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Cyanotoxins, related health hazards on animals and their management: A Review

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ABSTRACT

Cyanobacteria are one of the largest subgroups of Gram-negative prokaryotic micro-organisms showing oxygenic photosynthesis. Under favorable environmental condition and nutrient enrichment of the water bodies these microorganisms may develop into toxic blooms. Nearly 25 to 75% of the cyanobacterial blooms produce secondary metabolites named cyanotoxins that may harm life forms like zooplankton, shellfish, fish, birds, and mammals. Cyanotoxins belong to a diverse group of chemical substances and can be grouped into: cyclic peptides, alkaloids or lipopolysaccharides; and may be neurotoxic, hepatotoxic, and dermatotoxic. The cyclic peptide toxins of the microcystin, nodularin and cylindrospermopsin family are the most frequent. *Microcystis* is the most predominant hepatotoxin producing cyanobacterium found in blooms and microcystin-LR is the most frequent microcystin variant and a major toxin in bloom. Anatoxins-a is a neurotoxic alkaloid and mimics acetylcholine activity. LD_{50} values of microcystins vary widely and range from 20 to 1,500 µg/kg body weight in fish whereas; the LD_{50} of Anatoxin-a is about 0.2 mg kg^{-1} of body weight of mouse. However, correct identification of cyanotoxins is complicated due to production of more than one type of cyanotoxins in the same bloom. Moreover, these toxins exist in several variants and many of them are yet undescribed. Therefore, this article emphasizes the correct detection of cyanotoxins including their extraction, concentration/cleanup, preservation and determination methods besides, current knowledge about toxicological mechanisms of these cyanotoxins, treatments, and control of fatalities related to cyanotoxins by proper monitoring through prescribed guidelines and standards.

Key words: Animals, Blooms, Cyanotoxins, Monitoring, Toxin detection

Cyanobacteria or blue-green algae belong to a diverse group of photosynthetic prokaryotes that possess mainly chlorophyll-A (Whitton and Potts 2000). These are frequently found growing in marine, brackish- and fresh-waters. Sometimes they produce massive annual growth as a consequence of nutrient enrichment of natural waters from agricultural fields by run off, or from domestic, industrial and sewage effluents resulting into eutrophication of water and formation of blooms and scums of planktonic species, mats and biofilms of benthic species (Codd *et al.* 2005a, Singh *et al.* 2008). Most of the blooms forming cyanobacteria produce various types of bioactive/toxic secondary metabolites commonly known as cyanotoxins which are injurious to animal health (Ferrao-Filho and Kozlowsky-Suzuki 2011). These cyanotoxins show specific toxic mechanism and belong to a diverse group of chemicals like, cyclic peptides (microcystins, nodularins), alkaloids (anatoxins, cylindrospermopsin, saxitoxins, lyngbyatoxin) or lipopolysaccharides (LPS); and may possess hepatotoxic, neurotoxic or dermatotoxic effects (Blaha *et al.* 2009). These toxin producing cyanobacteria may threaten animal health and can disrupt aquatic ecosystems both in freshwater and marine environments. Hence, it is imperative to review the various cyanobacteria producing toxins and their effects on animals, factors affecting toxin production, symptoms, toxin detection, treatment, prevention and control.

Cyanobacterial blooms: Development of artificial water reservoirs, diverting natural streams and increased pollution of the freshwater bodies interfere with their natural flushing. The situation becomes more alarming by anthropogenically increased nutrient levels (especially phosphorous) and temperature resulting into development of freshwater cyanobacterial blooms (Wulff *et al.* 1990). Algal blooms adversely affecting the environmental, animal or plant health are called cyanobacterial harmful algal blooms (CyanoHABs) (Backer 2002). Nearly 25 to 75% of the cyanobacterial blooms are toxic in nature (Blahova *et al.* 2009, Chorus 2001). These blooms normally float on the surface and can be many inches thick, especially near the shoreline. They

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normally can occur at any time, but are most often in late summer or early fall, and appear blue, bright green, brown, or red like paint floating on the water surface.

Blooms develop in warm, slow-moving waters that are rich in nutrients such as fertilizer runoff, sewage disposal or septic tank overflows. Both heterocystous and non-heterocystous cyanobacteria have the capability to scavenge available nitrogen (NO^{3-} and NH^{4+}) and phosphorous from secondary treated sewage effluents. A positive correlation co-efficient was also observed between available nitrogen removal with dry weight and chlorophyll content which shows the usefulness of sewage effluent for bloom like growth of microalgae (Singh and Dhar 2006, 2007). The cyanobacterial genera producing toxins include mainly, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis, Anabaena*, *Nodularia, Oscillatoria, Synechococcus, Aphanothece*, *Chroococcus*, *Snowella, Merismopedia*, *Lyngbya*, *Planktothrix* etc. (Codd *et al.* 2005a, 2005b). The blooms that occur in freshwater reservoirs are of great concern especially in the tropical regions. Moreover, the geographical extent and magnitude of cyanobacterial bloom population are suspected to have increased due to enhanced nutrient concentrations and changed nutrient ratios.

Cyanotoxins: Cyanotoxins belong to a rather diverse group of chemical substances which show specific toxic mechanisms (Carmichael 1994). These toxins reportedly affect liver (hepatotoxins: microcystins, nodularin and cylindrospermopsin), nervous system (neurotoxins: anatoxin-a, homoanatoxin-a, anatoxin-a(s), saxitoxins) and skin (dermatotoxic alkaloids and lipopolysaccharides) besides causing problems like skin itch and gastroenteritis (Sivonen and Jones 1999). However, cyanobacterial toxins may be classified chemically into (a) cyclic peptides – microcystins and nodularins, (b) alkaloids – anatoxin-a, anatoxin-a(s), homoanatoxin-a, cylindrospermopsin, saxitoxin and neosaxitoxin, aplysiatoxin and lyngbyatoxin-a, and (c) lipopolysaccharides (LPS).

