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Review Article

An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact and detection by molecular marker tools

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Abstract

Toxic cyanobacteria found in eutrophic, municipal and residential water supplies are an increasing environmental hazard in South Africa. Cyanobacteria produce lethal toxins, and domestic and wild animal deaths are caused by drinking water contaminated by these toxins. Among the species causing death of livestock, blooms of *Microcystis aeruginosa* are the most common in South Africa. More than 65 microcystins have been isolated to date and they are the most abundant cyanobacterial toxins. Hazards to human health may result from chronic exposure via contaminated water supplies. Microcystins are powerful tumour promoters and inhibitors of protein phosphatase 1 and 2A and they are suspected to be involved in the promotion of primary liver cancer in humans. In this minireview, we discuss the significance of toxic cyanobacteria in South Africa as well as the detection of potential microcystin-producing cyanobacteria strains in South African reservoirs with a *mcyB* molecular marker. It would be of economic and public health value to be able to detect early stage blooms of cyanobacteria, especially if it is on a sufficiently timely basis for municipalities and recreation facilities to implement a response plan.

Key words: water quality, *Microcystis aeruginosa*, longterm exposure, purification processes

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Introduction

Southern Africa is generally an arid to semi-arid region, with an average rainfall of a little under 500 mm per annum. There are practically no freshwater lakes in South Africa; exploitable water supplies are therefore confined to rivers, artificial lakes behind dams, and groundwater. The total runoff from South Africa is estimated at 53 500 million m^3 per annum, of which about 33 000 million m^3 could practically be exploited. The many demands for water, and the erratic flow of most South African rivers, have led to the creation of artificial lakes, i.e. impoundments on all the major rivers, in order to stabilize flow and therefore guarantee annual water supply. The total capacity of state impoundments amounts to more than 50 per cent of South Africa's total average annual river runoff[1].

Urban complexes in Gauteng especially Pretoria and Johannesburg, generate large amounts of sewage, which even if treated give rise to effluents that are high in salts, phosphates and nitrates. When effluents containing high levels of nutrients reach artificial lakes, they stimulate growth of algae including cyanobacteria leading to accelerated eutrophication, disturbances of relationships among organisms, biodiversity and levels of oxygen concentrations. Extensive growth of cyanobacteria in compound reservoirs can create severe problems in the maintenance of water supplies and in meeting the ever-increasing demand for potable water[2,3].

Large cyanobacteria blooms may rapidly clog not only the fine sand filters but even the primary coarse fast filters of water treatment plants. Secondly, cyanobacteria may release substances in the water that are harmful or toxic, which cause unnatural colouration of the raw water or which add an objectionable odour or taste to drinking water[2].

Cyanobacterial toxins and health effects

Toxins of cyanobacteria are grouped in two main categories by Carmichael[4] namely, biotoxins and cytotoxins based on the types of bioassays used to screen for their activity. Cytotoxins are detected by mammalian cell lines and biotoxins are assayed with small animals, e.g. mice or aquatic invertebrates. Because cytotoxins are not highly lethal to animals, and no reports in South Africa have been published indicating they were responsible for livestock deaths in the field, they will not be discussed further. In the toxicity standards, biotoxins are considered supertoxic (Table 1). Biotoxins of cyanobacteria are water-soluble and heat stable and they are released upon aging or lysis of the cells. The primary types of cyanobacterial biotoxins include hepatotoxin (microcystins, nodularins, cylindrospermopsins), neurotoxin (anatoxins, saxitoxins) and dermatotoxins (lyngbyatoxin A, aplysiatoxins, lipopolysaccharides) (Fig. 1).

Hepatotoxins

Hepatotoxins are low molecular weight cyclic peptide toxins that affect the liver and have been the predominant toxins involved in the case of freshwater algae toxicosis.

Microcystins and nodularins

Microcystin are a cyclic heptapeptides with about 65 different isoforms identified, with diverse levels of toxicity[5]. Nodularins are pentapeptides with only four forms been described. The hepatospecificity of these toxins is due to the requirement for uptake by a bile acid transporter. Microcystin and nodularins have been shown to be inhibitors of serine/threonine protein phosphatase 1 and 2A. This inhibition leads to hyperphosphorylation of proteins associated with the cytoskeleton in hepatocytes[6].

