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# Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa

P.J. Oberholster [a,*], J.G. Myburgh [b], P.J. Ashton [a], A.-M. Botha [c]

[a] CSIR Natural Resources and the Environment, P.O. Box 395, Pretoria 0001, South Africa
[b] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, P/Bag X04, Onderstepoort 0110, South Africa
[c] Department of Genetics, University of Pretoria, Hillcrest, Pretoria ZA0002, South Africa

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ABSTRACT

The relationships between water quality and the phytoplankton community within Lake Loskop were studied during the late summer and autumn of 2008 to evaluate the impacts of acid mine drainage and high nutrient concentrations. The higher concentrations of metal ions and sulphate had adverse effects on certain phytoplankton species in the inflowing riverine zone of Lake Loskop, in comparison to the reference site in the lacustrine zone of the lake, which was dominated by the larger and slower growing late summer species of *Coelastrum reticulum* Nägeli, *Straurastrum anatinum* Meyen *ex* Ralfs and *Ceratium hirundinella* Müller. The high nutrient concentrations (nitrogen: 17 mg $l^{-1}$ and orthophosphate: 0.7 mg $l^{-1}$) during the mid-summer peak of the rainy season were associated with the development of a bloom of the cyanobacterium *Microcystis*. Water quality data associated with the development of the *Microcystis* bloom suggest that the aquatic system of Lake Loskop has now entered an alternate, hypertrophic regime. This change overshadowed the adverse effects of high concentrations of heavy metal ions and low pH. Throughout this study, the reference site in the lacustrine zone of Lake Loskop had lower concentrations of metal ions and sulphate, and higher pH values. The response of phytoplankton bioassays on integrated water samples from the different sampling sites did provide potential answers to the reasons for the absence of the algal group Chlorophyceae in the phytoplankton community structure in the riverine zone of the lake.





## 1. Introduction

South Africa's fresh water resources have been almost completely allocated for existing uses and the quality of most water resources is declining as a result of pollution caused by industry, urbanisation, acid mine drainage, inflows of treated and untreated sewage, deforestation, and agricultural return flows and energy use (Lusher and Ramsden, 1992; DWAF, 2004). The situation is aggravated by the fact that South Africa has one of the lowest conversions of precipitation to runoff and groundwater recharge (<9%) in the world (O'Keeffe et al., 1992). The Olifants River is one of the main river systems in South Africa, and has been described as one of the most polluted rivers in southern Africa, with Lake Loskop acting as a repository for pollutants from the upper catchment of the Olifants River system (Grobler et al., 1994). In the upper Olifants River catchment, acid mine drainage, sewage pollution and extensive agriculture are the mean sources of anthropogenic stressors on the aquatic environment (Driescher, 2008). Over time, these activities have had progressively greater detrimental effects on water quality, as well as a notable adverse impact on the aquatic environment of Lake Loskop (DWAF, 2004; Driescher, 2008).

Over the past 15 years isolated incidents of fish mortality have been recorded at different times in Lake Loskop. These incidents have become more frequent during the past 5 years (2003–2008) (Driescher, 2008) and have coincided with Nile crocodile (*Crocodylus niloticus*) mortalities; the crocodile population in Lake Loskop has declined from approximately 30 animals to a total of 6 in 2008 (Paton, 2008). Crocodile mortality in Lake Loskop during this period of time was ascribed to pansteatitis, which is associated with the intake of rancid fish fat after a fish die-off, which appears to have resulted from sporadic incidents of acid mine drainage flowing into the lake (Paton, 2008).

Apart from a few scanty details reported in an earlier study on the bioaccumulation of metals in different fish species in Lake Loskop (Kotze et al., 1999), little is known about the current phytoplankton community of this impoundment. Interestingly, an earlier study on the limnology of Lake Loskop (Walmsley and Butty, 1980) reported frequent population peaks of the cyanobacterium *Microcystis aeruginosa*. This cyanobacterium species is known to disrupt the normal patterns of phytoplankton succes-

* Corresponding author. Fax: +27 124202954.
*E-mail address:* poberholster@csir.co.za (P.J. Oberholster).

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sion, decrease phytoplankton diversity, and alter virtually all of the interactions between organisms within the aquatic community (Figueredo and Giani, 2001; Oberholster et al., 2009a). One of the most serious effects of a *M. aeruginosa* bloom is the production of harmful secondary metabolites that can have a serious adverse effect on the health and of humans and animals (Oberholster et al., 2009b).

Previous studies on acid mine drainage (DeNicola, 2000; Niyogi et al., 2002) reported that the presence of *Nitella* sp. and *Euglena mutabilis* acted as indicators of acid mine drainage. Therefore, a study of the phytoplankton community of Lake Loskop may reveal insights into the water quality of the lake and the possible impluence of inflows containing toxic metal ions and untreated sewage. Importantly, chemical compounds that accumulate in the phytoplankton can enter the algae–herbivore fish food chain and, through biomagnification, could pose a serious health threat to animals and humans (Zhou et al., 2008).

From an ecotoxicological perspective, it is difficult to use a single phytoplankton test species under regulated laboratory conditions to assess the levels of ecosystem toxicity posed by a mixture of different chemical compounds—as in the case of Lake Loskop. This is because the species-specific responses of controlled test species to individual toxins cannot closely mimic those of the diverse phytoplankton species that characterize natural plankton communities living under highly variable conditions in a natural or man-made aquatic ecosystem (Chapman, 1995).

However, the central assumption of a bioassay is that pollution acts first at the biochemical level, then at physiological and perhaps morphological levels, and finally at the population or community level (Ward et al., 1995). While it is accepted that the ecological consequences of water quality degradation are best examined at higher levels of biological organisation (e.g., at phytoplankton community level), the ideal is to identify links between the latter and the outcome of changes at the lowest biological level (e.g., a phytoplankton bioassay), which can then be translated into likely impacts on the aquatic system, as measured at phytoplankton community level in the field (Bunn, 1995). Therefore, the responses of phytoplankton observed in a bioassay may provide us with potential answers to the reasons for subsequent changes in phytoplankton community structure in the field. The objectives of this study were to: (1) link data generated from phytoplankton bioassays to the taxonomic diversity and distribution of the phytoplankton assemblage in time and space, as well as with the physical and chemical features of Lake Loskop, and (2) identify the most likely reason for the sudden emergence of a *Microcystis* bloom in the riverine zone of Lake Loskop.

## 2. Materials and methods

### 2.1. Study area

Lake Loskop (25° 26′ 57. 05″ S; 29° 19′ 44. 36 E) is situated in the Mpumalanga Province of South Africa and its main inflow is the Olifants River (Fig. 1), which flows through a confined gorge before entering the main basin of Lake Loskop. Construction of the dam wall that eventually impounded Lake Loskop started in the mid-1930s and was completed in the early 1940s. The impoundment has a surface area of 24.27 $km^2$ and a volume of 374.3 $Mm^3$ at full supply capacity, and was designed primarily to supply water for irrigated agriculture downstream of the dam wall (DWAF, 2004). The total catchment area that drains into Lake Loskop is 11,464 $km^2$, with a mean annual precipitation of 683 mm and a mean annual runoff of 10,780 $mm^3$ (Midgley et al., 1994). Land use in the catchment is dominated by extensive coal mining in the Witbank Coalfields, which are located in the headwaters of the Olifants River, upstream of Lake Loskop, as well as mineral processing and dryland plus irrigated agricultural activities (Basson et al., 1997).

