# The use of remote sensing and molecular markers as early warning indicators of the development of cyanobacterial hyperscum crust and microcystin-producing genotypes in the hypertrophic Lake Hartbeespoort, South Africa

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**Abstract:** In this study we monitor the formation of cyanobacterial hyperscum in Lake Hartbeespoort, consisting of 99% *Microcystis aeruginosa* cells, using satellite images. The hyperscum which formed near the reservoir wall was characterised by a distinctive white surface layer of crust exposed to photo-oxidizing due to high radiation, inflicting irreversible damage to the genetic constitution of the upper layer of *Microcystis* cells. Under the 3 mm thick layer of crust dark (<0.93 µmol of photons $m^{-2}s^{-1}$) anaerobic conditions (0.4 $mg.l^{-1}$, 3% saturation) prevailed with high levels of microcystin (12,300 $\mu g.l^{-1}$) in the absence of sunlight irradiation and photolysis by UV light. Real time PCR analysis indicated low levels of transcription of the *mcy*A, B and D genes responsible for sythesis of cyanotoxins, under these low light intensity conditions. At other sampling sites where cyanobacterial scum occurred and hyperscum was absent, only the *mcy*B and D genes were transcripted. The only explanation for the transcription of the *mcy*A gene in the hyperscum and not at the other sampling sites was possibly due to the presence of environmental stress-inducing factors e.g. low light intensity (0.93 µmol of photon $m^{-2}$ $s^{-1}$) and pH 6.1. At sampling site 4 where no cyanobacterial scum was visible on the satellite images, low cell abundance ($2.4 \times 10^4$ $\mu g.l^{-1}$) and chlorophyll *a* (12.2 $\mu g.l^{-1}$) was measured compared to sites 2 and 3 where cyanobacterial scum (chl *a*: 96 $\mu g.l^{-1}$; 90.1 $\mu g.l^{-1}$) was visible on the satellite images.

**Key words:** hyperscum, RT-PCR, *mcy*A transcript, microcystin, satellite imaging.

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## INTRODUCTION

Eutrophication of waters can result in blooms of especially toxic cyanobacteria. Such algae form dense surface films, and scum's. Cyanobacteria blooms have several consequences in terms of water quality and frequently, their collapse causes mortality of aquatic animal populations (Skulberg et al. 1984). Oxygen depletion due to the decomposition of the cyanobacteria is often the main cause for mortality of aquatic organisms and the toxins released by the cyanobacteria are also known to produce intoxication (Negri et al. 1995).

The cyclic heptapeptide microcystin, first isolated from the freshwater cyanobacterium *Microcystis aeruginosa*, is a potent hepatotoxin (Theiss et al. 1985). This toxin is produced nonribosomally *via* a multifunctional enzyme complex, consisting of both peptide synthetase and polyketide synthase modules coded for by the *mcy* gene cluster (Kaebernick et al. 2000). In addition to causing death and illness in animals, fish, and livestock, recreational exposure can cause diarrhoea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers and skin and eye irritation (Pilotto et al. 1997). Symptoms reported after recreational exposure might be the responses of inner or outer body surfaces to irritation of varying degree, possibly as a result of the lipolysaccharides contained in the cell walls of all cyanobacterial cells (Pilotto et al. 1997).

Rantajärvi et al. (1998) showed in their study that spatial and temporal frequencies of conventional water-sampling programs are not adequate to report changes in phytoplankton biomass, especially during bloom conditions, when spatial and temporal variability in

phytoplankton density is particularly high. Furthermore, they indicated from their observation that routine monitoring data is not sufficient for early warning due to the time lag between the formation of the cyanobacterial scum and the availability of relevant information for risk management (Rantajärvi et al. 1998). However, due to the fact that cyanobacteria can regulate their buoyancy, and in calm weather tend to keep themselves close to the water surface, surface scums are easily observed. Accumulation of aggregated cyanobacterial cells, just below the water surface and surface scums are so distinct that the extent of the blooms can be mapped using almost any remote sensing instrument (Kutser 2004). Moreover, the implementation of risk assessment requires not only rapid and reliable methods for analysis of ambient cyanotoxin concentrations, but also tools for identifying factors leading to the production of hazardous levels of toxicity. Knowledge of the regulation of microcystin biosynthesis in the South African context could facilitate water management strategies that would avoid chronic exposure of consumers to drinking water containing microcystins.

The objective of this study was to use RT-PCR methodology as an early warning indicator for water resource management by using a combination of primer sets for the detection of *Microcystis* strains that bear the *mcy* gene cluster in Lake Hartbeespoort. This gene cluster, if present, can be responsible for the synthesis of cyanotoxins within the cyanobacterial hyperscum of Lake Hartbeespoort. The targeted genes (*mcy*A, B and D) were chosen as representatives of the microcystin peptide synthetase and polyketide synthase genes. The transcriptional regulation of these genes will give us clear indications of the effect of aphotic, anaerobic stress conditions within the cyanobacterial hyperscum and their effect on the synthesis of cyanotoxins. We further evaluated remote sensing as an early warning system for the detection of the development of cyanobacterial hyperscum crust in combination with physical and chemical parameters on ground level.

## MATERIALS AND METHODS

### Study area

Lake Hartbeespoort (25º43'S, 27º51'E) is a warm, monomictic man-made impoundment with a mean depth of 9.6 metres and a maximum depth of 32.5 metres (**Fig. 1**). The impoundment has a capacity of 195 million metres$^2$ at full supply level and is situated south-west of the city of Pretoria, South Africa. The lake has a surface area of about 20 km$^2$ within a watershed of approximately 4,144 km$^2$ in area extent. Excessive nutrient loads (80-300 metric tonnes of phosphorus as P), originating largely as point source (16 sewage treatment works) discharged annually into the Crocodile-Jukskei River system that supplies the lake, resulting in the reservoir becoming hypertrophic (Harding et al. 2004).