On the basis of bioassay, cyanotoxins are classified as cytotoxin and biotoxin (Sivonen and Jones 1999). Cytotoxins show wide range of bioactivity against algae, bacteria, fungi and mammalian cell lines (Carmichael 1997), and include acutiphycins, indocarbazoles, mirabilene, isonitriles, paracyclophane, scytophycins, tentazoles, tolytoxin, toyocamycins and tubercidin (Patterson *et al.* 1991). They possess structures different from the molecules already discovered from other natural sources (Carmichael 1997). Biotoxins are produced by species and strains of several planktonic cyanobacteria (*Anabaena*, *Microcystis, Nodularia, Nostoc* and *Oscillatoria*). Acute poisoning was not observed from biotoxins because animals are repelled by the intention of using water containing an earthy and musty odour caused by geosmin and 2-methyl isoborneol compounds produced by cyanobacteria (Gorham and Carmichael 1988).

Hepatotoxic cyclic peptides: The most frequently cyanobacterial toxins found in blooms are the cyclic peptide toxins of the microcystin, nodularin and cylindrospermopsin family. Microcystins are produced by *Microcystis, Anabaena, Oscillatoria (Planktothrix), Nostoc, Anabaenopsis* and *Hapalosiphon* species, nodularins are produced only by *Nodularia* species, and cylindrospermopsin by *Cylindrospermopsis raciborskii, Aphanizomenon flos-aquae, Anabaena planctonica, Anabaena lapponica, Lyngbia wollei etc*. *Microcystis* is reported as the most predominant hepatotoxin producing cyanobacterium and was observed in several water bodies, for example, Lake Erie (Brittain *et al.* 2000, Murphy *et al.* 2003), Lake Huron (Vanderploeg *et al.* 2001) and Lake Ontario (Murphy *et al.* 2003). The cyclic peptides are large natural products of about 800–1,100 molecular weight (Rinehart *et al.* 1994), water soluble and unable to penetrate the animal cells and lipid membrane of animals directly. Therefore, their toxic effect is elicited only after their uptake into cells through membrane transporters. These toxins usually remain contained within the cyanobacterial cells and are only released in substantial amounts after cell lysis hence are called intracellular toxins (Sivonen and Jones 1999). However, these toxins are occasionally liable to chemical, physical, and biological degradation (Hrudey *et al.* 1999).

Microcystins are present in high amounts in cyanobacterial biomass (up to 1% of dry weight) (Blaha *et al.* 2009). All microcystins detected till date are cyclic heptapeptide, constitute a much larger group of compounds, and show a greater diversity in their structures (Rinehart *et al.* 1994). *Microcystis* strains appear to contain chiefly microcystin-LR, -RR and -YR, with some cultures showing all three variants while some others are being dominated by only one of them (Rinehart *et al.* 1994, Watanabe 1996). Microcystin-LR is often mentioned as the most frequently occurring microcystin variant and has been reported to be the major toxin in bloom (Vezie *et al.* 1997). These are synthesized via multifunctional enzymes that include polypeptide synthetase and polyketide synthase modules. However, as such, these potentially toxic cells have no unique morphometric traits (Chorus and Bartram 1999). LC50 (72 h) of microcystins LR was determined to be 0.242 µg/mL and LD50 (72 h) as 49.19 g kg^{-1} body weight in fish, *Astyanax bimaculatus*, a native species from South America (Da Silva *et al.* 2010). However, the LD50 values of microcystins vary widely in fish and range from 20 to 1500 µg microcystin LR/kg body weight.

Nodularia spumigena is a bloom forming cyanobacterium and its toxin is characterized as nodularin (Rinehart *et al.* 1988). The most remarkable characteristic of nodularin is the presence of the C20 amino acid, 3-amino-9-methoxy-2, 6, 8 -trimethyl-10-phenyl-4, 6-decadienoic acid (abbreviated *Adda*), with its þ-amino group, multiple methyl substitution and diene unit (Namikoshi *et al.* 1989, Rinehart *et al.* 1988). The structure of these two cyanobacterial toxins is similar

and they possess similar IC_{50} and LD_{50} values (Runnegar *et al.* 1988). However, Microcystin-LR differs from nodularin in having two additional amino acids: D-alanine and L-leucine (Rinehart *et al.* 1994). The two-letter suffix is derived from the variability of the molecule as result of amino acid substitutions at positions 2 and 4 of the heptapeptide ring (Metcalf and Codd 2004).

The mode of toxicity is hepatotoxicity in which blood is concentrated in the liver, which gets swollen several times its normal size (Hooser *et al.* 1989). The cyclic hepatotoxins exert their hepatotoxicity through protein phosphatases inhibition and may cause haemorrhage into liver (Matsushima *et al.* 1990, Runnegar *et al.* 1988). Microcystin is also believed to cause damage to the DNA by activation of the endonucleases (Jochimsen 1998). Regarding tumour promotion microcystin-LR and nodularin function in the similar manner as okadaic acid (Fujiki *et al.* 1991, Nishiwaki-Matsushima *et al.* 1992). Symptoms of acute hepatotoxin poisonings in animals has been described as whitening of the mucous membranes, vomiting, cold extremities, diarrhoea and death which occur by haemorrhage within the liver tissues (Carmichael 1992).

However, cylindrospermopsin is a protein synthesis inhibitor (Froscio *et al*. 2003, Kinear *et al*. 2008), genotoxic (Humpage *et al.* 2000) and probably carcinogenic (Falconer and Humpage 2006) and results in widespread necrosis of the tissues of many organs and at lower doses may cause enteritis and hepatitis shortly after the ingestion of these toxins (Fastner *et al*. 2003, Manti *et al.* 2005).

Alkaloids: Alkaloids, in general belong to a broad group of heterocyclic nitrogenous compounds with low to moderate molecular weight (<1000). The cyanobacterial alkaloids are mainly neurotoxic, cytotoxic and dermatotoxic. Neurotoxic alkaloids are extremely potent toxins, termed as "very fast death factors" (VFDF) (Gugger *et al.* 2005). Although, anatoxin-a and anatoxin-a(S) are unique to the cyanobacteria, saxitoxins and neosaxitoxins are also produced by dinoflagellates (Carmichael 1994). Three types of cyanobacterial neurotoxins known are anatoxins, saxitoxins and neosaxitoxins.

