ENVIRONMENTAL FACTORS AFFECTING THE PERSISTENCE OF TOXIC PHYTOPLANKTON IN THE HARTBEESPOORT DAM

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Report to the Water Research Commission

by

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CD containing all the tables used in the appendices as well as colour photos used in the report, attached.

EXECUTIVE SUMMARY

BACKGROUND AND MOTIVATION

Cyanobacteria are a group of extraordinarily diverse Gram-negative prokaryotes. They are considered to be one of the potential organisms which can be useful to mankind in food, fuel, fertilizer, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and pollution abatement. However, problems may occur as a result of algal overgrowth and production of toxins. This species periodically blooms in Hartbeespoort Dam, a popular recreational dam and a source of domestic and irrigation water in North West Province. Consequently, there was a need to conduct a study to assess the appearance pattern and persistence of the *Microcystis aeruginosa* in the Dam. The review examines numerous aspects that influence cyanobacterial growth in Hartbeespoort Dam, their persistence and various options that have been used to prevent and control the bloom. Critical among them include complex and dynamic relationships between physical, chemical and biological factors such as water temperature, pH, and nutrient availability (nitrogen, phosphorus, iron). Sometimes when aquatic systems are highly enriched because of eutrophication, plants produce and over accumulate organic material. Eutrophication problems, which often result from increased anthropogenic activities, have been widely accepted as the main problem in Hartbeespoort dam. The nutrients are continuously generated in the catchment areas draining into the dam and the load from Jukskei /Crocodile rivers.

Apart from growth requirements of macronutrients and micronutrients such as phosphorous, nitrates, and trace metals respectively, other abiotic factors such as pH, electrical conductivity, dissolved oxygen, turbidity and temperature are influential in bloom formation. These parameters were also determined in this study.

AIMS AND OBJECTIVES

The primary aim of this work was to investigate environmental factors that affect the occurrence, persistence and bloom formation of phytoplankton species with particular emphasis on the *Microsystis aeruginosa* in the dam. This broad aim had the following specific objectives:

a) to investigate the physical and chemical parameters of the dam and any possible effects to bloom persistence;
b) to investigate species composition and blooming with specific reference to *Microcystis* species;
c) to determine the toxins produced by the identified species.

d) to determine the possibility of over-wintering mechanism and therefore possible sources of perennial algal inoculum feeding into the dam yearly after reported absence in winter months;

All the aims stated above were achieved during the study with the exception of aim *c)*. This was not performed due to insufficient or lack of materials e.g. calibration standards required for the quantification of toxins produced by different algal species identified in the dam. However, some studies on trace metal i.e. iron, manganese, zinc, copper and lead were included as part of the chemical quality parameters. Information from major studies conducted were also investigated and correlations drawn if any changes might have occurred in the dam.

METHODOLOGY

Water and sediment samples for algal identification and quantification were collected fortnightly using a polythene hose for an integrated surface to 5 meters sample and a Van Dorn sampler, sampling at discrete depths of 0, 1, 2, 5, 10, 15 and 20 meters respectively. Water samples were also collected for the determination of selected chemical water quality parameters such as, nitrates, phosphates, sulfates using standard methods. Some trace metals, iron, manganese, zinc, copper and lead were also quantified. The study also retraced past data on physical and chemical quality parameters. Statistical *t*-test analyses were then conducted on the observed values and past data to determine significant differences that might have occurred over the years.

RESULTS

Results showed that *Microcystis aeruginosa* consistently dominated the impoundment at bloom levels during summer months. Other algal species such as *Pseudanabaena* and *Oscillatoria spp*, often associated with the *Microcystis* colonies and the planktonic diatom *Melosira granulata* var. *angustissima* were also observed. It was observed that these species were more abundant in water samples than in the sediment samples throughout the season. This was attributed to the favourable physical conditions such as temperature and light penetration required for photosynthesis in the upper water surface. However, four times more *Microcystis aeruginosa* colonies were identified in the sediment samples during the winter months than in late summer of 2003. This could be due to over-wintering behaviour. More so, unlike in the previous years (1980's and 1990's), *Microcystis aeruginosa* never disappeared in winter months from the water column for three consecutive years of study after the turnover of the dam. In addition, the average levels of basic water parameters have remained fairly the same except significant changes in the levels of sulphate (1096 µg/L), pH (8.35) and dissolved nitrates as NO_3-NO_2 (51.94 µg/L) for this study and 1680 µg/L, 8.56 and 71.25 µg/L for previous studies respectively. However, values obtained in this study for the period of 2003 and 2004, also revealed increased Kjeldahl nitrogen (1961 µg/L) and total phosphorus, PO_4-TOT (73 µg/L) in 2003 compared to 1155 µg/L and 48 µg/L in

2004 respectively. The surface water temperatures were also higher in 2003. Minimum temperatures increased from 9 to 13°C and maximum from 24 to 27°C for winter and summer periods respectively.

CONCLUSION AND RECOMMENDATION

Changes in phosphate, nitrate and temperature during the year 2003 provided favourable environmental factors for the cyanobacterial bloom observed in the dam. These could explain the reasons for 2003 and early 2004 unacceptable high bloom season in the dam. In order to minimize effects of such blooms It is recommended that sustained and pro-active integrated approach looking at WHO models used in Australia (Burch, 1993) also cited by Du Preez & Van Baalen (2006) based on continuous monitoring efforts as currently being practiced in this dam should continue.

CAPACITY BUILDING

Master's in Technology (MTech). Kennedy Onyango Owuor (black male): "Environmental factors affecting the persistence of toxic phytoplankton in the Hartbeespoort dam", Tshwane University of Technology

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1. INTRODUCTION

Cyanobacteria (aka "blue-green algae") are one of the earth's most ancient life forms. Evidence of their existence on earth, derived from fossils records, encompasses a period of some 3.5 billion years. They are considered to be the first photosynthetic organisms on earth (Harding & Paxton, 2001). Their prokaryotic characteristics group them together with the bacteria, but they are nevertheless considered to belong to the algae, the simplest members of the plant kingdom.

The autotrophic (*auto* = "self" *tropho* = "nourishment", Greek) cyanobacteria were once classified as "blue green algae" because of their superficial resemblance to eukaryotic green algae. Although both groups are photosynthetic, they are only distantly related: cyanobacteria lack internal organelles, a discrete nucleus and the histone proteins associated with eukaryotic chromosomes. Like all eubacteria, their cell walls also contain peptidoglycan. In many species, however, the external membrane has been folded to increase total surface area. The "packets" created in the cell membrane by these foldings are the surfaces across which the chemical reactions of photosynthesis take place (Soil & Water Conservation, 2006). Unlike most organisms that use photosynthesis for energy, cyanobacteria carry out photosynthesis directly in the cytoplasm of the cell, rather than in specialised organelles (chloroplasts). Photosynthesis in cyanobacteria uses water as an electron donor and produces oxygen as a byproduct. The process occurs in membranes called thylakoids, with chlorophyll being employed to absorb the sun's rays (Soil & Water Conservation, 2006). Chlorophyll *a* and several accessory pigments (phycoerythrin and phycocyanin) are embedded in these photosynthetic lamellae, the analogs of the eukaryotic thylakoid membranes. These photosynthetic pigments impart a rainbow of possible colours: yellow, red, violet, green, deep blue and blue-green which cyanobacteria are known for.

They are referred to in literature by various names, among which are Cyanophyta, Myxophyta, Cyanochloronta, Cyanobacteria, blue-green algae, blue-green bacteria (Soil & Water Conservation, 2006). Based on their shapes (morphologies), cyanobacteria have been classified into five groups: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales. The majority of blue-greens are aerobic photoautotrophs: their life processes require only oxygen, light and inorganic substances (Echlin, 1966).

Cyanobacteria may be single-celled or colonial. Depending upon the species and environmental conditions, colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into three different cell types. Vegetative cells are the normal, photosynthetic cells formed under favorable growing conditions. Climate-resistant spores may form when environmental conditions become harsh. A third type of cell, a thick-walled heterocyst, contains the enzyme nitrogenase, vital for nitrogen fixation. Heterocyst-forming species are able to "fix" nitrogen gas, which under normal circumstance may not be absorbed by plants, into ammonia (NH_3), nitrites (NO_2) or nitrates (NO_3). These can be

absorbed by plants and converted to protein and nucleic acids (Soil & Water Conservation, 2006). Cyanobacteria are therefore, natural purifiers of surface waters through assimilation of nitrogen and other nutrients during photosynthesis and in the process; oxygen is released as a by–product. However, problems occur as a result of algal overgrowths (blooms) accelerated by eutrophication, which often results from increased anthropogenic activities.

Eutrophication is the enhancement of the natural process of biological production in rivers, lakes and reservoirs, caused by increases in levels of nutrients, usually phosphorus and nitrogen compounds (WHO, 1999). Eutrophication therefore, can result in visible cyanobacterial or algal blooms, surface scum, floating plant mats and benthic macrophyte aggregations. The decay of this organic matter may lead to depletion of dissolved oxygen in the water, which in turn can cause secondary problems such as fish mortality from lack of oxygen and liberation of toxic substances or phosphates that were previously bound to oxidised sediments (WHO, 1999).

1.1 Nuisance/Noxious Conditions

Algal blooms are unwanted for many reasons:

- they can block sunlight and use up all the oxygen in the water, killing other plants and animals.
- they are known to produce toxins that are among the most powerful natural poisons. These toxins have no known antidotes and;
- they are objectionable for recreational activities, and more importantly, create great nuisance in the management of water reservoirs.

Many of these conditions in freshwater are produced by three blue-greens, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*.

An oversupply of nutrients, especially phosphorus and possibly nitrogen, can often result in excessive growth of blue-greens because they possess certain adaptations that enable them to out-compete true algae. Perhaps the most important adaptation is their positive buoyancy, which is regulated by their gas vesicles and are absent in true algae (Carmichael, 1992).

1.2 Poisonous Conditions

Algae do not produce substances that are toxic to humans or animals. In contrast, some cyanobacteria produce substances that are extremely toxic, and are capable of causing serious illness or even death if consumed. These substances are called cyanotoxins. Most of the recorded toxic blooms are caused by *Microcystis aeruginosa*, which manufactures "microcystin", which yields 7 (or 14) amino acids upon hydrolysis (Carmichael, 1996). The microcystins are a group of cyclic heptapeptide hepatotoxins produced by a number of cyanobacterial genera. As shown in Figure 1.1, the peptide ring is made up of two protein amino acids and five non-protein amino acids. It is the two protein amino acids that distinguish

microcystin types from each other, while the other amino acids are relatively constant. By using amino acid single letter code classification, each microcystin is designated a name depending on the variable amino acids which complete their structure. For instance, one of the most common toxins found in water supplies around the world, microcystin-LR contains the amino acids Leucine (L) and Arginine (R) in these variable positions.

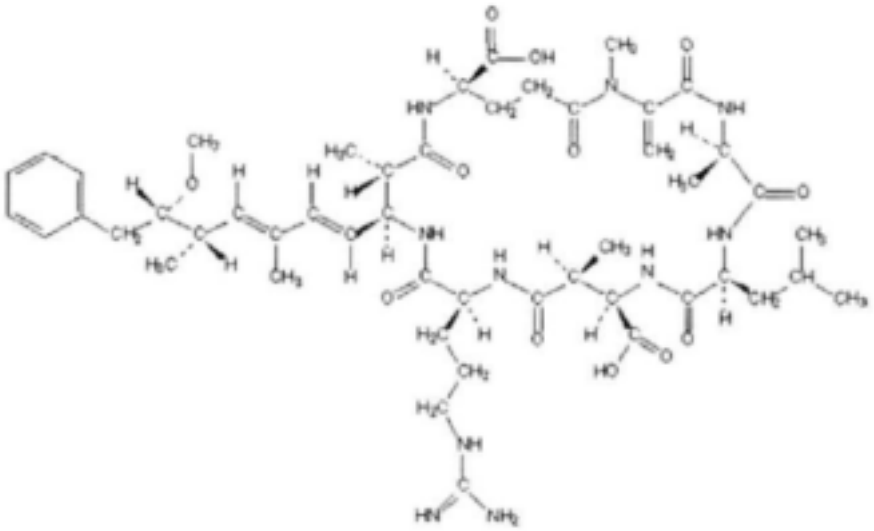


Figure 1.1: Chemical structure of Microcystin LR, (Carmichael, 1996)

To date, approximately more than 75 different kinds of microcystins have been discovered (Sigma-Aldrich, 2006). Poisonous blue-greens occur in ponds, dams and lakes throughout the world. Toxicity may be experienced in mammals, birds and fish (Van der Westhuizen, 1984). Although humans ordinarily avoid drinking water that displays a blue-green bloom or scum, they may be affected by toxic strains when they swim or ski in recreational water bodies during a bloom or via the consumption of foods previously exposed to water containing *Microcystis aeruginosa* (Codd et al., 1999) after spray irrigation of such crops. Typical symptoms include redness of the skin and itching around the eyes; sore, red throat; headache; diarrhea; vomiting; and nausea.

A number of human deaths have been reported through exposure to cyanobacterial toxins through renal dialysis (Carmichael, 1996; Jochimsen et al., 1998), and also implicated in drinking-water (Teixera et al., 1993). The microcystins cause enlargement and congestion of the liver followed by necrosis and hemorrhage, and may also exhibit neurotoxic activity. These hepatotoxins inhibit the protein phosphatases inside hepatocytes. They damage the liver by affecting the maintenance of the cytoskeleton where upon disruption of the balance of phosphate groups on cytoskeletal proteins occur. The hepatotoxins cause the cytoskeleton to collapse, which may result to the hepatocytes to equally collapse inwards (Sigma-Aldrich, 2006). The hepatocytes and the capillary cells then pull apart, spilling blood into the liver that may cause death. This is because

the chief pathway for microcystins entry into cells is the bile acid carrier, which is found in liver cells and, to a lesser extent, in intestinal epithelia (Falconer, 1993).

It is speculated that low-level exposure to these toxins may promote the development of cancer in the liver and other chronic disorders of the gastrointestinal tract. This is likely to occur because the protein phosphatases that are inhibited by hepatotoxins play an important role in regulating cell division. While not initiating the cancers, these toxins have been shown to hasten the development of cancer in animals. Carmichael (2001) suggests, "the extraordinarily high rates of liver cancer in parts of China may be tied to the cyanobacterial toxins in water."

1.3 Colour and identification

The blue-green colour of cells (cyan means blue-green) is due to the combination of green chlorophyll pigment and a unique blue pigment (phycocyanin). However, not all blue-greens are blue-green. Their pigmentation includes yellow-green, green, grey-green, grey-black, and even red specimens (Fay, 1983). The blue-greens are microscopic life forms that exhibit several different types of organization. Some grow as single cells enclosed in a sheath of slime-like material, or mucilage. Other species may aggregate into colonies that are flattened, cubed, rounded, or elongated into filaments (Haynes, 1988).

1.4 Reproduction:

Cyanobacteria are able to reproduce through a variety of methods: binary fission, budding, or fragmentation. These forms of reproduction explain to a great extent the various appearances that cyanobacteria take on: patches, slimy masses, strings, filaments, and branched filaments (Fay, 1983).

Binary fission is the reproduction that involves duplicating the DNA and dividing in half. It does not involve a complex cell cycle like that shown by eukaryotic cells. Budding or cell fission on the other hand, involves the formation of smaller cells from larger ones while fragmentation involves breaking into fragments, each of which then regenerates into a complete organism (Haynes, 1988). However, photosynthesis plays an important role in the reproduction and growth of cyanobacteria. The wavelength of the light available determines what form of cyanobacteria will grow.

Nutrients such as phosphates, iron and dissolved organic carbon are also known to play some roles in growth and reproduction.

1.5 Blue-greens in freshwater bodies

Some cyanobacterial blooms can look like foam, scum, or mats on the surface of fresh water lakes, ponds, dams or in eutrophic (nutrient rich) waters.

The blooms can be blue, bright green, brown, or red and may look like paint floating on the water. Some blooms may not affect the appearance of the water. However, as algae in a cyanobacterial bloom die, the water may smell bad (Soil & Water Conservation, 2006).

In temperate lakes, there is a characteristic seasonal succession of the bloom-forming species, due apparently to their differing responses to the physical-chemical conditions created by thermal stratification. Usually the filamentous forms, *Anabaena* species for example, develop first soon after the onset of stratification in late spring or early summer, while the unicellular-colonial forms (like *Microcystis* species) typically bloom in mid-summer or in autumn (Soil & Water Conservation, 2006).

1.6 Attached and benthic populations in lakes

Many cyanobacteria grow attached on the surface of rocks and stones (epilithic forms), on submerged plants (epiphytic forms) or on the bottom sediments (epipelic forms, or the benthos) of lakes. They can occur in marine, estuarine, and fresh waters, but the blooms of greatest concern are the ones that occur in fresh water, such as drinking water reservoirs or recreational waters (Lambou et al., 1977).

1.7 Distribution

Toxic cyanobacteria are distributed worldwide and water blooms of noxious species cause health concerns in many countries including South Africa (Codd, 1985). The development of toxic blue-green algae in inland waters adds to the difficulties of providing safe water for drinking and recreational purposes.

During bloom conditions, a critical phase occurs when dense cell masses decompose naturally (Berg and Skulberg, 1987). Besides possessing the ability to produce toxins, cyanobacteria can also impart non-toxic but unpleasant tastes and odours to the surrounding water. The removal of bad taste and odour require the use of additional and more expensive water treatment procedures. Moreover, such eutrophied water bodies and its surrounding may experience economic losses e.g. decrease in property values, interference with water transport system should phytoplankton and other undesirable weeds persist over a long period of time.
In South Africa, recent studies show that most common blooms forming toxic species are *Microcystis spp* and *Anabaena spp*, although a number of other species may also produce toxins on occasions (DWAF, 1996).

Table 1.1 shows some of the toxins of concern from *Microcystis* and *Anabaena* species, which may sometimes inhabit South African water systems.

Table 1.1: Toxins of concern from *Microcystis* and *Anabaena*

Cyanobacteria	Cyanotoxins		LD_{50} [µg/kg bw]
Microcystis sp.	Hepatotoxins	(Microcystin-LR)	25 - 50
Anabaena sp.		(Microcystin-RR)	200 - 800
Anabaena sp.	Neurotoxin	Anatoxin-a	200 – 500

Microcystis aeruginosa blooms have been linked to mortality in humans (Carmichael et al., 2001), livestock and fish, widespread gastroenteritis in humans as well as skin irritations in swimmers (Rashidan & Bird, 2001). In 1973 and in 1974 cattle deaths were linked to *Microcystis aeruginosa* blooms in the Hartbeespoort Dam (Quibell et al., 1995). These effects and risks associated with cyanobacterial toxins are bound to increase with increased human activities promoting eutrophication.

1.8 Problem statement

Hartbeespoort Dam has over so many years been affected by high algal bloom, which mostly attracted bad publicity. This has in many occasions drawn substantial attention to the dam from the local inhabitants, dam managers and researchers. Due to the persistent blooming and bad publicity, the stakeholders have continuously been trying various management options and research studies that may conclusively solve the Hartbeespoort Dam bloom problem. Despite the fact that a lot of studies have been conducted in the past regarding the status of the algae in the dam and more so with specific reference to *Microcystis spp (*Scott et al., 1980; Roberts, 1984; Rossouw & Grobler, 1988; Chutter, 1989; Chutter and Rossouw, 1991; van Ginkel et al., 2000), there still exists widespread and persistent blooming.

Many of the studies conducted in the 1980's and early 1990's focused on the assumption that nitrogen and phosphorus nutrients are the determinants of blooming of the dam.

Studies conducted in the early 1990's pointed out that the 1988/89 and 1989/90 physical conditions of the dam were more favourable to the thriving of the *Microcystis* than would have been in the case of 1984/85 and 1985/86 periods. The latter conditions had included cooler surface water temperatures and an overall less stable water column. Nevertheless, these were the years in which *Microcystis* dominated the phytoplankton profile (Chutter and Rossouw, 1991). The study therefore, concluded that it was the nitrogen and phosphorus nutrient ratio that enhanced blooming of the dam. So far, little attention has been given to the contribution of temperature, major trace metals, the quantity and source of inoculums (mother cells) towards blooming and persistence of cyanobacteria in the dam. Considering the fact that algae generally need optimum environmental

conditions to flourish, the contribution of temperature, other nutrients, pH and major trace metals should not be ignored.

