

**Monitoring toxicity in raw water of the Cache la Poudre River and Sheldon Lake, Colorado, USA using biomarkers and molecular marker technology.**

by

**Paul Johan Oberholster**

Submitted in partial fulfilment of the requirements for the degree

*Philosophiae Doctor*

In the Department of Microbiology and Plant Pathology,  
Faculty of Natural and Agricultural Sciences  
University of Pretoria  
Pretoria

December 2005

Supervisor: Prof TE Cloete

## **Acknowledgements**

I would like to thank the following people and institutions:

- Prof. T.E. Cloete for his guidance, support and advise during this study and preparation of this manuscript.
- Prof. A-M. Botha-Oberholster for suggestions, guidance and bearing with me during the course of the study.
- The Colorado State University for their facilities.
- The University of Pretoria; Department of Genetics for their facilities and materials provided.
- The Water Research Commision and National Research Foundation for funding this project.
- The National Research Foundation for the bursary provided.
- Leanne Coetzee, City Council of Tshwane and Karin van Ginkel, Department of Water Affairs and Forestry for providing research materials.
- Much appreciation to my sons, family and friends for their interest and support.

## **DECLARATION**

I the undersigned hereby declare that the work carried out in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Paul Johan Oberholster  
20<sup>th</sup> December 2005

## Table of Contents

	Page Number
List of Abbreviations	x
List of Units	xi
List of Figures	xiii
List of Tables	xviii
<b>Chapter 1 Introduction</b>	<b>1</b>
<b>Outline of Chapters</b>	<b>5</b>
Chapter 2	5
Chapter 3	5
Chapter 4	6
Chapter 5	7
Chapter 6	8
References	9
<b>Chapter 2 An overview of the impact of anthropogenic activity on water quality with special reference to human health, risks and detection of toxic compounds present in water bodies.</b>	<b>12</b>
2.1. Introduction	13
2.2 Water quality	14
2.3 Urban runoff	15
2.4 Eutrophication	15
2.5 Human health risks of long-term exposure to toxic compounds	18
2.5.1 Health risks of long-term exposure to low levels of microcystin	18
2.5.2 Effect of long-term exposure to PAHs	21
2.6 Methods for detection of toxicity as a result of chemical pollutants and biological toxic compounds in raw water	22

2.6.1 Bioassays for detection of PAHs	22
2.6.2 Methods for detection of microcystin	24
2.6.2.1 Alternative bioassays	24
2.6.2.2 Use of animal and/or cell biopassays	25
2.6.2.3 Enzyme-linked assays	26
2.6.2.4 Chromatographic analysis	27
2.6.2.5 Monitoring toxigenicity of cyanobacterial strains by molecular assay	28
2.7 Raw water treatment processes	31
2.8 Conclusion	33
References	34

**Chapter 3** Using a battery of bioassays, Benthic Phytoplankton and the AUSRIVAS method to monitor long-term coal tar contaminated sediment in the Cache la Poudre River, Colorado **50**

Abstract	51
3.1 Introduction	51
3.2 Material and methods	53
3.2.1 Study area	53
3.2.2 Toxicity testing	55
3.2.2.1 <i>Selenastrum capricornutum</i> test	55
3.2.2.2 <i>Daphnia magna</i> test	57
3.2.2.3 <i>Chironomus tentans</i> test	58
3.2.2.4 Data analysis of biotests endpoints	58
3.2.2.5 Sampling of macroinvertebrates and phytobentos	59
3.3 Results	61
3.3.1 Physicochemical characteristics	61
3.3.2 <i>Selenastrum capricornutum</i> biotest	62
3.3.3 Freshwater <i>Daphnia magna</i> and <i>Chironomus tentans</i> biotests	64
3.3.4 Macroinvertebrate response	65
3.3.5 Benthic phytoplankton response	67
3.4 Discussion	70
3.4.1 Physicochemical characteristics	70
3.4.2 <i>Selenastrum capricornutum</i> biotest	70
3.4.3 Freshwater <i>Daphnia magna</i> and <i>Chironomus tentans</i> biotests	71
3.4.4 Macroinvertebrate response	72
3.4.5 Benthic phytoplankton response	74

