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Full Length Research Paper

Use of remote sensing and molecular markers to detect toxic cyanobacterial hyperscum crust: A case study on Lake Hartbeespoort, South Africa

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In this study, we monitored the formation of cyanobacterial hyperscum and crust formation in Lake Hartbeespoort using satellite images and ground monitoring. The hyperscum that formed near the reservoir wall was characterised by a distinctive white surface layer of crust. Hyperscum is the result of exposure of the cells to high radiation, inflicting irreversible damage to the genetic constitution of the upper layer of *Microcystis aeruginosa* 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, 3% saturation) prevailed with high levels of microcystin (12,300 µg/l) in the absence of sunlight irradiation and photolysis by UV light. Real time polymerase chain reaction (PCR) analysis indicated low levels of transcription of the *mcyA*, *mcyB* and *mcyD* genes which are responsible for synthesis of cyanotoxins under these low light intensity conditions. At other sampling sites where cyanobacterial scum occurred and hyperscum crust was absent, only the *mcyB* and *mcyD* genes were transcribed. A plausible explanation for the transcription of the *mcyA* gene in the hyperscum and not at the other sampling sites, was 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 the sampling site where no cyanobacterial scum was visible on the satellite images, low cell abundance (2.4 x 10^4 µg/l) and chlorophyll *a* (12.2 µg/l) was measured in comparison with sites where cyanobacterial scum was visible on the satellite images.

Key words: Hyperscum crust, reverse-transcription PCR, *mcyA* levels, microcystin, satellite imaging, cyanobacteria.

INTRODUCTION

Eutrophication of waters can result in blooms of toxic cyanobacteria. Such algae form temporary dense surface films and scums. However, under certain environmental conditions, these temporary surface scums can develop into cyanobacterial hyperscum (Zohary and Breen, 1989; Zohary and Madeira, 1990). Hyperscum is defined as crusted and buoyant cyanobacterial mats that are formed when floating cyanobacterial colonies drift and accumulate at wind protected areas (Zohary and Breen, 1989). These hyperscums can form thick, crusted and surface scums under which chlorophyll *a* (chl-*a*) concentrations can exceed 100 mg/l (1 x 10^5 µg/l) (Zohary and Breen, 1989). In the case of Lake Hartbeespoort, South Africa, hyperscum can often exceed a hectare in area, measuring several centimetres in thickness with a distinctive white crusted surface. This phenomenon can remain in the same areas for between a few days to

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Abbreviation: ELISA, Enzyme-linked immunosorbent assay; chl-*a*, chlorophyll *a*; **PCR,** polymerase chain reaction.

several months, depending on environmental conditions (Louw, 2003). Cyanobacteria blooms in South Africa have several consequences in terms of water quality and frequently toxic bloom formation can cause animal mortalities (Oberholster et al., 2009b). 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 (Oberholster et al., 2009a).

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 (Dittmann et al., 1999; Kaebernick et al., 2000). In addition to causing death and illness in animals, fish and livestock, recreational exposure (i.e. via swimming, sailboarding, canoeing, paddling and to a lesser extent boating and angling) can cause diarrhoea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers and skin and eye irritation, with the latter symptoms, possibly as a result of the lipolysaccharides contained in the cell walls of all cyanobacterial cells (Pilotto et al., 1997).

During a previous occurrence of hyperscum in 2003 in Lake Hartbeespoort, 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 (Van Ginkel et al., 2006). Long-term chronic exposure by residents 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, 1976). This health risk increases with the presence of the hyperscum that accumulate near the reservoir wall which is in higher concentrations than the limiting range of 10 to 400 µg/l suggested for the safe practice when managing recreational waters (Oberholster et al., 2004).

Accumulation of aggregated cyanobacterial cells, just below the water surface and surface scums are so distinct, that the extent of a bloom can easily be mapped using almost any remote sensing instrument. However, little is known about mapping the development of hyperscum and the environmental conditions that prevail during the formation of hyperscum on ground level.