In addition to the study of most of the abiotic factors, there was an urgent need to find a source of perennial inoculums that have constantly fed the dam with the algal species every year even after winter absence of close to three months in a year. It is possible that the location of the source of such reproductive mother cells can explain their re-appearance after harsh winter months.

1.9 Aims:

This study therefore aimed;

To investigate environmental factors that affect the occurrence, persistence and blooming of phytoplankton species with particular emphasis on the *Microsystis aeruginosa* in the dam. This broad aim had the following specific objectives;

- to investigate species composition with specific reference to *Microcystis species;*
- to investigate the physical and chemical parameters of the dam and any possible effects to bloom persistence;
- to investigate the extent of bloom formation;
- to determine whether measures undertaken in the past to reduce blooming in the dam yielded desired results and;
- to determine the possibility of over-wintering mechanism and therefore possible sources of perennial algal inoculum feeding into the dam yearly after reported absence in winter months;

1.10 Hypotheses:

In order to conduct an investigation in the dam a number of hypotheses were drawn which included:

- over wintering mechanism provide inoculums for the subsequent season's growth and hence yearly persistent occurrence of *Microcystis* species in the dam;
- *Microcystis* and other phytoplankton are present at various depths in the dam throughout the year due to buoyancies and;
- bloom formation and algae growth patterns correlate to other abiotic factors such as temperature, pH and light penetration.

2. LITERATURE REVIEW

2.1 Cyanobacteria and the bloom formation.

Cyanobacteria are a group of extraordinarily diverse Gram-negative prokaryotes. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, freshwater to marine including hypersaline. They are found both free living and as endosymbionts. They are considered to be one of the potential organisms which can be useful to mankind in mariculture, food, feed, fuel, fertilizer, colourant, production of various secondary metabolites including vitamins, toxins, enzymes, pharmaceuticals, pharmacological probes and pollution abatement (Thajuddin and Subramanian, 2005).

In many environments, cyanobacteria are the primary producers at the base of the food web of the ecosystem, e.g. marine waters, freshwater, paddy fields and in soils. Cyanobacteria are symbionts of a variety of other organisms, viz. the marine diatom *Rhizosolenia*, leaves of *Azolla* and the roots of *Cycas*.

Cyanobacteria are basically microscopic, although large colonies or mats are quite conspicuous. Coccoid species occur as single cells colonies or masses of various shapes wherein cells are arranged in rows resulting in a flat plate, or are arranged radially in spherical colonies. Cell numbers may range from few to many. Colonies may be loosely attached to the substrata without polarity or remains firmly attached with a distinct base and apex (Geitler, 1932). However, these are enclosed in a gelatinous sheath that varies in consistency and thickness. Filamentous forms produce a row of cells, referred to as trichome. Trichomes may be simple, straight, as aggregated bundles and or permanently spirally coiled. The trichome with the enclosing sheath is referred to as a filament. Some filamentous species are characterized by true cell differentiation and form heterocysts, which unlike vegetative cells lack an oxygenic photosystem, possess extraordinarily thick cell wall, and lack biliprotein pigments and carboxysomes. These cells are considered as the sites of nitrogen fixation, provide vegetative cells with combined nitrogen and are not viable when disconnected from the trichome (Thajuddin and Subramanian, 2005). Many heterocystous cyanobacteria also form a second cell type, an akinete, which can germinate when conditions are suitable for growth.

Cyanobacteria, belonging to the order Chroococcales, and families Oscillatoriaceae and Nostacaceae occur ordinarily as planktonic forms. Several species grow in abundance and colour the entire body of water, forming the so-called water blooms. Huber-Pestalozzi (cited by Thajuddin and Subramanian, 2005) lists as many as 41 genera of 251 species of cyanobacteria that occur as freshwater planktons.

Microcystis is one of the dominant organisms that is associated with almost permanent blooms (Frankelin, 1972) in tropical freshwaters that are exposed to constant sunshine, warmth, carbon dioxide, pH and nutrients like phosphate and nitrates. The source of nitrogen for freshwaters includes drainage from the surrounding areas, precipitation bringing in dissolved free ammonia from atmosphere and release of nitrates from the dead matter. Nitrates form a preferred non-toxic source of combined nitrogen for most cyanobacteria and are produced as a result of high rate of nitrification under waterlogged conditions (Cristofir et al., 1981).

Formation of cyanobacterial blooms in freshwater bodies is essentially aided by buoyant nature of these organisms. Buoyancy is imported by the gas vacuoles and the rate of surface accumulation of these organisms is dependent upon the number of gas vacuoles within their cells. Gas vacuole containing cyanobacteria form dense growth on the water surface in ponds, reservoirs, and lakes and cause serious nuisance because of visual appearance, production of toxins (Carmichael, 1994).

2.2 *Environmental factors and bloom formation*

The changes that occur in a phytoplankton community, and ultimately the formation of cyanobacterial blooms, are a result of complex and dynamic relationships between physical, chemical and biological factors (Chorus & Bartram, 1999; Harding & Paxton, 2001). Some of these factors include water temperature, pH, and nutrient availability (nitrogen, phosphorus, iron).

The overwhelming dominance of *Microcystis* in various lakes of the world has been explained by water temperature, underwater light climate (turbidity or suspended solids or Secchi-depth), nutrients, buoyancy regulation and zooplankton grazing or fish grazing (Chen et al., 2003).

2.2.1 Temperature

Cyanobacteria have a wide range of temperature tolerance, but rapid growth rates are usually achieved when water temperatures exceed 20^0C (Du Preez & Van Baalen (2006). Batchelor et al., (1992) observed that the influence of temperature is interrelated to other environmental factors, particularly light, although the direct relationship can be variable and rapid adaptations to changing conditions influencing the development of low and high temperature tolerant strains.

Ashton (1976) cited by Harding and Paxton (2001) while describing the succession of algal blooms in the Rietvlei dam observed that after winter overturn, the concentration of dissolved nitrogenous and phosphorus compounds in the surface waters are high, but concomitantly low water temperatures prevented the growth of green and blue-green algae. Certain cold tolerant species were, however, able to survive (e.g. *Melosira granulata* var. *angustissima)*. As temperature rose,

non-nitrogen fixing species became dominant (e.g. *Volvox rousseletti*), and reduced the amount of available nitrogenous compounds – resulting in a phosphorus - rich environment suitable for the onset of nitrogen fixing blue-green algae e.g. *Anabaena circinalis*. Cooler weather and heavy rainfalls caused a disappearance of *Anabaena circinalis* bloom and the release of large quantities of nitrogen. This led to the creation of an environment suitable for the onset of noxious nitrogen- requiring blue-green algae, e.g. *Microcystis aeruginosa* that are able to tolerate lower water temperatures.

2.2.2 Turbidity/ Light penetration

Several studies on shallow lakes discussed the effect of turbidity on phytoplankton assemblages (Scheffer, 1998). Dokulil (1984) cited by Chen et al., (2003) suggested that the most obvious feature of turbid lakes like Neusiedlersee was the profound influence on underwater light, which was rapidly attenuated and altered in its spectral composition according to the nature of the suspended particles. In turbid underwater environments, algal species with gas vesicles, such as *Microcystis*, can either move down to avoid the high light intensity at the water surface, or float up when underwater light conditions are poor.

In water systems where Secchi-depth and suspended solids data showed the high turbidity like in Lake Taihu (Chen et al., 2003) or Hartbeespoort Dam, *Microcystis spp.* generally dominated. Underwater light climate therefore, seems to be one of the selective environmental factors that strongly influence the species composition and biomass of phytoplankton in the aquatic systems.

Scott et al., (1981) suggested that *Microcystis* adapts to high light intensities by reducing the chlorophyll content of the cells while at lower light intensities and in darkness more chlorophyll is synthesized.

2.2.3 pH values

Cyanobacteria and other photosynthetic prokaryotes are exceptional among micro-organisms in their inability to grow at low pH values. Cyanobacterial growth appears to be inhibited completely in habitats with pH values 4 to 5. With growth optima of between pH 7.5 and pH =10, cyanobacteria can therefore be considered alkalopiles (Thajuddin and Subramanian, 2005 cited Brock, 1973). Their preference for alkaline conditions suggests that there is an acid barrier that these micro-organisms have not been able to overcome, as a consequence of which prokaryotic algae have been excluded from acidic environment. Although it is not known why acid is detrimental to cyanobacteria, lack of control over internal pH might be responsible for growth limitations at low external pH, since bacterial cell function seems to depend on the maintenance of an appropriate intracellular pH (Thajuddin and Subramanian, 2005). The pH of the median influences the algal metabolism directly through enzymatic controls and indirectly through the availability of nutrients, minerals and trace metals (Bachelor et al., 1992). Under

alkaline conditions, (pH>8.6) the solubility of phosphate decreases as it combines as salts of calcium and magnesium, whilst ammonia may be lost through volatilisation to the atmosphere and conversion from the NH_4^+ -N form into more toxic NH_3 form. Growth limitation can be correlated to low CO_2 at high pH, or to high CO_2 at low pH, which can inhibit cell division and directly influence the production of biomass and carotenoids.

2.2.4 Nutrients

A nutrient is a chemical compound or element that can be used directly by plant cells (algae and aquatic macrophytes) for growth (Wamsley, 2000). In the context of eutrophication, nutrients are mostly inorganic elements that are assimilated by plants and, in conjunction with the process of photosynthesis, are utilised to produce and accumulate organic material in aquatic ecosystems. Algae and aquatic macrophytes require about 20 different elements. However, the rate and extent of aquatic plant growth is dependent on the concentration and ratios of nutrients present in the water. Plant growth is generally limited by the concentration of that nutrient that is present in the least quantity relative to the growth needs of the plant. This is known as the limiting nutrient concept and usually becomes the basis of eutrophication management policy.

The overall composition of aquatic plant tissue is $C_{106}H_{263}O_{110}N_{16}P$ yielding a C: N: P (Wamsley, 2000). Growth and luxury uptake in algal nitrogen (N) content is generally taken to be 9 to 12% by weight with phosphorus (P) 0.9 to 1.5% and carbon 35 to 60% (Bachelor et al., 1992). Because of nutrient supply and demand in nature, it has been observed that phosphorus (P) and nitrogen (N) are the most frequent limiting nutrients in fresh water systems. Increases in the level of these two nutrients in a water body will raise the risk (and frequency) of experiencing eutrophication problems. The availability of carbon (C) to plants is controlled by the calco-carbonate equilibrium in the water, and C limitation is normally restricted to short-term and transient circumstances (hourly) under conditions of high aquatic plant population.

Other nutrients can be important, but usually only under special circumstances. Micronutrients such as iron (Fe) are shown to be limiting in extremely pristine waters and some marine areas (Wamsley, 2000). Nitrogen occurs in surface waters in several forms (e.g. ammonium, nitrite, nitrate, urea and nitrogen gas). The ability to use any of the nitrate, nitrite and ammonia appears to be common, as does the limited uptake of organic forms. However, the report (Bachelor et al., 1992) indicated that when taken in the oxidised form, the nitrogen must be reduced before it can be incorporated into organic molecules. This requires additional energy and accounts for the preferential utilisation of ammonia over other nitrogen forms.

Algae assimilate phosphorus as inorganic phosphate although orthophosphate can be obtained from organic and inorganic polyphosphates of low molecular weight and is generally considered to be the most immediately available form of

phosphorus. however, mineralisation, sorption onto suspended material or sediment, desorption under aerobic conditions, luxury up take by plants, and precipitation with calcium or iron, are all processes that play important roles in influencing the concentration of available phosphorus in fresh and marine waters.

Biological growth and decay processes in aquatic ecosystems are important in determining the levels of available nutrients. Plants accumulate nutrients while bacteria mineralize them, but the processes occur at different rates depending on systems and conditions. It is therefore important to have a holistic ecosystem perspective of the situation. A eutrophic ecosystem is one where the potential concentration of nutrients is excessive even if instantaneous concentrations might be low.

2.3 Situation in Hartbeespoort Dam

For a number of years, this dam has been under persistent bloom of cyanobacteria with resultant consequences whenever blooms occur.
The main factors, which appear to determine the development of phytoplankton populations, are light, temperature, pH and nutrient concentrations.

The nutrients are continuously generated in the catchment areas draining to the dam and the overwhelming burden of the load from Juskei /Crocodile rivers. Apart from growth requirements of macronutrients such as phosphorous and nitrates, other abiotic factors such as pH, trace metals are also essential microelements, which are required by plants. Their presence in the dam may influence the growth dynamics of the cyanobacteria present and hence persistence.

A possibility of any correlations between the presence and levels of trace metals entering this dam; species composition and extent of such bloom formation could exist. Other conditions, such as patterns of wind, sheltering and a semblance of biological structure play an influential role in sustaining acceptable water quality.

However, once this semblance of biological structure for example macrophytes or fish population is lost, it may only minimally function, the balance swings in favour of algal species that are known strategists in dominating nutrient rich environments (Hartbeespoort Dam remediation project, 2004).

A technical report from Hartbeespoort Dam Remediation Project (2004) confirmed that not only is this dam hypertrophic, with more than 200 metric tones of phosphorus as P for several years but also that the semblance of the biological structure in this dam has been interfered with. In a report (Water Wheel 2005), it was noted that coupled with other environmental factors such as low rainfall and warm temperature, windless weather, the influx of nutrients generally led to rapid and excessive growth of cyanobacteria and aquatic weeds in this dam. The same report pointed out that Hartbeespoort Dam is effectively a massive nutrient trap with approximately 16 sewage works and many industries discharging wastewater effluents from the high density Johannesburg and Pretoria area into the Crocodile

River, the main system flowing into the dam. However, the release of wastewater into catchment areas is a worldwide practice and that with management options and legislation in place under South Africa's Water Act this dam should not attract negative publicity it has generated over the years. It reported further, that the dam has nutrients trapped in the sediments at the bottom and this may remain inactive for extended periods. In summer however, different thermal layers form in the water column. The deeper layer becomes anaerobic (oxygen depleted).

Under these conditions phosphorous is released from the sediment, a process known as internal loading. This provides an optimal cyanobacterial growth in the reservoir. However, for reasons not yet known, nowhere else in South Africa do these "hyperscums" produce so rapidly or in such quantities than in Hartbeespoort Dam (The Water Wheel, 2005).

Figure 2.1 shows a picture of a typical bloom underway next to the bridge crossing at Hartbeespoort Dam. Marked by a distinct algal bloom in the background and a relatively algae water free at the foreground, situations like these are common and often result in poor aesthetic water quality; strong smell, public outcry and sometimes fish kill when blooms occur in the dam.

Figure 2.1: A typical bloom underway at Hartbeespoort Dam next to the bridge.

According to the media release in the Water Wheel (2005), Hartbeespoort Dam suffers from massive seasonal growth of cyanobacteria, which accumulates on the dam surface and rots in the sunlight. It releases offensive odours and often looks and smells like raw sewage- but it is not. The sludge is caused by the natural

biodegradation of the cyanobacteria and not human excreta. The bloom not only affect the taste and smell of the water supplied to local residents, the foul smell around the dam wall puts off prospective buyers looking to purchase up market homes in the area. Understandably, the residents are up in arms whenever such rotting cyanobacteria are smelly with reports that raw sewage is spilling into the dam. This has resulted in management problems for all the stakeholders involved. Among such stakeholders include the Hartbeespoort Water Action Group (HWAG), the Department of Water Affairs and Forestry (DWAF) and the general public who reside around the dam and are all concerned in trying to find an approach hence a solution to this perennial problem. An investigation into the source of mother cell was therefore necessary in understanding the behaviour of this species.

The re-appearance of algal species in the dam after reported absence during winter has always been a subject of question. Tow (1979) observed that little was known of the fate of *Microcystis aeruginosa* during absence from the phytoplankton in winter months, only that they sometimes immediately re-appear and dominate certain water systems. In a subsequent investigation, microscopic examination of the sediment samples collected from Jan Smuts Park Dam, a natural shallow pan to the North of Brakpan did not reveal the presence of *Microcystis aeruginosa*. When sediments were incubated to determine the presence or absence of the algae, *Microcystis aeruginosa* – colonies appeared in all the sediment samples after a period of incubation.

Tow (1979) citing Reynolds (1973) stated that colonies settle on the bottom sediments during autumn due to reduction in the number of vacuoles. *Microcystis aeruginosa* break up into individual cells by sloughing off the mucilaginous sheath, settle in the sediments and later, under favourable environmental conditions, redevelop into colonies. They may also reform colonies from the release of nannocytes (Harding & Paxton, 2001; Tow, 1979; Pretorius et al., 1977). Investigation on this phenomenon was therefore necessary more so, for management strategies.

In order to rehabilitate and manage this dam however, DWAF as a lead agent have from time to time chosen certain management policies aimed at reducing the nuisance of the phytoplankton. Among such measures included the promulgation of the effluent phosphate-P standard of 1 mg/L, which was introduced in the catchment of Hartbeespoort Dam in 1985 (Chutter, 1989). A subsequent study (Rossouw & Grobler, 1988) after this initiative revealed that, while achievement of the effluent phosphate standard would result in decline in the availability of this nutrient to algae in the dam, the standard alone would not be sufficient to reduce the phytoplankton abundance to desirable levels. Furthermore, because of financial and capacity constraints in the metropolitan areas, sewage treatments works do not always comply with the 1 mg/L phosphate effluent standard and enforcement to the standard may not be possible in such an expansive catchment area. There are also numerous informal settlements in the catchment, as well as

intensive farming activities, all of which contribute to the extent of eutrophication of the aquatic system (van Ginkel et al., 2000).

2.3.1 Concept of eutrophication

Eutrophication is caused by the enrichment of an ecosystem with chemical nutrients typically compounds containing nitrogen or phosphorus. Phosphorus is often regarded as the main culprit in cases of eutrophication in lakes or dams subjected to point source pollution from sewage. The concentration of algae and the trophic state of lakes correspond well to phosphorus levels in water. Eutrophication may occur on land or in the water. It is frequently a result of nutrient pollution such as the release of sewage effluent into natural waters (estuaries, rivers, dams, lake or coasts) although it may occur naturally in situations where nutrients accumulate (e.g. depositional environments) or where they flow into systems on an ephemeral basis (e.g. intermittent upwelling in coastal systems).

Figure 2.2 shows how eutrophication process occurs naturally. Eutrophication generally promotes excessive plant growth and decay, favours certain weedy species over others, and is likely to cause severe reductions in water quality.

In aquatic environments, enhanced growth of choking aquatic vegetation or phytoplankton (that is, an algal bloom) disrupts normal functioning of the ecosystem, causing a variety of problems (WHO, 1999).

Health-related problems can occur where eutrophic conditions interfere with drinking water system. Although eutrophication is a natural process in the aging of lakes, dams and some estuaries, human activities can greatly accelerate it by increasing the rate at which nutrients and organic substances enter aquatic ecosystems from their surrounding watersheds (Horne and Goldman, 1994). Agricultural runoff, urban runoff, leaking septic systems, sewage discharges, eroded stream banks, and similar sources can increase the flow of nutrients and organic substances into aquatic systems. These substances can over stimulate the growth of algae. Such stimulation may create conditions that interfere with the recreational use of lakes and estuaries, and the health and diversity of indigenous fish, plant, and animal populations depending on the trophic state of the water system. The trophic state of a dam or lake refers to the nutrient status of the water, the biological production that occurs in the water and the morphological characteristics of the lake basin itself.