Neurotoxic alkaloids: Anatoxins are a group of neurotoxic alkaloids produced by a number of cyanobacterial genera including *Anabaena*, *Oscillatoria* and *Aphanizomenon*. Anatoxin-a, a secondary amine alkaloid and a structural analogue of cocaine, has a molecular weight of 165. The toxicity (LD_{50}) of these compounds is found to be about 0.2 mg kg^{-1} of body weight of mouse (Hjelmgaard *et al.* 2005). It mimics acetylcholine activity and binds to neuromuscular nicotinic receptors with greater affinity than acetylcholine (Karlin 2002). Anatoxin-a (S) (S means "salivation factor") is quite different from anatoxin-a in structure and toxicity and is the only naturally occurring organophosphate isolated from *Anabaena* species, including *A. flos-aquae*, *A. spiroides*, and *A. circinalis* (Carmichael 1992, 2001). It is a phosphate ester of cyclic N-hydroxy guanine with the molecular weight of 252 (Henriksen *et al.* 1997). The toxicity of these compounds (LD_{50}) has been observed to be about 20 µg kg^{-1} body weights in mouse (Carmichael 2001). These toxins act as post-synaptic acetylcholine antagonist, resulting in paralysis, asphyxiation, decreased movement, collapse, exaggerated abdominal breathing, cyanosis, convulsion, and ultimately death (Kuiper-Goodman *et al.* 1999).

Saxitoxins and neosaxitoxins are neurotoxic alkaloids and are also known as paralytic shellfish poisons (PSPs) due to their occurrence and association with seafood. These were isolated from *Anabaena flos-aquae*, *Anabaena circinalis*, *Lyngbya wollei* and *Cylindrospermopsis raciborskii* (Carmichael *et al.* 1997, Negri *et al.* 1995). These are highly toxic with LD_{50} as low as 10 µg kg^{-1} of body weight in mice (Carmichael 1994). The alkaloids produce neurotoxic effects by inhibiting nerve conduction by blocking sodium channels.

Cytotoxic alkaloids: This is a cyclic guanidine alkaloid having a molecular weight of 415 and was isolated from *Cylindrospermopsis raciborskii* (Hawkins *et al.* 1997), *Umezakia natans* (Harada *et al.* 1994) and *Aphanizomenon ovalisporum* (Banker *et al.* 1997). It is a potent inhibitor of protein synthesis and causes fatty liver and centrilobular necrosis. Crude extracts of *C. raciborskii* also induce pathological symptoms in the kidneys, spleen, thymus and heart in mouse (Chiswell *et al*. 1999). Pure cylindrospermopsin has an LD_{50} of 1–2.1 mg kg^{-1} body weight at 24 h in mice (Ohtani *et al.* 1992).

Dermatotoxic alkaloid: Aplysiatoxins are produced by some marine cyanobacteria such as *Schizothrix*, *Lyngbya,* and *Oscillatoria*. The compound is a protein kinase C activator, inflammatory and tumor promoter (Mynderse *et al.* 1977). The toxins cause headache and dizziness upon exposure. Debromoaplysiatoxin was also found associated with filamentous cyanobacterial species including *Schizothrix calcicola* and *Oscillatoria nigroviridis*. These are known for their dermatotoxic activity, cause inflammation of the skin, and may also act as tumor promoters. However, lyngbyatoxin-a is an alkaloid that was characterized from the cyanobacterium *Lyngbya majuscula* and causes dermatitis and severe skin, oral and gastrointestinal inflammation (Cardellina *et al.* 1979).

Lipopolysaccharides: Lipopolysaccharides (LPS) were reported for the first time from *Anacystis nidulans* by Weise *et al.* (1970). Lipopolysaccharides are found in the outer membrane of cyanobacteria where they form complexes with proteins and phospholipids and are considered to be pyrogenic and toxic (Codd *et al.* 2005a). These are usually complex products of a hexose sugar with a hydroxyl fatty acid (C14-C18). These are phylogenetically conserved among various groups of cyanobacteria. Thus, different genera have distinct lipopolysaccharides compositions that are largely conserved within a specific genus (Kerr *et al.* 1995). It is the fatty acid component of the lipopolysaccharides which affect

the exposed tissues and produce irritant of allergenic response in mammals (Sivonen and Jones 1999).

Some reports indicated that cyanobacterial lipopolysaccharides are less toxic than that of other bacteria, such as *Salmonella* (Raziuddin *et al.* 1983). However, the cyanotoxins are considered to have great industrial importance for production of several bioactive compounds. The compound tanikolide 1 from the lipid extract of *Lyngbya majuscula* has been reported to be antifungal and toxic to brine shrimp (Singh *et al.* 1999). The lipophilic extract of *Phormidium ectocarpi* yielded a new natural product hierridin B, which showed antiplasmodial activity towards *Plasmodium falciparum* (Papendorf *et al.* 1998).

Animal intoxication: The animals can experience acute or lethal toxicity depending upon the bloom density, toxin content and the quantity of water ingested. In 1878, George Francis presented the first scientific report of the toxic nature of cyanobacteria. *Anabaena* related cattle and other animal deaths at an Ontario Lake have been discussed by Howard and Berry (1933). *Anabaena flos–aquae* blooms in the Storm Lake in Iowa in 1952 resulted in an estimated death of about 7,000 gulls, 560 ducks, 400 coots, 200 pheasants, 50 squirrels, 18 muskrats, 15 dogs, 4 cats, 2 hogs, 2 hawks, 1 skunk, 1 mink, and many songbirds (Firkins 1953). Domestic animals and wildlife consuming water contaminated with toxic blue-green algal blooms may die from acute neurotoxicity and/or hepatotoxicity (Jochimsen 1998, Negri *et al.* 1995). A large number of birds, wild animals, cattle, sheep, pigs and horse intoxication have been ascribed to cyanotoxins (Bury *et al*. 1995, Carmichael 1992, Soll and Williams 1985). The death of white rhinoceroses (Soll and Williams 1985) and bats (Pybus *et al*. 1986) was attributed to the hepatotoxic *Microcystis aeruginosa* and neurotoxic *Anabaena flos-aquae* bloom, respectively.