### Sampling strategy

Samples of water were collected from four sampling sites (**Fig. 1**) on a two weekly basis – from November 2004 to April 2005 (n = 12) – the mid-summer and lake overtune period when cyanobacterial blooms are most commonly present in Lake Hartbeespoort. For all sampling sites a syringe sampler modified after Baker et al. (1985) was used. Duplicate samples were taken at the surface and at 1 metre depth intervals down to 5 metres at all 4 sampling sites. Integrated water samples were transferred from the field to the laboratory in a dark cooler box within 2 h. Duplicate samples (5 litres) were preserved in the field by addition of acid Lugol's solution to a final concentration of 0.7 %, followed by addition of buffered formaldehyde to a final concentration of 2.5 % one hour later.

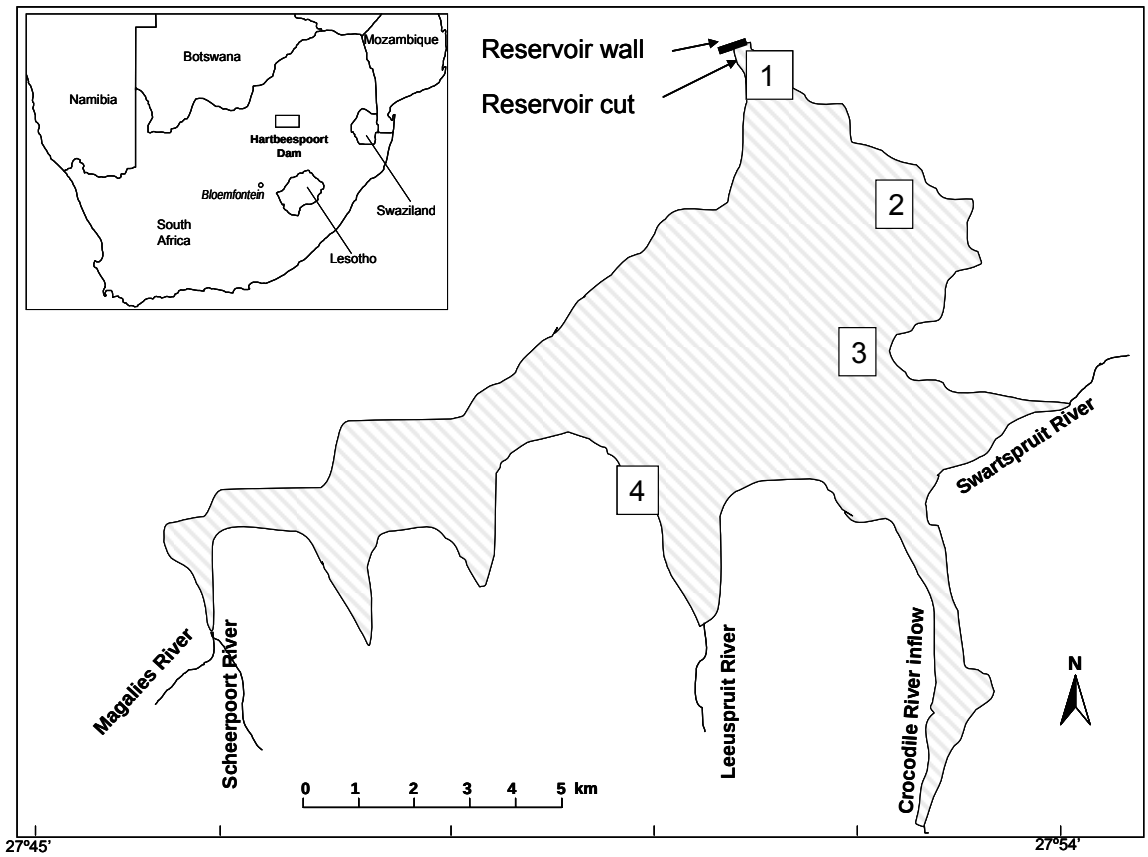


**Fig. 1.** Map of Lake Hartbeespoort, showing the location of the four sampling sites, and the positions of inflowing rivers. Inset shows the location of the map area in South Africa.

### Microscopy and enumeration

Species identification and enumeration were done using an Olympus inverted light microscope with a 1,250 x magnification (Wehr & Sheath 2001). Strip counts were made until at least 100 individuals of each of the dominant phytoplankton species were counted. Colonies of *Microcystis* were disintegrated by ultrasonication prior to counting (40 impulses per s over 4 min for a 10-ml sample) (Kurmayer et al. 2003). All counts were based on numbers of cells observed and the individual data grouped into major algal classes at each sampling site. Community comparisons were made using percent community similarity (the sum of the minimum relative abundance for all taxa between any two samples) to compare all 4 study sites in each sampling event (Brower et al. 1990).

### Chlorophyll *a*

Chlorophyll *a* (chl *a*) was extracted from lyophilized GF filters using N, N-dimethylformamide for 2 h at room temperature and measured spectrophotometrically at 647 and 664 nm according to the calculations of Porra et al. (1989).

### Physicochemical measurement

Nutrients dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) was determined using classical spectophotometric methods (American Public Health Association, American Water Work Association, and Water Pollution Control Federation 1980). Temperature profiles (ºC), pH and dissolved Oxygen (DO) of the water column were measured with a Hach$^{TM}$ sension 156 portable multiparameter (Loveland, USA). Wind velocity was measured at each of the sampling sites 1 m above the water surface with a Weather monitor 2 (Hayward, USA). Light intensity was measured with a model LI 1000 (Li-Cor, USA) light meter at a depth of 1 m at all 4 sampling sites. Sampling in this study was

carried out between 10h00 and 12h00 to eliminate possible variations in light intensity in the surface water.

