IMPACT OF CYANOBACTERIAL TOXINS ON WATER QUALITY AND SUPPLY

By

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DECLARATION

I declare that the dissertation hereby handed in for the qualification Master of Science in Botany at the University of the Free State is my own work and that I have not previously submitted the same work for a qualification at/in another university/faculty. I furthermore concede copyright of the dissertation to the University of the Free State.

Signed

L.R. Mohale

DEDICATION

I dedicate this work to my wonderful and loving husband, Thato Williams and my lovely son, Keabetsoe Williams. Without your emotional support, I wouldn’t be where I am today and this dream wouldn’t have become a reality. Thank you guys!

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CHAPTER 1

INTRODUCTION

1.1) BACKGROUND

Water, especially surface water, is an important resource for human life and activities hence why its quality is currently arousing considerable concern (Briand *et al*., 2003). Water quality is defined as the suitability of water to sustain various uses, and many water uses have specific requirements with regard to physical and chemical or biological variables or contaminants (Meybeck *et al*., 1996). Increasing human population growth, coupled with industrialisation and urbanisation, has resulted in an increase in the range of demands for water together with greater demands for higher quality water (Shuval, 1980; Meybeck & Helmer, 1996; Ashton, 2002). This, according to Shuval (1980), has led to a greater demand of the limited renewable freshwater resources.

There is growing evidence that human activities, including abstraction of water and discharge of wastes, may have specific and predictable effects on the quality of an aquatic system since these activities are changing the distribution and movement of the major nutrients resulting in increased nutrient loading to receiving waters (Shuval, 1980; Meybeck & Helmer, 1996; Murrell & Lores, 2004; Davies *et al*., 2009). This has led to accelerated eutrophication.

The introduction of the terms oligotrophic, mesotrophic, and eutrophic by the German biologist Weber in 1907 gave rise to the description of eutrophication (Connell & Miller, 1984; Harper, 1992; Connell, 2005). Eutrophication is defined as the enrichment of water by plant growth nutrients, usually phosphorus and nitrogen compounds, causing accelerated growth of algae and higher forms of plant life (Connell & Miller, 1984; Harper, 1992; Horne &

Goldman, 1994; Rast & Thornton, 1996; Connell, 2005; Tett *et al*., 2007). An undesirable disturbance to the balance of organisms and the impairment of quality of the water concerned are some of the undesirable effects of eutrophication (Krüger, 1978; Tett *et al*., 2007). Run-off and erosion from fertilized agricultural areas, erosion resulting from deforestation, and sewage are implicated as the major contributors to eutrophication (Chorus & Mur, 1999). Eutrophication is recognized as a serious and growing threat to lakes, rivers and estuaries (Hart, 2006), and it is expected that during the coming decades the driving force for eutrophication (the losses of nutrients from agriculture, organic wastes, sewage, sludge and ashes) will increase as a result of continuous world population growth (Rorsberg, 1998).

The combination of a complex set of factors related to eutrophication and climate change has resulted in the rise in the frequency and geographic spread of phytoplankton blooms (Adolf *et al*., 2009; Kouzminov *et al*., 2007). Global change is described by Huntley & Baxter (2006) as the changes that are currently taking place in various aspects of the global environment as a consequence of human activities. According to Davis *et al.* (2009), the earth's surface temperature has increased by approximately 1 ^{o}C during the 19th century and this is attributed to the burning of fossil fuels and subsequent rise in atmospheric carbon dioxide. Global warming leads to the lengthening of the optimal growth periods of cyanobacteria because it causes the lakes to stratify earlier in spring and destratify again later in autumn (Paerl & Huisman, 2008).

If eutrophication is allowed to continue, plant growth nutrient levels rise to a point where they do not restrain plant growth, and physical factors such as temperature and light availability become limiting, and this ultimate stage of eutrophication, according to Robarts (1984), is referred to as hypertrophy. Development in the Third World countries often results in eutrophication, and increasingly hypertrophy, threatening their water resources (Robarts, 1984). Although South Africa is generally not considered to be a Third World country,

Hartbeespoort Dam, South Africa, is an example of a hypertrophic system that has been under persistent blooms of cyanobacteria (Owuor *et al*., 2007). The phosphorus loading rates observed in Hartbeespoort Dam are amongst the highest recorded in South and southern Africa, thus the major cause of its hypertrophic status (Thornton & Ashton, 1989). Apparently, the trophic state of the dam is due to the fact that it is effectively a massive nutrient trap with approximately 16 sewage works and many industries discharging wastewater effluents from the high density Johannesburg and Pretoria into the Crocodile river, the main river system flowing into the dam (Owuor *et al.*, 2007). Thus high concentrations of total phosphorus (about 0.5mg/l) and soluble inorganic nitrogen (1-2mg/l) in this impoundment (which exceed algal growth requirements) are maintained throughout the year (Zohary & Breen, 1989).

In an attempt to reduce the existing hypertrophic conditions, Hartbeespoort Dam and its catchment have, since August 1985, been included under regulations promulgating a 1mg/l effluent phosphate discharge standard for sensitive catchments (Thornton & Ashton, 1989). According to Oberholster & Ashton (2008), this effluent phosphate standard is inappropriately high and since its promulgation, the water quality in South Africa's rivers and reservoirs has deteriorated rapidly. There is conclusive evidence that in order for the adverse effects of eutrophication to be minimized, a far lower effluent phosphate concentration (<0.1 mg/l as P) was needed (Oberholster & Ashton, 2008). Reducing nutrient loading rates was the initial approach that was globally used as the primary corrective measure for the control of eutrophication. However, as an isolated approach, the phosphate standard is a global failure (Hart, 2006). Even though the approach would reduce the availability of phosphate to algae, the standard alone would not be sufficient to achieve the reduction of the phytoplankton abundance to desirable levels (Chutter & Rossouw, 1992; Owuor *et al*., 2007). Consequently, in many countries, the approach is being pushed aside in favour of integrated treatment approaches with a strong biological component (Hart, 2006). However, in South Africa, reducing nutrient loading is still a preferred approach.

Oberholster & Ashton (2008) consider South Africa's freshwater resources to be moderately to highly eutrophic, and this is based on the estimated values for the freshwater pollution (in the form of chemical oxygen demand) and average phosphorus (as orthophosphates). These values are 4.74 tonne/ km^3 and 0.73 mg/L respectively (Oberholster & Ashton, 2008). According to them, the eutrophication of rivers and water storage reservoirs in South Africa is a result of large-scale changes to the aquatic ecosystems which were brought about by a combination of factors namely:

(i) The country's climatic conditions.

(ii) The discharge of treated and untreated sewage effluent.

(iii) Excessive nutrient loads in return flows from agriculture.

(iv) Modification of river flow regimes.

(v) Changing land use or land-cover patterns.

Many countries are faced with a challenge to develop policies to reduce cyanobacterial blooms by improved water body management (Codd, 2000) and South Africa is no exception. According to Shuval (1980), preserving and managing water resources should be coupled with preserving and managing water quality. Oberholster *et al*. (2008) forecasted that by 2025 the demand for water in South Africa will exceed its supply; therefore it is of utmost importance that the quality and availability of the country's water is managed. The South African government has recognized that water is a scarce and precious resource, and hence the need for monitoring and assessment of the quality of water. This is clearly stipulated by the National Water Act of 1998 which requires that monitoring of water quality should be an integral part of resources management (Government Gazette, 1998). The Act recognizes, among others, the necessity for protection of the quality of water resources in the interest of all water users. One of the requirements of the Act is the establishment of the National Water Resource Strategy (NWRS) whose

purpose is, among others, to set out a national framework for protection, use, development, conservation, management and control of water resources, and that is the responsibility of the Department of Water Affairs and Forestry (DWAF). The department's name has recently (2009) changed to Department of Water Affairs.

In an attempt to meet the requirements of the Act, DWAF has developed a number of National Water Quality Programs and one such program is the National Eutrophication Program (NEMP). The program was established to measure, assess and report regularly on, among others, the current eutrophication problems (Van Ginkel *et al*., 2002). One recent initiative by DWAF is the introduction of the Blue-Drop Certification Programme whose purpose is, amongst others, to introduce key requirements for effective and efficient management of drinking water quality by water services institutions (DWAF, 2008).

1.2) ROLE OF PHYTOPLANKTON

Phytoplankton are photosynthetic, free-floating organisms found in all lakes, slow-flowing rivers, estuaries and oceans, and they are probably the most common photosynthetic organisms on the planet (Horne & Goldman, 1994). According to these authors, virtually all the dynamic features of a lake (colour, clarity, trophic state, water chemistry, the taste and odour of water, animal plankton and fish production) depend to a large degree on phytoplankton. Conducive conditions of temperature, light and nutrient availability result in surface waters supporting increased growth of phytoplankton (Paerl, 1988; Quiblier *et al*., 2008; Swanepoel *et al*., 2008). If the increase in phytoplankton growth involves undesirable species of algae, for example, cyanobacteria, this could result in the destruction of the potential of the water body for recreation or as a drinking water supply (Horne & Goldman, 1994). Increases in human population density, agriculture and industrial activities has led to the increase in the nutrient loading rates into many freshwater ecosystems and often

results in a shift in the phytoplankton community towards dominance by cyanobacteria (Davies *et al*., 2009).

1.3) AIM OF THE PROJECT

This study aims to establish the safety of water supplied to Bloemfontein and surrounds for recreational and household purposes.

1.4) HYPOTHESIS

It is hypothesized that microcystin-producing blooms occur mostly in water bodies which are used for water supply, and that conventional water treatments methods are ineffective in removing these toxins from potable water supplies.

1.5) SPECIFIC OBJECTIVES

In order for the aim to be achieved and the hypothesis to be investigated, the following specific objectives were identified:

1. To establish the prevalence of cyanobacterial toxins in the reservoirs supplying potable water to Bloemfontein and surrounds.
2. To verify the efficiency of the treatment process in water treatment plants, concerning the reduction or removal of microcystins.

1.6) PROBLEM STATEMENT

Microcystins are known to be chemically stable compounds so conventional water treatment methods such as flocculation, sedimentation, rapid sand filtration and chlorination have only limited efficacy in removing dissolved microcystins to WHO recommended safe concentrations (Izaguirre *et al*., 1982; Lawton *et al*., 1999b; Cornish *et al*., 2000; Liu *et al*., 2002, 2003, 2009; Schijven *et al*., 2003; Oberholster *et al*., 2005; Valeria *et al*., 2006; Swanepoel *et al.,* 2008). On top of that, there is a possibility that the toxin from the

cyanobacteria may be released by treatment processes that include potassium permanganate or chlorine, and this could result in these toxins reaching the people through potable water supplies (Chow *et al*., 1998; Peterson *et al*., 1995; Van Apeldoorn *et al*., 2007).

According to Oberholster *et al*. (2005), only the advanced treatment methods like granular activated carbon filtration can effectively remove cyanobacterial toxins from water. Apparently, granular carbonated carbon is able to remove more than 80% of the microcystins (Hurtado *et al*., 2008). However, as indicated by Lawton & Robertson (1999), performance may be reduced with normal water treatment practices and on top of that, not much is known about the fate of microcystins adsorbed onto activated carbon. Another alternative method is the photocatalytic degradation of microcystins using a titanium dioxide (TiO_2) photocatalyst, and this has been found to be an extremely effective process (Lawton & Robertson, 1999; Lawton et al., 1999b; Cornish *et al*., 2000; Liu *et al*., 2002, 2009). When compared to other semiconductors, TiO_2 is suitable as a photocatalyst for water treatment because it is highly photo-reactive, cheap, non-toxic, chemically and biologically inert, and photostable (Mills *et al*., 1993; Robertson *et al*., 2005). According to Lawton & Robertson (1999), ozonation is also effective for the removal of microcystins from potable water but it is costly.

Observations by Yoo *et al*. (1995) suggested that analyzing specific cyanobacterial toxins in the laboratory is a complex exercise and most water treatment plant laboratories do not perform them. On top of this, only a few purification water treatment plants in South Africa are equipped with granular activated carbon systems while the rest rely on the conventional methods of purification which only remove cyanobacterial cells and debris but not biotoxins dissolved in water (Oberholster *et al*., 2005). It is therefore reasonable to assume that humans are, on occasion, exposed to very high and potentially lethal concentrations of cyanobacterial toxins in potable water. With increasing world population growth, climate change and eutrophication,

the occurrence of cyanobacterial blooms has increased world-wide and this has led to the increase in the threat posed by toxins produced by these organisms to water supplies. It is, therefore, important to conduct a study like this one in order to establish how safe our potable water is.

1.7) SIGNIFICANCE OF THE PROJECT

The presence of toxic cyanobacterial blooms in water bodies used either as drinking water reservoirs or for recreational purposes represents a serious health risks for human population. Drinking water consumption is a potentially significant route of exposure to cyanotoxins. This calls for evaluation of water treatment methods for their effectiveness at removing cyanotoxins from drinking water supplies. Drinking water facility operators are faced with the challenge of supplying safe portable water (water free from cyanotoxins) to consumers. This study will help raise the awareness of the concerned facilities about the dangers imposed by cyanobacteria and their toxins and hopefully help them make informed decisions, especially, in cases of cyanobacterial blooms.

1.8) DISSERTATION OUTLINE

- Chapter one covers background on water quality, purpose of the study as well as its significance.
- Chapter two gives extensive literature on cyanobacteria and their metabolites.
- Chapter three describes the study area and the methods used in the study.
- Chapter four presents the results of the study and their discussion.
- Chapter five presents the conclusions derived from the study and recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1) CYANOBACTERIA

Cyanobacteria, commonly known as blue-green algae, are photosynthetic prokaryotes possessing both bacterial and algal characteristics, (Rae & Moollan, 1999; Duy *et al*., 2000; Chorus, 2001). Like bacteria, they do not possess membrane-bound sub-cellular organelles such as nucleus, chloroplasts and mitochondria, but like algae, they are autotrophic and have cell walls made of peptidoglycan and polysaccharides instead of cellulose as in algae (Rae and Moollan, 1999; Chorus, 2001; Graham *et al*., 2008). They are a very diverse group of prokaryotes in terms of their morphology, physiology and metabolism. For example, they range from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensively pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophylic, planktonic to barophilic, freshwater to marine including hypersaline species (Owuor *et al*., 2007). The gelatinous sheath surrounding the cell wall layers in most species is believed to be responsible for the ability of the cyanobacteria to survive in extreme conditions (Duy *et al*., 2000).

Evidence from fossil record indicates that these organisms originated in the Precambium (Van der Westhuizen, 1984, Henson *et al*., 2002, 2004), and it is believed that they have played an important role in introducing oxygenic photosynthesis, thereby (together with plants) increasing the level of oxygen in the atmosphere to that of today (Henson *et al*., 2002, 2004; Thomas *et al*., 2005; Osswald *et al*., 2007).

Cyanobacteria are considered by phycologists as algae and this is based on the fact that cyanobacterial photosynthetic pigments and mechanisms are

more similar to those of true algae than those of the photosynthetic green and purple sulfur bacteria (Yoo, 1995). The photosynthetic apparatus of cyanobacteria has two reaction centres, Photosystem I (PS I) and Photosystem II (PS II), and it contains chlorophyll-a, carotenoids, and phycobiliproteins (blue phycocyanin and red phycoerythrin), which serve as accessory pigments to chlorophyll-a (Stolz, 1990; Neilan *et al*., 1995; Yoo, 1995; Duy *et al*., 2000). Although phycocyanin is present in both photosystems, it is particularly important in photosystem II (Fogg *et al*., 1973). Phycocyanin traps light energy in the red wavelength band of the visible light spectrum and transfers it to chlorophyll-a, while chlorophyll-a traps light in both the red and the blue wavelengths, and this pigment compliment gives cyanobacteria a distinct ecological advantage in that it enables them to utilize light at both extremes of the visible spectrum (Mur *et al*., 1999; Yoo, 1995). Cyanobacteria or blue-green algae owe their name to the combined visual effects of the blue phycocyanin and the green chlorophyll-a (Lau *et al*., 1977; Yoo, 1995). However, blue-green is not the only cyanobacterial colour. Other colours include yellow-brown, purplish and red, and this is due to the presence of diverse range of pigments in cyanobacteria (Duy *et al*., 2000).

Cyanobacteria are often associated with the occurrence of blooms and hence the dominance of phytoplankton communities (Quiblier *et al.*, 2008). Cyanobacterial bloom formation is stimulated by, among others, elevated levels of nutrients especially phosphorus, nitrate or ammonia, water temperatures between 15 ºC and 30 ^{o}C and a pH between 6 and 9 or higher (Wicks & Thlel, 1990). Apparently, climatic conditions govern the timing and duration of the bloom season of cyanobacteria (Van Apeldoorn *et al*., 2007). In temperate climates, cyanobacterial dominance is often evident during mid-summer to early fall, however, this dominance may occur any time throughout the year, even under ice during winter (Graham *et al.*, 2008). In subtropical and tropical climates cyanobacteria may dominate at any time, and dominance may persist year-round because the seasonal differences in environmental factors are often not great enough to induce the replacement of cyanobacteria by other phytoplankton species (Bartram *et al*., 1999).

Persistent blooms of toxic cyanobacteria imply considerable water quality problems and health risks and may also lead to an impoverishment of the fauna in lakes (Lindholm *et al.*, 1989).

A key feature of the success of cyanobacteria in bloom formation is their ability to out-compete other members of the phytoplankton at a time of year when conditions of temperature, light and nutrient status are favourable for their growth (Sigee, 2006; Jang *et al*., 2007). The competitive success of cyanobacteria is partly due to their versatile physiology and wide ecological tolerance (Cohen & Gurevitz, 2006). According to Osswald *et al*., (2007), cyanobacteria are able to achieve this dominance because they have some characteristics which permit them to out-compete other microalgae, and these features are summarized by Sigee (2006) as:

(i) Their optimum growth at high temperature. Most cyanobacteria attain maximum growth rates at temperatures above 25^{o}C, higher than for green algae and diatoms (Mur *et al.*, 1999).

(ii) Low light tolerance. Cyanobacteria can maintain relatively higher growth rates than other phytoplankton organisms when light intensities are low because they require little energy to maintain cell function and structure (Havens *et al*., 1998; Mur *et al.*, 1999). In addition, species which possess phycoerythrin are able to carry out photosynthesis at depths that receive only green light (Briand *et al*., 2003)

(iii) Tolerance of low N/P nutrient ratios. The development of cyanobacterial blooms may be favoured by a low ratio between nitrogen and phosphorus concentrations because many species have the ability to fix N_2, and those that cannot fix N_2 may have greater storage abilities for N than do other phytoplankton species (Fogg *et al*., 1973; Yoo, 1995; Mur *et al*., 1999).

(iv) Depth regulation by buoyancy. Many, but not all, cyanobacteria have gas vacuoles that allow them to maintain a favourable position in the water column by regulating buoyancy (Fogg *et al.,* 1973; Yoo

et al., 1995; Briand *et al*., 2003; Mwaura *et al*., 2004; Graham *et al*., 2008).

(v) Resistance to zooplankton grazing. Most zooplankton are deterred by the size, taste, nutritional inadequacy or toxicity of cyanobacteria (Horne & Goldman, 1994; Jang *et al*., 2003 & 2007; Work & Havens, 2003).

(vi) Tolerance of high pH/low CO_2 concentrations. They have optimum growth at pH values between 7.5 and 10 (Owuor *et al*., 2007). Van der Westhuizen (1984) indicated that at high pH levels, cyanobacteria are able to utilize carbon dioxide or carbonate more efficiently than green algae.

Colonial cyanobacteria form the major nuisance-algae of freshwater systems and have the potential to cause deterioration in water quality and adverse environmental effects (Sigee, 2006). Though cyanobacteria are notorious for their undesirable effects on aquatic systems, they are also useful, and the beneficial features of cyanobacteria as outlined by Bartram *et al*. (1999), include:

(i) They are important primary producers and their nutritive value is high. However, it has been documented that cyanobacteria generally lack long-chained polyunsaturated fatty acids, rendering them sources of low quality food (Von Elert & Wolffrom, 2001; Jang *et al*., 2007).

(ii) The nitrogen-fixing species contribute globally to soil and water fertility.

(iii) The use of cyanobacteria in food production and solar energy conversion holds promising potential for the future. Some strains of cyanobacteria have a very high content of proteins, vitamins and other essential growth factors (Mur *et al*., 1999).

However, cyanobacterial blooms are notorious for causing water-treatment, supply, conservation, and health problems, hence attracting the attention of

water authorities and utilities, environmental and health agencies, and water-user groups (Codd, 2000). In Steffensen's (2008) view, the water quality, environmental and ecological status of water bodies and most of the uses of water are affected by cyanobacterial blooms. The extent of the impact is dependent on the type, size and frequency of blooms, the size of water body affected, the uses made of water, and the treatment options available to respond to the blooms (Steffensen, 2008).

2.2) EFFECTS OF CYANOBACTERIA IN FRESHWATER SYSTEMS

The potable water production industry in South Africa and in many other countries is faced with problems caused by algal blooms, especially cyanobacteria (Swanepoel *et al.*, 2008). The problems caused by cyanobacteria with respect to water quality include:

(i) Production and release of toxins which can be detrimental to a wide range of animals, including humans (Mankiewicz *et al*., 2005; Sigee, 2006; Oberholster & Botha, 2007; Owour *et al*., 2007). This has been the object of scientific attention and concern because of associated public health and environmental hazards (Yoo *et al*., 1995; Rae & Moollan, 1999; Osswald *et al.*, 2007).

