical data for Vaalkop dam (sampling point M-RAW_VAALKOP), and it was found that the dinoflagellate *C. hirundinella* seem to be favoured by low temperature and turbidity, associated cyanobacteria, and high DIN, Fe, M-alkalinity, Cd, PO_4, Conductivity, pH, hardness and SO_4 concentrations. The biomass of *C. hirundinella* (and cyanobacteria) has decreased in Vaalkop Dam over the study period from 2004 to beginning 2011. Source water sampled from Vaalkop Dam during a *C. hirundinella* bloom; the source water had a high conductivity, low turbidity and low concentrations of nutrients which is similar with what was found from the analysis of Rietvlei Dam data, as well as previous studies (Grigorszky *et al*, 2003a; Janse van Vuuren and Pieterse, 2005a). However, some discrepancies with DIN are found in literature, e.g. Van Ginkel *et al* (2001) found *C. hirundinella* in source water containing high concentrations of nutrients (DIN). No correlation was found between *C. hirundinella* and temperature. This non-correlation of *C. hirundinella* with extreme high or extreme low temperatures may indicate that *C. hirundinella* most probably occur during moderate temperature conditions, e.g. during spring and autumn, as was found during this study. Similar findings have been found by Van Ginkel *et al* (2001) and Hart and Wragg (2009). However, results from multivariate analysis with Vaalkop Dam data and Rietvlei Dam data (Coetzee *et* al, 2010) indicated that *C. hirundinella* favours water with lower temperature conditions (11 - 24°C).

Coetzee *et al* (2010) performed multivariate statistical analysis with historical data of Rietvlei Dam, the authors found a close correlation of *C. hirundinella* with environmental variables such as chlorophyll-*a* and PO_4. The authors also found that *C. hirundinella* seemed to be favoured by lower temperature, which is similar to what was found with multivariate analysis of Vaalkop Dam historical data. It is unclear whether a correlation exists between *C. hirundinella* and cyanobacteria, since a reduction in *C. hirundinella* concentrations were found from sampling point C-VRB5T, but *C. hirundinella* occurred in high concentrations with *Microcystis* sp. in Rietvlei Dam and as found in many other studies by Nichols *et al* (1980) and Van Ginkel *et al* (2001).

The source water (e.g. Vaalkop, Rietvlei and Forebay) used for the jar testing experiments differed chemically and biologically. Forebay source water had high turbidity, low conductivity

and TDS, low concentrations of nutrients, and low concentrations of specifically chlorides and sulphates. The chemical composition of M-CANAL_VD source water (upstream from the Forebay) was found to be very similar to the Forebay source water. Source water from Vaalkop Dam (M-RAW_VAALKOP) had low turbidity and nutrient concentrations (PO_4, nitrate and nitrite), high conductivity, high TDS and high concentrations of sulphates and chlorides. The algal concentration and diversity were very low in the Forebay source water in comparison with Vaalkop Dam source water. Rietvlei Dam has many similarities with Vaalkop Dam, such as low turbidity, high conductivity (sodium and chlorides especially), high sulphate concentrations and pH range between 7 and 8. In Rietvlei Dam the nutrient concentrations (PO_4, nitrate and nitrite) were present in low concentrations during the presence of *C. hirundinella.*

The source water from sampling point M-FOREBAY has been studied during April to October 2010, and very low concentrations of *C. hirundinella* was found. However, high concentrations of the algal taxa Cyanophyceae and Chlorophyceae were found during warmer conditions and had a negative correlation with conductivity and DIN. The cyanobacterium *Anabaena* sp. reached high concentrations during lower water temperature, low pH and high PO_4. High concentrations of algal taxa such as Bacillariophyceae and Cryptophyceae were found during lower temperature conditions. The diatoms reached high concentrations during lower water temperature, low pH, low DOC, high conductivity and high turbidity. The dominant algae found in the Forebay source water (M-FOREBAY, April to October 2010) were the cyanobacteria *Anabaena* sp. and *Microcystis* sp., the green alga *Chlamydomonas* sp. and the Cryptophyte *Cryptomonas* sp., that were still present in the supernatant in high concentrations after performing jar testing experiments regardless of the coagulant chemical or concentration. Cyanobacteria species such as *Microcystis* sp. and *Anabaena* sp. are very small algal cells found in colonies that contain mucilage that may render it less susceptible to sedimentation. The species *Chlamydomonas* sp. and *Cryptomonas* sp. avoided flocculation due to their motility. The species that were removed efficiently from the Forebay source water were diatoms and some of the other green algae such as *Scenedesmus* sp. and *Coelastrum* sp. Source water from Vaalkop Dam (M-RAW_VAALKOP, November 2010) had much higher concentrations and diverse algal species; the dominant species during that time include the *Ceratium hirundinella*, *Peridinium pernardiforme*, *Chlamydomonas* sp., *Pediastrum duplex*, *Scenedesmus* sp., *Sphaerocystis* sp., *Merismopedia minima*, *Aulacoseira* sp., *Anabaena* sp., *Microcystis* sp., *Cryptomonas* sp. and diatoms. Species such as *Ceratium hirundinella, Aulacoseira* sp., *Cryptomonas* sp., *Chlamydomonas* sp., *Microcystis* sp. and *Sphaerocystis* sp. were not efficiently removed after performing jar testing experiments. However, algae such as *Scenedesmus* sp., *Pediastrum duplex* and the diatoms were successfully removed, which could be due to its size or presence of spines. Rietvlei Dam was dominated by *Ceratium hirundinella, Merismopedia minima* and *Microcystis* sp. during the sampling period.

It is important to note that drinking water treatment plants should investigate the best coagulant chemical(s) for their particular source water. In order to optimise treatment processes and resolve problems associated with high *C. hirundinella* concentrations during the production of potable water, jar testing experiments were performed to investigate three different coagulant chemicals namely poly-electrolyte, poly-electrolyte in combination with 10 mg/L CaO and different concentrations of CaO with 2.5 mg/L activated silica. The jar testing experiments using the poly-electrolyte alone or poly-electrolyte in combination with CaO on M-FOREBAY and M-CANAL_VD source water, showed a decrease of turbidity, chlorophyll-665 concentration, and total algal biomass, with an increase in coagulant chemical concentration. When the poly-electrolyte alone was used on Vaalkop Dam and Rietvlei Dam source water, very small flocs were formed and the chlorophyll-665 concentrations did not decrease to such a great extent as with M-FOREBAY and M-CANAL_VD source water. When poly-electrolyte as only coagulant chemical was used on Vaalkop and Rietvlei Dam source water it did not remove high concentrations of *C. hirundinella* efficiently. This was due to the chemical and physical characteristics of the source water, especially turbidity and conductivity. It was found that poly-electrolytes are successful coagulants in water with a high turbidity, such as M-FOREBAY and M-CANAL_VD source water in contrast with Vaalkop Dam source water (M-RAW_VAALKOP) with a very low turbidity. Poly-electrolytes do not influence the pH of water, and it is known that algae are relatively sensitive to pH changes and therefore, when raising the pH with CaO, it "shocks or stresses" the algae (in this case of *C. hirundinella*), rendering it immobile by detaching the flagellae and thereby enhancing the coagulation and flocculation processes (Steynberg *et al*, 1994). The relatively weak removal of *C. hirundinella* when poly-electrolyte was used as the only coagulant chemical indicates that *C. hirundinella*, when not inactivated by high pH (e.g. when lime is use as coagulant chemical) is able to interfere with the coagulation, flocculation unit processes. It is speculated that this is most probably because of its large cell size and ability to swim out of flocs and thereby breaking up the flocs.

However, when CaO (in combination with poly-electrolyte or activated silica) was added to the Vaalkop Dam source water a significant decrease of chlorophyll-665 concentration and *C. hirundinella* were found. When CaO in combination with poly-electrolyte was dosed to Rietvlei Dam source water, the turbidity and chlorophyll-665 results indicated that this procedure was ineffective in removing algal material from the source water, as algal taxa Dinophyceae were still present in high concentrations as the coagulant chemical dosage increased.

When CaO in combination with activated silica was used on M-FOREBAY, M-CANAL_VD, Vaalkop Dam source water, it was found that the residual turbidity of the lime increased the turbidity of the water to such an extent that it affected coagulation negatively, resulting in high turbidity values in the supernatant. Regardless of the turbidity values, the chlorophyll-665 concentration and *C. hirundinella* biomass however decreased significantly (in a greater extent

than poly-electrolyte in combination with CaO). Jar testing experiments with source water from sampling point M-CANAL_VD were done to investigate the reasons behind the high turbidity in the supernatant when CaO in combination with activated silica was used. It was suspected that the recovered water released from Panfontein was responsible for this finding, due to the chemical composition of the source water such as high alkalinity and high turbidity. After jar testing with source water from M-CANAL_VD, it seems that the recovered water released from Panfontein did not have an influence on the results of jar testing experiment with Forebay source water when CaO in combination with activated silica was used, as was previously suspected, since the results were practically identical when compared to the results from Forebay source water. It is known that activated silica is a successful coagulant in source water with low turbidity, however, the effect of activated silica on source water with high turbidity, should be investigated. When CaO in combination with activated silica were dosed to Rietvlei Dam source water, a decrease in turbidity (dissimilarity with Vaalkop Dam results) and chlorophyll-665 concentration was found with an increasing coagulant chemical concentration. Dominant algae remaining in the supernatant included *Actinastrum* sp. and *Microcystis* sp.

The results from the jar testing experiments indicate the negative effect that the high pH of CaO (whether dosed with poly-electrolyte or activated silica has on the physiology of the algae) have on algae, causing the death thereof and/or rendering it immobile for more effective removal during coagulation, flocculation and sedimentation. Turbidity is commonly used as an indicator to determine the optimum or appropriate dosage or to evaluate the coagulant chemical efficiency. The Rand Water plant laboratory uses turbidity only, to determine the “appropriate dosage”. The appropriate coagulant dosage as determined by the turbidity, chlorophyll-665 and the total algae biomass is usually different, due to the occurrence of small algae that are not picked up by the turbidity reading. Therefore, although turbidity is a quick and easy way of determining water clarity, it is not always the most appropriate way to determine the appropriate coagulant dosage. In the case of using CaO with activated silica, regression analysis between turbidity and the coagulant chemical CaO with activated silica did not reveal a good correlation, but chlorophyll-665 concentrations gave a very good correlation, indicating that chlorophyll-665 is a much better indication of algal removal than turbidity. It is recommended that the plant include the chlorophyll-665 concentration as an indicator of the effectiveness of the simulated coagulation/flocculation process, at least during periods of high algal blooms in the source water or the presence of problematic algae such as *Microcystis* sp.

Since *C. hirundinella* has unique characteristics that prevent its removal by conventional water treatment processes, it was imperative to investigate the effect of chlorine on high concentrations of this dinoflagellate in the source water, for the purpose of pre- or intermediate chlorination with the aim to render the cells immobile for more effective coagulation. Steynberg *et al* (1994), found an improvement (85 – 95 % removal) of the sedimentation and filtration

stage when flagellate algae were first rendered immobile by pre-oxidation. Chlorine exposure experiments were carried out to determine the effect of chlorine on the freshwater dinoflagellate *C. hirundinella*. It is important to determine the exact chlorine concentration to inactivate *Ceratium* cells and not cause them or other algal cells to lyse and release toxins or other organic compounds into the potable water. For the source water sampled on two occasions from Vaalkop Dam, it was found that the effective concentration where 50 % of *Ceratium* sp. cells were rendered immobile was at approximately 1.16 mg/L. It was found as the chlorine concentration increased the concentration of immobilised cells also increased (R = 0.95). It is evident that the chlorine concentrations for both sampling dates' differed, however, very similar findings were made regardless of the concentration *Ceratium* cells in the source water. For the source water sampled from Rietvlei Dam, it was found that the effective concentration where 50 % of *Ceratium* cells were rendered immobile was at approximately 0.87 mg/L would seem to be an effective treatment for the dinoflagellate *C. hirundinella* to be rendered immobile and thereby assisting in its coagulation process. It was evident that with an increase of chlorine exposed to the sample containing *Ceratium* sp., the total organic carbon concentration (TOC) increased, and a slight increase of MIB and trihalomethane ($CHCl_3$) was found as the chlorine concentration increased. However, it should be noted that the chlorine concentration to be dosed as part of pre- or intermediate chlorination will differ for each type of source water as the chemical and biological composition of each water body are unique. Therefore, pre- or intermediate chlorination would seem to be an effective treatment for the dinoflagellate *C. hirundinella* to be rendered immobile and thereby assisting in its coagulation process.

The objectives and aims set out to be achieved at the beginning of this study were met to a great extent. The *Ceratium* species in the Vaal River was identified as *Ceratium hirundinella* as proposed by Van Ginkel *et al* (2001). The Multivariate statistical analyses that were performed on sampling point C-VRB5T, identified the main driving forces behind the occurrence and increase of *C. hirundinella* in the source water and similarities are found in literature. The influence of high *C. hirundinella* concentrations on the water treatment process specifically the coagulation, flocculation and sedimentation unit processes indicated that CaO in combination with activated silica or poly-electrolyte gave the best results due to the pH "shock" experienced by the algae. The chlorine exposure experiments performed in this study gave an indication of the possibility of increasing the removal efficiency of *C. hirundinella* during coagulation, flocculation and sedimentation and the potential for implementing it into the Rand Water water treatment plants.

For this research project a number of recommendations regarding the 1) implementation in the water purification plant as well as 2) future research, are discussed in the next chapter.

CHAPTER 7

RECOMMENDATIONS

The following recommendations are made regarding the implementation in the water purification plant:

- Although turbidity is used as a variable in the plant laboratory to determine the most appropriate coagulant dosage, if problems with high algal concentrations in the source water are experienced, it would be advisable to also determine the chlorophyll-665 concentrations of the supernatant of the different jar tests, since it will give a better indication of algae removal.
- Investigating the possibility of implementing pre- or intermediate chlorination as an effective treatment for the dinoflagellate *C. hirundinella* and thereby improving the coagulation/flocculation processes.

Future research in the following should be considered:

- Environmental data from other sampling points within the catchment should also be used to gain more clarity on the environmental drivers associated with the occurrence of *C. hirundinella* in the Vaal River.
- Isolation and growth studies to determine growth requirements of *C. hirundinella*.
- Investigation into the application value of the rule-based HEA model developed by Van Ginkel *et al* (2007) to predict high concentrations (blooms) of dinoflagellates as biomass volume.
- Due to the expensive nature of coagulant chemicals, it is advised to investigate other coagulant chemicals (such as aluminium sulphate) that can remove high concentrations of *C. hirundinella* as an alternative coagulant for Rand Water during dinoflagellate blooms.
- Perform more chlorine exposure experiments to determine the exact effective chlorine concentrations to immobile *C. hirundinella*.
- Research alternative oxidation methods Rand Water may apply to eliminate high concentrations of *C. hirundinella* before the coagulation stage, e.g. ozone (O_3) or ultraviolet light.
- Perform tests to determine the release of organic compounds, toxins, THM's, geosmin and MIB during chlorination.
- Investigate the floc structure by means of microscopy with each chemical coagulant, to determine if the floc is compacted or porous. Thereby, investigating at which

concentration coagulant chemical the floc is most stable when high concentrations of *C. hirundinella* are present in the source water.

- Culture certain problematic algae that cause problems in water treatment and perform jar tests and pre-chlorination experiments to determine the most effective coagulant chemical and concentration.
- Investigate the zeta potential before, during and after coagulation which may give an indication of the effectiveness of different coagulant chemicals and appropriate dosages.

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APPENDIX A

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-1111/2222
Web: http://www.nwu.ac.za

Botany
Tel: (018) 299 2514
EMail Sanet.JanseVanVuuren@nwu.ac.za

Analytical Services: Operations
Rand Water
Vereeniging

18 March 2010

For attention: Rand Water Analytical Services

Identification of *Ceratium hirundinella* (O.F. Müller) Dujardin

Hereby I confirm that the *Ceratium* species present in the sample C-B9-25/01/2010 is *Ceratium hirundinella*, a common species find in freshwaters, although it may have a wide salinity tolerance. They are widely distributed in enriched eutrophic lakes and ponds where they may dominate. During bloom formation *Ceratium* often produces a brown discoloration of the water.

Cells of *Ceratium hirundinella* are generally 80-400 µm in length (cells in the sample received for identification were approximately 223 µm in length). They are strongly compressed dorsoventrally. The apical horn is long and narrow with a blunt tip. The three antapical horns are straight with pointed, closed tips. The antacpical horns are slightly diverging from each other distally.

The classification of this species can be summarised as follows (classification according to AlgaeBase: Listing the World's Algae (http://www.algaebase.org/):

Classification:
Empire Eukaryota
Kingdom Protozoa
Subkingdom Biciliata
Infrakingdom Alveolata
Phylum Myzozoa
Class Dinophyceae
Order Peridiniales
Family Ceratiaceae
Genus Ceratium

Yours sincerely

Dr. Sanet Janse van Vuuren
Phycologist

APPENDIX B

Vaalkop Dam

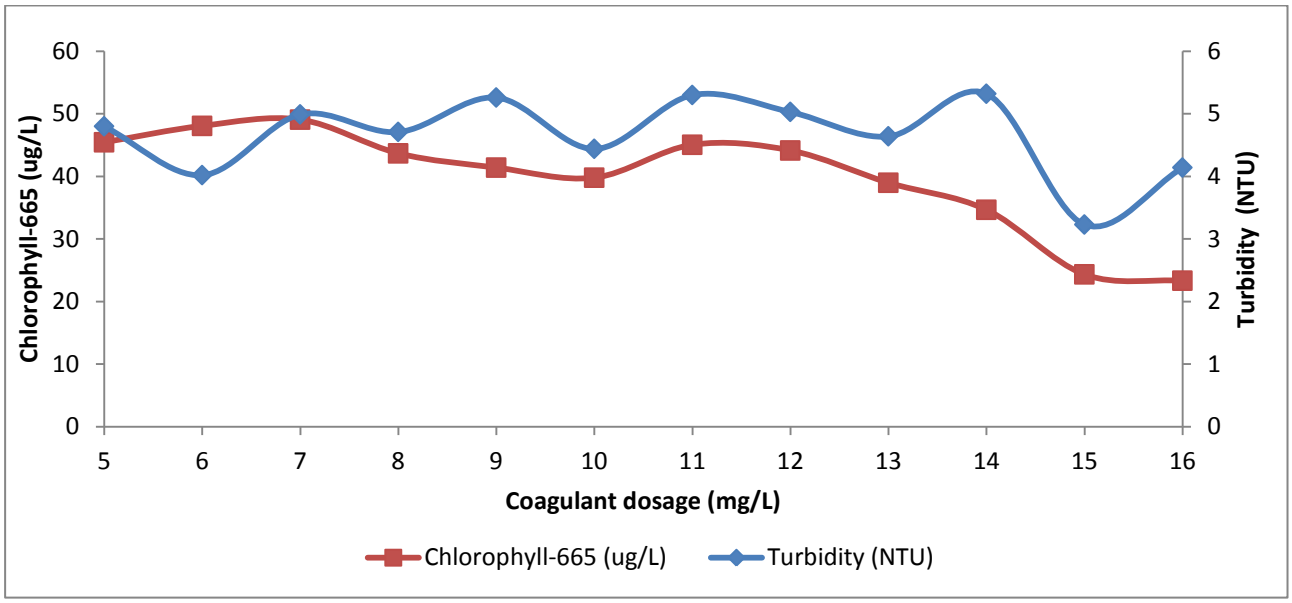

Figure B.1: The comparison between turbidity and chlorophyll-665 in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **03/11/2010**.

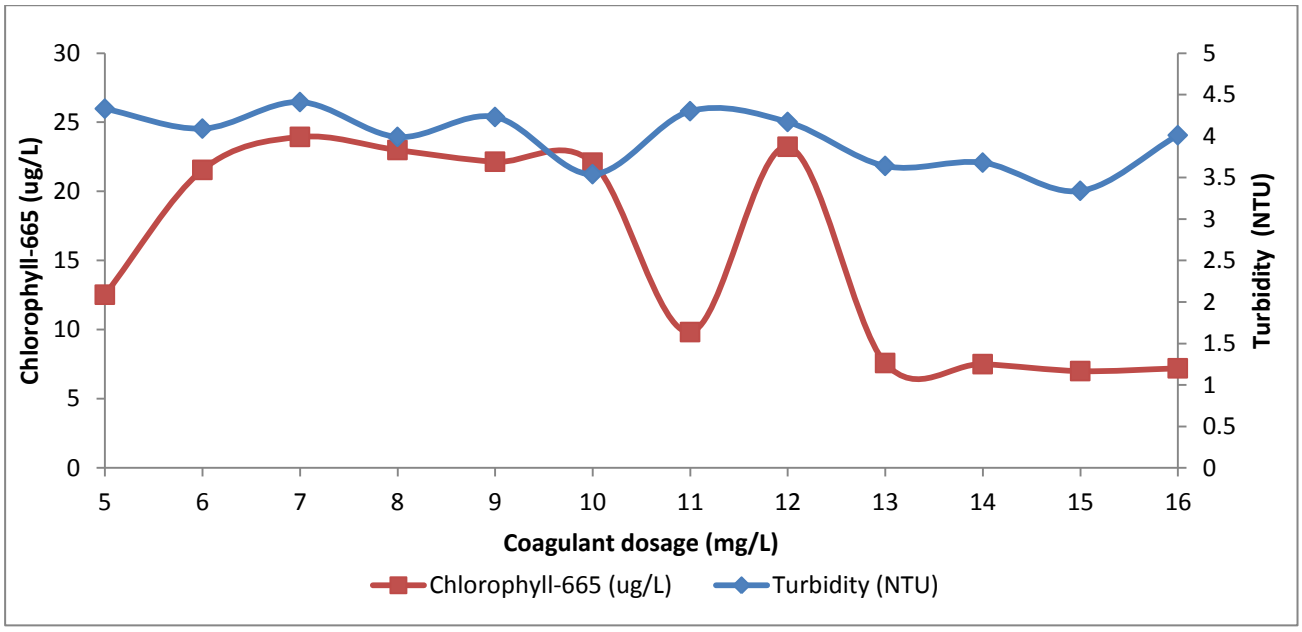

Figure B.2: The comparison between turbidity and chlorophyll-665 in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **23/11/2010**.

