A RISK ASSESSMENT OF CYANOBACTERIA AND CYANOTOXINS IN HARTBEESPOORT DAM

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Exact wording of the title of the dissertation as appearing on the copies submitted for examination:

A Risk Assessment of Cyanobacteria and Cyanotoxins in Hartbeespoort Dam.

I, **Cassidy Kuiper**, declare that this **Dissertation** is my own, unaided work. It is being submitted for the Degree of **MSc Environmental Sciences** at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

C Kuiper _____ 18/06/2021 _____

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Abstract

Water is an important natural resource for the human population. Nutrient build-up through cultural eutrophication, warm water temperatures, and high insolation in Hartbeespoort Dam have historically resulted in the dam becoming hypertrophic accompanied by frequent and persistent cyanobacterial blooms. The decreased quality of water in Hartbeespoort Dam is a public health threat as water from the dam is used for irrigation, recreational activities, and is a source of potable water. Many cyanobacteria genera produce toxins (cyanotoxins) that pose a health risk to animals and humans. Cyanobacterial bloom formation is a function of complex interactions between system-specific physical, chemical, and biological factors occurring simultaneously in freshwater bodies**.** The aim of this study was to determine the human health risk associated with exposure to cyanotoxins produced by cyanobacteria based on an ecological health assessment of Hartbeespoort Dam. Water quality was determined based on *in situ* physico-chemical measurements and laboratory-based measurements of nutrient concentrations. Additionally, phytoplankton community structure and biomass, and total microcystin concentrations were determined.

The rapidly growing population and poor water management in the Gauteng Province has resulted in a build-up of nutrients in Hartbeespoort Dam. Inorganic nutrient concentrations increased as the study progressed into winter positively correlating to cyanobacteria cell density, reaching a maximum density during mid-winter. Dissolved oxygen correlated to the photosynthetic activity of phytoplankton instead of water temperature changes in the dam. Additionally, microcystin concentration increased as the study progressed into winter due to a poor dilution capacity in Hartbeespoort Dam. It was assumed that condition required for bloom formation did not occur simultaneously in Hartbeespoort Dam during the study. Water hyacinth in Hartbeespoort Dam appears to control phytoplankton and cyanobacterial growth thus preventing bloom formation in the dam as it is a useful tool for removing excess inorganic nutrients from eutrophic waterbodies. Microcystin concentration in Hartbeespoort Dam, however, exceeded the recommended guidelines for recreational waters. Hartbeespoort Dam therefore posed a significant risk to human health, and it was advised to approach the dam with caution.

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Abbreviation List

ALS-PDC	Amyotrophic lateral sclerosis-Parkinsonism/dementia complex
AMD	Acid mine drainage
ANOVA	Analysis of variances
APES	Animal, Plant, and Environmental Sciences
BMAA	β-methylamino-L-alanine
BOD	Biological oxygen demand
CMTSI	Carlson Modified Trophic State Index
DWA(F)	Department of Water and Forestry
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DWS	Department of Water and Sanitation
ELISA	Enzyme-linked immunosorbent assay
MC	Microcystin
NEMP	National Eutrophication Management Programme
NWA	National Water Act
RDA	Redundancy constrained analysis
SAWS	South African Weather Services
TDS	Total dissolved salts
tRNA	Transfer-RNA
TSS	Total suspended solids
TWQR	Target Water Quality Range
WHO	World Health Organisation
WWTWs	Wastewater treatment works

1. Introduction

1.1. Water and the South African context

1.1.1. Water as a resource in South Africa

Water is a critical natural resource essential for all living organisms (Sanchez *et al*., 2007; Reichwaldt and Ghadouani, 2012; Mitchell *et al.*, 2014). As water is a scarce and unevenly distributed resource, the National Water Act (NWA No. 36 of 1998) was established (King and Pienaar, 2011; Matthews and Bernard, 2015). The Act is an overarching framework that recognises that water is a natural resource belonging to all people (Matthews and Bernard, 2015). Additionally, the Act specifies the minimum quality requirements to meet the intended use of the water (potable, agricultural, industrial, recreational, and other uses) (Matthews and Bernard, 2015). Water management is crucial for sustaining the growing population of South Africa (Oberholster *et al.,*2008; King and Pienaar, 2011; Reichwaldt and Ghadouani, 2012).

Water is a natural resource belonging to all people and the discriminatory acts of the past prevented the equal access to water resources (NWA, 1998; Matowanyika, 2010). Every person in South Africa has the constitutional right to the access of sufficient water, where sufficient refers to the quality and the quantity of the water provided (Constitution of the Republic of South Africa, 1996).

South Africa is a semi-arid country with high evaporation rates and an average annual rainfall of 450 mm, almost half the world's average of 860 mm per annum (DWAF, 1996: 5; Oberholster *et al.,* 2005; Oberholster *et al.,* 2008; King and Pienaar, 2011; Mitchell *et al.*, 2014; Harding, 2015). Climatic gradients exist across the country with the western half being mostly arid whereas the eastern half experiences summer rainfalls (DWAF, 1996: 5; Matthews, 2014; Mitchell *et al.,* 2014). Seasonal rainfalls often occur as short and intense storm events resulting in increased runoff of silt, and organic and inorganic material from catchment areas into waterbodies (DWAF, 1996: 5). Freshwater in South Africa is therefore a scarce resource vulnerable to environmental degradation (Oberholster *et al.*, 2005; Sanchez *et al.*, 2007; King and Pienaar, 2011; Mitchell *et al.*, 2014; Matthews and Bernard, 2015).

Degradation of surface water quality is caused by natural and anthropogenic processes, particularly eutrophication, acid mine drainage (AMD), mineralisation, pollutants from urban runoff, and increased rural dwellers in major cities (WHO, 2003;

Sanchez *et al*., 2007; Matowanyika, 2010; Van Ginkel, 2011; Harding, 2015; Matthews and Bernard, 2015; Dube *et al.,* 2017). Water quality refers to the physical components, chemical composition, biological characteristics, and aesthetic properties of the water (DWAF, 1996: 5; NWA, 1998; Matowanyika, 2010; King and Pienaar, 2011; Matthews and Bernard, 2015). Water of sufficient quality and quantity is essential to meet basic human needs and the demands of agricultural, industrial, and conservational and ecosystem uses (DWAF, 1996: 5; Sanchez *et al.*, 2007; Reichwaldt and Ghadouani, 2012; Banna *et al.*, 2014; Matthews, 2014; Mitchell *et al.*, 2014; Matthews and Bernard, 2015).

Most water in South Africa is used for irrigation (62%) followed by domestic and urban consumption (27%) (King and Pienaar, 2011; Harding, 2015). The remaining 11% of South Africa's water is consumed by the mining, power, and forestry industries (King and Pienaar, 2011; Harding, 2015). Poor water quality is one of South Africa's major threats to providing sufficient water as it poses a risk to public health and safety and has negative aesthetic and economic impacts (Oberholster *et al.*, 2005; Van Ginkel, 2011; Mitchell *et al.*, 2014; Matthews and Bernard, 2015).

Exploitable water resources in South Africa are limited to groundwater, rivers, and artificial impoundments (Oberholster *et al.*, 2005; King and Pienaar, 2011). Due to the aridity, relative scarcity of water, high water demands, and the almost complete absence of large natural lakes, approximately 497 artificial impoundments exist to improve yield (Oberholster *et al.*, 2005; King and Pienaar, 2011; Matthews, 2014; Matthews and Bernard, 2015). Additionally, many rivers in South Africa exhibit erratic, variable, and seasonal flow rates varying annually (DWAF, 1996: 5; Oberholster *et al.*, 2005; King and Pienaar, 2011). Artificial impoundments therefore stabilise the flow of major rivers and ensure a continual supply of water to the continually increasing demand (Oberholster *et al.*, 2005; King and Pienaar, 2011).

Aquatic ecosystems exist in surface water resources such as rivers, artificial impoundments, and wetlands and are defined as the abiotic (physical and chemical) and biotic (biological) components, habitats, and ecological services of these waterbodies (DWAF, 1996: 5). Aquatic ecosystems are complex systems consisting of many interlinked and interdependent habitats, species, and processes (DWAF, 1996: 5).

1.1.2. Rapidly growing South African population

The mid-year population estimate for South Africa in 2018 was 57.73 million with most of the population (14.7 million people, 25.4%) living in the Gauteng Province (Stats SA, 2018). The mid-year population for South Africa increased from 56.52 million in 2017 to 57.73 million in 2018 and the proportion of the population living in Gauteng Province increased from 14.3 million to 14.7 million during the same period (Stats SA, 2017; Stats SA, 2018). The Gauteng Province had the greatest in- and out-flows of migrants and had the highest number of international migrants entering when compared to the other provinces (Stats SA, 2018).

The estimated population growth rate increased from 1.04%, between 2002 and 2003, to 1.55%, between 2017 and 2018 (Stats SA, 2018). This increase in population growth rate can be attributed to an increase in life expectancy at birth (more people over 60 years), an increase in birth rate between 2002 and 2008 (more children under 15 years) and a decrease in death rate (Stats SA, 2017).

1.1.3. Poorly functioning Wastewater Treatment Works (WWTWs)

Due to the scarcity and the deteriorating quality of water in South Africa, the NWA requires all effluents to be purified and returned to natural waterbodies to ensure maximised utilisation of water (DWAF, 1996: 5; NWA, 1998; Mitchell *et al.,* 2014). As South Africa is a semi-arid country, the quantity of water supplied to the population is threatened (Oberholster *et al.*, 2005; Sanchez *et al.*, 2007; Mitchell *et al.*, 2014; Matthews and Bernard, 2015). Wastewater treatment works (WWTWs) function in treating wastewater, polluted through urban and industrial use, and returning water of acceptable quality back into the environment (Mitchell *et al.*, 2014). Treating and returning water back into the environment ensures that water is reused therefore enhancing water availability (DWAF, 1996: 5). Effluent treatment, however, adversely affects the quality of water as pollutants within effluent streams are discharged into waterbodies (DWAF, 1996: 5).

An increased supply of inorganic nutrients to surface waterbodies has been directly linked to the inadequate treatment of domestic sewage, and urban, industrial, agricultural, and mining wastewater (Xu *et al.*, 2010; King and Pienaar, 2011; Mitchell *et al.,* 2014; Harding, 2015, Mitchell and Crafford, 2016). Disposal of poorly treated/untreated wastewater into aquatic systems increases the spread of disease,

incidence of eutrophication, and water treatment costs for downstream users as water quality is reduced (Xu *et al.,* 2010; King and Pienaar, 2011; Mitchell *et al.,* 2014; Harding 2015). A large percentage of the wastewater generated by the Gauteng Province is inadequately treated (Mitchell *et al.,* 2014; Harding, 2015). Society depends on good quality water for urban, agricultural, and industrial uses (Mitchell *et al.,* 2014; Matthews and Bernard, 2015). Adequate treatment of wastewater is therefore essential for the health and livelihoods of downstream water users (Mitchell *et al.,* 2014).

The South African Department of Water Affairs (DWA), now the Department of Water and Sanitation (DWS) identified effluent from failing WWTWs to be the main contributing factor of eutrophication in South African waterbodies thus contributing to the deteriorating quality of water resources in the country (Harding *et al.*, 2009; Harding, 2015). In 2009, it was found that of the 850 WWTWs in South Africa, 32 WWTWs were fully compliant achieving Green Drop Status (King and Pienaar, 2011; Harding, 2015). Inadequately treated sewage is therefore being discharged into the environment by approximately 96% of the WWTWs in South Africa (King and Pienaar, 2011; Harding, 2015). Malfunctioning WWTWs have been caused by a combination of underinvestment in infrastructure, insufficient maintenance budgets, hydraulic and/or biological overloading, and a lack of technically skilled staff (Harding, 2015). Additionally, population growth and rapid urbanisation has further exacerbated the problem as the already impaired WWTWs are unable to handle the increasing wastewater loads (Mitchell *et al.,* 2014; Dube *et al.,* 2017). The current level of wastewater treatment is therefore inadequate in controlling eutrophication of inland waters, particularly obvious in Hartbeespoort Dam (Mitchell *et al.*, 2014).

1.1.4. Local problems affecting Hartbeespoort Dam

Hartbeespoort Dam (Figure 1.1) is an artificial impoundment located within the Brits magisterial district, approximately 40 km north of Johannesburg and approximately 40 km west of Pretoria, in South Africa (Steyn *et al.,* 1975; Pearce, 1987; Conradie and Barnard, 2012; Ballot *et al.*, 2014). Hartbeespoort Dam was completed in 1923 and was originally built for irrigation purposes, supplying the surrounding agricultural activities and farmlands (Steyn *et al*., 1975; Scott *et al*., 1980; Pearce, 1987; Ballot *et al.,* 2014). Additionally, the dam has subsequently been used as a recreational reserve

and to supply domestic water to Brits (Steyn *et al*., 1975; Pearce, 1987; Conradie and Barnard, 2012; Ballot *et al.*, 2014).

As a result of rapid urban development and industrial growth as well as the occurrence of agricultural activities (e.g., livestock production) within the catchment area, Hartbeespoort Dam receives high water volumes and high inorganic nutrient loads, namely inorganic phosphorus (orthophosphate) and inorganic nitrogen (ammonia, nitrate plus nitrite) (Steyn *et al*., 1975; Conradie and Barnard, 2012). Hartbeespoort Dam is therefore representative of a hypertrophic system and is globally well-known for its frequent and persistent cyanobacterial blooms and the formation of, often toxic, surface scums (Conradie and Barnard, 2012; Ballot *et al.,* 2014; Matthews, 2014; Matthews and Bernard, 2015).

Hartbeespoort Dam receives 90.4% of its inflow from the Crocodile River (Figure 1.1); 9.3% from the Magalies River (flowing mainly through agricultural land) (Figure 1.1) and 0.3% from the Swart River (Pearce, 1987; Roux *et al.*, 2010). The water that flows into the dam includes wastewater from Johannesburg, Pretoria, Krugersdorp, and Kempton Park and includes contributions from urban, industrial, agricultural, and mining activities (Steyn *et al*., 1975; Roux *et al.,* 2010).

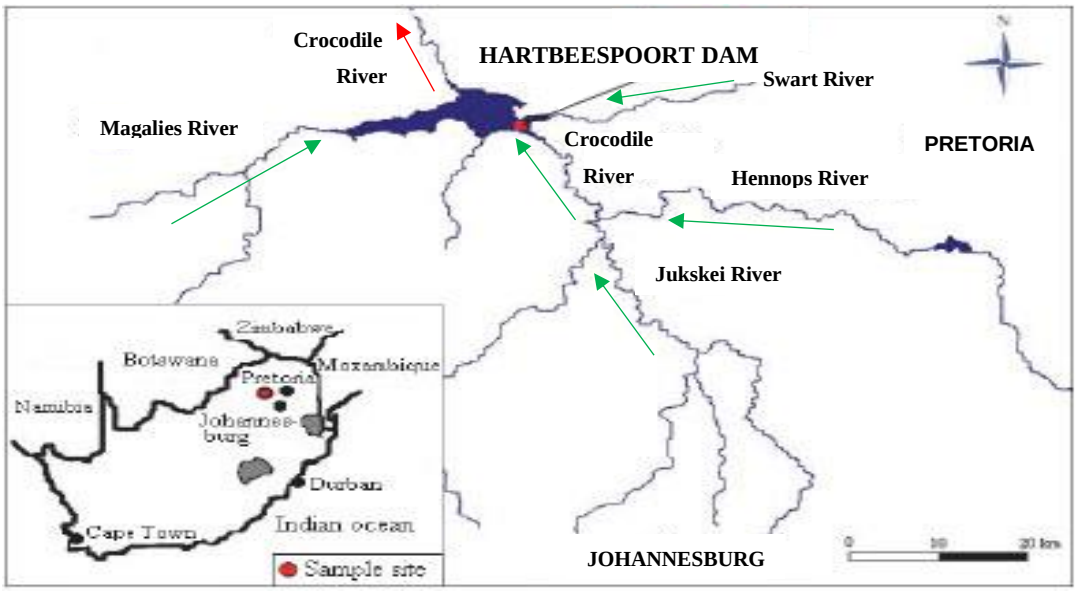


Figure 1.1: Line diagram illustrating how the Hennops and the Jukskei rivers join the Crocodile River before flowing into Hartbeespoort Dam. The figure additionally illustrates how the Magalies River and the Swart River also drain into the dam and how the largest outflow of the dam is the Crocodile River. The arrows represent the direction of flow with the green arrows representing inflow and the red arrows representing outflow of the dam. *Image adapted from Amandy et al. (2014)*.

The Crocodile River, originating near Krugersdorp and Roodepoort, is joined by the Jukskei and Hennops rivers before it drains into Hartbeespoort Dam (Figure 1.1), carrying > 95% of the inorganic nutrient load (Wittmann and Förstner, 1976; Pearce, 1987; Matowanyika, 2010; Roux *et al*., 2010; Dube *et al.,* 2017). The Crocodile River is joined by the Bloukrans River that flows past Randfontein and Krugersdorp carrying water polluted with mining effluent from Randfontein (Roux *et al*., 2010). The main outflow of the dam is the Crocodile River (Figure 1.1), which carries 61% of the water downstream towards Brits (Roux *et al*., 2010). This outflow of water supports the inhabitants of Brits and the surrounding farming activity (Roux *et al*., 2010). The remaining 39% of water loss is attributed to evaporation, consumptive uses, and two canals supplying mining and agricultural activities to the north of the dam (Pearce, 1987; Roux *et al*., 2010).

The Jukskei River continues north from Johannesburg and is the southern inflow of the Crocodile River, contributing 56.7% inflow to the river (Figure 1.1) (Roux *et al*., 2010; Dube *et al.*, 2017). Prior to draining into the Crocodile River, the Jukskei River is joined by smaller rivers such as the Braamfonteinspruit and the Klein Jukskei (Wittmann and Förstner, 1976; Roux *et al*., 2010). The Jukskei River flows through urban areas and carries pollution emanating from these areas and any other informal settlements formed along the river (Wittmann and Förstner, 1976; Matowanyika, 2010; Roux *et al*., 2010; Mitchell *et al.*, 2014; Dube *et al.,* 2017). The Jukskei River is therefore the most polluted river within the catchment area and contributes 72% of the inorganic nitrogen and 65.1% of the inorganic phosphate entering the Crocodile River before it drains into Hartbeespoort Dam (Matowanyika, 2010; Roux *et al.*, 2010; Mitchell *et al.,* 2014).

The Hennops River contributes an additional 27.5% inflow to the Crocodile River and is formed by the Sesmylspruit, a stream discharging from the Rietvlei Dam, which joins the Kaalspruit and flows through Centurion (Roux *et al*., 2010). Secondary purified effluents from the Kempton Park WWTWs drains into the Hennops River (Wittmann and Förstner, 1976). The Hennops River is joined by Rietspruit and is the eastern inflow of the Crocodile River (Figure 1.1) (Wittmann and Förstner, 1976; Roux *et al*., 2010). Together, these rivers and streams contribute to the overall volume and pollution load entering Hartbeespoort Dam (Roux *et al*., 2010)

The nutrient build-up, warm water temperatures and high insolation in the dam have contributed to the dam becoming hypertrophic accompanied by periodic cyanobacterial blooms consisting of the toxin producing *Microcystis aeruginosa* (*M. aeruginosa*), the most common cyanobacterial bloom former (Steyn *et al*., 1975; Pearce, 1987; Robarts and Zohary, 1987; WHO, 2003; Oberholster *et al.,* 2009; Matowanyika, 2010; Xu *et al.*, 2010; Van Ginkel, 2011; Conradie and Barnard, 2012; O'Neil *et al.*, 2012; Ballot *et al*., 2014; Matthews, 2014; Matthews and Bernard, 2015; Quevedo-Castro *et al.*, 2019).

Cultural eutrophication of the dam has negative impacts that include inconvenience to recreational users, unpleasant odours and tastes, and prolific water hyacinth growth (*Eichhornia crassipes*) (Steyn *et al*., 1975; Scott *et al*., 1980; Pearce, 1987; DWAF, 1996: 2; Oberholster *et al.*, 2009; Xu *et al.*, 2010; Van Ginkel, 2011; Matthews and Bernard, 2015; Miller and Russel, 2017; Quevedo-Castro *et al.*, 2019; Hill *et al.,* 2020). Cyanobacterial blooms threaten the quality of drinking water supplied to the South African population and are a public health concern (Van Ginkel, 2004; Oberholster *et al.,* 2009; Xu *et al.*, 2010; Matthews, 2014; Mitchell *et al.*, 2014; Matthews and Bernard, 2015; Lee *et al.*, 2017).

1.2. Cultural eutrophication

Eutrophication is the change in the biological and chemical processes of an aquatic ecosystem because of nutrient enrichment, particularly with nitrogen and phosphorus (Figure 1.2) (Oberholster *et al.*, 2005; Van Ginkel, 2011; Ballot *et al.,* 2014; Harding, 2015; Matthews and Bernard, 2015; Quevedo-Castro *et al.*, 2019). Eutrophication is part of the natural ageing process of waterbodies which is accelerated by anthropogenic activities thus altering the natural biogeochemical nutrient cycling within a waterbody (Oberholster *et al.*, 2009; Van Ginkel, 2011; Quevedo-Castro *et al.*, 2019). Natural eutrophication is the slow process of nutrient enrichment from natural resources (e.g., soil and rocks) within the catchment area of a waterbody and is therefore neither controllable nor reversible (Figure 1.2) (Van Ginkel, 2011). Alternatively, cultural eutrophication is the process of nutrient enrichment from human activities (agricultural, industrial, and urban runoff) and anthropogenic (i.e., wastewater effluents) sources (Figure 1.2) (WHO, 2003; Oberholster *et al.*, 2005; Xu *et al.,* 2010; Van Ginkel, 2011; Ballot *et al.*, 2014; Harding, 2015; Matthews and Bernard, 2015; Quevedo-Castro *et al.*, 2019).

The trophic status of a waterbody can be determined based on the degree of nutrient enrichment (Harding, 2015; Matthews and Bernard, 2015; Quevedo-Castro *et al.,* 2019). With increasing nutrient enrichment, the trophic status of water is either oligotrophic (slightly nutrient enriched), mesotrophic (medium nutrient enriched), eutrophic (well nutrient enriched), or hypertrophic (very well nutrient enriched) (Figure 1.2) (Harding, 2015; Matthews and Bernard, 2015; Quevedo-Castro *et al.*, 2019). Negative impacts of eutrophic and hypertrophic waterbodies include turbid water conditions, taste and odour problems, oxygen depletion (hypoxia and anoxia), increased phytoplankton blooms, increased primary growth of macrophytes (i.e., water hyacinth), loss of biodiversity, increased fish deaths, and decreased aesthetic value (Figure 1.2) (Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Xu *et al.,* 2010; Van Ginkel, 2011; Janse van Vuuren and Taylor, 2015; Matthews and Bernard, 2015; Turner *et al.*, 2018; Hill *et al.*, 2020).

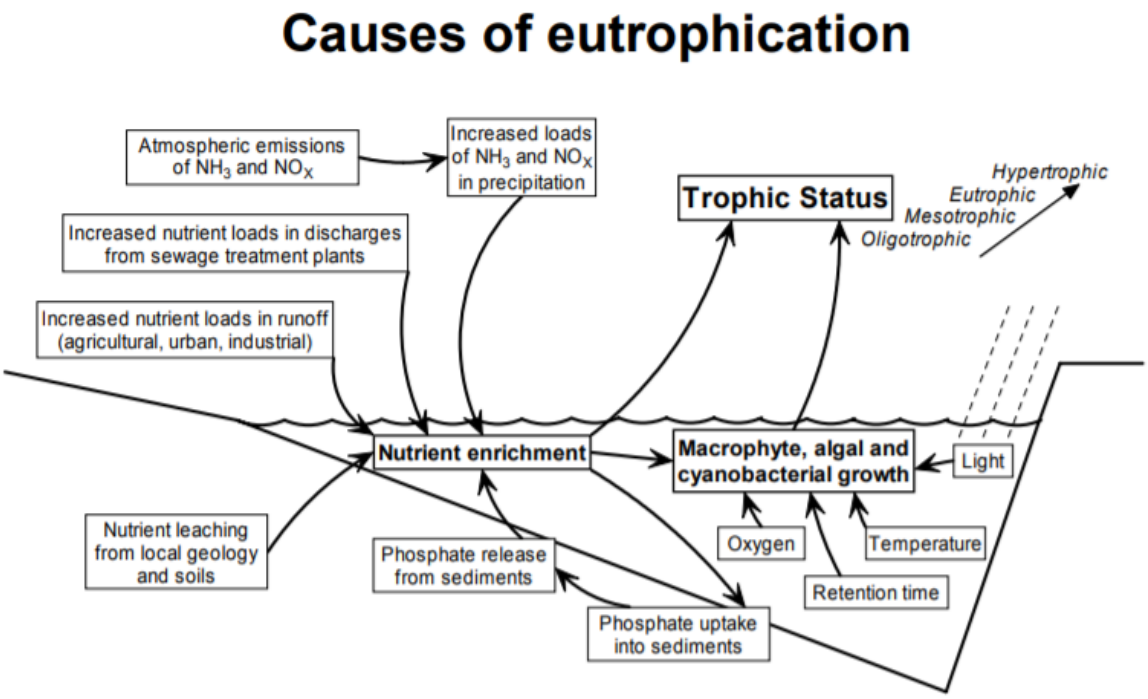


Figure 1.2: The process of nutrient enrichment (eutrophication) of a waterbody, primarily by nitrogen and phosphorus. The figure illustrates how the primary growth of algae, cyanobacteria, and macrophytes is increased as a result of environmental changes in a waterbody. Additionally, the figure illustrates how eutrophication and increased plant growth alters the trophic status of a waterbody. With increasing nutrient enrichment, the trophic status of a waterbody ranges from oligotrophic to hypertrophic. *Image adapted from DWAF (Department of Water Affairs and Forestry, South Africa) (2002).*

Cyanobacterial growth is stimulated in waterbodies with high nutrient concentrations (thriving in eutrophic and hypertrophic waters) and with suitable temperatures, oxygen concentration, and light availability (Figure 1.2) (Harding *et al.*, 2009; Xu *et al.,* 2010; Van Ginkel, 2011; Ballot *et al.*, 2014; Matthews, 2014; Matthews and Bernard, 2015; Turner *et al.*, 2018). Accumulations of cyanobacteria in surface water are known as cyanobacterial blooms and surface scums are dense accumulations of cyanobacteria on the surface of a waterbody or at the water's edge (WHO, 2003; Tonk *et al*., 2007; Swanepoel *et al.,* 2008; Davis *et al.*, 2009; Van Ginkel, 2011; O'Neil *et al*., 2012; Matthews and Bernard, 2015). Surface scums have negative ecological impacts on the diversity and the functioning of the phytoplankton community and higher order organisms (Matthews, 2014). Surface scums are therefore an important ecological indicator of over-enrichment and meteorological warming and senescence (WHO, 1998; Tonk *et al*., 2007; Swanepoel *et al.,* 2008; Davis *et al.*, 2009; Oberholster *et al.*, 2009; O'Neil *et al*., 2012; Matthews, 2014; Matthews and Bernard, 2015).

In South Africa, eutrophication and cyanobacterial blooms are extensive and widespread (Van Ginkel, 2011; Matthews, 2014). According to data collected from 75 of South Africa's major impoundments, by the then DWA for the National Eutrophication Management Programme (NEMP), approximately 3% of surface water samples were oligotrophic, 37% were mesotrophic, 33% were eutrophic, and 28% were hypertrophic during April and September 2013 (Matthews and Bernard, 2015). Cyanobacteria was present in all 75 major impoundments at concentrations dependent on the impoundment's trophic status (Matthews and Bernard, 2015). The incidence and extent of cyanobacteria in South Africa's waterbodies are therefore symptomatic of eutrophic and hypertrophic conditions (Harding *et al.*, 2009; Matthews, 2014)

The widespread, frequent, and seasonal cyanobacterial blooms threaten the supply of safe potable water, mainly sourced from surface water resources, in South Africa as many cyanobacterial genera have the ability to produce a range of toxins (cyanotoxins) (Oberholster *et al.,* 2005; Oberholster *et al.*, 2009; Xu *et al.,* 2010; Van Ginkel, 2011; Ballot *et al.*, 2014; Matthews, 2014; Mitchell *et al.,* 2014; Matthews and Bernard, 2015). Toxic cyanobacterial blooms pose a risk to human and animal health (Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Van Ginkel, 2011; Matthews, 2014; Matthews and Bernard, 2015). The quantity and quality of water supplied to the South

African population is therefore limited (Conradie and Barnard, 2012; Ballot *et al.,* 2014; Harding, 2015; Matthews and Bernard, 2015).

1.3. Phytoplankton

Phytoplankton are a diverse group of free-floating photosynthetic organisms that are mostly microscopic (Swanepoel *et al*., 2008). Phytoplankton are present in every waterbody exposed to sunlight and are a normal and common component of these waterbodies (DWAF, 1996: 1; Swanepoel *et al*., 2008). Phytoplankton are photosynthetic organisms (autotrophs) that use light energy to produce carbohydrates and oxygen from water and carbon dioxide (Swanepoel *et al*., 2008). Many phytoplankton cellular processes and algal growth rates are temperature-dependent, and the phytoplankton community composition tends to shift from diatoms to green algae to cyanobacteria with increasing temperature (Robarts and Zohary, 1987; Davis *et al.,* 2009; O'Neil *et al.*, 2012). Naturally occurring phytoplankton communities are therefore strongly influenced by temperature with maximum cellular processes occurring between 25ºC and 40ºC (Robarts and Zohary, 1987; O'Neil *et al.,* 2012).

The composition and abundance of phytoplankton within a waterbody can be used as indicators of water quality, to an extent, as phytoplankton are part of the first level of the food chain and are sensitive to minor changes in the aquatic environment (Pearce, 1987; Swanepoel *et al*., 2008; Matthews, 2014). Interactions between phytoplankton species within waterbodies are complex (Pearce, 1987). Oligotrophic waterbodies are generally dominated by non-flagellates as they support low levels of phytoplankton biomass lacking species diversity (Swanepoel *et al*., 2008). On the other hand, eutrophic and hypertrophic water bodies are generally dominated by fewer taxa with high levels of phytoplankton biomass (Swanepoel *et al*., 2008). This is due to a low concentration of nutrients and a high concentration of nutrients in these waters, respectively (Swanepoel *et al.,* 2008).

Changes in temperature, light, water column stability, salinity, and nutrient availability can enhance phytoplankton growth in waterbodies with this growth being most apparent in eutrophic waters (Robarts and Zohary, 1987; Tonk *et al.*, 2007; Swanepoel *et al*., 2008; Davis *et al.*, 2009; O'Neil *et al.,* 2012; Quevedo-Castro *et al.,* 2019). Excessive growth of certain phytoplankton species (e.g., cyanobacteria) in eutrophic waters can result in the formation of blooms and surface scums (Swanepoel *et al*.,

2008; Davis *et al.*, 2009; Van Ginkel, 2011; O'Neil *et al*., 2012; Ballot *et al.*, 2014; Quevedo-Castro *et al.*, 2019). As previously outlined, surface scums are an important ecological indicator of over-enrichment and meteorological warming and senescence (WHO, 1998; Tonk *et al*., 2007; Swanepoel *et al.,* 2008; Davis *et al.*, 2009; Oberholster *et al.*, 2009; O'Neil *et al*., 2012; Matthews, 2014; Matthews and Bernard, 2015).

Between one and two percent of phytoplankton's dry weight is attributed to chlorophyll *a,* a component of all green plants as it is an essential component of photosynthesis (DWAF, 1996: 1, 5; Swanepoel *et al.,* 2008; Davis *et al*., 2009). Chlorophyll *a* concentration in water is used to express phytoplankton biomass but is not a suitable analysis for waters with low chlorophyll *a* concentration (e.g., potable water) (DWAF, 1996: 1, 5; Chorus and Bartram, 1999; Swanepoel *et al*., 2008; Ballot *et al.,* 2014; Matthews, 2014; Matthews and Bernard, 2015). Chlorophyll *a* content per cell varies between species and phyla of phytoplankton (DWAF, 1996: 1,5; Swanepoel *et al*., 2008; Davis *et al.*, 2009). Chlorophyll *a* concentration as a measure of eutrophication in South Africa has been positively correlated with the incidence of cyanobacterial blooms (Matthews, 2014). High chlorophyll *a* concentration therefore increases the likelihood of severe and extensive cyanobacterial blooms in an aquatic system (Matthews, 2014).

Oligotrophic waterbodies typically have a chlorophyll *a* concentration of between 1 µg/L and 10 µg/L whereas chlorophyll *a* concentrations in eutrophic waterbodies can reach up to 300 µg/L and up to 3 000 µg/L in hypertrophic waterbodies (Zohary and Robarts, 1990; Chorus and Bartram, 1999; Matthews, 2014). Low concentrations of chlorophyll *a,* however, are not necessarily indicative of low phytoplankton biomass when the phytoplankton community is dominated by cyanobacteria (Swanepoel *et al*., 2008).

1.4. Cyanobacteria

Cyanobacteria (previously known as blue-green algae) are prokaryotes with similar characteristics of some algae and bacteria (WHO, 2003). Cyanobacteria contain photosynthetic pigments (WHO, 2003; O'Neil *et al*., 2012; Miller and Russell, 2017; Turner *et al.*, 2018) and perform oxygenic photosynthesis and respiration simultaneously in the same compartment, making cyanobacteria evolutionary hardy organisms (Vermaas, 2001). Many cyanobacteria species (~ 2 000 to 8 000 species

worldwide) are able to fix atmospheric nitrogen (diazotrophic cyanobacteria), enabling cyanobacteria to thrive under many environmental conditions (Steyn *et al.*, 1975; Vermaas, 2001; Davis *et al.*, 2009; O'Neil *et al.*, 2012; Miller and Russell, 2017).

The optimum temperature for other phytoplankton groups tends to be lower than that of cyanobacteria (Robarts and Zohary, 1987). Cyanobacteria therefore generally dominate freshwater phytoplankton communities in temperate, eutrophic waters during warmer periods forming potentially harmful blooms (WHO, 2003; Davis *et al.*, 2009; Oberholster *et al.*, 2009; O'Neil *et al*., 2012; Matthews, 2014; Lee *et al.*, 2017; Miller and Russell, 2017). These blooms are referred to as cyanobacterial blooms in this study (O'Neil *et al.*, 2012). Cyanobacterial bloom formation is a function of complex interactions between physical, chemical, and biological factors in freshwater bodies (WHO, 1998; O'Neil *et al.*, 2012; Reichwaldt and Ghadouani, 2012).