(ii) Fish kills due to development of acute anoxia as a result of large-scale death of algal cells, affecting the ecology of the freshwater environment (Yoo *et al*., 1995; Rae & Moollan, 1999; Mankiewicz *et al*., 2005; Sigee, 2006; Owuor *et al*., 2007).

(iii) Blockage of the filtration systems of water-treatment works, affecting the efficiency of the extraction processes (Sigee, 2006), and resulting in increased filter maintenance with associated cost implications (Rae & Moollan, 1999; Swanepoel, *et al*., 2008).

(iv) Increase in the metal complexes entering the water supply due to the chelating of iron or aluminium coagulants added to the water

during treatment processes by mucopolysaccharides produced by algal breakdown (Harper, 1992; Sigee, 2006).

(v) Unpleasant changes in the odour and taste of water (Yoo *et al*., 1995; Mankiewicz *et al*., 2005; Sigee, 2006; Owuor *et al*., 2007). The most troublesome odours are usually those described as muddy or earthy-musty, and these are said to be caused by two organic compounds, 2-methylisoborneol (2-MIB) and geosmin (Izaguirre *et al*., 1982; Harper, 1992; Yoo *et al*., 1995; Rae & Moollan, 1999; Oestman *et al*., 2004; Tung *et al*., 2008).

(vi) Accumulation of ammonia with the collapse of the bloom, affecting the oxidation and disinfection capacity of chlorine and converting iron and manganese to soluble forms that can lead to discolouration of water (Harper, 1992; Sigee, 2006).

(vii) Unsightly appearance of cyanobacterial blooms makes them unappealing for any water supply that is used for recreational purposes, resulting in loss of recreation amenities (Rae & Moollan, 1999; Sigee, 2006; Owuor *et al*., 2007).

2.3) CYANOTOXINS, TASTE AND ODOUR

Cyanobacteria naturally produce cyanotoxins and taste and odour compounds as their by-products (Graham *et al.*, 2008), and these compounds usually remain contained within cells, and are only released in large quantities on cell lysis which could be due to breakdown of natural cyanobacterial bloom or the artificial lysis of blooms by application of copper sulphate (Duy *et al*., 2000; van Apeldoorn *et al*., 2007). Toxin production, as well as the production of taste-and-odour compounds is strain, rather than species dependent (Graham *et al.*, 2008), hence why there may be a mixture of toxic and non-toxic strains within a single-species bloom (Sivonen & Jones, 1999).

There are more than 50 genera of freshwater cyanobacteria, 19 of which are capable of producing toxins (Yoo *et al*., 1995), and these include *Anabaena, Microcystis*, *Planktothrix, Schizothrix, Nostoc, Anabaenopsis, Nodularia,*

Aphanizomenon, Cylindrospermopsis, Umezakia, and Lyngbya. Cyanotoxins can be classified into three broad groups on the basis of chemical structure, and these groups are peptides, alkaloids, and lipopolysaccharides (LPS) (Sivonen & Jones 1999; Kabernick & Neilan, 2001). Hepatotoxins, microcystins and nodularins are cyclic hepta- and pentapeptides, respectively, containing the same unusual C_{20} amino acid (Codd, 2000; Heresztyn & Nicholson, 2001; Kabernick & Neilan, 2001). Unlike other hepatotoxins, cylindrospermopsins is an alkaloid containing tricyclic guanidine combined with hydroxymethyl uracyl (Oberholster *et al*., 2005). Neurotoxins, anatoxins and saxitoxins are non-sulphated alkaloid toxins, and aplysitoxins and lyngbyatoxin are dermatotoxic alkaloids (Sivonen & Jones, 1999).

However, the cyanotoxins are commonly classified based on their mode of action in animals or animal-derived organs or cells, and they are dermatoxins (e.g. lyngbyatoxin A), neurotoxins (e.g. anatoxin-a), and hepatotoxins (e.g. microcystins) (Sivonen & Jones, 1999; Codd, 2000; Briand *et al*., 2003; Galczynski & Ociepa, 2008). The effects of dermatoxins include acute, often severe, dermatis and inflammation of the gastro-intestinal tract (Sivonen & Jones, 1999). Lyngbyatoxin A is described by Briand *et al.* (2003) as a potent tumor promoter. Neurotoxins, anatoxin-a and anatoxin-a(s) inhibit transmissions at the neuromuscular junction (Kabernick & Neilan, 2001), but they are rarely associated with human illness and death (Kuiper-Goodman *et al*., 1999). Hepatotoxins on the other hand have been associated with human toxicoses that have occurred after consumption of drinking water, contact with cyanobacteria during recreational activities, and haemodialysis using tainted water (Kuiper-Goodman *et al.*, 1999). Microcystins and nodularins inhibit eukaryotic protein phosphatases type 1 and type 2A resulting in excessive phosphorylation of cytoskeletal filaments, ultimately leading to liver failure (Falconer, 1998; Kabernick & Neilan, 2001). It is believed that the recognition and inhibition of protein phosphatases in initiated by the Adda side chain and possibly the planar portion of the peptides (Hitzfield *et al*., 2000; Neilan *et al*., 2008). Microcystins are also known to promote liver tumours and of significant concern is the possibility that chronic exposure to low

concentrations of microcystins in drinking water supplies may contribute to life threatening illnesses such as liver cancer (Bourne *et al*., 1996; Kuiper-Goodman *et al*., 1999; Cornish et al., 2000; Liu *et al*., 2009). However, as indicated by Briand *et al*. (2003), it is documented that nodularin is a more potent tumour promoter than microcystin.

2.3.1) MICROCYSTINS

Microcystins are the most prevalent, potent and destructive liver toxin produced by several strains of the genera *Microcystis, Oscillatoria, Anabaena, Nostoc*, and *Planktothrix* and to date, more than 70 different variants have been identified (Briand *et al*., 2003; Babica *et al*., 2006). All these variants share a general structure, (Figure 1), consisting of cyclo -(D-Ala-X-D-MeAsp-Y-Adda-D-Glu-Mdha) where Adda is an unusual 20 carbon amino acid, Mdha is N-methyldehydroalanine, and X and Y are variable amino acids (Falconer, 1998; Lawton *et al,* 1999b; Lawton *et al*., 2003b). These variable amino acids are found in position 2 and 4 of the cyclic structure and they are responsible for the variety in the microcystins (Oberholster *et al*., 2004). Microcystins are named according to the variable amino acids they contain and it has been documented that microcystin-LR (variant containing leucine (L) and Arginine (R) in positions 2 and 4 respectively) is the most frequently occurring microcystin variant (Lawton & Robertson, 1999; Cornish *et al*., 2000). However, Sivonen & Jones (1999) believe that the fact that a chemical standard for the analysis of microcystin-LR was the earliest to be commercially available renders the observation biased.

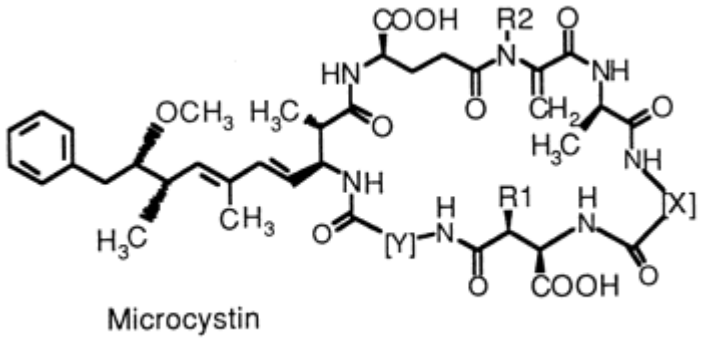

Figure 2.1: Structure of microcystins (Falconer, 1998; Lawton et al., 2003)

Once these toxins are released into water they can potentially persist for long periods because they are non-volatile and relatively stable (Harada, 1996; Lawton & Robertson, 1999; Cornish *et al*., 2000; Duy *et al*., 2000; Metcalf & Codd, 2004). Apparently, cooking is not sufficient to destroy extracellular microcystins because these toxins can withstand boiling and extremes of pH (Lawton & Robertson, 1999; Cornish *et al*., 2000; Metcalf & Codd, 2004; Butler *et al*., 2009). The dissolved microcystin concentration is mostly low in natural waters because of dilution, adsorption by clay particles, thermal decomposition aided by temperature and pH, photodegradation by UV and visible light, and biodegradation by some bacterial proteases (Harada, 1996; Harada *et al*., 1996; Ozawa *et al*., 2003; Metcalf & Codd, 2004; Butler *et al*., 2009). According to Butler *et al*. (2009), microcystins have a half-life of 10 weeks at typical ambient conditions, but if they are released into cooler, dark natural waters, they can persist for months or even years.

2.3.2) TOXIN PRODUCTION

Scientists and responsible authorities are still faced with a challenge of establishing why cyanobacteria produce toxins, and the physiological functions of cyanotoxins (Osswald *et al*., 2007). Some authors came up with what they believe could be the functions of the cyanotoxins and these are outlined by Osswald *et al.* (2007) as:

(i) Cyanotoxins may be produced as a strategy to avoid grazing by other organisms such as zooplankton and higher animals.

(ii) Inducing alteration of population structures to gain ecological advantage.

(iii) Mediating cell signaling allelopathy and chemotoxy to establish trophic relationships with other cyanobacteria or other organisms.

There seems to be a close relationship between the factors that influence toxin production and those that influence bloom formation. The influence of environmental factors on toxicity of some cyanobacterial species has been investigated by a number of researchers and it has been documented that environmental factors have a major impact on the physiological processes of toxin production (Van der Westhuizen, 1984; Yoo *et al*., 1995; Rae & Moollan, 1999; Kabernick & Neilan, 2001; Ortelli *et al*., 2008). Temperature, aeration rate and nutrient composition are given by Krüger (1978) as the important factors in toxin production by *Microcystis aeruginosa.* However, a report on a study conducted in Hartbeespoort Dam, South Africa showed a positive correlation between concentration of toxins in *Microcystis aeruginosa* and primary production per unit of chlorophyll-a, solar radiation, surface water temperature, pH and percentage oxygen saturation; and a negative correlation between the toxins and surface water organic and inorganic nutrient concentrations (Duy *et al*., 2000).

Knowledge of the biosynthetic pathways of cyanotoxins is in its early stage (Sivonen & Jones, 1999). However, according to Kabernick & Neilan (2001), it is believed that anatoxin-a, anatoxin-a(s), and cylindrospermopsin are synthesized via arginine derivatives involving a retro-Claisen condensation. It has been documented that microcystins and nodularin are synthesized nonribosomally and their biosynthesis gene clusters for microcystin and nodularin have been characterized and sequenced while the gene cluster for cylindrospermpsin biosynthesis is partially derived (Neilan *et al*., 2008). The gene clusters encoding the biosynthetic enzymes for microcystins (mcyS) and

nodularin (ndaS) consist of peptide synthetase and polyketide synthetases, a putative ABC transporter, and tailoring enzymes (Tillett *et al*., 2000; Yoshida *et al*., 2006; Schatz *et al*., 2007; Neilan *et al*., 2008; Pearson & Neilan, 2008). According to Neilan *et al*. (2008), the mcyS region of the genome spans 55kb and consists of 10 genes (mcyA-J) while that of ndaS spans 48kb and consists of 9 genes (ndaA-I). The small size, cyclic structure and content of unusual amino acids possessed by peptides (microcystin and nodularin) are given by Hitzfeld *et al*. (2000) as evidence for the nonribosomal synthesis of these peptides.

2.3.4) TASTE AND ODOUR COMPOUNDS

As mentioned earlier, cyanobacterial blooms release odour and taste compounds, geosmin and 2-methylisoborneol (2-MIB) in source water when they decompose (Lanciotti *et al*., 2003). Geosmin is known to be produced by *Microcystis sp.*, *Aphanizomenon sp., and Oscillatoria sp*., while *Oscillatoria sp., Pseudoanabaena sp., and Synechococcus sp*. are known to produce 2-MIB (Swanepoel *et al*., 2008). Apparently, there is another odour compound produced by *Microcystis* called β-cyclocitral which causes tobacco or chocolate odour in water (Yoo, 1995; Yoo *et al*., 1995). Geosmin and 2-MIB are detectable by humans at concentrations between 5-10ng/l. Therefore these compounds may be noticeable before the potential cyanobacterial producers become apparent (Graham *et al*., 2008). The production of these metabolites seems to be governed by environmental variables such as nutrient concentrations, water temperature, light intensity, hydraulic residence time of the reservoir and water quality (Tung *et al*., 2008). According to these authors, of all these parameters, nitrogen and phosphorus are the most important.

Water treatment facilities are often faced with the complaints of bad taste and odour, due to the presence of geosmin and 2-methylisoborneol (2-MIB) in drinking waters (Swanepoel *et al.*, 2008). The presence of taste and odour

compounds in water results in unpalatable drinking water and increased treatment costs (Graham *et al.*, 2008), since both geosmin and 2-methylisoborneol (2-MIB) are saturated cyclic tertiary alcohols and thus resistant to oxidation by conventional water treatment methods (Izaguirre *et al*., 1982; Chow *et al*., 1998, 1999; Swanepoel *et al*., 2008). Water treatment plants often use powdered activated carbon (PAC) to control the problem of odours since it is relatively inexpensive and can be applied only when required (Pendleton *et al*., 1997; Cook *et al*., 2001). The results from the study by of Lawton *et al*., (2003a) have shown that TiO_2 photocatalysis can be used to rapidly remove the odour compounds from water. Seasonal odour problems are not considered a direct threat to public health, however, since the consumers generally rely on the taste of their water as the primary indicator of its safety, they are perhaps the single most important public health relation issue many water utilities face (McGuire, 1995; Yoo *et al*., 1995; Young *et al*., 1996; Pendleton *et al*., 1997; Davies *et al*., 2004; Oestman *et al*., 2004; Watson, 2004; Tung *et al*., 2008).

2.4) CYANOTOXINS AND HEALTH

Potential human risks of cyanobacteria have earned them a place on the U.S. Environmental Protection Agency drinking water contaminant candidate list (CCL) (Graham, *et al*., 2008). There are discrepancies in the incidence of mortalities and illness due to cyanobacteria because, as indicated by Codd *et al*. (2004), there is inadequate recognition and case definition, analytical epidemiology and notification. However, recently, epidemiology evidence, which depends upon good case definition, good characterization of exposure and a reporting system that enables these data to be compared, has proven to be of special importance in directly demonstrating the link between toxin exposures and human health outcomes (Kuiper-Goodman *et al.*, 1999). This includes:

(i) An outbreak of severe hepatitis at a Brazilian haemodialysis centre, where 100 patients developed acute liver failure, and 50 of them died. This was attributed to microcystins exposure.

(ii) A severe gastro-enteritis epidemic in Brazil, where 2000 cases, 88 of which resulted in death, were reported; and again, this was attributed to toxin produced by cyanobacteria present in water.

(iii) A severe outbreak of cyanobacterial toxicity in a human settlement in Australia, where 140 children and 10 adults were hospitalized due to severe hepatoenteritis. This was attributed to *Cylidrospermopsis raciborskii* (cyanobacteria).

(iv) Symptoms shown by 13 people who swam in a lake with cyanobacterial bloom in Canada, and these were attributed to *Microcystis* spp. and *Anabaena circinalis*.

(v) Symptoms indicating intoxication in 10 of 20 United Kingdom army recruits after swimming and canoe training in water with a dense bloom of *Microcystis* spp.

(vi) Epidemiological evidence of adverse health effects after recreational water contact established in a study involving 852 participants.

There is also epidemiological evidence of animal poisonings, and this includes:

(i) A serious outbreak of livestock and other animal poisonings occurring during the summers of 1942 and 1943 around the Vaal Dam which resulted in the death of thousands of cattle, sheep and other animals, and *Microcystis aeruginosa* was identified as the cause (Van der Westhuizen, 1984).

(ii) The deaths of cattle, sheep, dogs, horses and pigs after drinking a scum of *Nodularia spumigena* in Lake Alexandrina (Kuiper-Goodman *et al*., 1999).

(iii) The deaths of sheep drinking from a farm dam contaminated with the neurotoxin *Anabaena circinalis* in Australia (Kuiper-Goodman *et al.,* 1999).

Poisoning episodes have been reported more often from South Africa and Australia because these countries have arid climates and this makes access to drinking water the limiting feature of livestock production.

2.5) MONITORING

The occurrence of cyanobacterial blooms in freshwater can create significant water quality problems as certain species of cyanobacteria are capable of producing toxins (McElhiney & Lawton, 2005). This is why the detection of toxic cyanobacteria and their toxins is very important (Oulette & Wilhelm, 2003).

2.5.1) IDENTIFICATION AND ENUMERATION OF CYANOBACTERIA

For one to obtain a clear indication of water quality at a certain site, it is important that identification and enumeration of algal taxa from that site are performed (Sigee 2006). Apparently, identification and quantification of cyanobacteria in water resources can provide an effective early warning system for the development of potentially toxic blooms (Lawton *et al*., 1999a). There are two major techniques used universally to perform phytoplankton identification and enumeration, and these are sedimentation techniques, where phytoplankton are sedimented by gravity or centrifugation; and membrane filtration techniques, where phytoplankt*on* are sedimented onto a membrane filter using vacuum pump (Swanepoel *et al*., 2008). The sedimented phytoplankton is then analyzed by using an inverted microscope with chambers, the approach regarded by Lawton *et al.* (1999a) as generally the best for estimating cyanobacterial numbers. According to Oullette & Wilhelm (2003), microscopic identification of toxic and non-toxic algae is an important component of water quality and harmful algae monitoring programs. However, as indicated by Shaw & Smith (2000), accurate identification and enumeration requires an experienced taxonomic phycologist and a good-quality microscope.

The above-mentioned techniques rely primarily on the morphological characteristics, and according to Shaw & Smith (2000), morphology is dependent on environmental conditions and the organism's phase of growth. This implies that morphology may change and this may lead to difficulty and

errors in cyanobacterial identification. This problem may be minimized by the use of standard bench references as well as in-house taxonomic reference documents (Shaw & Smith, 2000). However, these authors have indicated that there are two other techniques whose development was prompted by the limitations of the traditional methods, and these are molecular biological techniques as well as flow cytometry. Molecular techniques involve ribosomal RNA (rRNA) repeat units. As indicated by Garcia-Pichel (2008), rRNAs are universal molecules present in all organisms with well-conserved regions as well as variable regions, and the variability of the rRNA repeat units is used to distinguish between various genera and species of cyanobacteria; Flow cytometry relies on florescence signals resulting from the excitation of individual cells at certain wavelengths which give an indication of the pigment composition of organisms present (Shaw & Smith, 2000).

2.5.2) TOXIN MONITORING

The identification of potentially toxic bacteria in water bodies may be enough to warn authorities of probable toxin release in water (Viera *et al*., 2005), but this alone cannot be used to determine whether or not these metabolites will be there, although genera that contain strains producing these compounds can be identified (Graham *et al*., 2008). The safety and quality of water for human and animal consumption, and for recreational activities can only be ensured by toxicity testing (Masango *et al*., 2008), and microcystins should be among the parameters to be analyzed (Hurtado *et al*., 2008).

The increased awareness of the toxicity of microcystins has raised the need for the development of fast, sensitive and reliable methods for their detection and quantification (Rapala *et al*., 2002; Campàs *et al*., 2005; McElhiney & Lawton, 2005). There is a wide range of methods available for monitoring microcystins but none of them is ideal, i.e. no single technique is able to provide precise measurement of toxicity as well as accurate profile of the microcystin variants present (Campàs *et al*., 2005; McElhiney & Lawton, 2005). According to Msagati *et al*. (2006), the methods employed in the

detection and identification of microcystins vary in terms of principles of detection, information they provide and simplicity/complexity.

According to McElhiney & Lawton (2005), high performance liquid chromatography (HPLC) has been the widely used technique both in research and for routine analysis of microcystins. HPLC coupled to UV detector is highly sensitive and is capable of providing both qualitative and quantitative data (Campàs *et al*., 2005; McElhiney & Lawton, 2005) However, this technique is time consuming, technically demanding and expensive (Rapala et al., 2002; Campàs *et al*., 2005; McElhiney & Lawton, 2005; Swanepoel *et al*., 2008). According to Rapala et al. (2002), HPLC is suitable for confirmation and identification of the toxin variants present in the sample. However, as indicated by Harada (1996), a definite conclusion on the structure cannot always be drawn from HPLC because it relies on retention time of each microcystin variant.

Biological-based assays are a simple, rapid and cheaper alternative for assessing toxicity of a sample (Campàs *et al*., 2005; McElhiney & Lawton, 2005). Mouse bioassay has been used in most laboratories for detection of hepatotoxins until recently, but the technique lacks sensitivity and specificity (Harada *et al*., 1999; Campàs *et al*., 2005; McElhiney & Lawton, 2005; Masango *et al*., 2008), and it is not convenient from an ethical point of view (Shaw & Smith, 2000; Campàs et al., 2005; McElhiney & Lawton, 2005). However, according to Masango *et al*. (2008) and Shaw & Smith (2000), the mouse bioassays provide natural physiological and biochemical responses for toxicological assessments. Apparently, there is a simple and inexpensive alternative to mouse bioassays, i.e. brine shrimp bioassays, which is an invertebrate bioassays specific for microcystins (McElhiney & Lawton, 2005). According to these authors, this method requires little expertise though, like mouse bioassay, it lacks specificity.