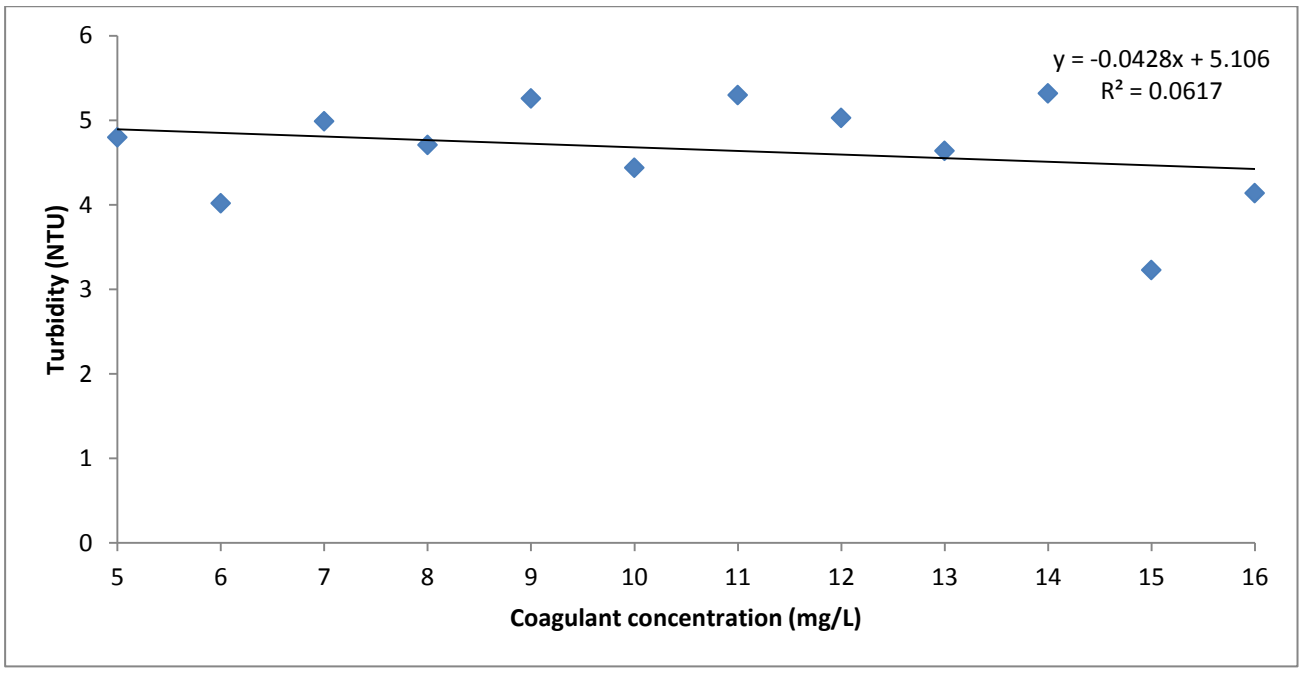

Figure B.3: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on **03/11/2010**.

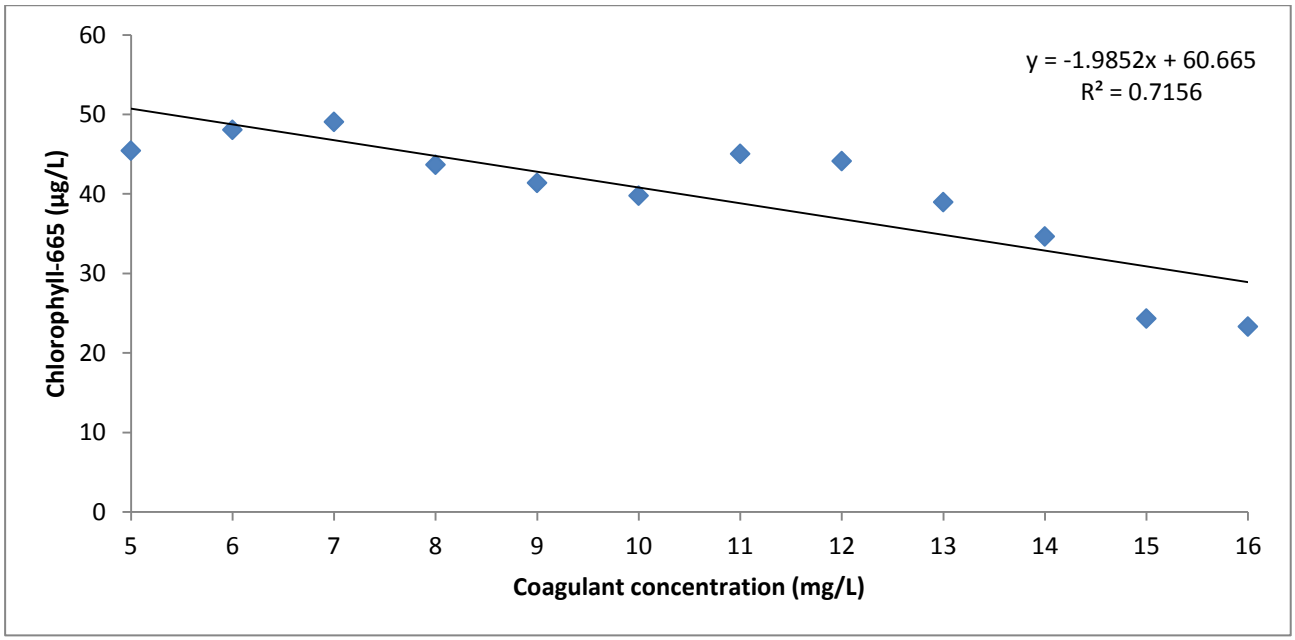

Figure B.4: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on **03/11/2010**.

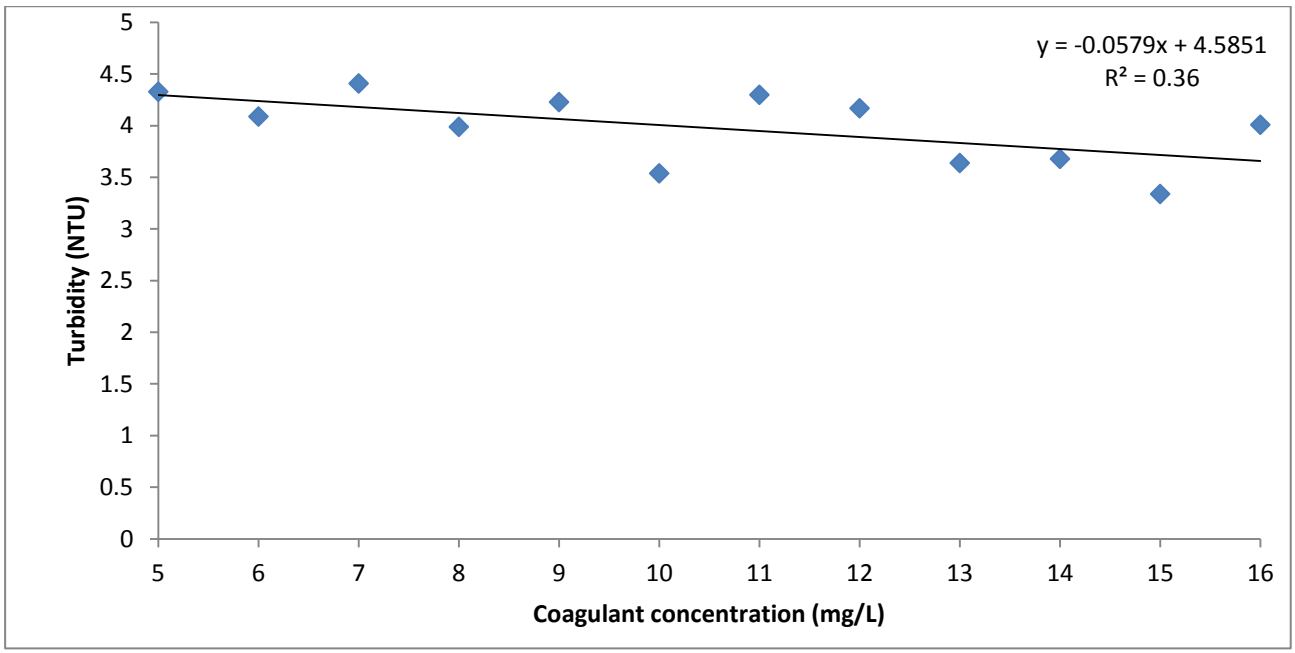

Figure B.5: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on **23/11/2010**.

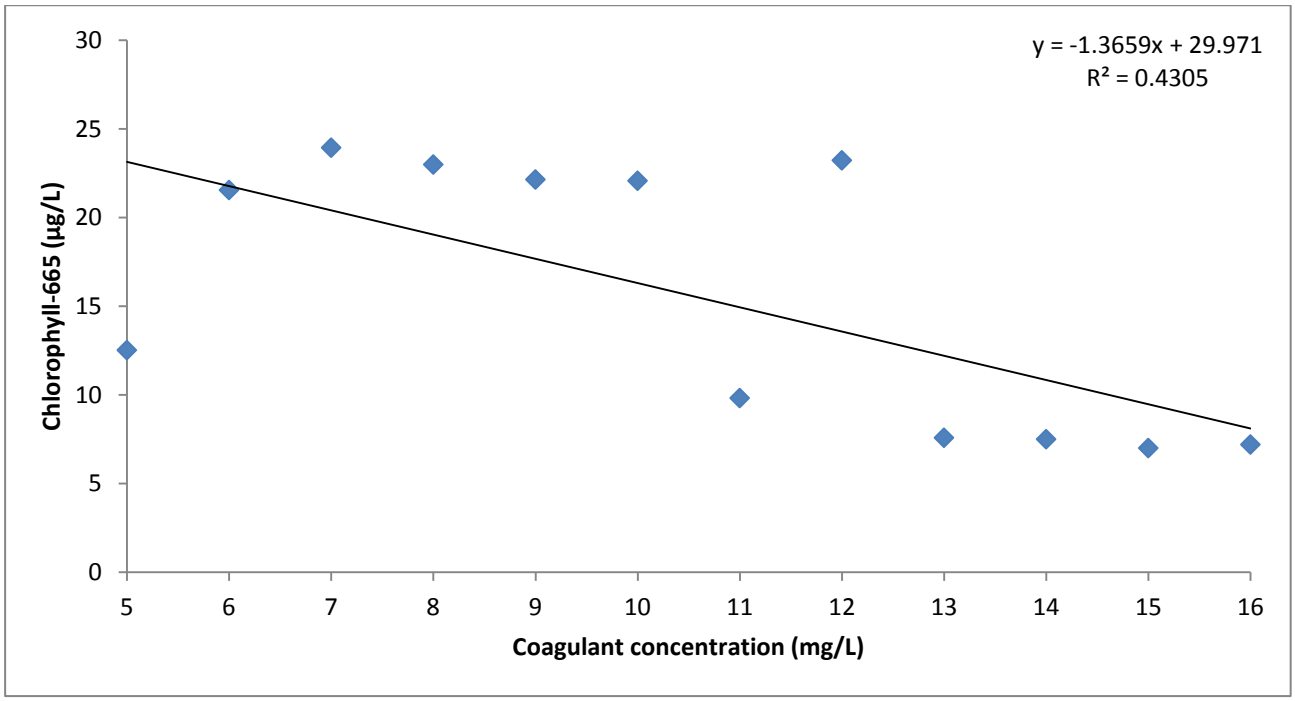

Figure B.6: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L for sampling point M-RAW_VAALKOP on **23/11/2010**.

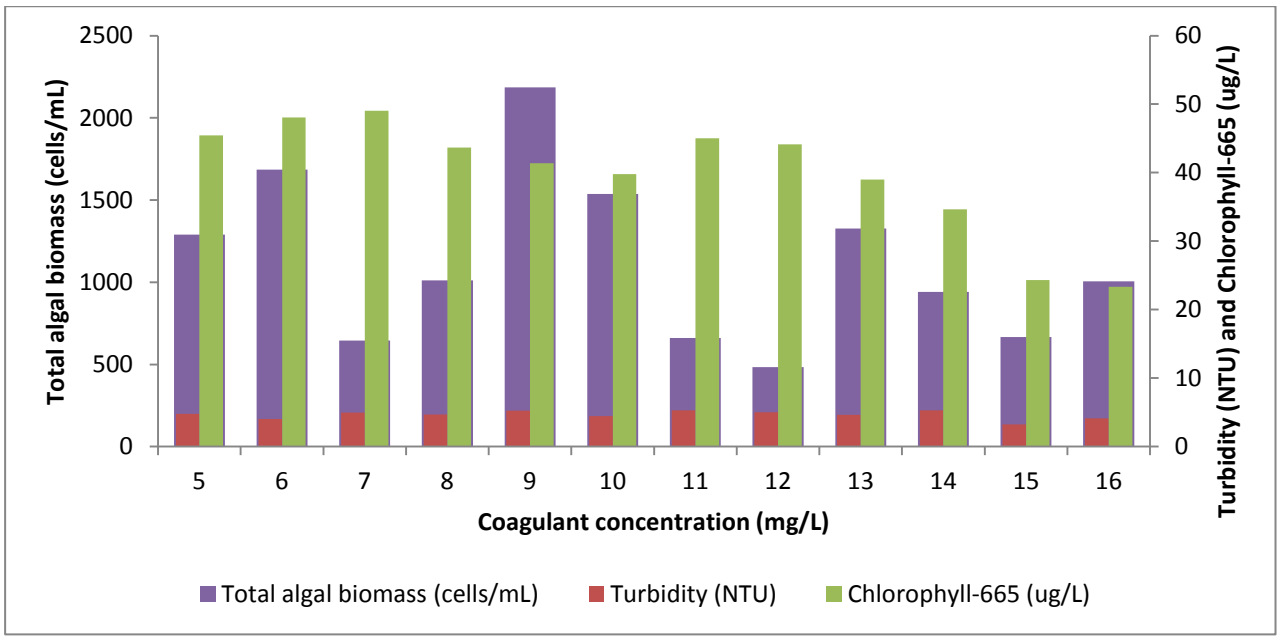

Figure B.7: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **03/11/2010**.

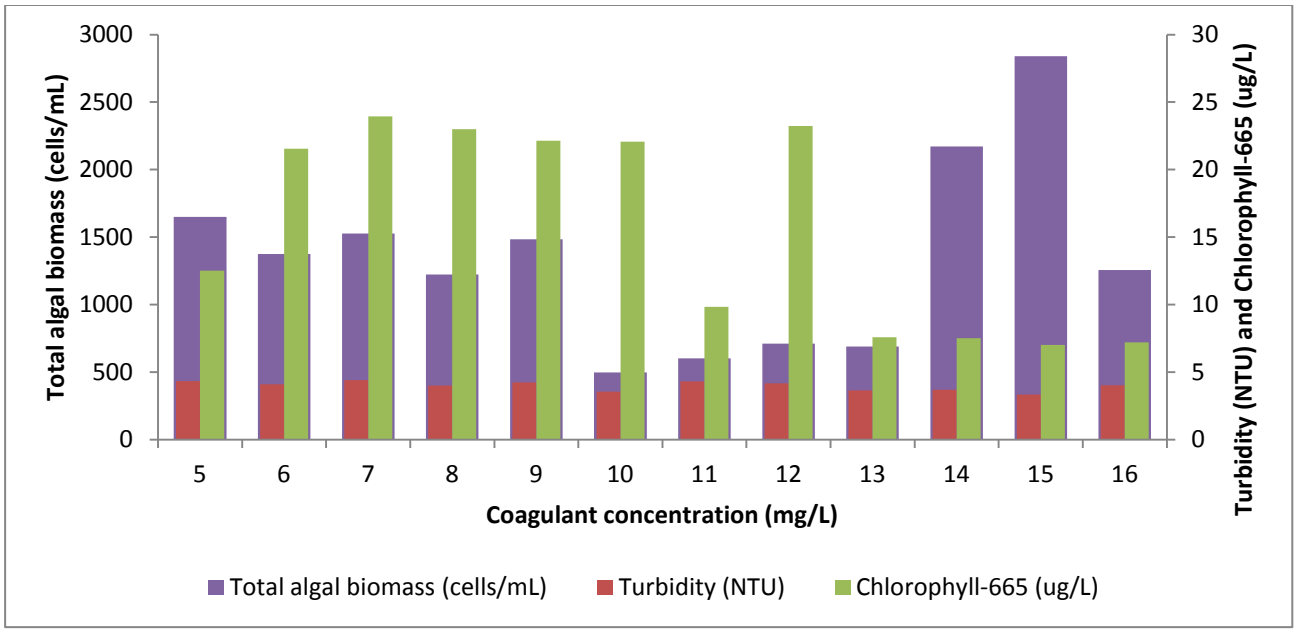

Figure B.8: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **23/11/2010**.

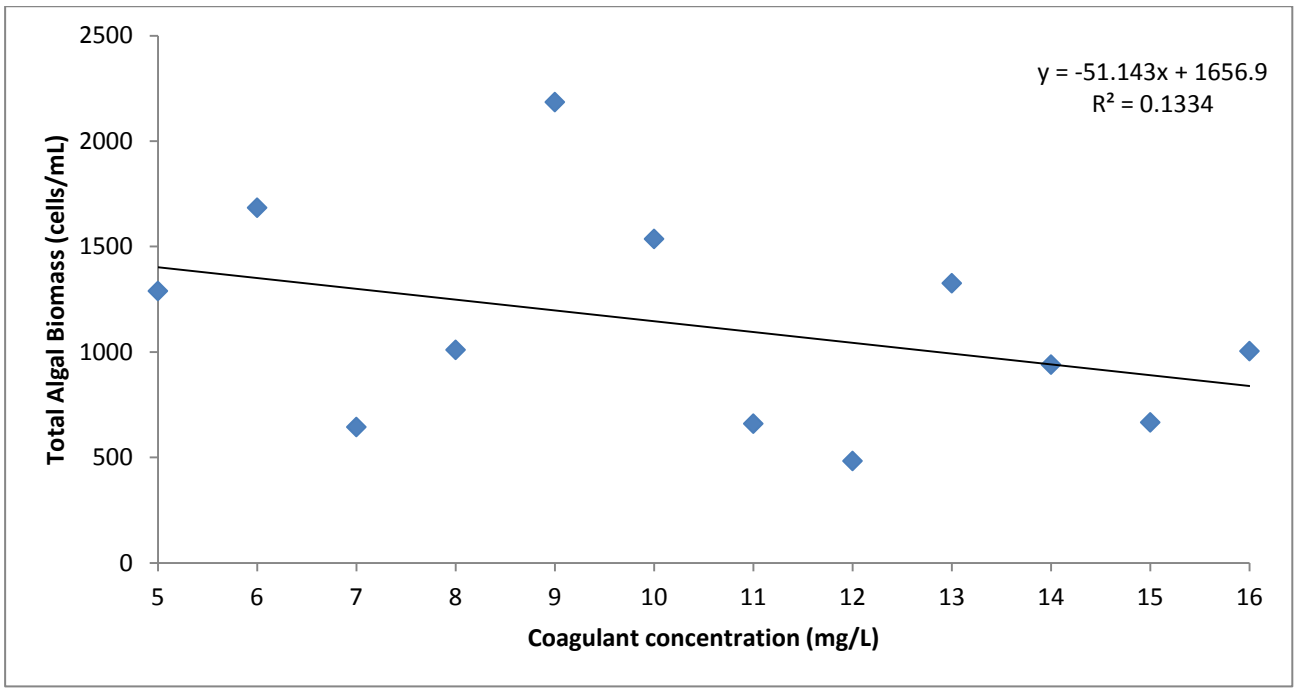

Figure B.9: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **03/11/2010**.

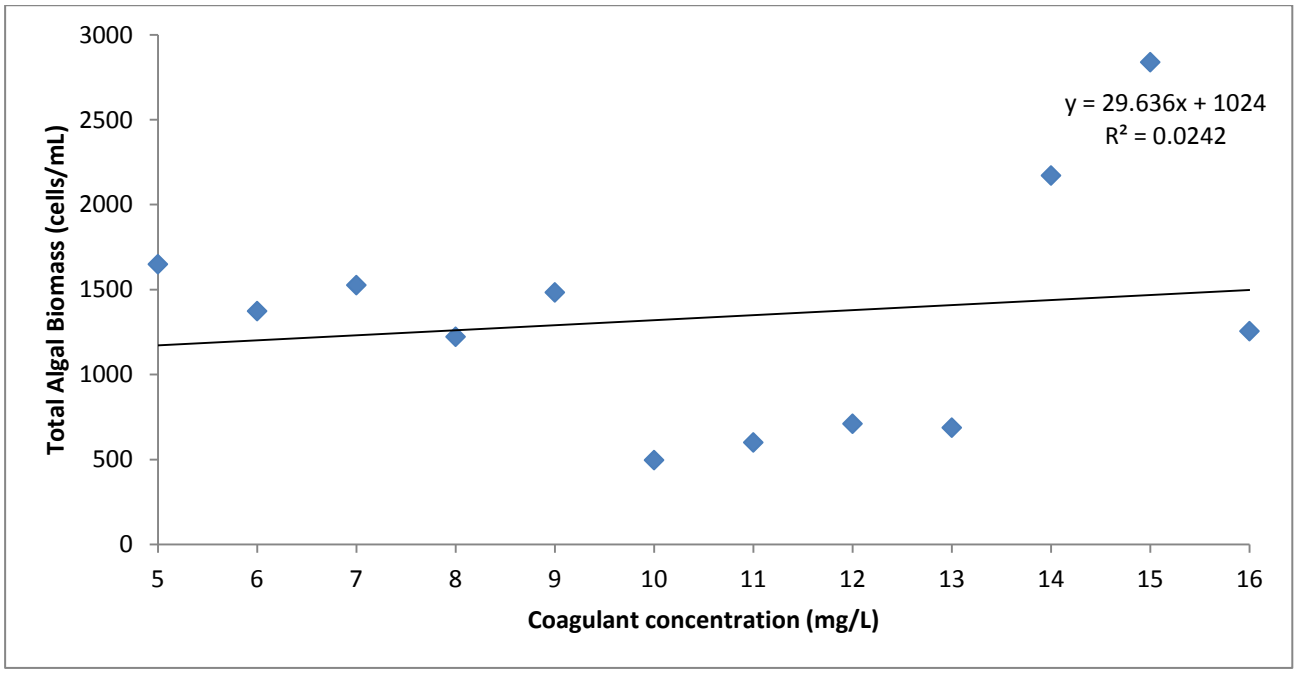

Figure B.10: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP on **23/11/2010**.