Historically, cyanobacteria in South Africa were abundant between January and April (late summer to early autumn) (WHO, 1998; Van Ginkel, 2011; Conradie and Barnard, 2012; Ballot *et al.,* 2014; Matthews and Bernard, 2015), in eutrophic and/or hypertrophic waterbodies (WHO, 1998; Ballot *et al.*, 2014; Matthews and Bernard, 2015). Matthews (2014) found that cyanobacterial blooms in South African impoundments reached their maximum coverage during autumn, with peak coverage occurring during April and then again in June. Winter cyanobacterial blooms appear to be a common occurrence in South Africa, however, the environmental conditions driving these events are unclear (Oberholster *et al.*, 2009; Matthews, 2014). In some South African impoundments, water temperature alone does not seem to control cyanobacterial bloom formation (Oberholster *et al.*, 2009; Matthews, 2014).

Furthermore, during the winter months of July 2005, 52 wildlife carcasses were discovered by rangers around Lake Nhlanganzwane in the Kruger National Park (Oberholster *et al.*, 2009). Clinical tests conducted by the State Veterinary Services concluded that these mortalities were attributed to the ingestion of cyanotoxins from a *M. aeruginosa* bloom that occurred in the lake (Oberholster *et al.*, 2009). A further 22 carcasses were discovered by rangers in June 2007 (Oberholster *et al.*, 2009). Additionally, cyanobacterial bloom formation occurred during autumn 2008 in Lake Loskop with concentrations of *M. aeruginosa* and *M. flos-aquae* reaching maximum concentrations during April (Oberholster *et al.*, 2009). Bloom formation in South Africa

therefore appears to have shifted from summer to autumn and early winter (Oberholster *et al.*, 2009; Matthews, 2014).

Cyanobacterial blooms are complex events that are typically caused by multiple environmental, physical, and chemical system-specific factors occurring simultaneously (Davis *et al*., 2009; Conrad and Barnard, 2012; O'Neil *et al.*, 2012).

1.4.1. Physical system-specific variables

1.4.1.1. Temperature

Temperature is an important factor in controlling the distribution of aquatic organisms as it impacts the rate of chemical reactions in waterbodies and the metabolic rates of organisms (DWAF, 1996: 5). As water temperature increases, the solubility of oxygen decreases thereby reducing the concentration of dissolved oxygen available to aquatic organisms (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). As temperature decreases, the rate of metabolic processes, including respiration, decreases therefore reducing oxygen utilisation by these organisms (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019).

Furthermore, the toxicity of certain chemical constituents, including ammonia, increases with increasing water temperatures (DWAF, 1996: 5; Matowanyika, 2010). Aquatic organisms therefore experience compound stress in waters with high temperatures due to the decreased availability of dissolved oxygen and the increased toxicity of certain chemical constituents (DWAF, 1996: 5; Matowanyika, 2010).

The optimum rates of photosynthesis, respiration, and growth of bloom-forming cyanobacteria occur at or above 25ºC (Robarts and Zohary, 1987; O'Neil *et al.*, 2012). Between October 2004 and May 2005, toxic *Microcystis* cell densities reached a maximum in water temperatures between 23ºC and 26.5ºC, dominating the phytoplankton community, in Hartbeespoort Dam (Conradie and Barnard, 2012). The optimum temperature of other phytoplankton groups tends to be lower than that of cyanobacteria (Robarts and Zohary, 1987), giving cyanobacteria a competitive advantage, particularly in water where temperature exceeds 30ºC (Fujimoto *et al.,* 1997; O'Neil *et al.,* 2012). A study performed by Davis *et al.* (2009) showed that *Microcystis* dominated all study sites as temperatures reached their maximum.

Cyanobacterial toxin production is influenced by water temperature and is abundant in water temperatures between 20ºC and 25ºC (WHO, 1998). Six different

microcystins within a cyanobacterial bloom were at their maximum concentrations during summer in Hartbeespoort Dam (WHO, 1998). This is supported by the finding that toxic *Microcystis* strains in Hartbeespoort Dam increased during the summer period (Conradie and Barnard, 2012). Elevated temperatures therefore tend to promote the growth of toxic *Microcystis* strains, and it is suggested that under these conditions, toxic *Microcystis* strains may outgrow non-toxic strains (Davis *et al.,* 2009; O'Neil *et al*., 2012). It is also suggested that the production of microcystin synthetase (the enzyme required to produce microcystins) increases with elevated temperatures (Davis *et al.*, 2009; O'Neil *et al*., 2012). Toxic cyanobacterial bloom formation is therefore influenced by increased water temperatures (Davis *et al.,* 2009; O'Neil *et al*., 2012).

1.4.1.2. Turbidity and Total Suspended Solids (TSS)

Turbidity is an optical property of water affected by the suspension of solid particles in the water column that scatter and absorb light, negatively affecting water clarity (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Suspended particles comprise of inorganic (silts and clays) and organic (algae, zooplankton, and bacteria) materials (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017). The scattering of light is caused by the presence of inorganic material in the water column whereas the absorption of light is caused by the presence of organic material in the water column (DWAF, 1996: 5). Suspended solids reflect more heat from the surface reducing the absorption of heat by an aquatic system (DWAF, 1996: 5). Turbid waterbodies therefore tend to be cooler (DWAF, 1996: 5).

1.4.1.3. Weather conditions, turbulence, and water flow

The formation of cyanobacterial blooms is influenced by weather conditions, turbulence, and water flow (WHO, 1998). The concentration of pollutants in aquatic systems is affected by seasonal variations in precipitation, surface runoff, and surface and groundwater flows (DWAF, 1996: 5; Matowanyika, 2010; Reichwaldt and Ghadouani, 2012). Rainfall events disturb aquatic ecosystems through alteration of environmental factors (Reichwaldt and Ghadouani, 2012). Surface runoff decreases during the dry months in South Africa thereby reducing electrical conductivity, turbidity, and inorganic nutrient concentrations in aquatic systems (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017).

Conversely, seasonal rainfall and storm events result in increased turbulence, and therefore increased aeration of aquatic systems (DWAF, 1996: 5; Davis *et al*., 2009; Roux *et al*., 2010; O'Neil *et al.,* 2012). Furthermore, rainfall and storm events increase surface runoff and the subsequent discharge of inorganic nutrients, total dissolved salts (TDS), and TSS (DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Dissolved oxygen, nutrient loads, electrical conductivity, and turbidity increase in aquatic systems during the wet months in South Africa (DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; O'Neil *et al.,* 2012; Dube *et al.*, 2017; Quevedo-Castro *et al*., 2019). Bloom formation is enhanced by rainfall as nutrient concentrations within the waterbody increase (WHO, 1998).

Some species of cyanobacteria possess specialised intracellular vesicles enabling these cyanobacteria to alter their buoyancy (WHO, 2003). Cyanobacteria species that can alter their buoyancy often gain a competitive advantage over other phytoplankton species as they are actively able to access light, nutrients, and inorganic carbon resources (all required for photosynthesis) across a wider range within the water column (WHO, 2003; Oberholster *et al.*, 2005; O'Neil *et al.*, 2012; Reichwaldt and Ghadouani, 2012). Additionally, cyanobacteria can maintain their position in the water column if there is constant water circulation, light to moderate winds, and high barometric pressure (WHO, 1998; Paerl and Huisman, 2009). Cyanobacteria are, however, unable to maintain their position in the water column in aquatic systems with high turbulence and waterflow (WHO, 1998; Paerl and Huisman, 2009; O'Neil *et al.,* 2012; Reichwaldt and Ghadouani, 2012). Cyanobacterial bloom formation is therefore enhanced by calm weather conditions (WHO, 1998; O'Neil *et al.*, 2012).

1.4.2. Chemical system-specific variables

1.4.2.1. pH

The pH value in natural waters is a measure of the hydrogen ion activity in water, usually ranging between 4 and 11 (DWAF, 1996: 5; Matowanyika, 2010). Most freshwaters in South Africa have pH values ranging between 6 and 8 as they are usually well buffered (DWAF, 1996: 5; Matowanyika, 2010). The introduction of acids and bases into aquatic ecosystems affects the equilibrium between hydrogen ions (H^+) and hydroxyl ions (OH^-) (DWAF, 1996: 5). As the pH of a system decreases below 7,

the system becomes more acidic whereas as the pH increases above 7, the system becomes more basic (DWAF, 1996: 5).

The main system controlling alkalinity in aquatic systems is the carbon dioxide-bicarbonate-carbonate equilibrium system (DWAF, 1996: 5). Carbon dioxide briefly dissolves in water forming carbonic acid (H_2CO_3) (DWAF, 1996: 5). Depending on the pH in the system, H_2CO_3 dissociates to form bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and H^+ (DWAF, 1996: 5).

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

Carbonic acid dominates the carbonate species in waters with a pH value < 4.0 whereas HCO_3^- dominates the carbonate species in water with a pH value between 6.4 and 8.6 (DWAF, 1996: 5). The proportion of CO_3^{2-} increases and dominates the carbonate species in water with a pH value > 8.6 (DWAF, 1996: 5). At a pH value > 10.3, CO_3^{2-} is the predominate ion in the system (DWAF, 1996: 5).

The pH value in aquatic ecosystems can be influenced by the biological activity of aquatic biota in productive and eutrophic systems (DWAF, 1996: 5; Dube *et al.*, 2017). Variations in the pH value can be attributed to the natural 24-hour cycle of respiration and photosynthesis in aquatic systems and can fluctuate daily between 6 and 10 (DWAF, 1996: 5). Algae undergo photosynthesis during the day removing CO_2 from the aquatic system whereas algae undergo respiration at night adding CO_2 to the aquatic system (DWAF, 1996: 5). As photosynthesis removes CO_2, the pH of the aquatic system increases becoming more alkaline (DWAF, 1996: 5). Alternatively, the production of CO_2 during respiration drives the carbonate/bicarbonate equilibrium towards the production of H_2CO_3 decreasing the pH making the aquatic system more acidic (DWAF, 1996: 5). The pH of the aquatic system therefore increases during the day reaching the highest at midday and decreases during the night reaching the lowest before dawn (DWAF, 1996: 5). Increased biological activity in eutrophic systems can therefore result in a more alkaline system as pH increases (DWAF, 1996: 5).

The pH of a system affects the availability and toxicity of certain constituents including ammonium ions (NH_4^+) (DWAF, 1996: 5; Dube *et al.*, 2017). At pH values > 8, the non-toxic NH_4^+ ions are converted to the highly toxic unionised ammonia (NH_3), which is exacerbated by increasing temperature (DWAF, 1996: 5; Dube *et al.*, 2017).

1.4.2.2. Dissolved Oxygen

Dissolved oxygen is essential for the survival and functioning of aquatic biota as respiration, performed by aerobic organisms, is an oxygen-dependent process (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.,* 2017; Quevedo-Castro *et al.*, 2019). Dissolved oxygen enters water through natural diffusion of gaseous oxygen (O_2) from the atmosphere continuing until oxygen saturation is achieved (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Additionally, dissolved oxygen is produced by aquatic biota (including phytoplankton) during photosynthesis (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Factors resulting in a hypoxic state include increased water temperature and salinity, respiration of aquatic organisms, increased suspension of solids (turbidity), chemical degradation of pollutants, and the decomposition of organic matter by microorganisms (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). The concentration of dissolved oxygen in water is therefore an important indicator of aquatic ecosystem health (DWAF, 1996: 5; Dube *et al.,* 2017; Quevedo-Castro *et al.*, 2019).

A natural daily variation in dissolved oxygen concentration exists in waterbodies due to the natural 24-hour cycle of respiration and photosynthesis in aquatic biota (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Photosynthesis is a process that occurs when light is available (DWAF, 1996: 5). During the night, when photosynthesis is halted, dissolved oxygen is utilised by aerobic organisms for respiration without being replenished through photosynthesis (DWAF, 1996: 5; Matowanyika, 2010). Dissolved oxygen concentrations therefore decrease during the night, reaching a minimum at dawn, and increase during the day, reaching a maximum at mid-afternoon (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.,* 2017).

Super-saturated states favour, the more tolerant, cyanobacteria as super-saturated states tend to inhibit phytoplankton photosynthesis (DWAF, 1996: 5).

1.4.2.3. Total Dissolved Salt (TDS) and Electrical Conductivity

Electrical conductivity refers to the ability of water to conduct an electrical current due to the presence of certain ions including magnesium, nitrate, potassium, and sulphate (DWAF, 1996: 1, 5; Matowanyika, 2010; Dube *et al.*, 2017). The TDS concentration refers to the quantity of compounds dissolved in water carrying an electrical charge (DWAF, 1996: 1, 5; Dube *et al.*, 2017). As water travels downstream, salts are

continuously added via natural and anthropogenic sources whilst very little is removed via evaporation and other natural processes (DWAF, 1996: 1, 5; Dube *et al.*, 2017). Effluent discharges and urban, agricultural, and industrial runoff contribute to the concentration of TDS in surface waters (DWAF, 1996, 1, 5; Dube *et al.*, 2017). Electrical conductivity is directly proportional to the concentration of TDS and is therefore used as an approximation of TDS concentration (DWAF, 1996: 1, 5; Dube *et al.*, 2017).

Salinity is a strong contributor to electrical conductivity as it refers to the total concentration of dissolved salts in water (DWAF, 1996: 1, 5; Conradie and Barnard, 2012). Most cyanobacteria do not survive in brackish or seawater but do exist across a range of salinities (Tonk *et al.,* 2007). Salinity of freshwater ecosystems is increasing due to agricultural practices, long-term droughts, water management processes, and rising seawater levels (Tonk *et al*., 2007; O'Neil *et al.*, 2012). It has been suggested that increasing salinities may cause a shift in the phytoplankton composition of freshwater ecosystems as many freshwater phytoplankton are salt-sensitive (Tonk *et al*., 2007; O'Neil *et al.,* 2012).

A study conducted by Tonk *et al.* (2007) showed that *M. aeruginosa* has a high salt tolerance for a freshwater cyanobacterium as its growth rate and microcystin production were unaffected up to a salinity of 10 g/L. Above salinities of 10 g/L, extracellular microcystin concentration initially increased due to cell lysis and cell leakage as the osmoregulation capacity of these cells was exceeded (Tonk *et al.*, 2007). Several weeks after exposure to salinities greater than 10 g/L, both the growth rate and microcystin production began to decrease (Tonk *et al.,* 2007). *M. aeruginosa* therefore thrive in freshwater salinities below 10 g/L and may have a competitive advantage over other freshwater phytoplankton in brackish waters (Tonk *et al.,* 2007; O'Neil *et al.*, 2012).

1.4.2.4. Inorganic Nutrients

Dissolved inorganic phosphorus and inorganic nitrogen (ammonia, ammonium, nitrite, and nitrate) both contribute to eutrophication and both have a stimulatory effect on the growth of algae and aquatic plants (DWAF, 1996: 5; Matowanyika, 2010; Xu *et al.*, 2010; Ballot *et al.*, 2014; Mitchell *et al.*, 2014; Matthews and Bernard, 2015; Quevedo-Castro *et al.*, 2019). A shift in the phytoplankton community structure towards

cyanobacteria dominance occurs when freshwaters become enriched with inorganic nutrients (Oberholster *et al.*, 2005; Davis *et al*., 2009; Xu *et al.*, 2010; Van Ginkel, 2011; O'Neil *et al*., 2012; Ballot *et al.*, 2014; Mitchell *et al.*, 2014; Gobler *et al.,* 2016). Potential toxin-producing cyanobacteria species are generally favoured in eutrophic and hypertrophic waterbodies due to their higher affinity to nutrients and their ability to adjust buoyancy compared to other phytoplankton species (Oberholster *et al.*, 2005; Reichwaldt and Ghadouani, 2012; Matthews and Bernard, 2015). The shift to cyanobacteria dominance occurs especially when freshwater becomes enriched with phosphorus as many bloom-forming cyanobacteria in warm waters are capable of fixing atmospheric nitrogen thus balancing nitrogen deficiencies in the ecosystem (DWAF, 1996: 5; Davis *et al*., 2009; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al*., 2016).

Cyanobacteria tend to dominate phytoplankton communities in temperate freshwater ecosystems with phosphorus concentrations of ~ 0.1 mg/L – 1.0 mg/L (Trimbee and Prepas, 1987; Jensen *et al.*, 1994; Watson *et al*., 1997; Downing *et al*., 2001; Xu *et al.*, 2010). Additionally, some cyanobacteria have displayed increased microcystin content per cell in environments with increased phosphorus concentrations (Utkilen and Gjølme, 1995; DWAF, 1996: 5; Rapala *et al*., 1997; Oh *et al.,* 2000; O'Neil *et al*., 2012). Phosphorus therefore tends to be the more growth-limiting nutrient in freshwater systems and is considered the main nutrient controlling eutrophication (Schindler, 1974; Utkilen and Gjølme, 1995; DWAF, 1996: 5; Rapala *et al*., 1997; Oh *et al.,* 2000; Xu *et al.*, 2010; O'Neil *et al*., 2012).

Toxic *Microcystis* strains have a higher nitrogen requirement than non-toxic *Microcystis* strains (Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012). Nitrogen is required by the cell to produce the enzymes (protein molecules) required for microcystin synthesis – microcystin synthetase (Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012). Microcystins are nitrogen-rich toxins, which further suggests a higher nitrogen requirement by toxic *Microcystis* strains (Conradie and Barnard, 2012). High nitrogen levels in the environment therefore enable toxic *Microcystis* strains to outgrow non-toxic *Microcystis* strains as growth and toxicity increases with increasing nitrogen loads (Davis *et al*., 2009).

In the case of bloom-forming cyanobacteria that cannot fix atmospheric nitrogen (non-diazotrophic), such as *M. aeruginosa*, nitrogen may limit cyanobacterial proliferation as non-diazotrophic cyanobacteria depend on exogenous sources of nitrogen for growth and toxin production (Davis *et al*., 2009; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al.,* 2016). It has been suggested that nitrogen is as important as phosphorus in controlling the proliferation and toxicity of cyanobacterial blooms and increased loads of both nitrogen and phosphorus results in a shift from diazotrophic to non-diazotrophic cyanobacteria (Davis *et al*., 2009; Xu *et al.*, 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al.,* 2016).

i. Inorganic Phosphorus

Phosphorus is an essential nutrient for proper cell functioning that is actively taken up by aquatic biota (Matowanyika, 2010). Natural sources of phosphorus in waterbodies include the decomposition of organic matter, the weathering of rocks, and the leaching of phosphate salts into surface waters (DWAF, 1996: 5). Phosphorus is exchanged between the sedimentary and aquatic compartments (the phosphorus cycle) (DWAF, 1996: 5) and is dissolved in water columns or is absorbed onto sediment as phosphate (Schindler, 1974; DWAF, 1996: 5; Matowanyika, 2010). Phosphorus is removed from the water column onto the sediment in the presence of oxygen via the settlement of particulate matter and biotic uptake (Schindler, 1974; DWAF, 1996: 5). Orthophosphate is the only form of soluble inorganic phosphorus utilised by aquatic biota (DWAF, 1996: 5). As orthophosphate is actively utilised by aquatic algae and plants, concentrations are therefore usually low in unimpacted waters (Schindler, 1974; DWAF, 1996: 5; Matowanyika, 2010).

Surface waters receiving domestic and industrial effluents (point source discharge) or surface runoff from urban and agricultural land (diffuse discharge) are at risk of elevated phosphorus levels (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015). As a result of increased surface runoff and resuspension of deposited materials into the water column, rainfall events also increase phosphorus concentrations in surface waters (DWAF, 1996: 5; Matowanyika, 2010).

ii. Inorganic Nitrogen

Inorganic nitrogen includes all forms of dissolved and absorbed inorganic nitrogen compounds (ammonia, ammonium, nitrite, and nitrate) available for utilisation by algae

and other aquatic biota (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Upon entering aquatic systems, organic nitrogen (e.g., amino acids and urea) is converted into ammonium (NH_4^+) (DWAF, 1996: 5; Matowanyika, 2010). Through the assimilation by bacteria, ammonium is converted to nitrite (NO_2^-), an inorganic intermediate, and then to nitrate (NO_3^-), the final product of nitrification (DWAF, 1996: 5; Matowanyika, 2010). Nitrate is more stable than nitrite therefore tends to be more abundant in surface waters (DWAF, 1996: 5). Nitrite and nitrate are often considered together due to their co-occurrence and rapid interconversion (DWAF, 1996: 5). Nitrification is an oxidation reaction producing the more water-soluble nitrate from organic nitrogen and ammonium therefore decreasing dissolved oxygen concentrations in aquatic systems (DWAF, 1996: 5; Matowanyika, 2010).

Ammonia, a common pollutant, is an essential component of the nitrogen cycle and exists in either unionized (NH_3) or ionized (NH_4^+) states (DWAF, 1996: 5). Small concentrations of ammonia are found in soil, water, and air, whereas large concentrations are found in decomposing organic matter, produced naturally when nitrogenous matter is biologically degraded (DWAF, 1996: 5). Both forms of ammonia are naturally produced when nitrogenous matter is biologically degraded and are both therefore reduced forms of inorganic nitrogen (DWAF, 1996: 5). The toxicity of inorganic ammonia is determined by the concentration of ammonia (NH_3) as the ammonium (NH_4^+) ion has little to no effect on aquatic organisms (DWAF, 1996: 5).

1.4.3. Nitrogen fixation by cyanobacteria

Molecular nitrogen (N_2) is abundant in the atmosphere but is not in a soluble form (Syiem *et al*., 2010). Nitrogen is therefore only integrated into the ecosystem once it has been converted into nitrogenous compounds that can be metabolised by organisms (Syiem *et al*., 2010). The process of converting atmospheric nitrogen into organic (e.g., urea, amino acids, and DNA/RNA) or inorganic (e.g., ammonia, nitrite, and nitrate) forms of nitrogen is called nitrogen fixation (Syiem *et al*., 2010). Cyanobacteria are one of the few prokaryotes that can perform nitrogen fixation using the enzyme nitrogenase (Syiem *et al*., 2010). Nitrogenase is sensitive to oxygen (O_2), both at the synthesis level and during activity (Syiem *et al*., 2010). Upon exposure to O_2, nitrogenase is irreversibly inactivated and degraded (Syiem *et al*., 2010). The type of nitrogen fixation occurring in cyanobacteria is therefore O_2-sensitive nitrogenase-dependent nitrogen fixation (Syiem *et al*., 2010).

Cyanobacteria either spatially or temporally separate O_2-sensitive nitrogenase-dependent nitrogen fixation and O_2-evolving photosynthesis, two seemingly mutually exclusive processes (Syiem *et al*., 2010). Two forms of cyanobacteria exist: heterocystous forms and non-heterocystous forms (Syiem *et al*., 2010). Cyanobacteria have therefore evolved an efficient way of protecting O_2-sensitive nitrogenase from the O_2 generated during photosynthesis (Syiem *et al*., 2010).

Heterocystous forms of cyanobacteria spatially separate the two processes and consist of both heterocysts and vegetative cells (Syiem *et al*., 2010). Nitrogenase is present in the heterocysts, the site of nitrogen fixation, whereas vegetative cells are the site of photosynthesis (Syiem *et al*., 2010). A mutualistic metabolite interchange relationship exists between the heterocysts and the vegetative cells as the vegetative cells transfer carbohydrates, produced during photosynthesis, to the heterocysts while relying on the heterocysts for the supply of fixed nitrogen (Syiem *et al*., 2010). Heterocystous cyanobacteria can enter symbiotic relationships with some plants (Syiem *et al*., 2010). In this case, the cyanobiont is almost always diazotrophic and is almost entirely responsible for fixing and providing nitrogen to the eukaryotic partner (Syiem *et al*., 2010).

Non-heterocystous forms of cyanobacteria temporally separate the two processes as these cyanobacteria alternate between cycles of nitrogen fixation and photosynthesis (Syiem *et al*., 2010). Nitrogenase in the non-heterocytsous cyanobacteria is responsible for the conversion of molecular nitrogen to a more soluble form (Syeim *et al*., 2010).

1.5. Cyanotoxins

1.5.1. Overview of cyanotoxins

Many cyanobacteria genera produce a range of potent toxins (cyanotoxins) that are usually concentrated intracellularly and released when cells senesce and die – released into the environment when cells rupture (DWAF, 1996: 1; WHO, 1998; WHO, 2003; Oberholster *et al.*, 2005; Tonk *et al.*, 2007; Oberholster *et al.*, 2009; Xu *et al.*, 2010; Van Ginkel, 2011; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Ballot *et al.*, 2014; Matthews, 2014; Lone *et al.,* 2015; Matthews and Bernard, 2015; Lee *et al.*, 2017; Miller and Russell, 2017). The extracellular cyanotoxin concentrations therefore remain low (Tonk *et al.*, 2007). Young, growing cells can, however, actively release

cyanotoxins (WHO, 1998). These toxins are compounds that are poisonous or pose a health risk to animals and humans, alter the physico-chemical features of aquatic ecosystems, and alter the interactions between aquatic organisms (DWAF, 1996: 1; WHO, 2003; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Xu *et al.*, 2010; Van Ginkel, 2011; O'Neil *et al.,* 2012; Ballot *et al.*, 2014; Matthews, 2014; Matthews and Bernard, 2015; Lee *et al.*, 2017). Cyanotoxins are therefore a public health threat for the potable and recreational use of water (WHO, 2003; Oberholster *et al.,* 2009; Xu *et al.*, 2010; Ballot *et al.*, 2014; Mathews, 2014; Matthews and Bernard, 2015).

Cyanotoxins can be classified based on chemical structure, mechanism of toxicity, and grouped according to which physiological system they target (Oberholster *et al.*, 2005; Swanepoel *et al.,* 2008; Oberholster *et al.*, 2009; Ballot *et al.*, 2014). Some cyanobacteria species, not all strains, within a surface water bloom can produce hepatotoxins (e.g., microcystins and nodularins), cytotoxins, dermatoxins, or neurotoxins (e.g., β-N-methylamino-l-alanine and anatoxins) (WHO, 1998; WHO, 2003; Oberholster *et al.*, 2005; Swanepoel *et al*., 2008; O'Neil *et al.*, 2012; Ballot *et al*., 2014). Hepatotoxins, or microcystins, are the primary toxin associated with freshwater toxicosis as they are the most prevalent cyanotoxin worldwide, followed by neurotoxins (WHO, 2003; Oberholster *et al.*, 2005; O'Neil *et al.*, 2012; Ballot *et al.*, 2014; Turner *et al.*, 2018). Microcystins are protein phosphatase inhibitors entering cells chiefly via the bile acid transporter found in hepatocytes (liver cells) (WHO, 2003; Oberholster *et al.*, 2005). Hepatotoxins therefore disturb hepatocyte integrity and functioning (WHO, 2003; Oberholster *et al.*, 2005). Cytotoxins damage genetic material and induce cell necrosis by inhibiting protein synthesis, whereas neurotoxins affect the nervous system (WHO, 2003; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009). Cyanotoxins are therefore a limitation to the use of water from contaminated waterbodies (WHO, 2003; Conradie and Barnard, 2012; Ballot *et al*., 2014; Gobler *et al*., 2016).

Cyanobacteria are capable of allelopathy, a process whereby chemicals produced by a plant inhibit the growth, germination, or metabolism of another plant (Pearce, 1987). Cyanotoxins have been shown to inhibit the growth of diatoms and can negatively affect other bloom-forming phytoplankton by inhibiting their growth (Pearce, 1987). Toxin-producing cyanobacteria can therefore gain a competitive advantage in aquatic ecosystems (Pearce, 1987).

Between 50% and 75% of cyanobacteria species within a bloom can produce one or more cyanotoxins (WHO, 1998; WHO, 2003; Conradie and Barnard, 2012). Toxic and non-toxic strains of the same cyanobacteria species can co-exist within a cyanobacterial bloom (WHO, 2003; Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012; Ballot *et al.*, 2014). These strains are morphologically indistinguishable thus determining toxin producing and non-toxin producing strains within a bloom is difficult (WHO, 1998; Oberholster *et al.*, 2005; Davis *et al.*, 2009; Conradie and Barnard, 2012; O'Neil *et al.*, 2012). A bloom is therefore always potentially dangerous and suspect (Ressom *et al.*, 1994; WHO, 1998; Swanepoel *et al*., 2008).

It has been found that cyanotoxins, particularly microcystins, can accumulate in consumable animal and plant tissues (Lee *et al.,* 2017; Turner *et al.*, 2018). Agricultural plants accumulate cyanotoxins when irrigated with cyanobacteria-contaminated water thus acting as a potential cyanotoxin reservoir (Lee *et al.*, 2017; Miller and Russell, 2017). Cyanotoxin accumulation in plants is dependent on the plant type, the concentration of cyanotoxins in the irrigation water, the type of irrigation method, the amount of irrigation water used, and the duration of exposure to cyanotoxins (Lee *et al.*, 2017; Miller and Russell, 2017). Cyanotoxin accumulation in edible plants is dose-dependent and reduces the quality and productivity of the crop (Lee *et al.*, 2017; Miller and Russell, 2017). It is therefore important to monitor cyanotoxin levels in irrigation water (Lee *et al.*, 2017; Miller and Russell, 2017).

Previous studies of Hartbeespoort Dam have focussed mainly on *Microcystis* spp. (Conradie and Barnard, 2012; Ballot *et al*., 2014) and it was hypothesised that the number of cyanobacterial species and cyanotoxins in Hartbeespoort Dam were underestimated (Ballot *et al*., 2014).

1.5.2. Mechanisms of exposure and associated health risks

The major routes of exposure include drinking of contaminated water, dermal contact during swimming or showering, inhalation, and consumption of animal and plant tissue exposed to contaminated water (WHO, 2003; Van Ginkel, 2011; Matthews, 2014; Oberholster *et al.*, 2009; Lee *et al.,* 2017; Miller and Russell, 2017; Turner *et al.*, 2018).

Exposure to cyanotoxin-contaminated water can cause acute and chronic health effects (Turner *et al.*, 2018). Acute health risks include, but are not limited to, nausea,

vomiting and diarrhoea, fever, headaches, respiratory or muscular paralysis, incoordination, and in some cases death (DWAF, 1996: 1; Oberholster *et al.*, 2005; Matthews and Bernard, 2015; Lee *et al.*, 2017; Miller and Russell, 2017), as seen by livestock and wildlife deaths on the shores of Hartbeespoort Dam and Lake Nhlanganzwane, respectively (Steyn, 1973; DWAF, 1996: 1; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Ballot *et al*., 2014; Matthews, 2014; Matthews and Bernard, 2015). Microcystin-LR is considered a Group 2B possible carcinogen to human beings (Miller and Russell, 2017). The chronic health risks therefore include possible tumour-promoting activity (WHO, 2003; Miller and Russell, 2017). A study performed by Li *et al.* (2015) on the chronic exposure of mice to MC-LR found that chronic exposure resulted in membrane, mitochondrial and cytoskeletal damage, DNA mutations, and the loss of cell morphology (Lee *et al.*, 2017).

1.5.3. Common cyanotoxins in freshwater systems

1.5.3.1. Microcystin-LR

Most microcystins are hepatotoxins and are produced by various cyanobacteria species (WHO, 1998; WHO, 2003; Oberholster *et al.*, 2005; Lone *et al.,* 2015). Microcystins displays hepato-specificity as they are actively transported into the liver by a bile acid transporter (WHO, 2003; Oberholster *et al.*, 2005). Microcystins are cyclic heptapeptides with seven amino acids and the most variable amino acids being in positions 2 and 4 (WHO, 2003; Oberholster *et al.*, 2005; USEPA, 2006; Tonk *et al.*, 2007; Ballot *et al*., 2014; Turner *et al.*, 2018). Different microcystin variants have different amino acids on the peptide structure and are named accordingly (Oberholster *et al.*, 2005; USEPA, 2006). A specific amino acid (ADDA) side chain is, however, common to all microcystin variants (WHO, 2003). Microcystins are produced by *Microcystis* spp. and members of other genera including *Nodularia, Anabaena,* and *Nostoc* (WHO, 1998; WHO, 2003; Oh *et al.,* 2000; Oberholster *et al.*, 2005; Ballot *et al.,* 2014). Worldwide, over 90 microcystin isoforms have been detected and altogether different studies have identified over 10 microcystin variants in Hartbeespoort Dam including: MC-LR, MC-YA, and MC-WR (Oberholster *et al.*, 2005; Ballot *et al*., 2014; Lone *et al.*, 2015).

Microcystins have caused several public health events as they bioaccumulate in aquatic organisms and thus can be passed up the food chain (Lone *et al*., 2015; Dube *et al.*, 2017). Microcystin-LR was the first microcystin to be chemically identified (WHO,

1998; WHO, 2003) and is the most toxic and most abundant microcystin (Oh *et al.*, 2000; Lone *et al.,* 2015; Russell and Miller, 2017). The presence of MC-LR in drinking water poses a problem as it is soluble in water and is resistant to temperatures up to 300ºC, pH extremes, and chemical oxidation or hydrolysis at near-neutral pH (WHO, 1998; Oberholster *et al.*, 2005; Lone *et al*., 2015).

The most vulnerable organ to MC-LR is the liver as it can damage the liver by inhibiting protein phosphatases (important molecular switches in eukaryotic cells) (WHO, 2003) therefore disturbing the cytoskeletal integrity of hepatocytes (Oberholster *et al.*, 2005; Tonk *et al.,* 2007; Lone *et al*., 2015; Turner *et al.*, 2018). Other organ systems vulnerable to MC-LR include the kidney, nervous system, heart, gastrointestinal tract, and the male reproductive system (Lone *et al*., 2015). The presence of microcystins in potable water is therefore a threat to public health (Oberholster *et al.*, 2005; Oberholster *et al.,* 2009; Xu *et al.*, 2010; Ballot *et al.*, 2014; Mathews, 2014; Matthews and Bernard, 2015).

During laboratory-based studies, low doses of microcystins administered to mice resulted in degeneration of hepatocytes, chronic liver inflammation and injury, increased tumour activity, and increased mortality rates (particularly in male mice) (WHO, 2003; Oberholster *et al.*, 2005). Increased incidence of liver cancer in China has been linked to the ingestion and use of cyanobacteria-contaminated surface water (Yu, 1995; Oberholster *et al.*, 2005). Additionally, seasonal *M. aeruginosa* blooms in an Australian reservoir was linked to patients with liver complaints, a correlation specific to the patients who had used the reservoir as a source of potable water (Falconer *et al.*, 1999; Oberholster *et al.*, 2005).

Microcystin-LR has been identified as a potential human carcinogen as it is able to cross the blood-testis barrier and cause morphological damage of the testis (Lone *et al*., 2015; Russell and Miller, 2017). Microcystin-LR is able to increase the expression of proto-oncogenes; disrupt the DNA damage repair pathway and has been shown to cause testicular atrophy and apoptosis (programmed cell death) of germ cells, spermatogenic cells, Sertoli cells, and Leydig cells, with Leydig cells being the most sensitive to MC-LR (Lone *et al*., 2015). Leydig cells damage and/or apoptosis induced by MC-LR results in the decreased production of testosterone thus affecting the hypothalamic-pituitary-gonadal axis (Lone *et al*., 2015). It has therefore been

suggested that the gonads are the second most vulnerable organ to MC-LR as studies have shown MC-LR accumulating in the gonads of invertebrates (Lone *et al*., 2015).

