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REVIEW

Cyanobacterial Toxins: The Current Status

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Cyanobacteria (blue-green algae), an ancient group of O_2-evolving photosynthetic prokaryotes, are known to inhabit a wide spectrum of habitats including terrestrial, freshwater, and marine ones. In aquatic environment, a rich supply of nutrients, especially nitrogen, phosphorous, and carbon, from domestic and agricultural run-off can stimulate an abundant, dense surface growth (water blooms) of these organisms (Fig. 1). Such overgrowth of these nuisance algae results in an unpleasant odour and taste and the loss of recreational and fishing value. But of particular concern for animal and human health is the production of toxins by these organisms [16]. The majority of the bloom forming species are known to produce bioactive secondary metabolites having toxic properties which periodically and frequently contaminate the water supply of wild and domestic animals and are responsible for sporadic and recurrent cases of poisoning and death among wild life and domestic animals [4, 14, 16, 23].

Since the first report of toxic cyanobacteria (*Nodularia spumigena*) in the late 19th century [38], studies in several countries have revealed the wide occurrence of toxic cyanobacterial blooms causing most of the problems in freshwater environments [5, 15, 23, 41]. Two types of secondary metabolites, viz., cytotoxins and biotoxins (Table 1), are known to be produced by cyanobacteria [15]. Of these, cytotoxins are not lethal to animals and show toxicities only to algae, bacteria, fungi, and mammalian cells [77, 78]. On the other hand, biotoxins are the most dangerous as they cause animal poisoning [14, 27].

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Toxins are produced by a number of other algae in addition to blue-green algae (cyanobacteria) but toxins of red-tide organisms called dinoflagellates (paralytic shellfish poisons) are rated as the most toxic ones. However, these are mostly produced in marine environments. Thus, in freshwater environments, cyanobacteria are the main toxic forms associated with animal poisoning. In general, researches on cyanobacteria have remained confined to N_2-fixation but during the last two decades this group of organism has proved to be an excellent material for studies on toxins [15, 16, 40, 93]. In addition to *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* which were thought to be potential toxic species, several new genera and species have been added to the list of toxicogenic forms [14, 93]. Our understanding in the area of cyanotoxins is increasing rapidly and this field of research is becoming one of the hot areas of study. Although a number of excellent reviews [15, 16, 27, 40, 90] are available covering various aspects of toxins, the informations gathered in recent years aroused our interest to update the current status of cyanobacterial toxins. In the present article, we have given a brief and recent account of cyanobacterial toxins and their known effects on animal and human health.

Fig. 1. Photomicrograph of natural *M. aeruginosa* colonies (×100).

Table 1. Bioactive compounds produced by cyanobacteria.

Type	Name	Organism	Reference
I. Cytotoxins			
	Anti-algal/herbicidal		
	Cyanobacterin	*Scytonema hofmanni*	68
	D-hydroxy-cyanobacterin	*Scytonema hofmanni*	64
	Unidentified	*Oscillatoria*	26
	Antifungal		
	Scytophycins	*Scytonema, Tolypothrix*	12, 53
	Tjipanazoles	*Tolypothrix tjipanasensis*	7
	Laxaphycins	*Anabaena laxa*	39
	Ambigols A,B	*Fischerella ambigua*	35
	Antiviral		
	Indolocarbazoles	*Nostoc sphaericum*	59
	Ambigols A,B	*Fischerella ambigua*	35
	Anti-mammalian cells		
	Acutiphycins	*Oscillatoria*	79
	Indolocarbazoles	*Nostoc sphaericum*	79
	Mirabiline isonitriles	*Scytonema*	13, 79
	Paracyclophanes	*Cylindrospermum*	79
	Scytophycins	*Nostoc*	12, 79
	Tantazoles	*Scytonema, Tolypothrix*	11, 79
	Toyocamycin	*Scytonema*	79
	Tubercidin	*Tolypothrix*	79
	Westiellamide	*Plectonema, Tolypothrix*	81
II. Biotoxins			
	Neurotoxins		
	Anatoxin-a	*Anabaena flos-aquae*	21, 31
		Anabaena	95
		Oscillatoria	95
	Anatoxin-a(s)	*Anabaena flos-aquae*	19
	Homoanatoxin	*Oscillatoria rubescens*	99
	Saxitoxin & Neosaxitoxin	*Aphanizomenon flos-aquae*	88, 52
		Anabaena circinalis.	50
		Lyngbya wollei	23
	Hepatotoxins		
	Microcystins	*Microcystis aeruginosa*	6
		M. viridis, M. wesenbergii	106
		Anabaena	97
		Anabaena flos-aquae	45
		Hapalosiphon hibernicus	81
		Nostoc	94
		Oscillatoria	95, 67
	Nodularin	*Nodularia spumigena*	38, 83
	Cylindrospermopsin	*Cylindrospermopsis raciborskii*	76
		Umezakia natans	44