The objective of this study are (1) To evaluate the feasibility of using remote sensing in the detection of hyperscum crust formation; (2) to correlate remote sensing imagery with ground level monitoring for the detection and characterization of the development of cyanobacterial hyperscum crust over a period of 6 months; (3) to use a combination of molecular tools to confirm the presence of genotoxic strains of *Microcystis* in Lake Hartbeespoort associated with the hyperscum crust. The targeted genes (*mcyA*, *mcyB* and *mcyD*) were chosen as representatives of the microcystin peptide synthetase and polyketide synthase genes. Previous laboratory studies suggested that the transcription of these genes may be associated with light intensity (Kaebernick et al., 2000), thus the transcriptional regulation of these genes may give us some indication of stress conditions within the cyanobacterial hyperscum underneath the crust layer and their effect on the synthesis of cyanotoxins under natural conditions.

MATERIALS AND METHODS

Study site

Lake Hartbeespoort (25°43'S; 27°51'E) is a warm, monomictic man-made impoundment with a mean depth of 9.6 m and a maximum depth of 32.5 m (Figure 1). The impoundment has a capacity of 195 million m^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 to 300 metric tonnes of phosphate (P), originating largely as point source (16 sewage treatment works) are discharged annually into the Crocodile-Jukskei River system that supplies the lake with water, resulting in the reservoir becoming hypertrophic (The Water Wheel, 2005). During summer months, different thermal layers form in the water column of Lake Hartbeespoort causing the deeper thermal water layer to become anaerobic. Under these conditions, phosphorus is released from the sediment which provides optimal cyanobacterial growth in the lake. However, for reason not yet known, nowhere else in South Africa are hyperscums produced so rapidly or in such quantities as in the case of Lake Hartbeespoort (The Water Wheel, 2005).

Sampling strategy and analysis methods

Lake water samples were collected from four permanent sampling stations (Figure 1) at 16-day-time cycle intervals that overlapped with the orbiting of the Landsat-5 satellite over the study area. Landsat-5 images were used in this study, since it is more affordable than the MERIS and MODIS systems in the long run, especially if it is used for national water quality monitoring programs in developing countries. Sampling occurred from November 2004 to April 2005 (n = 12), the mid-summer and lake overturn period when cyanobacterial blooms are most commonly present in Lake Hartbeespoort. Duplicate water samples were collected from each of the four sampling stations. The duplicate water samples were collected at the lake surface and at 0.5 m intervals down to 1 m, using a 6 L capacity Von Dorn sampler. The two water samples from each depth interval at each site were then mixed to form two integrated 5 L samples for the respective sites. Three 1 L sub-samples were taken from each of the two integrated 5 L samples at each site in the field. The first 1 L sub-sample was frozen on dry ice in the field for testing genotoxicity in the strains using molecular markers. The second sub-sample was poured gently through a 934 AH glass fiber filter which was frozen on dry ice for later analysis of cyanobacterial toxicity by enzyme-linked immunosorbent assay (ELISA). The last sub-sample of 1 L was preserved in the field by addition of acid Lugol's solution to a final concentration of 0.7%, followed after one hour by addition of buffered formaldehyde to a final concentration of 2.5% for the determination of phytoplankton assemblage. The remaining 2 L of each of the 5 L integrated water samples were used for the determination of water chemical and chl-*a* analyses. All samples were kept in a cooler box and in the dark during the 1 h period of transfer from the field to the laboratory.