Microcystin-LR also disrupts the motility and morphology of sperm (Lone *et al*., 2015). Studies have shown that long term exposure to MC-LR in male rats resulted in decreased sperm motility; testis weight; concentration of sperm; and decreased hormone levels of the male reproductive system (follicule-stimulating hormone, luteinizing hormone, and testosterone) (Lone *et al.,* 2015). The seminiferous tubules in these male rats also atrophied and became obstructed (Lone *et al*., 2015). Microcystins are therefore toxic to the testes and the epididymis (Lone *et al*., 2015).

1.5.3.2. β-methylamino-L-alanine

Neurotoxins (including β-N-methylamino-l-alanine and anatoxins) affect the nervous system resulting in a depolarizing neuromuscular blockade followed by fatigue and paralysis (Oberholster *et al.*, 2005, Oberholster *et al.*, 2009). Paralysis of peripheral skeletal muscles is followed by paralysis of smooth muscle, including respiratory muscles, resulting in respiratory arrest and death (Oberholster *et al.*, 2005, Oberholster *et al.*, 2009). A correlation between the exposure to cyanobacteria and an increased incidence of neurodegenerative diseases has been found (Dunlop, 2013).

Recent studies have found a link between the non-protein amino acid β-methylamino-L-alanine (BMAA) and amyotrophic lateral sclerosis-Parkinsonism/dementia complex (ALS-PDC) (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; O'Neil *et al.,* 2012; Dunlop, 2013). ALS-PDC, also known as motor neuron disease, is a neurodegenerative disease that causes paralysis, shaking, and dementia (Holtcamp, 2012). ALS affects motor neurons and paralyses patients, causing the patients to die when they can no longer breathe or swallow (Holtcamp, 2012). Ten percent of ALS cases are familial (caused by inherited genetic mutations) and ninety percent are sporadic with the causes being unknown (Holtcamp, 2012; Dunlop, 2013). Nerve cells are unable to regenerate and accumulate damaged proteins until they reach a critical point and undergo apoptosis (Holtcamp, 2012; Dunlop, 2013). Neurodegenerative diseases are characterised by aggregates of misfolded proteins in the nerve cells (Holtcamp, 2012).

BMAA is a cyanotoxin that is produced by most tested cyanobacterial genera, including *Microcystis* (Fristachi and Sinclair, 2008), and is found in potable water,

recreational waterbodies, and in contaminated seafood and shellfish (Holtcamp, 2012). BMAA can co-occur with other cyanotoxins in naturally forming cyanobacterial blooms and is found in both fresh and marine waters (Cox, 2009). BMAA is produced by *Nostoc,* a cyanobacterial species living in symbiosis with cycad trees, and is absorbed into the fruits of these trees (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013). In Guam, the seeds of the cycad tree are used as food and medicine by the Chamorro people and are eaten by fruit bats (flying foxes) and feral pigs (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013). The seeds are ground to make flour that is used to make tortillas and dumplings (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013). Fruit bats (a fruit bat coconut soup is made) and feral pigs also form part of the Chamorro people's diet (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013). BMAA therefore bioaccumulates in the food chain and is considered the most likely cause of elevated cases of ALS in the Chamorro population (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013).

In 2002, it was hypothesized that a neurotoxic reservoir of BMAA in brain tissues was due to biomagnification – a process whereby a toxin accumulates in an organism (Cox *et al.,* 2003; Holtcamp, 2012; Dunlop, 2013). BMAAs are bound into proteins as they exist as free water-soluble molecules (Cox, 2009; Holtcamp, 2012). BMAA is able to cross the blood-brain barrier and accumulates in the nerve cell proteins providing a mechanism for how BMAA might biomagnify in neural tissue (Cox *et al.,* 2003; Holtcamp, 2012). Analysis of flying fox tissue found that BMAA can bind to proteins enabling it to biomagnify in the tissue (Holtcamp, 2012; Dunlop, 2013). When these bats are consumed by the Chamorro people, they therefore receive a high toxic load of BMAA (Cox *et al.,* 2003; Holtcamp, 2012; Dunlop, 2013).

BMAAs are misincorporated into nerve cell proteins resulting in protein misfolding and cell death when a critical point is reached (Cox, 2009; Holtcamp, 2012; Dunlop, 2013). This misincorporation is due to a translation malfunction, during protein synthesis, where the transfer-RNA (tRNA) synthetase enzyme mistakes BMAA for serine, a proteinogenic amino acid (Cox, 2009; Holtcamp, 2012; Dunlop, 2013). This translation malfunction can result in the formation of protein aggregates of incorrectly misfolded proteins, a character of neurodegenerative disorders, which impede normal cell functioning (Cox, 2009; Holtcamp, 2012; Dunlop, 2013). BMAA is therefore selectively toxic to neurons.

BMAA is, however, not limited to the Chamorro people in Guam. High concentrations of BMAA have been found in the brains of Canadians who died of Alzheimer disease (AD) (Cox *et al.,* 2003; Cox, 2009; Holtcamp, 2012; Dunlop, 2013). In New Hampshire, USA, it was shown using GIS software mapping that people living within a half a mile radius of cyanobacteria-contaminated lakes had a 2.32 times greater chance of developing ALS than the rest of the population (Holtcamp, 2012; Dunlop, 2013). The presence of cyanobacteria in freshwater bodies is therefore a threatening source of BMAA to the greater population.

Based on previous literature, it was assumed the trophic status of Hartbeespoort Dam was hypertrophic. As a result, the dam was dominated by phytoplankton with cyanobacteria being the most abundant phytoplankton present in the water (Davis *et al.*, 2009; O'Neil *et al*., 2012; Lee *et al.*, 2017; Miller and Russell, 2017). The cyanobacteria present in the dam were producing cyanotoxins that posed a risk to human health.

The aim of this study was to determine the human health risk associated with the exposure to cyanotoxins produced by cyanobacteria based on an ecological health assessment of the Hartbeespoort Dam. The objectives were:

1. To determine the quality of water in Hartbeespoort Dam, where quality was based on *in situ* physico-chemical measurements and laboratory-based measurements of nutrient concentrations.
2. To determine the phytoplankton community structure and biomass in Hartbeespoort Dam, focussing on cyanobacteria.
3. To determine the cyanotoxins present in Hartbeespoort Dam and to link these cyanotoxins to human health risk, using latest available literature.

2. Materials and Methods

2.1. Study Site

This study was conducted at the Hartbeespoort Dam in the Brits magisterial district, South Africa (25.7401°S, 27.8592°E) from April 2018 to July 2018. The dam has an area of approximately 15.6 km^2 with an altitude of 1165 m above sea level (Matthews, 2014). It is globally well-known for its high chlorophyll *a* concentrations, persistent *M. aeruginosa* cyanobacterial blooms, and the production of toxic surface scums (Allanson and Gieskes, 1961; Steyn *et al*., 1975; Zohary, 1985; Pearce, 1987; Robarts and Zohary, 1987; Conradie and Barnard, 2012; Ballot *et al*., 2014; Matthews and Bernard, 2014; Harding, 2015). The catchment area of the dam is 4 033 km^2 and it includes parts of the southern slopes of the Magaliesberg Mountain range, the Rietvlei Dam, and northern slopes of the Witwatersrand (Steyn *et al*., 1975; Pearce, 1987; Conradie and Barnard, 2012).

The maximum depth of the dam is 30 m with a mean depth of 9.6 m (Scott *et al*., 1980; Pearce, 1987; Ballot *et al*., 2014). Monthly meteorological data for Hartbeespoort Dam during the period of August 2017 to July 2018 were obtained from the South African Weather Service (SAWS) (Hartbeespoort Weather Station 0512554 4; 25.7480°S, 27.8320°E; Appendix A). The average ambient temperature was 28.5ºC, reaching a maximum of 34.1ºC in January 2018 (Appendix B). The total rainfall between August 2017 and July 2018 was 673.4 mm mainly occurring during the summer season. Maximum rainfall of 157.6 mm occurred in March 2018 (Appendix A).

Both the average ambient temperature and the average rainfall at Hartbeespoort Dam decreased as the study progressed from autumn into winter. The average ambient temperature decreased from 27.5ºC in April to 21ºC in July 2018, averaging 23.5ºC across the study period (Appendix A). Similarly, average rainfall decreased from 100.4 mm in April to 0.4 mm in July 2018, averaging 30.1 mm per month across the study period (Appendix A). An average humidity of 81.6% was obtained during the study (Appendix A).

Where possible, water samples were collected from six different locations in the dam to ensure that a broad spatial range of samples were collected. Additionally, a broad temporal range of samples were collected as sampling sites overlapped with previous studies. This study attempted to locate the six different sites used by Steyn *et al.*

(1975), as shown in Figure 2.1. Sampling sessions occurred on three different occasions during the sampling period: 10 April 2018 (Sampling Trip 1), 12 June 2018 (Sampling Trip 2), and 6 July 2018 (Sampling Trip 3). Sampling Trip 1 was cut short due to an impenetrable mat of water hyacinth and a thunderstorm (total rainfall = 10 mm) over Hartbeespoort Dam resulting in only four of the six sites being sampled.

Figure 2.1: Google Earth satellite image of Hartbeespoort Dam showing approximate locations of the six sampling sites that were used in this study. The red pin indicates the location of the Hartbeespoort Weather Station 0512554 4. *Image obtained on the 2nd of December 2020.*

2.2. Sampling methods

Water measurements of *in situ* physico-chemical parameters (temperature, pH, dissolved oxygen, and electrical conductivity) and water samples collected for inorganic nutrients (orthophosphate, ammonia, nitrate + nitrite), phytoplankton community structure and biomass, and total microcystins were conducted on three occasions from the dam between April 2018 and July 2018. Emphasis was placed on changing seasons from autumn to mid-winter as cyanobacterial growth is heavily influenced by water temperature (Robarts and Zohary, 1987; WHO, 1998; O'Neil *et al.,* 2012). In addition, the sampling period should have captured a change in nutrient load entering the dam because of decreased rainfall during the winter months. These measurements and samples were taken from the same six locations in the dam on

each collection day, except for the first sampling trip where four sampling sites were sampled.

Water samples at each sampling site were collected using a 2 L phytoplankton Van Dorn sampler. Water samples were collected from just below the surface, 1 m, and then at 5 m intervals to the bottom of the dam (Table 2.1). Water samples were taken to the aquatic ecology laboratory within the School of Animal, Plant, and Environmental Sciences (APES), University of the Witwatersrand, where the samples were analysed.

Table 2.1: Depths at which *in situ* physico-chemical measurements and water samples were taken, where possible, at the six different locations in the dam on the three different sample collection days.

Reading/Water Sample	Water Depth (m)
1	0
2	1
3	5
4	10
5	15
6	20
7	25

Samples for nutrient and total microcystin analyses were collected and placed into sterile 50 mL plastic containers and stored in a dark cooler box on ice bricks until they could be frozen in the laboratory. Samples for the measurement of phytoplankton community composition were collected in 100 mL plastic containers and fixed with 1 mL Lugol's iodine solution on site. They were stored in a dark place in the laboratory until analysis. Samples for the measurement of chlorophyll *a* concentration were collected in 500 mL plastic containers and stored in a dark cooler box on ice bricks until they could be stored in the laboratory refrigerator.

2.2.1. *In situ* physico-chemical methods and nutrient analyses

At each of the six different locations, a YSI Professional Plus handheld multiparameter meter was used to measure *in situ* temperature, pH, electrical conductivity, and dissolved oxygen, as it was lowered from the water surface to the bottom of the dam. Readings were obtained across the water column from just below the surface, 1 m, and then at 5 m intervals to the bottom of the dam (Table 2.1). A Secchi disk depth was determined by the three people on the boat at the six different sampling sites and the mean was calculated to determine the water clarity for each site (Matthews and Bernard, 2015).

On the day of water sample collection, water for the analysis of inorganic nutrients was filtered *in situ* through 0.45 µm glass fibre syringe filters, to remove any potential contaminants. Within three months of sampling, the frozen samples were defrosted and analysed for inorganic nutrients using standard colorimetric methods for ammonium and orthophosphate (Parsons *et al*., 1984), and for nitrate + nitrite (Bate and Heelas, 1975). Dissolved inorganic phosphorus (DIP) concentrations were equivalent to orthophosphate concentrations whereas dissolved inorganic nitrogen (DIN) concentrations were obtained by combining ammonium and nitrate + nitrite concentrations.

The DIN:DIP ratio was determined to identify the nutrient prevailing in Hartbeespoort Dam and therefore the nutrient potentially controlling algal growth and biomass (Janse van Vuuren and Taylor, 2015; Quevedo-Castro *et al*., 2019). The nutrient controlling algal growth will be consumed faster therefore reaching a minimum value before the other nutrient (Quevedo-Castro *et al.,* 2019). Nitrogen typically controls algal growth and biomass in marine waters whereas phosphorus typically controls algal growth and biomass in freshwater systems (Janse van Vuuren and Taylor, 2015). For this study, nitrogen was considered the limiting nutrient if a DIN:DIP ratio < 16 was obtained whereas phosphorus was considered the limiting nutrient if a DIN:DIP ratio > 16 was obtained (Janse van Vuuren and Taylor, 2015).

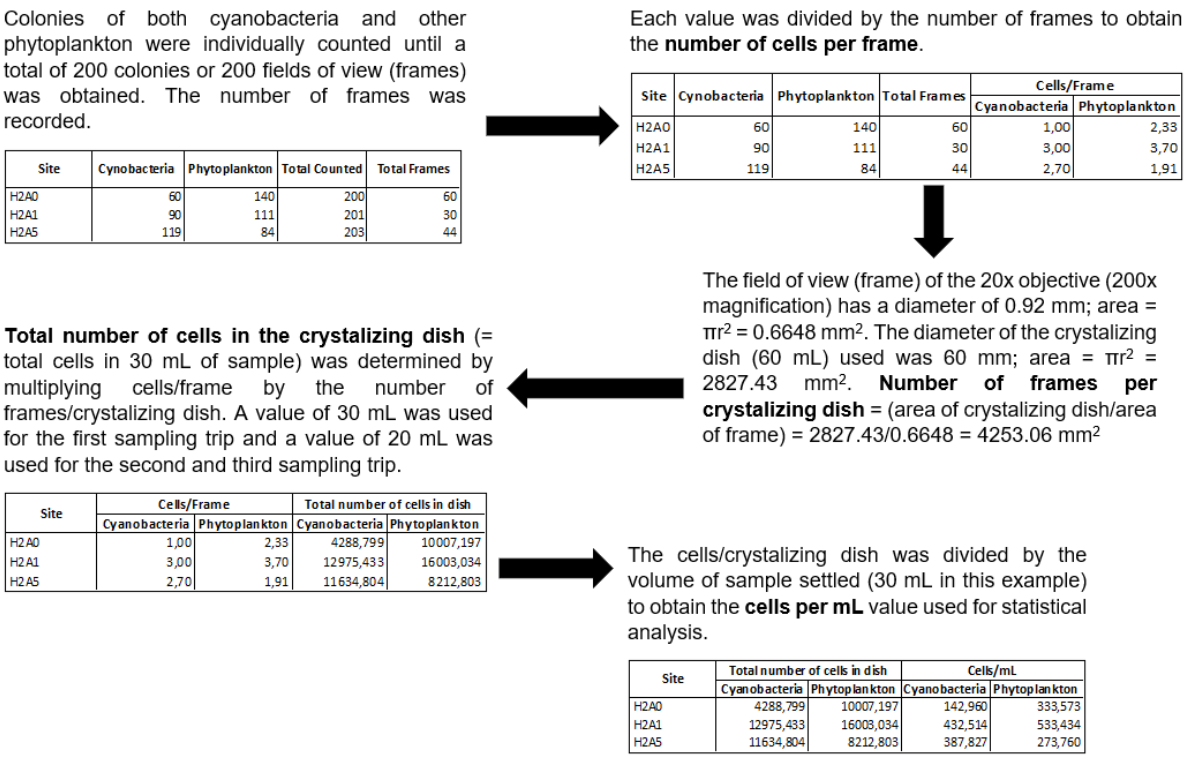
2.2.2. Phytoplankton identification, cell density, and biomass

A known volume of each Lugol's-fixed sample was poured into a 60 mL crystalizing dish and left to settle for ten minutes before being viewed under an inverted light microscope. A crystalizing dish was used instead of Utermohl settling chambers as

these were not available during this study. For the first sampling trip, 30 mL of the sample was used whereas for the second and third sampling trips, 20 mL of the sample was used. The samples from the second and third sampling trips were too turbid to view under the microscope therefore were diluted with 10 mL deionized water prior to viewing under the microscope.

The samples in the 60 mL crystalizing dish were viewed on an Olympus CK 2 Inverted Microscope at a maximum magnification (x 200 magnification; x 20 objective). The number of cyanobacteria units (a single colony or filament) within the sample were counted, and collectively grouped as “Cyanobacteria”, and all other phytoplankton taxa were collectively counted and grouped as “Other phytoplankton” (Figure 2.2.A). This method does not account for the difference in cyanobacterial colony sizes. At least a total of 200 units or 200 fields of view (frames) were counted for each sample, whichever came first (Davis *et al.*, 2009). Algal identification was performed using a freshwater algae identification manual by Janse van Vuuren *et al.* (2006).

For each sample, the number of algal cells per frame was determined by dividing the counted number of cyanobacteria and other phytoplankton by the number of frames used for that sample (Figure 2.2.B). The total number of cells in the crystalizing dish was obtained by multiplying the number of frames per crystalizing dish (area of the crystalizing dish divided by the area of the frame) (Figure 2.2.C) by the number of algal cells per frame (Figure 2.2.D). The volume of sample settled for Sampling Trip 1 was 30 mL whereas the volume of sample settled for Sampling Trips 2 and 3 was 20 mL. The total algal colonies per 1 mL of sample were determined for both cyanobacteria and other phytoplankton taxa by dividing the total number of cells in the crystalizing dish by the volume of sample settled (Figure 2.2.E). This method of phytoplankton enumeration was adapted from Snow (2007).



Site	Cynobacteria	Phytoplankton	Total Counted	Total Frames
H2A0	60	140	200	60
H2A1	90	111	201	30
H2A5	119	84	203	44

Site	Cynobacteria	Phytoplankton	Total Frames	Cells/Frame	
				Cyanobacteria	Phytoplankton
H2A0	60	140	60	1,00	2,33
H2A1	90	111	30	3,00	3,70
H2A5	119	84	44	2,70	1,91

Site	Cells/Frame		Total number of cells in dish	
	Cyanobacteria	Phytoplankton	Cyanobacteria	Phytoplankton
H2A0	1,00	2,33	4288,799	10007,197
H2A1	3,00	3,70	12975,433	16003,034
H2A5	2,70	1,91	11634,804	8212,803

Site	Total number of cells in dish		Cells/mL	
	Cyanobacteria	Phytoplankton	Cyanobacteria	Phytoplankton
H2A0	4288,799	10007,197	142,960	333,573
H2A1	12975,433	16003,034	432,514	533,434
H2A5	11634,804	8212,803	387,827	273,760

Figure 2.2: A flow chart showing the method used to determine the number of cyanobacterial and phytoplankton cells per 1 mL of sample**.**

To determine chlorophyll *a* concentration, a known volume of water (500 mL) from the collected water samples was filtered through Whatman GF/C glass-fibre filters (diameter = 47 mm; pore size = 1.2µm) the day after water sample collection. The filter, with the filtered phytoplankton, was then folded and placed in 95% ethanol to extract the chlorophyll *a* pigment. The abovementioned filters, submerged in 95% ethanol, were stored in a fridge overnight. The concentration of chlorophyll *a* was measured the following day using a spectrophotometer (model number: UV-1100) according to the modified method described by Nusch (1980).

2.2.3. Total microcystin analysis

For the analysis of total microcystins in the water samples, an Enzyme-Linked Immunosorbent Assay (ELISA) was performed at the Health Sciences Campus, University of the Witwatersrand, under the supervision of Dr. M. Killick. ELISA is a non-specific analytical method. As there are over 90 microcystin isoforms that have been detected in freshwater systems worldwide (Oberholster *et al.*, 2005; Tonk *et al.*, 2007; Ballot *et al.*, 2014; Lone *et al.*, 2015), total combined microcystin (cell bound microcystin and released microcystin) in the water samples were measured using the

ELISA MC-ADDA method. This method is based on ADDA, a chemically cleaved amino acid side chain common to all microcystin variants (WHO, 2003; Birbeck *et al.*, 2019). This study therefore did not distinguish between different microcystin variants present in Hartbeespoort Dam during this study (Ballot *et al.*, 2014).

Before analysis, the cells were lysed to ensure total combined microcystin was measured. Due to *Microcystis* spp. being hardy organisms, samples of cyanobacteria were frozen and thawed three times to ensure cell lysis. ELISA was performed using the Abraxis-Microcystin ELISA kits following the protocol within the kits (Appendix B). The ELISA test plate was loaded onto the tray of the ELISA microplate photometer and the analysis of the tray was initiated by the analysis software (Model BioRad iMark plate reader). The ELISA microplate photometer quantified absorbance at 450 nm.

2.3. Trophic status determination

The mean values of orthophosphate concentration (µg/L), chlorophyll *a* concentration (µg/L), and Secchi disk depth (m) were used to determine the trophic status of Hartbeespoort Dam using the Carlson Modified Trophic State Index (CMTSI) (Quevedo-Castro *et al.*, 2019). The natural logarithm (ln) of the mean values was used in the equations. The following equations were used to determine the CMTSI of the Hartbeespoort Dam:

CMTSI for Orthophosphate (P) = [14.42 * ln (P)] + 4.15 (1)

CMTSI for Chlorophyll *a* (CA) = [9.81 * ln (CA)] + 30.6 (2)

CMTSI for Secchi disk depth (SD) = 60 – [14.4 * ln (SD)] (3)

Total CMTSI = [CMTSI(P) + CMTSI(CA) + CMTSI(SD)]/3 (4)

The trophic status of Hartbeespoort Dam was determined for each individual sampling trip and then for all three sampling trips.

2.4. Statistical analysis

Correlations between different datasets were determined using RStudio (Version 1.2.5033). All data were organised and inserted into Microsoft Excel (Windows 10) spreadsheets prior to being imported to RStudio for statistical analysis. Boxplots used to graphically represent datasets were created using RStudio (Version 1.2.5033). Unequal sample sizes were generated during this study due to the first sampling trip (10/04/2018) being cut short thus resulting in four of the six sampling

sites being sampled. Additionally, because of decreased rainfall as the study progressed from autumn to mid-winter, sampling depth intervals at sampling sites varied between the sampling trips. Analysis between different datasets was performed using the sampling trips, sampling sites, and sampling depth intervals outlined in Table 2.2.

Table 2.2: Sampling trips, sampling sites (H1 – H6), and sampling depth intervals (0 m – 25 m) used for the analysis of differences between datasets. Cells highlighted in grey indicate the following exclusions: analysis of *in situ* physico-chemical measurements did not include sample H5A0; analysis of microcystin concentration did not include sample H2A0, and analysis of phytoplankton community structure did not include sampling site 6 (H6).

Sampling Trips	Sampling Site	Sample Name	Sampling Depth Interval (m)
Sampling Trip 1	Site 2 (H2)	H2A0	0
		H2A1	1
		H2A5	5
	Site 3 (H3)	H3A0	0
		H3A1	1
	Site 4 (H4)	H4A0	0
		H4A1	1
		H4A5	5
		H4A10	10
	Site 5 (H5)	H5A0	0
		H5A1	1
		H5A5	5
		H5A10	10
		H5A15	15
Sampling Trip 2	Site 1 (H1)	H1B0	0
		H1B1	1
		H1B5	5
		H1B10	10
		H1B15	15
	Site 2 (H2)	H2B0	0
		H2B1	1
		H2B5	5
		H2B10	10
	Site 3 (H3)	H3B0	0
		H3B1	1
	Site 4 (H4)	H4B0	0
		H4B1	1
		H4B5	5
	Site 5 (H5)	H5B0	0
		H5B1	1

		H5B5	5
		H5B10	10
		H5B15	15
		H5B20	20
	Site 6 (H6)	H6B0	0
		H6B1	1
		H6B5	5
Sampling Trip 3	Site 1 (H1)	H1C0	0
		H1C1	1
		H1C5	5
		H1C10	10
		H1C15	15
		H1C20	20
		H1C25	25
	Site 2 (H2)	H2C0	0
		H2C1	1
		H2C5	5
	Site 3 (H3)	H3C0	0
		H3C1	1
	Site 4 (H4)	H4C0	0
		H4C1	1
		H4C5	5
		H4C10	10
	Site 5 (H5)	H5C0	0
		H5C1	1
		H5C5	5
		H5C10	10
	Site 6 (H6)	H6C0	0
		H6C1	1
		H6C5	5

In situ physico-chemical parameters, inorganic nutrient concentrations, Secchi disk depths (across sampling trips only), cyanobacteria cell densities, phytoplankton cell densities, chlorophyll *a* concentration, and microcystin concentration datasets were analysed to identify differences across the sampling trips, sampling sites, and sampling depths. Correlations between sampling trips, sampling sites, and sampling depth intervals were determined by:

- Organising data into three separate datasets based on sampling trip regardless of sampling site and sampling depth interval.
- Organising data into six separate datasets based on sampling site (H1 – H6) regardless of sampling trip and sampling depth interval.

- Organising data into five separate datasets based on sampling depth interval (0 m, 1 m, 5 m, 10 m, and 15 m – 25 m) regardless of sampling trip and sampling site. Sampling depth intervals 15 m, 20 m, and 25 m were collectively grouped into one sampling depth interval (15 m – 25 m).

An alpha value of $p = 0.05$ was selected for this study. The Shapiro-Wilks test was used to determine if the datasets were parametrically distributed. The null hypothesis of the Shapiro-Wilks test states that a variable is parametrically distributed within a population. For both parametric and non-parametric datasets, the Fligner-Killeen test for homogeneity of variances was used to assess the variances of more than two groups of data. The Fligner-Killeen test was chosen over Bartlett's test (used for parametric data) and Levene's test (used for non-parametric data) as it is robust against non-parametric data. The null hypothesis of the Fligner-Killeen's test states that the variances in populations are equal. The null hypotheses of the Shapiro-Wilks and Fligner-Killeen tests were therefore rejected if a p value < 0.05 was obtained.

In parametric datasets where homogeneity was obtained ($p > 0.05$), the analysis of variances (ANOVA) was used to determine differences in datasets between sampling trips, sampling sites, and sampling depth intervals. The null hypothesis of ANOVA states that the mean values are the same for all groups. The null hypothesis of ANOVA was therefore rejected if a p value < 0.05 was obtained thus indicating significant differences in the mean values obtained across the groups of different datasets. Where significance was obtained, a post-hoc Tukey test was performed to identify which specific groups were significantly different from the others.

In non-parametric datasets ($p < 0.05$) where homogeneity was obtained, the Kruskal-Wallis test (non-parametric ANOVA) was used to determine differences in datasets between sampling trips, sampling sites, and sampling depth intervals. The Kruskal-Wallis test was chosen as it is a hardy test against uneven sample sizes, as found in this study. The null hypothesis of the Kruskal-Wallis test states that the mean ranks are the same for all groups. The null hypothesis of the Kruskal-Wallis test was therefore rejected if a p value < 0.05 was obtained thus indicating a significant difference in the mean ranks across the groups of different datasets. Where significance was obtained, a post-hoc Dunn's test was performed to identify which

specific groups were significantly different from the others. P values were adjusted using the Bonferonni method to correct for any Type I errors.

In cases where either parametric or non-parametric datasets were found to be non-homogenous, data was logarithmically transformed [log(x+1)] prior to statistical analysis.

2.4.1. Multivariate analysis of ecological data

Aquatic ecosystems are complex systems consisting of many interlinked and interdependent habitats, species, and processes (DWAF, 1996: 5). Multivariate analysis of ecological data was conducted using RStudio (Version 1.2.5033) to investigate the variation of the phytoplankton community structure across a range of environmental conditions (Lepš and Šmilauer, 2003). Variations in community composition are often related to the differing demands of individual species for specific environmental conditions (Lepš and Šmilauer, 2003). Ordination methods are used to describe these community structure variations (Lepš and Šmilauer, 2003).

A redundancy constrained analysis (RDA) was used to identify the extent of which environmental variables (explanatory data) explained the distribution of species data (primary data consisting of response variables) in Hartbeespoort Dam during the study (Lepš and Šmilauer, 2003). Redundancy constrained analysis is a direct gradient analysis and was chosen for this study as RDA can identify the relationships between more than one response variables and explanatory variables (Lepš and Šmilauer, 2003). An RDA biplot ordination diagram was generated using RStudio (Version 1.2.5033). The ordination diagram was used to compare the suggested gradients with results obtained from statistical analyses and with independent knowledge of the environmental conditions in Hartbeespoort Dam during the study.

An RDA was performed using combined data for all sampling trips using data obtained at the six sampling sites and five sampling depth intervals. As the RDA was performed on a combination of the sampling trips, the results therefore reflected the conditions in Hartbeespoort Dam during the study period. The following environmental variables were included in the RDA ordination: water temperature, pH, electrical conductivity, dissolved oxygen, Secchi disk depth, dissolved inorganic nitrogen, dissolved inorganic phosphorus, the DIN:DIP ratio, and chlorophyll *a* concentration. Chlorophyll *a* concentration is not an environmental variable, however, it was included in the

analysis to determine the effect of environmental variables on total algal biomass (Janse van Vuuren *et al.*, 2007).

3. Results

3.1. Trophic Status of Hartbeespoort Dam

A CMTSI value of 70.5 was obtained for Sampling Trip 1 (mean orthophosphate = 511 µg/L; mean chlorophyll *a* concentration = 22.5 µg/L; and mean Secchi disk depth = 1.3 m) whereas a CMTSI value of 65.1 was obtained for Sampling Trip 2 (mean orthophosphate = 518 µg/L; mean chlorophyll *a* concentration = 9.6 µg/L; and mean Secchi disk depth = 2.3 m) (Table 3.1). Additionally, a CMTSI value of 67.7 was obtained for Sampling Trip 3 (mean orthophosphate = 562 µg/L; mean chlorophyll *a* concentration = 9.9 µg/L; and mean Secchi disk depth = 1.5 m) (Table 3.1).

Moreover, a CMTSI value of 67.7 was obtained for the entire study period at Hartbeespoort Dam (mean orthophosphate = 553 µg/L; mean chlorophyll *a* concentration = 12.7 µg/L; and mean Secchi disk depth = 1.7 m) (Table 3.1).

Table 3.1: The mean orthophosphate, chlorophyll *a* concentration, and Secchi disk depths used to calculate the CMTSI value for each individual sampling trip and for all three sampling trips. ST 1 to ST 3 refers to sampling trips 1 to 3, respectively. The trophic status relating to the obtained CMTSI value is outlined.

	Orthophosphate (µg/L)	Chlorophyll *a* concentration (µg/L)	Secchi disk depth (m)	CMTSI value	Trophic status
ST 1	511	22.5	1.3	**70.5**	Hypertrophic
ST 2	518	9.6	2.3	**65.1**	Eutrophic
ST 3	562	9.9	1.5	**67.7**	Eutrophic
All	553	12.7	1.7	**67.7**	Eutrophic

3.2. *In situ* physico-chemical parameters

3.2.1. Sampling trips: *in situ* physico-chemical parameters

As *in situ* pH and temperature datasets were non-parametric, a Kruskal-Wallis test was used to determine differences in mean ranks across the three sampling trips. *In situ* pH varied significantly in the dam across the three sampling trips ranging from a mean of 7.51 ± 0.03 for Sampling Trip 2 to a mean of 7.64 ± 0.05 for Sampling Trip 1 ($H = 10.016$, 2 df, $p < 0.05$) (Figure 3.1). Additionally, *in situ* temperature significantly decreased as the study progressed from autumn to mid-winter (Figure 3.1). *In situ*

temperature significantly decreased from a mean temperature of 22.34 ± 0.23ºC during Sampling Trip 1 to a mean temperature of 13.56 ± 0.11ºC during Sampling Trip 3 ($H = 42.873$, 2 df, $p < 0.05$). *In situ* pH in Hartbeespoort Dam ranged from 7.37 to 7.95 and *in situ* temperature in Hartbeespoort Dam ranged from 12.10ºC to 23.48ºC for the entire study.

Pairwise comparisons using Dunn's test indicated that Sampling Trip 2 was significantly different from Sampling Trip 3 in terms of *in situ* pH ($p < 0.05$) (Table 3.2; Figure 3.1). No other differences were statistically significant in terms of *in situ* pH. Pairwise comparisons using Dunn's test, however, indicated that all three sampling trips were significantly different from each other in terms of temperature ($p < 0.05$) (Table 3.2; Figure 3.1).

Table 3.2: Unadjusted and adjusted p values obtained from performing post hoc Dunn's Tests on *in situ* pH and temperature. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of *in situ* pH and temperature, where ST 1 to ST 3 refers to sampling trips 1 to 3, respectively.

	Unadjusted p value		**Adjusted p value**	
	pH	**Temperature**	**pH**	**Temperature**
ST 1 - ST 2	0.018	5.80E-04	0.053	1.74E-03
ST 1 - ST 3	0.928	9.00E-11	1.000	2.70E-10
ST 2 - ST 3	0.004	3.44E-04	0.011	1.03E-03

A Shapiro-Wilk's test for normality indicated that the *in situ* dissolved oxygen dataset met the assumption of normality ($p = 0.338$). An ANOVA was therefore used to determine differences in means across the three sampling trips. Mean *in situ* dissolved oxygen significantly ranged from 2.37 ± 0.29 mg/L for Sampling Trip 2 to 3.97 ± 0.64 mg/L for Sampling Trip 1 [$F(2, 56) = 4.037$, $p < 0.05$] (Figure 3.1). *In situ* dissolved oxygen in Hartbeespoort Dam ranged from 0 mg/L to 7.40 mg/L for the entire study. Pairwise comparisons using a post hoc Tukey test indicated that *in situ* dissolved oxygen for Sampling Trip 2 was significantly lower than Sampling Trip 1 ($p < 0.05$) (Table 3.3; Figure 3.1). No other differences were statistically significant.

Table 3.3: Adjusted p values obtained from performing a post hoc Tukey test on *in situ* dissolved oxygen. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of *in situ* dissolved oxygen, where ST 1 to ST 3 refers to sampling trips 1 to 3, respectively.

Dissolved oxygen concentration (mg/L)	
	Adjusted p value
ST 2 - ST 1	0.018
ST 3 - ST 1	0.315
ST 3 - ST 2	0.263

Additionally, mean *in situ* electrical conductivity decreased from 54.61 ± 1.18 mS/m for Sampling Trip 1 to 15.87 ± 0.05 mS/m for Sampling Trip 3 (Figure 3.1). *In situ* electrical conductivity in Hartbeespoort Dam ranged from 15.66 mS/m to 59.70 mS/m for the entire study. *In situ* electrical conductivity did not meet the assumption of normality nor homogeneity despite having been logarithmically transformed.