**Satellite remote sensing**

The satellite images used in this study were obtained from the Council of Science and Industrial Research (CSIR) Satellite Application Centre, Hartbeeshoek, South Africa and were taken by sensors on the Landsat-5 satellite. Landsat-5 has a circular, sun-synchronous orbit which has a 16 day repeat cycle (Lillesand et al. 2004). The satellite orbits at 705 km above the surface of the earth and has both multi-spectral scanners (MSS) and thematic mapper (TM) sensors. The resolution for the spectral bands is 30 m by 30 m (except for the thermal band which has a resolution of 120 m). Light intensity, Secchi disc, chl *a*, Total Nitrogen, Total Phosphorus and phytoplankton composition were measured concurrently with satellite flybys.

**RT-PCR and ELISA analysis**

**Reference cyanobacterial culture**

The axenic *Microcystis aeruginosa* strain PCC 7806 was obtained from the Institute Pasteur (PCC; Paris, France). The strain was cultured in liquid MA (Ichimura, 1979) medium at 25 ± 2 ºC under continuous illumination of 25 µmol photons $m^{-2}$ $s^{-1}$. At 21 days growth, 2 ml of the culture was transferred to a serum vial and lyophilized (freeze dried) for 48 h. The sample was then stored under vacuum until DNA was extracted.

**Sampling of *Microcystis* cells for RT-PCR**

For RT-PCR, cyanobacterial cells were collected from the cyanobacterial hyperscum, 1 m below the surface crust at site 1 as well as at all the other 3 sampling sites at the end of February and the beginnig of March 2005 (n = 2). Before resuspension in distilled water to define volume, the cells were washed three times with distilled water and subjected to a freeze-thaw treatment for PCR template preparation (Baker et al. 2001). DNA was extracted from the environment samples as well as from the reference culture strain PCC 7806 using DNAzol®-Genomic DNA Isolation reagent following the manufacturer's procedures (Molecular Research Center, Inc., USA).

**PCR amplification**

PCR was performed in a GeneAmp2400 thermocycler (Perkin-Elmer Cetus, USA). The thermal cycling protocol included an initial denaturation at 94 °C for 2 min, followed by 35 cycles. Each individual cycle began with 10 s at 93 °C followed by 20 s at the annealing temperature at $T_m$°C for the specific primer pairs, and ended with 1 min at 72 °C. When extracted DNA was used, the amplification reactions contained a 10 x amplification buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmol of each primer and 1 U Taq DNA polymerase, and 3-5 ng purified DNA in a final volume of 50 µl (Dittmann et al. 1999). The PCR amplification for whole cells started with 6 µl of a crude cyanobactertial sample, pretreated subsample with an approximate cell density of 5 x $10^8$ cells.$ml^{-1}$, or 0.1 µg lyophilized (freeze dried) cyanobacterial cells. The sample was added directly to a 20-µl-reaction solution containing bovine serum albumin (0.1 mg.$ml^{-1}$) and a 10 x amplification buffer that contained 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmol of each primer, and 0.5 U Taq DNA polymerase (Howitt 1996). The PCR amplifications conditions were identical to those for the samples described above. An extra ramp rate of 3 s/°C between the denaturing and annealing steps was set when a GeneAmp9600 cycler instead of GeneAmp2400 was used for PCR amplification.

For analysis of the *mcy*A gene the primer set was as follows: McyA NMT (MSF: 5'-ATCCAGCAGTTGAGCAAGC-3', 59 °C; MSR: 5'-TGCAGATAACTC CGCAGTTG-3', 60 °C,

MSI: 5'-GAGAATTAGGGACACCTAT-3', 48 °C)(Tillett et al. 2001). For analysis of the *mcy*B gene the primers sets was as follow: Tox 1P: 5'-CGATTGTTACTGATACTCGCC-3', 57.9 °C; Tox1M: 5'-TAAGCGGGCA GTTCCTGC-3', 58.2 °C; Tox 3P: 5'-GGAG AATCTTTCATGGCAGAC-3', 62.4 °C; Tox1M: 5'-CCAATCCCTATCTAACACAGTACCTCGG-3', 65.1 °C; Tox 7P: 5'-CCTCAGACAATCAACGGTTAG-3', 53.7 °C; Tox 3M: 5'-CGTGGATAATAGT ACGGGTTTC-3', 58.4 °C; Tox 10Pf: 5'-GCCTAATATAGAGCCATTGCC-3', 59.8 °C; Tox 4Mr: 5'-CCAGTGGGTTAATTGAGTCAG-3', 57.9 °C)(Grobbelaar et al. 2004); McyB1-L: 5'-AGGCAAGCAGAAATTCAGGA-3', 55.9 °C; McyB1-R: 5'-ATAGCAACCACCGTCAAAGG-3', 55.9 °C; McyB3-L: 5'-TCA TCCCAACGTTGAACAAA-3', 55.2 °C; McyB3-R: 5'-CACCATATAAGC GGGCAGTT-3', 55.2 °C)(Botha-Oberholster and Oberholster 2007). For analysis of the *mcy*D gene the primer set was as follows: McyDF2: 5'-GGTTCGCCTGG TCAAAGTAA-3', 50 °C; McyDR2: 5'-CCTCGCTAAAGAAGGGTTGA-3', 50 °C)(Kaebernick et al. 2000). PCR amplicons were analyzed by gel electrophoresis on 2% agarose.

**RNA extraction and RT- PCR**

Cells were homogenized using liquid nitrogen and RNA extracted using the Qiagen RNAeasy kit (Qiagen Inc., USA) according to the manufacturers' instructions, and using DEPC-treated equipment and solutions.

