CYANOBACTERIA IN SOUTH AFRICA: A REVIEW

REPORT TO THE
WATER RESEARCH COMMISSION

By

William (Bill) Harding and Bruce Paxton

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Cover illustration:

Background image: *Anabaena solitaria* bloom at Theewaterskloof (Villiersdorp, Western Cape, December 1993); Insert: Shoreline scum of *Microcystis aeruginosa* (Malmesbury District, Western Cape, April 1994); Chemical structure: Microcystin-LR (heptapeptide).
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AUTHOR'S OVERVIEW

Cyanobacteria enjoy a notorious reputation. Blooms rapidly emerge from apparent obscurity, wreaking havoc on water quality and ecosystem integrity, and often recede abruptly. Some waters exhibit frequent and even sustained aggregations of these algae, while others experience evanescent, but often extremely noxious, growth events. Seriously toxic incidents that evoke public and occasionally governmental attention are rare. None has received international coverage via non-scientific media. The events of the 1989 summer in the United Kingdom, the sustained blooms in the Australian Darling-Barwon River system during the early summer of 1991, and the South African Tsitsikamma-Kareedouw poisoning of an entire dairy herd in 1996 were, however, catapulted to the national attention of the affected countries. Surprisingly, the cyanobacterial-related deaths of dozens of dialysis patients in Caruaru, Brazil during 1996 were not regarded as being internationally newsworthy. While there are, undoubtedly, many cases of sustained algal growths that result in persistent and often worrying problems, these remain largely hidden in the records of water quality managers and potable supply utilities. It is probable that numerous cases from the agricultural and rural sectors of many countries have gone unnoticed, and the noxious effects undiagnosed.

While the sporadic nature of cyanobacterial blooms is a blessing in that associated incidents of toxicosis are relatively few, the perceived low intensity of toxic events gives an illusion of the problem being too transitory to warrant any serious or sustained input in terms of funding.

In developing countries, such as South Africa, resistance to the provision of funding for cyanobacterial research is often based on the argument that there are far greater health problems and that funding needs to be directed to the alleviation of diseases such as HIV-AIDS and tuberculosis. While this is inarguably legitimate, the threats posed by cyanobacteria are also very real and are a consequence of the insidious and burgeoning problem caused by the eutrophication of scarce water resources. This renders selection of the "do nothing" option invalid.

Globally, eutrophication receives second-rate status in developing countries, not least in South Africa. To illustrate, the simple and effective implementation of phosphorus stripping from wastewater effluents continues to be ignored on the basis of perceived high capital and operating costs, but to the detriment of the long-term cost to the environment and to

society as a whole. This is compounded by the harsh reality that South Africa suffers from a dearth of suitably trained and experienced professionals in the disciplines of limnology and river ecology who can provide meaningful support to water quality managers. Despite being an arid country with identified and serious future limitations for water quality and quantity, South Africa lacks tertiary institutions that offer limnology or river ecology, or even algology, as formal major subject training courses. Out of 26 final year (2000) zoology undergraduate students attending a final year semester course in freshwater ecology at a leading South African university, none expressed the intention to pursue a career in the limnological or (aquatic) ecological sciences. This is a damning indictment for the future of aquatic ecosystem management in this country.

Notwithstanding the best intentions of integrated catchment management philosophy and policy, the problem of eutrophication, with its wide-ranging associated evils, is unlikely to be meaningfully resolved in the short-to-medium term. Cyanobacterial blooms and toxin production constitute a subset of this greater problem. In the absence of deliberate eutrophication management, the reality is that increasing numbers of a given population will be exposed to waters containing cyanobacterial metabolites that pose very real, acute and chronic implications for their health, and especially for those whose physical condition may already be compromised. The risk increases exponentially as one moves from the relative safety provided by tertiary water treatment in the cities, to rural communities or areas serviced by less-sophisticated water treatment technologies.

A somewhat pessimistic implication is that we will have to learn to live with the impacts of unattenuated pollution and eutrophication, and rely on water treatment for the protection of our health in areas where the impoundments are eutrophic. However, this constitutes an “end-of-pipe” approach that precludes the greater portion of the South African population from this protection.

While South Africa no longer enjoys the scientific prominence in cyanobacterial research that it held during the 1980s, the continued development of cyanobacterial understanding constitutes a crucial need. Efforts to ensure prevention of excessive cyanobacterial growth should underpin practical research efforts and the primary allocation of funding. Management of the problem must now concentrate objectively on mitigating the underlying causes of eutrophication and degradation of aquatic ecosystems, supported in parallel by investigations of regional and local cyanobacterial demographics and incidence of toxicosis. Examples here

would be the geographic expansion of genera such as *Oscillatoria*, and the appearance of hitherto rare cyanobacteria such as *Cylindrospermopsis*. Expansion of research into the realms of the molecular pathways driving toxin production, and the sourcing and provision of funding for this purpose, might well be more pragmatically served through the persuance of collaborative international research links with centres of excellence that have established proven track records in this regard. There are relatively few funded programmes dealing with issues pertaining to cyanobacteria in the freshwater environment, with the consequence that huge benefits stand to be gained from aggregate efforts.

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HOW TO USE THIS DOCUMENT

Of necessity a review document makes multiple references to the published and unpublished (grey literature) work of researchers. In many cases, articles cite the work of others in drawing the basis for their arguments, or to substantiate their findings. The documenting of reference material has been dealt with in three ways in this document:

- Firstly, a bibliography of Suggested Reading is provided. As this review concentrates on South Africa in particular, this section contains references to books and publications that will provide additional in-depth understanding of the topic at hand;

- Secondly, a section of Supporting Literature, which, for specific sections of the review, lists important references, cited by South African researchers in their work. Such sections are clearly marked with a "see Supporting Literature" flag;

- Finally, a bibliography of references used for the compilation of this review.

Certain references may be duplicated in the above, or the reader may need to consult more than one thereof in order to locate the details for a publication.

1. INTRODUCTION

1.1 The cyanobacteria

Cyanobacteria (aka "blue-green algae") are one of the earth's most ancient life forms. Evidence of their existence on earth, derived from fossil records, encompasses a period of some 3.5 billion years. They are considered to have been the first photosynthetic organisms and, consequently, as having contributed to the generation of oxygen in the earth's atmosphere. These organisms are not true algae at all, but rather ancient bacteria belonging to the procaryotic groupings of organisms. The common name of "blue-green algae" is derived from the colouration (hence "cyan") that many of this algal division exhibit. They do not occur only in marine and freshwater environments, but also in a variety of habitats ranging from hot springs to beneath sandstone layers in the Golden Gate National Park, and artificially-illuminated cave systems such as the Cango Caves near Oudtshoorn, South Africa, to ice layers in the polar regions.

The cyanobacteria are entirely cosmopolitan organisms, species of which may commonly occur in relatively small numbers in most aquatic environments. However, during times of environmental imbalance, usually caused by eutrophication, cyanobacteria tend to gain a selective advantage and out-develop the remainder of the algal assemblage in terms of numbers and biovolume. The result is a phenomenon referred to as a "bloom" of cyanobacteria, more correctly described as dense aggregations of cells that are sometimes buoyant, float to the surface and form thick, paint-like scums on the downwind shores of lakes and ponds, or along the banks of rivers. In cases where toxins are present, it is these scums of algal cells that pose the greatest risk to animals, as well as to humans who may come into contact with the material (see below).

The most alarming characteristic of the cyanobacteria is the ability of many genera and species to produce a range of extremely potent biological toxins. Toxins, encompassing hepato- and neurotoxic secondary metabolites, are most commonly known from the cyanobacterial genera *Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, Nodularia, Nostoc* and *Oscillatoria* (*Planktothrix*). The majority of poisoning incidents are the result of acute or sub-acute liver toxicity caused by the largest group of toxins known as the microcystins. In general, the toxins are produced in a random and unpredictable fashion, and pose a significant threat in terms of acute and chronic health risks to both animals and humans. In addition, certain genera and species produce taste and odour compounds, typically geosmin

and 2-methyl isoborneol, which cause non-hazardous but unpleasant problems for suppliers and users of potable water.

1.2 The first scientific links between cyanobacteria and toxicity

Although relatively sound descriptions of cyanobacterial "blooms" exist from as far back as the 12th century, the first conclusive demonstration of and links to toxicity in this group of organisms originated from Australia during the late 1800s. During 1878, George Francis, a chemist from the town of Adelaide, reported the poisoning of animals around Lake Alexandrina in Australia. By feeding a calf with algal scum from the surface of the lake, he was able to determine that it was the alga (*Nodularia spumigena*) in the lake that had caused the deaths.

This quaint description of a toxic algal bloom by Francis (1878) is cited in Soll and Williams (1985):

'A conferva that is indigenous and confined to the lakes has been produced in excessive quantities, so much as to render the water unwholesome. It is, I believe Nodularia spumigena, allied to Protococcus. Being very light, it floats on the water, except during breezes when it becomes diffused. Thus floating, it is wafted to the lee shores, and forming a thick scum like green oil paint, some two to six inches thick and as thick and pasty as porridge, it is swallowed by cattle when drinking, especially such as suck their drink at the surface like horses. This acts poisonously and rapidly causes death.'

2. OBJECTIVES AND TERMS OF REFERENCE

2.1 Background

Concerns within the South African aquatic science community, pertaining to cyanobacteria, were heightened by the 1996 incident of massive stock loss in the Tsitsikamma-Kareedouw district of the southern Cape (Harding, 1997a). A resolution passed by the 1997 Annual General Meeting of the Southern African Society of Aquatic Scientists (SASAQS) led to the submission of recommendations calling for heightened attention to the problems posed by cyanobacteria in South Africa, to the national departments of Water Affairs and Forestry, Agriculture, Health and Welfare, and Environmental Affairs and Tourism.

Subsequently, during 1998, a group of concerned managers and scientists, led by Dr Machiel Steynberg of Rand Water, convened the Toxic Algal Forum.

2.2 Objectives of this review

During 1999, the Water Research Commission (WRC) convened two workshops at the Rhoodevallei Conference Centre in Pretoria. The first placed the problems facing South Africa in both international and local perspective. Professor Geoff Codd of the Department of Biological Sciences, Dundee University, Scotland attended this meeting as international specialist in the field of cyanobacteria and their associated toxins. The workshop resolved that a review of the South African experiences relating to cyanobacteria was necessary in order to be able to meaningfully evaluate new research proposals and needs. This was supported at a second workshop dealing with the assessment of research needs pertaining to cyanobacteria.

2.3 Terms of Reference

This review was tasked with drawing together relevant information pertaining to the history of cyanobacterial occurrence, associated toxicity, poisonings, taste and odour generation, research and management in South Africa during the 20th century to December 1999. The review proposal included the need to document key international linkages, and relevant photographic material.

3. OVERVIEW OF THE CYANOBACTERIA AND THEIR TOXINS

As indicated above, the purpose of this review is to detail the South African experiences of cyanobacteria, and the contributions made by South African researchers to the understanding of these organisms. Accordingly, while this short summary of cyanobacteria and their toxins is included to aid readers who may be unfamiliar with the topic, it is by no means intended as an exhaustive review. The reader is referred to the many excellent texts and summaries listed in the section on Recommended Reading. The publication of the World Health Organization (WHO) entitled "*Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*" (WHO, 1999) is strongly recommended as essential reading for anyone wishing to have a detailed background to the topic. As a supportive text, "*The Ecology of Cyanobacteria: Their Diversity in Time and Space*" (Whitton and Potts, eds., 2000) provides an excellent, albeit disappointingly already out of date with respect to toxins, overview of the cyanobacteria. Texts and reports identified in the unpublished "grey literature" are referenced in this document, but have not been included in the section on Recommended Reading.

3.1 Factors leading to cyanobacterial dominance ("blooms")

The information provided below is drawn from arid climate experience and, accordingly, may differ from the understanding of similar factors in the north-temperate zones. It must also be stressed that factors that may influence the growth and development of one alga over another do not act singly, but rather in concert with a constantly changing suite of parameters – the combination of which determines the outcome of a successional phase of algal growth. It is only when a single parameter becomes limiting that it begins to exert definitive control.

3.1.1 Physical factors

Temperature

As water temperatures increase, algal successional patterns typically follow a progression from diatoms through chlorophytes to cyanobacteria (Robarts and Zohary, 1987). While cyanobacterial dominance usually occurs at temperatures in excess of 20°C, different genera have wide ranges of temperature extremes. The genus most commonly associated with problems relating to toxin production, namely *Microcystis*, copes well with temperatures over a wide range of water

temperatures. In South Africa, prevailing water temperatures are generally suitable for cyanobacterial growth during the greater portion of the year.

Water column stability and stratification

Wind-induced turbulence, flow and temperature, or combinations may variously affect the physical stability of a body of water thereof. Furthermore, otherwise stable conditions may be disrupted through large-scale recreational use by powercraft, or by the regular disruption of conditions in canals and waterways caused by the passing of barges and ferries. When cyanobacteria are present in numbers, stable water column conditions are visibly evident by the formation of surface scums of buoyant algal cells.

Thermal stratification, resulting from a layer of water becoming thermally distinct from the remainder of the water column, is a characteristic of deep lakes and impoundments, but does also occur for short periods in shallow lakes and ponds. Thermal stratification ultimately results in nutrient stratification, with the upper, light-rich layer of water being rendered nutrient-poor, and with nutrients in the deeper layers of water being unavailable to phytoplankton.

Turbulence is generally considered to reduce cyanobacterial development, the argument being that the algal cells will be mixed out of the light rich environment for periods of time that exceed their relatively-long growth response times. While this is certainly true for deeper waters, wind-induced turbulence has been shown to have an apposite effect in nutrient-rich shallow and warm lakes. Here, regular mixing mimics the principles of a chemostat with the result that much higher levels of production are attained than would otherwise be the case in systems with less mictic action (e.g. Harding, 1997b). Varying frequencies of mixing in the aquatic environment have been shown to support those selective processes driven by frequency of disturbance (e.g. Connell, 1978; Reynolds, 1994; Harding, 1996).

Evaporation

Evaporation plays two roles. As water volumes reduce, nutrient concentrations increase, and so does the concentration of dissolved salts (TDS). Thus, the process alters nutrient availability and those environmental characteristic of salinity that would support successional sequences favouring, for example, mildly halo-tolerant cyanobacterial genera (e.g. *Nodularia*).

Turbidity

The level of inorganic turbidity is influenced by the factors that govern

stability, and also by the processes of bioturbation. As turbidity increases, either as a consequence of runoff bearing eroded soils, or from the entrainment of sediments, so light availability is reduced with depth below the surface. At low to moderate levels, ambient turbidity often favours cyanobacterial development, especially in rivers. This is due to the fact that many cyanobacterial genera have light harvesting mechanisms that impart to them a selective growth advantage in light-limited situations. Incidences of *Oscillatoria* in muddy, turbid rivers are a case in point. This advantage is progressively reduced as the level of turbidity increases.

Organic turbidity, or that turbidity occurring as a consequence of algal development, constitutes a self-attenuating feature. This is most apparent in stable water bodies where calm conditions can allow the formation of extremely thick scums of algae that limit the availability to the cells below. The South African studies on algal development in the Hartbeespoort Dam, and the characteristics of encrusted scums of *Microcystis aeruginosa*, are well documented and are referred to elsewhere in this review. The negative influence of organic turbidity has been offset in eupolymictic environments such as Zeekoevlei (Harding, 1997b).

Colour

The level of ambient colour in water reduces the ability of photosynthetically active radiation (PAR) to penetrate deep into the water column. In South Africa, intensely coloured waters are a common feature of the western and southern Cape, and photic zone depths are generally quite shallow ($K_d \cong 1.2$ m). Low levels of pH and nutrient availability typically characterize these waters. Notwithstanding this, some are well known for their propensity to support sustained and problematical blooms of cyanobacteria.

Morphology

Lake morphology, or the shape and depth of the lake basin or impoundment, can play a profound role in the development of cyanobacterial blooms through dictating where, and the manner in which, algal growth may occur. As should be apparent from the foregoing, the development of aggregations of cyanobacterial cells tends to be favoured by shallow, warm and sheltered conditions. Thus, this is more likely to occur in sheltered bays of dendritic lakes.

3.1.2 Chemical factors

Nutrients

The relationship between the excessive enrichment of aquatic ecosystems with plant nutrients (eutrophication) and the consequent positive effect on algal growth is well-known. While certain genera of filamentous, nitrogen-fixing blue-greens can overcome low levels of nitrogen availability, phosphorus is accepted as being the essential growth-limiting nutrient. With respect to the relationship between phosphorus availability and cyanobacterial development, it must be stressed that the simple non-limiting availability of phosphorus does not, in itself, provide this group of algae with the ability to become dominant in a particular body of water. For this to occur, a suitable combination of both nutrients, and the other abiotic and biotic factors described, have to prevail in order for the cyanobacteria to gain the selective advantage.

Myths of N:P ratios

Much has been made of the relationship between prevailing ratios of nitrogen and phosphorus (N:P) and the composition and density of phytoplankton assemblages that may occur. While certain broad categories generally and accurately support prediction of which algal division may predominate, other biophysical features and attributes should not be excluded from the equation. It is becoming increasingly apparent that, notwithstanding the prevailing N:P ratio, the phytoplankton assemblage may be significantly altered through biomanipulation, and without any change whatsoever to the ambient availability of nitrogen and phosphorus (e.g. Harding and Wright, 1999).

Furthermore, the issue of N:P ratio as a controlling factor in cyanobacterial development only comes into play when one or both elements are present in concentrations that are limiting for further algal development. Hence the value of N:P ratios should be linked to nutrient availability, and prediction of which element is likely to become limiting during the algal growth phase (e.g. Reynolds, 1992).

The foregoing is by no means intended to imply that eutrophic lakes and vleis can simply be left in their enriched state while superimposed or “piggy-back” techniques are used to address the management of noxious algal populations. The management of eutrophication, focussed on the attenuation of nutrient enrichment, should and must remain the foundation of water resource management. In most cases where eutrophication has impacted on an aquatic ecosystem it is not only the phytoplankton that has been affected. More often than not, one or more feedback loops or switches have been impacted to

the point where imbalance occurs at more than one level in the trophic web. What is implied, therefore, is that far greater improvements may be attained through the concomitant application of nutrient reduction and biomanipulation techniques, and that the two measures should be applied in concert with each other wherever possible.

Sediments and seepages

The internal recycling of nutrients can play a profound role in confounding attempts to manage eutrophication through the attenuation of external loading. In certain cases what may seem to be relatively minor accumulations of sediments can produce autochotonous loads of phosphorus that impart a high level of resilience to rehabilitation efforts.

Management of phosphorus loads that originate from sediments is often ignored in shallow oxic lakes on the basis that active phosphorus release will not prevail in the absence of anoxic, reducing conditions. While these conditions certainly do drive P-release from hypolimnetic sediments, in shallow lakes the passive release of phosphorus into the interstitial pore water, and its movement into the overlying water facilitated through wind- or bioturbation-induced sheer stresses, causes significant quantities of this element to be made available for plant development.

pH and CO_2

Conditions of high pH or a low availability of CO_2 generally favours the growth of cyanobacteria. Cyanobacterial physiology and growth, in relation to these factors, is described in detail by Shapiro (1990). On a diel basis, dense aggregations of cyanobacteria cause the pH to increase from early morning minima to late afternoon maximum levels, a phenomenon mirrored by a similar cycle of dissolved oxygen concentrations.

Salinity

Cyanobacterial genera exhibit a wide range of tolerance to salinity that influences both the likely assemblage composition and opportunities for management – particularly in estuarine and coastal wetland environments. Many of the common 'freshwater' genera tend to exhibit low salinity tolerances (< 3 ppt), while others such as *Nodularia spumigena*, and certain members of the *Planktothrix* genus, tend to become dominant as the ambient salinity increases. The freshening of estuary environments due to increased catchment runoff, or the increased availability of nutrients in the nearshore zone as a consequence of land-based activities, can result in eutrophic estuaries and algal blooms. In many cases the negative effects impact on estuary-based aquaculture or recreational pursuits, with concomitant financial losses and

ecosystem devastation. Manipulation of optimum salinity levels, or levels that are unfavourable to the growth of the problematical alga, has been attempted on various scales, ranging from the management of the Australian Peel-Harvey Inlet (McComb and Lukatelich, 1995; Hearn, 1995) to the alleviation of a toxic *Microcystis* bloom in a South African coastal wetland (Harding, 1998).

Dissolved oxygen

Most well mixed, shallow and warm eutrophic water bodies seldom suffer from low levels of dissolved oxygen. In systems, oxygen availability may directly affect the development of other populations of organisms such as zooplankton and fish and, consequently, affect the trophic web structure. Where algal bloom collapse and decay occurs, the resultant loss of oxygen through degradative processes can result in fish kills.

3.1.3 Biological factors

Trophic web interactions and ecosystem degradation

Imbalances in trophic web structure can and does have profound impacts on the functioning of aquatic ecosystems, and especially in shallow environments. In recent years it has become profoundly apparent that shallow lake systems exist in one of two alternative and stable conditions, either dominated by macrophytes (see below), or by phytoplankton. The eutrophication-associated progression from the one condition to the other is usually influenced by the regulation of water levels that previously exhibited a high level of natural variability. These effects typically become apparent as amplified "top-down" control, for example with over-grazing of zooplankton by fish, leading to excessive and uncontrolled development of algae. Restructuring of the "top-down" control pathways through the deliberate management of fish populations can have profound and beneficial consequences (e.g. Moss 1990; Scheffer et al. 1993; Harding and Wright, 1999). Similarly, the relief of seemingly intangible cause-and-effect pathways through the imposition of water level variation in regulated systems serve to strengthen ecosystem functioning.

Macrophytes

The presence of healthy stands of rooted aquatic macrophytes plays a crucial and frequently overlooked role in determining the extent of phytoplankton, including cyanobacterial development. The presence of submerged pondweeds is most often challenged by the need to be able to enjoy unhindered recreational pursuits. Examples of the ill-advised or unwitting eradication or over-control of plants such as *Potamogeton* spp. resulting in noxious

algal dominance are legio. What is not realized is the suite of benefits provided by these plants – an array of functions that encompasses much of the foregoing summary of factors that influence phytoplankton, and particularly cyanobacterial dominance, namely:

- Stabilization of sediments by the plant roots;
- Uptake of nutrients through both the root zone and the leaves of the plant;
- Provision of refugia for zooplankton that feed on algae;
- Provision of shelter and habitat for juvenile fish;
- Provision of physical attachment surface for a host of beneficial epiphytes;
- Provision of food and nesting material for species of waterfowl.

The health of hydrophytes is often severely impacted by water level regulation, and the destruction of natural variability with respect to water level variations. This leads to impoverished stands of rooted submerged plants, and consequent loss of faunal habitat, and concomitant strengthening and encroachment of monospecific stands of emergent species such as *Typha*, again with few opportunities for habitat development. Accordingly, regulated eutrophic lakes in the arid to semi-arid zones tend towards turbid, phytoplankton-dominated open water areas surrounded by dense and largely impenetrable bands of reeds, and with an abrupt interface between the shoreline and the open water, and with the latter dominated by coarse species of fish. This condition lends itself to self-reinforcement and perpetuation.

3.2 Consequences of cyanobacterial aggregations ("blooms")

Cyanobacterial blooms impact on the health of aquatic ecosystems, and the users thereof, in a number of ways. These impacts include loss of aesthetic appeal, turbid water, increased sedimentation, cost implications for water treatment, health threats as a consequence of toxicity, reduced recreational use value and ecosystem degradation, including that of downstream environments. Some of the dynamics that are involved are self-reinforcing, with the consequence that the problem becomes increasingly ingrained and resistant to rehabilitation.

3.3 Implications for human health

The health-related impacts of cyanobacterial toxins on human and animal health have been extensively documented, and have been referenced in the Suggested Reading section of this document. Sufficient for the purposes of this document, is that more than 60 cyanobacterial toxins are now known to exist. This suite of compounds includes hepatotoxic and neurotoxic alkaloids, hepatotoxic peptides and lipopolysaccharide endotoxins. A wide range of fatal incidents of toxicosis, involving domestic and stock animals, has occurred around the world. Dramatic incidents of human fatalities occurred recently in South America, and reports of non-fatal, cyanobacterial toxin induced symptoms are legio.

3.4 The monitoring and management of algal blooms

A variety of management strategies for cyanobacterial blooms have emerged during the past decade. Almost without exception, these are based on the UK and Australian models that resulted from the incidents of toxicosis that occurred in these countries (NRA, 1990; Ressom et al. 1993 and UKEA, 1998a), and detailed in WHO (1999). These protocols are based on a step-wise response to the exceedance of "Alert levels" based on cyanobacterial presence, numerical density and evidence of toxins. A similar protocol was formulated for South Africa (Quibell et al. 1995). Similar in-house response procedures have been adopted by the larger South African water utilities.

4. A GLOBAL PERSPECTIVE OF CYANOBACTERIA

Cyanobacterial problems have emerged during the past decade as a problem of international consequence. The cause-and-effect pathways of eutrophication and water level regulation do not recognize international boundaries, and the issues dealt with in this review are indeed cosmopolitan. In arid regions such as Southern Africa, cyanobacterial problems pose a considerable and significant threat to the sustainable use and management of scarce and diminishing water resources.

An examination of the global situation clearly shows that the problem, although evident through infrequent but sudden blooms of varying toxicity and magnitude, is certainly not static. Research efforts continue to document the discovery of new variants of toxins, and incidents of poisoning continue to occur. It is perhaps unfortunate that no central registry of verified poisoning events is available.

Considerable effort is being made on a number of fronts to delve deeper into the physiology and bimolecular functioning of the potentially toxic cyanobacterial genera, and to seek the initiators and pathways that trigger production of toxins by these organisms. Work on the relationships to cancer initiation and promotion are also receiving attention. Given the intermittent nature of the problem, funding for cyanobacterial research is difficult to come by, and research effort is fragmented to very few institutions located around the world. Often the research is driven more by personal interest than by grant funding provided for the specific purpose. As the problem is greater than the sum of the parts of the whole, it is essential that inter-programme communication and the dissemination of information can overcome the temporal limitations of publishing first via the scientific literature.

On a global scale, incidents of toxicosis remain largely confined to the poisoning of domestic animals, livestock and, occasionally, wild animals. An incident involving 2 000 cases of gastroenteritis and the associated 88 deaths of dialysis patients in Brazil, and the more recent 1996 case in Caruaru, Brazil where more than 60 dialysis patients died, constitute the only verifiable and documented fatal cases of cyanobacterially-linked human poisonings (Codd and Bell, 1998; Jochimsen et al. 1998; Pouria et al. 1998).

The ability to detect and resolve cyanobacterial toxins in a variety of sample matrices advanced by leaps and bounds during the 1990s. The methodologies remain costly and were confined to use within relatively well-equipped laboratories. This level of sophistication often initiates a call for an

easy-to-use field method, possibly coupled to some form of colour reaction. While this has not been forthcoming to-date, the apparent rarity of toxic incidents, the likely need to verify any field assessment in a quantitative manner, the availability of animal (mouse) testing and the probable high cost and limited shelf life of the envisaged test kit should be offset against the costs of transporting samples to distant laboratories. In many cases the use of international courier services for the sending of samples to laboratories on other continents provides a cost-effective means of analysis.

Much effort, time and expense is being spent on developing predictive ability, i.e., the ability to forecast whether or not a lake or impoundment has the propensity to develop potentially problematical cyanobacterial blooms. While this approach has some validity, it remains an "end-of-pipe" symptomatic and most often futile means of living with the problem. The prevention and management of the causes and consequences of eutrophication are neither easy nor cost-friendly, but they cannot be ignored. A practical and pragmatic two-pronged preventative approach, aimed at both eutrophication and the understanding of the cyanobacteria, needs to be encouraged and interlaced on a global scale.

5. THE SOUTH AFRICAN PERSPECTIVE

5.1 Historical overview – the early discoveries

The pioneering work of D G Steyn

In South Africa, Dr D.G. Steyn (1945) noted that over a period of twenty-five to thirty years, the deaths of 'many thousands' of stock (horses, sheep, cattle and rabbits) around pans in the north-eastern Free State and south-eastern Transvaal had been reported by farmers in the region who referred to the condition as 'pan sickness'. The first deaths suspected to be due to algal poisoning were brought to the attention of staff at Onderstepoort Veterinary Laboratories by farmers from the Amersfoort district in 1927 (Steyn *ibid*) and another case was reported from Wakkerstroom in 1942.

Veterinary officers sent to investigate the Wakkerstroom pan found that once the pan had been fenced off, the apparent disease ceased immediately. However, water samples taken from this pan failed to reach Onderstepoort in a condition that would have allowed reliable experiments to be carried out. Algal poisoning could not definitively established as being the cause of death. It was only after the construction of the Vaal dam in 1938 that this was achieved.

As the newly constructed dam began to fill, extensive areas of fertile farmland were inundated by the rising waters, resulting in eutrophic conditions and the appearance of a bloom of blue-green algae which Edith Stephens (1949) estimated as covering up to 98% of the dams' surface area. During the summers of 1942 and 1943, numerous stock deaths were being reported on farms adjacent to the dam and those located along the banks of the Vaal and Wilge rivers where the waters from these rivers overflowed into pans and vleis.

In 1943 Dr Douw Steyn, then Head of the Department of Pharmacology and Toxicology at Onderstepoort Laboratories, was sent to investigate. He reported seeing many animals (horses, mules, donkeys, dogs, hares, poultry, waterbirds and fish) lying dead near the banks and described the water as being the colour of 'green pea soup' with dense mats of algae floating on the surface (Steyn 1945). A light breeze was sufficient to drive the floating mats of algae to the shallows where they would decay, changing colour to a dark purple and sometimes staining the water red. It was found that high incidences of stock mortality were usually associated with prevailing winds - thus mortality would occur on the western banks on one day, and on the following day, on the eastern banks as the direction of the wind changed. Through experiments conducted on-site and later,

through controlled feeding of animals at Onderstepoort, Steyn was able to confirm that it was indeed the algae that were responsible for mortality. From these experiments, he reached the following astute and entirely accurate conclusions:

- the algal-infested water was poisonous;
- fresh growing algae was poisonous and the poison was discharged into the water when the organism died;
- the process of decay reduced toxicity and dried algae was less poisonous than fresh algae;
- the toxicity could not be reduced by boiling;
- experimental animals administered with polluted water displayed the same symptoms as the cattle, sheep and dogs which had died at the Vaal Dam;
- control of the bloom could be effected by the application of copper sulphate;
- the degree of poisoning depended upon the quantity of algae consumed.

Steyn identified two toxic components associated with the alga: (1) a toxin, which affected the nervous system and liver and (2) a toxin that caused photosensitisaton of the skin (Steyn 1943). He described the first toxin as 'one of the most potent liver toxins known', which, in acute cases, destroyed the liver cells within a few hours. The second substance, causing staining of the water in the pans, was identified by Polson (Steyn 1943) as the phycocyan chromoprotein. Steyn (1943) suggested that the phycocyan reached the blood of the animal and absorbed the ultra-violet rays of the sun, causing the skin lesions (photosensitisation) which were so characteristic in cases of algal poisoning. Typical symptoms of poisoning included constipation, blood in the faeces, a drop in milk yield, lesions on the skin and jaundice as a result of liver damage (Steyn 1945). Symptoms of more severe poisoning included progressive paralysis sometimes accompanied by 'strychnine-like convulsions' (Steyn 1945), coma and eventually death (Louw 1950). Typical post mortem symptoms included necrosis of the liver cells and haemorrhaging (Smit, cited in Louw 1950).

The blue-purple, fluorescent-coloured, material was identified by Dr Polson as phycocyanin, and it was found that if the liver was damaged by the first poison, this was taken up directly by the blood.

At that point of these incidents, the alga responsible had only been recorded from the eastern Transvaal and north eastern Free State.

Steyn categorized the cases into peracute, acute, sub-acute and chronic, depending on the quantity of algae consumed. Peracute cases were generally found dead near the water's edge. The symptoms

were deemed to be similar to those of strychnine poisoning. In other cases, the animals were overcome by an encroaching paralysis. In acute and subacute cases, the symptoms described above were prolonged over a period of one to two weeks. Jaundice-like conditions were evident in some cases, and inflammation and light sensitivity were evident in the nasal membranes, ears and parts of the skin. The affected animals' faeces were hard and often coated in a bloody slime. In chronic cases, these symptoms were recorded to last several weeks or months. Other symptoms included emaciation, a poor appetite and reduced milk production.

Post-mortem analysis revealed blood in the lungs, an enlarged liver that was often dark red in colour (sometimes black). Blood was occasionally found in the intestines. In chronic cases, a light yellow, bloody liquid was found in the pericardium, chest cavity and peritoneum. Steyn proposed methods of controlling the algae, including copper sulphate, suggesting a concentration of 'one pound for every 200 000 gallons of water. A year prior to the writing of Steyn's article, the then Department of Irrigation treated the dam by dosing it with copper sulphate, released from a motorboat. Between the 1st of June and 30th September 1943, 360 tonnes were dissolved into the dam (equivalent to 1-pound/300 000 gallons of water). This cleared the algae from the dam, until six months later, when a new bloom developed. No further deaths were recorded from September 1943 onwards.

It was believed that further infection of the major irrigation dams could be avoided only if the minor dams and pans in their catchments were kept clear of cyanobacteria. The alga was declared a weed and a campaign was launched by the Department of Agriculture and Forestry to eradicate it. Unfortunately, no records of this campaign appear to have been kept.

The American scientist, Theodore Olsen, was the first person to document the association between cyanobacterial genera and the mortality of stock animals. It is interesting to note that Olsen's work was conducted during the period 1948 to 1950, i.e. during the same period that Steyn was publishing the findings of his South African experiences.

(see also Steyn 1944 and 1949).

The contributions of Edith Stephens

Edith Stephens, the doyen of South African limnologists, examined the algae from several locations including the Vaal Dam, Bon Accord Dam and Spaarwater Mine Dam in the Transvaal, and Zeekoevlei and Rondevlei on the Cape Flats (Stephens, 1949). She established that the alga responsible for the poisoning was a previously undescribed species of blue green algae similar to *Microcystis aeruginosa* Kutz. and named this new species *Microcystis toxica*. Like *M. aeruginosa*, *M. toxica* had spherical cells containing 3-8 gas vesicles and cells formed colonies which were bound by a colourless gelatinous matrix which was secreted by the individual cells. However, she found that *M. toxica* differed from *M. aeruginosa* in several respects (Stephens, 1949):

- *M. toxica* formed sponge-like colonies which became hollow with peripherally arranged cells;
- *M. toxica* formed a framework of close-packed cells which joined to form a well-defined reticulum;
- *M. toxica* colonies attained a size of 2.5 mm, larger than *M. aeruginosa* which averaged 0.5 mm;
- *M. aeruginosa* was found not to be poisonous;
- Decaying *M. toxica* was found to emit a strong, unpleasant odour and dry in a blue-purple scum, whereas *M. aeruginosa* was found to be less strong smelling, forming a milky-white scum upon decay.

Herbarium records of Stephens' collections are still housed in the Bolus Herbarium at the University of Cape Town.

5.2 Scientific research in South Africa

5.2.1 Introduction

The following introductory excerpt is extracted *verbatim* from a chapter prepared by Pieter Thiel of the Medical Research Council for a chapter published in *Environmental Medicine* (Thiel, 1999).

'Attempts to isolate the toxic principles responsible for [these] dramatic hepatotoxic effects started in the 1950s. Most of the attempts in the USA, Australia and South Africa were aimed at the isolation of the hepatotoxic secondary metabolites of strains of *Microcystis aeruginosa*. Although the first indication that these toxins were peptides was obtained in 1959, it was not until 1982 that the breakthrough was made in the isolation and structural clarification of the first hepatotoxins from *M. aeruginosa* by Dr Dawie Botes and colleagues from the South African Council for Scientific and Industrial Research in Pretoria, South Africa. The group of cycloheptapeptide toxins isolated was given the name cyanoginosins by Botes, but was subsequently renamed microcystins. Six years after the identification of the microcystins another cyclic peptide hepatotoxin was isolated and identified from the filamentous cyanobacterium *Nodularia spumigena*. Professors Carmichael and Rinehart from the USA and Dr Kaarina Sivonen from Finland described the structure of a cyclic pentapeptide hepatotoxin, nodularin, which had some features in common with the cyclic heptapeptide toxins from *M. aeruginosa* (this discovery was made in 1988, some 110 years after Francis linked *Nodularia* to the deaths of animals at Lake Alexandrina).