Cylindrospermopsins

Cylindrospermopsins is an alkaloid containing a tricyclic guanidine combined with hydroxymethyl uracyl and is stable to boiling. Studies on the mechanism of action of cylindrospermopsin have shown that in mouse hepatocytes *in vivo* the toxin disrupts protein synthesis[7]. The main target of this toxin is the liver, but unlike the microcystins, it can affect other organs such as the lungs, kidneys, adrenals and intestine[8]. Genotoxic activity is caused by the ability of cylindrospermopsins to induce strand breaks at the DNA level and loss of whole chromosomes [9].

Table 1: Comparison of toxicities of some biological toxins

Toxins	Sources	Lethal doses (LD50)	Reference
Saxitoxin	*Aphanizomenon flos-aquae*	10	Oshima, 1995.[98]
Anatoxin-a(s)	*Anabaena flos-aquae*	20	Falconer, 1998.[8]
Cobra toxin	*Naja naja*	20	Bagchi, 1996.[69]
Nodularin	*Nodularia spumigena*	30	Rhinehart *et al*., 1994.[70]
Microcystin-LR	*Microcystis aeruginosa*	50	Rhinehart *et al*., 1994. [70]
Anatoxin-a	*Anabaena flos-aquae*	200	Carmichael, 1992.[10]
Brevetoxin	*Karenia brevis (dinoflagellate)*	500	Morohashi *et al*., 1999.[71]
Ciguatoxin	*Gambierdiscus toxicus (dinoflagellate)*	0.25	Bagnis *et al*., 1980.[72]
Cylindrospermopsins	*Cylindrospermopsins raciborskii*	2 100	Ohtani *et al*., 1992.[73]
Styrchnine	*Strychnos nuxvomica*	2 000	Bagchi, 1996.[69]

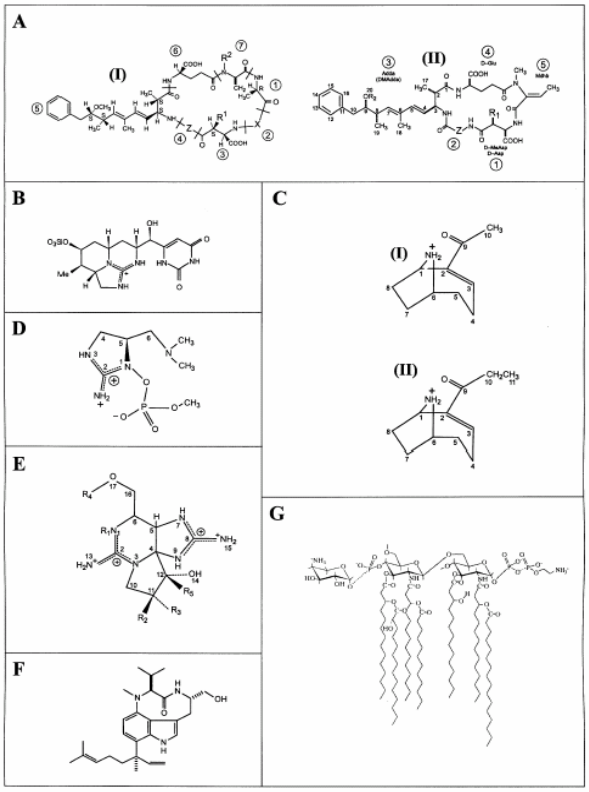

Figure 1. Chemical structures of **A**: microcystins (I) and nodularins (II) (X and Z are variable amino acids, R = H or CH3), **B**: cylindrospermopsin, **C**: anatoxin-a (I) and homoanatoxin-a (II), **D**: anatoxin-a(s), **E**: PSPs, **F**: lyngbyatoxin A, **G**: lipopolysaccharides (LPS)[54].