South Africa possesses approximately 5% of the global recoverable coal reserves and is the world's sixth largest coal producer (220 Mt coal per year) (DME, 2004). The Witbank coal fields represent the largest conterminous area of active coal mining in South Africa and mine de-watering activities result in the discharge of approximately 50 Ml/d of mine water into the upper Olifants catchment (Maree et al., 2004). Other sources of pollution in the upper Olifants River and its major tributary the Wilge River are acid mine drainage emanating from a number of abandoned coal mines and the discharge of untreated and partially treated domestic and industrial sewage from municipal sewage treatment works located upstream of Lake Loskop.

Lake Loskop is a mainstream reservoir which exhibits pronounced longitudinal zonation, which is largely absent from the typically shallow and windswept storage reservoirs that do not occupy a well-defined river valley (Kalff, 2001). For the purpose of this study, Lake Loskop was divided into three zones: the inflowing riverine zone (R: sampling sites 1 and 2, representing high flow and rapid water flushing rates); the transitional zone (T: sampling site 3, representing reduced flow and flushing rates) and the lacustrine zone (L: sampling sites 4 and 5, representing slowest flows and water flushing rates). The presence of a large group of resident hippopotamus (*Hippopotamus amphibious*) in the transitional zone meant that only one suitable sampling site could be accessed safely in this zone (Fig. 1). Sampling site 5 in the main lake basin was used as the reference site in this study. The study was undertaken during the latter part of the summer season (when rainfalls are usually highest) through to early winter, from January to June 2008. Although low pH values and high metal concentrations are restrictive to most aquatic life forms, the study period was characterized by the development of a *Microcystis* spp. bloom. The nutrient content of the water in Lake Loskop throughout the study can be considered as high when compared with a sewage-polluted system such as Lake Hartbeespoort, which was reported to have a maximum total nitrogen concentration of 13.5 $mg\,l^{-1}$ and total phosphorus of 0.4 $mg\,l^{-1}$ (Owuor et al., 2007). Although certain saline lakes are amongst the most productive lakes globally, Lake Hartbeespoort in South Africa appears to be the most productive lake on record (Kalff, 2001).

### 2.2. Selenastrum capricornutum bioassay

In this study, we used a 96-h growth standard freshwater algal toxicity test with the unicellular green alga *S. capricornutum* (syn. *Raphidocelis subcapitata*). It has been reported that photosynthetic activity is a less sensitive indicator of toxic effects than population growth measurements (Versteeg, 1990; Pardos et al., 1998); therefore, we used cell growth as the endpoint for the 96-h bioassay and the chlorophylls *a* and *b* concentrations as indicators of sub-lethal endpoints. Both of these tests were conducted with 5 replicate flasks for each treatment, plus triplicate controls. *S. capricornutum* (strain ATCC 22662, Canada) was cultured in

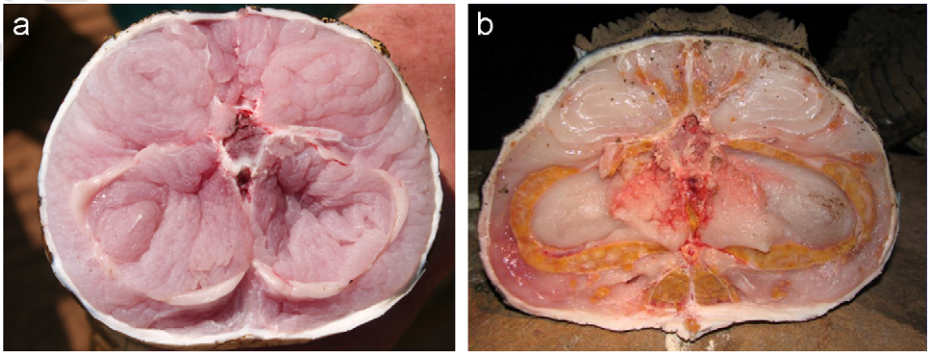


**Fig. 1.** Cross-section of crocodile tails from: (A) a healthy crocodile, and (B) a crocodile with pansteatitis. Note the yellowish appearance of the fat layers.

standard AAP media under axenic conditions in 250 ml glass Erlenmeyer flasks (USEPA, 1989). The AAP meduim had the following chemical composition, per litre: 25 mg $CaCl_2.2H_2O$; 0.78 μg $CoCl_2.6H_2O$; 0.009 μg $CuCL_2.2H_2O$; 12.16 mg $MgCl_2.6H_2O$; 96 μg $FeCL_3.6H_2O$; 185.64 μg $H_3BO_3$; 175 mg $K_2HPO_4$; 75 mg $MgSO_4$; 264.27 μg $MnCL_2.4H_2O$; 7.26 μg $Na_2MoO_4.2H_2O$; 15 mg $NaHCO_3$; 250 mg $NaNO_3$; 32.7 μg $ZnCL_2$; and 333 μg $Na_2EDTA.2H_2O$. Culture flasks were shaken continuously at 100 rpm and incubated at 24±2 °C under constant light (255 μmol photon $m^{-2}$ $s^{-1}$) at a pH = 7.5±0.1. Algal inocula were prepared for each lake water sample from fresh culture stocks sampled during the exponential growth phase (Fig. 2). The algal cells were concentrated by gentle centrifugation and the inoculum density was adjusted to 1 × $10^5$ cells $ml^{-1}$ (Ross et al., 1988). Each flask was inoculated with 1 ml of stock algal culture that was resuspended in an appropriate volume (100 ml) per flask of nutrient-spiked (USEPA, 1989) filtered lake water (filtered through a 0.45 μm mesh Whatman GF filter). Quintuplicate replicate tests (i.e., five replicates of each sample) were conducted on samples of water that had been collected from each of the 5 sampling sites during each of the 6 field trips, plus the controls. The following water quality parameters: pH, alkalinity, hardness and temperature, were measured at the start and end of each bioassay test. Cell growth inhibition or stimulation as endpoints were quantified (as cells $ml^{-1}$) after 96-h exposure using an electronic cell counter (Coulter® Z series).

### 2.3. *Chlorophylls a and b concentrations as sub-lethal indicator of physiological changes*

Every 24 h, for a period of 96 h, one-millilitre sub-samples of water containing *S. capricornutum* cells were removed from each of the five replicate flasks prepared for each of the 5 sampling sites plus the control flasks for analyses of their chlorophyll (chl) *a* and *b* content. Chlorophyll was extracted with 80% acetone at 4 °C. The chl *a* and *b* content of each sample was determined spectrophotometrically at 647 and 664 nm wavelengths according to the method of Porra et al. (1989).