The protein phosphatase (PP) inhibition assay is an enzymatic method based on the ability of microcystins and nodularin to inhibit serine-theorinine protein phosphatase enzymes (Rapala *et al*., 2002; Campàs *et al*., 2005; McElhiney & Lawton, 2005). There is a radiometric assay which involves the measurement of the release of acid-soluble ^{32}P from ^{32}P-labelled glycogen phosphorylase (Harada *et al*., 1999; Ward *et al*., 1997; Oh *et al*., 2001; McElhiney & Lawton, 2005), and a colorimetric assay utilizing the ability of PP-1 to dephosphorylate ρ-nitrophenyl phosphate (Ward *et al*., 1997; Oh *et al*., 2001). The colorimetric version of the assay is the most commonly used because it simple, cost effective, sufficiently sensitive and more convenient as it does not involve use of radioactive materials (Harada *et al*., 1999; Rapala *et al*., 2002; Campàs *et al*., 2005). PP inhibition assays are rapid and sufficiently sensitive to detect microcystins below the WHO guideline (Campàs *et al*., 2005; McElhiney & Lawton, 2005). However, these assays lack specificity, i.e. they are unable to distinguish between microcystins and other non-cyanobacterial toxins and metabolites such as calyculin A and okadaic acid (Campàs *et al*., 2005; McElhiney & Lawton, 2005; Masango *et al*., 2008). Another drawback suffered by PP inhibition assays is given by McElhiney & Lawton, (2005) as variable sensitivity shown by these assays for all microcystin variants.

Enzyme-linked immuno sorbent assays (ELISAs) have been developed using either polyclonal or monoclonal antibodies for microcystins (Rapala et al., 2002; Campàs et al., 2005; McElhiney & Lawton, 2002). These assays are specific, simple and sufficiently sensitive to monitor microcystins within WHO guideline levels (Rapala et al., 2002; Campàs et al., 2005; McElhiney & Lawton, 2005; Masango et al., 2005). However, according to these authors, ELISAs often show poor cross-reactivity against different microcystin variants. According to McElhiney & Lawton (2005) the reason is that there are no standards for different microcystin variants and the commercially available ELISA kits are only capable of determining toxicity in terms of microcystin-LR equivalence. Falconer (2005) indicated that this drawback results in underestimation of natural mixtures of microcystins.

2.5.3) GEOSMIN AND 2-METHYLISOBORNEOL

Usually, the presence of odour suggests higher than normal biological activity, and is a simple test for the suitability of drinking water (Meybeck & Helmer, 1996). Water suppliers are expected to provide water that is inoffensive to the consumer and to achieve this, they are bound by law to carry out frequent qualitative, and less frequent quantitative determinations of taste and odour (Young *et al*., 1996). Taste and odour compounds dramatically impact the aesthetic quality and consumer acceptability of drinking water and this makes their identification and quantification essential (Watson *et al*., 2000).

These compounds have odour threshold concentrations at ng/L and the methods currently in use though effective, they are expensive, time consuming and labour intensive (Lloyd *et al*., 1998; Watson *et al*., 2000). These techniques include closed-loop stripping, liquid-liquid extraction, simultaneous steam distillation extraction and purge and trap (Watson *et al.*, 2000). However, according to Lloyd *et al*. (1998), there are three new techniques namely membrane-based extraction, solid phase extraction and solid phase micro-extraction (SPME). The latter has been tested and compared to conventional and other extraction methods, the approach is simple, rapid, inexpensive and reliable (Lloyd *et al*., 1998; Watson *et al*., 2000; Lin *et al*., 2003). Based on the results of their study, Watson *et al*. (2000) concluded that headspace solid phase micro-extraction (HSPME) coupled with gas chromatography mass spectrometry (GC/MS) detection is able to detect geosmin and 2-methylisoborneol in natural and treated drinking water at concentration levels several times lower than the threshold concentrations for human consumption.

2.6) CYANOTOXIN TREATMENT

The effective removal of microcystin from potable drinking water is, and should be, a major goal for all water utilities because these toxins are prevalent in reservoirs and have the potential to compromise human health

severely (Edwards *et al*., 2008). It has been established that most conventional water treatment methods are not effective in removing cyanotoxins, microcystins in particular, from potable water. Consequently, there is a requirement for routine reliable methods for the removal of microcystins (Lawton & Robertson, 1999; Lawton *et al*., 2003b). Coagulation can efficiently eliminate cyanobacterial cells from water but not soluble cyanotoxins and the efficiency of the cyanobacterial removal depends on an optimization of chemical doses and coagulation pH (Hitzfeld *et al*., 2000; Jurczak *et al*., 2005). Exposure to highly oxidizing conditions such as the presence of high levels of chlorine or ozonation has proven to be effective in degrading microcystins (Lawton *et al*., 1999b; Cornish *et al*., 2000; Lawton *et al*., 2003b). Lawton & Robertson (1999) reviewed various water treatment methods that have been used to remove microcystins from potable waters and found that:

- Adsorption with activated carbon seemed reasonably effective though little is known about the fate of the microcystins adsorbed onto activated carbon.
- Chlorination appeared effective but its performance is dependent on the dose used. The application needs to be carefully monitored and little is known about the potential health implications of the by-products.
- Ozonation was also found to be effective though the process is costly. However, the by-products and their potential health implications need to be characterized.
- Photocatalytic degradation appeared to be a suitable method for the removal of microcystins from drinking water. Apparently, it is simple and easy to operate, mineralizes most organics with a limited chance for the production of by-products, and it is potentially sustainable and clean.

However, according to Ho *et al*. (2006), the effectiveness of activated carbon and ozonation is reduced by the presence of natural organic material (NOM). Apparently, NOM decrease the adsorption capacity for microcystins to activated carbon through competitive adsorption and/or pore blockage mechanisms and for ozonation, NOM can consume ozone thus reducing its concentration (Ho *et al*., 2006).

The use of biological filtration systems for the removal of toxins is one approach that is receiving attention (Ho *et al*., 2006; Edwards *et al*., 2008). These systems involve the use of selected biodegrading bacteria to compliment the natural microbial flora of the filter (Edwards *et al.,* 2008). The findings from the study by Ho *et al*. (2006) showed that biological sand filtration is an effective means for the complete removal of microcystin-LR and microcystin-LA. These systems are believed to be a low cost solution for the provision of safe potable water because:

- They are of low technology.
- They require little maintenance and infrastructure.
- Their mechanism does not add other chemicals that might have the potential to produce undesirable by-products (Ho *et al*., 2006; Edwards *et al*., 2008)

2.7) CHAPTER SUMMARY

Cyanobacteria (blue-green algae) are a diverse group of autotrophic prokaryotes. They are often associated with the occurrence of blooms because they have characteristics which allow them to out-compete other microalgae at a time of year when environmental conditions are favourable for their growth. These organisms form the major nuisance-algae of freshwater systems. Their undesirable effects on freshwater systems are, among others, production and release of toxins (e.g. microcystins) and water and taste metabolites (e.g. geosmin and 2-methylisoborneol), resulting in deterioration of quality of water. The reason for the production of these metabolites has not

yet been established, but it has been established that it is influenced by environmental conditions.

Microcystins, especially microcystin-LR, are the most prevalent and potent cyanobacterial hepatotoxins. They have been implicated in a number of livestock, wildlife and human poisonings. Microcystins are relatively chemically stable thus, not easily removed by conventional water treatments methods. Treatment methods which have so far proved to be efficient in removing microcystins from potable waters are adsorption with activated carbon, chlorination, ozonation, photocatalytic degradation and biological sand filtration. However, some of these methods have some limitations.

The increased awareness of the toxicity of microcystins has raised the need for the development of fast, sensitive and reliable methods for their detection and quantification. Methods employed include HPLC coupled to UV detector, mouse bioassays, ELISAs and protein phosphatase inhibition assays. These methods vary in terms of principles of detection, information they provide and simplicity/complexity. However, none of these methods is ideal.

CHAPTER 3

STUDY AREA, MATERIALS AND METHODS

3.1) STUDY AREA

The study was conducted on three impoundments in the Upper Modder River, namely Rustfontein, Mockes and Maselspoort Dams (**Figure 3.1**). Location, morphological and hydrological data of these impoundments is given in **Table 3.1**. Water stored in Rustfontein Dam is treated at the Rustfontein Treatment Works owned and operated by Bloemwater, and then distributed to Botshabelo and Thaba Nchu by Mangaung Local council. The impoundment was constructed in 1955 and is located approximately 50km east of Bloemfontein, just off the Bloemfontein/Thaba Nchu road (R64). Besides being used for water supply, the dam is also used for recreational activities including sport angling, sailing, skiing, wind surfing and canoeing. According to Slabbert (2007), Rustfontein Dam also forms part of a nature reserve with animal species such as springbok, zebra, blesbok and wildebeest.

Mockes Dam is located in the Phillip Saunders Resort, about 25km north east of Bloemfontein. This impoundment was constructed in 1948 and it serves to regulate the flow through to the Maselspoort Dam. Maselspoort Dam is situated in the Maselspoort holiday resort, 23km north east of Bloemfontein and just 13km from the airport. The water treatment plant at Maselspoort Dam is currently owned and operated by the Mangaung Local Municipality and it supplies the city of Bloemfontein with 31 % water. The other 69 % is supplied by Welbedacht Dam, an impoundment on the Caledon River, owned and operated by Bloemwater.

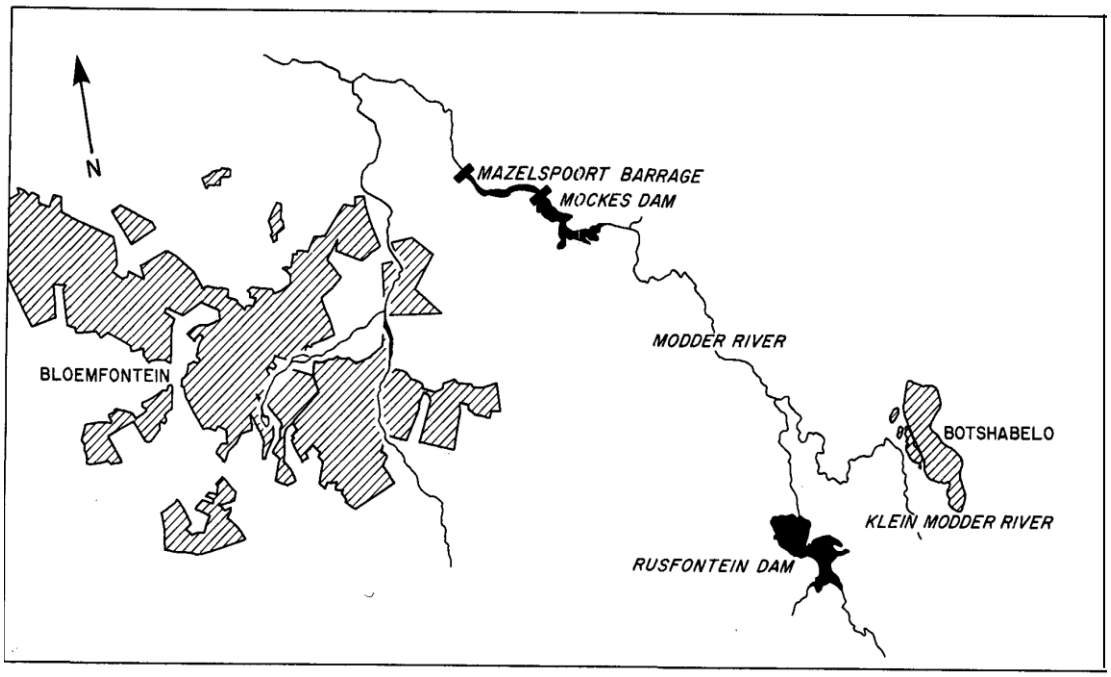

Figure 3.1: Schematic map showing three impoundments in the upper Modder River (Grobler & Toerien, 1986).

Table 3.1: Location, morphological and hydrological data of impoundments in the upper Modder River (Grobler & Toerien, 1986; Grobbelaar, 1992)

	Rustfontein	Mockes	Maselspoort
Latitude	29°18'43.9''S	29°03'57.8''S	29°02'19.4''S
Longitude	26°37'24.4''E	26°28'35.5''E	26°26'03.9''E
Catchment area (km^2)	950	2960	3059
Mean depth (m)	6.5	1.8	2
Capacity at FSL ($m^3 10^6$)	76	6	0.8
Width (m) x Height (m)	6577 x 10413	3886 x 3871	5908 x 3180
Mean retention time (annum)	2.2	0.06	0.008
Mean annual runoff ($m^3 10^6$)	35	106	106

However, according to resent data by DWAF on weekly state of reservoirs, Rustfontein Dam has 71.2 m^3x10^6 as its capacity at FSL due to sedimentation.

3.2) MATERIALS AND METHODS

3.2.1) SAMPLING

Sampling was done twice a month, from May to December 2009 and March to April 2010 at Rustfontein Dam (raw and treated water), Mockes Dam and Maselspoort Dam (raw and treated water). Sampling in Rustfontein and Maselspoort Dams was done as close to the treatment plants' abstraction points as possible so that the samples would be the true representatives of the water entering the plants. *In situ* measurements were made during the sampling visits and subsurface water samples (1L) were taken and transported on ice to the laboratory for chemical and other analyses. The samples were stored in a dark cold room to limit chemical and biological changes and the analyses were done within 48 hours, except for the toxin analysis. Samples for toxin analysis were deep frozen and analysed when sufficient numbers of samples were accumulated to use all 96 wells of the ELISA test kit.

3.2.2) SELECTION OF VARIABLES

Traditionally, water quality monitoring has been driven by the need to verify whether the observed water quality is suitable for intended use (Mäkelä & Meybeck, 1996). According to Chapman & Kimstach (1996), for the objectives of any water quality assessment programme to be met efficiently and in the most cost effective way, it is important to select the variables appropriately. As indicated by Mäkelä & Meybeck (1996), water quality can be described by certain physical, chemical and biological characteristics and they gave temperature, electrical conductivity, pH, dissolved oxygen (DO) and total suspended solids (TSS) as the simplest combination of variables. Apparently, these variables can provide a basis for crude assessment of overall water

quality. Other variables that could be included are nutrients causing eutrophication and chlorophyll *a*.

3.2.3) TEMPERATURE

Latitude, altitude, season, time of day, air circulation, cloud cover and the flow and the depth of the water body are the factors influencing the temperature of surface waters (Chapman & Kimstach, 1996). According to these authors, temperature measurement should be included in a sampling regime because it has an influence on physical, chemical and biological processes in water bodies. Increase in temperature has the following effects:

(i) Increase in the rate of chemical reactions.
(ii) Decrease in the solubility of gases in water.
(iii) Increase in respiration rates leading to increased oxygen consumption and increased decomposition of organic matter.
(iv) Increase in growth rates leading to increased water turbidity, macrophyte growth and algal blooms, when nutrient conditions are suitable (Chapman & Kimstach, 1996).

Temperature of water bodies should be measured *in situ* because a water sample will gradually reach the same temperature as the surrounding air (Ballance, 1996; Chapman & Kimstach, 1996). Temperature measurement was done during each sampling visit. This was performed by dipping the Hanna HI 9828 multiparameter probes into the water at the sampling point after which the readings were recorded. The device was calibrated once a month with HI 9828-25 Calibration Solution and tested before each sampling visit.

3.2.4) CONDUCTIVITY

This refers to the ability of water to conduct an electric current and according to Balance (1996) and Chapman & Kimstach (1996), it is influenced by:

(i) The degree to which dissolved solids dissociate into ions.
(ii) The amount of electrical charge on each ion, ion mobility.
(iii) Temperature of solution.

Electrical conductivity of water samples was measured at the sampling points during each sampling visit using the Hanna HI 9828 multiparameter device.

3.2.5) pH

This refers to a measure of the acid balance of a solution and it is controlled by the dissolved chemical compounds and biochemical processes in the solution. According to Chapman & Kimstach (1996), pH is an important variable in water quality assessment because it influences many biological and chemical processes within a water body and all processes associated with water supply and treatment. When continuously measured, changes in pH can give an indication of the presence of certain effluents. It should also be determined *in situ*, if possible.

pH was measured during each sampling visit at the sampling point. This was performed by dipping the Hanna HI 9828 multiparameter probes into the water at the sampling point after which the readings were recorded.

3.2.6) DISSOLVED OXYGEN (DO)

Oxygen is essential to all forms of aquatic life. Dissolved oxygen levels in natural and wastewaters depend on the physical, chemcal, and biochemical activities in the water body (Chapman & Kimstach, 1996), and these include temperature, salinity, turbulence, the photosynthetic activity of algae and plants, and atmospheric pressure. Oxygen influences nearly all chemical and biological processes within water bodies, so its measurement provides a good indication of water quality (APHA, 1985; Ballance, 1996). According to

Balance (1996), changes in dissolved oxygen concentrations can be an early indication of changing conditions in the water body.

The measurement dissolved oxygen was performed at the time of collecting water samples using Hanna HI 9828 multiparameter device. The measurement was perfomed by dipping the probes into water at sampling points and recording the readings once they are stabilized.

3.2.7) NITRATE

Nitrate plus nitrite concentrations in surface waters give a general indication of the nutrient status and level of organic pollution (Ballance, 1996). According to Chapman & Kimstach (1996), when nitrate concentrations in the source water are high, it is important to analyze treated water because the normal processes for drinking water treatment remove little nitrate.

The method used for nitrate determination was based on the principle that nitrate is reduced to nitrite on exposure to cadmium chips. The nitrite is then combined with reagents to form a red/pink azo dye which can then be measured spectrophotometrically.

3 mL of NH4Cl, 1 mL of $Na_2B_4O_7.10H_2O$ and 0.5-0.6 g spongy cadmium were added to 10 mL Whatman GF/C filtered water, prepared standards and blank (deionised water) and mixed on a mechanical shaker for 20 minutes afterwhich 1.4 mL of each sample was transferred to a 10 mL test tube and 0.2 ml of sulphanilamide and 0.2 mL of N-1 naphthylethylene diamide dihydrochloride were added to each tube. This resulted in the development of a pink azo dye which was then measured spectrophotometrically at 543 nm with the Cary 3 UV-Visible Spectrophotometer. A calibration curve was

prepared by plotting absorbance of the standards against concentration and this was used to determine the concentration of nitrate in the samples.

3.2.8) PHOSPHATE

Levels of phosphate in lakes and reservoirs need to be determined in order to assess the capacity of the water body to carry a cyanobacterial population (Lawton *et al*. 1999a). High concentrations of phosphates can indicate the presence of pollution and are largely responsible for eutrophic conditions (Chapman & Kimstach, 1989).

The method used for phosphate determination is based on the reaction of the phosphate ion in an acid medium with ammonium molybdate and antimonyl tartrate to form antimony-phosphomolybdate complex, which on reduction with ascorbic acid, yields an intense blue colour suitable for photometric measurement (APHA, 1985; Van Vliet *et al*., 1988).

5 mL of molybdate-antimony solution was added to 40 mL Whatman GF/C filtered water, standards and blank followed by the addition of 2 mL ascorbic acid which resulted in the development of a blue colour. The absorbance of the samples and the standards was measured at 882 nm with the Cary 3 UV-Visible Spectrophotometer.

3.2.9) CHLOROPHYLL

The use of chlorophyll-*a* as an index of trophic status is based on the fact that chlorophyll-*a* is normally the most abundant and important pigment in phytoplankton cells. Thus its measurements provide a convenient estimation of the algal biomass. Chlorophyll-*a* in the three impoundments investigated was measured using the modified method as described by Sartory (1982). It involved filtering 200 mL of water samples (20 mL of water sample from

Rustfontein Dam on 15 April 2010) whereafter the cells entrapped on a filter were boiled in 10 mL of 95 % ethanol at 78 ºC in Kimax screw-capped glass tubes. The extracts were then centrifuged for 15 minutes at 4000 rpm with the MSE Centrifuge after which the absorbance was scanned from 750 to 400 nm and measurements taken at 665 nm using the Cary 3 UV-Visible Spectrophotometer. The extracts were then acidified with 100 µL of 0.3N HCL and the absorbance scanned from 750 to 400 nm after 2 minutes and measurements taken at 665 nm again. Acidification is required to correct the interference by phaeophytin, a degradation product of chlorophyll- *a* because this pigment absorbs light and fluoresces in the same region of the spectrum as chlorophyll-*a* (APHA, 1985). The absorbance values were used to calculate the chlorophyll concentration using the equation:

Chlorophyll-*a* (mg/L extract) = (E_{665}^{o} – E_{665}^{a}) x 28.66

Where E_{665}^{o} = absorbance at 665nm before acidification (less absorbance at 750 nm)

E_{665}^{a} = absorbance at 665nm after acidification (less absorbance at 750 nm)

The concentration in the original sample was calculated using the equation:

$$Chlorophyll\ a\ (\frac{\mu g}{L}) = \frac{(Ca \times v)}{V}$$

Where Ca = concentration of chlorophyll-*a* in extract in mg/L.

v = volume of extract in mL.

V = volume of original sample in L.

3.2.10) PHYTOPLANKTON

Phytoplankton communities are sensitive to changes in their environment and, therefore, phytoplankton biomass and many phytoplankton species are used

as indicators of water quality (Vuorio *et al*., 2007). According to them, phytoplankton communities give more information on changes in water quality than mere nutrient concentrations or chlorophyll-*a* concentrations. The dominant algal species in the three impoundments investigated were identified with a Zeiss light microscope after fixation with Lugol's iodine solution. The illustrated guides of Truter (1987) and Dillard (1999) were used to identify the phytoplankton.