Neurotoxins

The neurotoxins are known to be produced by freshwater cyanobacteria strains include anatoxin-a, anatoxin-a(s) and saxitoxins. Neurotoxins producing death by paralysis of peripheral skeletal muscles, then respiratory muscles leading to respiratory arrest in a few minutes to a few hours following exposure.

Anatoxins

Anatoxin-a are produced by species and strains of the genera *Anabaena* and *Oscillatoria* and is a secondary amine, 2-acetyl-9-azabicyclo(4.2.1)non-2-ene. This alkaloid, a

structural analogue of cocaine, is a potent post-synaptic cholinergic nicotinic agonist, which causes a depolarizing neuromuscular blockade, followed by fatigue and paralysis[10].

Anatoxin-a(s) is unrelated to anatoxin-a. Structurally it is a unique N-hydroxyguanidine methyl phosphate ester. It can be called a natural organophosphate because of its ability to irreversibly inhibit acetylcholinesterase, causing the same clinical end result as anatoxin-a[10]. Blood, lung and muscle acetylcholinesterases are inhibited, whereas retina and brain acetylcholinesterase activities are normal[11].

Saxitoxins

Saxitoxins or paralytic shellfish poisons are produced by species and strains of freshwater cyanobacteria *Anabaena* and *Aphanizomenon*, but are better known as the products of dinoflagellates, the marine algae responsible for red-tide paralytic shellfish poisoning. The saxitoxins or paralystic shellfish poisons inhibit nerve conduction by blocking sodium channels in axons, thereby preventing the release of acetylcholine at neuromuscular junctions with resultant muscle paralysis. The paralysis of the respiratory muscles leads to the death of animals within a few minutes[12].

Dermatotoxins

Dermatotoxins lyngbyatoxin A and aplysiatoxin are produced by the cyanobacterium *Lyngbya majuscula*, a marine benthic cyanobacterium with different metabolite constituents in deep and shallow water varieties. While the deep water varieties produce inflammatory substances and tumor promoters, the shallow water forms produce lipophilic substances, malyngamides A, B and C. Clinical signs include skin, eye and respiratory irritation[13].

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Historical perspective in South Africa

The genus of most concern for toxin-producing strains is the cosmopolitan *Microcystis*, predominantly *Microcystis aeruginosa*, with other genera being *Oscillatoria*, *Anabaena*, *Aphanizomenon* and *Nodularia*[14,15]. In South Africa almost all cases of animal poisoning have been associated with *Microcystis aeruginosa*[16,17](Table 2). Early researchers on the algal flora of South Africa have commented on the ubiquitous nature of *Microcystis* throughout the country[18,19]. It was around this period (1927) that the first cattle intoxications by *Microcystis* in the Transvaal province were recorded. In agricultural practice, poisoning of farm animals occurs when the animals are prevented from reaching clean water by the specific layout of fences restricting them to shorelines contaminated by cyanobacteria. Because access to drinking water may be the limiting feature of livestock production in arid climates such as those of South Africa or Australia, poisoning episodes have been reported more often from those countries[20,21,22]. Yearly duration of exposure is also shorter (3-5 months) in countries where the water bloom growth season is shorter, like the United States and Canada compared to those with milder climates such as Australia and South Africa (6-10 months)[23]. As far as can be ascertained, cases of poisoning in South Africa have only been described from the former Transvaal (currently Gauteng and Mpumalanga), former Orange Free State (currently Free State)[16,17] and Western Cape provinces[24]. In Gauteng, cyanobacteria poisoning periodically occurs around the Bon Accord and Hartbeespoort Dams[25]. Kellerman *et al*. [25] ranked the plant poisonings and mycotoxicoses occurring in South Africa in order of importance and regarded *Microcystis aeruginosa* poisoning as the fifth most important type of poisoning in the Gauteng Province, and the tenth most important in Mpumalanga.