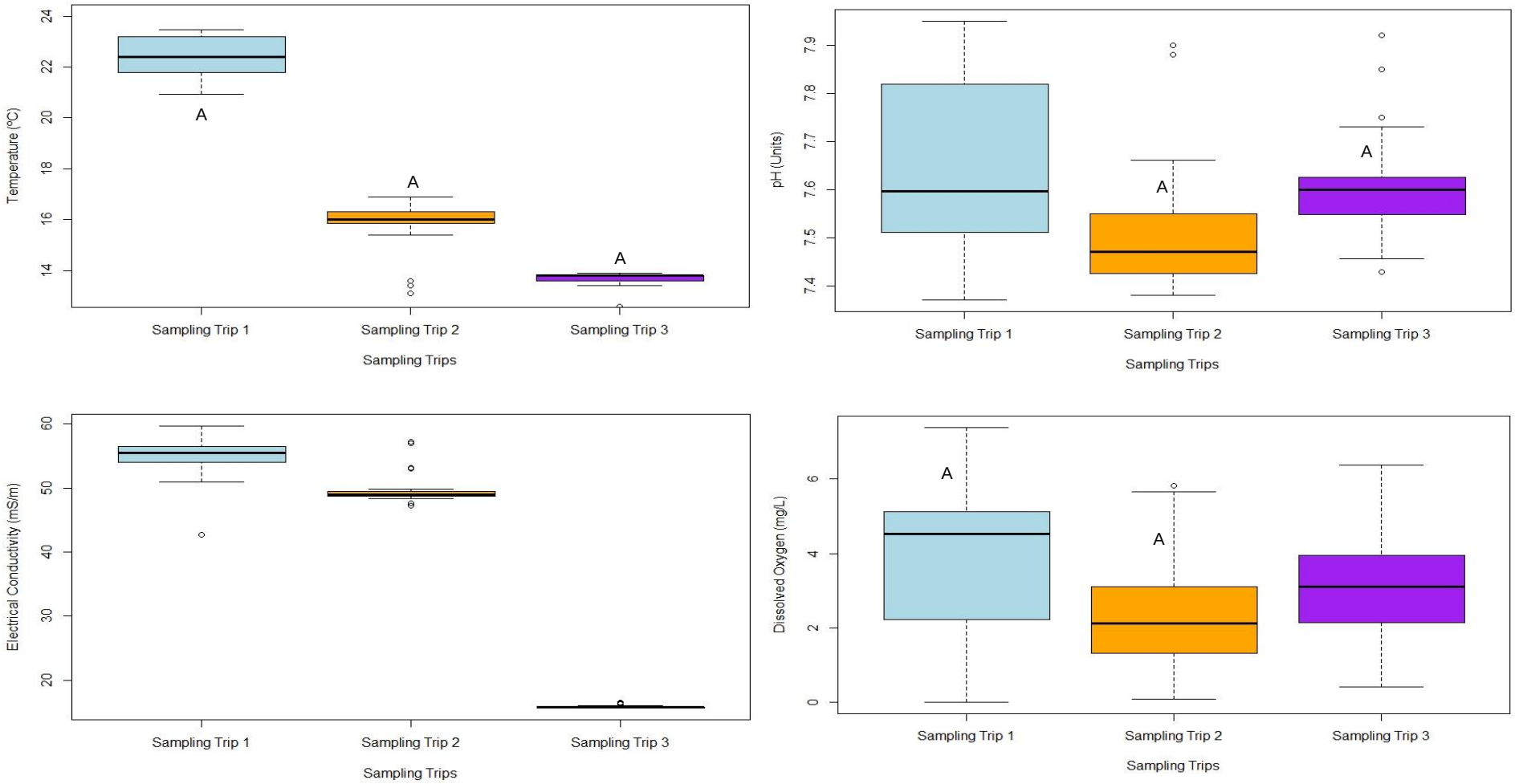


Figure 3.1: Boxplots of *in situ* temperature (ºC), pH (Units), electrical conductivity (mS/m), and dissolved oxygen (mg/L) in the dam for each sampling trip. Significant differences between sampling trips are indicated by the letter A. *In situ* electrical conductivity did not meet the assumption of normality nor homogeneity despite having been logarithmically transformed.

3.2.2. Sampling sites: *in situ* physico-chemical parameters

As *in situ* temperature, pH, and electrical conductivity datasets were non-parametric, a Kruskal-Wallis test was used to determine differences in mean ranks across the six sampling sites. An ANOVA was, however, used to determine differences in means across the six sampling sites in terms of *in situ* dissolved oxygen as the dataset was parametric.

In situ temperature did not differ significantly between the six sampling sites during the study period ($H = 11.036$, 5 df, $p = 0.05$) (Figure 3.2). Mean *in situ* temperature ranged from 14.55 ± 0.37°C at Site H1 to 17.59 ± 1.15°C at Site H4. *In situ* pH, however, varied significantly between the six sampling sites ranging from a mean pH of 7.45 ± 0.02 at Site H1 to a mean pH of 7.87 ± 0.01 at Site H3 ($H = 29.603$, 5 df, $p < 0.05$) (Figure 3.2). Pairwise comparisons using Dunn's test indicated *in situ* pH readings obtained at Site H1 were significantly lower than Sites H2, H3, H4, and H6 whereas *in situ* pH readings obtained at Site H3 were significantly higher than Sites H1, H2, H4, and H5 ($p < 0.05$) (Table 3.4; Figure 3.2). Additionally, Site H5 was significantly lower than Sites H2 and H6 in terms of *in situ* pH ($p < 0.05$) (Table 3.4; Figure 3.2). No other differences were statistically significant.

Both *in situ* electrical conductivity and dissolved oxygen differed significantly between the six sampling sites during the study period. Mean *in situ* electrical conductivity ranged from 29.60 ± 4.71 mS/m at Site H1 to 44.39 ± 8.06 mS/m at Site H3 ($H = 15.692$, 5 df, $p < 0.05$) (Figure 3.2). Additionally, mean *in situ* dissolved oxygen ranged from 1.59 ± 0.12 mg/L at Site H1 to 4.87 ± 0.71 mg/L at Site H3 [$F (5, 53) = 5.171$, $p < 0.05$] (Figure 3.2). Pairwise comparisons using Dunn's test indicated *in situ* electrical conductivity at Site H1 was significantly lower than Site H3 ($p < 0.05$) (Table 3.4; Figure 3.2). Additionally, pairwise comparisons using a post hoc Tukey test indicated that *in situ* dissolved oxygen at Site H1 was significantly lower than Sites 2 and 3, and Site H3 was significantly higher than Site H5 ($p < 0.05$) (Table 3.5; Figure 3.2). No other differences were statistically significant.

Table 3.4: Unadjusted and adjusted p values obtained from performing post hoc Dunn's Tests on *in situ* pH and electrical conductivity. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of *in situ* pH and electrical conductivity. H1 to H6 refers to sampling sites 1 to 6, respectively.

	Unadjusted p value		**Adjusted p value**	
	pH	**Electrical conductivity (mS/m)**	**pH**	**Electrical conductivity (mS/m)**
H1 - H2	1.62E-03	0.019	2.43E-02	0.284
H1 - H3	1.44E-06	0.002	2.17E-05	0.028
H2 - H3	4.01E-02	0.287	6.02E-01	1.000
H1 - H4	3.80E-02	0.033	5.70E-01	0.492
H2 - H4	2.68301e	0.794	1.00E+00	1.000
H3 - H4	2.36E-03	0.191	3.54E-02	1.000
H1 - H5	1.59E-01	0.500	1.00E+00	0.978
H2 - H5	5.47E-02	0.089	8.21E-01	1.000
H3 - H5	1.44E-04	0.681	2.15E-03	1.000
H4 - H5	4.39E-01	0.782	1.00E+00	1.000
H1 - H6	1.93E-03	0.782	2.89E-02	1.000

H2 - H6	6.97E-01	0.027	1.00E+00	0.403
H3 - H6	1.37E-01	0.003	1.00E+00	0.050
H4 - H6	1.78E-01	0.043	1.00E+00	0.639
H5 - H6	4.12E-02	0.077	6.18E-01	1.000

Table 3.5: Adjusted p values obtained from performing a post hoc Tukey test on *in situ* dissolved oxygen. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of *in situ* dissolved oxygen. H1 to H6 refers to sampling sites 1 to 6, respectively.

Dissolved oxygen concentration (mg/L)	
	Adjusted p value
H2 - H1	0.006
H3 - H1	0.001
H4 - H1	0.182
H5 - H1	0.477
H6 - H1	0.219
H3 - H2	0.845
H4 - H2	0.722
H5 - H2	0.265
H6 - H2	0.944
H4 - H3	0.171
H5 - H3	0.035
H6 - H3	0.435
H5 - H4	0.980
H6 - H4	1.000
H6 - H5	0.947

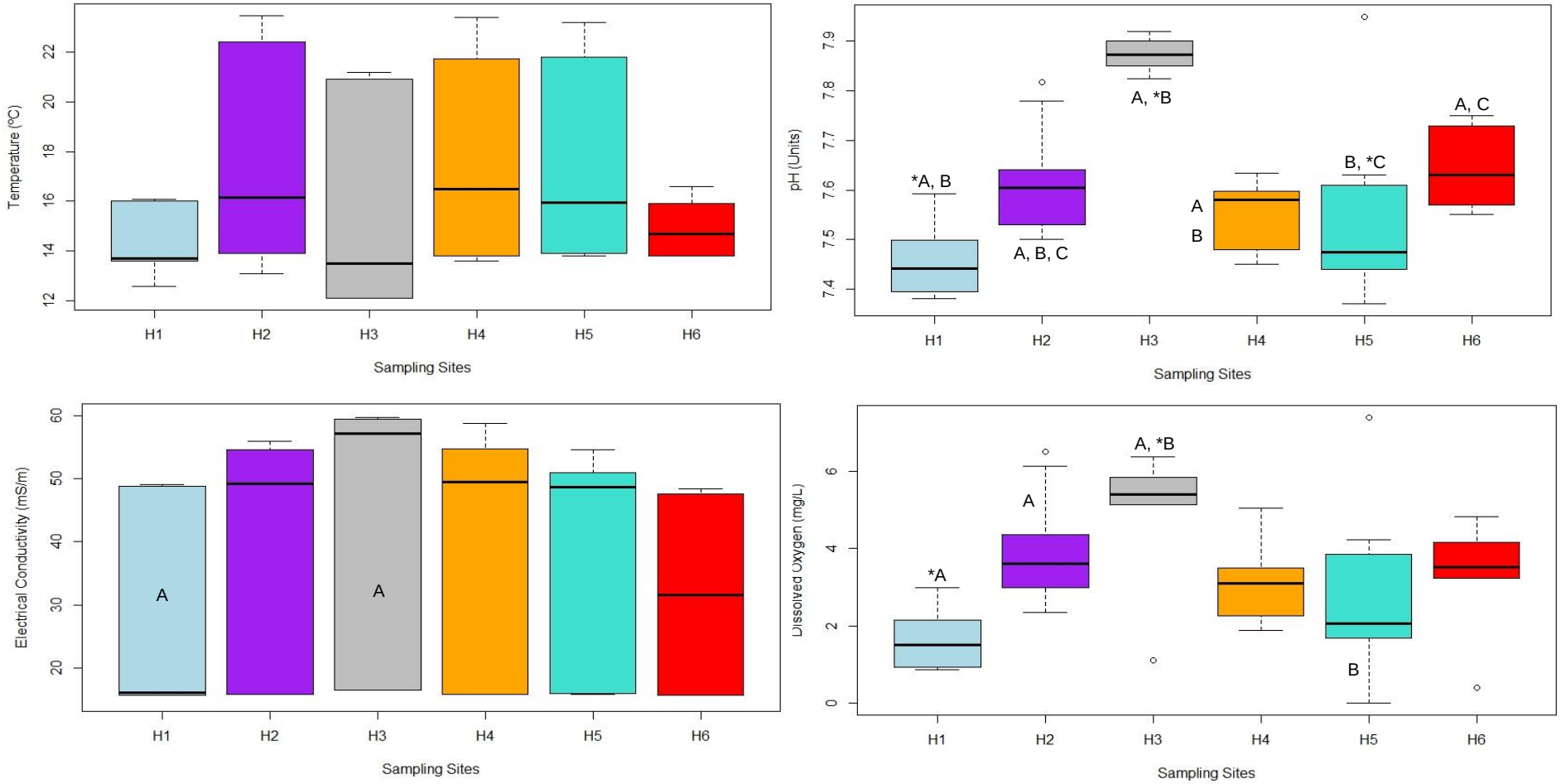


Figure 3.2: Boxplots of *in situ* temperature (ºC), pH (Units), electrical conductivity (mS/m), and dissolved oxygen (mg/L) between the six sampling sites (H1 – H6), across all sampling depth intervals, during the study period. Significant differences between sampling sites are indicated by the letters A - C. Starred letters indicate the sampling site from which sites with corresponding letters significantly differ i.e., sites H2, H3, H4 and H6 (A) differ significantly from site H1 (*A) in terms of *in situ* pH.

3.2.3. Sampling depths: *in situ* physico-chemical parameters

A Kruskal-Wallis test was used to determine differences in mean ranks across the five sampling depth intervals as both *in situ* temperature and electrical conductivity were non-parametric. Both *in situ* temperature and electrical conductivity did not differ significantly between the five sampling depth intervals. Mean *in situ* temperature ranged from 15.51 ± 1.07ºC (15 – 25 m depth interval) to 16.62 ± 0.91ºC (1 m depth interval) (H = 2.525, 4 df, p = 0.64) (Figure 3.3). Additionally, mean *in situ* electrical conductivity ranged from 35.56 ± 6.44 mS/m (15 – 25 m depth interval) to 38.68 ± 6.32 mS/m (10 m depth interval) (H = 1.031, 4 df, p = 0.91) (Figure 3.3).

In situ pH was logarithmically transformed to meet the assumptions of homogeneity. A Kruskal-Wallis test yielded significant differences in terms of *in situ* pH from the 0 m depth interval to the bottom of the dam across the study. Mean *in situ* pH across the sampling depth intervals ranged from 7.47 ± 0.03 (15 - 25 m depth interval) to 7.65 ± 0.04 (1 m depth interval) (H = 11.427, df = 4, $p < 0.05$) (Figure 3.3). Pairwise comparisons using Dunn's test was performed and the P values were adjusted using the Bonferroni method to correct for any Type I errors. Despite the Kruskal-Wallis test indicating significant differences between the five sampling depth intervals in terms of *in situ* pH, adjusted P values indicated that there were no statistically significant differences between the sampling depth intervals.

As *in situ* dissolved oxygen was parametrically distributed, an ANOVA was used to determine differences in means across the five sampling depth intervals. *In situ* dissolved oxygen varied significantly across the five sampling depth intervals. Mean *in situ* dissolved oxygen ranged from 1.52 ± 0.41 mg/L (15 – 25 m depth interval) to 4.06 ± 0.45 mg/L (1 m depth interval) [$F(4, 54) = 4.67$, $p < 0.05$] (Figure 3.3). Pairwise comparisons using a post hoc Tukey test indicated that *in situ* dissolved oxygen at the 1 m depth interval was significantly higher than the 15 – 25 m depth interval ($p < 0.05$) (Table 3.6; Figure 3.3). No other differences were statistically significant.

Table 3.6: Adjusted p values obtained from performing a post hoc Tukey test on *in situ* dissolved oxygen. Blue-highlighted cells indicate sampling depth intervals (SD) that were significantly different from each other in terms of *in situ* dissolved oxygen. SD 1 refers to the 0 m depth interval, SD 2 refers to the 1 m depth interval, SD 3 refers to the 5 m depth interval, SD 4 refers to the 10 m depth interval, and SD 5 refers to the 15 m – 25 m depth interval.

Dissolved oxygen concentration (mg/L)	
	Adjusted p value
SD 2 - SD 1	0.832
SD 3 - SD 1	0.423
SD 4 - SD 1	0.412
SD 5 -SD 1	0.056
SD 3 - SD 2	0.057
SD 4 - SD 2	0.077
SD 5 - SD 2	0.005
SD 4 - SD 3	0.999
SD 5 - SD 3	0.685
SD 5 - SD 4	0.865

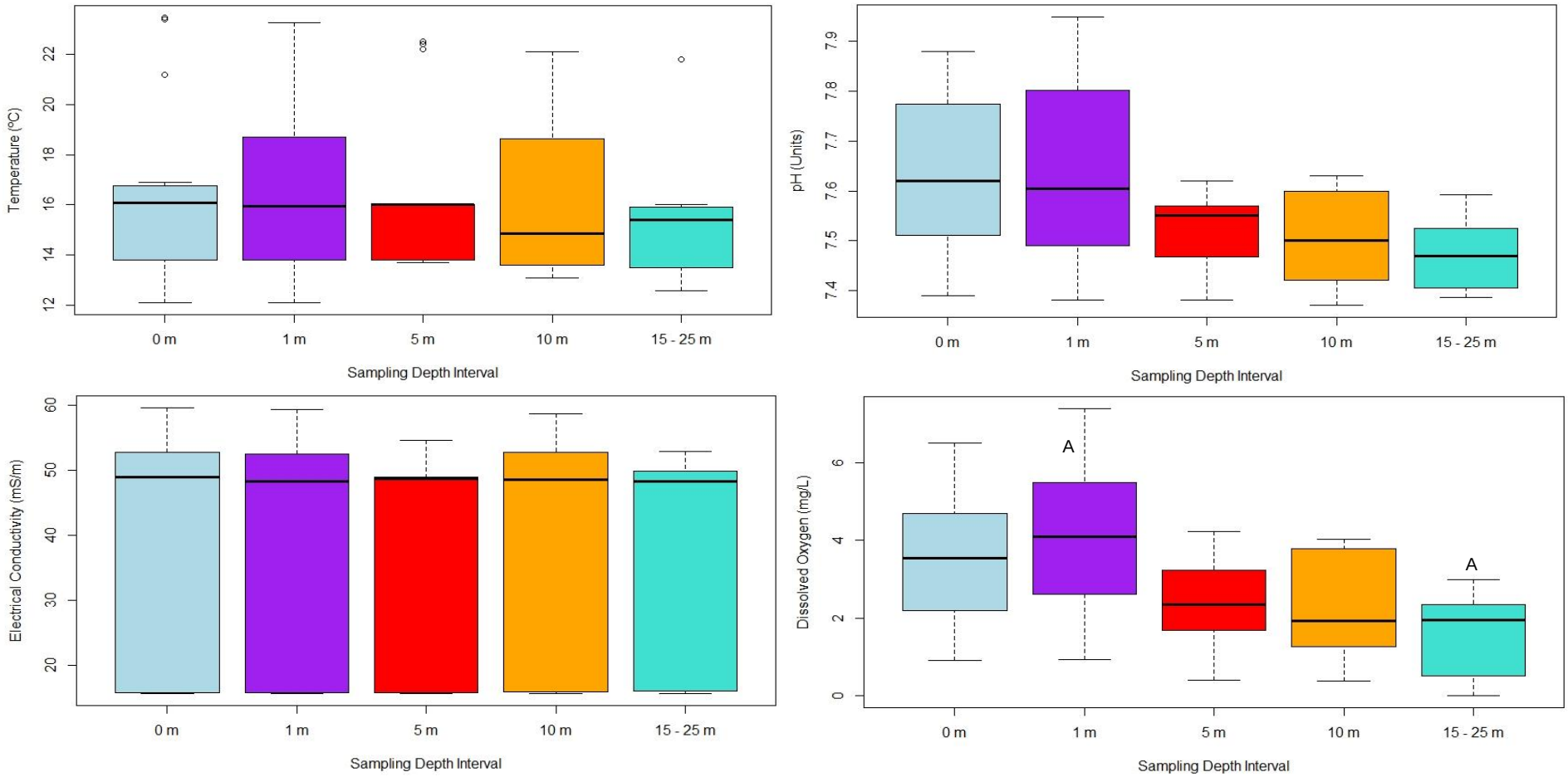


Figure 3.3: Boxplots of *in situ* temperature (ºC), pH (Units), electrical conductivity (mS/m), and dissolved oxygen (mg/L) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m and 15 – 25 m), across all sampling sites, during the study period. Significant differences exist between 1 m (A) and 15 – 25 m (A) in terms of *in situ* dissolved oxygen. No other significant differences exist.

3.3. Inorganic nutrients

As inorganic nutrient concentrations were non-parametrically distributed across the dam during the study, a Kruskal-Wallis test was used to determine the differences in mean ranks across the three sampling trips, six sampling sites, and five sampling depth intervals.

3.3.1. Sampling trips: inorganic nutrients

All four inorganic nutrient concentrations significantly increased in Hartbeespoort Dam as the study progressed from autumn to mid-winter. Across the three different sampling trips, mean orthophosphate concentrations ranged from 0.511 ± 0.02 mg/L to 0.562 ± 0.03 mg/L ($H = 8.148$, 2 df, $p < 0.05$) (Figure 3.4) and mean ammonium concentrations ranged from 1.363 ± 0.12 mg/L to 2.033 ± 0.44 mg/L ($H = 8.687$, 2 df, $p < 0.05$) (Figure 3.4). Orthophosphate concentrations across Hartbeespoort Dam ranged from 0.323 mg/L to 0.959 mg/L whereas ammonium concentrations ranged from 0.671 mg/L to 8.713 mg/L during the sampling period.

Pairwise comparisons using Dunn's test indicated that the orthophosphate concentration of Sampling Trip 2 was significantly lower than orthophosphate concentration of Sampling Trip 3 ($p < 0.05$) (Table 3.7; Figure 3.4) and that the ammonium concentration of Sampling Trip 1 was significantly lower than ammonium concentration of Sampling Trip 3 ($p < 0.05$) (Table 3.7; Figure 3.4). No other differences were statistically significant.

Across the three different sampling trips, mean nitrate + nitrite concentrations ranged from 2.773 ± 0.5 mg/L to 4.201 ± 0.13 mg/L ($H = 21.389$, 2 df, $p < 0.05$) (Figure 3.4) and mean DIN concentrations ranged from 4.136 ± 0.66 mg/L to 6.234 ± 0.5 mg/L ($H = 19.652$, 2 df, $p < 0.05$) (Figure 3.4). Nitrate + nitrite concentrations across Hartbeespoort Dam ranged from 0.887 mg/L to 7.201 mg/L whereas DIN concentrations ranged from 1.842 mg/L to 13.781 mg/L during the sampling period.

Pairwise comparisons using Dunn's test indicated that all three sampling trips were significantly different from each other in terms of nitrate + nitrite concentrations ($p < 0.05$) (Table 3.7; Figure 3.4) and that the DIN concentration of Sampling Trip 3 was significantly higher than the DIN concentration of Sampling Trips 1 and 2 ($p < 0.05$) (Table 3.7; Figure 3.4). Statistically significant differences between DIN concentrations of Sampling Trips 1 and 2 were not observed.

Table 3.7: Unadjusted and adjusted p values obtained from performing post hoc Dunn's Tests on orthophosphate, ammonium, nitrate + nitrite, and DIN concentrations. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of inorganic nutrient concentrations, where ST 1 to ST 3 refers to sampling trips 1 to 3, respectively.

	Unadjusted p value				**Adjusted p value**			
	Orthophosphate	**Ammonium**	**Nitrate + Nitrite**	**DIN**	**Orthophosphate**	**Ammonium**	**Nitrate + Nitrite**	**DIN**
ST 1 - ST 2	0.434	0.454	2.48E-01	1.89E-01	1.000	1.000	7.43E-01	0.568
ST 1 - ST 3	0.096	0.007	2.37E-05	3.58E-05	0.288	0.021	7.12E-05	0.001
ST 2 - ST 3	0.005	0.025	4.17E-04	1.19E-03	0.015	0.075	1.25E-03	0.004

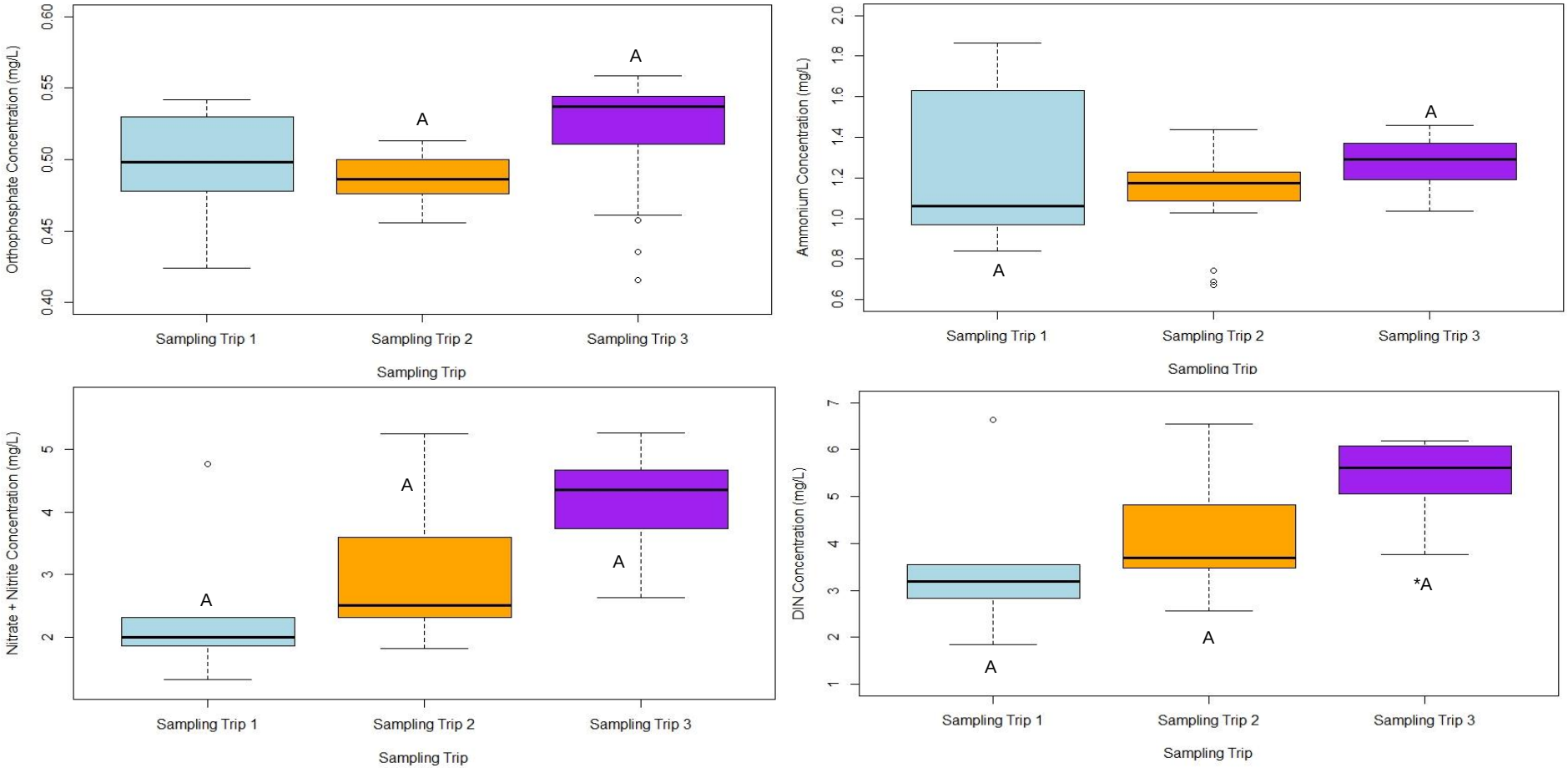


Figure 3.4: Boxplots of orthophosphate, ammonium, nitrate + nitrite, and dissolved inorganic nitrogen (DIN) concentrations (mg/L) in the dam for each sampling trip. All four inorganic nutrient concentrations increased in the dam as the study progressed from autumn to mid-winter. For orthophosphate, ammonium, and nitrate + nitrite concentrations, significant differences between sampling trips are indicated by the letter A. For DIN concentration, sampling trip 3 (*A) was significantly different from sampling trips 1 and 2 (A).

3.3.2. Sampling sites: inorganic nutrients

Ammonium concentrations were logarithmically transformed prior to statistical analysis to meet the assumption of homogeneity. Concentrations of orthophosphates, ammonium, nitrate + nitrite, and DIN significantly differed across the dam between the six sampling sites. Mean orthophosphate concentrations across the sampling sites ranged from 0.390 ± 0.02 mg/L to 0.835 ± 0.06 mg/L ($H = 27.426$, 5 df, $p < 0.05$) (Figure 3.5) and mean ammonium concentrations across the sampling sites ranged from 0.933 ± 0.1 mg/L to 6.319 ± 1.05 mg/L ($H = 20.329$, 5 df, $p < 0.05$) (Figure 3.5). For both orthophosphate and ammonium concentrations, the lowest mean concentrations were obtained at Site H6, located near to the Magalies River entrance, whereas the highest mean concentrations were obtained at Site H3, within the Crocodile River.

Pairwise comparisons using Dunn's test indicated that Sites H3 and H6 were significantly different from each other in terms of orthophosphate concentration ($p < 0.05$) (Table 3.8; Figure 3.5). Additionally, pairwise comparisons using Dunn's test indicated that Sites H3 and H6 were significantly different from the remaining four sampling sites in terms of orthophosphate concentration ($p < 0.05$) (Table 3.8; Figure 3.5). Furthermore, pairwise comparisons using Dunn's test indicated that Site H3 was significantly higher than Sites H4, H5, and H6 in terms of ammonium concentration ($p < 0.05$) (Table 3.8; Figure 3.5). No other statistically significant concentration differences were observed.

Mean nitrate + nitrite concentrations across the dam ranged from 2.622 ± 0.24 mg/L to 5.459 ± 0.46 mg/L ($H = 18.942$, 5 df, $p < 0.05$) (Figure 3.5) and mean DIN concentrations ranged from 3.841 ± 0.29 mg/L to 11.778 ± 0.69 mg/L ($H = 21.445$, 5 df, $p < 0.05$) (Figure 3.5). For both nitrate + nitrite and DIN, the lowest mean concentrations were obtained at Site H2 whereas the highest mean concentrations were obtained at Site H3 (Figure 3.5). Pairwise comparisons using Dunn's test indicated that concentrations at Site H3 were significantly higher than concentrations at Sites H2, H5, and H6 in terms of nitrate + nitrite and DIN ($p < 0.05$) (Table 3.8; Figure 3.5). No other statistically significant concentration differences were observed.

Table 3.8: Unadjusted and adjusted p values obtained from performing post hoc Dunn's Tests on orthophosphate, ammonium, nitrate + nitrite, and DIN concentrations. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of inorganic nutrient concentrations. H1 to H6 refers to sampling sites 1 to 6, respectively.

	Unadjusted p value				**Adjusted p value**			
	Orthophosphate	**Ammonium**	**Nitrate + Nitrite**	**DIN**	**Orthophosphate**	**Ammonium**	**Nitrate + Nitrite**	**DIN**
H1 - H2	8.760E-01	9.095E-01	2.60E-02	6.27E-02	1.000E+00	1.000	0.389	0.941
H1 - H3	1.982E-03	3.391E-01	3.25E-02	8.21E-03	2.972E-02	0.051	0.488	0.123
H2 - H3	4.165E-03	3.378E-01	8.99E-05	4.08E-05	6.248E-02	0.051	0.001	0.001
H1 - H4	5.675E-01	9.181E-01	8.59E-01	7.59E-01	1.000E+00	1.000	1.000	1.000
H2 - H4	6.941E-01	9.895E-01	4.42E-02	1.26E-01	1.000E+00	1.000	0.663	1.000
H3 - H4	9.969E-03	2.968E-01	2.43E-02	4.28E-03	1.495E-01	0.045	0.365	0.064
H1 - H5	7.712E-01	4.643E-01	1.54E-01	2.28E-01	1.000E+00	1.000	1.000	1.000
H2 - H5	6.602E-01	5.652E-01	3.26E-01	4.19E-01	1.000E+00	1.000	1.000	1.000
H3 – H5	5.931E-04	2.952E-01	7.88E-04	2.14E-04	8.897E-03	0.004	0.012	0.003
H4 - H5	3.762E-01	5.446E-01	2.28E-01	3.94E-01	1.000E+00	1.000	1.000	1.000
H1 - H6	4.185E-03	4.019E-01	1.72E-01	9.49E-02	6.278E-02	0.603	1.000	1.000
H2 - H6	3.704E-03	5.843E-01	6.00E-01	9.41E-01	5.556E-02	0.877	1.000	1.000

H3 - H6	2.486E-07	1.601E-01	2.42E-03	1.87E-04	3.729E-06	0.0002	0.036	0.003
H4 - H6	9.959E-04	5.277E-01	2.31E-01	1.64E-01	1.494E-02	0.792	1.000	1.000
H5 - H6	6.311E-03	1.243E-01	7.88E-01	4.46E-01	9.466E-02	1.000	1.000	1.000

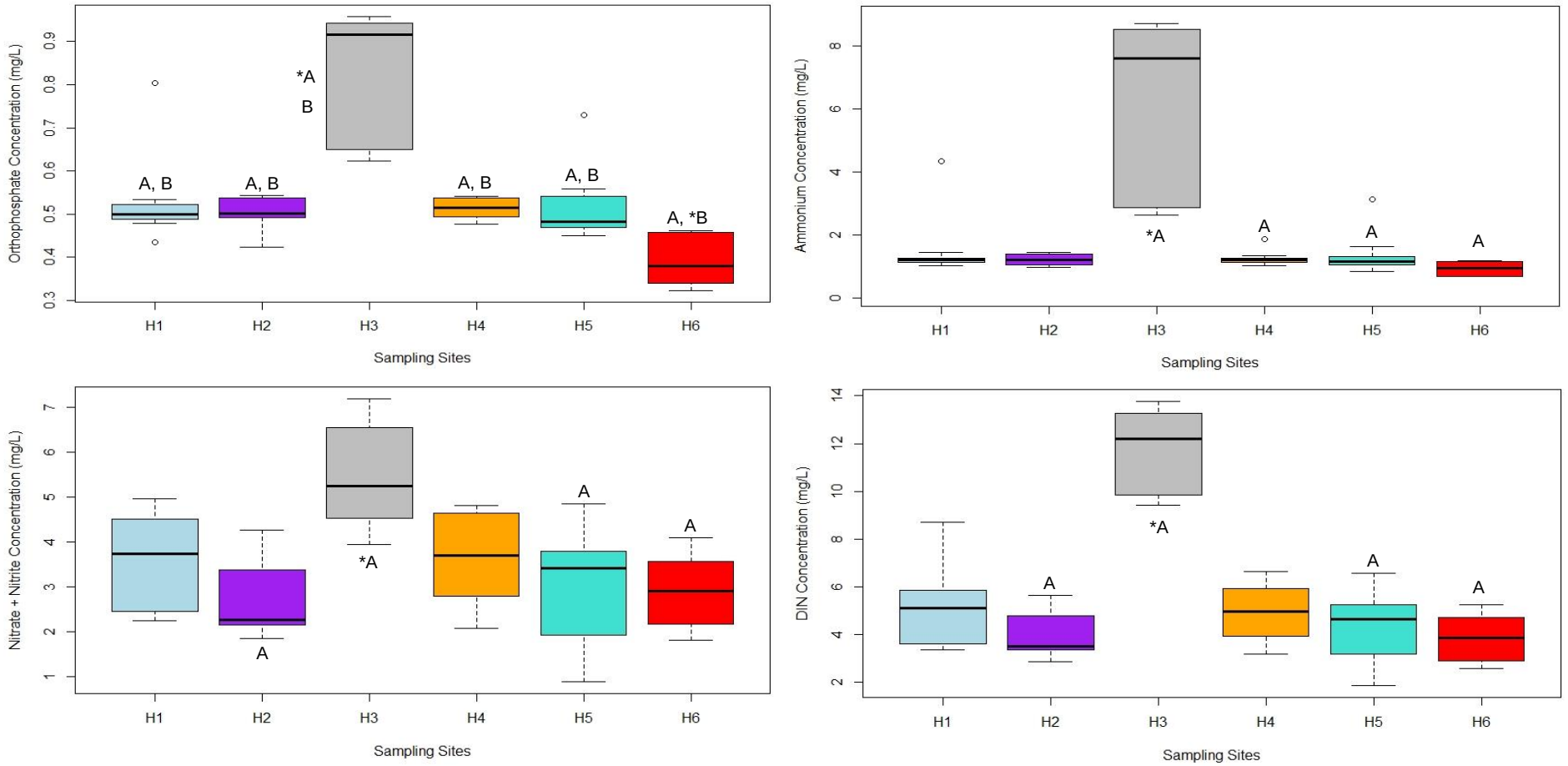


Figure 3.5: Boxplots of orthophosphate, ammonium, nitrate + nitrite, and dissolved inorganic nitrogen (DIN) concentrations (mg/L) between the six sampling sites (H1 – H6) and across all sampling depth intervals, during the study period. Significant differences between sampling sites are indicated by the letters A and B. Starred letters indicate the sampling site from which sites with corresponding letters significantly differ i.e., sites H1, H2, H4, H5 and H6 (A) differ significantly from site H3 (*A) in terms of orthophosphate concentration.