RT-PCR was performed using 5 ng of total RNA per reaction and with 10µM of each primer (as above). RT-PCR was employed, using the iScript One-Step RT-PCR Kit (Bio-Rad, USA) and analysed using the iCycler iQ Real-Time PCR Detection Instrument (Bio-Rad, USA). The cycling parameters consisted of 1 cycle at 95 °C for 10 min; 40 cycles starting with 1 cycle at 95 °C for 10 s, primer specific annealing T°C for 5 s, 72 °C for 10 s; followed by the melting curve analysis (95 °C for 0 s, 65 °C for 15 s, 95 °C for 0 s), and cooling (40 °C for 30 s). A minimum of 7 reactions was done for each fragment analyzed. Standard curves were generated using a dilution series (1:1, 1:10, 1:100, 1:1000) and repeated. In order to calculate relative expression ratios for target genes, these were normalised with the expression of the unregulated 16S chloroplast rRNA transcript (Pfaffl 2001).

**Protein Phosphatase Inhibition and ELISA Assays**

Toxicity was determined by using the method of Boyer et al. (2004). Briefly, samples were collected from all 4 sites, 1 metre below the surface accept for sampling site 1 where integrated water samples were taken — during the end of February and the beginning of March 2005 (n = 2) — when the cyanobacterial hyperscum crust started to develop. The water was poured gently through a 934 AH glass fibre filters in the field, frozen on dry ice, and returned to the laboratory in a cooler box. Filters for toxin analysis were extracted by grinding with 10 ml of 50 % methanol containing 1 % acetic acid and clarified by centrifugation. This cyanobacterial extract was used for analysis of microsystins using the protein phosphatase inhibition assay (PPIA) as described in Carmichael and An (1999).

The ELISA assay was conducted with a Quanti™ Kit for Microsystins (EnviroLogix, USA). The limit of detection (LOD) of the kit is 0.147 ppb. The results were obtained by reading it on a multiskan ascent plate reader (Thermo Labsystems, USA) at 450 nm within 30 min after the addition of the stop solution.

**Statistical analysis**

All data were recorded on standard Excel spreadsheets for subsequent processing. The statistical analysis was conducted using the SYSTAT® 7.0.1 software package (SYSTAT, 1997). Statistical differences were analyzed using the Pearson correlation coefficient and *t* test Sigma Stat (Jandel Scientific) program. Values of $p \leq 0.05$ were regarded as significant.

## RESULTS

### Satellite imaging

Although floating scums of cyanobacteria were visible as a green mass on the water surface in the LANDSAT imagery at all 4 sampling sites, during late November 2004 to the end of April 2005 (n = 12), cyanobacterial hyperscum crust only started to develop at the end of February during the beginning of the overtune period of the lake (**Fig. 2**). The average environmental conditions at site 1 in the beginning of February were comparable to sites 2 and 3 (i.e. cell density of 2.1 x $10^8$ cells $ml^{-1}$, pH of 8.1 and chl *a*: 102 $\mu g\ l^{-1}$) before the hyperscum crust started to form at the end of February (**Fig. 3 and 4**). The satellite images of late February beginning March did reveal eutrophic conditions in the form of a cyanobacteria bloom present in the main basin of the lake as a single point in time which was comparible with the ground monitoring data of each of the specific 4 sampling points in time. The cyanobacteria *Microcystis aeruginosa* which made up the dominant species in the phytoplankton composition at sites 1 – 4, during the end of February 2005, dominated the spectrum of the satellite images by a typical cyanophyte signature which made the red absorbance of chl *a* readily apparent at sites 2 to 4 but — not at site 1 which gave a distinctive white image due to the development of hyperscum crust. The average ground monitoring data from late February to the end April 2005 – high average chl *a* (90 $\mu g\ l^{-1}$ and 96 $\mu g\ l^{-1}$) and *Microcystis* cell abundance (3.1 x $10^8$ $\mu g\ l^{-1}$, 1.4 x $10^8$ $\mu g\ l^{-1}$) of sampling sites 2 and 3 where confirmed by the satellite images during this sampling period (**Fig. 3**).

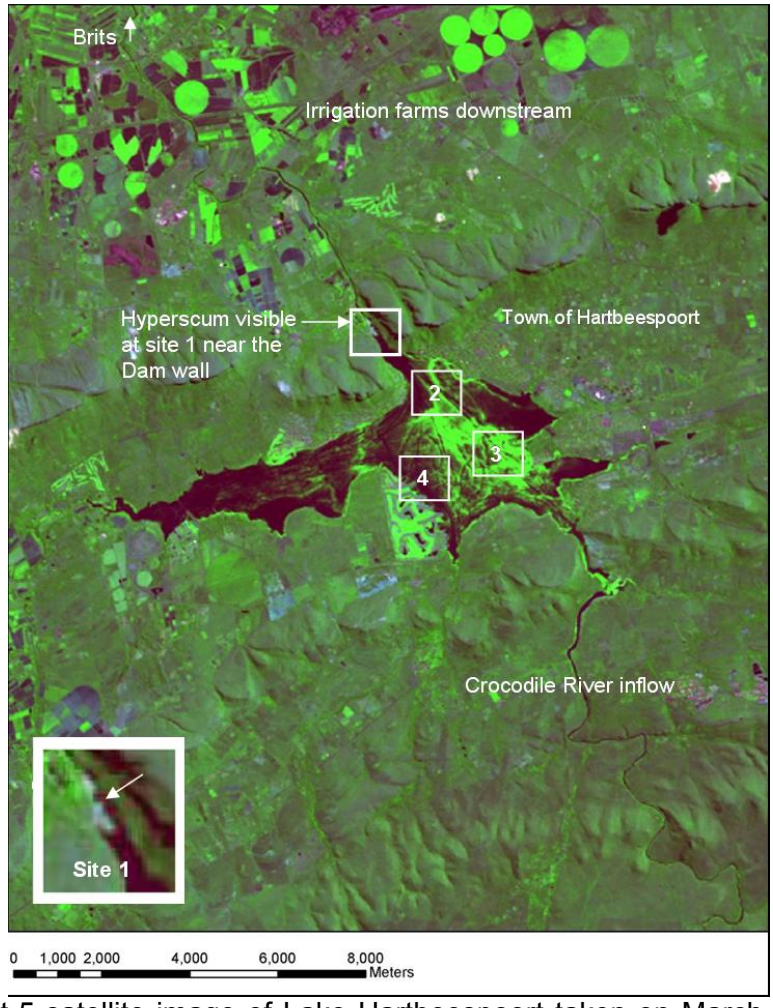


**Fig. 2.** A landsat-5 satellite image of Lake Hartbeespoort taken on March 8, 2005 showing the location of the four sampling sites and the cyanobacterial bloom. Inset shows the distinctive white hyperscum crust at sampling site 1.