In 1959 Bishop and co-workers were the first to demonstrate the peptide-like nature of the hepatotoxins from *M. aeruginosa*. Subsequent workers over the next two decades in the USA, Australia and South Africa were in agreement about the peptide nature of the toxins, but reported conflicting results on the composition of the toxins they isolated (probably from impurities or from the presence of more than one toxin in their preparations). Contributing to the difficulties in elucidating the structure of the hepatotoxins was the fact that they proved to be cyclic peptides and contained unknown amino acids in their structures. The toxins proved to have no free amino groups and yielded some methylamine upon hydrolysis that eventually proved to be a hydrolytic breakdown product of N-methyldehydroalanine. The absence of free amino groups and resistance towards enzymatic digestion precluded the use of the classical approach, using the Edman procedure, in sequencing the amino acids in the structure of the toxins.

The major breakthrough by Botes and co-workers in elucidating the structures of the hepatotoxins necessitated the use of a variety of chemical and physical techniques. Apart from the classical use of chromatographic techniques to isolate and identify hydrolysis products, use was made of nuclear magnetic resonance (NMR) and both electron impact and fast atom bombardment mass spectrometry (MS). They eventually showed that one strain of *M. aeruginosa* was capable of producing more than one toxin, and that these toxins were all monocyclic heptapeptides containing both D- and L-amino acids.

5.2.2 A chronology of cyanobacterial research in South Africa

Three distinct periods of activity can be distinguished in South Africa's history of studies on cyanobacteria and their toxins:

1927 to 1970

The first cases of cyanobacterial poisoning recorded in South Africa were diagnosed by Dr DG "Douw" Steyn of the Onderstepoort Veterinary Institute. These occurred at a pan in the Wakkerstroom area of the then south-eastern Transvaal. The algae were responsible for the deaths of horses, sheep, cattle, rabbits and water birds. Thereafter, during the following 25 years, thousands of stock animals died from algal poisoning. These incidents were carefully documented by Dr Steyn. The most serious cases were recorded during the period 1941 to 1943, shortly after the Vaal Dam was filled for the first time. Many thousands of animals died, including mules, donkeys, dogs, rabbits and poultry. Steyn undertook a series of pioneering investigations, and formulated a number of erudite and entirely correct assumptions regarding the toxins produced by this group of organisms (see elsewhere in this review). The vast blooms present in the dam were subsequently treated with copper sulphate, and no further poisonings were reported from the area.

During the 1950s, PG Louw, also working from Onderstepoort, succeeded in isolating the poison from cyanobacteria present in the nearby Bon Accord Dam. He tentatively identified it as an alkaloid.

Subsequent to Louw's investigations no other cyanobacterial-related findings emerged during the remainder of the period to the end of the 1960s.

1970 to 1989

The onset of the 1970s saw a flurry of activity on a number of fronts in the cyanobacterial field. Central to this was the reporting of an increased incidence of poisonings during the early years of the decade (e.g. Tustin et al. 1973; Scott, 1974). An awareness of the burgeoning threat to the scarce water resources of an arid South Africa, and the apparent cause and effect links between this phenomenon and cyanobacteria, was just cause for attention. It may be argued, however, that greater effort should have, and still needs to be, paid to the ravages of our surface waters through the ill-managed causes of nutrient enrichment. During the same period the foundations of the later multi-disciplinary studies of the Hartbeespoort Dam limnology (eutrophication) study (NIWR, 1985) were being laid through the interests of researchers based at the then National Institute for Water Research of the CSIR.

During 1977, a multi-departmental investigation was initiated to undertake the following tasks:

- To obtain information on the types of cyanobacteria capable of producing toxins, and to describe the conditions under which they did so;
- To investigate methods for the control of toxic algae;
- To determine the chemical structures of the toxin(s) involved, and to develop methods to measure these toxins in water;
- To investigate the pathology of the toxins on test animals in order to determine safe exposure levels for humans;
- To investigate which water treatment processes were necessary in order to provide adequate levels of protection for consumers of potable water.

The passage of time was to reveal just how ably these goals were met.

This study was afforded a ten-year time span, funded by the Department of National Health and Population Development and the NIWR, and was prepared under the direction of W E (Willem) Scott of the NIWR. The core team of researchers comprised:

- Dr DP (Dawie) Botes (National Chemical Research Institute, and later University of Cape Town);
- Mr CA (Charl) Bruwer (Hydrological Research Institute – now the Institute for Water Quality Studies);
- Professor JN Eloff (University of the Orange Free State and later the Kirstenbosch Botanical Gardens);
- Miss PS (Sally) Gathercole (Research Insitute for Nutritional Diseases, later with the PROMEC division of the Medical Research Council);
- Mr JH Haumann (Microbial Genetics Unit of the Medical Research Council);
- Dr K Jaskiewicz (Research Insitute for Nutritional Diseases);
- Dr E (Ella) Johanssen (National Chemical Research Institute);
- Dr TS Kellerman (Onderstepoort Veterinary Research Institute);
- Dr R (Rifka) Kfir (National Chemical Research Institute, later with the NIWR);
- Professor GHJ (Gert) Kruger, (University of the Orange Free State);
- Dr PG (Pieter) Thiel (Research Institute for Nutritional Diseases, later with the PROMEC division of the Medical Research Council);
- Dr AJ van der Westhuizen (University of the Orange Free State).

This mammoth effort spawned a number of concomitant and interrelated investigations and studies, as will be apparent from the contents of this review. The project was completed in 1987 (Scott, 1987), and it is not known whether a final

report was ever prepared. Rationalization of the activities of the CSIR during the same period saw an abrupt end to a groundbreaking period of research into both cyanobacteria and the limnology of impounded waters (NIWR, 1985), and with the research team disbanding to pursue new career options or directions. Nonetheless, the efforts of Scott's team had ensured that all of the project goals were met, and the conclusions drawn and technical achievements made were recognized internationally as being of enormous significance to the understanding of a global problem. Willem Scotts' final publication on the subject (Scott, 1991) detailed the range of cyanobacterial genera that had been found in South Africa, and the geographic distribution.

1990 to 1999

Insofar as South Africa was concerned, the closing years of the 1980s yielded little for the cyanobacterial field. The country was also now without any formal means of having cyanobacterial or water samples analysed for toxins. In the western Cape, and despite opinions to the contrary, it was becoming increasingly apparent that the acid waters of the region were showing alarming signs of potential cyanobacterial problems. The recently commissioned Theewaterskloof Dam, the single largest water storage for the Cape metropole had been plagued from the outset by taste and odour problems caused by *Anabaena solitaria*. This caused WR (Bill) Harding to begin to lobby the then City of Cape Town authority to heed the signs and to put in place mechanisms for determining whether or not toxins were also present. At that time, an ill-advised myth had led many to believe that if taste and odours were present, toxins would not be!

At the same time, the then Umgeni Water Board was also experiencing problems with cyanobacterial blooms in certain of its impoundments. The need to be able to screen for the presence of toxins was becoming increasingly essential. During 1992 six South Africans attended a working meeting on detection methods for cyanobacterial toxins convened at the University of Bath, UK. Those attending were:

- Dr Henk van Vliet (IWQS, Department of Water Affairs and Forestry);
- Dr Bill Harding (Department of Scientific Services, City of Cape Town);
- Dr Bruce Rae (Umgeni Water);
- Dr Pete Ashton (CSIR);
- Dr Clark (CSIR);
- Dr Thys Pieterse (Water Research Commission)

The proceedings of this symposium (Codd et al. 1994) indicated that the concerns

being experienced in South Africa were by no means unique, and that scientists were wrestling with similar problems in many different countries. A variety of potential analytical methods were proposed, the most suitable of these appearing to be the HPLC technique developed in the laboratory of Professor Geoff Codd of the Department of Biological Sciences, University of Dundee (Lawton et al. 1994). Subsequent to the Bath symposium, Bill Harding and Bruce Rae spent time separately at Dundee to study the technique used in order to use it under South African conditions. The so-called "Codd" method was later to become the UK "Blue Book" reference method for resolving and quantifying cyanobacterial hepatotoxins, and is now used widely throughout the world (see WHO 1999, or UKEA, 1998b) for full details of the analytical procedure).

During 1994 the City of Cape Town, following the undisputed appearance of toxins in the Theewaterskloof Dam, established the then only laboratory in Africa that was fully dedicated to the analysis and quantification of cyanobacterial hepatotoxins. Shortly thereafter, Dr Elsie Meintjies of Rand Water established a mirror facility at their laboratories in Vereenigning, but it was some years before this came into full operation. Harding also assisted the Water Utilities Corporation in Gaborone, Botswana, to set up and commission an identical procedure. Umgeni Water adapted existing HPLC operations to accommodate the method in support of their studies on cyanobacteria in their impoundments and pipelines. The laboratories of IWQS were utilizing a similar technique, but using an isocratic method. During the late 1990s, Dr Gordon Shephard of the MRC in Parow, Cape Town, upgraded his HPLC technique from that used by Sally Gathercole and Pieter Thiel (1988) to the "Codd" method.

The establishment of the laboratory in Cape Town took place not a moment too soon. The mid-1990s saw an explosion of incidents of cyanobacterial toxicosis throughout the western and southern Cape, culminating in the largest single stock death since the incidents described by Steyn during the 1940s (see elsewhere in this review).

During 1994, Professor Codd visited South Africa to evaluate the performance of the City of Cape Town's laboratory, and to assist the IWQS with the formulation of an action plan, similar to those already in place in the UK and Australia, for dealing with the threats and problems posed by cyanobacteria. The resultant document, authored by Gavin Quibell, stalled at the draft stage, only to be revisited after the formation of the Toxic Algal Forum (Quibell et al. 1995).

The closing years of the 90s saw a worrying lull in attention to issues pertaining to cyanobacteria. The swansong of the CSIR-driven programmes was a short course entitled Algae in Water – Problems and Treatment held during August 1989. This meeting dealt, essentially, with the last objective of the 10-year programme, namely water treatment options for dealing with cyanobacterial in raw potable water sources. Research activities were now largely confined to the grey-area records of water utility operations; the proposals made to national government had apparently fallen on deaf ears, and no 'torch-carriers' prepared to champion the problem were coming to the fore.

During 1998 a group of concerned scientists met at Rand Water in Alberton to convene what became known as the Toxic Algal Forum (TAF).

During 1999, the Water Research Commission (WRC) convened two workshops at the Rhoodevallei Conference Centre in Pretoria. The first placed the problems facing South Africa in both international and local perspective. Professor Geoff Codd of the Department of Biological Sciences, Dundee University, Scotland attended this meeting as an international specialist in the field of cyanobacteria and their associated toxins. The second workshop, addressed cyanobacterial research needs, and enlisted the input of Professor Ian Falconer from Australia. Both workshops resolved that a review of the South African experiences relating to cyanobacteria was necessary in order to be able to meaningfully evaluate new research proposals and needs. The need for this document was born.

5.2.3 International working links

Between 1980 and 1999, South African researchers maintained working contacts with three overseas specialists, namely Dr Wayne W. Carmichael of Wright State University, Ohio, USA; Professor Geoff A. Codd, University of Dundee, Scotland; and Professor Ian Falconer, University of Adelaide, Australia. Their involvement, connections and involvement are summarised below:

Wayne W. Carmichael

Wayne Carmichael's links to South Africa began at a conference he organised in Dayton, Ohio, during 1980. Several South African scientists, *viz*. Eloff, Haumann, Kruger, Scott and van der Westhuizen were invited to attend. Subsequently, Wayne visited South Africa during 1982 and 1985, the latter visit being for the 6th IUPAC symposium on Mycotoxins and Phycotoxins. On that occasion he visited Willem Scott, Dawie Botes and Pieter Thiel. His contact with South African researches was renewed during 1992 at the Bath symposium on detection methods for algal toxins, and subsequently, through visits to Dayton by Johan Grobbelaar and Machiel Steynberg.

Geoffrey A. Codd

Geoff Codd's influence in South Africa has been mainly through the application of the HPLC method, developed in his laboratory, for the detection and resolution of cyanobacterial hepatotoxins (see elsewhere in this review). He also made contributions towards the development of cyanobacterial awareness and related policy development in South Africa. Following the 1992 Bath Symposium, two South African delegates, *viz*. Bill Harding and Bruce Rae, independently visited Dundee to view the HPLC technique before utilising it locally.

Geoff visited South Africa on several occasions during 1993, 1994 and 1999, during which visits he contributed to various meetings, seminars and workshops, provided specialist advice and guidance to Umgeni Water, Rand Water, the Water Research Commission, Cape Town City Council and the Department of Water Affairs and Forestry (DWAF). His input was instrumental towards the development of the as yet unpublished DWAF policy for a national surveillance programme for cyanobacteria (see elsewhere in this review). At the 1999 Rhoode Vallei symposium Geoff delivered the plenary lecture dealing with the global perspective on cyanobacteria, and was appointed as the Overseas Correspondent for the Toxic Algal Forum.

The first Western Cape toxin analyses, from the 1993 incident at Theewaterskloof (see elsewhere) were undertaken in the Dundee laboratories, as were the samples from the 1994 animal poisoning incident at Zeekoevlei. Subsequently the City of Cape Town established its own dedicated toxin analysis and detection facility at its Athlone laboratories during 1994, and Geoff Codd continued to provide collaborative support and guidance. He also visited the Cape Town laboratory during 1994 to evaluate its performance.

Ian Falconer

Ian Falconer and Maria Runnegar (Armidale, University of New South Wales, Australia), and the South African group of Dawie Botes and Willem Scott et al. were in contact from the mid-1970s. The joint interest of this work was the elucidation of the structure of the microcystins (see Botes et al. 1985). Runnegar and Falconer (1982) had published the structure of their variant, the tyrosine methionine form, but missed the dehydroalanine because it broke down, and the ADDA moiety was unknown at the time. Dawie Botes subsequently sent the compounds to Cambridge for analysis, and derived the first accurate structure (see elsewhere in this review). Ian Falconer's direct links with South Africa were restored during 1999 when he was invited to give a plenary address and expert input to the WRC workshop on Algae and Endocrine Disruptors held at Rhoodevallei during October 1999.

6. SOUTH AFRICAN CONTRIBUTIONS TO THE GLOBAL UNDERSTANDING OF THE ECOLOGY AND TOXICOLOGY OF CYANOBACTERIA

Subsequent to the pioneering work by Steyn (see above), South African research into cyanobacteria spanned the following:

- Nature and structure of the microcystins
- Ecology and physiology
- Isolation and cultivation
- Ecological field studies on algal blooms
- Hartbeespoort Dam project (NIWR)
- Vaal River studies
- Management of blooms
- Researched incidents of toxicosis
- Toxin removal from water supplies
- Cyanobacterial investigations in South Africa post-1990

6.1 Elucidation of the nature and structure of the microcystins

Prior to the definitive work on the structure of microcystins performed in South Africa by Eloff, Botes and colleagues, the following research had been conducted elsewhere.

- Bishop (1959) isolated an endotoxin from *Microcystis* NRC-1 isolated from a strain cultured from a natural bloom from Little Ridean Lake, Ontario, Canada. The toxin was found to be of a peptide nature, and comprising seven amino acids and ten residues.

- Murthy and Capindale (1970) isolated toxin from same *Microcystis* strain as did Bishop. He found fourteen different amino acids in the acid hydrosylate of the toxin.

- Rabin and Darbre (1975) purified the toxin and found most of the common amino acids and ornithine to be present.

- Kirpenko et al. (1975) isolated a toxin of molecular weight 19 400 ± 1 400 from a bloom of *Microcytis aeruginosa* from Dnieper basin. Consisted of acetomethylene, isothiocyanate, carbohydrate groups, 16.6% peptide and disulphide cross-linking bonds.

- Elleman et al. (1978) isolated a toxin from a bloom of *M. aeruginosa* in Malpas Dam Reservoir, New South Wales, Australia. Using hydrolysis he determined this to be a pentapeptide (i.e. a nodularin-type toxin) with equimolar quantities D-Ala, D-Glu, erythro-β-CH_3- Asp, L-Tyr and methylamine.

The active constituent of the poisonous algae, *Microcystis toxica* Stephens (Louw, 1950)

In 1950, P.G.J. Louw, a biochemist at Onderstepoort, succeeded in isolating the poison and tentatively identified it as an alkaloid. The experiments were carried out on *M. toxica* (Stephens) collected from the Bon-Accord Dam near Onderstepoort. Louw found that, when separated from fresh algae, the watery filtrate was inactive and that only once the algae began to decay did the toxin leach into the water. Experiments on rats and rabbits confirmed that the concentration of toxins in the water varied considerably from season to season - being highest at the end of summer. He also found that the rabbits appeared to develop immunity after several doses of the poison. Louw did not elaborate on this very interesting, yet incorrect, observation.

Experimental cases of algae poisoning in small animals (Smit, 1950)

Smit (1950) observed that sudden death could occur within half an hour after injection or oral administration of the alkaloid in small animals. Symptoms included restlessness, anaemia, dyspnoea, progressive paralysis, coma and eventually death. Chronic cases revealed cirrhosis of the liver.

Post mortem analysis revealed bloodstained ascites, tumour splenis, hyperaemia of the kidneys and necrosis of the liver, blood stasis and haemorrhages. The liver was dark red. Histopathological examination of test animals revealed:

- *Spleen*: well-defined malphigian tubules
- *Kidney*: blood stasis, slight nephrosis in acute cases
- *Liver*: severe necrosis of the hepatic cells in acute cases. Karyorrhexis, blood stasis, haemorrhages and fatty degeneration.

Examination of toxic and non-toxic Microcystis aeruginosa in the field and laboratory culture (Scott 1979 and 1986)

Zehnder and Gorham (1960, cited in Scott 1986) demonstrated that *M. aeruginosa* loses its colonial habit when grown under laboratory conditions. Eloff (1981) indicated that, in some cases, *M. aeruginosa* lost its ability to form gas vacuoles in laboratory cultures, rendering the *Microcystis* cultures indistinguisable from other genera such as *Synechocystis* and *Aphanocapsa*. Thus, while field samples may be adequately identified using morphology, identification of laboratory cultures with altered morphology may present problems.

Scott further showed that two forms of *Microcystis*, namely *M. aeruginosa* forma. *aeruginosa* and forma *flos-aquae* were easily distinguished on the basis of colony morphology from the freshly collected field material. Percentage composition of the two strains from fixed sampling point on the Hartbeespoort Dam showed that the toxic *M. aeruginosa* forma *aeruginosa* was dominant, whereas the non-toxic *M. aeruginosa* forma *flos-aquae* formed a significant proportion of the phytoplankton population only during the winter months. Harding (1996) has observed that in many eutrophic vleis in the western Cape, dominance by *Microcystis* is typically in the form of the non-toxic forma flos-aquae variant, and appearance of *M. aeruginosa* forma *aeruginosa* almost always associated with toxicity.

Scott found that laboratory cultures of *M. aeruginosa* were found to undergo changes including loss of colonial growth

habit and sometimes gas vacuoles, making it impossible to distinguish toxic and non-toxic strains.

Loss of colonial growth habit is associated with changes in the hydrophobicity of the surface of the cells and reduced ability to form mucopolysaccharides that may be required for colony formation. The reasons for the loss of the colonial habit could not be explained, but demonstrated the need for physiological, chemical and genetic tools was demonstrated in order to be able to undertake laboratory identification.

Microcystis toxins: isolation, identification, implications (Toerien et al. 1976)

Toerien et al. (1976) cite Louw (1950) as suggesting that the *M. toxica* toxin was an alkaloid. Hughes et al. (1958) suggested, however, that *M. toxica* may be related or the similar to *M. aeruginosa* forma *aeruginosa*, and that the toxin may be an acidic cyclic peptide. In this paper Toerien and co-workers set out to clarify the nature of the toxin produced by *M. aeruginosa* forma *aeruginosa* from the Hartbeespoort Dam.

Results suggestsed that Louw's (1950) conclusion that the toxins were alkaloid was erroneous and that the toxins were similar to those identified by Hughes et al. (1958) as being polypeptides.

Preliminary study of the toxins of different Microcystis strains (Amann and Eloff 1980)

Amann and Eloff (1980) report that the characterisation of the toxins found in *Microcystis* blooms had, at the time of writing, led to widely divergent results from different researchers. They investigated toxins of six *Microcystis* strains*: M. aeruginosa* NRC-1 (UV-001) (Rideau Lake, Ontario), *M. aeruginosa* (UV-006) (Hartebeespoort Dam), *M. aeruginosa* (UV-007) (Gottingen culture), *M. aeruginosa* (UV-010) (Witbank Dam), *M. aeruginosa* (UV-018) (Gottingen culture) and *M. aeruginosa* (UV-019) (Allemanskraal Dam), using gel chromatographic separation.

The quantity of phycocyanin extracted from the strains differed significantly, but this was not correlated with toxicity. The toxic extracts from the Allemanskraal Dam (UV-019) were found to have the greatest difference in absorption behaviour from all other extracts. This was ascribed to the effect of laboratory cultivation on the presence of the UV-absorbing components. The difference in the absorption patterns of UV-018 and UV-007 (which originally came from the

same culture) was suspected to be due to the difference in the cultivation history of the two cultures and its effect on the UV absorbing components.

The elution volume of the toxic compounds was found to correspond to vitamin B_{12}, suggesting that if the compounds were peptides, they would have a molecular weight of 1300 daltons or less. Based on the elution characteristics, the authors suggest that the toxins were similar or had similar molecular weights. The similarity between toxin from NRC-1 that had been in cultivation for 25 years, and UV-001 that had been collected from a living bloom in the Allemanskraal Dam, suggested that toxin production is a genetically stable characteristic. The authors further proposed that the differences in the toxins found by other researchers were due to variations in natural environmental conditions, the extraction of mixed toxins and differences between the extraction and fractionation systems used.

Plasmid(s) involvement in the toxicity of Microcystis aeruginosa (Hauman, 1981a,b)

Hauman investigated the possible involvement of plasmids in toxin production by *M. aeruginosa*. Plasmids from *M. aeruginosa* WR70 were obtained using mass lysis methods. Two plasmids were found in lysates of *M. aeruginosa* WR70 with molecular masses of 2.05 Md and 5.02 Md, with the smaller plasmid being more abundant. A plasmid of 2 Md had been isolated from a non-toxic strain of *M. aeruginosa* PCC 7005. Hauman suspected that the 5.02 Md plasmid was involved in toxicity, but this could not be confirmed.

Four agents were used to test the toxicity of *M. aeruginosa* (NIWR strain WR 70) including acridine orange, which eliminates the sex factors from *E. coli* (Hirota, 1960) and cures toxigenic cultures of *Clostridium botulinum* of their prophages and toxicity (Eklund and Poysky, 1973). Acridine orange interferes with plasmid replication without interfering with chromosomal replication (Yamagata and Uchida, 1969). Sodium dodecyl sulphate (SDS) selects for plasmid-free cells in *E. coli* (Tomoeda et al. 1968); *Staphylococcus aereus* (Sonstein and Baldwin, 1972); chloramphenicol, which eliminates the plasmid-coded haemolysin of *E. coli* (Mitchell and Kenworthy, 1977), and streptomycin which acts as a mutagen in the non-chromosomal genes of *Chlamydomonas* (Sager 1962) (all cited in Hauman, 1981a).

All agents tested eliminated the toxicity of *M. aerugninosa* at relatively low doses.

No explanation could be suggested for this. Hauman suggested that the effectiveness of acridine orange at low doses indicated that the genetic determinant of toxicity could be a non-integrated plasmid rather than a prophage, as suggested by Vance (1977) for *M. aeruginosa* NRC-1 (see also Vakeria et al. 1985).

(see *Supporting Literature*)

Amino acid sequence of two toxic peptides from Microcystis aeruginosa UV-006 (Eloff et al, 1982a)

Eloff et al. (1982a) isolated two major toxins from *Microcystis aeruginosa* UV-006 using gel chromatography and high performance liquid chromatography. Toxin 'A' was found to comprise: β-methyl aspartic acid, D-glutamic acid, D-alanine, L-alanine, L-leucine and methylamine in a 1:1 molar ratio. Toxin 'B' was found to have a similar composition with L-arginine replacing L-alanine. From the molecular weights of toxins 'A' and 'B' (1 550 and 1 650), the authors concluded that 15 amino acids were present.

Partial acid hydrolysis of 'A' and 'B', with HPLC and amino acid analysis suggested the following sequence of amino acids:

A = D-glx → L-ala → D-ala → L-leu → mas

B = D-glx → L-arg → D-ala → L-leu → mas

where glx is glutamic acid coupled to methylamine and mas is β-methyl aspartic acid.

Extraction, isolation and stability of toxins from Microcystis aeruginosa (Eloff et al. 1982b)

Toxins were extracted from cells of *Microcystis* after pre-treatment by freeze drying and extraction using acetone, sodium carbonate (0.1M), methanol and ethanol (at different concentrations), methanol-chloroform-water (12:5.3), 0.01M HCl, 1% Triton in 1.25M K_2HPO_4 and water (at different temperatures). Toxicity was determined by intraperitoneal injection of white mice. Some loss of toxicity was found to take place after the removal of interferring pigments. Using gel chromatography, Eloff et al. (1982b) found a direct linear relationship between toxicity and the absorbance 240 nm at 1.0 (1 cm light path), i.e. 1 mℓ of the toxic fraction at this absorbance was sufficient to kill 19 mice.

High performance liquid chromatography, using different gradient and isocratic systems, was investigated to purify the toxins. Good separations were achieved using octadecyl-substituted columns with 55% MeOH in 25 mM ammonium. The

toxins were found to be unstable at pH 4 at room temperature and loss of 50% toxicity was found to occur by heating the toxins to 100°C.

An HPLC method for detecting and resolving the microcystins (Gathercole and Thiel, 1987)

Sally Gathercole and Pieter Thiel developed a very functional mono-wavelength HPLC method that separated six microcystin variants with a high degree of resolution. This method provided a basis for the development of the "Codd" method (described elsewhere in this report).

Isolation and characterization of four toxins from the blue-green alga, Microcystis aeruginosa (Botes et al. 1982a,b)

Toxins were isolated from two strains of *Microcystis aeruginosa* from the Hartebeespoort Dam (90% toxic *Microcystis aeruginosa* forma *aeruginosa* and 10% of the non-toxic *Microcystis aeruginosa* forma *flos aquae*), and from a toxic strain of *Microcystis aeruginosa* forma *aeruginosa* grown in culture originally from the Witbank Dam.

Amino acid analysis showed compositional similarities in the structures of the toxic variants, as well as with the toxin isolated by Elleman (1978) from *M. aeruginosa*. Botes and co-workers subsequently demonstrated the configuration assignments of the amino acid residues, and demonstrated the presence of N-methyldehydroalanine in the toxins from *M. aeruginosa* (Botes et al. 1982b).

A specific ultraviolet absorbance band of Microcystis aeruginosa (Eloff, 1982)

Eloff (1982) cites Amann and Eloff (1980) as stating that the nature of toxins extracted from *M. aeruginosa* strains isolated from Canada, Australia and USSR suggested wide differences in amino acid composition and molecular weight, possibly due to divergent isolation procedures. In this paper Eloff (1982) reported that toxins from several isolates are always associated with a 240 nm absorbance band. He postulated that the ratio of the absorbance at 240 nm to other wavelengths could provide a measure of toxin purity.

Figures presented in the text (not reproduced here) show the elution profile of *M. aeruginosa* toxins purified by gel chromatography on a Sephadex G-25 superfine column. Fractions 68-87 were found to be toxic, the combined pools of fractions A, C, D, E and F concentrated 40

fold were not toxic when 0.4 m*l* was injected into mice. Also shown are the absorbance spectra for fraction 81 and five non-toxic pools. The 240 nm absorbance maximum was only evident in the toxic fraction.

Fractions 78, 79, 80 and 81 were used for LD_{50} determination. A direct linear relationship was obtained for toxicity and 240nm absorbance. One m*l* of a toxic fraction with an $A^{1.0cm}_{240nm}$ - 1.0 is sufficient to kill 19 mice (LD_{50} = 0.88 A $A^{1.0cm}_{240nm}$/kg).

Absorbance ratios of 240/220 nm or 240/260 nm were found to provide an estimate of toxin content and/or purity. Elution patterns of *M. aeruginosa* isolates NRC-1, UV-006 and UV-027, suggested similarities and differences. Eloff suggested that elution patterns could be used for fingerprinting *M. aeruginosa* isolates for taxonomic purposes.

Comparative study on the toxins from several Microcystis aeruginosa isolates (Eloff et al. 1982c)

In this study Eloff et al. extracted the toxins (solvent extraction) of sixteen clonal isolates of *Microcystis aeruginosa* from different parts of the world. Toxin composition was found to be similar but variations in toxin composition were found. Seven major toxins were identified in all. In most cases one or two toxins accounted for >90% of the toxin in a single isolate, but, in some organisms, up to six toxins were found. β-methyl aspartic acid, glutamic acid, alanine, methylamine and two other amino acids in a 1:1 molar ratio were found in all cases. Other amino acids in the major toxins included leucine and arginine, leucine and alanine, tyrosine and arginine, methionine and arginine, leucine and tyrosine, alanine and tyrosine or arginine and arginine. From toxin composition, seven groups could be identified, suggesting that toxin composition may have taxanomic significance. Differences between toxic and non-toxic strains were found to be quantitative and not qualitative.

Microcystis aeruginosa toxin: cell culture toxicity, hemolysis, and mutagenicity assays (Grabow et al. 1982)

During the early 1980s, the chemical and toxicological properties of the compounds in certain cyanobacterial species of algae had not been fully established. It had been ascertained that certain strains of *Microcystis aeruginosa* produced toxins which may have consisted of several peptide or peptide containing toxins of undefined structure with hepatotoxic or neurotoxic activity (e.g. Collins 1978; Kirpenko et al. 1970; Gorham and

Carmicheal, 1979). It had also been established that the toxin now known as microcystin (or fast-death factor) killed mice in 1–3 h, and may have consisted of a cyclic polypeptide containing up to 16 amino acids with a molecular weight of between 654 to 19 400 (Collins 1978, Gorham and Carmichael, 1979, Murthy and Capindale, 1970; Toerien et al. 1976). The death of mice and vervet monkeys had been ascribed to circulatory failure due to liver damage (Falconer et al. 1981; Toerien et al. 1976; Tustin et al. 1973). The short survival time suggested that liver damage might not be the primary cause, and that neurotoxic activity could also be a factor (Gorham and Carmichael, 1979). Kirpenko et al. (1970) concluded that the toxins lead to degenerative changes in parenchymatous organs and brain cells. Toxins had also been reported to agglutinate erythrocytes (Carmichael and Bent, 1981) and to be cytopathogenic for cultures of mammalian cells (Kirpenko et al. 1970).

Grabow and co-workers set out to investigate the response of cells to *Microcystis* toxin that had previously been untested. They dealt with the development and evaluation of biological assays for the toxin and the testing of the toxin for mutagenic activity using the Ames/*Salmonella* microsome mutagenicity assay (Ames test). Human hepatoma cells, hamster ovary cell, fetal rhesus monkey kidney cells, human embryo lung cells, human cervical carcinoma, African green monkey kidney cells and primary vervet monkey kidney cells were cultured, and subjected to the following tests:

- Microtiter cell culture assays
- Hemagglutination and hemolysis tests
- Mouse toxicity tests
- Test for *E. coli* heat-labile enterotoxin
- Trypan blue test of cell membrane permeability
- Bacteria
- Ames test
- Chemical toxicants and pesticides

It was found that the cells from all of the cultures were severely damaged or disintegrated after overnight incubation in the presence of the toxin. Cytopathogenic effects were detected in 6 h in cells seeded in the presence of the undiluted toxin and after 8 h when added to cells in established 24 h cultures. Serum had no effect on the action of the toxin. The cell culture assays were more sensitive than the mouse toxicity test. The hemolysis tests were the most sensitive for toxin obtained from the Hartbeespoort Dam. For tests of *M. aeruginosa*, WR70 toxin and toxins from the Vaal and Roodeplaat Dams, cell culture assays were more sensitive than the mouse toxicity test, but the hemolysis test was less sensitive than both.

In response to *E. coli* enterotoxin cell elongation could be distinguished from

cytopathogenic effects of *Microcystis* toxin. The mouse toxicity test was found to be less sensitive than the microtiter cell culture assays, while the Trypan blue test: with mammalian tissue exhibited disruption of membrane permeability 5 min after application of the *Microcystis* toxin. This technique was not, however, found to be particularly sensitive as an assay for the toxin.

The results from the haemagglutination and haemolysis test resembled the agglutination of erythrocytes. Haemolysis tests revealed that apparent diffusion of erythrocytes and the absence of settling were probably due to lysis of the cells. Both reactions were found to be temperature dependent: tests at 4°C yielded negative results, tests at 10°C yielded marginally positive results, at 35°C positive results yielded an end point after 5 h. There was no evidence for agglutination in addition to lysis. The absence of evidence for agglutination suggests that previous studies (e.g. Carmichael and Bent 1981) mistook haemagglutination for haemolysis.

The average count of *E. coli* cells cultured in the presence of the toxins was found to increase, while in the absence of the toxin, the count decreased. Similar results were obtained from the test on *S. faecalis*. This suggested that these bacteria thrive in toxin preparations. In the Ames tests no mutagenic activity was detected in the assays in the presence or absence of activating liver enzymes. This indicated that no mutagenic or carcinogenic compounds were present.

The absence of significant differences in the sensitivities of liver, lung, cervix, ovary or kidney cells suggested that the toxin was not specific to liver cells. Damage to liver cells was a consequence of the toxin being directly transported to the liver. Epithelial cells were shown to be slightly more sensitive than fibroblast cells.

The trypan blue tests and haemolysis tests suggested that the microcystin toxin inactivated the cell membrane permeability barrier almost instantaneously. This confirmed that the destruction of liver cells is the earliest histological sign of the toxic effect on animals and that this action is probably the only mechanism of action of the *Microcystis* toxin. Immediate disruption of the cell membrane permeability also suggested that protein synthesis is not involved in the cytolytic action of the toxin.

The pattern of early disintegration caused by the toxin was found to be different from the effects of heavy metal and pesticide toxin effects that caused gradual rounding of the cells and death.

The researchers also concluded that the temperature dependency of the toxic effect suggested that the toxin may be specific to warm-blooded animals. The absence of any correlation between the haemolysis tests, mouse toxicity and cell culture tests, suggested that these assays measured different components, and that the ratio of the components are not the same for all strains of *M. aeruginosa*. The observation that various compounds with different molecular weights, physical properties and amino acid composition exist substantiates this. Grabow et al. (1982) correctly warned that the toxicity noted in their study might have been due to the combined effects of different compounds.

(see Supporting Literature)

Effect of growth conditions on toxicity and chemical composition of the toxin of *Microcystis aeruginosa* (van der Westhuizen 1984; van der Westhuizen and Eloff 1982, 1985; van der Westhuizen et al. 1986).

Van der Westhuizen investigated, under laboratory conditions, the role of various growth parameters, namely culture age, temperature, irradiance and pH, on the toxicity of *M. aeruginosa*, in an attempt to determine how they influenced the chemical composition and concentration of the toxin. He further wished to endeavour to explain of variability in toxicity and to define optimal growth conditions for production of toxins for subsequent chemical analysis. Van der Westhuizen's study was divided into two main areas, namely the influence of growth conditions on (a) cell growth and toxicity, and (b) on the chemical composition of toxin produced by UV-006. He drew the following conclusions from his work:

(a) Influence of growth conditions on cell growth and toxicity

Effect of culture age on growth and toxicity:

Toxicity per cell mass of UV-006 and UV-010 increased during the exponential growth phase to a maximum (LD_{50} = 18 $mg.kg^{-1}$) at the start of the stationary phase, before declining rapidly. This supported the finding of Paul Gorham (1962, cited in van der Westhuizen, 1984) that production of the toxin declines when growth ceases. Decreasing toxicity in the stationary phase is generally attributed to the release of the toxin into the cell medium through cell lysis.

