AN ASSESSMENT OF THE TOXICITY OF CYANOBACTERIA IN THE KRUGER NATIONAL PARK

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

It is possible that significant numbers of wild animals succumb to cyanobacterial poisoning every year as no formal data on mortality are recorded. The major problem in diagnosis of cyanobacterial poisoning in wild animals is that carcasses of these animals are usually found decomposed or partially consumed by scavengers and therefore the cause of death can seldom be established.

The lack of continuous assessment of the surface waters for cyanobacteria and their toxins in the Kruger National Park (KNP) is a major challenge. Assessment of the surface water for cyanotoxins occurs only when there are deaths of wild animals in the Park, and sometimes the levels of the cyanobacterial toxins of the nearby rivers/dams to which the animals were exposed, may have changed completely by the time of sampling, thus not reflecting the toxin levels that may have caused mortality.

Unaccountable mortalities were observed by the section ranger in the vicinity of Mpanamana and Nhlanganzwane dams in the south-eastern corner of the KNP during the autumn and early winter of 2005. At both of these dams a heavy algal bloom was visible, and water samples showed the presence of *Microcystis*. Carcasses that were found around these dams included ruminants, hind-gut digesters and predators. Several zebra (*Equus burchelli*) deaths along the Shiloweni Dam in the KNP were also reported (Bengis et al., 2006). It has been postulated that the high concentration of hippopotamus (*Hippopotamus amphibius)* influence the formation of the algal blooms in the dams because the hippo dung and urine lead to high levels of nitrates and phosphates in the water. The algal scum usually floats around the edges of the water and animals that wade into the water beyond the scum line, such as elephants (*Loxodonta africana*) and buffalo (*Syncerus caffer*), are less affected by the algal scum. However, animals that drink on the edges of the dams such as rhino (*Ceratotherium simum*), wildebeest (*Connochaetes gnou*) and zebra (*Equus burchelli*) are the more frequent victims.

Death of wild animals due to cyanobacteria has a negative impact on the growing economy of South Africa, as ecotourism relies on wildlife as the main tourist attraction. Early detection of cyanobacteria and their toxins in water may facilitate appropriate actions by the KNP authorities that mitigate the possible impacts of cyanotoxins on animals.

The KNP has implemented a monitoring programme to reduce the risk of further animal deaths caused by blue-green algal blooms in the man-made dams in the Park. The ARC-OVI, Toxicology Section was approached by Veterinarians at the Park to start a surveillance study for cyanobacteria and their toxins, following the deaths of wild animals, close to algal blooms in specified dams. It is hoped that the results obtained from this study will contribute to the understanding of the current status on cyanobacteria and their toxins, and also the main factors involved in promoting eutrophication at the KNP dams.

OBJECTIVES

The main aim of this project was to generate information (database) on the extent to which cyanobacteria (bluegreen algae) and their toxins affect wildlife in the South African game reserve (Kruger National Park). This was done by: (i) Identifying cyanobacteria present in dams in the Kruger National Park, and (ii) Assessing wildlife exposure and death due to cyanotoxins in the Kruger National Park by examining carcasses.

RESULTS AND CONCLUSIONS

Water samples were collected from 13 KNP dams, namely, Sunset Dam, Shiloweni Dam, Piet Grobler Dam, Mazithi Dam, Nyamundwa Dam, Shitlhave Dam, Mestel Dam, Kumana Dam, Transport Dam, Mpondo Dam, N'watindlopfu Dam, Mpanamana Dam and Nsemane Dam, between 2009-2011 (June 2009, September 2009, February 2010, July 2010, October 2010 and February 2011). The following physicochemical parameters were determined for each dam throughout the study period: algal biomass, water temperature and pH, dissolved oxygen, chlorophyll-*a*, total phosphorus, total nitrogen, turbidity, total dissolved solids and microcystin toxin levels. The strip test, ELISA and HPLC methods were used in the determination of microcystin toxin levels.

The phytoplankton of all 13 KNP dams consisted of a total of 42 taxa in June, 37 taxa in September 2009, 43 taxa in February 2010, 36 taxa in July 2010 and February 2011, and 34 taxa in October 2010 all belonging to Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae and Chrysophyceae divisions. The highest cyanobacteria cell numbers were observed at Nsemane Dam (about 4.4×10^7 cells/mℓ) in June 2009. Microcystin-producing algae were detected in some of the 13 KNP dams throughout the study period. *Microcystis aeruginosa* was the most dominant cyanophyceae, especially during the incidents of bloom formation. Algal blooms were experienced mostly at the Sunset, Shiloweni and Nsemane dams during the study period.

The water temperature recorded for all the 13 KNP dams ranged between 15.5 and 36.1°C with the lowest temperature determined at Nyamundwa and Kumana dams in June 2009 and July 2010, respectively, and the highest temperature determined at N'watindlopfu Dam in October 2010. The water pH recorded for all the 13 KNP dams ranged between 6.5 and 9.0 with the lowest pH determined at Mestel Dam in October 2010 and the highest pH determined at Transport Dam in September 2009. Although the temperature and pH levels recorded at the KNP dams were favourable for promoting the growth of cyanobacteria, no linear correlation was observed between the cyanobacteria biomass and the two parameters.

The dissolved oxygen values recorded for all the 13 KNP dams ranged between 0.1 and 13.6 mg/ℓ with the lowest dissolved oxygen measured at Shiloweni Dam in February 2010 and the highest dissolved oxygen measured at Nyamundwa Dam in September 2009. The chlorophyll-*a* values recorded for all the 13 KNP dams were between 1.0 and 44710 µg/ℓ with the highest chlorophyll-*a* values determined at Sunset Dam in September 2009. Chlorophyll-*a*, which is a biomass-related variable, was positively correlated to the cyanobacteria cell numbers.

The total phosphorus and nitrogen levels reported in this study are among the highest levels recorded in South Africa. The total phosphorus levels recorded for all the 13 KNP dams ranged between 0.2 and 155 mg/ℓ with the highest phosphorus levels determined at Sunset Dam in February 2010. The total nitrogen levels recorded for all the 13 KNP dams ranged between 0.92 and 1870 mg/ℓ with the highest nitrogen levels determined at Sunset Dam in February 2010. A positive correlation was observed between the cyanobacteria biomass and the nutrients (total phosphorus and nitrogen). This shows that nutrient supply is the main driving factor in promoting the growth of cyanobacteria at the KNP dams. It is postulated that nutrient supply is influenced by the urine and faecal material excreted by the high population of Hippopotami living in most of the KNP dams.

Results of the qualitative determination of microcystins using the ABRAXIS strip test showed that 60% of the 13 specific KNP dams sampled in June 2009 were positive for microcystins, whereas 55% in September 2009; 85% in February 2010; 62% in July 2010 and 82% in October 2010 were also positive for microcystins. All the 13 KNP dams sampled in February 2011 were negative for microcystins. The microcystin toxin levels recorded for all the 13 KNP dams using the ELISA ranged between 0.10 ng/mℓ and 33645.29 ng/mℓ with the highest microcystin levels determined at Sunset Dam in September 2009. The highest microcystin toxin levels were detected (ELISA) at the KNP dams in the following decreasing

order over the sampling period: September 2009 > June 2009 > July 2010 > February 2010 > October 2010 > February 2011. The strip test results were comparable to the ELISA results with only 3.1% overall disagreement observed between the two immunoassays.

The presence of the seven microcystin toxins variants, -RR, -YR, -LR, -LA, -LY, -LW and -LF, was found to vary between the different 13 KNP dams and the sampling periods when HPLC was used. The microcystin toxin levels recorded for all the 13 KNP dams using the HPLC ranged between 0.02 and 1954.03 ng/mℓ with the highest microcystin levels determined at Shiloweni Dam in June 2009. The highest microcystin toxin variants were detected (HPLC) at the KNP dams in the following decreasing order over the sampling period: June 2009 > February 2010 > September 2009 > February 2011 > July 2010 > October 2010. The highest microcystin toxin variants were recorded at Shiloweni Dam, in June 2009, in the following decreasing order: -YR > -LR > -LA > -LF > -LY > -RR.

A significant positive linear correlation was found between the cyanobacteria biomass and the total microcystin for all the 13 KNP dams during this study when using the ELISA and HPLC methods. The HPLC method was found to be more sensitive than the immunoassays when quantifying microcystin toxins. However, the HPLC method was limited in its quantification only to the seven microcystin variants, whereas the immunoassays detected all the microcystin variants and nodularin toxins.

Dissolved oxygen, which is one of the major physical water quality variables, was negatively correlated to the cyanobacteria biomass. There was no correlation between the total dissolved solids and the cyanobacteria biomass. It seems that the size of the KNP dams did not influence the cyanobacteria cell numbers and microcystin levels because no correlation was found between the size of the KNP dams and the two parameters.

During this study, a number of carcasses were found within the vicinity of Shiloweni Dam in June 2010 and they included 11 zebras (*Equus burchelli*), 2 rhinos (*Ceratotherium simum*) and 1 wildebeest (*Connochaetes taurinus*). The water samples collected from Shiloweni Dam in June 2010 had high algal concentrations of 4.61×10^5 cells/mℓ, which were dominated by *Microcystis aeruginosa*, and high microcystin levels (ELISA, 4.06×10^5 ng/mℓ). Post mortem and histopathology performed on the intact zebra carcasses revealed signs (hepatic haemorrhaging and necrosis) that are consistent with *Microcystis* bloom intoxication.

RECOMMENDATIONS FOR FUTURE RESEARCH

The following actions are recommended:

- Continuous monitoring of the KNP dams to ensure that proper preventive actions are taken to prevent poisoning of the wild animals.

- The use of dipstick immunoassays (e.g. ABRAXIS Strip Test kit or Agdia ImmunoStrip kit) in monitoring the toxin levels in the KNP dams, because the dipsticks provide almost instant results and the users require no previous training.

- Control of eutrophication at the KNP should focus on maintaining the nutrients at low levels and also controlling the Hippopotamus population.

- Future studies to focus on :
    - Using molecular techniques to further characterise the cyanobacteria at the KNP dams.
    - The role of Hippopotami in maintaining high levels of nutrients at the KNP dams and also the possible intervention strategies that could be used.
    - The extension of assessment of the toxicity of cyanobacteria to include the northern part of the KNP, because the current study focused only on the southern part of the KNP.

CAPACITY BUILDING

This project has formed the basis of an MTech degree (RG Matshoga) and skills development in the HPLC technique (RG Matshoga and MG Masango).

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ARC	Agricultural Research Council
Chl	Chlorophyll-*a*
DO	Dissolved Oxygen
DoAF	Department of Agriculture and Fisheries
DWAF	Department of Water Affairs and Forestry
ELISA	Enzyme-Linked ImmunoSorbent Assay
HPLC	High Performance Liquid Chromatography
KNP	Kruger National Park
MC-LR	Microcystin-LR
OVI	Onderstepoort Veterinary Institute
PP1	Protein Phosphatase 1
PP2A	Protein Phosphatase 2A
SANParks	South African National Parks
TDS	Total Dissolved Solids
Temp	Temperature
TN	Total Nitrogen
TP	Total Phosphorus
Turb	Turbidity
UV	Ultraviolet

1 BACKGROUND

1.1 General introduction

This project is the first extensive study in the Kruger National Park to investigate the identity and abundance of cyanobacteria and their toxins, factors driving the growth of cyanobacteria, and the role of cyanotoxins in the wild animal mortality incidents. This study covered both the winter and summer seasons from June 2009 to February 2011 and included 13 specific man-made dams in the southern part of the KNP. The use of microcystin strip test kits (Abraxis product) as the primary screening tool for microcystins and nodularins in the field was also evaluated and the results were compared with those of the other more technically advanced analytical detection methods (ELISA and HPLC).

The limitations of this project relate to the sampling strategy and sampling frequency. For the sampling strategy, only surface grab samples were collected from the edges of the specific dams. This was influenced by the location and design of the dams, which made it difficult to access the dams, as well as the presence of dangerous animals (crocodiles, hippos, etc.) inside and around the dams which posed significant risks during sample collection. In respect of sampling frequency, only six field samplings (June 2009, September 2009, February 2010, July 2010, October 2010 and February 2011) were undertaken and more field samplings would have provided a much greater perspective on the data presented in this report. The field samplings were influenced by the costs and other logistics such as the availability of game guards and accommodation at the KNP.

1.2 Target audience

It is hoped that this report will, in some way, contribute to and broaden our understanding on the current status of cyanobacteria and eutrophication and its role in the wild animal deaths in the KNP. This report is targeted at the following readers:

- South African National Parks (SANParks)
- Department of Water Affairs and Forestry (DWAF)
- Department of Agriculture and Fisheries (DoAF)
- Researchers
- Educators and trainers

1.3 Literature review

The review of literature presented in this report is not exhaustive but briefly highlights the most common cyanobacteria and their toxins; the eutrophication process and the factors driving it; how animals are exposed to cyanotoxins; and the methods commonly used in the detection and quantification of cyanotoxins.

For more detailed background information, readers are encouraged to consult the relevant references provided in the reference list of this report.

1.3.1 Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs and recreational waters has become a worldwide problem. Some of the cyanobacterial genera such as *Microcystis*, *Anabaena*, *Nostoc*, *Oscillatoria* and *Aphanizomenon* can produce a wide range of potent toxins, including a family of hepatotoxins called microcystins that comprise the most frequently encountered cyanotoxins in fresh water (**Table 1**) (Carmichael, 1992; Chorus & Bartram, 1999; Song et al., 2007).

Microcystins are cyclic heptapeptides (they contain seven peptide-linked amino acids) that share the common structure cyclo-(D-Ala^{1}-X^{2}-MeAsp^{3}-Z^{4}-Adda^{5}-D-Glu^{6}-Mdha^{7}), where X and Z are variable L-amino acids, D-Ala is D-Alanine, MeAsp is D-*erythro*-β-methylaspartic acid, Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, D-Glu is D-Glutamic acid and Mdha is *N*-methyl-dehydroalanine (**Figure 1**). The toxins are named according to the two L-amino acids at positions X and Z (e.g. microcystin-LR refers to leucine and arginine in the variables positions of this peptide). Currently there are over 80 structural variants of microcystin that have been characterised from field samples and isolated strains, and the structural variation has been reported most frequently in positions 2, 4 and 7 of the microcystin molecule (Dawson, 1998; Falconer & Humpage, 2005; Okello et al., 2010).

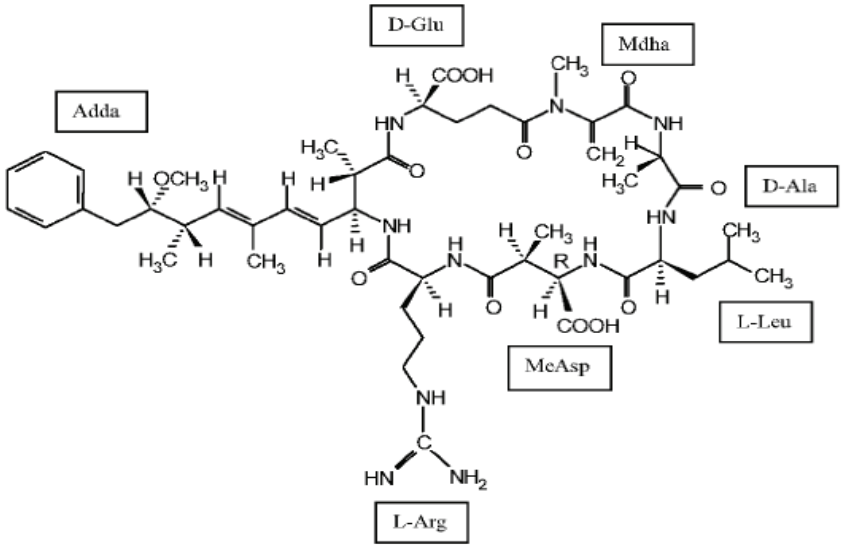


Figure 1. Chemical structure of microcystin-LR (MC-LR). D-Ala = D-Alanine; L-Leu = L-Leucine (L-amino acid); MeAsp = D-*erythro*-β-methylaspartic acid; L-Arg = L-Arginine (L-amino acid); Adda = 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; D-Glu = D-glutamic acid; Mdha = N-methyl-dehydroalanine (Mankiewicz et al., 2003).

Table 1. Principal groups of cyanobacterial toxins and the cyanobacterial genera that produce them, their target and activity (Chorus & Bartram, 1999; Bláha et al., 2009).

Toxin	Cyanobacterial genera	Primary target organ in mammals	Activity
Hepatotoxins:			
Microcystins	*Microcystis*, *Anabaena*, *Planktothrix* (*Oscillatoria*), *Nostoc*, *Hapalosiphon*, *Anabaenopsis*	Liver	Protein phosphatase inhibition, tumour promoters
Nodularins	*Nodularia*	Liver	Protein phosphatase inhibition, tumour promoters, carcinogenic
Cylindrospermopsins	*Cylindrospermopsis*, *Umezakia*, *Aphanizomenon*, *Raphidiopsis*	Liver, kidneys, spleen, lungs	Protein synthesis inhibitor, genotoxic
Neurotoxins:			
Anatoxin-a	*Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermum*	Nerve synapse	Postsynaptic, depolarizing neuromuscular blockers

Anatoxin-a(S)	*Anabaena*	Nerve synapse	Acetylcholinesterase inhibitor
Saxitoxins	*Anabaena*, *Aphanizomenon*, *Lyngbya, Cylindrospermopsis*	Nerve axons	Sodium channel blockers
Dermatotoxins:			
Lyngbyatoxin-a	*Lyngbya, Schizotrix, Oscillatoria*	Skin, gastrointestinal tract	Inflammatory agent, protein kinase C activator
Aplysiatoxin	*Lyngbya, Schizotrix, Oscillatoria*	Skin	Inflammatory agent, protein kinase C activator
Endotoxins:			
Lipopolysaccharides	All cyanobacteria	Potential irritants, affects any exposed tissue	Inflammatory agent, gastrointestinal irritants

1.3.2 Factors affecting bloom formation

The effect that environmental factors have on the growth of cyanobacteria has been demonstrated in batch and continuous cultures (Sivonen, 1996). The existence of a direct correlation between the environmental factors and growth of cyanobacteria has also been observed in field studies (Robarts & Zohary, 1984; Wicks & Thiel, 1990). The environmental factors include temperature, pH, nutrients (nitrogen and phosphorus), light and buoyancy. Each of these factors alone may only partly determine bloom formation and it has been demonstrated that certain combinations of these factors may strongly favour the formation of a bloom (Oberholster et al., 2004).

1.3.2.1 Temperature

Cellular processes of many phytoplanktons are dependent on temperature with their rates accelerating exponentially when the temperature increases. For optimal growth, cyanobacteria prefer warmer conditions with temperatures ranging from 20°C to 30°C. The growth rate of *Microcystis* has been shown to be severely limited at temperatures below 15°C and optimum at temperatures above 25°C. *Oscillatoria* tolerates the widest range of temperature (Robarts & Zohary, 1987). A survey on the effects of temperature on algal growth rate demonstrated that as the temperature increased, the algal group with the highest growth rate changed from diatoms to green algae to blue-green algae (cyanobacteria) (Konopka & Brock, 1978). As a result, cyanobacteria are able to out-compete the other

species when subjected to high summer temperature conditions. This also explains why in temperate water bodies during summer most cyanobacteria form blooms (Msagati et al., 2006).

1.3.2.2 Salinity and pH

Salinity influences the physiology of cyanobacteria and has the potential to disturb ion balance or induce nutrient deficiencies. The pH levels are related to nutrient dissolution which can cause a change in the species composition and biomass of the phytoplankton. Wangwibulkit et al. (2008) showed under laboratory conditions that the optimal salinity levels for growth of *Oscillatoria* sp. ranged from 0-20 ppt and from 0-6 ppt for *Microcystis* sp. Decreased growth rates were observed at high salinity levels for both species. Cyanobacteria favour a pH range that is between 6 and 9 for their optimal growth (Wicks & Thiel, 1990). Wangwibulkit et al. (2008) showed that water pH levels from 7.5 to 9.0 were suitable for *Oscillatoria* and *Microcystis* growth, while pH levels above or below this range caused a significant decrease in growth of these cyanobacteria (pH at these levels affects the morphology of the cyanobacteria).

1.3.2.3 Nutrients

Nutrients such as phosphorus and nitrogen are essential for the growth of cyanobacteria. Phosphorous exists in a wide variety of forms in natural waters both in dissolved and particulate phases, and in inorganic and organic forms, including biomass (Jarvie et al., 2002). Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) are the most common ionic forms of dissolved inorganic nitrogen found in aquatic ecosystems (Carmargo & Alonso, 2006). Cyanobacteria have been shown to have higher affinity for nitrogen or phosphorus than other photosynthetic organisms (Metting & Pyne, 1986; Kaebernick et al., 2001), meaning that they can survive better than other phytoplanktonic organisms under limiting conditions of phosphorus or nitrogen (Msagati et al., 2006). Downing et al. (2005) have demonstrated that phosphorus is the growth limiting nutrient for *Microcystis aeruginosa* at high N:P ratios. *Microcystis* blooms were found to be associated with higher N:P ratios in the Huron River (Lehman, 2007).