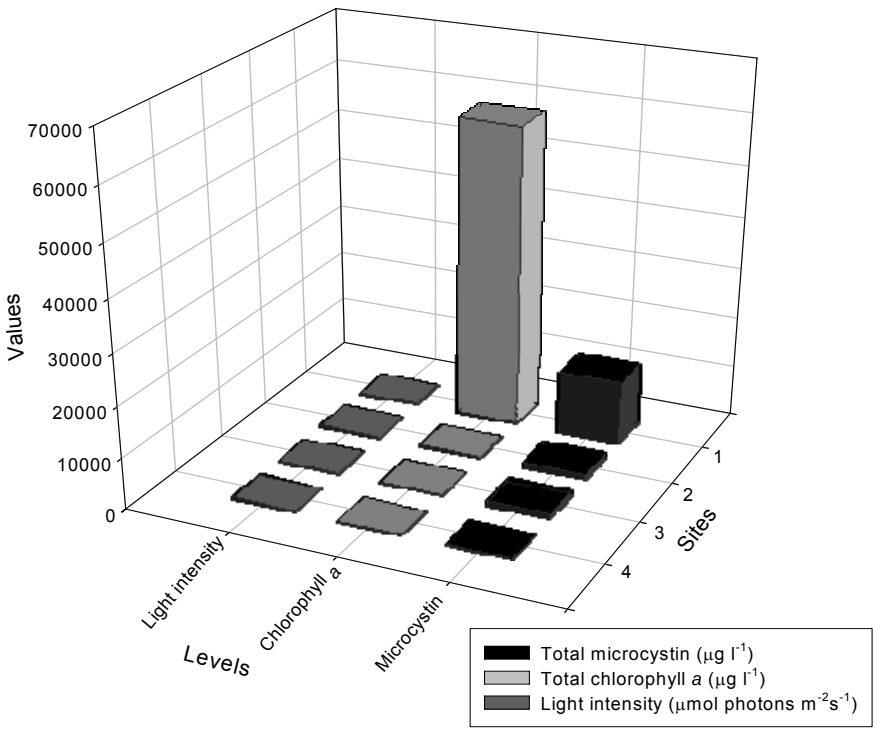


**Fig. 3.** Comparison of the levels of average light intensity (µmol photons $m^{-2}s^{-1}$); total chlorophyll *a* (µg $l^{-1}$) and total microcystin (µg $l^{-1}$) recorded at the four sampling sites in Lake Hartbeespoort from late February to early March 2005 (n = 2).

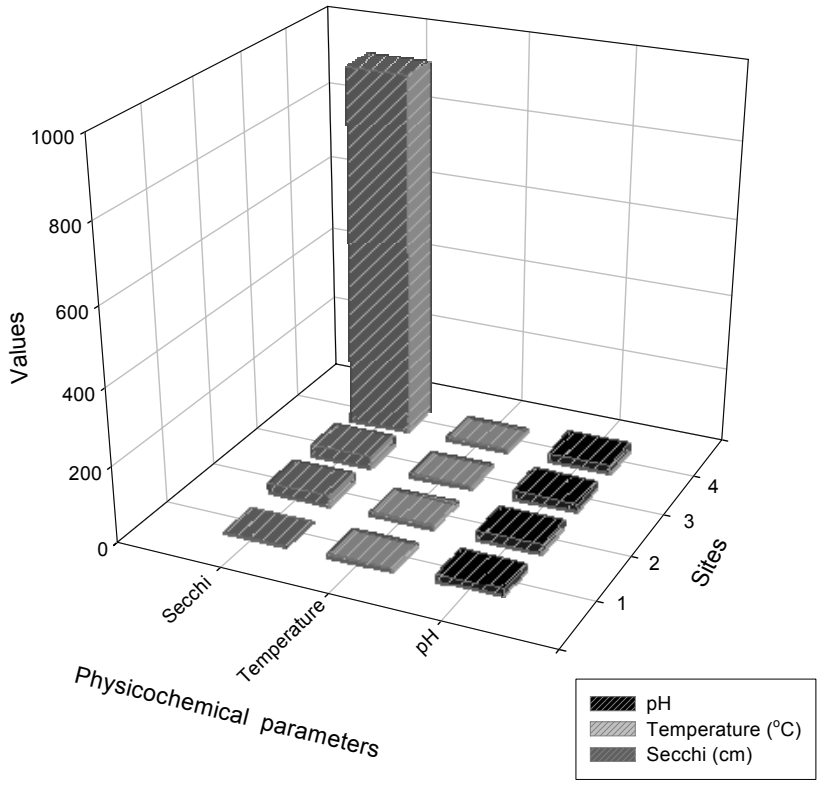


**Fig. 4.** Comparison of the average physicochemical parameters: pH; Temperature (º C) and Secchi depht (cm) recorded at the four sampling sites in Lake Hartbeespoort from late February to early March 2005 (n = 2).