Chronic studies of cyanotoxins; implications for humans

Where climate and other environmental factors permit, there may be continuous water blooms of toxic cyanobacteria in drinking water reservoirs and other surface water supplies (Table 3). The dominance of cyanobacteria may be due to their low need for uptake of nutrients during the benthic life phase and overwintering[26] or additionally, buoyancy control enables them to outcompete other algal species for light and nutrients[27]. While water supply authorities often control these blooms, the conventional method of algicide treatment lyses the organisms, releasing toxic cell contents into the water. The chronic administration of *Microcystis*

Table 2: Some reported animal poisoning incidents related to cyanobacterial blooms in South Africa

Cases attributed to cyanotoxins in raw drinking water

Date	Description
1913-1943	Location; Free State and Southeast Transvaal. Affected animals; Thousands of livestock (horses, sheep, cattle and rabbits); Symptoms and findings; liver damage, photosensitivity Organism; *Microsystis toxica* (=*aeruginosa*)[21,74].
1973-1974	Location; Hartbeespoort Dam. Affected animals; cattle deaths, Symptoms and findings; microcystin poisoning Organism; *Microcystis aeruginosa*[75].
1979	Location; Klipvoor Dam. Affected animals; Death of 3 White Rhinoceroses (*Ceratotherium simum*). Symptoms and findings; Necrosis of the liver. Organism; *Microcystis aeruginosa*[76].
1980	Location; Vaal Dam. Affected animal; Cattle deaths Symptoms and findings; *Microcystis* poisoning Organism; *Microcystis aeruginosa*[77].
1984	Location; Willem Pretorius Game Reserve (Free State). Affected animals; Death of several Black Wildebeest (*Connochaetes gnou*) Symptoms and findings; *Microcystis* poisoning, Organism; *Microcystis aeruginosa*[76].
1987	Location; Eastern Transvaal. Affected animal; Death of 47 cattle. Symptoms and findings; Microcystin poisoning Organism; *Microcystis aeruginosa* [17].
1989	Location; Bloemhof Dam, Sandveld Nature Reserve (Free State). Affected animals; Seven Giraffe deaths Symptoms and findings; Microcystin poisoning Organism; *Microcystis aeruginosa*[78].
1989	Location; Klipdrif Dam. Affected animals; Livestock Symptoms and findings; *Microcystis* poisoning Organism; *Microcystis aeruginosa*[79].
1994	Location; Zeekoevlei. Affected animal; Bull terrier bitch Symptoms and findings; Hepatic necrosis, first reported incident of nodularin in South Africa. Organism; *Nodularia spumigena*[15].
1994	Location; Paarl, Western Cape. Affected animals; Death of 11 sheep and induced-photosensitivity in a further 20 animals Symptoms and findings; Hepatotoxin, microcystin-LR Organism; *Microcystis aeruginosa*[24].
1996	Location; Tsitsikamma-Kareedouw district, South Cape. Affected animals; Death of 290 dairy livestock and induced-photosensitivity in a further 70 Symptoms and findings; Microcystin poisoning Organism; *Anabaena* spp. and *Oscillatoria* spp.[80].
1998	Location; Erfenis Dam, Free State Affected animals; Death of livestock Symptoms and findings; Neurotoxicosis Organism; *Anabaena* spp.[81].
2000	Location; Orange River. Affected animals; Fish kills along the rivier Symptoms and findings; First reported incident of *Cylindrospermopsis raciborskii* in South Africa. Organism; *Cylindrospermopsis raciborskii*, *Anabaena* sp., *Oscillatoria* sp.[82].

extract in the drinking water of mice resulted in increased mortality, particularly in male mice, together with chronic active liver injury. The deaths were largely due to endemic bronchopneumonia, indicating an impairment of disease resistance. Only six tumors were seen in the 430 mice killed at intervals up to 57 weeks of age; however, four of the six tumors were in females that ingested the highest *Microcystis* concentration[23].

This result led to an investigation of the tumor-promoting activity of orally administered *Microcystis* in mice that had dimethylbenzanthracene applied to their skin. Results of these trials showed that there were significant increases in the growth of skin papillomas in mice given *Microcystis* but not *Anabaena* to drink[28]. The finding that microcystin activated phosphorylase A preceded studies showing that microcystin-LR, -YR, and -RR, and nodularin are potent inhibitors of protein phosphatases type 1 and type 2A[29]. This inhibition leads to hyperphosphorylation of proteins associated with the cytoskeleton in hepatocytes. The rapid loss of the sinusoidal architecture and attachment to one another leads to the accumulation of blood in the liver, and death most often results from hemorrhagic shock. These experiments clearly indicate that microcystin are a health threat in drinking water supplies[10].