Effect of pH (CO_2 concentration) of the medium on growth and toxicity:

pH was controlled by CO_2 addition and, therefore, the effects could be ascribed either pH or CO_2 concentration. The highest growth rate of UV-006 occurred at

pH 9, and highest levels of toxicity occurred at pH values above and below pH 8. pH could not be correlated with maximum growth rates, suggesting that growth-inhibiting conditions under certain pH conditions promoted toxin production. The reasons for this could not be determined, but van der Westhuizen suggested that toxin production might be related to stress conditions at higher and lower pH levels.

Effect of temperature on growth and toxicity:

Temperature was found to have the most pronounced effect on toxicity. Highest growth rate (t_d = 1.23 d) was obtained at 32°C, while the highest toxicity was found at 20°C (LD_{50} = 25.4 $mg.kg^{-1}$), but reduced at temperatures in excess of 28°C. Toxicity was 1.6 and 4 times less in cells cultured at 32 and 36°C than cells cultured at 28°C, suggesting that highest growth rate is not correlated with highest toxicity. This could not be attributed to leaching of toxins into the medium since cells were actively growing, but van der Westhuizen (1984) considered the decreased toxin production to be possibly related to decreased stress levels at temperatures above 20°C.

Effect of irradiance level on growth and toxicity:

The level of irradiance levels was not found to have any significant effect on toxicity. Toxicity levels were lowest at low irradiance, increased through intermediate and declined thereafter at the highest irradiance levels. Maximum toxicity was not associated with the highest irradiance-driven growth rate.

Van der Westhuizen (1984) postulated that a well aerated culture of *Microcystis aeruginosa* (UV-006) grown at pH 9.5, 20-24°C, 145 $\mu mol.photons.m^{-2}.s^{-1}$ and harvested during the late logarithmic growth phase should supply the maximum quantity of toxin per unit time.

(b) <u>Influence of growth conditions on the chemical composition of toxin from UV-006</u>

Influence of different growth conditions of chemical composition of M. UV-006 toxin

In this further study, van der Westhuizen determined the amino acid composition of the toxic peptides using gas chromatography. This revealed that 90% of the toxins were comprised of peptides labelled as ‘A’ and ‘B’ by van der Westhuizen (see table below) (assuming that the toxins consisted of peptides A and B only). The relative amino acid content of these peptides, differing by only the

addition of one other amino acid, is summarised in Table 1.

Table 1:

Relative amino acid content of toxins (peptides) H (A) and J (B).			
Amino Acids		**Relative mole % ( ) = mole ratio**	
		H (A)*	**J(B)***
ALA	(Alanine)	23.0 (1.1)	33.8 (1.80
LEU	(Leucine)	26.3 (1.3)	24.5 (1.3)
β-CH_3-ASP	(β-CH_3 aspartic acid)	20.7 (1.0)	19.0 (1.0)
GLU	(Glutamic acid)	23.6 (1.1)	22.1 (1.2)
ARG	(Arginine)	6.30 (0.3)	
* The labels 'H' and 'J' are referred to as A and B in subsequent discussions.			

Effect of culture age:

Young cells were found to contain less of peptide A as compared with mature cells - toxicity increased with culture age from the 4th to the 9th day, primarily due to an increase in the concentration of toxin A. Toxicity decreased on the 14th day due to an equal decrease in toxins A and B, and thereafter the composition of A and B remained unchanged. Declining growth, possibly due to die-off and consequent leaching of the toxin into the medium, occurred on the 14th day.

Effect of temperature:

Temperature-changes were found to induce variations in both the concentration and peptide composition of the toxin. A third toxic peptide (C) was discovered in higher concentrations than either peptides A or B at 16°C. Peptide C was suspected of containing aspartic acid rather than β-methylaspartic acid. Small amounts of phenylalanine and arginine were detected in peptide C, as well as alanine (23%), leucine (26%), aspartic acid (23%) and glutamic acid (27%). The percentage content of peptide A increased between 16°C and 36°C, while overall toxicity decreased sharply – this being due to a decrease in the concentration of peptides A and B. Peptide C disappeared gradually at higher temperatures, this ascribed by van der Westhuizen as a consequence of reduced synthesis or increased decomposition, rather than leaching since cells were still growing after the growth phase.

Effect of irradiance:

Increased levels of toxicity at lower irradiance were attributed to an increase in the concentration of peptide B. The concentration of peptide A increased at higher irradiance levels. At still higher levels, a reduction in overall toxicity was due to an equal decrease in the concentrations of both peptides A and B.

Effect of pH (CO2 concentration) of the culture medium:

Total toxicity declined over the pH range 6.5 to 8 due to a rapid decrease in the concentration of peptide B. Increased overall toxicity was detected at pH 10.5, and found to be due to an increase in the concentrations of both peptides.

Amino acid composition:

Amino acid composition of the peptides A and B was found to remain unchanged at differing growth conditions, indicating that changes in toxicity were related primarily to the concentration of the peptides.

Derivation of a partial structure for microcystin-LA (Santikarn et al. 1983)

Working on the material isolated from Witbank Dam (see Botes et al. 1982c), Sitthivet Santikarn, a member of the NCRL research team later led by Dawie Botes (see below), determined a partial structure for what was then known as Toxin BE-4, later to be designated cyanoginosin (= microcystin) –LA (Santikarn et al. 1983).

The structure of Cyanoginosin-LA, and –LR, -YR, -YA & -YM (Botes et al. 1984 & 1985; Botes, 1986).

At the time of this definitive work by Dawie Botes and his colleagues, other laboratories throughout the world had, for more than three decades, been attempting to characterize the toxic metabolites isolated from blooms of *Microcystis*. Botes' laboratory had previously reported on a partial structure for one of the toxins, and the N-methyldehydroalanine (Mhda) residue had been tentatively positioned as an exocyclic branch of the cyclohexapeptide (Santikarn et al. 1983). In their 1984 paper, Botes and co-workers corrected this tentative placement, and established the position of the Mhda moiety with a monocyclic heptapeptide structure for cyanoginosin-LA. They also proposed use of the term cyanoginosin and a two-letter suffix, as opposed to the previously used designations of microcystin or aeruginosin. Cyanoginosin had an LD_{50} of 0.05 μg g^{-1}, and a molecular weight of 909.

In subsequent work, this group of researchers derived full or partial structures for four additional toxic analogues, viz. cyanoginosin (= microcystin) –LA, -YR, -YA and –YM.

Preparation of antibodies to cyanoginosin-LA (Kfir et al. 1985)

Due to their very low molecular weights, the ability of microcystin-type toxins to induce immune responses was deemed to be very low. Based on the fact that immunological studies could assist in identifying the organ or cells targeted by the toxins, Kfir and colleagues raised antibodies against cyanoginosin-LA using mice. They were able to purify and characterize the resultant monoclonal antibodies, and mooted the potential for use of the antibody material for studying both the biological activity of the toxins, and their detection in water.

Environmental factors affecting the production of peptide toxins by Microcystis aeruginosa (Wicks and Thiel, 1990).

Following the formulation of an analytical procedure that allowed detection and resolution of a range of microcystin variants (Gathercole and Thiel, 1988), Richard Wicks and Pieter Thiel monitored six microcystins in Hartbeespoort Dam over a period of two and a half years. They found that the production of the toxins was both positively and strongly correlated with primary production, solar radiation, water temperature, pH and oxygen saturation. No strong relationships were found that linked toxin concentrations and the availability or concentration of organic or inorganic compounds. Wicks and Thiel concluded that the specific rate of photosynthesis of Microcystis aeruginosa was closely linked to the concentration of peptide toxins extracted from algal scums. They concluded that studies conducted in the laboratory could provide an indication of those environmental factors likely to influence cyanobacterial toxin production in naturally occurring algal populations.

Toxic and non-toxic variants of Microcystis aeruginosa (Eloff and van der Westhuizen, (1981)

A number of studies and observations have alluded to the fact that toxic and non-toxic variants of *M. aeruginosa* exist. Some, alluded to in this review, made this association in relation to distinct aerotypical variation in colony structure, e.g. the forma *aeruginosa* vs forma *flos-aquae* variants of *Microcystis*.

Eloff and van der Westhuizen set out to test the hypotheses that the apparent existence of toxic vs non-toxic variants was due to:

- *Microcystis*- bacterial interactions;
- Environmental influences;
- Existence of different strain variants.

These researchers found that environmental factors only influenced toxicity in “toxic” variants, and “non-toxic” isolates lost the ability to form gas vacuoles. They concluded that the phenomenon was driven by the existence of different taxons.

6.2 Cyanobacterial ecology and physiology

The photo-oxidation of laboratory cultures of Microcystis under low light intensities (Eloff, 1978).

Abeliovich and Shilo (1972) postulated that sudden die-off of cyanobacterial blooms in nature were the result of photo-oxidation. Allinson et al. (1937) found that laboratory cultures were much more sensitive to light intensities than natural blooms. Zehnder and Gorham (1960) noted that *Microcystis* growing under low light intensities lyse for no apparent reason. Also, laboratory cultures of *Microcystis* reach cell concentrations of 5×10^6 cells/mℓ, whereas in nature they can reach concentrations of 10^9 cells/mℓ.

(see Supporting Literature)

Photo-oxidation of cyanobacteria in natural conditions (Eloff et al. 1976)

Abeliovich and Shilo (1972) showed that CO_2 depletion sensitizes certain laboratory strains of cyanobacteria to photo-oxidative conditions. Photo-oxidation causes the activity of superoxide dismutase (SOD) to decrease (Abeliovich et al. 1974). Eloff et al. suspected that this could be the cause of sudden die-off observed in nature when blooms are at peak development and concentrated in scums. Under these conditions the cyanobacteria are subjected to high dissolved oxygen saturation (200–300%) and rapid CO_2 depletion. Their paper discusses the possible mechanisms underlying varying levels of resistance to photooxidation and the factors that may lead to photo-oxidative death. Samples were collected from Israeli fishponds using several cyanobacteria types (*Microcystis aeruginosa* strain 7005, *Synechococcus* sp. 6311, *Aphanocapsa* sp. 6714, *Anabaena* sp B-381, *Plectonema boryanum* 594, *Nostoc* sp, *Microcystis* sp NRC-1, *Microcystis* Bethulie strain).

In field photo-oxidation experiments dialysis tubing filled in the laboratory with cyanobacterial culture was suspended in pond water within 1 cm of the surface. Dark controls were wrapped in a black polythene sleeve. In the laboratory, photooxidation experiments, cell suspensions were placed in Klett test tubes and incubated. A range of parameters, namely: viable count, SOD activity (see above), protein content, turbidity, chlorophyll-*a* and phycocyanin contents were measured and correlated.

Different strains of cyanobacteria showed variable resistance to photo-oxidation. To test whether the self-shading effect protects the microorganisms against photo-oxidation, the effect of cell density on photo-oxidative sensitivity was

examined. It was noted that bleaching occurred in all the *Microcystis* strain 7005 cultures, but that it occurred less rapidly in the more concentrated suspensions. Culture age appeared to have no effect on sensitivity to photo-oxidation. It was noted that SOD levels dropped rapidly upon the onset of photooxidation in laboratory strains. Initial SOD levels in pond *Microcystis* were found to be low, but all initial SOD activity was retained after exposure to photo-oxidative conditions. The results suggested a difference in sensitivity to photo-oxidative conditions between laboratory strains and pond samples, emphasising the danger in extrapolating data from laboratory conditions to natural conditions and drawing ecological conclusions.

The effects of photo-oxidative conditions and chloramphenicol on SOD activity on pond *Microcystis* suspensions were investigated. SOD levels declined after 5 h in the suspensions exposed to light and chloramphenicol. Turbidity and cell protein decreased, lagging behind the SOD drop. Phase-constrast micrographs showed that these cells had bleached and lysed.

In several experiments it was demonstrated that the concentration, breaking up of the colonies and effecting the collapse of the gas vacuoles had no effect on the viable count or turbidity of the cell suspensions maintained under photo-oxidative conditions in the field. *(see Supporting Literature section)*

The fine structure of the cyanobacterium Microcystis aeruginosa Kutz. emend Elenkin in natural and controlled environments (Barlow, 1978)

Fine structure of cells grown under natural conditions

Barlow sampled several *Microcystis* waterblooms from six Transvaal impoundments (Hartbeespoort Dam, Rietvlei Dam, Roodeplaat Dam, Buffelspoort Dam, New Doringpoort Dam and the Vaal Dam) for ultrastructural study. An electron microscope was used to study the cytological structures of *Microcystis* cells in an attempt to link season, locality and fine structure to toxicity.

Barlow found the colonial morphologies and cell diameters of *M. aeruginosa* forma *aeruginosa*, *M. aeruginosa* forma *flos-aquae and M. wesenbergii* easily distinguishable using a light microscope, but differences were less apparent at cytological level. *M. wesenbergii* was found to have prominent polyglucoside rods and scalloped L_{IV} of the cell wall (this was also found in *M. aeruginosa* forma *aeruginosa* from Buffelspoort Dam).

Barlow suggests, however, that such structures may depend on metabolic conditions or be preparative artefacts. *M. aeruginosa* forma *aeruginosa* was ultrastructurally indistinguishable from forma *flos-aquae*. As a result, Barlow distinguishes only two species with certainty: *M. aeruginosa* and *M. wesenbergii*. Barlow did not associate *M. wesenbergii* with toxicity.

Although Barlow could not link the presence of toxicity to season or any one environmental factor he found that in Buffelspoort Dam, which had the lowest mean phosphorus and nitrogen concentrations, was never found to have a toxic bloom. Barlow suggested that eutrophic conditions (high phosphorous and nitrogen concentrations) might be important for toxin induction.

Toxicity was usually associated with *M. aeruginosa* f. *aeruginosa*, but this form was not always toxic. Toxic *M. aeruginosa* f. *aeruginosa* could be distinguished from non-toxic *M. aeruginosa* f. *aeruginosa* by the presence of a prominent sheath composed of globular acid mucopolysaccharide with fibrils condensed into striae. Some toxic *M. aeruginosa* f. *aeruginosa* cells were found to possess microvilli-like structures, membranous whorls and crystal lattices on L_{III} and L_{IV} of the cell wall. Barlow suggested that the whorls may be implicated in toxin production, while the lattices may be the crystallized protein structures of the toxin itself or viral particles involved in toxin induction. Notwithstanding this, the almost consistent presence of toxicity in *M. aeruginosa* forma *aeruginosa*, as opposed to its absence in aggregations of *M. aeruginosa* forma *flos-aquae*, remains an extremely valuable distinguishing diagnostic feature (author's observation).

Other differences included variations in the type and number of storage granules (related perhaps to cell age or environmental differences) and thykaloid arrangement (possibly related to light intensity).

The effect of light intensity

The effect of light intensity on the fine structure of *M. aeruginosa* under laboratory conditions was investigated. The optimal growth rate for *M. aeruginosa* cells was 3 600 - 18 000 lux. Lag phases lasted approximately 5 d, followed by an 11 d period of exponential growth. Growth rate declined rapidly at light levels in excess of 18 000 lux.

Visual pigmentation and pigment ratios were found to change considerably at different light intensities. At 3 600 lux and lower, cultures were green for the duration of the experiment (28 d). At 5700 lux, cultures were yellow, at 18 000 lux they were orange. The ratio of

chlorophyll *a* to carotenoid, plotted against light intensity showed that as light intensity increased, carotenoid pigments increased relative to chlorophyll *a*. A reduction in this ratio occurred with ageing. Carotenoid pigments shield cells from detrimental light levels, preventing the destruction of chlorophyll *a* and the photo-oxidation of photosynthetic pigments (Abelovich and Shilo, 1972, cited in Eloff, 1978).

Gas vacuole content and thykaloid number were also found significantly affected by light intensity. Gas vacuole content increased as light intensity increased to 6 000 lux, thereafter decreasing between 6 000 and 8 000 lux. Waaland et al. (1971, cited in Eloff, 1978) suggested that the vesicles could act as light shields in addition to their possible buoyancy functions. The absence of gas vacuoles grown at low light intensities of 400 lux supports this observation.

The amount of thykaloid membranes in the cell was found to be directly proportional to the amount of chlorophyll *a*, suggesting that these were sites of photosynthesis. Light intensity affected thykaloid number and arrangement. Thykaloids numbered 25 - 30 units at light intensities of 3 600 lux and lower and were peripherally arranged, parallel to the cell wall and in stacks of 3 to 6. Thykaloid units were shorter and fewer at lower light intensities and were arranged peripherally, singly and inwardly oriented in relation to the cell wall. From this evidence Barlow (1978) suggests that *M. aeruginosa* is able to orient the thykaloids to maximise the amount of light available.

The effects of ageing included: slight decrease in the number of thykaloid units, decrease in polyhedral bodies and gas vacuole content and cytoplasmic shrinkage and an increase in the cyanophycin granules and lipid droplets.

Carotenoid composition as taxonomic character for Microcystis isolates (Smit et al. 1983)

Smit et al. (1983) emphasised the fact that cell size and colony habit had previously been the main taxonomic criteria to distinguish different *Microcystis* species. However, since both these characteristics are variable under different environmental conditions, they could not be relied upon as absolutes. The purpose of this study was to examine the pigment composition - the carotenoids being the main lipid pigments - in relation to their usefulness as taxonomic markers. Nineteen *Microcystis aeruginosa*, one *M. incerta* and one *Synechococcus* isolate were investigated.

β-carotene was the major carotenoid present and was found in all of the *Microcystis* isolates (concentration:

100–1 577 $\mu g.g^{-1}$ dry mass). Zeaxanthin concentrations were found to be very variable among the isolates (concentrations: 2-871 $\mu g.g^{-1}$). Echinone and myxoxanthophyll were found to be the second most abundant carotenoids present (concentrations: 475-1 399 $\mu g.g^{-1}$ and 1 740-2 954 $\mu g.g^{-1}$ respectively). These pigments were found to be absent from *M. incerta* and only low concentrations were found in the *Synechococcus* isolate. Canthaxanthin was found in eight of the isolates, while 3-hydroxy-echinone was found as a trace pigment and only one isolate had more than trace quantities of cryptoxanthin. 4-hydroxy-4-keto β carotene was identified in only three isolates. Caloxanthin and nostoxanthin were only identified in the *M. incerta* and the *Synechococcus* isolates.

The ratio of occurrence of the main carotenoids in *Synechoccus* and *M. incerta* was found to differ significantly from all other *M. aeruginosa* isolates, prompting Smit and co-workers to suggest that the carotenoid composition may have value for distinguishing between different genera.

Smit et al. (1983) found that there was little qualitative difference between the *M. aeruginosa* isolates, but quantitative differences did appear to be present. A clustering of the isolates into 7 groups was evident from the data. Apart from isolate UV-017, all of the isolates in groups III and IV were non-toxic, while all isolates in group I had a similar toxin composition that was different from all of the others. From these results, the researchers suggested that quantitative carotenoid composition might be taxonomically useful in studies of cyanobacteria.

The effect of phosphate concentration. (Barlow et al. 1977, 1979)

Barlow investigated the effect of changing levels of phosphate on the fine structure of *M. aeruginosa* cells in a laboratory culture.

Increased external phosphate concentration was found to result in faster initial growth rate, but lower final cell yield. Highest final yields were obtained from phosphate concentrations of 2 $mg.\ell^{-1}$. In the absence of phosphate, growth proceeded slowly for 8 days before ceasing. Phosphate starved cells showed a decrease in the number of thykaloids and a change from grouped peripherally arranged orientation to shorter, single and inwardly oriented units (similar to the symptoms displayed by cells under low light conditions). Cyanophycin granules and lipid droplets were only present in phosphate-starved cells.

Polyphosphate granules are indicative of 'luxury uptake' of phosphate (Shapiro, 1968, cited in Barlow, 1978) in conditions of unlimited available phosphorous. After introduction of cells into a new medium, the number of polyphosphate granules decreases before re-assimilation of granules occurs. This suggested to Barlow that stored polyphosphates needed to be used initially for physiological activation of the cells before external phosphate could be assimilated. After 20 d, the number of polyphosphate granules was the same in all cells irrespective of the concentration of external phosphate in the growth medium, suggesting that a critical concentration of polyphosphate could be stored. In the phosphate-starved cells, polyphosphate granules were still present after 20 d, suggesting that these cells were senescent and incapable of metabolizing the stored polyphosphate.

Barlow (1978) made a distinction between ageing due to nutrient depletion (when cells could be re-stimulated by addition of fresh media), and senescence due to phosphate starvation, when restimulation of growth may not have been possible.

Barlow (1978) suggested that the reason for *M. aeruginosa's* success in eutrophic waters was due to its ability to store phosphate during times of abundance, its ability to regulate its position in the water column and shield itself from harmful light intensities using gas vesicles, and its ability to orient itself its photosynthetic apparatus to maximise light availability. However, he pointed out that the fine structure of the cells could not be used to interpret the environment in which the cells were growing.

However, in a second series of experiments, Barlow and co-workers demonstrated ultrastructural differences in cells grown in the presence and absence of phosphate (Barlow et al. 1979).

Barlow et al. (1979) cite Brown (1974) as stating that the increase and decrease of external phosphorous concentration enhanced the toxicity of *Microcystis aeruginosa*. Luxury uptake of phosphorous occurs when phosphorous is abundant in the medium (Shapiro 1968, cited in Barlow et al. 1979) and levels of acid soluble and insoluble polyphosphates increase to a critical concentration in the cell (Sicko-Goad and Jensen 1976, cited in Barlow, 1979). Luxury uptake was found to be exaggerated in cells initially starved of phosphate (Shapiro 1967, cited in Barlow).

Cultures grown at four phosphate concentrations (0, 2, 4 and 8 $mg.\ell^{-1}$ $HPO_4^{=}$) were used. Cells grown in the phosphate-starved medium showed reduced growth rates after approximately 96 h and growth ceased after 8 d. Barlow et al. (1978) suggested that the exponential growth rate for the first four days was due

to the use of stored polyphosphate. Other cultures grown in the presence of external phosphate showed similar growth curves.

No ultrastructural differences could be found in the cells grown in the presence of external phosphate - no statistical difference was found in the number of thykaloid units or polyphosphate granules (Table 2). In phosphate starved cells, polyphosphate deposits decreased after 20 d.

Table 2:

Ultrastructural differences between cells grown in the presence of phosphate and those grown in a phosphate-starved medium (compiled from Barlow et al. 1979).

	Phosphate media	**Phosphate-starved medium**
Thykaloid units	Peripherally oriented in groups parallel to cell wall	reduced and oriented perpendicular to cell wall
Polyhedral bodies	Present in centroplasm	reduced or lost
Cyanophycin granules	Not observed	Increased
Polyglucoside rods	Restricted to interthykaloid regions of the cytoplasm	increased and scattered throughout cytoplasm
Polyphosphate granules	Three sites of polyphosphate deposition: centroplasmic granules most evident, also fibrillar deposits and intrathykaloid deposits	reduced and then increased slightly to half the numbers in the control and only centroplasmic, fibrillar deposits observed

Simon (1971), cited in Barlow et al. (1979) found cyanophycin granules to be comprised of a proteinaceous reserve of aspartic acid and arginine copolymers and Stewart (1972) cited *ibid*. suggested that they may be involved in nitrogen metabolism. Hence, Barlow et al. (1979) suggests that they may play a role in storing proteins under unfavourable conditions (in this case, phosphate starvation).

Reduced thykaloid number suggests a decreased rate of photosynthesis in phosphate-starved cells. Allen (1968, cited in Barlow et al. 1979) found a direct correlation between chlorophyll-*a* content and the expanse of thykaloid membranes in *Anacystis nidulans*.

Polyhedral bodies are the site of the ribulose-1.5 diphosphate carboxylase enzyme (Stewart and Codd 1975 cited in Barlow et al. (1979). Their loss indicated a decline in photosynthetic activity.

Barlow et al. (1979) suggested that the initial decline in polyphosphate granule numbers observed at all phosphate concentrations indicates that stored material is used for metabolism. Phosphate starvation would have been induced by the 24 h rinse period conducted on all the

cultures at the start of the experiment. The period of maximum restorage was approximately 8 d. The slight increase in polyphosphate after the initial decrease observed in the phosphate-starved cells, suggests that restorage of phosphate had been derived from other sources in the cell such as RNA (Harold 1960 cited in Barlow et al. 1979).

Barlow et al. (1979) hypothesised that the ability of *Microcystis aeruginosa* to continue growth for 8 d after phosphate starvation using internal reserves may partially explain its success in producing waterblooms.

(see *Supporting Literature*)

The release of nannocytes during the growth cycle of <u>*Microcystis*</u> (Pretorius et al. 1977; Pretorius and Eloff, 1977)

Pretorius et al. observed small bodies forming within *Microcystis* cells that were similar to those observed by Canabaeus (1929), and which she referred to as 'nannocytes'. It was suggested that these nannocytes may play a role in reproduction as endospores, possibly enabling *Microcystis* to endure unfavourable environmental conditions.

Microcytis aeruginosa (UV-007 – a strain of *Microcystis* that has become something of an icon in cyanobacterial research, and has travelled around the world from the University of the Free State (UV = Universiteit Vrystaat) to several research laboratories – see elsewhere in this review.) was grown in BG 11 medium at two light intensities: 9.5 and 21 $\mu Einsteins.m^{-2}.sec^{-1}$. At 9.5 $\mu Einsteins.m^{-2} sec^{-1}$ the logarithmic growth phase of the culture lasted from day 0-10, while the stationary phase began at day 30. At 21 $\mu Einsteins.m^{-2}.sec^{-1}$ the logarithmic growth phase continued to day 6 followed by a death phase due to the stress effect of high light intensity. Cell numbers oscillated until day *ca* 20, whereafter a new growth was entered that was more rapid than the first.

The hypothesis that this second growth phase was due to the release of nannocytes during or just prior to the death phase was not confirmed - nannocytes were found to form throughout the growth cycle and not mainly under light stress conditions. Thus while nannocytes play a role in reproduction, they do not function as means of surviving unfavourable environmental conditions. Nannocytes were released by rupturing the cell wall, the cells becoming swollen before release, suggesting a possible decrease in the osmotic potential of the cell that triggered the release.

Effect of lethal concentrations of copper on the ultrastructure and growth of Microcystis (Verhoeven and Eloff, 1979)

Verhoeven and Eloff (1979) reported that copper was an effective algicide that was used in natural waters to control blue-green algae. *Microcystis aeruginosa* Berkeley strain 7005 (UV-007) and *M. aeruginosa* isolated from the Hartbeespoort Dam (UV-006) were used to test the effects of copper on the ultrastracture of cells. Copper sulphate was added once cultures had been grown to a turbidity of 80, 150, 220, 310 Klett units to give concentrations of 0.3, 0.4, 0.5, 0.6 and 0.7 ppm Cu^{2+}. It was found that toxicity of the copper depended on cell concentration. Cell concentrations of 1.8×10^{7} cells.mℓ^{-1} (148 Klett units), 0.3 and 0.4 ppm Cu^{2+} decreased growth rate temporarily, whereas 0.5 ppm Cu^{2+} was lethal.

Copper was found to decrease the electron-density of the nucleoplasm as well as cause aggregation of the DNA fibrils. Membrane-bounded inclusions and polyphosphate bodies disappeared, and thykaloids were present as short membrane structures. Carboxysomes remained present.

Cyanobacterial biocontrols (Scott and Chutter, 1981).

As part of an investigation reviewing controls for cyanobacterial blooms, Willem Scott and Mark Chutter investigated possible biological agents capable of reducing algal populations in ponds

Viruses

Scott and Chutter suggest that viruses may be important in controlling blue-green algae (e.g., review by Padan and Shilo 1973). The first virus isolated that was capable of lysing a filamentous blue-green alga *Plectonema* sp., was isolated from an oxidation pond. The authors assumed that viruses were not important in controlling eukaryotic algae in large cultures, this on the basis of there being no apparent evidence to the contrary (e.g. reviews by Lemke 1976; Hoffman and Stanker 1976; Dodds 1979).

Bacteria

Stewart and Brown (1969, 1971) first reported a myxobacterium capable of lysing freshwater algae. Bacteria are generally aerobic, flagellate, gram-negative rods. Scott and Chutter suggest that myxobacteria are more important than viruses in controlling algal populations

than viruses since they are less host-specific.

Fungi

Fungal parasites of freshwater algae are referred to 'chytrids', and belonging to the families Phlyctidiaceae, Rhizidiaceae and Chytridiaceae. Pioneering work was conducted by Lund (1957) and Canter (1950, 1951, 1954) in the English Lake District. Up to 70% of the individuals in an algal population could be infected by fungal parasites. A large proportion of chytrids are host-specific, suggesting that, in some instances, they may prevent 'desirable' species from growing while allowing 'less desirable' species to proliferate.

Algae

Some Chrysophyta and Pyrrophyta are capable of phagotrophic nutrition. In some cases, smaller algae such as *Chlorella* may be ingested. Cole and Wynne (cited in Scott and Chutter, 1981) noted that when the chrysophyte *Ochromonas danica* was mixed in culture with *Microcystis aeruginosa*, the *M. aeruginos*a numbers were reduced by 30% in 10 min. as a result of ingestion by *Ochromonas*.

Protozoa

Bader, Tsuchiya and Frederickson (1976a & b, cited in Scott and Chutter) noted that when the ciliate *Colpoda steinii* was added to the blue-green alga *Anacystis nidulans*, the ciliate could double every 4.3 hours – it was estimated that approximately 1 000 algal cells were needed to produce one ciliate. Ciliates formed cysts as soon as the algal population was depleted. Lund and Canter (1968) reported a 99% reduction in the algal population within 7 to 14 days in the Lake District as a consequence of protozoan predation.

Zooplankton

Uhlmann (1971) noted that zooplankton grazing could completely suppress algal growth in sewage lagoons and hyperfertilised ponds – 5 mass units of algae are required to produce 1 mass unit of *Daphnia*. Uhlmann found that the Volterra model does not hold for the complex grazing relations between *Daphnia* and *Chlorella*. Gliwicz and Hillbricht-Ilkowska (1972) reported that plankton feeders which filter larger particles (10–15 μm) occurred in eutrophic waters, while smaller filter feeders (filtering 3–5 μm nanoplankton) occurred in oligotrophic waters. Rotifers were found to predominate in algal ponds.

Others

Little information is available on grazing by benthic herbivores. The tadpoles of *Xenopus laevis* are filter feeders of planktonic algae, and where they occur in large numbers they were assumed by Chutter and Scott to have a considerable impact on algal populations. Their contribution to the nitrogen budget in the form of ammonia is probably also assumed to be significant.

(*see Supporting Literature*)

Katagnymene mucigera, a new planktonic blue-green alga from South Africa (Compére et al. 1979).

A filamentous planktonic cyanophyte was found in a 1976 study of Lindleyspoort Reservoir in the (then) Transvaal province, South Africa. The sample was identified as *Oscillatoria ornata* Gom. f. *planctonica* Elenk. A specimen of the sample was sent to Trebon for a comparison with Elenkin's original description. The alga from Lindleyspoort corresponded well with *Oscillatoria ornata* f. *planctonica* described by Elenkin (1949) but differed by having straight or flexuose trichomes, whereas those described by Elenkin are often spirally coiled at the extremities. The alga was then compared to several other algal species from around the world including: *Oscillatoria lacustris* (Kleb); *T. iwanoffianum* Nygaard; *Oscillatoria lacustris* var. *solitaria* Behre. Compére *et. al* decided to revive the old genus *Katagnymene* and place the South African form as a new species in this genus. The South African species differed mainly by having relatively longer cells and was named *mucigera*.

K. mucigera was also prominent in the Spitskop Dam on the Harts River, southern Transvaal, and Harding recorded this species from a small western Cape irrigation dam during the early 1990s.

An unusual occurrence of biogenic (cyanobacterial) carbonate sediments for a sinkhole lake in the western Transvaal, South Africa (Gow, 1981).

Bottom samples to depths of 60m were collected from Wondergat, a sinkhole near Mafikeng, Western Transvaal. Several forms of biologically-accreted calcium carbonate deposits up to a centimetre thick were recovered. The complete encrustation of the rock suggested that they accreted sub-aquaeously *in situ*. Gow describes the structure as 'cauliflower like' in shape, with evidence of internal banding. A second structure had a discoidal shape, bearing a resemblance to fungi, often with two lobes in close association. The presence of banding and

uniformity of shape suggested biological origin. Regularly distributed pits were evident on the uppermost surfaces. A third structure was identified and described as being white with low cohesion when dry, crumbling easily to a fine powder.

Gow suggested that the first two structures were stromatolites created by the deposition of mucilaginous sheaths by cyanobacteria. The identity of the third type of structure was less evident.

To investigate the nature of the deposits, encrustations were sectioned with a diamond saw, and polished on glass and prepared for microscopy. The powder was found to contain irregular rounded grains of similar size with numerous microtubules. Two stromatolites showed evidence of prokaryote unicells and filaments – no organic material had been preserved. No cellular structures could be observed, but uniformity of size and layered domed structure suggested typical stromatolites. The presence of microtubules in the powder suggested biological origin. The size of the powder grains approximated the size of small cyanobacterial colonies and Gow suggested that the microtubules represent the tracks of cyanobacteria as the carbonate was deposited.

The unicells in the stromatolites suggested a solitary coccoid cyanobacteria, whereas the filaments suggested filamentous cyanobacteria. Gow inferred that these structures were incidental and that the more numerous microtubules were the main carbonate accreting agents.

Cyanobacteria in the sandstone cliffs of the Golden Gate National Park (Wessels and Büdel, 1995).

Cyanobacteria are known to occur from just about any type of environment. They are also known to contribute to the processes of weathering in sandstone. Wessels and Büdel discovered a cryptoendolithic community of *Chroococcidiopsis* in Clarens sandstone in the north-eastern Orange Free State. Such communities contribute to soil formation, and the formation of hollows. The sandstone also provides localised areas in which organic material is protected from harsh environmental (surface) conditions, including fires. The algal cells live between the soil grains at what appear to be optimal, light-dependent depths.

6.3 Isolation and cultivation under laboratory conditions

Isolation methods

The isolation of Microcystis (Scott, 1974)

Scott attempted the isolation of three types of *Microcystis* from dams in the Transvaal to aid future studies on bloom formation, toxicity and taxonomy. These dams contained *M. aeruginosa* forma *flos-aquae*, *M. aeruginosa* forma *aeruginosa* (which may have been identical to *M. toxica* Stephens. described by Stephens in 1949) and a third that was found to be the dominant form in the Rietvlei dam and identified by Komarek (1958, cited in Scott, 1974) as *M. wesenbergii*.

Reynolds (1972), cited in Scott (1974) noted that differences in colony size and gas vacuole content - which is determined by the chemical environment (Canabaeus 1929, cited in Scott 1974), light intensity (Lemmermann 1910, cited in Scott 1974) and age of the cells (Reynolds 1972, *ibid*.) led to variations in buoyancy between species. Buoyancy may be used to separate out different species of blue-green algae. However, problems arise when trying to isolate *Microcystis* from contaminants such as other algae and bacteria embedded in the mucilage of the colony.

Scott homogenised colonies of the various species in a test-tube using a glass piston to break the material into smaller units and separate out any contaminating bacteria or algae. Thereafter, buoyant cells were separated from non-buoyant cells by centrifugation for one hour at 4 500 x *g*. The buoyant material was removed and inoculated into sterile BG-11 medium with 0.1 $mg.m\ell^{-1}$ Actidione to inhibit the growth of eukaryotic algae and the culture was incubated. Homogenisation and centrifugation was repeated several times and a unialgal culture of the three types of *Microcystis* was obtained. The procedure was, however, not successful in removing contaminating bacteria.