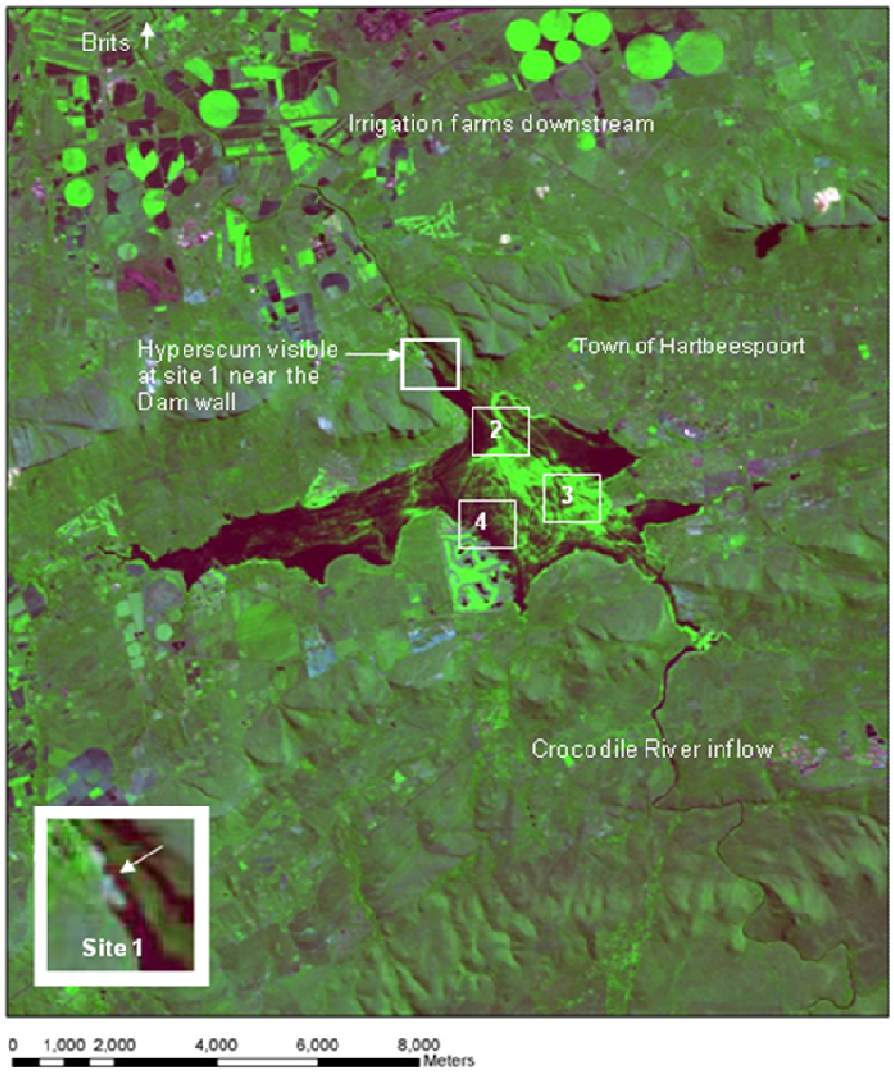

Figure 1. A landsat-5 satellite image of Lake Hartbeespoort taken on March 8, 2005, showing the location of the four sampling sites and the cyanobacterial bloom. Inset shows the distinctive white hyperscum crust at sampling site 1.

Microscopy and enumeration

All phytoplankton identifications were done using an Olympus compound microscope with 1,250 x magnification (Van Vuuren et al., 2006). Strip counts were made until at least 300 individuals of the dominant phytoplankton species were counted. Colonies of *Microcystis* were disintegrated by ultrasonication prior to counting (Kurmayer et al., 2003). Biovolume was calculated by measuring the corresponding dimensions using the geometric formulas given by Willen (1976).

Pigment and physical/chemical determination

Concentrations of chlorophyll*a* (Chl-*a*) and physical/chemical parameters were determined as previously reported (Oberholster et al., 2009a, b). 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 10 and 12 h 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. In this study, the concentrations of the main photosynthetic pigment chl-*a* and the ratio of a near infra-red band (around 700 nm) over a band near the red absorption maximum of chl-*a* at 675 nm was used (Mittenzwey et al., 1992). The band ratio was used to target chl-*a* absorption and serves as an indicator for phytoplankton biomass (algae and cyanobacteria) in productive waters (Gons, 1999).

Detection of toxicity and toxigenic strains

The axenic *M. aeruginosa* strain PCC 7806 was obtained from the Institute Pasteur (PCC; Paris, France). The strain was cultured according to Ichimura (1979). The sample was then stored under vacuum until DNA was extracted.

Sampling of *Microcystis* cells for polymerase chain reaction (PCR) and quantitative PCR analyses

Sampled water with cyanobacterial cells collected at the four sampling stations was filtered through a 45 µm Whatman filter after which the cyanobacterial cells was rinsed 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). Extracted DNAs were purified once (culture strain) or twice (environmental strains) with a Prep-A-Gene DNA Purification Kit (Bio-Rad) according to the manufacturer's instructions and eluted in 60 µl (Botha-Oberholster and Oberholster, 2007).

PCR amplification

PCR was performed in a GeneAmp2400 thermocycler (Perkin-Elmer Cetus, Emeryville, Calif., USA) as previously described (Botha-Oberholster and Oberholster, 2007; Oberholster et al., 2009a, b). The thermal cycling protocol included an initial denaturation at 94°C for 2 min, followed by 35 cycles. Each 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 as previously described (Oberholster et al., 2009a; Tillett et al., 2001; Grobbelaar et al., 2004; Botha-Oberholster and Oberholster, 2007; Kaebernick et al., 2000) and ended with 1 min at 72°C.