### 2.4. *Phytoplankton sampling*

Water samples for analysis of the phytoplankton population structure were collected from the water column at each of the 5 sampling sites during the 6 monthly sampling trips from January to June 2008. A random sampling procedure was used to reduce hydrobiological variability between sites. At each site, duplicate water samples were collected at the lake surface and at 0.5 m intervals down to a depth of 2 m using a 6-l capacity Von Dorn sampler. At each site, the pairs of water samples from each depth were separated and each set of five samples was then pooled to form two integrated samples for the site. One of these samples was preserved in the field by addition of acidic Lugol's solution to a final concentration of 0.7%, followed after 1 h by the addition of buffered formaldehyde to a final concentration of 2.5%. The integrated water samples were kept cool and in the dark during the 3-h period of transfer from the field to the laboratory.

All algal identifications were made with a compound microscope at 1250× magnification (Van Vuuren et al., 2006; Taylor et al., 2007). Strip counts were made until at least 100 individuals of each of the dominant phytoplankton species had been counted. All counts were based on the numbers of cells observed and the individual species were grouped into major algal groups (Lund et al., 1958; Willen, 1991). The total number of phytoplankton taxa was recorded after careful examination for at least 15 min and after not finding additional taxa. Algal biovolume was calculated by measuring the corresponding dimensions using the geometric formulae given by Willen (1976). The relative abundance of phytoplankton taxa at each sampling site was categorised according to Hörnström (1999): 1 = ≤250, 2 = 251–1000, 3 = 1001–5000, 4 = 5001–25,000 cells $l^{-1}$. Equilibrial phytoplankton species (*sensu* Naselli-Flores et al., 2003) in the main lake basin (lacustrine zone, sampling sites 4 and 5) were determined over the study period of 6 months, using the following criteria: (1) 1, 2 or 3 species of phytoplankton contributed more than 80% of the total biomass or biovolume, (2) their existence or coexistence persisted for more than 1–2 weeks, and (3) during this period, the total phytoplankton biomass did not increase significantly.

### 2.5. *ELISA assays*

Microcystin concentrations within the cyanobacterial bloom were determined according to the methods described in Boyer et al. (2004). In summary, water samples from sampling site 2 where the cyanobacterial bloom occurred during March 2008 were poured gently through a 934 AH glass fibre filter in the field, after which the filters were stored on dry ice and returned to the laboratory for toxin analysis. Filters for toxin analysis were extracted by grinding with 10 ml of 50% methanol containing 1% acetic acid and clarified by centrifugation. This extract was used for the analysis of total microcystin levels using the ELISA assay. The ELISA assay was conducted with a Quanti$^{tv}$ Kit for Microsystems (EnviroLogix, USA).

### 2.6. *Physical and chemical parameters*

On each of the six field visits, dissolved oxygen, water temperature, pH and electrical conductivity values were measured at the water surface and at 0.25 m

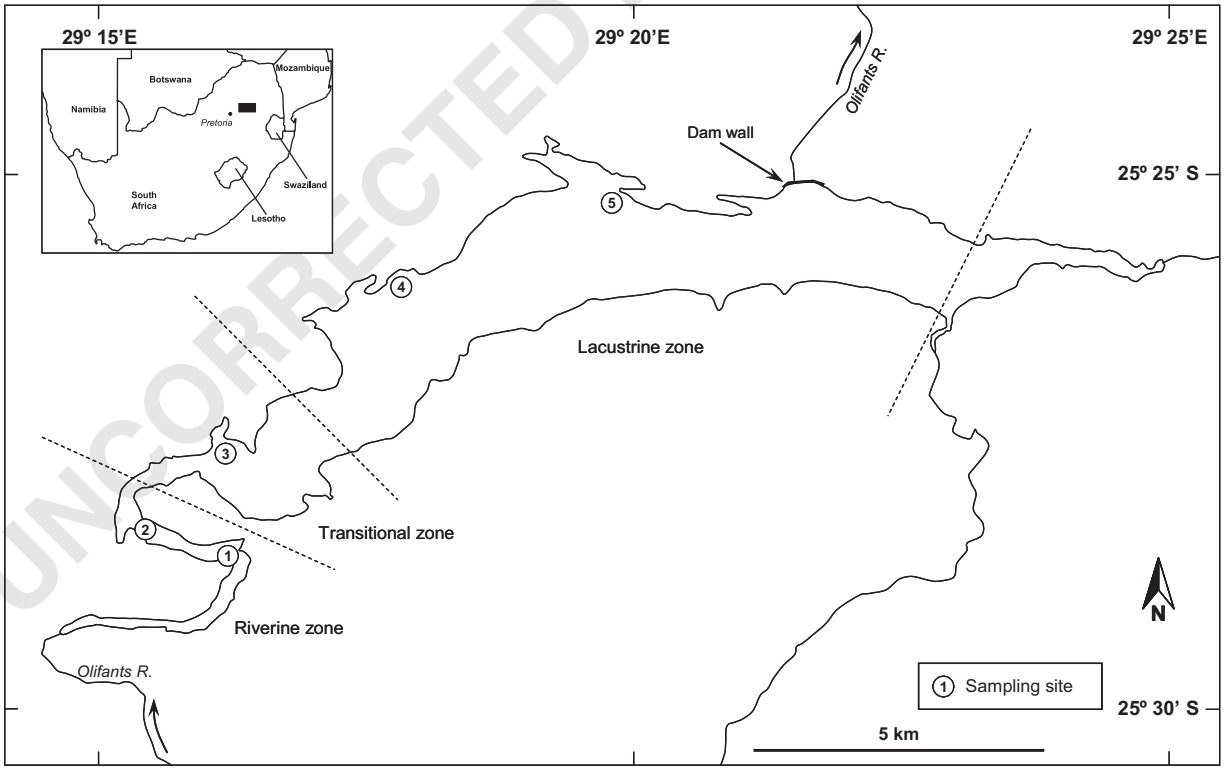


**Fig. 2.** Sketch map of Lake Loskop showing the positions of the five sampling sites in relation to the different zones of the lake. Inset shows the location of the map area within South Africa.



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intervals down to a depth of 2 m at each of the 5 sampling sites, using a Hach sension™ 156 portable multiparameter (Loveland, USA). The data for each sampling site were then pooled to give an average value for the upper portion of the surface water at each sampling site, on each sampling date. Samples of the lake water from the 5 sampling sites were filtered through 0.45 μm pore size Whatman GF/filters and stored in polyethylene bottles that had been pre-rinsed with dilute sulphuric acid (to pH 2.0) for analysis of dissolved nutrients. All analyses were carried out according to standard methods (USEPA, 1983; APHA, AWWA and WPCF, 1992) in the CSIR's accredited analytical laboratory. Concentrations of total nitrogen (TN) and total phosphorus (TP) were determined with the persulphate digestion technique. Nitrate concentrations were determined on an autoanalyzer with the cadmium reduction method, while soluble reactive phosphorus concentrations were determined by the ascorbic acid method (APHA, AWWA and WPCF, 1992). Sulphate concentrations were analysed turbidimetrically, while alkalinity concentrations were analysed by titrimetry (USEPA, 1983). Total heavy metal concentrations within the water samples were determined with inductively coupled plasma (ICP). We only analysed aluminium (Al); copper (Cu); iron (Fe) and zinc (Zn) since an earlier study by Driescher (2008) indicated that the concentrations of these metals was problematic in the upper catchment of Lake Loskop and that other metal ions were only present at low concentrations ($\leq$0.1 mg l$^{-1}$). Water transparency was measured with a 25 cm Secchi disc with black and white quadrants. The summer near-surface average nutrient concentration index (Vollenweider, 1968; Forsberg and Ryding, 1980) was used to classify the trophic state of Lake Loskop for the period January–June 2008.