The influx of untreated sewage result into sudden appearance of toxic bloom of *Microcystis aeruginosa* and unnatural death of the spot-billed ducks in Shin–ike pond in Japan (Matsunaga *et al*. 1999). Sheep exposed to *Microcystis aeruginosa* in a lake in Australia for six months showed a mortality rate of 34% without clear etiology (Carbis *et al.* 1995). The microcystins can cause tumor promotion in animals exposed to low level non-lethal doses (Carmichael 1994). Development of hepatic tumors by microcystins in rats is mediated through the inhibition of protein phosphatase type 1 and type 2A activity (Hong-Bing and Hui-Gang 1996). Lyngbyatoxin-a, a dermatotoxic alkaloid, has also been detected to be a potent tumor promoter in mouse (Fujiki *et al*. 1984). Many reports of animal poisonings were found associated with *Nodularia* blooms (Kuiper-Goodman *et al.* 1999). Harding *et al.* (1995) reported death of dog after drinking of water from an urban lake having a bloom of 95% *Nodularia spumigena* and the remainder of *M. aeruginosa*. The most important diseases caused by red tides are paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP) and ciguatera fish poisoning (CFP) (Christian and Luckas 2008, Lueger *et al.* 1999).

Cyanobacterial toxins like microcystins and anatoxin–a caused mortalities in flamingos in the Kenyan soda lakes (Codd *et al.* 2003). Similar flamingo poisonings were also reported from alkaline lakes in Tanzania from toxic *Arthrospira fusiformis* (Lugomela *et al.* 2006). Negri *et al.* (1995) reported death of 13 ewes and 1 ram in Australia from a bloom of *Anabaena circinalis*. The toxins detected were C–toxins and gonyautoxins, with low levels of saxitoxin and decarbamoyl saxitoxin. *Anabaena lemmermannii* associated death of waterbirds at Lake Knud in Denmark in 1993 was reported from toxic bloom (Henriksen *et al.* 1997). Intra peritoneal injections of the gut and gut contents of boiled edible mussels from a water bloom of *Nodularia* in Western Australia to mice were lethal. The *Nodularia* bloom LD_{50} was reported to be 24.4 mg dry weight/kg of body weight (Falconer *et al*. 1992). Thus, it was concluded that the hepatotoxins produced by blue-green algal blooms are potential toxins which may result into mass killing of animals in contaminated waters.

Factors influencing toxin production: Temperature, light, and nutrients level and composition are the main factors affecting toxin production. Variation of cellular toxin levels under different growth conditions have been studied mainly with the batch cultures of *Microcystis*, *Oscillatoria*, *Anabaena, Anabaena*, *Aphanizomenon,* and *Planktothrix* (Rapala 1998, Sivonen and Jones 1999). Environmental factors control toxin concentration in *Microcystis* by affecting the rate of cell division and not through any direct effect on the metabolic pathways of toxin production (Sivonen and Jones 1999). The intracellular microcystin concentration is related to N: P ratio of the medium. However, extracellular cyanotoxins in water body shows seasonal fluctuation depending upon the physico-chemical factors of water bodies and cyanobacterial species dominating at particular time (Jiang *et al.* 2008). They used statistical approach to study the effect of various environmental factors including light intensity, temperature and iron on the growth and microcystins production by *Microcystis aeruginosa*.

Temperature controls hepatotoxin concentrations in many blue-green algae, such as *Anabaena*, *Microcystis*, and *Oscillatoria*. Highest toxin concentrations (Sivonen 1990) or toxicities (Ohtake *et al*. 1989) were detected from cyanobacterial cells grown between 18–25°C whereas; *Nodularia* cells produce highest toxin concentrations at temperatures of about 20°C (Sivonen 1990). The effects of temperature on toxin concentrations in *Microcystis*-blooms in eutrophic lakes show contradictory results. In the Hartbeespoort Dam (South Africa), microcystin-YR, -LR, -YA and -LA concentrations were reported to have positive correlation with the solar radiation and water temperature

whereas; microcystin-YR and -YA correlated negatively with PO_4^{3-} concentration (Wicks and Thiel 1990). However, in the Coal, Driedmeat, and Little Beaver lakes (Canada), no correlation between microcystin-LR and water temperature was discovered whereas; the toxin concentration correlated positively with the total and dissolved P and negatively with NO^{3-} content (Kotak *et al.* 1995).

In Babtiste, Narrow, Skeleton, Steele, the Coal, Little Beaver and Driedmeat lakes (Canada), microcystin-LR correlated positively with total P and total N levels (Zurawell *et al.* 1999). In Lake Grand-Lieu (France), microcystin-LR and one of microcystin-RR variants correlated negatively with solar radiation, and both microcystin-RR variants correlated positively with the dissolved P and NO^{3-} (Vezie *et al*. 1998). Again, in Lake Tuusulanjärvi (Finland), microcystin-LR correlated positively with total N and total P and negatively with the dissolved NO^{3-} (Lahti *et al.* 1997). The corresponding analysis of field data on environmental factors during 78 water blooms from 72 Finnish lakes revealed that hepatotoxic *Microcystis* blooms correlated with high PO_4^{3-} concentrations (Rapala and Sivonen 1998).

Some studies have suggested that light had no marked effect on hepatotoxicity (Codd and Poon 1988) whereas other studies noticed a considerable change in toxicity or toxin concentration with variations in light intensity (Watanabe and Oishi 1985, Utkilen and Gjolme 1992). *Nodularia* strain BY1 was reported to produce low concentration of nodularin at light intensity of 2 μmol $m^{-2}s^{-1}$ than at higher light levels (Holswilder 1999). Light limitation was also reported to decrease the concentration of hepatotoxin in *Anabaena* (Rapala and Sivonen 1998) and neurotoxin in *Aphanizomenon* (Rapala *et al*. 1993). However, the concentration of microcystin in *Oscillatoria* (Sivonen 1990) decreased at high light levels; and that of *Anabaena* at both high and low light levels (Rapala *et al.* 1993). Light control microcystins content and also regulate the composition of microcystin variants (Rapala *et al.* 1997).