RNA extraction and quantitative PCR

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

Quantitative PCR was performed using 5 ng of total RNA per reaction and with 10 µM of each primer as previously described (Oberholster et al., 2009a; Tillett et al., 2001; Grobbelaar et al., 2004; Botha-Oberholster and Oberholster, 2007; Kaebernick et al., 2000). Quantitative PCR was performed using the iScript One-Step RT-PCR Kit (Bio-Rad, USA) and analysed using the iCycler iQ Real-Time PCR Detection Instrument (Bio-Rad) as previously described (Botha-Oberholster and Oberholster, 2007, Oberholster et al., 2009a, b).

ELISA assays

Cyanobaterial toxicity was determined according to Boyer et al. (2004). The ELISA assay was conducted with a Quanti™ Kit for Microsystins (EnviroLogix, USA). The results were obtained by reading on a multiscan 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. Statistical differences between different environmental variables and *Microcystis* cell abundance 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 at site 1 during the beginning of the overturn period of the lake (Figure 1). This was also the only site where hyperscum crust was detected during the whole period of the study. Although hyperscum crust was not visible at site 1 on the LANDSAT image at the end of February, it was detected from ground level monitoring and exceeded 2 m in diameter (Figure 2). However, data generated from the satellite band ratio targeting chl-*a* absorption indicated an increase in phytoplankton biomass from the time period of January to March which shows a decrease in normalized surface reflectance (January – 2.5 to March – 1.75). The average environmental conditions at site 1 in the beginning of February were comparable to sites 2 and 3 (average cell density of 2.1 x 10^7 cells/ml, average pH of 8.1 and chl-*a* = 102 µg/l) before the hyperscum crust started to develop at the end of February (Table 1). The satellite images of late February to beginning of March did reveal eutrophic conditions in the form of a cyanobacteria bloom presence in the main basin of the lake as a single point in time which was comparable with the ground monitoring data of each of the specific sampling sites (Figure 1). The cyanobacteria *M. aeruginosa* (Kützing) were the dominant species (71%) in the phytoplankton composition at sites 1 to 4, during the end of February 2005. However, a distinctive irregular white pattern near the dam wall was only revealed on the satellite images during the mid of March when the hyperscum crust exceeded 50 m in diameter. This pattern of hyperscum crust extended to ±100 m in diameter at the end of March to April 2005. The ground monitoring data generated from late February to the end of April 2005 revealed high average chl-*a* (90 and 96 µg/l) and *Microcystis* cell abundance (3.1 x 10^7 µg/l and 1.4 x 10^7 µg/l) at sampling sites 2 and 3 which were confirmed by the satellite images during this sampling period (Table 1).

Cyanobacterial population structure within the hyperscum

The cyanobacteria taxonomic composition of the biovolume within the hyperscum that started to develop at the end of February 2005 at site 1 comprised of 71% densely packed cyanobacterial *M. aeruginosa* (Kützing) cells, 25% *M. aeruginosa* forma *flos aquae* (Wittrock) and to a much lesser extent (4%) *Oscillatoria* sp. cells (verified microscopically). Although cyanobacterial hyperscum

Figure 2. (A) Ground monitoring image showing the formation of white hyperscum crust at the dam wall of Lake Hartbeespoort during late February 2005. (B) Ground monitoring image March 26, 2005 showing the distinctive white hyperscum crust at sampling site 1.

crust formation was not observed at sampling sites 2, 3 and 4 for the duration of this study, the latter sites contained *M. aeruginosa*, *Oscillatoria* sp., *Pseudoanabeana* sp. and the diatom species *Melosira granulata* (Agardh) cells. The buoyancy mechanism of the *M. aeruginosa* cells at the surface of sampling site 1, led to increasing compaction of the *Microcystis* colonies which decreased down to a depth of 1 m at the beginning of March 2005. A distinct patch of dry surface crust (3 mm thick) developed at the end of February 2005 which was possibly due to the exposure of the *Microcystis* cells to photo-oxidizing conditions at the surface. The apparent cell bleaching on the surface of the hyperscum was probably caused by the exposure of the surface layer of cells to high light intensities (2,000 µmol of protons $m^{-2}.s^{-1}$) during this time period. Environmental conditions underneath the crust during March were continuous dark (<0.93 µmol of protons $m^{-2}.s^{-1}$) anaerobic (DO, 0.4 µg/l, 3% saturation) conditions. The compact layer of *Microcystis* cells (1.1 x 10^9 cells/ml) correlated significantly ($r = 0.9897$; $p \leq 0.01$) with the average chl-*a* concentration of 59 mg/l measured underneath the crust during this time period.