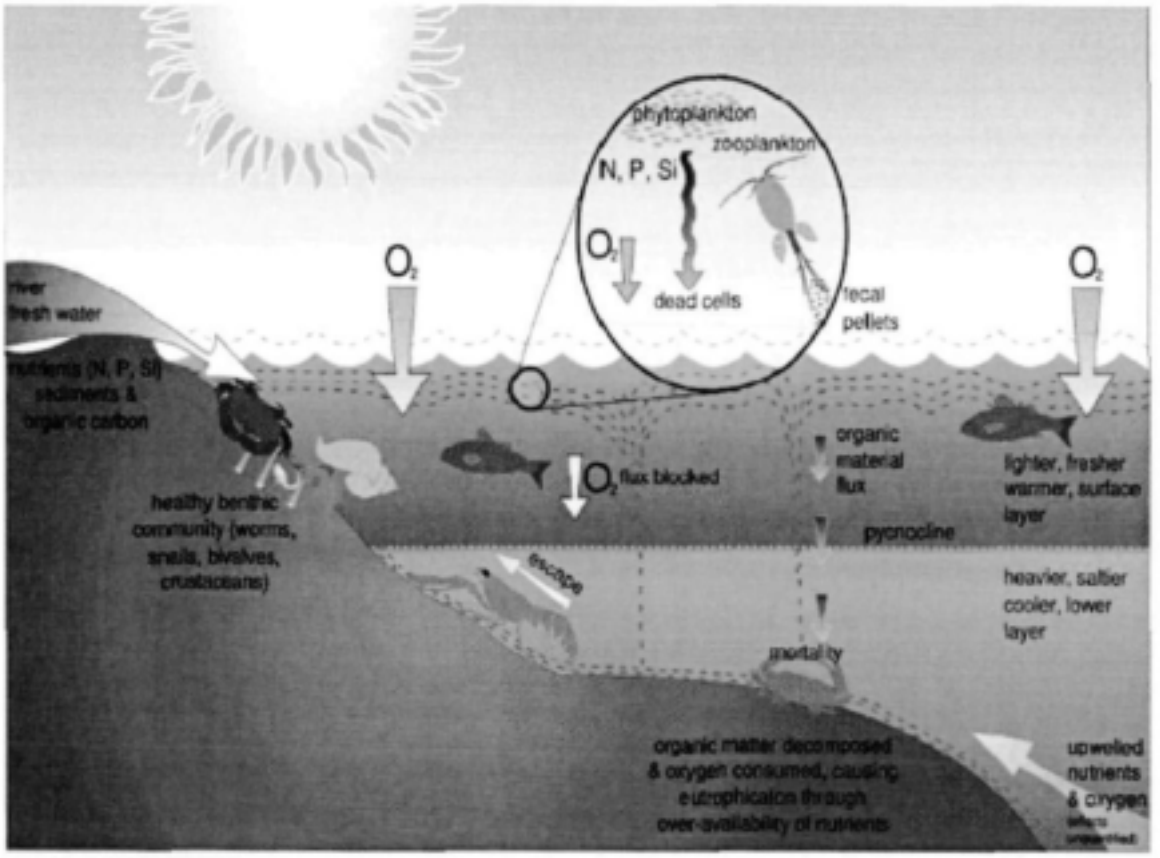


Figure 2.2: How eutrophication works in an estuary (Wikimedia, 2006)

Table 2.1 (Henry, 1993) indicates various levels of trophic states that many water systems may be classified depending on the nutrient load in the basin. Various nutrients, macro and micronutrients such as nitrogen, phosphorous, iron may reach water systems and contribute to different trophic levels. Some of the cyanobacteria can exist from oligotrophic (low nutrient) to hypereutrophic (very high nutrient) environments.

Changes in the ratio of nitrogen to phosphorus (N: P ratio) can affect algal species composition within a given water system. Some species of cyanobacteria (blue-green algae) have a competitive advantage over other algae by having the ability to fix nitrogen, whereas other types of algae cannot.

Nitrogen fixation is the process of converting unusable nitrogen (atmospheric nitrogen) into usable nitrogen (ammonia). This characteristic allows these species to exist in areas where low nitrogen availability inhibits growth. Therefore, under phosphorus-rich conditions, when nitrogen may be limited, blue-green cyanobacteria algae have a competitive advantage because they can utilize ("fix") nitrogen directly.

Table 2.1: Different trophic levels in water systems.

Trophic States	
Oligotrophic	Clear waters with little organic matter or sediment and minimum biological activity.
Mesotrophic	Waters with more nutrients, and therefore, more biological productivity.
Eutrophic	Waters extremely rich in nutrients, with high biological productivity. Some species may be choked out.
Hypereutrophic	Murky, highly productive waters, closest to the wetland status. Many clear water species cannot survive.
Dystrophic	Low in nutrients highly coloured with dissolved humic organic material. (Not necessarily a part of the natural trophic progression.)

Cyanobacteria can also successfully compete against other groups such as green algae and diatoms because they can store phosphorus for later use, and are not preferred as food by zooplankton (microscopic animals), larval fish and other animals that graze on many kinds of algae. As little as 15 parts per billion of total phosphorous can encourage excessive production of algae. Undesirable aquatic plant growth results from additions of phosphorus to the water. The net effect of the eutrophic condition and excess plant growth in water is the depletion of oxygen in the water due to the heavy oxygen demand by microorganisms as they decompose the organic material. It severely impacts the lakes natural ability to support aquatic life.

Center for Disease Control and Prevention (CDC-USA) recommends that in order to reduce the occurrence of cyanobacteria, a reduction of nutrient loading of ponds and lakes by using only the recommended amounts of fertilizers and pesticides on yards or catchments, properly maintained household septic system and maintaining a buffer of natural vegetation around ponds and lakes to filter incoming water should be considered. This is practical in this dam because in addition to external nutrient loading, the water body traps and recycles nutrients, thus rendering ineffective attempts to eliminate or reduce external loading (Karen, 2003).

Furthermore, internal nutrient loading can severely extend the response time of the restoration strategies as nutrients, particularly phosphorus, which are retained efficiently within sediments. Internal nutrient loading in the Hartbeespoort Dam has been estimated to be as high as 6.5 – 10 tones of phosphorus (Karen, 2003). In addition to both internal and external nutrient loading, water bodies trap and recycle nutrients and may as well do so to resident phytoplankton in the sediments throughout seasons.

Other control mechanisms may include aerated water systems by feeding air through pipes along the dam floor. This mixes the water and increases the oxygen levels and hence interferes with mobilisation of phosphorus, a limiting nutrient in algal growth. This method has reportedly been tested in South Africa with limited success. Chemical additives, such as iron or aluminium sulphate, or copper to prevent phosphorus from being released or seeping out of sediments can be undertaken and have been proposed. However, this could pose a problem for domestic water use. In late August 1993, a large algal bloom developed in Deacon Reservoir, Canada, Winnipeg's main storage facility for water from Shoal Lake. In an attempt to control algal density, taste and odour problems, municipal officials isolated the reservoir and treated it with copper sulphate. However, this action raised the concern that if the algal bloom contained toxin-producing algae, significant quantities of the toxins may have been released into the reservoir (Kenefick et al., 1993).

Other strategies DWAF initiated included bio-manipulation (or food web manipulation) in conjunction with the re-establishment of macrophytes (aquatic plants). Bio-manipulation involves analysing the impoundment's ecosystem and removing any natural elements that somehow contribute to the release of phosphorus or control the cyanobacterial growth. In Hartbeespoort Dam for example, harvesting surplus fish in the dam such as carp, a bottom feeder that disturbs the sediment to such an extent that phosphorus is released from the sediment has been started. Bio-manipulation theory is based also on the assumption that increased zooplankton abundance and grazing pressure will result in reductions in phytoplankton abundance and improved water clarity (Aroviita et al., 2000). Among the advantages of using bio-manipulation are cost effectiveness and the potential to be a long-term solution (Karen, 2003).

This strategy has been commissioned with progress being reported (Hartbeespoort Dam Remediation Project, 2004). It is however, a long-term solution that may not appeal to short to medium term needs that this dam may require whenever massive blooms occur.

In USA, other forms of bioremediations have been considered. Based on the fact that the growth of algae can be manipulated by nutrient dynamics, bioaugmentation, a process called biological nutrient removal (BNR) is practiced. The method explores the limiting element and the bacteria used in bioaugmentation, which are selected specifically for their ability to degrade organic material and detritus, and nitrify ammonia. These bacteria consume the nutrients in the water. The microbes ingest carbon, nitrogen and phosphorus.
In the presence of calcium carbonate and high pH, an insoluble phosphorus particulate is created by the death of the microbes. This reduces the amount of bioavailable nutrients that are then available for the algae.

To date however, physical removal of cyanobacteria has been the most successful management option used in Hartbeespoort dam. This is a short term and a

reactionary measure. It involves pumping the algae through pipes to a safe dry area where it is treated to mitigate the odours and accelerate the natural decomposition process. It may not address the root cause of the problem and has been used whenever the dam is experiencing massive bloom with decomposition of organic material already underway. Together with other measures already in practice, this study proposed further strengthening of the process of physical removal. Further study, may in future yield a mathematical model, which will predict possible bloom conditions. As pointed out in the model proposal by Grobler and Silberbauer (1984), any dynamic model presupposes a fair knowledge of water body concerned before prediction can be made. This study therefore was conducted to add to the body of limnological information on the current status of this dam.

3. MATERIALS AND METHODS

3.1 Location, morphology and hydrological characteristics:

Hartbeespoort Dam as shown in Figure 3.1, is a hypertrophic, warm, monomictic water body situated downstream of the cities of Pretoria and Johannesburg (25° 43'S, 27° 51'E). The Crocodile (Jukskei-Hennops), the Magalies Rivers as well as two minor streams of Leeuspruit and Swartspruit serve the dam. The dam is known to receive very high nitrogen and phosphorus loads (Scott et al., 1980). These sources ensure an over-abundance of algal growth throughout the year. The dam receives, in a typical year, about 160,000 kg of phosphorus, about 120,000 kg more than it can handle (Hartbeespoort dam remediation project, 2004). Overturn in Hartbeespoort Dam occurs in April and the whole lake, may become anaerobic (Robarts, 1984). The main outflow of water is through sluices in the wall. Alternatively, secondary overflow takes place through the spillway (Hely-Hutchinson & Schumann, 1997). Water abstraction from the dam occurs as well for domestic use and riparian irrigation. At full supply, the volume of the dam is 195 x 10^6 m^3, with a maximum depth of 32.5 m, mean depth of 9.6 m, surface area of 20 km and a length of 5.6 km. The dam has a catchment area of 4144 km^2 and exists at 1162 m above sea level (Middleton et al., 1981).

Figure 3.1: Hartbeespoort Dam and its catchments. Rivers, Dams and Topography

3.2 Sampling points

The sampling sites (Figure 3.2) were considered in consultation with Resource Quality Services (RQS) of Department of Water Affairs based on their sampling site positions in the dam. Site A was a permanent sampling point (main water extraction point for the local village) for all the chemical and physical water quality parameters. Site B was at the Dam wall near exit sluices where samples taken for algal identification was carried out. Site C, randomly considered within the basin was occasionally sampled to determine similarities or differences of species types in the dam. Site D, mean depth of about 6m, positioned at the confluence with Crocodile River was the sediment sampling point.

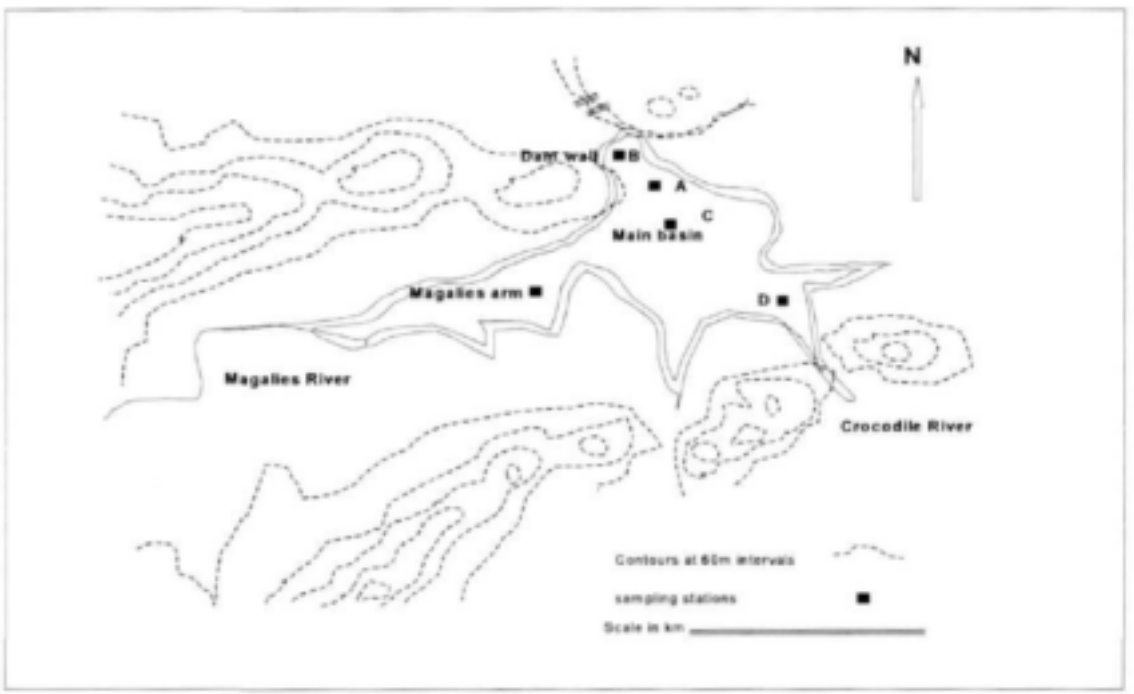


Figure 3.2: Locality of sampling points

3.3 Samples treatment

3.3.1 Water sample

3.3.1.1 Physical and chemical water quality parameters

Water samples for chemical and physical water quality parameters were collected using a polyethylene hosepipe for an integrated surface to 5 meters sample and a Van Dorn sampler for the specified depths. Sampling was done at 1, 2, 5, 10, 15 and 20 meters.

Physical water quality parameters such as temperature, dissolved oxygen (DO) electrical conductivity (EC), light penetration were determined on site using portable equipment and a Secchi disk. The disc was painted in black and white quadrants. Readings were taken at 0.1 m intervals until total light extinction was obtained.

1L water samples for microanalysis were preserved with 2 mg mercury chloride ($HgCl_2$) for major basic water quality parameters such as total phosphates, nitrates, dissolved ammonia, total alkalinity and sulphates. These samples were filtered after collection and were then stored for a few hours at temperatures below 20°C before determinations were carried out using published methods of Institute of Water Quality Studies (IWQS, 1999). Some selected dissolved trace metals such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and lead (Pb) whose sources could be anthropogenic in nature from the catchments area of the dam were also determined (Radojevic and Vladimir, 1999).

3.3.1.2 Algal species and Chlorophyll determination

Freshly prepared unpreserved water samples for algal identification, quantification and chlorophyll *a* content were collected at the water surface using a 1L-sampling bottle and integrated (0-5 m) samples were taken with a Van Dorn sampler at sites shown above. The containers were then capped and thereafter transported in a cooler bag for laboratory analyses. These samples were identified immediately in the laboratory after collection as algae die and cells rupture in a short time, making identification impossible. Whenever algal concentration was low, samples were concentrated by letting the algae to settle out onto the bottom of a measuring cylinder and thereafter upper water was carefully siphoned off. However, in cases of highly dense bloom condition, dilution with distilled water was performed.

3.3.2 Sediment samples

3.3.2.1 Algal species identification and quantification

Sediment samples were also collected with an Ekmann Grab Sampler and the content transferred in a polythene bag packed in a cooler bag for laboratory

microscopic analyses. The preparation for sample analyses was according to IWQS (1998).

3.3.2.2 Blocks as surface attachments

For a period of more than one and half years, blocks of bricks were laid submerged in shallow water on the bank of the dam. These were checked every two months to establish a possible sheltering of phytoplankton on the underside of these blocks. The snapshots and further laboratory microscopical identification of species were conducted to determine the presence of phytoplankton on the underside of arranged blocks. This was conducted in order to reveal empirical information as to whether some of these species survive winter conditions hiding under structures such as blocks, rocks or sediment in the water and provide a possible source of inoculum for the next season's crop.

3.4 Analytical methods

3.4.1 Physical and Chemical quality parameters

The physical water quality parameters were determined on site while the chemical parameters were determined using IWQS (1999). The results obtained were then presented as tables, figures and data analyses discussed.

A Perkin Elmer 3030 atomic absorption spectrophotometer equipped with a single slot burner was used for the analyses of the trace metals studied using Radojevic and Vladimir (1999). Normal linear fit working calibration curves were prepared for metal determinations.

3.4.2 Chlorophyll analysis

An analysis of chlorophyll *a*, a measure of phytoplankton abundance, was conducted using spectrophotometer as described in the test methods manual of the IWQS (1998). The values obtained bimonthly were then compared with the total phytoplankton counts during those respective sampling dates for a given period.

3.4.3 Algal identification and quantification

Freshly prepared unpreserved samples were used for microscopical algal identification and quantification (Truter, 1987). Polycarbonate filters (Millipore 3 µm) and plastic syringes were used to retain a known volume of phytoplankton onto the filters, which were placed in a drop of immersion oil on slides. The filters thereafter were subjected to high power (400x objectives) microscopic examination. Cells or colonies were then counted using transects covering the entire surface area of the slide and approximated as percentages in relations to the other species of algae present in the sample (Scott et al., 1980). The biological

analyses were conducted both in the water column and in the sediment samples. The seasonal phytoplankton pattern was then obtained from the bimonthly values and compared for winter and summer months for the three-year study. However, because of unavailability of a camera mounted on the microscope at the time, relevant plates showing different algal species observed could not be captured and are not included in the study report.

3.5. Previous studies

Some data from Department of Water Affairs (DWAF, 2005) referred in this study as earlier studies (1990 – 2000) and others (Chutter, 1989; Scott et al., 1979) were considered with respect to those relevant to the aims of this study in order to compare changes that might have occurred in the dam with any corresponding changes to phytoplankton community, physical and chemical quality parameters.

3.6 Statistical analysis

The data collected were then subjected to student *t* - test to determine significant differences that may have occurred over the years and within the study period. A pooled method was used at probability ($p=0.05$).

4. RESULTS AND DISCUSSION

4.1 Physical quality parameters

Table 4.1, shows the mean and corresponding range for some physical parameters observed during the study period (2003/ 2004).

TABLE 4.1: Mean and range for physical water quality parameters

Year	2003		2004	
Parameter	Mean	Range	Mean	Range
Temps	20.3	14.6 - 26.6	19.7	12.4 - 26
pH	8.4	6.4 - 9.1	8.3	9.8 - 7.4
EC (ms/cm)	527	461 - 573	540	446 - 587

EC= Electrical conductivity (ms/cm), Temps (^{0}C) = Temperature at surface (0 m)

4.1.1 Temperature

As can be seen from Table 4.1, the mean temperatures for 2003 (20.3^{0}C) were higher than 2004 (19.7^{0}C) at the surface level of the dam. This indicated warmer conditions for the year 2003 as opposed to 2004. Furthermore, temperature measurements were also recorded in Figures 4.1 up to 4.4 (see also Table A, Appendix 2).

As can be seen from Figures 4.1 and 4.2, the temperature decreased from March to August 2003 in the dam. From Figure 4.1, there was minimal temperature variation between the surface (0 m) to 5 m depth in March 2003. However, there was temperature difference at depths between 5 m to 15 m indicating stratification. These temperature differences decreased progressively until May 2003 when almost uniform values were observed in the entire water column. It was also observed that there was an almost equal temperature measurement ranging from 14°C to 18.5°C during winter period (Figure 4.2). This slight temperature variation was as a result of overturn in the dam that occurred in April 2003.

Figure 4.3 shows increasing temperature measurements in late September and the beginning of re-stratification of the dam. By November 2003, the dam had undergone a marked stratification below 2 m depth. Figure 4.4 again shows surface temperatures below 18°C during winter period. It also indicates an overturn with almost uniform temperature measurements recorded from surface to the bottom of the dam from May to July. However, the lowest winter temperature of 2003 (13.1°C) is higher than the winter period of 2004 (11.9°C). Equally, in Figure 4.4, winter months of 2004 showed almost uniform temperature values in the water column until the end of July as was recorded in winter months the previous year showing minimal temperature differences for the two seasons.