Utkilen and Gjolme (1995) studied the effect of iron on toxin production by *Microcystis aeruginosa* and concluded that the influence of iron uptake on toxin concentration of *Microcystis* is light dependent. The influence of physiological factors on the production of heptapeptide microcystin from *Nostoc ktalis* sp. strain 152 has been studied by Kurmayer (2011), who observed maximum microcystin content per cell under low P and irradiance. Subsequently, the dynamics of toxic and non-toxic cyanobacterium *Microcystis* in correlation with environmental factors were measured using molecular techniques (Joung *et al.* 2011).

Marked differences in nodularin concentrations between cultures grown under different temperatures, salinities and P concentrations can be observed in *Nodularia* (Blackburn and Jones 1995, Heresztyn and Nicholson 1997). However, it decreased with low and high salinities and high inorganic N (Sivonen 1990). It has been observed that nodularin concentrations decrease on both the gravimetric and cellular basis at a salinity of 35% (Blackburn *et al.* 1996; Holswilder 1999). Nodularin production reported to decrease under light limitation and increase under P limitation (Holswilder 1999). However, N addition was observed to promote growth and increase the nodularin content of *Nodularia* (Carmichael *et al.* 1988). But at the highest N concentration, there was a negative effect of N on the nodularin concentration. Moreover, N-addition changes the proportion of different microcystin variants in *Anabaena*, decreases the total intracellular concentration, and increases the concentrations of extracellular toxins in growth media (Rapala *et al.* 1997). The sewage effluent, being rich in N and P when used as a medium for growth of cyanobacteria, resulted in increase of cellular total nitrogen, nitrate reductase and glutamine synthetase which are important factors favoring rapid multiplication of biomass (Singh and Dhar 2007).

Toxin detection: The same cyanobacteria can produce more than one type of toxin during a bloom and very often the presence of toxin is masked by the premature death of the animal (Chorus *et al.* 2000, Falconer 1996). In addition, there exist other toxins (including lipopolysaccharides, endotoxins and additional neurotoxins) as well as yet undescribed cyanobacterial toxins including additional tumor promoters (Chorus *et al.* 2000, Falconer 1994, 1996). Some toxins like microcystins exist in several variants and these differ markedly in toxicity (Carmichael 1992), which generally interferes with the applicability of a particular method of toxin identification and quantification (Sivonen and Jones 1999). HPLC along with mass spectral analysis (HPLC-MS) may facilitate more accurate detection of toxin variants but as several microcystins share the same molecular mass, definitive identification may be difficult (Lawton *et al.* 1995). Bioassays were assumed to be one of the most common methods for the determination of biotoxins (Christian and Luckas 2008).

Currently, routine analysis of cyanotoxins is most commonly carried out using high-performance liquid chromatography with photodiode array detection (HPLC-PDA), although more sensitive biological assays such as antibody-based Enzyme Linked Immunosorbent Assays (ELISAs) and protein phosphatase inhibition assays have also proven useful (Ikawa *et al.* 1999, Watanabe *et al.* 1988). New measurements for detecting the presence and levels of cyanobacterial toxins in natural waters using HPLC coupled to tandem mass spectrometry has been discovered (Hedman *et al.* 2008). The immunological examination technique is fast, easy and accurate even when trace amount of phycotoxins are present. The prospects of these methods and their application in phycotoxin determination lead to the introduction of the theory of immunological diagnosis (Guo 1999). New technologies employing recombinant antibodies and molecularly imprinted polymers have been exploited to develop assays and biosensors for cyanotoxins. These novel

Table 1. Extraction, concentration, preservation and determination of various cyanobacterial toxins

Cyanotoxins	Procedures	Methods	References
Neurotoxins [Anatoxins anatoxin a(s), anatoxin-a, homoanatoxin-a]	Extraction	Extracted using aqueous acid containing an alcohol.	Haugen *et al.* 1994, Matsunaga *et al.* 1989
	Concentration/cleanup	By ultracentrifugation after solvent and solid-phase extraction at alkaline pH or using cation exchange cartridges or styrene-divinylbenzene cartridges in the presence of an ion-pairing reagent at neutral pH.	Harada *et al.* 1989, Haugen *et al.* 1994, Hormazabal *et al.* 2000
	Sample preservation	Rapid degradation in sunlight at alkaline pH. In dark at pH 6, it remains stable for several months. So samples should be preserved in dark at pH around 6 if analyses are not performed immediately.	Stevens and Krieger 1991
	Determination of anatoxins	Anatoxin-A has an absorption maxima of 227nm, detected mainly by High Performance Liquid Chromatography (HPLC). Other methods are Thin Layer Chromatography (TLC) for qualitative or semi-quantitative analysis or Gas chromatography/Mass spectrometry (GC/MS) analysis following conversion of anatoxin-a to more volatile derivatives.	Harada *et al.* 1989, Haugen *et al.* 1994, Himberg 1989, Hormazabal *et al.* 2000, James *et al.* 1998, Stevens and Krieger 1988, Takino *et al.* 1999
Neurotoxins (Saxitoxins)	Extraction	Cell lysis in aqueous acetic acid.	Rositano *et al.* 1998, Ravn *et al.* 1995
	Concentration/cleanup	Direct analysis using High Performance Liquid Chromatography with post-column derivatisation and fluorescence detection.	Rositano *et al.* 1998
	Sample preservation	During preservation it turns more toxic; aqueous acetic acid solution may be the best choice for preservation of saxitoxins.	Jones and Negri 1997, Rositano *et al.* 1998
	Determination of saxitoxin	Saxitoxins (PSPs-Paralytic Shell-fish Poisoning) are determined analytically by High Performance Liquid Chromatography. Other methods include ultraviolet (UV) or mass spectrometry, Enzyme Linked Immunosorbent Assays (ELISAs), neuroreceptor and cytotoxicity assays in neuroblastoma cells of cultured mouse.	Cembella and Lamoureux 1993, Cembella *et al.* 1995, Jellett *et al.* 1995, Lawrence *et al.* 1995. Onodera *et al.* 1996, Oshima *et al.* 1989, Quilliam 1996, Sullivan 1990, Thibault *et al.* 1991
Peptide hepatotoxin (Microcystins and nodularin)	Extraction	Toxin is extracted after its release by cell lysis with algicide such as $CuSo_4$ and freeze-thawing. These may also be extracted in mixture of acetic acid and water or alcohol (75% methanol) in conjunction with sonication, simple lysis and heating in water bath or microwave oven using water as solvent.	Fastner *et al.* 1998, Harada *et al.* 1988b, Jones and Orr 1994, Lawton *et al.* 1994, Metcalf and Codd 2000, Rositano and Nicholson 1994, Ward *et al.* 1997
	Concentration/cleanup	Concentrated in volume by evaporation of solvents such as methanol after solid-phase extraction usually using an octadecyl (C18) coated silica adsorbent cartridge.	Gathercole and Thiel 1987, Harada *et al.* 1988a, Kondo *et al.* 2000, Lawton *et al.* 1994, Ojanpera *et al.* 1995; Pyo and Lee 1994, Tsuchiya and Watanabe 1997, Tsuji *et al.* 1994b