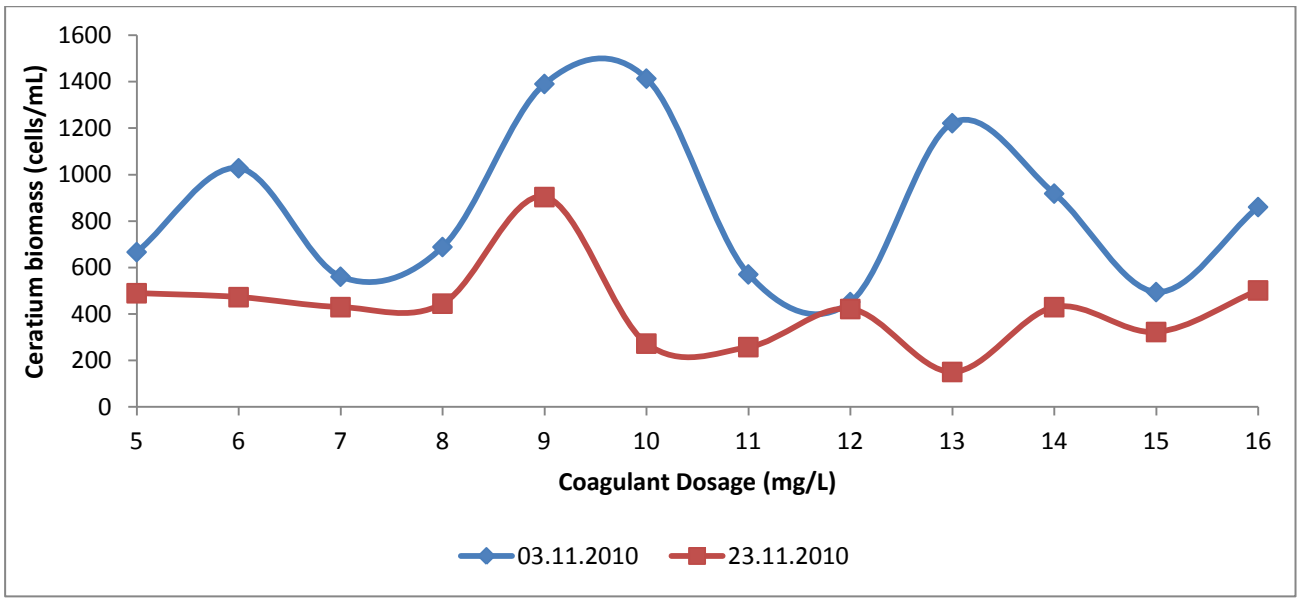

Figure B.11: Biomass (cells/mL) of *Ceratium hirundinella* when only poly-electrolyte is dosed at concentrations of 5 – 16 mg/L for sampling point M-RAW_VAALKOP for 03/11/2010 and 23/11/2010.

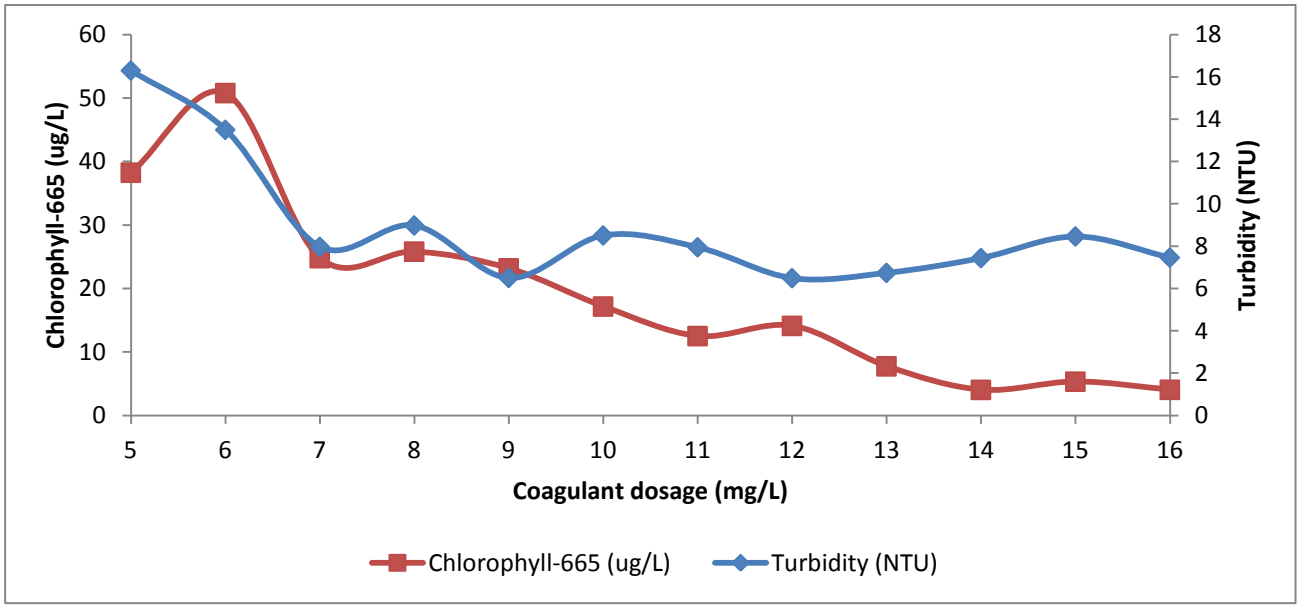

Figure B.12: The comparison between turbidity and chlorophyll-665 in the supernatant when poly-electrolyte was dosed at a range of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **03/11/2010**.

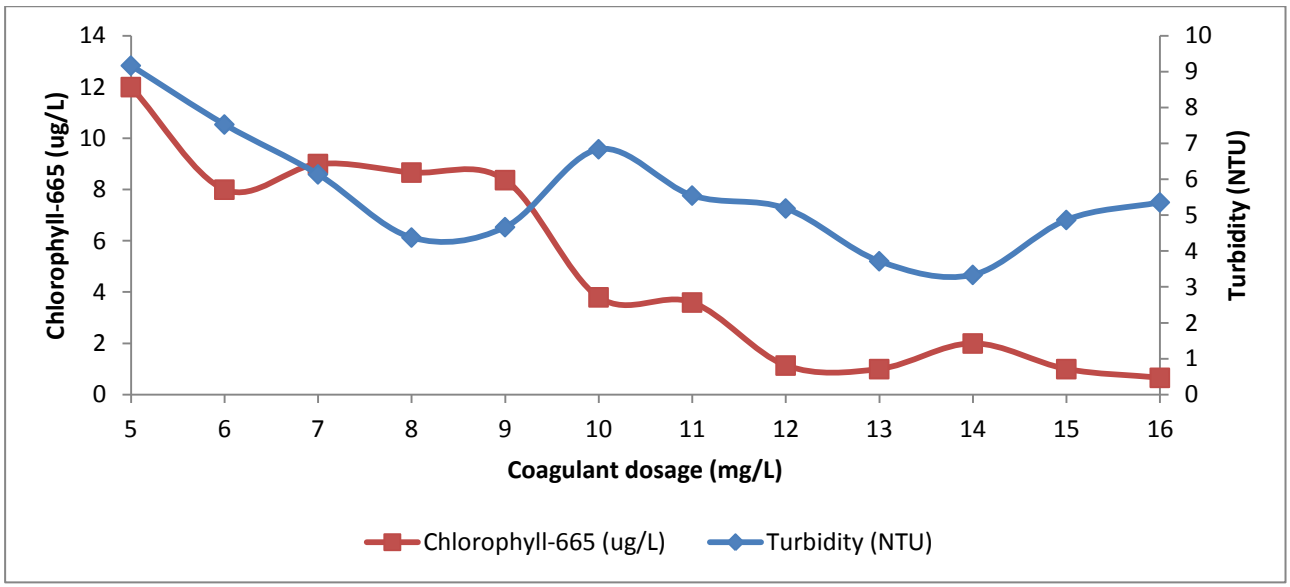

Figure B.13: The comparison between turbidity and chlorophyll-665 in the supernatant when poly-electrolyte was dosed at a range of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **23/11/2010**.

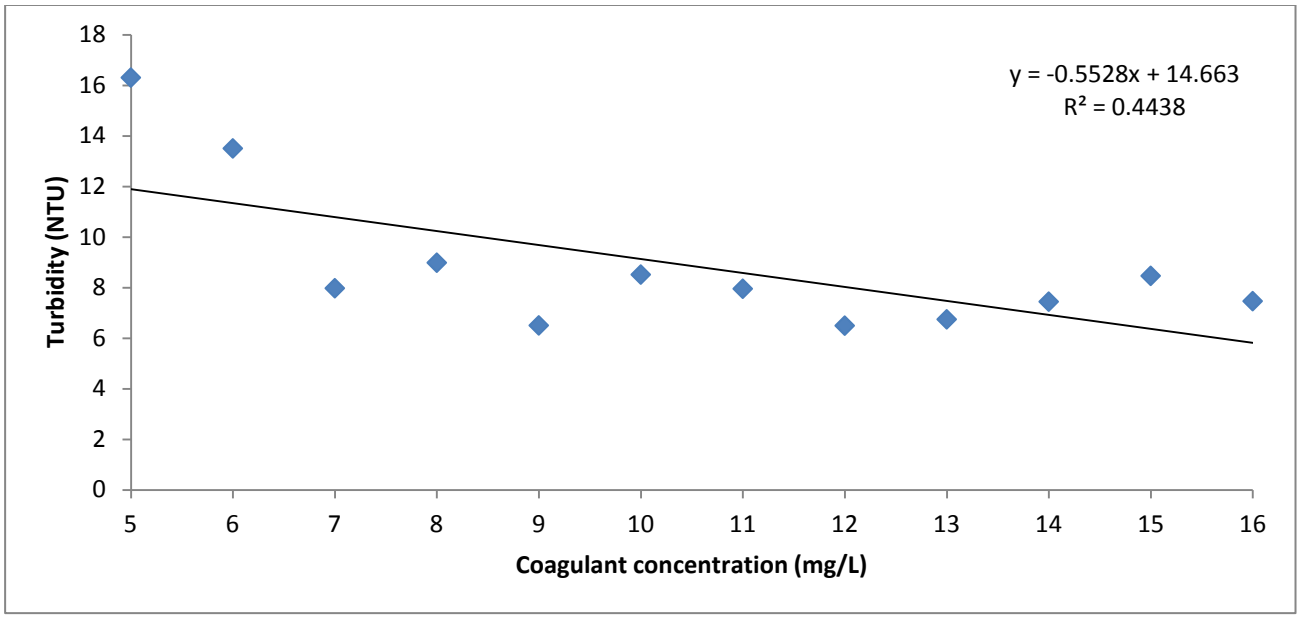

Figure B.14: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **03/11/2010**.

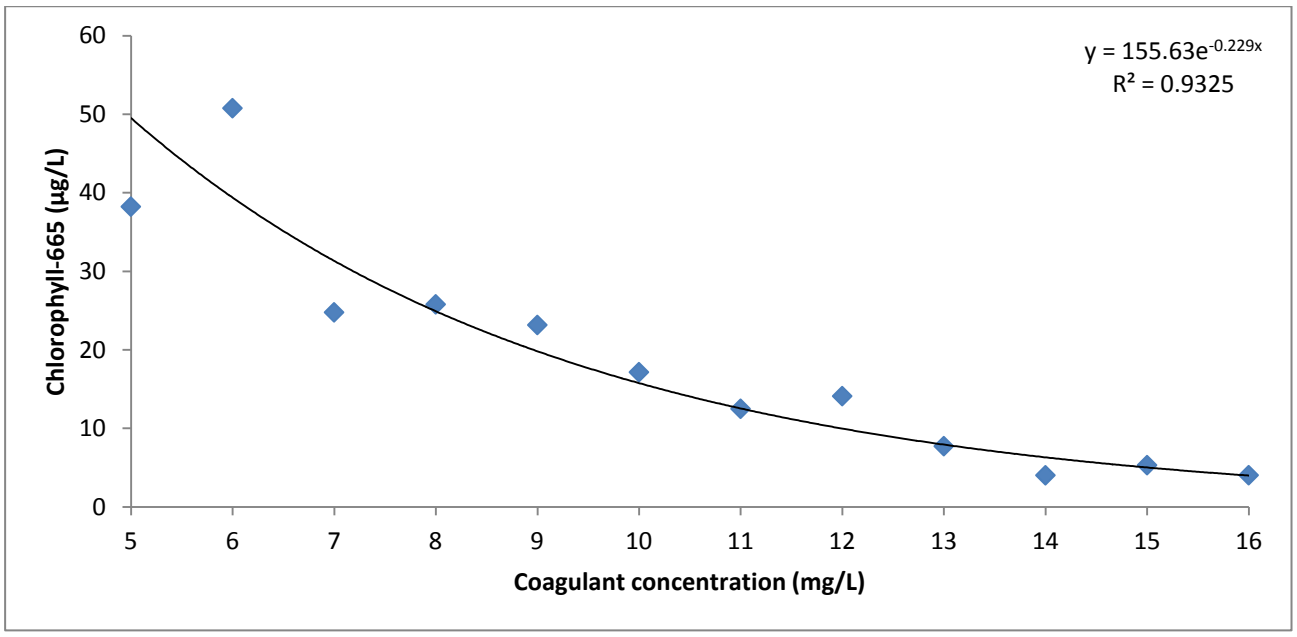

Figure B.15: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **03/11/2010**.

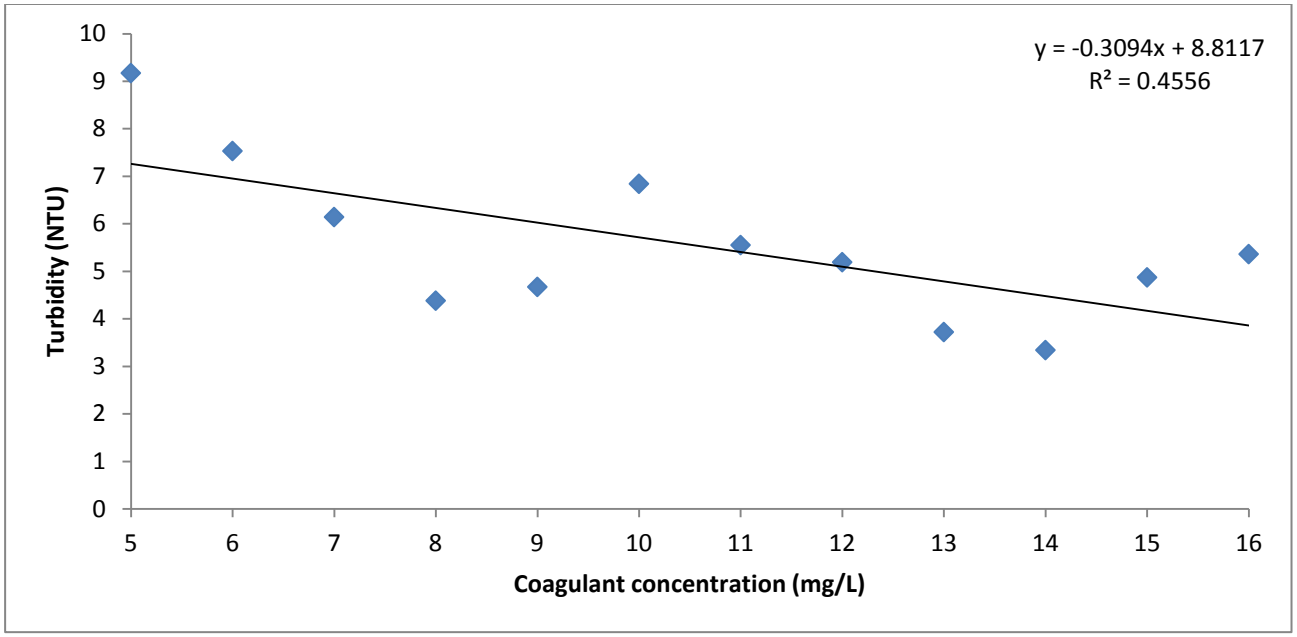

Figure B.16: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **23/11/2010**.

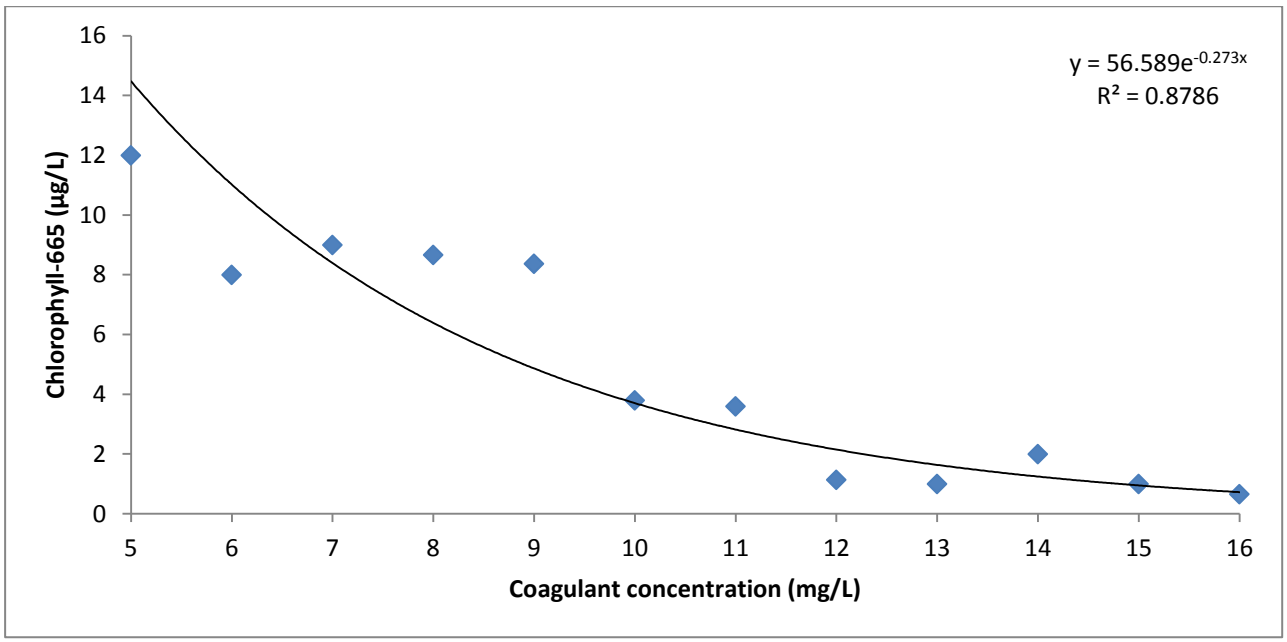

Figure B.17: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **23/11/2010**.

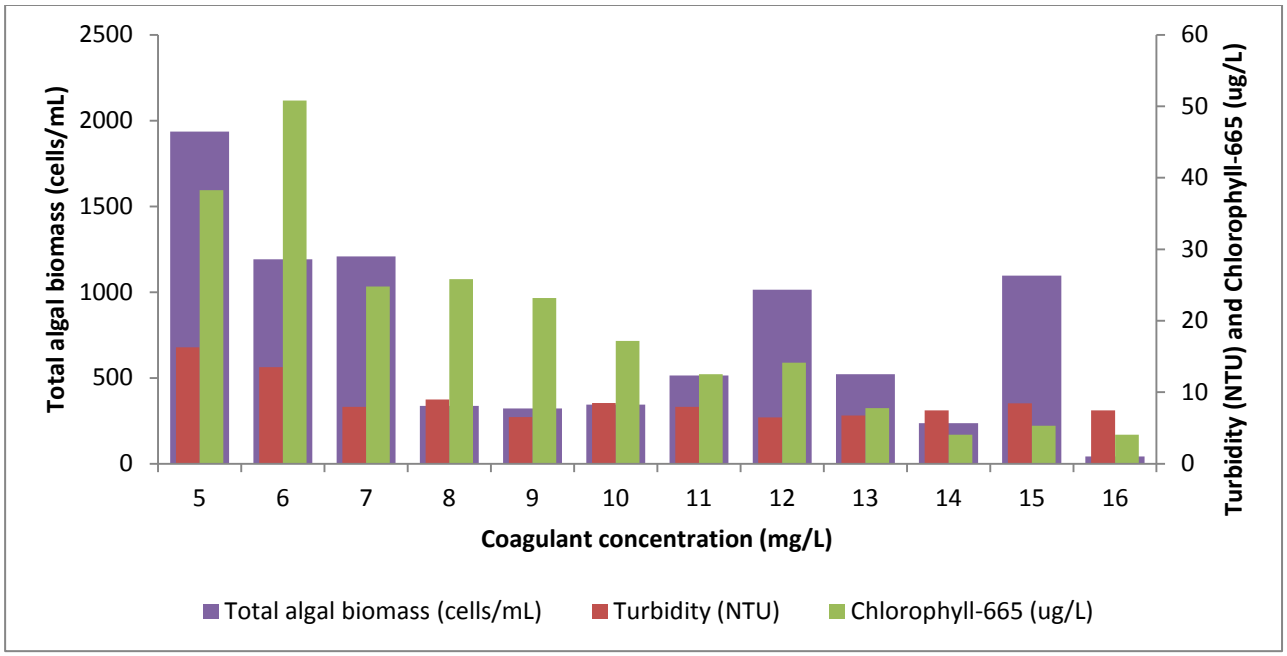

Figure B.18: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when poly-electrolyte was dosed at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **03/11/2010**.

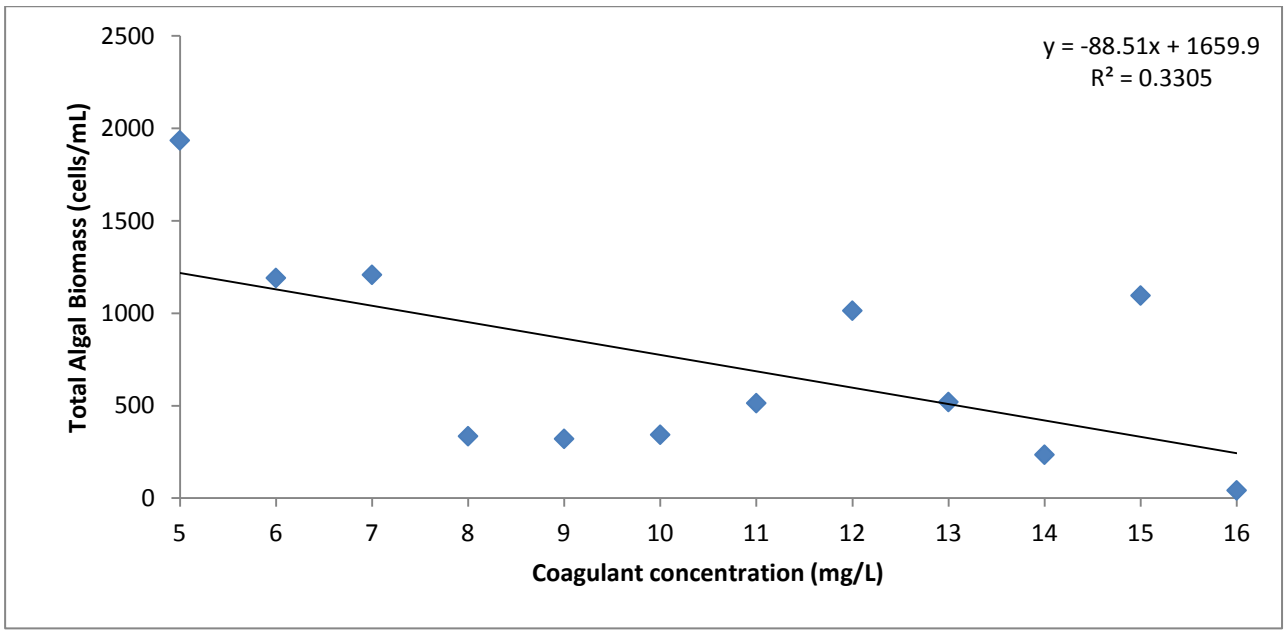

Figure B.19: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **03/11/2010**.