3.3.3. Sampling depths: inorganic nutrients

Concentrations of all four inorganic nutrients did not differ significantly in the water column from 0 m to the bottom of the dam during the study. Across the sampling depth intervals, mean orthophosphate concentrations ranged from 0.488 ± 0.01 mg/L to 0.564 ± 0.05 mg/L (H = 0.344, 4 df, p = 0.987) (Figure 3.6) and the mean ammonium concentrations ranged from 1.137 ± 0.05 mg/L to 2.098 ± 0.59 mg/L (H = 4.68, 4 df, p = 0.322) (Figure 3.6). Both orthophosphate and ammonium concentrations reached a minimum at the 5 m depth interval. Orthophosphate concentrations reached a maximum at the 15 – 25 m depth interval whereas ammonium concentrations reached a maximum at the 1 m depth interval.

Additionally, across the sampling depth intervals, mean nitrate + nitrite concentrations ranged from 3.265 ± 0.27 mg/L to 3.58 ± 0.34 mg/L (H = 0.354, df = 4, p = 0.986) (Figure 3.6) and mean DIN concentrations ranged from 4.403 ± 0.3 mg/L to 5.657 ± 0.78 mg/L (H = 1.413, 4 df, p = 0.842) (Figure 3.6). Both nitrate + nitrite and DIN concentrations reached a minimum at the 5 m depth interval and a maximum at the 0 m depth interval.

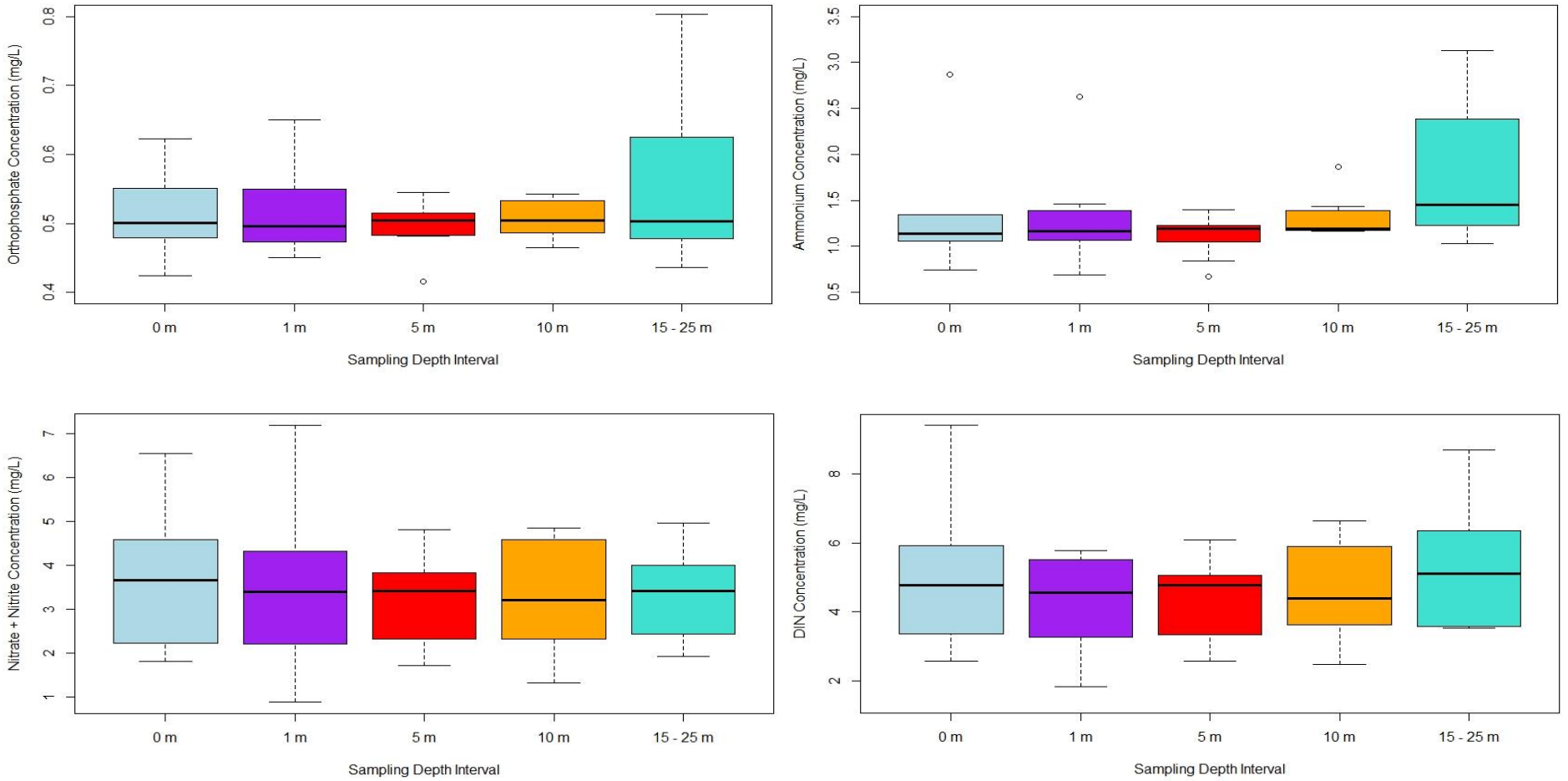


Figure 3.6: Boxplots of orthophosphate, ammonium, nitrate + nitrite, and dissolved inorganic nitrogen (DIN) concentrations (mg/L) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m, and 15 – 25 m) and across all sampling sites, during the study period. No significant differences exist between sampling depth intervals in terms of inorganic nutrient concentrations.

3.4. Water clarity: Secchi disk depth

3.4.1. Sampling trips: Secchi disk depth

A Shapiro-Wilk's test for normality indicated that the Secchi disk depth dataset met the assumption of normality ($p = 0.315$). An ANOVA did not, however, yield significant variations in Secchi disk depths across the three sampling trips. Mean Secchi disk depth ranged from 1.31 ± 0.21 m for Sampling Trip 1 to 2.26 ± 0.3 m for Sampling Trip 2 [$F(2,13) = 2.819$, $p = 0.1$] (Figure 3.7). Secchi disk depths in Hartbeespoort Dam ranged from 0.685 m to 3.208 m during the study.

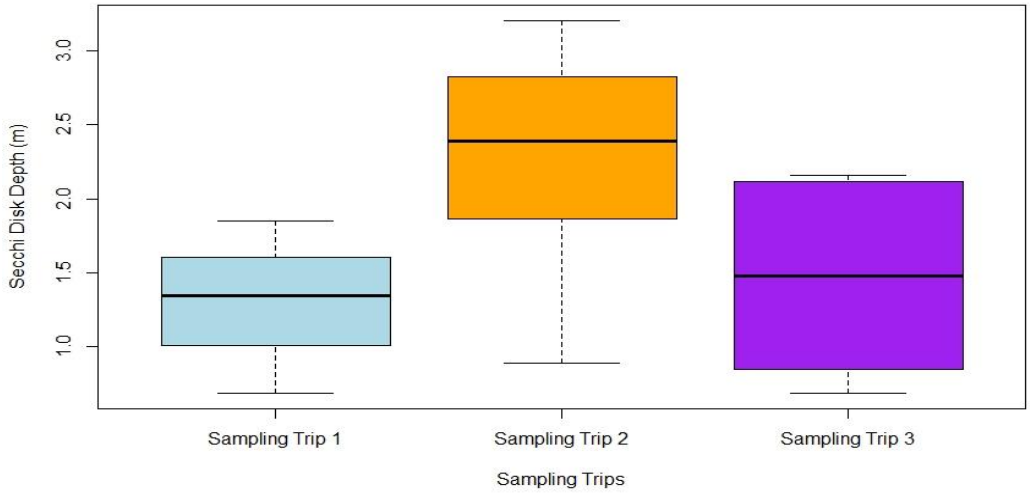


Figure 3.7: Boxplots of Secchi disk depths (m) obtained during each sampling trip. No significant differences exist between sampling trips in terms of Secchi disk depth (m).

3.5. Phytoplankton community composition: cyanobacteria and phytoplankton cell densities

Both cyanobacteria and phytoplankton cell density datasets were logarithmically transformed to correct for non-homogeneity. Where homogeneity was obtained, a Kruskal-Wallis test was performed to determine differences in mean ranks of cyanobacteria and phytoplankton cell density datasets across the three sampling trips, six sampling sites, and five sampling depth intervals as both cyanobacteria and phytoplankton cell density datasets were non-parametric.

3.5.1. Sampling trips: cyanobacteria and phytoplankton cell densities

Both cyanobacteria and phytoplankton cell densities varied significantly across the dam during the study. Mean cyanobacteria cell density ranged from 273 ± 114.9 cells/mL for Sampling Trip 2 to 902 ± 139.57 cells/mL for Sampling Trip 3 ($H = 21.461$, 2 df, $p < 0.05$) (Figure 3.8). Mean phytoplankton cell density ranged from

34 ± 21.16 cells/mL for Sampling Trip 2 to 294 ± 57.84 cells/mL for Sampling Trip 1 ($H = 21.129$, 2 df, $p < 0.05$) (Figure 3.9). Cyanobacteria cell densities in Hartbeespoort Dam ranged from 26 cells/mL to 2 363 cells/mL and phytoplankton cell densities in Hartbeespoort Dam ranged from 0 cells/mL to 745 cells/mL during the study.

Pairwise comparisons using Dunn's test indicated that Sampling Trip 2 was significantly lower than Sampling Trips 1 and 3 in terms of cyanobacteria cell densities ($p < 0.05$) (Table 3.9; Figure 3.8). Pairwise comparisons using Dunn's test, however, indicated that all three sampling trips differed significantly in terms of phytoplankton cell density (Table 3.9). Sampling Trip 1 was significantly higher than Sampling Trips 2 and 3 whereas Sampling Trip 3 was significantly higher than Sampling Trip 2 in terms of phytoplankton cell densities ($p < 0.05$) (Table 3.9; Figure 3.9).

Table 3.9: Unadjusted and adjusted p values obtained from performing post hoc Dunn's Tests on cyanobacteria and phytoplankton cell densities. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of cyanobacteria and phytoplankton cell densities, where ST 1 to ST 3 refers to sampling trips 1 to 3, respectively.

	Unadjusted p value		**Adjusted p value**	
	Cyanobacteria	**Phytoplankton**	**Cyanobacteria**	**Phytoplankton**
ST 1 - ST 2	1.83E-03	4.83E-06	5.50E-03	1.45E-05
ST 1 - ST 3	3.98E-01	1.71E-02	1.00E+00	5.12E-02
ST 2 - ST 3	9.92E-06	1.87E-02	2.97E-05	5.62E-02

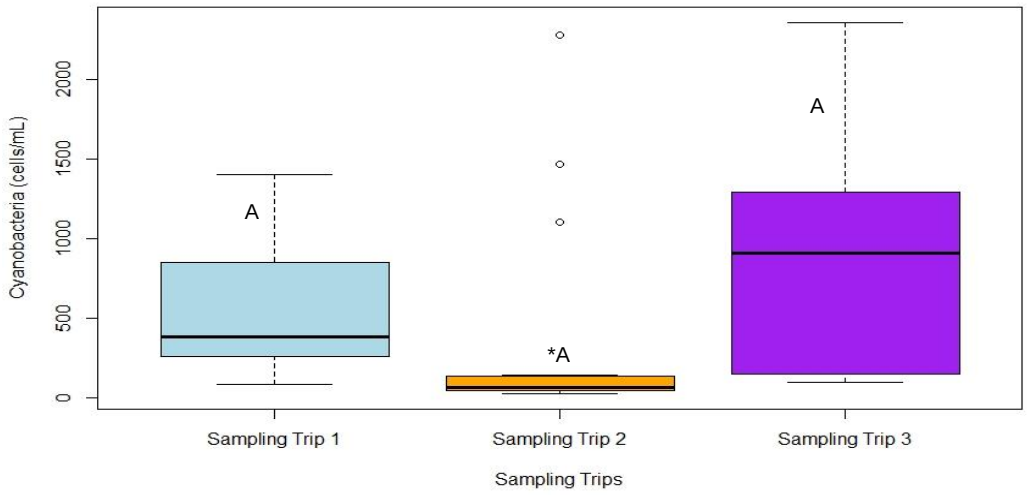


Figure 3.8: Boxplots of cyanobacteria cell density (cells/mL) in the dam for each sampling trip. For cyanobacteria cell density, sampling trip 2 (*A) was significantly different from sampling trips 1 and 3 (A).

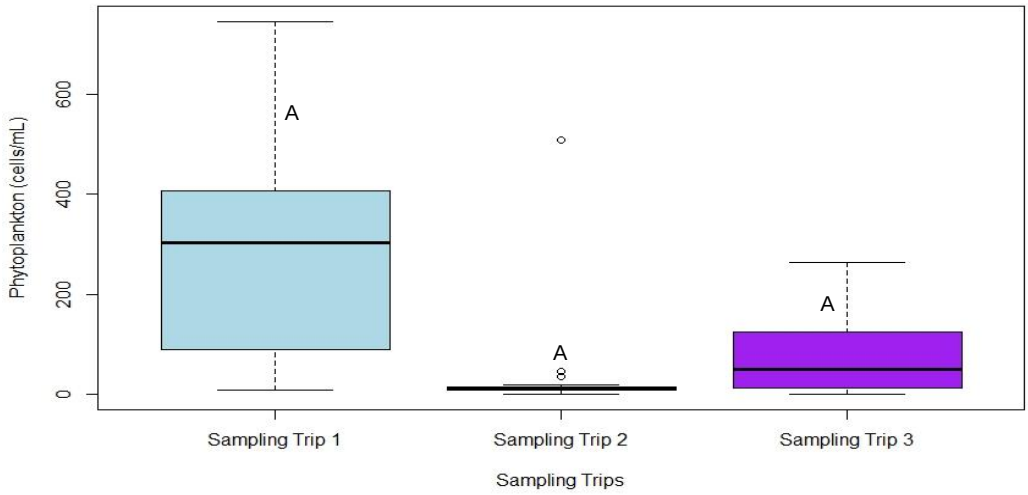


Figure 3.9: Boxplots of phytoplankton cell density (cells/mL) in the dam for each sampling trip. All three sampling trips were significantly different from each other in terms of phytoplankton cell density as represented by the letter A.

3.5.2. Sampling sites: cyanobacteria and phytoplankton cell densities

Despite logarithmic transformation, cyanobacteria cell densities did not meet the assumption of homogeneity ($p = 0.01$). Mean cyanobacteria cell density ranged from 250 ± 81.74 cells/mL at Site H2 to 1 460 ± 186.86 cells/mL at Site H3 (Figure 3.10). Mean phytoplankton cell density, however, varied significantly between the six sampling sites during the study period ($H = 11.013$, 4 df, $p < 0.05$) (Figure 3.11). Mean phytoplankton cell density ranged from 18 ± 13.2 cells/mL at Site H3 to

182 ± 60.82 cells/mL at Site H5 (Figure 3.11). Pairwise comparisons using Dunn's test indicated phytoplankton cell density at Site H3 was significantly lower than phytoplankton cell density at Site H5 ($p < 0.05$) (Table 3.10; Figure 3.11). No other differences were statistically significant.

Table 3.10: Unadjusted and adjusted p values obtained from performing a post hoc Dunn's Test on phytoplankton cell density. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of phytoplankton cell density. H1 to H6 refers to sampling sites 1 to 6, respectively.

	Phytoplankton density	
	Unadjusted p value	**Adjusted p value**
H1 - H2	0.226	1.000
H1 - H3	0.082	0.822
H2 - H3	0.007	0.072
H1 - H4	0.198	1.000
H2 - H4	0.965	1.000
H3 - H4	0.006	0.056
H1 - H5	0.176	1.000
H2 - H5	0.990	1.000
H3 - H5	0.004	0.039
H4 - H5	0.972	1.000

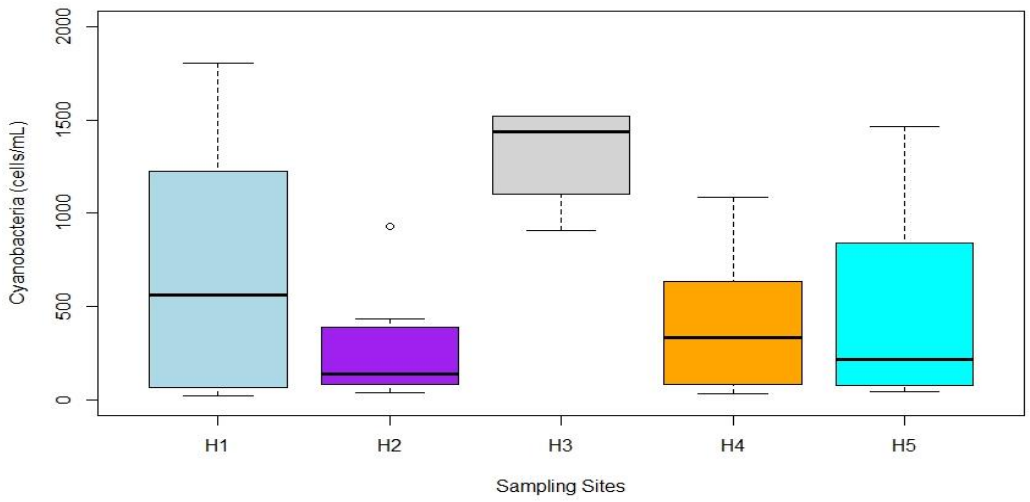


Figure 3.10: Boxplots of cyanobacteria cell density (cells/mL) in the dam between the six sampling sites (H1 – H6), across all sampling depth intervals, during the study period.

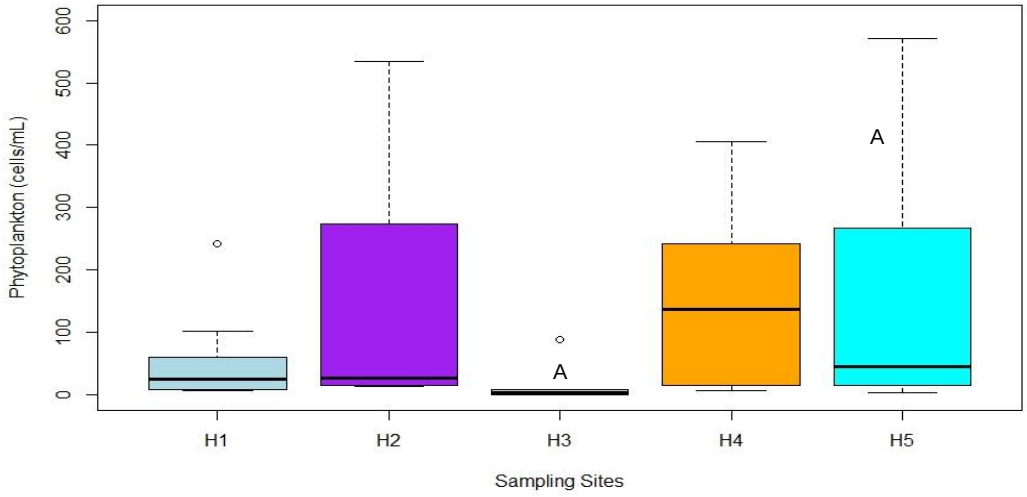


Figure 3.11: Boxplots of phytoplankton cell density (cells/mL) in the dam between the six sampling sites (H1 – H6), across all sampling depth intervals, during the study period. Significant differences between sampling sites are indicated by the letter A.

3.3.3. Sampling depths: cyanobacteria and phytoplankton cell densities

Both cyanobacteria and phytoplankton cell densities did not differ significantly between the five sampling depth intervals. Mean cyanobacteria cell density ranged from 320 ± 102.05 cells/mL at the 5 m depth interval to 835 ± 288.36 cell/mL at the 15 – 25 m depth interval ($H = 1.575$, 4 df, $p = 0.81$) (Figure 3.12). Mean phytoplankton cell density ranged from 55 ± 22.34 cells/mL at the 10 m depth interval to 148 ± 52.99 cells/mL at the 0 m depth interval ($H = 0.122$, 4 df, $p = 1.00$) (Figure 3.13).

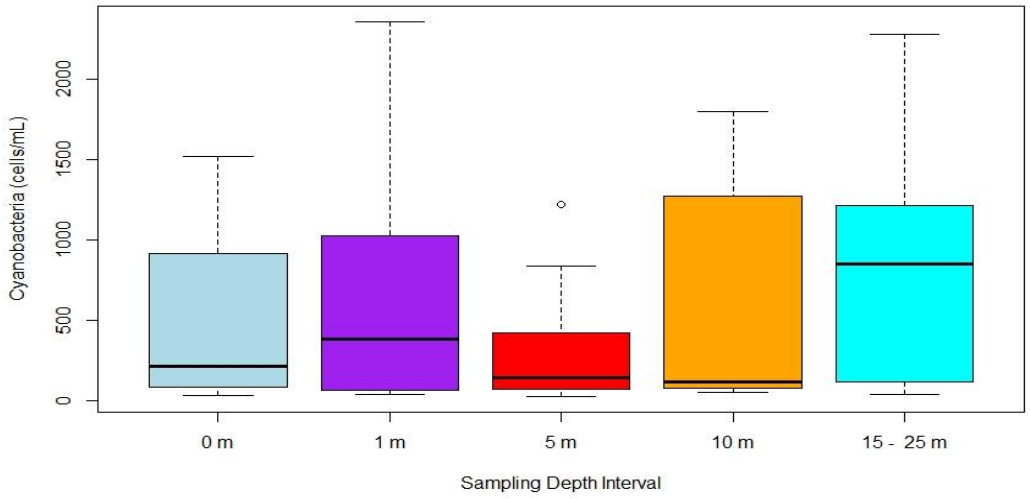


Figure 3.12: Boxplots of cyanobacteria cell density (cells/mL) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m and 15 – 25 m), across all sampling sites, during the study period. No significant differences exist between sampling depth intervals and cyanobacteria cell density (cells/mL).

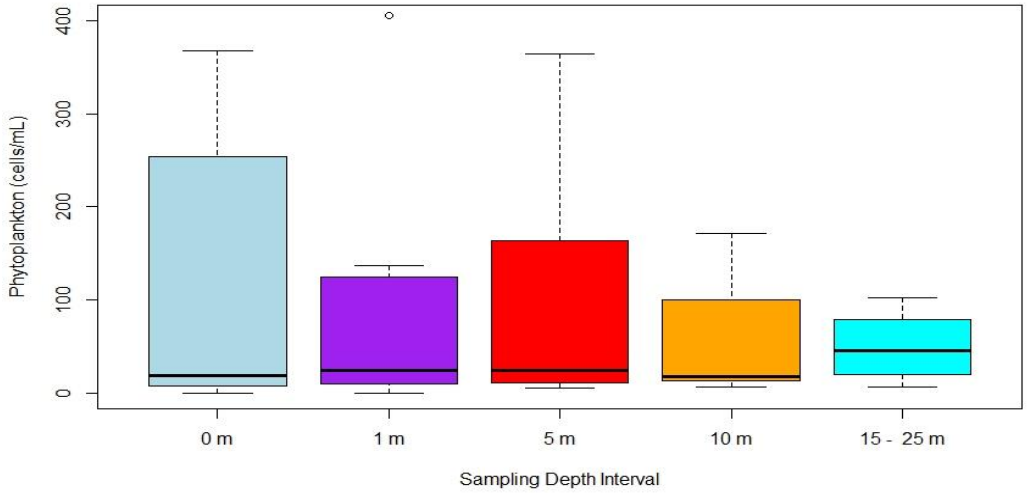


Figure 3.13: Boxplots of phytoplankton cell density (cells/mL) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m and 15 – 25 m), across all sampling sites, during the study period. No significant differences exist between sampling depth intervals and phytoplankton cell density (cells/mL).

3.4. Phytoplankton biomass: chlorophyll *a* concentration

Chlorophyll *a* concentration for sampling trips and sampling sites was logarithmically transformed to meet the assumption of homogeneity. As chlorophyll *a* concentration was non-parametric, a Kruskal-Wallis test was used to determine differences between mean ranks of chlorophyll *a* concentration across the three sampling trips, six sampling sites, and five sampling depth intervals.

3.4.1. Sampling trips: chlorophyll *a* concentration

Chlorophyll *a* concentration varied significantly in the dam across the three sampling trips ranging from a mean of 9.645 ± 2.36 µg/L for Sampling Trip 2 to a mean of 22.519 ± 3.39 µg/L for Sampling Trip 1 ($H = 12.085$, 2 df, $p < 0.05$) (Figure 3.14). Chlorophyll *a* concentration in Hartbeespoort Dam ranged from 3.439 µg/L to 60.759 µg/L during the study period. Pairwise comparisons using Dunn's test indicated that chlorophyll *a* concentration obtained during Sampling Trip 2 was significantly lower than Sampling Trip 1 ($p < 0.05$) (Table 3.11; Figure 3.14).

Table 3.11: Unadjusted and adjusted p values obtained from performing a post hoc Dunn's Test on chlorophyll *a* concentration. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of chlorophyll *a* concentrations, where ST 1 to 3 refers to sampling trips 1 to 3, respectively.

	Chlorophyll *a* concentration	
	Unadjusted p value	**Adjusted p value**
ST 1 - ST 2	0.0005	0.002
ST 1 - ST 3	0.04	0.119
ST 2 - ST 3	0.104	0.312

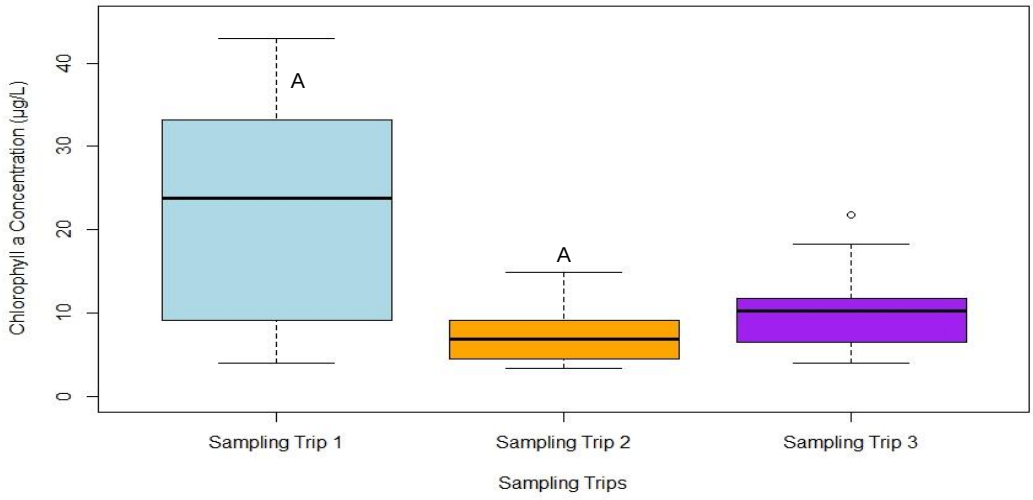


Figure 3.14: Boxplots of chlorophyll *a* concentration (µg/L) in the dam for each sampling trip. Sampling Trip 1 and Sampling Trip 2 were significantly different from each other in terms of chlorophyll *a* concentration, as represented by the letter A.

3.3.2. Sampling sites: chlorophyll *a* concentration

Chlorophyll *a* concentration varied significantly in the dam across the six sampling sites ranging from a mean of 6.353 ± 0.73 µg/L at Site H1 to a mean of 18.228 ± 3.3 µg/L at Site H2 ($H = 21.702$, 2 df, $p < 0.05$) (Figure 3.15). Pairwise comparisons using Dunn's test indicated that chlorophyll *a* concentration obtained at Site H2 was significantly higher than Sites H1 and H3 ($p < 0.05$) (Table 3.12; Figure 3.15).

Table 3.12: Unadjusted and adjusted p values obtained from performing a post hoc Dunn's Test on chlorophyll *a* concentration. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of chlorophyll *a* concentration. H1 to H6 refers to sampling sites 1 to 6, respectively.

	Chlorophyll *a* concentration	
	Unadjusted p value	**Adjusted p value**
H1 - H2	0.0002	0.003
H1 - H3	0.916	1.000
H2 - H3	0.003	0.042
H1 - H4	0.005	0.080
H2 - H4	0.322	1.000
H3 - H4	0.029	0.431
H1 - H5	0.004	0.062
H2 - H5	0.235	1.000
H3 - H5	0.029	0.429
H4 - H5	0.894	1.000
H1 - H6	0.535	1.000
H2 - H6	0.013	0.193
H3 - H6	0.655	1.000

H4 - H6	0.093	1.000
H5 - H6	0.098	1.000

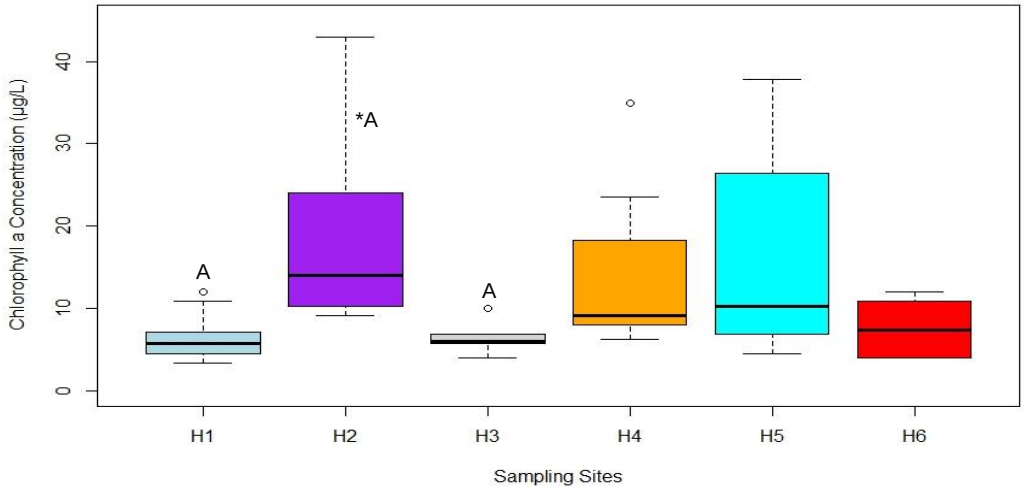


Figure 3.15: Boxplots of chlorophyll *a* concentration (µg/L) between the six sampling sites (H1 – H6), across all sampling depth intervals, during the study period. The letter A indicates significant differences in chlorophyll *a* concentration between sampling sites. Starred letters indicate the sampling site from which sites with corresponding letters differ significantly i.e., sites H1 and H3 (A) differ significantly from site H2 (*A) in terms of chlorophyll *a* concentration.

3.3.3. Sampling depths: chlorophyll *a* concentration

Chlorophyll *a* concentrations did not differ significantly from 0 m to the bottom of the dam during the study. Mean chlorophyll *a* concentration across the sampling depth intervals ranged from 10.318 ± 1.82 µg/L at the 0 m depth interval to 14.418 ± 3.11 µg/L at the 5 m depth interval ($H = 2.492$, 4 df, $p = 0.65$) (Figure 3.16).

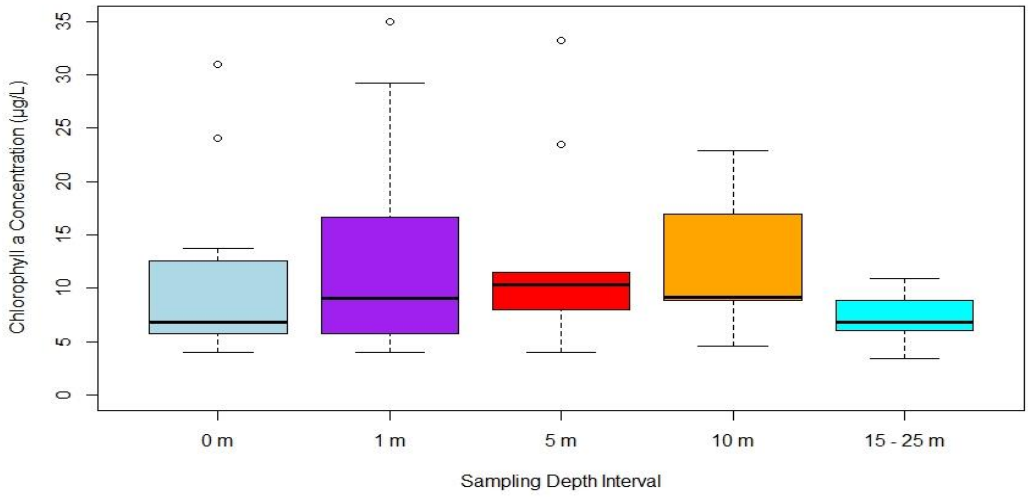


Figure 3.16: Boxplots of chlorophyll *a* concentration (µg/L) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m and 15 – 25 m), across all sampling sites, during the study period. No significant differences exist between sampling depth intervals in terms of chlorophyll *a* concentration.

3.5. Analysis of microcystin concentrations

As microcystin concentration datasets were non-parametric, a Kruskal-Wallis test was used to determine differences between mean ranks of microcystin concentrations across the three sampling trips, six sampling sites, and five sampling depth intervals.