Removal of *Microcystis* toxins in water purification processes

Hoffman[30] demonstrated that dissolved substances like microcystin deriving from *Microcystis aeruginosa* samples of the Hartbeespoort Dam were not removed to below 'active levels' by conventional water treatment like flocculation, sedimentation, rapid sand filtration and chlorination. These findings are in accordance with results presented by James and Fawell[31] and Rositano and Nicholson[32] that flocculation was effective in removing cells, but not in eliminating free microcystins and other extra-cellular secondary metabolites which remained constant after flocculation with aluminium sulphate[33,34] or ferric chloride[35]. In another study Pietsch *et al.*[36] reported that flocculation and filtration resulted in an increase of extracellar toxin after experiments with *Microcystis aeruginosa* and *Planktothrix rubescens*. The researchers suggested turbulences in pipes and pressure gradients in the filter as reasons for the increase of the toxin level. The efficacy of chlorine (0.5 mg/l) to eliminate microcystin is also doubtful[37]. Water treatment studies conducted at the laboratory and pilot plant-scale have concluded that granular activated carbon filtration is effective in removing the cyanobacterial toxins from water[38,39]. This treatment add considerably to the expenses of water treatment and only a few purification water treatment plants in South Africa is equipped with granular activated carbon systems, the rest make use of conventional water treatment practices that remove live cyanobacterial cells and debris but not biotoxins in solution. In rural areas the choice of water supply may be limited, depending on the stage of development of the country. Similarly, in urban areas if the reticulated drinking water is of doubtful quality, the only choice may be bottled water, which is financially out of reach for the poorer majority of the population. Thus, the potential for injury from cyanobacteria toxins in water supplies will to some extent depend on the level of development of the country and to some extent on the socio-economic status of the family[40].

Survey analysis of utility waters in the United States and Canada were confirmed to contain microcystin during the sampling period of June 1996 to January 1998. Of the 677 samples collected, 539 (80 percent) were positive for microcystin when tested using ELISA. Of the positive samples, 4.3 percent were higher than the WHO drinking water guideline levels of 1µg/L. Only two of the plant outlet samples submitted exceeded the 1-µg/L WHO drinking water guideline. This indicates that, although almost all water treatment plants had adequate procedure to reduce microcystin to safe levels in the finished water during the test period, the majority of source waters with cyanobacteria do contain microcystin[23]. Surveys of different cyanobacterial blooms for given geographical areas have shown that the frequencies of toxic cyanobacterial blooms in raw water ranged from 22 to 95%[5]. For example, the frequency is an average of 74% for some Mediterranean countries including Portugal,

France (Brittany) and Greece[41,42,43]. The screening of cyanobacterium strains isolated from rice fields, irrigation and drainage water canals in the Nile Delta in Egypt showed that 23% of these isolates were found as active producers of microcystins with an amount of more than 500 ng-1[44]. A survey of cyanobacterial water blooms carried out from 2000 to 2004 in South Africa reservoirs confirmed an average frequency of 95% toxicity in field samples tested by the ELISA method[45](Table 3).