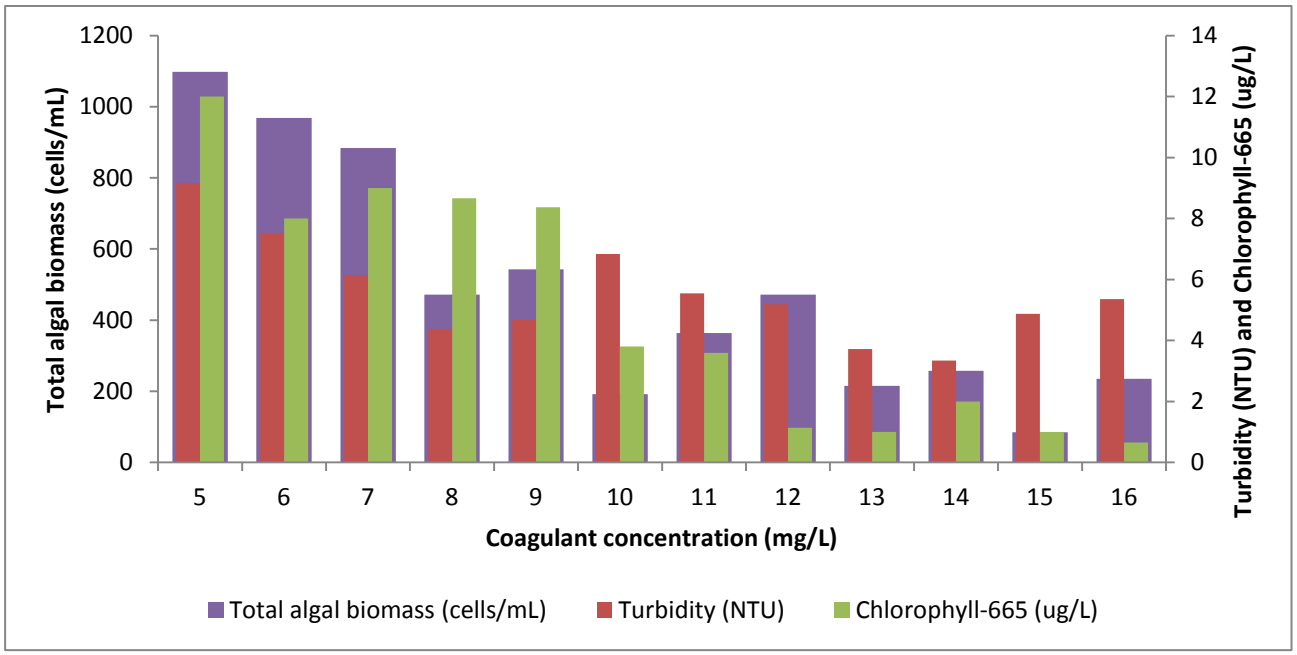

Figure B.20: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when poly-electrolyte was dosed at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **23/11/2010**.

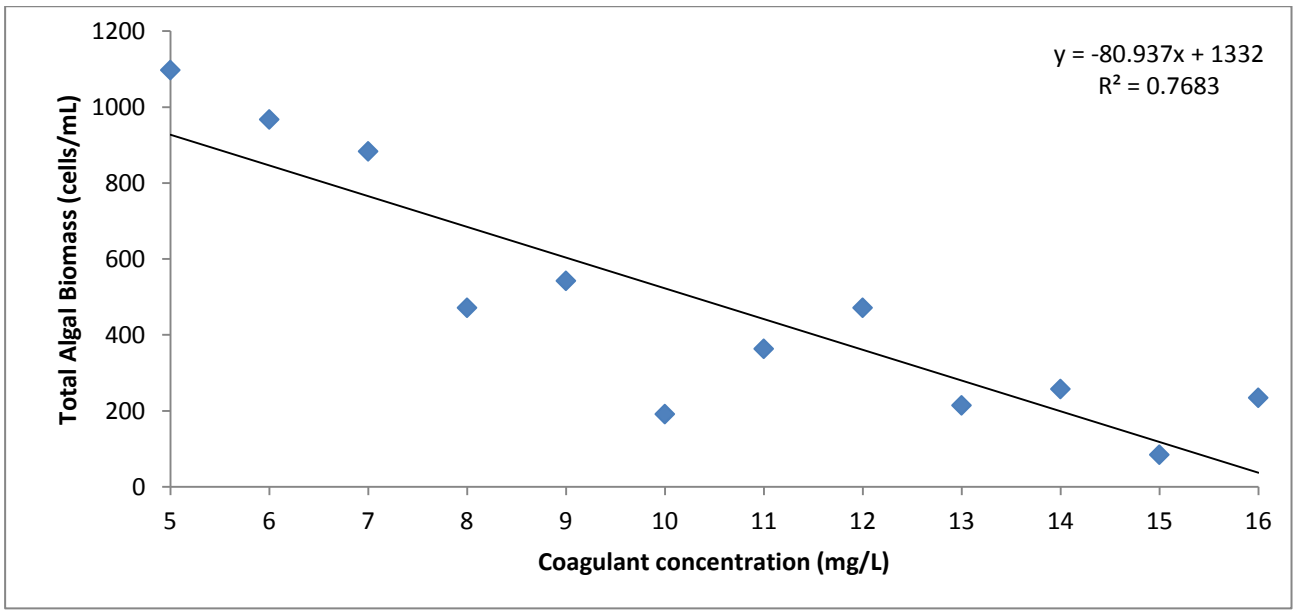

Figure B.21: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for sampling point M-RAW_VAALKOP on **23/11/2010**.

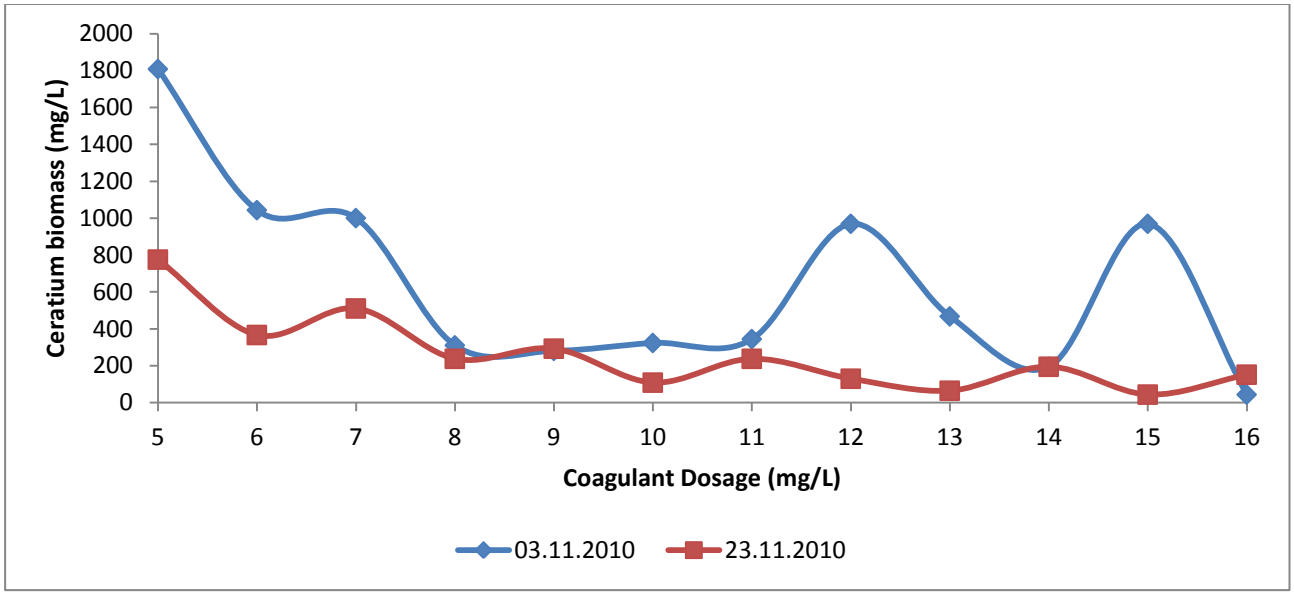

Figure B.22: Biomass (cells/mL) of *Ceratium hirundinella* when only poly-electrolyte is dosed at concentrations of 5 – 16 mg/L with 10 mg/L CaO for sampling point M-RAW_VAALKOP for 03/11/2010 and 23/11/2010.

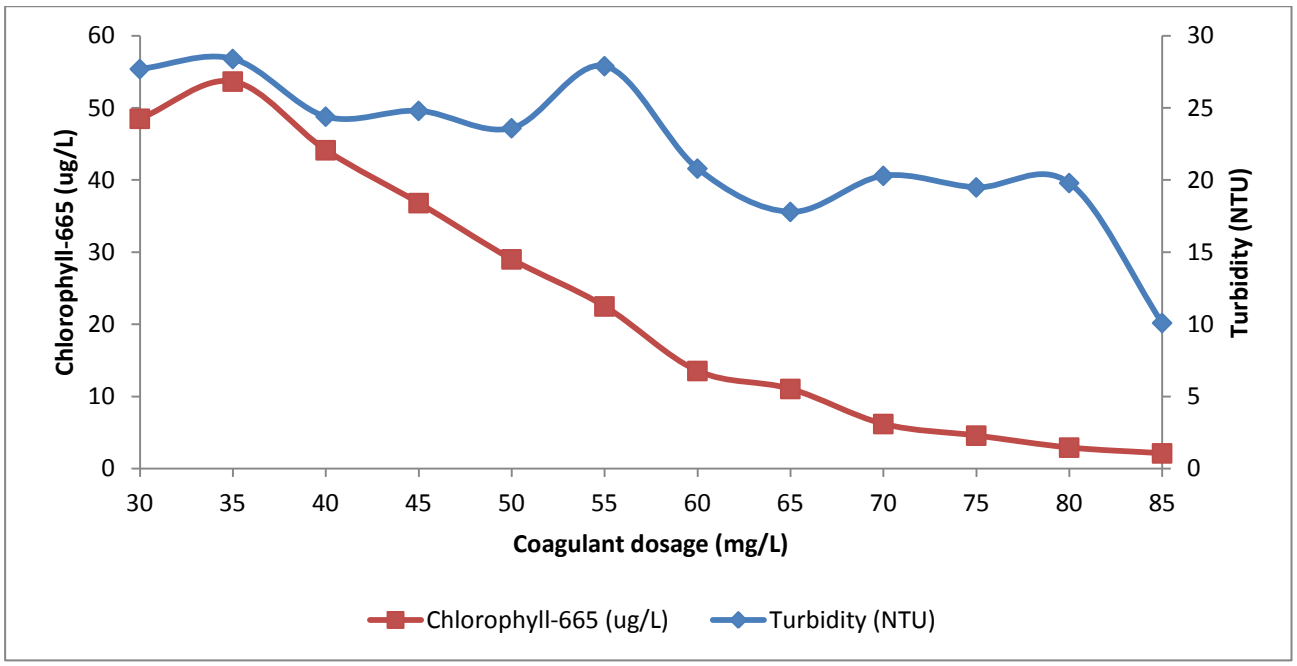

Figure B.23: The comparison between turbidity and chlorophyll-665 in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **03/11/2010**.

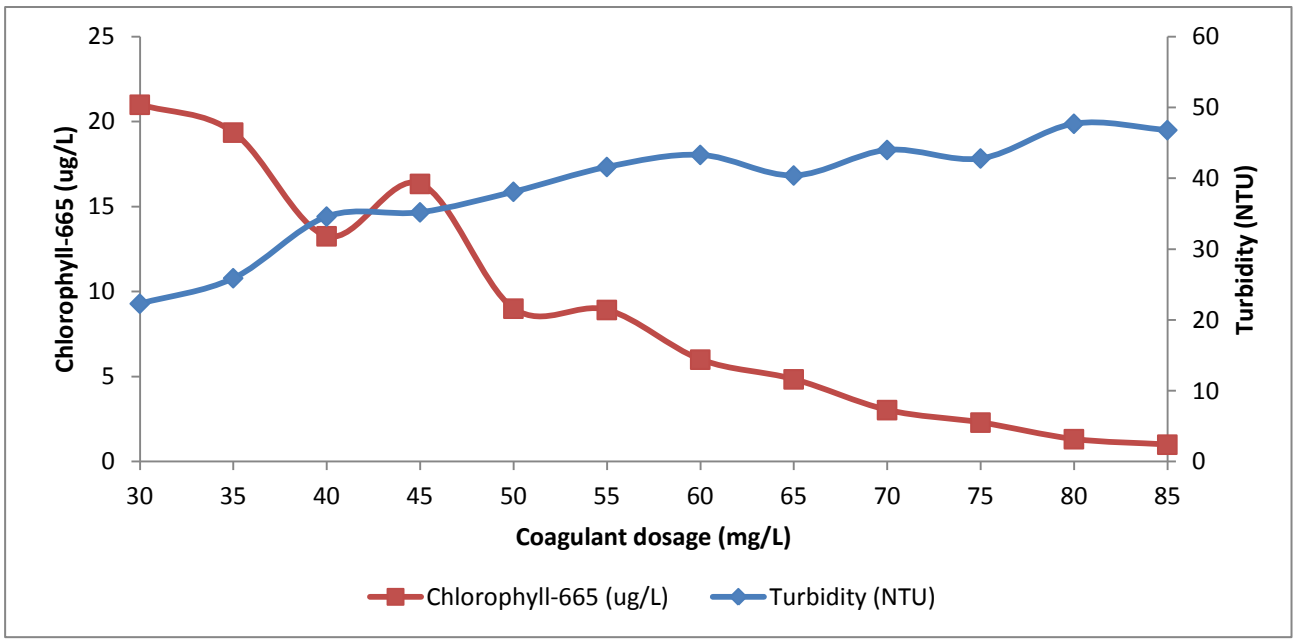

Figure B.24: The comparison between turbidity and chlorophyll-665 in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **23/11/2010**.

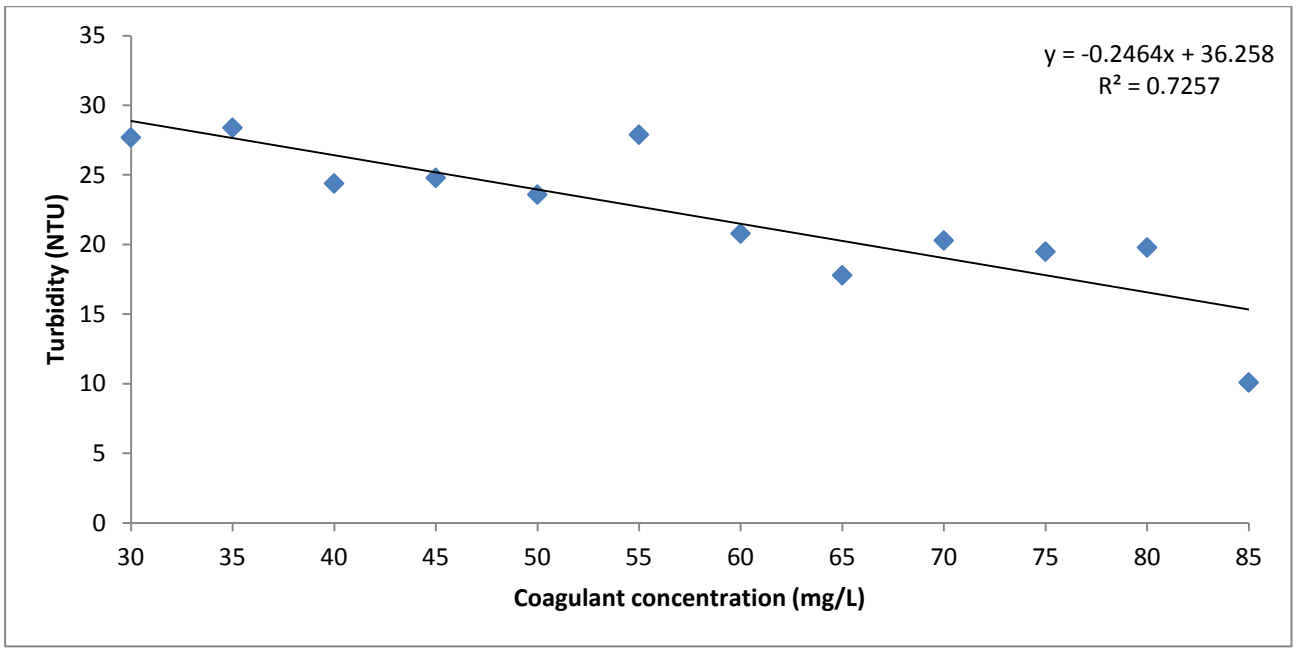

Figure B.25: Regression analysis between turbidity and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **03/11/2010**.

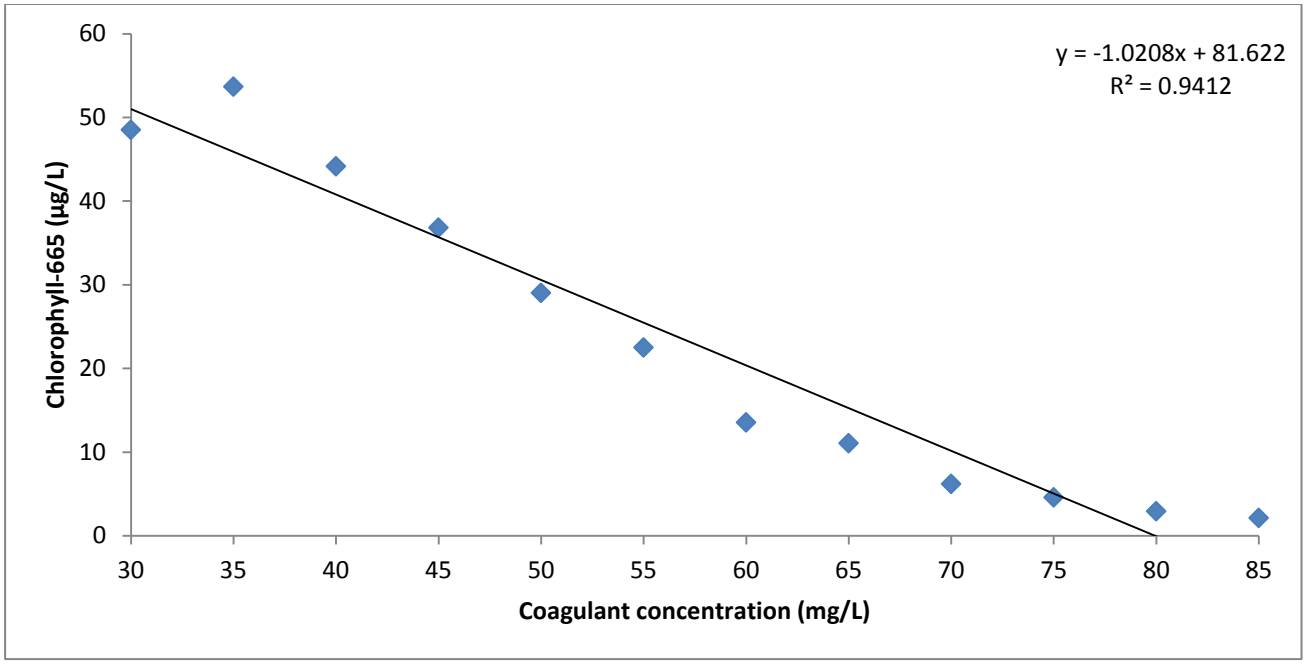

Figure B.26: Regression analysis between chlorophyll-665 and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **03/11/2010**.

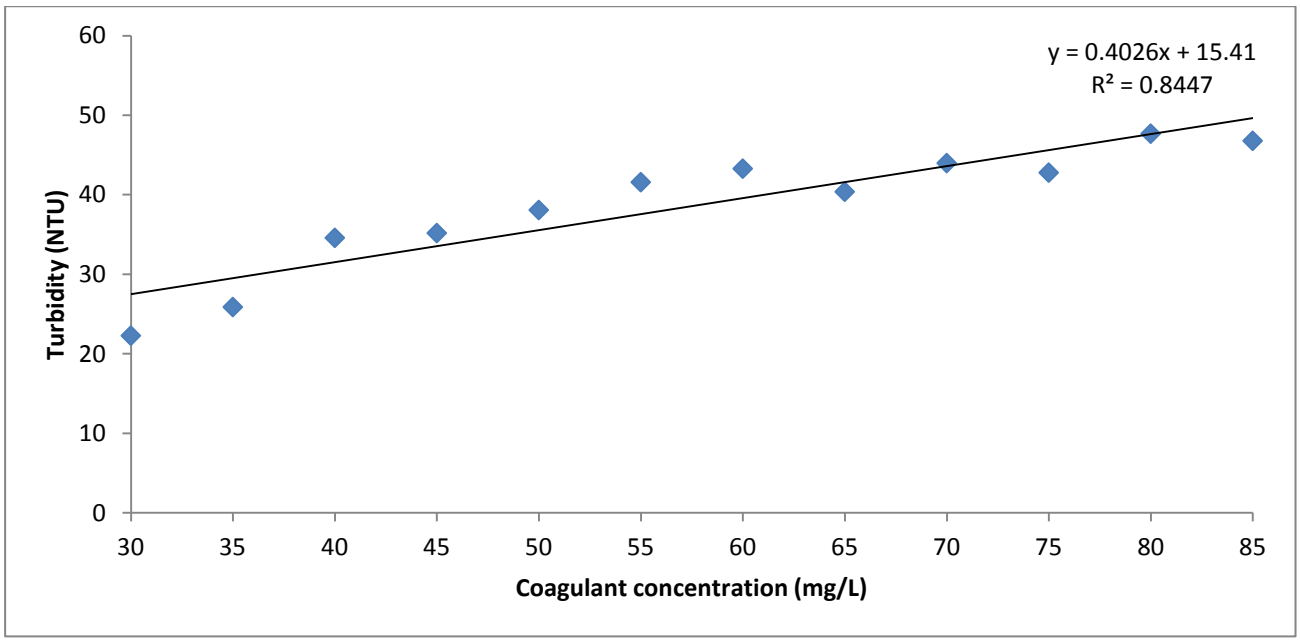

Figure B.27: Regression analysis between turbidity and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **23/11/2010**.