3.2.11) CYANOTOXINS

There is a diverse range of laboratory methods used to detect and identify cyanotoxins in water and these methods vary in their degree of sophistication and information they provide (Harada *et al*., 1999). The availability of facilities and expertise, together with the type of information required are the factors governing the choice of methods, though selectivity and sensitivity of these methods are the important criteria for selection of methods (Harada *et al.,* 1999; Msagati *et al* 2006). Two methods commonly employed for the detection of microcystin are enzyme-linked immunosorbent assay (ELISA) (biological screening method) and high performance liquid chromatography (HPLC) (analytical technique).

3.11.1) ELISA

ELISA is currently the most promising method for rapid sample screening for microcystins because of its sensitivity, specificity and ease of operation (Harada *et al*., 1999; Swanepoel *et al*., 2008). The technique involves the competition between microcystin toxin in the sample and enzyme-labeled microcystin for a limited number of antibody binding sites on the inside of the test wells, and according to Flury *et al*. (2001), the technique is based on the structure of the microcystin molecule. The information provided by ELISA assay is total toxin concentration of the sample (Flury *et al*., 2001; Rapala *et al*., 2002).

All the reagents except the water came with the Quantiplate kit for microcystin supplied by Envirologix. Since the water samples were very clear (filtered), the analyses were performed following assay protocol with increased sensitivity where the negative control and the three microcystin-LR calibrators, 0.16, 0.16, and 2.5 ppb, were diluted 1:3 in distilled water by adding 100 µL of each to 200 µL of distilled water resulting in concentrations of calibrators as 0.05, 0.2 and 0.83 ppb respectively. The procedure involved the addition of 50 µL of negative control, each calibrator and each sample to the wells to which 50 µL of microcystin assay diluent was added and incubated for 30 minutes on a Heidolph vortex shaker. Thereafter, 100 µL of microcystin-enzyme conjugate was added to each well and the wells were once again incubated for 30 minutes after which the wells were flooded four times with wash solution (prepared by dissolving the wash solution salts, phosphate-buffered saline-Tween 20, pH 7.4, in distilled water and made up to 1 L). The wash step solution was followed by the addition of substrate to each well and incubation for further 30 minutes. After incubation 100 µL of the stop solution was added to each well turning the contents of the wells yellow and their optical density was measured with Bio-Rad 3550 Microplate Reader at 450 nm. From the optical density values given by the reader, toxin concentration in the samples was calculated from the standard curve from the 3 calibrators.

3.2.11.2) HPLC

Reversed phase HPLC coupled with photo-iodide array detection is the technique that is commonly used for routine analysis of microcystins and this method involves the separation of toxins on a stationary phase using a mobile phase (McElhiney & Lawton, 2005). The composition of the mobile phase and the stationery phase used in the analysis influence the separation of microcystin variants within a sample (McElhiney & Lawton, 2005; Msagati *et al*., 2006). Typical HPLC analysis employs a reverse-phase C18 silica column with separation achieved over a gradient of water and acetonitrile, both containing 0.05% trifluoroacetic acid (TFA) (Harada *et al.*, 1999; McElhiney & Lawton, 2005; Msagati *et al*., 2006). The separated microcystins are recognized on the basis of their retention times and characteristic UV absorption spectra (Rapala *et al.*, 2002).

HPLC was supposed to have been used as an alternative method to confirm the results from ELISA but unfortunately it could not be performed because the standards were not available throughout the study period. Attempts were made to obtain them but to no avail.

CHAPTER 4

RESULTS AND DISCUSSION

4.1) CONDUCTIVITY

The concentration of total dissolved salts (TDS) in a water sample has been described by Dallas & Day (2004) as one of the major descriptors of its quality. It can be measured as electrical conductivity (EC) or as salinity and, they correlate closely in most waters. Of the three parameters, EC is commonly used because it is easy to measure and because inexpensive, portable meters for its measurement are available (Dallas et al., 1994; Dallas & Day, 2004).

As mentioned, EC and TDS usually correlate closely in a particular type of water and according to Chapman & Kimstach (1996), the TDS (in mg/L) of most natural waters can be estimated by multiplying EC (in mS/m) by a factor. This factor generally falls between 5.5 and 7.5 and according to the South African Department of Water Affairs, it is approximately 6.5 for the inland waters of South Africa (DWAF, 1996a). However, this factor has to be determined for each water body and once done, it remains relatively constant overtime (Chapman & Kimstach, 1996). The HANNA HI 9828 multiparameter instrument used in this study converted EC into TDS and the factor was found to be approximately 5 for the study sites.

Most freshwaters have EC values ranging between 1 and 100 mS/m (Chapman & Kimstach, 1996) and according to the South African water quality guidelines for domestic use (DWAF, 1996b), no health, aesthetic or treatments risks are associated with the EC ranging from 0-70 mS/m. The EC in Rustfontein Dam ranged from 25 to 30.8 mS/m (average was 26.3mS/m), for Mockes Dam the range was 21.6 – 45.6 mS/m (average was 32.7 mS/m) and for Maselspoort Dam the range was 21 -39.2 mS/m (average was 32.6

mS/m) (Figures 4.1 & 4.2). These are well within the target range proposed by DWAF (1996b) for waters with no risks.

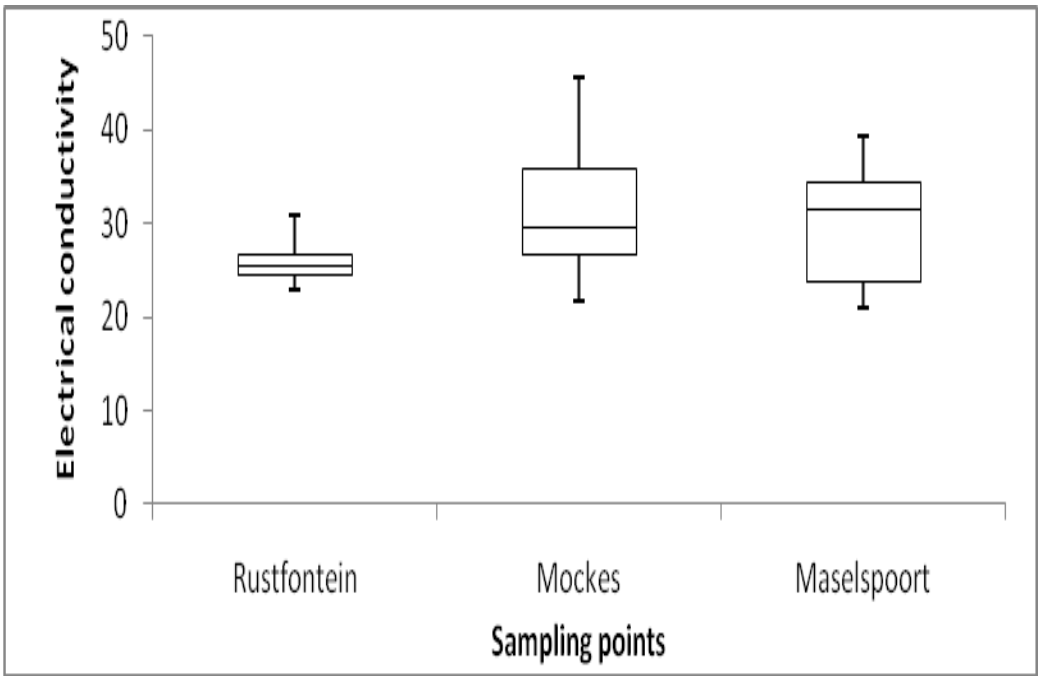

Figure 4.1: Variation in conductivity in the three impoundments in the upper Modder River. The horizontal lines of the box plots mark the median (50th percentile), 1st Quartile (25th percentile) and 3rd Quartile (75th percentile). The whiskers (vertical lines ending with a horizontal strokes) indicate the maximum and the minimum values.

The EC in the impoundments investigated was much lower than those recorded by Oberholster *et al*. (2009) at different sites in Krugersdrift Dam, an impoundment downstream to the three impoundments investigated in this study. They found average EC's of between 70 and 77 mS/m for this impoundment. According to Oberholster *et al*. (2009), a possible reason for these high EC values could be because Krugersdrift Dam is situated downstream of Bloemfontein and it also receives the urban runoff and treated domestic and industrial effluent from the city, via Modder River. Koning (1998) found the average EC for the Modder River to be 36 mS/m. This is higher than the average EC in the three impoundments investigated. This

could be ascribed to the fact that the water which is stored in impoundments comes from peak floods that is usually low in EC (Koning, 1998). According to van Vuuren & Pieterse (1997) and Dallas & Day (2004) the lowest EC values recorded in South African inland freshwater systems are about 0.9 to 3.6 mS/m (Waterkloof stream, Gauteng) and 1.8 to 3.1 mS/m (Swartboskloof stream near Stellenbosch) while the highest recorded value is 9790 mS/m (Burgerspan, south western Cape).

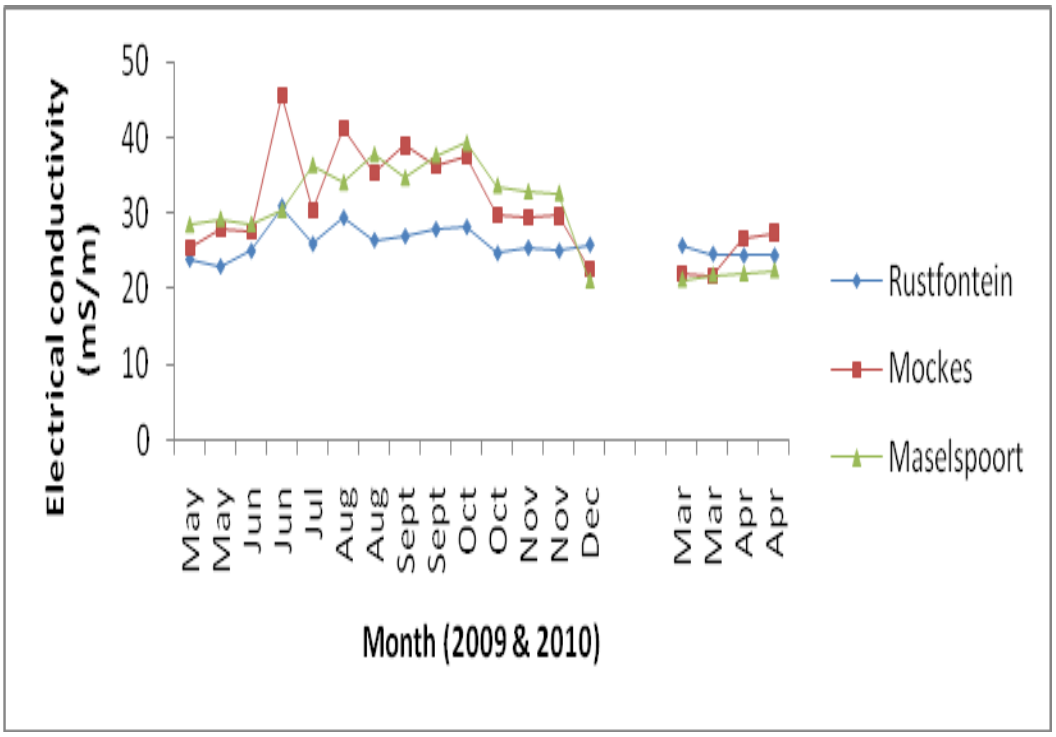

Figure 4.2: Variation in the monthly conductivity in the three impoundments during the study period.

Figure 4.1 shows that EC increased from Rustfontein Dam to Mockes Dam, whereafter a decrease occurred downstream to Maselspoort Dam. This observation is also seen in the data for Rustfontein and Mockes Dams (2005 – 2009) compiled by the Centre for Environmental Management, UFS (personal communication). According to their data, average ECs in Mockes Dam from 2006 to 2009 were 24, 39, 33 and 29 mS/m for the various years respectively. This was higher than the average EC in Rustfontein Dam which

was 21, 27, 28 and 24 mS/m for the same years. The 2005 data is the only exception where the average EC in Rustfontein Dam (35 mS/m) was higher than the average EC in Mockes Dam (30 mS/m). The high conductivity values for Mockes Dam could be ascribed to the effluents from Botshabelo because Grobler & Toerien (1986) have indicated that effluents from Botshabelo might result in the increase in the TDS content of the Upper Modder River as these have a higher TDS content than natural runoff. It should also be remembered that Mockes Dam is situated downstream from Rustfontein, allowing concentration of salts through evaporation.

Increased cation concentrations, indicated by increased EC should enhance flocculation of clay particles and should therefore result in a reduction of water turbidity due to increased settling rate of suspended clay particles (Grobler *et al*., 1983). In Maselspoort Dam EC values were higher than those in Rustfontein Dam (Figure 4.2) which could explain the greater clarity of the water in Maselspoort Dam. However, Mockes Dam, with the highest EC values, was also the most turbid impoundment of the three investigated (from suspended solids rather than algal biomass). This is ascribed to the fact that Mockes Dam is shallower than the other two and thus subjected to wind-driven resuspension. Mockes Dam also acts as a sediment trap upstream of Maselspoort Dam, and this could also explain the greater clarity of the water in Maselspoort Dam.

4.2) DISSOLVED OXYGEN

Oxygen influences nearly all chemical and biological processes within water bodies, so its measurement provides a good indication of water quality (APHA, 1985; Ballance, 1996). According to Ballance (1996), changes in dissolved oxygen (DO) concentrations can be an early indication of changing conditions in a water body. Adequate DO is vital for the survival of aquatic organisms and it is, therefore, an important variable in the assessment and monitoring of water quality. The source of oxygen in the aquatic environments

is the atmosphere together with the photosynthetic activity of algae and higher plants.

The DO levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body (APHA, 1985; Dallas *et al*., 1994; Chapman & Kimstach, 1996; Dallas & Day, 2004) and they outlined them as:

1. Atmospheric re-aeration – dissolved oxygen concentrations can be increased by natural diffusion of gaseous oxygen from the atmosphere into water.
2. Temperature and salinity – increased temperatures and salinities reduce the solubility of oxygen in water, decreasing the amount that can physically dissolve and hence be available to aquatic organisms.
3. Photosynthesis and respiration by aquatic plants – increased photosynthetic activity results in increased dissolved oxygen concentrations whereas increased respiration decreases concentrations of dissolved oxygen.
4. Respiration by aquatic animals – the rate of oxygen uptake by aquatic animals is affected by species, size, age, activity, physiological condition, nutritional status and level of stress.
5. Organic waste – the presence of high levels of organic matter reduces the amount of oxygen dissolved in water because their bacterial decomposition consumes oxygen.

The DO was in the range 5.24 – 11.9 mg/L (average, 7.28 mg/L) for Rustfontein Dam, 4.16 – 12 mg/L (average, 7.28 mg/L) for Mockes Dam and 2.6 -9.05 mg/L (average, 5.34 mg/L) for Maselspoort Dam (Figures 4.3 & 4.4). According to Chapman & Kimstach (1996), functioning and survival of biological communities is adversely affected by concentrations below 5 mg/L and Lind (1974) has indicated that 3 mg/L DO is considered stressful to most aquatic vertebrates. The concentration of DO in Mockes Dam was lower than 5 mg/L in November and December 2009 when the water temperatures were high. The similar behavior was observed in Maselspoort Dam except that in

this dam the low DO concentrations were also recorded in March and April 2010 (Figure 4.4). This could be ascribed to the low solubility of oxygen in warm waters coupled with increased rate of decomposition of organic matter derived from dead leaves from trees along the banks. In Mockes Dam, organic matter could have also been derived from phytoplankton production (high chlorophyll-*a* concentrations). In Maselspoort Dam, low photosynthetic activity (low chlorophyll-*a* concentrations) could be a contributing factor to low DO concentrations. The presence of organic matter in these impoundments was confirmed by the presence of phytoplankton like *Euglena, Phacus* and *Trachelomonas* species (Wetzel, 1983, 2001), as well as the presence of copepods, which are believed to feed on organic matter.

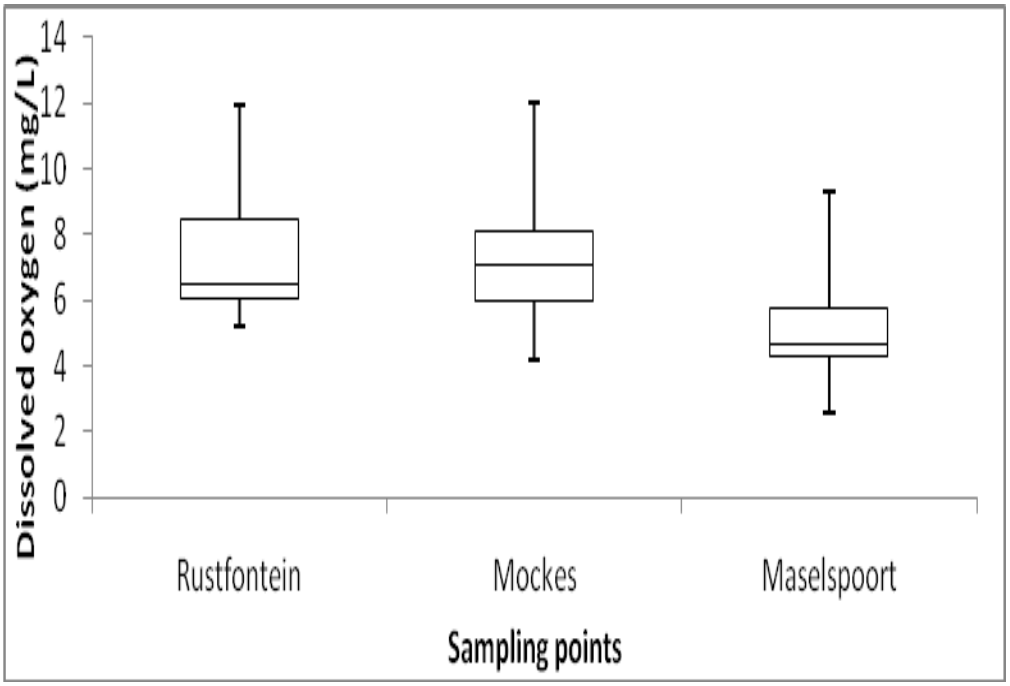

Figure 4.3: Variation in the dissolved oxygen concentration in Rustfontein, Mockes and Maselspoort Dams. For explanation of box plots, see Figure 4.1.

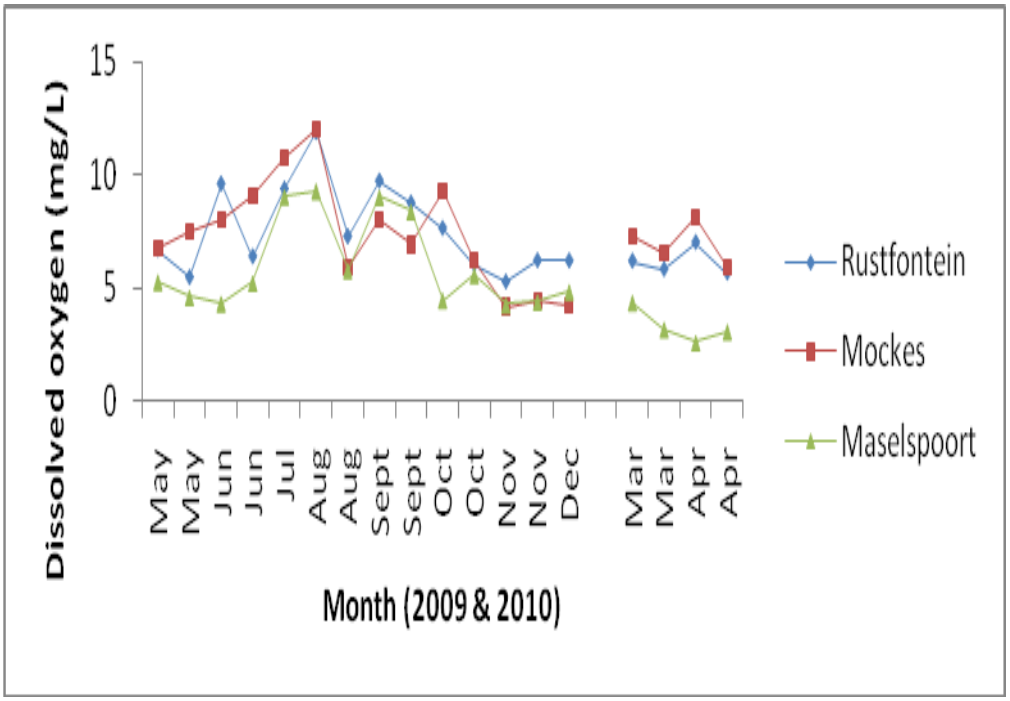

Figure 4.4: Monthly dissolved oxygen variation in Rustfontein, Mockes and Maselspoort Dams.

The DO seemed to follow a pattern with higher concentrations in winter than in the summer months. This could be ascribed to the fact that the solubility of oxygen in cold water is higher than in warmer water, and that the metabolic processes of aquatic organisms are slower in winter than in summer and therefore need less oxygen during the winter for respiration (Wetzel, 1983; Horne & Goldman, 1994). An increase in water temperature results in increased metabolic rates of aquatic organisms which places an additional demand on the oxygen of aquatic ecosystems (Horne & Goldman, 1994). Figure 4.4 shows that the highest concentrations of dissolved oxygen was observed in all the three impoundments in August 2009 (11.9 mg/L for Rustfontein Dam; 12 mg/L for Mockes Dam; 9.30 mg/L for Maselspoort Dam) and this could be ascribed to the possible photosynthetic activity associated with the onset of spring. An inverse correlation between dissolved oxygen and surface water temperature (Figure 4.5) was found in the three impoundments (where DO is dissolved oxygen, T is the surface water temperature and r^2 is the coefficient of determination).