Microcystin levels and environmental conditions

The total average microcystin levels measured within the first 1 m depth underneath the crust at site 1, at the end of March was very high (12,300 µg/l). During this sampling period, there existed a significant correlation ($r = 0.9975$; $p \leq 0.01$) between the high average total

Table 1. Average physical and chemical data of the four sampling sites pooled together to give a monthly average over the study period (n = 12 sampling trips).

Parameter	Unit	Months					
		November	December	January	February	March	April
Chl *a*	µg/l	13.0 ± 1.1	19.1 ± 3.2	78.8 ± 11.1	29,600 ± 10.1	33,280 ± 8.1	24,300 ± 120.5
Secchi	Depth/m	2.1 ± 0.4	1.1 ± 0.29	0.8 ± 0.2	0.6 ± 0.1	0.3 ± 01	0.5 ± 0.2
Temperature	℃	23.4 ± 1.9	25.1 ± 1.2	25.8 ± 0.9	25.3 ± 0.7	24.6 ± 0.4	23.2 ± 0.9
Microcystis-LR	µg/l	10.8 ± 1.8	23.1 ± 1.1	49.8 ± 6.3	2,550 ± 241	4,270 ± 109	3,780 ± 213
Conductivity	µ/S/cm	561 ± 31	593 ± 11	583 ± 24	576 ± 31	566 ± 22	548 ± 28
Dissolved oxygen	mg/l	7.3 ± 0.8	7.7 ± 0.3	5.8 ± 1.0	4.2 ± 0.9	3.1 ± 0.4	3.5 ± 0.2
PO_4	mg/l	0.01 ± 0.006	0.01 ± 0.002	0.01 ± 0.009	0.02 ± 0.005	0.03 ± 0.002	0.03 ± 0.005
Kjel-N	mg/l	0.89 ± 0.11	1.05 ± 0.23	1.21 ± 0.31	1.44 ± 0.25	1.56 ± 0.33	1.68 ± 0.61
$NO_3 – NO_2$	mg/l	0.61 ± 0.10	0.62 ± 0.08	0.60 ± 0.11	0.77 ± 0.07	1.35 ± 0.13	1.34 ± 0.19
NH_4-N	mg/l	0.06 ± 0.007	0.08 ± 0.004	0.06 ± 0.01	0.09 ± 0.03	0.11 ± 0.009	0.14 ± 0.05
pH		8.8 ± .0.2	8.9 ± 0.6	8.1 ± 0.3	8.3 ± 0.5	7.1 ± 0.7	7.2 ± 0.2

It must be noted that the abnormal high chl *a* and microcystin-LR data during February to April was due to conditions that exist under the hyperscum at site 1.

microcystin levels (12,300 µg/l) and the high *Microcystis* cell abundance (1.1 x 10^9 cells/ml) underneath the hyperscum crust at this sampling site (Tables 1 and 2). From February to April, microcystin levels at all 4 sampling sites exceeded the safety limit for drinking water (1.0 µg/l) set by the World Health Organization for water recreation activities (WHO, 1996). The average total *Microcystis* sp. cell abundance measured at the end of February and beginning of March at sites 2, 3 and 4 were 3.1 x 10^7, 1.4 x 10^7 and 2.4 x 10^4 µg/l, respectively, whereas average Secchi depth measurements were 25, 28 and 945 cm, respectively (Table 1). The average total nitrogen (2.075 µg/l) and phosphate (0.181 µg/l) during the period in late February and beginning of March 2005, were high with a TN : TP ratio of 11.5:1 indicating that the lake was hypertrophic (OECD, 1982). Average wind speed during late February and beginning of March 2005 was predominant from a ESE and WNW direction (2.1 $m.s^{-1}$) and correlated positively ($r = 0.9797$; $p \leq 0.05$) with the high *Microcystis* cell abundance (1.1 x 10^8 cells/ml) in the water column at site 1 near the dam wall which act as a shelter, but negatively ($r = -0.9867$; $p \leq 0.01$) with the much lower cell abundance (2.4 x 10^4 µg/l) measured at site 4.