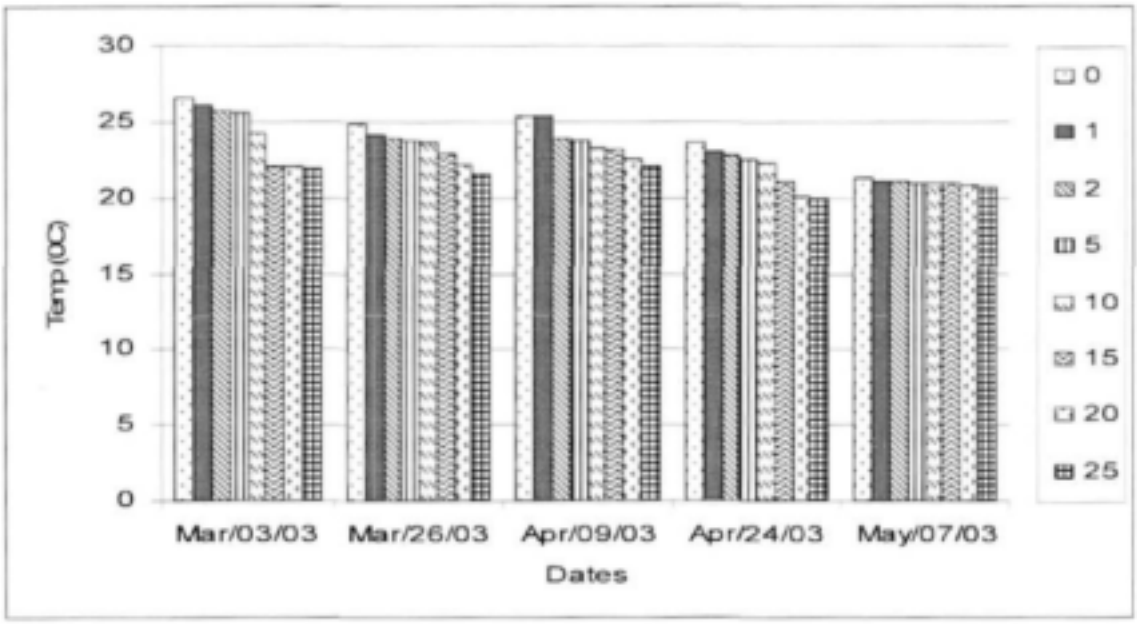


Figure 4.1: Temperature values for early months of 2003

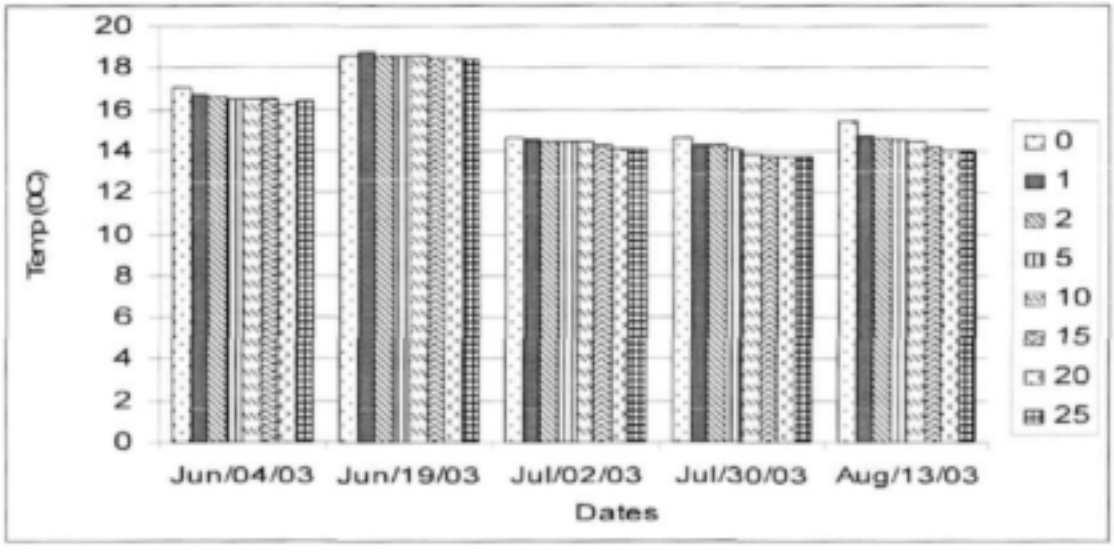


Figure 4.2: Temperature values for winter Months of 2003

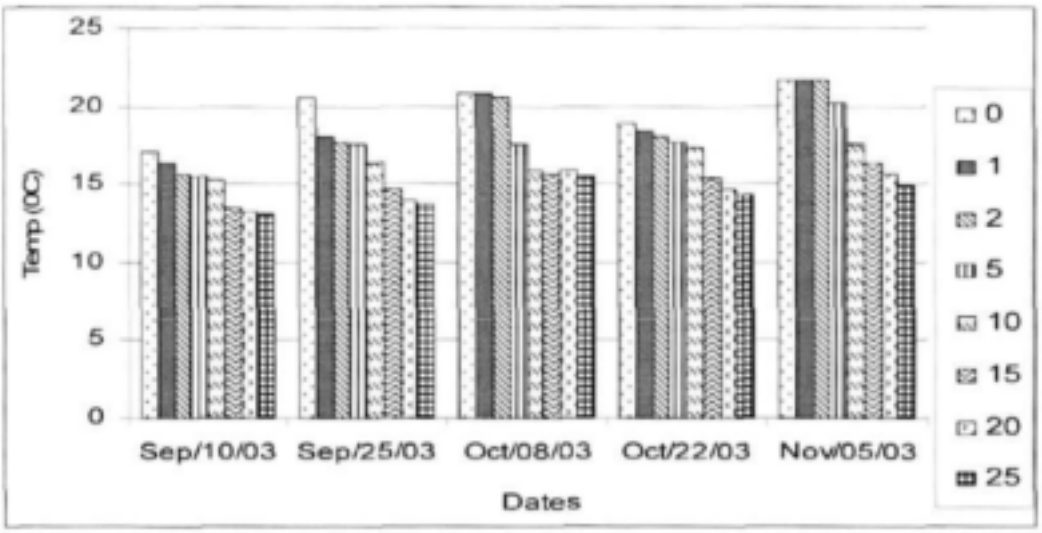


Figure 4.3: Temperature values for late months of 2003

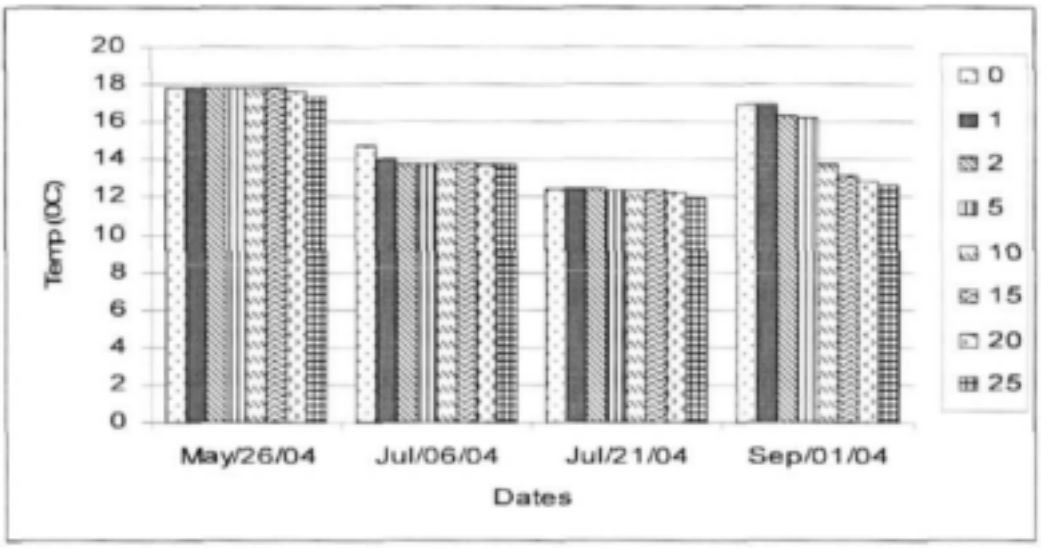


Figure 4.4: Temperatures of winter months of 2004

When temperature readings were subjected to statistical analysis to determine levels of significance in pooled mean values at various depths for this study (2003 and 2004) and the values obtained in earlier studies (DWAF, 2005), there was no significant difference (Table 4.2) at all the depths considered.

4.1.2 Dissolved Oxygen

Dissolved oxygen measurements are recorded in Figures 4.5 to 4.7. (See Table B, Appendix 2,). Oxygen levels ranged from 4 to 6 mg/L early in June 2003 in the

water column with the surface level concentration reaching a level of 12 mg/L later in June the same year

Table 4.2: Statistical Analysis for Temperature (^{0}C) at different Depths

			Mean Temps (^{0}C) ± SD		
Depth (m)	2003/2004 (n)	1996-2000 (n)	2003/2004	1996-2000	$p^\dagger$
0	24	20	20.07± 4.19	20.98 ± 5.05	NS
2	24	20	19.43 ± 4.13	19.82 ± 4.81	NS
5	24	16	19.09 ± 4.03	19.21 ± 4.51	NS
15	24	20	17.52 ± 3.53	16.47 ± 3.59	NS

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^\dagger$ value. NS = Not Significant
This study (2003/2004) and Earlier studies (1996-2000)

The dam was well oxygenated at all depths during July and August (Figure. 4.5). A marked anaerobic hypolimnion was visible in November 2003 (Figure. 4.6) and in January 2004; oxygen levels were extremely low throughout the dam. A possible explanation was that a lot of dissolved oxygen was being used up during the decomposition of the algae at the time and hence low level concentration in water at these depths in the dam.

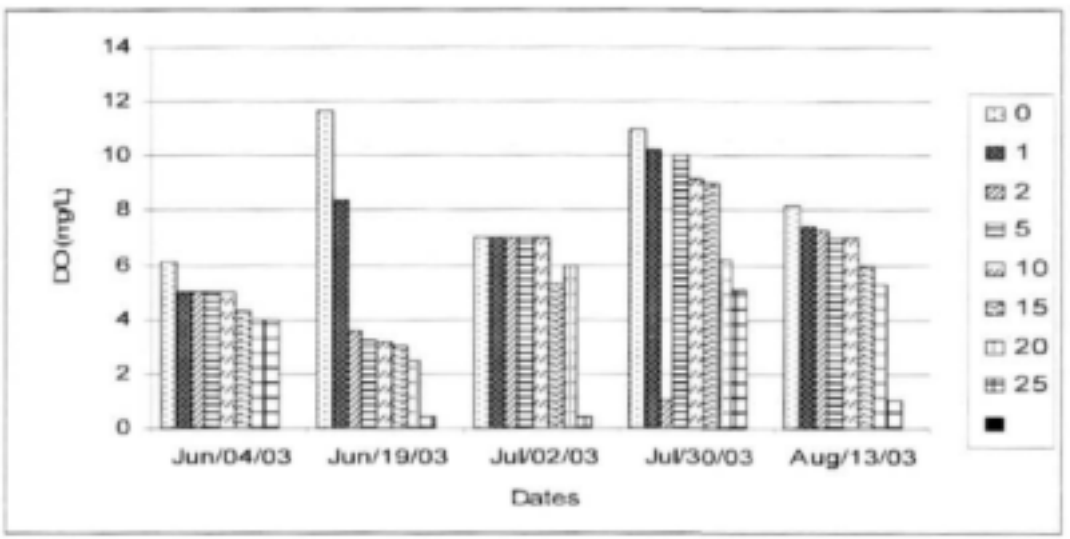


Figure 4.5: Dissolved Oxygen for winter 2003

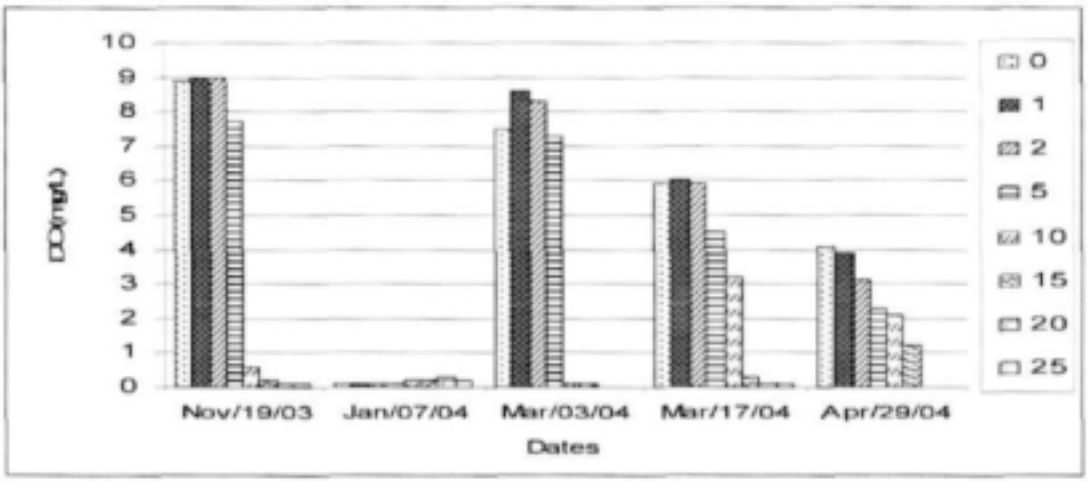


Figure 4.6: Dissolved Oxygen for November 2003 and the early months of 2004

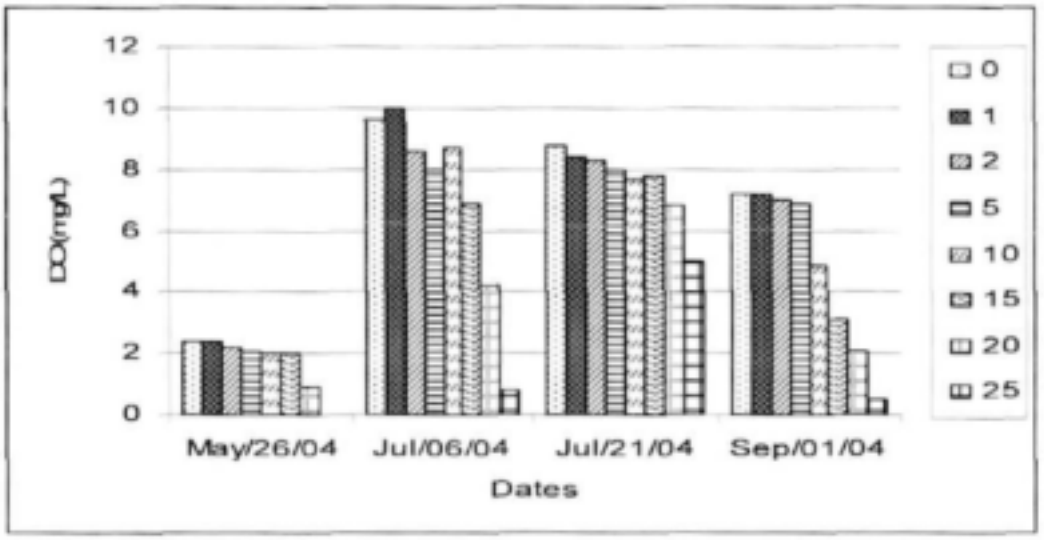


Figure 4.7: Dissolved Oxygen for winter Months of 2004

However, by March 2004, dissolved oxygen levels increased again followed by a declined in April and May 2004 (Figures 4.6 and 4.7). The months of July-September 2004, however, saw an increase to a depth of 10 m. A possible explanation could have been due to inflow of water from the feeder-rivers hence mixing of the water in the dam. Low temperatures in winter may have also contributed to increased dissolution of molecular oxygen in water.

Figure 4.8 showed the relationship between dissolved oxygen, temperature and chlorophyll *a* changes. It indicated a normal phenomenon that as temperature of the water increased, the concentration of the dissolved oxygen decreased. Low

chlorophyll *a* concentration was observed after physical removal of algae in the dam, which later appeared to have increased with temperature increase and vice versa.

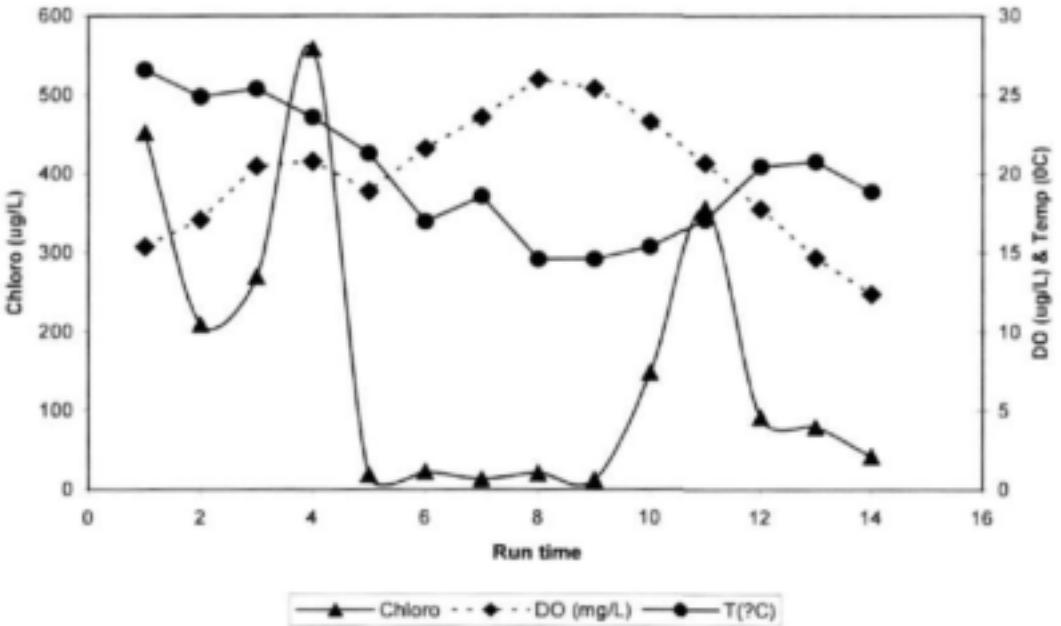


Figure: 4.8: Correlation between dissolved oxygen, temperature and chlorophyll *a* changes.

4.1.3 Electrical conductivity

In Table 4.1, the electrical conductivity ranged from 461 to 573 ms/cm in 2003 and 446 to 587 ms/cm in 2004. A pattern of low (461 ms/cm) levels in winter and higher values in summer (573 ms/cm) was observed in the year 2003.

Table 4.3: Statistical Analysis for Electrical Conductivity

		Mean Electrical Conductivity (ms/cm) ± SD		
2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
20	112	537.9 ± 29.77	527.4 ± 61.12	NS

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value, NS = Not Significant
This study (2003/2004) and Earlier studies (1990-2000)

This was similar to the year 2004. It should be noted that this pattern was also observed for values obtained in the past ten years with data being insignificantly different when their mean values were pooled (Table 4.3)

4.1.4 pH value

The pH values as shown in Table 4.1 indicated that the dam was slightly more alkaline in 2003 (pH = 8.4) than was in the year 2004, pH (8.3). However, the opposite was true for the pH range for 2003 (9.1 - 6.4) compared to the year 2004 (9.8 - 7.4) with 9.8, the highest recorded value during the study period. Harding and Paxton (2001) while citing the work of Pieterse and Janse van Vuuren (1997), stated that pH increased with increasing chlorophyll *a* concentrations and higher algal biomass, a consequence of elevated photosynthetic activity. pH values above 8 would then be favourable for algal growth. On average, both years had pH values above 8, making the dam alkaline. The contributions on alkalinity made by bicarbonate ion, carbonate ion, and hydroxide ion depend upon the pH in water. However, for the calculated statistical mean pH values obtained in this study and those from the previous studies (DWAF, 2005) the values were found to be significantly different (Table 4.4).