1.3.2.4 Light and buoyancy

Cyanobacteria contain chlorophyll-*a* and other pigments, such as phycobiliproteins, phycocyanin and phycoerythrine, which are used for harvesting light and conducting photosynthesis. These pigments are able to harvest light in the spectrum (green, yellow and

orange) that is hardly used by other phytoplankton. The phycobiliproteins with chlorophyll-*a* enable cyanobacteria to harvest light energy efficiently and to live in an environment with only green light. Cyanobacteria have a low maintenance constant, meaning that they require little energy to maintain cell function and structure; as a result they can maintain a relatively higher growth rate than other phytoplankton when light intensities are low (Chorus & Bartram, 1999).

Movement of cyanobacteria within the water column is due to the presence of specialised gas-filled vesicles which give them a lower density than that of water, thus making them buoyant (Walsby et al., 2006). Cyanobacteria which are able to form surface blooms seem to have a higher tolerance for high light intensities. High light intensities increase the cellular iron intake, since Fe^{3+} is converted to Fe^{2+} by light before it is transported into algal cells, which may be responsible for the higher growth rate of cyanobacteria (Sunda et al., 1991; Msagati et al., 2006).

1.3.3 Toxin production

Cyanobacteria may produce several toxins simultaneously and a single cyanobacterial strain is capable of producing more than one type of microcystin. Although many cyanobacterial strains produce several microcystins simultaneously, usually only one or two microcystins are dominant in any single strain (Sivonen, 1996). All *Oscillatoria*, *Nodularia* and some *Microcystis* seem to produce one major microcystin toxin. *Microcystis*, *Anabaena* and *Oscillatoria* produce the same microcystins (Sivonen et al., 1989; Sivonen et al., 1992). Simultaneous neurotoxin and hepatotoxin production has been observed in *Anabaena flos-aquae* strain NRC 525-17 which produces anatoxin-a(s) and several microcystins (Harada et al., 1991). In natural samples, which usually contain many strains or more than one toxin-producing species, different combinations of microcystins can be found (Chorus & Bartram, 1999).

The climate condition of a region determines the timing and duration of the bloom season of cyanobacteria. Mass occurrences of cyanobacteria are most prominent during the late summer and early autumn and may last two to four months in temperate zones. The bloom season may start early and persist longer in regions with more subtropical climates (Kotak et al., 1995; Vezie et al., 1998). The toxigenic and non-toxigenic strains of cyanobacteria have been found to exist together when grown in the laboratory (Bolch et al., 1997).

1.3.4 Mechanism of action of microcystins

Microcystins (and nodularins) are water soluble and most of them are unable to directly penetrate the lipid membranes of animals, plants and bacterial cells. Therefore, in order to elicit their toxicity, uptake into the cells occurs through the carrier-mediated transport system known as the bile acid transporters. As a result, toxicity of microcystins is restricted to organs expressing the bile acid transporter on their cell membranes, such as the liver (Falconer et al., 1981; Eriksson et al., 1990; Honkanen et al., 1990).

Uptake of microcystins into the liver cells is followed by the inhibition of serine/threonine protein phosphatases type 1 and 2A (PP1 and PP2A) enzymes. The consequent protein phosphatase imbalance results in the cytoskeleton disruption of the cells, which then leads to the massive intrahepatic haemorrhage of the cells. This is characterised by the large increase in liver weight due to pooling of blood in the damaged liver cells (Honkanen et al., 1990; Carmichael, 1992).

1.3.5 Exposure of animals to cyanotoxins

Exposure of animals to cyanobacterial toxins occurs mainly through ingestion of contaminated waters. Cases of animal deaths due to cyanotoxin poisoning have been reported worldwide, including South Africa (Steyn, 1945; Bell & Codd, 1994; Kellerman et al., 2005; Oberholster et al., 2009; Masango et al., 2010). Ruminants, especially cattle, are more often affected probably because they do not hesitate to drink water that is covered with a thick cyanobacterial scum. Besides the consumption of cyanobacteria from water, it has been shown that an additional source of intoxication for terrestrial animals is cyanotoxins that have bioaccumulated in the food chain (Negri & Jones, 1995; Prepas et al., 1997).

Most cases of animal poisoning worldwide have been caused by the genera *Microcystis*, *Anabaena*, *Aphanizomenon*, *Nodularia*, *Nostoc* and *Oscillatoria*. Although these genera occur in South Africa, poisoning of animals is mainly due to *Microcystis aeruginosa* (Steyn, 1945; Soll & Williams, 1985: Harding et al., 1995; Van Halderen et al., 1995; Oberholster et al., 2009; Masango et al., 2010). In most cases of animal mortality, post mortem examinations reveal evidence of cyanobacterial ingestion as well as characteristic liver pathology. The other main cause of animal deaths due to cyanotoxins is from acute neurotoxicity leading to respiratory failure with no post mortem indications of organ injury (Gunn et al., 1992).

1.3.6 Methods for cyanotoxin detection

Two methods are generally used in the detection of cyanobacterial toxins, namely biological/biochemical screening assays and physicochemical methods. These methods differ in terms of principles of detection, information they provide and complexity of the set-up. Selection of technique depends on the availability of facilities and expertise as well as the type of information required. However, specificity and sensitivity of these techniques are important criteria that should be used to select the most reliable method (Chorus & Bartram, 1999; Msagati et al., 2006).

1.3.6.1 Biological/biochemical methods for cyanotoxin screening

Biological/biochemical screening methods commonly used for cyanotoxins include the mouse bioassay, in vitro (primary and continuous cell lines) assays, enzyme linked immunosorbent assay (ELISA), protein phosphatase inhibition (PPi) assay and dipsticks.

ELISAs are sensitive methods commonly used for detecting microcystins. Several kits are commercially available and easy to use. As with other biological assays, the ELISA reports the total microcystin content of a sample. The ELISA allows a simple assessment of risk and also eliminates the use of a wide range of analytical standards since individual microcystins are not identified. The ELISA has a detection limit lower than 1 µg/ℓ, a guideline set by the World Health Organisation (WHO) for drinking water, and can be performed without sample pre-concentration. Problems in the ELISA assay arise from the high structural variation of microcystins since the cross-reactivity of antibodies with different microcystin variants may be variable and does not always correlate with toxicity. Also the configuration of the 96-well plate makes it more suited to screening multiple samples at one time (An & Carmichael, 1994; Nagata et al., 1995; Rapala et al., 2002; Mountfort et al., 2005; Lawton et al., 2010).

Lateral flow dipsticks have been successfully used for a wide range of applications including the detection of drugs of abuse, pregnancy and many environmental contaminants. Dipsticks provide almost instant results and users require no previous training. The dipstick format of the ImmunoStrip® has been used successfully in the detection of microcystins (Lawton et al., 2010). This technique has been shown to be easy to perform in the field and provides a simple monitoring tool giving ‘real-time’ detection of microcystin toxins.

1.3.6.2 Physicochemical methods for cyanotoxin detection

These are analytical methods which use the physicochemical properties of cyanotoxins such as the presence of UV chromophores within their structures, molecular weights, and their reactivities due to specific functional groups present in their structures. Among the methods used are high performance liquid chromatography (HPLC), liquid chromatography/mass spectrophotometry (LC/MS), capillary electrophoresis (CE) and nuclear magnetic resonance (NMR) (Meriluoto, 1997). HPLC and LC/MS are the most commonly used analytical methods for the detection of microcystins (Gathercole & Thiel, 1987; Lawton et al., 1994; Bateman et al., 1995; Meriluoto et al., 1998).

Typical HPLC analysis uses a reverse-phase C18 column with separation achieved over a gradient of water and acetonitrile, both containing trifluoroacetic acid. A photodiode array (PDA) detector is used to collect the spectral data between 200-300 nm (Lawton et al., 1994; Moollan et al., 1996; Spoof et al., 2001). When further confirmation and identification of microcystins is required, the LC/MS is very useful as it enables the simultaneous separation and identification of microcystins in a mixture (Kondo et al., 1992; Poon et al., 1993; Mountfort et al., 2005). These analytical methods have the disadvantage of being expensive and only a small range of standards is available for the large known congeners. The methods also require highly skilled personnel and pre-concentration of the water samples (Mountfort et al., 2005).

HPLC, retrofitted with UV detection or mass spectrometry, is a powerful tool for identification of microcystins, capable of providing both qualitative and quantitative data. In mass spectrometry (MS), molecules of biological samples are converted to desolvated ions, which are resolved by mass analysers on the basis of mass and charge (Meriluoto, 1997; Msagati et al., 2006; Sangolkar et al., 2006). Given the molecular weights of toxin variants, HPLC/MS offers the advantage of monitoring for only those specific masses of interest as well as identification by retention times. HPLC coupled with different MS methods (e.g. fast atom bombardment, FAB; electrospray ionisation, ESI-MS and atmospheric pressure ionisation, API-MS) has resulted in accurate determination of microcystins (Kondo et al., 1992; Msagati et al., 2006; Sangolkar et al., 2006). Mass spectra of microcystin variants show different fragmentation patterns ($[M+H]^+$, $[M+2H]^+$ ions) depending on the amino acid residues present. Toxins and related peptides, in absence of standards, can be tentatively identified by comparing mass spectrum provided by HPLC/MS with those described in the literature (Sivonen & Jones, 1999; Sangolkar et al., 2006).

2 MATERIALS AND METHODS

2.1 Study site

The Kruger National Park was established in 1926 and is one of the largest conservation areas (19,485 km^2) in Africa. One of the challenges that emerged many years after the establishment of the Park was the scarce and unreliable supply of water needed to sustain the wild animals. This depended mostly on seasonal rainfall. To address this challenge, more than 300 boreholes were drilled and 50 earth dams were constructed to ensure all year round reliable supplies of surface water in the Park (Eckhardt et al., 2000; Oberholster et al., 2009). The first official case of *Microcystis* poisoning was recorded in 2005 in the KNP where several carcasses were found near one of the artificial water points (Nhlanganzwane Dam, **Figure 2**), which at the time had a visible cyanobacterial bloom (Bengis et al., 2006). Since then cases of *Microcystis* poisoning of wild animals have been reported around the constructed water impoundments (Nhlanganzwani, Makhohlola, Mapanamana and Sunset dams) in the south-eastern part of the Park (Oberholster et al., 2009; Masango et al., 2010).

In this study, the sampling schedule included 13 dams situated in the southern part of the KNP (**Figure 2**), namely 1) Sunset Dam, 2) Shiloweni Dam, 3) Piet Grobler Dam, 4) Mazithi Dam, 5) Nyamundwa Dam, 6) Shitlhave Dam, 7) Mestel Dam, 8) Kumana Dam, 9) Transport Dam, 10) Mpondo Dam, 11) N'watindlopfu Dam, 12) Mpanamana Dam and 13) Nsemane Dam. Aerial photos, by Google Earth, showing the 13 KNP dams are given in **Figure 3**. The size of the dams ranged between 0.14 and 24 ha, with N'watindlopfu Dam having the smallest surface area and Piet Grobler Dam having the largest surface area (**Table 2**).

2.2 Field sampling

Water samples were collected in June 2009, September 2009, February 2010, July 2010, October 2010 and February 2011 to cover as much as possible both the winter and summer seasons. Sampling of surface water near the shoreline targets toxins that are at the point of contact with terrestrial animals. Sarnelle et al. (2010) proposed that microcystin concentrations are generally higher at the surface than in depth-integrated samples from the mixed layer because cyanobacteria can regulate their buoyancy and form scums at the surface during calm weather. Surface grab samples were collected from at least three different locations at the edges of the specific dams. Sampling was focused on cyanobacterial scums in cases where they were visible. Clean 1 ℓ plastic containers were used. Water samples were transported from the field in a dark cool box to the laboratory where they were immediately transferred to a freezer and kept frozen until analysed (toxin and chemical analysis). Additional water samples were collected from Shiloweni Dam in

June 2010 following the death of wild animals (11 zebras – *Equus burchelli*, 2 rhinos – *Ceratotherium simum* and 1 wildebeest – *Connochaetes taurinus*) within the vicinity of this dam. Liver samples were also obtained from the zebra carcasses and stored at -80°C.

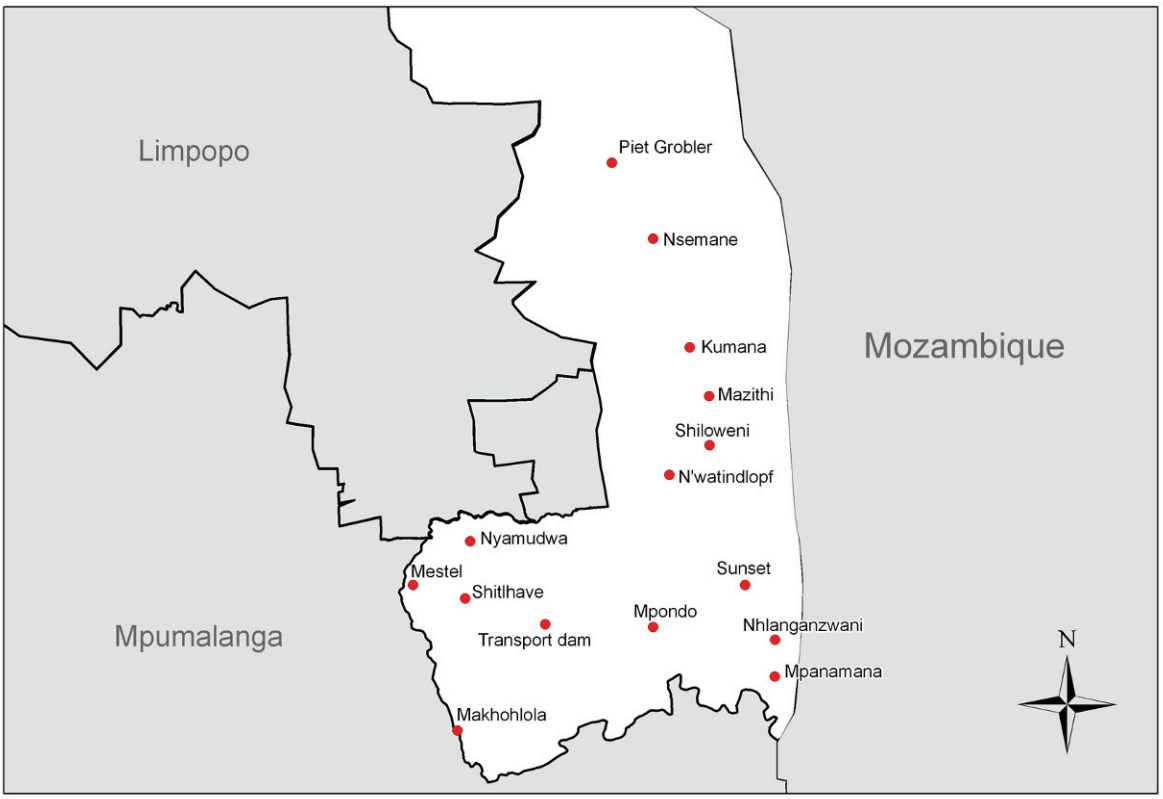
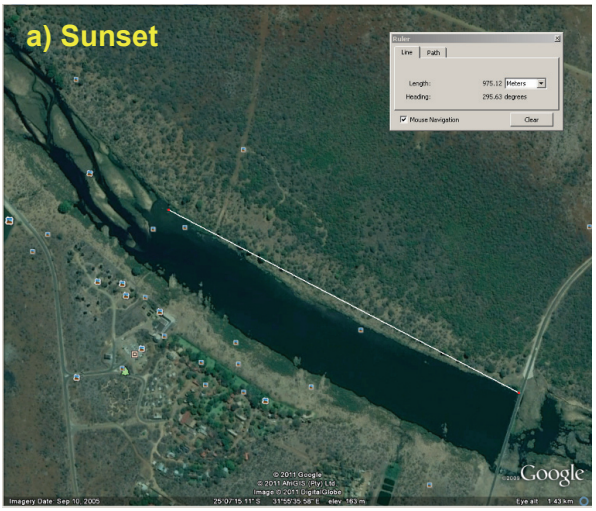
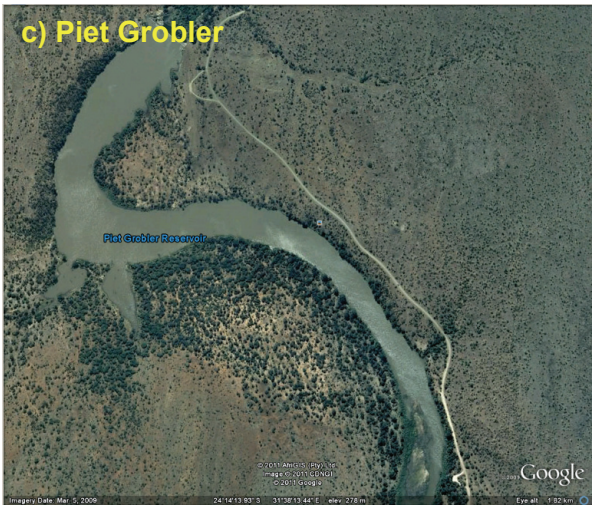
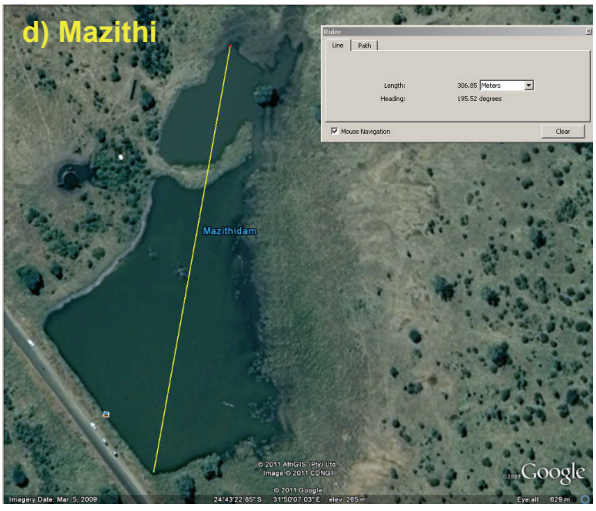


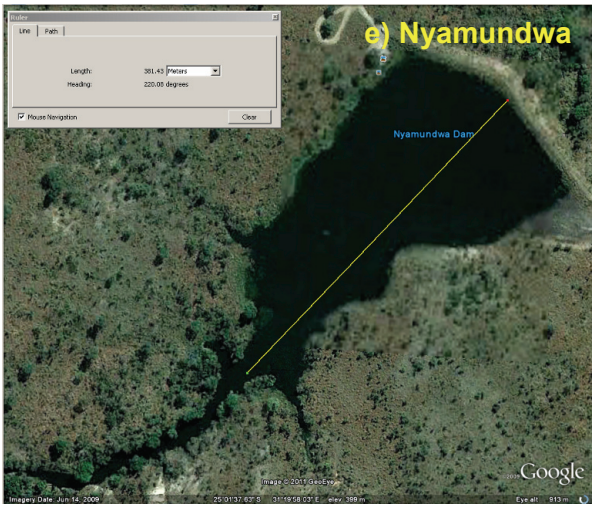
Figure 2. Map showing dams (•) that were sampled during the current study.


a) Sunset


b) Shiloweni
Shiloweni Reservoir
Xiloweni dam

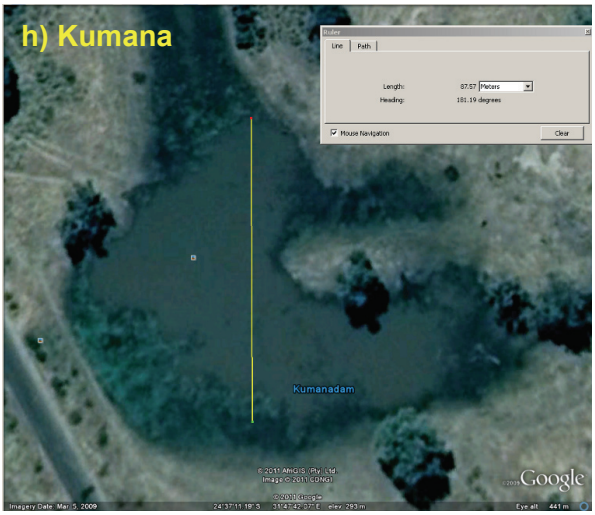

c) Piet Grobler
Piet Grobler Reservoir

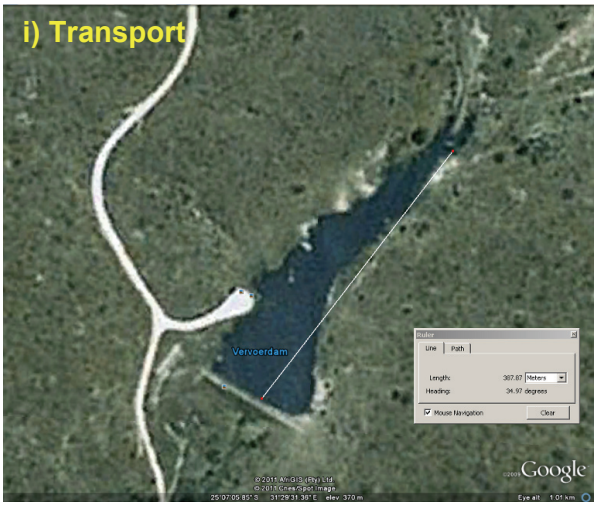

d) Mazithi
Mazithidam

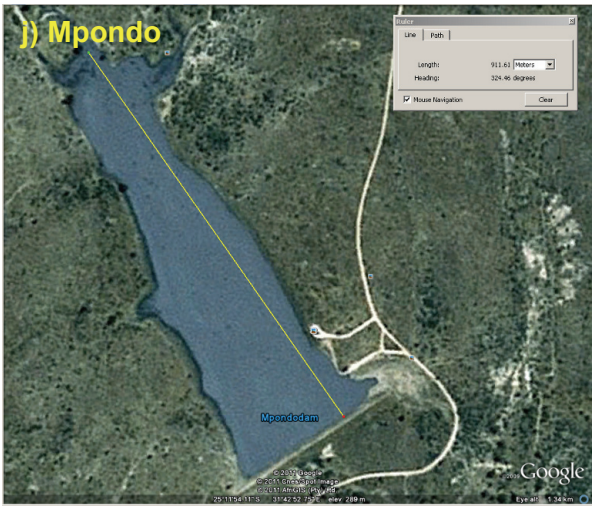

e) Nyamundwa
Nyamundwa Dam


f) Shitlhave
Shitlhave Reservoir
Shitlhave


g) Mestel
Mestel Reservoir
Mestelpan


h) Kumana
Kumanadam


i) Transport
Vervoerdam


j) Mpondo
Mpondodam


k) N'watindlopfu


l) Mpanamana

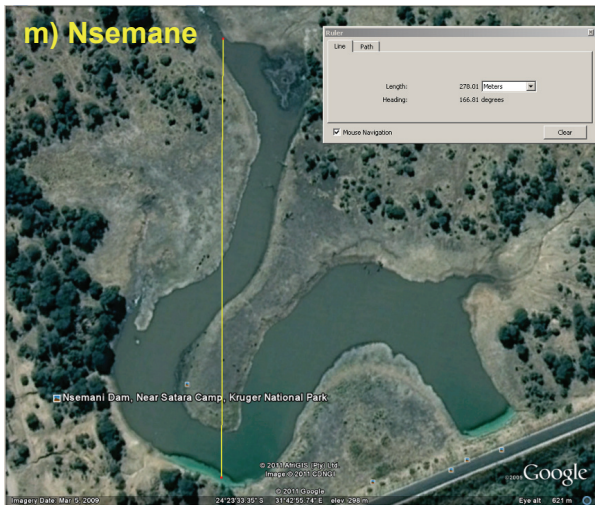


Figure 3. Aerial photos of the 13 KNP dams as shown by Google Earth – a) Sunset, b) Shiloweni, c) Piet Grobler, d) Mazithi, e) Nyamundwa, f) Shitlhave, g) Mestel, h) Kumana, i) Transport, j) Mpondo, k) N'watindlopfu, l) Mpanamana and m) Nsemane.