Toxin-producing Cyanobacteria

Toxic cyanobacterial waterblooms are found in many eutrophic to hypereutrophic lakes, ponds, and rivers throughout the world. Multiple interacting physical, chemical, and biotic factors such as nutrient loading, retention time of water within the waterbody, stratification, and temperature lead to the formation of these blooms [15]. It is during these water blooms that the concentration of toxins exceeds a level such that any animal can ingest acutely lethal doses of toxic cells or toxins [27]. Systematic studies done in U.S.A., Scandinavia, and a few areas of Europe have reported about 40% of all cyanobacterial blooms to be toxic [73, 84, 94, 98]. Since cyanobacterial waterblooms result from eutrophication, it may be presumed that with increase in eutrophication, the occurrence of toxic blooms is likely to increase in size and duration [25].

Of the known 50 genera and more than 250 species of freshwater cyanobacteria, only a few are shown to be toxic. Species and strains in all of the common planktonic cyanobacterial genera including *Anabaena, Aphanizomenon, Microcystis, Nodularia, Nostoc*, and *Oscillatoria* produce biotoxins (Table 1). Most of them produce more than one type of toxins. Besides these, other genera including *Coelosphaerium, Cylindrospermopsis, Fischerella, Gloeotrichia, Gomphosphaeria, Hapalosiphon, Microcoleus, Schizothrix, Scytonema, Spirulina, Symploca, Tolypothrix*, and *Trichodesmium* have also been reported to be toxic [17, 89, 93, 98].

Microcystis aeruginosa, Anabaena flos-aquae, and *Aphanizomenon flos-aquae* are the most important toxin-producing cyanobacteria reported from all over the world. Species of *Microcystis* are the most studied and most widely distributed among the toxigenic cyanobacteria (Fig. 1). *Microcystis aeruginosa* kutz. emend, Elenkin is the most widely distributed species and is also the first toxic cyanobacterium to be isolated, cultured, and studied in the laboratoy [6]. Other common toxic species in this genus are *M. viridis* and *M. wesenbergii* [17].

Within the genus *Anabaena* six toxic species, viz., *A. circinalis, A. hassallii, A. lemmermanni, A. spiroides, A. flos-aquae*, and *A. variabilis*, have been recognized [16, 17, 93]. Toxins produced by these species are responsible for sickness and death of livestock, pets, and wildlife following ingestion of water containing toxic algal cells or the toxin(s) released by the aging cells [5, 27].

Nature and Type of Toxins

The toxins produced by cyanobacteria show a range of toxicities depending upon the type of toxin, experimental conditions, and the test organisms used. Although less toxic than the most of bacterial toxins, cyanobacterial biotoxins show more toxicity than fungal or plant toxins [15]. Two types of cyanobacterial biotoxins are known:

Table 2. Clinical symptoms produced by cyanobacterial toxins and their median lethal doses (LD_{50}) {After Carmichael, [16]}.

Toxin's type	Symptoms	LD_{50}* (μg/kg body wt.)	Test organism(s)
Neurotoxins			
Anatoxin-a	Muscular fasciculation, decreased movement, Collapse, cyanosis, convulsions, death	200	mice, rat
Anatoxin-a(s)	Hypersalivation, mucoid nasal tremors, diarrhoea, cyanosis, death	20	mice, pig, rat, duck
Saxitoxin and Neosaxitoxin	Irregular breathing, spasm, gasping, loss of coordination, tremors, death	10	mice, rat
Hepatotoxins			
Microcystins	Slow movement, increase in liver weight, hypovolemic shock, intrahepatic haemorrhage, death	50~100	mice, rat
Nodularin	- do -	50~100	mice
Cylindrosermopsin	Liver swelling, hepatic necrosis, congestion in kidney and heart, death	2000	mice

*Toxin was injected intraperitoneally (i.p.).

(i) the alkaloid neurotoxins which interfere with the functioning of the nervous system and cause very fast death (within minutes) due to paralysis of respiratory muscles, and (ii) the cyclic peptide hepatotoxins which damage the liver and result in excessive blood pooling in the liver ultimately leading to fatal circulatory shock within few hours or death within a few days by liver failure (Table 2). Clinical symptoms produced by cyanobacterial toxins vary depending upon the specific toxin administered to test organisms (Table 2). A brief description of a few toxins is presented below.

NEUROTOXINS

Neurotoxins are produced by the species and strains of *Anabaena* [23], *Aphanizomenon* [71], *Oscillatoria* [95, 99], and *Trichodesmium* [17]. In addition to neurotoxins, many of these species are also known to produce peptide hepatotoxins, but because of the more rapid action of neurotoxins, in field and clinical syndromes signs of neurotoxicoses are more dominant [28, 41, 57, 65].