Table 4.4 also indicated a drop in mean pH values over two decades, though still favourable for growth of algae.

Table 4.4: Statistical Analysis for pH values

		Mean pH value ± SD		
2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
20	112	8.35 ± 0.62	8.58 ± 0.39	Significant

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value.
This study (2003/2004) and Earlier studies (1990-2000)

4.1.5 Light penetration

Figure 4.9 shows the Secchi depth readings for August-November 2003 and March-September 2004. The early summer months in 2003 (October) and 2004 (September) showed the highest readings of 3.4 m and 4.7 m respectively. The value for the September 2003 (3.0 m) was lower than that of the corresponding month in 2004 (4.7 m). It was at the same time when little phytoplankton was observed in water. It appeared that in Hartbeespoort dam, light penetration into the water was greatly influenced by phytoplankton, rather than by inorganic suspended solids. This was because of higher secchi disk values whenever less

bloom or no bloom was present as opposed to low secchi disk values in hypertrophic conditions in the dam.

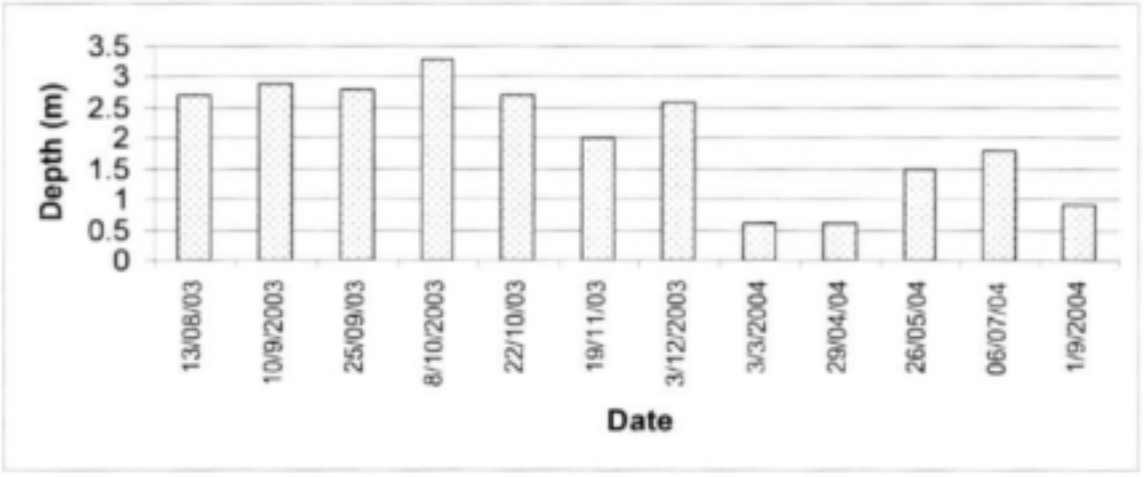


Figure 4.9: Secchi disk reading (Light penetration with depth)

When the data from this study were compared to those obtained previously as earlier studies (DWAF, 2005) and subjected to statistical analysis, the result revealed a significant difference in the data (Table 4.5). From the table, it can be seen that the mean values for the past data were also lower than the current study, which signified a probable high levels of bloom formation, or other influential factors such as dissolved solids, inorganic suspended solids present in the dam during the periods.

Table 4.5: Statistical Analysis for Secchi Depth reading

		Mean Secchi Disk (m) ± SD		
2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
13	22	2.23 ± 1.17	1.28 ± 0.46	Significant

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value.
This study (2003/2004) and Earlier studies (1990-2000)

4.2 Chemical water quality parameters

Some of the data obtained in this study (the year 2003 and 2004) on nutrients in the dam were also considered (Table 4.6a) and then compared to those obtained as earlier studies (DWAF, 2005) and other publications (Chutter, 1989; Scott et al., 1979). Furthermore, some of the data were subjected to statistical tests (Table 4.6b) to reveal any significant differences in their pooled mean values and the following observations were made;

Table 4.6a: Annual averages for chemical water parameters

Chemical water quality parameters concentrations ($\mu g \cdot l^{-1}$)				
Year	2003		2004	
Parameter	Mean	Range	Mean	Range
PO_4-TOT	73	17 - 402	48	13 - 284
Kjeldahl-N	1961	40 - 13520	1155	355 - 2877
NO_3-NO_2	574	11 -1473	1330	13 - 2764
NH_4-N	197	23 - 2015	196	10 - 1904
SO_4^{2-}	5400	4700 - 7100	5000	4100 - 6200
T-AL	11700	10000 - 14000	11600	8400 - 13800

4.2.1 Total phosphorous

The average total phosphorous concentration (Table 4.6a) was higher in 2003 (73 µg/L) than 2004 (48 µg/L). Similarly, higher concentrations were determined in summer with reduction in phosphate levels in winter months. Within this period the total phosphorous concentrations varied between (284 µg/L) during the winter, July 2004 with an exceptional increase of 402 µg/L in April 2003 and reduction, 13 µg/L in summer (March 2004). Generally, concentrations observed in the summer were unacceptably high with the mesotrophic total phosphorous levels less than 47 µg/l. This is an indication of highly enriched water. On the statistical analysis for the two sets of data (Table 4.6b), it was revealed that whereas the pooled mean for the current study (38.9 µg/L) was lower than those obtained from the earlier studies (66.6 µg/L), there was no significant difference in both data.

Table 4.6b: Statistical Analysis for selected Chemical parameters

			Mean concentrations ($\mu g \cdot l^{-1}$) ± SD		
Constituents	2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
PO_4-TOT	20	112	38.9± 13.68	66.58 ± 101.8	NS
Kjeldahl-N	20	112	1140 ± 445.3	1235 ± 438.6	NS
NO_3-NO_2	20	112	1096 ± 801.8	1020 ± 96.4	S
NH_4-N	20	112	126.35 ± 113	93.0 ± 78.8	NS
SO_4^{2-}	20	112	51940 ± 4.09	71250 ± 18.51	S

This study (2003/2004) and Earlier studies (1990-2000)
n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value. S= Significant, NS = Not Significant

4.2.2 Kjeldahl-nitrogen

A Kjeldahl nitrogen concentration of 13520 µg/L in April 2003 coincided with the high total phosphorous, 402 µg/L. Mean averages for the year 2003 were also higher than 2004 (Table 4.6a, See also Table C, Appendix 2). Statistical analysis (Table 4.6b) for the parameter also revealed no significant difference for this and earlier studies in the dam.

4.2.3 Nitrogen – nitrate

The concentration of dissolved NO_3 - NO_2 as nitrate almost doubled in September 2004 (2764 µg/L) as compared to October 2003 (1473 µg/L). There was also an increase in winter months of 2003 as opposed to summer months of the same year. However, 2004 had higher average concentration of nitrate (1330 µg/L) compared to (574 µg/L) in 2003 (Table 4.6a). At the epilimnion, nitrite and ammonium showed higher concentrations in 2003 than in 2004. Higher concentrations may signify deterioration in water quality. But in comparison to the earlier set of data, the analysis revealed significant difference (Table 4.6b) in the two studies. The overall lower mean concentrations of the NO_3 - NO_2 levels may suggests an improvement in water quality over the past two decades in the dam or that increased quantities of these nutrients were tied up in the algal cell material. Death and decomposition of these materials was therefore a major source of dissolved ammonium in the water.

4.2.4 Dissolved ammonium

Table 4.6a, showed that on average, there was a constant value (197 µg/L) for dissolved ammonia in two years, an indication of the presence of materials with organic loading. Possible fates of ammonium (NH_4^+) within the system include biotic uptake, nitrification, volatilisation and absorption (Melody & Dodds, 2002). Dissolved ammonium concentration in the dam varied from 10 µg/L on 5/7/2004 to 2015 µg/L on 9/4/2003 (1192 µg/L) compared to (562 µg/L) in 2003 in the impoundment. These lower and higher concentrations were observed in the upper surface (0-5 m), epilimnion compared to the lower part (hypolimnion) of the dam (20 m) respectively. This confirmed that hypolimnion is a region of reduced chemical species (ammonia), while epilimnion is dominated by oxidised (nitrate - nitrogen) species. However, both oxidised and reduced forms of nitrogen were present interchangeably depending on the level of denitrification, which was possible in partially anaerobic systems like Hartbeespoort Dam. When ammonia volatilises, it is subsequently lost immediately from the water surface and hence less concentration may be observed in water. Whereas the statistical analysis (Table 4.6b) showed increased pooled mean values for ammonia, the differences were not significant.

4.2.5 Total alkalinity

Total alkalinity values varied between 83760 µg/L in 2004 to 138670 µg/L in 2003 as can be seen in Table 4.6a. Higher average values were observed in 2003 than was observed in 2004. Alkaline waters signified higher pH values as was observed in the dam. Alkalinity therefore, served as a pH buffer and a reservoir for inorganic carbon and this helped to determine the ability of water to support algal growth and other aquatic life. Apart from the usual contributors of alkalinity, bicarbonate ion, carbonate ion and hydroxide ion, ammonia, are also minor contributors.

4.2.6 Sulphate ion

Table 4.6a, showed that while there was a marginal difference in mean annual concentrations for sulphate ion (54000 and 50000 µg/L) for the year 2003 and 2004 respectively, comparison with earlier data revealed an overall decrease in concentrations over the period with their pooled mean showing 51940 µg/L and 71250 µg/L respectively (Table 4.6b). These pooled mean values were significant in their differences, an indication of less sulphate ions reaching the dam. This suggested a reduction from point or non-point sources of the anion in the catchment areas.

4.3 Trace metals

Trace metals are part of the essential micronutrients, which by nature are required by plants even though in small or trace quantities. Their presence together with other parameters in this impoundment was a factor considered for a possible explanation of growth dynamics of the cyanobacteria present and hence persistence in the dam. The results of the total monthly trace metal concentrations are shown in Table 4.7 as current and previous data (Chutter, 1989).

Table 4.7: Current /past trace metal levels: Annual mean averages

	Year and concentrations ($\mu g \cdot l^{-1}$)			
	2004/2005		1985/86	1986/87
Metal	Mean	Range		
Cu	56	45 - 69	67	<25
Fe	86	78 - 94	87	50
Mn	59	34 - 91	No data	No data
Pb	47	42 - 53	56	56
Zn	78	71 - 86	269	77

Zinc concentration ranged from 71 µg/L to 86 µg/L in 2004. Manganese on the other hand had the highest concentration value (91 µg/L) in that year. However, copper and lead had relatively lower concentrations as compared to other trace metals during the period of study.

When the annual trace metal values (Figure 4.10) obtained in this study (2004 – 2005) were compared to the values obtained in the 1985/86 and 1986/87 (Figure 4.11), except for manganese, little or no change was observed. Due to lack of available past raw data in other metals except for iron, a statistical analysis for these metals was not possible as only mean values were reported.

Although copper concentration in 1986/87 was low (25 µg/L) the values obtained in the 1985/6 averaged 67 µg/L, which was close to those obtained in this study. Similarly, zinc concentrations were approximately the same value (77 µg/L) in 1986/7 as compared to the current value (78 µg/L). Whereas the available data (Table 4.7) indicated a higher concentration of zinc metal (269 µg/L) in the impoundment for the period 1985/6, it has been pointed out (Scott, 2006) that this may have been a statistical error. Nonetheless, there was slightly higher concentration of lead metal (56 µg/L) in 1970's (Scott *et al.*, 1979) as compared to the current study (47 µg/L). Iron concentrations ranged from 78 µg/L to 94 µg/L in 2004.

Whereas there was lowest concentration of iron in October 2004, comparing these values to those obtained in earlier studies revealed that the two sets of data had a significant difference in mean concentration. The mean pooled values were higher for current study (Table 4.8), a possible indication that more iron reached the dam during or before this study commenced.

Table 4.8: Statistical Analysis for iron concentration

			Mean iron concentration ($\mu g \cdot l^{-1}$) ± SD		
Constituent	2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
Iron	12	12	85.75 ± 4.81	39.42 ± 23.0	Significant

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value.
This study (2003/2004) and Earlier studies (1990-2000)

Based on these two studies, it was not possible to determine any direct correlation between metal levels and phytoplankton growth or their persistence in the dam. However, increased iron concentration in the dam over the years was noted. This view was also shared by Harding (2005), who pointed out that the level of limnological understanding should have indicated that this was unlikely to be the case for an impoundment receiving so massive an effluent component from the catchments.

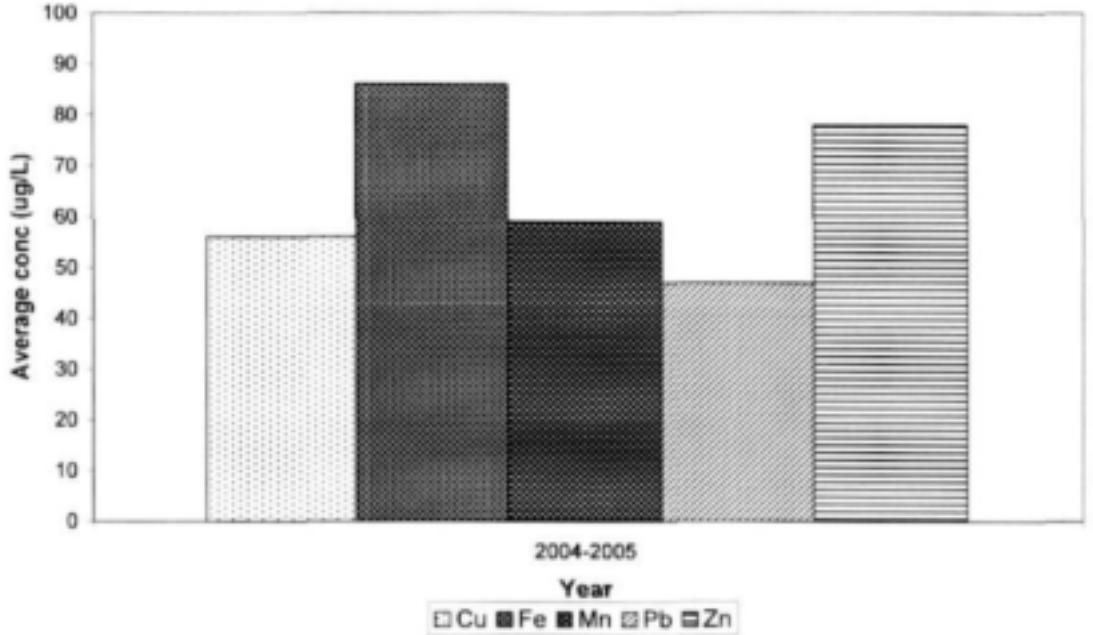


Figure 4.10: Annual mean averages for trace metal levels (µg/L)

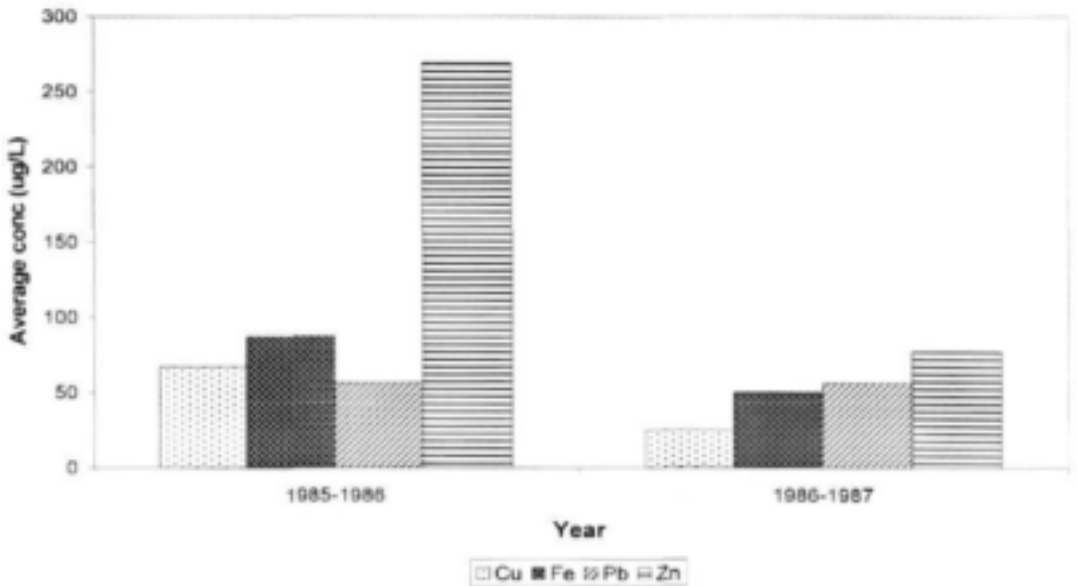


Figure 4.11: Annual mean averages for past trace metal levels (µg/L)

4.4 Chlorophyll quantification

One of the measures of quantifying phytoplankton abundance is chlorophyll *a* concentration (Chutter, 1989). Figures 4.12a and 4.12b report Chlorophyll *a* values for this study.

A massive algal bloom was observed between August and September 2003 with the highest values (Figures 4.12a) in September 2003, although a decrease vertically down the water column was noted. This was followed by a dramatic reduction (Figure 4.12b) until December, after some of the algae were physically removed from the dam. There was an increase of chlorophyll *a* content again in the summer months of January 2004, followed by reduced levels (79 µg/L) in July 2004 during winter.

Figure 4.13 however, indicates a possible direct relationship between temperature increases in water and the concentration of chlorophyll *a* observed in the dam. Except for periods when physical removal of algae took place, temperature appeared to be among the important environmental factors that influenced the bloom hence increased chlorophyll *a* quantity. It is commonly observed that warm temperatures favour cyanobacteria (Wood, 1993). A study by Robarts and Zohary (1987) concluded that temperature was the second most important factor affecting the *Microcystis* bloom in Hartbeespoort Dam.

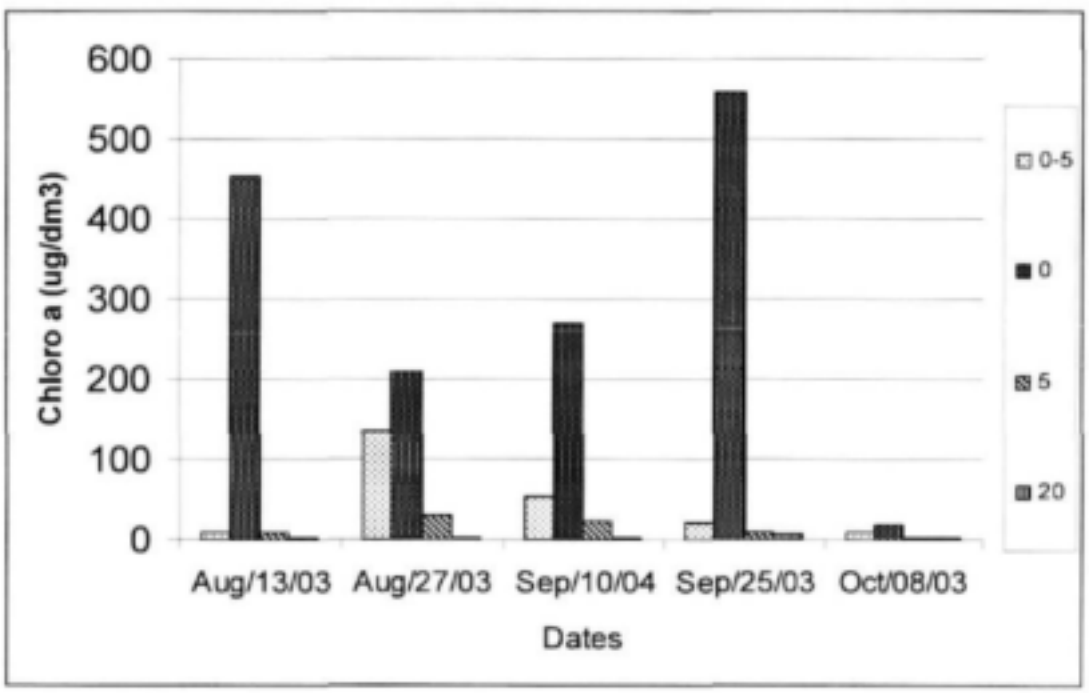


Figure 4.12 (a): Chlorophyll *a* concentration in the water column

However when values obtained in this study were compared to those obtained in earlier studies, the mean averages indicated no significant differences (Table 4.9). This suggested that the high levels of bloom formation have continued for many years until when this study was conducted.