Table 2. Location and size of the KNP dams.

KNP Dam	South (˚)	East (˚)	Area (ha)
Sunset	-25.1170	31.9114	16.01
Shiloweni	-24.8240	31.8363	8.31
Piet Grobler	-24.2328	31.6319	24.00
Mazithi	-24.7213	31.8359	2.35
Nyamundwa	-25.0250	31.3346	4.53
Shitlhave	-25.1449	31.3248	1.07
Mestel	-25.1164	31.2149	2.93
Kumana	-24.6196	31.7954	0.53
Transport	-25.1990	31.4920	2.46
Mpondo	-25.2045	31.7183	20.24
N'watindlopfu	-24.8861	31.7519	0.14
Mpanamana	-25.3079	31.9729	2.40
Nsemane	-24.3921	31.7182	1.70

2.3 Phytoplankton analysis

Phytoplankton samples were preserved with Lugol's solution and the Lugol's concentration was dependent on the density of cyanobacteria scum. Identification and enumeration of

phytoplankton was done at the Centre for Environmental Management, University of Free State. The dominant algal species were identified with an inverted Zeiss Light Microscope after being fixed and placed in a sedimentation chamber for at least 24 h. The number of specific algal species was determined in a known volume of water, counting the individuals (cells, filament and colonies) occurring in 20 blocks of known dimensions. The result was multiplied by a constant to obtain the total counts. Algal species were determined as a percentage of the total community. Cyanobacteria genera were identified using universally accepted taxonomic keys based on cell structure and dimension, colony morphology and mucilage characteristics.

2.4 Chemical and physical parameters

Water temperature, pH and dissolved oxygen were measured *in situ* using the Hach HQ40d digital multiparameter. After collection of the water samples, the multiparameter probes were immediately placed in the water and readings were recorded. The air temperature and rainfall information at the KNP was kindly provided by the South African Weather Services (**Figure 11**).

2.4.1 Chlorophyll-*a*

Chlorophyll-*a* was measured by shaking the water samples vigorously and filtering a 5 mℓ aliquot through a glass microfiber filter. The filters were then extracted with 5 mℓ methanol followed by fluorometric detection (630 nm excitation and 660 nm emission) of the extracted chlorophyll-*a* according to the method described by Welschmeyer (1994).

2.4.2 Total phosphorus and total nitrogen

Total phosphorus and total nitrogen were determined following the method of Maher et al. (2002). The stock Kjeldahl digestion reagent was composed of sulphuric acid (2.4 M), potassium sulphate (0.39 M) and copper sulphate (0.023 M). Samples were digested at 160°C for 90 min and then 360°C for 120 min following addition of 10 mℓ digestion reagent to 25 mℓ sample in a digestion tube. Samples were then analysed following cooling and addition of water. Phosphorus was measured at 880 nm. Nitrogen was determined following cadmium column reduction by reaction with sulphanilamide coupled to N-1-napthylethylene dihydrogen chloride. Measurements were recorded at 520 nm.

2.4.3 Turbidity

Turbidity was determined following the Nephelometric method which involves the use of a Hach 2100 AN turbidimeter. Water samples were agitated gently and then poured into sample cells. Turbidity was read directly from the instrument display. The primary standards (0.1-4000 NTU) constituted 5 mℓ hydrazine sulphate in deionised water and 5 mℓ hexamethylenetetramine in deionised water (EPA, 1994).

2.4.4 Total dissolved solids

Gravimetric determination of the total dissolved solids (TDS) was done following the method of Atekwana et al. (2004). A 100 mℓ of a well-mixed sample was filtered through a 0.45 µm glass fibre filter and washed with three successive aliquots of 10 mℓ deionised water. Complete drainage was allowed between washings and the suction continued for about 3 min after filtration was complete. The filtrate was transferred into a pre-weighed 200 mℓ beaker and evaporated to dryness in an oven for 24 h at 105°C. The residue was then dried at 180°C for exactly 2 h, cooled in a desiccator and immediately weighed. The TDS for each sample was determined as the mass of solid normalised to the volume of water filtered.

2.5 Quantification of cyanotoxins

Commercial microcystin strip test kit (Abraxis product), ELISA kit (Abraxis product) and HPLC were used to quantify the microcystin toxins present in the KNP water samples.

2.5.1 Microcystin strip test

Quantification of microcystin in the KNP water samples was performed *in situ* following the manufacturer's instructions (Abraxis microcystin strip test). The Abraxis microcystin strip test is a rapid immunochromatographic test used in the qualitative screening of microcystins and nodularins in water. The test device consists of a vial with specific antibodies for microcystins and nodularins labelled with a gold colloid and a membrane strip to which a conjugate of the toxin is attached. A control line, produced by a different antibody/antigen reaction, is also present on the membrane strip. The control line is not influenced by the presence or absence of microcystins in the water samples, and therefore, it should be present in all valid reactions. In the absence of toxin in the water sample, the colloidal gold labelled antibody complex moves with the water sample by capillary action to contact the immobilised microcystin conjugate. An antibody-antigen reaction occurs forming a visible line in the test area. The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect the toxin at or above the cut-off point established for

the toxin. If microcystin is present in the water sample, it competes with the immobilised toxin conjugate in the test area for the antibody binding sites on the colloidal gold labelled complex. If a sufficient amount of the toxin is present, it will fill all the available binding sites, thus preventing attachment of the labelled antibody to the toxin conjugate, therefore preventing the development of a coloured line. If a coloured line is not visible in the test line region, or if the test line is lighter than the negative control line, microcystin is present at the levels of concern (> 1 µg/ℓ).

Briefly, 200 µℓ of water sample was dispensed into a conical test vial and shaken gently for 30 sec. The sample was incubated for 20 min and a colour change (purple) was observed. A test strip was inserted into the conical vial containing the sample/antibody mixture and the test was allowed to run for 10 min. The test strip was removed from the conical vial and placed on a flat surface; the test was allowed to develop further for 5 min. Results were read visually following the interpretation of the results in **Figure 4** and **Table 3** (Abaraxis microcystin strip test technical bulletin).

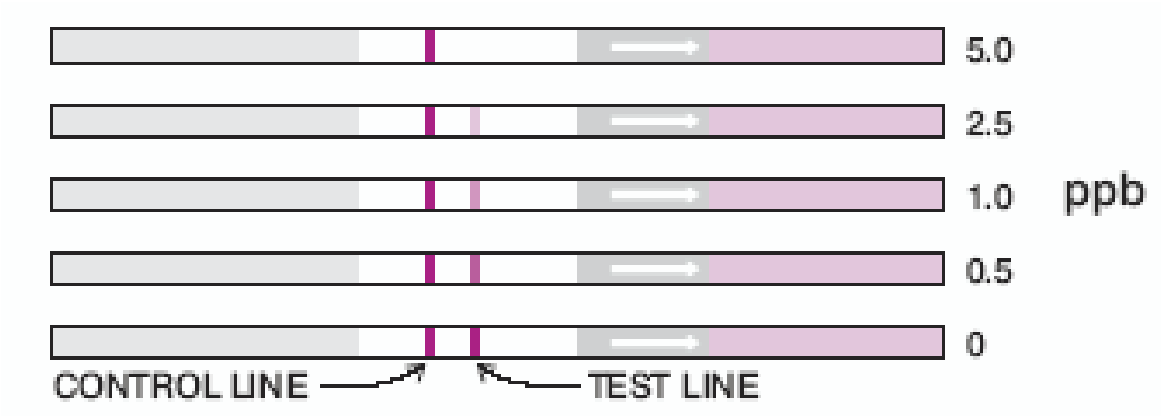


Figure 4. Illustration on how to interpret the Abraxis microcystin strip test results.

Table 3. Interpretation of the Abraxis microcystin strip test results.

Control line	Test line	Interpretation
No control line present	No test line present	Invalid result
Control line present	No test line present	> 5 µg/ℓ
Control line present	Moderate intensity test line present	Between 0 & 5 µg/ℓ

2.5.2 ELISA

The ELISA (Abraxis product) test was done in the laboratory according to the manufacturer's instructions. The ELISA is an indirect-competitive method used for the quantitative analysis of microcystins and nodularins in water. The sensitivity of the method was 0.1 ng/mℓ and the method was applied to the water samples without filtration or extraction. After addition of the standard solutions and thawed water samples (50 µℓ) into the wells of the microtitre plate, an antibody solution (50 µℓ) was added to the wells. The microtitre plate was incubated for 90 min at room temperature. At the end of the incubation period, wells of the plate were washed three times using the washing buffer solution (250 µℓ). An enzyme conjugate solution (100 µℓ) was added to each well; the microtitre plate was incubated for 30 min at room temperature. After another washing step, a substrate solution (100 µℓ) was added to individual wells followed by incubation for 20-30 min at room temperature. At the end of the incubation period, a stop solution (50 µℓ) was added and absorbance was measured at 450 nm using a microplate ELISA spectrophotometer (Bio-Tek µQuant product). Each test was carried out in duplicate. A standard curve was constructed using microcystin-LR at concentrations of 0, 0.15, 0.4, 1, 2 and 5 ng/mℓ (GraphPad Prism Software), and concentrations of the water samples were determined from this standard curve. Water samples showing concentrations lower than 100 ng/ℓ are considered to be negative (ABRAXIS Microcystin Kit Technical Bulletin). Water samples with concentrations higher than 5 µg/ℓ were diluted to obtain values that fall within the standard curve.

2.5.3 HPLC

The SPE HyperSep C18 columns (100 mg/mℓ, 40-60 µm particle size, 60Å pores size) were used. The columns were conditioned with 1 mℓ of methanol and 1 mℓ of water. Sample volume (8-12 mℓ) was noted and sample mixed on a Whirley mixer. The samples were then centrifuged at 3000 rpm for 5-7 min. The supernatant was loaded onto the column and vacuum regulated at 1 drop/sec in a vacuum manifold. The sample was eluted from the column by 2 mℓ of methanol. The samples were diluted 1:1 with methanol/water before analysis by LC-MS.

Separation and quantification of the microcystin variants were performed with a Liquid chromatography system consisting of a 100x2.0 mm Luna 3µ C18 100Å column (Phenomenex, Torrance, CA, USA). An Accela pump equipped with autosampler (Thermo Scientific, San Jose, CA, USA) was used for providing a mobile phase flow of 300 µL min^{-1}, and for the injection of 10 µℓ sample aliquots, respectively. Separation was achieved using

a linear gradient starting with acetonitrile-water (20:80, both containing 0.1% formic acid) rising to 55% acetonitrile over 12 min, and then to 100% acetonitrile over 3 min. Isocratic elution with 100% acetonitrile was maintained for 5 min before the mobile phase composition was returned to the starting conditions and the column re-equilibrated for 5 min. The HPLC was coupled to a TSQ quantum access tandem quadrupole mass spectrometer (Thermo Scientific) operating with an electrospray interface (ESI) in the positive mode. The mass analyser was operated in the multiple reaction monitoring mode (MRM). The parameters for the ESI as well as optimum collision energies were set using automatic and semi-automatic tuning procedures while aqueous tuning solutions of microcystin-RR, -LR and -LA standards (35-701 ng/mℓ) was continuously infused into the mobile phase at starting condition for the gradient. The parameters for the ESI interface were adjusted as follows: a spray voltage of 4.5 kV, a capillary temperature of 180°C, a skimmer offset of 10 and a sheath gas and auxiliary gas flow of 40 and 20 arbitrary units, respectively. The argon pressure in Q2 was set to 1.3 mTorr, and divert valve was set to waste during the first 2 min of the run. The other TSQ MS method settings are given in **Table 4**.

Table 4. The TSQ Quantum instrument method.

Compound	Parent $[M+H]^+$	Parent $[M+2H]^{++}$	Parent $[M+Na]^+$	Centre	Collision energy
Microcystin-RR		519.7		135.0	62
Microcystin-YR	1045.5			135.0	60
Microcystin-LR	995.5			135.0	63
Microcystin-LA	910.5		932.5		0
Microcystin-LY	1002.0		1024.0		0
Microcystin-LW	1025.2		1047.2		0
Microcystin-LF	986.2		1008.2		0

Microcystins were quantified using external four-point calibration curves in the concentration range 0.01-710 ng/mℓ. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as the minimum concentration resulting in two diagnostic MRM traces with signal-to-noise ratios (S/N) equal to 3 and 10, respectively. The limits of detection (ng/mℓ) for each microcystin were: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW =

0.53 and -LA = 0.53. The HPLC analysis was done by the National Veterinary Institute (Department of Feed and Food Safety, Section for Toxicology) in Norway.

2.6 Statistical analysis

Pearson's correlation (r) was used to determine the linear correlation between cyanobacterial numbers, water temperature, water pH, dissolved oxygen, chlorophyll-*a*, total phosphorus, total nitrogen, turbidity, total dissolved solids, strip test, microcystin toxin (ELISA and HPLC), the individual microcystin variants (-RR, -YR, -LR, -LA, -LY, -LW and -LF) and the size of the KNP dams. Correlation was considered significant when $p \leq 0.0001$.

3 RESULTS AND DISCUSSION

3.1 Phytoplankton analysis

The phytoplankton of all 13 KNP dams consisted of a total of 42 taxa in June, 37 taxa in September 2009, 43 taxa in February 2010, 36 taxa in July 2010 and February 2011, and 34 taxa in October 2010 all belonging to Cyanophyceae, Bacillariophyceae, Chlorophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae and Chrysophyceae divisions. The recorded taxa and their concentrations for water samples collected from the 13 KNP dams are shown in **Appendix 1**.

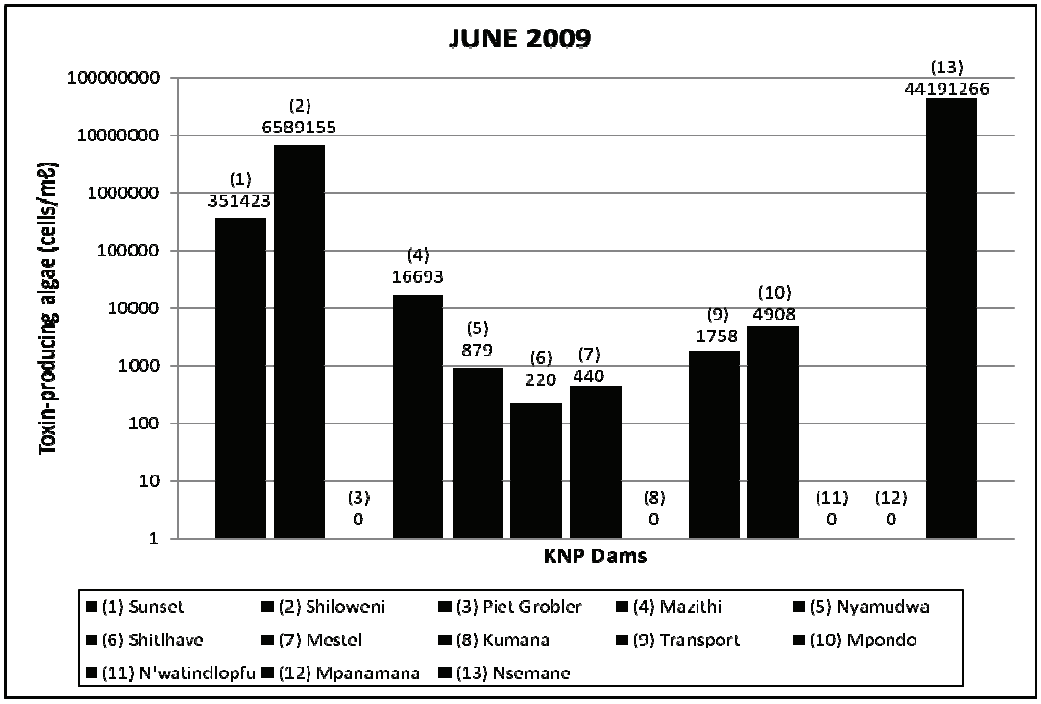


Figure 5. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in June 2009.

In June 2009, Nsemane (441.91x10^5 cells/mℓ), Shiloweni (65.89x10^5 cells/mℓ) and Sunset (3.51x10^5 cells/mℓ) dams had very high concentrations of the known toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis* – belonging to the Cyanophyceae division) (**Figure 5**). During this period, Nsemane Dam was dominated by 99.97% *Microcystis aeruginosa*; Shiloweni Dam was dominated by 97.7% *Microcystis aeruginosa* and Sunset Dam was dominated by 81.6% *Microcystis aeruginosa* and *Anabaena* sp. Algal blooms were visible in the three dams during this period. Mazithi, Nyamundwa, Shitlhave, Mestel, Transport and Mpondo dams contained the known toxin-producing algae and their concentrations ranged between 220 and 16693 cells/mℓ with Mazithi Dam having the highest algal concentration (visible as blue-green streaks during

sampling) and Shitlhave having the lowest algal concentration. Piet Grobler, Kumana, N'watindlopfu and Mapanamana dams did not contain the known toxin-producing algae in June 2009.

In September 2009, Sunset (221.98x10^5 cells/mℓ), N'watindlopfu (26.41x10^5 cells/mℓ), Shiloweni (20.28x10^5 cells/mℓ) and Mpanamana (5.95x10^5 cells/mℓ) dams had very high concentrations of the known toxin-producing algae (**Figure 6**). During this period, Sunset Dam was dominated by 99.87% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria* sp.; N'watindlopfu Dam was dominated by 99.27% *Microcystis aeruginosa* and *Anabaena* sp.; Shiloweni Dam was dominated by 96.85% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria* sp. and Mpanamana Dam was dominated by 99.40% *Microcystis aeruginosa* and *Anabaena* sp. Algal blooms were visible in the four dams during this period. Piet Grobler, Nyamundwa, Mestel, Transport and Mpondo dams contained the known toxin-producing algae and their concentrations ranged between 74 and 1318 cells/mℓ with Transport Dam having the highest algal concentration and the lowest algal concentrations were observed in Piet Grobler and Nyamundwa dams. Mazithi Dam did not contain any of the known toxin-producing algae, however, a slight green discolouration was observed in the water during sampling. Kumana Dam was dry and empty in June 2009 and Nsemane Dam was not sampled during this period.