Some of the most extreme cases of cyanobacterial poisoning on record have been attributed to the blooms of *Anabaena flos-aquae* [15], the strains of which produce anatoxins (antx). Five chemically defined neurotoxins, anatoxin-a, anatoxin-a(s), homoanatoxin, saxitoxin, and neosaxitoxins, are known to be produced by cyanobacteria (Table 1). Of these, anatoxin-a and anatoxin-a(s) are unique to cyanobacteria whereas, saxitoxin and neosaxitoxin are better known from marine dinoflagellates. Table 3 depicts the chemical nature of various neuro- and hepatotoxins.

Anatoxin-a, formerly known as the very fast death factor (VFDF), was the first toxin from a freshwater cyanobacterium to be chemically and functionally defined [31, 49]. It is a bicyclic secondary amine of alkaloid origin, and is a potent post-synaptic neuromuscular blocker [2, 22] with an LD_{50} (i.p. mouse) of 200 μg/kg body weight. Most reports of antx-a occurrence are associated with *Anabaena flos-aquae, A. spiroides*, or *A. circinalis*. However, *Oscillatoria* sp. and *Aphanizomenon flos-aquae* are also known to produce antx-a [95, 96]. A strain of *Oscillatoria rubescens* produces homoanatoxin-a which is a methyl homologue of antx-a having toxicity somewhat lesser than antx-a [99], with an LD_{50} of 200~250 μg/kg body weight (Table 2).

The other neurotoxin, anatoxin-a(s), is a unique N-hydroxyguanidine methyl phosphate ester [69] isolated from *Anabaena flos-aquae* NRC 525-51 [20]. It is a naturally occurring organophosphate which causes marked salivation in laboratory mice, hence named as antx-a(s) ('s' for salivation). Other signs in affected animals include lacrymation, chromodacryorrhoea, and urinary defection. Antx-a(s) is about ten times more toxic than antx-a with an LD_{50} (i.p. mouse) of 20 μg/kg body wt. with survival time of 10~30 min (Table 2).

The two other neurotoxins, saxitoxin and neosaxitoxin, generally termed as aphanotoxins are produced by the strains of *Aphanizomenon flos-aquae* NH-1 and NH-5 [71]. In addition, strains of *Anabaena circinalis* and *Lyngbya wollei* are also known to produce these toxins [17]. These toxins are better known as a product of dinoflagellates (paralytic shellfish poisons) that cause red tides in several coastal areas of the world. They are even more lethal (LD_{50} i.p. mouse, 10 μg/kg body wt.) than antx-a(s). Shimizu *et al.* [91] have studied the biosynthetic pathways for production of these toxins in *Aphanizomenon flos-aquae* NH-1. The occurrence of these very peculiar compounds in both prokaryotes and eukaryotes suggests their evolutionary trend [90].

Table 3. Chemical nature and structure of certain cyanotoxins.

Types	Name	Nature	MW	Formula
Neurotoxins				
Alkaloids				
	Anatoxin-a	2-Acetyl-9-azabicyclo(4-2-1)non-2-ene	165	$C_{10}H_{15}NO$
	Homoanatoxin-a	2-Propionyl-9-azabicyclo(4-2-1)non-2-ene	179	$C_{11}H_{17}NO$
	Anatoxin-a(s)	N-Hydroxyguanidine methyl phosphate ester	252	$C_7H_{17}N_4O_4P$
	Saxitoxin	Tricyclic perhydropurine	299	$C_{10}H_{17}N_7O_4$
	Neosaxitoxin	Tricyclic perhydropurine	315	$C_{10}H_{17}N_7O_5$
Hepatotoxins				
Cyclic peptides				
	Microcystins	Heptapeptide	909~1067	Cyclo-(D-Ala^1-X^2-D-MeAsp^3-Z^4-Adda^5-D-Glu^6-Mdha^7).
	Nodularin	Pentapeptide	824	Cyclo-(D-MeAsp^1-L-Arg^2-Adda^3-D-Glu^4-NMdhb^5).
Alkaloid				
	Cylindro-spermopsin	Tricyclic guanidine moiety combined with hydroxymethyl uracil.	415	$C_{15}H_{21}N_5O_7S$

HEPATOTOXINS

Hepatotoxins are the most ubiquitous cyanotoxins that have been responsible for killing animals in virtually every corner of the earth [16, 17, 85, 93]. The hepatotoxins-producing cyanobacteria are strains of species within the genera *Microcystis, Anabaena, Nodularia, Oscillatoria, Nostoc, Cylindrospermopsis*, and *Umezakia natans*. In addition, clinically undefined hepatotoxins have been demonstrated in *Aphanizomenon, Gloeotrichia*, and *Coelosphaerium* [10, 16, 45, 93]. They mainly damage the hepatocytes of the liver and thus owe their name [75]. Death occurs within a few hours to a few days after initial exposure due to intrahepatic haemorrhage and hypovolemic shock [102]. The toxin is transported preferentially into the hepatocytes and an increase in liver weight of up to 100 percent in small animals under laboratory conditions has been recorded [36, 48, 54, 86].