- For Rustfontein: DO = -0.22 T + 11.04, r^2 = 0.27;
- For Mockes: DO = -0.24 T 11.89, r^2 = 0.26;
- For Maselspoort: DO = -0.25 T + 9.84, r^2 = 0.32;

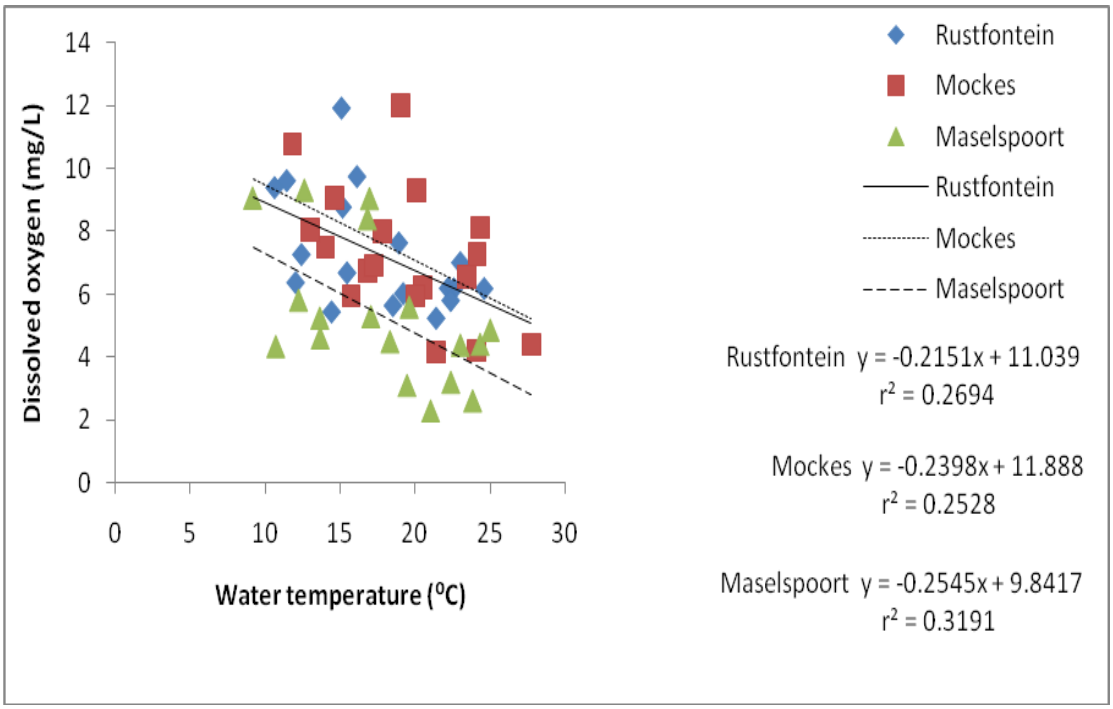

Figure 4.5: Relationship between dissolved oxygen and surface water temperature in Rustfontein, Mockes and Maselspoort Dams during the study period.

The coefficient of determination suggests that 27 %, 26 % and 32 % of the variation in DO in Rustfontein, Mockes and Maselspoort Dams respectively is explained by the variation in surface temperature. The influence of temperature on DO concentrations in these impoundments was partially masked by the photosynthetic activity of the algae as well as organic matter decomposition.

4.3) pH

pH of water is the measure of the concentration of hydrogen (H^+) ions (Horne & Goldman, 1994), and together with DO, it directly or indirectly influences other limnological parameters such as transparency, viscosity, total dissolved solids and conductivity (Lind, 1974; Araoye, 2009). In natural water pH is governed to a large extent by an increase in H^+ ions arising from the dissociation of H_2CO_3 or OH^- ions produced during the hydrolysis of bicarbonate (Wetzel, 1983, 2001). pH determines the ionic species, and thus availability and toxicity, of metals and non-metallic ions in water (Dallas *et al*., 1994; Dallas & Day, 2004). The examples given by these authors are: aluminium is highly toxic, but only in very acidic waters, where the low pH results in the formation of the toxic aqua-Al^{3+} ion; and ammonium ions (NH_4^+), which are not toxic, are gradually converted to highly toxic un-ionised ammonia (NH_3^+) at a pH above about 8. They also reported that changing pH of water alters the concentrations of both H^+ and OH^- ions, which in turn affects the ionic and osmotic balance of aquatic organisms.

Most natural waters have pH values between 6.0 and 9 (Lind, 1974; Dallas *et al*, 1994; Horne & Goldman, 1994; Chapman & Kimstach, 1996; Dallas & Day, 2004) and this is due to the carbonate and bicarbonate buffering system (Wetzel, 1983, 2001; Dallas *et al*, 1994; Horne & Goldman, 1994; Dallas & Day, 2004). pH in natural waters is controlled by several reactions and the most important one is the series of reversible chemical changes shown by the equation below (Wetzel, 1983, 2001; Horne & Goldman, 1994):

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

According to Horne & Goldman (1994), the consumption of CO_2 during photosynthesis or its dissolution from the atmosphere result in the removal or addition of carbonic acid and this should cause a shift in the pH. However, the

pH remains essentially unaltered due to the presence of huge reservoirs of carbonate and bicarbonate.

pH in Rustfontein Dam ranged from 7.46 to 9.48 with an average of 8.65. In Mockes Dam the range was 7 - 9.71 with an average of 8.37 while in Maselspoort the range was from 6.9 to 9.19 with an average of 7.97 (Figures 4.6 & 4.7). These values were not very different from the values recorded by Oberholster *et al*. (2009) at different sites in Krugersdrift Dam (6.4, 6.9, 7.9 and 8.1). They were also in agreement with those found for a number of Dams in South Africa, e.g. Sterkfontein Dam, 7.34; Gariep Dam, 8.25; Van der Kloof Dam, 8.0; Hartbeespoort Dam, 8.15; Bloemhof Dam, 7.7; Loskop Dam, 8.11; Roodeplaat Dam, 8.0; Midmar Dam, 7.4 (Dörgeloh *et al*., 1993). Variations in pH values are attributed to factors such as the removal of CO_2 by photosynthesis through bicarbonate degradation, decomposition of organic matter and reduction of temperature (Saravanakumar *et al*., 2008).

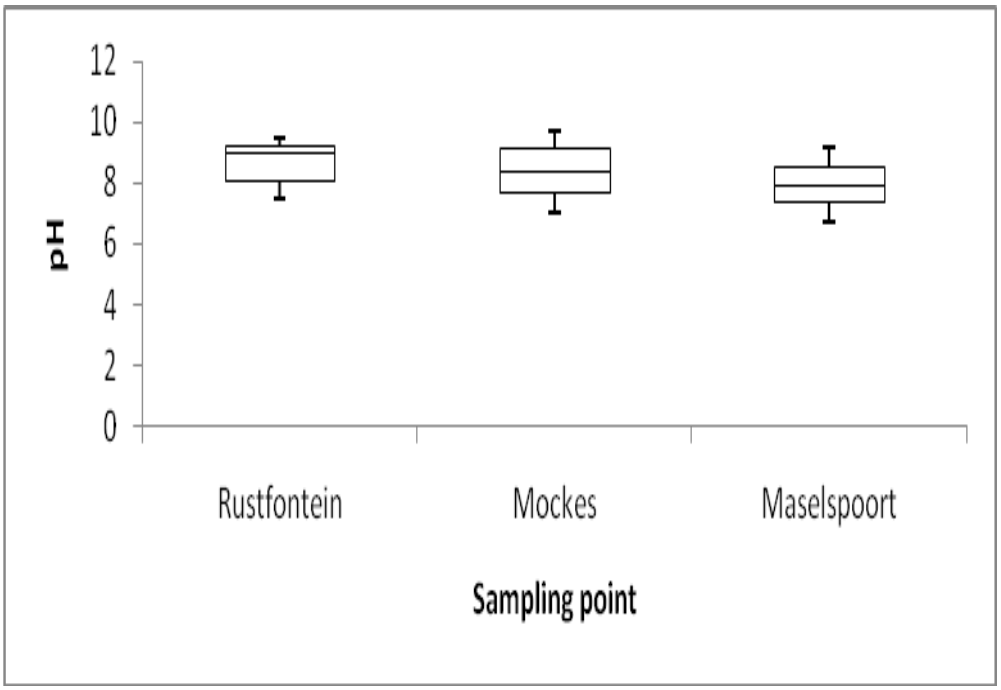

Figure 4.6: pH variations in Rustfontein, Mockes and Maselspoort Dams during the study period. See Figure 4.1 for explanation of box plots.

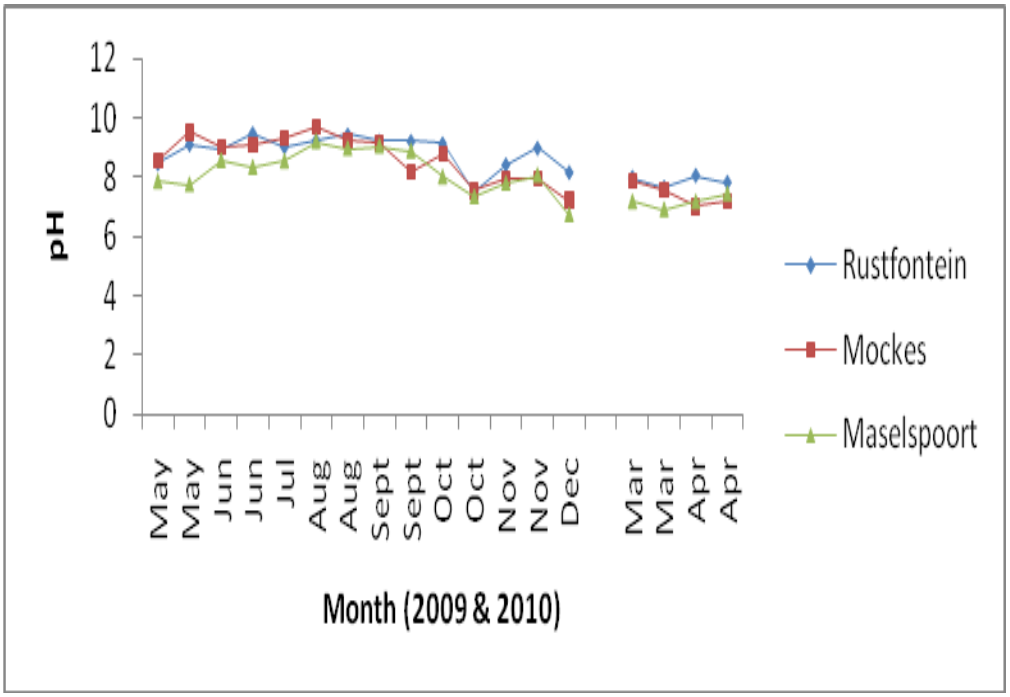

Figure 4.7: Seasonal variation in monthly pH values in Rustfontein, Mockes and Maselspoort Dams during the study period.

In this study, a statistically significant inverse correlation between pH and water temperature (Fig. 4.8) was calculated in all three impoundments:

- pH = -0.10 T + 10.42, r^2 = 0.47 for Rustfontein;
- pH = -0.15 T + 11.19, r^2 = 0.56 for Mockes;
- pH = -0.11 T + 10.00, r^2 = 0.54 for Maselspoort

Furthermore, a statistically positive correlation was found between pH and dissolved oxygen concentration in all the three impoundments (Figure 4.9), i.e.

- pH = 0.19 DO + 7.26, r^2 = 0.28 for Rustfontein;
- pH = 0.26 DO + 6.50, r^2 = 0.39 for Mockes;
- pH = 0.25 DO + 6.63, r^2 = 0.53 for Maselspoort.

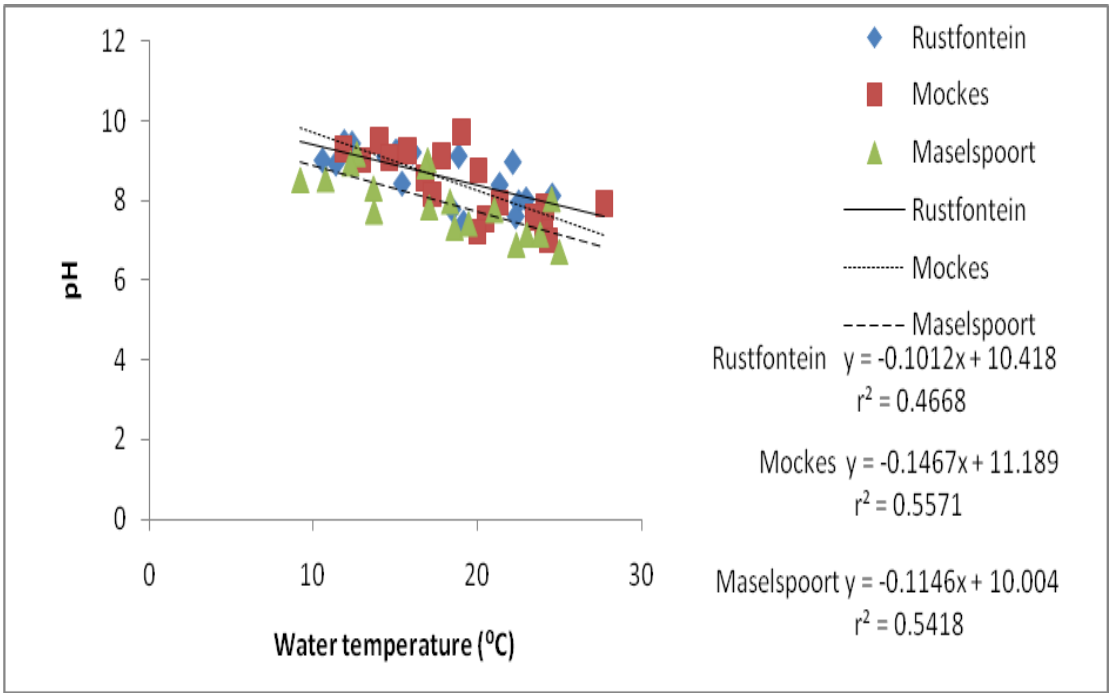

Figure 4.8: Relationship between pH and surface water temperature in Rustfontein, Mockes and Maselspoort Dams during the study period.

This means that variations in pH explained 28 % of the DO in Rustfontein, 39 % in Mockes and 53 % in Maselspoort. This could be explained by the fact that the photosynthetic activity of the algae influences the DO and pH. The higher the photosynthetic activity, the higher the DO will be, with a concomitant increase in pH and *vice versa*. However, the effect of photosynthesis on DO and pH could be masked by decomposition of organic matter. Decomposition of organic matter reduces the amount of oxygen, which increases the amount of carbon dioxide, thereby decreasing the pH (Araoye, 2009).

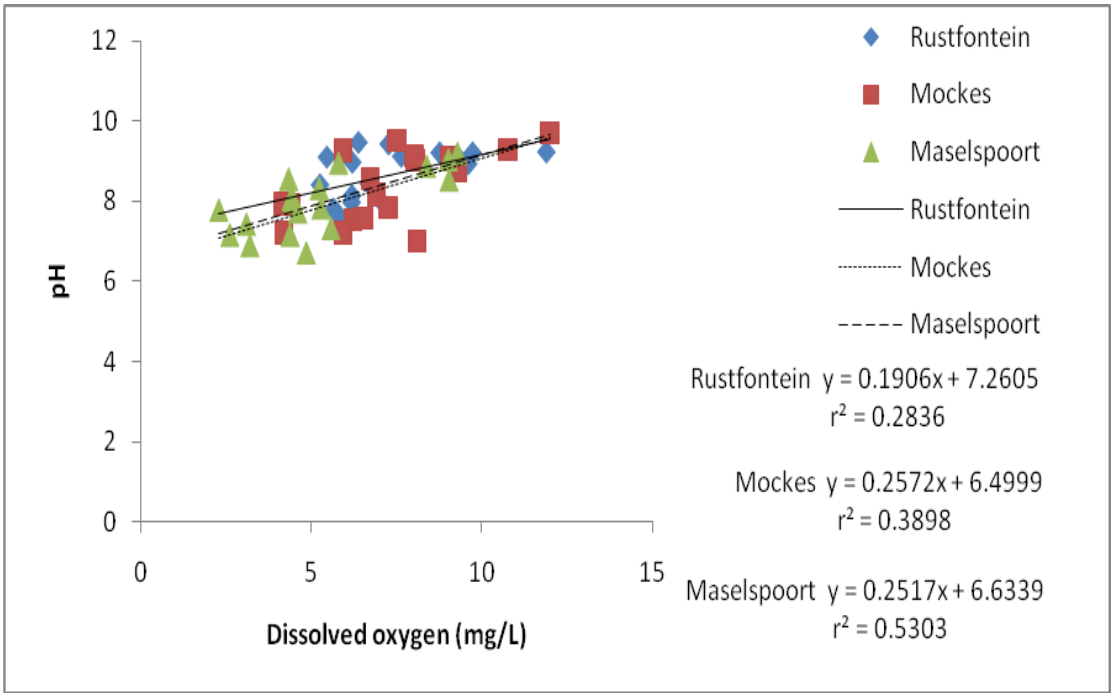

Figure 4.9: Relationship between pH and dissolved oxygen in Rustfontein, Mockes and Maselspoort Dams during the study period.

4.4) TEMPERATURE

Water temperature is one of the most important physical properties of aquatic systems because it influences many physical and chemical characteristics of water, which include:

(i) Vapour pressure,
(ii) Surface tension,
(iii) Density and viscosity,
(iv) The solubility of oxygen and other gases,
(v) Sediment concentrations and transportation,
(vi) Chemical and biological reaction rates,
(vii) The presence and absence of pathogens (Webb, 1996).

According to Webb (1996), the considerable sensitivity of water temperature to modification by natural factors and human activities enhances its

importance as a parameter of water quality. The temperature range for most surface waters is 0 ºC to 30 ºC and these fluctuate seasonally with minimum values occurring in winter and maximum values occurring in summer (Chapman & Kimstach, 1996). This is in agreement with the observation by Walling & Webb (1992), that strong relationships can often be established between air and water temperature because in winter the air is cold and in summer it is warm. However, Wetzel (1983, 2001), Wetzel & Likens (2000) and Saravanakumar *et al*. (2008) have indicated that surface water temperature is generally influenced by, among others, intensity of the solar radiation. Apparently, some transfer of heat from the air and sediments does occur, but this input is usually small compared to direct absorption of solar radiation by water, dissolved organic compounds and suspended particulate matter (Wetzel, 1983 & 2001; Wetzel & Likens, 2000).

The variations in surface water temperatures were similar in the three impoundments, with lower values in winter and higher values in summer (Figure 4.11). They ranged from 10.6 ºC in July to 24.6 ºC in November in Rustfontein dam; from 11.8 ºC in July to 27.7 ºC in November in Mockes Dam; and from 9.2 ºC in July to 25 ºC in November in Maselspoort Dam (Figures 4.10 & 4.11). Surface water temperatures in Mockes Dam were higher than in the other two impoundments throughout the study period (Figures 4.10 & 4.11). This could be ascribed to the fact that Mockes Dam is shallowest of the three. Chapman & Kimstach (1996) indicated that depth of the water body also influences temperature of surface waters.

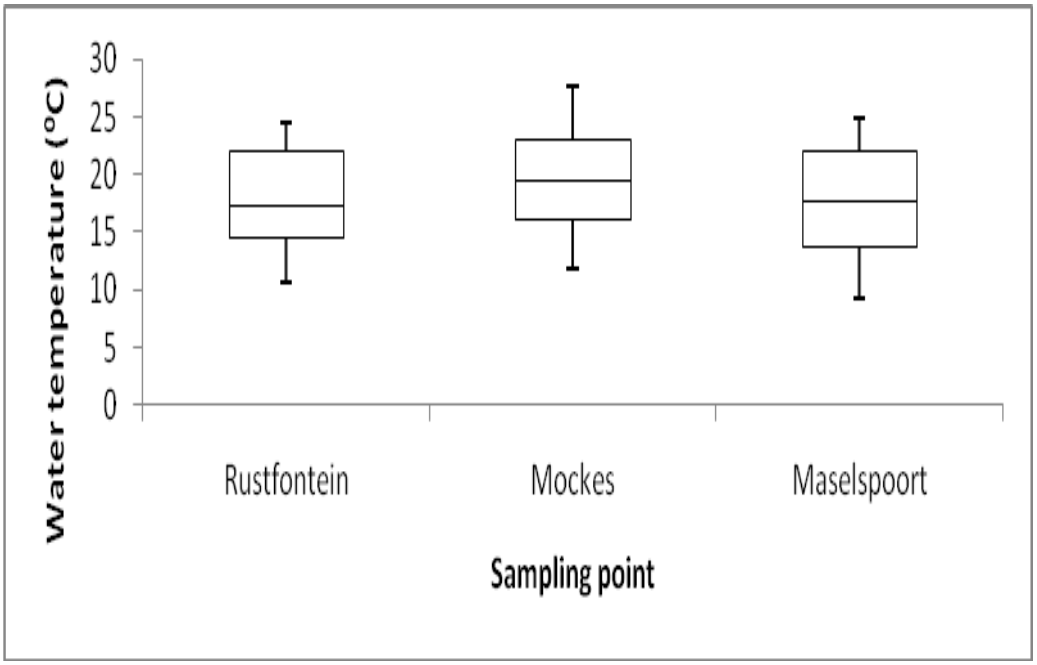

Figure 4.10: Variation in surface water temperature during the study period in Rustfontein, Mockes and Maselspoort Dams. See Figure 4.1 for explanation of box plots.