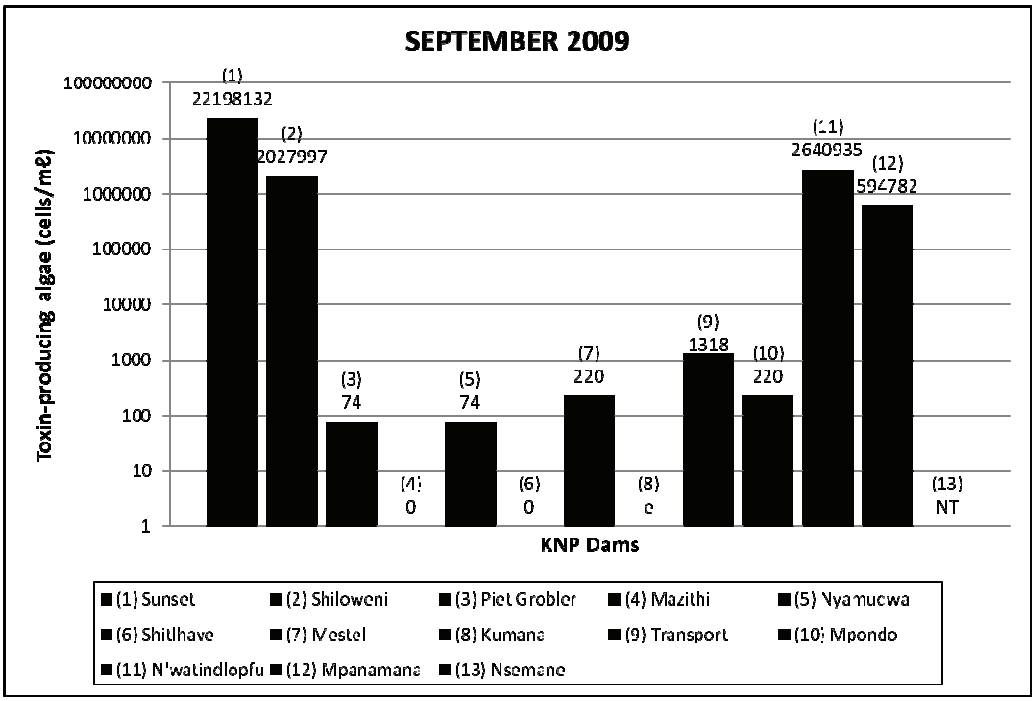


Figure 6. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in September 2009.

In February 2010, Sunset (141.89x10^5 cells/mℓ), Shiloweni (137.05x10^5 cells/mℓ), N'watindlopfu (14.72x10^5 cells/mℓ), Mazithi (3.3x10^5 cells/mℓ), Mpanamana (0.56x10^5 cells/mℓ) and Nsemane (0.46x10^5 cells/mℓ) dams had very high concentrations of the known toxin-producing algae (**Figure 7**). During this period, Sunset Dam was dominated by 99.70% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria* sp.; Shiloweni Dam was dominated by 99.40% *Microcystis aeruginosa*; N'watindlopfu Dam was dominated by 95.71% *Microcystis aeruginosa* and *Anabaena* sp.; Mazithi Dam was dominated by 89.10% *Microcystis aeruginosa* and *Oscillatoria* sp.; Mpanamana Dam was dominated by 72.70% *Microcystis aeruginosa* and Nsemane Dam was dominated by 81.20% *Microcystis aeruginosa*. Algal blooms were visible only in Sunset and Shiloweni dams during this period. The water appeared muddy (brown in colour) in N'watindlopfu and Nsemane dams, whereas the water was clear in Mazithi and Mpanamana dams. Piet Grobler, Nyamundwa, Shitlhave, Mestel, Transport and Mpondo dams contained the known toxin-producing algae and their concentrations ranged between 440 and 14643 cells/mℓ with Nyamundwa Dam (water appeared muddy) having the highest algal concentration and Shitlhave Dam having the lowest algal concentration. Kumana Dam did not contain any of the known toxin-producing algae.

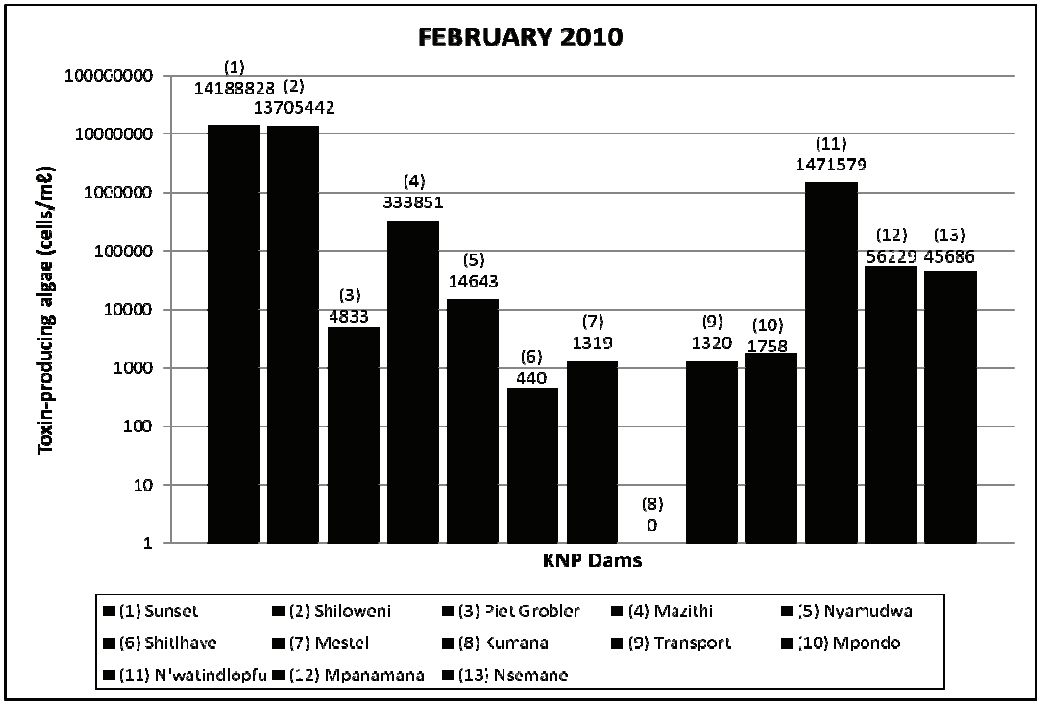


Figure 7. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in February 2010.

In July 2010, Sunset ($0.20x10^5$ cells/mℓ) and Shiloweni ($0.11x10^5$ cells/mℓ) dams had very high concentrations of the known toxin-producing algae (**Figure 8**). During this period, Sunset Dam constituted 32.90% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria* sp. and Shiloweni Dam constituted 30.77% *Microcystis aeruginosa* and *Oscillatoria* sp. Algal blooms were visible in the two dams during this period. Piet Grobler, Shitlhave, Mestel and Mpondo dams contained the known toxin-producing algae and their concentrations ranged between 147 and 879 cells/mℓ with Piet Grobler (water appeared muddy) and Mpondo dams having the highest algal concentration and Shitlhave Dam having the lowest algal concentration. Mazithi, Nyamundwa, Kumana, Transport, N'watindlopfu, Mpanamana and Nsemane dams did not contain any of the known toxin-producing algae and the water appeared muddy in Mazithi, Kumana, N'watindlopfu and Mpanamana dams.

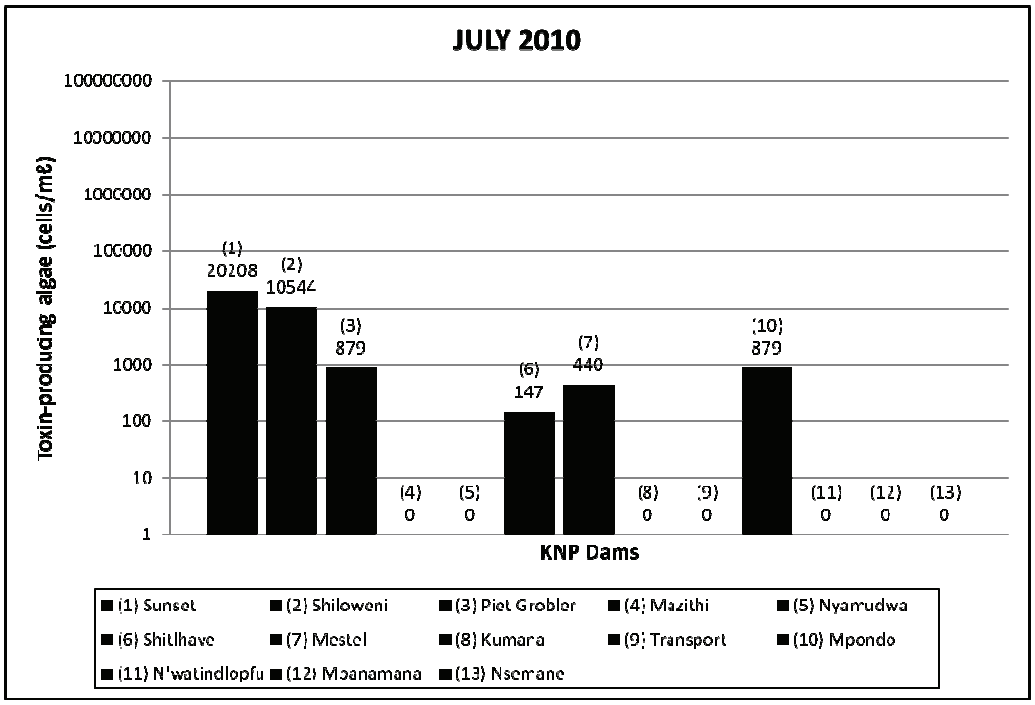


Figure 8. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in July 2010.

In October 2010, Nsemane ($4.69x10^5$ cells/mℓ), Mazithi ($0.079x10^5$ cells/mℓ), N'watindlopfu ($0.073x10^5$ cells/mℓ), Sunset ($0.070x10^5$ cells/mℓ) and Mestel ($0.066x10^5$ cells/mℓ) dams had very high concentrations of the known toxin-producing algae (**Figure 8**). During this period, Nsemane Dam was dominated by 98.46% *Anabaena* sp.; Mazithi Dam constituted 23.69% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria* sp.; N'watindlopfu Dam constituted 11.11% *Anabaena* sp.; Sunset Dam constituted 4.98% *Anabaena* sp. and *Oscillatoria* sp. and Mestel Dam constituted 28.57% *Microcystis aeruginosa*, *Anabaena* sp. and *Oscillatoria*

sp. Algal bloom was visible in Nsemane Dam during this period. Piet Grobler (water appeared slightly muddy), Nyamundwa, Shitlhave and Transport (water appeared slightly muddy) dams contained the known toxin-producing algae and their concentrations ranged between 368 and 1758 cells/mℓ with Transport Dam having the highest algal concentration and Piet Grobler Dam having the lowest algal concentration. Mpondo and Mpanamana dams did not contain any of the known toxin-producing algae and the water appeared muddy in the two dams. Shiloweni and Kumana dams were dry and empty. Shiloweni Dam was emptied by the KNP authorities following the June 2010 wild animal deaths.

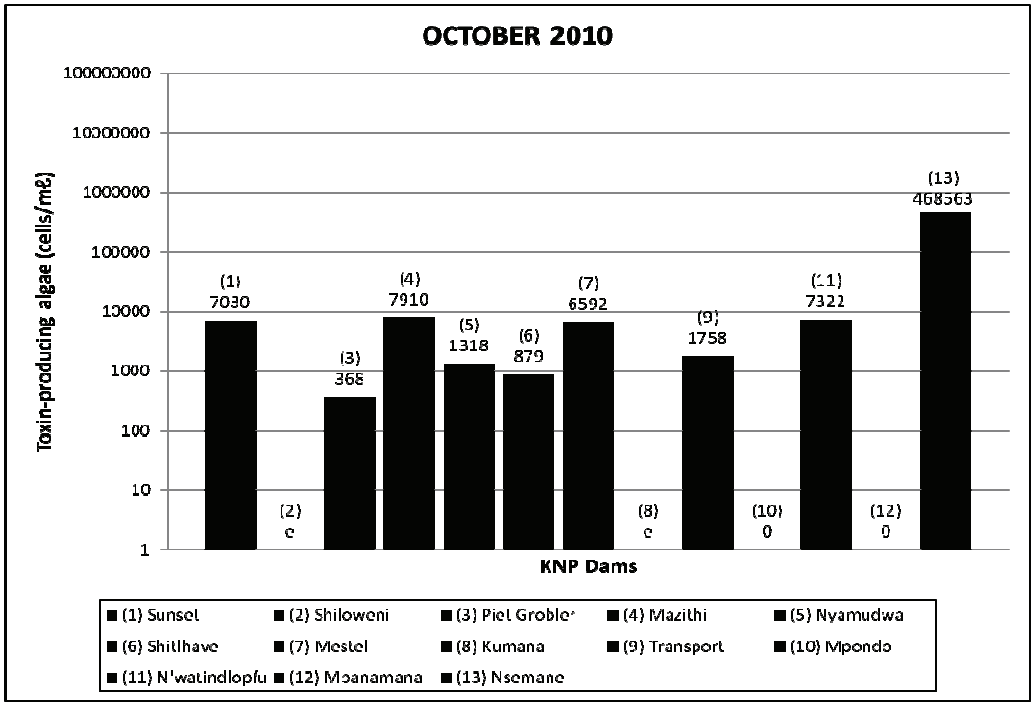


Figure 9. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in October 2010.

In February 2011, Sunset ($0.053x10^5$ cells/mℓ), Transport ($0.035x10^5$ cells/mℓ) and Mazithi ($0.026x10^5$ cells/mℓ) dams had high concentrations of the known toxin-producing algae (**Figure 10**). Sunset Dam constituted 11.49% *Microcystis aeruginosa*, *Anabaena* sp., *Oscillatoria* sp. and *Rhaphidiopsis* sp.; Transport Dam constituted 32.14% *Microcystis aeruginosa*, *Anabaena* sp., *Oscillatoria* sp. and *Rhaphidiopsis* sp. and Mazithi Dam was dominated by 45.00% *Microcystis aeruginosa*, *Anabaena* sp., *Oscillatoria* sp. and *Rhaphidiopsis* sp. Nyamundwa, Shitlhave, Mestel and Mpondo dams contained the known toxin-producing algae and their concentrations ranged between 440 and 1758 cells/mℓ with Mpondo Dam having the highest algal concentration and Shitlhave Dam having the lowest algal concentration. Algal blooms were not visible in these dams during this period. Piet

Grobler, Kumana, N'watindlopfu, Mpanamana and Nsemane dams did not contain any of the known toxin-producing algae. The water was clear only in Mestel and Shitlhave dams. Shiloweni Dam was dry and empty.

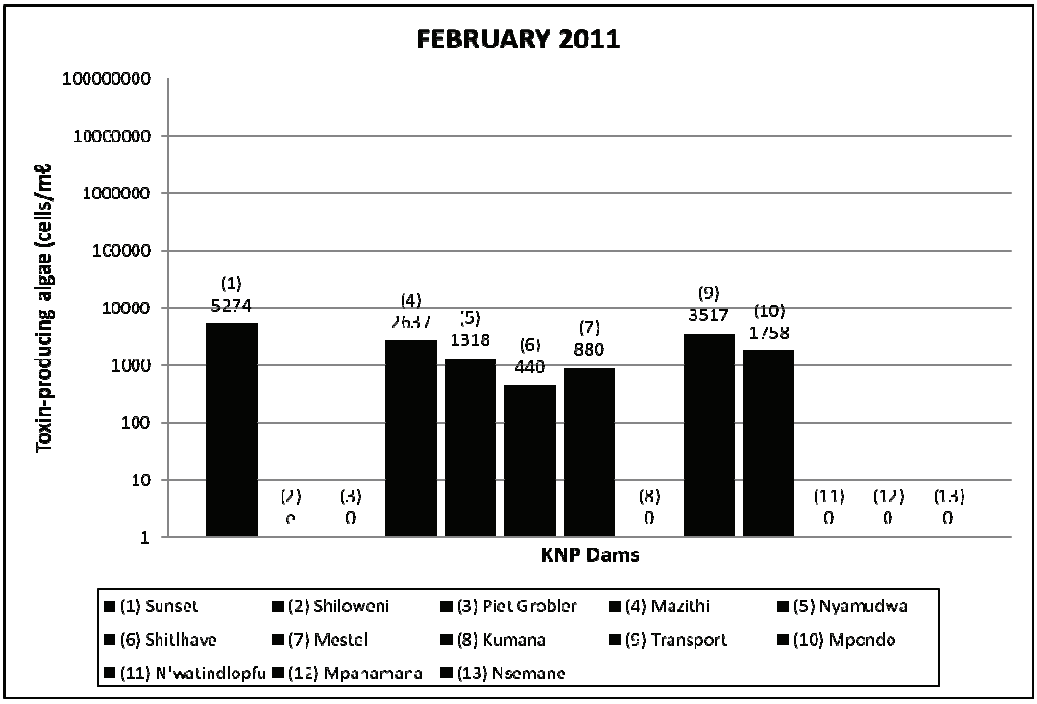


Figure 10. Concentration (cells/mℓ) of the potential toxin-producing algae (e.g. *Microcystis*, *Anabaena*, *Oscillatoria* and *Anabaenopsis*) from the different KNP dams sampled in February 2011.

3.2 Chemical and physical parameters

3.2.1 Temperature

The water temperature recorded during sampling of the 13 KNP dams is given in **Table 5**. The water temperature varied between 15.5-20.5°C in June 2009, 20.1-31.2°C in September 2009, 24.2-34.9°C in February 2010, 15.5-25.3°C in July 2010, 22.6-36.1°C in October 2010 and 23.9-32.0°C in February 2011. The lowest temperature was determined at Nyamundwa and Kumana dams in June 2009 and July 2010, respectively, whereas the highest temperature was determined at N'watindlopfu Dam in October 2010. In general, cyanobacteria prefer warmer conditions for optimal growth (temperatures between 20°C and 30°C) and temperatures below 15°C are limiting to the growth of *Microcystis*. Even though the surface water temperatures observed in this study at the different 13 KNP dams are high, they are comparable with observations made by Wicks and Thiel (1990) at the Hartbeespoort Dam where they observed maximum water temperatures of 27°C in summer and minimum water temperatures of 13°C in winter. Oberholster and co-workers (2009)

recorded surface water temperatures varying between 20-22°C at the Nhlanganzwane and Makhohlola dams in the KNP in July 2007. The rainfall data showed that the winter seasons in 2009 and 2010 were generally dry and characterised by monthly mean rainfall of less than 5 mm (**Figure 11**).

There was no linear correlation (r=0.0098) found between the cyanobacteria concentrations and temperatures for all the 13 KNP dams during this study (**Appendix 2**). The correlation results are consistent with those of Te and Gin (2011) who did not observe a significant correlation between *Microcystis* and temperature at the Kranji Reservoir in Singapore. However, other investigators have observed a positive correlation between the cyanobacteria biomass and temperature (Robarts & Zohary, 1987; Wicks & Thiel, 1990; Li et al., 2010; Liu et al., 2011). Although the temperatures recorded at the KNP dams in the current study were favourable in promoting the growth of cyanobacteria, no correlation was observed between the cyanobacteria cell numbers and temperature. This could be due to the small temperature variation relative to the wide variability in the other parameters.

3.2.2 pH

The water pH recorded during sampling of the 13 KNP dams is given in **Table 5**. The water pH ranged between 6.9-7.8 in June 2009, 7.2-9.0 in September 2009, 6.8-7.7 in February 2010, 7.2-8.2 in July 2010, 6.5-8.5 in October 2010 and 7.7-8.3 in February 2011. The lowest water pH was determined at Mestel Dam in October 2010 and the highest water pH was determined at Transport Dam in September 2009. Wangwibulkit and co-workers (2008) found that water pH influenced the growth of cyanobacteria especially when the pH was higher than 9.0 or lower than 6.0. At these levels, pH could inhibit photosynthesis and adversely affect the morphology of the cyanobacteria. The pH levels of this study are comparable with those reported by Wicks and Thiel (1990) who recorded pH levels in the range of 7.7-9.4 at the Hartbeespoort Dam. Other investigators have also recorded similar pH values (Li et al., 2010; Te & Gin, 2011).

There was no linear correlation (r=-0.02483) found between the cyanobacteria concentrations and pH for all the 13 KNP dams during this study (**Appendix 2**). Other investigators have reported a positive correlation between *Microcystis* and pH (Wicks & Thiel, 1990; Liu et al., 2011). Although the pH recorded at the KNP dams in the current study was favourable in promoting the growth of cyanobacteria, no correlation was observed between the cyanobacteria cell numbers and pH. This could be due to the small pH variation relative to the wide variability in the other parameters.

3.2.3 Dissolved oxygen

The dissolved oxygen values recorded during sampling of the 13 KNP dams are given in **Table 5**. The dissolved oxygen values ranged between 3.5-9.8 mg/ℓ in June 2009, 0.3-13.6 mg/ℓ in September 2009, 0.1-8.7 mg/ℓ in February 2010, 3.4-10.1 mg/ℓ in July 2010, 4.1-11.5 mg/ℓ in October 2010 and 2.5-9.3 mg/ℓ in February 2011. The lowest dissolved oxygen was determined at Shiloweni Dam in February 2010 and the highest dissolved oxygen was determined at Nyamundwa Dam in September 2009. Heavy or dense blooms use large amounts of dissolved oxygen at night and on very cloudy/overcast, windless days causing oxygen depletion and fish kill. The dissolved oxygen levels recorded in this study are comparable with the levels reported by Oberholster and co-workers (2009) who recorded dissolved oxygen levels of 2.13 mg/ℓ at the Nhlanganzwane Dam in the KNP. Dissolved oxygen levels ranging between 3.0-9.0 mg/ℓ were recorded by Li et al. (2010) at the Yanghe Reservoir, North China.

There was a negative linear correlation (r=-0.40912) found between the cyanobacteria concentrations and the dissolved oxygen for all the 13 KNP dams during this study (**Appendix 2**). A positive correlation between cyanobacteria biomass and dissolved oxygen has been previously reported by other investigators (Liu et al., 2011; Te and Gin, 2011).