Microcystis in pure culture (Scott, 1976, 1980)

Scott (1976) subsequently reported success in isolating *Microcystis* from the contaminating bacteria in the mucilage surrounding the cells. This was achieved by controlled homogenisation to yield single cells of both *Microcystis* and bacteria. The homogenate was then repeatedly filtered through a polycarbonate filter with a pore size large enough to retain *Microcystis* cells, but which allowed most other contaminating bacteria to pass through. The filters were then placed on nutrient medium. Scott noted that the procedure was not always

successful as most of the *Microcystis* cells lost their viability after treatment (see also Pretorius 1977). It is not known whether Scott attempted the inclusion of anti-bacterial compounds (e.g. Actidione or cycloheximide) on the assumption that these may not have inhibited the procaryotic cyanobacteria.

Growth on Agar

Growth of Microcystis aeruginosa on agar media (Pretorius, 1977)

By inoculating several agar media with decreasing cell concentrations of *Microcystis* UV-001, UV-005 and UV-007, Pretorius (1977) was able to confirm that culture viability was dependent upon the cell concentration of the initial inoculum – i.e., that the number of colonies which were obtained was directly proportional to the size of the initial inoculum. Table 3 shows the times when the cultures from different initial inocula became visible. The results shown in the table suggest that UV-001 and UV-005 are not subject to the same inhibitory factors as UV-007 – the latter apparently needing considerably larger inocula to form a viable culture. Due to the greater sensitivity of UV-007 to inhibitory factors, it was decided that the strain should be used in all subsequent experiments.

Table 3:.

Times after which dilution's of UV-001, UV-005 and UV-007 formed visible colonies.			
	Time (days)		
	13	24	34
Culture	**Dilution**		
UV-001	10^{-1}	10^{-4}	10^{-6}
UV-005	10^{-5}	10^{-8}	10^{-8}
UV-007	-	-	10^{-3}

Pretorius investigated algal growth on two agar media, one pure (Ion-agar) and one less-pure (Merck Agar Agar). The fastest growth was observed on the pure agar medium, suggesting that impurities have an inhibitory effect on growth. In each treatment, agar media with different concentrations were used (3%, 2.5%, 2%, 1.5%, 1% and 0.5%). Those treatments using higher agar concentrations displayed less growth than those with lower concentrations. This may have been attributed to the lower humidity levels in media with higher agar concentrations.

The effect of media components, sterilization and ageing

The effect of ageing on the physical and chemical characteristics of the medium after sterilization was investigated since it had been observed that UV-007 displayed poor growth in growth media older than 3 days. One of the problems that had been identified in the laboratory culture of blue-green algae was the formation of a white or yellow precipitate on the medium after approximately 72 h, whereafter the capacity of the medium to support growth was reduced. Iron has been identified as a principal problem in the growth of algae at a pH above 7 when using the N-11 medium (Zender and Gorham, 1960, cited in Pretorius, 1977) - primarily because it is insoluble and may be the cause of an iron deficit. Other elements which may also precipitate in the algal medium include Mg^{2+}, Ca^{2+} and PO_4^{3-}.

To determine whether different oxidation states of Fe may have different effects on precipitation of Fe in blue-green algal cultures, the replacement of $Fe_2(SO_4)_3$ (Fe 3+) with Fe SO_4 (Fe 2+) in the growth medium N-11 was investigated. In both cases a white precipitate formed in the medium after preparation. After 72 h, a yellow precipitate formed in the $Fe_2(SO_4)_3$ medium, but not in the Fe SO_4 medium. The precipitate in the Fe SO_4 medium had little influence on the growth of UV-007, suggesting that the precipitated elements were in equilibrium with the solution. On the basis of these observations, it was concluded that the Fe SO_4 medium was more suitable to sustain the growth of UV-007 than the $Fe_2(SO_4)_3$ medium and was used in all subsequent experiments.

The effect of the timing of inoculation before and after sterilization was also investigated and it was determined that the duration of time before sterilization had no influence on the growth of UV-007, while the duration of time after sterilization did have an effect. The longer the duration of time after sterilization, the more growth was limited and the lag phase was prolonged. The best growth was obtained by inoculating 72 h after sterilization.

Experiments confirmed the presence of peroxide (H_2O_2) in the medium, but Pretorius (1977) was not able to determine whether this had an inhibitory effect on the growth of individual cells.

Aspects of the growth of Microcystis aeruginosa on agar (Pretorius and Eloff, 1981)

Pretorius and Eloff (1981) noted that less research has been done on *Microcystis* than on *Synechococcus* or *Anabaena* since axenic cultures of these algae were more readily grown on an agar medium and their growth rates are faster than

Microcystis - colonies of *Synechococcus* become visible after 5 d (Allen, 1968, cited in Pretorius and Eloff, 1981) whereas *Microcystis* colonies only become visible after 10 d.

They also encountered variable growth on different types and concentrations of the growth mediums - the highest growth rates were obtained on 'Difco' Bacto agar, and where lower agar concentrations were used. These lower growth rates on more concentrated agar media, they suggested, could be attributable to the susceptibility of *Microcystis* to growth inhibiting factors in the agar medium.

Effect of light

The effect of light intensity (Pretorius, 1977)

The effect of light intensity on algal cultures grown in liquid cultures was investigated at three intensities (8, 17 and 30 μEinsteins.m^{-2}.s^{-1}). It was found that at high light intensities (17 and 30 μEinsteins.m^{-2}.s^{-1}) initial growth was rapid, followed by a stationary phase and finally a die-off phase. At low light intensities (8 μEinsteins.m^{-2}.s^{-1}) and despite a slow growth rate, no die-off was observed. Light intensities of 8 μEinsteins.m^{-2}.s^{-1} were found to be optimal and used in all subsequent experiments.

The influence of light intensity on the growth of different Microcystis isolates (Krüger and Eloff, 1977)

Krüger and Eloff stressed that an understanding of the regulatory role played by light in the growth and death of *Microcystis* species was important for controlling their growth in the environment. They grew seven different *Microcystis* isolates at light intensities varying between 4.5-38 μEinsteins.m^{-2}.s^{-1}. By correlating cell death with turbidity they were able to use turbidity as a measure of growth. Growth was associated with increased turbidity, whereas the death phase was associated with decreased turbidity due to cell bleaching and lysis.

Microcystis cultures were found to be sensitive to higher light intensities. Cultures grown at high light intensities (>14 μEinsteins.m^{-2}.s^{-1}) had high growth rates but maintained these over shorter periods and cells began to die off after 5 to 15 d. The maximum turbidity values (i.e. low maximum cell numbers) for these cultures was low (89 Klett units at 36 μEinsteins.m^{-2}.s^{-1} after 6 d).

Cultures grown at low light intensities had lower growth rates and took longer to achieve maximum turbidity values (514 Klett units at 9.3 μEinsteins.m^{-2}.s^{-1} after 47 d).

Krüger and Eloff (1977) also suggested, but did not investigate, that early initiation of the death phase in cultures grown at higher light intensities may have been attributable to

(i) increase in pH;
(ii) decrease in CO2 concentration due to photosynthesis;
(iii) photooxidative bleaching of cell pigments and
(iv) growth factors formed under stress light conditions.

Microcystis aeruginosa (UV-004) from Hendrik Verwoerd Dam was found to have the highest specific growth rate (= 0.7 d^{-1}, t_d = 0.99 d), while *Microcystis aeruginosa* (UV-006) from the Hartbeespoort Dam had the lowest (= 0.52 d^{-1}, t_d = 1.33 d).

To measure sensitivity to high light intensity, the light intensity at which light saturation was reached was determined. Optimal light intensity was defined as the light intensity at which the highest yield was obtained for a particular culture (i.e. highest turbidity value). The highest turbidity was calculated as $\log_2$ of the ratio of final turbidity to initial turbidity (T_t /T_0) to make comparisons between the cultures possible.

Optimal light intensity values measured over a period of 22 d for all *Microcystis* cultures ranged between 7.5 and 22 μEinsteins.m^{-2}.s^{-1}. Specific growth rate at optimal light intensities were lowest for *Microcystis* (UV-008) 0.14 d^{-1} and highest for *Microcystis incerta* (UV-003) was 1.32 d^{-1}. Maximum specific growth rate varied between 0.52 d^{-1} and 1.32 d^{-1} for *Microcystis* (UV-003) (Krüger and Eloff 1977).

Krüger and Eloff (1977) found that *Microcystis* (UV-007) grown at low light intensities (below 10 μEinsteins.m^{-2}.sec^{-1}) were still growing after three months, suggesting the suitability of this species for stock cultures. No correlation was found between light intensity and toxicity. The growth rate of the toxic *Microcystis* (UV-001) was found to be higher than the growth rate of the other toxic species *Microcystis* (UV-006) making *Microcystis* (UV-001) more suitable for mass cultivation.

The interaction between cell density of Microcystis batch cultures and light induced stress conditions (Krüger and Eloff, 1979)

Krüger and Eloff (1979) also investigated whether the growth inhibition of *Microcystis* at light intensities > 10 μEinsteins.m^{-2}.sec^{-1} may have been due to the accumulation or depletion of some substance in the growth medium, rather than the direct effect of light *per se*. *Microcystis aeruginosa* (Berkeley strain 7005) referred to as *Microcystis* UV-007 was used in a culture of BG 11. Four different inoculations were prepared at different cell concentrations and incubated for 11 days. The concentrations after this period were calculated from turbidity values at 3.8, 6.8, 14.5, 21.6 × 10^6. The cells were then subjected to light intensities of 20, 29 and 39 and 8 μEinsteins.m^{-2} .sec^{-1} (the lowest value as a control).

The results from the figures suggest:

(i) higher cell concentrations exhibited growth inhibition sooner than lower cell concentrations at all light intensities and;

(ii) cultures grown at higher light intensities exhibited growth inhibition sooner than lower light intensities at all cell concentrations.

The performance (growth) of a particular treatment was represented as the turbidity values of the control ($T_{contr.}$) for all time intervals for the duration of the experiment, subtracted from the corresponding turbidity values of the respective light treatments (T_{exp}) and added: $\sum(T_{contr} - T_{exp})$. The same was done for cell concentration treatments. From these observations, it was clear that inhibition of growth increases with increasing light intensity and cell concentration.

Krüger and Eloff (1979) cite Eloff et al. (1976) as reporting that bleaching and inhibition of growth occurred at all cell concentrations, but was less rapid in more concentrated suspensions. However, in their experiments, Eloff et al. kept pH, CO_2 and O_2 and growth medium concentrations in equilibrium with the environment through dialysis tubing in which the cultures were kept.

The discrepancy between the above observations of Eloff et al. (1976) and those of Krüger and Eloff suggest that it is not light *per se* which is causing growth inhibition, but the accumulation or inhibition of some substance in the culture medium which leads to light-induced inhibition and the enhancement of

inhibition due to an increase in cell concentration.

Krüger and Eloff suggested that factors leading to light-induced inhibition may include: increased O_2, decreased CO_2 or pH.

Morphological and ultrastructural changes in Microcystis grown at different irradiance levels (Krüger, 1978).

Krüger revealed that age of the culture and irradiance level influenced the size of the cells and that cell size can be correlated with toxicity. The influence of ageing and light intensity on cell morphology was also investigated using cultures of UV-007.

Cell size and size distribution

This study showed there was:

(i) an increase in the variation of cell size at different irradiance levels;

(ii) an increase in cell volume;

(iii) an increase in the rate at which cell volume increased concomitant with increasing light intensity. The researchers observed an initial decrease in cell volume at all light intensities. Thereafter cell volume increased, reaching a maximum volume dependant upon light intensity - the higher the light intensity, the sooner the maximum cell volume was reached. Maximum cell volume (139 μ^3) was achieved at 17 μEinsteins.m^{-2}.s^{-1} after 16 d growth.

Krüger did not ascribe the changes in cell volume and the variation of volume directly to differences in light intensity, but to the development of stress conditions in the medium due to different growth rates. The stress conditions were believed to be created by depletion of CO_2 in the medium (findings elsewhere in Kruger's thesis support this conclusion where – e.g. 'Influence of aerating gas' where no increase in cell volume took place in cultures aerated with CO_2).

Krüger suggested that an increase in cell volume was caused by the inhibition of cell division and the accumulation of certain substances within the cell, suggesting that production of the toxin may also increase. Krüger also suggests that growth conditions should be taken into account when cell size is used as a criterion for identifying *Microcystis* strains. A narrow distribution curve and small cell volume could be regarded as being indicative of a healthy culture.

Changes in ultrastructure

No changes were found to take place in the ultrastructure of the cells in the control cultures grown at light intensities of 9.5 μEinsteins.m^{-2}.s^{-1}. No changes were found in cells grown at stress light intensity (24 μEinsteins.m^{-2}.s^{-1}) during the logarithmic growth phase. After the 6th day, the thykaloid lamellae showed some deterioration and a change in orientation. Lipid droplets, carboxysomes and poly-β-hydroxybutyric acid granules remained present and inter-thykaloidal pigmentation was still visible, suggesting a possible protective mechanism against high light intensity. 50% of the cells were dead after 8 d. Cell structure started to collapse, thykaloids were completely deteriorated and the remains of carboxysomes and lipids were faintly visible. 70% of the cells were dead by the 9th day.

Morphological changes in toxic and non-toxic Microcystis isolates at different irradiance levels (Krüger et al. 1981)

Krüger hypothesized that cell sizes amongst algae may be environmentally determined and thus may vary within specific taxa. The cell size of *Synechococcus lividus* had previously been found to be influenced by temperature, light intensity and dissolved solids (Kullberg 1977, cited in Krüger et al. 1981), while Krüger (1978) noted that irradiance and culture age both had significant impacts on cell size. Barlow (1978) found that toxicity varied according to cell size and that cell diameters of toxic *Microcystis* vary from 4.1 to 5.0 nm, whereas toxic *Microcystis* was found to be 4.8 nm. In this paper, Kruger and co-workers state that it is unclear whether differences in cell size and toxicity may be related to environmental factors, or whether this may be due to different *Microcystis* taxa being involved. They cite Toerien (1976) as stating that *M. aeruginosa* forma *aeruginosa* is toxic, whereas *M. aeruginosa* forma *flos-aquae* (Komarek, 1958) is non-toxic (see comments and observations elsewhere in this review). Since distinction based on colony formation is not possible for these two forms, cell size may be a suitable alternative. Krüger and co-workers tested the relationship between cell morphology, ageing and light intensity as well as die-off of *Microcystis* at light intensities >10 μEinsteins.m^{-2}.s^{-1}, as follows:

Two axenic cultures of *Microcystis* were used: *Microcystis aeruginosa* UV-007 (originally from the Gottingen culture collection 1450-1) which does not possess gas vacuoles and is non-toxic. It probably corresponds to *M. aeruginosa* forma *flos-aquae*. The second strain they used was *Microcystis aeruginosa* UV-006 (isolated from the Hartbeespoort Dam) which does

possess gas vacuoles and is toxic. This strain, they suggested, may correspond to *M. aeruginosa* forma *aeruginosa* (Komarek, 1958).

The cultures were grown at seven different light intensities (ranging from 4.5 to 40 μEinsteins.m^{-2}.s^{-1}) and size range curves were plotted. The peak of the size distribution curve was assumed to represent average cell volume at a particular light intensity. For the UV-007 cultures, average cell volume increased with increasing light intensity up to 17 μEinsteins.m^{-2}.s^{-1}. Above 17 μEinsteins.m^{-2}.s^{-1} a rapid increase in cell volume preceded cell lysis. A good linear correlation was found to exist between cell volume and light intensities.

Changes in cell volumes of the toxic isolate UV-006 were less than those of UV-007. Cell size distribution of UV-006 remained relatively constant and stayed within a narrower range than UV-007 at corresponding light intensities. At 6.2 μEinsteins.m^{-2}.s^{-1} UV-006 cell volumes ranged between 10-50 m^3, whereas UV-007 cell volumes ranged between 20-70 m^3 at similar light intensities. At higher light intensities (26.5 μEinsteins.m^{-2}.s^{-1}), UV-006 cell volumes ranged between 25-110 m^3, while UV-007 cell volumes ranged between 20- >200 m^3 at 12 μEinsteins.m^{-2}.s^{-1}.

Krüger and co-workers ascribed the increase in cell size at light intensities greater than 10 μEinsteins.m^{-2}.s^{-1} to stress conditions resulting from an increase in CO_2 concentration (and possibly a change in the CO_2/O_2 ratio). This was confirmed by the fact that no increase in cell size took place in *Microcystis* cultures aerated with 0.5% CO_2, despite light intensities of 98 Einsteins m^{-2} s^{-1} (Krüger 1978). They suggested that cell size may be a better indicator of physiological stress than turbidity, chlorophyll content or cell number - small average cell size and narrow size distribution curve would indicate a healthy culture - and conclude from the above that the lower variability in cell size of the toxic isolate UV-006 suggests that it is better able to cope with stress conditions than the non-toxic isolate UV-007.

Non-toxic cells (UV-007 - *M. aeruginosa* forma *flos-aquae*) were found to be larger (3.4-7.5 μm) on average than toxic cells (UV-006 - *M. aeruginosa* forma *aeruginosa*) which ranged between 1.8 and 6.4 μm. Non-toxic cells became larger as light intensity increased to 18 μEinsteins.m^{-2}.s^{-1}, toxic cell size remained constant or became smaller. They concluded that cell size should not be used as a taxonomical character unless careful account is taken of the environmental conditions. Thus, cells collected near the water surface would be expected to be

larger than those lower down due to higher light intensities and low CO_2 and O_2 concentrations.

Chemical changes in the growth medium of Microcystis batch cultures grown at stress and non-stress light intensities (Krüger and Eloff, 1979b)

Inhibition of the growth of *Microcystis* under laboratory conditions occurs at light intensities >10 μEinsteins.m^{-2}.s^{-1}, whereas under natural conditions, *Microcystis* can endure without adverse effect light intensities of up to (and exceeding – see work by Harding, 1996) 1 500 μEinsteins.m^{-2}.s^{-1}. Therefore, light could not be the limiting factor for growth in laboratory batch cultures. Krüger and Eloff investigated the buffer ability of the growth medium BG 11, changes in pH, total alkalinity, CO_2 and O_2 concentration as a result of the growth of *Microcystis* UV-007 to identify the reasons for growth inhibition of batch cultures at higher light intensities.

Krüger and Eloff noted that cultures exposed to lower light intensities (6.0, 9.5 and 12 μEinsteins.m^{-2}.s^{-1}) increased continuously to maximum pH values of after 14 to 19 d, whereas cultures exposed to higher light intensities (19 and 26 Einsteins m^{-2} s^{-1}) reached a maximum pH values after 7 and 6.5 d respectively, thereafter the pH dropped markedly. From their work it is clear that the time of maximum growth (i.e. highest turbidity values) corresponded with the highest pH values in both cultures, and that the decrease in growth after 7 and 6.5 d for cultures grown at higher light intensities corresponded with a decrease in the pH.

A cessation of growth after 6.5 to 7 d occurred in cultures grown at higher light intensities (26 μEinsteins.m^{-2}.s^{-1}). A depletion of CO_2 and a decline in the concentration ratio CO_2:O2 indicating a higher rate of removal of CO_2 by photosynthesis than replacement by diffusion, appeared to be a cause. The concentration ratio CO_2:O_2 in the cultures grown at low light intensities (9.5 μEinsteins.m^{-2}.s^{-1}) decreased from 3.5 to 3.0 on the 9^{th} day, and thereafter remained constant, suggesting that the rate of CO_2 removal was compensated for by diffusion and CO_2 stress was not evident.

Krüger and Eloff pointed out that assimilation of CO_2 by algae occurs by the C_3 pathway and ribulose diphosphate carboxylase with a low affinity for CO_2, suggesting that *Microcystis* would not effectively assimilate CO_2 at the low levels occurring in these cultures. They also point out that RudP carboxylase shows an oxygenating activity which, under conditions of low CO_2, high O_2 concentrations and high pH (conditions

found in these cultures), accumulates glycolic acid which may be excreted by the cells. They also suggested that this may also play a role in the die-off of cells, but that this remains to be investigated.

Krüger and Eloff confirmed that pH does not appear to be implicated in cessation of growth at higher light intensities (pH = 10.75) since pH reached higher levels (pH = 10.78) after 13 d at lower light intensities, with sustained growth beyond this period. The same patterns are evident in O_2 concentrations. Die-off could not be explained by 'photooxidative death' described by Eloff as supported by data from van Vuuren (1979).

Effect of temperature on cell growth

The effect of temperature on specific growth rate and activation energy of Microcystis and Synechococcus isolates relevant to the onset of natural blooms (Krüger and Eloff, 1978a)

Krüger and Eloff found a correlation between the water temperature and the development of *Microcystis* blooms in small eutrophic impoundments in the Vaal River catchment. They reported that *Microcystis* blooms started to develop in open lake water once temperatures reach 16-17°C (Connell et al. 1977, cited in Krüger and Eloff).

The following organisms were used to test the effect of temperature on growth: *Microcystis aeruginosa* NRC-1 (UV-001); *Microcystis aeruginosa*, Berkeley strain 7005 (UV-007); *Microcystis aeruginosa*, Hartbeespoort Dam (UV-006); *Microcystis incerta*, Cambridge culture (UV-003); *Synecoccus sp*, Berkeley strain 6301, previously *Anacystis nidulans* (UV-005).

Their results show the effect of temperature on specific growth rate. A rapid decline in the growth rate occurs after the upper temperature limit is surpassed (light intensities were kept below saturating intensity).

The mean value of the optimal temperature range was used to estimate the optimal temperature value. UV-001 exhibited the lowest lower temperature limit (10.5°C), while UV-006 (from Hartbeespoort Dam) had the highest upper temperature limit (40.0°C) - 3.6 to 5°C higher than for any of the other *Microcystis* isolates investigated. They suggested that this might have been attributable to the fact that the other isolates had been grown under laboratory conditions for much longer periods at lower temperatures and had lost their ability to adapt to higher temperatures. Also, the other forms originated from cooler European and Canadian lakes as a opposed to the much warmer conditions in South African impoundments. Optimal

growth temperatures remained relatively constant for all *Microcystis* isolates ranging between 28.8 and 30.5°C. *Synechococcus* displayed the widest temperature tolerance (lower temperature, 10.3°C; upper temperature 44.3°C) as well as the highest thermal growth optimum (34.5°C). The lower temperature limits of 10.5-13.5°C for the various *Microcystis* isolates suggested that the temperatures in South African highveld impoundments in winter (7-8°C) may be a barrier to growth and that overwintering may be related to the production of nannocytes (Pretorius and Eloff 1977, cited in Kruger and Eloff 1978a; see also elsewhere in this review).

Arrhenius plots prepared by Krüger and Eloff show the specific growth rate as a function of absolute temperature). Inflection points are evident for all organisms, suggesting different Arrhenius relationships for different temperature relationships. The changes in slope (i.e. changes in activation energy) can be attributed to the existence of master reactions that limit the rate of growth over different temperature ranges. Sorokin (1960), cited in Krüger and Eloff (1978a), suggested that the regulating reaction in the lower temperature range is related to cell division, whereas the regulating reaction in the upper temperature range is related to the accumulation of cell material.

The most notable change in slope for all organisms occurred at an inflection point that occurred between 16-17.5°C. For UV-006 (ex Hartbeespoort dam) this corresponded to the temperatures recorded during the occurrence of blooms in South African impoundments noted by Scott et al. (1977). Other reported inflection points could be attributed to the fact that the light intensities used for the duration of the experiment were well below saturating intensity - thus light intensity rather than temperature may have been limiting over this range, or that corresponding with the upper temperature limits of the organisms.

Effect of temperature and light on <u>M. aeruginosa</u> UV-006 (van der Westhuizen and Eloff, 1985).

Microcystis aeruginosa UV-006 was originally isolated from Hartbeespoort Dam. In this laboratory study it was determined that cells were most toxic when grown in well-aerated cultures at 20°C, a pH of 9.5, maintained using CO_2, and harvested during the late logarithmic phase of growth. The 20°C LD_{50} (mice) of 25.4 $mg.kg^{-1}$ was markedly reduced at temperatures above 28°C. The fluence rate induced a much smaller effect, but toxin production was noted to be less at the extremes of light availability.

Photo-oxidation

The absorption of oxygen from liquid cultures of blue-green algae by alkaline pyrogallol (Eloff, 1977a)

In this work Eloff investigated the growth of *Microcytis* under different oxygen tensions in the presence of alkaline pyragallol, and described the absorption of oxygen from the growth medium by alkaline pyrogallol in order to determine whether photo-oxidation takes place under relatively low light intensities. Three forms were used: *Microcytis aeruginosa* strain 7005, *Synechococcus* sp. strain 6311 and *Microcystis aeruginosa* strain NRC-1.

It was determined that pyrogallol mixed with equimolar parts sodium hydroxide and sodium carbonate could absorb up to 70% of the oxygen in a bicarbonate-carbonate buffered medium without changing the pH. While this method could be used to obtain partially anaerobic conditions for the growth of *Microcystis* cultures, at least 20% of the oxygen remained in the medium. It is evident that the higher the pyrogallol concentration, the higher the yield in the *Microcystis* culture. Eloff ascribed the increase in yield of *Microcystis* grown in closed containers in the presence of pyrogallol to reduced photo-oxidation due to lowered oxygen tensions rather than an increase in the carbon dioxide concentration. The experiments also confirm that photo-oxidation could take place at low light intensities of $1\text{-}4 \times 10^3$ ergs.cm^{-2}.s^{-1}.

The influence of CO_2

Chemical changes in the growth medium with regard to pH, CO_2 concentration, O_2 concentration, alkalinity and buffer ability of Microcystis cultures grown at stress and non-stress light intensities (Krüger, 1978; Krüger and Eloff, 1981).

Krüger hypothesized that the inhibition of growth of cultures could not be ascribed to depletion of nutrients in the commonly used BG-11 growth medium (since this medium contains an excess of mineral nutrients), pH or high O_2 concentrations, but to the depletion of the total inorganic carbon content of the medium. Krüger knew that from work conducted by Wildman et al. that *Microcystis* might release phosphate into the surrounding medium and thereby increase its buffer capacity. Since cyanophytes are not known to grow at low pH levels - pH values of 10 have been found to be optimum (Gerloff, Fitzgerald and Skoog 1950 cited in Krüger 1978a), this may serve to maintain pH levels within a suitable range. However, Krüger (1978a) points out that poor growth at very low (<6.5) and very high pH levels (>10) has more to do with the changes in the

composition of the medium than with the pH *per se*.

Krüger also carried out tests on the buffering ability of the growth medium and found this to be about the same as tap water. An increase in pH occurred at all irradiance levels, suggesting that *Microcystis* does not excrete sufficient organic substances to buffer the medium and prevent major pH changes. Turbidity and pH values were found to correspond to one another - highest turbidity values correspond with the highest pH values. Turbidity and pH values at 27 and 19 μEinsteins.m^{-2}.s^{-1} decreased sharply after 6.5 and 7 d.

This paper reports changes with time in turbidity, pH, O_2 concentration, ΣCO_2, CO_2:O_2 ratio and total alkalinity. The concentration of H_2CO_3 after 5 d was calculated at 1.5×10^{-3} μmol.ℓ^{-1} - when the minimum concentration of ΣCO_2 ($\Sigma CO_2 = H_2CO_3 + HCO^-_3 + CO^{2-}_3$) and maximum pH values were reached. King (1970, cited in Krüger, 1978a) suggests that the minimum concentration of CO_2 (aq) required to support photosynthesis is 2.5 μmol.ℓ^{-1} – 1 600 times higher than the value measured. From these results, Krüger concluded that the cessation of growth in cultures grown at 26 μEinsteins.m^{-2}.s^{-1} after 6 d was therefore due to the depletion of ΣCO_2 in the medium. Krüger, furthermore, indicated that cessation of growth could not have been caused by pH *per se* since pH reached higher levels (pH =10.78) after 13 d at a lower light intensity, and where healthy growth of cells was maintained. This led him to conclude that the inability of *Microcystis* to grow for extended periods at light intensities > 10 μEinsteins.m^{-2}.s^{-1} is due to a higher rate of removal of CO_2 by photosynthesis than replenishment by diffusion. The poor buffering capacity of the medium would have further reduced the free CO_2 concentration. At 9.5 μEinsteins.m^{-2}.s^{-1} the rate of CO_2 removal by photosynthesis was in equilibrium with the rate of replenishment, thus stress CO_2 concentrations did not develop in these cultures.

The influence of CO_2 concentration of the aerating gas, on the growth of Microcystis in batch cultures (Krüger, 1978a).

In further work, Krüger suggested that standardization of the CO_2 concentration and pH in batch cultures would be important to obtain reproducible data for investigating aspects such as nutrition. Aeration with CO_2 enriched air is the most feasible way of achieving this. Different concentrations of CO2-air mixtures were bubbled through autoclaved medium at a constant rate and the pH was determined. *Microcystis* UV007 was used.

A correlation was found to exist between μ max (d^{-1}) and the CO_2 concentration of the aerating gas. The differences, however, were not marked. The CO_2 concentration of the aerating gas was found to have a significant effect on the final cell number and degree of multiplication obtained in the culture after 6.6 days. Bleaching of cells was found to occur at all irradiance levels when cultures were aerated with air only or not aerated at all. A correlation was found between CO_2 concentration of the aerating gas and the light intensity that the cultures could withstand before bleaching occurred. CO_2 concentration of the aerating gas was also found to have an influence on cell diameter - the lower the CO_2 concentration, the larger the cell diameter. Low CO_2 concentrations appeared to inhibit cell division. Healthy cells thus tend to be smaller than cells under CO_2 or light stress (cell size is not directly influenced by light stress but by the growth rate of the cells which affects the CO_2 concentration of the medium under different light intensities).

From his work, it was possible for Krüger to determine the mathematical relationship between specific growth rate and light intensity using light intensities as high as 100 μEinsteins.m^{-2}.s^{-1}, since growth inhibition and bleaching at these light intensities did not occur in aerated batch cultures.

Krüger concluded that it was possible to prevent bleaching and die off of cells at higher light intensities through aeration with an appropriate concentration of CO_2. The CO_2 concentration gradient between the sparged gas and the medium was found to be more important than the volume of gas sparged per unit time.

Role of CO_2 in culture of axenic <u>Microcystis</u> for isolation of the toxin (Krüger and Eloff, 1982)

Krüger and Eloff (1982) compared three different cultures of *Microcystis* with respect to their sensitivity to light. The condition of the cultures was determined using turbidity, cell number, chlorophyll-a concentration and rate of photosynthesis.
They found that bleaching and die-off of cells subjected to high light intensities could be prevented if they were aerated with CO_2. The CO_2 concentration gradient between the aerating gas and cell suspension was found to be more important than the volume of gas sparged per unit time. Krüger and Eloff (1982) postulated that, as well as playing a nutritional function, the CO_2 may play a catalytic role in protecting the cells against photoinhibition. They suggest that oxygenation instead of carboxylation by ribulose diphosphate carboxylase may be induced by the low CO_2:O_2 ratio just prior to bleaching and lysis.

CO_2 concentration was found to influence cell size - the higher the CO_2 concentration the smaller were the cells.

The effect of agitation and turbulence

The effect of agitation and turbulence of the growth medium on the growth and viability of Microcystis (Krüger and Eloff, 1978a)

Eloff cites observations by Shillinglaw and Pieterse (1977) that *Microcystis aeruginosa* forma *flos-aquae* was the dominant alga in pond systems having a retention period of longer than 20 d, suggesting that the turbulence associated with shorter retention periods may regulate growth. *Microcystis* blooms are also known to develop after several wind-free days. The purpose of this study was to investigate the effect of agitation on the growth of *Microcystis* in order to determine the possible impacts of a pumping system on the axenic mass culture of *Microcystis* and the importance of turbulence for water management.

Turbulence was found to have a slight beneficial effect on the growth of UV-007 (*M. aeruginosa* Berkeley strain 7005) reflected in the increased μ (maximum specific growth rate measured during the exponential growth phase) and the highest turbidity values reached within 20 days ($\log_2 T_t/T_0$). Krüger and Eloff found that these observations could be attributable to more efficient aeration, improved nutrient uptake and accessibility and exposure of individual cells to light in the turbulent cultures.

Turbulence increased the viability of the cells (expressed as percentage recovery). A significant correlation (r - 0.94, n = 5) was obtained between the mean of the percentage recovery values and linear velocity. The researchers ascribed this to: better accessibility to CO_2, improved nutrient uptake and accessibility, more efficient illumination and a physiological change in the cells leading to the production of nannocytes.

The adverse effects on *Microcystis* of water bodies with a short retention period could not be ascribed to turbulence. Krüger and Eloff proposed two explanations for this:

(i) that the increased agitation of a water body may lead to increased CO_2 availability thus making green algae more competitive; and

(ii) (ii) that the dispersion of a growth factor in the water by higher circulation levels. These results should be compared with those of Harding (1997b), determined

in situ in a polymictic hypertrophic lake.

Mass cultivation

Mass culture of Microcystis under sterile conditions (Krüger and Eloff, 1978c)

In this study Krüger and Eloff state their reasons for attempting to develop the mass culture of algae as being the high protein content relative to other plants (Fisher and Burlew, 1953, cited in Krüger and Eloff, 1978c), and the ability of algae to photobiosynthesise valuable organic compounds which are difficult to synthesize *in vitro* (such as sterols). Surprisingly, they do not mention the value of mass production of toxic secondary metabolites for analytical purposes.

But the primary aim of developing mass culture techniques was to study the biochemical characteristics of algae, which requires access to large quantities of axenic cell material. Natural algal blooms which are subject to seasonal variation, impurities (bacteria, zooplankton etc.) and variations in the toxicity from bloom to bloom could not be relied upon for the isolation of biologically active substances (Scott, 1987). One of the main reasons why the structure and properties of the toxins produced by Cyanophycaea could not be studied were the difficulties involved in mass culture. Problems associated with mass culture included self-shading and aeration (provision of CO_2 and removal of O_2).

Based on the system built by Juttner et al. (1971), which they used to cultivate *Chromatium*, Krüger and Eloff built a similar tubular mass culture system to grow *Microcystis.* Prior to the publication of this work, no successful attempts to grow a mass culture of *Microcystis* in this type of system had been reported. *Microcystis aeruginosa* (UV-007) Berkeley strain 7005 was grown at 28°C in BG-11 medium modified by altering the citrate concentration and using 0.006 $g.\ell^{-1}$ $FeSO_4$ instead of ferrous citrate.

This system yielded 8.5 $g.\ell^{-1}$ fresh mass per litre per day over 7.6 d with a growth rate of 0.6 doublings per d (μ = 0.41 d^{-1}) and a total yield of 507.9 g. The high light intensity (130 $\mu Einsteins.m^{-2}.s^{-1}$) did not appear to have an inhibitory effect on the cells and Krüger and Eloff suggested that the same yield may be obtained in a shorter space of time with increased light intensity. pH was controlled between 8.5 and 10 by the addition of CO_2.

They found that the O_2 concentration of the aerating gas, as well as that produced by the cells, had no inhibitory effect on cell growth despite high light intensities,

as long as the CO_2 concentration remained high. This suggested that a high CO_2 concentration might be important for protecting the cells against photooxidative death (Eloff *et. al*. 1976 cited in Krüger and Eloff 1978c).

6.4 Ecological field studies on algal blooms

Algal blooms in the Vaal catchment area (Tow, 1976)

Tow made observations on the chemical parameters in several dams in the Witwatersrand area between August 1974 and August 1975. He attempted a correlation of the physical and chemical parameters of the dams with the onset of blooms of *Microcystis aeruginosa*. Sodium, potassium, calcium and magnesium concentrations were monitored and found not to be limiting. Monitoring of the seasonal variation in primary productivity revealed highest levels over spring and summer. The vertical distribution of *Microcystis* in the water column over a 24-h period was also monitored and *Microcystis* colonies were found to concentrate at the surface during mid-morning. A downward movement occurred at noon, and the colonies were found in deeper water at night. Apparently Tow did not attempt any correlations with prevailing wind directions.