3.5 Conclusion	75
Acknowledgements	78
References	78
<b>Chapter 4 Population dynamics and ecological changes in an urban artificially mixed shallow lake Colorado, one year after restoration</b>	<b>88</b>
Abstract	89
4.1 Introduction	90
4.2 Materials and methods	91
4.2.1 Study area	91
4.2.2 Sampling protocol	93
4.2.3 Data analyses	98
4.3 Results	99
4.3.1 Phytoplankton	99
4.3.2 Zooplankton	103
4.3.3 Benthic macroinvertebrate	104
4.3.4 Vertebrates	106
4.3.5 Waterbirds and nearshore birds	107
4.4 Discussion	109
4.4.1 Phytoplankton	109
4.4.2 Zooplankton	112
4.4.3 Macroinvertebrates	115
4.4.4 Vertebrates	117
4.4.5 Waterbirds and Nearshore birds	118
4.5 Conclusion	120
References	121
<b>Chapter 5 Toxic cyanobacterial blooms in a shallow artificially mixed urban lake in Colorado, US.</b>	<b>131</b>
Abstract	132
5.1 Introduction	133

5.2 Study site description and background	135
5.3 Materials and methods	138
5.3.1 Bloom Sampling	138
5.3.2 Bloom toxicity confirmation	139
5.3.2.1 Pretreatment of environmental samples for whole-cell PCR	140
5.3.2.2 PCR amplification	140
5.3.2.3 RNA extraction and RT-PCR	142
5.3.2.4 Fish bioassay	142
5.3.2.5 Light microscopy	143
5.3.2.6 Protein Phosphatase Inhibition and ELISA Assays	143
5.3.2.7 Data analysis	144
5.4 Results	144
5.4.1 Species composition and physical/chemical measurements	144
5.4.2 Artificial destratification	147
5.4.3 Detection of toxicity	149
5.5 Discussion	153
5.5.1 Incomplete mixing	154
5.5.2 Toxicity	156
5.6 Conclusion	159
Acknowledgements	159
References	158

**Chapter 6** Assessment of the genetic diversity of geographical unrelated *Microcystis aeruginosa* strains using Amplified fragment length polymorphisms (AFLPs) **169**

Abstract	170
6.1 Introduction	171
6.2 Material and Methods	174
6.2.1 Chemicals, Strains and Culture Conditions	174
6.2.2 DNA extraction	178
6.2.3 AFLP analysis	179
6.2.4 Gel electrophoresis and scoring	181
6.2.5 Data analysis	182

6.3 Results	183
6.3.1 Fast screening of AFLP primer combinations	183
6.3.2 Genetic diversity as defined by AFLP fingerprinting	184
6.4 Discussion	187
Acknowledgements	192
References	192
Summary	<b>201</b>
References	206
Appendixes	<b>208</b>



## List of Abbreviations

aa	Amino acid
ABS	Absorbed photon flux
Adda	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
AFLP	Amplified Fragment Length Polymorphism
ATP	Adenosine triphosphate
AMP	Adenosine monophosphate
AP	Alkaline phosphatase
APHA	American Public Health Association
ASTM	American Standard Test Method
AUSRIVAS	Australian river bioassessment system
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bp	Base pair
CCAP	Culture Collection of Algae and Protozoa, UK
Co	Company
CTAB	N-cetyl-N-N-N-trimethyl ammonium bromide
DAF	DNA amplification fingerprinting
dATP	Deoxyadenine triphosphate
dCTP	Deoxycytidine triphosphate
ddH <sub>2</sub> O	Double distilled water
dGTP	Deoxyguanosine triphosphate
DIG	Digoxigenin
DIN	Dissolved inorganic nitrogen
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
dNTP	Deoxynuclein triphosphate
DTE	Dithioerythritol
DTT	Dithiothreitol
dTTP	Deoxythymine triphosphate
dUTP	Deoxyuracil triphosphate
EC	Enzyme code
EDTA	Ethylenediamine tetra-acetic acid, disodium magnesium
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
ET	Electron transport past Q <sub>A</sub> -
e-value	expectancy value
F <sub>0</sub>	Minimal fluorescence of a dark adapted sample
F <sub>m</sub>	Maximal fluorescence of a dark adapted sample
GC	Gas chromatography
Hepes	4-(2-Hydroxyethyl)iperazine-1-ethanesulfonic acid
HPLC	High performance liquid chromatography
I <sub>k</sub> ,	the light intensity at the onset of light saturated photosynthesis in $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$
i.p.	intraperitoneally
IPTG	Isopropyl- $\beta$ -D-galactoside