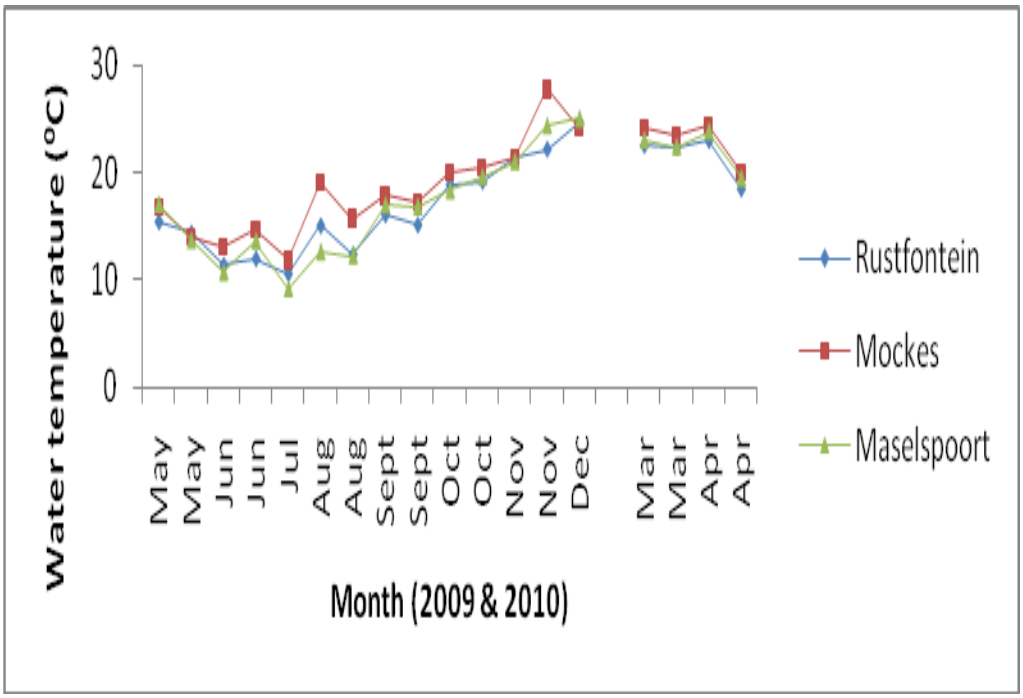

Figure 4.11: Seasonal variation in surface water temperature in Rustfontein, Mockes and Maselspoort Dams during the study period.

Van Vuuren & Pieterse (1997) reported that in the majority of South African impoundments, maximum chlorophyll-*a* concentrations coincide with high water temperatures. In their study on Vaal River, they observed that maximum chlorophyll-*a* concentrations occurred when the water temperature started to increase after winter minimum was reached, as well as during periods when high water temperatures were recorded. However, in Mockes Dam the opposite happened because the maximum chlorophyll-*a* concentration occurred in June when the water temperature was low. During the study by Roos (1991) on the Vaal River, algal blooms and high photosynthetic rates occurred during the winter-spring months (June to August) when the water temperature was about 14 ºC. Roos (1991) argued that the stimulation of temperature on algal growth in the Vaal River was probably obscured by the impact of high nutrient levels after floods and subsequent improved light availability as well as the ability of diatoms to maintain high growth rates at low temperatures.

4.5) NITRATE-NITROGEN (NO_3-N)

Nitrate and nitrite levels in natural waters are among the most important indicators of water quality since they give a general indication of the nutrient status and level of organic pollution. In many countries, there has been an increase in the concentration of nitrate-nitrogen in underground and surface waters due to the extensive use of chemical fertilizers and improper treatment of waste-water from domestic and industrial sources (Mizuta *et al*., 2004). Nitrate in drinking water supplies is a health concern since it can be readily converted to nitrite in the gastrointestinal tract, resulting in methemoglobinemia in new born infants (APHA, 1985; Ballance, 1996; DWAF, 1996; Morrison *et al*., 2001). Though this condition occurs rarely, only with water containing more than 30 mg NO_3-N/L, it is still a cause for concern (Morrison *et al*., 2001).

In South Africa, mainly three provinces (Northern Cape, Northwest Province and Limpopo Province) are seriously affected by accumulation of nitrate in ground water (Tredoux *et al*., 2000). Schoeman (2009) reported that the nitrate-nitrogen concentration of many borehole waters near clinics in the Limpopo Province is very high (30-70 mg NO_3-N/L). This concentration is too high to be fit for human consumption, though in many cases it is used for potable purposes (Schoeman, 2009). Tredoux *et al*. (2000) identified anthropogenic activities such as fertilizer application to land, sewage sludge and application to soil, waste water irrigation, deforestation and mobilization of natural soil nitrogen by ploughing as the sources of nitrate in groundwater. Schoeman (2009) also believes that the high nitrate-nitrogen concentrations are due to the location of pit latrines and other community sewage-disposal systems in the vicinity of production boreholes.

Nitrate is seldom abundant in natural surface waters (normally < 0.1 mg NO_3-N/L) in spite of their many sources because photosynthetic action is constantly converting it to organic nitrogen in plant cells or reduced by microbes and converted into atmospheric nitrogen (APHA, 1985; Dallas *et al*., 1994; Dallas & Day, 2004).

The nitrate + nitrite concentrations in the three impoundments investigated were generally lower than 1 mg/L (except on two occasions, in May 2009 in Rustfontein Dam and in November 2009 in Maselspoort where the concentrations were 1.079 and 1.088 mg NO_3-N/L) respectively (Figures 4.12 & 4.13). The range in Rustfontein Dam was 0.38-1.079 mg NO_3-N/L (average was 0.72 mg NO_3-N/L); the concentrations in Mockes Dam ranged from 0.175 to 0.998 mg NO_3-N/L (average was 0.62 mg NO_3-N/L); the concentration range in Maselspoort Dam was 0.26-1.088 mg NO_3-N/L with the average of 0.65 mg NO_3-N/L. The concentrations in the three impoundments investigated were higher than the average concentration recorded in the Modder River (0.230 mg NO_3-N/L) by Koning (1998), but they were much lower than the average concentrations recorded by Oberholster *et al*. (2009) at different sites

in Krugersdrift Dam (2.9, 2.1, 2.2 and 1.9 mg NO_3-N/L). However, they were higher than the average concentration (0.13 mg NO_3-N/L) recorded by Dörgeloh *et al*. (1993) at Sterkfontein Dam.

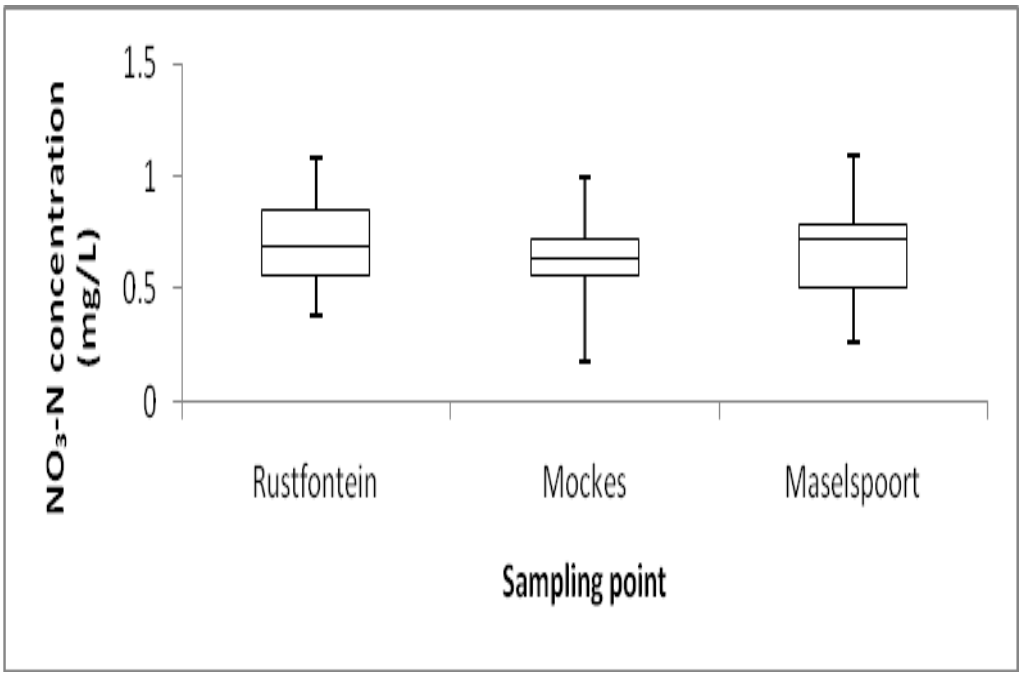

Figure 4.12: Variation in the NO_3-N concentration in Rustfontein, Mockes and Maselspoort Dams during the study period. For explanation of box plots, see Figure 4.1.

Concentration of NO_3-N in the three impoundments investigated was the lowest in June 2009 (Figure 4.13). Fogg (1980) indicated that during blooms of planktonic algae in freshwater concentrations of phosphate and nitrogen are at their lowest. This seemed to be the case in Mockes Dam, where the lowest concentration of nitrate in June coincided with the highest concentration of chlorophyll-*a*. In Maselspoort Dam, low NO_3-N and PO_4-P concentrations accompanied by high algal biomass occurred during the end of September. This tendency of high algal blooms being accompanied by low concentrations of phosphorus and/or nitrogen was also observed by van Vuuren & Pieterse (1997) during their study on Vaal River.

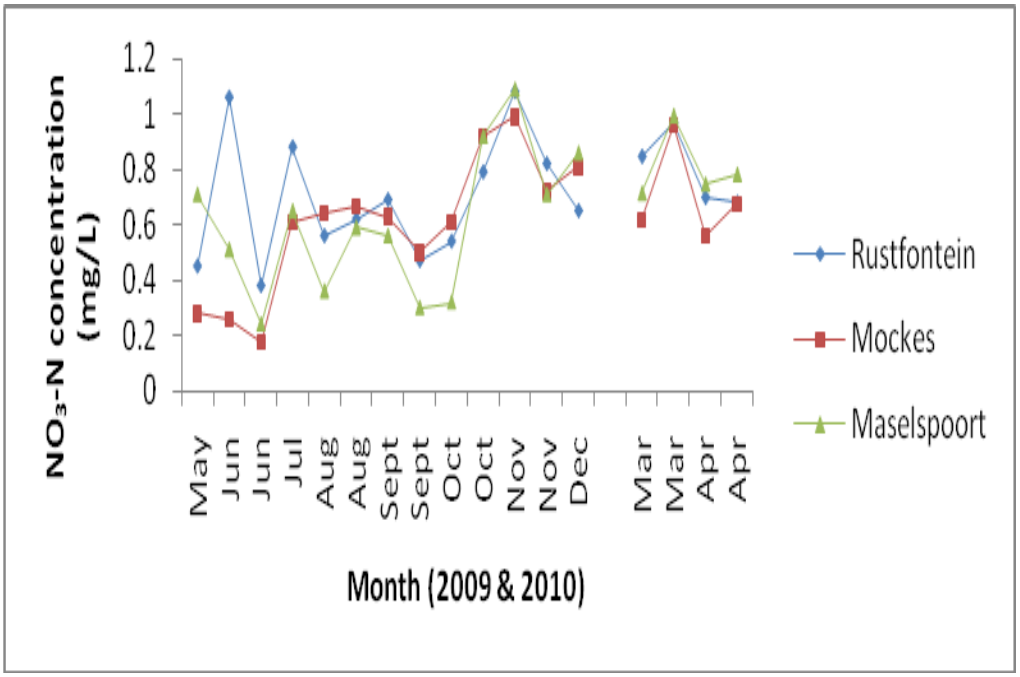

Figure 4.13: Seasonal variation in NO_3-N concentration in Rustfontein, Mockes and Maselspoort Dams during the study period.

Figure 4.13 shows that NO_3-N concentration in the three impoundments increased from the middle of October 2009 and peaked by the middle of November 2009. The concentrations dropped during the end of November and this coincided with the increase in chlorophyll-*a* concentration (Figure 4.17). It is believed that the depletion was a result of uptake by phytoplankton.

4.6) ORTHOPHOSPHATE-PHOSPHORUS (PO_4-P)

Phosphorus is an essential component of the biological cycle in waterbodies hence why it is often included in basic water quality surveys (Chapman & Kimstach, 1996). Acording to these authors, it occurs in natural waters and wastewaters almost solely as phosphates which could either be orthophosphates, condensed phosphates or organically bound phosphates.

Determination of phosphate content of natural waters is of great importance in water quality assessments since it is generally regarded as the best indicator of the trophic status of natural waters (Heron, 1962; Wetzel & Likens, 2000). Phosphorus is rarely found in high concentrations in freshwater because it is actively taken up by plants (Dallas & Day, 2004). High concentration of phosphates can indicate the presence of pollution and eutrophication (Ballance, 1996; Chapman & Kimstach, 1996). Elevated phosphate concentrations in water have been linked to the increasing rates of plant growth, changes in species composition and proliferation of planktonic, epithytic and epibenthic algae (Zui & Birks, 2000; Shen & Song, 2007).

According to Chapman & Kimstach (1996), phosphorus in most natural waters ranges from 5 to 20 µg PO_4-P/L. In Rustfontein Dam, the concentrations ranged from 43 to 166 µg PO_4-P/L (average = 88 µg PO_4-P/L). The range in Mockes Dam was 52 – 259 µg PO_4-P/L (average = 109 µg PO_4-P/L); and the range was 25 – 200 µg PO_4-P/L (average = 105 µg PO_4-P/L) in Maselspoort. The average PO_4-P concentration in Mockes Dam was about five times higher than the average concentration (22.42 µg PO_4-P/L) recorded by Grobbelaar (1991) in the same Dam. The average concentration recorded by Koning & Roos (1999) in the Modder River (66 µg PO_4-P/L) was also lower than the averages in the three impoundments investigated. However, the concentrations in those impoundments were much lower than the average concentrations observed by Oberholster *et al*. (2009) at different sites in Krugersdrift Dam (510, 470, 300 and 390 µg PO_4-P/L). The variance in Rustfontein was lower than in the other two impoundments (Figure 4.14) and this could be ascribed to the fact that Rustfontein Dam is deeper and has a longer residence time than the other two.

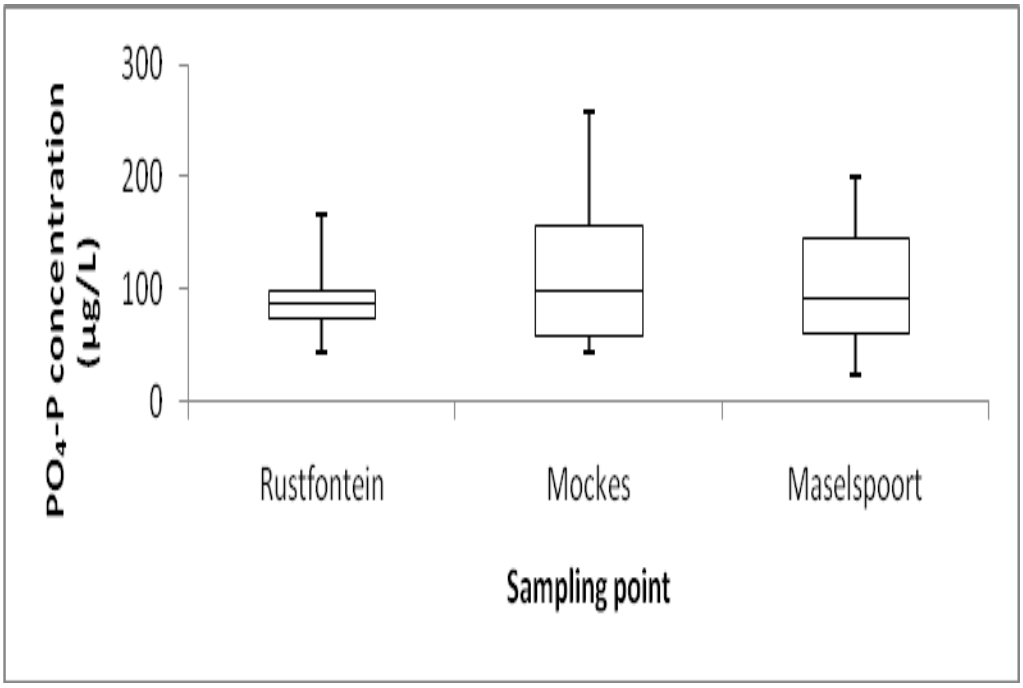

Figure 4.14: Variation in the PO_4-P concentrations in Rustfontein, Mockes and Maselspoort Dams during the study period. For explanation of box plots, see Figure 4.1.

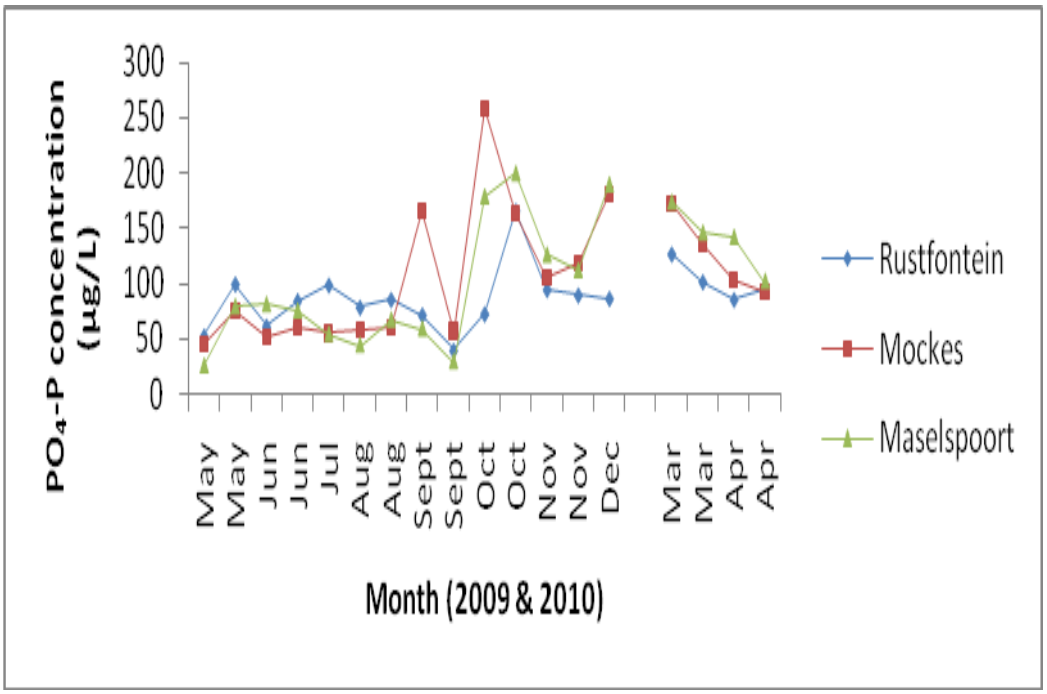

Figure 4.15: Seasonal variation in PO_4-P concentration in Rustfontein, Mockes and Maselspoort Dams during the study period.

Van Vuuren & Pieterse (1997) have indicated that aquatic plants and plants in general are integrators of environmental variables and that they respond to changes in environmental factors with a certain lag period occurring between the environmental stimulation and its response. During the present study, blooms of phytoplankton were, in several occasions, preceded by high phosphate concentrations (with a time lag of 3 to 6 weeks). There was a high concentration of PO_4-P in the water in Rustfontein Dam during the end of October 2009 (Fig. 4.15), and this could have been responsible for an increase in chlorophyll-*a* concentration which peaked during the middle of November 2009 (Fig. 4.17). Another peak of chlorophyll-*a* concentration observed during the middle of April 2010 (Fig. 4.17) could also be ascribed to the high PO_4-P concentrations recorded during the middle of March 2010 (Fig. 4.15).

In Mockes Dam, chlorophyll-*a* concentrations were often high, but there were distinct peaks and these were preceded by high PO_4-P concentrations. For example, the peaks during the end of June 2009, the middle of October 2009, the middle of November 2009 and the middle of April 2010 (Fig. 4.17) were all preceded by high concentrations of PO_4-P. These high PO_4-P concentrations occurred during the end of May 2009, middle of September 2009, middle of October 2009 and during the middle of March 2010 (Fig. 4.15). In Maselspoort Dam, a distinct peak of PO_4-P was observed during the end of October, 2009 (Fig. 4.15) and this is believed to be responsible for the highest chlorophyll-a concentrations recorded during the middle of November 2009 (Fig. 4.17). Van Vuuren & Pieterse (1997) also had similar observations (i.e. the precedence of high algal biomass by high phosphorus concentrations) during their study on Vaal River.