The enrichment of the Jan Smuts Park Dam was found to be due to sewage discharge, combined with a high evaporation rate and the absence of a natural outlet, creating conditions most favourable for the growth of *Microcystis*. Similar conditions prevailed in the Kleinfontein Dam. However, the absence of a bloom during the summer of the study period was assumed to be due to the high turnover rate and low ammonium and nitrogen levels. While Homestead Park, Middle and Civic Dams were found to be relatively unpolluted, although the development of a bloom during the study period was assumed to be due to an influx of raw sewage. A comparison of the Jan Smuts Park and Kleinfontein Dams indicated the importance of nitrogen and ortho-phosphate in bloom development.

During the period when *Microcystis* was absent from the phytoplankton, the algae was found to be present in the benthic sediments of the dam, indicating that it overwintered in the sediments.

Nitrogen fixation in a nitrogen-limited impoundment (Ashton, 1976)

Ashton (1976) investigated conditions in the Rietvlei Dam in terms of the succession of algal blooms and nitrogen fixation (the fixation of atmospheric nitrogen by blue-green algae in freshwater lakes can be a major factor in nitrogen budgets) - especially in terms of the heterocystous, nitrogen-fixing blue-green algae *Anabaena circinalis*. The dam frequently experienced large algal blooms

due to nutrient loading emanating from the Hennops River.

Ashton reported changes in nitrogen fixation rates with depth and time in the top 6 m of the dam over the duration of two 12-h studies. Fixation rates were found to increase to a maximum at around noon and then to decrease again between noon and 18:00. With increasing depth, the time of maximum fixation rates also increased. Also, maximum nitrogen fixation rates at depths of 1 and 2 m were found to be less than maximum values at 0.25 m, indicating the importance of light intensity in nitrogen fixation.

The total amount of nitrogen fixed per hour on two days: (a) November 25th, 1975 and (b) December 9th of the same year. Nitrogen fixation levels recorded for December 9th were approximately 2% of those recorded on 25 November, and corresponded to the numbers of *A. circinalis* colonies recorded at the time of the surveys. Approximately 29% of the total daily nitrogen fixation occurred before noon - the largest proportion was fixed between 12:00 and 15:00.

A comparison of the nitrogen and phosphorus sources identified by Ashton with the cell counts of *A. circinalis* for the top 6m was undertaken. Low Kjeldahl-N, NO_3-N, NO_2-N, PO_4-P and total P measured on November 25th suggested that large quantities of these nutrients were tied up in the algal cell material. High NH_3-N levels, that are indicative of the liberation of large amounts of ammonia by algae as products of nitrogen fixation, were considered. The increase in Kjeldahl-N, NO_3-N, NO_2-N, PO_4-P and total P values after the disappearance of the bloom provided evidence of the subsequent release of these elements from dead and lysing algal material. The decrease in Kjeldahl-N, NO_3-N and NH_3-N during December was due to the onset of a *Microcystis* bloom following the *A. circinalis* bloom.

Based on daylight fixation rates, Ashton estimated the total amount of nitrogen fixed during the period of the *Anabaena* bloom as being 1.13 metric t. (19 Kg N fixed day^{-1}). This is of some significance when compared with the nitrogen loading of the Hennops River (35.8 metric t a^{-1}). The fixation of nitrogen by algae is thus equivalent to 3.2% of the annual loading. Ashton pointed out that this value was determined from a (temporally) short period, and that the loads could vary considerably from year to year.

Ashton further described the succession of algal blooms in the Rietvlei dam as follows: after winter overturn the concentration of dissolved nitrogenous and phosphorous 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 (eg. *Melosira granulata* var. *angustissima*). As temperatures rose, non-nitrogen fixing species became dominant (eg. *Volvox rousseletti*), and reduced the amount of available nitrogenous compounds – resulting in a phosphorous-rich environment suitable for the onset of nitrogen-fixing blue-green algae i.e. *A. circinalis*. Cooler weather and heavy rainfalls caused a disappearance of the *A. 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.

Ashton emphasised the importance of phosphorous loading rather than nitrogen loading in governing algal production, since nitrogen is readily available from the atmosphere. Thus, any treatment or event that limits the availability of nitrogen and not phosphorous would not be effective in controlling problems related to eutrophication.

The occurrence of Microcystis aeruginosa in the bottom sediments of a shallow eutrophic pan (Tow, 1979)

In a subsequent investigation, Tow observed that little was known of the fate of *Microcystis aeruginosa* during its absence from the phytoplankton in the winter months (here it should be noted that this observation pertained to the pan systems he was studying – perennial dominance or presence of *M. aeruginosa* is quite common in many parts of South Africa). Tow collected sediments and water samples over the period 27th July to 3 November 1975 from Jan Smuts Park Dam, a natural shallow pan to the north of Brakpan. Microscopic examination of the sediments did not reveal the presence of *M. aeruginosa*. The sediments were therefore incubated to determine the presence or absence of the alga. *M. aeruginosa* - colonies appeared in all the sediment samples after a period of incubation.

Tow cites Reynolds (1973) as stating that colonies settle on the bottom sediments during autumn due to a reduction in the number of their gas vacuoles. From the results of his culture experiments, Tow decided that *M. aeruginosa* colonies 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 (Pretorius et al. 1977 cited in Tow, 1979). Tow further postulated that water temperatures might be the most important factor in stimulating the development of summer populations.

Studies on the ecology, growth and physiology of toxic Microcystis aeruginosa in South Africa (Scott et. al., 1981)

Growth of *Microcystis* at different light intensities

Scott and co-workers concluded that the ability of *Microcystis* to grow well at relatively low light intensities (3 600 lux) was indicative of a competitive advantage that *Microcystis* has over other algae. They were of the opinion that a further competitive advantage is the presence of gas vacuoles that prevent the cells sinking to a depth where light may be limiting (for a detailed review of cyanobacterial buoyancy mechanisms see Walsby 1987).

Scott and co-workers recorded sigmoidal growth curves for light intensities of 3 600, 5 700 and 18 000 lux. The initial lag phased decreased with increasing light intensity. Cultures grown at 3 600 lux (corresponding to a depth of 4-5 m in a typical non-silty Transvaal impoundment) were observed to remain green, while cultures grown at 5 700 and 18 000 lux turned yellow-green and yellow respectively. After returning these cultures to lower light intensities (720 and 230 lux respectively), they turned green again, but were unable to resume high growth rates. Gas vacuole content was also observed to increase with increasing light intensities and age of the culture, supporting the hypothesis that the vacuoles act as light shields.

Fine structural observations

Thykaloid numbers decreased as the light intensity increased: 4-6 long thykaloids stacked parallel to the cell wall were observed at lower light intensities. At higher light intensities, thykaloids were more fragmentary and arranged perpendicular to the cell wall. Ageing appeared to increase the number of cyanophycin granules and lipid droplets in the cells.

Field measurements.

Scott's team compared the laboratory observations on the pigmentation of *Microcystis aeruginosa* with observations of colonies kept in dialysis tubes for 48 h in the Hartbeespoort Dam. Cells at the surface of the dam showed a linear drop in the chlorophyll:carotenoid ratio due to a decrease in the chlorophyll content and a more gradual decrease in the carotenoid content. Cells at 3 m and 28 m showed an increase in chlorophyll content, while carotenoid content showed a slight increase resulting in a lower chlorophyll:carotenoid ratio.

Scott et al. (1981) suggested that *Microcystis* adapts to high light intensities by reducing the chlorophyll content of the

cells - at lower light intensities and in darkness more chlorophyll is synthesised.

Disruption of Microcystis colonies for enumeration as single cells (Zohary and Pais Madeira, 1987)

Many researchers have been confronted by the frustration of needing to disaggregate colonies of *Microcystis* in order to be able to count and measure single cells, and accurately determine biovolumes. Tamar Zohary and Cangela Pais Madeira developed a simple and effective means of disrupting the colonies using a high speed "Ultra-Turrax"-type blender process. Concomitant effects on the counts of other phytoplankton present in the sample were found to be minimal. The technique has been and is still used in many laboratories.

Ecological associations of cyanobacteria in a shallow, hypertrophic lake (Harding, 1991, 1992a,b, 1994, 1996, 1997a, 1999).

Over a period of 8 years, Bill Harding conducted a series of in-depth investigations of cyanobacteria in shallow coastal lakes, and in particular Zeekoevlei, a hypertrophic, continuously mixed system. From his work Harding was able to show that:

- conditions in the well-mixed lake were akin to those in a chemostat, supporting significantly higher levels of primary production than would otherwise be the case;
- that *Microcystis aeruginosa* forma *flos aquae* is capable of acclimating and attaining high levels of dominance in conditions of rapidly fluctuating light availability, and where mean wind speeds were sufficient to otherwise offset the gas vacuolate upward driven motion of the alga;
- that high frequencies of mixing select for the generally non-toxic *Microcystis aeruginosa* forma *aeruginosa*;
- Levels of primary production in Zeekoevlei were comparable with, or exceeded, those of the most productive systems yet studied;
- Despite sustained, non-limiting availability of nutrients, phytoplankton succession in Zeekoevlei was dominated by large ornamented chlorophyte species during the spring and autumn, and by *Microcystis* during the winter and spring. The co-dominance of chlorophyte species occurred despite the high mean pH (9.3) and low N:P (< 10) ratios;
- Phytoplankton diversity was low, with between 1 and 2 species comprising in excess of 80% of the total phytoplankton biovolume at any time. This finding was consistent with the

tenets of the Intermediate Disturbance Hypothesis (Connell, 1978).

6.5 The NIWR Hartbeespoort Dam study

The presence of toxic Microcystis in Hartbeespoort Dam (Scott, 1983).

During the early 1980s the Technical sub-Committee of the Hartbeespoort Dam study decided that a sensitive assay for microcystins should be developed, and that mouse assays would only give a yes or no answer. Scott examined weekly samples from the Hartbeespoort Dam to determine the relative proportions of *Microcystis aeruginosa* forma *aeruginosa* (the toxic form) and *M. aeruginosa* forma *flos-aquae* (Scott et. al., 1981) (non-toxic) between 2nd November 1982 and 27th September 1983.

Microcystis aeruginosa forma *aeruginosa* was found to be present in the dam for at least 10 months of the year. Toxicity tests on *Microcystis aeruginosa* forma *aeruginosa* using mouse assays confirmed that it was always toxic.

Factors controlling primary production in a hypertrophic lake (Hartbeespoort Dam, South Africa) (Robarts, 1984)

Robarts (1984) investigated the physical factors controlling algal primary production (large populations of *Microcystis aeruginosa*) in the Hartbeespoort Dam, a hypertrophic, warm, monomictic lake with high nitrogen and phosphorous loading from the surrounding suburbs. A_{max}, the maximum rate of photosynthesis in the depth profile was found to range between 12.4 $mg.C.m^{-3}.h^{-1}$ during April 1981 (winter overturn) and 5916 $mg.C.m^{-3}.h^{-1}$ during December 1981 (summer). Planimetrically-determined integral rates of primary production (ΣA) ranged between 46.9 $mg.C.m^{-2}.h^{-1}$ during April 1981 and 3381 $mg.C.m.^{-3}.h^{-1}$ during December 1981.

Robarts separated the factors controlling primary production in the Hartbeespoort Dam into two categories: Category (1) included nutrient (N and P) and wind factors which affected the algal standing crop: high N and P loading rates ensured that nutrients did not become limited and were therefore initially responsible for high primary production. Wind patterns were responsible for considerable spatial and temporal changes in the standing crop.

Category (2) included biological, chemical and physical factors that affected rate processes. The buoyancy mechanism of *M. aeruginosa* enabled the bulk of the population to remain within the euphotic zone.

Microcystis aeruginosa and underwater light attenuation in a hypertrophic lake (Hartbeespoort Dam, South Africa) (Robarts and Zohary 1984)

As part of the Hartbeespoort Dam Research Programme (see elsewhere in this review) Richard Robarts and Tamar Zohary investigated underwater light attenuation, primary productivity and phytoplankton dynamics in the hypertrophic Hartbeespoort Dam. The dominant component of the phytoplankton assemblage during most of the year was *Microcystis aeruginosa*. Nitrogen and phosphorus availability was never found to be limiting, but was always in excess of requirements.

The researchers found that blue light (443 nm) was found to attenuate more rapidly (1% of the sub-surface value remained at values ranging from 0.3-4.2 m) than green (550 nm) (1% of the sub-surface value between 0.6-8.5 m). A variable light regime was suggested by euphotic zone depth variation (z_{eu}) (1% PAR) which ranged between 0.6 and 6.4 m in 1982-83.

Surface chlorophyll *a* concentrations ranged between 3.4 and 1 000 $mg.m^{-3}$. Most of the algal pigment was confined to the upper 4 m of water column – constituting a major factor constraining light availability.

M. aeruginosa, was found to be the dominant alga in the upper 8 m of the lake during most of the year. Lowest algal biovolumes were reached in late winter to spring (10-30% of the total phytoplankton volume during August to October). During September and October species of Chlorophyta appeared and became dominant. Between the end of October and December, *M. aeruginosa* increased from comprising 50% of the phytoplankton population to levels in excess of 90%. By May, it comprised 98-99% of the population. Decline occurred in June and July and *M. aeruginosa* was replaced as the dominant species by *Melosira granulata*. A wide range of colony sizes was recorded for *M. aeruginosa*, ranging from 10 × 10 μm to 50 mm in calm weather. After dispersal of the scums by wind, colonies of 10-100 mm in diameter were measured which fractured easily when water turbulence increased.

PAR and underwater light attenuation were strongly affected by chlorophyll *a*. However, only 68% of the variance accounted for the relationship between chlorophyll *a* concentration and light attenuation, suggesting that other factors were involved.

Primary productivity (measured by uptake of radiolabelled $^{14}C\text{-}CO_2$) ranged between 46.3 $mg.C.m^{-2}.h^{-1}$ and 2 290 $mg.C.m^{-2}\ h^{-1}$. Production was restricted to upper 4 m of

water column due to vertical stratification of the phytoplankton and algal self-shading (see comments above).

Two principal reasons for dominance of *M. aeruginosa* were proposed by Robarts and Zohary. Firstly, the ability of the alga to maintain position in the upper part of the water column. Robarts and Zohary cite Humphries and Imberger (1982) as suggesting that the alga could maximise the photic depth to mixed layer depth quotient (z_{eu}/z_m). The buoyancy mechanism of the alga would remain in the diurnally mixed layers (except during high wind), z_m in the quotient and the time the algae spent in the euphotic zone would be reduced. The second advantage that *M. aeruginosa* has over other lake competitors is its ability to increase in colony size with a decrease in mixing and turbulence (it was noted that when calm weather followed a windy period, *M. aeruginosa* moved to the surface and small colonies started to clump together). The increase in colony size would moderate light attenuation and, hence, reduce self-shading.

Hyperscums of the cyanobacterium Microcystis aeruginosa in a hypertrophic lake (Hartbeespoort Dam, South Africa) (Zohary, 1985)

Tamar Zohary proposed that densely packed scums of blue-green algae covered by a crust of photo-oxidized cells and found on the surface of hypertrophic impoundments after prolonged periods of warm weather be referred to as 'hyperscums'. The physical, chemical and biological characteristics of an extensive hyperscum of *M. aeruginosa* covering 1-2 ha of the Hartbeespoort Dam in South Africa for a period of 103 days were investigated in this study. Zohary was of the opinion that these hyperscums are rare and do not occur in smaller hypertrophic freshwater bodies. She postulated that the conditions driving their development include: hypertrophic conditions, a large volume of water, stable weather conditions and lake morphometry with wind-protected sites for cyanobacterial accumulation such as that provided by impoundment structures such as the Hartbeespoort Dam wall.

M. aeruginosa was the dominant component of the hyperscum community of cyanobacteria, comprising >99% of the phytoplankton biomass. At 10 cm depth, the number of *M. aeruginosa* cells per unit volume ranged between 0.86×10^9 and 1.76×10^9 cells.mℓ^{-1}. The chlorophyll *a*

concentration of the colonies exceeded 100 mg.ℓ^{-1} (1 x 10^5 μg.ℓ^{-1}) - the highest value reported for any planktonic cyanobacteria in freshwater systems!

A surface crust on the top few centimetres of the scum was identified as comprising of a layer of *Microcystis* cells exposed to photo-oxidizing conditions with high light intensities, oxygen supersaturation and CO_2 depletion. Photo-oxidative damage was evident as bleaching of the pigments, which occurred after 2-3 hrs of exposure to midday radiation. The buoyant crust, 1-3 mm thick covered the underlying cyanobacteria. Light intensity below this crust was found to be <0.001 $\mu E.m^{-2}.s^{-1}$, making the interior of the scum aphotic. Diel temperature variation within the hyperscum ranged between 4.2 and 23.4°C compared with 12.5 and 16.7°C within the main basin of the dam. Dissolved oxygen within the scum was undetectable. pH ranged between 6.2-6.8 in the scum compared with 8.8-9.6 in the main basin of the impoundment. Redox potential ranged from highly negative (-240 mV) within the scum to positive (+51 mV) just below it, while chlorophyll concentrations ranged from 224 mg.ℓ^{-1} in the hyperscum to 2.4 mg.ℓ^{-1} just below it (Table II, p.404 and Fig 4. p.405). Ammonium-N concentration increased over time to a maximum of 273 mg.ℓ^{-1} – a level ten times the level of raw domestic sewage (Reeves 1972, cited in Zohary 1985). Nitrate-N and nitrite-N concentrations were negligible. From the above observations Zohary suggested that the bacteria are involved in denitrification and ammonification processes.

Thus the conditions inside the hyperscum are aphotic, anaerobic, highly reducing with fluctuating temperatures and reduced water movement. Zohary was able to determine that, despite this, the cyanobacteria were able to remain viable under these conditions for as long as 77 days (11 weeks). Zohary suggested that *M. aeruginosa* is capable of chemoheterotrophic growth in the dark, as has been reported for other cyanobacteria (e.g., Khoja and Whitton, 1971, cited in Zohary, 1985). The ecological function(s) (if any) of the hyperscums could not be determined by Zohary, but she felt that they could serve as inocula for the following season (in the same way as overwintering in benthic sediments).

Factors favouring the formation of Microcystis hyperscums (Zohary and Breen, 1989)

Hyperscums were defined by Zohary (1985) as crusted and buoyant cyanobacterial mats that could persist at a given location for extended periods of up to several weeks. Cyanobacterial bloom formation depends on the pre-existence of

a large enough and buoyant population of cyanobacterial cells that, should wind-induced mixing or turbulence be insufficient to offset upward cell movement, will aggregate at or near the surface of a body of water (Reynolds and Walsby, 1975). Zohary and Breen examined the phenomenon of hyperscum formation in further detail, and concluded that, in addition to the above pre-requisites for bloom formation, for hyperscums to form prolonged periods of low wind speed, suitable lake morphometry, large standing crops of cyanobacteria and high insolation were necessary.

Digestion of Microcystis aeruginosa by Oreochromis mossambicus (de Moor and Scott, 1985)

In this investigation De Moor and Scott (1985) reported on the digestion of *Microcystis aeruginosa* by *Oreochromis mossambicus* and described its passage through the gut.

The protein content of *Microcystis* used in the feeding experiments, as determined by de Moor and Scott (1985), was found to be high (56%), whereas the phosphorus content was always less than 1%. The mean ash content was 7.0%. Experiments designed to determine assimilation efficiencies revealed a 5% decline in the protein content of *Microcystis* fed to fish over a 24 hr period, suggesting some breakdown of cells and leaching of soluble proteins. The analysis of faecal matter (using total organic matter and protein estimates) showed a wide range of assimilation efficiencies for protein (32-92%). The digestion of *M. aeruginosa* by *O. mossambicus* was confirmed by electron microscopical examination (EM) of the faecal matter.

This EM of the faeces revealed cell-content disintegration of *Microcystis*. 20-25% of green faeces contained ultrastructurally undamaged cells, while less than 1% of the reddish brown faeces contained undamaged cells. The colour of the faeces is determined by the gastric pH at the time of ingestion: low gastric pH results in the cell walls becoming permeable, allowing the entry of proteolytic enzymes and leaching of cell contents.

Comparison between the growth of *O. mossambicus* fed on trout pellets and fish fed on *M. aeruginosa* exclusively showed a 2% change from the start to the end of the experiment of fish fed on *M. aeruginosa.* Fish fed on trout pellets showed a 19% mass change, i.e. indicating that *M. aeruginosa* is inadequate as a sole food source for *O. mossambicus*.

De Moor and Scott concluded that, although the digestibility of *M. aeruginosa* was confirmed by the results of these

experiments, it was not adequate as the sole food source.

Zooplankton community grazing in a hypertrophic lake (Hartbeespoort Dam, South Africa) (Jarvis, 1986)

Andrew Jarvis investigated the seasonal population dynamics of the dominant cladoceran species in terms of grazing rates and food availability in the warm, hypertrophic Hartbeespoort Dam.

Large cladocerans, *Daphnia pulex* and *D. longispina* (up to 2.25 mm long), dominated zooplankton biomass from autumn to mid-summer. From January to April (summer) of 1981 and 1982, *Daphnia* was replaced by *Ceriodaphnia reticulata* as the dominant crustacean. The integrated community grazing rates were highest in December (260% day^{-1}) when *Daphnia* dominated the grazing community and edible phytoplankton were diminishing through grazing pressure. Lowest community grazing rates occurred between Jan - Feb (19.8 - 35.3%) when the phytoplankton comprised primarily *Microcystis*, and with *Ceriodaphnia* dominating grazing community during this time. The specific grazing rate reflected changes from *Daphnia* to *Ceriodaphnia* - integrated specific grazing rates were highest when *Ceriodaphnia* dominated the herbivore community.

Species succession ranged from spring dominance by edible Chlorophyceae and Cryptophyceae to summer, autumn and winter dominance by inedible *Microcystis*. The most abundant, partially edible, genus was *Melosira*. Midsummer decline in *Daphnia* population and shift to smaller cladoceran community (*Ceriodaphnia*) was accompanied by a change from edible phytoplankton species to *Microcystis*.

Several studies cited in Jarvis (e.g., Webster and Peters 1978; Gliwicz 1980; Porter and McDonough 1984) indicate that increased concentrations of large cyanobacterial colonies reduce the filtration rates of large cladocerans, and increase particle rejection rates, leading to increased energetic cost of their feeding response and reduced brood size. Thus, feeding by large *Daphnia* may have been reduced by the presence of *Microcystis*. Jarvis did not, however, rule out the possibility of predation by fish fry as being a possible explanation for the decline in large *Daphnia* at the time of the change to cyanobacterial dominance. Lampert (1982), cited in Jarvis (1986) reports that toxic inhibition of *in situ* CGRs and SGRs by *Microcystis* does not appear to occur.

Gliwicz (1977) cited by Jarvis indicated that small-bodied cladocerans (e.g. *Ceriodaphnia*) have a lower particle size limit to ingestion and are able to replace

large cladocerans (e.g. *Daphnia*) in water with large Cyanophyceaen particles.

Jarvis concluded that large filter feeders such as *Daphnia* unlikely to retard Cyanophycaea blooms by high grazing pressure (see also the results of studies by Combrink, 1994; and Harding and Wright, 1999).

See Supporting Literature

Apparent predation of Microcystis aeruginosa Kütz. emend Elenkin by a Saprospira-like bacterium in a hypertrophic lake (Hartbeespoort dam, South Africa) (Ashton and Robarts, 1987)

In this paper, Ashton and Robarts described the colonisation and apparent lysis of *Microcystis aeruginosa* by a *Saprospira*-like organism in the Hartbeespoort Dam. They noted that a bloom of *M. aeruginosa* obtained from the Hartbeespoort Dam had been colonised by a colourless, unbranched gram-negative, helically-shaped organism. A DAPI (a DNA fluorescing stain) resolved the organism into a series of bow- or comma-shaped, prokaryotic cells. Colonisation commenced on the ends of protruding lobes and advanced along the colony. Those portions of the colony that appeared to have been parasitised were colourless and the cell contents lysed, whereas unparastised portions of the colony appeared to be healthy. The filaments of the organism appeared to penetrate the outer sheath of the colony.

On the basis of morphological data, Ashton and Robarts tentatively identified the organism as *Saprospira*, possibly *S. albida*. Their observations suggested that this organism might be parasitic and not dependent on the autolytic products of *Microcystis*. They were only found in the upper epilimnion, corresponding to the depth distribution of *Microcystis*. It was estimated that between 10 and 20% of the *Microcystis* colonies in the upper 3 m of the water column had been affected.

The researchers decided that *Saprospira* only attacked the toxic form of *Microcystis* (*M. aeruginosa* forma *aeruginosa*) and that bioengineering techniques may be used to produce a persistent form that could aid in the management of these blooms.

Ecological problems in Hartbeespoort Dam (Jarvis, 1987).

Due to nutrient loading in the Hartbeespoort Dam, the phosphorous concentration is high and results in an N:P ratio of between 5 and 2:1 (the ideal ratio of carbon, nitrogen and phosphorous being 100:16:1 – the ‘Redfield’ ratio). Although

low nutrient ratios may suggest nitrogen limitation of *Microcystis aeruginosa* (not known to be capable of fixing its own nitrogen), nitrogen levels in the Hartbeespoort Dam never decrease to the point where nitrogen limitation occurs.

Suitable nutrient conditions in the Hartbeespoort Dam enable *Microcystis* to form large buoyant colonies. Extremely high standing crops enable *Microcystis* to dominate by up to 90-99% of the biovolume in the late summer (Robarts and Zohary 1984). High species diversity only occurs when green algae and flagellates become more abundant in the spring (August – October) (see also the findings of Harding (1996) from the hypertrophic Zeekoevlei – Western Cape).

The buoyancy mechanism of *Microcystis* allows it to monopolise the upper euphotic zone, effectively reducing the light available to other species. This process is compounded by stable stratification, calm wind conditions and a reduction in the mixing depth. Dense hyperscums build up in sheltered sites (Zohary 1985 – see elsewhere in this review). Anaerobic conditions, decomposition and photo-oxidation within these hyperscums result in the production and release of foul-smelling gases.

The standing crop of zooplankton in the lake is low when compared to the standing crop of phytoplankton (Jarvis 1986). High maximum integrated grazing rates (260% d^{-1}) have been recorded during the spring-early and early summer when species diversity is high and *Microcytis* abundance is low. When *Microcystis* is abundant, grazing rates are lowest. Jarvis (1986) suggested clogging of the filter feeding mechanism as a reason for this. Schindler (1971) and Hanazato & Yasuno (1987) have shown that the assimilation efficiency of zooplanton fed on *Microcystis* is low. The reduced growth potential of *Oreochromis mossambicus* (the blue kurper) has also been attributed to the poor nutritive quality of *Microcystis* (see elsewhere in this review).

The underutilisation of *Microcystis* by the zooplankton shifts the trophic pathway to a detrital-based trophic structure. The sedimentation and decomposition of phytoplankton is at its highest following the break-up of the hyperscums (NIWR 1985) accompanied by an increase in heterotrophic activity per bacterial cell and increase in bacterial numbers (Robarts & Zohary 1986).

(see Supporting Literature)

Diurnal mixed layers and <u>*Microcystis*</u> (Zohary and Robarts, 1989).

Microcystis aeruginosa remains dominant in shallow South African waterbodies for extended annual periods. In this

investigation, Tamar Zohary and Richard Robarts set out to show that *M. aeruginosa* remained dominant because of its ability to maintain itself within the light-rich shallow diurnally mixed layers of Hartbeespoort Dam. Their work showed that the formation of shallow layers mixed on a daily frequency were indeed important, while in deeper warm water lakes, stability of the water column was a greater determinant in the dominance of the algal assemblage by *Microcystis*. In work reported on elsewhere in this review, Harding (1997b) showed that high frequencies of mixing supported very high levels of *Microcystis* productivity and dominance.

6.6 Studies on cyanobacterial behaviour and associations in the Vaal River

*An investigation into the phytoplankton blooms in the Vaal River and the environmental variables responsible for their development (*Pieterse, 1986, 1987; Pieterse and Janse van Vuuren, 1997*)*

During the late 1980s Braam Pieterse reported on changes in phytoplankton diversity in the Vaal River near Balkfontein. The initial work examined which indicators of diversity were most appropriate to the environment being examined, and reported that the Hurlbert, Shannon-Wiener or Simpson's measures were best suited to describing phytoplankton community structure in the river.

A subsequent series of investigations were reported as a compilation of work in a report to the Water Research Commission (Pieterse and Van Vuuren, 1997). This work is summarised below:

In response to development of phytoplankton blooms along certain sections of the Vaal River, resulting in aesthetic problems, health hazards and interfering with water treatment and distribution systems, Braam Pieterse and Sanet Janse van Vuuren undertook an investigation of the causes and consequences. Their work included the effect of water transfer and stream regulation on the algological and environmental variables in the river. They considered the project unique in the South African context and to be of both local and international scientific significance. The work may be divided into several sub-components that are summarised as follows:

Phytoplanton production and photosynthetic characteristics in relation to environmental variables in the Vaal river at Balkfontein (*Pieterse and Janse van Vuuren, 1997,* Chapter 2).

In this component of the study, the environmental variables that influenced the production of phytoplankton in the middle reaches of the Vaal River (at Balkfontein) during 1993 were investigated. It was found that discharge was the most important variable influencing transparency of the Vaal River –increased discharge being associated with episodic inputs from summer rain. A higher discharge resulted in higher concentrations of total suspended solids (TSS) and, hence, turbidity, and, thus, a reduced Z_{eu} depth that impacted negatively on algal growth. High discharges were also related to higher nutrient and chlorophyll-*a* concentrations, but phytoplankton could not attain maximum

productivity because of the elevated turbidity prevailing during floods. Only after a lapse of several weeks after high discharge, was the phytoplankton able to bloom as the turbidities decreased.

During the winter-spring period (June to September), a high TDS value, low discharge and a possible increase in water hyacinth infestation over the study period was associated with low turbidity and favourable light conditions for photosynthesis and algal biomass build up. However, increased water clarity was associated with lower nutrient concentrations (N, P and Si), thus limiting production even when the light environment was favourable. It was suggested that the role that water hyacinth plays in reducing nutrient concentrations (especially DIN) and its ability to limit phytoplankton production in the Vaal River should be investigated further.

Reduced I_k (irradiance at the onset of light saturation) and P^B_m (photosynthetic capacity – ratio of carbon fixed at light saturation to chl-*a* concentration) values were associated with a reduced euphotic zone in the Vaal River, but increased dP_d (daily rates of areal photosynthesis) and P_m (photosynthesis in depth profile) values were ascribed to an increase in chlorophyll-*a* levels. The photosynthetic efficiency (PSE) increased with decreased concentrations of algal pigment.

Interestingly, the Vaal River at Balkfontein is reported as switching from a phosphorus-limited system (1986-1990) to a nitrogen-limited system (1991-1993), as suggested by reduced annual DIN:DIP ratios. Roos et al. suggested that reduced silica availability (1990-1993) was responsible for reduced peak levels of chlorophyll-a during the winter-spring period.

Environmental variables, abundance and seasonal succession of phytoplankton populations (*Pieterse and Janse van Vuuren, 1997*, Chapter 3)

This component of the study aimed to describe the types of algae present, seasonal occurrence patterns and environmental effects controlling growth and succession at four sites including the Rand Water Barrage, Parys Municipality, Western Transvaal Regional Water Company and Goldfield Water at Balkfontein. The findings, largely consistent with extant understanding of algal behaviour from the lentic environments, may be summarised as follows:

- Discharge was found to be the most important variable affecting phytoplankton blooms in the Vaal River. Increased discharge increased turbidity, and reduced light

penetration. Flooding also had the capacity to wash phytoplankton blooms out of the system, whereas reduced discharges led to the settling out of sediment and an associated decrease in inorganic turbidity.

- Increased discharge diluted the concentrations of dissolved solids (TDS) resulting in lower concentrations of major ion and hence electrical conductivity. Decreases in electrical conductivity results in reduced flocculation of clay particles and increasing turbidity, reducing the quality of the underwater light climate and resulting in conditions that are unfavourable for algal growth.

- Increased discharge resulted in higher particulate-borne nutrient availability (nitrogen and phosphorus). The disposal of industrial, mining and domestic wastes at the level of the watershed was found to increase dissolved phosphorous and nitrogen concentrations leading to eutrophication of the river.

- Water temperature determined dominance by specific algal species. Summer periods promoted cyanobacterial growth (e.g. the filamentous *Oscillatoria simplicissima*) and the filamentous centric diatom *Aulacoseira* (formerly *Melosira) granulata*. Winter blooms were dominated by a succession of unicellular centric diatoms and green algae (e.g. *Chlamydomonas incerta*).

- Temperature and diatom concentration had an impact on silicon and oxygen concentrations – in the form of increased water temperatures, decreased oxygen solubility and decreased silicon concentrations. Diatom concentration increased with high Si:DIP ratios – diatom numbers led to decreases in Si concentrations.

- High temperatures and nutrient values and low DIN:DIP and TN:TP ratios (less than 5) and low Si:DIP ratios were found to promote cyanobacterial growth.

- pH increased with increasing chl-*a* concentrations and higher algal biomass – a consequence of elevated photosynthetic activity. Similarly, increased oxygen concentrations (usually low because of higher temperatures) were associated with increasing algal biomass (chlorophyll-*a* concentrations).

- Approximately 124 algal species from seven major groups were identified during the study. Some groups were more prevalent at certain localities than others. The highest diversity of

Cyanophyceae was found at the Barrage, Euglenophyceae at Balkfontein, and with biomass increasing downstream from the Barrage to Balkfontein.

The scarcity of Chrysophyceae and Cryptophyceae and abundance of Cyanophycaea demonstrated that the Vaal River is a polluted and eutrophic system. Maximum chlorophyll-*a* concentrations occurred from January to March (summer) and July to November (winter-spring). Highest levels of algal pigments were found at Parys due to high N and P concentrations at this site.

The effect of increased concentrations of total dissolved salts on algal species from the Vaal River (Pieterse and Janse van Vuuren, 1997, Chapter 4).

Irrigation return flows below the Barrage are known to have a major impact on salinity levels below the barrage. Atmospheric pollution with a dominant sulphate constituent also contributes to TDS concentrations. Higher TDS levels result in lower turbidities, increased light penetration and elevated primary productivity. Prinsloo reported that little is known of the relationship between salinity and algal communities in running water. The change in salinity levels was monitored at two study sites on the Vaal River (Stilfontein and Balkfontein) between 1984 and 1993. Average TDS values were determined using an historical overview and used to do growth and carbon assimilation experiments.

An increase in salinity levels of 29 $mg.\ell^{-1}.a^{-1}$ was observed between 1984-1993 at Balkfontein. Mean annual TDS concentration was 516 $mg.\ell^{-1}$. Stilfontein TDS concentrations were observed to increase by 21 $mg.\ell^{-1}.a^{-1}$ and mean annual concentrations were lower, 504 $mg.\ell^{-1}$. Highest salinities occurred during the winter-spring period. Lowest salinities were recorded during floods as a consequence of dilution.

Nutrient availability: phosphatase and nitrate reductase activity in the Vaal River and its phytoplankton (Pieterse and Janse van Vuuren, 1997, Chapter 5).

The aim of this study was to determine the availability of inorganic nitrogen and phosphorus to algae using estimates of enzyme activities. The role of nitrate reductase (NR) and alkaline phosphatase in phytoplankton was examined in samples collected from the Vaal River at Balkfontein. Since NR plays a role in reducing NO_3^- to NH_4^+ before nitrogen can be incorporated in the cells, NR activity has been used to predict (Hochman et al. 1986) the use of nitrate in cells rather than

NH_4^+ (which is the preferred form). Inorganic orthophosphate (PO_4-P) is the main source of P for phytoplankton. Acid and alkaline orthophosphatase is used by phytoplankton to liberate orthophosphate for metabolism in cells from organophosphate esters (Wynne, 1981). The presence of alkaline phosphatase in lake water appears to reflect the degree of eutrophication (Jones 1972) because it corresponds to maximum rates of cell division (Pollinger & Serruya 1976).