### 2.7. Data analyses

A two-way ANOVA was performed to test whether there exist a significant relationship between the mean maximum phytoplankton abundance at the different sampling sites and nutrient and metal concentrations at these sites. Subsequent multiple comparisons were done with the Tukey posthoc test at $P<0.05$ to compare different mean phytoplankton species abundance between sampling sites. All statistical calculations were done with the statistical package SPSS (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Physical and chemical parameters

The slightly acidic pH values (5.9 and 6.1) of the water in the riverine zone of Lake Loskop differed significantly from the alkaline values recorded in the transitional (8.1 and 8.2) and lacustrine zones (8.6) of the lake during January 2008. However, pH values at all the sites changed gradually up to the end of April, when the average pH in the riverine zone was 7.1 while the average pH in the lacustrine zone was 9.1. Total nitrogen concentrations (3.2 and 2.67 mg l$^{-1}$) in January at sampling sites 1 and 2, respectively, were much higher than the concentrations (1.98; 1.96 and 1.78 mg l$^{-1}$) at sites 3, 4 and 5, respectively (Table 1). However, total nitrogen concentrations rapidly increased to an average of 16.5 mg l$^{-1}$ at sites 1 and 2 in February and March 2008, when the ratio of total phosphate to total nitrogen was 1:12 indicating that the area within the riverine zone was becoming hypertrophic. The aluminium (Al), sulphate ($SO_4$) and iron (Fe) concentrations remained high at sampling sites 1 and 2 throughout the study, with the highest concentrations recorded during February and April 2008 at sampling sites 1 and 2 (Al = 1.56 mg l$^{-1}$; $SO_4$ = 440 mg l$^{-1}$ and Fe = 1.2 mg l$^{-1}$) (Table 1). The maximum concentrations of Al and Fe detected at sites 1 and 2 were higher than allowed by the South African Water Quality Guideline values for Al ($\leq$5 μg l$^{-1}$) and Fe ($<$100 μg l$^{-1}$) for domestic water consumption (DWAF, 1996).

Surface water electrical conductivity values varied between 297 and 492 μS cm$^{-1}$ throughout the study ($n=6$) at all 5 sampling sites. The average surface water temperature measured during the 6 sampling trips was 22.5 °C, while Secchi disc measurements fluctuated between 1.63 m at sampling site 1, –0.82 m at sampling site 5 (main lake basin). In our study, $SO_4$ was the dominant anion in Lake Loskop with concentrations ranging between 111 and 481 mg l$^{-1}$, while Al was the dominant metal cation in the riverine zone ranging from 0.09 to 1.6 mg l$^{-1}$ (Table 1). The average nutrient concentration index (Vollenweider, 1968; Forsberg and Ryding, 1980) revealed that the lake was mesotrophic in January 2008 with a TN:TP ratio >25. However, from February to June 2008, the trophic state of the lake changed to hypertrophic with a TN:TP ratio (<12). A significant relationship ($r^2=0.98805$, $p\leq0.001$) between the high cyanobacterial cell abundance ($2.2\times10^7$ cells ml$^{-1}$) and total phosphorus concentration (0.7 mg l$^{-1}$) were observed during March 2008 at sampling site 2 (Table 1). The average DO were 5.5 mg l$^{-1}$ at all sampling sites throughout the study except for site 2 where DO was 0.38 mg l$^{-1}$ due to the cyanobacterial bloom during February and March.

### 3.2. Phytoplankton community structure and cyanobacterial toxicity

A succession of phytoplankton species was recorded for the main basin of Lake Loskop from January to February 2008, when the dominant community of the small and rapidly reproducing species *Trachelomonas intermedia* Ehrenberg was replaced by the larger, slower growing late summer species *Coelastrum reticulum* Nägeli, *Staurastrum anatinum* Meyen *ex* Ralfs and *Ceratium hirundinella* Müller. Low numbers of diatoms ($\leq$250 cells l$^{-1}$) were observed throughout the study at all 5 sampling sites, with *Eunotia flexuosa* Ehrenberg as the dominant diatom species (250–1000 cells l$^{-1}$) in the riverine zone. *Eunotia formica* Ehrenberg and *Eunotia bilunaris* Ehrenberg also occurred in the riverine zone, though these species were present in much lower numbers (125–210 cells l$^{-1}$), while *Fragilaria ulna* Lynbye (178–195 cells l$^{-1}$) was the dominant diatom in the lacustrine zone (Table 2). There exist a significant relationship ($r^2=0.9756$, $p\leq0.001$) between the higher numbers (250–1000 cells l$^{-1}$) of the diatom *E. flexuosa* in the riverine zone (site 1) and the slightly acidic surface water pH (5.9) in January. This diatom species is reported to occur most frequently in acidic habitats and appears to act as an indicator of low pH (Taylor et al., 2007). The low average biovolume (2.1 mm$^3$ l$^{-1}$) of the different species of diatoms showed a relationship ($r^2=0.5747$, $p\leq0.05$) with the average silica concentration (209 mg l$^{-1}$) during the 6-month study period (Table 2). The abundance of the phytoplankton species *T. intermedia* at sampling sites 1, 2 and 3 decreased when compared with the reference site (*t*-test, $p\leq0.05$).

The dominant cyanobacterial species present during January at sites 1 and 2 (i.e., *Merismopedia tenuissima* Meyen, 1001–5000 cells l$^{-1}$), had a significant relationship ($r^2=0.9875$, $p\leq0.001$) with the low average pH of 6.0 at these sites. During February, a mixed bloom of *M. aeruginosa* Kützing *ex* Lemmermann and *Microcystis flos-aquae* ($2.3\times10^5$ cells ml$^{-1}$; 6.5 mm$^3$ l$^{-1}$ of total biovolume) occurred at site 2 in the shallow riverine zone, and floating surface scums of this bloom drifted to the nearby bays. Eventually, this bloom declined to very low densities ($1.2\times10^2$ cells ml$^{-1}$) in the lacustrine zone of Lake Loskop. A maximum cyanobacterial cell count of $2.2\times10^7$ cells ml$^{-1}$ (18 mm$^3$ l$^{-1}$ of total biovolume) was observed in late March when the chlorophyll *a* concentration increased to 196 μg l$^{-1}$ at site 2.