Human health risks of long-term exposure to low levels of microcystin

Little information is available on the effects of long-term exposure to low levels of microcystin toxins in humans. We know that in experiments performed on a time-scale of minutes or hours, microcystin has obvious effects on the functions of plant and animal cells at concentrations as low as 3-10 nM that is equivalent to 3-10 µg for an adult female liver. In cells that take up microcystin freely, the maximum effects are visible at concentrations of around 1µM, the point at which all of the cellular PP1 and PP2A is saturated with toxin. This means that approximately 1 mg (equivalent to drinking two liters of water per day at 32 µg/L microcystin over two weeks) would bind all of the PP1 and PP2A in an adult female human liver, provided that the PP-microcystin complexes were stable[46]. However most of the available data about uptake and turnover of microcystins has been obtained from experiments carried out with rodents. In this regard, it should be noted that PP1 and PP2A from mice and humans amino acid sequences are 100 percent identical[47]. In the case of mice low doses of microsystin cause progressive changes in liver tissue over time, including chronic inflammation, focal degeneration of hepatocytes and the accumulation of metabolites such as bilirubin in the blood, and tend to increase mortality[48]. In South Africa, liver damage and death of vervet monkeys has occurred following toxic *Microcystis* administration with signs of poisoning similar to those observed in live stock and mice[49]. These demonstrations of the susceptibility of primates to cyanobacterial poisoning are consistent with the results of an epidemiological study of a human population of the city of Armidale, New South Wales, Australia, which obtains its drinking water from the Malpas Dam reservoir. A clear pattern of admission of patients to the local hospital with liver complaints was identified which coincided with the seasonal production of a hepatotoxic *Microcystis aeruginosa* bloom in the reservoir. This correlation was confined to patients who had taken their drinking water from the Malpas Dam[50].

Yu[51] in 1995 reported that the incidence of liver cancer is significantly higher for populations using cyanobacteria-infested surface water than those drinking groundwater in China. In Shanghai and its nearby regions where epidemiological studies showed that increased incidence of primary liver cancer is related to the consumption of microcystin contaminated water, the concentrations of microcystins in samples of pond-ditch water were within the range of 0.09-0.46 µg/l[52]. However, Zegura *et al*.[53] showed that microcystin-LR induced oxidative DNA damage in HepG2 human cells at low concentrations (0.01µg/ml) and this might be a mechanism by which chronic exposure to low concentrations of mycrocistins contribute to increase the risk for liver cancer development. A recent study in mice has shown that *Microcystis aeruginosa* extract provided in drinking water increased the area of aberrant crypt foci in the colon, suggestive that microcystins promote preneoplastic colonic lesions[55].

Monitoring toxigenicity of cyanobacterial strains by molecular assay

Monitoring the quality of water destined to public supply includes identification of potentially toxic cyanobacteria and their population density. Identification of such microorganisms based on morphological features only, though widespread, has proven problematic, mainly for the genus *Microcystis*, due to its extensive phenotypic plasticity[56]. Identification of a cyanobacterial genus by microscopic morphology or molecular analysis does not indicate the potential for toxin production. Different strains of one species can be morphologically identical but differ in toxigenicity. *Microcystis aeruginosa* for example has both toxic and nontoxic strains[57].