3.5.1. Sampling trips: microcystin concentration

Microcystin concentration significantly increased from Sampling Trip 1 to Sampling Trip 3 as the study progressed from autumn to mid-winter ranging from a mean microcystin concentration of 222.92 ± 22.27 µg/L to 424.15 ± 19.9 µg/L ($H = 22.059$, 2 df, $p < 0.05$) (Figure 3.17). Microcystin concentrations in Hartbeespoort Dam ranged from 59.33 µg/L to 832.62 µg/L during the study period. Pairwise comparisons using Dunn's test indicated that all three sampling trips were significantly different from each other in terms of microcystin concentration ($p < 0.05$) (Table 3.13; Figure 3.17).

Table 3.13: Unadjusted and adjusted p values obtained from performing a post hoc Dunn's Test on microcystin concentration. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling trips (ST) that were significantly different from each other in terms of microcystin concentrations, where ST 1 to 3 refers to sampling trips 1 to 3, respectively.

	Microcystin concentration	
	Unadjusted p value	**Adjusted p value**
ST 1 - ST 2	1.08E-01	3.25E-01

ST 1 - ST 3	9.82E-06	2.95E-05
ST 2 - ST 3	9.21E-04	2.76E-03

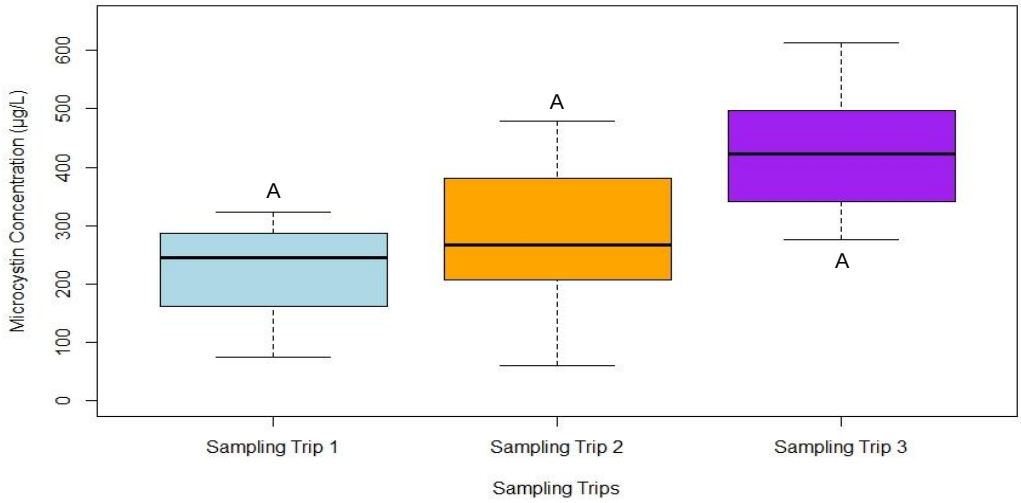


Figure 3.17: Boxplots of microcystin concentration (µg/L) in the dam for each sampling trip. Microcystin concentrations increased in the dam as the study progressed from autumn to mid-winter. The letter A indicates significant differences in microcystin concentrations between sampling trips.

3.5.2. Sampling sites: microcystin concentration

Microcystin concentrations significantly differed across the dam between the six sampling sites. Mean microcystin concentration across the sampling sites ranged from 234.33 ± 30.43 µg/L at Site H3 to 559.21 ± 58.7 µg/L at Site H6 ($H = 12.971$, 5 df, $p < 0.05$) (Figure 3.18). Pairwise comparisons using Dunn's test indicated that Sites H3 and H6 were significantly different from each other in terms of microcystin concentration ($p < 0.05$) (Table 3.14; Figure 3.18). No other differences were statistically significant.

Table 3.14: Unadjusted and adjusted p values obtained from performing a post hoc Dunn's Test on microcystin concentration. P values were adjusted using the Bonferroni method to correct for any Type I errors. Blue-highlighted cells indicate sampling sites that were significantly different from each other in terms of microcystin concentration. H1 to 6 refers to sampling sites 1 to 6, respectively.

	Microcystin concentration	
	Unadjusted p value	**Adjusted p value**
H1 - H2	0.7413	1.0000

H1 - H3	0.2214	1.0000
H2 - H3	0.1510	1.0000
H1 - H4	0.9747	1.0000
H2 - H4	0.7239	1.0000
H3 - H4	0.2386	1.0000
H1 - H5	0.6198	1.0000
H2 - H5	0.9120	1.0000
H3 - H5	0.0962	1.0000
H4 - H5	0.6049	1.0000
H1 - H6	0.0062	0.0931
H2 - H6	0.0203	0.3052
H3 - H6	0.0006	0.0091
H4 - H6	0.0065	0.0973
H5 - H6	0.0149	0.2235

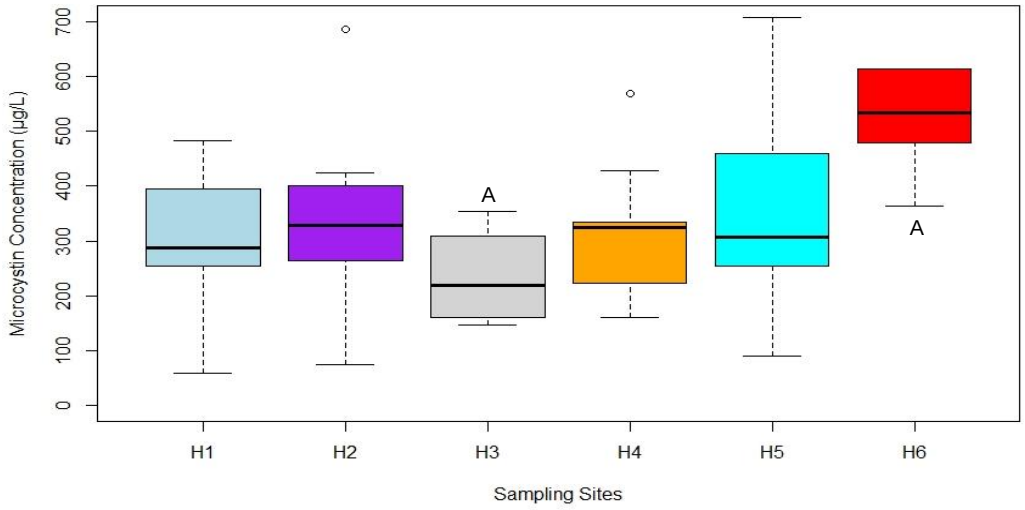


Figure 3.18: Boxplots of microcystin concentration (µg/L) between the six sampling sites (H1 – H6), across all sampling depth intervals, during the study period. The letter A indicates significant differences in microcystin concentrations between sampling sites.

3.5.3. Sampling depths: microcystin concentrations

Microcystin concentrations differed significantly from 0 m to the bottom of the dam. Mean microcystin concentration across the sampling depth intervals ranged from 275.53 ± 35.18 µg/L at the 0 m depth interval to 453.26 ± 59.34 µg/L at the 10 m depth interval (H = 10.669, 4 df, p = 0.03) (Figure 3.19). Pairwise comparisons using Dunn's test were performed and the P values were adjusted using the Bonferroni method to correct for any Type I errors. Despite the Kruskal-Wallis test indicating significances between the five sampling depth intervals, adjusted p values indicated there were no statistically significant differences between the sampling depth intervals in terms of microcystin concentration.

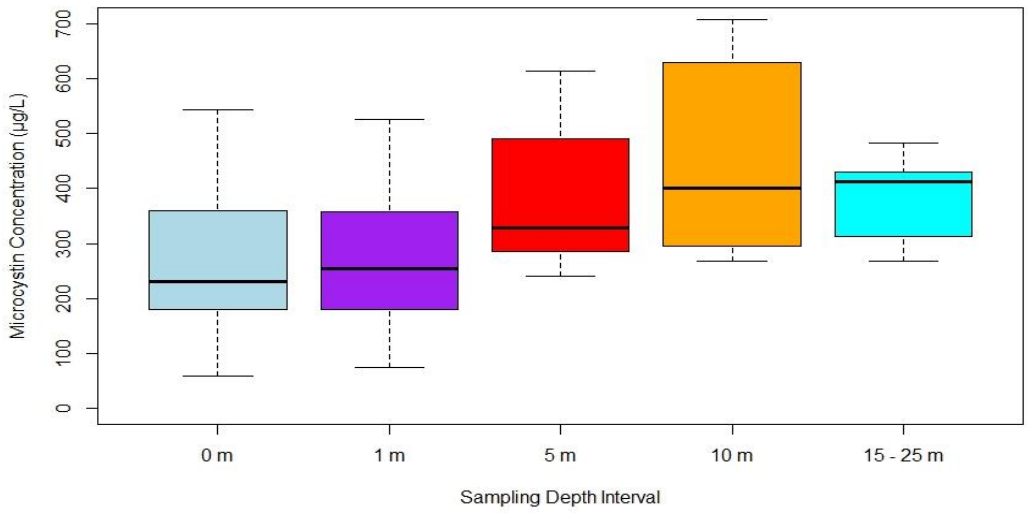


Figure 3.19: Boxplots of microcystin concentration (µg/L) between the five sampling depth intervals (0 m; 1 m; 5 m; 10 m and 15 – 25 m), across all sampling sites, during the study period. No significant differences exist between sampling depth intervals in terms of microcystin concentrations.

3.6. Multivariate Analysis

The RDA (Figure 3.20) demonstrated the ranking of phytoplankton density, cyanobacteria density, and environmental variables in Hartbeespoort Dam across the entire study. The eigenvalues for the different axes are 0.8225; 0.6036; 0.4043; and 0.1696 with a total variance of 2.000 (Table 3.15). The RDA results indicated that 71.31% of the variance in the data was explained by the first two axes (Table 3.15). The ordination plot illustrated that phytoplankton cell density positively correlated to chlorophyll *a* concentration, dissolved oxygen, pH, and water temperature (Figure 3.20). Additionally, phytoplankton cell density was negatively correlated to Secchi disk depth (Figure 3.20). Although cyanobacteria cell density positively

correlated to chlorophyll *a* concentration, dissolved oxygen, and pH, cyanobacteria cell density strongly correlated to inorganic nutrient (DIN and DIP) concentrations (Figure 3.20). Additionally, cyanobacteria cell density negatively correlated to water temperature and weakly correlated to MC-ADDA concentration (Figure 3.20). A positive correlation between MC-ADDA and both inorganic nutrient concentrations was obtained whereas MC-ADDA concentration negatively correlated to water temperature (Figure 3.20).

Table 3.15: Results from an RDA ordination on the phytoplankton density, cyanobacteria density, and environmental variables in Hartbeespoort Dam across the three sampling trips.

	RDA 1	**RDA 2**	**PC 1**	**PC 2**
Eigenvalue	0.8225	0.6036	0.4043	0.1696
Proportion explained	0.4112	0.3018	0.2021	0.0848
Cumulative proportion	0.4112	0.7131	0.9152	1.0000

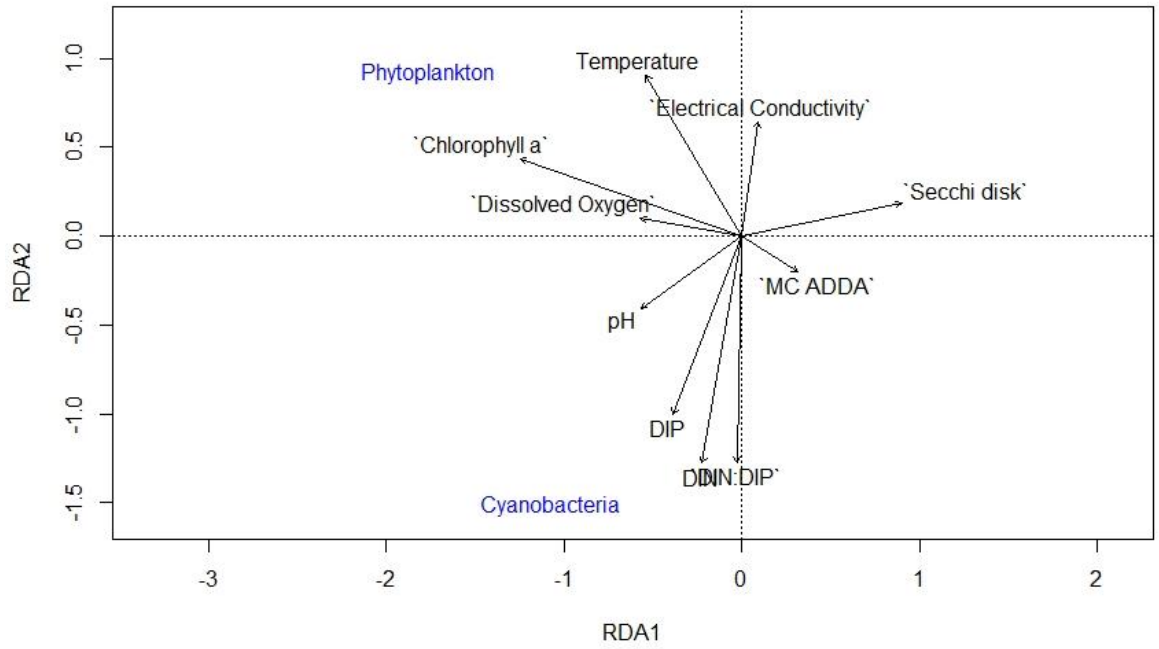


Figure 3.20: An RDA biplot ordination diagram showing the phytoplankton density, cyanobacteria density, and environmental variables in Hartbeespoort Dam across the three sampling trips. The eigenvalues for the different axes are presented in Table 3.15. The cumulative percentage variance of the species data for the different axes is 41.12, 71.31, 91.52, and 100.0.

4. Discussion

4.1. Overall ecological health of Hartbeespoort Dam

4.1.1. Eutrophication and nutrient build-up in Hartbeespoort Dam

This study was performed at Hartbeespoort Dam from autumn to mid-winter in 2018 to investigate the potential human health risks based on the ecological health of the dam. Hartbeespoort Dam is geographically located in the upper Crocodile River catchment (Mitchell *et al*., 2014), and rapid urbanisation, industrial growth, and agricultural activities have occurred within the catchment area (Steyn *et al*., 1975; Conradie and Barnard, 2012). Surface runoff from these activities has resulted in Hartbeespoort Dam receiving high inorganic nutrient loads, namely inorganic phosphorus and inorganic nitrogen (DWAF, 1996: 5; Davis *et al.,* 2009; Matowanyika, 2010; Roux *et al*., 2010; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Hartbeespoort Dam is an artificial impoundment that is representative of a hypertrophic system due to the nutrient enrichment of the impoundment (Conradie and Barnard, 2012; Matthews, 2014; Matthews and Bernard, 2015).

Water resources in South Africa are protected under the National Water Act (NWA) No. 36 of 1998 (NWA, 1998; Matthews and Bernard, 2015). Due to the scarcity and the deteriorating quality of water in South Africa, the NWA requires all effluents to be purified and returned to natural waterbodies ensuring maximised utilisation of water (DWAF, 1996: 5; NWA, 1998; Mitchell *et al.,* 2014). Wastewater treatment works (WWTWs) function in treating wastewater, polluted through urban and anthropogenic activities, therefore returning water of acceptable quality back into the environment (Mitchell *et al.*, 2014). Inadequately treated sewage is being discharged into the environment by approximately 96% of the WWTWs in South Africa (King and Pienaar, 2011; Harding, 2015), a problem that has been further exacerbated by the increased wastewater loads generated because of population growth and rapid urbanisation (Mitchell *et al.,* 2014; Dube *et al.,* 2017). Water quality of aquatic systems is either directly or indirectly (point and diffuse pollution sources) impacted by human activity (Matowanyika, 2010; Dube *et al.*, 2017).

The South African population is rapidly and continually growing (Oberholster *et al.,* 2008; Reichwaldt and Ghadouani, 2012; Stats SA, 2018). The mid-year population

estimates increased from 56.52 million in 2017 to 57.73 million in 2018 (population growth rate of 1.55%) with the largest proportion of the population (14.7 million) residing in the Gauteng Province (Stats SA, 2018). The Gauteng Province had the greatest in- and out-flows of migrants and had the highest number of international migrants (47.5%) entering when compared to the other provinces (Stats SA, 2018). Both international and domestic (from rural provinces such as Limpopo, Eastern Cape, and Kwa-Zulu Natal) migrants are attracted to the Gauteng Province as it is the economic hub of the country (Oberholster *et al.,* 2008; Mitchell *et al.*, 2014; Stats SA, 2018). Tributaries of Hartbeespoort Dam carry surface runoff from urban, industrial, agricultural, and mining activities in the Gauteng Province (Steyn *et al.*, 1975; Matowanyika, 2010; Roux *et al.*, 2010; Conradie and Barnard, 2012; Mitchell *et al.*, 2014).

Urban complexes, such as those existing in the Gauteng Province, generate large amounts of sewage and wastewater (Oberholster *et al.*, 2005; Dube *et al.*, 2017). This sewage and wastewater, treated or untreated, contributes to the increased salt and nutrient loads entering waterbodies as discharged effluents contain excrement, detergents, fertilisers, and organic industrial waste (DWAF, 1996: 5; Oberholster *et al.,* 2005; Matowanyika, 2010; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Hartbeespoort Dam receives inadequately treated wastewater (Mitchell *et al.*, 2014; Harding, 2015) and surface runoff from the Gauteng Province (Dube *et al.*, 2017). The increasing population growth and rapid urbanisation of the Gauteng Province therefore contributes to eutrophication and the frequent cyanobacterial blooms of Hartbeespoort Dam (Conradie and Barnard, 2012; Matthews, 2014; Matthews and Bernard, 2015).

Sewage, typically a point source pollutant (De Jonge *et al.*, 2002; Janse van Vuuren and Taylor, 2015), is a type of wastewater generated by domestic dwellings consisting of blackwater (wastewater from toilets containing urine and excrement) and greywater (wastewater from all domestic sources except toilets) (Friedler *et al*., 2013). Excrement of living organisms is high in phosphate and nitrogenous wastes generated through metabolic processes (DWAF, 1996: 5; Matowanyika, 2010; Friedler *et al*., 2013). Sewage contains high concentrations of urea (produced by the liver during protein metabolism) (Huntington and Archibeque, 2000), the main nitrogen-containing compound found in mammalian urine (Matowanyika, 2010). Upon entering aquatic systems, urea is converted into ammonium (NH_4^+) (DWAF, 1996: 5; Matowanyika,

2010). Through the assimilation of bacteria, ammonium is converted to nitrite (NO_2^-) and then to nitrate (NO_3^-), the final product of nitrification (DWAF, 1996: 5; Matowanyika, 2010). This nitrification process utilises oxygen (O_2) therefore decreasing dissolved oxygen concentrations in aquatic systems (DWAF, 1996: 5; Matowanyika, 2010). Additionally, domestic detergents contain phosphates (Matowanyika, 2010; Van Ginkel, 2011; Schindler *et al.,* 2016; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Sewage, treated or untreated, therefore contributes to the nutrient enrichment of aquatic systems (DWAF, 1996: 5; Xu *et al.*, 2010; Quevedo-Castro *et al.*, 2019).

Inadequate treatment of urban and anthropogenic wastewater by malfunctioning WWTWs has been directly linked to the increased supply of inorganic nutrients to surface waterbodies (Xu *et al.*, 2010; Mitchell *et al.,* 2014; Harding, 2015) and has been identified as one of the main contributing factors of eutrophication in South Africa (King and Pienaar, 2011; Harding, 2015). The Johannesburg Metropolitan Municipality's Northern Works achieved a Green Drop Rating of 92.4% in 2011 as it employs capable personnel and modern technologies (Mitchell *et al*., 2014). The Northern Works discharges effluent into the Jukskei River and is designed to treat 450 ML of influent per day, treating effluent from approximately half of Johannesburg (Mitchell *et al*., 2014). Hartbeespoort Dam receives 600 ML of treated sewage per day from WWTWs in the catchment area (Mitchell *et al.*, 2014), of which approximately 70% (420 ML/day) comes from the Northern Works (Mitchell *et al.*, 2014).

Phosphorus concentrations are usually low in unimpacted aquatic systems as phosphorus is actively taken up and utilised by aquatic biota (DWAF, 1996: 5; Matowanyika, 2010). Phosphorus is difficult and expensive to remove from sewage and wastewater effluents (Mitchell *et al*., 2014). Effluents generated from the denitrification of domestic sewage are therefore phosphorus-rich (Mitchell *et al*., 2014). According to Mitchell *et al.* (2014), of the daily 600 ML treated sewage discharging into Hartbeespoort Dam, a mean daily orthophosphate load of approximately 547.95 kg (200 tonnes per annum) is discharged into Hartbeespoort Dam. The performance of well-managed WWTWs in South Africa (such as the Northern Works) is therefore not always adequate in preventing eutrophication of downstream reservoirs (Mitchell *et al*., 2014).

Furthermore, population growth and rapid urbanisation have resulted in the increased discharge of inorganic nutrients into aquatic systems via diffuse sources (De Jonge *et al.*, 2002; Matowanyika, 2010), including land drainage of cultivated land and surface runoff from urban and industrial areas (DWAF, 1996: 5; De Jonge *et al.*, 2002; Davis *et al.*, 2009; Matowanyika, 2010; Roux *et al.*, 2010; O'Neil *et al.*, 2012; Mitchell *et al.*, 2014; Harding, 2015; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019; Hill *et al.*, 2020). Diffuse pollutant sources discharge pollution into aquatic systems via dispersed, poorly defined ways whereas point pollutant sources discharge pollution from a single identifiable source, including industrial discharge and WWTWs effluents (De Jonge *et al.*, 2002; Matowanyika, 2010). Surface runoff from urban areas is associated with contaminated stormwater from roads, roofs, and open spaces (DWAF, 1996: 5; Matowanyika, 2010).

Common agricultural fertilisers supplement essential nutrients (nitrogen, phosphorus, and potassium) in the soil required for plant growth (Matowanyika, 2010). Commonly used agricultural fertilisers applied to cultivated land contain highly soluble ammonia and ammonium salts (DWAF, 1996: 5; Matowanyika, 2010). Excess nutrients are transported into aquatic systems via precipitation or irrigation water if the nutrient concentrations exceed the immediate requirement of the plant following the application of fertilisers (DWAF, 1996: 5). Effluent streams containing animal excrement generated during livestock production further increases inorganic nutrient concentrations in aquatic systems (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Organic nitrogen is transformed to inorganic nitrogen by many bacteria during the biological degradation of organic material, including manure (DWAF, 1996: 5). Surface runoff of cultivated land and livestock farms is therefore a diffuse source of inorganic nutrients entering aquatic systems (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017).

Most rivers flowing through urban areas are susceptible to degradation and are under pressure because of the large and dense populations depending on their ecological services (Matowanyika, 2010). Historically, various laws restricted the movement of people in South Africa resulting in rapid urbanisation following the passing of the Abolition of Influx Control Act 86 of 1968 (Matowanyika, 2010; Dube *et al.*, 2017). Mass migration of people from rural to urban areas occurred in the pursuit of employment (Matowanyika, 2010). The rate of population growth in urban areas was, and still is,

greater than the infrastructure available to support the population (Matowanyika, 2010; Dube *et al.*, 2017). Informal settlements have therefore been established in urban areas to accommodate the ever-increasing urban populations (Matowanyika, 2010).

The rapid urbanisation of the Gauteng Province, South Africa's economic hub (Stats SA, 2018), has resulted in the establishment of informal settlements in communities such as Diepsloot, Cosmo City, and Alexandra (Oberholster *et al.,* 2008; Matowanyika, 2010; Mitchell *et al.*, 2014; Dube *et al.*, 2017). The Jukskei River, a major tributary of the Crocodile River (Wittmann and Förstner, 1976; Pearce, 1987; Matowanyika, 2010; Roux *et al*., 2010; Dube *et al.,* 2017), flows through and supports these communities and their associated informal settlements (Matowanyika, 2010). The establishment of these settlements has resulted in the overloading of infrastructure (often resulting in overflowing sewers) and overcrowding (often rendering waste removal difficult) (Dudula, 2008; Matowanyika, 2010; Dube *et al.*, 2017). Informal settlements are not connected to sewer infrastructure therefore dwellers consequently discard sewage and household waste directly into stormwater drains and rivers (Matowanyika, 2010; Mitchell *et al*., 2014; Dube *et al.*, 2017). The Jukskei River is the most polluted river in the Hartbeespoort Dam catchment area as it is frequently contaminated by urban runoff and sewage effluents (Matowanyika, 2010; Roux *et al.*, 2010; Mitchell *et al*., 2014).

Hartbeespoort Dam receives 90.4% of its inflow from the Crocodile River (Pearce, 1987; Roux *et al.*, 2010). The Crocodile River (Site H3) flows through and supports urban and rural areas, and other anthropogenic activities, and is joined by the Jukskei and Hennops rivers before it drains into Hartbeespoort Dam (Pearce, 1987; Matowanyika, 2010; Roux *et al.*, 2010; Dube *et al.*, 2017). These rivers do not have the assimilative capacity to dilute contaminated wastewater entering these systems from activities in the catchment area (Matowanyika, 2010; Mitchell *et al*., 2014). The Crocodile River is therefore the most polluted and problematic tributary of Hartbeespoort Dam carrying > 95% of the inorganic nutrient load entering the dam (Wittmann and Förstner, 1976; Pearce, 1987; Matowanyika, 2010; Roux *et al*., 2010; Mitchell *et al.*, 2014).

Across the study period, the concentrations of inorganic nutrients (namely inorganic phosphorus and inorganic nitrogen) in the Crocodile River (Site H3) water samples

were significantly higher when compared to the other five sampling sites in the dam. Using mean daily flow rates (m^3/s) and mean inorganic nutrient concentrations (mg/L) obtained in the Crocodile River during this study, approximate inorganic nutrient loads (kg/day) entering Hartbeespoort Dam via the Crocodile River were calculated (Appendix C). Daily flow rates were obtained from the Department of Water and Sanitation's (DWS) Crocodile River flow gauge A2H012 (Crocodile River at Kalkheuwel). Based on these calculations, Hartbeespoort Dam received daily inorganic nutrient loads of approximately 15 tonnes DIN and approximately 1 tonne DIP from the Crocodile River during this study period (Appendix C). The Crocodile River therefore discharges high water volumes and significantly high nutrients loads into Hartbeespoort Dam (Steyn *et al.*, 1975; Matowanyika, 2010; Conradie and Barnard, 2012); a finding similar to the conclusion outlined in Roux *et al.* (2010).

Electrical conductivity is the ability of water to conduct an electrical current due to the presence of certain ions (compounds carrying an electrical charge) (DWAF, 1996: 1, 5; Matowanyika, 2010; Dube *et al.*, 2017). Electrical conductivity is directly proportional to the concentration of total dissolved salts (TDS) in water (DWAF, 1996: 1, 5; Dube *et al.*, 2017). A significantly high electrical conductivity reading was obtained at the Crocodile River site (Site H3) when compared to the other five sampling sites. As water travels downstream, salts are continuously added via natural and anthropogenic sources whilst very little is removed via evaporation and other natural processes (DWAF, 1996: 1, 5; King and Pienaar, 2011; Dube *et al.*, 2017). As previously discussed, the Crocodile River carries wastewater from urban, industrial, agricultural, and mining activities in the Gauteng Province (Steyn *et al*., 1975; Roux *et al.,* 2010; Dube *et al.*, 2017). Across this study, electrical conductivity at Site H3 was therefore significantly higher than the other five sampling sites.

Turbidity is an optical property of water measuring water clarity (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Turbidity is equivalent to the concentration of total suspended solids (TSS) as it is affected by the suspension of solid particles in the water column (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Suspended particles comprise of inorganic (silts and clays) and organic (algae, zooplankton, and bacteria) materials (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017). Secchi disk depth is a measure of water clarity (DWAF, 1996: 1, 2, 5; Oberholster *et al.*, 2009; Molisani *et al.*, 2010; Matthews and

Bernard, 2015). The lowest Secchi disk depth reading across all three sampling trips was obtained in the Crocodile River (Site H3) when compared to the other five sampling sites. A low Secchi disk depth reading is indicative of murky, or turbid, water (Matowanyika, 2010). Anthropogenic sources of suspended solids, and turbidity, in water include discharges of domestic sewage and effluents from industrial and mining activities (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). The high turbidity of water entering Hartbeespoort Dam via the Crocodile River was therefore attributed, like electrical conductivity, to the heavily polluted wastewater generated by urban, industrial, and mining activities in the Gauteng Province (Steyn *et al*., 1975; Roux *et al.,* 2010; Dube *et al.,*2017).

As previously mentioned, Hartbeespoort Dam receives 9.3% of its inflow from the Magalies River (Pearce, 1987; Roux *et al.*, 2010). The Magalies River supports agricultural activities in the catchment area prior to draining into the dam (Pearce, 1987; Roux *et al.*, 2010). Surface runoff of cultivated land and livestock farms is a diffuse source of inorganic nutrients and dissolved salts entering aquatic systems (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017). Inorganic nutrient concentrations, particularly inorganic nitrogen, and electrical conductivity were therefore expected to be high at the Magalies River entrance (Site H6) (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Across the study period, orthophosphate concentration was significantly lower at the Magalies River entrance when compared to the other five sampling sites in the dam. Additionally, ammonia, nitrate + nitrite, and DIN concentrations at the Magalies River entrance were significantly lower than concentrations obtained in the Crocodile River. High water volumes and significantly high nutrient loads entering Hartbeespoort Dam can therefore be attributed to the heavily polluted Crocodile River, and its tributaries (Steyn *et al*., 1975; Roux *et al.,* 2010; Dube *et al.*, 2017).

Furthermore, the second lowest turbidity (after Site H2) and electrical conductivity (after Site H1) readings were obtained at the Magalies River entrance (Site H6) during this study. Low turbidity is indicative of clearer water due to low concentrations of suspended solids in the water column (Matowanyika, 2010). As electrical conductivity is impacted by dissolved salts added to an aquatic system via agricultural activities (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017), a significantly lower electrical conductivity reading, when compared to the Crocodile River (Site H3), was not

expected at the Magalies River entrance (Site H6). As nutrient concentrations and electrical conductivity readings obtained at the Magalies River entrance were significantly lower than those obtained in the Crocodile River, the Magalies was therefore not considered to be a significantly polluted tributary of Hartbeespoort Dam during this study.

The only large outflow of the dam is the Crocodile River, carrying 61% of the water downstream towards Brits (Roux *et al*., 2010). The remaining 39% of water loss is attributed to evaporation, consumptive uses, and two canals supplying mining and agricultural activities to the north of the dam (Pearce, 1987; Roux *et al*., 2010). The quality of water at the Hartbeespoort Dam Wall (Site H1) can be used as an indication of the water quality flowing out of the dam into the Crocodile River (Harding, 2015). Following the Crocodile River (Site H3), maximum orthophosphate, ammonium, and DIN concentrations across the dam were obtained at the Hartbeespoort Dam Wall. Electrical conductivity at the Hartbeespoort Dam Wall was, however, significantly lower than the Crocodile River (Site H3). Despite inorganic nutrient concentrations being lower at the Hartbeespoort Dam Wall than the Crocodile River (Site H3), Hartbeespoort Dam did not serve as an effective ecological filter as high nutrient loads flowed out of the dam downstream towards Brits.

Poor water quality has detrimental impacts on ecosystems (DWAF, 1996: 5) and the health and livelihoods of downstream users as poor water quality reduces the ability of the environment to provide the ecological services upon which society depends (Mitchell *et al.*, 2014; Matthews and Bernard, 2015). Additionally, poor water quality increases water treatment costs for downstream users (Xu *et al.,* 2010; King and Pienaar, 2011; Mitchell *et al.,* 2014; Harding 2015). High nutrient loads flowing out of Hartbeespoort Dam via the Crocodile River therefore reduces the availability of clean water for urban, industrial, and agricultural activities by the inhabitants of Brits and other downstream users (Roux *et al.*, 2010; Mitchell *et al.*, 2014; Matthews and Bernard, 2015).

The rapidly growing population in the Gauteng Province has resulted in increased surface runoff and anthropogenic nutrient pollution entering Hartbeespoort Dam (Steyn *et al.*, 1975; DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*,

2017; Quevedo-Castro *et al.*, 2019). Based on the daily nutrient loads that flowed into Hartbeespoort Dam via the Crocodile River during this study, approximately 365 tonnes of orthophosphate entered the dam via the Crocodile River annually. Annual orthophosphate loads entering Hartbeespoort Dam therefore increased from 200 tonnes, as outlined by Mitchell *et al.* (2014), to 365 tonnes during 2018.

Additionally, based on the findings by Roux *et al.* (2010), out of the total 24 798 tonnes of nitrogen that entered the dam, 19 184 tonnes (77%) remained and out of the 2 362 tonnes of phosphate that entered the dam, 1 934 tonnes (82%) remained. The study showed that these nutrients were trapped in the dam and contributed to a reduction in the quality of water (Roux *et al*., 2010). Rapid urbanisation of the Gauteng Province as well as increased industrial and agricultural activities within the catchment area have therefore led to a build-up of nutrients in Hartbeespoort Dam (Steyn *et al.*, 1975; DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019).

Hartbeespoort Dam is therefore representative of a hypertrophic system and is globally well-known for its frequent and persistent cyanobacterial blooms and the formation of, often toxic, surface scums (Conradie and Barnard, 2012; Ballot *et al.,* 2014; Matthews, 2014; Matthews and Bernard, 2015). The decreased quality of water in Hartbeespoort Dam is problematic as the water is used for irrigation in farming, recreational activities, and is a source of potable water (Steyn *et al.*, 1975; Pearce, 1987; Roux *et al*., 2010; Conradie and Barnard, 2012; Ballot *et al.*, 2014).

4.1.2. Trophic status of Hartbeespoort Dam

The trophic status of Hartbeespoort Dam during the study was determined using the Carlson Modified Trophic State Index (CMTSI) (Quevedo-Castro *et al.*, 2019). A CMTSI value of 67.7 across the three sampling trips was obtained indicating that Hartbeespoort Dam was eutrophic (50 - 70) during this study (Quevedo-Castro *et al.*, 2019). Additionally, Hartbeespoort Dam was eutrophic during the winter months as CMTSI values of 65.1 and 67.7 (Quevedo-Castro *et al.*, 2019) were obtained for Sampling Trips 2 and 3, respectively. Hartbeespoort Dam was, however, hypertrophic (> 70) during autumn as a CMTSI value of 70.5 (Quevedo-Castro *et al.*, 2019) was obtained for Sampling Trip 1. Chlorophyll *a* concentration (used as an indicator of algal

biomass in surface waters) obtained during Sampling Trip 1 was significantly higher than Sampling Trip 2. A higher CMTSI value during Sampling Trip 1 can therefore be attributed to the increased phytoplankton biomass during the autumn months.

Negative impacts of eutrophic and hypertrophic waterbodies include turbid water conditions, taste and odour problems, oxygen depletion (hypoxia and anoxia), increased phytoplankton blooms, increased primary growth of macrophytes (i.e., water hyacinth), a loss of biodiversity, increased fish deaths, and decreased aesthetic value (Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Xu *et al.*, 2010; Van Ginkel, 2011; Janse van Vuuren and Taylor, 2015; Matthews and Bernard, 2015; Hill *et al.*, 2020).