The *Microcystis* bloom at site 2 disappeared gradually between January and April, but *Microcystis* cells remained present from April until June at a moderately low density of $4.1\times10^3$ cells ml$^{-1}$ in the riverine zone. *M. aeruginosa* was the dominant (69% of total cyanobacterial biovolume) species present in the mixed cyanobacterial bloom in the riverine zone during February and March. However, during the same period, the drifting cyanobacterial scum at site 2 that had floated into the transitional zone (site 3) and the lacustrine zone (sites 4 and 5) was dominated by *M. flos-aquae*, where this species comprised an average of 54% of the total

**Table 1**
Physical and chemical water quality characteristics prior to and during the different monthly stages (February–March = bloom development and peak; April–June = degradation of bloom) of the cyanobacterial bloom in Lake Loskop (±) = standard deviation.

| Parameter | Units | Site 1—Riverine zone | | | | Site 2—Riverine zone | | | | Site 3—Transitional zone | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | January | February and March | April and May | June | January | February and March | April and May | June | January | February and March | April and May | June |
| Total alkalinity ($CaCO_3$) | $mg\,l^{-1}$ | 49(2) | 55 (4) | 56(2) | 66(7) | 51(3) | 58(1) | 52(4) | 69(2) | 64(6) | 68(3) | 42(5) | 76(2) |
| Total nitrogen (N) | $\mu g\,l^{-1}$ | 3200 (120) | 17000 (57) | 8180 (211) | 4177 (41) | 2670 (211) | 15980 (90) | 8110 (141) | 4161 (98) | 1980 (32) | 12321 (110) | 6500 (71) | 3510 (42) |
| Total phosphate (P) | $\mu g\,l^{-1}$ | 183 (21) | 711 (51) | 469 (11) | 377 (54) | 157 (10) | 700 (61) | 411 (13) | 364 (11) | 134 (5) | 457 (13) | 321 (37) | 331 (9) |
| Aluminium (Al) | $\mu g\,l^{-1}$ | 98 (18 ) | 1610 (51) | 79 (32) | 72 (20) | 91 (33) | 1511 (72) | 71 (24) | 76 (11) | 76 (21) | 191 (12) | 66 (8) | 61 (3) |
| Copper (Cu) | $\mu g\,l^{-1}$ | 22 (2) | 105 (11) | 21 (9) | 20 (13) | 12 (4) | 92 (17) | 18 (1) | 19 (12) | 12 (7) | 26 (13) | 18 (3) | 19 (1) |
| Iron (Fe) | $\mu g\,l^{-1}$ | 75 (15) | 1220 (39) | 285 (11) | 295 (31) | 76 (2) | 1213 (29) | 216 (21) | 221 (14) | 65 (7) | 175 (11) | 112 (12) | 111 (9) |
| Zinc (Zn) | $\mu g\,l^{-1}$ | 17 (2) | 23 (7) | 19 (2) | 20 (6) | 14 (1) | 19 (6) | 19 (13) | 18 (3) | 13 (7) | 12 (2) | 11 (3) | 10 (1) |
| Sulphate ($SO_4$) | $mg\,l^{-1}$ | 397 (45) | 481 (39) | 291 (21) | 290 (11) | 391 (9) | 398 (21) | 271 (31) | 262 (10) | 283 (27) | 167 (14) | 113 (9) | 101 (16) |
| Silica (Si) | $mg\,l^{-1}$ | 3.4 (0.3) | 2.1 (0.1) | 2.4 (1) | 2.3 (0.5) | 2.4 (0.8) | 2.1 (0.1) | 2.5 (0.5) | 2.7 (0.9) | 2.1 (0.8) | 2.0 (1) | 1.9 (0.4) | 2.01 (0.3) |
| pH | – | 5.9 (0.5) | 6.1 (0.7) | 6.2 (0.2) | 6.8 (0.3) | 6.1 (0.1) | 6.3 (0.5) | 7.1 (0.3) | 7.3 (0.6) | 8.2 (0.9) | 8.9 (0.2) | 8.2 (0.2) | 8.1 (0.5) |
| Conductivity | $\mu S\,cm^{-1}$ | 464 (12) | 492 (23) | 364 (11) | 369 (17) | 338 (5) | 490 (21) | 338 (12) | 345 (18 ) | 322 (9) | 333 (21) | 322 (8) | 312 (28) |
| Dissolved oxygen | $mg\,l^{-1}$ | 6.64 (0.9) | 6.64 (2) | 5.61 (0.3) | 6.11 (0.6) | 5.61 (0.1) | 0.38 (0.9) | 3.18 (0.6) | 5.19 (0.5) | 5.12 (0.7) | 5.32 (1) | 5.29 (0.8) | 6.21 (0.4) |
| Temperature | °C | 25 (1) | 24 (0.5) | 21 (1) | 15 (2) | 25 (0.6) | 25 (0.3) | 23 (1) | 18 (0.5) | 25 (0.9) | 25 (0.3) | 22 (1) | 17 (0.8) |

| Parameter | Units | Site 4—Lacustrine zone | | | | Site 5—Lacustrine zone | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | January | February and March | April and May | June | January | February and March | April and May | June |
| Total alkalinity ($CaCO_3$) | $mg\,l^{-1}$ | 75(2) | 79(7) | 41(8) | 77(3) | 71(2) | 77(3) | 41(5) | 76 |
| Total nitrogen (N) | $\mu g\,l^{-1}$ | 1961 (21) | 9641 (43) | 3951 (86) | 3421 (101) | 1777 (123) | 9754 (76) | 3210 (44) | 3449 |
| Total phosphate (P) | $\mu g\,l^{-1}$ | 132 (23) | 359 (45) | 322 (17) | 342 (27) | 129 (2) | 340 (19) | 311 (11) | 329 |
| Aluminium (Al) | $\mu g\,l^{-1}$ | 78 (14) | 76 (21) | 57 (9) | 59 (17) | 71 (6) | 73 (8) | 51 (13) | 50 |
| Copper (Cu) | $\mu g\,l^{-1}$ | 10 (2) | 21 (8) | 12 (1) | 10 (5) | 9 (3) | 22 (6) | 13 (4) | 13 |
| Iron (Fe) | $\mu g\,l^{-1}$ | 44 (3) | 64 (13) | 98 (11) | 109 (24) | 41 (6) | 67 (9) | 99 (17) | 97 |
| Zinc (Zn) | $\mu g\,l^{-1}$ | 12 (7) | 10 (3) | 12 (4) | 11 (8) | 12 (5) | 12 (2) | 10 (4) | 12 |
| Sulphate ($SO_4$) | $mg\,l^{-1}$ | 180 (21) | 144 (27) | 108 (1) | 102 (12) | 181 (25) | 151 (18) | 111 (13) | 109 |
| Silica (Si) | $mg\,l^{-1}$ | 1.1 (1) | 2.1 (0.2) | 2.6 (0.4) | 1.9 (0.1) | 1.3 (0.6) | 1.9 (0.4) | 2.8(1) | 1.9 |
| pH | – | 8.1 (0.5) | 8.8 (0.4) | 8.5 (0.2) | 8.6 (0.7) | 8.6 (0.3) | 8.9 (1) | 8.4 (0.5) | 8.3 |
| Conductivity | $\mu S\,cm^{-1}$ | 297 (13) | 314 (29) | 297 (17) | 301 (8) | 299 (11) | 317 (15) | 299 (12) | 293 |
| Dissolved oxygen | $mg\,l^{-1}$ | 5.32 (0.3) | 4.97 (0.1) | 5.87 (0.9) | 6.77 (0.7) | 5.71 (0.2) | 5.24 (0.5) | 5.79 (1) | 6.12 |
| Temperature | °C | 26 (0.7) | 27 (1) | 23 (0.5) | 16 (0.1) | 26 (0.7) | 26 (0.5) | 23 (0.6) | 17 |