Table 3. Acute intoxications of humans from cyanobacteria

Year	Report
	Cases attributed to cyanotoxins in drinking water
1931	United States; A massive *Microcystis* bloom in the Ohio and Potomac rivers caused illness in 5 000 to 8 000 persons whose drinking water was taken from these rivers. Low rainfall has caused the water of a side branch of the river to develop a cyanobacterial bloom, which was then washed by new rainfall into the main river. Drinking water treatment by precipitation, filtration, and chlorination was not sufficient to remove the toxins[83, 84].
1960 to 1965	Zimbabwe, Harare; Cases of acute gastroenteritis among European children admitted to the local hospital in Salisbury, Rhodesia (now Harare, Zimbabwe). In this instance, several supply reservoirs provided water to different regions of the city, but only the reservoir containing blooms of *Microcystis* supplied water to the affected population[85].
1968	United States; Numerous cases of gastrointestinal illness after exposure to mass developments of cyanobacteria were compiled by Schwimmer and Schwimmer (1968)[86].
1975	United States; Hindman *et al*. (1975)[87] reported the results of an investigation into 49 pyrogenic reactions in patients undergoing haemodialysis treatment in Washington, DC. They concluded that 'the cause of these reactions was traced to an increase in endotoxin contamination of the tap water used to prepare dialysate, possibly caused by an increase in the algae levels in the local water source.
1979	Australia; Combating a bloom of *Cylindrospermopsis raciborskii* in a drinking water reservoir on Palm Island with copper sulfate led to liberation of toxins from the cells into the water, thus causing serious illness with hospitalization of 141 persons supplied from this reservoir[88,89].
1981	Australia; In the city of Armidale, liver enzyme activities were elevated in the blood of the population that was supplied from surface water polluted by *Microcystis* spp.[50].
1992	United States; Carmichael (1992)[10] compiled case studies on nausea, vomiting, diarrhea, fever and eye, ear, and throat infections after exposure to mass developments of cyanobacteria.
1993	Australia; Ressom *et al*. (1994)[28] estimated that more than 600,000 person-days are lost annually due to absence of their water source due in turn to toxic cyanobacterial blooms. China; The incidence of very high rates of liver cancer is related to water sources. The incidence is significantly higher for populations using cyanobacteria-infested surface waters than those drinking ground water. A cohort study showed that people who drank pond and ditch water had 121 deaths per 100 000 compared with 0 for those who drank well water[51, 90].
1994	Sweden Near Malmo; Illegal use of untreated river water in a sugar factory led to an accidental cross-connection with the drinking water supply for an uncertain number of hours. The river water was densely populated by *Planktothrix agardhii*, and samples taken a few days before and a few days after the incident showed these cyanobacteria contained mycrocistins. Of 304 inhabitants of the village, 121 became ill with vomiting, diarrhea, muscular cramps, and nausea[91].
Date	**Description**
	Cases attributed to cyanotoxins in recreational water
1959	Saskatchewan, Canada; In spite of livestock deaths and warnings against recreational use, people did swim in a lake infested with cyanobacteria. Thirteen persons became ill (headaches, nausea, muscular pains, painful diarrhea). In the excreta of one patient – a medical doctor who had accidentally ingested 300 ml of water-numerous cells of *Microcystis* spp. And some trichomes of Anabaena circinalis could be clearly identified[92].
1989	England; In Staffordshire ten out of 20 soldiers became ill after swimming and canoe-training in water with a heavy bloom of *Microcystis* spp.; two of them develop severe pneumonia attributed to the inhalation of a *Microcystis* toxin and required hospitalization and intensive care. Sixteen develop sore throat, headache, abdominal pain, dry cough, diarrhoea, vomiting and blistered mouths[93]. Swimming skills and the amount of water ingested appear to have been related to the degree of illness.
1995	Australia; Epidemiological evidence of adverse health effects after recreational water contact from a prospective study involving 852 participants who showed elevated incidence of diarrhea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers, and eye or ear irritations within 2 to 7 days after exposure. The sensitivity of individuals to allergic-type reactions at low cyanobacteria cell densities is greater than can be attributed to the toxin content of cyanobacteria[94].
Date	**Description**
	Cases due to other exposure routes
1996	Caruaru in Brazil; One hundred and twenty six dialysis patients were exposed to microcystin through the water used for dialysis, and 60 of them eventually died, principally of liver failure, 6 had died by 2 weeks after exposure, 30 by 6 weeks, 44 by 10 weeks, and 55 by 27 weeks. At least 44 of these victims showed the typical common symptoms associated with microcystin, now referred to as 'Caruaru Syndrome' and the liver microcystin content corresponded to that of laboratory animals that received a lethal dose of microcystin[95,96,97].

There have been numerous attempts to refine the identification of strains by using amplified fragment length polymorphism markers[58], and specific gene analysis. Examples include the use of PCR-based methods for amplification of the phycocyanin intergenic spacer (PC-IGS) between the α and β subunits of the phycocyanin operon in environmental samples[59], the 16S-23S rRNA internally transcribed spacer region[60] and the DNA-dependent RNA polymerase (rpoCI) gene[61]. Although these molecular techniques have improved the accuracy of strain identification, they have not been able to distinguish toxigenic from nontoxigenic strains of the same species.

The biosynthetic pathway for production of *microcystin* has now been elucidated[62] and this has enabled the development of specific oligonucleotide primers for gene common to production of *microcystins*[62]. To better detect microcystin-producing cyanobacterial strains, Neilan *et al.*[63] and Nishizawa *et al.*[64] have developed genetic probes directed, respectively, to the *mcyB* gene and to adenylation domains within the microcystin synthetase gene cluster. The *mcy* gene cluster contains 55kb of DNA encoding six large open reading frames, *mcyA-E* and *-G*, together with a further four small open reading frames *mcyF* and *H-J,* placed in the chromosome[62]. The insertional inactivation of microcystin peptide synthetase gene *mcyB* of a *Microcystis aeruginosa* strain (PCC 7806) resulted in loss of microcystin production, showing their involvement in microcystin synthesis. It was also observed by Dittmann *et al.*[65] that all isoforms of the cyclic heptapeptide were disrupted by inactivation of the microcystin synthetase gene sequence *mcyB*.