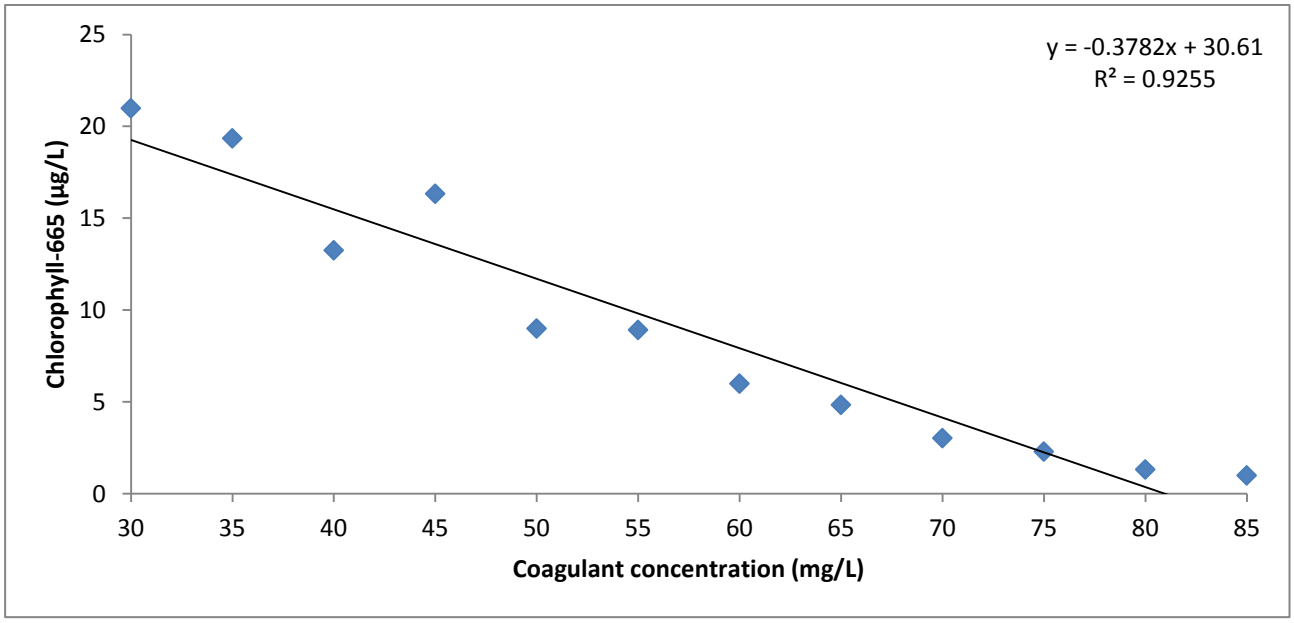

Figure B.28: Regression analysis between chlorophyll-665 and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **23/11/2010**.

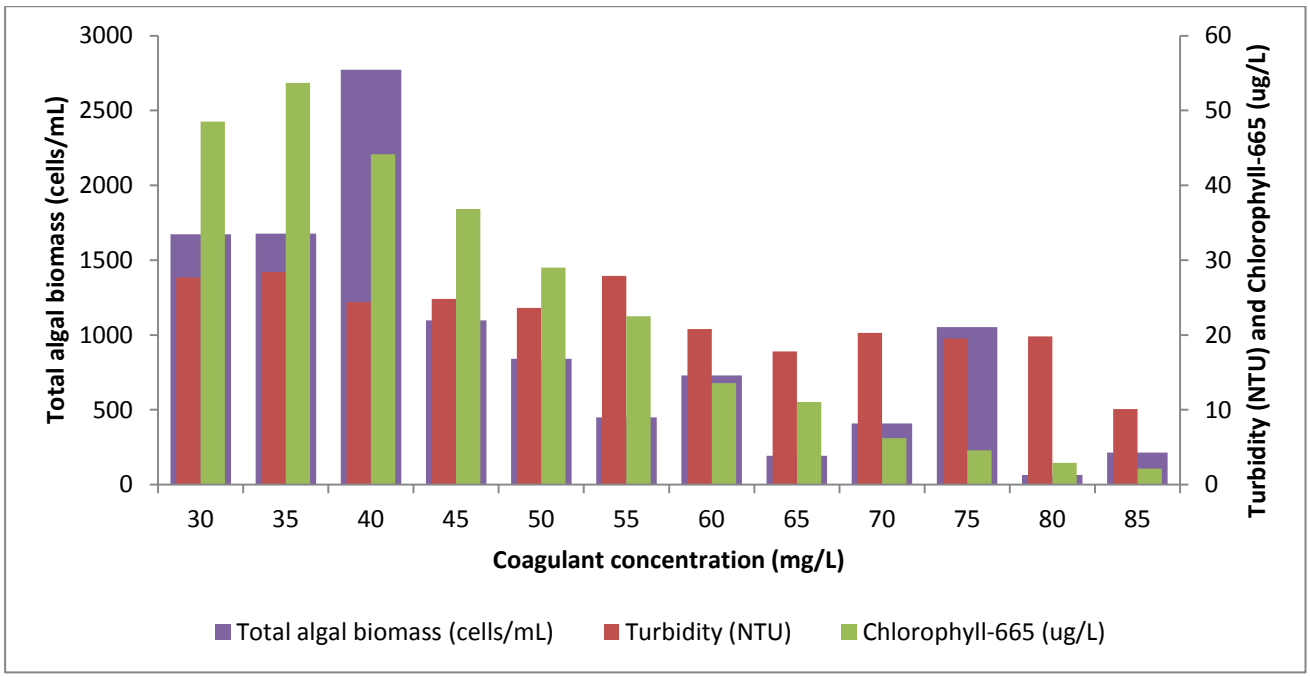

Figure B.29: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **03/11/2010**.

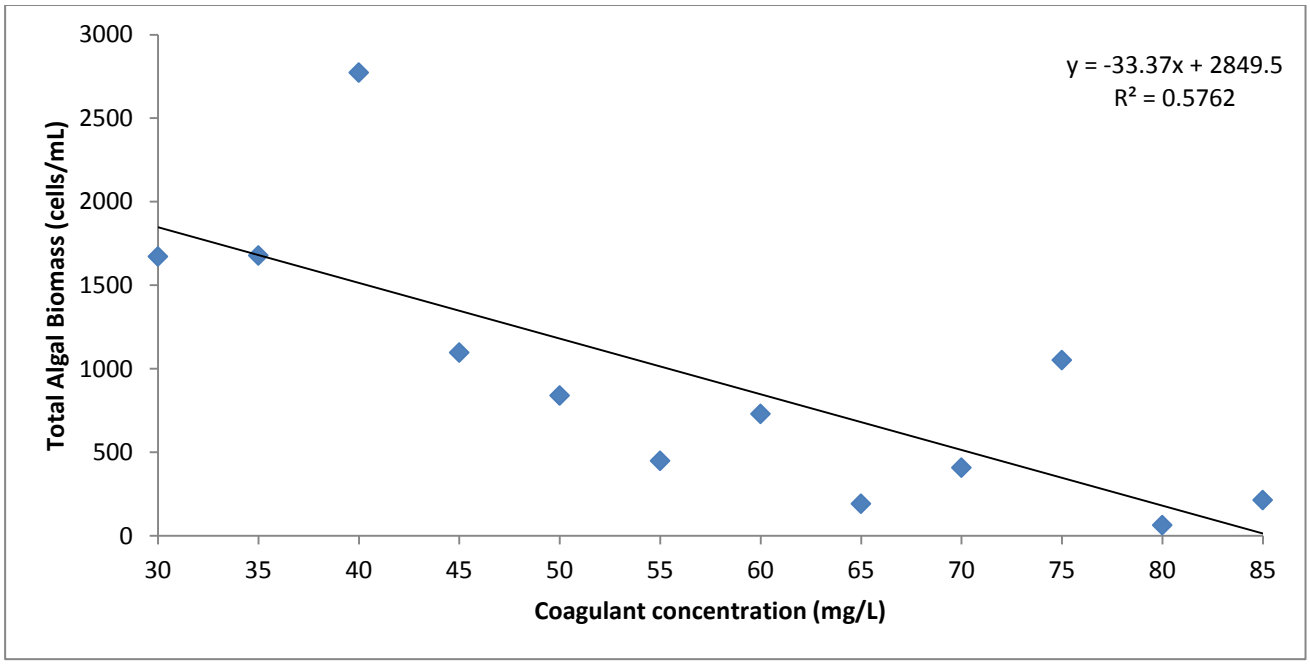

Figure B.30: Regression analysis between total algal concentration and coagulant dosage when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **03/11/2010**.

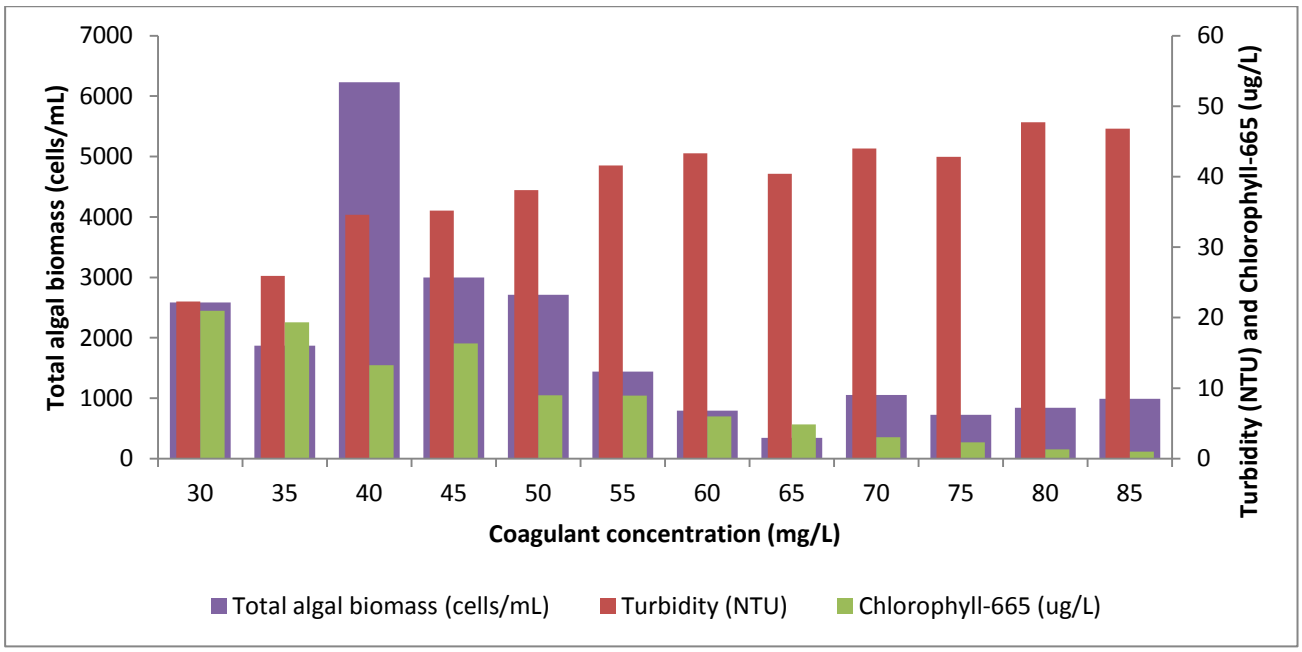

Figure B.31: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **23/11/2010**.

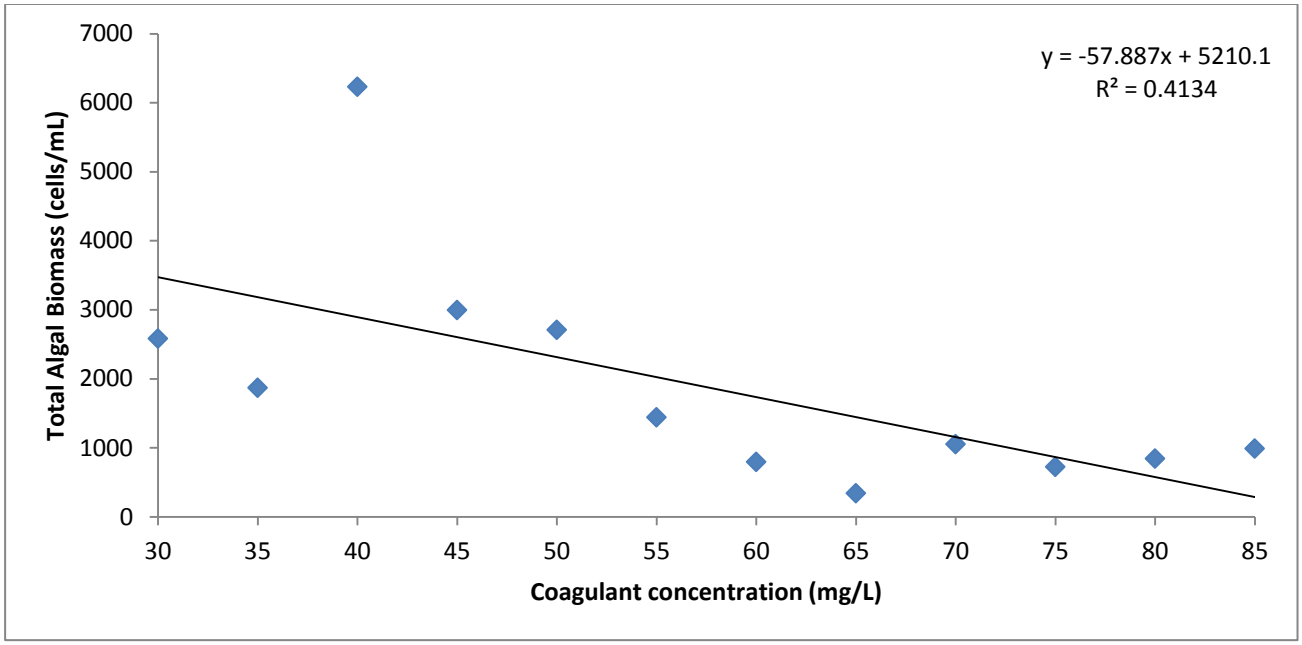

Figure B.32: Regression analysis between total algal concentration and coagulant dosage when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for sampling point M-RAW_VAALKOP on **23/11/2010**.

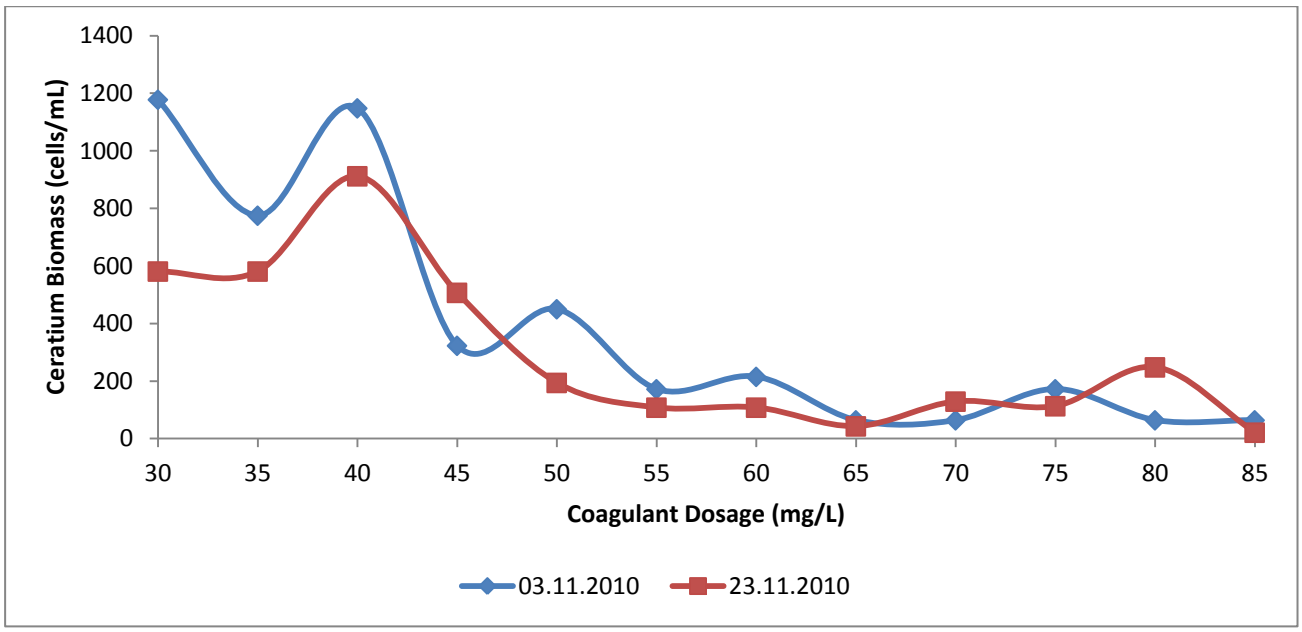

Figure B.33: Biomass (cells/mL) of *Ceratium hirundinella* when 2.5 mg/L activated silica are dosed with varying concentrations of CaO for sampling point M-RAW_VAALKOP for 03/11/2010 and 23/11/2010.

Rietvlei Dam

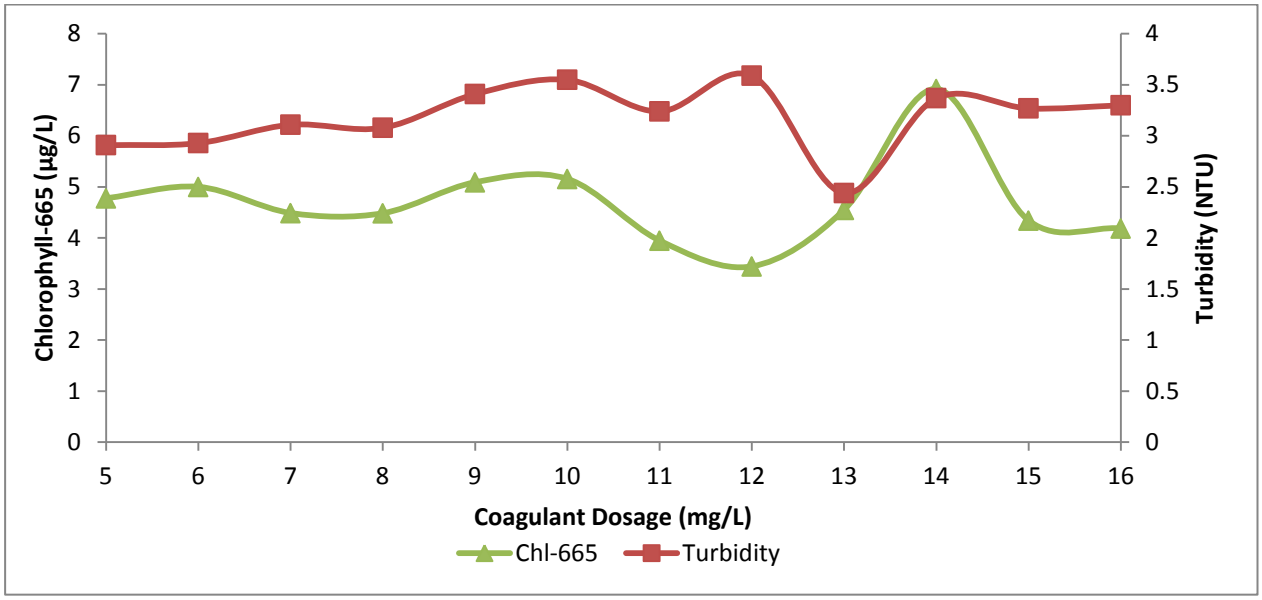

Figure B.34: The comparison between turbidity and chlorophyll-665 in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for Rietvlei Dam on 21/02/2011.

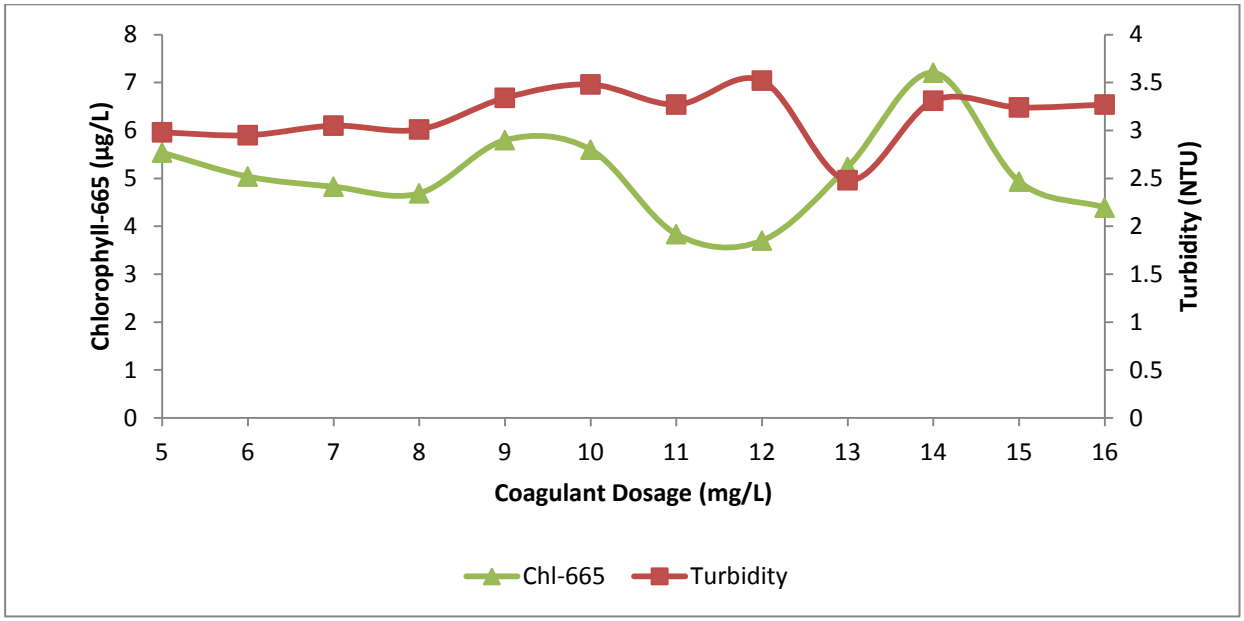

Figure B.35: The comparison between turbidity and chlorophyll-665 in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for Rietvlei Dam on 22/02/2011.

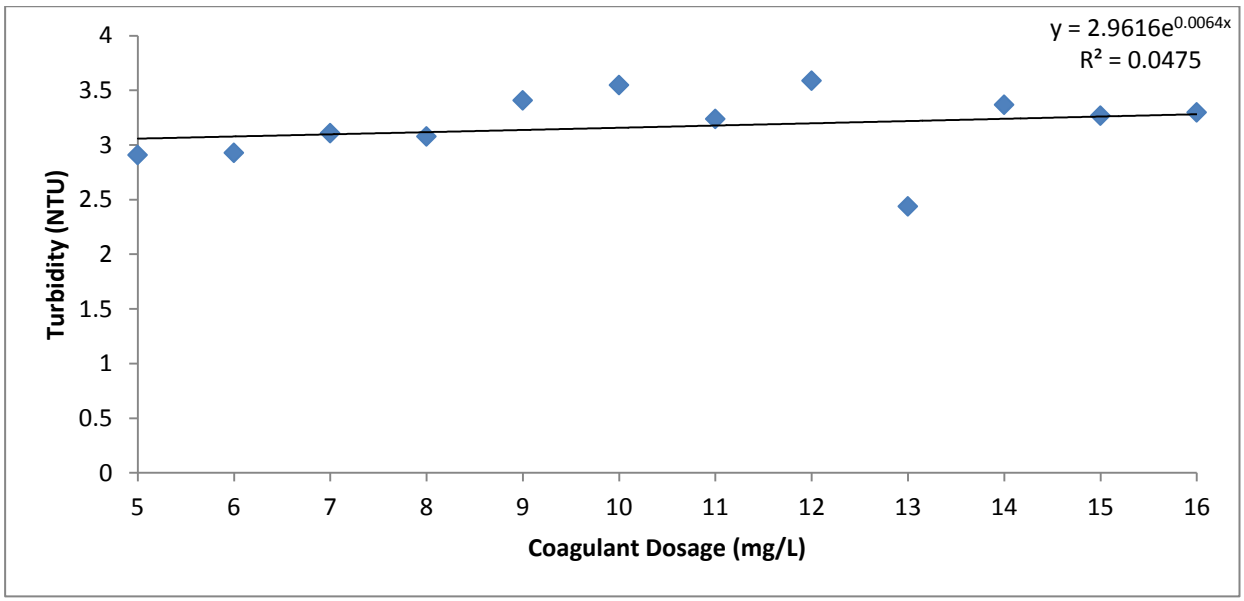

Figure B.36: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L for Rietvlei Dam on 21/02/2011.