(Contd..)

(Concluded Table 1.)

Cyanotoxins	Procedures	Methods	References
	Sample Preservation	These toxins are relatively stable in aqueous solutions of high purity or sterile water. However, in the presence of pigments, photochemical degradation can occur rather quickly. Also susceptible to microbiological degradation.	Gjolme and Utkilen 1996, Harada *et al.* 1996b, Heresztyn and Nicholson 1997, Hyenstrand *et al.* 2001, Jones *et al.* 1994, Watanabe *et al.* 1992, Tsuji *et al.* 1994a
	Determination	By HPLC- reversed phase C18 packed column/ ion exchange column and an aqueous mobile phase containing methanol or acetonitrile. Most microcystins and nodularin have absorption maxima at 238 nm. Other detection methods include photo-diode array (PDA) detector, mass spectrometry (MS) followed by High Performance Liquid Chromatography separation, MALDI-TOF mass spectrometry, Capillary Electrophoresis (CE), Thin Layer Chromatography (TLC), gas chromatographic (GC) method followed by mass spectrometry (as its methyl ester), fluorescence detection (after conversion to a fluorescent derivative), Enzyme Linked Immunosorbent Assays, phosphatase inhibition assays etc.	Boland *et al.* 1993, Chu *et al.* 1989, 1990, Harada *et al.* 1996a, 1988a, Heresztyn and Nicholson 2001, Ikawa *et al.* 1999, Isobe *et al.* 1995, Lambert *et al.* 1994, Lawton *et al.* 1994, 1995, Li *et al.* 1999, Metcalf *et al.* 2001, Nagata *et al.* 1995, Ojanpera *et al.* 1995, Rivasseau *et al.* 1999a, 1999b, Robillot *et al.* 2000, Sherlock *et al.* 1997, Sim and Mudge 1993, Tanaka *et al.* 1993, Tsuji *et al.* 2001, 1994b, Ueno *et al.* 1996, Ward *et al.* 1997, Watanabe *et al.* 1988, *Zeck et al.* 2001, Zweigenbaum *et al.* 2000
Cylindrospermopsin	Extraction	By freeze thawing of water samples in aqueous methanol or 5% acetic acid for freeze-dried material.	Banker *et al.* 1997, Eaglesham *et al.* 1999, Harada *et al.* 1994, Hawkins *et al.* 1997
	Concentration/Cleanup	While water probably is an adequate extraction solvent for this hydrophilic toxin, extraction for quantitative determination of intracellular toxin as a component of the total water concentration requires further attention.	Bailey *et al.* 1999, Cullum 2001, Eaglesham *et al.* 1999
	Sample Preservation	Strongly adsorbed by polyethylene. Cylindrospermopsin is stable in the dark in water and little loss by chemical decomposition occurs over several weeks between pH 4 and 10, and at temperatures up to 50°C. However, sunlight rapidly degrades cylindrospermopsin in the presence of cellular material.	Chiswell *et al.* 1999
	Determination	It is hydrophilic, and hence, is extracted and concentrated from water samples with solid phase extraction cartridges such as C18 and determined by HPLC. Absorption maxima of cylindrospermopsin are 262nm. A method for monitoring cylindrospermopsin using liquid chromatography/ Mass spectrometry (LC/MS) has also been reported.	Eaglesham *et al.* 1999, Harada *et al.* 1994

detection systems are highly sensitive, often do not require sample processing, and offer a simpler, less expensive alternative to analytical techniques (McElhiney and Lawton 2005). A detailed description of extraction, concentration/ cleanup and preservation and determination method for various cyanotoxins reported by various workers is given in Table 1.

Treatment: Specific treatment does not exist for the

Table 2. Important cyanotoxins, their effects, and symptoms

Cyanotoxins	Effects	Symptoms	References
Anatoxin-a	Neurotoxicity	Muscle fasciculations, decreased movement, abdominal breathing, cyanosis, convulsions, death, Opisthotonos ("s"-shaped neck) in birds.	Fawell *et al.* 1999, Hjelmgaard *et al.* 2005, Karlin 2002
Anatoxin-a (s)	Neurotoxicity	Hypersalivation, mucoid nasal discharge, tremors, fasciculations, ataxia, diarrhea, recumbency in pigs; regurgitation, paresis, opisthotonos, clonic seizures in rabbits; lacrimation, hypersalivation, urination, defecation, death from respiratory arrest in mice, red-pigmented ears in rats.	Carmichael 1992, 2001, Henriksen *et al.* 1997, Kuiper-Goodman *et al.* 1999
Saxitoxin, neosaxitoxin	Neurotoxicity	Animals suffer incoordination and death by respiratory failure.	Carmichael *et al.* 1994, 1997, Negri *et al.* 1995
Cylindrospermopsin	Hepatotoxicity, renal toxicity, chromosome breakage, aneuploidy	Mice show huddling, anorexia, diarrhoea, gasping respiration.	Falconer and Humpage 2006, Fastner *et al*. 2003, Froscio *et al*. 2003, Kinear *et al*. 2008, Manti *et al.* 2005
Microcystins	Hepatotoxicity	Mice show elevated alanine aminotransferase level; embryo lethality, teratogenicity in rats; mammals exhibit weakness, reluctance to move, anorexia, pallor of extremities and mucous membranes, mental derangement, survivors may be photosensitized.	Brittain *et al.* 2000, Carmichael 1992, Hrudey *et al.* 1999, Metcalf and Codd 2004, Murphy *et al.* 2003, Namikoshi *et al.* 1989, Rinehart *et al.* 1988, 1994, Sivonen and Jones 1999, Vanderploeg *et al.* 2001; Vezie *et al.* 1997
Nodularin	Hepatotoxicity	Animals suffer inhibition of protein phosphatases, tumor-promotion.	Rinehart *et al.* 1988, 1994, Runnegar *et al.* 1988, Metcalf and Codd 2004