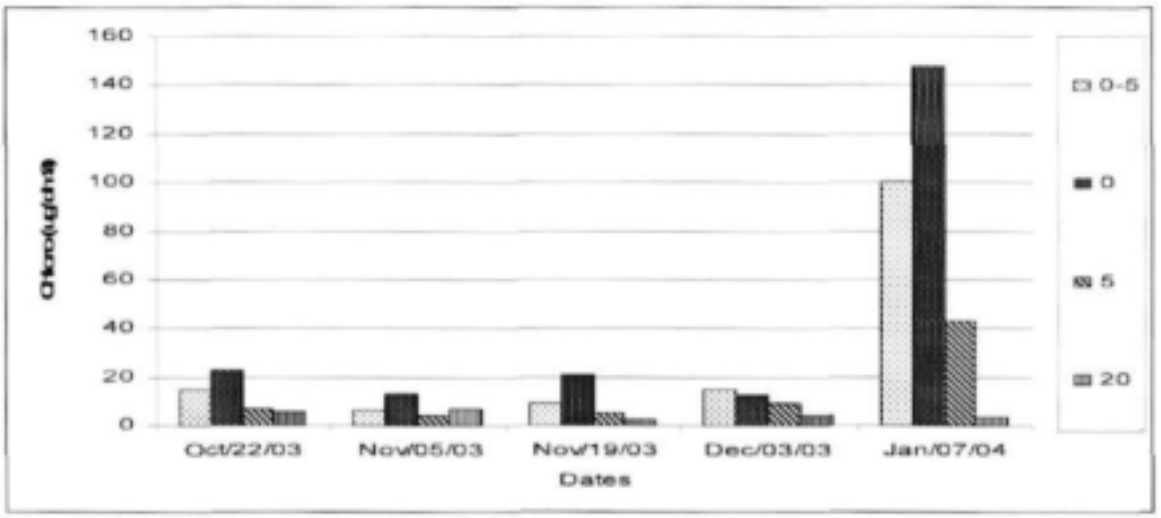


Figure 4.12 (b): Chlorophyll *a* concentration in the water column

Table 4.9: Statistical Analysis for Chlorophyll *a* concentration (0-5m)

		Mean Chlorophyll *a* concentration ($\mu g \cdot l^{-1}$) ± SD		
2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
14	12	55.78 ± 77.04	261.92 ± 158.18	Not Significant

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value.
This study (2003/2004) and Earlier studies (1990-2000)

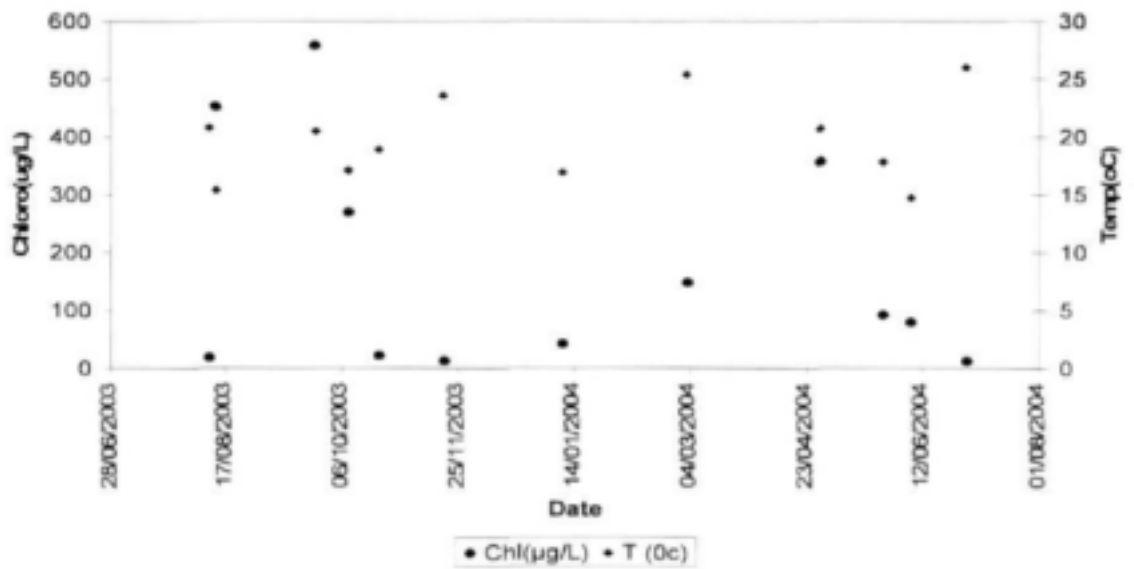


Figure 4.13: Temperature effect on Chlorophyll *a* concentration.

4.5 Algae population

Six different algal phyla were identified as major assemblage present in the dam although a number of some individual species (Figure 4.14) varied in their biovolume percentage counts seasonally in water column (Figure 4.15) and in the sediments (Figure 4.16). They were identified as:

4.5.1 Blue green algae (Cyanophytes) as shown in Figure 4.14 was represented by:

4.5.1.1 *Microcystis aeruginosa;*

The species was found in the Hartbeespoort Dam and appeared in one of two known forms: the net-shaped colonies of *Microcystis aeruginosa* forma *aeruginosa* and the spherical or lens-shaped colonies of *Microcystis aeruginosa* forma *flos aquae* (Zohary et al., 1996). At times though, relatively smaller sizes and colonies were observed in water. These two recognizable forms continued to dominate the impoundment with toxic strains, *Microcystis aeruginosa* accounting for over 80% presence in summer months (Figure 4.15). However, there was a decrease in algal count since May 2004 as compared to the year 2003. It was observed that as winter approached in April 2004, *Microcystis* were increasing their presence in the sediment (Figure 4.16). Apparently, this phenomenon started earlier in the year 2004 as opposed to June 2003. This indicated that *Microcystis* inoculums have an over wintering mechanism by moving into the sediment.

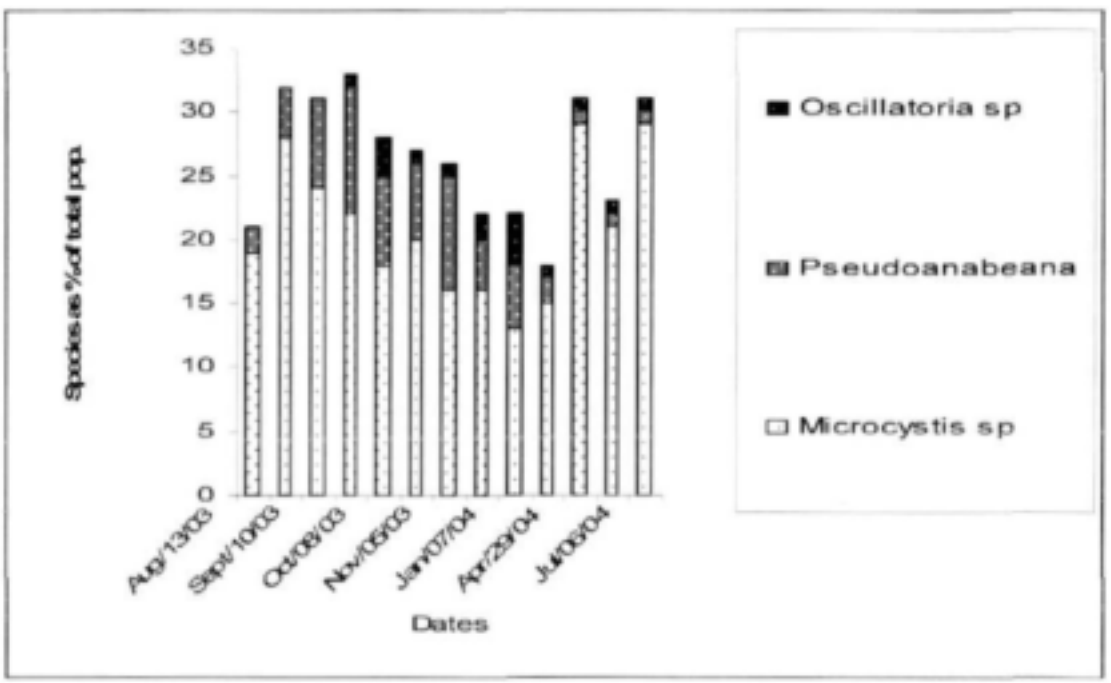


Figure 4.14: Different cyanobacterial species

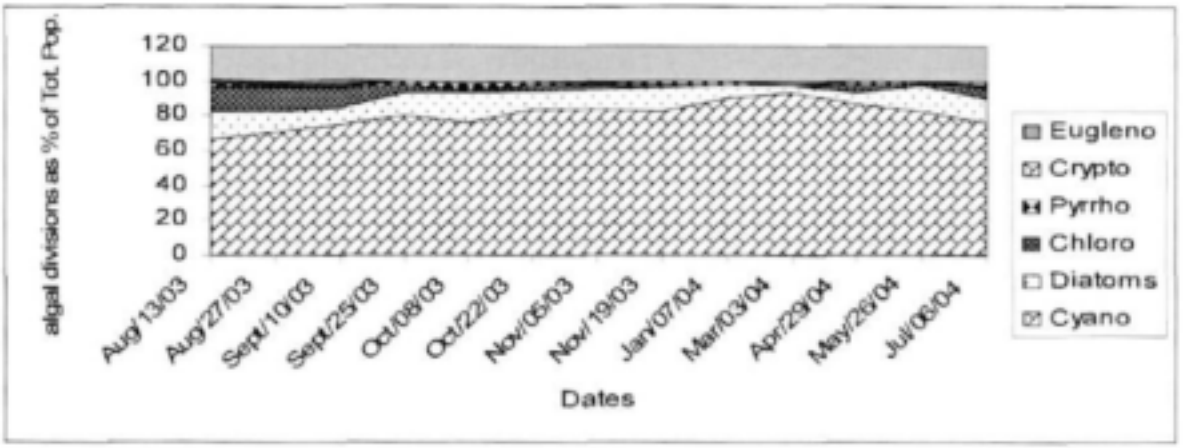


Figure 4.15: Different algal species in the water Column

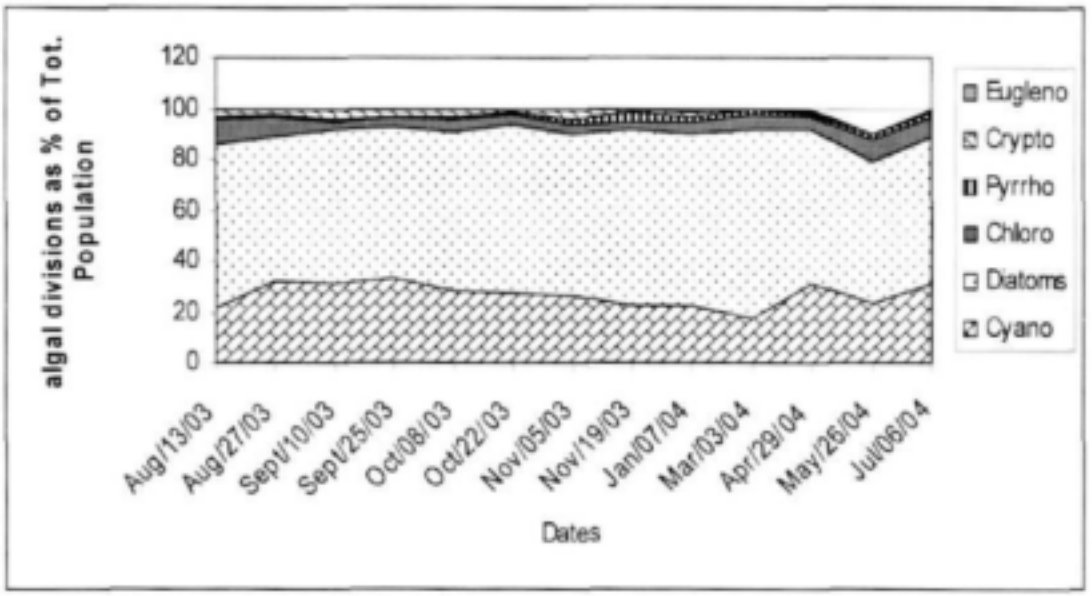


Figure 4.16: Different algal species in the sediment

The presences of numerous colonies of *Microcystis aeruginosa* were observed on the underside of immersed rocks and bricks in the shallow water along the dam bank. This phenomenon was more pronounced in the year 2003 than in 2004. However, the colonies observed appeared viable and intact under the microscope when directly observed. This confirmed Harding (2001) while citing Reynolds (1973), Tow (1979) that algae may move to the sediment, an indication of overwintering behaviour. When the *Microcystis aeruginosa* count in the water column were compared to those obtained in earlier studies through pooled mean, no significant difference (Table 4.10) was observed. And with high *Microcystis*

species count observed in the study, it is possible to attribute continued similar high levels of the phytoplankton species with assuming dominance over the years.

Table 4.10: Statistical Analysis for *Microcystis Species* as (%) in relations to other phytoplankton in water column.

		Mean (%) value ± SD		
2003/2004 (n)	1990-2000 (n)	2003/2004	1990-2000	$p^{\dagger}$
14	12	72.47 ± 5.86	79.25 ± 24.34	Not Significant

n= number of determinations, ± = Standard Deviation (SD), Pooled $p^{\dagger}$ value.
This study (2003/2004) and Earlier studies (1990-2000)

4.5.1.2 *Pseudoanabeana;*

The species was observed associated with *Microcystis* colonies and had almost uniform presence in the water column as observed in Figure 4.14. While some of the species had straight trichomes, others had slightly curved trichomes with barrel shaped cells. The species were observed more in the sediment during late winter but the number decreased as summer approached.

4.5.1.3 *Oscillatoria;*

The species was filamentous, elongated and had straight trichomes. When observed in groups, these trichomes were entangled with one another. They were always observed gliding through in the sample. They were present uniformly in the water column as observed in Figure 4.14 with few species recorded during late summer in the sediment sample. However, no species was observed towards the end of the year 2003 (Figure 4.14).

4.5.2 Chrysophyta (Yellow green algae) included:

4.5.2.1 *Melosira granulata;*

The species were also present in the water column as observed in Figure 4.15 (see Table G (i), Appendix 2). They were often the most prolific diatom species with the assuming numerical dominance in the sediment (Figure 4.16). The species had cells that were cylindrical and united into long filaments. The lengths of the cells were greater than the breath.

4.5.2.2 *Cyclotella;*

The cells were decoid, drum shaped and were either in pairs or solitary (Truter, 1987). This species was frequently present in the diatom-dominated sediment in

2003. However, no observations were made regarding the species between March and May 2004.

4.5.3 Chlorophyta (Green algae) was represented by:

4.5.3.1 *Oocystis*;

This species had cells that appeared ovoid with smooth walls. They were present in colonies with few cells. The species were greater in winter months than in summer. However, they were observed more in the water column in the year 2003 than in 2004.

4.5.3.2 *Scenedesmus*;

The species consisted of flat colonies of a few cells, which were ovoid, fusiform or oblong in shape. The single chromatophore was parietal in nature. The flat colonies were in most cases lying side by side in double rows. They were irregularly observed throughout the study period in the water column but increased presence in the sediment samples was noticed in the year 2004.

4.5.4 Crytophyta (Cryptomonads);

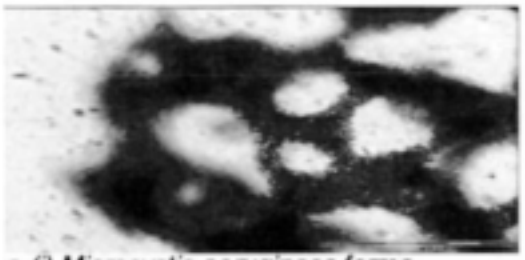
This species had cells that were oval in shape. Variedly, it had one or two parietal chloroplasts, with broader cells at the anterior end (Truter, 1987). The two flagella were unequal. Although the species were observed both in the water column (Figure 4.15) and sediment sample (Figure 4.16) they were more present in the sediment than in the water column.

4.5.5 Pyrrophyta (Dinoflagellates);

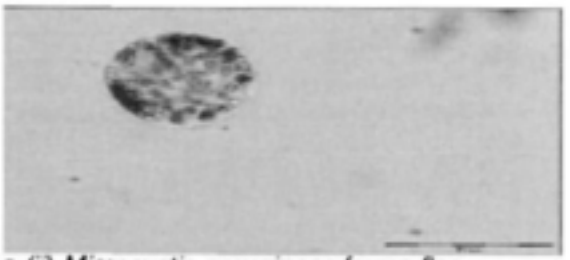
Ceratium were observed consistently throughout the 2 years in the water column and in the sediment samples. These are unicellular dinoflagellated motile algae. They had a conspicuous cell wall that bore large spines and elaborate cell-wall processes. The species is capable of sexual reproduction, but asexual reproduction occurs by the formation of aplanospores (van Ginkel et al., 2001). The algae was primarily a mid summer species and was scarce or absent in winter months (Moore 1981; Van Ginkel et al., 2001). Figures (a) up to (i) below shows some of the microscopic templates of different algal species in the dam.

4.5.6 Euglenophyta;

Euglena, a member of was not well represented and was only recorded sometimes in the water column. Rarely was this species observed in the sediment sample (Figure 4.16). The species had cells that were fusiform, cylindrical and were usually round in cross section. The periplast was either firm with fine spiral striations or rows of granules, or sometimes soft or pliable, changing shape when in movements.

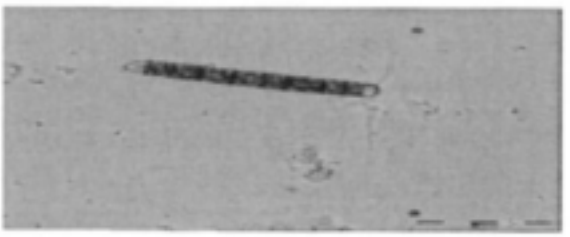


a *(i) Microcystis aeruginosa* forma *aeruginosa*

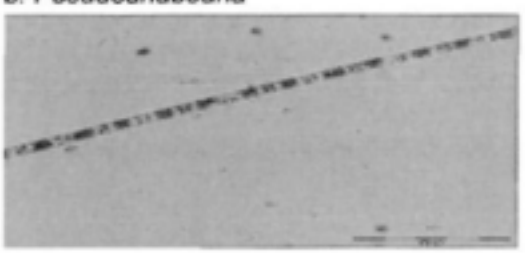


a *(ii) Microcystis aeruginosa* forma *flos aquae*

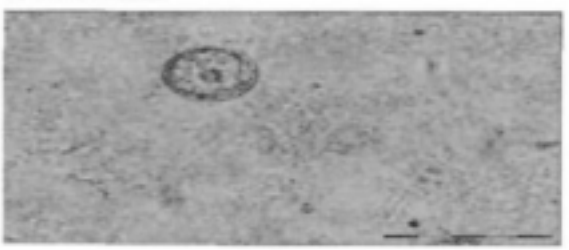
b. *Pseudoanabeana*



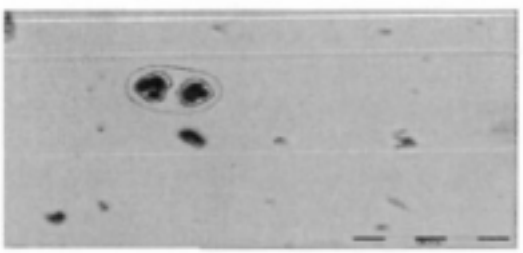
c. *Oscillatoria*



d. *Melosira granulata*

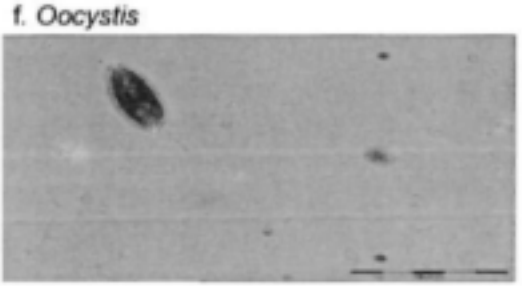


e. *Cyclotella*

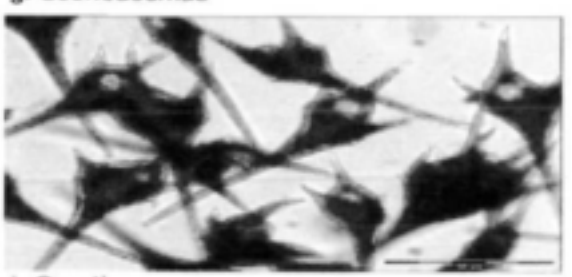


f. *Oocystis*

g. *Scenedesmus*



h. *Cryptomonas*



i. *Ceratium*

Figure 4.17 (a-i) microscopic templates of algal population in Hartbeespoort dam.