**Cyanobacterial population structure within the hyperscum**

The cyanobacteria taxonomic composition within the hyperscum that started to develop at the end of February 2005 at site 1 — comprised of 99 % densely packed cyanobacterial *Microcystis aeruginosa* colonies and 0.1 % *Oscillatoria* sp. (verified microscopically) — accumulated near the reservoir wall of Lake Hartbeespoort. No cyanobacterial hyperscum was observed at the other 3 sampling sites for the duration of this study. The buoyancy mechanism of the *Microcystis aeruginosa* cells at the surface of sampling site 1, led to increasing compaction of the *Microcystis* colonies which decreased down to a depth of 5 metres at the beginning of March 2005. A distinct patch of dry surface crust (3 mm thick) developed at the end of February 2005 possibly due to the exposure of the *Microcystis* cells to photo-oxidizing conditions. The apparent cell bleaching on the surface of the hyperscum was possibly due to exposure to high light intensities (2,000 µmol of protons $m^{-2}.s^{-1}$). Immediately below the crust, continuously dark (< 0.93 µmol of protons $m^{-2}.s^{-1}$) anaerobic (DO, 0.4 mg $l^{-1}$, 3 % saturation) conditions prevailed. The compact layer of *Microcystis* cells (1.1 x $10^9$ cells $ml^{-1}$) correlated significantly with the chl *a* concentration of 59 mg $l^{-1}$ , dissolved organic carbon of 9.1 mg $l^{-1}$ and a pH value of 6.1 ($r$ = 0.9897; $p \leq 0.01$).

**Microcystin concentration and RT-PCR assays**

The total average microcystin concentration measured within the first 10 cm depth underneath the crust at site 1, in the beginning of March was 12,300 µg $l^{-1}$, decreasing to 312 µg $l^{-1}$ at a depth of 5 m. There were a significant correlation ($r$ = 0.9975; $p \leq 0.01$) between the high average total microcystin concentration (12,300 µg $l^{-1}$) and the high *Microcystis* cell abundance (1.1 x $10^9$ cells $ml^{-1}$) underneath the hyperscum crust at this site (**Fig. 3**). In all cases, microcystin concentrations exceeded the safety limit (1.0 µg $l^{-1}$) set by the World Health Organization for drinking water (WHO 1996). The average total *Microcystis* cell abundance measured at the end of February and beginning of March (n=2) at sites 2, 3 and 4 were 3.1 x $10^8$ µg $l^{-1}$, 1.4 x $10^8$ µg $l^{-1}$ and 2.4 x $10^4$ µg.$l^{-1}$, respectively, whereas secchi depth measurements were 25 cm, 28 cm and 945 cm, respectively. The variable average total microcystins to chl *a* ratio was not a reliable indicator of microcystin toxicity at these sites. Since the ratios of microcystin to chl *a* concentration calculated for sites 2-4 were not within the range of 0.1-0.4 µg microcystins (µg chl *a*)$^{-1}$ typically measured for *Microcystis* spp. (Sivonen and Jones 1999) (**Fig. 3**). The average total nitrogen (TN) (2.075 µg $l^{-1}$) and phosphorus (TP) (0.181 µg $l^{-1}$) during the period in late February, beginning March 2005, were high and nonlimiting with a TN : TP ratio of 11.5 : 1 indicating that the lake was hypertrophic (OECD 1982). Average wind speed during late February, beginning March 2005 was predominant from a ESE and WNW direction (2.1 $m.s^{-1}$) and correlated positive ($r$ = 0.9797; $p \leq 0.05$) with the high *Microcystis* cell abundance (1.1 x $10^9$ cells $ml^{-1}$) at site 1 near the dam wall, but negative ($r$ = -0.9867; $p \leq 0.01$) with the low cell abundance (2.4 x $10^4$ µg $l^{-1}$) at site 4. PCR analyses and Real time PCR analyses indicated the presence of the nonribosomal peptide synthetase gene *mcy*B and the polyketide synthase gene *mcy*D at all 4 sampling sites (**Table 1**, **Fig. 5**). At site 1, within the hyperscum low transcript levels (4-fold change) of the nonribosomal peptide synthetase gene *mcy*A was detect by RT-PCR but no transcript levels of the *mcy*A was express at sampling sites 2, 3 and 4 **(Table1, Fig. 5).**

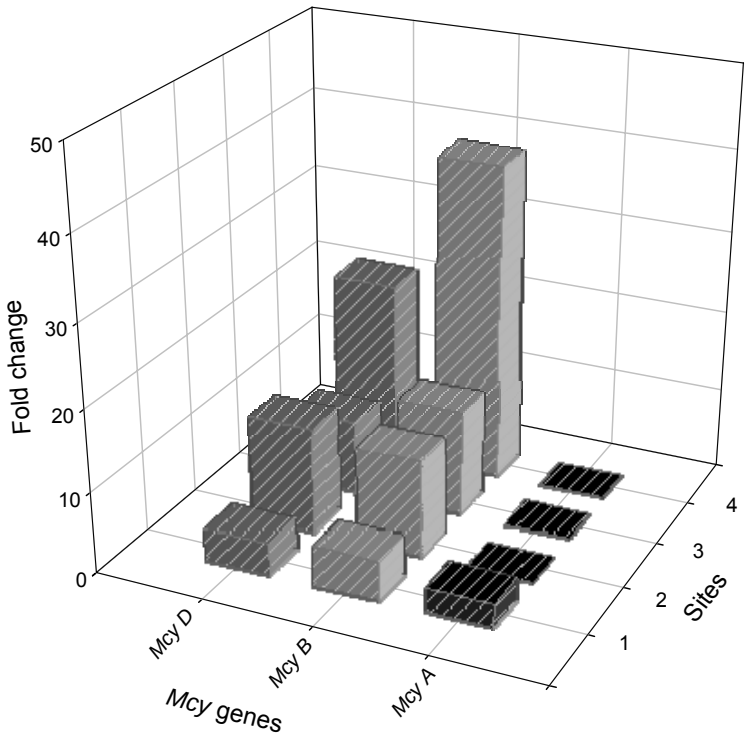


**Fig. 5.** Comparison of the average fold changes of the *mcy* genes A, B and D recorded at the four sampling sites in Lake Hartbeespoort from late February to early March 2005 (n = 2).