Hepatotoxins constitute a family of at least 53 related cyclic or ring peptides [16]. Those consisting of seven amino acids are 'microcystins' (MCYSTs), the name being derived from the most common producer, *Microcystis aeruginosa* [15]. The others consisting of five amino acids are pentapeptides 'nodularin', originally isolated from *Nodularia spumigena* [96]. Both groups exhibit similar biological activity but differ in the number and types of amino acids that make up their cyclic skeleton (Table 3).

The first report that hepatotoxins are involved in the liver toxicosis was by Bishop *et al.* [6], who isolated the fast death factor from *M. aeruginosa* NRC-1 (SS-17). The toxin was later termed 'microcystin' and the blooms of *M. aeruginosa* were found in Canada, U.S.A., South Africa, Australia, USSR, Finland, Norway, England, Sweden, Germany, Japan, Bangladesh, India, etc. [15, 101]. Later, different degrees of toxicity exhibited by *Microcystis* isolates and their hydrolysates confirmed the presence of many MYCST variants that differ mainly in their amino acids composition. However, a single strain was capable of producing more than one MCYST [74].

Botes *et al.* [8, 9] worked out the structure of toxin (BE-4) from S. African *M. aeruginosa* (strain WR-70) and found the toxin to be monocyclic peptide, containing both D and L amino acids (Table 3). Since then, the structure of a number of microcystins have been worked out. All microcystins are monocyclic heptapeptides having the general structure cyclo (-D-Ala^1-X^2-D-Me Asp^3-Z^4-Adda^5-D-Glu^6-Mdha^7) where X and Z are variable L-amino acids, D-MeAsp is D-erythro-β methylaspartic acid, Mdha is N-methyldehydroalanine, and Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid [87]. Adda is the most remarkable structural feature of these toxins and is important in the toxicity of these compounds [60]. To-date, over 47 variants of microcystins have been identified. These microcystins differ in their L-amino acid combinations, with microcystin-LR (leucine-arginine) being the most common and in being with or without methyl group on amino acid 3, 5, and 7. For the BE-4 toxin, the L-amino acids were leucine and alanine [93]. Botes *et al.* [10] have reported the structure of four other related toxins having L-amino acids combination of -LR (leucine-

arginine), -YR (tyrosine-arginine), -YA (tyrosine-alanine), and -YM (tyrosine-methionine). Namikoshi *et al.* [74] have reported the occurrence of L-glutamic acid in microcystin from *Anabaena* sp.

The molecular weight of MCYSTs varies from 909 to 1067 depending upon the variable L-amino acids present (Table 3). Of the total 47 variants of microcystin, 32 have been reported from bloom samples or laboratory strains of *Microcystis* [82]. Other known genera producing microcystin include *Anabaena, Nostoc*, and *Oscillatoria*. The LD_{50} (i.p. mouse) for the most toxic microcystin has been reported to be 50~100 μg/kg body weight. Variations in the L-amino acids do not affect toxicity, but rather glutamic acid at position 6 is critical in affecting toxicity. All *Microcystis* studied (water bloom as well as laboratory grown) so far, produce more than one type of microcystin.

Although of less common occurrence, nodularin has been found to be produced by *Nodularia spumigena* in brackish water habitats [65, 96]. The pentapeptide hepatotoxin has a mol. wt. of 824 and is composed of Adda, β-linked D-erythro-β methyl aspartic acid, y-linked D-glutamic acid, L-arginine, and N-methyl Z-dehydrobutyrine [83, 96].

Cylindrospermopsin, a unique alkaloid hepatotoxin has been isolated from *Cylindrospermopsis raciborskii* and *Umezakia natans* [44]. The toxin possesses a sulfate ester at the hydroxylmethyl uracil moiety and, unlike MYCST and nodularin, it showed congestion in kidney and heart along with hepatic necrosis. Recently, occurence of cylindrospermopsin in *Aphanizomenon ovalisporum* has been reported from Israel [3].