i.v.	intravenous
kb	Kilobase
kDa	Kilodalton
LB	Luria Bertrani
LD <sub>50</sub>	Lethal dose
LDH	Lactate dehydrogenase
MC	Microcystin
Mdha	N-methyl-dehydroalanine
MI	Marker Index
MMPB	3-methoxy-2-methyl-4-phenylbutric acid
mRNA	Messenger ribonucleic acid
NBT	Nitroblue tetrazolium salt
NIES	National Institute for Environmental Studies, Japan
PAH	polycyclic aromatic hydrocarbons
P <sub>max</sub> <sup>B</sup>	maximum biomass specific photosynthetic rate in $\mu\text{mol O}_2 \text{ mg chl } a^{-1} \text{ h}^{-1}$
PCC	Pasteur Culture Collection
PCR	Polymerase Chain Reaction
PCR-RFLPs	Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms
PcoA	Principal coordinate analysis
PIC	Polymorphic Information Content
PP	Protein phosphatase
PPi	Inorganic pyrophosphate
RC	Reaction Centre
rDNA	Ribosomal deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulfate
SSC (20X)	0.3 M NaCitrate, 3 M NaCl, pH 7.0
SRP	soluble reactive phosphorus
STET	0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 5 % Triton®X-100
TAE (1X)	40 mM Tris-acetate, 1 mM EDTA, pH 8.0
TE	10mM Tris-HCl, 1 mM EDTA, pH 8.0
Tes	N-[Tris(hydroxymethyl)methyl]-2-aminoethane-sulfonic acid
TOC	Total organic carbon
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
tRNA	Transfer ribonucleic acid
UP	University of Pretoria
UPGMA	Unweighted Pair Group Method using Arithmetic averages
UV	Ultraviolet
UV	Strain in the University of the Free State Culture collection
WHO	World Health Organization
X-gal	5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside
X-phosphate	Toluidinium salt

## List of Units

LD<sub>50</sub> Dose of toxin that kills 50 % of the animals tested.

LC<sub>50</sub> Lethal concentration of toxin that kills 50% of the tested organisms.

LT<sub>50</sub> Lethal time that toxin take to kill 50% of the tested organisms.

### Restriction Enzyme

One unit is the enzyme activity that completely cleaves 1µg λDNA in 1 h at enzyme specific temperature in a total volume of 25 µL.

### Taq DNA Polymerase

One unit is the quantity of enzyme required to catalyse the incorporation of 10 nmol of dNTP's into acid insoluble material in 30 minutes at 74 °C.

## List of Figures

	Page Number
<b>Figure 1.1</b> Map indicating the catchment area of the Cache la Poudre River (Adapted from Collins & Sprague 2005).	3
<b>Figure 2.1</b> PCR fragments obtained after amplification of <i>Microcystis</i> strains with primer pairs specific for <i>mcyB</i> . Lanes 1-13 represents <i>Microcystis aeruginosa</i> strains, and lanes 14-18 (UP04) represents <i>Microcystis wesenbergii</i> . M = Marker III (lambda DNA restricted with <i>EcoRI</i> and <i>HindIII</i> ), 1 = PCC7813 (0.2 µl DNA); 2 = SAG1 (0.2 µl DNA); 3 = CCAP1450/1 (0.2 µl DNA), 4 = UV027 (0.2 µl DNA), 5 = PCC7813 (0.4 µl DNA); 6 = PCC7813 (0.5 µl DNA); 7 = SAG1 (1 µl DNA), 8 = SAG1 (0.8 µl DNA); 9 = water control; 10 = UP01 (0.2 µl DNA); 11 = UP03 (0.2 µl DNA); 12 = UP03 (1 µl DNA); 13 = UP04 (1 µl DNA); 14 = UP02 (0.2 µl); 15 = UP02 (0.2 µl); 16 = UP02 (1 µl); 17 = UP02 (1 µl); 18 = water control (Oberholster 2004).	31
<b>Figure 3.1</b> Map of the study area in Fort Collins showing the sampling sites on the banks of the Cache la Poudre River, Colorado.	54
<b>Figure 3.2</b> Substratum composition of the ten sampling sites in the Cache la Poudre River, Colorado.	61