5. CONCLUSION

The data in the Tables and Figures indicated that Hartbeespoort dam is not a reservoir in a steady state between seasons i.e., winter and summer periods with constant changes in chemical and physical characteristics, some marginal at times. From this study, as shown in Table 4.6a, it can be concluded that while other parameters such as dissolved ammonia, total alkalinity and electrical conductivity, marginally changed there was a noticeable mean increase in dissolved nitrogen as nitrates (1961 µg/L) and phosphorus (73 µg/L) compared to 1155 µg/L and 48 µg/L in 2004 respectively. Furthermore, on a longer period of more than ten years, statistical analyses also revealed some significant changes. Those changes occurred in SO_4^{2-}, pH, NO_3 - NO_2, and trace metal, iron. Other parameters did not significantly change. In addition to warmer conditions experienced in the same year (14.6°C and 12.4°C minimum temperatures respectively), the year 2003 was Hartbeespoort Dam's one of the heavy bloom conditions in the current study, which continued into early 2004. It can be deduced therefore, that phosphorous, nitrogen and temperature and probably iron may have been important environmental factors that contributed to the massive bloom formation in the dam in this particular year.

Low observed oxygen values either in seasons or at various depths were due in part to very low numbers of algae or partly to the consumption of oxygen during decomposition of organic debris (Scott et al., 1979). Also noted was availability of inoculums of the cyanobacterial species in the sediment or sheltered rock surfaces, which provided favourable conditions for the blooming in the dam. An overwintering mechanism observed, therefore, ensures supply of the innoculum in this dam yearly.

There was no observable correlation between bloom conditions and the selected trace metals in this study though considerable high levels of iron metal were detected as compared to the past studies.

It is possible to assume that with the current hypertrophic conditions in the dam, trace metals may not be a trigger to bloom formation in this dam for now.

Six different algal phyla were identified as major assemblage present in the dam although a number of some individual species were sporadic. *Microcystis aeruginosa* was the dominant phytoplankton species in the Hartbeespoort Dam for most of the year. The number of *Microcystis aeruginosa* observed had lowest count in winter, 2003 (60%) and highest in summer, 2004 (86%) of the total phytoplankton biovolume in the water column. There was a remarkable variation in phytoplankton biomass in the year 2004 compared to the year 2003.

The year 2003 showed heavy algal bloom throughout the year with *Microcystis species* massively dominating the impoundment up to August. This domination was observed at different depths except in the sediment where diatoms were the

dominant species. Suitable nutrient conditions in the Hartbeespoort Dam enable *Microcystis species* to form large buoyant colonies (Harding 2001). Roberts and Zohary (1984) concluded that extremely high standing crops enable *Microcystis species* to dominate by up to 90-99% of the biovolume in the late summer. High species diversity only occurs when green algae and flagellates become more abundant in the spring. The abundance of cyanophycea demonstrated that Hartbeespoort Dam is a polluted and eutrophic system. However, this dominant position observed may not be the case with the previous study where blooms of *Microcystis* disappeared altogether and other phytoplankton species e.g. *Ceratium* emerged and dominated the impoundment for months as reported by Van Ginkel CE (2001). The same study observed that in 1998, the July to November algal population was dominated by green algae (*Oocystis* and *Coelastrum*) while the following year in 1999, the algal population was dominated by *Ceratium hirundinella*.

Water depths/columns determined quantities in algal counts with higher percentages at the epilimnion level. According to Haider et al., (2003), the *Microcystis species* posses intracellular gas vacuoles that allow them to regulate their position in the water column and hence make them buoyant. This gives them an ecological advantage allowing *Microcystis* to exploit spatial separations between nutrients and light, and provides access to atmospheric carbon dioxide when supplies in the water column are limited (Zohary, 1987). The buoyancy mechanism also allows cyanobacteria species to accumulate at the surface, thereby exploiting the moderate underwater light and effectively excluding other phytoplankton species (Robarts & Zohary, 1984).

The sizes of the colony on aggregation also varied depending on frequency and intensity of the wind (Harding, 1997). During windy conditions smaller colonies were found, as large colonies were easily fragmented (Robarts, 1984), while calm conditions promoted the formation of larger colonies (blooms) at the surface (Robarts, 1984). A similar case occurred in the dam towards the end of the year 2003. This meant that thick colonies of patches or stretches of several meters formed at various sites depending on the sheltering of the impoundment from wind direction (Plate 1). This resulted in high algal counts when quantified. Similarly, some disappeared when strong winds blew in other directions. However when calm weather prevailed, the blooms developed into crusted, buoyant mats of several centimeters thick that covered large areas. This condition if not interfered with may exist for weeks or months (Zohary, 1985). This thick mat often results in serious odours when decomposition takes place and can be felt several hundreds of meters away as witnessed in September 2003.

Figure 5.1 showed an aerial picture captured when a similar mat of decomposed algae was underway. At this stage, the process normally demands an enormous amount of dissolved oxygen rendering such water systems hypoxic, a condition of low concentration of dissolved oxygen. Dissolved oxygen in the water is essential to most organisms living in the water, such as fish and crabs. Hypoxic systems

therefore may result in death of such plants and animals. Fish kills occurred in the dam during this period as some dead fish floated on the surface of the water. This may have been as a result of either hypoxic condition or toxins in water at the time.

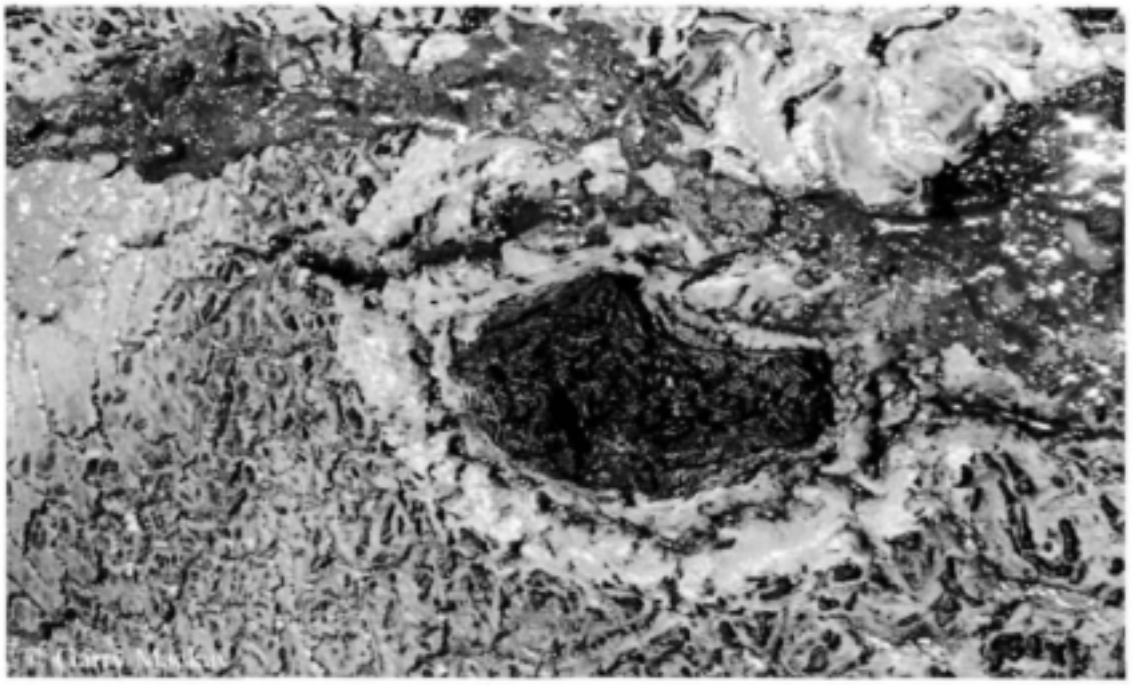


Figure 5.1: Thick, decomposing mat on the surface of the water

In the euphotic zone, chlorophyll *a*, which is a measure of phytoplankton abundance, was variable but greatest on 25/09/2003. The discrepancy between total algal counts and chlorophyll *a* concentrations can be ascribed to the presence of other less or no yielding chlorophyll *a* species like *Melosira*, resulting in high algal counts but low chlorophyll *a* values and vice versa. However, higher chlorophyll *a* concentrations always followed increased algal conditions in the dam. The study showed an over-abundance of nutrient levels in the dam over the years. Other variables such as temperature variations as observed in the year 2003 may simply act as an immediate determining environmental factor for bloom formation. Whenever, such favourable conditions are realized through constant monitoring, it may be possible to predict whether there is likelihood of cyanobacterial bloom.

6. RECOMMENDATION

This study therefore is proposing both sustained pro-active and integrated approach towards short-term physical removal. Based on the study and WHO models used in Australia (Burch, 1993), New Zealand, Brazil, Canada, the European Union, Du Preez & Van Baalen (2006) for Rand Water, the proactive approach recognises the continuous monitoring effort as currently practiced in this dam. Monitoring of the dam should therefore continue as it helps track impending bloom conditions. This triggers immediate warning signs based on risk assessment and allow relevant information to be processed to the stakeholders timeously. At the same time it prepares the relevant authorities' task with both preventative and treatment options to begin preparing early enough to combat massive blooms and public protest that this dam has attracted over decades.

There is also much more potential for community involvement in monitoring strategies, to report on the appearance of blooms and scum as this will help to promote community action and joint responsibility for the causes and cures of cyanobacterial bloom problems (Du Preez & van Baalen, 2006).

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8. APPENDIX 1: A plate

Plate 1: The bloom sparsely existing with vegetation in the dam

APPENDIX 2: TABLES OF FIELD RESULTS

TABLE A

TEMPERATURE (TEMP. ^{O}C)

Date	2/3/03	26/03/03	9/4/03	24/04/03	7/5/03	4/6/03	19/06/03	2/7/03	30/07/03	13/08/03	10/9/03	25/09/03	8/10/03	22/10/03
Depth (m)														
0	26.6	24.9	25.4	23.6	21.3	17	18.6	14.6	14.6	15.4	17.1	20.5	20.8	18.9
1	26.1	24.1	25.4	23	21.1	16.7	18.7	14.5	14.3	14.7	16.4	18	20.8	18.4
2	25.7	23.9	23.9	22.8	21.1	16.6	18.6	14.4	14.3	14.6	15.6	17.7	20.5	18
5	25.6	23.8	23.7	22.5	21	16.5	18.6	14.4	14.1	14.5	15.5	17.5	17.6	17.7
10	24.2	23.6	23.3	22.3	21	16.5	18.6	14.4	13.8	14.4	15.3	16.4	15.9	17.3
15	22.1	22.9	23.1	21.1	21	16.5	18.5	14.3	13.7	14.2	13.5	14.7	15.6	15.4
20	22.1	22.2	22.5	20.1	20.8	16.2	18.5	14.1	13.7	14	13.2	13.9	15.9	14.7
25	21.9	21.6	22	20	20.7	16.4	18.4	14.1	13.7	14	13.1	13.7	15.5	14.3

(TEMP. ^{O}C) Cont'

Date	5/11/03	19/11/03	7/1/04	3/3/04	17/03/04	29/04/04	26/05/04	6/7/04	21/07/04	1/9/04
Depth (m)										
0	21.6	23.6	26	25.4	23.3	20.7	17.8	14.7	12.4	16.9
1	21.6	23.5	25.9	25.4	22.7	20.1	17.8	14	12.4	16.9
2	21.6	23.3	25.6	25.3	22.1	20.5	17.8	13.7	12.4	16.3
5	20.2	22.5	25	25	21.9	20.5	17.8	13.7	12.3	16.2
10	17.5	18.8	23.5	22.7	21.8	20.4	17.8	13.8	12.3	13.7
15	16.4	16.9	20	21.7	21.6	20.4	17.8	13.8	12.3	13.1
20	15.6	16	17.5	20.9	20.4	20.1	17.6	13.7	12.2	12.8
25	14.9	15.6	17.1	19.3	19.8	19.7	17.3	13.7	11.9	12.6

TABLE B

DISSOLVED OXYGEN (DO mg/L)

Date	2/3/03	26/03/03	9/4/03	24/04/03	07/05/03	4/6/03	19/06/03	2/7/03	30/07/03	13/08/03	10/9/03	25/09/03	8/10/03	22/10/03
Depth (m)														
0	7.3	7.1	6.8	15	3.3	6.1	11.7	7	11	8.2	3.5	8.98	9.9	8.2
1	7.1	5	6.8	12.9	3.1	5	8.4	7	10.2	7.4	4.4	10.5	9.5	7.7
2	5	4.2	3.6	10.9	3	5	3.6	7	10	7.3	4.4	13.4	9.4	7.3
5	5	4.1	3.3	9.5	3	5	3.2	7	10	7	5.2	13	6.8	7.4
10	1	3.1	0.2	7.2	3	5	3.2	7	9.1	7	7.4	10.8	4.3	6.6
15	1	1	0.3	0.1	3	4.3	3	5.3	9	6	6.8	7.1	3.7	0.7
20	1	0.5	0.1	0	3.3	4	2.5	6	6.2	5.3	6.6	4.2	4.5	0.5
25	0.5	0.3	0.1	0	0.1	4	0.4	0.4	5.1	1	5.8	1.6	3	3.1

DISSOLVED OXYGEN (DO mg/L) Cont'

Dates	5/11/03	19/11/03	7/1/04	3/3/04	17/03/04	29/04/04	26/05/04	6/7/04	21/07/04	1/9/04
Depth (m)										
0	9	8.9	0.1	7.5	5.9	4.1	2.4	9.6	8.8	7.2
1	8.9	9	0.1	8.6	6	3.9	2.4	10	8.4	7.2
2	8.8	9	0.1	8.3	5.9	3.1	2.2	8.6	8.3	7
5	6.2	7.7	0.1	7.3	4.5	2.3	2.1	8	8	6.9
10	2.7	0.6	0.2	0.1	3.2	2.1	2	8.7	7.7	4.9
15	0.3	0.2	0.2	0.1	0.3	1.2	2	6.9	7.8	3.1
20	0.2	0.1	0.3	0	0.1	0	0.9	4.2	6.8	2.1

TABLE C: PHYSICAL AND CHEMICAL PARAMETERS OF THE HARTBEESPOORT DAM

Date	Depth(m)	pH	Total KJel-N(µg/L)	NO_3 . NO_2 Diss. (µg/L)	NH_4-N(µg/L)	T- AL.Diss (mg/L)	PO_4- Tot(µg/L)	SO_4^{-2} Diss (mg/L)	EC (ms/cm)
9/4/03	0	7.6	274	31	264	100	72	46.5	481
	5	8.7	149	66	391	101	63	53.3	482
	2	8.9	1913	71	399	103	55	51.1	484
	1	8.6	2312	26	296	103	71	50.7	479
	10	8.5	1485	40	32	106	39	52.0	482
	15	8.4	2022	37	28	112	81	55.3	489
	20	8.0	3104	321	2015	121	19	38.9	513
	0-5	8.7	1851	44	294	100	72	49.5	489
24/04/03	0-5	6.4	40	11	371	111	38	62.4	504
	0	7.7	13520	183	298	122	402	71.0	527
	1	8.0	7802	174	163	109	252	64.0	482
	2	7.8	5103	155	228	107	107	59.2	464
	5	8.3	2327	82	216	108	79	51.6	461
	10	8.6	3133	193	521	105	66	50.5	479
	15	8.3	2327	142	266	107	89	52.7	473
	20	8.3	1953	146	538	115	194	55.8	491
30/07/03	0-5	8.3	1087	1052	31	120	34	52.2	541
	0	8.0	973	994	41	114	72	56.8	540
	1	8.5	856	1185	496	110	35	51.4	544
	2	8.3	849	1081	26	114	67	58.5	539
	5	8.4	833	1031	41	119	41	50.2	538
	10	7.4	769	996	37	117	36	54.0	536
	15	8.3	1094	1069	33	109	52	63.1	535
	20	8.4	791	1047	59	119	19	54.2	535

TABLE C CONT'

8/10/03	0-5	8.7	1273	141	42	134	46	55.0	568
	0	8.0	1413	1005	41	134	33	57.9	565
	1	8.8	1824	1039	36	125	39	49.1	566
	2	8.8	1904	962	41	122	43	49.6	569
	5	8.6	1673	1173	36	124	59	46.9	572
	10	8.4	1753	1427	499	121	64	49.1	569
	15	8.4	1265	1473	215	121	72	49.0	560
	20	8.4	1436	1214	347	136	55	56.4	554
22/10/03	0-5	8.9	1148	127	38	140	29	54.4	554
	0	8.8	952	633	23	130	46	50.1	560
	1	8.4	1295	826	41	127	49	54.6	573
	2	8.7	992	728	23	131	29	56.8	569
	5	9.0	1261	793	41	126	35	60.0	572
	10	8.5	1773	922	28	126	76	54.8	563
	15	7.9	1528	1128	44	132	73	52.7	563
	20	8.1	1632	1284	214	125	129	46.7	526
3/12/03	0-5	9.0	1384	799	39	107	35	51.8	518
	0	9.1	1294	119	28	106	27	57.8	510
	1	8.9	1844	109	31	108	278	58.2	512
	2	8.4	1473	103	33	104	17	54.4	518
	5	8.4	2365	193	296	100	18	55.8	524
	10	8.9	1629	109	147	119	52	52.7	531
	15	7.8	2216	227	34	125	91	54.7	555
	20	8.8	2231	842	41	130	52	49.5	543

TABLE C CONT'

4/2/04	0-5	8.6	1687	185	273	90	39	50.4	499
	0	9.8	2523	117	48	92	41	48.4	499
	1	9.3	2293	121	29	95	28	52.5	495
	2	9.1	1852	126	265	99	32	42.8	493
	5	9.3	1428	161	26	100	37	53.5	499
	10	8.9	1078	512	51	105	63	47.7	503
	15	8.5	1563	531	62	99	127	44.4	502
	20	8.5	2671	143	769	108	136	44.8	511
3/3/04	0-5	9.3	1829	21	54	88	38	52.5	499
	0	9.2	1873	17	68	87	39	44.8	482
	1	9.5	1902	15	22	84	52	51.8	489
	2	9.2	1822	13	25	84	42	56.8	488
	5	8.8	1952	47	68	89	47	56.0	479
	10	8.5	1478	55	165	99	13	42.7	479
	15	8.0	2177	169	1175	105	157	39.5	496
	20	7.8	2877	159	1941	107	284	33.7	483
29/4/04	10	8.4	771	799	69	106	66	43.5	528
	0-5	8.7	952	863	21	104	74	44.7	514
	15	8.3	902	944	114	107	81	49.8	521
	0	8.1	1161	641	40	110	63	50.6	496
	20	8.3	1132	1489	462	116	45	54.8	506
	1	9.0	636	913	65	110	81	52.4	508
	2	8.4	782	933	83	102	89	48.0	503
	5	8.2	883	718	52	105	79	49.3	519
26/5/04	20	8.8	1229	1682	385	118	39	41.0	528
	10	8.0	597	1490	153	114	63	47.4	527
	5	8.3	759	1120	191	115	62	37.8	504
	2	7.8	862	1064	217	116	71	40.7	525
	0	7.6	2638	1318	547	116	69	44.4	546