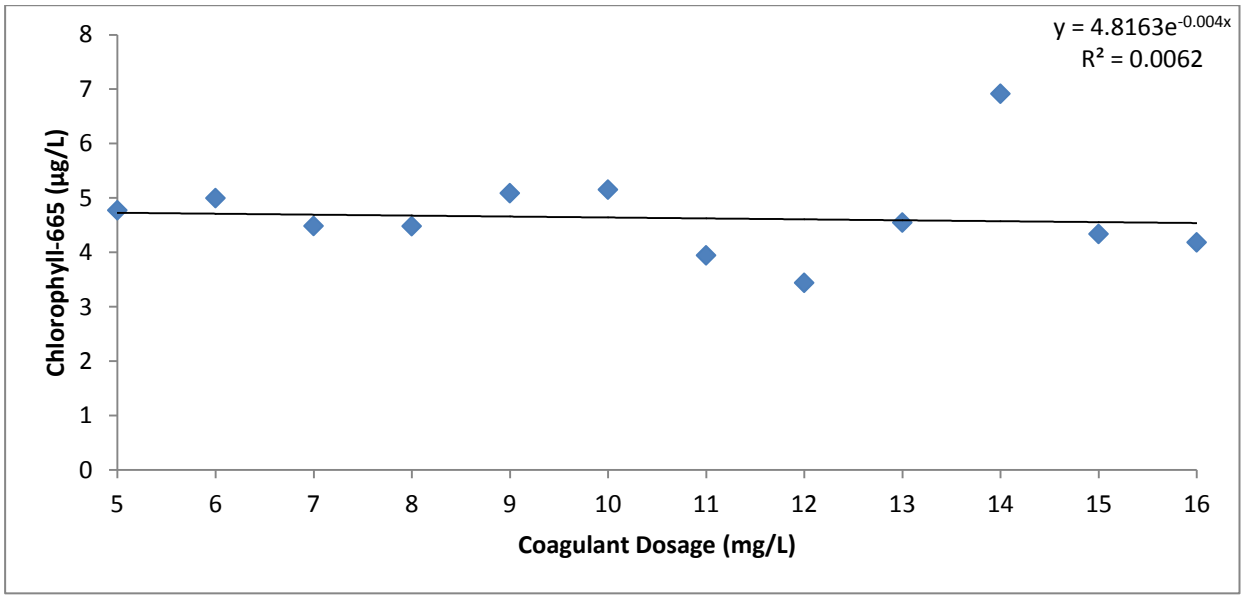

Figure B.37: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L for Rietvlei Dam on 21/02/2011.

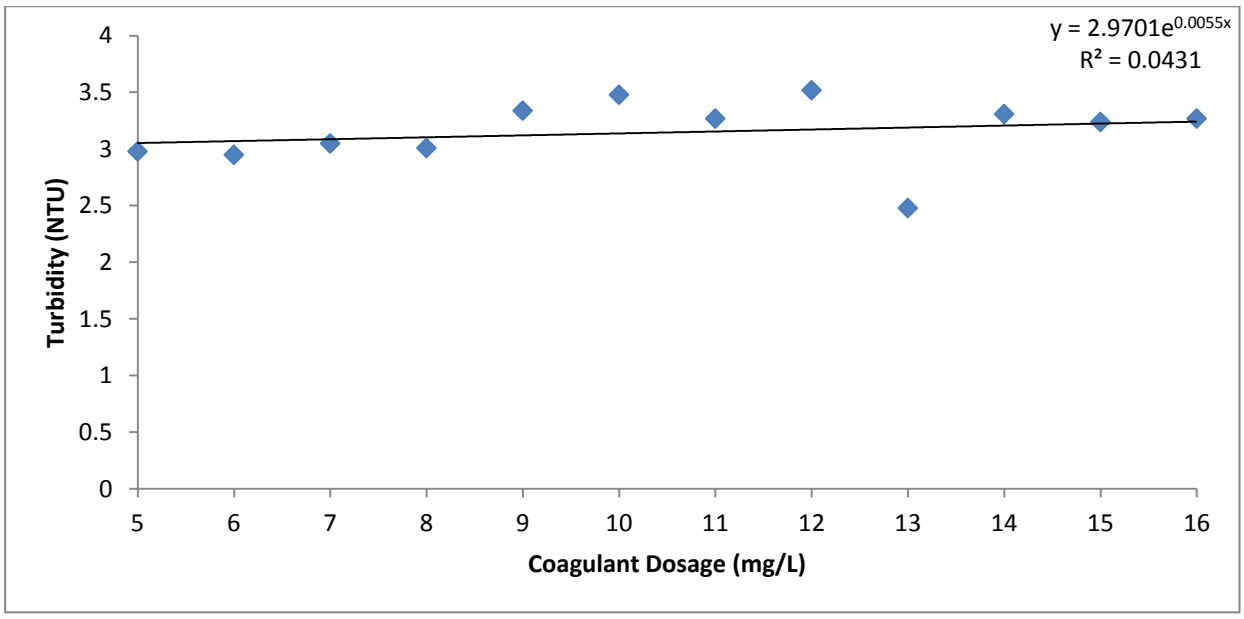

Figure B.38: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L for Rietvlei Dam on 22/02/2011.

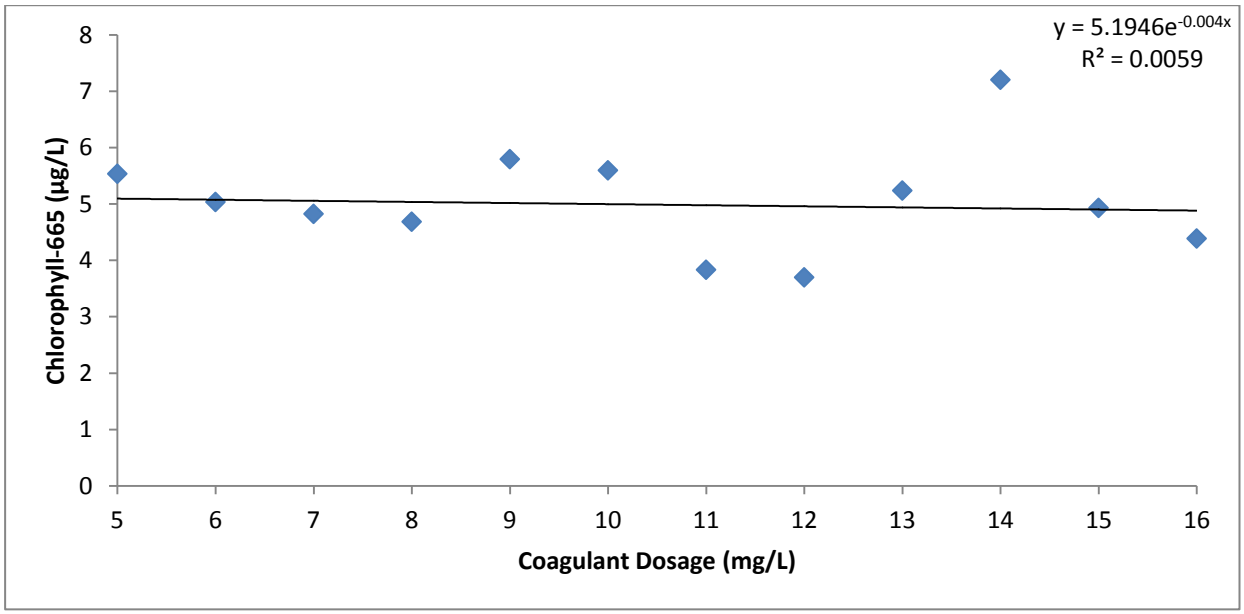

Figure B.39: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L for Rietvlei Dam on 22/02/2011.

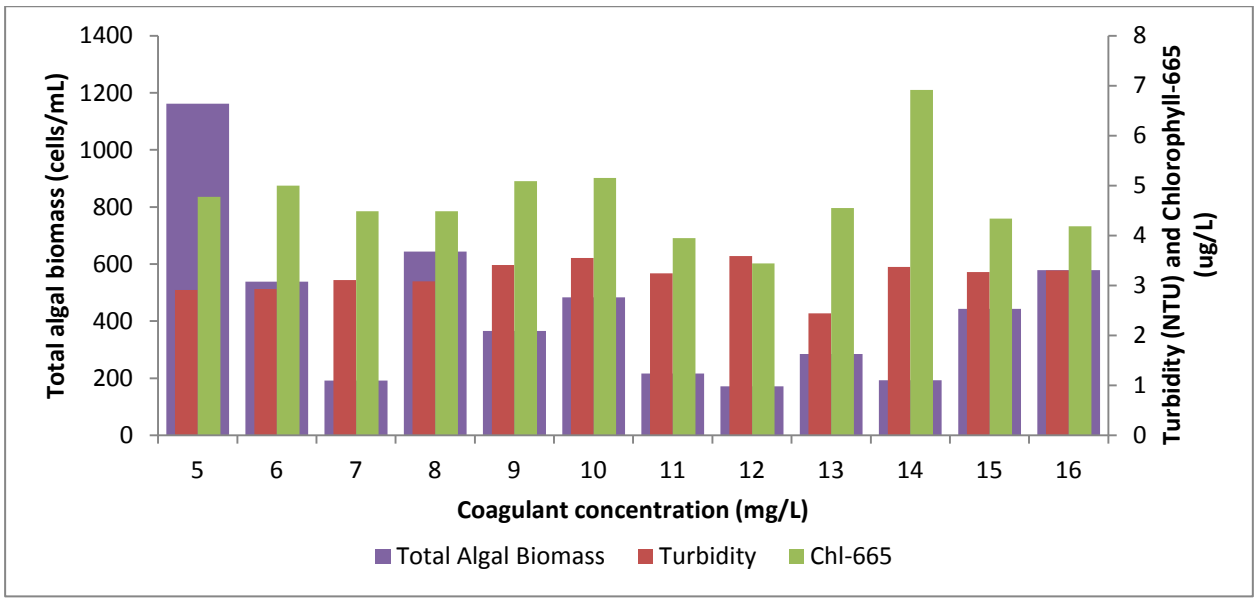

Figure B.40: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for Rietvlei Dam on 21/02/2011.

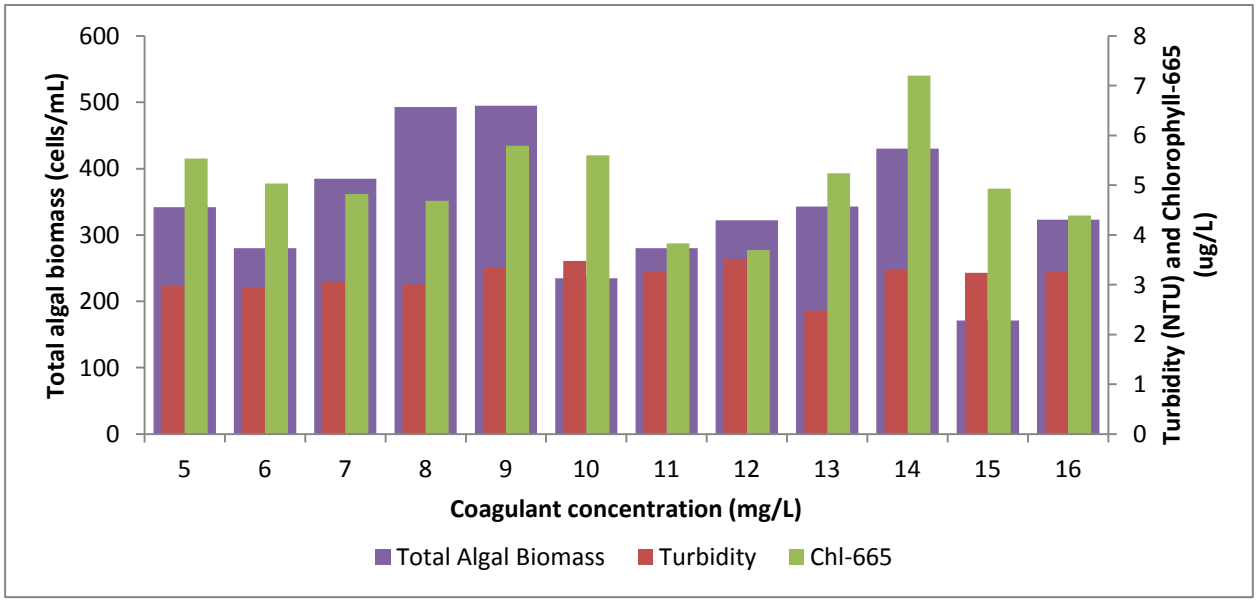

Figure B.41: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when only poly-electrolyte was dosed at concentrations of 5 – 16 mg/L for Rietvlei Dam on 22/02/2011.

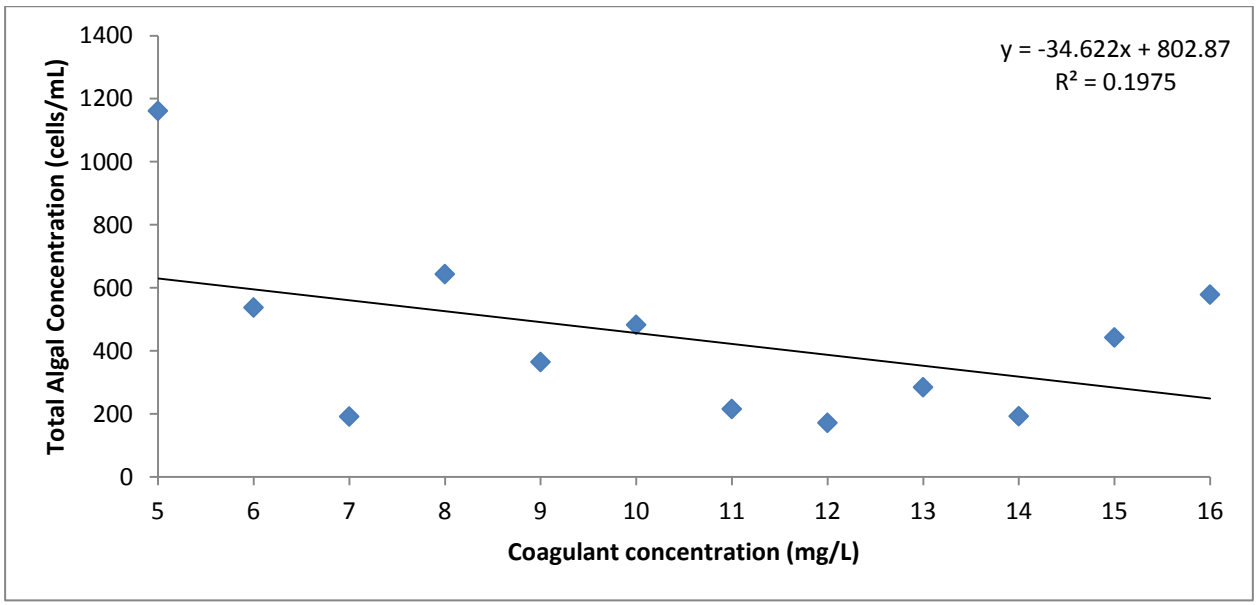

Figure B.42: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L for Rietvlei Dam on 21/02/2011.

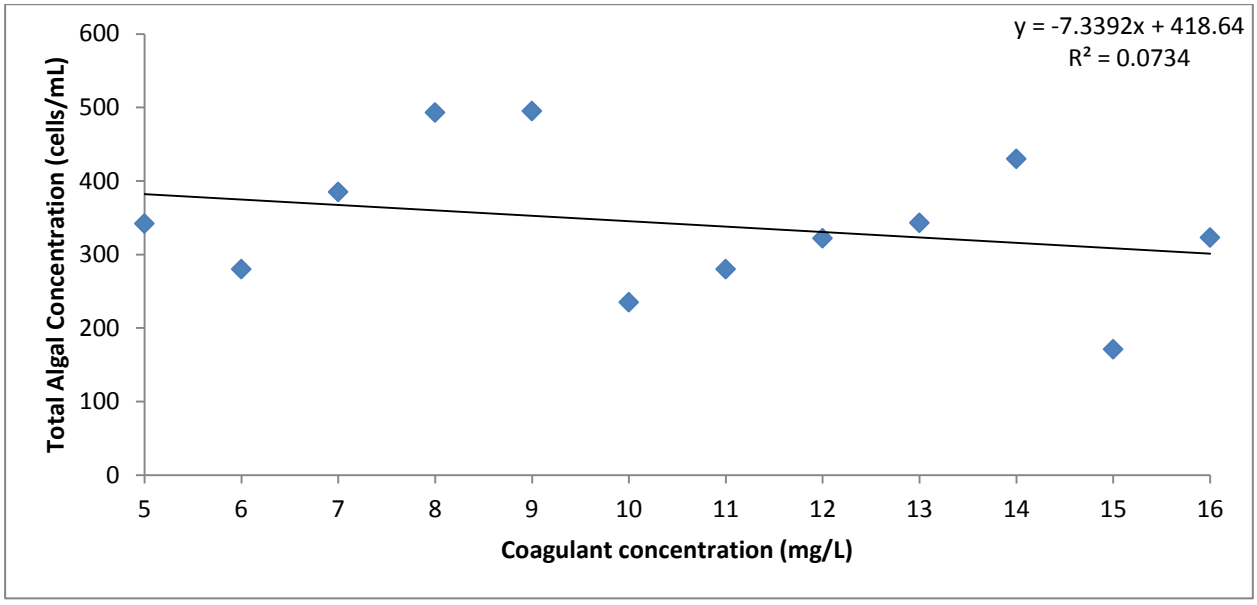

Figure B.43: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L for Rietvlei Dam on 22/02/2011.

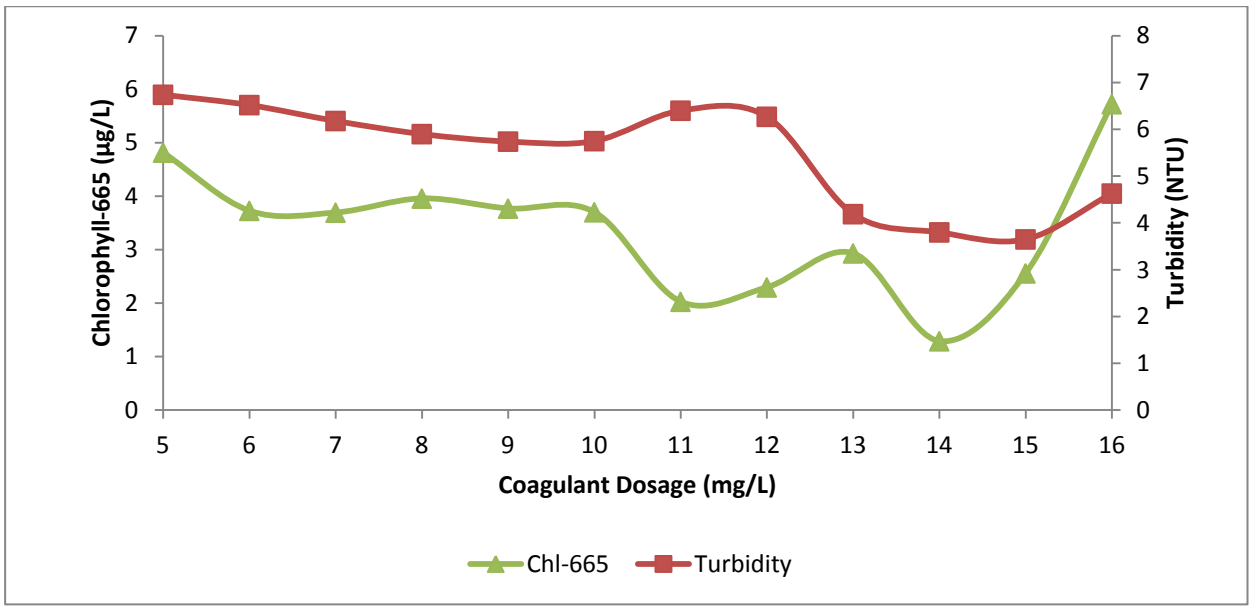

Figure B.44: The comparison between turbidity and chlorophyll-665 in the supernatant when poly-electrolyte was dosed at a range of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 21/02/2011.

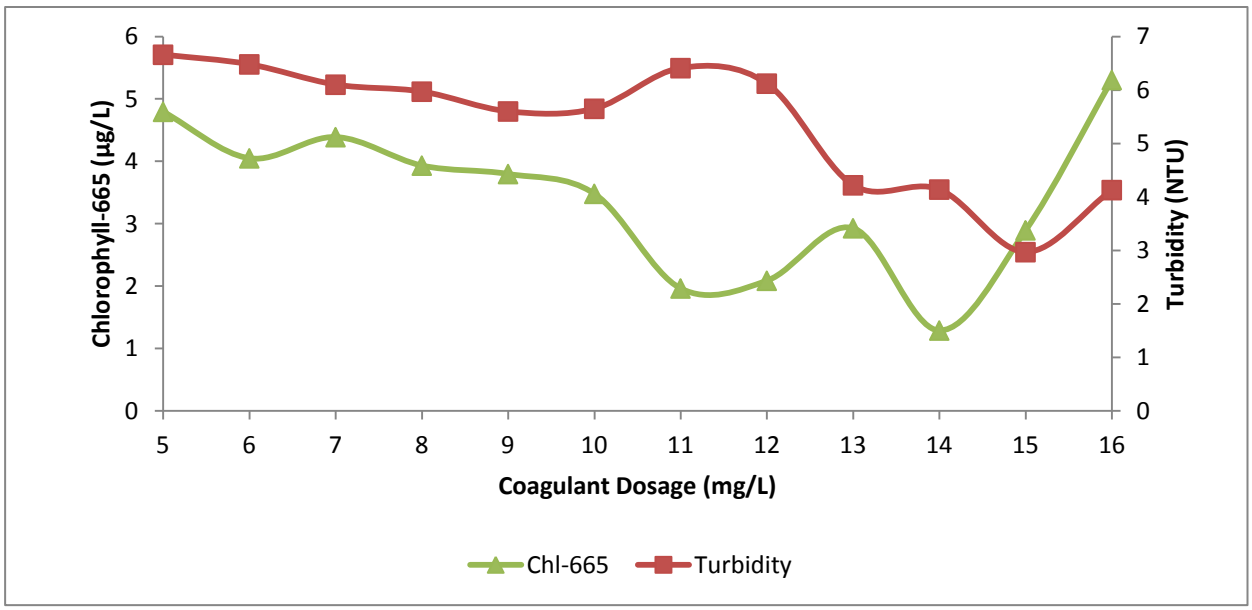

Figure B.45: The comparison between turbidity and chlorophyll-665 in the supernatant when poly-electrolyte was dosed at a range of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 22/02/2011.