3.2.4 Chlorophyll-*a*

The chlorophyll-*a* values determined for the 13 KNP dams are given in **Table 6**. The chlorophyll-*a* values varied between 1.0-13986 µg/ℓ in June 2009, 1.0-44710 µg/ℓ in September 2009, 1.0-18629 µg/ℓ in February 2010, 1.0-906 µg/ℓ in July 2010, 18-4012 µg/ℓ in October 2010 and 1.0-214 µg/ℓ in February 2011. The highest chlorophyll-*a* was determined at Sunset Dam in September 2009. Chlorophyll-*a* is one of the major phytoplankton biomass-related variables. The chlorophyll-*a* levels reported in this study are among the highest levels recorded in South Africa. Oberholster et al. (2009) recorded chlorophyll-*a* levels of 580 µg/ℓ and 3.8 µg/ℓ at the Nhlanganzwane and Makhohlola dams in the KNP in July 2007. The highest chlorophyll-*a* levels recorded by Te and Gin (2011) and Lehman et al. (2009) were 1584 µg/ℓ and 41.6 µg/ℓ, respectively.

A significant positive linear correlation (r=0.53935; p<0.0001) was found between the cyanobacteria biomass and the chlorophyll-*a* levels for all the 13 KNP dams during this study (**Appendix 2**). The results of this study are comparable with the results of Te and Gin (2011)

who also observed a positive correlation between the cyanobacteria biomass and chlorophyll-*a*.

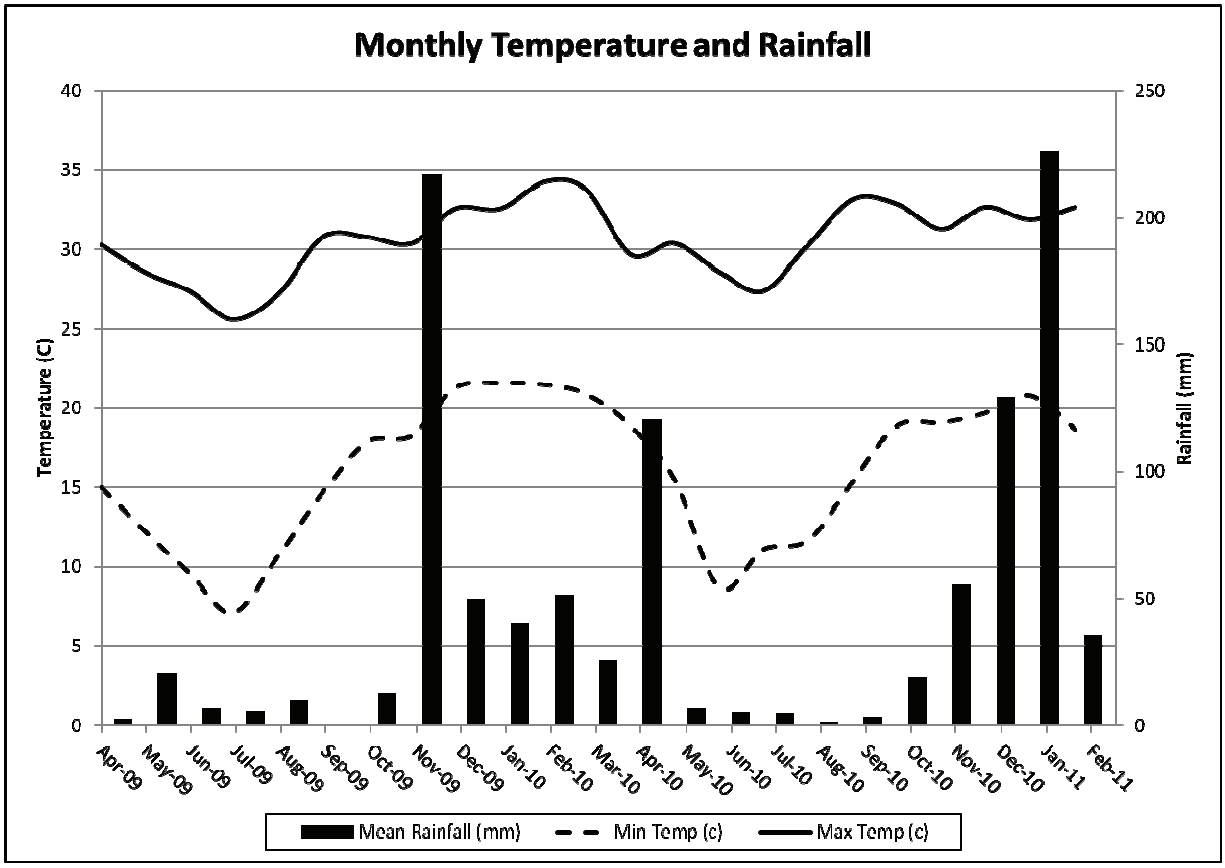


Figure 11. Minimum and maximum air temperature and mean rainfall recorded monthly from April 2009 to February 2011 at the Kruger National Park (South African Weather Services).

Table 5. Water temperature, pH and dissolved oxygen (DO) of the KNP dams.

KNP Dam	June 2009			September 2009			February 2010			July 2010			October 2010			February 2011		
	Temp. (°C)	pH	DO (mg/ℓ)	Temp. (°C)	pH	DO (mg/ℓ)	Temp. (°C)	pH	DO (mg/ℓ)	Temp. (°C)	pH	DO (mg/ℓ)	Temp. (°C)	pH	DO (mg/ℓ)	Temp. (°C)	pH	DO (mg/ℓ)
Sunset	20.5	7.8	9.6	20.1	7.2	0.3	27.7	6.8	0.2	21.7	7.3	6.5	25.4	7.1	7.1	29.0	8.0	9.3
Shiloweni	16.8	6.9	6.4	20.5	7.8	6.8	24.2	6.8	0.1	16.1	7.2	3.6	e	e	e	e	e	e
Piet Grobler	18.9	7.3	7.0	27.6	8.4	8.7	33.7	7.7	8.7	20.8	7.7	6.2	25.6	7.4	8.2	29.2	8.2	7.3
Mazithi	16.9	7.5	8.8	21.0	8.6	7.1	26.0	7.5	6.2	17.2	8.2	6.7	23.2	7.5	4.5	25.8	8.2	4.9
Nyamundwa	15.5	7.1	3.5	24.9	8.7	13.6	34.9	7.7	6.8	16.9	8.2	9.0	24.4	7.4	6.4	25.5	7.7	3.7
Shitlhave	17.1	7.3	9.8	24.9	8.1	7.9	28.1	7.4	6.2	18.1	8.0	8.4	25.3	7.5	7.3	26.2	7.8	6.4
Mestel	16.5	7.0	5.8	23.8	7.3	6.1	30.0	7.3	5.6	16.6	7.4	7.4	24.1	6.5	7.5	25.4	7.7	5.0
Kumana	16.0	7.5	8.3	e	e	e	26.0	7.3	6.2	15.5	7.7	5.4	e	e	e	23.9	8.3	6.6
Transport	18.3	7.7	9.6	27.2	9.0	9.0	27.4	7.3	5.4	19.3	8.1	8.8	26.4	6.8	5.4	28.6	7.9	6.3
Mpondo	20.3	7.5	9.4	24.6	8.3	8.6	32.6	7.4	7.6	20.4	8.2	8.6	22.6	7.5	4.5	32.0	8.0	7.6
N'watindlopfu	17.3	7.2	6.8	31.2	8.8	10.3	32.0	7.3	6.2	22.1	8.0	10.1	36.1	8.4	7.0	31.8	8.1	7.9
Mpanamana	19.6	7.7	5.1	25.5	8.4	8.5	33.3	7.5	5.7	25.1	7.8	3.4	26.4	8.5	11.5	29.0	7.8	2.5
Nsemane	NT	NT	NT	NT	NT	NT	32.9	7.1	3.8	25.3	7.7	5.6	27.7	8.5	4.1	28.2	7.8	5.9

e = empty dam; NT = not tested

3.2.5 Total phosphorus

The total phosphorus determined for the 13 KNP dams is given in **Table 6**. The phosphorus values ranged between 0.33-19.0 mg/ℓ in June 2009, 0.25-29.0 mg/ℓ in September 2009, 0.28-155 mg/ℓ in February 2010, 0.30-2.90 mg/ℓ in July 2010, 0.20-5.70 mg/ℓ in October 2010 and 0.30-0.80 mg/ℓ in February 2011. The highest phosphorus was determined at Sunset Dam in February 2010. Total phosphorus was below the detection limit at Mpondo Dam (June 2009 and February 2010), Piet Grobler Dam (September 2009, October 2010 and February 2011), Shitlhave Dam (February 2010, July 2010 and February 2011), Mestel Dam (February 2010, July 2010 and February 2011), Nyamundwa Dam (July 2010 and February 2011), Transport Dam (October 2010 and February 2011) and Mazithi Dam (February 2011). Phosphorous is delivered to the water-body systems from a range of sources, varying in its bioavailability from source to source. The phosphorus load from point sources, which are dominated by sewage treatment works, is typically highly bioavailable and is delivered along with considerable loads of readily degradable organic material. Much higher proportions of soluble phosphorous occur when livestock excreta or soluble inorganic fertilisers are washed off the land soon after application.

The total phosphorus levels reported in this study are among the highest levels recorded in South Africa. The highest total phosphorus level recorded at the Nhlanganzwane and Hartbeespoort dams was 1.21 mg/ℓ and 0.5 mg/ℓ, respectively (Wicks & Thiel, 1990; Oberholster et al., 2009). In the Yanghe Reservoir, total phosphorus levels varied between 0.04-0.08 mg/ℓ (Li et al., 2010), whereas in the Kranji Reservoir total phosphorus varied between 0.03-0.21 mg/ℓ (Te & Gin, 2011). A significant positive linear correlation (r=0.42574; p=0.0001) was found between the cyanobacteria biomass and the total phosphorus levels for all the 13 KNP dams during this study (**Appendix 2**). The results of this study are comparable with the results of Te and Gin (2011) who also observed a positive correlation between the cyanobacteria biomass and total phosphorus.

3.2.6 Total nitrogen

The total nitrogen determined for the 13 KNP dams is given in **Table 6**. The nitrogen values ranged between 1.50-328 mg/ℓ in June 2009, 1.20-738 mg/ℓ in September 2009, 0.92-1870 mg/ℓ in February 2010, 1.30-26.0 mg/ℓ in July 2010, 1.70-28.0 mg/ℓ in October 2010 and 1.40-5.60 mg/ℓ in February 2011. The highest nitrogen was determined at Sunset Dam in February 2010. Total nitrogen was below the detection limit at Nyamundwa Dam in February 2011. Reduced organic and inorganic nitrogen forms, such as urea and ammonium, are the

favoured nitrogen source for phytoplankton (Msagati et al., 2006). The total nitrogen levels reported in this study are high when compared to those reported by other investigators. Total nitrogen levels reported at the Nhlanganzwane and Makhohlola dams in the KNP are 13.6 mg/ℓ and 0.66 mg/ℓ, respectively (Oberholster et al., 2009). In the Yanghe Reservoir, total phosphorus levels varied between 3.0-7.0 mg/ℓ (Li et al., 2010), whereas in the Kranji Reservoir total phosphorus varied between 0.61-4.36 mg/ℓ (Te & Gin, 2011).

A significant positive linear correlation (r=0.48788; p<0.0001) was found between the cyanobacteria biomass and the total nitrogen levels for all the 13 KNP dams during this study (**Appendix 2**). The results of this study are comparable with the results of Te and Gin (2011) who also observed a positive correlation between the cyanobacteria biomass and total nitrogen. In their study, Wicks and Thiel (1990) could not find a strong correlation between the nutrients and total toxin concentration of *M. aeruginosa*. The high levels of nutrients, especially the total nitrogen, have been linked to the urine and faecal matter released into the water by the high population of Hippopotami inhabiting some of the KNP dams. In their study, Oberholster et al. (2009) stated that the release of nitrogen from the decaying dung and urine of the hippopotami at Nhlanganzwane Dam, possibly supplied the cyanobacteria with a slow but steady supply of NH_4^+-N which is sufficient for maximum growth and bloom formation.

3.2.7 Turbidity

The turbidity values determined for the 13 KNP dams are given in **Table 7**. The values ranged between 6.10-948 NTU in June 2009, 3.80-833 NTU in September 2009, 1.0-725 NTU in February 2010, 2.20-441 NTU in July 2010, 6.50-144 NTU in October 2010 and 2.10-34.0 NTU in February 2011. The lowest turbidity was determined at Shitlhave and Mpondo dams in February 2010, whereas the highest turbidity was determined at Nsemane Dam in June 2009. Turbidity levels reported by Te and Gin (2011) were ranging between 5.76-224.5 NTU. A significant positive linear correlation (r=0.79156; p<0.0001) was found between the cyanobacteria biomass and the turbidity levels for all the 13 KNP dams during this study (**Appendix 2**). The results of this study are comparable with the results of Te and Gin (2011) who also observed a positive correlation between the cyanobacteria biomass, expressed as *Microcystis* 16S rRNA, and turbidity.

Table 6. Chlorophyll-*a* (Chl), total phosphorus (TP) and total nitrogen (TN) levels of the KNP dams.

KNP Dam	June 2009			September 2009			February 2010			July 2010			October 2010			February 2011		
	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)	Chl (µg/ℓ)	TP (mg/ℓ)	TN (mg/ℓ)
Sunset	986.0	3.20	32	44710	29.0	738	1863	155	1870	218.0	0.70	6.0	202.0	0.8	7.2	214	0.6	5.6
Shiloweni	13986	19.0	328	3124	5.40	69	18629	57.0	157	906.0	2.90	26	e	e	e	e	e	e
Piet Grobler	21.00	0.57	1.6	1.00	< DL	1.5	14.00	0.28	1.8	1.00	0.30	1.8	18.00	< DL	2.4	16	< DL	1.8
Mazithi	183.0	0.75	4.8	72.00	1.20	3.2	75.00	0.44	3.3	29.00	0.40	2.3	212.0	0.8	6.7	19	< DL	2.4
Nyamundwa	8.00	0.77	3.5	16.00	1.00	1.5	41.00	0.44	4.6	17.00	< DL	2.0	34.00	0.5	2.4	4.0	< DL	< DL
Shitlhave	14.00	0.53	1.7	1.00	0.89	1.2	6.00	< DL	0.92	9.00	< DL	1.4	21.00	< DL	1.7	14	< DL	1.4
Mestel	1.00	0.36	1.5	20.00	1.00	1.4	34.00	< DL	1.6	1.00	< DL	1.3	41.00	< DL	1.7	22	< DL	1.6
Kumana	57.00	1.90	7.0	e	e	e	18.00	1.20	4.6	40.00	1.10	7.4	e	e	e	34	0.6	2.2
Transport	78.00	0.36	2.7	25.00	0.25	3.7	27.00	1.20	1.4	121.0	0.50	3.2	202.0	< DL	2.8	42	< DL	2.3
Mpondo	53.00	< DL	2.1	5.00	1.00	1.6	16.00	< DL	1.2	15.00	0.30	1.9	36.00	0.2	1.9	36	0.3	2.2
N'watindlopfu	2.00	0.33	2.2	969.0	1.80	22	34.00	0.61	6.6	62.00	0.30	12	599.0	1.5	12	24	0.4	2.4
Mpanamana	17.00	0.55	4.4	608.0	2.40	20	1.00	0.33	2.5	10.00	0.70	7.1	26.00	0.8	12	1.0	0.5	2.8
Nsemane	3669	8.40	101	NT	NT	NT	7.00	1.40	6.6	19.00	0.40	2.5	4012	5.7	28	19	0.8	2.7

e = empty dam; NT = not tested; < DL = less than the detection limit (0.20 mg/ℓ)

3.2.8 Total dissolved solids

The total dissolved solids (TDS) determined for the 13 KNP dams are given in **Table 7**. The values ranged between 131-1421 mg/ℓ in June 2009, 129-849 mg/ℓ in September 2009, 106-1400 mg/ℓ in February 2010, 160-527 mg/ℓ in July 2010, 162-940 mg/ℓ in October 2010 and 107-339 mg/ℓ in February 2011. The lowest TDS was determined at Nsemane Dam in February 2010, whereas the highest TDS was determined at Shiloweni Dam in June 2009. Suspended solids in the water column contribute to the underwater climate that favours growth of *Microcystis*. The TDS results observed in this study are comparable to those observed by Te and Gin (2011) at the Kranji Reservoir, where they reported TDS levels ranging between 60.0-180 mg/ℓ. There was no linear correlation (r=0.1594) found between the cyanobacteria biomass and the TDS for all the 13 KNP dams during this study (**Appendix 2**).

3.3 Quantification of microcystins

Since no *Nodularia* spp. were detected in any of the 13 KNP dams, the strip test and ELISA immunoassays, measured only the microcystin concentration. Lateral flow dipsticks have been successfully used for a wide range of applications including the detection of drugs of abuse, pregnancy and many environmental contaminants (Lawton et al., 2010). Results of the qualitative determination of microcystins using the ABRAXIS strip test kit are given in **Table 8**. The results showed that 60% of the 13 KNP dams sampled in June 2009 were positive for microcystins, whereas 55% in September 2009; 85% in February 2010; 62% in July 2010 and 82% in October 2010 were also positive for microcystins. All the 13 KNP dams sampled in February 2011 were negative for microcystins. The dipstick format of the ImmunoStrip® has been used successfully in the detection of microcystins (Lawton et al., 2010). There was no linear correlation (r=0.236) found between the cyanobacteria biomass and the strip test results for all the 13 KNP dams during this study (**Appendix 2)**.

ELISAs using either polyclonal or monoclonal antibodies for microcystins have been most widely utilised because they are highly specific, sensitive and quick methods to detect microcystins and nodularins (An & Carmichael, 1994; Nagata et al., 1995; Rapala et al., 2002; Lawton et al., 2010). The microcystin concentrations determined for the 13 KNP dams using the ELISA are given in **Table 8**. The concentrations ranged between 0.15-4728.23 ng/mℓ in June 2009, 0.32-33645.29 ng/mℓ in September 2009, 0.10-3275.53 ng/mℓ in February 2010, 0.12-3475.74 ng/mℓ in July 2010, 0.11-2.87 ng/mℓ in October 2010 and 0.13-0.28 ng/mℓ in February 2011. The highest microcystin concentration was determined at Sunset Dam in September 2009. The highest microcystin toxin levels were observed at the

KNP dams in the following decreasing order over the sampling period, when using the ELISA: September 2009 > June 2009 > July 2010 > February 2010 > October 2010 > February 2011. The microcystin levels reported in this study are among the highest levels reported in South Africa. The highest microcystin levels that have been previously reported were at Sunset Dam, 125000 ng/mℓ, in June 2007 (Masango et al., 2010). Oberholster et al. (2009) recorded microcystin levels of 23.7 ng/mℓ at Nhlanganzwane Dam in July 2007. A significant positive linear correlation (r=0.53247; p<0.0001) was found between the cyanobacteria biomass and the total microcystin (ELISA results) for all the 13 KNP dams during this study (**Appendix 2**). The results of this study are comparable with the results of Te and Gin (2011) who observed a positive correlation between the cyanobacteria biomass, expressed as *Microcystis* 16S rRNA, and the microcystin levels. In their study, Li et al. (2010) also found a positive correlation between microcystin toxins and the cyanobacterial cell numbers.

Microcystins are a large group of toxic peptides characterised by differing hydrophobicity that can be readily chromatographed by reversed-phase HPLC. Seven microcystin toxin standards designated -RR, -YR, -LR, -LA, -LY, -LW and -LF were used. The two-letter suffix in the cyclic heptapeptides denote: R=arginine, Y=tyrosine, L=leucine, A=alanine, W=tryptophan and F=phenylalanine. A linear calibration for all the seven microcystins was achieved and the examples of the typical calibration curves and the corresponding correlation coefficients for the microcystin variants are shown in **Figure 11**. Separation of the seven microcystins by the HPLC procedure is shown in **Figure 12**. A chromatogram indicating the different microcystins present in one of the field water samples collected from Nyamundwa Dam at the KNP is shown in **Figure 13**. The different microcystin variants and their concentrations determined for the 13 KNP dams using the HPLC are given in **Tables 9** to **14**.