PCR and quantitative PCR assays

PCR and quantitative PCR analyses indicated the presence of the nonribosomal peptide synthetase gene *mcyB* and the polyketide synthase gene *mcyD* at all 4 sampling sites during the period of the study (Table 2). At site 1, within the hyperscum low transcription levels of the nonribosomal peptide synthetase gene, *mcyA* was measured by quantitative PCR. However, no detectable levels of the *mcy*A were measured at sampling sites 2, 3 and 4 (Table 2).

DISCUSSION

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 irreversible genetic damage causing phototoxicity in the *Microcystis* cells.

Earlier work by Zohary (1985) reported that light intensity below the crust layer of *Microcystis* in Lake Hartbeespoort was <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 (light intensity <0.93 µmol of photons $m^{-2}.s^{-1}$) measured under the hyperscum in this study. In a previous laboratory study by Kaebernick et al. (2000), an increase in microcystin content was reported when light intensity increased from 2 to 40 µmol of photons $m^{-2}s^{-1}$. This led to the conclusion that different light intensities may influence the toxicity levels of *M. aeruginosa* cells at less than 40 µmol of photons $m^{-2}s^{-1}$ (Utkilen and Gjolme, 1992). Unfortunately, direct comparison of light intensities and toxicity were not possible in this study, due to different environmental conditions within the cyanobacterial hyperscum underneath the crust. However, studies by Utkilen and Gjolme (1992) indicated that light intensity had a pronounced effect on both toxicity and toxin production rate in the continuous culture of *M. aeruginosa.* It was found that toxicity and toxin production rate increased with light

Table 2. A comparison of amplification products obtained after PCR and RT-PCR analyses with different primers, as well as ELISA assays as determinants of toxicity, in strains of *M. aeruginosa*.

M. aeruginosa	Geographic origin	PCR					RT-PCR (relative fold change)					ELISA
		McyA-Tox3P/2M	McyA-MSR/MSF	McyB-Tox1P/1M	McyB-Tox7P/3M	McyD-F2/R2	McyA-Tox3P/2M	McyA-MSR/MSF	McyB-Tox1P/1M	McyB-Tox7P/3M	McyD-F2/R2	
PCC7806	The Netherlands	+	+	+	+	+	*	*	*	*	*	+
UP29 (Site 1)	Hartbeespoort, ZA	+	+	+	+	+	3.3 ± 0.7	2.5 ±1.3	5.3 ± 1.5	6.2 ± 2.5	4 ± 2.2	+
UP30 (Site 2)	Hartbeespoort, ZA	-	-	+	+	+	nd	nd	12.2 ± 3.6	13.4 ± 2.8	13 ± 3.8	+
UP31 (Site 3)	Hartbeespoort, ZA	-	-	+	+	+	nd	nd	13.5 ± 4.2	11.2 ± 3.6	9 ± 3.3	+
UP38 (Site 4)	Hartbeespoort, ZA	-	-	+	+	+	nd	nd	10.1 ± 2.6	8.5 ± 3.2	7.9 ± 1.1	+

+, Positive/product; -, negative/no product; nd, not detectable; *, not determined – cultured strain of the 4 sampling sites (n = 12).

intensity up to an intensity of 40 µmol of photons $m^{-2}s^{-1}$ and decreased at higher light intensity. These observations by Utkilen and Gjolme (1992) is in agreement with our data and may explain why the lowest average total microcystin levels (13.1 µg/l) was observed at site 4 where a light intensity of 61 µmol of photons $m^{-2}s^{-1}$ was measured at a depth of 1 m. Through conducting PCR and quantitative 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 reverse transcriptase (RT)-PCR analyses showed low levels of transcription of the *mcyA*, *mcyB* and *mcyD* genes under very low light intensity (<0.93 µmol of photon m^{-2} s^{-1}) at site 1, while much higher levels of the *mcyB* and *mcyD* genes were expressed at site 2 and 3 with light intensities of 19 and 21 µmol of photons $m^{-2}s^{-1}$ at a depth of 1 m. The low levels of expression of the *mcyB* and *mcyD* 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 (Table 1). A possible explanation for the low level of transcription of the *mcyA* gene at sampling site 1, and its apparent absence at the other sites may also be related to the role of different environmental stresses within the hyperscum underneath the crust or that the *M. aeruginosa* strains is genetically distinct and has an *mcyA* gene sequence that was not amplified by the primers used.