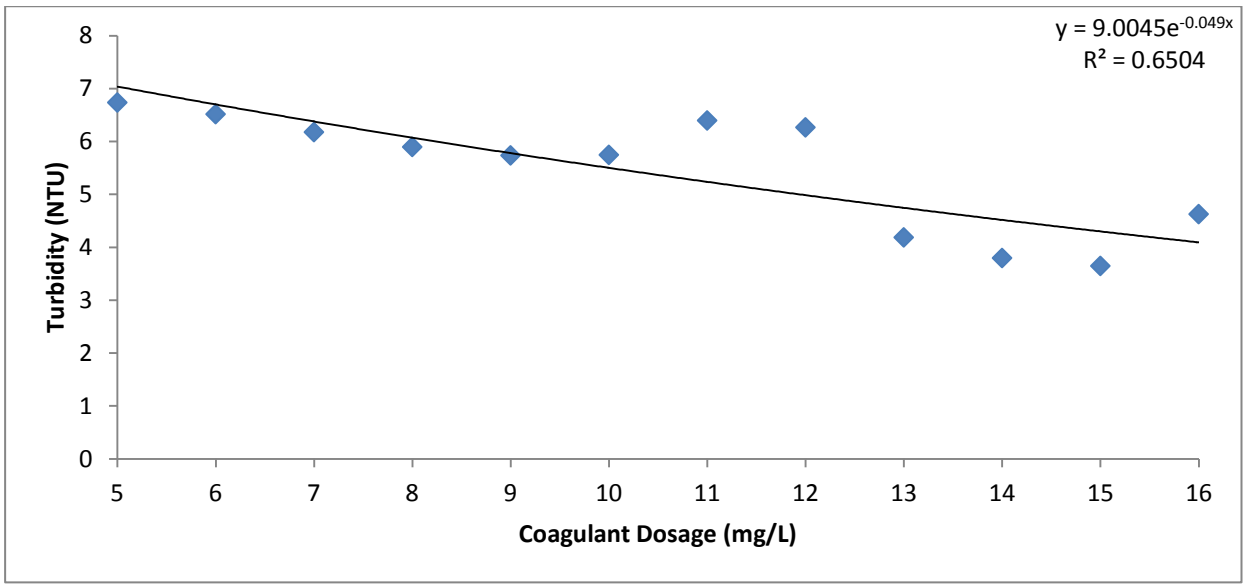

Figure B.46: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 21/02/2011.

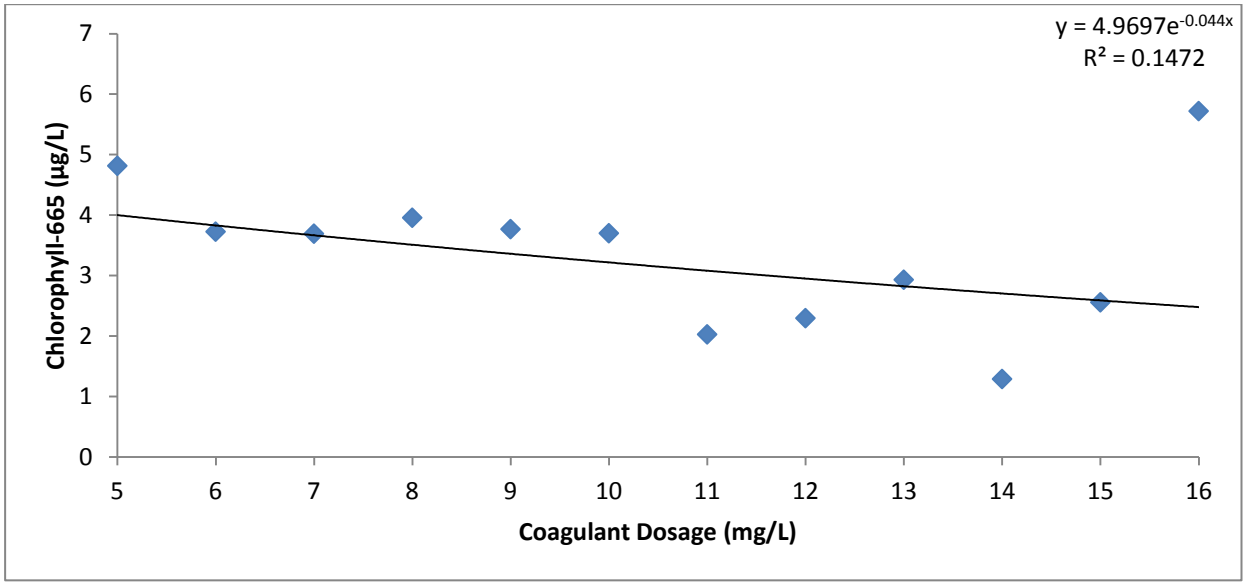

Figure B.47: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 21/02/2011.

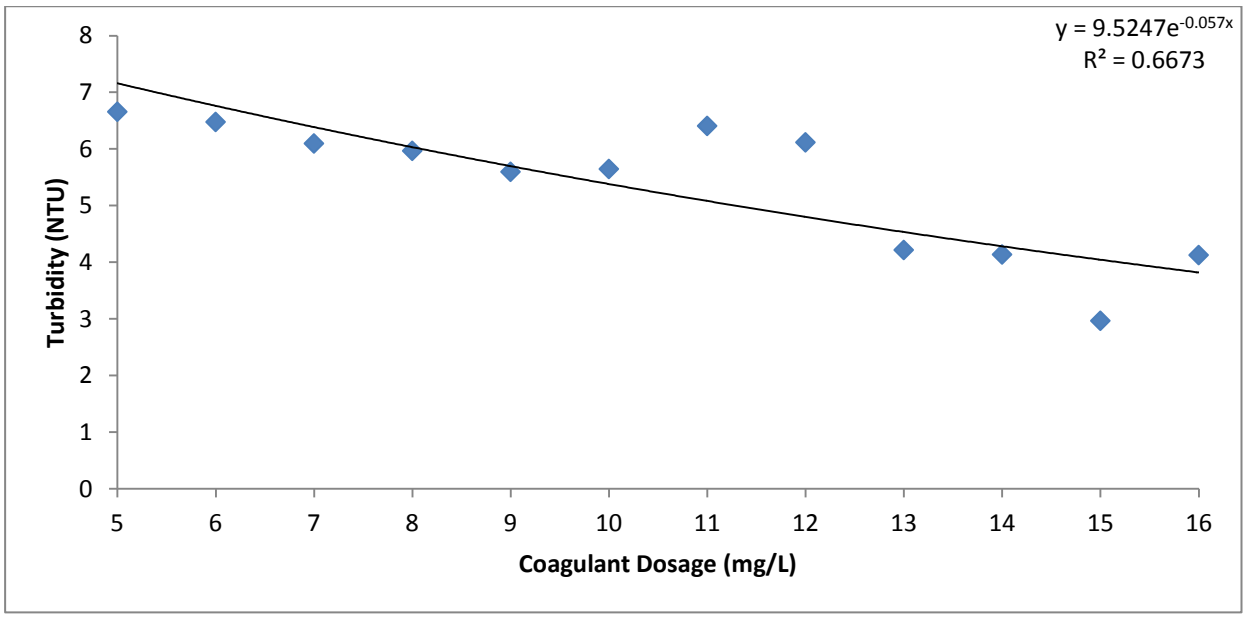

Figure B.48: Regression analysis between turbidity and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 22/02/2011.

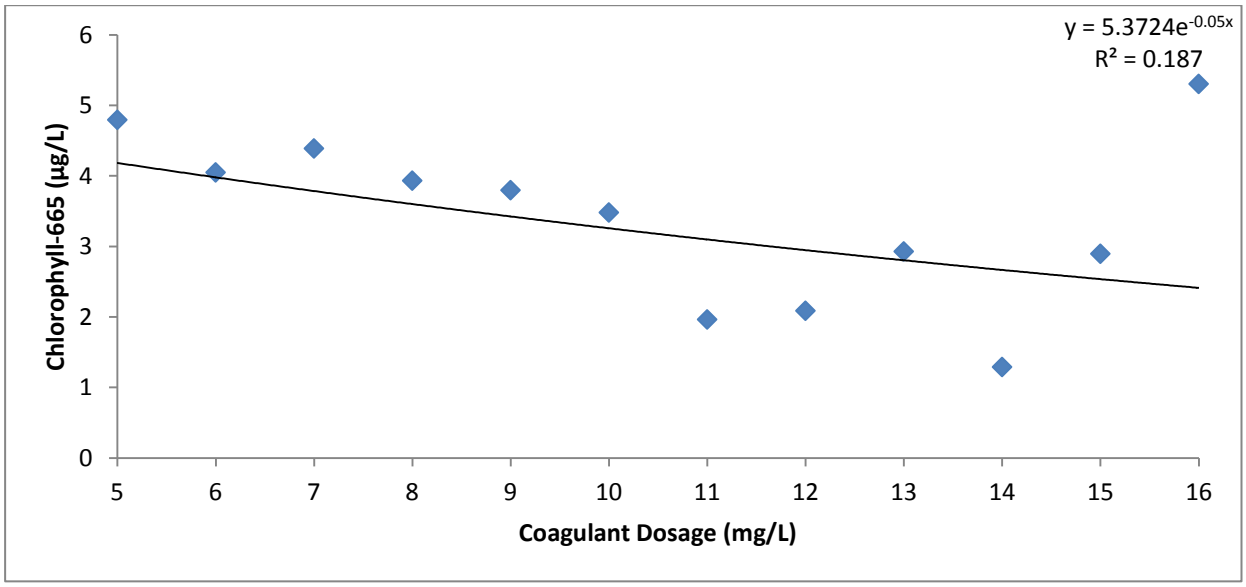

Figure B.49: Regression analysis between chlorophyll-665 and poly-electrolyte at concentrations of 5 - 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 22/02/2011.

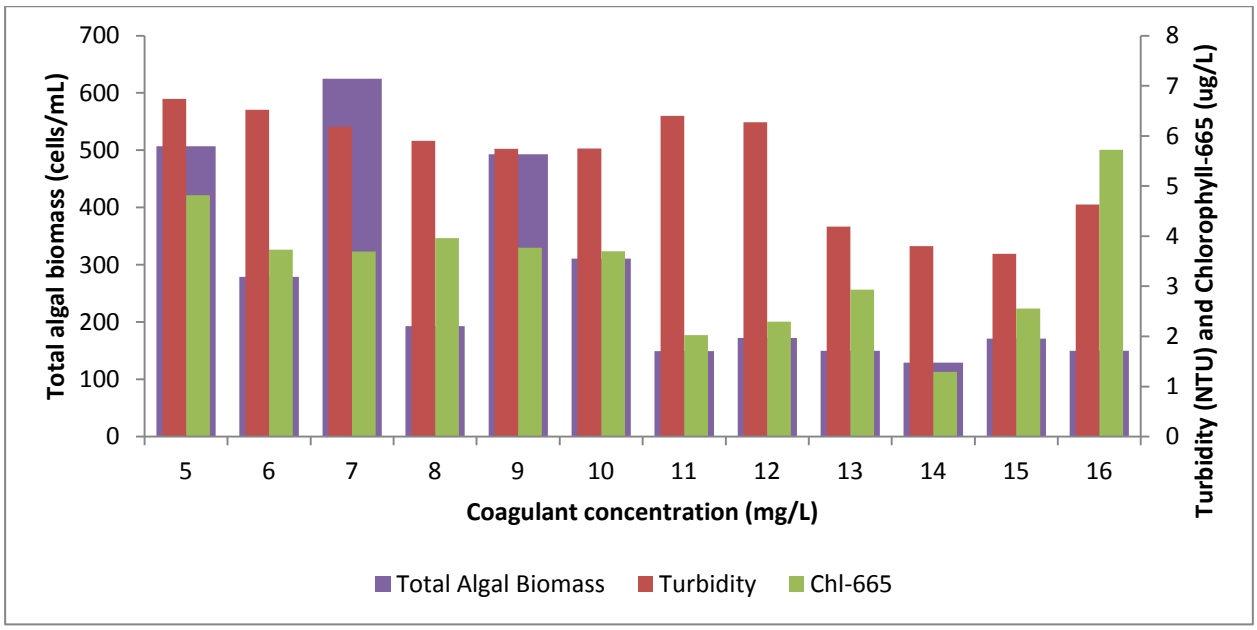

Figure B.50: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when poly-electrolyte was dosed at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 21/02/2011.

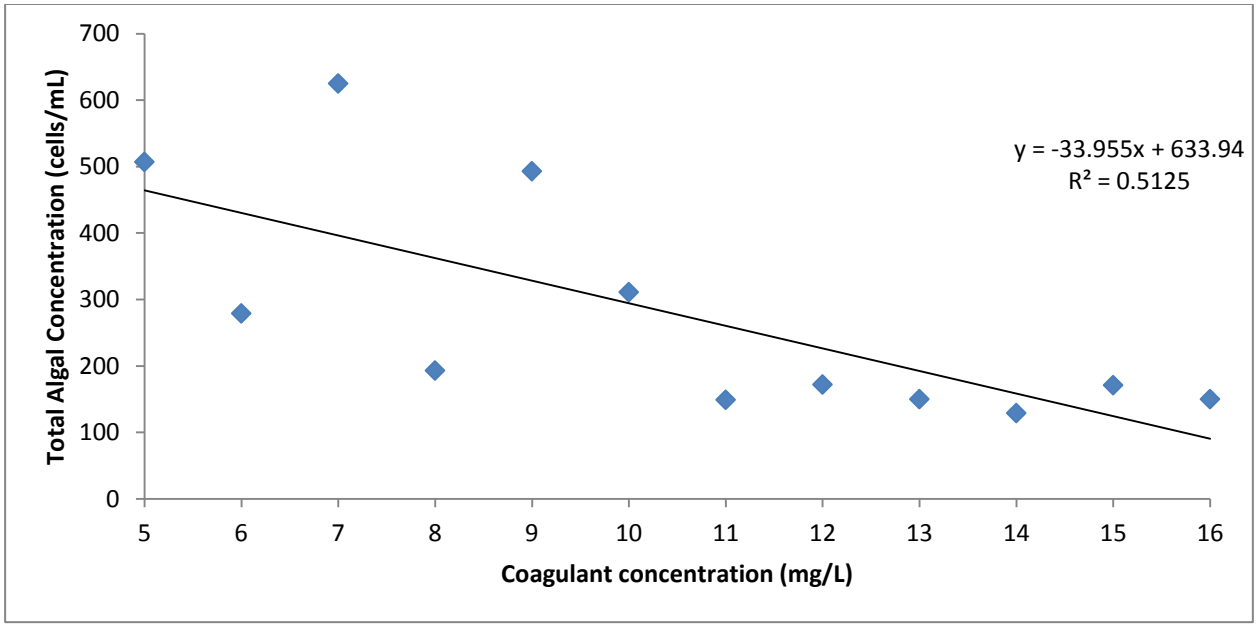

Figure B.51: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 21/02/2011.

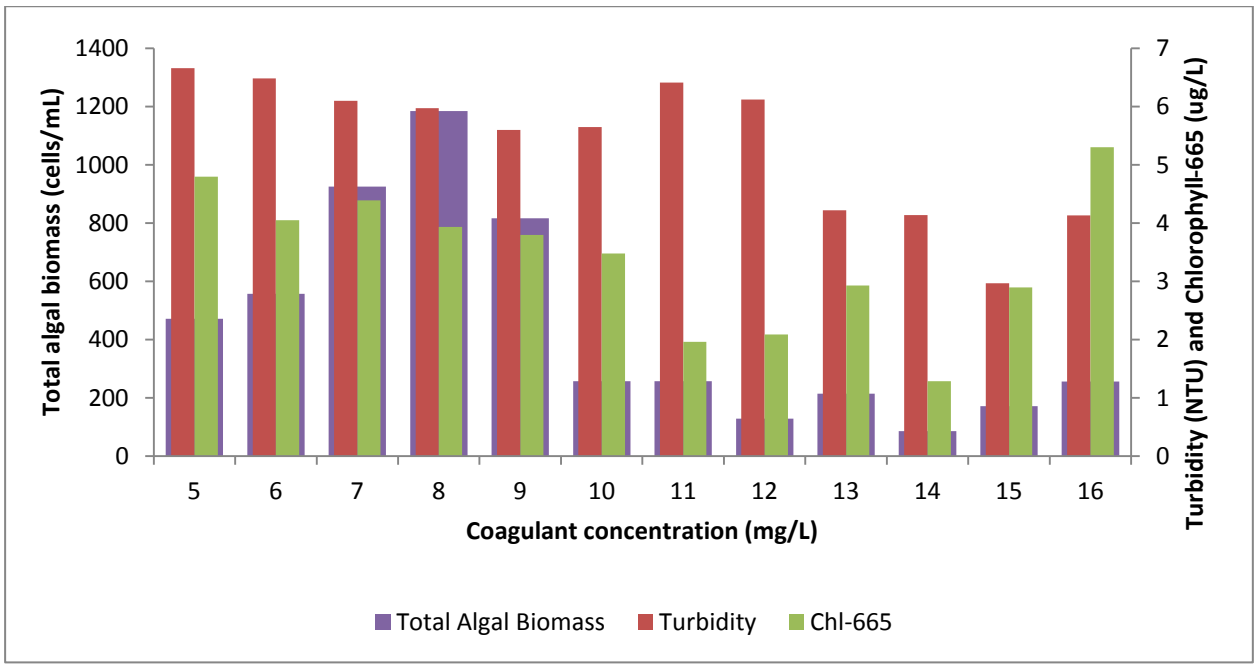

Figure B.52: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when poly-electrolyte was dosed at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 22/02/2011.

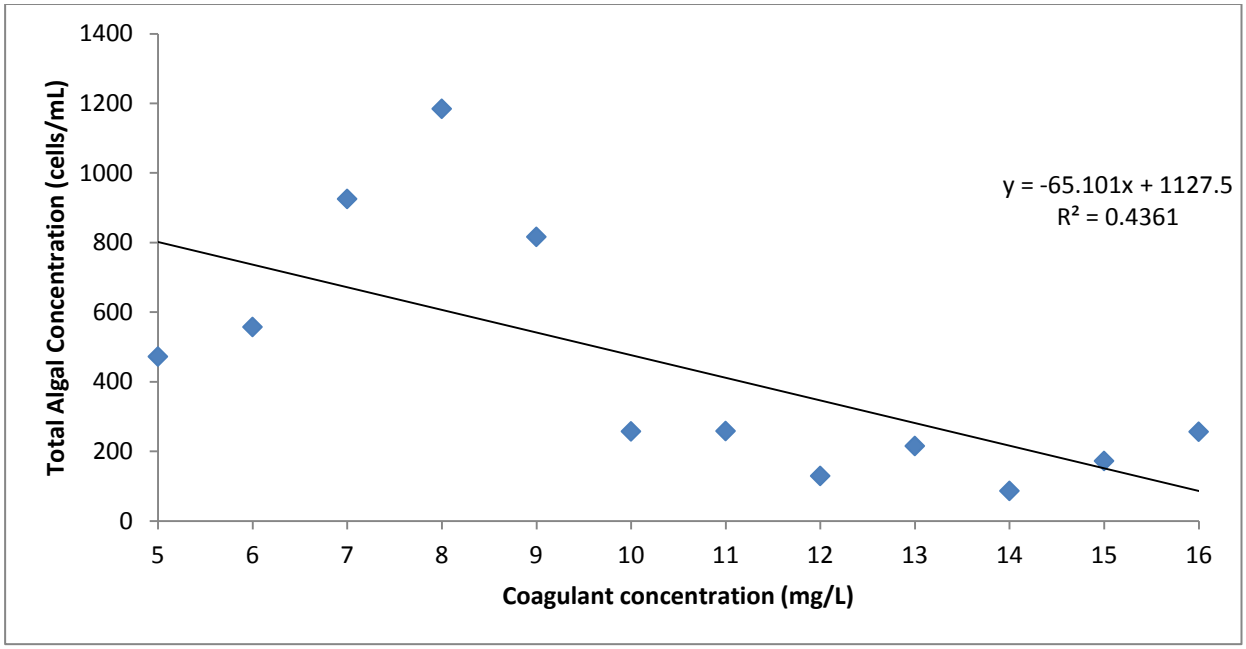

Figure B.53: Regression analysis between total algal concentration and poly-electrolyte at concentrations of 5 – 16 mg/L in combination with 10 mg/L CaO for Rietvlei Dam on 22/02/2011.

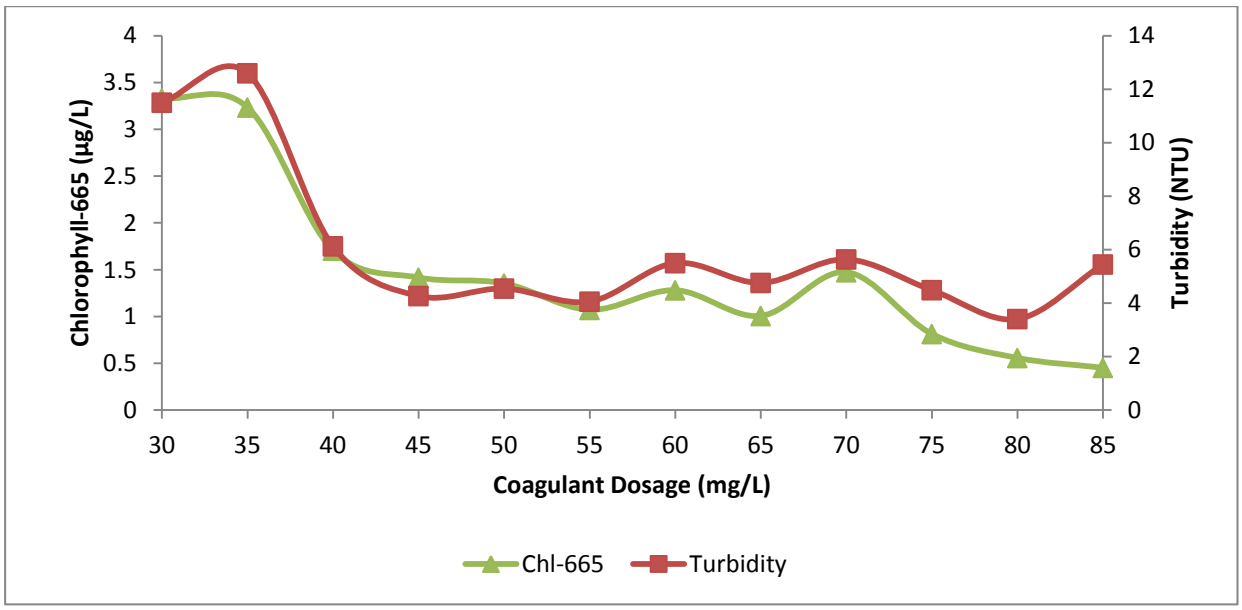

Figure B.54: The comparison between turbidity and chlorophyll-665 in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 21/02/2011.