**Table 2**
Comparison of the phytoplankton species composition and relative abundance at the five sampling sites in Lake Loskop ($n = 6$ sampling dates).

| Algal group | Genus and species | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
|---|---|---|---|---|---|---|
| Bacillariophyceae | *Eunotia flexuosa* | 2 | 1 | 1 | 0 | 0 |
| | *Ctenophora pulchella* | 0 | 0 | 0 | 1 | 1 |
| | *Amphora ovalis* | 0 | 0 | 1 | 0 | 1 |
| | *Cymatopleura solea* | 1 | 0 | 0 | 0 | 0 |
| | *Fragilaria tenera* | 0 | 0 | 0 | 1 | 1 |
| | *Fragilaria ulna* | 0 | 0 | 1 | 2 | 1 |
| | *Gryrosigma rautenbachiae* | 0 | 0 | 1 | 0 | 0 |
| Euglenophyceae | *Trachelomonas intermedia* | 1 | 1 | 1 | 2 | 2 |
| Chlorophyceae | *Coelastrum reticulum* | 0 | 0 | 1 | 0 | 1 |
| | *Staurastrum anatinum* | 0 | 0 | 1 | 2 | 1 |
| Dinophyceae | *Ceratium hirundinella* | 0 | 0 | 4 | 4 | 4 |
| Chrysophyceae | *Dinobryon divergens* | 3 | 1 | 0 | 0 | 0 |
| Cyanophyta | *Microcystis aeruginosa* | 1 | 4 | 2 | 1 | 1 |
| | *Microcystis flos-aquae* | 0 | 3 | 2 | 2 | 2 |
| | *Merismopedia tenuissima* | 3 | 3 | 0 | 0 | 0 |

The numbers (1–4) represent the maximum relative abundance of the phytoplankton taxa at each site where 1 = ≤250, 2 = 251–1000, 3 = 1001–5000 and 4 = 5001–25,000 cells $l^{-1}$.

**Table 3**
Phytoplankton bioassay response as cell growth or inhibition on exposure to undiluted lake water from 5 sampling sites and the control[a].

| Sampling sites and different zones | *S. capricornutum* cells $ml^{-1}$ |
|---|---|
| 1. Riverine | $2.1 \times 10^4$ |
| 2. Riverine | $2.6 \times 10^4$ |
| 3. Transitional | $2.2 \times 10^6$ |
| 4. Lacustrine | $2.8 \times 10^6$ |
| 5. Lacustrine | $1.3 \times 10^6$ |
| Control | $4.3 \times 10^5$ |
| SD[b] | $1.17 \times 10^5$ |
| CV[c] | 104.015 |

Lake water was sampled from January to June ($n = 6$).

[a] Algal stimulation or inhibition after 96 h (cells $ml^{-1}$).
[b] Standard deviation.
[c] Coefficient of variation (CV) = (standard deviation/mean) × 100.

cyanobacterial biovolume. A relatively low average microcystin concentration of 8.2 μg $l^{-1}$ was recorded in February 2008, followed by a higher peak concentration of 46.7 μg $l^{-1}$ during March, when *Microcystis* spp. dominated ($1.2 \times 10^7$ cells $ml^{-1}$) the phytoplankton community at site 2. The green alga *S. anatinum*, which was absent from the riverine zone (sites 1 and 2) throughout the study, had a low total average biovolume of 1.1 $mm^3 l^{-1}$ in the transitional and lacustrine zones (Table 3). The Dinophycean *C. hirundinella* was the only species that dominated the phytoplankton assemblage in both the transitional zone (site 3) and lacustrine zone (sites 4 and 5) after January until June 2008. This species accounted for more than 80% of the biovolume of the phytoplankton community for this period, indicating that it was an "equilibrial species" (*sensu* Naselli-Flores et al., 2003).

### 3.3. Responses of *S. capricornutum* bioassay

Water samples collected in the riverine zone (sites 1 and 2) of Lake Loskop were found to be the most toxic in the 96-h phytoplankton bioassay (Table 3). Although the *S. capricornutum* algal bioassay indicated algal growth inhibition ($2.1 \times 10^4$ and $2.6 \times 10^4$ cells $l^{-1}$) compared with the control ($2.3 \times 10^6$ cells $l^{-1}$) after exposure to lake water from sites 1 and 2, growth stimulation ($5.2 \times 10^6$, $4.8 \times 10^6$ and $1.3 \times 10^7$ cells $l^{-1}$) was observed in water from sites 3, 4 and 5, respectively in Lake Loskop (Table 3). The average high concentrations of Al, Fe and $SO_4$ at sites 1 and 2 had a significant relationship ($r^2 = 0.9978$, $p \leq 0.05$) with cell growth inhibition ($2.1 \times 10^4$ and $2.6 \times 10^4$ cells $l^{-1}$) in the algal bioassay. Hence, the growth inhibition observed in the algal bioassay of *S. capricornutum* (a member of the Chlorophyceae), can be linked to the absence of this algal group in the phytoplankton community recorded at sites 1 and 2.

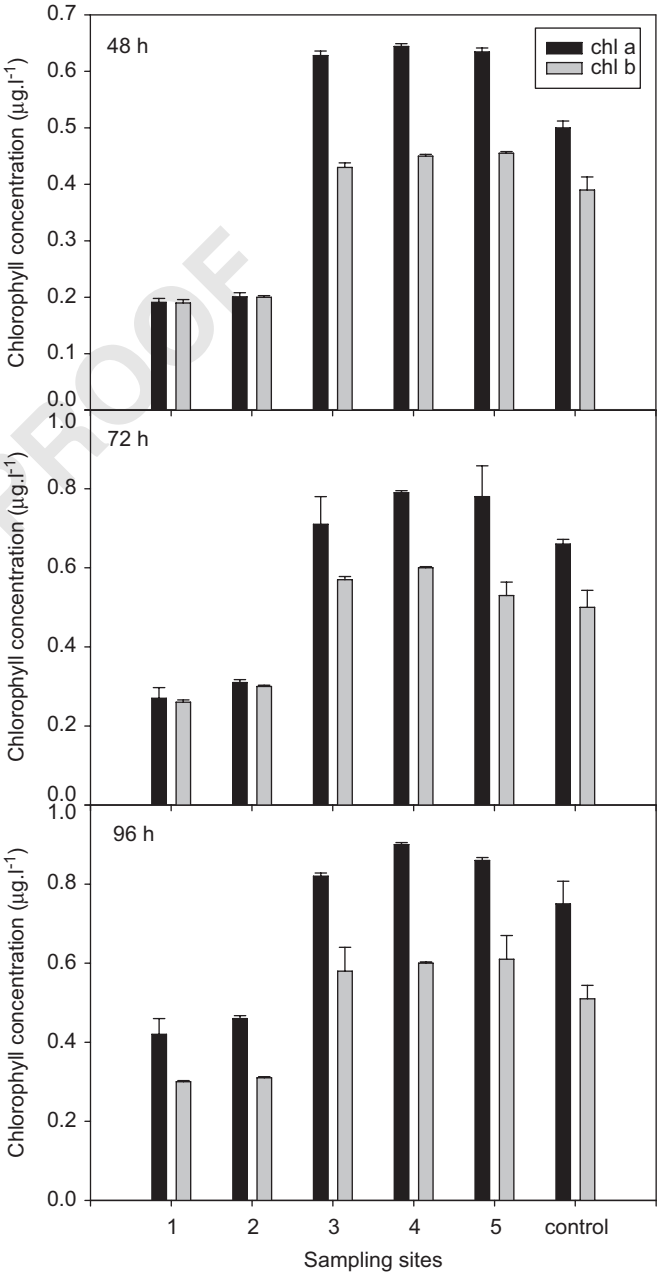


**Fig. 3.** Changes in chlorophyll (chl *a* and chl *b*) content over a 96 h exposure time period to a screen (100% concentration) of lake water from the 5 different sampling sites as well as the control.