cyanobacterial toxins till date, the only treatment available for exposure to these toxins is supportive medical treatment after complete removal from the toxin exposure (Chorus and Bartram 1999). In acute gastroenteritis associated with cyanotoxins, monitoring of volume, electrolytes and liver and kidney function should be considered. Administration of activated carbon is efficacious if given within hours of oral exposure due to reduction of the gut absorption of toxins. However, activated carbon is not an effective antidote for preventing effects from subsequent toxin administration (Beasley *et al.* 1989). Artificial respiration can also be considered in case of exposure to the neurotoxins (NHMRC 1994). Monoclonal antibodies show protective effects against hepatotoxicity and inhibition of protein phosphatase of microcystin LR (Nagata *et al.* 1995). Chemoprotectants can be used in pre- treatment prior to exposure of experimental mice to microcystin. Phenobarbital (neither calcium channel blockers nor water soluble anti-oxidants) provide partial protection while, the hydrophobic antioxidants (Vitamin E and silymarin), glutathione active compounds (glutathione), and immuno suppressive agents (rifampin and cyclosporine A) provide significant protection, if administered 48 h prior to exposure to microcystin in the laboratory animals (Hermansky *et al.* 1991). Although, prevention of exposure of animals is the best way to avoid adverse health effects of the cyanotoxins, Important cyanotoxins, their effects, symptoms and therapy are given in Table 2.

Monitoring of harmful cyanobacteria: Monitoring of these harmful microorganisms and their bioactive compounds is considered crucial for prevention of ill-health and other toxin related problems (NHMRC 1994). Monitoring of recreational and surface drinking water supplies in Florida with algal blooms have found 87 out of 167 samples with significant levels of toxin producing blue green algae (SJRWMD 2000). Monitoring for cyanotoxins should include visual monitoring for blooms, cell counts, cyanobacterial identification, toxin identification and toxicity testing. Other monitoring indices like phosphorus levels in the water, surveillance for ill-health effects in human and animal populations were also considered (Chorus and Bartram 1999). A cell density of 20,000 cyanobacterial cells ml^{-1} is recommended as the maximum safe limit in the top meter of open water in recreational water bodies and if the bloom is toxic, swallowing or bathing in these waters should be considered hazardous (Falconer 1994).

Australia and the UK have developed such monitoring and response programs for surface drinking water sources (Burch 1993, NHMRC 1994) with alert levels and corresponding responses based on the number of cyanobacterial cells ml^{-1} in routine sampling. For example,

Burch (1993) and Chorus and Bartram (1999) proposed Alert Levels 1 (500–2,000 cells ml^{-1} or offensive odor or taste), Level 2 (2,000–15,000 cells ml^{-1}, confirmed toxic bloom, persistent odor/taste, and obvious bloom), and Level 3 (>15,000 cell ml^{-1}, toxic for species, persistent bloom, and only partial success of control measures). A guideline was also derived for acute non-cumulative health effects resulting in discomfort, not amounting to serious health outcomes (Chorus and Bartram 1999). However, the current threshold safety standard of 20,000 cells ml^{-1} for exposure to Cyanotoxins might be too high for occurrence of significant health problems.

Standards for water: About 75% of all cyanobacteria sampled contain toxins. It is the huge conglomerations of cells that present a great concern of development of large blooms in late summer and early fall (Chorus *et al.* 2000). The information available till date is considered inadequate for the calculation of a tolerable daily intake for the majority of the cyanobacterial toxins (Chorus and Bartram 1999). In particular, data are not available on metabolic disposition, acute and sub-acute toxicity, repeated administration, developmental effects, carcinogenicity and genotoxicity. The WHO (1998) has adopted a provisional guideline (tolerable daily intake × body weight × proportion of total daily intake of the contaminant ingested from drinking water divided by the daily water intake in litres) for microcystin-LR of 1.0 μgL^{-1}. Key procedures in the risk management of cyanobacterial toxins and cells have been reviewed for tolerable daily intakes and guideline values with reference to the cyanotoxins in drinking and bathing waters (Codd *et al.* 2005b). The subchronic exposure data used in pigs showed the lowest observed effect level of 280 $\mu gkg^{-1}day^{-1}$ (Falconer 1994).

There is a possibility of exposure to these toxins through the consumption of contaminated food (Vasconcelos 1999). Microcystin has been reported to be toxic to a wide range of zooplankton (Duy *et al*. 2000), and such toxin may accumulate in the bodies of fish and other organisms in the food chain. Mussels and clams concentrate the toxin in their gut, but are themselves unaffected by the microcystin (Falconer *et al.* 1992). Both carp and crayfish may possess detectable levels of toxin in their muscle as well as in the viscera, and hence, may pose a threat of chronic low level exposure (Vasconcelos 1999). Moreover, use of potentially contaminated water for irrigation is controversial because terrestrial plants including food crops can take up microcystins (Codd 1999, MacKintosh *et al.* 1990). A study of commercially sold blue-green algal supplements prepared from algae gathered routinely from a lake in Oregon tested positive for toxins, most notably microcystins in 85 out of 87 samples (Gilroy *et al.* 2000). Hence, consumption of cyanobacteria like, *Spirulina* and *Aphanizomenon flos-aquae* as food supplement and therapeutic products should be done with great caution (NHMRC 1994). It is because most of the cyanobacterial species are nontoxic and, the etiology and conditions for toxicity are not well understood, neither health risks and benefits of long term consumption of cyanobacteria have been studied.