According to the Department of Water Affairs and Forestry (DWAF, 2002), the trophic status of a waterbody is largely related to the phosphorus

concentration. This in turn determines the level of eutrophication. The terms used to describe the state of enrichment are oligotrophic, mesotrophic, eutrophic and hypertrophic. Thomas *et al* (1996) defines these terms as follows:

- Oligotrophic - low levels of nutrients and no water quality problems.
- Mesotrophic - intermediate levels of nutrients, with emerging signs of water quality problems.
- Eutrophic - high levels of nutrients and an increasing frequency of water quality problems.
- Hypertrophic - excessive levels of nutrients, plant growth is determined by physical factors. Water quality problems are almost continuous.

Toerien *et al* (1975), in a survey of 98 South African impoundments established that:

- Rustfontein Dam was mesotrophic on the basis of its algal growth potential (20.5 mg/L) (the potential to sustain algal growth) and phosphorus was the growth-limiting nutrient.
- Mockes and Maselspoort Dams were also mesotrophic with algal growth potentials of 32 mg/L and 49.1 mg/L respectively and that nitrogen was the primary limiting nutrient for algal growth.

According to DWAF (1996), the average summer levels of inorganic phosphorus (measured as PO_4-P) are less than 5 µg/L in oligotrophic systems, 5-25 µg/L in mesotrophic systems, 25-250 µg/L in eutrophic systems and above 250 µg/L in hypertrophic systems. Based on these, all the three impoundments investigated in this study are eutrophic. However, this is based on a single sampling site and according to Thornton *et al*., 1996, classifying the whole reservoir on the basis of single sampling site can be misleading.

4.7) CHLOROPHYLL-*A*

Chlorophyll is a green pigment in all photosynthetic organisms and it exists in three forms, chlorophyll *a*, *b*, and *c*. All green plants contain chlorophyll-*a* and planktonic algae owe about 1-2% of its dry weight to chlorophyll *a* (APHA, 1985; Van Vliet *et al*., 1988; Lawton *et al*., 1999; Swanepoel *et al*., 2008). Chlorophyll-*a* concentrations have been accepted as an indirect measurement of phytoplankton biomass and this was brought about by the various observations on the correlations between algal biovolume and chlorophyll-*a* (Vörös & Padisák, 1991). However, Watson *et al*. (1992) have indicated that the relationship between chlorophyll-*a* concentration and algal biovolume is thought to be highly variable and unpredictable. They pointed out that variability is attributed to species- or size-specific differences in cell chlorophyll content and/or the influence of environmental factors such as light and nutrient levels.

Estimation of chlorophyll-*a* concentrations is important in the monitoring of water quality because it is, therefore an indicator of the trophic state, phytoplankton abundance and biomass (Flemer, 1969; Dillon & Rigler, 1974; Sartory & Grobbelaar, 1984; APHA, 1985; Van Vliet *et al*., 1988; Chapman & Kimstach, 1996; Moses *et al*., 2009; Huang *et al*., 2010). According to Roos (1991), it is the best reliable trophic status indicator because:

1. It is relatively easy to measure.
2. It gives a quantitative indication of the problem condition.
3. It can be qualitatively related to other eutrophication and water quality characteristics such as phosphorus and nitrogen loads/concentrations and primary productivity.

Huot *et al*. (2007) observed that chlorophyll-*a* concentration is the most widely used proxy for phytoplankton biomass because it is coloured, specific to, and shared amongst all primary producers.

According to Donohue & Molinos (2009), light attenuation by inorganic turbidity decreases the fraction of light absorbed by photosynthesising organisms in lakes consequently reducing the density, growth rates and phytoplankton production considerably. The Modder River is turbid as a result of suspended sediments and it is possible that the algal (phytoplankton) growth might be limited by light rather than nutrient availability. Grobbelaar (1992) reported that the productivity in the impoundments in the Modder River could not be ascribed to increased nutrient loading because turbidity influences overall productivity and nutrients are only important when more favourable water light regime prevails. However, Grobler & Toerien (1986) indicated that studies on Wuras Dam, a shallow turbid impoundment 80 Km south of Bloemfontein showed its phytoplankton productivity was relatively high. They believed that the response of Mockes Dam to increased nutrient loading would not differ from that of Wuras Dam because they are in the same geographical area.

Of the three impoundments studied, Mockes Dam displayed relatively higher chlorophyll-*a* concentrations (Figure 4.16) throughout the study period (minimum = 7.2 µg/L; maximum = 521 µg/L; mean = 76.4 µg/L). This is more than twice the average concentration of chlorophyll-*a* (33.6 µg/L) in Mockes Dam observed by Grobbelaar (1991). Grobler & Toerien (1986) had predicted that by the year 2010, average chlorophyll-*a* concentration for Mockes Dam would be 56 µg/L, and be 66 µg/L by 2020. However, this study showed that they underestimated the impact of the sewage treatment at Botshabelo because their 2020 predictions have already been superseded. The study conducted by Koning (1998) indicated that the sampling sites just below the confluence of the Modder and Klein Modder Rivers experienced most algal blooms. Mockes Dam is the first impoundment after the confluence of the

Modder and the Klein Modder Rivers and the high chlorophyll-*a* concentrations in this impoundment can be ascribed to the nutrient-rich water that flows from the Klein Modder River into the Modder River. Dillon & Rigler (1974), Schindler (1977,1978), and many others have indicated that increased nutrient levels frequently result in increased phytoplankton standing crops.

The highest concentration of chlorophyll-*a* in this impoundment was recorded at the end of June 2009 (Figure 4.17), and this coincided with low concentrations of NO_3-N and PO_4-P and the highest EC (Figures 4.13, 4.15 & 4.2). The low concentrations of the nutrients coupled with high chlorophyll-*a* concentration indicated that the nutrients were used by phytoplankton for photosynthesis. The high EC, hence high TDS could have caused floc formation which in turn resulted in the light limitation being lifted thus permitting algal growth.

It is stated in the South African Water Quality Guidelines for Recreational use (DWAF, 1993) that chlorophyll-*a* concentrations above 30 µg/L for phytoplankton are indicative of bloom conditions. According to Grobler & Toerien (1986), surface scums develop when chlorophyll-*a* concentrations are between 10 and 20 µg/L, nuisance conditions develop between 20 and 30 µg/L, and severe nuisance conditions develop at 30 µg/L. This is collaborated by Raschke (1993) who indicated that:

1. Chlorophyll-*a* concentrations in the range 0 – 10 µg/L causes no discolouration of water and no problems.
2. A range 10 – 15 µg/L can cause discolouration to water and algal scums could develop.
3. At the range 20 -30 µg/L, the water is deeply discoloured, frequency of scums increases and matting of algae can occur.
4. Beyond 30 µg/L of chlorophyll-*a*, the intensity of discolorations and the frequency of mats increase.

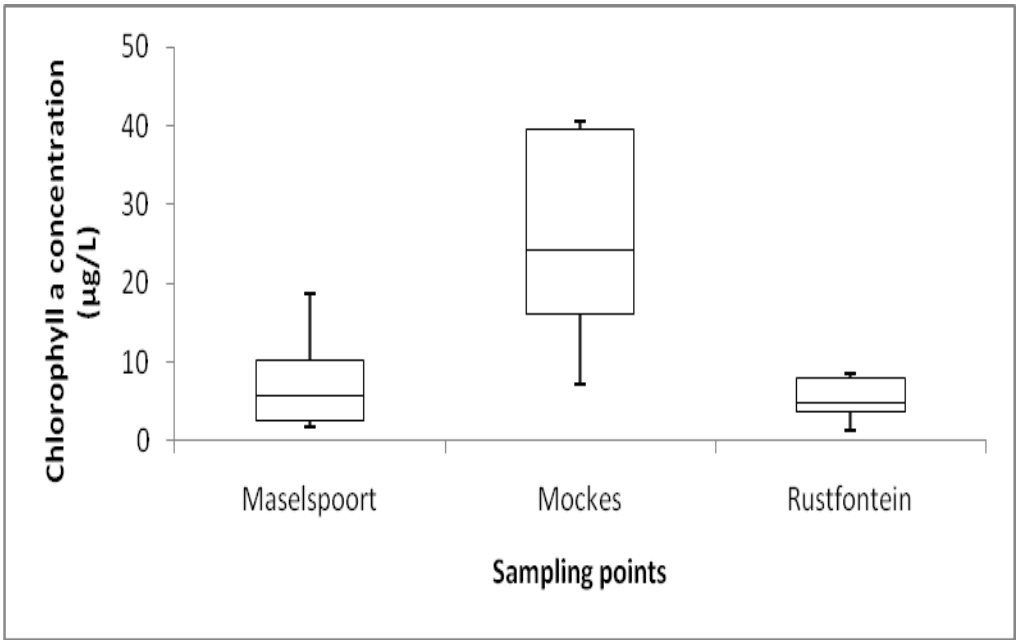

Figure 4.16: Variation in chlorophyll-*a* concentration in Rustfontein, Mockes and Maselspoort Dams during the study period (extreme values are not included). For explanation of box plots, see Figure 4.1.

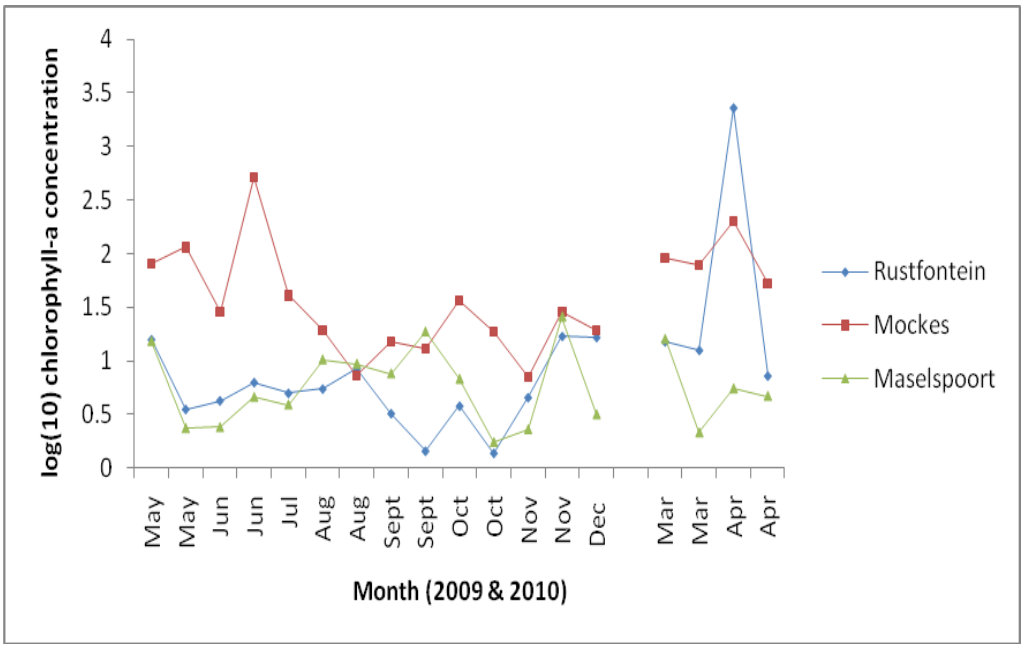

Figure 4.17: Seasonal variation in chlorophyll-*a* concentration in Rustfontein, Mockes and Maselspoort Dams during the study period.

In Mockes Dam about 55 % of the samples had chlorophyll-*a* concentration levels higher than 30 µg/L, and 39 % had chlorophyll-*a* concentration levels above 60 µg/L. This is in accordance with the prediction by Grobler & Toerien (1986) who stated that as the treated sewage from Botshabelo will contribute to the P load in Mockes Dam and that severe nuisance conditions could be expected to occur more than 40 % of the time.

The chlorophyll-*a* concentration in Rustfontein Dam was below 20 µg/L throughout the study period except in April 2010 when there was a bloom and the concentration of chlorophyll-*a* was 2 275 µg/L (Figure 4.17). This lasted for a short period of time because the following week, the bloom had disappeared. In Maselspoort Dam chlorophyll-a concentration was also below 20 µg/L except in November 2009 when the concentration was 25.5 µg/L. As with Mockes Dam, the highest concentrations of chlorophyll-a in these two impoundments were accompanied by low concentrations of NO_3-N and PO_4-P, and these were preceded by very high concentrations, indicating that the depletion was due to assimilation by the phytoplankton.

As already mentioned, chlorophyll-*a* concentration is used as a general indicator of the trophic status of water body (Jones *et al*., 1979; Roos, 1991). According to Jones *et al* (1979), oligotrophic waterbodies tend to have chlorophyll-*a* concentrations of about 5 µg/L or less while eutrophic waterbodies have chlorophyll-*a* concentrations greater than 6 µg/L. However, according to OECD (1982), 8 µg/L of chlorophyll-*a* is considered the boundary between mesotrophy and eutrophy. Dodds *et al*., (1998) described oligotrophic waterbodies as being characterised by low nutrients, low algal counts, high clarity and deep photic zones while eutrophic waterbodies are characterised by frequent cyanobacterial blooms, high total nutrients and wide swings in dissolved oxygen concentrations and pH. They indicated that mesotrophic lakes have intermediate characteristics.

Based on the chlorophyll-*a* concentrations measured for these three impoundments in the present study, Rustfontein can be classified as eutrophic, Maselspoort as mesotrophic and Mockes Dam as hypertrophic.

4.8) ALGAL SPECIES COMPOSITION

It has been shown that the algal species composition change with varying nutrient levels and that these changes are related to differences among taxa in nutrient uptake, storage, growth and loss rates (Watson *et al*., 1997). According to Anderson *et al*. (2002), phytoplankton species composition is affected by, among others, the composition of the nutrient pool (the forms of the nutrients supplied) as well as the relative abundance of the major nutrients. However, as indicated by these authors, the rate of nutrient supply will not necessarily correlate with the rate of nutrient assimilation by the algae, as the latter is influenced by nutritional preferences, uptake capabilities, and physiological or nutritional status (for example, dinoflagellates are mostly associated with low nitrate concentrations, higher ammonium, urea or a dissolved organic nitrogen supply, and consistent physiological preference for reduced N forms).

The study carried out by Van Ginkel (2004) highlighted the fact that cyanobacteria are present in all water sources but their dominance during summer periods as well as the extent of cyanobacterial blooms that occur are determined by the trophic status of impoundment. The cyanobacteria (especially *Microcystis* and *Anabaena* spp) were found in all three impoundments. The other algal species that were often found impoundments included:

1. *Aphanocapsa* sp - occasionally present in Maselspoort Dam
2. *Ceratium* sp. - present in these impoundments throughout the study period and were dominant in Mockes Dam in October 2009, November 2009, March 2010 and April 2010.

3. *Chlamydomonas* sp. - found in all the impoundments in small numbers.
4. *Cryptomonas* sp. - occurred mostly in August 2009, September 2009 and October 2009 in all three impoundments.
5. *Cyclotella* sp. – found to be occurring in high numbers in the three impoundments in November 2009 and March 2009.
6. *Euglena* sp. – found throughout the study period in all three impoundments.
7. *Melosira* sp. – occurred mostly in July 2009, August 2009 and September 2009 in Mockes Dam.
8. *Oscilltoria* sp. – occurred in the three impoundments throughout the year and was dominant in Mockes Dam in June 2009.
9. *Phacus* sp. – found in low numbers throughout the study period in all three impoundments.
10. *Trachelomonas* sp. – also found in low numbers.

Reynolds (1998) indicated that the composition of phytoplankton from a given water body is very often an excellent indication of the trophic state of the water. The phytoplankton in Rustfontein Dam was mostly dominated by cyanobacteria, especially *Microcystis* species and the taxonomic diversity was relatively low. In Mockes Dam, the phytoplankton was mostly dominated by the dinoflagellates, though there were periods of cyanobacterial dominance (*Oscillatoria*). The taxonomic diversity in Maselspoort Dam was higher though the algal biomass was low. Most algal taxonomic groups, especially Bacillariophyceae (diatoms), Chlorophyta (green algae), Cryptophyta, Dinophyta, Cyanobacteria and Chrysophyceae are represented over the growing season in the mesotrophic systems, whereas eutrophic or hyper eutrophic lakes are usually dominated by Cyanobacteria, diatoms, Chlorococalles or Dinoflagellates (Watson *et al*., 1997).

The occurrence of *Oscillatoria* in Mockes Dam during the study period is in agreement with the statement by Havens *et al* (2003) that shallow eutrophic lakes are typically dominated by *Oscillatoria* which have adaptations to

maintain net growth at low underwater irradiance. According to them, *Oscillatoria* have adaptations to permit effective light harvesting under low irradiance and these include high chlorophyll-to-biovolume ratios, high concentrations of accessory photopigments, and a large surface-to-volume.

According to Swanepoel *et al*. (2008), phytoplankton in source water are known to be sensitive indicators of water quality and thus affect the production of potable water, the aesthetic aspect of recreational waters and consumer health. The reasons cited by these authors are:

1. Phytoplankton and their cellular products interfere with the physical and/or chemical water purification processes.
2. Phytoplankton are able to pass through the purification processes resulting in water of aesthetically unacceptable quality (odour, taste and colour).
3. Excessive growth of phytoplankton species in source water can create aesthetically unacceptable recreational and potable water and may pose a health risk to consumers (taste, odour, scum and toxins).

Some of the species found in the three impoundments studied (Rustfontein, Mockes and Maselspoort Dams) have the above mentioned features and Table 4.1 lists some of their characteristics.

Table 4.1: Some characteristics and problems caused by some of the phytoplankton species found in Rustfontein, Mockes and Maselspoort Dams (Truter, 1987).

SPECIES	**CHARACTERISTICS**
Anabaena	-common in the plankton of lakes and ponds -capable of producing toxins -capable of producing taste and odour compound -filter-clogging algae -indicative of hard water with high nutrient content

Ceratium	-abundant in hard water -filter-clogging algae
Cyclotella	-distributed in all types of surface waters -an indicator of clean water -filter-clogging algae
Euglena	-indicative of high organic matter
Melosira	-widely distributed -indicative of particular environmental conditions -filamentous, thus filter-clogging
Microcystis	-frequent component of algal blooms -produce toxins poisonous to animals and humans -cause bad tastes and odours in drinking water
Oscillatoria	-widely distributed -filamentous, thus filter-clogging -indicator of polluted water
Trachelomonas	-indicative of high content of organic matter -filter-clogging -can colour water brown

The majority of the phytoplankton species identified in the three impoundments were also identified by Koning (1998) in the Modder and the Klein Modder Rivers. However, cyanobacterial species and *Ceratium* species, which seemed to be abundant sometimes in the impoundments, were not identified in those two rivers during that study. *Phacus*, *Euglena* and *Trachelomonas* species are known to be associated with eutrophic waters (Wetzel 1983, 2001) and were occasionally identified in low numbers in these impoundments.

4.9) CYANOBACTERIAL TOXINS

Providing the human population with safe drinking water is one of the most important issues in public health. As indicated by Hitzfeld *et al*. (2000), the

presence of cyanobacterial toxins in drinking water supplies poses a serious threat to water treatment facilities, since not all technical procedures are able to effectively remove these toxins to below acceptable non-toxic levels. The most commonly occurring cyanobacterial toxins in surface waters are microcystins with the variant microcystin-LR as the most prevalent one.

Table 4.2 shows that microcystin-LR was detected in Rustfontein Dam throughout the study period except on four occasions (29 August 2009, 16 September 2009, 16 November 2009 and 3 December 2009). The highest concentration of microcystin-LR recorded in Rustfontein Dam was on 15 April 2010 (1.19 µg/L) and this coincided with the highest concentration of chlorophyll-*a* measured (2 275.8 µg/L). The high chl-*a* concentration was due to a *Microcystis* bloom.

In Mockes Dam the microcystin-LR was detected on a number of occasions except on 5 November 2009, 16 November 2009, 3 December 2009, 19 March 2010, 15 and 21 April 2010 (Table 4.2). The highest concentration of microcystin-LR was recorded on 29 June 2009 (0.093 µg/L) and again this coincided with the highest chlorophyll-*a* concentration (521.77 µg/L). The concentration of NO_3-N on that day was the lowest recorded (0.175 µg/L). This could be due to the depletion of NO_3-N by the phytoplankton. The phytoplankton on that day was dominated by *Oscillatoria*. Mockes Dam experienced high phytoplankton biomass during the study period but these periods of high chlorophyll-*a* concentration were not usually associated with cyanobacterial dominance. As indicated by Van Ginkel (2004), toxins will be detected at chlorophyll-*a* concentrations higher than 30 µg/L when *Microcystis* is the dominant species. This explains why no toxins were detected in Mockes Dam even when chlorophyll-a concentrations were higher than 50 µg/L (for example on 15 March 2010, 15 April 2010 and 21 April 2010 when the chlorophyll-a concentrations were 91.7, 199.2 and 52.6 µg/L respectively). At that time, the phytoplankton was dominated by *Ceratium* species.

In Maselspoort Dam the toxins were detected on a few occasions (11 June 2009, 20 July 2009, 6 August 2009, 29 September 2009, 15 October 2009, 26 October 2009, 19 March 2010 and 15 April 2010). The highest concentration (0.097 µg/L) was recorded on 15 October 2009 and again it coincided with the lowest concentration of NO_3-N (0.03 µg/L). *Anabaena* was part of the phytoplankton on that date, and even though the concentration of NO_3-N was low, Anabaena species were able to grow probably because it is known that they can fix N_2. According to Havens *et al*. (2003), *Anabaena* species are strong resource competitors under conditions of nitrogen limitation because they can fix N_2.