4.1.3. System variables in Hartbeespoort Dam

Based on the Hartbeespoort Dam meteorological data obtained from the South African Weather Service (SAWS), air temperature decreased as the study progressed from autumn to mid-winter. The thermal conditions of a waterbody are influenced by the climate, anthropogenic activities, and the structural features of its catchment area (Matowanyika, 2010). Heat is transferred in either direction from a high to a low temperature between the atmosphere and the surface of water (Jiji, 2006). As air temperature decreases into winter, heat is transferred from the warmer water to the air thereby decreasing surface water temperature (Jiji, 2006). Surface water temperature therefore significantly decreased as the study progressed into winter. Additionally, surface water temperature was uniform across the dam for each sampling trip.

Phytoplankton are photosynthetic organisms (autotrophs) that use light energy to produce carbohydrates and oxygen from water and carbon dioxide (Swanepoel *et al.*, 2008). Between one and two percent of phytoplankton's dry weight is attributed to chlorophyll *a,* a component of all green plants as it is an essential component of photosynthesis (DWAF, 1996: 1, 5; Swanepoel *et al.*, 2008; Davis *et al.*, 2009). Chlorophyll *a* concentration is therefore used as an indicator of phytoplankton biomass in surface waters (DWAF, 1996: 1, 5; Chorus and Bartram, 1999; Swanepoel *et al*., 2008; Ballot *et al.*, 2014; Matthews, 2014; Matthews and Bernard, 2015). During this study, both chlorophyll *a* and dissolved oxygen concentrations reached a maximum and a minimum during the first and second sampling trips, respectively. Phytoplankton biomass and photosynthetic activity in Hartbeespoort Dam were therefore most

abundant during autumn and minimal at the beginning of winter as phytoplankton cellular processes (including photosynthesis) and growth-rates are strongly influenced by temperature (Robarts and Zohary, 1987; Davis *et al.,* 2009; O'Neil *et al.*, 2012).

The pH value in aquatic ecosystems can be influenced by the biological activity of aquatic biota in productive and eutrophic systems (DWAF, 1996: 5; Dube *et al.*, 2017. The pH in Hartbeespoort Dam exhibited similar trends to chlorophyll *a* and dissolved oxygen concentrations reaching a maximum and minimum during the first and second sampling trips, respectively. As photosynthetic activity was abundant in autumn, carbon dioxide was removed from the aquatic system increasing the pH and making the system more alkaline (DWAF, 1996: 5). Alternatively, decreased photosynthetic activity during the winter months would have driven the carbonate/bicarbonate equilibrium towards the production of carbonic acid (H_2CO_3) (DWAF, 1996: 5) therefore decreasing the pH in Hartbeespoort Dam. A positive correlation between chlorophyll *a* concentration, dissolved oxygen concentration, and pH was therefore attributed to the seasonal variations in biological activity in the eutrophic Hartbeespoort Dam.

The concentrations of pollutants in aquatic systems are affected by seasonal variations in precipitation, surface runoff, and surface and groundwater flows (DWAF, 1996: 5; Matowanyika, 2010; Reichwaldt and Ghadouani, 2012). Seasonal rainfall and storm events result in increased aeration of aquatic systems and increased surface runoff of organic and inorganic materials from urban, industrial, agricultural, mining activities within a catchment area (DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Furthermore, fast-flowing water during rainfall and storm events increases the eroding power of rivers resulting in increased sediment particles being deposited into aquatic systems and transported downstream (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Seasonal rainfall and storm events therefore increase dissolved oxygen, nutrient loads, TDS concentrations, and turbidity of aquatic systems (DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Conversely, surface runoff decreases during the dry months in South Africa therefore reducing inorganic nutrient concentrations, electrical conductivity, and turbidity in aquatic systems (DWAF, 1996: 5; Matowanyika, 2010).

Rainfall events in the catchment area of Hartbeespoort Dam occur predominantly in summer (November to April) and are scarce during the winter months (May to October) (Matowanyika, 2010). This study was conducted at Hartbeespoort Dam from April 2018 to July 2018 encapsulating the end of the rainy season as well as the dry season in the catchment area. Electrical conductivity, turbidity, and inorganic nutrient concentrations in Hartbeespoort Dam were therefore expected to decrease as the study progressed into winter (DWAF, 1996: 5; Matowanyika, 2010)

As previously discussed, effluent discharges from urban, industrial, agricultural, and mining activities in the Gauteng Province resulted in a significantly high salt load entering the dam via the Crocodile River (Site H3) (Steyn *et al*., 1975; Roux *et al.,* 2010). As surface runoff from these activities decreases when rainfall and storm events are scarce (DWAF, 1996: 5; Matowanyika, 2010), the electrical conductivity of Hartbeespoort Dam decreased as the study progressed into winter. Water temperature significantly decreased as the study progressed into winter as a result of the ambient temperature decreasing during winter months. A positive correlation between water temperature and electrical conductivity was therefore attributed to rainfall and storm events in the catchment area predominantly occurring during the summer months.

The primary suspended material found in most natural waters consists of soil particles derived from land surfaces (DWAF, 1996: 5). Fast-flowing water during rainfall and storm events increases the eroding power of rivers resulting in increased deposition of sediment particles into aquatic systems being transported downstream (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Additionally, rainfall events can result in the resuspension of deposited sediment in the water column (DWAF, 1996: 5). As flow and rainfall decrease, the suspended solids within the water column settle out (DWAF, 1996: 5). Seasonal rainfall and storm events therefore increase the turbidity of water (DWAF, 1996: 5; Matowanyika, 2010).

Based on the Hartbeespoort Dam meteorological data obtained from the SAWS, a total rainfall of 258 mm occurred between March and April 2018 whereas a total rainfall of 19.4 mm occurred between May and June 2018. The lowest and highest Secchi disk depth readings across the dam were obtained during the first and second sampling trips, respectively. A negative correlation between water temperature and

Secchi disk depth in Hartbeespoort Dam was obtained during this study as summer rainfall and storm events increase the turbidity of water decreasing the Secchi disk depth (DWAF, 1996: 5). Turbidity of Hartbeespoort Dam was therefore attributed to seasonal variations in rainfall and storm events, like electrical conductivity.

Additionally, particles suspended within the water column affecting turbidity comprise of both inorganic (silts and clays) and organic (algae, zooplankton, and bacteria) materials (DWAF, 1996: 5; Matowanyika, 2010; Janse van Vuuren and Taylor, 2015; Dube *et al.*, 2017). A negative correlation between phytoplankton cell density, and chlorophyll *a* concentration, and Secchi disk depth in Hartbeespoort Dam was obtained. A negative correlation indicated that turbidity in Hartbeespoort Dam was affected by phytoplankton abundance as phytoplankton contributed to the organic materials suspended within the water column. Turbidity in Hartbeespoort Dam could therefore also be linked to the seasonal variations in phytoplankton biomass.

On the contrary, intense rainfall events can result in the dilution of nutrient concentrations in a waterbody as a result of higher volumes of water entering the waterbody (Pearce, 1987; Harding and Paxton, 2001; Reichwaldt and Ghadouani, 2012; Mitchell *et al.*, 2014). Population growth and rapid urbanisation have resulted in increased nutrient-loads being discharged into surface waterbodies (Steyn *et al.*, 1975; DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). At times, in well-watered countries, the dilution capacity of waterbodies lowers nutrients to near-natural concentrations (Matowanyika, 2010; Mitchell *et al.*, 2014). The dilution capacity of waterbodies therefore reduces the impacts of increased nutrient discharges caused by population growth and rapid urbanisation (Mitchell *et al.*, 2014).

Between August 2017 and July 2018, maximum rainfall of 157.6 mm occurred during March 2018 at Hartbeespoort Dam. Rainfall during April 2018 was 100.4 mm and decreased as the study progressed into winter with a total rainfall of 0.6 mm occurring between June and July 2018. The dilution capacity of Hartbeespoort Dam therefore decreased as the study progressed into winter.

As intense rainfall and storm events occur during the summer months in the Hartbeespoort Dam catchment area (Matowanyika, 2010), the daily flow rates of the

Crocodile River (obtained from the DWS's Crocodile River flow gauge A2H012) were highest during April and decreased as the study progressed into winter. As surface runoff from the catchment area into Hartbeespoort Dam was highest during autumn, inorganic nutrient concentrations were expected to decrease as the study progressed into winter. Concentrations of dissolved inorganic phosphorus (orthophosphate) and dissolved inorganic nitrogen (ammonia, nitrate, and nitrite) in Hartbeespoort Dam, however, significantly increased as the study progressed into winter. As a negative correlation between water temperature and inorganic nutrient concentrations was obtained, the increased inorganic nutrient concentrations obtained in Hartbeespoort Dam during the winter months were therefore attributed to the lack of dilution factor in the dam during winter rather than an increased surface runoff load from the catchment area.

The solubility, and therefore the concentration, of oxygen increases with decreasing temperatures (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Dissolved oxygen is essential for the survival and functioning of aquatic biota as it is utilised by aerobic organisms during respiration (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.,* 2017; Quevedo-Castro *et al.*, 2019). Water temperature directly impacts the rate of metabolic processes of aquatic organisms. As temperature decreases, the rate of metabolic processes, including respiration, decreases therefore reducing oxygen utilisation by these organisms (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Dissolved oxygen concentrations therefore increase in waters with lower temperatures (DWAF, 1996: 5).

As temperature influences dissolved oxygen concentration, a negative correlation between the two variables was expected. A positive correlation between water temperature and dissolved oxygen concentration was, however, obtained as water temperature and dissolved oxygen concentration exhibited similar trends in Hartbeespoort Dam across the study. Dissolved oxygen concentration obtained during autumn (Sampling Trip 1) was significantly greater than the dissolved oxygen concentration obtained at the beginning of winter (Sampling Trip 2). Additionally, within the water column, both temperature and dissolved oxygen concentration reached a maximum at the 1 m depth interval and a minimum at the 15 - 25 m depth interval. The significantly low dissolved oxygen concentration at the 15 - 25 m depth interval could be attributed to reduced photosynthetic activity of aquatic biota due to minimal light

availability (DWAF, 1996: 5). The significantly low dissolved oxygen concentration at the 15 – 25 m depth interval could also be attributed to the bacterial degradation of organic material in the sediment, a process that requires oxygen (DWAF, 1996: 5; Matowanyika, 2010). Temperature therefore appears to not have impacted the dissolved oxygen concentration in Hartbeespoort Dam during the study.

Dissolved oxygen enters water through natural diffusion of gaseous oxygen (O_2) from the atmosphere continuing until oxygen saturation is reached (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Additionally, dissolved oxygen is produced by aquatic biota (including phytoplankton) during photosynthesis (DWAF, 1996: 5; Matowanyika, 2010; Quevedo-Castro *et al.*, 2019). Dissolved oxygen concentration positively correlated to phytoplankton cell density, and chlorophyll *a* concentration, in Hartbeespoort Dam during this study. Dissolved oxygen in Hartbeespoort Dam therefore appeared to be primarily affected by the photosynthetic activity of phytoplankton rather than water temperature.

As a result of the diel cycle of photosynthesis and respiration in aquatic biota, a natural diel variation in dissolved oxygen concentration occurs in waterbodies (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Aquatic biota continuously utilise dissolved oxygen to perform respiration, a process that does not require light unlike photosynthesis (DWAF, 1996: 5). During the day when light is available, photosynthetic activities replenish the dissolved oxygen utilised in respiration (DWAF, 1996: 5; Matowanyika, 2010). During the night, when photosynthesis is halted, dissolved oxygen is utilised by aerobic organisms for respiration without being replenished through photosynthesis (DWAF, 1996: 5; Matowanyika, 2010). Dissolved oxygen concentrations therefore decrease during the night, reaching a minimum near dawn, and steadily increase during the day, reaching a maximum at mid-afternoon (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017).

Photosynthetic activity of aquatic biota is affected by the degree of light availability (DWAF, 1996: 5). A symptom of eutrophic and hypertrophic waterbodies is the increased primary growth of invasive macrophytes (Van Ginkel, 2011; Matthews and Bernard, 2015; Hill *et al.*, 2020). The dominant macrophyte present in Hartbeespoort Dam is water hyacinth (*Eichhormia crassipes*) (Makofane, 2018), an invasive free-floating macrophyte that forms dense floating mats on the surface of waterbodies

(DWAF, 1996: 5; Hill *et al.*, 2020). These dense mats limit the light available to submerged phytoplankton species thereby reducing dissolved oxygen in the aquatic ecosystem (Hill *et al.*, 2020). Dense hyacinth mats extended from the Hartbeespoort Dam Wall (Site H1) into the body of the dam throughout the study. Both chlorophyll *a* and dissolved oxygen concentrations were significantly low at Site H1. As a result of decreased photosynthetic activity at Site H1, a significantly lower pH value was obtained as the carbonate/bicarbonate equilibrium was driven towards the production of H_2CO_3 (DWAF, 1996: 5). These impenetrable hyacinth mats therefore limited phytoplankton growth and photosynthetic activity at the Hartbeespoort Dam Wall (Site H1) site.

Solid particles suspended in the water column scatter and absorb light affecting photosynthetic activity as light penetration is reduced (DWAF, 1996: 5; Matowanyika, 2010; Dube *et al.*, 2017). Turbidity was lowest at Site H2 across the three sampling trips as the highest Secchi disk depth reading was obtained when compared to the other five sampling sites. Light availability was therefore maximal at Site H2 compared to the rest of the dam during this study. Phytoplankton was most abundant at Site H2 as chlorophyll *a* concentration was significantly higher than the Hartbeespoort Dam Wall (Site H1) and the Crocodile River (Site H3) during this study. Additionally, dissolved oxygen concentration was significantly higher at Site H2 than at the Hartbeespoort Dam Wall despite being lower than the Crocodile River. Increased phytoplankton abundance and photosynthetic activity at Site H2 was therefore attributed to greater light availability due to low turbidity (DWAF, 1996: 5).

Chlorophyll *a* concentration peaked at the 5 m depth interval during the study indicating that phytoplankton was most abundant at the 5 m depth interval within the water column. Conversely, chlorophyll *a* concentration reached a minimum at the 0 m depth interval within the water column. All inorganic nutrient concentrations were at their lowest at the 5 m depth interval during the study. Inorganic nutrients are essential for the growth and proliferation of aquatic biota, including phytoplankton and cyanobacteria (DWAF, 1996: 5; Davis *et al.,* 2009; Matowanyika, 2010; Xu *et al.*, 2010). As inorganic nutrients are actively taken up and utilised by aquatic biota, including phytoplankton and cyanobacteria (Matowanyika, 2010), the low inorganic nutrient concentrations at the 5 m depth interval were attributed to the abundant algal biomass. Additionally, light is often too bright at the surface of a waterbody for optimal

primary production. Phytoplankton in Hartbeespoort Dam therefore positioned themselves in the water column, particularly the 5 m depth interval, where both inorganic nutrient concentrations and light availability were optimal (Jäger *et al.*, 2008; Winder and Sommer, 2012).

Phosphorus is dissolved in water columns or is absorbed onto sediment as phosphate as phosphorus is exchanged between the sedimentary and aquatic compartments in the phosphorus cycle (Schindler, 1974; DWAF, 1996: 5; Matowanyika, 2010). Phosphorus is removed from the water column onto the sediment in the presence of oxygen via the settlement of particulate matter and biotic uptake (Schindler, 1974; DWAF, 1996: 5). Absorbed phosphorus is therefore released back into the aquatic compartment under conditions of anoxia and high flow (Schindler, 1974; DWAF, 1996: 5).

As previously mentioned, dissolved oxygen concentration was significantly low at the 15 - 25 m depth interval. The significantly low dissolved oxygen concentration was attributed to reduced photosynthetic activity of aquatic biota due to minimal light availability and the bacterial degradation of organic material in the sediment, a process the requires oxygen (DWAF, 1996: 5; Matowanyika, 2010). Orthophosphate concentration, however, reached a maximum at the 15 – 25 m depth interval. A maximum orthophosphate concentration was therefore attributed to absorbed phosphorus being released from the sedimentary compartment back into the aquatic compartment as conditions at the 15 – 25 m depth interval were anoxic (Schindler, 1974; DWAF, 1996: 5).

4.2. Shift in the phytoplankton community

Phytoplankton are a diverse group of free-floating photosynthetic organisms that are present in every waterbody exposed to sunlight (DWAF, 1996: 5; Swanepoel *et al*., 2008). The composition and abundance of phytoplankton within a waterbody can be used as an indicator of water quality as phytoplankton are a normal and common component of these waterbodies and are sensitive to minor changes in the aquatic environment (Pearce, 1987; Swanepoel *et al*., 2008; Matthews, 2014). Across the study period, total combined phytoplankton (“Cyanobacteria” and “Other Phytoplankton”) cell density in Hartbeespoort Dam was equivalent to 38 076 cells/mL, of which 31 573 cells/mL were cyanobacteria. Cyanobacteria dominated 82.9% of the

phytoplankton community structure in Hartbeespoort Dam during this study. Additionally, Ballot *et al* (2014) found that cyanobacteria dominated 96.9% of the phytoplankton community structure during April 2011. Cyanobacteria, however, dominated 63.8% of the phytoplankton community structure during April 2018. Cyanobacteria abundance in Hartbeespoort Dam therefore appears to have decreased since 2011.

An RDA indicated the 71.31% of the variance in phytoplankton species data was explained by the environmental data in Hartbeespoort Dam during this study. The effect of environmental variables on total algal density was determined through the inclusion of chlorophyll *a* concentration in the RDA ordination despite chlorophyll *a* concentration not being an environmental variable (Janse van Vuuren *et al.*, 2007). Chlorophyll *a* concentration was an effective measure of phytoplankton cell density in Hartbeespoort Dam during this study as a positive correlation between the two variables was obtained. Dissolved oxygen is a product of photosynthesis performed by aquatic biota, including phytoplankton and cyanobacteria. As previously mentioned, dissolved oxygen correlated to the degree of photosynthetic activity of phytoplankton in Hartbeespoort Dam as a positive correlation between phytoplankton cell density, chlorophyll *a* concentration, and dissolved oxygen concentration was obtained. Dissolved oxygen variations in Hartbeespoort Dam could therefore be attributed to phytoplankton abundance rather than temperature changes as phytoplankton are primary producers.

Interactions between phytoplankton species within waterbodies are complex and community composition changes in response to temperature variations (Pearce, 1987; Robarts and Zohary, 1987; Davis *et al.,* 2009; O'Neil *et al.*, 2012). In previous studies, increasing temperature resulted in the phytoplankton community composition shifting from diatoms to green algae to cyanobacteria (Robarts and Zohary, 1987; Davis *et al.,* 2009; O'Neil *et al.*, 2012). Phytoplankton cell density, and chlorophyll *a* concentration, positively correlated to water temperature during the study. Phytoplankton cell density in Hartbeespoort Dam during autumn was significantly greater than phytoplankton cell density during winter. Phytoplankton was therefore more abundant during the warmer autumn period in Hartbeespoort Dam as phytoplankton cellular processes (including photosynthesis) and growth-rates are temperature-dependent (Robarts and Zohary, 1987; Davis *et al.,* 2009; O'Neil *et al.*, 2012).

Cyanobacterial growth is stimulated by multiple environmental factors occurring simultaneously in waterbodies including suitable temperatures, oxygen concentration, light availability, and high nutrient concentrations (thriving in eutrophic and hypertrophic waters) (Harding *et al.*, 2009; Van Ginkel, 2011; O'Neil *et al.*, 2012; Matthews and Bernard, 2015). Phytoplankton community composition in freshwater systems is influenced by the concentration of inorganic nutrients in the waterbodies in which they exist (Swanepoel *et al.*, 2008). As previously discussed, the rapidly growing population in the Gauteng Province has resulted in increased surface runoff and anthropogenic nutrient pollution, which have led to a build-up of nutrients in Hartbeespoort Dam (Davis *et al*., 2009; Roux *et al*., 2010; O'Neil *et al.,* 2012). Freshwater phytoplankton community structures tend to shift towards cyanobacterial dominance when waterbodies become enriched with inorganic nutrients as these nutrients have a stimulatory effect on the growth of algae and aquatic plants (DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Xu *et al.*, 2010; O'Neil *et al*., 2012; Janse van Vuuren and Taylor, 2015; Gobler *et al.,* 2016).

As a shift in the phytoplankton community composition towards cyanobacteria dominance occurs when water becomes enriched with nutrients (DWAF, 1996: 5; Davis *et al*., 2009; Xu *et al.*, 2010; O'Neil *et al*., 2012; Janse van Vuuren and Taylor, 2015; Gobler *et al.,* 2016), cyanobacteria cell density strongly correlated to DIN and DIP concentrations as well as the DIN:DIP ratio. Cyanobacteria in Hartbeespoort Dam therefore strongly responded to changes in nutrient concentrations compared to other growth-promoting environmental variables (e.g., temperature).

It has been suggested that phosphorus is the primary nutrient controlling cyanobacterial proliferation and the degree of eutrophication (Utkilen and Gjølme, 1995; DWAF, 1996: 5; Rapala *et al*., 1997; Oh *et al.,* 2000; Xu *et al.*, 2010; O'Neil *et al*., 2012) as many bloom-forming cyanobacteria species in warm waters are able to fix atmospheric nitrogen (diazotrophic) thus balancing any nitrogen deficiencies in the ecosystem (DWAF, 1996: 5; Davis *et al*., 2009; Xu *et al.*, 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al*., 2016). Phosphorus is therefore the more growth-limiting nutrient in freshwater systems and is considered the main nutrient controlling eutrophication (Utkilen and Gjølme, 1995; DWAF, 1996: 5; Rapala *et al*., 1997; Oh *et al.,* 2000; Xu *et al.*, 2010; O'Neil *et al*., 2012).

Cyanobacteria tend to dominate phytoplankton communities in temperate freshwater ecosystems with phosphorus concentrations of ~ 0.1 mg/L – 1.0 mg/L (Trimbee and Prepas, 1987; Jensen *et al.*, 1994; Watson *et al*., 1997; Downing *et al*., 2001; Xu *et al.*, 2010). During this study, orthophosphate concentrations across Hartbeespoort Dam ranged from 0.323 mg/L to 0.959 mg/L. Phosphorus concentrations in Hartbeespoort Dam were therefore optimal for a shift in the phytoplankton community structure towards cyanobacterial dominance.

Non-diazotrophic cyanobacteria, including *Microcystis* spp., depend on exogenous sources of nitrogen for growth and toxin production (Davis *et al*., 2009; Xu *et al*., 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al*., 2016). A shift in the cyanobacteria community structure from diazotrophic to non-diazotrophic cyanobacteria occurs when there are increased loads of both phosphorus and nitrogen (Davis *et al*., 2009; Xu *et al*., 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al*., 2016). Previous studies have therefore hypothesised that nitrogen is as important as phosphorus in controlling the proliferation and toxicity of cyanobacterial blooms (Davis *et al*., 2009; Xu *et al*., 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al*., 2016).

Orthophosphate concentration was used an indicator of the DIP concentration within the dam as orthophosphate is the only form of soluble inorganic phosphorus utilised by aquatic biota (DWAF, 1996: 5). DIN concentrations were obtained by combining ammonium and nitrate + nitrite concentrations. The DIN:DIP ratio was determined to identify the nutrient prevailing in Hartbeespoort Dam and therefore the nutrient controlling algal growth and biomass (Quevedo-Castro *et al*., 2019). The nutrient controlling algal growth will be consumed faster reaching a minimum value before the other nutrient (Quevedo-Castro *et al.,* 2019). For each sampling trip, the DIN:DIP ratio obtained was < 16 indicating that nitrogen was the main nutrient controlling cyanobacterial growth in Hartbeespoort Dam (Janse van Vuuren and Taylor, 2015). It is, however, unlikely that one nutrient controlled algal growth and biomass in Hartbeespoort Dam as both inorganic phosphorus and inorganic nitrogen concentrations were high during this study.

Hartbeespoort Dam is globally known for its periodic cyanobacterial blooms predominantly consisting of the non-diazotrophic *Microcystis aeruginosa (M.*

aeruginosa), the most common cyanobacterial bloom former (Steyn *et al*., 1975; Pearce, 1987; Robarts and Zohary, 1987; WHO, 2003; Xu *et al.*, 2010; Conradie and Barnard, 2012; O'Neil *et al.*, 2012; Ballot *et al*., 2014; Matthews, 2014). During the algal identification process, it was noted that *M. aeruginosa* dominated the cyanobacteria community in Hartbeespoort Dam, a finding similar to that of previous studies (Conradie and Barnard, 2012; Ballot *et al.*, 2014). As *M. aeruginosa* is non-diazotrophic, inorganic nitrogen concentration strongly correlated to cyanobacteria cell density during this study.

As cyanobacteria dominated 82.9% of the phytoplankton community structure in Hartbeespoort Dam during the study, a weak correlation between cyanobacteria cell density and chlorophyll *a* concentration was obtained. A low concentration of chlorophyll *a* is, however, not necessarily indicative of low phytoplankton biomass when the phytoplankton community is dominated by cyanobacteria (Swanepoel *et al.*, 2008).

Freshwater bodies with temperatures between 15ºC and 30ºC, the optimal temperature range for *Microcystis* spp., typically have persistent cyanobacterial blooms (Chorus and Bartram, 1999). Historically, cyanobacteria in South Africa have been most abundant between January and April (late summer to early autumn) (WHO, 1998; Van Ginkel, 2011; Conradie and Barnard, 2012; Ballot *et al.*, 2014; Matthews and Bernard, 2015), in eutrophic and/or hypertrophic waterbodies (WHO, 1998; Ballot *et al.*, 2014; Matthews and Bernard, 2015). Emphasis was placed on the changing seasons as this study progressed from the austral autumn (April) to mid-winter (July) as cyanobacterial growth is heavily influenced by water temperature (Robarts and Zohary, 1987; WHO, 1998; O'Neil *et al.,* 2012). It was therefore hypothesised that cyanobacteria cell density would be most abundant during the first sampling trip and decrease as the study progressed into winter.

Matthews (2014) found that cyanobacterial blooms in South African impoundments reached their maximum coverage during autumn, with peak coverage occurring during April and then again in June. Cyanobacteria cell density of the June sampling trip was significantly lower than both the April and July sampling trips, however, cyanobacteria biomass obtained during the July sampling trip was significantly higher than both the April and June sampling trips. Cyanobacteria cell density therefore negatively

correlated to water temperature in Hartbeespoort Dam during this study. Winter cyanobacterial blooms appear to be a common occurrence in South Africa although the environmental conditions driving these events are unclear (Oberholster *et al.*, 2009; Matthews, 2014). In some South African impoundments, water temperature alone therefore does not seem to control cyanobacterial bloom formation (Oberholster *et al.*, 2009; Matthews, 2014).

Cyanobacteria are capable of allelopathy, a process whereby chemicals produced by a plant inhibit the growth, germination, or metabolism of another plant (Pearce, 1987). In the RDA ordination, phytoplankton and cyanobacteria cell densities were separated by the RDA 2 axis as cyanotoxins have been shown to inhibit the growth of diatoms and other bloom-forming phytoplankton (Pearce, 1987). Toxin-producing cyanobacteria therefore appeared to gain a competitive advantage in Hartbeespoort Dam.

Cyanobacterial blooms are complex events that are typically caused by multiple physical and chemical system-specific factors occurring simultaneously (Davis *et al*., 2009; Conrad and Barnard, 2012; O'Neil *et al.*, 2012). As cyanobacterial blooms did not occur in Hartbeespoort Dam during the study, it can therefore be assumed that suitable conditions required for bloom formation did not occur simultaneously.

4.3. Toxicity of Hartbeespoort Dam

Many cyanobacteria genera produce a range of potent toxins (cyanotoxins) that are poisonous or pose a health risk to animals and humans, alter the physico-chemical features of aquatic ecosystems, and alter the interactions between aquatic organisms (DWAF, 1996: 1; WHO, 1998; Oberholster *et al.*, 2005; Tonk *et al.*, 2007; Oberholster *et al.*, 2009; Xu *et al.*, 2010; Van Ginkel, 2011; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Ballot *et al.*, 2014; Matthews, 2014; Lone *et al.,* 2015; Matthews and Bernard, 2015; Lee *et al.*, 2017; Miller and Russell, 2017; Turner *et al.*, 2018). Microcystins are the most prevalent cyanotoxins worldwide and altogether different studies have identified between 10 and 41 microcystin variants in Hartbeespoort Dam (Oberholster *et al.*, 2005; Ballot *et al*., 2014; Lone *et al.*, 2015).

Based on previous literature and the knowledge that *Microcystin* spp. tend to dominate the phytoplankton community of Hartbeespoort Dam (Conradie and Barnard, 2012; Ballot *et al.*, 2014), total combined microcystin (cell bound microcystin and released

microcystin) concentration in the water samples was determined using the MC-ADDA method. Light microscopy is not sufficient for the detection of toxin concentrations within a waterbody despite light microscopy being a useful tool for determining the presence and the density of potentially toxin-producing cyanobacterial species within a waterbody (Turner *et al.*, 2018). As over 90 microcystin variants exist in freshwater systems worldwide (Oberholster *et al.*, 2005; Ballot *et al*., 2014; Lone *et al.*, 2015), the MC-ADDA method was used to determine microcystin concentration in Hartbeespoort Dam as ADDA is a chemically cleaved amino acid side chain common to all microcystin variants (WHO, 2003; Birbeck *et al.*, 2019).

Increased formations of high-density cyanobacterial blooms and high toxin concentrations could be attributed to increased water temperatures and nutrient concentrations within the water column (Turner *et al.*, 2018). It has been suggested that elevated temperatures promote the growth of toxic *Microcystis* strains, and that toxin production is abundant in water temperatures between 20ºC and 25ºC (WHO, 1998; Davis *et al.,* 2009; O'Neil *et al*., 2012). It has been suggested that toxic *Microcystis* strains may outgrow non-toxic *Microcystis* strains in waterbodies with elevated temperatures as elevated temperatures tend to promote the growth of toxic *Microcystis* strains (Davis *et al.,* 2009; O'Neil *et al*., 2012). It has also been suggested that the production of microcystin synthetase (the enzyme required to produce microcystins) increases with elevated temperatures (Davis *et al.*, 2009; O'Neil *et al*., 2012). Toxic cyanobacterial bloom formation is therefore influenced by increased water temperatures (Davis *et al.,* 2009; O'Neil *et al*., 2012).

Previous studies found that toxin production in Hartbeespoort Dam was at its maximum during the summer periods as microcystin concentrations reached a maximum of 3 200 µg/L in March 2005 (WHO, 1998; Conradie and Barnard, 2012). Mean water temperature in Hartbeespoort Dam decreased from 22.3°C in autumn to 13.6°C in winter. Microcystin concentration was therefore expected to reach a maximum during the first sampling trip and decrease as the study progressed into winter. Microcystin-ADDA concentration in Hartbeespoort Dam, however, negatively correlated to water temperature as MC-ADDA concentration significantly increased as the study progressed into winter. As previously discussed, the dilution capacity of Hartbeespoort Dam was poor during the winter months. Increased concentrations of MC-ADDA during the winter months could therefore be attributed to a lack of dilution

factor in the dam as water temperature during winter was unfavourable for toxin production.

Furthermore, Turner *et al.* (2018) suggested that the growth and toxicity of cyanobacterial blooms are influenced by specific localised environmental factors such as nutrient concentrations. Toxic *Microcystis* strains have a higher nitrogen requirement than non-toxic *Microcystis* strains as nitrogen is required by the cell to produce the enzyme required for microcystin synthesis (Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012). Microcystins are nitrogen-rich toxins which further suggests a higher nitrogen requirement by toxic *Microcystis* strains (Conradie and Barnard, 2012). High nitrogen levels in the environment therefore enable toxic *Microcystis* strains to outgrow non-toxic *Microcystis* strains as growth and toxicity increases with increasing nitrogen loads (Davis *et al*., 2009).

Additionally, in the case of bloom-forming, non-diazotrophic cyanobacteria, such as *M. aeruginosa*, nitrogen may limit cyanobacterial proliferation as non-diazotrophic cyanobacteria depend on exogenous sources of nitrogen for growth and toxin production (Davis *et al*., 2009; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al.,* 2016). It has been suggested that increased loads of both phosphorus and nitrogen result in a shift from diazotrophic to non-diazotrophic cyanobacteria (Davis *et al*., 2009; Xu *et al.*, 2010; O'Neil *et al*., 2012; Mitchell *et al.*, 2014; Gobler *et al.,* 2016).

Both inorganic nutrient and microcystin concentrations significantly increased in Hartbeespoort Dam as the study progressed from summer into winter. As *Microcystis* spp. dominated the phytoplankton community structure in Hartbeespoort Dam during this study, cyanobacteria strongly correlated to both DIP and DIN concentrations in the dam. As growth and toxicity of *Microcystis* spp. increases with increasing nitrogen concentrations (Davis *et al.*, 2009), a positive correlation between DIN and MC-ADDA concentrations was obtained. A weaker correlation between DIP and MC-ADDA concentrations was attributed to increased concentrations of both DIP and DIN, resulting in a shift in the cyanobacteria community structure from diazotrophic to non-diazotrophic cyanobacteria (Davis *et al*., 2009; Xu *et al*., 2010; O'Neil *et al*., 2012; Gobler *et al*., 2016). Microcystin concentration in Hartbeespoort Dam was therefore influenced by concentrations of both inorganic phosphorus and inorganic nitrogen.

Across the six sampling sites, microcystin concentration did not correlate to inorganic nutrient concentrations. Microcystin concentration at the Crocodile River (Site H3) was significantly lower than microcystin concentration at the Magalies River entrance (Site H6) whereas inorganic nutrient concentrations at the Crocodile River were significantly higher than inorganic nutrient concentrations at the Magalies River entrance during the study. Additionally, MC-ADDA concentration was significantly low in the Crocodile River whereas maximum cyanobacteria cell density in the Crocodile River was obtained. Microcystin-ADDA concentrations therefore did not correlate to cyanobacteria cell density and inorganic nutrient concentrations in the Crocodile River during this study.

If microcystin-producing cyanobacteria dominate the community in recreational water samples containing 20 000 cells/mL, a microcystin concentration of 2-4 µg/L can be expected whereas a microcystin concentration of 20 µg/L can be expected in water samples containing 100 000 cells/mL (WHO, 2003). Microcystin concentrations of 2-4 µg/L and 20 µg/L represent the WHO's guideline for relatively low probability and moderate probability of adverse health effects, respectively (WHO, 2003; Turner *et al.*, 2018; Hartnell *et al.*, 2020). The WHO's guideline value of 20 µg/L for MC-LR concentration has therefore been used in this study as an indication of the suitability of Hartbeespoort Dam as a recreational reserve (WHO, 2003).