Axenic cultures of natural phytoplankton samples from the Vaal River were examined. Ambient concentrations of nitrogen and phosphorous (as DIN, TN and DIP and TP) were measured once a month as part of a long-term management programme.

Nitrate reductase (NR) activity

Coetzee and Pieterse used both centrifugation and filtration techniques to harvest cells and collect the NR enzyme. They found that the filtration method produced higher NR activity values than centrifugation, but that the two methods produced the same general trend.

NR activity was found to be inversely proportional to chlorophyll-*a* concentrations. Peaks in September 1993 (chl-a 79 $\mu g.\ell^{-1}$), November 1993 (chl-a 50.7 $\mu g.\ell^{-1}$) and March 1994 (chl-a 116 $\mu g.\ell^{-1}$) corresponded to low NR activities on these same dates.

No correlation could be demonstrated between NR activity and NO_3-N or TN concentration (similar to findings of Wynne and Berman, 1990). Coetzee and Pieterse concluded that the control of NR activity is not directly dependent on NO_3^- /NH_4^+ concentrations, but may be dependent on several environmental factors. A positive correlation was found between NR activity, DIP (PO_4-P) and TP concentrations. The conclusion drawn was that high P levels activate NR.

Phosphatase (PASE) activity

Four phosphatase enzyme activities were studied: alkaline soluble and particulate phosphatase and acid soluble and particulate phosphatase. All four were found to show the same trend. PASE activity appeared to be indirectly proportional to chl-a concentrations. Hence, both NR and PASE appear to be indirectly proportional to chlorophyll-*a* concentrations, suggesting both enzymes respond to similar conditions. PASE corresponds positively to NR activity. Phosphatase activity also corresponded positively to DIP (PO4-P) and TP concentrations. No correlations were found between N:P ratios and phosphatase activity.

(see Supporting Literature)

Phytoplankton biomass and environmental variables in the Vaal River at Barrage and Stilfontein (Pieterse and Janse van Vuuren, 1997, Chapter 6).

The study aimed to investigate the relationship between physical and chemical environmental variables and phytoplankton in the Vaal River. Using statistical correlations, Pieterse and co-workers compared water variables upstream of the Rand Water Barrage sluice gates with the 7 m deep water released in to the Vaal River, the water at Lindeques Drift 9 km downstream from the barrage, and the water 140 km downstream at Stilfontein.

At the Barrage and Lindeques Drift, NO_3, TDS, chlorophyll, pH, conductivity, alkalinity and ionic species were negatively correlated with turbidity and orthophosphate. The water was turbid, and orthophosphate concentrations were found to be high when the concentration of dissolved ions, chlorophyll, pH and alkalinity were low.

Temperature, suspended solids, turbidity and phosphorous were related to ionic species (Na, Cl, SO_4, etc.) and associated properties of conductivity, TDS and alkalinity. Erosion due to the summer rains resulted in higher siltation loads, with the converse being true during winter. Longer water retention times in winter led to high concentrations of dissolved salts.

The chlorophyll concentration was greatest in winter when the water was clear and cold, and highest pH levels, a direct consequence of algal photosynthesis, were recorded during this period. Algal growth was lowest in summer when the water was turbid and warm.

Depth related processes over a depth of 7 m were found to have a more significant effect on variables than longitudinal processes over a distance of 9 km.

Strong correlations between suspended solids and turbidity and correlations between conductivity, TDS and alkalinity suggested that only one of each group would be necessary for modelling purposes (see below).

Modelling algal growth in the Vaal River (Pieterse and Janse van Vuuren, 1997, Chapter 7).

Cloot et al. described the foundation for a mathematical model to describe the development of algal blooms. Cloot's model for algal growth was based on the assumptions that the water is eutrophic

and that the growth and death of algae are primarily dependent on available light and temperature. The parameters of the equations include environmental variables (maximal irradiance, temperature, concentration of suspended inorganic material, etc.) and on variables specific to a particular algal group (optimal light intensity temperature etc. for growth, algal settling rate etc.).

Weekly-averaged temperature and total suspended solids concentration were used as the inputs to the model. The agreement between calculated chlorophyll-a concentrations and measured (observed) values over a three-year period (1985 – 1987) were fairly good, but some discrepancies remained. An additional factor – the effects of dissolved silicon concentration on the growth coefficient – was included in order to improve the quality of the simulation. The modelling was based on the dissolved silicon concentration as a mechanical and chemical process, the uptake of silicon by diatoms, the effect this (uptake) has on silicon concentration in the water, and on the growth coefficients of diatoms.

Applications of the model include: the splitting of global data between smaller groups responsible for blooms, identifying important environmental variables for specific algae in a bloom, describing mechanisms when numerical data are not available, a means of testing hypotheses and as a planning tool in water resource management.

6.7 Management of blooms

The feasibility of managing the nitrogen to phosphorous ratio in the Hartbeespoort Dam as a means of controlling Microcystis scums (Conradie and Quibell, 1992).

Conradie and Quibell (1992) investigated the possibility of managing *Microcystis* populations by increasing the total nitrogen: total phosphorous (TN:TP) ratio.

Factor influencing dominance of Microcystis

Rhee and Gotham (1980) describe a model of algal competition for nitrogen- and phosphorus- limited growth where two algal species are growth-limited for different TN:TP ratios. They suggested that *Microcystis* becomes nitrogen-limited at a TN:TP ratio of 9. At ratios less than this, *Microcystis* would be expected to dominate the algal community. This explanation was the basis of Chutter and Rossouw's (1992) paper suggesting that the shift in dominance away from *Microcystis* in the Hartbeespoort dam during 1988/89 was because of an increase in the TN:TP ratio.

However, studies by Bierman et al. (1974) contradict the above hypothesis. In studies of competition between *Microcystis* and *Chlorella*, Bierman et al. (1974) noted that the phosphorous transport system of *Microcystis* was more efficient than that for *Chlorella*, but that the growth rate of *Chlorella* was faster. Thus at a TN:TP ratio of 6, *Microcystis* may not dominate, but will always dominate at a TN:TP ratio of 30. Dominance by *Microcystis* was related to the availability of phosphorus – at increased phosphorus concentrations, *Chlorella* was able to utilise its faster growth rate and outcompete *Microcystis*.

This contradicts the view that *Microcystis* is able to dominate at lower TN:TP ratios. Conradie and Quibell argued that this view is too simplistic and that it was rather the low concentrations of ortho-phosphate triggered the shift away from a *Microcystis* dominated community in the Hartbeespoort Dam. This latter view is deemed to be the most relevant in the argument surrounding N:P ratios and algal dominance. As has been so clearly pointed out by Reynolds (1992), elemental ratios are only of significance when the availability of one or both thereof is limiting, or potentially so.

De Noyelles and O'Brien (1978) stated that when carbon concentrations are low and pH is high, blue-green algae might predominate since they may be better assimilators of carbon (see also Shapiro 1990). Novak and Brune (1985) also argued that the half saturation constants (K) and maximum rate of uptake (V_m) for

carbon is species-specific and varies with temperature and light. This could provide an alternative explanation for the shift in algal community composition in the Hartbeespoort Dam. They cited numerous other factors other than TN:TP ratios cited by Chutter and Rossouw (1992) that may have precipitated this shift.

(See Suggested Reading)

Algal regrowth in the holding dam at the Withoogte water treatment works (Quibell and du Plessis, 1991)

Taste and odour problems were associated with algal regrowth in the holding dam at Withoogte treatment works that forms part of the Berg River-Saldanha Government Water Scheme in the Western Cape. The study was initiated to assess the extent of the problem and the effect on the DOC content (dissolved organic carbon). Water is abstracted at a depth of 4 m from the Misverstand weir and pumped via a 12.5 km pipeline to the Withoogte holding dam where it is fed into the treatment works.

The algal population was found to consist of the blue-green algae *Anabaena* sp., the diatom *Melosira* sp. and the dinoflagellate *Peridinium* sp. These organisms, when they occur in sufficient quantity in eutrophic waters fix large amounts of carbon. The death, decay of these cells combined with bacterial activity produce micro-pollutants that produce taste and odour in the chlorinated water. *Anabaena* produces a characteristic 'mouldy' or 'muddy' taste (geosmin and/or 2-methyl isoborneol) even when present in low concentrations. *Anabaena* occasionally forms a significant proportion of the community in the Misverstand weir and taste and odour problems were noted in the potable water. This appeared to be the most likely source of the taste and odour problems. Other likely sources of taste and odour suggested by Quibell and du Plessis (1991) included bacterial activity in the holding dam and treatment works. They suggested the use of activated carbon to combat the problem (see also work by Harding relating to taste and odour problems in the Theewaterskloof and Voelvlei impoundments.

Health aspects of eutrophication (Scott et al. 1985)

The paper highlights the role of phosphate as a key nutrient in eutrophication and its contribution towards health problems associated with water supplies, particularly the development of cyanobacterial blooms. A brief history of outbreaks (in Queensland Australia), the kinds of toxins (structure) and their effects is given. Also, possible links between Legionnaires disease, Haff's disease and

the occurrence of cyanobacteria and their extracellular products is discussed.

The possible contribution of the extracellular products of *M. aeruginosa* to the formation of organohalogens (potentially carcinogenic and/or mutagenic) after chlorination is not known.

Total organohalogen formation potential (TOHp) was estimated after chlorination of three unialgal NWIR laboratory cultures of *M. aeruginosa*, strain WR 133. Preliminary results showed that TOHp increased with time in cultures augmented with phosphorus. The third culture died on the 8th day, and TOHp value has increased to 6 700 μg.$CHCl_3$.ℓ^{-1} at the time. After 14 days, all three cultures indicated TOHp concentrations in excess of 1 000 μg $CHCl_3$.ℓ^{-1}.

Scott et al. summarise organohalogen concentrations from several studies that indicate that cyanobacterial cultures show much higher TOHp values than green algae.

Scott and co-workers point out the possible beneficial effects of algae and point out the use of floating mats of *Spirulina* sp., *Phormidium tenue*, *Chroococcus turgidus* and *Nostoc commune* for food in Mexico (Ortega 1972). They also point out that some species (*Hydrodictyon reticulatum* and *Aphanothece nidulans*, Graf and Baier 1981) had antibacterial effects on a range of pathogenic organisms.

(see Supporting Literature)

The management of phosphate concentrations and algae in Hartebeespoort Dam (Chutter and Rossow, 1992).

This report aimed, firstly, to investigate the impact of the so-called 1 mg.ℓ^{-1} special orthophosphate standard for effluents on the phosphorous load in the Hartbeespoort Dam, and, secondly, to investigate the cause of the disappearance of a bloom of *Microcystis* during the summers of 1988/89 and 1989/90. The dam had been full during April and May of 1988 and deliberate flushing of the *Microcystis* scums at the sluice gates contributed to the major disappearance of *Microcystis* during this time. However, the reason for the failure of *Microcystis* to reappear thereafter was the main reason for the study.

Temperature and stability of the water column were not outside the normal range observed over the previous two summers, precluding instability of the water column as a possible factor.

The external load of total phosphate per unit area declined in 1989 and 1990 relative to previous levels, with the flow-weighted mean and annual concentration of total phosphorus in the Crocodile river having declined. The reduction in mean total phosphate concentration in the dam was deemed to be due to the reduced incoming load and a greater proportion of this load having sedimented from the water column. The external load and the flow-weighted mean annual concentration of total nitrogen also declined, but not has much as the total phosphorous load and concentration. These changes resulted in an increase in the TN:TP ratio and it was suggested that the disappearance of *Microcystis* was a consequence of this.

Recommendations were made that the 1 $mg.\ell^{-1}$ effluent phosphate standard be strictly enforced, nutrient ratios and composition of the phytoplankton community in the dam should be regularly monitored, and that nitrogen standards for nitrogen effluents could be relaxed in order to increase the TN:TP ratio.

Assessment of the phosphate standard (DWAF, 1998)

The 1 $mg.\ell^{-1}$ standard for phosphate was promulgated in August 1980 as the first step in a national eutrophication control programme. Initially, the standard was introduced in seven sensitive catchments (the so-called Special Standard Rivers), and local authorities were given five years to achieve compliance. During 1997/8, compliance with the 1 $mg.\ell^{-1}$ phosphate standard was assessed at the Baviaanspoort and Zeekoegat wastewater treatment plants (Gauteng, Roodeplaat Dam). Both treatment works were found to not comply, with the standard being exceeded in greater than 50% of samples analysed. The investigation concluded that, while the standard would no doubt result in an improvement in trophic condition of the receiving waters in the long term, internal loading from within the impoundment would prolong the recovery process. It was also concluded that a more stringent standard, viz. 0.5 $mg.\ell^{-1}$ was likely to be of far greater benefit.

Health Problems of Hartbeespoort Dam (Aucamp et al. 1987).

Aucamp and co-workers considered the impacts of increasing anthropogenic activity in the catchments draining to Hartbeespoort Dam, and *inter alia* on the development of toxic cyanobacterial blooms. They observed that, given the buoyant nature of the dominant cyanobacterial genera, the abstraction offtakes for raw potable water should be located as deep beneath the epilimnetic layers of the dam as possible.

Furthermore, they noted that the water treatment processes would require augmentation with activated carbon if the cyanobacterial toxins were to be successfully removed.

Use of dissolved air floatation to remove cyanobacteria (De Wet, 1980; Langenegger, 1985).

Th work by De Wet reports on some of the early investigations of dissolved air floatation as a means of removing algae from raw potable water. Using results obtained from an 800 $\ell\ h^{-1}$ pilot plant, De Wet concluded that there was no significant difference between floatation and sedimentation, other than that floatation appeared to be a more economical alternative. In subsequent work, Otto Langenegger showed that some of the problems encountered when treating eutrophic waters could be overcome using DAF technology.

(see also Williams et al. 1985)

Algal penetration of water treatment plants (Pieterse et al. 19--).

Penetration of algal cells through all of the stages of the potable treatment process can and does occur. In an examination of this phenomenon in South African waters, Pieterse and co-workers investigated algal penetration of the Goldfields treatment works at Balkfontein on the Vaal River. They found that the various secondary and tertiary treatment processes, viz. pre-chlorination, flocculation/sedimentation, sand filtration and post-chlorination reduced algal biomass and species richness, but did not always preclude passage of certain algal cells through the complete system. They found the following algae to commonly penetrate the purification process: *Synechococcus cedrorum*, *Chlamydomonas incerta*, *Oocystis lacustris*, *Scenedesmus opoliensis* and *pennate diatoms*. This work showed that even large, ornamented algal cells such as *S. opoliensis* pass through a apparently intact treatment system.

Tastes and odours (Wnorowski, 1993)

Although not health-threatening, the odours (geosmin and 2-methyl isoborneol) produced by certain species present a major problem to managers of many potable water treatment plants. In most cases addressing the problem entails the retro-fitting of activated carbon treatment, either in granular (GAC) or powdered (PAC).

Wnorowsji examined water from 54 dams, and found taste and odour problems to be present in 30% thereof. All of the lakes

experiencing odours were dominated by cyanobacteria, and with *Microcystis* as the dominant genus. Geosmin was exclusively found to be the odourous compound. The result is interesting as geosmin production is commonly associated with species of *Anabaena*, and not *Microcystis* (see also similar findings in work by Umgeni Water described later in this review).

6.8 Researched incidents of toxicosis in South Africa

Mortality of a white rhinoceros (Ceratotherium simum) suspected to be associated with the blue-green alga Microcystis aeruginosa (Soll and Williams 1985)

Soll and Williams (1985) reported the deaths of several black wildebeest (*Connochaetes gnou*) in the Willem Pretorius Game Reserve (Free State). These mortalities were suspected to have been caused by a toxic bloom of *Microcystis* (P le Roux, 1984, Division of Nature Conservation, Orange Free State, personal communication, cited in Soll and Williams 1985).

This account relates the death of a white rhinoceros *(Ceratotherium simum)* at Barakologadi Game Reserve in the Odi district of Bophuthatswana. The reserve, on the banks of the Klipvoor Dam, was approximately 9 000 ha in extent. Four animals had been introduced from the Umfolozi Game Reserve in Natal. Having paired off, the older pair remained closer to the dam than the younger pair. The younger animals were found dead and in an advanced state of decomposition in mid-July 1979 and a post-mortem could not be carried out. The condition of the older animals appeared to be poor. The female was subsequently found dead in sternal recumbancy during late July of the same year. Upon inspection, the animal showed signs of excessive lacrimation and dried soft faeces. The male appeared to be healthy but showed signs of mild diarrhoea. A necropsy was conducted on the female, and revealed the following:

Macroscopical pathology

Severe hepatomegaly. The liver was found swollen with rounded edges and deep purple colouration. The surface bulged when cut, red fluid seeped from incision, the parenchyma was soft and friable and evidenced advanced autolytic changes (even though no other organs showed signs of autolysis).

Petechiae and ecchymoses were apparent in the subcutaneous tissue and serosa of thorax, abdomen and diaphragm. Severe subendocardial and subepicardial haemorrhages were noted. Severe focal disseminate subcapsular haemorrhage was apparent in the spleen, and the blood vessels supplying intestines were congested. 5 ℓ of serous fluid was discovered in the abdominal cavity, along with serous atrophy of fat in the mesentrium and pelvic area. Localized congestion was also present in the lungs of the animal.

Microscopical pathology

90 100% of the hepatocytes in the liver had undergone lytic necrosis. The connective tissue supporting the liver cell plates was distorted or absent, and, hence, confluent sinusoids with erythrocytes admixed with cytoplasmic debris from lysed hepatocytes. The portal triads showed no lesions similar to those typically reported for mice, cattle and sheep in cases of *Microcystis* poisoning. The spleen showed severe subcapsular haemorrhage with atrophy of white pulp. Lung congestion was considered as being probably due to hypostasis.

Algal identification

A porridge-like green scum between 4 and 12 cm thick, and formed by blue-green alga was reported as covering the surface of the Klipvoor Dam. The Department of Botany at the University of Pretoria identified the bloom as comprising an almost pure culture of *M. aeruginosa*. A large amount of fertilizer had previously been added to the dam as part of a fish-breeding scheme, leading to the development of the eutrophic conditions.

In their analysis, Soll and Williams suggested a wide interspecies variation in susceptibility to *Microcystis* toxins since, despite the presence of many other species in the reserve (giraffe, zebra, wildebeest, impala, kudu, jackal and warthog), no mortalities other than that of the rhinoceros had been reported. This did not, however, exclude deaths where the carcass had not been detected, or where decomposition was too advanced for necropsy.

Soll and Williams cite Jackson et al. (1984) as reporting that up to 90% of the lethal dose from a bloom could be ingested without measurable effect. They suggest that, because rhinoceros would drink a larger volume of water in the reserve than other animals, it would ingest more toxic algae. They also suggest that variations in the toxicity of *Microcystis* (due to environmental factors such as light intensity, temperature, age of the cells and pH) might explain the poisoning of the rhinoceros, since they happened to drink the water when the bloom was most toxic.

Giraffe deaths associated with Microcystis in the Bloemhof Dam (Theron, 1990a)

During July 1989, Mr J. van Zyl of the Sandveld Nature Reserve in the Orange Free State reported the deaths of two giraffe in January, and a further five during March of that year. State veterinarians could not ascertain the cause of death, and the then Hydrological Research Institute (HRI - now IWQS) were asked to investigate.

Samples from the surface of the water of the Bloemhof Dam confirmed that between 10-60% of the algae in the dam were *Microcystis*, and that between 10-15% of these colonies were toxic. With hindsight, these characteristics were not in themselves indicative of a significant risk. Analysis using HPLC confirmed that there were two toxins present. A mouse injected with 0.5 mℓ of a low (unspecified) concentration of the toxin did not die within 24 h. This did not preclude the presence of a toxin since the concentration may have been too low, i.e less than the LD_{50} level.

Wind directions on the Bloemhof Dam were monitored: the wind was found to be predominantly south west to westerly between March and September, i.e., during the months that the deaths were reported. This would have driven any floating material, including the algae towards the shore of the dam bordering the Sandveld Nature Reserve. It was noted in the report that giraffe do not wade into the water like other wild animals, but drank water near the shore – where *Microcystis* scums would have been concentrated by the prevailing winds.

The giraffe that had died had evidence of muscle relaxation of the neck and large quantities of water in the stomach – typical symptoms of *Microcystis* poisoning. Liver samples analysed at Onderstepoort could, however, not confirm this. It was concluded that *Microcystis* poisoning had resulted in the deaths of these giraffe.

Toxicity associated with Microcystis in the Klipdrif Dam (Theron, 1990b).

During 1989, the HRI was again requested by the Klipdrif irrigation board to investigate the possible link between livestock deaths near the Klipdrif Dam and the occurrence of *Microcystis* in the dam. It was reported that since 1988 a periodic unpleasant smell was encountered near the dam together with high concentrations of a blue-coloured algae.

Samples were taken from the surface of the dam and taken to the HRI laboratories for analysis. Microscopic investigation of the samples confirmed that the algal community comprised of up to 80% *Microcystis*. Two guinea pigs injected with 10 ml of the sample and another two with 15 ml. All four guinea pigs died within 24 hours. Examination of the livers revealed that they were yellow and friable. This confirmed that the *Microcystis* isolated from the Klipdrif Dam was highly toxic.

Toxic algae in the Erfenis and Allemanskraal Dams (Van Ginkel and Hohls, 1999)

Algae were implicated in the deaths of livestock at the Erfenis Dam during April and May 1998. Toxicity was associated with the presence of *Anabaena* spp. During approximately the same period algal blooms in the Allemanskraal Dam were alleged to be the cause of malodours. Both dams are in the eutrophic to hypertrophic nutrient enrichment category, and both are deemed to have a high potential for cyanobacterial bloom development.

Animal death due to nodularin (Nodularia spumigena) toxicity (Harding et al. 1995)

During 1994, a short-lived bloom of *Nodularia spumigena* was recorded in Zeekoevlei, a hypertrophic shallow lake on the Cape Flats near Cape Town. The bloom was atypical in that *N. spumigena* is uncommon in the Western Cape, and Zeekoevlei is typically dominated by *Microcystis aeruginosa* forma *aeruginosa*. The bloom was found to contain 3470 mg nodularin g^{-1} of freeze-dried material, and resulted in the death of a bull terrier bitch that drank from the scum. This occurrence was the first reported incident of nodularin in South Africa, and the first documented case of cyanobacterial-related animal poisoning in the south-western Cape (see also Van Halderen et al. 1995). Previously the confirmed but unreported presence of *Anabaena solitaria* produced microcystin in Theewaterskloof Dam during November of 1993 was the only indication that cyanobacterial toxicity could become a problem in the province (Harding, 1997a).

Livestock poisonings in the western Cape (van Halderen et al. 1995).

The mid-1990s saw the widespread appearance of incidents of cyanobacterial toxicosis across the western and southern Cape, as well as the presence of hepatotoxins associated with an increasing number of algal blooms. These incidents culminated in a massive loss of dairy livestock near Kareedouw, possibly the single largest incident of stock death since the cases reported by Douw Steyn during the 1940s (Harding, 1997a). Van Halderen and co-workers researched and documented three incidents of stock losses, two involving *Nodularia spumigena*, and the third *Microcystis aeruginosa* forma *aeruginosa*. The presence of microcystins at concentrations in excess of 1000 mg toxin.g^{-1} f-d weight of scum, supporting the observation that concentrations in excess of this level pose a very real threat of lethal toxicosis. Together with the Zeekoevlei incident,

(Harding et al. 1995), these cases were investigated by a multi-disciplinary team of veterinarians, algologists and pathologists.

Biomagnification of microcystins by marine filter feeders (Harding, 1998; 1999)

While biomagnification of cyanobacterial hepatotoxins has been reported elsewhere (e.g. Vasconcelos, 1995), the first such reported incident in South Africa occurred during 1998. The collapse of Sago pondweed in Wildevoelvlei, a small coastal vlei near Kommetjie, was followed by a dense bloom of *Microcystis aeruginosa* forma *aeruginosa*. The microcystins –YR and –LR were detected in both the scum material, and later in the tissue of black mussels, *Choromytilus meridionalis* located in the intertidal zone where Wildevoelvlei drains to the sea. A ban on mussel collections threatened the livelihood of a community dependent thereon, and efforts were made to eradicate the algal bloom. Based on experiences drawn elsewhere regarding the sensitivity of *M. aeruginosa* to salinity (Harding, 1994), a decision was made to augment the salinity level in the Wildevoelvleis to approximately one-third that of sea water, viz. 10 ppt. This was carried out by "bombing" the vlei with coarse rock salt, and introducing salt in solution from an adjacent wastewater treatment works. The resultant ambient salinity of 8 ppt was sustained enough to bring about the decline of the *M. aeruginosa* bloom, and succession by *Kirchneriella* sp. Complete depuration of the mussels was observed two weeks later. The alleviation was, however, temporary, and efforts to reduce the level of nutrient enrichment in the vleis, and support the re-establishment of rooted macrophytes (*Potamogeton pectinatus*) have yet to bear fruit.

6.9 Removal of toxins from water supplies

Removal of Microcytis toxins in water purification processes (Hoffman, 1976)

Hoffman (1976) confirmed the results of previous studies that suggested that conventional purification plants using coagulation, flocculation, sedimentation, filtration or chlorination are not able to remove algal toxins. Toxicity assays on mice injected with toxins subjected to the above treatments showed that the processes had failed to remove the toxins to below active levels. However, treatment with powered activated carbon *was* able to remove the toxin. Bioassay tests on the toxins occurring in solution in the waters of the Hartbeespoort Dam (mean chlorophyll *a* value of 20 $\mu g.\ell^{-1}$) showed that the concentration of toxins in the water was too low to have any lethal effects (it was determined that 560 ℓ would have to be consumed by a human of 70 kg in order to be lethal).

Hoffman (1976) suggests that since the LD_{50} for the toxins had not been determined at the time of writing, the cumulative effects of ingestion could not be predicted.

(see also Section on post-1990 era for details of investigations undertaken by the Cape Metropolitan Council and Umgeni Water .)

6.10 Cyanobacterial Studies in South Africa post-1990

This section reviews the activities, developments and incidents as reported for various regions of the country during the 1990s, i.e., during the period following the cessation of formal cyanobacterial investigations at national level. During this period the emphasis on cyanobacterial research, and the detection of cyanobacterial toxins was centered in the local authority and water utility arenas. However, towards the end of the 1990s, fresh research studies conducted at tertiary education centres began to emerge.

6.10.1 Western Cape

Incidents of toxicosis

Although blooms of cyanobacteria are common in South Africa, animal deaths due to hepatotoxins were, prior to 1994, largely confined to the central and Northern Provinces. This pattern changed during the last weeks of 1993 when the first stock losses, attributable to cyanobacterial toxicosis, occurred in the south-western Cape. Previously, problems associated with cyanobacteria had been limited to the production of the taste and odour compound geosmin in Theewaterskloof Dam near Villiersdorp. The first detection of toxins was made from a sample of *Anabaena solitaria* collected from this same reservoir during November 1993, and analysed at the University of Dundee, Scotland. Subsequently, between the December months of 1993 and 1996, 7 stock and domestic animal deaths were recorded in this region and along the southern Cape coast. During the same period a single incident of stock loss was reported from the Northern Province. Together, these losses were estimated to represent an immediate financial loss of 1.1 million SA Rands (1996 value -1 US$ = R4.50).

The first south western Cape poisonings occurred between December 1993 and March 1994 in the Malmesbury-Darling area. In both cases the stock losses (3 cattle plus 5 exhibiting photosensitivity, and 29 sheep, respectively) occurred in camps where animal deaths with identical clinical signs had previously been observed. These earlier deaths, however, had not been linked to toxins produced by the cyanobacteria. Filaments of *Nodularia spumigena* were found in the drinking water supplies in both incidents. No analyses for the presence of nodularin were performed as appropriate methodology was not available locally at the time. These two incidents have been described in detail elsewhere (Van Halderen et al. 1995).

During March 1994, a bloom of *Nodularia spumigena* in Zeekoevlei, a shallow lake near Cape Town, resulted in the death of a bull terrier bitch. HPLC analysis of algal material collected from where the dog drank contained a nodularin concentration of 3479 $\mu g.g^{-1}$ of freeze-dried (f-d) material. This incident, described fully elsewhere (Harding et al. 1995), was interesting in that this was a short-lived appearance of *Nodularia* in a lake which has been dominated by *Microcystis aeruginosa* forma *flos-aquae* for the past five decades. At the time of writing this review, *N. spumigena* had not subsequently been recorded in Zeekoevlei.

In May of 1994 a bloom of *Microcystis aeruginosa* forma *aeruginosa* occurred in a farm dam near the town of Paarl, resulting in the death of 11 sheep and

induced-photosensitivity in a further 20 animals. The cyanobacterial hepatotoxin, microcystin-LR, was detected in the algal bloom at 1 340 $\mu g.g^{-1}$ f-d weight, and a second, unidentified, microcystin variant at 585 $\mu g.g^{-1}$ f-d weight. The case history of this incident is detailed in Van Halderen et al. (1995).

After an incident-free lull of two years, a *Microcystis aeruginosa* forma *aeruginosa* bloom in the George district was implicated in the deaths of three calves, and photosensitivity in a further 30. Analysis of lyophilized material from this bloom revealed the presence of the microcystins-LR and -YA at a combined concentration of 1 720 $\mu g.g^{-1}$ f-d weight. The George case was followed by a massive stock loss of 290 in-milk dairy cows on a farm in the Kareedouw district. A further 70 animals were diagnosed with acute photosensitivity and had to be slaughtered. Although the clinical signs and symptoms were consistent with acute hepatotoxicosis, the water supply was flushed out soon after the incident and no toxins could be detected at the scene of the incident. Microscopic examination of the rumen contents of two of the dead animals showed the presence of filaments of *Anabaena*, and remnants of a mat of *Oscillatoria* filaments was found growing on the walls of the cement-lined reservoir from which the animals had drunk. HPLC analysis of rumen and bile revealed a hydrophobic, microcystin-like component possessing a UV_{238} spectrum closely typical of the microcystins. The results of further tests on the rumen contents, performed by Professor Geoff Codd (University of Dundee, Scotland), using ELISA immunoassay and protein phosphatase inhibition, were consistent with the presence of microcystins.

During December 1996 another incident was reported, again from the Malmesbury district. Here three 8-month old lambs died after drinking water containing *Microcystis aeruginosa* forma *aeruginosa*, and containing the microcystins -LR, -YR and LY at a combined total concentration of 1 890 $\mu g.g^{-1}$ f-d weight. No clinical signs or symptoms were evident prior to the deaths. Post-mortem examinations of the affected animals showed severe lung congestion and oedema, widespread endo- and epicardial haemhorrages, severe liver damage with yellow colouration, and moderate swelling of the kidneys. There were also widespread haemhorrages in the skeletal muscles. The histopathological examination showed pannecrosis of the liver, and acute nephrosis of the kidneys.

During the period encompassed by these incidents, a case of a death of goats was reported from the Northern Province. Eighteen four-month old goats died after drinking from a cement-lined trough near the town of Alldays. As was presumed to be the case at Kareedouw, the causative organism was found to be *Oscillatoria*.

Post-mortem examination revealed swollen and congested livers, congestion of the kidneys with some nephrosis, and oedema of the lungs. The results of the histopathological examination were consistent with the effects of acute cyanobacterial poisoning. HPLC analysis of a water sample from this incident contained 71 $\mu g.\ell^{-1}$ of an unidentified, hydrophobic microcystin.

Although cyanobacterial blooms have long been common in South Africa, the incidents reported on here represented the first documented emergence of related poisonings in the southern and south-western regions of the country. They also heralded the appearance of *Oscillatoria*-related incidents in a country where, apart from the possible exception of the Vaal River, *Microcystis* and *Anabaena* spp. are the commonly expected dominants of cyanobacterial blooms.

The incidents involving *Oscillatoria* were found to differ from those typical of *Microcystis* or *Anabaena* in that a lesser amount of cyanobacterial biomass appears to be involved, and that the hydrophobic toxins produced appear to have a toxicity greater than equivalent amounts of the more hydrophilic, commonly-encountered microcystins. This observation has significant implications for the determination of toxicity based on equivalents of microcystin-LR.

Cyanobacterial investigations

Cape Metropolitan Council

During the period 1990–1997 a number of investigations were conducted to investigate the ecological associations of cyanobacteria in shallow well-mixed and eutrophic lakes (Harding, 1991, 1992a,b, 1994, 1996 1997a,b; Harding and Quick, 1992, Harding and Wright, 1999). These studies revealed *inter alia* that cyanobacteria, and in particular *Microcystis aeruginosa*, can adapt extremely well to very high (< 1 day) frequencies of intense mixing; that the combination of mixing, non-nutrient limitation and mild climatic conditions results in chemostat-like conditions being generated that support high levels of phytoplankton productivity; that the sustained effect of these conditions results in a plagioclimactic (near-equilibrium) dominated by, typically, one to three species of phytoplankton. It was also found that, despite the overwhelming availability of nutrients, that clear co-dominance of chlorophyte and cyanophyte algal genera occurred, and that impairment of the top-down control feedback dynamic (fish > zooplankton > phytoplankton) could be readily and effectively controlled.

Associated studies (Harding 1992a, 1994, 1996, 1998) clearly showed that the regulation of water levels in shallow lakes

constituted a serious impairment to ecosystem functioning, but that the effect could be markedly reduced through restoration of a greater degree of natural variability.

The production of geosmin by *Anabaena solitaria* has posed a problem for potable water treatment in the Western Cape for many years. Low level aggregations of this alga appeared rapidly in the newly commissioned Theewaterskloof Dam, and were numerous enough to necessitate the implementation of powdered activated carbon (PAC) geosmin stripping at water treatment facilities operated by the Cape Town City Council. Following an initial cyanobacterial bloom of *Microcystis robusta*, *A. solitaria* subsequently appeared in the Voëlvlei Dam near Villiersdorp, with similar consequences. Cyanobacterial hepatotoxins were detected in both impoundments during the mid-1990s, with low levels (chlorophyll-a <15 $\mu g.\ell^{-1}$) generating persistent extracellular microcystin-LR concentrations of the order of 1–3 $\mu g.\ell^{-1}$. Investigations conducted by the Department of Scientific Services of the Cape Town City Council revealed considerable differences in the ability of various batches of PAC to remove both geosmin and microcystins (SA Pieterse, CMC, pers. comm), and necessitated that a challenge-testing process be added to the tender for and procurement of PAC for use by the local authority.

The HPLC methodology developed by Lawton and co-workers (the "Codd Method") for the detection of cyanobacterial hepatotoxins was put to use at the Cape Town City Council's Athlone laboratories during 1994. This was a dedicated automated operation that subjected the methodology to exhaustive testing under local conditions, and using samples submitted from South and southern Africa. The method proved to be robust, sensitive and reproducible for a wide matrix of sample types including water (fresh and brack), muds, algal cells, blood and body fluids including rumen and bile.

Medical Research Council (PROMEC Division – Tygerberg (Shephard et al. 1998).

A joint project between the Photocatalytic Research Group at the University of Stellenbosch, and the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) of the Medical Research Council, was launched during July 1995. The main objective of this collaborative effort was to investigate the possible titanium dioxide (TiO_2) photocatalytic oxidative degradation of cyanobacterial microcystins in water.

Photocatalytic oxidation using TiO_2 is extremely effective in the chemical destruction of dissolved organic compounds, yielding only carbon dioxide, inorganic ions and mineral acids as the end products. The results of this project revealed that it is possible to remove microcystins –YR, -LR and –RR from water via this process. More importantly, no secondary degradation products were observed. This means that total chemical destruction (mineralization) of the parent molecules occurred. Similar results were achieved using both distilled water and natural lake water as spiking media for microcystins. The current research programme of the Photocatalytic Research Group aims to develop and optimize small-scale photocatalytic reactors for water treatment.