Detection, Separation, and Characterization

Several methods for screening, identification, separation, and quantification of cyanobacterial toxins have been developed in recent years [8, 15, 29, 36, 40, 58, 61, 93]. The use of mouse bioassay is still the most simple and cheap method to screen toxic cyanobacteria [93]. Many workers have used bioassays employing *Artemia salina*, mosquito larvae, hepatocytes, or seedlings of certain plants [58, 61, 93]. Whole cell agglutination has also been used to detect toxic cyanobacteria but could not get wide application [18]. The most promising method for the detection has been the development of certain immunological assays [15, 29, 61, 93]. Radio-immuno assay and ELISA based on polyclonal and monoclonal antibodies have been frequently used for the detection of toxins. These methods can detect even minute quantities of toxin [93]. Estimation of protein phosphatase and cholinesterase activity is now commonly used for the detection of toxins [69, 70, 103]. Protein phosphatase and cholinesterase activity are severely inhibited by microcystin and anatoxin-a(s), respectively. TLC is routinely used for screening and detection of toxins [43]. In fact, TLC has become the most common analytical tool for identification and separation of cyanotoxins. Partially purified toxin(s) by TLC is further purified and characterized by HPLC attached with specialized columns and detector [43, 45, 74, 93]. NMR and mass spectrometric methods such as FABMS/CID/MS are in wide use for the determination of structure of various cyanobacterial toxins [14, 45]. Altogether, the combination of mass spectrometry, gas liquid chromatography as well as capillary electrophoresis have played significant roles in elucidiating the structure of a variety of toxins [43, 45]. However, characterization of many toxins is still awaited solely due to the lack of proper standards [14, 93].

Regulation of Toxin Production

Most of the investigations on the regulation of toxin production have been made in the hepatotoxin producing cyanobacterium *M. aeruginosa* [15, 17]. Available informations show that the toxin production is influenced both by physical and nutritional factors in addition to the age of culture [15, 27, 40, 93]. Gorham and Carmichael [41] reported that toxin production in *M. aeruginosa* increases during exponential phase of growth and then decreases. A few reports reveal highest concentration of toxin during pre-stationary or stationary phase of growth [27, 101]. It is pertinent to mention that toxin is also liberated in the medium especially during stationary phase of growth and thus the real estimation is problematic [101]. Growth temperature markedly influenced the level of toxin in a few cyanobacteria. Toxin production in *M. aeruginosa* was found maximal at 25°C with a 60% reduction at 28°C and almost negligible production at 32.5°C [27]. On the other hand, the red strain of *Oscillatoria agardhii* elicited more or less similar amounts of toxin production at growth temperatures between 15°C to 25°C. Another strain (green) of *O. agardhii* produced highest amount at 25°C. However, both the strains showed lowest level of toxicity at 30°C [92]. Impact of temperature on toxin production varies in different strains; nevertheless, in the majority of the strains so far investigated, there does not exist any close correlation between optimal growth temperature and toxin production [92].

Four fold increase in *M. aeruginosa* toxicity by increasing the incident photon fluecence rate (PFR) from 7.5 to 200 $\mu m^{-2} sec^{-1}$ have been reported [27]. In *O. agardhii*, toxin production was favoured at low light intensities [92]. Furthermore, the response of light intensity towards toxin production showed differential behaviour with different strains. It has also been reported that the effect of light on toxicity in *M. aeruginosa* could be due to the effect of light intensity on iron uptake [104].

Among nutritional factors, availability of phosphorus and nitrogen sources play a major role in the regulation of toxin production [15]. Phosphorus limitations stimulated

toxin production both in *M. aeruginosa* and *O. agardhii*. Toxin content was highest at low levels of phosphorus (0.1 to 0.4 mg P/L) in *O. agardhii* and higher concentrations did not cause any additional effect. On the contrary high nitrogen (NO_3^-) concentration (0.42 to 84 mg N/L) caused higher toxin production in *O. agardhii* [92]. A direct relationship between the nitrate concentration and toxin production has been observed in certain other cyanobacteria [27, 105]. Owing to the peptide nature of toxin the requirement of excess nitrogen sources especially in non-N_2-fixing cyanobacteria seems physiologically sound. However, Utkilen and Gjolme [104] reported that NO_3^- and PO_4^{-2} have no influence on toxin production in *M. aeruginosa*. According to them, the ratio of toxin content to protien content is a much better way to express changes in toxin production than the ratio of toxin content to dry weight. Their findings suggest the role of iron on toxin production by *M. aeruginosa*. Iron limited conditions significantly decreased the cellular content of both -RR and -LR microcystins. Utkilen and Gjolme [104] have proposed that peptide toxin is an intra-cellular chelator which inactivates free cellular Fe^{2+} and the amount of Fe^{2+} controls the activity of the enzyme peptide synthetase which is probably responsible for the synthesis of microcystin.

Little, if any, information is available on the genetics of toxin production [73, 93]. The involvement of plasmid in toxin production was suggested in the South African isolate of *M. aeruginosa* WR 70. Loss of plasmid by curing agents in the above strain caused elimination of toxin production. On the other hand, the highly toxic *Microcystis* 7820 was found to produce high levels of toxin even after elimination of plasmid [27]. To date, it has not been possible to localize/identify the gene(s) responsible for microcystin production [16]. Available informations suggest that the production of toxin is not governed by plasmid.