- Figure 3.3** Variations in flow and pH from August 22 to October 8 in the Cache la Poudre River, Colorado. 62
- Figure 3.4** Changes in algal cell counts (A) and specific Chl $a$  content (B) at the different sampling sites in the Cache la Poudre River, Colorado. 63
- Figure 3.5** Results of the survival rates of *Daphnia magna* (48 h) and *Chironomus tentans* (240 h) after biotesting of sampling sites. 65
- Figure 3.6** (A) Abundance (individuals/m<sup>2</sup>) and (B) percentage composition of macroinvertebrate functional feeding groups recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004. 67
- Figure 3.7** Bacillariophyceae, *Didymosphenia geminata* (indicated by a arrow), the only taxon in large abundance in the contaminated sediment. Unstained, bright-field microscopy, 200 x. 68
- Figure 3.8** Percentage composition of different Bentic phytoplankton species recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004. 69
- Figure 3.9** Clean-up of the Cache La Poudre, 2004-2005. Before (A) and during (B) restoration. 77
- Figure 4.1** Map of Sheldon Lake and City Park recreational area, Colorado (Sheldon Lake Drainage Improvement Project 2002) (Scale: 10 m = 5 mm). 93

- Figure 4.2** Seasonal changes and contribution to phytoplankton species composition as percentage for the five major algal classes 2004-2005 from the four sampling sites. 99
- Figure 4.3** Seasonal changes and contribution to phytoplankton species composition as percentage for the Chlorophyceae, Cyanophyceae, and Bacillariophyceae in 2001 and then 2004-2005 for the four sampling sites. 100
- Figure 4.4** Specific Chla measured from May 2004 to April 2005, Sheldon Lake, Colorado. 102
- Figure 4.5** Abundance in *Bosmina* sp. during summer 2004 (A) Site with cyanobacterial surface bloom and sparse macrophyte growth; (B) Site with cyanobacterial surface bloom and sparse macrophyte growth; (C) Site with cyanobacterial surface bloom but without a macrophyte growth; and (D) Site without cyanobacterial surface bloom and macrophyte growth. 104
- Figure 4.6** Macroinvertebrate abundance (%) at the four sampling sites, Sheldon Lake, Colorado during summer 2004. 105
- Figure 4.7** Composition by volume of the gut contents of juvenile *Lepomis macrochirus* and yearling *Micropterus salmoides* of Sheldon Lake, Colorado during summer 2004. 106
- Figure 4.8** Northern shoveler's clustered around air bubbles that form surface 'boils' in the ice cover during artificial mixing. 107
- Figure 4.9** Monthly distribution of waterbirds and nearshore birds in Sheldon Lake, Colorado (a) count and (b) species. 108