The presence of the seven microcystin toxins varied between the different 13 KNP dams and the sampling periods. The total microcystin concentrations for all the 13 KNP dams determined in June 2009 are given in **Table 9**, and they ranged between 0.03-1954.03 ng/mℓ. Shiloweni Dam had the highest total microcystin concentration in June 2009 with microcystin-YR, 873.41 ng/mℓ, as the major variant. Shitlhave Dam had the lowest total microcystin concentration in June 2009 with microcystin-YR, 0.03 ng/mℓ, as the major variant. None of the seven microcystin toxins were detected in the water samples from Piet Grobler, Nyamundwa, Mestel, Kumana and N'watindlopfu dams in June 2010. The total microcystin concentrations for all the 13 KNP dams determined in September 2009 are given in **Table 10**, and they ranged between 0.04-169.61 ng/mℓ. Sunset Dam had the highest total

microcystin concentration with microcystin-LR, 78.73 ng/mℓ, as the major variant in September 2009. During the same period, Piet Grobler Dam had the lowest total microcystin concentrations with microcystin-LR, 0.04 ng/mℓ, as the major variant. The seven microcystin toxins were not detected in the water samples from Nyamundwa, Shitlhave and Transport dams in September 2010. The total microcystin concentrations for all the 13 KNP dams determined in February 2010 are given in **Table 11**, and they ranged between 0.03-937.20 ng/mℓ. The highest total microcystin concentration was observed at Shiloweni Dam in February 2010, with microcystin-RR, 346.60 ng/mℓ, as the main variant. The seven microcystin variants were not detected in Piet Grobler, Shitlhave, Transport, Mpondo, N'watindlopfu and Nsemane dams in February 2010. The total microcystin concentrations for all the 13 KNP dams determined in July 2010 are given in **Table 12**, and they ranged between 0.02-0.66 ng/mℓ. None of the seven microcystin toxins were detected in the water samples from Piet Grobler, Mazithi, Shitlhave, Mestel, Kumana, Transport, Mpondo, Mpanamana and Nsemane dams in July 2010. Sunset Dam had the highest total microcystin levels during the same period, with microcystin-LY, 0.63 ng/mℓ, as the main variant. The total microcystin concentrations for all the 13 KNP dams determined in October 2010 are given in **Table 13**, and they ranged between 0.06-1.0 ng/mℓ. Microcystin toxins were only detected in Shitlhave, Transport and Mpanamana dams in October 2010. Transport Dam had the highest total microcystin concentration in October 2010 with microcystin-LF, 1.0 ng/mℓ, as the major variant. Shiloweni and Kumana dams were dry and empty during this period. The total microcystin concentrations for all the 13 KNP dams determined in February 2011 are given in **Table 14**, and they ranged between 0.03-1.21 ng/mℓ. Transport Dam had the highest total microcystin levels with microcystin-LY, 1.07 ng/mℓ, as the major variant during the same period.

The HPLC results show a substantial variability in the individual toxin content at the different KNP dams in different seasons. This variability has been reported previously in South Africa at the Hartbeespoort Dam and also worldwide, as the common characteristic of cyanobacteria and their blooms (Wicks & Thiel, 1990). In the current study, the highest microcystin toxin variants were observed at the KNP dams in the following decreasing order over the sampling period: June 2009 > February 2010 > September 2009 > February 2011 > July 2010 > October 2010. The highest microcystin toxin variants were observed at Shiloweni Dam, in June 2009, in the following decreasing order: -LR > -YR > -RR > -LA > -LY > -LF > -LW.

As with the ELISA test, the microcystin levels detected in this study using the HPLC were high when compared to the results of other investigators. In their study, Li et al. (2010) found

the three microcystin toxin variant, -RR, -YR and -LR, at the Yanghe Reservoir and their total concentrations were: -RR, 71.7 ng/mℓ; -YR, 3.80 ng/mℓ and -LR, 25.1 ng/mℓ. At the Hartbeespoort Dam, six microcystin toxin variants were detected and they are -LR, -YR, -LA, -YA, -FR and -LAba (Wicks & Thiel, 1990). Okello et al. (2010) found the highest total microcystin levels at Lake Saka ranging between 0.50-10.2 ng/mℓ. A significant positive linear correlation (r=0.50437; p<0.0001) was found between the cyanobacteria biomass and the total microcystin (HPLC results) for all the 13 KNP dams during this study (**Appendix 2**). In addition to the correlation of the total microcystin, the microcystin toxin variants -LR (r=0.72107; p<0.0001), -LY (r=0.87671; p<0.0001) and -LW (r=0.86142; p<0.0001) had a significant positive linear correlation with the cyanobacteria biomass. The results of this study are comparable with the results of Okello et al. (2010) who observed a positive correlation between the *Microcystis* cell numbers and the total microcystin concentration. In their study, Li et al. (2010) also found a positive correlation between microcystin toxins and the cyanobacterial cell numbers.

The cyanobacteria cell numbers and microcystin levels were not influenced by the size of the KNP dams since there was no correlation found between the sizes of the KNP dams and the cyanobacteria biomass and microcystin toxins (**Appendix 2**).

Table 7. Turbidity and total dissolved solids (TDS) levels of the KNP dams.

KNP Dam	June 2009		September 2009		February 2010		July 2010		October 2010		February 2011	
	Turb. (NTU)	TDS (mg/ℓ)	Turb. (NTU)	TDS (mg/ℓ)	Turb. (NTU)	TDS (mg/ℓ)	Turb. (NTU)	TDS (mg/ℓ)	Turb. (NTU)	TDS (mg/ℓ)	Turb. (NTU)	TDS (mg/ℓ)
Sunset	178	358	833	260	725	1400	16.0	216	25.0	280	19.0	274
Shiloweni	818	1421	425	330	661	142	441	486	e	e	e	e
Piet Grobler	60.0	295	15.0	233	2.90	719	24.0	218	7.30	202	7.00	151
Mazithi	42.0	244	25.0	308	5.70	259	21.0	289	79.0	432	34.0	256
Nyamundwa	7.20	304	3.80	324	1.60	300	7.60	311	11.0	358	5.80	127
Shitlhave	20.0	131	4.70	129	1.00	120	7.50	160	11.0	170	2.40	123
Mestel	6.10	149	7.20	159	2.10	127	2.20	164	6.50	176	2.10	130
Kumana	514	273	e	E	3.50	185	124	336	e	e	15.0	339
Transport	47.0	184	27.0	190	1.70	201	31.0	222	7.50	162	5.10	107
Mpondo	15.0	149	4.80	185	1.40	180	47.0	322	19.0	238	15.0	160
N'watindlopfu	78.0	351	353	739	4.70	238	37.0	388	81.0	822	8.70	113
Mpanamana	146	526	161	849	2.20	347	4.60	527	31.0	940	13.0	229
Nsemane	948	410	NT	NT	1.50	106	59.0	244	144	739	9.10	112

e = empty dam; NT = not tested

Table 8. Analysis of microcystin in the KNP dams using the ABRAXIS strip test and ELISA.

KNP Dam	June 2009		September 2009		February 2010		July 2010		October 2010		February 2011	
	Strip Test	ELISA (ng/mℓ)	Strip Test	ELISA (ng/mℓ)	Strip Test	ELISA (ng/mℓ)	Strip Test	ELISA (ng/mℓ)	Strip Test	ELISA (ng/mℓ)	Strip Test	ELISA (ng/mℓ)
Sunset	+ve	4.49E+02	+ve	3.36E+04	+ve	1.00E+03	+ve	3.48E+03	+ve	2.15E+00	-ve	2.80E-01
Shiloweni	+ve	1.48E+03	+ve	1.21E+02	+ve	3.28E+03	+ve	2.38E+03	e	e	e	e
Piet Grobler	-ve	<DL	-ve	<DL	-ve	<DL	-ve	<DL	-ve	<DL	-ve	<DL
Mazithi	+ve	1.04E+00	-ve	3.20E-01	+ve	2.83E+00	+ve	3.00E-01	+ve	<DL	-ve	1.30E-01
Nyamundwa	NT	<DL	-ve	<DL	+ve	1.11E+00	+ve	4.10E-01	+ve	<DL	-ve	<DL
Shitlhave	+ve	<DL	-ve	<DL	+ve	<DL	-ve	1.34E+00	+ve	2.87E+00	-ve	<DL
Mestel	NT	<DL	-ve	<DL	-ve	<DL	-ve	<DL	+ve	<DL	-ve	<DL
Kumana	-ve	<DL	e	e	+ve	2.72E+00	-ve	<DL	e	e	-ve	<DL
Transport	-ve	3.40E+00	+ve	2.73E+00	+ve	1.70E-01	+ve	1.20E-01	+ve	2.54E+00	-ve	1.40E-01
Mpondo	+ve	8.30E-01	+ve	2.23E+00	+ve	<DL	+ve	<DL	-ve	<DL	-ve	<DL
N'watindlopfu	-ve	1.50E-01	+ve	<DL	+ve	4.67E+00	+ve	3.87E+00	+ve	1.00E+00	-ve	<DL
Mpanamana	+ve	6.40E-01	+ve	<DL	+ve	1.20E-01	+ve	1.40E-01	+ve	1.10E-01	-ve	<DL
Nsemane	NT	4.73E+03	NT	NT	+ve	1.00E-01	-ve	2.40E-01	+ve	8.70E-01	-ve	<DL

e = empty dam; NT = not tested; +ve = positive; -ve = negative; < DL = less than detection limit (0.10 ng/mℓ)

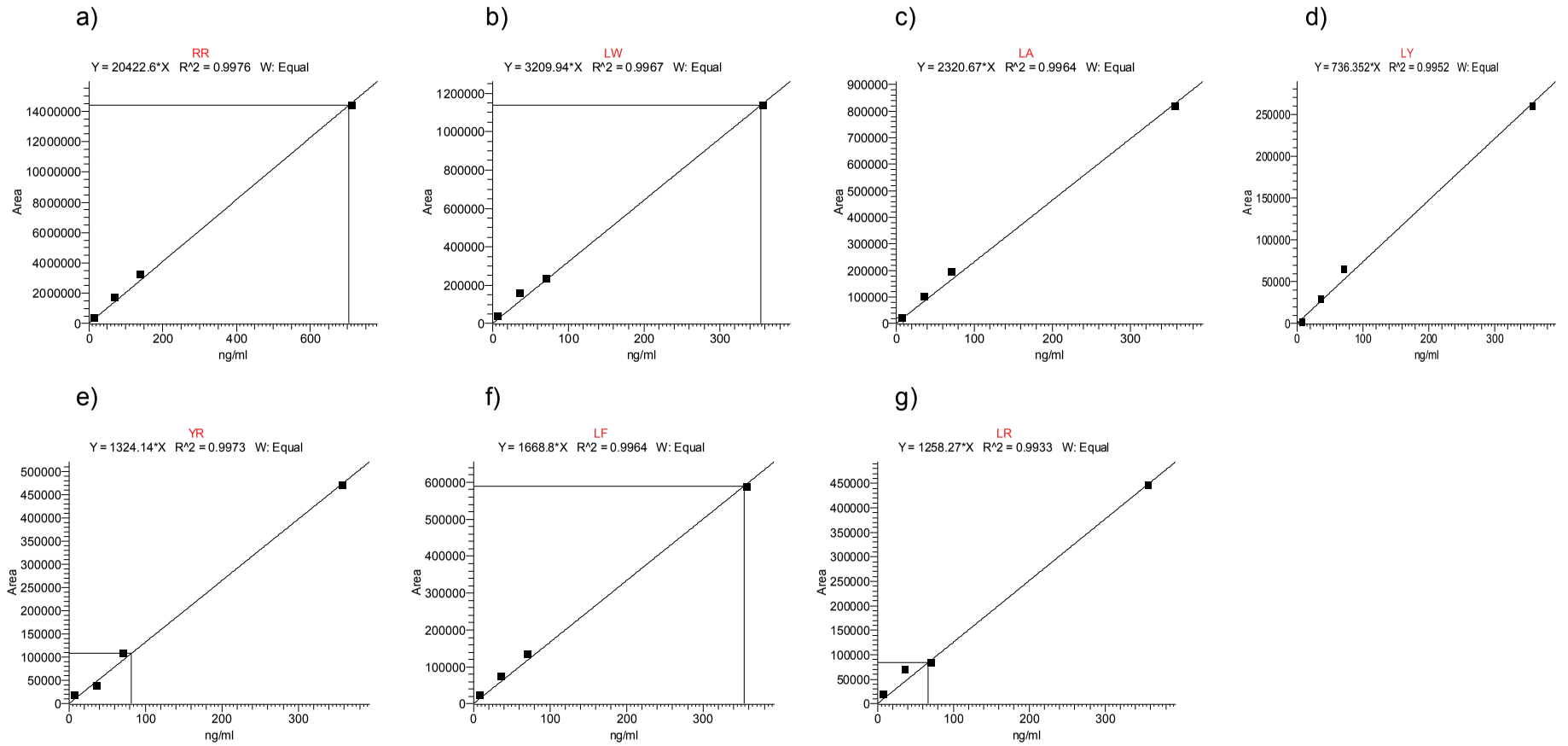


Figure 12. Linear calibration graphs by HPLC: a) microcystin-RR, b) microcystin-LW, c) microcystin-LA, d) microcystin-LY, e) microcystin-YR, f) microcystin-LF and g) microcystin-LR.

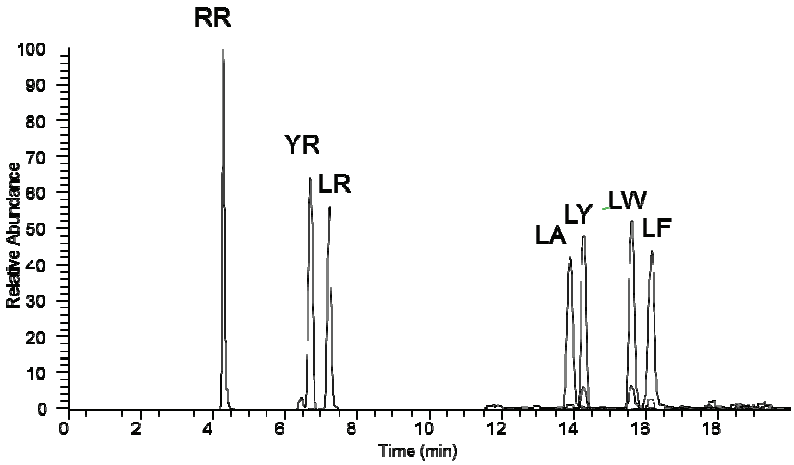


Figure 13. HPLC separation of a solution of standards containing the seven microcystin variants: microcystin-RR, microcystin-LW, microcystin-LA, microcystin-YR, microcystin-LF, microcystin-LR and microcystin-LY.

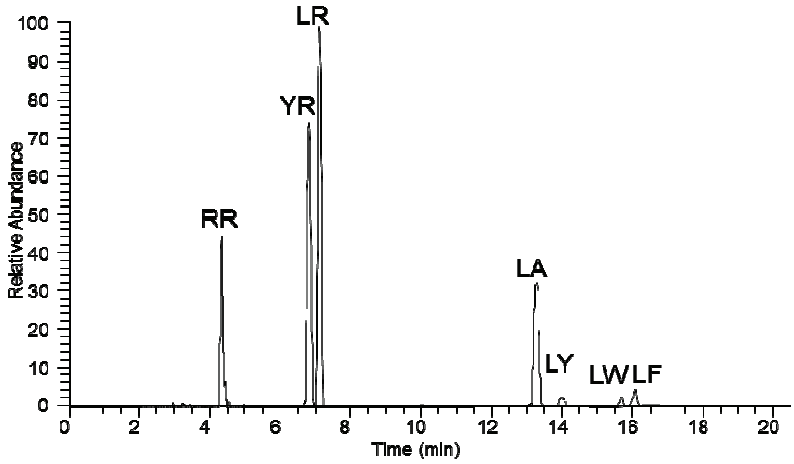


Figure 14. Chromatogram of microcystins present in the water sample from Nyamundwa Dam (June 2008) in the Kruger National Park.

Table 9. Analysis of microcystin in the KNP dams using the HPLC (June 2009).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	0.11	3.33	2.19	0.80	<DL	<DL	<DL	6.43
Shiloweni	13.74	873.41	742.37	277.71	19.19	<DL	27.61	1954.03
Piet Grobler	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mazithi	0.01	0.07	0.15	<DL	<DL	<DL	<DL	0.23
Nyamundwa	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Shitlhave	<DL	0.03	<DL	<DL	<DL	<DL	<DL	0.03
Mestel	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Kumana	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Transport	<DL	0.21	0.93	<DL	<DL	<DL	<DL	1.14
Mpondo	<DL	<DL	0.30	<DL	<DL	<DL	<DL	0.30
N'watindlopfu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpanamana	<DL	<DL	0.05	<DL	<DL	<DL	<DL	0.05
Nsemane	6.41	<DL	633.81	<DL	40.78	33.77	<DL	714.77

<DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

Table 10. Analysis of microcystin in the KNP dams using the HPLC (September 2009).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	<DL	52.84	78.73	26.83	6.85	2.62	1.74	169.61
Shiloweni	1.37	1.24	2.34	1.49	0.60	<DL	<DL	7.04
Piet Grobler	<DL	<DL	0.04	<DL	<DL	<DL	<DL	0.04
Mazithi	<DL	0.01	<DL	<DL	0.82	<DL	<DL	0.83
Nyamundwa	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Shitlhave	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mestel	0.01	0.68	0.52	0.36	<DL	<DL	<DL	1.57
Kumana	e	e	e	e	e	e	e	e
Transport	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpondo	<DL	0.10	0.83	<DL	<DL	<DL	0.70	1.63
N'watindlopfu	<DL	0.19	3.74	0.50	<DL	<DL	<DL	4.43
Mpanamana	0.03	0.05	0.18	<DL	<DL	<DL	<DL	0.26
Nsemane	NT	NT	NT	NT	NT	NT	NT	NT

e = empty dam; NT = not tested; ND = not detected; <DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

Table 11. Analysis of microcystin in the KNP dams using the HPLC (February 2010).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	1.84	118.46	144.53	44.55	<DL	1.46	<DL	310.84
Shiloweni	346.60	169.59	338.31	73.37	9.33	<DL	<DL	937.20
Piet Grobler	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mazithi	0.28	0.06	0.02	<DL	<DL	<DL	<DL	0.36
Nyamundwa	<DL	0.05	<DL	<DL	<DL	<DL	<DL	0.05
Shitlhave	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mestel	<DL	0.03	<DL	<DL	<DL	<DL	<DL	0.03
Kumana	<DL	<DL	0.03	<DL	<DL	<DL	<DL	0.03
Transport	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpondo	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
N'watindlopfu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpanamana	<DL	<DL	0.04	<DL	<DL	<DL	<DL	0.04
Nsemane	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

<DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

Table 12. Analysis of microcystin in the KNP dams using the HPLC (July 2010).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	<DL	<DL	0.03	<DL	0.63	<DL	<DL	0.66
Shiloweni	<DL	0.02	<DL	<DL	<DL	<DL	<DL	0.02
Piet Grobler	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mazithi	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Nyamundwa	<DL	<DL	0.03	<DL	<DL	<DL	<DL	0.03
Shitlhave	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mestel	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Kumana	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Transport	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpondo	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
N'watindlopfu	<DL	<DL	0.07	<DL	<DL	<DL	<DL	0.07
Mpanamana	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Nsemane	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

<DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

Table 13. Analysis of microcystin in the KNP dams using the HPLC (October 2010).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Shiloweni	e	e	e	e	e	e	e	e
Piet Grobler	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mazithi	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Nyamundwa	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Shitlhave	<DL	0.03	0.03	<DL	<DL	<DL	<DL	0.06
Mestel	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Kumana	e	e	e	e	e	e	e	e
Transport	<DL	0.05	0.30	<DL	<DL	<DL	0.65	1.0
Mpondo	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
N'watindlopfu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpanamana	<DL	<DL	<DL	<DL	0.44	<DL	<DL	0.44
Nsemane	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

e = empty dam; ND = not detected; <DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

Table 14. Analysis of microcystin in the KNP dams using the HPLC (February 2011).

KNP Dam	Microcystin (ng/mℓ)							
	-RR	-YR	-LR	-LA	-LY	-LW	-LF	Total
Sunset	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Shiloweni	e	e	e	e	e	e	e	e
Piet Grobler	<DL	<DL	0.03	<DL	<DL	<DL	<DL	0.03
Mazithi	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Nyamundwa	<DL	<DL	0.03	<DL	<DL	<DL	<DL	0.03
Shitlhave	<DL	0.02	0.02	<DL	<DL	<DL	<DL	0.04
Mestel	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Kumana	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Transport	<DL	<DL	0.14	<DL	1.07	<DL	<DL	1.21
Mpondo	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
N'watindlopfu	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
Mpanamana	<DL	0.03	<DL	<DL	<DL	<DL	<DL	0.03
Nsemane	<DL	<DL	0.06	<DL	0.80	<DL	<DL	0.86

e = empty dam; ND = not detected; <DL = less than detection limit; Detection limits (ng/mℓ) are: -RR = 0.01, -YR = 0.02, -LR = 0.02, -LA = 0.32, -LY = 0.32, -LW = 0.53 and -LF = 0.53

3.4 Wild animal deaths in June 2010 in the KNP

The water samples collected from Shiloweni Dam in June 2010 had high algal concentrations of 4.61×10^5 cells/mℓ which were dominated by *Microcystis aeruginosa*. Euglenophyceae (e.g. *Trachelomonas*) was also present but in low numbers (7.32×10^3 cells/mℓ). The physicochemical conditions measured at the Shiloweni Dam in June 2010 were chlorophyll-*a*, total nitrogen, total phosphorus, total dissolved solids, turbidity and microcystin concentration using the ELISA. The data measured in Shiloweni Dam in June 2010 are given in **Table 15**. A number of carcasses were found within the vicinity of Shiloweni Dam in June 2010 and they included 11 zebras (*Equus burchelli*), 2 rhinos (*Ceratotherium simum*) and 1 wildebeest (*Connochaetes taurinus*). Post mortem and histopathology performed on the intact zebra carcasses revealed symptoms (hepatic haemorrhaging and necrosis) that are consistent with *Microcystis* bloom intoxication.

Table 15. Algal concentration and physicochemical measurements of water samples from Shiloweni Dam and the wild animal deaths recorded in June 2010.

Variables:	**Shiloweni Dam (June 2010)**
Algal concentration	4.61×10^5 cells/mℓ
Microcystis aeruginosa	Present
Chlorophyll-*a*	6341 µg/ℓ
Total Nitrogen	187 mg/ℓ
Total Phosphorus	21 mg/ℓ
Total Dissolved Solids	851 mg/ℓ
Turbidity	461 NTU
Microcystin concentration (water):	
ELISA	4.06×10^5 ng/mℓ
Wild animal deaths	11 zebras (*Equus burchelli*), 2 rhinos (*Ceratotherium simum*), 1 wildebeest (*Connochaetes taurinus*)

Figure 15. Photos showing the carcass of a zebra (*Equus burchelli*) found within the vicinity of Shiloweni Dam in June 2010 (Photos taken by the KNP personnel).