6.10.2 Eastern Cape

University of Port Elizabeth

The Microbiology Section of the Department of Biochemistry at the University of Port Elizabeth is focusing on cyanobacteriology, with an emphasis on toxin-producing genera. The scope of these research projects include the ecophysiology of growth and toxin production, with emphasis on carbon and nitrogen fluxes. Methods for the mass production of toxin for research purposes, using mass culture and membrane bioreactor technology are also being investigated. The ecophysiological data contribute to a project investigating cyanobacterial intra- and interspecies competition. This work is aimed at developing predictive models for bloom prevention and control, and includes genetic tagging, laboratory-scale competition and mark and recapture-type field experimentation. Various toxicological experiments include the role of specific micronutrients on the effects of chronic exposure to microcystins. Related work encompasses the survey of water bodies for the presence of cyanobacteria, and the establishment of a comprehensive culture collection.

6.10.3 Gauteng and Northern Province

Rand Water

Over the past few decades, Rand Water has developed a physical, chemical and biological monitoring programme on source and drinking water. This information is used to protect source water, to proactively ensure that treatment changes are made to accommodate changes in water quality and to evaluate the performance of treatment process. To a bulk water supplier of more than 10 million people, this programme is essential for quality assurance and control and for an awareness of changing water quality. A good example of the practical application and need for the monitoring of especially blue-green algae was during the 1998 toxic algal bloom of *Microcystis aeruginosa* in the Vaal Dam, which resulted in toxin build-up in the backwash water of the purification plant. The presence of these toxins dramatically increased treatment costs as the backwash water could not be re-used and the chlorination dosage increased. At the same time, recreational warnings were constantly issued to inform the public of possible health risks when utilizing the Vaal Dam water at specific locations for recreation.

In order to be pro-actively aware of situations such as those of 1998, Rand Water has developed in-house competency to perform phytoplankton species identification, enumeration and chlorophyll-*a* determinations on source and drinking water as part of the biological monitoring programme. This source water monitoring includes:

- Daily monitoring of all intakes to the purification plants. When blue-green algal or associated toxin concentrations exceed set alert levels, the monitoring frequency is usually stepped-up.
- The Vaal, Sterkfontein and Katse Dams are monitored on a monthly schedule. The frequency of monitoring in the Vaal Dam can be increased when the blue-green algal or associated toxin concentrations exceed set alert levels.
- The Vaal River from Vaal Dam to Barrage and Loch Vaal is monitored weekly during summer and bi-monthly during winter. Because of the importance of this water for potable water generation and recreational utilization, the monitoring is also stepped up when blue-green algal or associated toxin concentrations exceed set alert levels.
- Phytoplankton monitoring of the Liebenbergsvlei and Ash Rivers is performed on a bi-monthly schedule, while phytoplankton monitoring is performed weekly on the Klip River, Suikerbosrant River, Natalspruit,

Rietspruit, Taaibosspruit and the outlet from the Barrage.

Due to the high importance placed on cyanobacteria and their associated toxins, an ELISA screening test laboratory has been established at Rand Water's Hydrobiology section. It performs screening tests on localities that are prone to high blue-green algal biomass concentrations on a set routine schedule, which is not totally dependent on the blue-green biomass concentrations at the time. Further ELISA screening tests are initiated as stipulated in the cyanophyceae monitoring protocol. HPLC analyses are also performed in-house as stipulated in the cyanophyceae monitoring protocol.

The cyanophyte monitoring protocol developed by Rand Water is based on the Australian model. It has recently been updated to incorporate the current trend described in WHO (1999), and to incorporate current information needs of Rand Water. This protocol has been optimised to serve both the drinking water production unit and the recreational use of the Vaal River Barrage, Loch Vaal and Vaal Dam effectively.

In collaboration with universities, Rand Water has focused some attention on research during this past decade. The research includes an investigation to determine toxin production in the blue-green alga *Oscillatoria simplicissima* under certain environmental conditions. In future this research will be expanded to include various other aspects for example rapid changes in environmental conditions. Research to determine the appropriateness and efficacy of treatment processes for the removal of algal toxins and associated tastes and odours from drinking water has also been performed, by evaluating different carbons, ozone, and chlorination dosages.

Institute for Water Quality Studies (Department of Water Affairs and Forestry)

Two periods of cyanobacterial-related data are available from the Institute for Water Quality Studies (IWQS- DWAF). These cover the testing performed prior to the formulation of the draft National Surveillance Protocol. The results are summarised in the following tables:

Pre-1995 results

Table 4:

Results of a microscopic survey of samples taken in January and February 1986. Toxic form refers to *M. aeruginosa* forma *aeruginosa*, and non-toxic to *M. aeruginosa* forma *flos aquae* sensu Scott. (Source: Quibell et al. 1995).

Water body	Toxic form	Non-toxic form	Chl *a* ($\mu g.\ell^{-1}$)
Bon Accord Dam	•		110
Boskop Dam	•		1
Bospoort Dam	•	•	62
Bronkhorstspruit Dam	ND		6
Buffelspoort Dam	•	•	9
Douglas Weir	ND		10
Grootdraai Dam	•	•	9
Klerkskraal Dam	•		6
Klipdrift Dam	ND		23
Klipvoor Dam	•		112
Kruger Dam	ND		1
Loskop Dam	ND		34
Potchefstroom Dam	•		4
Premier mine Dam	ND		7
Rietvlei Dam		•	31
Roodeplaat Dam	•		22
Rooikoppies Dam	ND		16
Rust de Winter Dam		•	13
Vaalkop Dam		•	10
Witbank Dam	•	•	4
Florida lake	•		19
Pretoria power station	•	•	109
Struben dam	ND		27
TUKS farm dam		•	61
Verwoerdburg lake	ND		50
Lake Banagher	ND		92
Lake Chrissie	ND		619

Chl *a* concentrations above 20 $\mu g.\ell^{-1}$ demonstrate significant amounts of algae, and the risk of scum formation. ND = *Microcystis* not detected.
Note: Toxicity was not actually determined in this survey, and the division between toxic and non-toxic forms was based on colony morphology alone.

Table 5:

Water bodies demonstrating positive toxicity either by the mouse bioassay, or by livestock deaths in the period 1976 to 1986. (Source: Quibell et al. 1995).			
Water body	**Livestock**	**Mouse**	**Genus**
Hartbeespoort Dam	•	•	*Microcystis*
Vaal Dam	•		*Microcystis*
Bon Accord Dam	•		*Microcystis*
Rietkuil Dam	•		*Microcystis*
Vanderkloof (PK Le Roux)	•		*Microcystis*
Klipvoor Dam	•		*Microcystis*
Allemanskraal Dam	•		*Microcystis*
Witbank Dam		•	*Microcystis*
Bronkhorstspruit Dam		•	*Microcystis*
Roodeplaat Dam		•	*Microcystis*
Rietvlei Dam		•	*Microcystis*
Bospoort Dam		•	*Microcystis*
Volksrus Dam		•	*Microcystis*
Erfinis Dam		•	*Microcystis*
Van Ryneveldspass Dam		•	*Microcystis*
Lake Arthur		•	*Microcystis*
Stink Pan		•	*Nodularia*

Table 6:

Results of the sampling for blue-green algal toxicity on impoundments in Gauteng and surrounds in the period November 1993 to November 1994. (Source: Quibell et al. 1995).			
Impoundment	**No. Samples**	**No. Qualify**	**No. Acutely Toxic**
Bon Accord Dam	27	2	0
Rietvlei Dam	32	8	2
Hartbeespoort Dam	45	21	9
Roodeplaat Dam	63	40	12
Bronkhorstspruit Dam	1	0	-
Roodekoppies Dam	2	2	0
Verwoerdburg Lake	1	0	0
Zoolake	2	2	1
Emmerentia pan	2	2	0
Florida lake	1	0	-
Witbank Dam	1	0	-
Middelburg Dam	1	0	-
Loskop Dam	1	0	-
Bloemhof Dam	2	1	0
Allemanskraal Dam	1	0	-
Boskop Dam	2	0	-
Erfinis Dam	1	0	-
Koppies Dam	1	0	-
Klipvoor Dam	6	6	2
TOTALS	192	84 (44%)	26 (31%)

Table 7:

The results of those samples analysed for blue-green algal toxicity, as microcystin-LR (M-LR) for the period November 1993 to November 1994 (Quibell et al.1995).

Dam	Date	Chl *a* ug.ℓ$^{-1}$	Phaeophytin ug.ℓ$^{-1}$	% Blue-green	m-LR ug.ℓ$^{-1}$	Mouse Test	Genus
Bloemhof	22-Nov-93	355	193	100%			*Microcystis*
Bon Accord	10-Mar-94	74.5	<1	96%			*Microcystis*
	24-Mar-94	45.8	9,5	85%			*Oscillatoria*
Emmerentia	09-Mar-94	36.7	<1	68%			*Microcystis*
Hartbeespoort	09-Dec-93	618	49.4	100%			*Microcystis*
	14-Dec-93	722	198	100%	1 965	+ve	*Microcystis*
	22-Dec-93	1 290	126	100%	1 861	+ve	*Microcystis*
	22-Dec-93	25.2	10.3	100%			*Microcystis*
	07-Jan-94	3 070	<1	100%	1 698	+ve	*Microcystis*
	12-Jan-94	5 850	<1	100%	414	+ve	*Microcystis*
	12-Jan-94	92.8	<1	100%			*Microcystis*
	19-Jan-94	1 530	17.2	100%	1 538	+ve	*Microcystis*
	02-Feb-94	148	27.1	100%			*Microcystis*
	02-Feb-94	2 080	202	100%	1 314	+ve	*Microcystis*
	10-Feb-94	NA	NA	100%	474	+ve	*Microcystis*
	16-Feb-94	688	<1	100%			*Microcystis*
	16-Feb-94	590	<1	100%			*Microcystis*
	23-Feb-94	48.7	2.45	100%			*Microcystis*
	09-Mar-94	35.0	0.11	100%			*Microcystis*
	16-Mar-94	573	8.34	100%			*Microcystis/Oscillatoria*
	16-Mar-94	646	52.9	100%			*Microcystis*
	16-Mar-94	835	<1	100%			*Microcystis*
	23-Mar-94	18 860	43.6	100%		+ve	*Microcystis*
	13-Apr-94	23 390	550	100%		+ve	*Microcystis*
	13-Apr-94	40.1	11.9	100%			*Microcystis*
	16-Nov-94	NA	NA	100%			*Microcystis*
	16-Nov-94	NA	NA	100%			*Microcystis*
Klipvoor	13-Nov-93	NA	NA	100%			*Microcystis*
	13-Nov-93	NA	NA	100%	22 330	+ve	*Microcystis*
	03-Dec-93	NA	NA	100%	21 100	+ve	*Microcystis*
	03-Dec-93	NA	NA	100%			*Microcystis*
	08-Mar-94	113	<1	100%			*Microcystis*
	08-Mar-94	6 020	<1	100%			*Microcystis*
	18-Nov-94	NA	NA	100%			*Microcystis*

Table 7 (continued)

Dam	Date	Chl *a* ug.ℓ$^{-1}$	Phaeophytin ug.ℓ$^{-1}$	% Blue-green	m-LR ug.ℓ$^{-1}$	Mouse Test	Genus
Rietvlei	04-Feb-94	103	14.9	70%			*Microcystis*
	09-Feb-94	4 170	<1	100%	198	+ve	*Microcystis*
	09-Feb-94	63.0	7.93	10%	184	+ve	*Microcystis*
	17-Feb-94	2 545	<1	100%			*Microcystis*
	14-Apr-94	88.3	3.35	100%			*Anabaena*
	20-May94	10 780	1560	90%			*Microcystis/ Anabaena*
	03-Jun-94	124	18.2	100%			*Microcystis*
Roodekoppies	08-Mar-94	4 300	<1	90%			*Microcystis*
	08-Mar-94	2 195	<1	100%			*Microcystis/ Anabaena*
	09-Mar-94	516	<1	100%		+ve	*Microcystis*
Roodeplaat	16-Nov-93	205	135	100%			*Microcystis*
	24-Nov-93	134	7.84	99%			*Microcystis*
	30-Nov-93	579	80.8	100%			*Microcystis*
	30-Nov-93	382	37.2	100%			*Microcystis*
	08-Dec-93	653	114	100%			*Microcystis*
	20-Dec-93	29.8	5.69	60%			*Microcystis*
	20-Dec-93	1 817	147	90%			*Microcystis*
	04-Jan-94	20.6	14.0	100%			*Microcystis/ Anabaena*
	11-Jan-94	63.0	3.81	100%			*Microcystis*
	18-Jan-94	3 210	315	100%			*Microcystis*
	14-Feb-94	32	1.74	100%			*Microcystis*
	14-Feb-94	252	<1	100%			*Microcystis*
	22-Feb-94	47.0	5.00	100%			*Microcystis*
	22-Feb-94	1 857	182	100%	981	+ve	*Microcystis*
	01-Mar-94	682	56.7	100%			*Microcystis*
	01-Mar-94	35.5	<1	92%			*Microcystis*
	01-Mar-94	347	<1	100%		+ve	*Microcystis*
	08-Mar-94	1 113	208	100%		+ve	*Microcystis*
	22-Mar-94	1 754	<1	100%			*Microcystis*
	22-Mar-94	1 582	<1	100%			*Microcystis*
	29-Mar-94	51.0	29.3	100%		+ve	*Microcystis*
	12-Apr-94	331	22.0	100%			*Microcystis*
	12-Apr-94	647	108	100%		+ve	*Microcystis*
	12-Apr-94	487	14.9	100%		+ve	*Microcystis*
	20-Apr-94	167	1.01	100%			*Microcystis*
	20-Apr-94	6 783	611	80%		+ve	*Microcystis*
	20-Apr-94	3 542	6.88	100%			*Microcystis*
	03-May-94	688	922	100%			*Microcystis*
	03-May-94	696	837	100%			*Microcystis*
	17-May-94	2 895	<1	70%			*Microcystis*
	17-May-94	65.4	0.69	100%			*Microcystis*

Table 7: (continued)

Dam	Date	Chl *a* ug.ℓ$^{-1}$	Phaeophytin ug.ℓ$^{-1}$	% Blue-green	m-LR ug.ℓ$^{-1}$	Mouse Test	Genus
Roodeplaat	17-May-94	4 092	<1	68%		+ve	*Microcystis*
	24-May-94	13 700	539	98%		+ve	*Microcystis*
	24-May-94	31 609	1408	100%		+ve	*Microcystis*
	08-Jun-94	2 568	106	100%			*Microcystis*
	14-Jun-94	860	6.88	100%		+ve	*Microcystis*
	14-Jun-94	13 184	64.2	100%		+ve	*Microcystis*
	12-Jul-94	502	858	100%			*Microcystis*

Post-1995

The following table summarises the incidents investigated by IWQS during the period 1997 to 1999:

Table 8:

Summary of cyanobacterial incidents reported by IWQS during the period 1997 to 1999 (C. van Ginkel, IWQS, pers. comm).

Location	Year	Causative organism	Impact
Erfenis Dam	05/1998	*Microcystis* sp.	Cattle and sheep mortality
	04/1999	*Anabaena* sp.	Fish kill
	06/2000	*Anabaena* sp.	Anatoxin-a identified (HPLC)
Bloemhof Dam	02-04/1997	*Microcystis* sp.	Presence of toxins
	1999	Unconfirmed	Death of 2 giraffe
Vaal Dam	04-05/1998		
Bospoort Dam	01-03/1999	*Microcystis* sp.	Positive presence of toxins in algal scum by mouse i-p. Reported incidence of diarrhoea in people living near the shore.
Roodeplaat Dam	12/1998 – 03-1999	*Microcystis* sp.	Positive toxicity by mouse test. Skin irritations reported by recreation users.
Hartbeespoort Dam	03/1999	*Microcystis* sp.	Taste and odour complaints.
Laing Dam	12/1999	*Microcystis* sp.	Scum tested positive for toxins.
Orange River*	01/2000*	*Cylindrospermopsis raciborskii** *Anabaena* sp. *Oscillatoria* sp.	Extended blooms and fish kills along the river. No analytical ability to identify cylindrospermopsin presence was available at the time*.
Reporting of this 2000 incident falls just outside of the period considered by this review. However, this record is significant as it documents the first incidence of a *C. raciborskii* bloom in South Africa. This organism was subsequently also reported from Spitskop and Boegoeberg Dams.			

Van Ginkel (IWQS, pers. comm.) conducted an ELISA-based screening for toxicity in 65 impoundments. The results, presented as cyanobacterial genus, dominant species, identified (HPLC) cyanobacterial toxin, and prevailing chlorophyll-a, are presented in Table 9.

Table 9:

ELISA-based one-off screening of cyanobacterial material isolated from selected South African impoundments during February and March 2000. Shading indicates positive test for toxicity, but does not indicate conclusive association with cyanobacterial toxins or with the genus present. Dark shading indicates the genus most likely to be associated with the presence of toxicity, and light shading the presence of toxicity in the sample as a whole. For example: The presence of toxicity in the Roodeplaat sample is unlikely to be assciated with *Spirulina* or *Pseudanabaena*, but rather with the *Microcystis* cells. (Source: CE van Ginkel, IWQS).

Anabaena	Chl*a*	*Merismopedia*	Chla	*Oscillatoria*	Chl*a*
Albert Falls	8	Boskop	8	Boegeoberg	27
Allemanskraal	430	Klipvoor	20	Buffelskloof	4
Belfast	6	Midmar	5	Donaldson	3
Bloemhof	6	Rhenosterkop	6	Douglas Weir	19
Boegoeberg	27	Rust de Winter	14	Hartbeespoort	232
Bon Accord	53	Spitskop	53	Loskop	6
Erfenis	5	Sterkfontein	1	Middelburg	9
Grootdraai	1	Vlugkraal	1	Nagle	5
Hartbeespoort	232	Woodstock	4	Rhenosterkop	7
Klipdrif	254			Roodekopjes	15
Klipvoor	20	*Microcystis*		Spioenkop	5
Koppies	330			Spitskop	53
Kromellenboog	3	Albert Falls	8	Vaalhartz Weir	11
Laing	1 510	Allemanskraal	430	Vlugkraal	1
Leeukraal	19	Boegoeberg	27		
Molatedi	6	Bon Accord	53	Pseudoanabaena	
Nagle	5	Groot Marico	8		
Piet Gouws	9	Grootdraai	1	Grootdraai	1
Roodekopjes	15	Hartbeespoort	232	Klipdrif	254
Shongweni	6	Klein Maricopoort	23	Laing	1 511
Spioenkop	5	Klipdrif	254	Roodeplaat	99
Twee Rivieren	6 358	Koppies	330	Witbank	16
Vaal	153	Kosterivier	11		
Vaalhartz Weir	11	Laing	1 510	Spirulina	
Wentzel	13	Loskop	6		
		Midmar	5	Bon Accord	53
Chroococcus		Piet Gouws	9	Klein Maricopoort	23
		Rietvlei	43	Nagle	5
Rust de Winter	14	Roodekopjes	15	Roodeplaat	99
		Roodeplaat	99	Spitskop	53
Cylindrospermopsis		Spioenkop	5	Twee Rivieren	6 358
		Spitskop	53		
Boegoeberg	27	Taung	2		
Spitskop	53	Twee Rivieren	6 358		
		Vaal	153		
		Wentzel	13		
		Witbank	16		

University of the Witwatersrand

This review would not be complete without inclusion of reference to the comprehensive study and description of South African marine cyanophytes undertaken by Simone Silva and Richard Pienaar of the Department of Botany at WITS (e.g. Silva and Pienaar, 1997a, b).

6.10.4 Kwa-Zulu Natal

Umgeni Water

During the 1990s a number of problems related to the occurrence of cyanobacteria in raw potable water supplies were investigated in the laboratories of Umgeni Water in Pietermaritzburg. During this period an extensive database was amassed, and several detailed reports were published via the Water Research Commission. These are summarised in this section.

Cyanobacterial investigations

Phytoplankton assemblages in water storages

The occurrence and abundance of cyanobacteria in the Umgeni Water operational area was examined over a 5-year period from 1990 to 1994 (Graham et al. 1998). The genera of *Microcystis, Anabaena* and *Oscillatoria,* were found to be the most abundant and significant from a water treatment perspective. Other genera encountered were *Chroococcus, Gloeocapsa, Gomphonema,* and *Merismopedia*.

Figure 1 (B-D) illustrates some summary abundance statistics for the three most important and dominant cyanobacteria. The total algal count (A) has also been included to provide some measure of how the individual genera contributed to the total algal count. Figure 2 illustrates the proportional composition (as a percentage of the total median count) of the major algal genera for the respective impoundments. *Microcystis* was found to be the dominant cyanobacterial genus, with secondary dominance by *Anabaena*, a finding that mirrors the typical occurrence of cyanobacteria in South African waters.

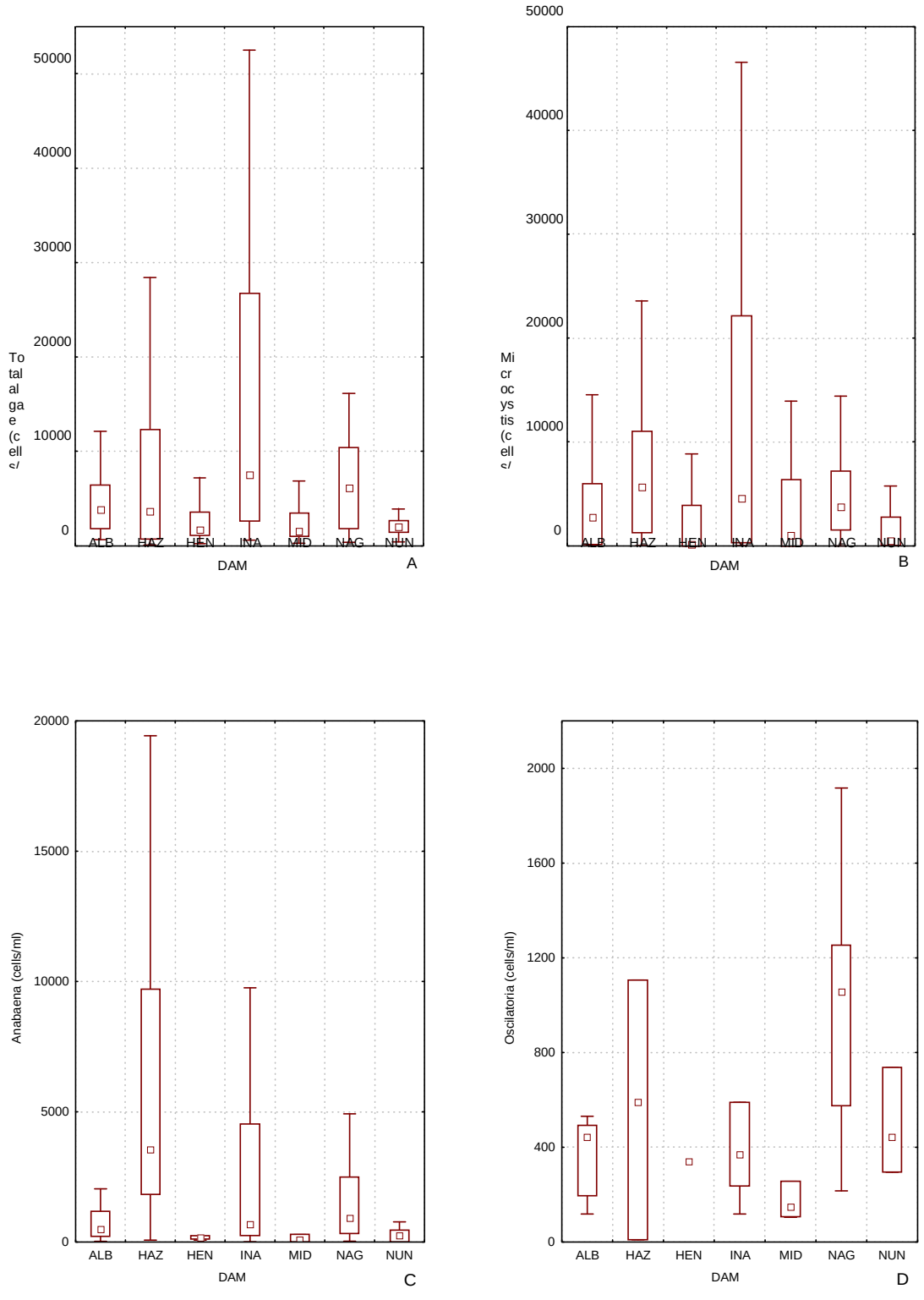


Figure 1 (A-D) Summary abundance statistics (median, 25 & 75 percentiles & non-outlier minimum & maximum) of Total Algae and key blue-green algal genera in impoundments within Umgeni Waters operational area for the period 1990-1994 (Impoundment acronyms are: ALB = Albert Falls; HAZ = Hazelmere; HEN = Henley; INA = Inanda; MID = Midmar; NAG = Nagle; NUN = Nungwane).

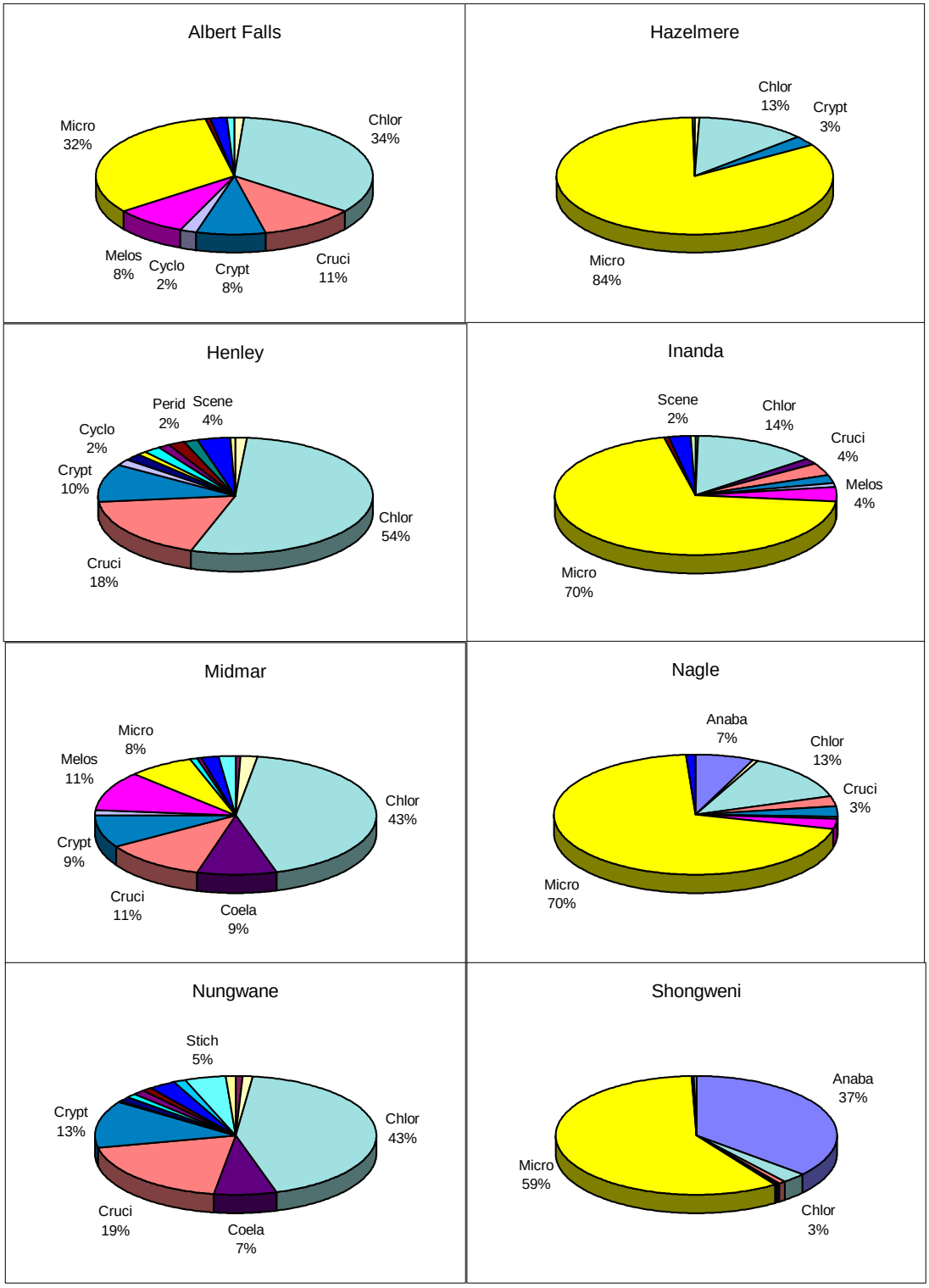


Figure 2: Proportional composition (as a percentage of the total median) for respective impoundments for the duration of the study period (Chlor=*Chlorella*; Crypt=*Cryptomonas*; Cruci=*Crucigena*; Melos=*Melosira*; Anab=*Anabaena*; Micro=*Microcystis*; Coel=*Coelastrum*; Stich=*Stichococcus*; Perid=*Peridinum*; Scene=*Scenedesmus*; Cyclo=*Cyclotella*).

Taste and odour problems

Umgeni Water has developed a Rapid Response System (RRS) that was set up to supersede normal channels when water quality problems are anticipated. The objectives of the RRS are:

- To provide the Operations Division with adequate warning of impending water treatment problems;
- To provide information on the management of water supply impoundments to ensure optimal raw water quality.

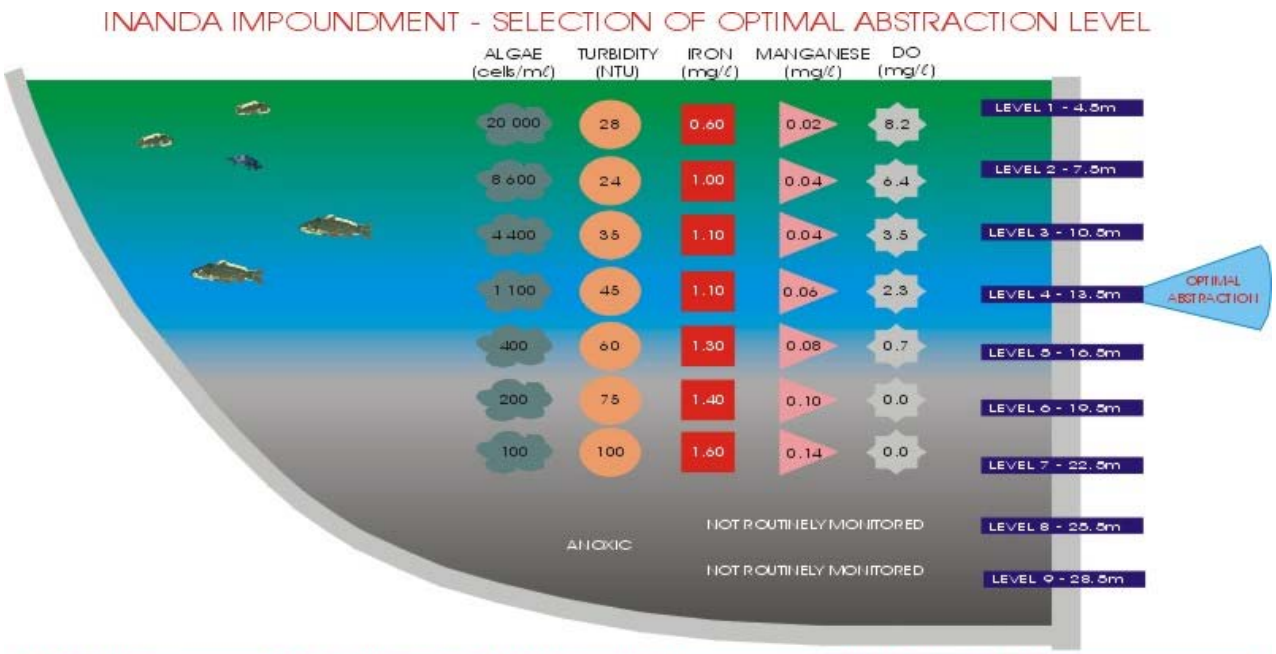
Warning limits for total algae as well as specific algal genera were established (see Table 10), and activated when exceeded. The extent of the exceedence, integrated with other data and information, is used to recommend optimal water quality management strategies.

Table 10:

Numbers and types of algae that trigger the issuing of operational warnings**.**

Description	Unit	Limit	Sites	Problem
Total Algal Count	Cells per ml	3 000 [1], 15 000 [2]	1=Raw/Abstraction; 2=Dam	*Taste and odours and toxins.*
Anabaena	Cells per ml	1 000	Raw/Abstraction/Dam	*Taste and odours and toxins.*
Chlorella	Cells per ml	3 000	Raw/Abstraction/Dam	*Filter clogging*
Cosmarium	Cells per ml	3 000	Raw/Abstraction/Dam	*Filter clogging*
Crucigenia	Cells per ml	3 000	Raw/Abstraction/Dam	*Filter penetration*
Cyclotella	Cells per ml	3 000	Raw/Abstraction/Dam	*Taste and odour; filter clogging*
Diatoma	Cells per ml	3 000	Raw/Abstraction/Dam	*Taste and odours.*
Dinobryon	Cells per ml	1 000	Raw/Abstraction/Dam	*Taste and odour; filter clogging*
Mallamonas	Cells per ml	100	Raw/Abstraction/Dam	*Taste and odour; filter clogging*
Melosira	Cells per ml	1 000	Raw/Abstraction/Dam	*Filter clogging*
Microsystis	Cells per ml	3 000	Raw/Abstraction/Dam	*Toxins and taste and odours.*
Ocillatoria	Cells per ml	1 000	Raw/Abstraction/Dam	*Taste and odour; filter clogging*
Peridinium	Cells per ml	1 000	Raw/Abstraction/Dam	*Taste and odour; filter clogging*
Synura	Cells per ml	1 000	Raw/Abstraction/Dam	*Taste and odours.*
Geosmin	ng/l	10	Raw/Abstraction/Dam	*Taste and odours*
Odours	no units	All geosmin odours	Raw/Abstraction/Dam	*Taste and odours*

A schematic representation of Inanda Dam and its abstraction levels is given in Fig. 3. This figure summarises the information that is assessed when advising Operations staff on the choice of abstraction level at the dam. From the figure, the general situation of high algae numbers at the surface, with poor chemical quality at greater depths can be seen, with the optimal mix of the two at some point in between.



VARIABLE	POSSIBLE TREATMENT PROBLEM	WARNING LIMIT
Algal count & genus diversity	Tastes and odours, filter clogging and filter penetration	Total count : 3 000 cells/mℓ *Anabaena* : 1 000 cells/mℓ
Turbidity	Increased coagulant dose and treatment costs	100 NTU
Iron and manganese	Reduced metal species precipitate in final waters causing staining of laundry/sanitaryware	Iron : 0.3 mg/ℓ (Apr - Jul) Manganese : 0.08 mg/ℓ (Apr - Jul)
Dissolved oxygen	Reduced metal species and other problems associated with anoxic water	2 mg/ℓ

Figure 3 Illustrative diagram of information that is assessed when advising operating staff on the choice of abstraction levels at a dam (diagram and text box provided by Umgeni Water).

Cyanobacterial rupture during abstraction and the consequences for water treatment

The rupture of algal cells in the pipeline between Nagle Dam and the Durban Heights Waterworks was observed to severely affect water treatment costs due to the release of taste and odour compounds into the water. The contributions of ruptured algae to coagulant and oxidant demand were also significant. As very few intact cells were present in the raw water reaching the works, an investigation was undertaken to examine the impacts of algal rupture. The increase in treatment cost was out of proportion to the number of algae entering the treatment plant and could only be anticipated by monitoring algal numbers in the impoundment. Chemical and physical determinands such as pH, turbidity, conductivity, total dissolved solids and total organic carbon did not provide any clear indication of the presence of a bloom of algae in the impoundment or of the rupture of the cells while in the aqueduct. This study was also somewhat unique as the genus *Microcystis* is not commonly-associated with the production of taste and odour compounds.