**Table 1**. A comparison of amplification products obtained after PCR analysis with different primers, as well as ELISA and PP1A assays as determinants of toxicity, in strains of *Microcystis aeruginosa* (+ = positive/product; - = negative/no product) of the 4 sampling sites.

| **Strain** | **Geographic origin** | **Primer sets** | | | | | | | | | **ELISA** | **PP1A** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Microcystis aeruginosa*** | | *McyB*-Tox3P/2M | *McyB*-Tox1P/1M | *McyB*-Tox7P/3M | *McyB*-Tox10P/4M | *McyB1*-L/R | *McyB3*-*L/R* | *McyA*-MSR/MSF | *McyD*-F2/R2 | *McyA*-MSI | | |
| PCC7806 | The Netherlands | + | + | + | + | + | + | + | + | + | + | + |
| UP29 (Site 1) | Hartbeespoort, ZA | - | + | + | - | + | + | + | + | + | + | + |
| UP30 (Site 2) | Hartbeespoort, ZA | - | + | + | + | + | + | - | + | - | + | + |
| UP31 (Site 3) | Hartbeespoort, ZA | - | + | + | + | + | + | - | + | - | + | + |
| UP38 (Site 4) | Hartbeespoort, ZA | - | + | + | + | + | + | - | + | - | + | + |

## DISCUSSION

### Hyperscum

Zohary & Breen (1989) defined hyperscum in Lake Hartbeespoort as crusted and buoyant cyanobacterial mats that formed when buoyant *Microcystis* colonies drifted and accumulated at the wind protected reservoir wall. These hyperscums forming thick, crusted, surface scums under which chl *a* concentrations exceeded 100 mg $l^{-1}$ ($1x10^5$ µg $l^{-1}$) the highest value reported for any plantonic cyanobacteria in freshwater systems (Harding & Paxton 2001). Hyperscum in Lake Hartbeespoort often exceeded a hectare in area, measured several centimetres in thickness and remained in the same site for between a few days to several months depending on environmental conditions (Louw 2003). This accumulation of cyanobacterial cells is termed hyperscums to distinguish them from the more temporary, thin, surface films of blue green algae that are known as water blooms or scum which were observed in this study at sites 2, 3 and 4 (Zohary & Breen 1989). The distinctive layer of white crust which is characteristic of hyperscum in Lake Hartbeespoort can possibly be attributed to the upper layer of *Microcystis* cells exposed to photo-oxidizing conditions during high light intensities. Photo-oxidative damage was evident as bleaching of the pigments occurred after exposure to high radiation (2,000 µmol of protons $m^{-2}.s^{-1}$) inflicting, causing irreversible genetic damage – phototoxicity – in the *Microcystis* cells.

### Cyanobacterial toxicity, DNA and transcript analysis

Earlier work by Zohary (1985) reported light intensity below the crust layer of *Microcystis* in Lake Hartbeespoort of <0.001 µmol of photons $m^{-2}.s^{-1}$ making the interior of the scum aphotic. These observations are comparable with the low light intensity measured in this study (light intensity <0.93 µmol of photons $m^{-2}.s^{-1}$). In a previous laboratory study by Kaebernick et al. (2000) an increase in microcystin content was observed when light intensity increased from 2 to 40 µmol of photons $m^{-2}s^{-1}$. This led to the conclusion that light intensities influencing the toxicity of *Microcystis aeruginosa* at less than 40 µmol of photons $m^{-2}s^{-1}$ (Utkilen & Gjolme 1992). Unfortunately, direct comparison of light intensities and toxicity were not possible in this study, due to different environmental conditions within cyanobacterial hyperscum and varying depth. Through conducting PCR and RT-PCR methodology we could not find any relationship between the DNA and RNA transcript analyses of site 1 and that of sites 2, 3 and 4. The PCR and RT-PCR analyses showed low levels of transcription of the *mcy*A, B and D genes under very low light intensity (<0.93 µmol of photon $m^{-2}$ $s^{-1}$) at site 1, while much higher levels of the *mcy*B and *mcy*D genes were expressed at site 2, 3 and 4 with a light intensity of (19; 21; 61 µmol of photons $m^{-2}s^{-1}$) at a depth of 1 m. The low levels of expression of the *mcy*B and D genes at sampling site 1, may be due to various stress-inducing environmental factors prevailing under the hyperscum e.g. low light intensity and pH (**Fig. 4 and 5**). The only possible explanation for the low level of transcription of the *mcy* A gene at sampling site 1, and its absence at the other sites may also be related to the role of different environmental stresses. Although previous surveys on environmental strains of *M. aeruginosa* in South African reservoirs demonstrated the absence of the *mcy* A is not uncommon, whereas *mcy*B and D was always associated with toxic *M. aeruginosa* strains (Botha-Oberholster and Oberholster (2007). In previous laboratory studies conducted by Kaebernick et al. (2000, 2002) they reported an increase in transcription of the *mcy*B and *mcy*D genes under high light conditions that may indicate higher toxin production. Furthermore, they found in a later study (Kaebernick et al. 2002) that the microcystin synthetase gene cluster (*mcy*ABCDEFGHIJ) consisted of two polycistronic transcripts, *mcy*ABC and *mcy*DEFGHIJ, which are transcribed from a central promoter between *mcy*A and *mcy*D and that both polycistronic transcripts have alternate transcription starting sites which are possibly light dependent. From the results of this study it is clear that the transcription of the *mcy*A, B and D genes, under environmental stress conditions, require only low light intensities (<0.93 µmol of photons $m^{-2}$ $s^{-1}$) for the upregulation of these genes. We further predict that microcystin synthetase production appears at low levels of light intensity, during unfavourable environmental conditions i.e. aphotic conditions in hyperscum

and dormant colonies in surface sediment of lakes (during overwintering periodes) to prevent possible grazing pressure by zooplankton (Oberholster et al. 2006a). These results are concurrent to suggestions made by Kaebernick et al. (2000) that toxins is constitutively produced under low and medium light intensities, but is exported only when a certain higher light threshold intensity is reached.