Previous surveys on environmental strains of *M. aeruginosa* in South African reservoirs demonstrated that the absence of the *mcyA* is not uncommon, whereas *mcyB* and *mcyD* was always associated with toxic *M. aeruginosa* strains (Botha-Oberholster and Oberholster, 2007). In laboratory studies conducted by Kaebernick et al. (2000, 2002), they reported an increase in transcription of the *mcyB* and *mcyD* genes under high light conditions that may indicate higher toxin production. Hence, their study also indicated that the microcystin synthetase gene cluster (*mcyABCDEFGHIJ*) consisted of two polycistronic transcripts, *mcyABC* and *mcyDEFGHIJ*, which are transcribed from a central promoter between *mcyA* and *mcyD* and that both polycistronic transcripts have alternate transcription starting sites which are possibly light dependent. From the results of this study, it seems that the transcription of the *mcyA*, *mcyB* and *mcyD* 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, that is, aphotic conditions in hyperscum and dormant colonies in surface sediment of lakes (during overwintering periods) 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 measured during the end of February and the beginning of March 2005 at sampling site 1, underneath the hyperscum crust may be: (i) Due to the collapse of great amounts of *Microcystis* colonies (high cell densities of 1.1 x 10^{9} µg/l) 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., 1999); (ii) 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 isomerisation to non-toxic forms (Tsuji et al., 1995); (iii) 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 *M. aeruginosa* cells under dark and aerobic conditions. In their study, a decrease to 58% in microcystin-YR at the end of the experiment (45th day) was measured, while 86% of microcystin- LR remained.

In the present study, it is suggested that the development of the compact layer of *Microcystis* colonies down to a depth of 5 m under the crust at the wind protected dam wall, which 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 these 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 and Mur, 1984) and in natural populations (Visser et al., 1996; Oberholster et al., 2006b).

In this study, the satellite imagery of Lake Hartbeespoort gives 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. However, the absence of hyperscum crust formation at sites 2 and 3 was possibly related to wind and surface water current movement, which prevented exposure of the surface layer of the hyperscum to long enough periods of UV light, as in the chase of site 1 (Figure 2).

In a previous study by Rinta-kanto (2005), they successfully applied a combination of ground level observations, LANSAT satellite images, toxin data and qualitative and quantitative PCR in the western basin of Lake Erie to evaluate the composition of cyanobacterial blooms within the lake. However, the tools used in our study provided novel insights into the development and composition of hyperscum crust in eutrophic and hypertrophic lakes. Furthermore, from observations in our study, we suggest that areas where hyperscum crust occurred and are detected through employing satellite imagery, may be the areas with the highest concentrations of cyanobacterial toxins and that light intensity had a pronounced effect on both toxicity and toxin production rate under natural environmental conditions in these areas. Therefore, water quality predictions like these are important for water quality management, especially if it happens on a sufficient timely basis, since large amounts of the water from Lakes are used directly or indirectly for human consumption. While lack of regulatory requirements for toxin monitoring limit most studies to research endeavors, increasing interest in toxin production and distribution makes such research valuable to future management efforts. In the case of Lake Hartbeespoort, the yearly total outflow for irrigation and municipal drinking water extracted near the reservoir wall amounts to 82 x 10^6 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 threat 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 et al. (2002) stated that irrigation water contaminated by cyanotoxins may lyse the cells and aerosolize the toxins which can then be inhaled by farm workers.

Conclusion

Results from the present study suggest that satellite imagery can be very useful during routine monitoring to detect the formation of hyperscum crusts (exceeding 10 m in diameter) that poses health risk. Although it is a known fact that cyanobacterial bloom formation can change within hours, days and weeks, hyperscum crusts on the other hand forms over a longer period of time, and prevails for several weeks making it easier to detect with remote sensing and preventing health risk.

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