A detailed investigation of long-term data indicated that the two blue-green algal genera *Microcystis* and *Anabaena* were prone to rupture (Table 11). Other genera monitored were also affected to a greater or lesser degree. A novel technique of adding *Microcystis* with a lithium marker to the head of the aqueduct was used to show that between 61% and 72% of the *Microcystis* cells were consistently lost due to rupture during passage through the aqueduct. An apparatus was subsequently designed to mimic the pressure and shear conditions experienced in pipelines, and was constructed and used to simulate aqueducts and to predict the percentage loss of algae. The apparatus was used to develop a model relating pressure and shear forces with algal rupture (Dickens and Graham, 1995; Dickens et al. 1996).

Table 11:

Results of Student's Paired Differences and the Wilcoxon Matched Pairs tests comparing the Nagle Dam flow weighted average for historical algal data with Durban Heights WW mean algal counts and the median % loss between the respective sample points.

Genus	Number of paired couplets	Significance (Student's test)	Significance (Wilcoxon test)	Nagle algae, flow weighted mean (cells.$m^{\ell-1}$)	WW algae, mean (cells.$m^{\ell-1}$)	Median % loss between Nagle & Dbn Hts WW *
Total	161	P < 0.001	P < 0.001	2 548	942	47
Anabaena	77	P < 0.001	P < 0.001	780	56	100
Ankistrodesmus	32	NS	P < 0.01	8	5	100
Chlamydomonas	104	NS	NS	15	13	26
Chlorella	159	NS	P < 0.001	458	345	24
Cocconeis	8	P < 0.01	P < 0.01	5	0	100
Coelastrum	42	P < 0.001	P < 0.001	94	34	100
Cosmarium	40	NS	P < 0.01	22	13	100
Crucigenia	135	P < 0.001	P < 0.001	118	57	70
Cryptomonas	151	NS	NS	47	63	+ 16
Cyclotella	128	NS	NS	53	39	25
Cymbella	12	NS	NS	23	2	100
Diatoma	17	NS	P < 0.01	7	3	100
Melosira	154	NS	NS	296	273	23
Microcystis	133	P < 0.001	P < 0.001	1 154	28	100
Navicula	109	NS	P < 0.01	13	9	41
Nitzschia	80	P < 0.001	P < 0.001	12	6	100
Oocystis	83	P < 0.001	P < 0.001	25	8	100
Oscillatoria	15	NS	P < 0.01	893	202	100
Pandorina	12	NS	NS	27	9	100
Pediastrum	22	NS	NS	50	15	100
Pteromonas	9	NS	----	3	0	100
Scenedesmus	127	P < 0.01	P < 0.001	55	34	63
Spermatozopsis	14	NS	P < 0.01	26	2	100
Stichococcus	21	NS	P < 0.001	28	4	100
Synedra	24	NS	P < 0.001	12	2	100
Tetraedron	28	P < 0.01	P < 0.001	14	5	100
Trachelomonas	23	P < 0.01	P < 0.001	4	0	100

(* Median % loss - calculated as the median of all of the percentage losses between Nagle Dam and Durban Heights WW. NS - results considered statistically non-significant at P > 0.05).

Using the large database of water quality (including algal) and water treatment cost data, available at Umgeni Water, a study was undertaken to explain the water quality relationships (particularly as they affected algae) in impoundments, and to see how this affected the cost of water treatment (Graham et al. 1998). The aims and findings of this study are summarised below:

Aims:

- To establish the key environmental variables influencing the distribution and abundance of problematic algae in impoundments in the Umgeni Water operational area.
- To establish a predictive model(s) relating algae to the environment for the major impoundments in the Umgeni Water operational area.
- To build an economic model relating water treatment costs to the types and numbers of algae likely to be found in impoundments.

Models were developed that described the relationship between algal abundance and key water quality variables. In most cases, the models developed were related to algae that were known to adversely-affect water treatment (traditionally certain blue-green and diatom algal genera). Thereafter impoundment water quality was examined to determine which factors were most impacting on water treatment, and, hence, treatment costs, at selected waterworks (WW). Models were subsequently developed relating raw water quality entering respective water works with the associated treatment costs.

Findings:

Algal environmental associations

As is a common feature of this type of study, many of the environmental variables investigated were highly correlated with each other. This is a consequence of process and functional relationships. The variation of the original large number of environmental variables (53) could be reasonably accounted for by a smaller number of 'key' environmental variables identified as conductivity, Secchi depth, silicon, total inorganic nitrogen, total phosphorus, inflow, temperature, dissolved oxygen and stability (stratification). These variables represented the primary aspects of variation in the environmental data and had intuitive biological appeal in that they are often implicated in the literature as influencing algal populations.

Low conductivity, low turbidity, 'inland' impoundments (Midmar, Albert Falls,

Nagle and Henley) were identified as distinct from more turbid 'coastal' impoundments (Hazelmere, and to a lesser extent, Nungwane). Inanda was characterised by generally higher conductivities but low turbidities. Midmar, Albert Falls and Nagle neatly demonstrate the change in water quality with progression down the Mgeni River catchment. This progression is characterised by increased conductivities. Inanda, the last impoundment on the Mgeni system cascade, had the highest conductivity.

Weaker gradients (characterised by total inorganic nitrogen, total phosphorus and inflows) distinguished between Midmar, Nagle and Albert Falls (higher total phosphorus) and Nungwane and Henley (higher total inorganic nitrogen). Inanda was relatively 'mixed', experiencing a range of values for total inorganic nitrogen, total phosphorus and inflows. Impoundments with similar water-quality are more likely to respond similarly to management/perturbation.

The reduced set of 'key' environmental variables explained a low (16%), but statistically significant ($P < 0.01$) portion of the variability in the algal data. This low amount of explained variation is not unusual for large multi-genera ecological data. Of these variables, total inorganic nitrogen, silicon, temperature, inflow and secchi were the most important in explaining algal variability. The ordination diagram, Fig. 4, illustrates the response of key algae to significant environment variables. Not all algae had appreciable amounts of their variability explained by analyses.

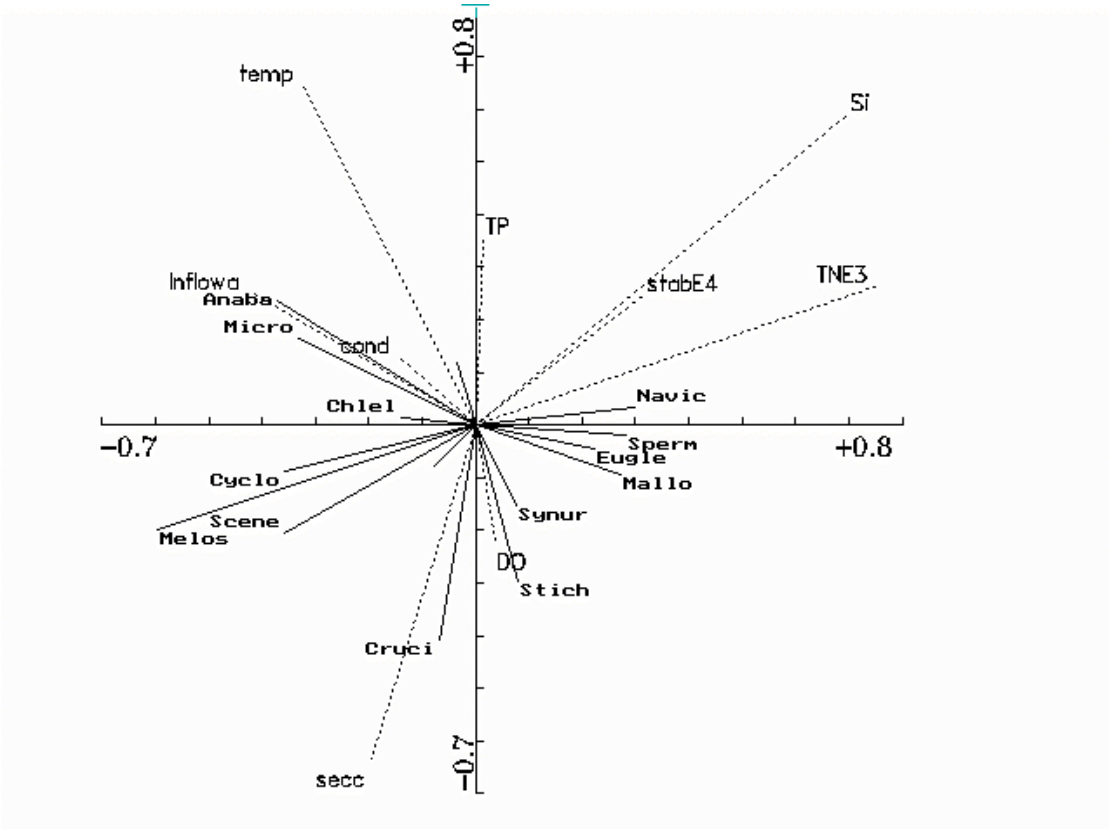


Figure 4 Biplot ordination of algae and key environmental variables for major impoundments within the UW operational area (1990 - 1994).
Algal genera are: Anaba=*Anabaena*, Micro=*Microcystis*, Navic=*Navicula*, Sperm=*Spermatozopsis*, Eugle=*Euglena*, Mallo=*Mallomonas*, Synur=*Synura*, Stich=*Stichococcus*, Cruci=*Crucigena*, Scene=*Scenedesmus*, Melos=*Melosira*, Cyclo=*Cyclotella* and Chlel=*Chlorella*. Environmental variables are: TP=Total Phosphorus, Si=Silicon, stabE4=stability, TNE3=Total Inorganic Nitrogen, DO=%Dissolved Oxygen, cond=conductivity, Inflowa=inflow and temp=temperature.

Changes in *Anabaena* and *Microcystis* (important cyanobacteria) were responsive to the higher (20–25°C) temperatures and inflow volumes associated with late summer conditions (January to April). These genera were also generally more abundant at the lower end of the total inorganic nitrogen (TN) gradient (low TN:TP ratio, <20). Under these conditions, they could become sufficiently abundant to cause water treatment problems in terms of taste and odour formation.

From an algal assemblage perspective, Hazelmere, Nungwane and Inanda (and to a lesser extent, Nagle) were found to be most dissimilar. The difference appears to be primarily associated with low water clarity (high turbidities or low Secchi depths). Given that they are all on entirely different river systems (except Nagle and Inanda on the Mgeni River), with different water qualities, this was not considered to be surprising.

Marked and predictable shifts in algal populations were observed during the late summer period (February/March) through to midwinter (June/July), and with another large shift again in early summer (September to November) through to late summer. These shifts were typified by changes in: *Chlorella* and *Anabaena* (increase in abundance in late summer), *Cyclotella* and *Cosmarium* (increase in autumn - March/April) and *Spermatozopsis* (increase in spring). The shifts were considered to be probable associations of stratification.

The cost implications of cyanobacterial development in a water storage is clear from examaination of Figs. 5a and 5b:

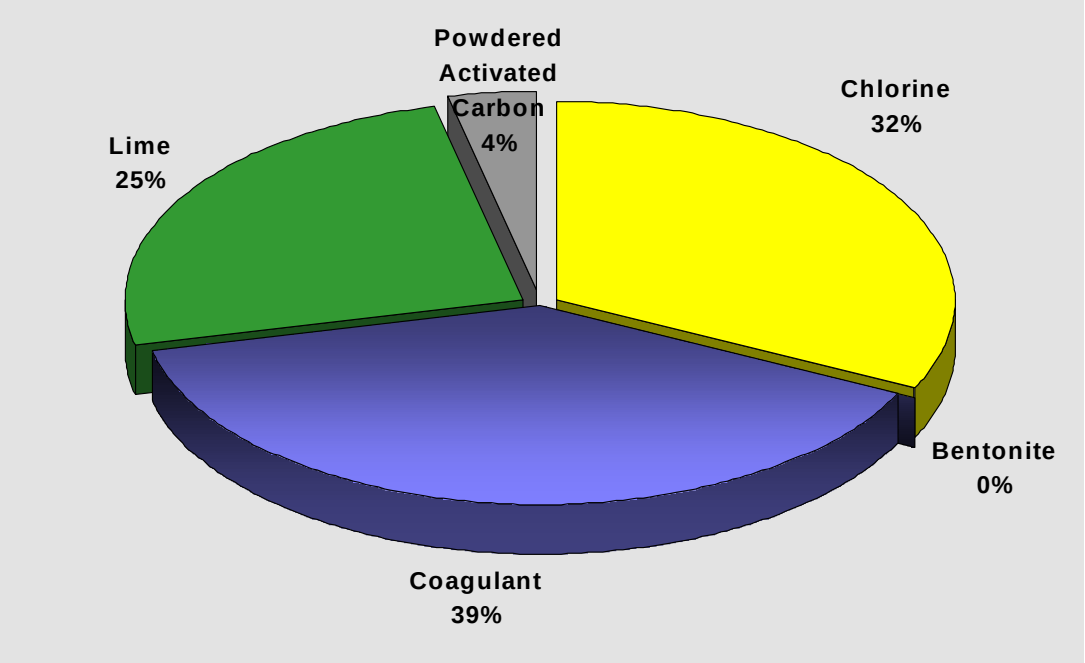


Figure 5a Average water treatment cost composition at Durban Heights WW (Jan 1990 to Feb 1997).

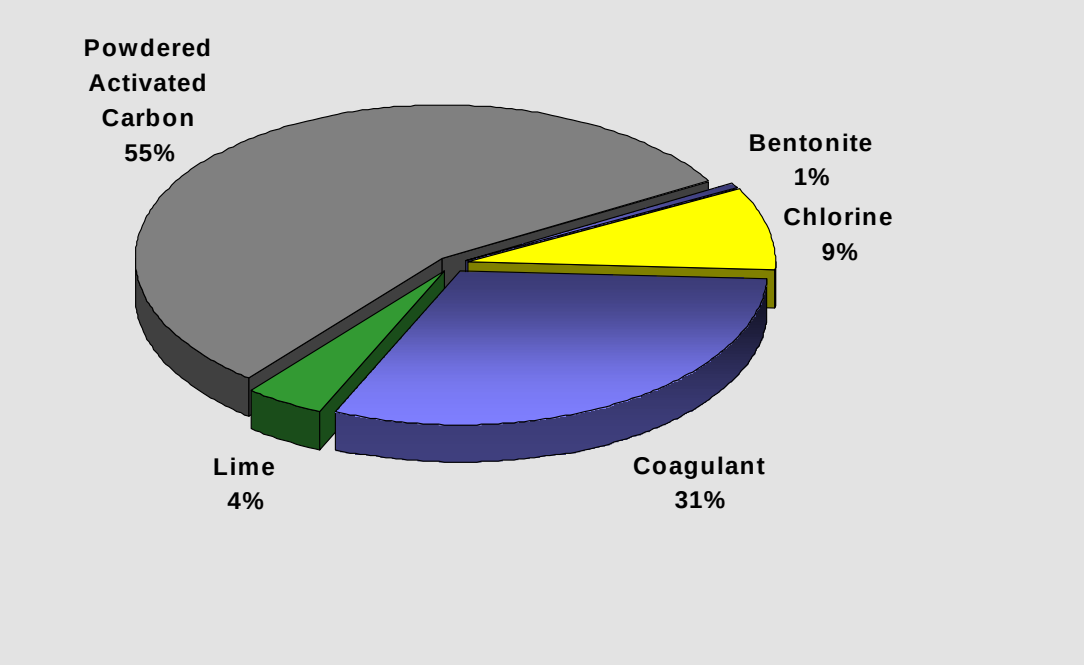


Figure 5b: Average water treatment cost composition at Durban Heights WW during a particularly severe period of taste and odour formation attributable to *Anabaena* (March 1994).

The model derived for the Hazelmere WW explained 79% of the variation in chemical treatment costs. The model accurately predicted real costs, and could be easily applied in simulation exercises. Abiotic water quality factors had a particularly significant impact on treatment costs at the Hazelmere WW. Treatment costs increased in relation to the concentration of turbidity, total aluminium, manganese, suspended solids, potassium, and sulphates in Lake Hazelmere. Likewise, costs rose with lower pH and alkalinity. Algae were found to have a relatively minor impact on treatment costs at the Hazelmere WW.

Similarly, the model created for the Durban Heights WW explained 64% of the variation in chemical treatment costs. Again, actual costs were accurately predicted, except during occasional peak cost periods (Fig. 6) and could easily be applied in simulation exercises (Fig. 7).

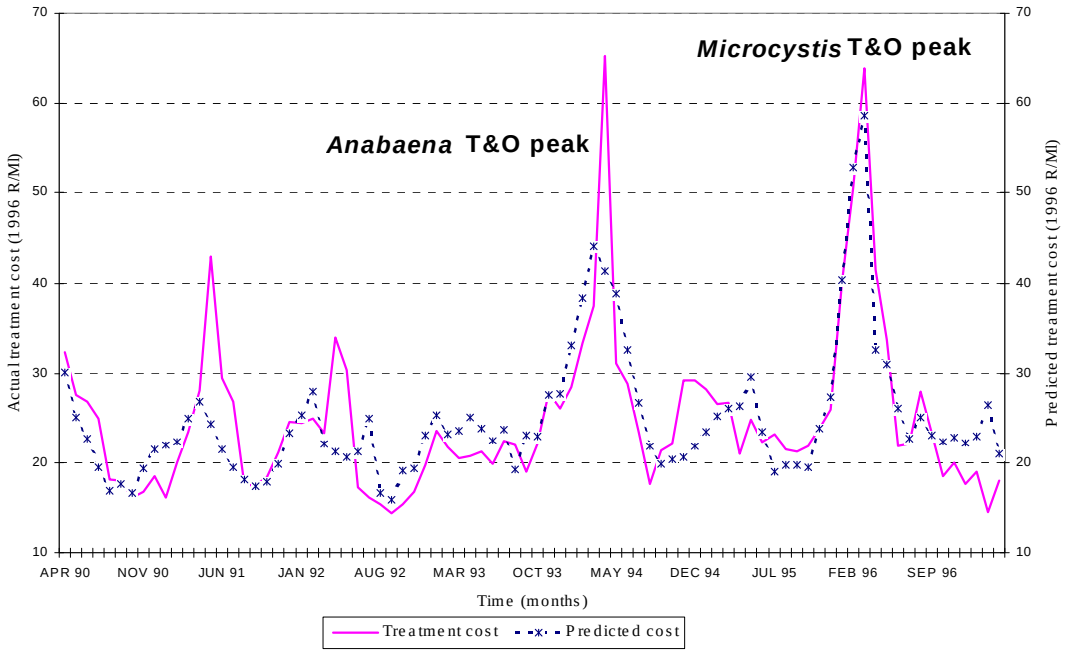


Figure 6: Actual versus predicted treatment costs at the Durban Heights Waterworks (note annotated peaks reflecting increased treatment costs associated with taste and odour incidents).

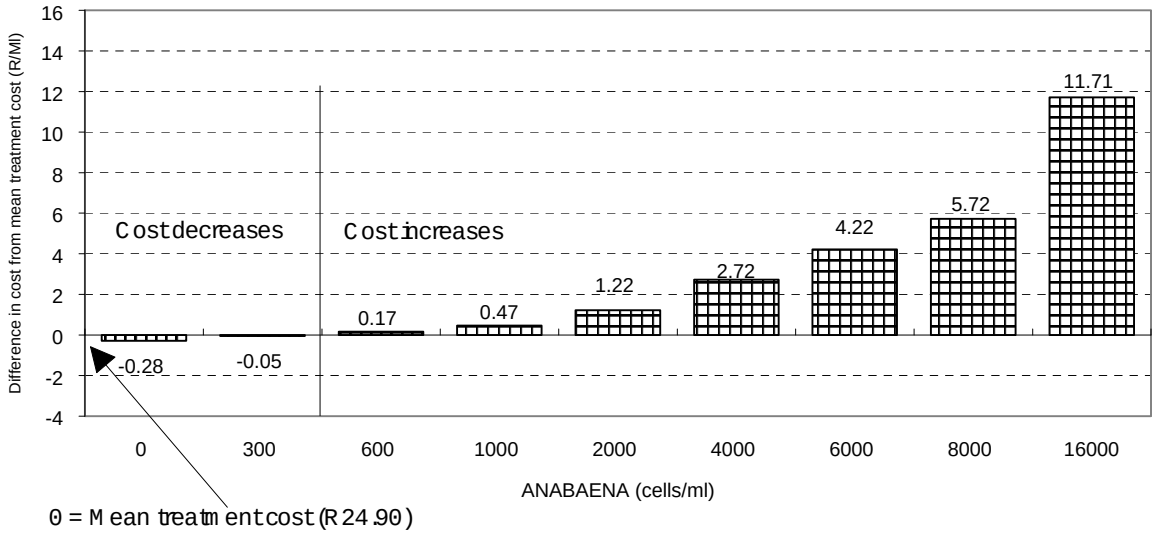


Figure 7: Differences in mean treatment cost with different abundances of *Anabaena* in the raw water treated at the Durban Heights WW.

Figure 7 illustrates that an increase in the abundance of *Anabaena* of >≈600 cells/mℓ would result in an increase in mean treatment cost. For example, treatment costs would increase by approximately R4.22/Mℓ (over the mean treatment cost of ≈R25/Mℓ) if the *Anabaena* counts in the raw water entering Durban Heights WW were 6 000 cells.mℓ$^{-1}$ and causing taste and odour problems. Currently at >1 000 cells.mℓ$^{-1}$ of *Anabaena* a warning is automatically generated advising operators of the WW to be aware of potential taste and odour problems associated with this algae. This 1 000 cells.mℓ$^{-1}$ of *Anabaena* cut-off was derived empirically and corresponds well with the results of this simulation.

High numbers of the algae *Anabaena* and *Microcystis* in Nagle Dam (which is the primary source of raw water for Durban Heights WW) had a major impact on treatment costs at this works, particularly when they are producing taste and odour compounds (see annotated peaks in Fig. 6).

Barley straw

Numerous reports exist in the literature documenting the success of using barley (or other) straw for the control of cyanobacteria. During the summer of 1998/9 the small coastal impoundment, EJ Smith dam at Mzinto, developed severe blooms of *Anabaena* followed by *Microcystis*, with associated very high concentrations of geosmin that proved to be difficult to treat. During March 1999 barley straw was added to the dam as recommended by Newman (1995). Unfortunately, this experiment was inconclusive as algal numbers were already declining when the straw was added. Nevertheless, algal number reduced dramatically to exceptionally low values. Of interest was that a few months later growths of *Oscillatoria* were noted - growing on the straw bales themselves! These growths (also on the base of reeds in the marginal vegetation) produced geosmin at low concentrations.

A second inconclusive trial saw the application of hay by a local municipality, to two small farm dams in Linfield Park near Pietermaritzburg that receive the bulk of their flow from a small sewage works. Nutrient concentrations were high and the two dams were dominated by blue-green scums. Reduction of algae populations in the upper of the two dams, closest to the sewage works, was total, with zero algae being detected within a few weeks of application of small quantities of hay. Interestingly, this dam also had a very healthy riparian vegetation zone with large numbers of grazers such as *Daphnia*. The lower dam was more unstable with a fluctuating water level and non-existent riparian zone. Control here was less.

Effect of cyanobacteria on advanced treatment processes

An investigation was conducted to assess the effect of advanced treatment processes, including ozonation, on the treatment of eutrophic waters. Samples of water were spiked with cyanobacterial scums consisting of either predominantly *Microcystis* or *Anabaena* to yield final cell concentrations of between 10 000 and 500 000 cells per mℓ. Samples of the spiked water were treated by different process combinations of ozone and polymeric or inorganic coagulants, and measurements of THMFP, DOC, algal cell concentration and optimum coagulant demand were compared.

A small increase in the THMFP was sometimes observed after ozonation of algal-laden waters and DOC was generally found to increase only slightly with increasing algal cell concentration. There also tended to be a slight increase in DOC after ozonation and DOC removal due to ferric chloride coagulation which generally deteriorated after ozonation. THM concentrations have been strongly correlated to DOC content, but the literature suggests that the THM yield per mg DOC is low and since the DOC generally rose by less than 1 mg.ℓ^{-1}, even when the cell concentration was increased to 500 000 cells.mℓ^{-1}, the THMFP was not expected to increase significantly as the cell concentration rose. These results would appear to be in conflict with those of van Steenderen et al. (1988) who reported that *Microcystis aeruginosa*, the species used in this study, gave rise to significant total organic halogen precursors on lysis, but this does not necessarily mean that these precursors were THM precursors.

The geosmin concentration of the water containing predominantly *Microcystis* sp. cells was in many cases below the detection limit of the analytical method. However, geosmin was usually detected in water containing predominantly *Anabaena* sp. cells, indicating that when *Anabaena* is the predominant genus, more severe taste and odour problems could be anticipated. *Microcystis aeruginosa* has been found to be the predominant species in the majority of taste and odour incidents that have occurred in South Africa, although it has been pointed out that only circumstantial evidence exists to suggest that it can produce geosmin (Wnorowski & Scott, 1992). On the other hand, there is scientific evidence to prove that *Anabaena circinalis*, the species of *Anabaena* that generally gives rise to taste and odour problems in the Umgeni Water operational area, produces geosmin (Bowner et al. 1992). Umgeni Waters experience has shown that the presence of *Anabaena circinalis* in significant concentrations generally gives rise to geosmin problems, whereas the presence of *Microcystis aeruginosa* in comparable numbers does

not always result in a taste and odour problem.

Ozonation of *Microcystis* sp. generally had a significant effect on the coagulant demand, although as mentioned above, this was dependent on the ozone concentration. At ozone concentrations that resulted in an increased coagulant demand, the demand increased almost exponentially with increasing cell concentration when *Microcystis* sp. predominated.

Ozonation of *Anabaena* sp. also resulted in increases in coagulant demand, but the effect of ozonation was much smaller than with *Microcystis* sp. As a result, the coagulant dose for an unozonated water containing predominantly *Microcystis* sp. was always lower than that for a water containing predominantly *Anabaena* sp. at similar cell concentrations. After ozonation, however, the coagulant demands for water containing predominantly *Microcystis* sp. or *Anabaena* sp. at comparative cell concentrations were much closer in value, especially at the greater cell concentrations. Despite this, the coagulant demand still tended to be lower when *Microcystis* was the dominant genus.

The differences observed between the two blue-green algal genera suggest that *Microcystis* sp. are more susceptible to lysis by ozonation. However, when *Anabaena* sp. predominated, the coagulant demand was always higher than for the same water containing comparable concentrations of *Microcystis* sp. implying that *Anabaena* sp. are less amenable to removal by coagulation.

Detection methods for algal toxins

In the early days of monitoring algal toxins (as late at the early 1990s), the lack of suitable laboratory techniques was a severe restriction. As standards became available (at great cost!) it was possible to use these to research the methods. A Water Research Commission funded project set out to identify novel techniques that would be quicker, easier and cheaper, and later to optimise techniques developed in other laboratories overseas. (Recently the laboratory has begun to use ELISA techniques to monitor algal toxins in raw water supplies. This technique of monitoring has proved to be robust and will be used for standard screening of blue-green algae rich samples in the future).

This project was based on a study of 4 microcystin toxins (MC RR, MC YR, MC LR, MC YA) and Nodularin. There were three broad sections, including methods of analysis, monitoring and remedial action.

Methods of Analysis

Two methods were investigated, namely HPLC with fluorescence detection and HPLC with photodiode array UV detection.

Fluorescence Detection

4-(2-phthalimidyl) benzoyl chloride (PIB-Cl) was used as the derivatisation reagent but the results from this study were inconclusive.

Photodiode Array UV Detection

This was essentially an assessment of the "Codd" method. The work showed clearly that the latter method was reliable for both quantitative and qualitative analysis. However, a number of limitations were observed. These include:

- poor detection sensitivity due to matrix interference (toxins not detected at 0.4 $\mu g.\ell^{-1}$ in some cases)
- low recoveries in spiked tap and raw water samples
- low recoveries as a result of turbidity in the sample
- co-elution of other organic compounds present in the sample matrix often masked toxin peaks
- the UV spectra was of limited use at low toxin concentrations
- sample preparation procedure long and laborious
- long sample analysis time

Using the Codd method as a basis for toxin analysis, the laboratory was able to achieve sample detection limits in the range 0.04 to 0.08 $\mu g\ell^{-1}$ for the various algal toxins.

Monitoring

Routine monitoring of Midmar, Albert Falls, Henley, Shongweni, Nagle, Inanda, Hazelmere, and Nungwane Dams was conducted during the period February 1995 to March 1997. No toxins were detected in main basin samples of water from these dams. However, concentrated algal scum samples from Nagle, Inanda, Albert Falls and Hazelmere Dams were found to contain microcystin toxins. Other sites with toxic scums included the Camps Drift Weir on the Umsunduzi River and the kwaMakutha wastewater works. The conclusion drawn from the algal toxin-monitoring programme was that microcystin toxins did not appear to be entering the water supply in the Umgeni Water catchment and as such did not pose an urgent threat.

Remedial Action

The three techniques for the removal of toxins in water, namely chlorination, ozonation and treatment with powdered activated carbon that have been reported in literature were assessed. The findings were as follows:

Chlorination

- toxin removal found to be pH-dependent
- doses up to 4 mg.ℓ^{-1} were required for toxin removal at pH 7-8

Ozonation

- toxin removal also pH dependent
- doses between 2.5 to 3 mg.ℓ^{-1} recommended for raw water with pH 7-8

Powdered Activated Carbon (PAC)

Three wood-based carbons and one coal-based carbon were tested. The Sutcliffe WF PAC (wood-based) was found to be most effective for toxin removal. A typical dose of 20 mg.ℓ^{-1} was used.

HPLC-MS

Attempts were made to identify an unknown toxin found in a Nagle Dam scum with the above technique. However, the experiment was dominated by the presence of impurities.

Sterile Culture of Toxic Microcystis

Microcystis was found to grow very slowly in BG-11 culture media. The cultures, however, remained toxic for 5 months. No tests were done after this period.

The above work is reported in Rae et al. (1999).

Algal identification and enumeration at Umgeni Water

As problems associated with algae are dependent on the size and composition of their population, it is necessary to quantify both the type and abundance of algae in the water.

The Hydrobiology laboratory at Umgeni Water uses the membrane filter method of determining both algal type (identified down to genus level) and algal abundance. This method has been refined “in house” and accredited in terms of ISO/IEC Guide 25 (1990). The membrane filter method is a rapid method capable of detecting algae at low concentrations and with adequate training has proven to be reliable and robust compared to the traditionally slower but more accurate sedimentation chamber approach. In principle, the

method involves filtering a known volume of sample (generally 20-50 mℓ for "raw" water samples and 1 200mℓ for "final" or potable water samples) under low vacuum onto a cellulose nitrate 0.45 μm membrane filter. The samples are then air dried and may then be examined under a standard compound light microscope with a 63X objective oil immersion lens. With a 10X eyepiece, normal counting magnifications are therefore 630X. If necessary, an algal count to genus level may be available within 15 min after receipt of a sample. Routinely however samples are available within 24 h after sample collection.

The laboratory participates in a quarterly inter-laboratory proficiency testing scheme (PTS) with all the major Southern African centres of algal identification and counting participating (both within the academic (university) and production (water authorities) environments). The method has been shown to be remarkably robust during these PTS exercises and within the production environment of Umgeni Water, which handles some 500 to 600 algal samples per month.

Dedicated computer software was developed at Umgeni Water to ensure statistical confidence in the algal counts produced by the Hydrobiology laboratory. Three criteria are specified to ensure the reliability of the algal count data produced viz:

1. The estimate of the total count of algae in a sample should be within prespecified precision;
2. The estimates of the count of different algal genera should be within prespecified precision;
3. The pre-specified proportion of algal genera present should be detected.

This software has certain basic user inputs, e.g. sample monitoring point, volume of sample filtered, magnification of microscope used and analyst. Algae observed under the microscope are entered into a database by the analyst via a standard PC keyboard, where each key is configured to represent a unique algal genus. Once sufficient fields of view under the microscope are counted, and the prespecified statistical criteria are met, the counting software prompts the analyst to stop counting. Results are converted to cells/mℓ and stored in the database with, amongst other details, relevant sample point, analyst, count, and statistical criteria. This software has recently been re-written and will be capable of running on a stand-alone PC. Umgeni Water have indicated that their intention is to make this software commercially available.

(*see Supporting Literature*)

7. South African national surveillance programme for cyanobacteria

A draft surveillance and monitoring protocol for cyanobacteria was drafted during 1995 by Quibell et al. with the assistance of Professor Geoff Codd from the University of Dundee, Scotland. The objectives of the review, which did not emerge from the draft stage, were the following:

- To provide a review of the blue-green algal toxins, their pathological effects, factors affecting their production, and the management of these algae;
- To select, test and establish the sampling and analysis procedures for a national surveillance programme;
- To present the results of a preliminary sampling network, as well as to provide a summary of previous incidents of toxic blue-green algal events in South Africa;
- To select and prioritise water bodies for the phased implementation of the national surveillance programme.

The draft report by Quibell and colleagues made the following conclusions:

- That the greatest priority facing management of the problems posed by cyanobacteria in South Africa was to determine the extent of the problem, and that a national surveillance and monitoring programme was required for this purpose;
- That the HPLC-based analysis technique developed by Lawton et al. (1994) was the best available analytical technique for the purpose of separating and quantifying the cyanobacterial hepatotoxins;
- That, from the initial surveillance screening, the occurrence of cyanobacteria in South African waters was both ubiquitous and present at a high level of (biovolume) dominance;
- That there was a strong likelihood that almost all water bodies in South Africa could support cyanobacterial growth;
- That approximately one-third of the samples examined as part of the preliminary screening were found to be toxic, and that this presented a definite threat to the users of both raw and treated water in South Africa.

The draft report also established a basis for the initiation of a formal monitoring programme, by ranking water storage impoundments into one of three priority categories, as follows:

Priority 1: All water bodies that serve as a source of drinking water for humans, and specifically including those water bodies served by minimal or no treatment. A pre-requisite for inclusion in the monitoring protocol was a phosphate (as P) concentration, at the 90th percentile,

greater than 30 $\mu g \ell^{-1}$. Thus categorized, the survey identified 70 'Priority 1' dams

Priority 2: All water bodies other than Priority 1, and with a 90th percentile phosphate (as P) concentration > 30 $\mu g.\ell^{-1}$. This category included irrigation dams used for contact recreation and livestock watering. A total of 42 'Priority 2' dams were identified.

Priority 3: All water bodies with a phosphate (as P) 90th percentile value of <30 $\mu g.\ell^{-1}$. 96 'Priority 3' dams were identified.

Although by no means onerous, the draft surveillance protocol acknowledged that the processing of the above numbers of samples on a routine basis (assuming that all contained identified cyanobacterial cells at any one time) was beyond the analytical capacity of the laboratories of the Department of Water Affairs and Forestry. An alternative to this would be to enlist the capacity of the analytical facilities of the major regional water supply utilities, viz. Umgeni Water, Rand Water and the City of Cape Town. This was an entirely reasonable suggestion as the division of the workload between 5 or more laboratories (other laboratories with developed analytical capacity for cyanobacterial toxins were being created in Bellville and Port Elizabeth, as well as in Gaborone, Botswana) would make the analytical load relatively easy to handle. Furthermore, the processing of samples by the regional water supply authorities themselves is a practical and sensible option. Once analysed, the results would be conveyed to Pretoria for storage in a central data base.

Akin to the monitoring and surveillance phased or step-wise monitoring and response protocols already created in the United Kingdom (NRA, 1990) and Australia (Ressom et al. 1993), the draft DWAF document incorporated a hierarchical approach with increasing levels and intensity of response.

It is most unfortunate that five years have been allowed to pass without the draft protocol having been subjected to detailed review and consideration, or without the methodology having been tested for at least one of the priority levels. However, the work performed by IWQS (see elsewhere in this review) has recently provided additional valuable data on the cyanobacterial status of many of South Africa's major water storages.

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