Morphologically indistinguishable toxic and non-toxic strains of the same cyanobacteria species can co-exist within a cyanobacterial bloom rendering a bloom as potentially dangerous and suspect at all times (Ressom *et al.*, 1994; WHO, 1998; Oberholster *et al.*, 2005; Swanepoel *et al.*, 2008; Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012; Ballot *et al.*, 2014; Turner *et al.*, 2018). Additionally, between 40 and 70% of cyanobacterial blooms worldwide are toxic (Turner *et al.*, 2018). As cell toxin content varies considerably between strains, toxin concentrations therefore do not necessarily correlate to cyanobacteria cell densities (Turner *et al.*, 2018).

Turner *et al.* (2018) found that of the samples containing cyanobacteria cell densities greater than 20 000 cells/mL, only 18% contained microcystin concentrations greater than the WHO moderate health guideline of 20 µg/L (Hartnell *et al.*, 2020). Cyanobacteria cell density weakly correlated to MC-ADDA concentrations in

Hartbeespoort Dam during this study. During this study, mean MC-ADDA concentrations in Hartbeespoort Dam exceeded the WHO's moderate health guideline of 20 µg/L whereas mean cyanobacteria cell densities were less than 100 000 cell/mL (WHO, 2003). The cyanobacteria cell density threshold within Hartbeespoort Dam was not triggered despite microcystin concentrations being high enough to cause adverse health effects (WHO, 2003; Turner *et al.*, 2018). It was therefore assumed that highly toxic cyanobacterial cells existed within the low-density bloom in Hartbeespoort Dam during the study (Turner *et al.*, 2018).

Potential routes of exposure to cyanotoxins in recreational waters include direct contact of exposed body parts (i.e., eyes, mouth, and ears), accidental swallowing of the water, inhalation of the water, or the inhalation of particles from desiccated cells that have been washed ashore and become airborne (Falconer *et al.*, 1999; WHO, 2003; Turner *et al.*, 2018). As previously mentioned, the WHO's moderate probability health alert guideline value of 20 µg/L for MC-LR concentration was used as an indication of the suitability of Hartbeespoort Dam as a recreational reserve (WHO, 2003). In relation to potential health effects, microcystin concentrations obtained across the six sampling sites and five sampling depth intervals during this study exceeded the WHO's moderate probability health alert threshold (WHO, 2003; Turner *et al.*, 2018). Mean MC-ADDA concentration during the study ranged from 309.03 µg/L (at Site H4) to 559.21 µg/L (at Site H6). Exposure to microcystin concentration > 20 µg/L can impair human health (WHO, 2003). The microcystin concentrations in Hartbeespoort Dam exhibited a significant risk to both humans and animals accessing the dam, a risk that could increase in the future with the predicted changes in temperature and rainfall patterns as a result of climate change (Turner *et al.*, 2018). Hartbeespoort Dam was therefore not suitable as a recreational reserve as cyanotoxins are a public health threat in the dam (Oberholster *et al.,* 2009; Mathews, 2014).

4.4. Suitability of Hartbeespoort Dam as a Water Resource

Hartbeespoort Dam was originally built for irrigation purposes, supplying the surrounding agricultural activities and farmlands (Steyn *et al*., 1975; Scott *et al*., 1980; Pearce, 1987; Ballot *et al.,* 2014). Additionally, the dam has subsequently been used as a recreational reserve and to supply domestic water to Brits (Steyn *et al*., 1975; Pearce, 1987; Conradie and Barnard, 2012; Ballot *et al.*, 2014). According to

CyanoLakes, the cyanobacterial health risk of Hartbeespoort Dam was low in 2018 as nutrient pollution was low (CyanoLakes, 2018). Full contact recreational activity was therefore allowed (CyanoLakes, 2018).

As part of the Department of Water Affairs and Forestry's mission to protect South Africa's water resources, the South African Water Quality Guidelines were developed (DWAF, 1996: 1-6). The guidelines serve as the primary source of information for determining the suitability of water for different water uses based on different water quality properties (DWAF, 1996: 1-6). The guidelines are technical documents that have been established and implemented to protect and maintain water resources in South Africa (DWAF, 1996: 1-6).

An acceptable ammonia range for agricultural use was not available and an acceptable inorganic phosphorus range for both domestic and agricultural uses were not available (DWAF, 1996: 1-6).

4.4.1. Domestic water use

Chlorophyll *a* concentration, algal cell densities, inorganic nutrient and total dissolved salt concentrations, pH readings, and microcystin concentrations obtained during the study at the Hartbeespoort Dam Wall (Site H1) were compared to the acceptable ranges outlined in the South African Water Quality Guidelines for Domestic Use (DWAF, 1996: 1) as the Crocodile River supplies water to the inhabitants of Brits (Roux *et al.*, 2010). Chlorophyll *a* concentration obtained at Site H1 across the study period was greater than the Target Water Quality Range (TWQR) of 1 µg/L (DWAF, 1996: 1). As the mean chlorophyll *a* concentration was < 7 µg/L, the water was unlikely to have a murky appearance (DWAF, 1996: 1). Taste and odour problems at Site H1 were, however, likely as a total cyanobacteria cell density of 8 103 cells/mL was obtained during the study (> 1 000 cells/mL) (DWAF, 1996: 1). No acute health effects were expected; however, chronic ingestion of water was discouraged (DWAF, 1996: 1).

Total oxidised nitrogen (nitrate + nitrite), total dissolved salt concentrations, and pH measurements obtained at Site H1 during the study fell within the TWQR (DWAF, 1996: 1). No associated adverse health and aesthetic effects were therefore expected (DWAF, 1996: 1). As the ammonium concentration obtained at Site H1 was greater than the TWQR, possible taste and odour complaints from consumers may have occurred (DWAF, 1996: 1).

The major oral and dermal exposure routes to cyanotoxins include potable water (Falconer *et al*., 1999; WHO, 2003; Van Ginkel, 2011; Matthews, 2014; Oberholster *et al*., 2009; Lee *et al.*, 2017; Miller and Russel, 2017). The WHO's guideline value of 1.0 µg/L for MC-LR concentration (WHO, 1998) has been adopted by the DWAF in the South African Water Quality Guidelines for Domestic Use (DWAF, 1996: 1). A mean MC-ADDA concentration of 300.12 µg/L at Site H1 across the study was obtained. Exposure to a microcystin concentration > 1 µg/L can result in acute hepatotoxic effects (DWAF, 1996: 1). Severe gastroenteritis and liver function impairment have been identified in populations supplied with potable water from waterbodies dominated by cyanobacteria (DWAF, 1996: 1; Oberholster *et al.*, 2005; Tonk *et al.*, 2007; Lone *et al.*, 2015; Matthews and Bernard, 2015; Lee *et al.*, 2017; Miller and Russell, 2017). Additionally, ingestion of sufficient concentrations of microcystins can result in liver haemorrhage and endotoxic shock (DWAF, 1996: 1; Oberholster *et al.*, 2005).

In terms of chlorophyll *a* concentration, algal cell densities, inorganic nutrient and total dissolved salt concentrations, and pH readings, water quality of water flowing out of Hartbeespoort Dam via the Crocodile River was suitable for domestic use (DWAF, 1996: 1). The microcystin concentration in the water flowing out of Hartbeespoort Dam, however, exceeded the guideline value posing a risk to downstream users (DWAF, 1996: 1; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Van Ginkel, 2011; Matthews, 2014; Matthews and Bernard, 2015). Water flowing out of Hartbeespoort Dam towards Brits was therefore not suitable for domestic use.

4.4.2. Recreational water use

Chlorophyll *a* concentration, water clarity, inorganic nutrient and total dissolved salt concentrations, pH readings, and microcystin concentrations obtained during the study at Site H2, Site H4, Site H5, and Site H6 within Hartbeespoort Dam were compared to the acceptable ranges outlined in the South African Water Quality Guidelines for Recreational Use (DWAF, 1996: 2). Recreational waters should have high clarity to ensure potential hazards to swimmers do not become obscured (DWAF, 1996: 2). Additionally, micro-organisms associated with suspended materials in turbid waters may pose a risk to the health of recreational users (DWAF, 1996: 2). Secchi disk depth (m) obtained at all four sites fell within the clarity range of 1.5 – 3 m (DWAF, 1996: 2). This clarity range is suitable for swimming and has a low risk of disease

transmission associated with particulate matter (DWAF, 1996: 2). Minimal eye irritations would have occurred at all four sites as the pH fell within the TWQR for recreational use (DWAF, 1996: 2).

Chlorophyll *a* concentrations obtained at Sites H4 and H6 were within the TWQR for full-contact exposure to water (DWAF, 1996: 2). Chlorophyll *a* concentrations obtained at Sites H2 and H5 were, however, greater than the TWQR indicating the potential for occasional occurrences of cyanobacterial scums (DWAF, 1996: 2). The water at Sites H2 and H5 therefore would have had a green colouration (DWAF, 1996: 2). All four sites fell within the TWQR for non-contact exposure to the water whereby accidental exposure was unlikely to result in adverse health effects (DWAF, 1996: 2).

Recreational use ranges for total dissolved salts, ammonia, and nitrate + nitrite concentrations are not available. The domestic use ranges were therefore used as an indication of the suitability of Hartbeespoort Dam for recreational use (DWAF, 1996: 1). Both TDS and nitrate + nitrite concentrations obtained at the four sites fell within the acceptable range for domestic and therefore recreational use (DWAF, 1996: 1). The ammonia concentrations obtained at Sites H2, H4, and H5, however, did not fall within the acceptable range for domestic use (DWAF, 1996: 1).

A microcystin concentration guideline value for recreational uses was not available therefore the WHO's moderate probability health alert guideline value of 20 µg/L for MC-LR concentration was used as an indication of the suitability of Hartbeespoort Dam as a recreational reserve. Water quality of Hartbeespoort Dam in terms of chlorophyll *a* concentration, water clarity, nitrate + nitrite and total dissolved salt concentrations, and pH readings was suitable as a recreational reserve, like Site H1 (DWAF, 1996: 1, 2). The microcystin concentration in Hartbeespoort Dam, however, exceeded the guideline value posing a significant risk to recreational users (DWAF, 1996: 1; WHO, 2003; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Van Ginkel, 2011; Matthews, 2014; Matthews and Bernard, 2015). As previously discussed, Hartbeespoort Dam was not suitable as a recreational reserve.

4.4.3. Agricultural water use

As water from Hartbeespoort Dam is used for irrigation (Steyn *et al*., 1975; Scott *et al*., 1980; Pearce, 1987; Ballot *et al.,* 2014), inorganic nutrient and total dissolved salt concentrations, and pH readings obtained during the study at Sites H1, H2, H4, H5,

and H6 were compared to the acceptable ranges outlined in the South African Water Quality Guidelines for Agricultural Use: Irrigation (DWAF, 1996: 3). The pH readings and inorganic nitrogen concentrations obtained at all five sites across the study fell within the TWQR (DWAF, 1996: 3). Furthermore, the total dissolved salt concentrations obtained at all five sites across the study period did not fall within the TWQR, however, were within the range suitable for maintenance of 90% relative crop yields of moderately salt-sensitive crops (DWAF, 1996: 3).

Furthermore, it has been found that cyanotoxins, particularly microcystins, can accumulate in consumable animal and plant tissues (Lee *et al.,* 2017). Agricultural plants accumulate cyanotoxins when irrigated with cyanobacteria-contaminated water thus acting as a potential cyanotoxin reservoir (Lee *et al.*, 2017; Miller and Russell, 2017). Cyanotoxin accumulation in edible plants is dose-dependent and reduces the quality and productivity of the crop (Lee *et al.*, 2017; Miller and Russell, 2017). Mean microcystin concentration obtained at Site H1 during the study was 300.12 µg/L which is greater than the WHO guideline of < 1 µg/L (WHO, 1998). Additionally, mean microcystin concentration at Site H2, H4, H5, and H6 ranged from 309.03 µg/L to 559.21 µg/L. Water from Hartbeespoort Dam was therefore not suitable to be used as irrigation water.

As the Crocodile River, and two associated irrigation canals, are the only large outflows of Hartbeespoort Dam supporting downstream farming activities (Roux *et al.*, 2010), algal cell densities and inorganic nutrient and total dissolved salt concentrations obtained at Site H1 across the study were also compared to the acceptable ranges outlined in the South African Water Quality Guidelines for Agricultural Use: Livestock Watering (DWAF, 1996: 4). Total cyanobacteria cell density of 8 103 cells/mL was obtained during the study (> 2 000 cells/mL) (DWAF, 1996: 4). Livestock watering water with a cyanobacteria cell density > 2 000 cells/mL has a low risk of acute toxic effects (DWAF, 1996: 4). Both the nitrate + nitrite and total dissolved salt concentrations obtained at Site H1 were within the acceptable range (DWAF, 1996: 4). Water from Hartbeespoort Dam was therefore suitable for livestock watering.

4.5. Water hyacinth (*Eichhornia crassipes*) in Hartbeespoort Dam

Water hyacinth (*Eichhornia crassipes*) is the most important freshwater macrophyte in South Africa and has become invasive in many South African waterbodies including

rivers and manmade impoundments (Hill, 2003; Hill *et al*., 2020; Auchterlonie *et al.*, 2021). Invasion of water hyacinth in waterbodies is as a result of anthropogenic distribution, and increased nutrient enrichment and cultural eutrophication (DWAF, 1996: 2; Van Ginkel, 2011; Matthews and Bernard, 2015; Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). Additionally, water hyacinth (and other aquatic macrophytes) undergoes rapid asexual reproduction and produce long-lived seeds contributing to their invasiveness (Hill and Coetzee, 2017; Hill *et al.*, 2020). Large impenetrable mats of water hyacinth were found on the surface of Hartbeespoort Dam throughout the study period. Negative impacts of eutrophication in Hartbeespoort Dam have therefore resulted in increased primary growth of water hyacinth (DWAF, 1996: 2; Oberholster *et al.*, 2005; Oberholster *et al.*, 2009; Van Ginkel, 2011; Matthews and Bernard, 2015; Auchterlonie *et al.*, 2021).

Invasive aquatic macrophytes are established in slow-flowing eutrophic impoundments as macrophytes rely on disturbances in the aquatic ecosystem (Hill *et al.*, 2020). Invasive aquatic macrophytes can gain a competitive advantage over native aquatic macrophytes as they lack natural enemies allowing them to rapidly proliferate (Hill *et al.*, 2020). Water hyacinth are invasive free-floating macrophytes that form dense floating mats on the surface of waterbodies limiting the light available to submerged aquatic biota, including phytoplankton species (DWAF, 1996: 3, 6; Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). These impenetrable hyacinth mats decrease dissolved oxygen in the aquatic ecosystem as phytoplankton growth and photosynthetic activity is limited (Pearce, 1987; Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). Decreased phytoplankton abundance alters invertebrate community composition therefore reducing the diversity of the aquatic ecosystem (Coetzee *et al.*, 2014; Hill *et al.*, 2020)

Additionally, large dense floating mats of invasive aquatic macrophytes have economic implications for tourism, recreational, and fishing industries (Pearce, 1987; Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). Dense floating mats limit access to the sporting and recreational capabilities of the waterbody and disturb the biodiversity of the aquatic ecosystem (Pearce, 1987; Hill, 2003; McConnachie *et al.*, 2003; Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). Additionally, property values along the shores of such waterbodies are affected as dense mats of invasive aquatic macrophytes are not aesthetically pleasing (McConnachie *et al.*, 2003; Hill *et al.*, 2020; Auchterlonie *et al.*,

2021). Invasive aquatic macrophytes in waterbodies therefore have negative economic impacts.

The presence of water hyacinth in the Vernon Hooper Dam, however, improved water quality as the cost of water treatment was reduced by 61% (Pearce, 1987). Water hyacinth removes large concentrations of nutrients (phosphorus, nitrogen, and potassium) from a waterbody while increasing sedimentation and lowering the biological oxygen demand (BOD) and TSS (Wolverton and McKnown, 1979; Pearce, 1987; Harding and Paxton, 2001; Auchterlonie *et al.*, 2021). Additionally, it has been found that water hyacinth plays a role in reducing algae present within a waterbody through settling, respiration, attachment, and growth suppression (Kim and Kim, 2000; Auchterlonie *et al.*, 2021). The phytoremediation potential of water hyacinth improves the quality of water (Pearce, 1987; Harding and Paxton, 2001; Auchterlonie *et al.*, 2021). Water hyacinth is therefore a useful tool for removing excess inorganic nutrients from eutrophic waterbodies such as Hartbeespoort Dam (Auchterlonie *et al.*, 2021).

During the study period, water hyacinth was not confined and was transported around the dam by wind and water currents as water hyacinth are free-floating aquatic macrophytes (Hill *et al.*, 2020; Auchterlonie *et al.*, 2021). According to previous literature, the conditions for cyanobacterial bloom formation were present in Hartbeespoort Dam, however, a bloom did not occur during the study period. As previously mentioned, water hyacinth plays a role in reducing algae present within a waterbody (Kim and Kim, 2000). It has been suggested that water hyacinth has allelopathic effects on phytoplankton communities as a negative correlation between water hyacinth and phytoplankton biomass, and chlorophyll *a* concentration, has been found (Pearce, 1987). When compared to previous studies, cyanobacteria dominance in Hartbeespoort Dam was lower in April 2018 when compared to April 2011 (Ballot *et al.*, 2014). Additionally, chlorophyll *a* concentrations between February 2017 and September 2018 were low (< 25 µg/L) when compared to other studies (Conradie and Barnard, 2012). Water hyacinth in Hartbeespoort Dam therefore appears to control phytoplankton and cyanobacterial growth thus preventing bloom formation in the dam (Auchterlonie *et al.*, 2021).

Cyanobacteria cell density in Hartbeespoort Dam was significantly higher during Sampling Trip 3, a finding that was not expected. Water hyacinth, however, naturally

dies during the winter months (Pearce, 1987). A decreased water hyacinth population during the winter months increases the availability of inorganic nutrients and light to cyanobacteria thus enabling cyanobacterial growth (Pearce, 1987). The unexpected increase in cyanobacteria cell density could potentially have been attributed to the dying water hyacinth population during the winter months. During the study, the percentage cover of aquatic vegetation (including algal blooms, water hyacinth, and other macrophytes) in Hartbeespoort Dam decreased from 18.04% during April 2018 to 16.5% during July 2018 (Singh *et al.*, 2020). The percentage cover of aquatic vegetation, including water hyacinth, decreased slightly as the study progressed into winter. The unexpected cyanobacteria cell density obtained during July 2018 was therefore unlikely as a result of the dying water hyacinth population in Hartbeespoort Dam.

As water hyacinth is an invasive aquatic macrophyte, it can be mechanically, chemically, and biologically controlled (Makofane, 2018; Auchterlonie *et al.*, 2021). Mechanical control involves the removal of water hyacinth by hand or using machines often resulting in plant matter being left behind increasing the potential for water hyacinth regrowth (Makofane, 2018; Auchterlonie *et al.*, 2021). Biological control of water hyacinth involves the release of natural enemies into waterbodies whereas the chemical control of water hyacinth involves the *in situ* application of herbicides, particularly glyphosphates (Makofane, 2018; Auchterlonie *et al.*, 2021).

Dying water hyacinth has negative impacts on the aquatic ecosystem as decaying plant matter results in increased nutrient loads within the waterbody (Pearce, 1987; Auchterlonie *et al.*, 2021) supporting the formation of cyanobacterial blooms. Additionally, dying water hyacinth results in hypoxic, and sometimes anoxic, aquatic environments as bacteria utilise dissolved oxygen during plant decomposition (DWAF, 1996: 5; Auchterlonie *et al.*, 2021). Chemical control of water hyacinth in waterbodies is therefore discouraged as decomposing water hyacinth adversely impacts the quality of the aquatic ecosystem.

As previously discussed, large dense mats of water hyacinth on the surface of a waterbody are, however, not an economically viable mechanism for improving water quality (Pearce, 1987; Auchterlonie *et al.*, 2021). It is recommended that a small population of water hyacinth in a confined area of Hartbeespoort Dam is maintained

at a size that will not negatively affect dissolved oxygen in the dam (Pearce, 1987). Water hyacinth harvesting programmes should be implemented, and water hyacinth should be harvested as rapidly as it proliferates and composted on-site (Pearce, 1987; Auchterlonie *et al.*, 2021). Harvesting and composting requires adequate on-site facilities and infrastructure increasing initial costs (Pearce, 1987). Water treatment costs should, however, decrease because of improved water quality in Hartbeespoort Dam. Confining a small population of water hyacinth in a waterbody therefore appears to be a more viable solution for maintaining water quality whilst allowing recreational activities to continue (Pearce, 1987).

5. Conclusion

The ecological health of Hartbeespoort Dam varied during the study. As the study progressed into winter, water temperature significantly decreased. As water temperature increases, the solubility of oxygen decreases thereby reducing the concentration of dissolved oxygen available to aquatic organisms (DWAF, 1996: 5; Matowanyika, 2010). Dissolved oxygen in Hartbeespoort Dam, however, correlated to the photosynthetic activity of phytoplankton as both phytoplankton cell density and dissolved oxygen reached a maximum at the beginning of autumn.

Rainfall events in the catchment area of Hartbeespoort Dam occur predominantly in the summer to autumn months (November to April) and are scarce during the winter months (May to October) (Matowanyika, 2010). Electrical conductivity decreased as the study progressed into winter as surface runoff from urban, industrial, agricultural, and mining activities decreased due to scarce rainfall and storm events during winter (DWAF, 1996: 5; Matowanyika, 2010). Inorganic nutrient concentrations, however, increased as the study progressed into winter as scarce rainfall and storm events during winter reduced the dilution capacity of Hartbeespoort Dam (Matowanyika, 2010; Mitchell *et al.*, 2014). This study identified that the high-water volumes and significantly high nutrient loads entering Hartbeespoort Dam were attributed to the heavily polluted Crocodile River, and its tributaries (Steyn *et al*., 1975; Roux *et al.,* 2010; Dube *et al.*, 2017). The rapidly growing population and urbanisation in the Gauteng Province has resulted in increased runoff from urban, industrial, agricultural, and mining activities delivering high nutrient loads to Hartbeespoort Dam (Steyn *et al.*, 1975; DWAF, 1996: 5; Davis *et al*., 2009; Matowanyika, 2010; Roux *et al*., 2010; Conradie and Barnard, 2012; O'Neil *et al.,* 2012; Harding, 2015; Dube *et al.*, 2017; Quevedo-Castro *et al.*, 2019). Hartbeespoort Dam was therefore representative of a eutrophic system during the study.

Cyanobacterial growth is stimulated by multiple environmental factors occurring simultaneously in waterbodies including suitable temperatures, oxygen concentration, light availability, and high nutrient concentrations (Harding *et al.*, 2009; Van Ginkel, 2011; O'Neil *et al.*, 2012; Matthews and Bernard, 2015). Cyanobacteria dominated the phytoplankton community throughout the study reaching a maximum cell density during mid-winter. As Hartbeespoort Dam is heavily enriched with both inorganic

phosphorus and inorganic nitrogen, cyanobacteria cell density strongly correlated to inorganic nutrients in the dam. Based on previous literature, cyanobacteria cell density was expected to be abundant during the summer months (WHO, 1998; Van Ginkel, 2011; Conradie and Barnard, 2012; Ballot *et al.,* 2014; Matthews and Bernard, 2015). Cyanobacteria cell density was, however, abundant in Hartbeespoort Dam during the winter months as winter cyanobacterial blooms appear to be a common occurrence in South Africa (Oberholster *et al.*, 2009; Matthews, 2014).

The concentration of microcystin significantly increased as the study progressed into winter possibly due to a lack of dilution capacity of Hartbeespoort Dam during the winter months (Matowanyika, 2010; Mitchel *et al.*, 2014). Microcystin concentration positively correlated to inorganic nitrogen concentration as toxic *Microcystis* strains have a higher nitrogen requirement than non-toxic strains and microcystins are nitrogen-rich toxins (Davis *et al.,* 2009; Conradie and Barnard, 2012; O'Neil *et al*., 2012). A weak correlation between cyanobacteria cell density and microcystin concentration was obtained, possibly because not all cyanobacterial strains within the cyanobacteria community are able to produce toxins (WHO, 1998; Oberholster *et al.*, 2005; Swanepoel *et al*., 2008; O'Neil *et al.*, 2012; Ballot *et al*., 2014). The microcystin concentrations in Hartbeespoort Dam exhibited a significant risk to both humans and animals accessing the dam, a risk that could increase in the future with the predicted changes in temperature and rainfall patterns as a result of climate change (Turner *et al.*, 2018). Hartbeespoort Dam was therefore not suitable as a recreational reserve as cyanotoxins are a public health threat in the dam (Oberholster *et al.,* 2009; Mathews, 2014).

According to previous literature, the conditions for cyanobacterial bloom formation were present in Hartbeespoort Dam, however, a bloom did not occur during the study period. Water hyacinth was present as large dense mats on the surface of the dam throughout the study. Water hyacinth removes large concentrations of nutrients (phosphorus, nitrogen, and potassium) from a waterbody and has been found to reduce algae present within a waterbody through settling, respiration, attachment, and growth suppression (Kim and Kim, 2000; Auchterlonie *et al.*, 2021). Water hyacinth in Hartbeespoort Dam therefore appears to control phytoplankton and cyanobacterial growth thus preventing bloom formation in the dam as it is a useful tool for removing excess inorganic nutrients from eutrophic waterbodies (Auchterlonie *et al.*, 2021). It

was recommended that a small population of water hyacinth in a confined area of Hartbeespoort Dam is maintained to improve the quality of water whilst ensuring the continuation of recreational activities.

Hartbeespoort Dam poses a risk to human health and it is advised to approach the dam with caution.

5.1. Limitations to this study:

Several limitations were experienced during this study, including:

- Sampling trip one was cut short due to an afternoon thunderstorm over Hartbeespoort Dam. This prohibited us from obtaining data for our last two sampling sites.
- Water hyacinth was present in the dam across all three sampling trips and limited our movement around the dam on sampling trips one and three. This prohibited us from obtaining data from certain sampling sites.
- Samples for the measurement of phytoplankton community composition were collected in 100 mL plastic containers and fixed with 1 mL Lugol's iodine solution on site. The samples from the second and third sampling trip were too dark to view under the microscope and were diluted with 10 mL of deionized water prior to viewing under the microscope. As the Lugol's solution was too dark, it is recommended that formalin or gluteraldehyde be used in future studies to fix phytoplankton in a water sample.
- This study tested for total MC concentrations and not for multiple cyanotoxins including BMAA.

Had the abovementioned limitations been overcome, this study could have been enhanced.

5.2. Future Works

Following this study, future work on the research topics is suggested:

- Determination of the potential correlation between cyanobacteria and water hyacinth as well as the role of water hyacinth in Hartbeespoort Dam.
- Aquatic macrophytes are closely related to freshwater snail such as *Bulinus africanus* (*B. africanus*), an intermediate host of bilharzia (*Schistosoma*

haematobium) (De Kock *et al.,* 2003). Perform risk surveys to confirm the presence, or lack thereof, of bilharzia host snails in Hartbeespoort Dam.

- Investigate the interactions between zoo- and phytoplankton, with a focus on the production of microcystins in response to predation pressure.
- Conduct a time lag response study of cyanobacterial bloom decay and microcystin release.

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7. Role of Each Author

[1] Cassidy Kuiper: collected all water samples using the 2 L phytoplankton Van Dorn sampler and distributed the water into the necessary containers during each sampling trip. During each sampling trip, determined Secchi disk depth at each sampling site. Performed all laboratory procedures used in this study including, but not limited to, the determination of chlorophyll *a* concentration, inorganic nutrient concentrations, and total microcystin concentrations using an ELISA. Performed all microscopy needed for this study and the statistical and quantitative analyses. This manuscript was drafted by the corresponding author.

[2] Dr Gavin Snow: collected all *in situ* physico-chemical parameters using a YSI Professional Plus Handheld Multiparameter meter during each sampling trip. During each sampling trip, determined Secchi disk depth at each sampling site and filtered water, for the analysis of inorganic nutrients, through 0.45 µm glass fibre syringe filters. Dr. Gavin Snow assisted the corresponding author with some laboratory procedures including the determination of chlorophyll *a* concentration and inorganic nutrient concentrations. Dr Gavin Snow supervised this study and has assisted with the editing of this manuscript.

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9. Appendix A

Table 9.1: Monthly meteorological data for Hartbeespoort Dam during the period of August 2017 to July 2018 were obtained from the South African Weather Service (SAWS) (Hartbeespoort Weather Station 0512554 4; 25.7480°S, 27.8320°E).

Year	Month	Average Temperature (°C)		Total Rainfall (mm)	Average Humidity (%)
		Minimum	Maximum		
2017	August	5.06	23.35	0.0	61.6
	September	10.16	31.48	6.0	53.7
	October	12.69	30.04	63.4	57.4
	November	14.35	32.63	87.8	48.4
	December	17.11	32.06	82.2	61.0
2018	January	16.99	34.09	80.4	52.7
	February	17.57	31.13	75.8	67.8
	March	15.58	30.65	157.6	73.3
	April	12.44	27.47	100.4	81.5
	May	6.67	23.66	19.2	82.5
	June	2.15	21.95	0.2	84.1
	July	2.28	20.97	0.4	78.3
Average		**11.09**	**28.46**	**56.1**	**66.9**

10. Appendix B

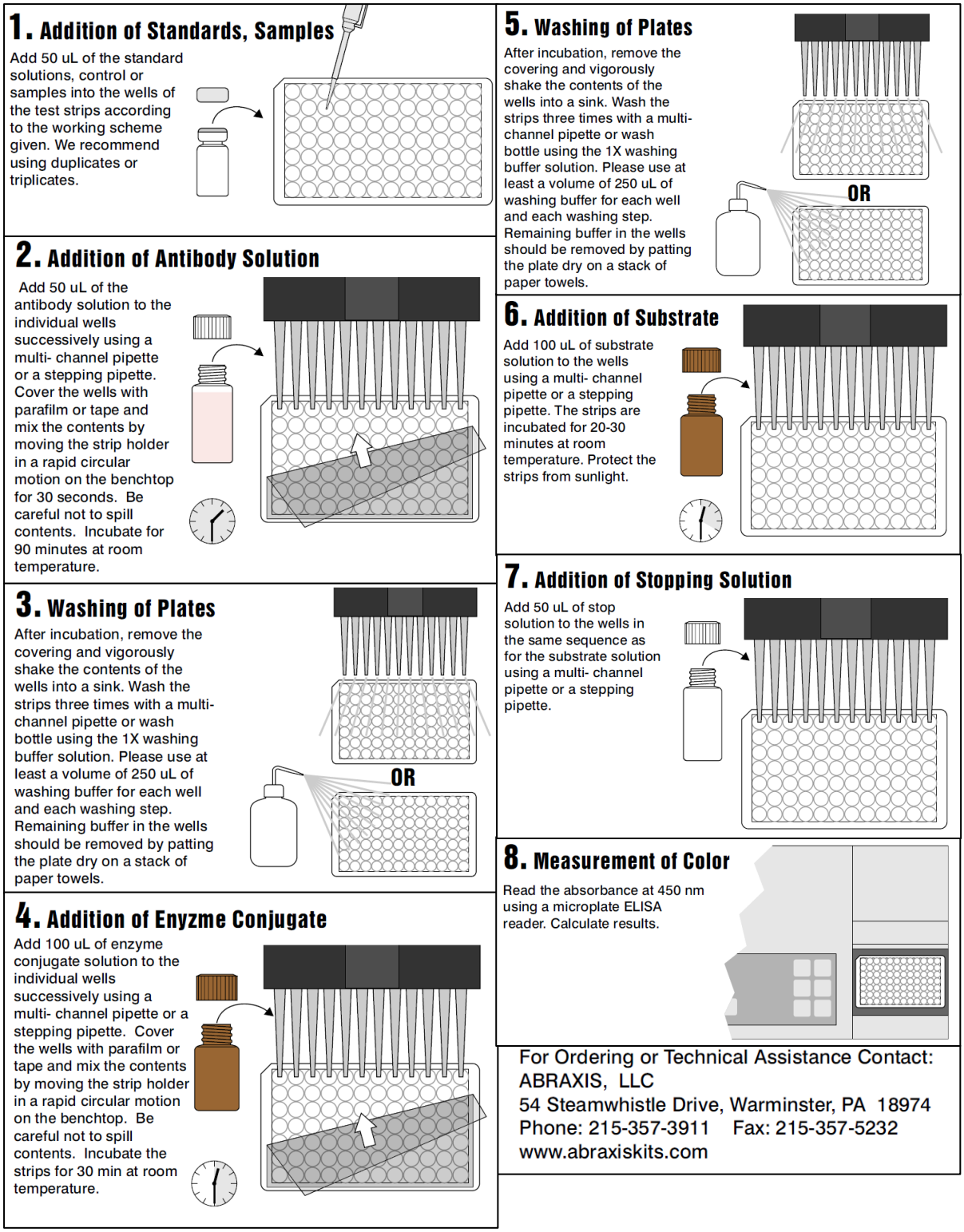

Figure 10.1: Abraxis Detailed ELISA Procedure used during this study to determine the MC-ADDA concentrations.

11. Appendix C

Table 11.1: Nutrient loads (kg/day) entering Hartbeespoort Dam via the Crocodile River were calculated for each sampling trip using nutrient concentrations (mg/L) obtained in the Crocodile River (Site H3). Crocodile River Daily Flow Rates (m3/day) were obtained from the Department of Water and Sanitation's (DWS) Crocodile River flow gauge A2H012 (Crocodile River at Kalkheuwel) on the day of each sampling trip.

Date	Crocodile River Daily Flow Rates (m^3/day)	Ammonium			Nitrate + Nitrite			Orthophosphate		
		Concentration		Load (kg/day)	Concentration		Load (kg/day)	Concentration		Load (kg/day)
		mg/L	kg/m^3		mg/L	kg/m^3		mg/L	kg/m^3	
2018/04/10	19.818	2.7476	0.0027	4704.65	6.8801	0.0069	11780.62	0.6364	0.0006	1089.69
2018/06/12	13.062	7.5927	0.0076	8568.79	4.5967	0.0046	5187.64	0.9510	0.0010	1073.26
2018/07/06	12.664	8.6162	0.0086	9427.58	4.9012	0.0049	5362.74	0.9164	0.0009	1002.70

Table 11.2: Nutrient loads (kg/day) entering Hartbeespoort Dam via the Crocodile River were calculated for each sampling trip. Dissolved inorganic phosphorus (DIP) loads (kg/day) were equivalent to orthophosphate loads whereas dissolved inorganic nitrogen (DIN) loads (kg/day) were obtained by combining ammonium and nitrate + nitrite loads. Mean loads entering Hartbeespoort Dam via the Crocodile River for this study were calculated.

Date	Ammonium Loads (kg/day)	Nitrate + Nitrite (kg/day)	DIN Loads (kg/day)	DIP Loads (kg/day)
2018/04/10	4704.65	11780.62	16485.27	1089.69
2018/06/12	8568.79	5187.64	13756.43	1073.26
2018/07/06	9427.58	5362.74	14790.33	1002.70
Mean Loads Entering Hartbeespoort Dam (kg/day)			**15010.68**	**1055.22**
Mean Loads Entering Hartbeespoort Dam (tonnes/day)			**15.01**	**1.06**