Mode of Action

The mode of action of cyanotoxins has been investigated in a number of laboratories. It was suggested that neurotoxins produced by cyanobacteria can disrupt normal signalling between neurons and muscles in a number of ways [16]. The disruption then leads to death by causing paralysis of respiratory muscles, followed by suffocation.

Antx-a is a mimic of acetylcholine and becomes bound to acetylcholine receptors on the muscle cell [16] triggering their contraction. However, unlike acetylcholine, it can not be degraded by the enzyme acetylcholinesterase and thus continues to act on muscle cells leading to exhaustion and over-excitation of the cells. If respiratory muscles are involved, the animal may suffer convulsion and die of suffocation.

The other neurotoxin, antx-a(s) exerts its physiological effects by inhibiting the enzyme acetylcholinesterase [51]. The enzyme is secreted by the muscle cells and is located in the synaptic cleft. It is a serine esterase with a nucleophilic serine residue as the active site. The unique feature of the antx-a(s) lies in its structural fit with the enzyme acetylecholinesterase. The bimolecular reaction occurs initially with the formation of an enzyme-antx-a(s)-complex which results in the phosphorylation of the enzyme. The enzyme in this condition becomes unable to degrade acetylcholine, which therefore remains continuously available to stimulate and overstimulate muscle cells [16].

Electrochemical ion-gradients, especially Na^+, K^+, or Ca^{2+}, across the cell membrane provides the basis for the propagation of impulses. Saxitoxin and neo-saxitoxin are fast acting neurotoxins acting as competitive inhibitors of the voltage gated Na^+-channel and prevent the influx of Na^+ into the axon. These neurotoxins are selective in binding the Na^+ channel only and do not affect the flow of K^+ and the resting potential of the membrane [41]. By blocking the inward flow of Na^+ ions across the membrane channel, they disrupt the propagation of impulse required for release of the neurotransmitter acetylcholine at the neuromuscular junction [16].

A number of possible modes of action of hepatotoxins have been reported [15, 16]. When injected orally, the toxin is preferentially absorbed across the ileum and is transported to the hepatocytes via bile acid carriers [33, 34] which results in hepatic necrosis [54]. In the liver, both hepatocytes and endothelial cells shrink or are destroyed with extensive fragmentation and vesiculation of the cell membrane [41]. As a result, the cells which are normally tightly packed together separate, causing the cells forming sinusoidal capillaries also to separate. This causes blood to spill into tissue leading to haemorrhagic shock [15, 16]. Many-fold increase in the levels of Y-glutamyltranspeptidase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were detected in plasma samples in mice administered with *M. aeruginosa* toxin [101]. The increase was more apparent in GGT levels [37]. It is pertinent to mention that GGT is characteristically released after toxin damage to cell membranes. An increase in GGT level was also detected in patients affected with liver damage following drinking of *M. aeruginosa* contaminated water [17].

Hepatotoxins distort liver cells by acting on the cytoskeleton, the network of proteins which give shape to the cell [90]. The most affected parts of the cytoskeleton are the protein polymers known as intermediate filaments and microfilaments. Microcystin and nodularin bring about shrinkage of these filaments by causing subunit loss and dissociation [16].

Several mechanisms have been proposed to explain the effects of microcystin on the cytoskeleton protein

frame work. The mechanism of action of microcystin LR resembles that of okadaic acid, a polyether fatty acid from marine sponges, both of which are known to inhibit protein phosphatases 1 and 2A. The most widely accepted mode of action is via the interaction with the enzymes, protein phosphatases PP1 and 2A [46, 70, 103]. Of the two, protein phosphatase PP1 has the major role in the maintenance of the cytoskeleton network, whereas, the inhibition of PP2A results in tumor promotion. These enzymes along with another enzyme protein kinase regulate the number of phosphate groups on proteins and are known to influence the structure and function of intermediate filaments and microfilaments. In isolated hepatocytes, MYCSTs are known to induce the overall phosphorylation of the cytoskeleton proteins. The resulting excessive phosphorylation of the cytoskeleton proteins increases the rate of subunit loss and dissociation [16].

MYSTs are reported to be the prominent tumor promoters both *in vivo* and *in vitro* [103]. Protein kinases and protein phosphatases play a major part in regulating cell division. Inactivation of protein phosphatases by hepatotoxins disturb the normal balance, resulting in cell proliferation and cancer production. However, it is not clear whether the toxin initiates the cell progression towards becoming cancerous or whether it needs some triggering substance [16]. Further investigations are required to establish the exact mode of action of microcystins.