4 CONCLUSIONS

Microcystin-producing algae were detected in some of the 13 KNP dams throughout the study period. *Microcystis aeruginosa* was the most dominant cyanophyceae, especially during the incidents of bloom formation. Algal blooms were experienced mostly at the Sunset, Shiloweni and Nsemane dams. Although the temperature and pH levels recorded at the KNP dams were favourable in promoting the growth of cyanobacteria, no correlation was observed between the cyanobacteria biomass and the two parameters.

The results of this study have shown that nutrient supply (total phosphorus and total nitrogen) is the main driving factor in promoting the growth of cyanobacteria at the KNP dams. It has been postulated that the nutrient supply is influenced by the urine and faecal material excreted by the high population of Hippopotami living in most of the KNP dams.

The use of the strip test, which can rapidly detect microcystins and nodularins in the field with minimal equipment or processing, has been successfully demonstrated in this study. The strip test results were comparable to the ELISA results with only 3.1% overall disagreement observed between the two immunoassays. The highest microcystin toxin levels (ELISA results) were observed at the KNP dams in the following decreasing order over the sampling period: September 2009 > June 2009 > July 2010 > February 2010 > October 2010 > February 2011. The presence of the seven microcystin toxins, -RR, -YR, -LR, -LA, -LY, -LW and -LF, varied between the different 13 KNP dams and the sampling periods. The highest microcystin toxin variants were observed at Shiloweni Dam, in June 2009, in the following decreasing order: -YR > -LR > -LA > -LF > -LY > -RR. A significant positive linear correlation was found between the cyanobacteria biomass and the total microcystin for all the 13 KNP dams during this study when using the ELISA and HPLC methods. The HPLC method was found to be more sensitive than the immunoassays when quantifying microcystin toxins. The water samples which were shown to be negative by the immunoassays were found to contain microcystin toxins, however in small quantities. The HPLC was limited in its quantification only to the seven microcystin variants thus resulting in the underestimation of the microcystin concentration, whereas the immunoassays detected all the microcystin variants and nodularin toxins.

The algal biomass and microcystin toxin levels at the KNP dams were variable throughout the sampling period; as a result the dominance of these parameters could not be ascertained based on the seasons. However, rainfalls appear to have had an influence/effect on the algal biomass and microcystin toxin levels. In general, the winter seasons of 2009

and 2010 were dry when compared to the summer months. Water samples collected in September 2009 were characterized by the highest algal biomass and microcystin toxin levels and there were no rains during the same month. Water samples collected in February 2011 were characterized by the lowest algal biomass and microcystin toxin levels and rains were observed days before sampling and during sampling in February 2011. It is highly possible that rainfalls have a dilution effect on the algal biomass and toxin levels.

The biomass-related variables, chlorophyll-*a* and turbidity, were positively correlated to the cyanobacteria cell numbers. Dissolved oxygen, which is one of the major physical water quality variables, was also negatively correlated to the cyanobacteria biomass. There was no correlation between the total dissolved solids and the cyanobacteria biomass. It seems that the size of the KNP dams did not influence the cyanobacteria cell numbers and microcystin levels.

The microcystin levels measured at the Shiloweni Dam were high during the incident of wildlife deaths which were observed around this dam. Post mortem and histopathology performed on the intact zebra carcasses revealed symptoms that are consistent with *Microcystis* bloom intoxication.

5 RECOMMENDATIONS

The following actions are recommended:

- Continuous monitoring of the KNP dams to ensure that proper preventive actions are taken to prevent poisoning of the wildlife.

- The use of dipstick immunoassays (e.g. ABRAXIS Strip Test kit or Agdia ImmunoStrip kit) in monitoring the toxin levels in the KNP dams, because the dipsticks provide almost instant results and the users require no previous training.

- Control of eutrophication at the KNP should focus on maintaining the nutrients at low levels and also controlling the Hippopotamus population.

- Future studies to focus on :

    - Using molecular techniques to further characterise the cyanobacteria at the KNP dams.
    - The role of Hippopotami in maintaining high levels of nutrients at the KNP dams and also the possible intervention strategies that could be used.
    - The extension of assessment of the toxicity of cyanobacteria to include the northern part of the KNP, because the current study focused only on the southern part of the KNP.

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APPENDIX 1: DETAILED ALGAL PROFILES (TAXA)

June 2009 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Kumana	Nsemane
1. CYANOPHYCEAE	**351 423**	**6 589 155**	**0**	**16 693**	**879**	**440**	**587**	**1 758**	**10 399**	**0**	**0**	**0**	**44 220 552**
	81.36%	**97.70%**	**0%**	**67.85%**	**6.70%**	**5.40%**	**6.70%**	**34.80%**	**90.39%**	**0%**	**0%**	**0%**	**99.97%**
Anabaena sp. (F)	153 747			6 150				659	74				
Anabaenopsis sp.									220				
Merismopodia sp. (col)						220	147		5 491				
Microcystis aeruginosa (single cells)	183 033	6 589 155		7 907	879		293	1099	4 540				44 191 266
Oscilatoria sp. (F)	14 643			2 636		220	147		74				
2. BACILLARIOPHYCEAE	**21 966**	**21 964**	**440**	**4 394**	**7 470**	**2 637**	**440**	**1 100**	**369**	**0**	**3 515**	**14 643**	**0**
	5.07%	**0.33%**	**13.30%**	**17.86%**	**56.70%**	**32.40%**	**5%**	**21.80%**	**3.21%**	**0%**	**16.70%**	**40%**	**0%**
Cocconeis sp.					440	220			74				
Cymbella sp.													
Gyrosigma sp.													
Melosira sp. (F)	7 322				2 197		147	220	74				
Cyclotella sp. (10-20 µm)								220	147				
Navicula sp. (pennate)				879		440		220					
Nitzschia sp. (pennate)	7 322	21 964	220	2 636	4 393	1 977	293	440	74		3 515	14 643	
Pennate diatoms (other)													
Stauroneis sp.	7 322		220	879									
Synedra sp.					440								
3. CHLOROPHYCEAE	**43 931**	**65 892**	**660**	**2 637**	**3 078**	**2 419**	**2 347**	**650**	**370**	**879**	**14 060**	**0**	**0**
	10.17%	**0.97%**	**20%**	**10.72%**	**23.30%**	**29.70%**	**26.70%**	**12.90%**	**3.22%**	**13.30%**	**66.70%**	**0%**	**0%**
Ankistrodesmus sp. (needle, 30 µm)						220	147		74				
Chlamydomonas sp. (15-20 µm)	7 322		220		440	440	733						

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Kumana	Nsemane
Chlorella sp. (3-6 µm)			220										
Chlorococcum sp. (12-18 µm)				879		220							
Closterium sp.		21 964	220			220					3 515		
Coelastrum microporum (col)													
Cosmarium sp.					879	220							
Crucigenia sp. (4x4 col.)						879	147			879			
Elakatothrix sp.													
Eudorina sp. (col.)								220					
Micratinium sp. (col. Round, spikes)											3 515		
Monoraphidium arcuatum (big sickle)					440		586		74		3 515		
M. contortum (s-sickle)													
Oocystis sp. (col.)													
M. circinale (short fat sickle)	7 322	21 964			440	220			74		3 515		
O. solitaria (single cell)	14 643												
Pandorina sp. (col.)								220					
Pediastrum sp. (col.)									74				
Scenedesmus sp. (col.)	7 322	21 964			879		293						
Staurastrum sp.							147						
Schroederia sp. (needle with foot)				1 758									
Sphaerocystis sp. (col.)								220					
Spirogyra sp. (F)							147						
Tetrastrum sp. (4cells with spikes)													
Tetraedron sp.(4-angular)	7 322								74				
4. CRYPTOPHYCEAE	**7 322** **1.70%**	**73 928** **1.10%**	**0** **0%**	**879** **3.57%**	**1 318** **10%**	**1 099** **13.50%**	**1 904** **21.70%**	**220** **4.40%**	**147** **1.27%**	**3 075** **46.70%**	**3 515** **16.70%**	**7 322** **20%**	**0** **0%**
Cryptomonas sp.	7 322	73 928		879	1 318	1 099	1 904	220	147	3 075	3 515	7 322	
5. DINOPHYCEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**440** **5%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Kumana	Nsemane
Ceratium sp.				0			147						
Peridinium sp.							293						
6. EUGLENOPHYCEAE	**7 322** **1.70%**	**0** **0%**	**2 200** **66.70%**	**0** **0%**	**440** **3.30%**	**1 319** **16.20%**	**2 345** **26.70%**	**1 099** **21.70%**	**220** **1.91%**	**2 637** **40%**	**0** **0%**	**14 644** **40%**	**14 643** **0.03%**
Euglena sp.						220	147	220				7 322	
Lepocinclis sp. (pear shaped)							147						
Phacus sp.	7 322					440	147			440			14 643
Trachelomonas sp.			2 200		440	659	1 904	879	220	2 197		7 322	
7. CHRYSOPHYSEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**220** **2.70%**	**733** **8.30%**	**220** **4.40%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Mallomonas sp. (Chrysophyta)						220		220					
Dinobyron sp.							293						
Synura sp. (single cells)							440						
Total	**431 964**	**6 750 939**	**3 300**	**24 603**	**13 185**	**8 134**	**8 796**	**5 057**	**11 505**	**6 591**	**21 090**	**36 609**	**44 235 195**

September 2009 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana
1. CYANOPHYCEAE	**22 198 132** **99.87%**	**2 027 997** **96..85%**	**74** **7.20%**	**0** **0%**	**148** **5%**	**0** **0%**	**294** **4%**	**1 318** **9%**	**220** **16.70%**	**2 640 935** **99.27%**	**594 782** **99.40%**
Anabaena sp. (F)	14 643	58 571	74					1 318	220	1 758	13 179
Anabaenopsis sp.							220				
Merismopodia sp. (col)					74		74				
Microcystis aeruginosa (single cells)	21 597 786	1 947 462								2 639 177	581 603
Oscilatoria sp. (F)	585 703	21 964			74						
2. BACILLARIOPHYCEAE	**0** **0%**	**14 643** **0.70%**	**148** **14.40%**	**2 929** **12%**	**222** **8%**	**2 491** **56.60%**	**1 760** **23%**	**3 078** **21%**	**660** **50%**	**3 516** **0.13%**	**1 758** **0.30%**
Cocconeis sp.			74	2 929	74				220		
Cymbella sp.							74				
Gyrosigma sp.						147					
Melosira sp. (F)					74			440	220	1 758	879
Cyclotella sp. (10-20 µm)											
Navicula sp. (pennate)						147	74	440			
Nitzschia sp. (pennate)		14 643			74	2 050	1 538	1 758	220		879
Pennate diatoms (other)			74					440		1 758	
Stauroneis sp.						147	74				
Synedra sp.											
3. CHLOROPHYCEAE	**0** **0%**	**43 932** **2.10%**	**221** **21.40%**	**21 089** **87%**	**2 275** **77%**	**1 173** **26.70%**	**4 618** **61%**	**1 318** **9%**	**0** **0%**	**1 758** **0.07%**	**879** **0.14%**
Ankistrodesmus sp. (needle, 30 µm)					74						
Chlamydomonas sp. (15-20 µm)		7 322	74		293	293	293			1 758	
Chlorella sp. (3-6 µm)					586	440	367	1 318			
Chlorococcum sp. (12-18 µm)											
Closterium sp.		7 322	147	19 329	74	220					879

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana
Coelastrum microporum (col)				74							
Cosmarium sp.						220	74				
Crucigenia sp. (4x4 col.)		7 322			220	879	220				
Elakatothrix sp.											
Eudorina sp. (col.)											
Micratinium sp. (col. Round, spikes)		7 322									
Monoraphidium arcuatum (big sickle)							147				
M. contortum (s-sickle)							293				
Oocystis sp. (col.)				440			74				
M. circinale (short fat sickle)		21 964			74						
O. solitaria (single cell)				1 172							
Pandorina sp. (col.)											
Pediastrum sp. (col.)		7 322		74	74						
Scenedesmus sp. (col.)					293	440	2 929				
Staurastrum sp.					74		74				
Schroederia sp. (needle with foot)											
Sphaerocystis sp. (col.)											
Spirogyra sp. (F)											
Tetrastrum sp. (4cells with spikes)		7 322									
Tetraedron sp.(4-angular)					513		147				
4. CRYPTOPHYCEAE	**0 0%**	**7 322 0.35%**	**220 21.30%**	**0 0%**	**74 2.50%**	**0 0%**	**74 1%**	**2 197 15%**	**220 16.70%**	**5 272 0.20%**	**0 0%**
Cryptomonas sp.		7 322	220		74		74	2 197	220	5 272	
5. DINOPHYCEAE	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**3 515 24%**	**0 0%**	**0 0%**	**0 0%**
Ceratium sp.								3 515			
Peridinium sp.											
6. EUGLENOPHYCEAE	**29 285**	**0**	**368**	**293**	**221**	**734**	**808**	**3 076**	**220**	**3 786**	**879**

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana
	0.13%	**0%**	**35.70%**	**1%**	**7.50%**	**16.70%**	**11%**	**21%**	**16.70%**	**0.14%**	**0.14%**
Euglena sp.	14 643		74			147	74	879			
Lepocinclis sp. (pear shaped)						147	74				
Phacus sp.	14 643		74		74	147	74				
Trachelomonas sp.			220	293	147	293	586	2 197	220	3 786	879
7. CHRYSOPHYSEAE	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**	**0 0%**
Mallomonas sp. (Chrysophyta)											
Dinobyron sp.											
Synura sp. (single cells)											
Total	**22 227 418**	**2 093 894**	**1 031**	**24 311**	**2 940**	**4 398**	**7 554**	**14 502**	**1 320**	**2 660 267**	**598 298**

*Kumana Dam and Nsemane Dam were dry and empty

February 2010 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
1. CYANOPHYCEAE	**14 188 828** **99.70%**	**13 705 442** **99.40%**	**5 053** **51.10%**	**340 880** **89.10%**	**14 643** **8.30%**	**440** **2.90%**	**1 319** **10.70%**	**0** **0%**	**1 320** **9.40%**	**1 758** **1.30%**	**1 471 579** **95.71%**	**56 229** **72.70%**	**45 686** **81.20%**
Anabaena sp. (F)	43 928		220				879		440		21 964		
Anabaenopsis sp.						220	440		440	147			
Merismopodia sp. (col)			220	7 029									
Microcystis aeruginosa (single cells)	14 100 972	13 705 442	4 393	323 308						293	1 449 615	56 229	45 686
Oscilatoria sp. (F)	43 928		220	10 543	14 643	220			440	1 318			
2. BACILLARIOPHYCEAE	**43 958** **0.30%**	**43 928** **0.30%**	**440** **4.40%**	**3 515** **0.91%**	**117 143** **66.70%**	**6 372** **42.00%**	**2 638** **21.40%**	**7 322** **25.00%**	**4 835** **34.40%**	**1 027** **0.80%**	**0** **0%**	**0** **0%**	**0** **0%**
Cocconeis sp.					14 643	220			440				
Achnanthes sp.					14 643								
Gomphonema sp.						220							
Melosira sp. (F)			220			220	440		440	147			
Cyclotella sp. (10-20 µm)							440			440			
Navicula sp. (pennate)			220			440			440				
Nitzschia sp. (pennate)	43 958	43 928		3 515	58 571	5 052	1 318	7 322	3 075	440			
Pinnularia sp.									440				
Pennate diatoms (other)							440						
Stauroneis sp.					14 643	220							
Synedra sp.					14 643								
3. CHLOROPHYCEAE	**0** **0%**	**0** **0%**	**3 079** **31.10%**	**38 663** **10.09%**	**43 929** **25.00%**	**5 274** **34.80%**	**3 515** **28.60%**	**14 644** **50.00%**	**4 837** **34.40%**	**124 905** **95.50%**	**21 964** **1.43%**	**14 060** **18.10%**	**7 030** **12.50%**
Ankistrodesmus sp. (needle, 30 µm)				7 029					879	115 677			
Chlamydomonas sp. (15-20 µm)			440			2 636	879	7 322	440	7 029	21 964	3 515	
Chlorella sp. (3-6 µm)			879		14 643	659	1 318		1 318	1 318			3 515
Actinastrum hantzchii (star-col)			220										

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
Closterium sp.			220	3 515		220			440			3 515	3 515
Coelastrum microporum (col)				3 515									
Carteria fornicata (4 flagellums)			220	3 515									
Crucigenia sp. (4x4 col.)				3 515	14 643	220			440	293			
Elakatothrix sp.				3 515									
Microspora										147			
Micratinium sp. (col. Round, spikes)									440				
Monoraphidium arcuatum (big sickle)			220							147			
M. contortum (s-sickle)			440										
Oocystis sp. (single cell)				7 029									
M. circinale (short fat sickle)			220										
Pediastrum sp. (col.)						220			440	147		3 515	
Scenedesmus sp. (col.)			220	3 515	14 643	659	1 318	7 322	440	147			
Staurastrum sp.						440							
Schroederia sp. (needle with foot)												3 515	
Tetraedron sp.(4-angular)				3 515		220							
4. CRYPTOPHYCEAE	**0** **0%**	**0** **0%**	**659** **6.70%**	**0** **0%**	**0** **0%**	**1 318** **8.70%**	**0** **0%**	**0** **0%**	**879** **6.20%**	**733** **0.60%**	**0** **0%**	**3 515** **4.60%**	**3 515** **6.30%**
Cryptomonas sp.			659			1 318			879	733		3 515	3 515
5. DINOPHYCEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**659** **4.40%**	**0** **0%**	**0** **0%**	**0** **0%**	**440** **0.30%**	**0** **0%**	**0** **0%**	**0** **0%**
Peridinium sp.						659				440			
6. EUGLENOPHYCEAE	**0** **0%**	**43 928** **0.30%**	**659** **6.70%**	**0** **0%**	**0** **0%**	**1 099** **7.20%**	**4 395** **35.70%**	**7 322** **25.00%**	**2 198** **15.60%**	**1 906** **1.50%**	**43 928** **2.86%**	**3 515** **4.60%**	**0** **0%**
Euglena sp.							440		440	440			
Lepocinclis sp. (pear shaped)							440			147			
Phacus sp.						220				147			
Trachelomonas sp.		43 928	659			879	3 515	7 322	1 758	1 172	43 928	3 515	

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
7. CHRYSOPHYSEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**440** **3.60%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Dinobyron sp.							440						
Total	**14 232 786**	**13 793 298**	**9 890**	**383 058**	**175 715**	**15 162**	**12 307**	**29 288**	**14 069**	**130 769**	**1 537 471**	**77 319**	**56 231**

July 2010 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
	21 966	**17 573**	**1 758**	**15 824**	**0**	**147**	**440**	**0**	**0**	**879**	**1 758**	**0**	**0**
1. CYANOPHYCEAE	**32.90%**	**30.77%**	**18.18%**	**44.24%**	**0%**	**4.36%**	**4.65%**	**0%**	**0%**	**12.50%**	**6.69%**	**0%**	**0%**
Anabaena sp. (F)	879		879			147				879			
Anabaenopsis sp.							440						
Merismopodia sp. (col)	1 758	7 029	879	15 824							1 758		
Microcystis aeruginasa (single cells)	5 272	3 515											
Oscilatoria sp. (F)	14 057	7 029											
	6 152	**7 909**	**879**	**294**	**2 785**	**733**	**660**	**14 643**	**1 758**	**879**	**0**	**0**	**0**
2. BACILLARIOPHYCEAE	**9.21%**	**13.85%**	**9.09%**	**0.82%**	**23.75%**	**21.73%**	**6.98%**	**14.29%**	**7.15%**	**12.50%**	**0%**	**0%**	**0%**
Cocconeis sp.	1 758												
Cymbella sp.					147		220						
Melosira sp. (F)		3 515	879		293		220		879				
Cyclotella sp. (10-20 µm)	3 515	879			147								
Navicula sp. (pennate)					440								
Nitzschia sp. (pennate)	879	3 515		147	1 611	440				879			
Pennate diatoms (other)				147		293	220	14 643	879				
Diatoma sp.					147								
	21 968	**28 118**	**2 637**	**2 079**	**1 320**	**880**	**3 078**	**14 643**	**3 516**	**2 637**	**1 758**	**5 274**	**1 758**
3. CHLOROPHYCEAE	**32.90%**	**49.23%**	**27.28%**	**5.82%**	**11.26%**	**26.09%**	**32.56%**	**14.29%**	**14.29%**	**37.50%**	**6.69%**	**50.00%**	**14.29%**
Actinastrum hantzchii (star-col.)			879										
Ankistrodesmus sp. (needle, 30 µm)	879			147	147	293					1 758		
Chlamydomonas sp. (15-20 µm)	4 393	3 515				147	1 538		879	879			
Chlorella sp. (3-6 µm)		3 515					220						
Chlorococcum sp. (12-18 µm)	879						440					1 758	
Closterium sp.	879	3 515	879	1 758					879				1 758
Crucigenia sp. (4x4 col.)	6 150	10 543	879		293		220						