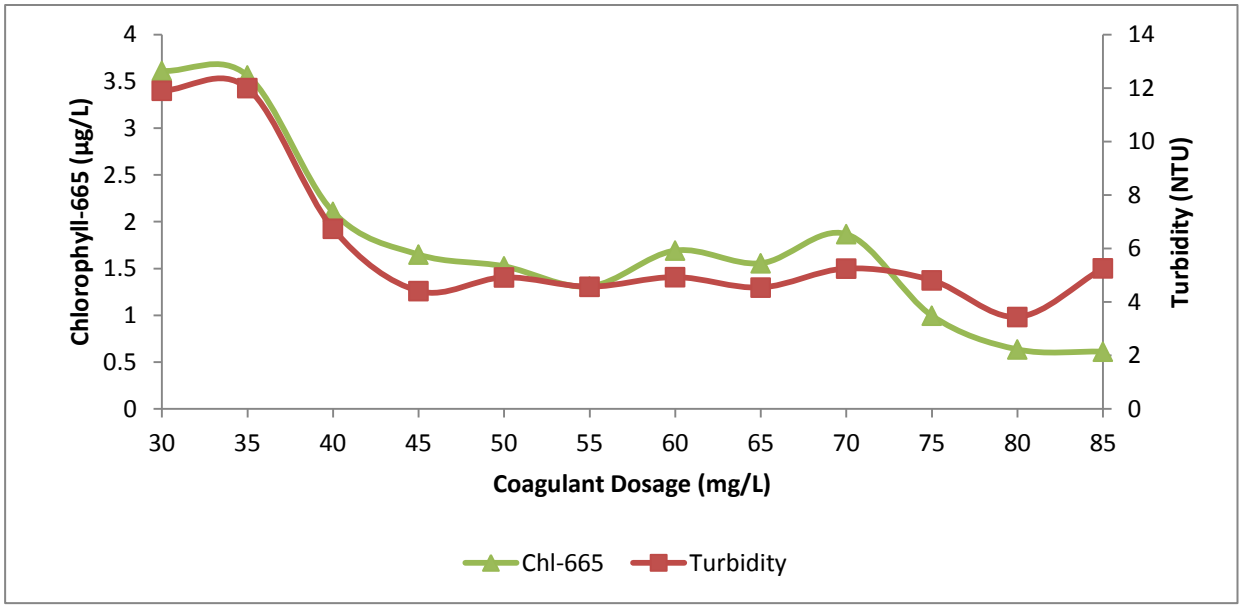

Figure B.55: The comparison between turbidity and chlorophyll-665 in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 22/02/2011.

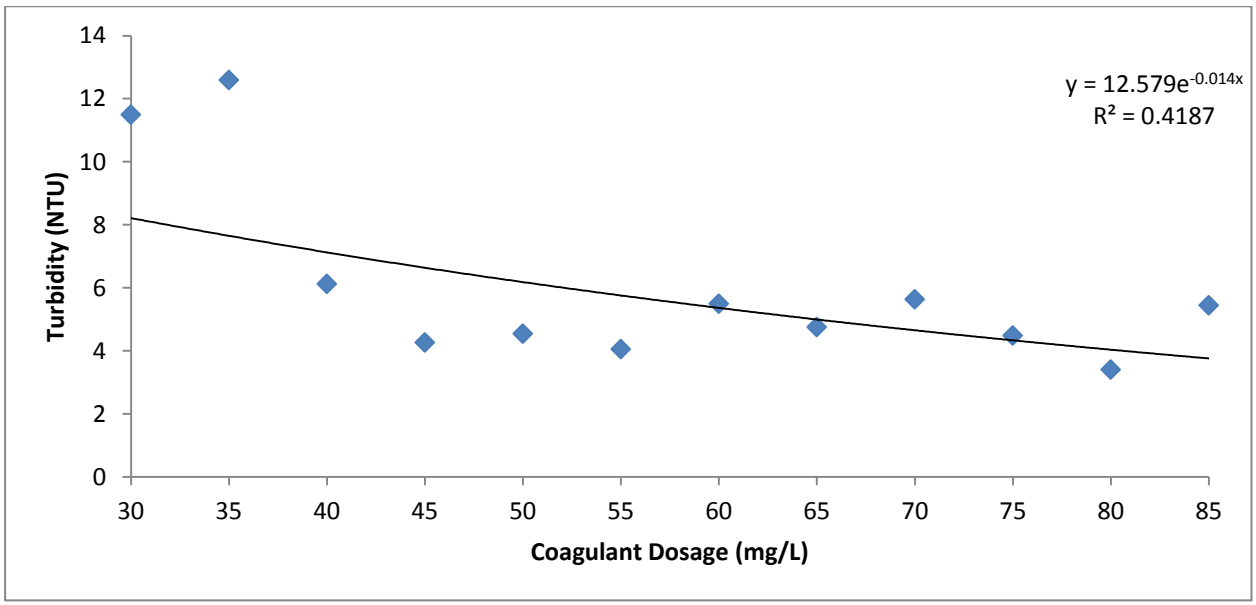

Figure B.56: Regression analysis between turbidity and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for Rietvlei Dam on 21/02/2011.

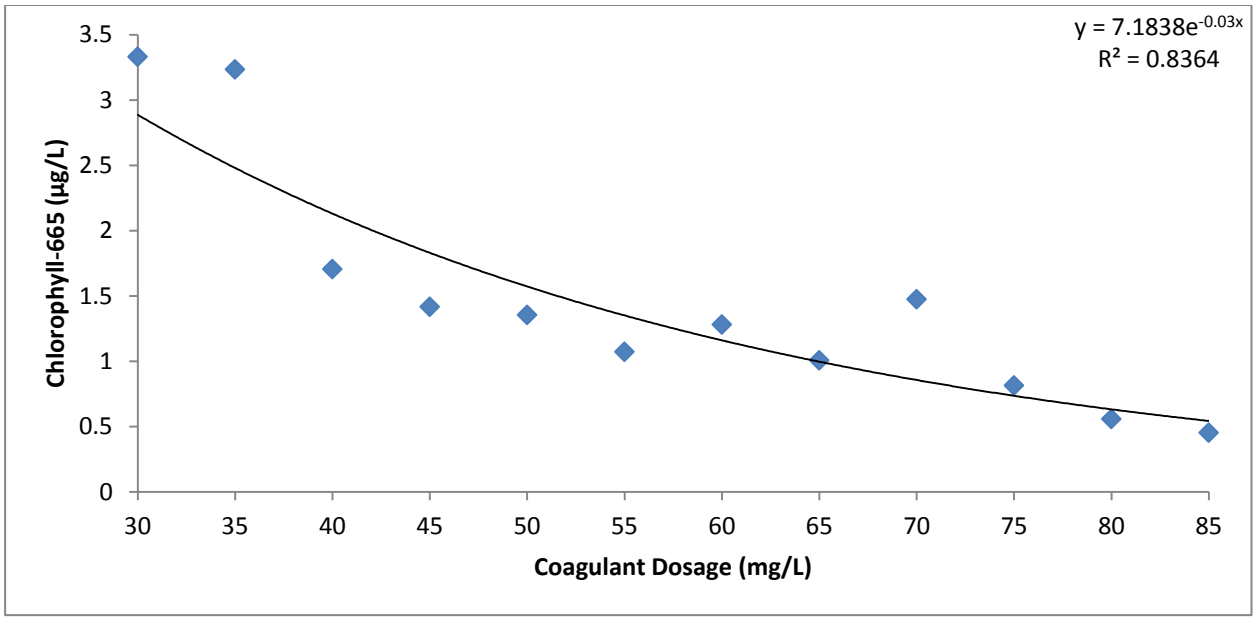

Figure B.57: Regression analysis between chlorophyll-665 and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for Rietvlei Dam on 21/02/2011.

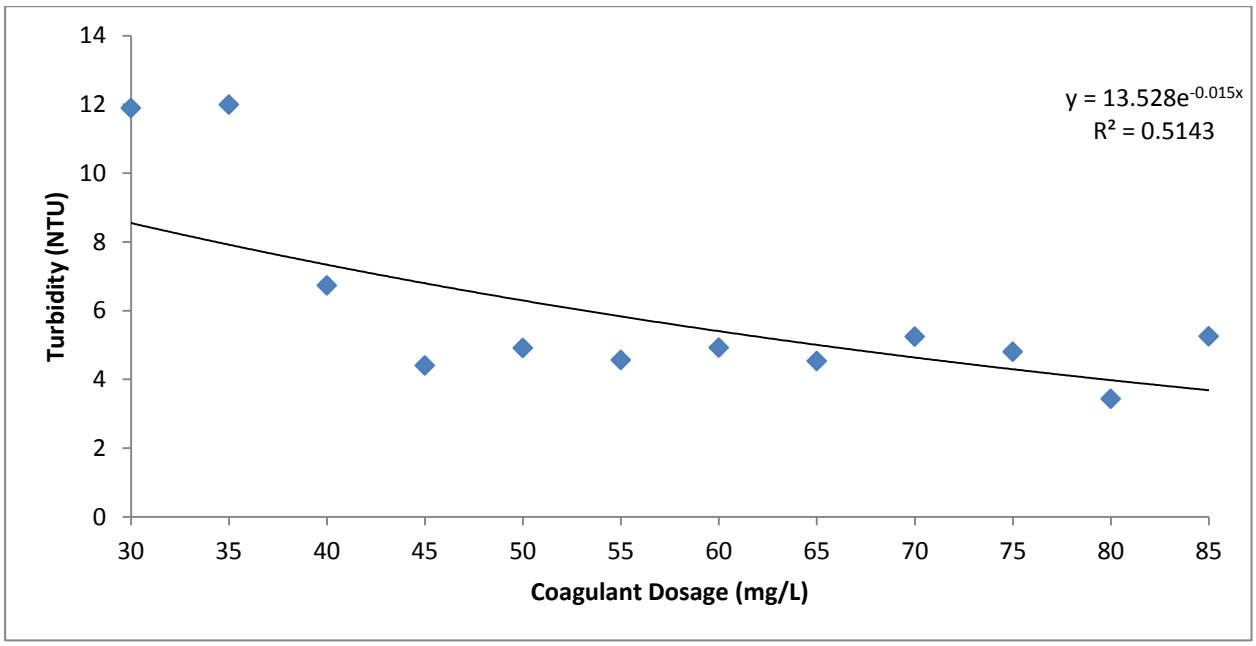

Figure B.58: Regression analysis between turbidity and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for Rietvlei Dam on 22/02/2011.

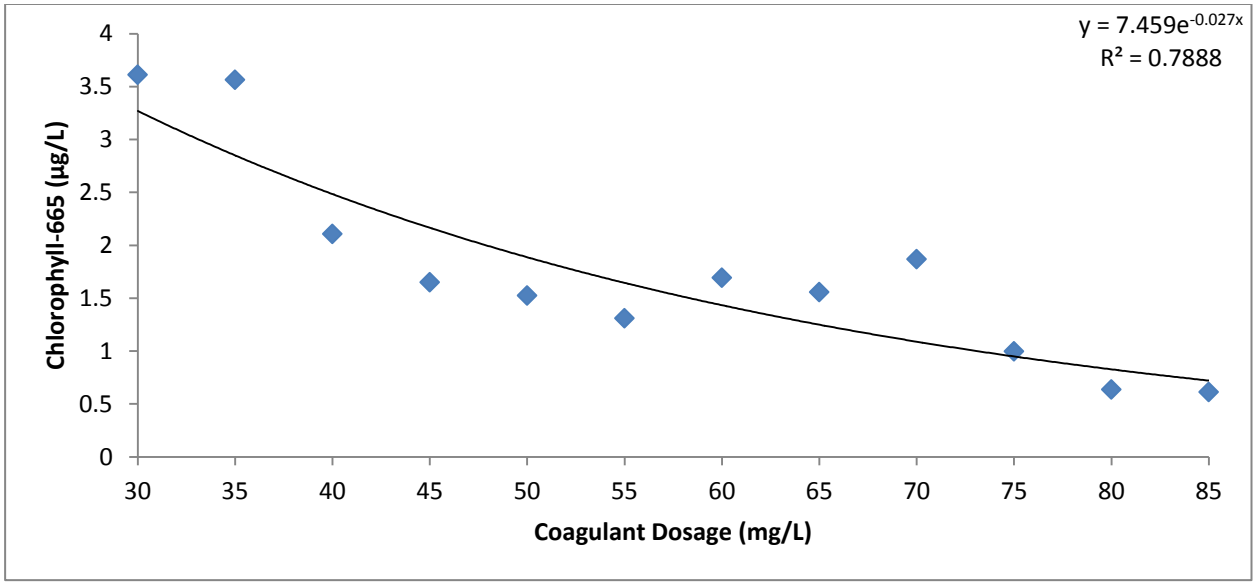

Figure B.59: Regression analysis between chlorophyll-665 and coagulant dosage when different concentrations of CaO in combination were dosed with 2.5 mg/L activated silica for Rietvlei Dam on 22/02/2011.

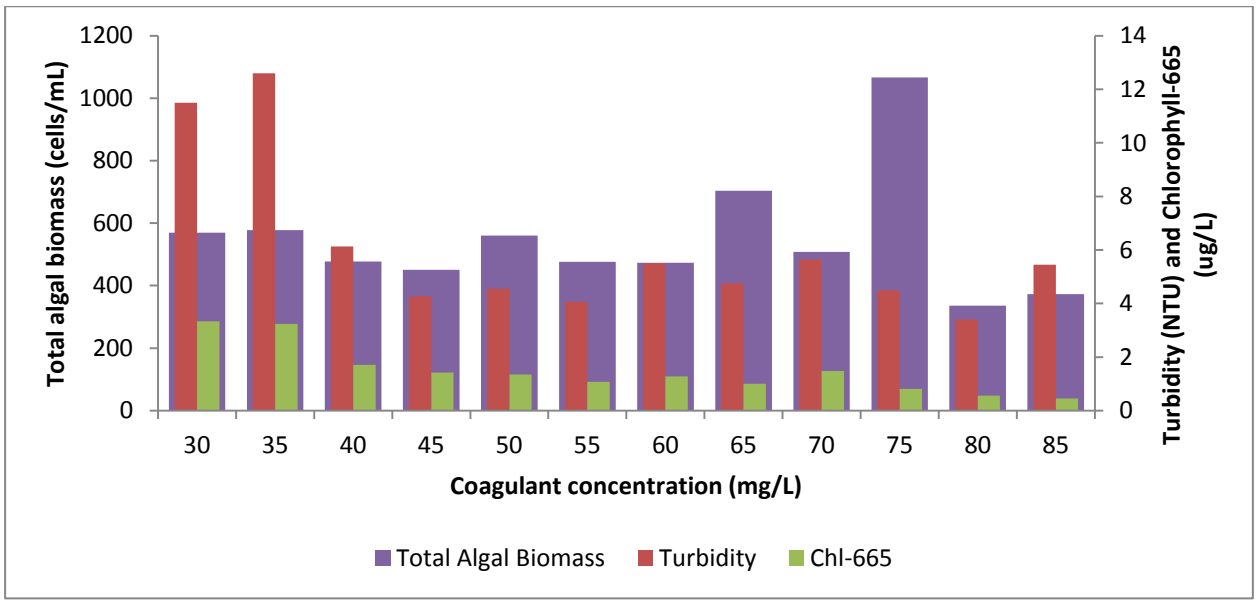

Figure B.60: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 21/02/2011.

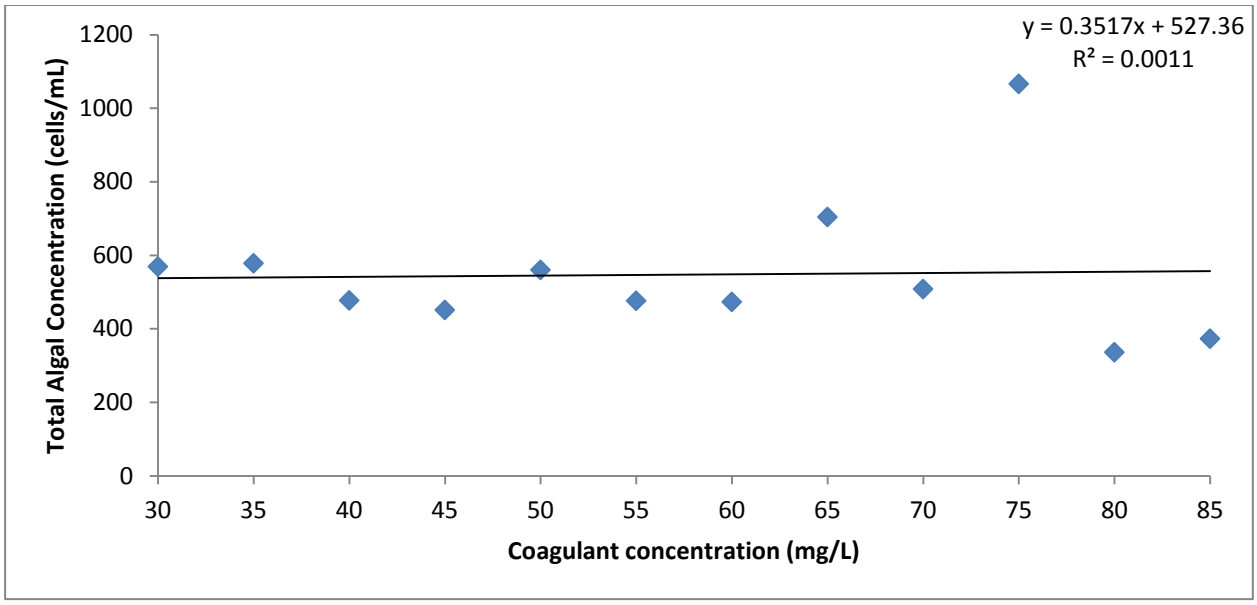

Figure B.61: Regression analysis between total algal concentration and coagulant dosage when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 21/02/2011.

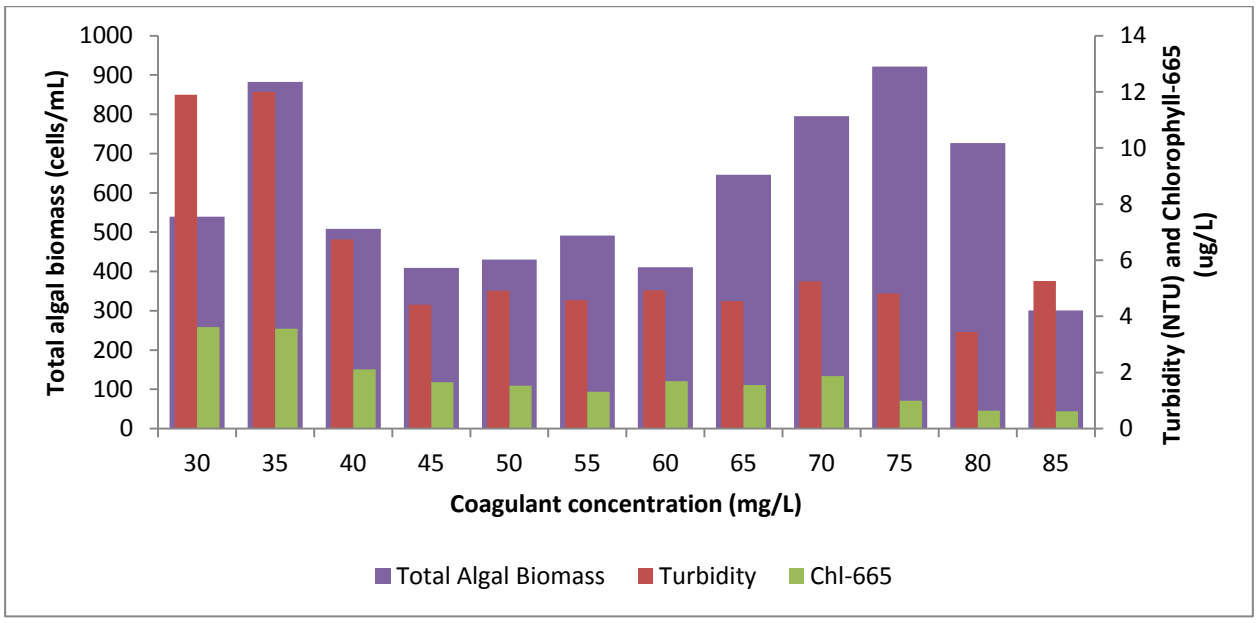

Figure B.62: Comparison between turbidity, chlorophyll-665 and total algal biomass in the supernatant when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 22/02/2011.

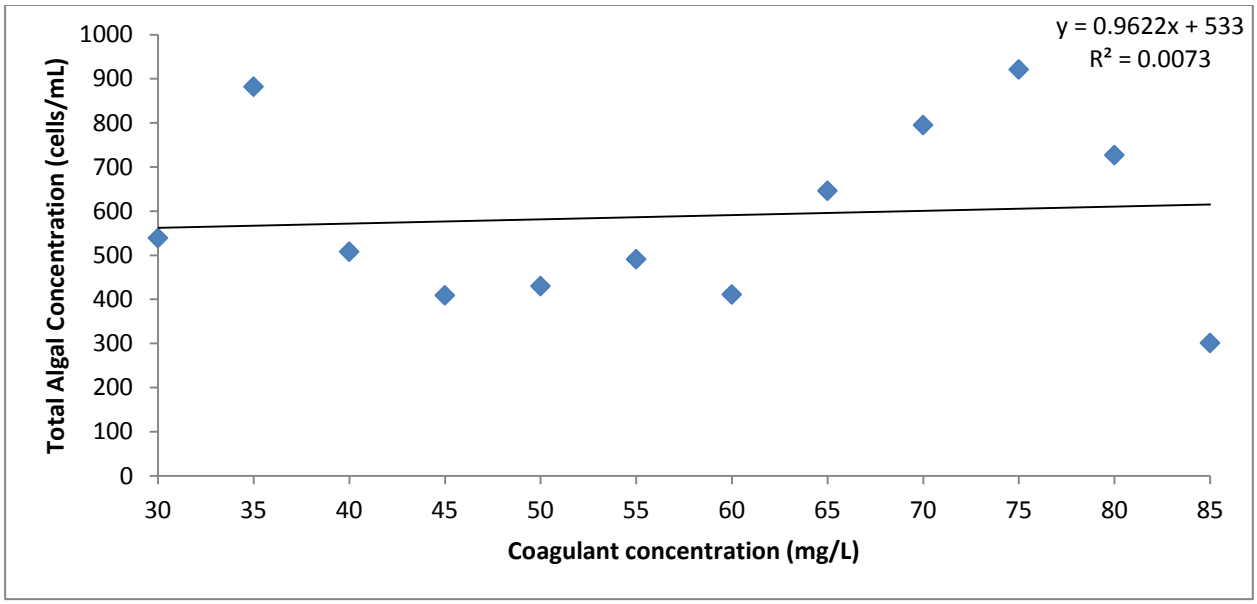

Figure B.63: Regression analysis between total algal concentration and coagulant dosage when different concentrations of CaO were dosed in combination with 2.5 mg/L activated silica for Rietvlei Dam on 22/02/2011.