Functions

During the last 20 years, extensive data have been collected pertaining to various aspects of cyanotoxins. However, the question "why cyanotoxins are produced" has remained enigmatic. Some suggest that the cyanotoxins are protective chemicals [15, 16]. Demott *et al.* [30] have reported that microcystins and nodularin are potent inhibitors of invertebrate grazers in the aquatic environment. However, toxins are formed even in the absence of grazers and other environmental stresses which raise doubts on the above suggested function. Furthermore, Carmichael's group observed that zooplanktonic species generally do not eat cyanobacteria capable of producing toxin, unless there is no other food around [17]. In extreme cases, even if they do eat, they do not take up lethal doses. It is possible that cyanotoxins had some primary function which they lost during the course of evolution, or the adaptive function of cyanotoxins may be multiple and may involve metabolic as well as defense functions. Future work on cyanotoxins may reveal their exact function.

Possible Threat on Humans and Other Species

The presence of toxic cyanobacterial blooms in a water body used for recreational or drinking purpose is of concern to animal and human health. Poisoning to both wild (amphibians, snakes, water fowl, rodents, bees, zebras, and rhinoceros) and domestic (cattle, sheep, pigs, horse, ducks, geese, and chickens) animals have been reported [15]. Since all animals in a herd and group often drink water from the same water supply, most or all of them will be affected within a similar time period.

Most cases due to cyanobacterial poisoning involve hepatotoxicosis. Death of the affected animal occurs within a few hours to a few days. Upon necropsy, affected animals show hepatic enlargement (2~3 fold increase in liver weight) and intrahepatic haemorrhage. Animals, especially cattle, that survive on acute cyanobacterial hepatotoxicosis also experience photosensitization and cows refuse to nurse their calves [17]. A summary of the signs of toxicosis occurring in laboratory studies due to various biotoxins is depicted in Table 3.

Several cases of fish toxicity due to cyanobacterial toxic blooms have also been reported. In Scandinavia, killing of fishes due to an *Oscillatoria* bloom occurred and the signs of poisoning were similar to that produced with hepatotoxins. Intraperitoneal injection of microcystins into rainbow trout [80], the common carp (*Cyprinus carpos*) [32], and antx-a injection into gold fish [15] resulted in mortalities with the same symptoms of poisoning as in mammals. Besides affecting wild and domestic animals, cyanobacteria also cause health risks to humans [20, 23, 27, 36, 40, 41]. There are a few reports from U.S.A. and Australia of cyanotoxins causing human illness, after the water blooms in municipal water supplies were treated by copper sulfate which resulted in lysis and release of more toxins. Cases of an adverse effect of freshwater cyanobacteria have been reported from countries like U.S.A., India, Canada, Zimbabwe, Norway, the Baltic Coast, USSR, Australia, and the UK. Such effects include gastroenteritis and hepatoenteritis due to ingestion of cyanobacteria [14, 16].

Cases of allergic reactions such as asthma, eye irritation, rashes, and blistering around the mouth and nose have been reported from Brazil, China, Europe, Norway, U.S.A., and the U.K. [42, 47, 72]. In Sewickley, Pennsylvania, an outbreak of gastroenteritis occurred due to growth of *Schizothrix calcicola* in the city's uncoverd water supply [66, 100]. Subsequently the lipopolysaccharide endotoxin was found to be responsible for gastrointestinal problems [56, 57]. *Microcystis aeruginosa* growing in a water supply reservoir of Harare, Zimbabwe also resulted in a serious outbreak of gastroenteritis, especially among children.

It is felt that the tumor promoting activity of hepatotoxins may lead to high rates of occurrence of liver cancer in human beings. It has been suggested that the occurrence of extra-ordinary high rates of liver cancer in China may be correlated to the cyanobacterial toxins in drinking water. Yu *et al.* [107] have shown that in China, people

who drank pond and ditch water had a higher risk of primary liver cancer than the people who drank well water. Subsequently, Carmichael *et al.* [24] showed that about 80% of all ponds in central China sampled showed dense cyanobacterial population, especially *M. aeruginosa.*

Besides microcystin, antx-a(s) and neosaxitoxin are known to possess clastogenic and gene mutation properties as well [17]. These compounds have been shown to be even more carcinogenic than benzene and sodium arsenite in laboratory conditions. Falconer *et al.* [37] have demonstrated teratogenic activity from chronic oral administration of *Microcystis* extracts in mice. Whether the tumor promoting effects of cyanobacteria are of public health significance awaits suitable epidemiological analyses of cancer deaths and birth defects frequency in populations exposed to this risk. Thus, a closer monitoring of drinking water supplies in many places is required.