Plausible explanations for the high levels of total microcystins during the end of February and the beginning of March 2005 at sampling site 1 underneath the hyperscum crust may be; due to the collapse of great amounts of *Microcystis* colonies (high cell densities of 1.1 x $10^9$ µg $l^{-1}$) in the compact layer of hyperscum underneath the hyperscum crust, of which one *Microcystis* cell might contain up to 0.2 pg of microcystin-LR (Codd et al. 2005). The second explanation is the absence of sunlight irradiation and photolysis by UV light under the layer of crust, since the latter have been implicated in microcystin decomposition and isomerization to non-toxic forms (Tsuji et al. 1995). The third plausible explanation came from a laboratorial study conducted by Watanabe et al. (1992). They reported changes in the amount of microcystins-YR and LR during decomposition processes of *Microcystis aeruginosa* cells under dark and aerobic conditions. It their study a decrease to 58 % in microcystin-YR at the end of the experiment ($45^{th}$ day) was measured, while 86 % of microcystin- LR remained. Although we did not measure the gas contents (accept for DO, 0.4 mg $l^{-1}$) within the hyperscum, Zohary & Madeira (1990) observed gas bubbles composed of 28 % methane, 19 % $CO_2$, 53 % $N_2$, and traces of $H_2$ trapped within the hyperscum. We speculate that the pressure of these gasses resulted in the collapse of the *Microcystis* cells setting microcystins free within the hyperscum underneath the crust, since gas vesicles are delicate structures (extremely thin, 2 nm proteinaceous membrane enclosing a gas-filled space) and tend to collapse when the cells are subjected to pressure (Fay 1983).

In the present study, it is suggested that the development of the compact layer of *Microcystis* colonies down to 5 metres under the crust at the wind protected dam wall, was possibly due to the action of horizontal surface currents and underwater circulation that down-drive surface scum under the hyperscum and layer of crust. Once the *Microcystis* colonies were under the hyperscum, they experience conditions of low light intensity (<0.93 µmol of photons $m^{-2}s^{-1}$) and reduced water movement. Under these conditions of low mean photon density, the *Microcystis* cells accumulate only small amounts of carbohydrates and increase their buoyancy by the formation of excess gas vesicles. However under dark conditions, *Microcystis* cells cannot reverse their buoyancy and thus gets “trapped” under the crust, since a linear relation between the loss of buoyancy and carbohydrate content, and buoyancy state and the light dose received, were reported for *Microcystis* cells in both cultured (Kromkamp & Mur 1984) and in natural populations (Visser et al. 1996; Oberholster et al. 2006b).

**Landsat satellite images and human health**

In this study the satellite imagery of Lake Hartbeespoort give a distinctive white pattern indicative of the development of surface crust on top of the hyperscum at the reservoir wall during late February and the beginning of March 2005, as well as indicating the development of cyanobacterial scum at sites 2 and 3 **(Fig. 2)**. Therefore, knowledge of the location, extent and possible genotoxicity of cyanobacterial blooms within the reservoir would allow the water resource manager to select a water withdrawal strategy that would minimize the risk posed by cyanobacterial toxins in drinking and irrigation water.

**Risk management and water quality threats posed by toxins**

Water quality predictions like these are important for water quality management, especially if it happen on a sufficient timely basis, since large amounts of the water from Lake Hartbeespoort are used directly or indirectly for human consumption. The yearly total outflow for irrigation and municipal drinking water extracted near the reservoir wall of Lake Hartbeespoort amounts to 82 x $10^6$ $m^3$ and 7 x $10^6$ $m^3$, respectively. This water is extracted

where toxic hyperscum with a distinct crust layer can accumulate for up to three months, and thus, pose a major thread to recreational activities and drinking water consumers. In a previous report, Codd et al. (1999) measured detectable amounts of microcystins and *Microcystis* cells in spray irrigation water and on sprayed-irrigated salad lettuce intended for human consumption. Another report by Hoppu & Samela (2002) stated that irrigation water contaminated by cyanotoxins may lyses the cells and aerosolizes the toxins which can then be inhaled by farm workers. It is also possible that dairy cows, after oral exposure, might secrete cyanobacterial toxins in their milk, although, tests to date after administration of microcystins to lactating cows by gavage have not confirmed the presence of microcystin in milk (Feitz et al. 2002).

During a previous occurrence of hyperscum in 2003, the drinking water distribution systems of two small towns, Hartbeespoort and Brits, receiving water from Lake Hartbeespoort exceeded the World Health Organisation total allowable daily intake of 1 µg $l^{-1}$ (Van Ginkel et al. 2006). Long-term chronic exposure by residence of these towns to low levels of microcystin may also occur as conventional water treatment processes are ineffective in the removal of cyanobacterial toxins from drinking water in these areas (Hoffman 1987). Furthermore the recreational activities near the reservoir wall of Lake Hartbeespoort, that involved direct contact with water (swimming, sailboarding, canoeing, paddling and to a lesser extent boating and angling) may result in incidental or accidental ingestion, inhalation, or skin contact from cyanobacteria scum can pose a health threat. This risk increases by the presence of the hyperscum that accumulate near the reservoir wall which is in much higher concentrations than the limiting range of 10 to 400 µg$l^{-1}$ suggested for the safe practice in managing recreational waters (Oberholster et al. 2004).

## CONCLUSION

We propose through the outcome of this study that by employing routine monitoring by ELISA, PCR and/or RT- PCR and satellite images, water utilities can predict the genotoxicity of *M. aeruginosa* cells, as well as the precise time and location of hyperscum formation near the reservoir wall of Lake Hartbeespoort and therefore can set a risk management plan in place before it become a health risk to the inhabitants of the area.

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