- Figure 5.1** (A) Map of Sheldon Lake, Colorado (Sheldon Lake Drainage Improvement Project 2002) (Scale: 10 m = 4 mm). (B) Sampling site D. 137
- Figure 5.2** Average (a) specific Chl<sub>a</sub>, (b) cell count and (c) transparency (Secchi depth) during surface blooms in the summer 2004 with artificial mixing, Sheldon Lake, Colorado. 145
- Figure 5.3** (A) *Woronichinia naegeliana* (after Smith 1950); (B) *Microcystis aeruginosa* (after Smith 1950); Unstained, bright-field microscopy, 200 x. 146
- Figure 5.4** Seasonal variations in the relative abundance of five phytoplankton groups with artificial mixing during the summer 2004, Sheldon Lake, Colorado. 147
- Figure 5.5** Cyanobacterial bloom still visible in the morning on the surface water during artificial mixing at sampling site B. 148
- Figure 5.6** The number of *Microcystis* colonies/ml of samples taken at 0.25-m depth intervals at different locations in the lake during artificial mixing, taken on the morning and afternoon of August 22, 2004. 149
- Figure 5.7** (A) Separation of PCR amplicons obtained after PCR of *Microcystis aeruginosa* strain UPUS1 (1); PCC7806 (2); UP37 (3) and *Woronichinia naegeliana* strain UPUS2 (4) using different primers on a 2% agarose gel. M = Hyperladder<sup>TM</sup> IV, Bionline, USA. (B) Quantitative PCR of RNA from *Microcystis aeruginosa* strains UPUS1 and *Woronichinia naegeliana* strain UPUS2 with selected primers. Actin was included as standard. 150
- Figure 5.8** Histopathological investigation of *Oncorhynchus mykiss* revealed damage to gills and fins during cyanobacterial surface blooms during August, 2004. 151

**Figure 6.1** (A) *Microcystis wesenbergii* (after Teiling 1941, and Wojciechowski 1971); (B) *Woronichinia naegeliana* (after Smith 1950); (C) *Microcystis aeruginosa* (after Smith 1950); and (D) *Chroococciopsis cubana* (after Komárek & Hindák 1975). Unstained, bright-field microscopy, 200 x. 176

**Figure 6.2** AFLP banding patterns generated using primer combinations *EcoR1-CC/Mse1-CT* (A) and *EcoR1-ATC/Mse1-CCA* (B). M = 100 bp ladder marker; 1 = NIES88; 2 = NIES89; 3 = NIES90; 4 = NIES99; 5 = NIES299; 6 = PCC7806; 7 = CCAP1450/1; 8 = SAG1; 9 = UV027; 10 = PCC7813; 11 = UP01; 12 = UP02; 13 = UP09, 14 = UP04, 15=UP04, 16 = UP10, 17 = UP13, 18 = UP15, 19 = UP26, 20 = UP37, 21 = UP38, 22 = UPUS1, 23 = UPUS2, 24 = UP06. 184

**Figure 6.3** Combined cluster analysis derived from AFLP analysis of 23 *Microcystis aeruginosa* and outgroup strains using eight AFLP primer combinations. 185



## List of Tables

	Page Number
<b>Table 2.1</b> Comparison of toxicities of some biological toxins.	17
<b>Table 2.2</b> Acute intoxications of humans from cyanobacteria.	20
<b>Table 3.1</b> O/E index and ecological health rating for the long-term contaminated sediment sampling sites in the Cache la Poudre River.	60
<b>Table 4.1</b> Dominant species associated with water of different trophic levels (Modified from Willen 2000).	97
<b>Table 4.2</b> Carlson's Trophic State Index and its associated parameters (Carlson 1977).	98
<b>Table 4.3</b> Annual mean values and ranges for selected variables for the years 2001 and May 2004 to May 2005 (Data for 2002-2003 is not available due to restoration; $\pm$ indicate standard error).	101
<b>Table 4.4</b> Dominant taxa in each functional feeding group.	105
<b>Table 5.1</b> Oligonucleotides used for RT and PCR.	141

<b>Table 5.2</b> Average wind velocity (m/s)	148
<b>Table 5.3</b> Comparison of PCR with different primers, quantitative PCR, ELISA and Protein Phosphatase inhibition (PP2A) assay as determinants of toxicity in strains from different geographical regions. (+ = positive/product; - = negative/no product; / not assayed).	152
<b>Table 6.1</b> Different strains used in the study and their origin.	175
<b>Table 6.2</b> Degree of polymorphism and average polymorphism information content (PIC) and marker index (MI) for the eight AFLP primer combinations used to analyse the 23 strains.	183
<b>Table 6.3</b> Geographical distance in kilometers between the reservoirs in Gauteng and North West Provinces, South Africa.	187