SPECIES (F = Filament)	Sunset	Shiloweni	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
Micratinium sp. (col. Round, spikes)		3 515				147							
Monoraphidium arcuatum (big sickle)				174	147		220	14 643	879			1 758	
Oocystis sp. (col.)	879									879			
Scenedesmus sp. (col.)	2 636				733	293	440		879			1 758	
Staurastrum sp.	879												
Schroederia sp. (needle with foot)	879												
Tetrastrum sp. (4cells with spikes)		3 515											
Tetraedron sp. (4-angular)	3 515									879			
4. CRYPTOPHYCEAE	**879** **1.31%**	**0** **0%**	**1 758** **18.18%**	**8 493** **23.74%**	**2 490** **21.24%**	**0** **0%**	**1 758** **18.60%**	**14 643** **14.29%**	**2 636** **10.71%**	**1 758** **25.00%**	**15 714** **59.84%**	**1 758** **16.67%**	**8 786** **71.42%**
Cryptomonas sp.	879		1 758	8 493	2 490		1 758	14 643	2 636	1 758	15 714	1 758	8 786
5. DINOPHYCEAE	**879** **1.31%**	**0** **0%**	**1 758** **18.18%**	**8 493** **23.74%**	**2 490** **21.24%**	**147** **4.36%**	**1 758** **18.60%**	**14 643** **14.29%**	**9 665** **39.28%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Ceratium sp.						147			879				
Peridinium sp.	879		1 758	8 493	2 490		1 758	14 643	8 786				
6. EUGLENOPHYCEAE	**14 937** **22.37%**	**3 515** **6.15%**	**879** **9.09%**	**586** **1.64%**	**2 492** **21.26%**	**1 466** **43.46%**	**1 759** **18.61%**	**43 929** **42.84%**	**6 151** **25.00%**	**879** **12.50%**	**7 032** **26.78%**	**3 516** **33.33%**	**1 758** **14.29%**
Euglena sp.	2 636	3 515			440	440	879	29 286	879		1 758		
Lepocinclis sp. (pear shaped)	879				147	147					1 758		
Phacus sp.	879				1 758		440		879		1 758	1 758	
Trachelomonas sp.	10 543		879	586	147	879	440	14 643	4 393	879	1 758	1 758	1 758
7. CHRYSOPHYSEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**147** **1.25%**	**0** **0%**	**0** **0%**	**0** **0%**	**879** **3.57%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Mallomonas sp. (Chrysophyta)					147				879				
Total	**66 781**	**57 115**	**9 669**	**35 769**	**11 724**	**3 373**	**9 453**	**102 501**	**24 605**	**7 032**	**26 262**	**10 548**	**12 302**

October 2010 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
	7 030	**661**	**7 910**	**1 977**	**879**	**7 032**	**1 758**	**0**	**7 322**	**0**	**468 563**
1. CYANOPHYCEAE	**4.98%**	**12.17%**	**23.69%**	**11.38%**	**5.00%**	**28.57%**	**5.01%**	**0%**	**11.11%**	**0%**	**98.46%**
Anabaena sp. (F)	1 758	220	3 515		879	440	1 758		7 322		468 563
Anabaenopsis sp.			879			440					
Merismopodia sp. (col)		293		659		440					
Microcystis aeruginosa (single cells)		74	1 758			5 272					
Oscilatoria sp. (F)	5 272	74	1 758	1 318		440					
	10 544	**1 320**	**7 029**	**2 637**	**2 637**	**5 713**	**3 516**	**5 273**	**7 322**	**14 644**	**0**
2. BACILLARIOPHYCEAE	**7.46%**	**24.31%**	**21.05%**	**15.19%**	**15.00%**	**23.21%**	**10.03%**	**50.00%**	**11.11%**	**25.00%**	**0%**
Cocconeis sp.											
Cymbella sp.						440	1 758				
Melosira sp. (F)		1 172		220		440	1 758	1 758		7 322	
Cyclotella sp. (10-20 µm)		74		220	879					7 322	
Nitzschia sp. (pennate)	8 786	74	6 150	1 977	879	4 833		3 515	7 322		
Pennate diatoms (other)	1 758		879	220	879						
	107 786	**1 837**	**13 182**	**4 835**	**8 790**	**8 790**	**9 055**	**3 516**	**43 929**	**36 609**	**0**
3. CHLOROPHYCEAE	**76.35%**	**33.82%**	**39.47%**	**27.86%**	**50.00%**	**35.71%**	**25.82%**	**33.34%**	**66.67%**	**62.50%**	**0%**
Actinastrum hantzchii (star-col.)		74									
Ankistrodesmus sp. (needle, 30 µm)	3 515	74	879	659		440					
Chlamydomonas sp. (15-20 µm)	57 985	879	2 636	879	879	879	7 297	1 758			

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
Chlorella sp. (3-6 µm)	22 843	293	1 758	879	1 758	1 318				14 643	
Chlorococcum sp. (12-18 µm)	5 272				879						
Cosmarium sp.					879	440					
Closterium sp.	1 758			220							
Crucigenia sp. (4x4 col.)	1 758		1 758	659		440				7 322	
Micratinium sp. (col. Round, spikes)	3 515	74							43 929		
Monoraphidium sp.	1 758	74	4 393	220	1 758	1 318					
Pediastrum simplex (col.)				220	879						
Sphaerocystis sp. (col.)		74									
Scenedesmus sp. (col.)	5 866	147	879	1 099	879	2 197				7 322	
Staurastrum sp.	1 758					440					
Schroederia sp. (needle with foot)										7 322	
Tetrastrum sp. (4cells with spikes)		74			879						
Tetraedron sp. (4-angular)	1 758	74	879			1 318	1 758	1758			
4. CRYPTOPHYCEAE	**1 758** **1.25%**	**1 392** **25.63%**	**1 758** **5.26%**	**7 468** **43.03%**	**0** **0%**	**440** **1.79%**	**10 192** **29.07%**	**1 758** **16.66%**	**0** **0%**	**7 322** **12.50%**	**0** **0%**
Cryptomonas sp.	1 758	1 392	1 758	7 468		440	10 192	1 758		7 322	
5. DINOPHYCEAE	**0** **0%**	**147** **2.71%**	**0** **0%**	**0** **0%**	**3 515** **20.00%**	**440** **1.79%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Peridinium sp.		147			3 515	440					
6. EUGLENOPHYCEAE	**14 059** **9.96%**	**74** **1.36%**	**3 515** **10.53%**	**440** **2.54%**	**1 758** **10.00%**	**1 319** **5.36%**	**10 545** **30.07%**	**0** **0%**	**7 322** **11.11%**	**0** **0%**	**7 322** **1.54%**
Euglena sp.	5 272		2 636	220	879						
Lepocinclis sp. (pear shaped)							1 758				
Phacus sp.	1 758			220		440	1 758				
Trachelomonas sp.	7 029	74	879		879	879	7 029		7 322		7 322
7. CHRYSOPHYSEAE	**0**	**0**	**0**	**0**	**0**	**879**	**0**	**0**	**0**	**0**	**0**

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
	0%	0%	0%	0%	0%	3.57%	0%	0%	0%	0%	0%
Dinobyron sp.						879					
Total	141 177	5 431	33 394	17 357	17 579	24 613	35 066	10 547	65 895	58 575	475 885

February 2011 (cells/mℓ)

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shitlhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
	29 875	0	23 723	3 735	440	880	0	3 957	1 758	0	0	0
1. CYANOPHYCEAE	11.49%	0%	45.00%	28.32%	5.56%	7.28%	0%	32.14%	33.33%	0%	0%	0%
Anabaena sp. (F)	1 758		879	659		220		1 758				
Anabaenopsis sp.			879	659		220		440				
Merismopodia sp. (col)	3 515		2 636	2 417								
Microcystis aeruginosa (single cells)	1 758				440			440				

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shithave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
Oscilatoria sp. (F)	1 758		879			440		879	1 758			
Raphidiopsis sp. (sicle-spiral)	21 086		18 450					440				
	14 061	**0**	**879**	**220**	**440**	**440**	**879**	**880**	**1758**	**0**	**3 515**	**0**
2. BACILLARIOPHYCEAE	**5.41%**	**0%**	**1.67%**	**1.67%**	**5.56%**	**3.64%**	**9.09%**	**7.15%**	**33.33%**	**0%**	**100.00%**	**0%**
Cocconeis sp.	1 758											
Cymbella sp.												
Navicula sp. (pennate)	3 515											
Melosira sp. (F)	5 272							440	879			
Cyclotella sp. (10-20 µm)	1 758								879			
Nitzschia sp. (pennate)	1 758			220		220	879	440				
Pennate diatoms (other)			879		440	220					3 515	
	80 004	**1758**	**15 818**	**1980**	**3 518**	**7 470**	**879**	**4 836**	**879**	**3 516**	**0**	**0**
3. CHLOROPHYCEAE	**30.78%**	**3.39%**	**30.00%**	**15.02%**	**44.45%**	**61.81%**	**9.09%**	**39.28%**	**16.67%**	**33.33%**	**0%**	**0%**
Ankistrodesmus sp. (needle, 30 µm)		879	1 758	220								
Chodatella sp.			879									
Chlamydomonas sp. (15-20 µm)	3 515			220					879			
Chlorella sp. (3-6 µm)	3 515					6 370				879		
Chlorococcum sp. (12-18 µm)				220								
Cosmarium sp.								440				
Closterium sp.			2 636									
Crucigenia sp. (4x4 col.)	3 515		3 515	220	440							
Golenkinia sp. (round with spikes)					440							
Cladophora sp. (F)								440				
Monoraphidium sp.	13 230		3 515	220	1 318			1 318		1 758		
Pediastrum sp. (col.)	1 758					220						
Oocystis sp. (col.)						220						
Scenedesmus sp. (col.)	47 442	879	3 515	220	440	440		1 758				
Planktosphaeria sp. (col.)					440			440				

SPECIES (F = Filament)	Sunset	Piet Grobler	Mazithi	Nyamundwa	Shilhave	Mestel	Kumana	Transport	Mpondo	N'watindlopfu	Mpanamana	Nsemane
Schroederia sp. (needle with foot)							879	440		879		
Tetrastrum sp. (4cells with spikes)				220								
Tetraedron sp. (4-angular)	7 029			440	440	220						
4. CRYPTOPHYCEAE	**35 143** **13.52%**	**47 442** **91.52%**	**9 665** **18.33%**	**5 931** **44.98%**	**1 318** **16.66%**	**2 856** **23.63%**	**5 272** **54.54%**	**440** **3.57%**	**0** **0%**	**2 636** **25.00%**	**0** **0%**	**10 543** **60.00%**
Cryptomonas sp.	35 143	47 442	9 665	5 931	1 318	2 856	5 272	440		2 636		10 543
5. DINOPHYCEAE	**0** **0%**	**0** **0%**	**0** **0%**	**659** **5.00%**	**440** **5.56%**	**220** **1.82%**	**0** **0%**	**879** **7.14%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Peridinium sp.				659	440	220		879				
6. EUGLENOPHYCEAE	**100 859** **38.80%**	**2 637** **5.09%**	**2 636** **5.00%**	**660** **5.01%**	**1 758** **22.21%**	**220** **1.82%**	**2 637** **27.28%**	**1320** **10.72%**	**879** **16.67%**	**4 394** **41.67%**	**0** **0%**	**7 029** **40.00%**
Euglena sp.		879		220	440		879	440				
Lepocinclis sp. (pear shaped)										879		
Phacus sp.	3 515	879						440				
Trachelomonas sp.	97 344	879	2 636	440	1 318	220	1 758	440	879	3 515		7 029
7. CHRYSOPHYSEAE	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**	**0** **0%**
Dinobyron sp.												
Total	**259 942**	**51 837**	**52 721**	**13 185**	**7 914**	**12 086**	**9 667**	**12 312**	**5 274**	**10 546**	**3 515**	**17 572**

APPENDIX 2: CORRELATION STATISTICS

		Cells	AreaD	Temp	pH	DO	CHL	TP	TN	TURB	TDS
Cells	**correlation**	1	0.0403	0.0098	-0.0248	-0.4091	0.5394	0.4257	0.4879	0.7916	0.1594
	probability		0.7314	0.9337	0.8337	0.0003	<.0001	0.0001	<.0001	<.0001	0.1719
	number	75	75	74	74	74	75	75	75	75	75
AreaD	**correlation**	0.0403	1.0000	0.0684	0.0346	0.0677	0.1422	0.1627	0.1647	0.0652	0.0121
	probability	0.7314		0.5628	0.7699	0.5664	0.2236	0.1631	0.1580	0.5786	0.9181
	number	75	76	74	74	74	75	75	75	75	75
Temp	**correlation**	0.0098	0.0684	1.0000	0.7357	0.3683	-0.0484	0.0625	0.0447	-0.0739	0.2325
	probability	0.9337	0.5628		<.0001	0.0012	0.6820	0.5970	0.7055	0.5314	0.0463
	number	74	74	74	74	74	74	74	74	74	74
pH	**correlation**	-0.0248	0.0346	0.7357	1.0000	0.6353	-0.0114	-0.0324	-0.0418	0.0334	0.3264
	probability	0.8337	0.7699	<.0001		<.0001	0.9234	0.7839	0.7236	0.7773	0.0045
	number	74	74	74	74	74	74	74	74	74	74
DO	**correlation**	-0.4091	0.0677	0.3683	0.6353	1.0000	-0.3302	-0.3652	-0.4189	-0.2768	0.1953
	probability	0.0003	0.5664	0.0012	<.0001		0.0041	0.0014	0.0002	0.0170	0.0955
	number	74	74	74	74	74	74	74	74	74	74
CHL	**correlation**	0.5394	0.1422	-0.0484	-0.0114	-0.3302	1.0000	0.3150	0.5192	0.6477	0.1513
	probability	<.0001	0.2236	0.6820	0.9234	0.0041		0.0059	<.0001	<.0001	0.1950
	number	75	75	74	74	74	75	75	75	75	75
TP	**correlation**	0.4257	0.1627	0.0625	-0.0324	-0.3652	0.3150	1.0000	0.9342	0.5699	0.2486
	probability	0.0001	0.1631	0.5970	0.7839	0.0014	0.0059		<.0001	<.0001	0.0315
	number	75	75	74	74	74	75	75	75	75	75

		Cells	AreaD	Temp	pH	DO	CHL	TP	TN	TURB	TDS
TN	**correlation**	0.4879	0.1647	0.0447	-0.0418	-0.4189	0.5192	0.9342	1.0000	0.6363	0.1858
	probability	<.0001	0.1580	0.7055	0.7236	0.0002	<.0001	<.0001		<.0001	0.1105
	number	75	75	74	74	74	75	75	75	75	75
TURB	**correlation**	0.7916	0.0652	-0.0739	0.0334	-0.2768	0.6477	0.5699	0.6363	1.0000	0.4438
	probability	<.0001	0.5786	0.5314	0.7773	0.0170	<.0001	<.0001	<.0001		<.0001
	number	75	75	74	74	74	75	75	75	75	75
TDS	**correlation**	0.1594	0.0121	0.2325	0.3264	0.1953	0.1513	0.2486	0.1858	0.4438	1.0000
	probability	0.1719	0.9181	0.0463	0.0045	0.0955	0.1950	0.0315	0.1105		<.0001
	number	75	75	74	74	74	75	75	75	75	75
STRIP	**correlation**	0.2364	-0.0512	0.2066	0.2159	0.0962	0.1989	0.1854	0.2053	0.2723	0.3728
	probability	0.0456	0.6695	0.0817	0.0686	0.4216	0.0940	0.1189	0.0837	0.0207	0.0013
	number	72	72	72	72	72	72	72	72	72	72
ELISA	**correlation**	0.5325	0.1570	-0.0494	-0.0094	-0.2875	0.9064	0.2072	0.3427	0.5091	-0.0069
	probability	<.0001	0.1785	0.6757	0.9364	0.0130	<.0001	0.0744	0.0026	<.0001	0.9532
	number	75	75	74	74	74	75	75	75	75	75
MC_RR	**correlation**	0.2678	0.0341	0.0201	-0.0340	-0.2729	0.3633	0.3736	0.6451	0.3491	-0.0426
	probability	0.0202	0.7716	0.8648	0.7737	0.0187	0.0014	0.0010	<.0001	0.0021	0.7166
	number	75	75	74	74	74	75	75	75	75	75
MC_YR	**correlation**	0.1973	0.0593	-0.0817	-0.0348	-0.0952	0.3719	0.2792	0.3284	0.5107	0.5508
	probability	0.0897	0.6135	0.4889	0.7687	0.4197	0.0010	0.0153	0.0040	<.0001	<.0001
	number	75	75	74	74	74	75	75	75	75	75
MC_LR	**correlation**	0.7211	0.0137	-0.0680	-0.0409	-0.1721	0.4004	0.3265	0.4045	0.7491	0.4354
	probability	<.0001	0.9070	0.5649	0.7296	0.1426	0.0004	0.0042	0.0003	<.0001	<.0001

		Cells	AreaD	Temp	pH	DO	CHL	TP	TN	TURB	TDS
	number	75	75	74	74	74	75	75	75	75	75
MC_LA	**correlation**	0.2291	0.0685	-0.0784	-0.0368	-0.1252	0.4194	0.3225	0.3891	0.5443	0.5399
	probability	0.0480	0.5592	0.5068	0.7559	0.2879	0.0002	0.0048	0.0006	<.0001	<.0001
	number	75	75	74	74	74	75	75	75	75	75
MC_LY	**correlation**	0.8767	-0.0301	-0.0820	-0.0252	-0.1776	0.3508	0.1450	0.2155	0.6948	0.2590
	probability	<.0001	0.7974	0.4873	0.8313	0.1302	0.0020	0.2147	0.0634	<.0001	0.0248
	number	75	75	74	74	74	75	75	75	75	75
MC_LW	**correlation**	0.8614	-0.0531	0.0107	0.0092	-0.3278	0.1153	0.0747	0.0638	0.5062	0.0607
	probability	<.0001	0.6509	0.9282	0.9378	0.0044	0.3247	0.5243	0.5865	<.0001	0.6048
	number	75	75	74	74	74	75	75	75	75	75
MC_LF	**correlation**	0.1174	0.0403	-0.0973	-0.0156	-0.0044	0.3077	0.0939	0.1154	0.4203	0.5566
	probability	0.3157	0.7312	0.4094	0.8948	0.9701	0.0072	0.4227	0.3244	0.0002	<.0001
	number	75	75	74	74	74	75	75	75	75	75
TOT_MC	**correlation**	0.5044	0.0411	-0.0976	-0.0824	-0.1924	0.4474	0.3595	0.4655	0.6878	0.4918
	probability	<.0001	0.7302	0.4146	0.4913	0.1054	<.0001	0.0018	<.0001	<.0001	<.0001
	number	73	73	72	72	72	73	73	73	73	73

		STRIP	ELISA	MC_RR	MC_YR	MC_LR	MC_LA	MC_LY	MC_LW	MC_LF	TOT_MC
MC_RR	**correlation**	0.1197	0.0797	1.0000	0.2179	0.3526	0.2810	0.2121	0.0029	0.0199	0.4371
	probability	0.3164	0.4966		0.0604	0.0019	0.0146	0.0677	0.9802	0.8653	0.0001
	number	72	75	75	75	75	75	75	75	75	73
MC_YR	**correlation**	0.1488	0.05909	0.21785	1	0.76644	0.99705	0.42889	-0.01172	0.9706	0.91912
	probability	0.2122	0.6146	0.0604		<.0001	<.0001	0.0001	0.9205	<.0001	<.0001
	number	72	75	75	75	75	75	75	75	75	73
MC_LR	**correlation**	0.1739	0.1653	0.3526	0.7664	1.0000	0.7772	0.8942	0.6052	0.7031	0.9407
	probability	0.1441	0.1565	0.0019	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
	number	72	75	75	75	75	75	75	75	75	73
MC_LA	**correlation**	0.1602	0.0971	0.2810	0.9971	0.7772	1.0000	0.4367	-0.0099	0.9523	0.9328
	probability	0.1789	0.4070	0.0146	<.0001	<.0001		<.0001	0.9326	<.0001	<.0001
	number	72	75	75	75	75	75	75	75	75	73
MC_LY	**correlation**	0.1677	0.2616	0.2121	0.4289	0.8942	0.4367	1.0000	0.8822	0.4016	0.6980
	probability	0.1591	0.0234	0.0677	0.0001	<.0001	<.0001		<.0001	0.0004	<.0001
	number	72	75	75	75	75	75	75	75	75	73
MC_LW	**correlation**	0.1216	0.1958	0.0029	-0.0117	0.6052	-0.0099	0.8822	1.0000	-0.0167	0.2999
	probability	0.3089	0.0923	0.9802	0.9205	<.0001	0.9326	<.0001		0.8868	0.0100
	number	72	75	75	75	75	75	75	75	75	73
MC_LF	**correlation**	0.1187	0.0428	0.0199	0.9706	0.7031	0.9523	0.4016	-0.0167	1.0000	0.8428
	probability	0.3208	0.7156	0.8653	<.0001	<.0001	<.0001	0.0004	0.8868		<.0001
	number	72	75	75	75	75	75	75	75	75	73
TOT_MC	**correlation**	0.1678	0.1302	0.4371	0.9191	0.9407	0.9328	0.6980	0.2999	0.8428	1.0000
	probability	0.1649	0.2722	0.0001	<.0001	<.0001	<.0001	<.0001	0.0100	<.0001	
	number	70	73	73	73	73	73	73	73	73	73