Control of bloom and related toxins: The recreational use of water bodies experiencing an algal bloom is becoming an increasing concern because animals are at risk of acute exposure to toxins while drinking, bathing or recreation in untreated water (Chorus *et al*. 2000). Dogs in particular can consume large quantities of water while playing and grooming themselves after water contact (Chorus *et al.* 2000). Prevention of development or exposure of water to cyanobacterial blooms at 'critical control points' is the first step in control of casualty from cyanobacteria (Hitzfeld *et al.* 2000). Several chemicals (algaecides) were tested widely for their effectiveness in controlling toxic cyanobacterial

Table 3. Chemicals (algicides) for control of cyanobacteria (modified after Burch *et al.* 1998)

Algicides	Formulation	References
Copper sulphate	$CuSO_4.5H_2O$	McKnight *et al.* 1983, Holden 1970, Casitas Municipal Water District 1987, Burch *et al.* 1987
Copper II alkanolamine complex	Cu Alkanolamine.$3H_2O^{++}$	Humberg *et al.* 1989
Copper - ethylenediamine complex	$[Cu(H_2NCH_2CH_2NH_2)_2(H_2O)_2]^{++}$ SO_4	Humberg *et al*. 1989, McGuire *et al.* 1984
Copper - triethanolamine complex	Cu $N(CH_2CH_2OH)_3.H_2O$	Humberg *et al*. 1989
Copper citrate	$Cu_3[(COOCH_2)_2C(OH)COO]_2$	Casitas Municipal Water District 1987, Raman 1988, McKnight *et al*. 1983.
Potassium permanganate	$KMnO_4$	Fitzgerald 1966; Holden 1970, Casitas Municipal Water District 1987.
Chlorine	Cl_2	Holden 1970
Lime	$Ca(OH)_2$	Zhang and Prepas 1996, Lam *et al*. 1995, Kenefick *et al*. 1993, Murphy and Prepas 1990.

blooms (Table 3). Copper sulphate is one of the important algaecides that can be added to water supplies to control toxic blooms, but such action can lead to cell lysis and substantial release of toxins into water (Burch *et al.* 2002). Additionally there is the possibility of copper toxicity, thus exacerbating the potential for health effects (Falconer 1999, NHMRC 1994), and hence, removal of intact cells is prescribed (Chorus and Bartram 1999).

Activated carbon, chlorination and ozonation in conjunction with other water treatment practices were used for treatment of water with potential blue-green algal contamination (NHMRC 1994). While treating the water contaminated with cyanobacterial bloom, the first priority should be removal of intact cells using separation techniques (coagulation or membrane filtration) (Chorus *et al.* 2000). Chlorination and ozonation are effective for the destruction of residual dissolved microcystins and cylindrospermopsin at moderate water temperatures (Drikas *et al.* 2002). Anatoxin-a can be effectively removed using ozone, whereas chlorine is relatively ineffective. Oxidation techniques do not appear to be the best method for the treatment of saxitoxins under normal treatment plant operating conditions. However, powdered activated carbon can be effective for the removal of most of the toxins, provided the appropriate carbon, and the correct dose is applied. Therefore, biodegradation of cyanotoxins across granular activated carbon filters shows great potential as a treatment process.

Conclusion: Toxic cyanobacterial blooms have occurred in many parts of the world as causes of livestock deaths and water unpalatability, and hence, cyanobacteria and their toxins are an important water quality issues for governments, health departments, providers of drinking water, and managers of waters used for recreational purposes. World Health Organization guideline prescribes a limit of $1\mu gL^{-1}$ for microcystin-LR (Burch 2002). Likewise, there should be specific guideline and threshold values for the presence of several bloom forming cyanobacteria and/or the toxins produced by them. Adverse health effects from the consumption of cyanobacterial toxins are now well understood and the health departments should aim to provide guideline values for safe water. Use of water supplies conforming to these guidelines may prevent injury from toxicity. However, due to the lack of adequate data, presently it is difficult to recommend a guideline for other toxins like nodularin, saxitoxins or cylindrospermopsin.

As traditional microscopic assessment of cyanobacteria is not an effective tool for assessment of potential toxicity and poisoning (acute or chronic) of consumers, research into the genetics and evolution of cyanobacteria should be undertaken for accurate identification of toxic species of cyanobacteria in water and sediment prior to proliferation of these species into a bloom. DNA-based tests employing genes encoding for phycocyanin, 16S rRNA, and various repetitive elements allow the identification of the toxin producing cyanobacterial species e.g. *Cylindrospermopsis raciborskii*, *Lyngbya majuscula*, *Microcystis aeruginosa* and *Nodularia spumigena* (Christopher and Fergusson 2002). However, neither all species of cyanobacteria are toxic nor all strains of such species have the potentiality to produce toxin. Therefore, it is better to directly target the potential for toxin production by detecting genes involved in toxin biosynthesis. Candidate genes involved in the biosynthesis of microcystin, cylindrospermopsin, nodularin and other toxins should be identified. These genes are found exclusively in the toxic strains of the cyanobacteria e.g. *Microcystis*, *Cylindrospermopsis, Nodularia*. Elucidation of the genetic basis of cyanotoxin production not only allows very accurate identification of toxic cyanobacteria but also provide clues that how toxin production might be regulated (Neilan 2002). There is a requirement of robust and sensitive analytical method for monitoring cyanotoxins and assessing their behavior during water treatment so that prompt action might be undertaken to effectively deal with any alarming situation. However, in view of inadequate specific treatment and lack of suitable antidote against a particular cyanotoxins, prevention of exposure is the best way to avoid adverse health effects of the cyanotoxins. Active monitoring and adoption of strict standards for the presence of these potentially toxic bioactive compounds in drinking water, recreational water, feeds and supplements can help us in reducing the related health problems.

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