Recently, Oberholster[66] reported for the first time in South Africa, the use of microcystin molecular markers for the detection of toxic cyanobacteria, both in cultivated strains and environmental samples in Gauteng and the North West provinces. *Microcystis aeruginosa* and *Microcystis wessenbergii* strains from Rietvlei, Hartbeespoort and Roodeplaat Dams in Gauteng and the North west provinces were analyzed by polymerase chain reaction (PCR) with oligonucleotide primers for the *mcyB* gene of the operon that encodes a microcystin synthetase (Fig. 2). The presence of the gene *mcyB* in three of the four environmental strains indicates that the strains produce microcystin.

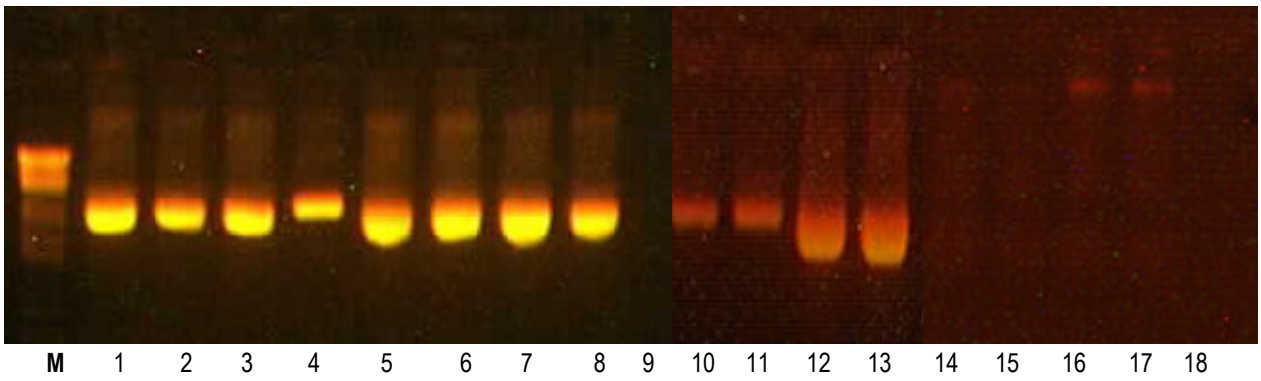

Figure 2: PCR fragments obtained after amplification of *Microcystis* strains with primer pairs specific for *mcyB*. Lanes 1-13 represents *Microcystis aeruginosa* strains, and lanes 14-17 represents *Microcystis wesenbergii*. M = Marker III (lambda DNA restricted with *Eco*RI and *Hind*III), 1 = PCC7813 (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[66]. Bright band indicates 1860 bp

By using the *mcyB* gene in PCR assays, applied directly to environmental samples provide a useful indicator that the analyzed strains have the genetic potential to produce microcystin. Although HPLC provides a direct

measure of toxins present, it does require a large capital investment and considerable sample preparation. The PCR-based assays detect toxigenic cells rather than toxins and require little sample preparation and modest capital costs. Detection of toxic *Microcystis aeruginosa* strains through molecular markers for microcystin may have great use-potential in routine analysis of aquatic ecosystems. Thus, it may make water monitoring more feasible and allow the early application of corrective action before cyanobacteria blooms start to die or disintegrate. The PCR-based assay is effective at a level of 10 cells ml^{-1} and can indicate a possible toxic bloom well before the cell count reaches the action alert at a cell density of 2 000 ml^{-1}, as recommended by the Australian Drinking Water Guideline[67], and a high alert level of 20 000 cell ml^{-1}, where blooms may contain sufficient toxin to be of concern for human health[68].

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