### 3.4. Phytoplankton chlorophylls a and b concentrations

In this study there was a decrease in the chl *a* concentration, compared with chl *b* and the positive control in *Selenastrum* cells exposed to water from sites 1 and 2 (Fig. 3). Conversely, *Selenastrum* cells exposed to water from sites 4 and 5 showed an increase in chl *a*, concentrations, compared with chl *b* and the control (Fig. 3). A significant relationship ($r^2 = 0.9547$, $p \leq 0.05$) was noted between chl *b* and the higher concentrations of Al, Fe and $SO_4$ at sites 1 and 2, while a chl *a* concentrations was associated with the higher concentrations of total phosphorus and total nitrogen at sites 1 and 2 ($r^2 = 0.9756$, $p \leq 0.05$) in comparison with the reference site.

## 4. Discussion

### 4.1. Physical and chemical parameters

The relatively low pH value (5.9) recorded in January at site 1 can be attributed to the formation of sulphuric acid as a product of reactions involving the oxidation of pyrite during the production of acid mine drainage (Bell et al., 2002). The relatively high concentrations of Al at site 1 in February and March (1.6 mg $l^{-1}$) were typical indicators of acid mine drainage and were possibly derived from alumino-silicate minerals associated with coal seams (Bell and Bullock, 1996). Hence, the increased solubility of minerals containing aluminium, silica and sulphate under acidic conditions at sites 1 and 2 would seem to explain the high concentrations of Al in the riverine zone of Lake Loskop. Furthermore, the higher sulphate concentrations in the inflowing river, compared with the lake reference site, were also a characteristic of water contaminated by acid mine drainage during this period (Bullock and Bell, 1997). In their study on the acidic seepage from an abandoned mine entering a nearby stream in the Witbank Coalfield area of South Africa, Bullock and Bell (1997) reported sulphate concentrations that were much higher than the South African Water Quality Guideline value of 400 mg $l^{-1}$ for domestic water supplies (DWAF, 1996).

In the inflowing riverine zone of Lake Loskop, Al concentrations remained fairly constant throughout the study, except for February and March when higher concentrations coincided with elevated rainfall in the upper catchment; this rainfall is likely to have increased the seepage of acidic water from abandoned coal mines in the catchment. However, Al concentrations in the main basin of Lake Loskop were considerably reduced, possibly through adsorbption of Al ions onto suspended sediment particles that would eventually sink to the bottom of the lake. The high concentrations of total nitrogen and phosphorus measured in Lake Loskop indicate that the lake water was hypertrophic and similar to the conditions recorded earlier in hypertrophic Lake Hartebeespoort (NIWR, 1985). However, we were unable to diagnose the precise trends in the nutrient-enrichment process in Lake Loskop over the last decade because large gaps in the available water-quality-monitoring data indicate that the monitoring of water quality in the lake water has been intermittent.

### 4.2. Phytoplankton community responses

While phytoplankton are amongst the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems, this study revealed that the species present in Lake Loskop consisted of those that displayed a mixture of sensitive, tolerant and resistant responses to anthropogenic stressors (Oberholster et al., 2005). The ability of algae to survive and reproduce in habitats that are polluted by metal ions may depend on genetic adaptations that have developed over time (Whitton and Kelly, 1995; Garcia-Villada et al., 2004).

Cyanobacterial interactions with and responses to metal ions have been frequently reported in laboratory studies, but very seldom in a bloom-forming context (Baptista and Vasconcelos, 2006). The presence of particular cations affects the accumulation of metals and decreases their toxicity to phytoplankton, probably due to competition and/or complexation and co-precipitation. The presence of high concentrations of phosphorus (0.7 mg $l^{-1}$) in Lake Loskop during January could also have decreased the toxicity of a number of heavy metals, while extracellular products of algal origin have also been reported to reduce metal toxicity (Reed and Gadd, 1990).

From the data generated in this study, we hypothesise that the presence of toxic secondary metabolites may have been one of the mechanisms that played a role in reducing heavy metal toxicity in *Microcystis* cells forming the cyanobacterial bloom within the riverine zone during February and March 2008. Toxic secondary metabolites – such as microcystin produced by the *Microcystis* species in this study – have the ability to bind metals in the water column, thereby influencing metal speciation (Oliveira et al., 2005). An earlier study by Butler et al. (1980) reported that extracellular products can only reduce metal toxicity in cyanobacterial cultures when the algae are present at high population densities; this occurred in our study, with cyanobacterial cell concentrations reaching $2.2 \times 10^7$ cells $ml^{-1}$ during March 2008. Furthermore, Hunter et al. (1965) report that acid tolerance appears to be closely related to metal-tolerance mechanisms, and the requirements for many trace elements by phytoplankton increase considerably in water with a relatively low (i.e., slightly acidic) pH. Since cyanobacteria depend upon a variety of metal cations to maintain their cellular metabolism, we speculate that some of these metals, e.g., iron which was present in high concentrations in the riverine zone during the cyanobacterial bloom, may have influenced the tolerance of the cyanobacterial cells higher levels of acidity (Andrews et al., 2003). This may explain why the cyanobacterial bloom started to develop in Lake Loskop – at a slightly acidic pH of 6.1 – when most incidents of *Microcystis* bloom-formation appear to occur at pH values above 7, as observed by Oberholster and Botha (2007) in Lake Midmar, South Africa, where the highest abundance of *Microcystis* cells occurred at a slightly alkaline pH of 7.9.

Another possible explanation for the acid tolerance shown by the *Microcystis* cells in the riverine zone of Lake Loskop, is the ability of the polypeptide mucus layer that is secreted by a variety of other algae to form complexes with metal ions and nutrients, and which may also help to exclude $H^+$ ions from cells as observed by Mohamed (2001) in a non-toxic strain of the freshwater cyanobacterium *Gloeothece magna*. Lackey (1968) reported that the mucilaginous secretion of euglenoids, mucin, serves as a protection against $H^+$ ions. The occurrence of surface cyanobacterial blooms and sub-surface blooms of dinoflagellates is most often recorded during the late summer months in mesotrophic and eutrophic lakes (Anderson et al., 2002). However, the development of a cyanobacterial bloom at an acidic pH (5.9) in the riverine zone of Lake Loskop was unusual, not only because of this species' tolerance for low pH values, but also because the formation of a cyanobacterial bloom in a lake appears to be most often favoured and controlled by stratified water column conditions (Oberholster and Botha, 2007). However, this was not the case in the riverine zone of Lake Loskop during the peak of the rainy season in February 2008. While the duration of the cyanobacterial bloom from February to March at site 2 in Loskop Dam was likely stimulated by the high concentrations of phosphate present in the water, it is possible that the higher



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concentrations of sulphate present at this site, compared with the reference site, site 5 (Table 1), also played a stimulatory role. Under anaerobic conditions underneath the cyanobacterial bloom at site 2, sulphate is converted to sulphite, which stimulates cyanobacterial growth as observed by Parker (1982) in laboratory cultures.