Table 4.2: Microcystin-LR concentrations (µg/L) measured in water samples from Rustfontein, Mockes and Maselspoort Dams and in treated water from Rustfontein and Maselspoort treatment plants. (nd stands for not detected)

	Rustfontein	Mockes	Maselspoort	Rustfontein treated	Maselspoort treated
5 May ‘09	0.088	0.03	nd	nd	nd
19 May ‘09	0.031	0.035	nd	nd	nd
11 Jun ‘09	0.051	0.048	0.045	nd	nd
29 Jun ‘09	0.044	0.093	nd	nd	nd
20 Jul ‘09	0.046	0.059	0.035	nd	nd
6 Aug ‘09	0.039	0.030	0.042	nd	nd
29 Aug ‘09	Nd	0.039	nd	nd	nd
16 Sep ‘09	Nd	0.037	nd	nd	nd
29 Sep ‘09	0.044	0.041	0.052	nd	nd

15 Oct ‘09	0.044	0.065	0.097	nd	0.043
26 Oct ‘09	0.041	0.033	0.034	nd	nd
5 Nov ‘09	0.049	Nd	nd	nd	nd
16 Nov ‘09	Nd	Nd	nd	nd	nd
3 Dec ‘09	Nd	Nd	nd	nd	nd
19 Mar ‘10	0.164	Nd	0.052	nd	nd
26 Mar ‘10	0.036	0.031	nd	nd	nd
15 Apr ‘10	1.191	Nd	0.037	nd	nd
21 Apr ‘10	0.035	Nd	nd	nd	nd

Though microcystin-LR was sometimes detected in these impoundments, the concentrations were generally very low (less than 0.1µg/L) except in March and April in Rustfontein Dam where the concentrations were 0.164 and 1.191 µg/L respectively (Table 4.2). Even though the highest concentration of microcystin-LR in Rustfontein was higher than the recommended concentration (TDI - Total Daily Intake, which is the allowable concentrations within drinking water.) set as the guideline for drinking water (1 µg/L) by the World Health Organization (WHO, 1998) it was still markedly lower than the concentrations recorded by Oberholster *et al*. (2009) at different sites in Krugersdrift Dam, an impoundment downstream to the three impoundments studied. They recorded the lowest concentration of 0.38 µg/L and the highest concentration of 43.7 µg/L. Rae & Moollan (1999) recorded the highest extracellular microcystin-LR (2.8 µg/L) at Inanda Dam in December 1995 and at Hazelmere Dam in March 1997. However, in May 1995, they recorded the microcystin-LR concentration of 1.0 µg/L at Inanda Dam, almost similar to the highest concentration of microcystin-LR recorded in Rustfontein Dam during this study. During their study, no massive blooms occurred and this was also

the case in this study and this explains why low concentrations of microcystin-LR were recorded.

Microcystin-LR was never detected in treated water from Rustfontein, even when there was a bloom and the concentration of microcystin-LR in source water was the highest. However, in Maselspoort, toxins were detected in treated water on 15 October 2009 (0.043 µg/L) and this coincided with the highest toxin concentration in source water (0.097 µg/L). According to the chief operators at both the water purification plants (Rustfontein and Maselspoort Dams), they use conventional treatment methods which consist of coagulation, flocculation, sedimentation, filtration and disinfection (chlorination). According to Hoffman (1976), if designed and operated properly, a purification plant using conventional methods produces good quality water, with respect to clarity (absence of turbidity and colour) and absence of possible pathogenic bacteria. However, dissolved substances (for example microcystins), are not affected by these process, unless they react chemically with the flocculants or disinfecting agent (Hoffman, 1976). Microcystins are known to be chemically stable compounds, thus the efficiency of their elimination by conventional treatment methods is very low, not exceeding 11-18 % (Duy *et al*., 2000).

The initial steps in the treatment process are designed to remove suspended particles in water. Coagulation is considered an efficient method for elimination of cyanobacterial cells from water, whereas soluble cyanotoxins are not very effectively removed by this method (Hitzfeld *et al*., 2000; Jurczak *et al*., 2005). Chlorine in the last step of treatment process is used as a disinfectant. Xagoraraki *et al*. (2006) found that extracellular microcystin-LR was inactivated by free chlorine and that the inactivation rate was influenced by pH. This is in agreement with the findings of Nicholson *et al*. (1994) and Tsuji *et al*. (1997) which suggested that chlorination using an adequate chlorine dose is very effective for the removal of microcystin in water. The efficiency of chlorination to remove microcystins in water depends on the

chlorine compounds and the concentration used and according to Hitzfeld *et al*. (2000) and Jurczak *et al* (2005), more than 95 % of microcystin is removed by aqueous chlorine and calcium hypochlorite at a concentration of 1 mg/L while sodium hypochlorite or chloramines at the same dose achieve 40-80 % removal at most. Hoeger *et al*. (2004) have reported that the effective removal of cyanobacterial cells and toxins is dependent on:

(i) Cyanobacterial species and density.

(ii) Additional organic load.

(iii) Concentration and type flocculant.

(iv) pH during flocculation and chlorination.

(v) Maintenance of the treatment system especially of the filter bed.

According to the chief operator at Maselspoort water treatment plant, they have never experienced cyanobacterial blooms in the source water, so they have never felt the need to use advanced treatment methods. However, when turbidity is high, they do pre-chlorination. As indicated by Jurczak *et al*. (2005), though effective removal of algal cells is achieved with pre-oxidation by chlorine and permanganate, pre-oxidation leads to cell lysis and toxin release into water which should be avoided. This is believed to be the reason why microcystin-LR was detected in treated water even though it was low in source water. It is, therefore, important to keep in mind that pre-chlorination should be stopped as soon as cyanobacterial bloom is apparent.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The three impoundments investigated (Rustfontein, Mockes and Maselspoort Dams) displayed no clear-cut seasonal trends as far as the physico-chemical variables are concerned, except for surface water temperature, as expected. The variability in DO concentration during the study can be ascribed to the photosynthetic activity of the phytoplankton, water temperature variations and wind induced mixing in these shallow impoundments. It is assumed that decomposition of organic matter was also a contributing factor, especially in Maselspoort Dam. The highest concentration of DO in all three impoundments investigated occurred in August 2009 and this ironically also coincided with the highest pH in those impoundments. A possible explanation could be that at this time the photosynthetic rates were high, causing the rapid uptake of CO_2 and evolution of O_2, the release of OH^- and thereby increasing water pH.

Hansson (1988) has indicated that temperature, light, turbulence and nutrient concentrations are usually considered the main factors influencing algal growth rates. However, Elliot *et al*. (2006) pointed out that water temperature and nutrient load are probably the two most important in determining the plant growth. In this study, concentrations of nutrients seemed to play a more crucial role in controlling phytoplankton growth than temperature. The observation was similar to that of Elliot *et al*. (2006) who found that the response of the community to the changes in water temperature was often greatly dependent on the level of nutrient supply.

In terms of the nutrient content of the water, the three impoundments investigated can be classified as eutrophic. These impoundments are on the

Upper Modder River, which Koning (1998) classified as eutrophic based on nutrient content of its waters, so it is no surprise that these impoundments are found to be eutrophic. Indication of pollution and eutrophication in these impoundments, especially Mockes and Rustfontein Dams, were confirmed by the chlorophyll-a concentrations that sometimes reached very high levels. The signs of eutrophication were more pronounced in Mockes Dam, than in the other two impoundments, where the chl-*a* concentrations were continually high. The presence of the eugelenoids *Phacus*, *Euglena* and *Trachelomonas* is also an indication of pollution because these species are associated with polluted water in which organic material is present (Wetzel, 1983; 2001)

It is believed that high water temperatures coupled with high nutrient concentrations result in increased phytoplankton growth. This seemed to have not been the case in Mockes Dam, where the highest chlorophyll-*a* concentration was recorded in June 2009, when the water temperature was low (it should be noted the lowest temperature of 11.8 ºC may not be severely limiting). However, the highest chlorophyll-*a* concentration coincided with the highest EC value, hence highest TDS concentrations. This could be ascribed to the reduction of turbidity due to floc formation, thereby improving the underwater light climate and thus algal algal growth. This was also observed by Koning and Roos (1999) in the Modder River, as well as by Roos (1991) and Roos *et al*. (1997) in the Vaal River.

According to Raschke (1993), a mean growing season limit of ≤15 µg/L chlorophyll-*a* is recommended for water supply impoundments because at this concentration few nuisance algal blooms or scums would be expected, thereby very few problems associated with filter clogging, taste and odour would be anticipated. Rustfontein and Maselspoort Dams are used for potable water supply and during the study period chlorophyll-*a* concentrations in the two impoundments exceeded 15 µg/L once. It can therefore be concluded that at the present, the two impoundments are suitable for supplying potable water. However, Rustfontein Dam seems to have a

potential to cause problems with filter clogging, cyanotoxins, taste and odour because the chlorophyll-*a* concentration as high as >2000 µg/L was measured, and the dominant species was *Microcystis*. Mockes Dam on the other had continually high chlorophyll-*a* concentrations throughout the study period. Fortunately, the impoundment is not used for potable water supply, but it regulates flow to Maselspoort Dam. The average chlorophyll-*a* concentration in Mockes Dam was much higher than the limit recommended by Raschke (1993) for other uses such as maintenance of aesthetic environmental conditions, safe swimming, good fishing and boating conditions. The results clearly show that Mockes Dam is not suitable for recreational activities.

According to Jurczak *et al*. (2005) mass occurrences of toxic cyanobacteria in reservoirs complicate production of safe drinking water and the diversity of produced toxins calls for advanced monitoring and processing techniques. According to Harada *et al*. (1999), a drinking water supply safe from cyanotoxins should either be free from cyanotoxins, or have treatment processes in place that will remove cyanobacterial cells (without rupturing them) and released cyanotoxins. Du Preez *et al*. (2007) emphasized the importance of cyanotoxins analysis on the source water and the final water. They indicated that cyanotoxins screening results from the source water will indicate if there are any cyanotoxins present and the screening results of the final supply water will be an indication of how well the process performs in removing these toxins and also indicate the potential risk to the consumer. They recommended that if the drinking water utility does not have the capacity to perform cyanotoxins analysis, it is important to outsource the samples for these analyses.

During the study, no massive cyanobacterial blooms occurred (except for one time in Rustfontein Dam in April 2010) and this could be the reason for the low incidence of microcystin in the water samples. According to Mankiewicz *et al*. (2003), in view of the toxic and genotoxic effect of cyanotoxins the World Health Organization established 1 µg/L microcystin-LR or microcystin-LR

equivalents as a guideline for acceptable levels of cyanotoxins in drinking water (WHO, 1998). During the monitoring period, this limit was exceeded once in raw water samples from Rustfontein Dam (1.19 µg/L in April 2010) but never in Maselspoort Dam. Microcystin-LR Dam was detected once in treated water from Maselspoort but the concentration was very low (0.043 µg/L). Based on these results, it can be concluded that water from these impoundments (Rustfontein and Maselspoort Dams) is safe for human consumption as far as microcystin-LR or microcystin-LR equivalents are concerned. However, it is recommended that the analysis for microcystin-LR be part of the monitoring in view of the trophic status and potential for the development of cyanobacterial blooms.

Conventional water treatment practices have been reported to be rather ineffective in microcystin removal. The results from the study showed an efficient removal of microcystin in water during treatment processes at both Rustfontein and Maselspoort water treatment plants. The health hazard posed by microcystins in the water used for drinking was potentially higher in Maselspoort waterworks systems due to prechlorination they perform when turbidity is high. Pre-oxidation by chlorine or permanganate leads to cell lysis and toxin release into water. According to Jurczak *et al*. (2005) pre-oxidation is not recommended if toxin removal is a priority and can only be considered acceptable when total toxin concentration is so low that lysing is irrelevant.

Jurczak *et al*. (2005) have indicated that the effective removal of microcystins depends, among other things, on the concentration of cyanotoxins entering the treatment system. Results from this study have shown that there is no threat of microcystin toxins entering the potable water treatment works because the occurrence of microcystin toxins is low in the two impoundments. However, there is a real concern that cyanobacterial blooms may occur more frequently in future.

According to Kotut *et al*. (2006), the presence of cyanotoxins in source waters means that the existing water quality assessment and monitoring strategies, that employ microbial and physico-chemical criteria will no longer be adequate, especially in water bodies showing evidence of progressive eutrophication. Of the two impoundments used for potable supply, Rustfontein Dam seemed to have a potential to experience massive cyanobacterial blooms, especially *Microcystis* blooms in future. Because all bloom-forming cyanobacteria genera are potentially toxic, as a precautionary measure, it is recommended that samples should be taken, and analysed whenever the cyanobacterial cell counts appear to increase rapidly. However, Rustfontein water utility does not have the capacity to carry out toxin analysis at the present. In South Africa, there are very few laboratories which have the capacity to perform cyanotoxins analysis (Swanepoel *et al*., 2008) and van Ginkel (2004) recommended that analytical laboratories for cyanotoxins be established on a regional, provincial or catchment management area (CMA) level for more efficient data acquisition and dissemination of information. An alternative would be the regular use of the so-called ‘dip stick’ testers for microcystins and nodularin. According to Harding *et al*. (2009) this much needed technology became available in 2008 and tests showed that they were suited for use in South Africa.

Van Apeldoorn *et al*. (2007) suggested that, although cyanobacterial blooms remain occasional events, most emphasis should be placed on the protection of drinking water supplies through the preparation of contingency plans and their activation when appropriate. Since the water treatment plants at the Rustfontein and Maselspoort Dams still use conventional methods, it is also recommended that they should consider introducing more advanced methods such as an activated carbon polishing step. Activated carbon also controls taste and odour compounds and according to Pendleton *et al*. (1997) and Cook *et al*. (2001), it is relatively inexpensive and can only be applied when required.

This study confirms the fact that South Africa's freshwater supplies are under threat and everything possible should be done to ensure the supply of safe potable water.

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SUMMARY

The occurrence of cyanobacterial blooms that may be toxic is one of the major consequences of eutrophication. The prevalence of cyanobacteria and their toxins in reservoirs create a significant water quality problem and complicates the water purification process for producing safe drinking water. Microcystin is the dominant group of cyanotoxins and the most commonly occurring variant is microcystin-LR. The purpose of this study was to determine the presence of cyanotoxins in the reservoirs and drinking water supplied to the city and towns of Bloemfontein, Thaba Nchu and Botshabelo, in the central region of South Africa. To achieve this, the microcystin-LR concentrations were determined in Rustfontein, Mockes and Maselspoort Dams and the efficiency of treatment processes at Rustfontein and Maselspoort treatment plants in removing this cyanotoxin were investigated.

In situ measurements of electrical conductivity (EC), dissolved oxygen (DO), pH and surface water temperature were done at two-weekly intervals over the study period. Water samples were collected and analysed upon return to the laboratory for NO_3-N, PO_4-P, chlorophyll-*a*, dominant algal species and microcystin-LR. Final treated water from Rustfontein and Maselspoort water treatment facilities was also analysed for microcystin-LR.

Physico-chemical variables displayed no clear-cut seasonal trends except for surface water temperature which followed a distinctive seasonal pattern with lower values in winter and higher values in summer. The concentration of DO seemed to be influenced by water temperature, photosynthetic activity of phytoplankton, wind induced mixing and decomposition of organic matter.

Based on the nutrient content, these impoundments were found to be eutrophic. However, in terms of chlorophyll-a concentrations, Maselspoort Dam was mesotrophic, Rustfontein Dam eutrophic and Mockes Dam hypertrophic. Cyanobacteria genera that were commonly found in the three impoundments were *Microcystis*, *Anabaena* and *Oscillatoria*. *Oscillatoria* was

occasionally dominant in Mockes Dam with *Ceratium* as co-dominant. *Microcystis* was occasionally dominant in Rustfontein Dam. Euglenoids such as *Euglena*, *Trachelomonas* and *Phacus*, that are indicative of presence of organic matter, were occasionally present, especially in Mockes and Maselspoort Dams.

There were no severe cyanobacterial blooms during the study period except for a single bloom of Microcystis in Rustfontein in April 2010. This explains why the concentrations of microcystin-LR or microcystin-LR equivalents in the three impoundments were generally low or undetectable. The highest concentration measured was 1.19 µg/L and this was in Rustfontein Dam during the bloom. Microcystin-LR was never detected in the final treated water from Rustfontein but was detected once in treated water from Maselspoort (0.043 µg/L). This is lower than the 1 µg/L proposed by WHO as an acceptable level of microcystin-LR or microcystin-LR equivalents in drinking water. Based on these results it was concluded that the treatment processes at both facilities were relatively efficient in removing microcystin-LR and that the water supplied to Bloemfontein, Botshabelo and Thaba Nchu is indeed safe. However, it is believed that the efficiency of these treatment processes might be compromised when high concentrations of microcystin-LR or microcystin-LR equivalents occur in source waters. Rustfontein Dam seemed to have the potential to develop massive cyanobacterial blooms and this could result in occurrence of high concentrations of microcystin-LR or microcystin-LR equivalents in the water. It is, therefore, recommended that the water treatment facilities at Rustfontein and Maselspoort should consider introducing more advanced treatments such as an activated carbon polishing step. It is also recommended that cyanotoxin analysis should be part of routine water quality monitoring.

Keywords: Water quality, Eutrophication, Cyanobacterial blooms, Microcystin-LR, Safe drinking water, Treatment processes, Rustfontein Dam, Mockes Dam, Maselspoort Dam

OPSOMMING

Die voorkoms van sianobakteriële opbloeie wat moontlik toksies kan wees, is een van die gevolge van eutrofikasie. Sianobakterieë en hulle toksiene is ‘n probleem in opgaardamme en bemoeilik die suiwering van water, asook die voorsiening van drinkwater. Mikrosistien is die groep sianotoksiene wat die meeste voorkom en mikrosistien-LR domineer hierdie groep toksiene. Die doel van die studie was om die voorkoms van sianotoksiene in die opgaardamme en drinkwater van die stad Bloemfontein en dorpe Thaba Nchu en Botshabelo in sentraal Suid-Afrika te ondersoek. Om dit te doen is die konsentrasies mikrosistien-LR in Rustfontein-, Mockes- en Maselspoortdamme bepaal, asook in die gesuiwerde water van die Rustfontein en Maselspoort suiweringsaanlegte.

In situ metings van konduktiwiteit (EC), opgeloste suurstof (DO), pH en oppervlak temperature is tweeweekliks gedurende die studietydperk gedoen. Watermonsters is versamel en na terugkeer in die laboratorium geanaliseer vir NO_3-N, PO_4-P, chlorofil-*a*, dominante alg spesies en mikrosistien-LR. Finaal gesuiwerde water van Rustfontein en Maselspoort suiweringsaanlegte is vir mikrosistien-LR getoets.

Geen duidelike seisoenale patroon was herkenbaar in die fisies-chemiese resultate nie, behalwe vir watertemperatuur wat duidelik laer in die winter en hoër in die somer was. DO was duidelik beïnvloed deur watertemperatuur, fotosintetiese aktiwiteit van die fitoplankton, windvermenging en die ontbinding van organiese materiaal.

Volgens die voedingstofkonsentrasies kan die opgaardamme as eutrofies geklassifiseer word. In terme van die chlorofil-*a* konsentrasies is Maselspoortdam mesotrofies, Rustfonteindam eutrofies en Mockesdam hipereutrofies. Die dominante sianobakteriese genera in die opgaardamme

was *Microcystis*, *Anabaena* en *Oscillatoria.* *Oscillatoria* was met tye dominant in Mockersdam gevolg deur *Ceratium*. *Microcystis* was met tye dominant in Rustfonteindam. Euglenoiede soos *Euglena*, *Trachelomonas* en *Phacus*, wat op die teenwoordigheid organiese materiaal kan dui, is met tye in Mockesdam en Maselspoortdam waargeneem.

Geen uitsonderlike opbloeie van sianobakterieë het gedurende die studietydperk voorgekom nie, behalwe vir ‘n *Microcystis* opbloei in April 2010 in Rustfonteindam. Dit verduidelik waarom die konsentrasies van mikrosistien of hulle derivate laag of onmeetbaar in die drie opgaardamme was. Die hoogste konsentrasie van 1.19 µg/L is in Rustfonteindam gedurende die opbloei gemeet. Mikrosistien-LR was nooit in die gesuiwerde water van die Rustfonteinaanleg gemeet nie, maar wel een keer in die behandelde water van Maselpoortsuiweringsaanleg (0.043 µg/L). Dit is heelwat laer as die 1 µg/L riglyn soos deur die Wêreld Gesondheidsorganisasie (WHO) voorgestel vir mikrosistien-LR en derivate in drinkwater. Hiervolgens kon afgelei word dat die suiweringsaanlegte relatief effektief is om mikrosistien-LR in die water wat aan Bloemfontein, Thaba Nchu en Botshabelo voorsien word, te verwyder. Dit word egter voorsien dat die suiwering onvoldoende sal wees indien hoë konsentrasies mikrosistien-LR in die rou water sou voorkom. Die moontlikheid bestaan dat groot opbloeie in Rustfonteindam sou kan voorkom wat weer tot hoë mikrosistienkonsentrasies in die rou water sou kon lei. Dit word voorgestel dat die suiweringsaanlegte gevorderde stappe soos ‘n geaktiveerde koolstof stap insluit. Verder word voorgestel dat daar vir sianotoksiene op ‘n roetine grondslag geanaliseer word.

Sleutelwoorde: Waterkwaliteit, Eutrofikasie, Sianobakteriese opbloeie, Mikrosistien-LR, Veilige drinkwater, Watersuiweringsaanleg, Rustfonteindam, Mockesdam, Maselspoortdam