Remedial Measures

Protection of water from further eutrophication is the most practical way to control the mass growth of cyanobacteria. In addition, development of awareness among the people about the toxic cyanobacteria and health hazards which they cause may eliminate the risk of exposure. Several methods to control the cyanobacterial water-bloom have been recommended [15]. These include nutrient deprivation and controlling through physical (reducing light, destratification), biological (planktivorous organisms), and chemical (algicide) means [55]. Nutrient deprivation (especially NO_3^- and PO_4^{-2}), although an expensive method, has been successfully used in Europe where phosphate stripping is used as a means to control phosphorous and thus water blooms. Besides, in order to keep shore areas free from surface scums, floating plastic booms or bags have also been used but these are not very successful [14, 15, 93].

Another method to control cyanobacterial population is the use of planktivorous fishes, zooplankton, cyanophages, and cyanobacteria lyzing bacteria. In China, successful control of cyanobacteria is achieved by using Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idellus*), Bighead Carp (*Aristichthys nobilis*), and Telapia (*Telapia nilotica*) [14, 15, 17]. These fishes feed upon and remove toxic *Microcystis* colonies. Ciliate Nassula has also been reported to graze a cyanobacterial population. Use of bacteria and cyanophages in controlling cyanobacterial population has been emphasized but it is difficult to maintain stocks of the lytic organisms [17, 93]. Moreover, extreme specificity of the lysing organism towards its host may pose some problem.

Algicides such as copper sulfate have also been used to control these nuisance algae. The usual treatment dose is from 0.2 to 0.4 ppm [4]. Since treatment causes cell death and the release ot the toxin into the water body, it is desirable to discontinue the use of $CuSO_4$-treated water for at least 5 days or, more safely, for 2~3 weeks. In order to minimize the effect on taste, odor, and release of toxin, it is better to use $CuSO_4$ during the early stages of bloom formation. Causes of human illness due to supply of water from municipal water supplies after treatment of the bloom by $CuSO_4$ and release of toxin into the water are also on record [17]. Toxins or cells of cyanobacteria in drinking water can be removed by filtration [63] or chemical flocculation. Expensive techniques such as activated carbon and ozonization also effectively eliminate toxicity [55]. Ozonization exerts its effect by cleaving the double bond on the Adda component of microcystins.

Problems and Perspectives

The impact of toxic blooms on aquatic ecology is an important, though less studied, subject. Dominant blooms may influence several trophic levels in food chain. Primary producers are often inhibited either due to shading or by the effect of toxic substances [30]. This ultimately influences the total energy production and oxygen content of water. These blooms are often associated with a decline of a number of secondary producers, the zooplanktons [30]. At higher trophic levels, fishes may also be killed due to deterioration of water quality or toxicity effects. Carnivorous fishes may acquire cumulative toxicity doses while feeding on zooplanktons.

Deterioration of water quality due to toxic blooms is of major concern for humans and animal health. Blooms as such can create difficulties to water treatment processes because of their direct interference with treatment units [55]. The other worrisome situation is the intoxication of the natural water that is used for municipal and recreational purposes. In such circumstances, animals ingesting the whole cells are not the only victims. They can, however, receive a fatal dose by drinking the water contaminated with toxins. This could raise the most threatening possibility of developing liver cancer since hepatotoxins are tumor promotors *in vitro* and *in vivo* [16].

Although a comprehensive overview on the toxins of cyanobacteria has been a subject of interest since the last few decades, the evolutionary aspects of toxin production and the chemical ecology of cyanobacteria has been largely ignored. However, considerable experimental evidences suggest that the toxins are originally produced for defence [30]. The assumption is supported by the results that these toxins are involved in the inhibition of feeding and increase the mortality in zooplanktons, which coexist with the toxic strains in natural habitats [58, 62]. Studies on the biogenesis of microcystins [1] and neurotoxins [90] may suggest an evolutionary trend of the toxins and will further reveal why and how so many

compounds are produced at a time, and why certain compounds are major toxic constituents.

The overture to the foregoing findings since more than 100 years was a public health concern that eventually led to a valuable research tool in biomedical research. Of profound importance is the possible implication of these novel molecules in pharmacological and agrochemical areas. Because of the high toxicity, they find only little application as such, but their chemically designed modified versions may provide many valuable products. Neurotoxins and their derivatives can be used in place of acetylcholine, the natural neurotransmitter, to study the binding action of acetylcholine to the receptors [16]. Modified versions of antx-a might be able to slow down the mental retardation of Alzheimer's disease [16]. MCYST and aphanotoxins may find wide applications as a molecular probe because of their selective inhibition of protein phosphatases in liver and electrochemical ion channels across the axon, respectively [16, 90].

Toxins also offer opportunity for their exploitation as pest control agents. Antx-a(s) is a natural organophosphate that behaves much like synthetic insecticides [51]. MCYSTs, which also showed mosquito-larvicidal property [58], can also be exploited in a similar manner. Chemical modification may provide a solution which would form the basis of a new generation of environmentally sound pesticides.

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