Earlier authors (e.g., Romo, 1998; Hörnström, 1999) have reported that phytoplankton species richness declines in areas where pH values decrease. This phenomenon was also observed in the riverine zone of Lake Loskop in this study. Detailed phytoplankton information collected from two lakes by Finlay and Saesura (1980) during experimental acidification with $H_2SO_4$ showed different trends to those reported here for the riverine zone of Lake Loskop. Finlay and Saesura (1980) reported that Bacillariophyceae and Chrysophyceae were replaced as dominant phytoplankton taxa by Chlorophyceae and Cyanobacteria during the first stage of acidification (from a pH of 6.5 to 5.6). In our study, the phytoplankton community structure in the riverine zone (sites 1 and 2) at a pH of 5.9 and 6.1 were dominated by Chrysophyceae (*Dinobryon divergens*), Bacillariophyceae (*E. flexuosa*) and Cyanobacteria (*M. tenuissima*) in January, but the population changed gradually to one where another cyanobacterium species (*Microcystis*) became dominant as the pH increased to an average of 6.7 at site 2 in February. Our data suggest that the development of the *Microcystis* bloom in the riverine zone of Lake Loskop at the end of February was possibly due to a switch in trophic states. This change appears to have caused the aquatic system to pass the upper critical threshold for high concentrations of nutrients and enter an alternate hypertrophic regime, which now overshadows the adverse effects of high concentrations of heavy metals, sulphate and low pH values. Possible drivers in the aquatic system that could have triggered the system to cross this threshold were nutrient-enriched runoff from agricultural land, as well as inflows of untreated and partially treated domestic sewage.

The dominance of *C. hirundinella* as an equilibrial species and the presence of very low numbers ($1.2 \times 10^2$ cells $ml^{-1}$) of *Microcystis* spp. in the lacustrine zone of Lake Loskop was possibly due to allelopathic interactions between these species. Although we did not test for allelochemicals in this study, Vardi et al. (2002) reported from their long-term growth experiment that the cyanobacterium *Microcystis* was hardly affected by the presence of the dinoflagellate *Peridinium gatunense* as long as the initial inoculum of the latter did not exceed 1000 cell $ml^{-1}$. However, the growth of *Microcystis* was severely hampered (loss of buoyancy and massive lysis of cells) when higher cell densities of the dinoflagellate *P. gatunense* were applied, even in the presence of adequate nutrients. The high average cell number for *C. hirundinella* recorded during our study (1200 cell $ml^{-1}$) could have had an adverse effect on the *Microcystis* colonies in the main lake basin.

It is known that the growth of diatoms can be inhibited by a low supply of silica. However, Willen (1991) reported that Si concentrations as low as 0.2 mg $l^{-1}$ – much lower than the concentrations measured at all 5 sites in our study – should be sufficient for diatom reproduction. Therefore, it seems unlikely that low silica concentrations would be the reason for the low number of diatoms observed, but rather the adverse effects of the high concentrations of mixed metal ions and sulphate (Willen, 1991). The dominance of the cyanobacterium *M. tenuissima* during January 2008 at sites 1and 2, followed by its later disappearance in February, can be related to the low pH value and the trophic status of the riverine zone of Lake Loskop. Rosen (1981) reported that this species is an excellent indicator of good water quality, preferring oligotrophic conditions and with an optimal occurrence at a pH of 5.6–6.2. The green alga *S. anatinum*, which was absent from the riverine zone of Lake Loskop (sites 1 and 2), and present only in very low numbers at sites 3–5 throughout the study, can be seen as a serious sign, suggesting that the mixture of chemicals present in the water had an adverse effect on the phytoplankton community in Lake Loskop.

### 4.3. Phytoplankton bioassay responses

The growth inhibition of *S. capricornutum* cells compared with the control after exposure to filtered lake water from sites 1 and 2 can possibly be related to the high concentrations of Al ions. Aluminium – the dominant trace metal cation in the riverine zone of Lake Loskop (Table 1) – tends to bind to thiol groups in algal cells causing inhibition of enzyme activity, or tends to interact with other cations by inhibiting their physiological functions (Nies, 1999). In his studies on changes of phytoplankton in acid and limed lakes in Sweden, Hörnström (1999) reported that Al concentrations around 100 µg $l^{-1}$ – occurring mainly as free ions – may cause serious adverse effects on phytoplankton. The growth inhibition of *S. capricornutum* cells after exposure to lake water from sites 1 and 2 was similar to the results of an earlier study by Regel et al. (2002), where esterase activity was adversely effected by acid mine drainage in *S. capricornutum* cells.

The changes in the chlorophylls *a* and *b* concentrations in *Selenastrum* cells within the first 72 h after exposure to high concentrations of metal ions (in filtered lake water from sites 1 and 2), were possibly due to the inhibition of the reductive steps in the biosynthetic pathway of photosynthetic pigments in this species. In a study conducted by de Filippis and Pallaghy (1976), pigment composition was reported to change within the green alga *Chlorella* after exposure to sub-lethal concentrations of mercury and zinc. Thompson and Couture (1990) reported changes in mitochondrial structure and reductions in the activity of at least four TCA (Krebs) cycle enzymes that were induced by some heavy metals. In addition, de Filippis and Ziegler (1993) reported that ATP levels are significantly decreased by heavy metals, which retard all ATP-dependent processes such as carbon fixation and nitrogen metabolism in the growth phase of the cyanobacterium *Nostoc*. Zhou et al. (2008) also reported a negative correlation between chlorophyll *a* content and heavy metal concentrations in purified strains of the alga *Chorella ellipsoidea*. Although growth inhibition was observed in *Selenastrum* cells exposed to the water from sites 1 and 2 in Lake Loskop, the stimulation of algal growth at sites 4 and 5 may be due to the fact that the concentrations of heavy metals and sulphate at these sites were below the toxic threshold for the test alga *S. capricornutum*, as observed by Chaudhary and Chandra (2005) in their study on the exposure of the cyanobacterium *Nostoc muscorum* to heavy metals.

## 5. Conclusion

From the data generated in this study, it is evident that the phytoplankton responses observed in the bioassays provided a potential link to the absence of the algal group Chlorophyceae in the phytoplankton community structure at sites 1 and 2 in Lake Loskop. When compared with the data from conventional chemical analyses, the phytoplankton communities in Lake Loskop exhibited rapid biological responses, indicating that these organisms were highly sensitive to the inflow of a complex mixture of pollutants. Because water quality directly affects the species abundance of phytoplankton in Lake Loskop, the phytoplankton species present can act as indicators for the evaluation of pollution or the success/failure of bioremediation attempts. Furthermore, the possible accumulation of metal ions in algal



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cells may adversely affect the algae–herbivore fish food chain through biomagnification in Lake Loskop.

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