	15	8.1	684	1386	214	112	78	42.4	543
	1	8.0	966	1383	169	113	49	41.5	519
	0-5	8.0	355	985	17	120	29	48.4	518
9/6/04	0-5	8.2	982	1633	73	119	41	54.7	529
	5	7.6	996	310	50	124	33	47.4	528
	0	7.4	1411	270	16	124	19	52.6	529
	10	8.4	702	1414	72	115	56	43.6	528
	2	8.3	731	1566	43	119	53	47.3	538
	20	7.5	1092	501	22	120	19	49.7	528
	15	8.0	662	769	29	116	66	49.2	524
	1	8.1	1318	1395	69	112	67	46.6	522
5/7/04	2	8.5	1844	1177	184	117	125	35.0	446
	5	8.7	729	1339	40	109	47	50.6	513
	0	8.2	1824	1106	55	113	39	46.4	521
	1	8.6	1339	1117	31	111	39	49.5	521
	2	8.9	1267	893	10	113	37	47.6	507
	10	8.4	1288	1286	62	110	58	44.4	523
	15	8.4	1269	1228	40	114	44	47.7	530
	0-5	7.7	1484	1019	46	114	46	48.6	523
	20	8.5	419	1382	259	111	30	49.1	514
21/7/04	1	8.6	551	1244	37	113	26	47.4	552
	10	8.3	688	1397	71	110	41	43.8	546
	0	8.7	1491	1522	530	111	28	53.9	531
	0-5	8.3	1338	1482	22	112	25	49.8	528
	2	8.5	599	1554	21	115	32	44.0	560
	15	8.2	865	1549	73	114	29	45.9	554
	20	8.3	922	1748	35	115	19	52.1	529
	5	8.5	1062	1376	28	117	28	43.9	528
4/8/04	0-5	8.4	977	1509	204	117	40	46.4	539
	1	8.4	1227	1726	69	117	27	55.4	528

	2	8.2	417	1550	131	120	28	54.3	554
	15	7.7	622	1294	202	114	27	60.0	553
	10	8.4	1558	1617	133	120	26	56.7	561
	20	8.3	1385	1885	164	118	35	58.2	557
	0	8.6	866	1811	271	117	32	55.3	535
	5	7.8	2163	1529	143	125	26	54.5	529
1/9/04	0-5	8.3	845	2044	252	125	29	48.5	573
	1	8.2	1262	290	303	115	32	59.0	557
	15	8.2	1354	1277	528	111	31	55.0	546
	2	8.3	1279	2764	305	115	38	56.5	569
	5	8.4	931	273	199	111	34	57.8	564
	10	8.3	968	249	281	113	29	56.5	553
	0	8.4	881	1577	244	116	31	56.1	561
15/9/04	10	7.7	1448	1652	299	123	35	49.4	569
	0	8.1	1244	1855	153	121	59	54.4	580
	15	8.3	1093	1463	398	125	46	50.0	554
	0-5	7.7	1481	2040	210	118	39	57.6	569
	1	8.4	703	2055	186	129	57	47.9	572
	20	8.2	1369	1582	436	124	39	52.7	550
	2	8.3	884	2328	132	123	71	48.8	581
	5	8.1	529	2177	178	124	43	49.4	565
29/9/04	0	8.6	881	2277	248	126	38	45.6	572
	10	8.2	769	2461	290	122	34	51.6	570
	1	8.2	608	2042	236	124	41	54.6	572
	15	8.8	711	1533	441	124	43	52.4	565
	0-5	8.1	992	2369	262	128	47	54.2	572
	5	8.3	727	1974	219	123	39	55.4	570
	20	8.0	1274	1642	490	127	41	47.5	561
	2	8.4	779	2211	284	124	44	53.6	571
13/10/04	2	8.3	571	1994	141	123	34	58.4	572

	15	7.9	1611	1744	269	128	44	56.5	568
	10	8.4	1402	2411	216	130	43	55.5	566
	5	8.5	783	2283	201	124	56	62.0	570
	0	7.9	992	2316	184	130	39	47.9	579
	0-5	8.2	913	2166	136	127	26	52.7	581
	1	8.4	1257	2162	098	129	35	60.7	574
	20	8.2	1068	392	496	132	52	50.6	552
27/10/04	2	8.6	1011	2277	22	131	34	52.7	578
	5	8.1	996	2381	43	131	34	55.9	580
	0	8.3	629	1966	51	124	22	53.6	587
	15	8.4	803	2180	356	130	45	58.9	583
	10	8.1	691	2174	53	129	36	54.4	577
	0-5	8.7	922	1620	70	124	25	55.2	575
	20	8.0	885	1644	448	127	49	56.1	581
	1	8.5	841	2352	19	130	29	55.7	586
10/11/04	10	8.1	993	1721	255	131	49	47.0	587
	0-5	8.8	1255	1799	72	118	26	49.8	565
	1	8.5	1514	1717	40	124	27	54.0	569
	2	8.8	795	1759	39	122	25	52.9	566
	0	8.5	615	1584	21	123	27	54.7	579
	20	8.2	1409	1371	715	138	81	55.6	575
	15	7.7	1039	1831	319	134	66	51.7	569
	5	8.7	979	1677	38	122	51	51.5	553

TABLE D (DWAF, 2005) – Early studies

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-90	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Feb-90	0-5	8.2	1.28	1.58	0.04	98.5	0.01	85.1	574
Mar-90	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Apr-90	0-5	8.6	0.77	0.04	0.04	85.7	0.01	78.7	544
May-90	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Jun-90	0-5	8.7	0.86	2.27	0.04	109.1	0.01	83.9	591
Jul-90	0-5	8.6	0.95	2.24	0.04	109.8	0.01	86.5	611
Aug-90	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Sep-90	0-5	8.6	0.88	0.94	0.04	96.3	0.01	87.7	582
Oct-90	0-5	8.9	1.31	1.41	0.08	109.9	0.01	87.3	598
Nov-90	0-5	8.8	1.16	1.04	0.07	106.4	0.01	87.4	590
Dec-90	0-5	9.2	1.26	0.8	0.07	104	0.01	84.9	595

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-92	0-5	8.0	1.75	0.67	0.08	98.4	0.01	88.9	588
Feb-92	0-5	8.7	1.2	0.62	0.06	100.7	0.02	85.6	587
Mar-92	0-5	8.4	1.01	0.63	0.11	111.2	0.03	82.2	594
Apr-92	0-5	8.2	1.05	0.77	0.1	114.0	0.01	85.4	611
May-92	0-5	8.0	0.98	1.15	0.05	119.5	0.01	90	618
Jun-92	0-5	8.4	0.98	1.35	0.06	115	0.01	90.1	649
Jul-92	0-5	8.3	1.3	1.52	0.17	121.4	0.02	92.4	648
Aug-92	0-5	8.6	1.23	1.76	0.12	130.6	0.01	93	667
Sep-92	0-5	9.1	1.44	1.61	0.08	135.8	0.02	88.7	680
Oct-92	0-5	8.9	1.55	1.11	0.16	133.6	0.02	100.0	687
Nov-92	0-5	8.6	1.82	1.06	0.34	135.4	0.14	92.0	682
Dec-92	0-5	9.3	1.73	1.13	0.08	115.3	0.09	84.7	613

TABLE *D cont'*

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-94	0-5	8.6	1.6	1.91	0.27	93.4	0.06	60.8	501
Feb-94	0-5	8.9	2.51	1.31	0.09	96.7	0.06	64	473
Mar-94	0-5	9.1	1.87	0.58	0.1	92.3	0.04	62	444
Apr-94	0-5	8.5	1.51	0.8	0.37	103.6	0.08	72.5	451
May-94	0-5	8.2	1.01	1.29	0.07	107.1	0.05	64.7	451
Jun-94	0-5	8.1	0.86	1.35	0.09	110.2	0.06	67.8	476
Jul-94	0-5	8.3	1.21	1.65	0.16	118.4	0.06	71.1	510
Aug-94	0-5	8.5	1.24	1.64	0.04	120.2	0.03	76.8	515
Sep-94	0-5	8.9	1.43	1.43	0.05	107.7	0.02	69.2	522
Oct-94	0-5	8.6	1.53	1.18	0.09	107.2	0.02	69.2	496
Nov-94	0-5	8.4	0.89	1.44	0.09	110.2	0.02	73.2	562
Dec-94	0-5	8.5	1.37	1.43	0.19	101.2	0.02	74.4	488

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-95	0-5	8.4	1.21	1.21	0.04	90.3	0.02	68.7	496
Feb-95	0-5	9.5	1.74	0.61	0.06	75.2	0.03	69.5	428
Mar-95	0-5	8.7	1.26	0.9	0.10	88.2	0.03	68.6	463
Apr-95	0-5	8.5	1.63	1.3	0.11	86.1	0.03	69.5	456
May-95	0-5	8.2	1.12	2.02	0.05	88.7	0.04	75.3	493
Jun-95	0-5	8.0	0.94	2.10	0.04	99.2	0.02	67	492
Jul-95	0-5	8.1	1.35	2.44	0.13	102.6	0.02	65.6	490
Aug-95	0-5	8.5	1.11	2.72	0.10	104.1	0.03	64.6	514
Sep-95	0-5	8.7	0.96	2.53	0.05	101.4	0.03	72.4	520
Oct-95	0-5	8.5	1.09	1.09	0.07	94.6	0.02	69	526
Nov-95	0-5	8.5	1.21	2.17	0.04	99.7	0.03	70.8	554

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-96	0-5	9.0	1.51	1.07	0.04	79.1	0.01	68.8	434
Feb-96	0-5	8.0	1.50	1.17	0.04	83.6	0.06	57	468
Mar-96	0-5	9.2	2.03	1.13	0.04	96.6	0.04	55.6	444
Apr-96	0-5	8.5	2.52	1.3	0.04	110.6	0.09	60.9	476
May-96	0-5	8.2	1.26	1.33	0.04	92.6	0.08	41.2	401
Jun-96	0-5	8.5	1.05	2.37	0.04	121.2	0.05	58.2	522
Jul-96	0-5	8.6	0.9	2.4	0.08	125.5	0.03	55.2	563
Aug-96	0-5	8.7	1.27	2.56	0.09	132.1	0.07	49.3	536
Sep-96	0-5	8.7	1.62	2.74	0.04	139.4	0.02	62.8	583
Oct-96	0-5	8.6	1.29	2.39	0.04	116.7	0.04	62.7	528
Nov-96	0-5	8.5	1.45	1.91	0.04	121.7	0.03	67.3	533
Dec-96	0-5	8.4	1.21	1.36	0.04	121.3	0.01	70.2	563

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-97	0-5	8.8	1.01	1.06	0.06	95.8	0.01	61.1	420
Feb-97	0-5	9.1	2.02	0.74	0.08	84.1	0.02	58.1	469
Mar-97	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Apr-97	0-5	No data	No data	No data	No data	No data	No data	No data	No data
May-97	0-5	No data	No data	No data	No data	No data	No data	No data	No data
Jun-97	0-5	8.4	0.9	2.41	0.12	113.1	0.04	50.4	496
Jul-97	0-5	8.8	0.48	2.77	0.06	121.9	0.03	56.6	472
Aug-97	0-5	8.9	1.09	2.81	0.08	128.3	0.02	65.7	521
Sep-97	0-5	8.6	0.84	2.91	0.12	141	0.03	63.3	546
Oct-97	0-5	8.7	0.91	2.54	0.12	128.6	0.02	56.1	516
Nov-97	0-5	8.8	1.0	2.39	0.06	133.2	0.02	59.2	536
Dec-97	0-5	8.7	1.08	2.19	0.05	124.5	0.02	61.8	500

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-98	0-5	8.8	1.09	1.8	0.05	101.4	0.02	56.0	507
Feb-98	0-5	8.7	1.32	0.96	0.04	92.2	0.02	59.8	446
Mar-98	0-5	8.4	1.67	0.42	0.04	96.9	0.02	58.9	446
Apr-98	0-5	8.9	1.50	0.22	0.04	88.8	0.02	57.0	470
May-98	0-5	7.8	1.12	0.65	0.04	104.9	0.01	60.7	491
Jun-98	0-5	8.3	1.11	1.18	0.08	114.7	0.03	60.2	490
Jul-98	0-5	8.3	1.01	1.6	0.01	126.3	0.03	60.4	453
Aug-98	0-5	8.2	1.14	1.89	0.12	122.2	0.07	66.4	518
Sep-98	0-5	8.6	0.97	1.86	0.04	118.4	0.02	61.1	480
Oct-98	0-5	8.3	1.25	1.65	0.10	113.2	0.01	62.2	553
Nov-98	0-5	8.4	0.91	1.92	0.10	115.3	0.02	65.4	546
Dec-98	0-5	8.4	1.09	1.92	0.04	113.3	0.03	61.4	528

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-99	0-5	8.6	1.33	1.42	0.04	102.9	0.04	59.8	476
Feb-99	0-5	9.0	1.05	0.34	0.04	79.3	0.03	59.2	435
Mar-99	0-5	9.2	1.14	0.06	0.04	83.8	0.04	58.2	441
Apr-99	0-5	8.9	1.67	0.07	0.38	91.0	0.05	62.3	454
May-99	0-5	8.3	0.90	0.90	0.09	98.6	0.03	58.4	484
Jun-99	0-5	8.0	0.85	1.63	0.05	109.1	0.03	51.6	507
Jul-99	0-5	8.4	0.72	1.64	0.06	112.6	0.02	59.0	522
Aug-99	0-5	8.4	1.41	1.71	0.12	115.5	0.05	58.7	538
Sep-99	0-5	8.0	2.06	1.34	0.04	107.8	0.03	65.8	538
Oct-99	0-5	8.1	2.90	0.58	0.44	109.5	0.07	65.1	537
Nov-99	0-5	8.4	2.05	1.3	0.09	111.6	0.08	61.5	554
Dec-99	0-5	8.9	1.32	0.9	0.04	99.2	0.02	64.1	533

TABLE D cont'

DATES	Depth (m)	pH	KJEL(mg/L)	$NO_3 - NO_2$ (mg/L)	NH_4-N (mg/L)	T-AL (mg/L)	PO_4-TOT (mg/L)	SO_4^{-2} (mg/L)	EC (ms/cm)
Jan-00	0-5	8.3	1.09	0.88	0.04	90.3	0.02	61.5	503
Feb-00	0-5	8.1	1.20	1.21	0.04	87.1	0.12	50.5	403
Mar-00	0-5	8.4	1.0	0.58	0.04	102.3	0.03	48.8	422
Apr-00	0-5	8.0	1.41	1.63	0.04	111.5	0.02	50.6	459
May-00	0-5	8.2	0.63	2.33	0.12	122.0	0.07	44.7	493
Jun-00	0-5	8.0	0.82	2.30	0.04	134.0	0.02	52.2	512
Jul-00	0-5	8.4	0.60	2.55	0.25	142.6	0.08	48.4	528
Aug-00	0-5	8.3	2.14	2.32	0.04	133.6	0.03	53.4	521
Sep-00	0-5	8.3	1.17	2.05	0.04	133.6	0.01	55.7	522
Oct-00	0-5	7.8	1.53	1.80	0.04	126.5	0.04	59.6	493
Nov-00	0-5	8.5	1.79	1.68	0.21	121.5	0.08	51.6	488
Dec-00	0-5	8.5	2.27	0.97	0.20	109.3	0.07	48.5	446

TABLE E

HARTBEESPOORT DAM. TRACE METALS (MONTHLY VALUES)

Dates	Iron (µg/L)	Manganese (µg /L)	Zinc (µg/L)	Copper (µg/L)	Lead (µg/L)
03/03/2004	86	62	72	45	49
27/04/2004	78	91	74	53	42
26/05/2004	93	34	86	69	53
06/07/2004	90	75	84	60	53
04/08/2004	82	53	71	58	44
01/09/2004	87	49	77	55	44
13/10/2004	94	72	84	59	46
10/11/2004	84	48	76	52	49
12/01/2005	86	57	79	54	48
09/02/2005	80	49	73	52	49
09/03/2005	84	63	76	56	44
11/04/2005	85	55	81	53	47

TABLE: F

CHLOROPHYLL a (µg/L) CONTENT

Dates	13/08/03	27/08/03	10/9/03	25/09/03	8/10/03	22/10/03	05/11/03	19/11/03	3/12/03	7/1/04	3/3/04	29/04/04	26/05/04	06/07/04	1/9/04
Secchi Depth	2.7	No data	2.9	2.8	3.3	2.7	2	2.6	No data	0.6	0.6	1.5	1.8	0.9	4.7
0-5	10.4	134.5	54	21.6	9.4	14.7	6.5	8.8	14.5	100.3	289.6	34.6	38.7	43.3	
0	452.6	209.4	269.8	558.1	18.7	22.5	13	20.6	12.6	147.8	357	91.8	79.4	42.1	
5	8.44	29.7	23.2	8.84	2	7	3.9	5	9	43.1	274.5	21.7	26.7	11.4	
20	2.01	3.4	2.2	6.43	1.3	5.4	6.6	2.3	3.7	3.7	19.1	8	13.6	3.6	

TABLE G (i)

PHYTOPLANKTONS POPULATION EXPRESSED AS PERCENTAGES (%) IN THE WATER COLUMN

Date	*Microcystis sp*	*Pseudoanabaena sp*	*Oscillatoria sp*	*Melosira granulata*	*Cyclotella sp*	*Oocytis sp*	*Scenedesmus sp*	*Cryptomonas sp*	*Ceratium sp*	*Euglena sp*
13/08/03	62	2	2	16	1	12	2	1	3	1
27/08/03	69	1	1	10	1	13	1	2	1	1
10/09/03	70	2	3	8	2	11	-	1	2	2
25/09/03	74	1	6	11	2	4	1	-	2	-
08/10/03	67	4	5	13	4	1	-	-	5	-
22/10/03	70	6	8	6	4	1	2	-	3	-
05/11/03	70	6	8	8	3	1	2	1	1	-
19/11/03	74	3	6	8	5	2	-	-	2	-
07/01/04	86	4	-	3	5	-	-	-	2	-
03/03/04	83	4	6	3	1	-	-	-	1	2
29/04/04	79	3	5	5	1	4	-	-	3	-
26/05/04	72	7	4	11	3	2	-	-	1	-
06/07/04	67	6	3	14	-	2	4	3	1	-
04/08/04	70	4	2	15	-	2	3	3	2	-
01/09/04	74	2	2	11	2	2	4	1	1	1
13/10/04	72	4	3	10	3	1	3	2	2	-
10/11/04	73	4	4	7	2	3	1	2	3	1

TABLE G (ii)

PHYTOPLANKTONS POPULATION EXPRESSED AS PERCENTAGES (%) IN THE SEDIMENT SAMPLE

Date	*Microcystis sp*	*Pseudoanabeana sp*	*Oscillatoria sp*	*Melosira granulata*	*Cyclotella sp*	*Oocytis sp*	*Scenedesmus sp*	*Cryptomonas sp*	*Ceratium sp*	*Euglena sp*
13/08/03	19	2	-	64	1	6	4	3	1	-
27/08/03	28	4	-	56	1	6	2	2	1	-
10/09/03	24	7	-	60	1	2	1	4	1	-
25/09/03	22	10	1	57	3	4	-	3	-	-
08/10/03	18	7	3	61	2	4	1	3	1	-
22/10/03	20	6	1	64	3	3	1	1	1	-
05/11/03	16	9	1	60	4	3	1	4	2	-
19/11/03	16	4	2	67	3	1	2	1	4	-
07/01/04	13	5	4	65	3	3	2	2	2	1
03/03/04	15	2	1	74	-	2	4	0	2	-
29/04/04	29	1	1	61	-	1	4	1	1	1
26/05/04	21	1	1	56	-	3	6	-	2	-
06/07/04	29	1	1	57	1	2	6	1	2	-
04/08/04	28	3	2	59	-	3	4	1	-	-
01/09/04	27	5	1	60	1	2	2	1	1	-
13/10/04	23	5	2	59	2	3	1	3	1	1
10/11/04	25	4	3	57	4	2	2	2	1	-