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Review Article

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Global health concern of cyanotoxins in surface water and its various detection methods

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ABSTRACT

Water is an absolutely required resource for life, nourishment which now became a worldwide threat due to unenviable changes in an environment that are mainly instigated by human influence. The foremost progressions intensify the consequence, permitting the growth of cyanobacteria that is blue-green algae in surface water. CyanoHABs (Cyanobacterial Harmful Algal Blooms) occurred in its adopting nature according to the temperature fluctuations in the earth. In this study, a basic introduction to cyanotoxins as well as the entanglement of public health that includes the route of exposure, health effects, and the pervasive impact of cyanotoxins and alleviation efforts in the water bodies along with the toxicosis were appraised. Cyanobacterial toxins with the conditions like hepatotoxicosis (liver toxicity), neurotoxicosis (brain toxicity) and gastrointestinal disturbances, respiratory and allergic reactions were reviewed. Their detection process and the treatment techniques with various physicochemical methods and bioassay methods were also reviewed. The assorted techniques and their combinatorial detection methods that are adopted in this review will help us to eradicate the toxins from the surface water.

KEYWORDS: Cyanobacteria, cyanotoxins, Bioassay, physicochemical methods, hepatotoxins and neurotoxins

INTRODUCTION

The algae, which are microscopically small, unicellular organisms form colonies and thus varies in sizes visible as minute green particles [1]. The term cyanobacteria are similar to algae in size, unlike other heterotrophic prokaryotes, they perform photosynthesis mechanism exhibit blue-green and green pigments. Harmful algal blooms are termed as HABs which reported globally due to several factors [2,3]. Predominantly because of anthropogenic activities like agricultural runoff, insufficient sewage treatment, industrial and uncontrolled use of fertilizer known as eutrophication [4]. Due to the excessive accumulation of nutrients in water bodies, causes an extensive growth of algae and cyanobacteria. Hence the water quality becomes diminished, which are hazardous to aquatic animals, plants and also implicated in humans [5,6].

Moreover, recent studies proved that anthropogenic global warming influences the toxic generating cyanobacteria also identified as cyanotoxins that exist over a prolonged period of time, a gradual increase in population, and geographical distribution [7]. The toxin discharged by cyanobacteria plays both deleterious and advantageous influences [8]. When centering on a profitable perspective, the compounds that are transmitted by blue-green algae are vital within the province of pharmaceutical and biotechnology intrigued.

The pharmacological properties of certain cyanobacteria on people incorporate anticancer, anti-inflammatory, and antibiotic bustles. It also plays a role in predator-prey models like their toxicity would be a nightmare to the dangerous species. This character sometimes useful to the ecosystem balancing. And at the same time, attributable to certain natural conditions can able to tolerate various climatic changes associated with anthropogenic impacts [9,10]. CyanoHABs can proliferate exponentially on surface waters and form complete blooms when the circumstances are favorable. These revolutions give rise to an increase in PH, temperature, oxygen demand, and accessibility of nutrients, especially the accumulation of phosphorous, nitrogen, etc. [11]. When the algae get perished, it utilizes oxygen completely and hence water becomes anoxic (Oxygen deprivation). This transpires exclusively after the complete growth of algal [12].

The physical constituents as well as chemical constituents blatantly enhance the development rate of poisonous algae.

There is a rich phosphorus backlog in the "Missisquoi Bay" that causes the complete development of algae [13]. Cyanobacteria from nine hot water/thermal springs located in the northwestern Himalayas. This unique adaptation of blue-green algal growth in consortium with other microbial community exhibit resistivity towards a thermal gradient of 74°C which is an upper threshold for photosynthesis.

In India, only a few studies carried out on cyanobacterial diversity from thermal/ hot water spring [14,15]; from Bihar [16,17]; from Uttarakhand and from West Bengal [18-22] from the western part of the country [23].

The cyanobacterial toxins (toxicosis) that include hepatic, neurotoxicosis, respiratory disorders, disruptions in both stomach and intestine (gastrointestinal disorder) and certain hypersensitivity reactions. So far these studies were unproven hence further studies needed [24].

In freshwaters, repeatedly acquired toxin-producing cyanobacterial strains are *Microcystis*, *Anabaena*, *Nodularia*, *Planktothrix*, *Aphanizomenon*, *Cylindrospermopsis* and *Lyngbya* etc. These colonizer or filamentous cyanobacteria generate a multiplicity of chemically and biologically distinctive toxic components such as hepatotoxins (microcystin and nodularin), neurotoxins (anatoxins and saxitoxins), paralytic shellfish toxins (as emitted by *Aphanizomenon*), cytotoxins (*cylindrospermopsin*) and dermatotoxins (lipopolysaccharides) [25]. The molecular structure, source of toxins and its mode of actions of the above said strains are discussed below in Table 1.

Microcystins and Nodularins: The Cyclic-Peptide

Nodularin, as well as microcystin poisons, are as often as possible happening with liver poisons (LD_{50} (i.p) 50-1000μg/Kg) [26]. The prevailing toxin of microcystin is specially unearthed in sources as mentioned in Table 1. As far as the toxin "Nodularin" is concerned, it could be found merely in "*Nodularia* sp" [27]. Polar compounds of microcystins having free carboxylic acid in its chemical composition and there will be arginine that occurred frequently. Non-ribosomal biosynthesis can take place with the aid of the MCS (microcystin synthetase) enzyme which is having multifunctional properties. These toxins are organ-specific, in which the cell membranes get affected by carrier molecule (organic anion). That is why the Liver influenced effectively due to the penetrability of such a molecule. In general, the peptides from a similar family are hydrophobic and usually do not own the ability to diffuse via the vertebrate cell membrane and hence they depend on ATP as a carrier molecule.

Anatoxin: Secondary Amine Alkaloids and Guanidium Methyl Phosphate Ester

Anatoxin causes an acute neurotoxic disorder. When living things exposed to the neural toxin Anatoxin (a)/ a(s) (i.p injection in mice-LD_{50} 20-250 μg/Kg b.wt) leads to immediate death by provoking expeditious tightening or relaxation of muscle fibers, Ataxia (an abnormal muscular movement), and hypoxemia (Paralyzed prodrome associated with respiration) [28]. Through the receptor "nAChRs" ("nicotinic acetylcholine") toxin enters into the cell, and the receptor imitates "Ach" (Acetylcholine) which is a natural ligand that amends the physical and biological responses with respect to neurons. The binding of anatoxin to the receptor is irrevocable and cannot be able to breakdown by the enzyme called "Ach esterase enzyme". The toxins which are accountable for the neuro-muscular blockades are *Anabaena flosaquae* and *Oscillatoria* species.

Saxitoxin: Alkaloid

In an existing freshwater/ brackish water, carbamate alkaloid compound present in the saxitoxin. These compounds are responsible for toxicity that is liberated by certain strains like *Anabaena circinalis*, *Aphanizomenon* sp, *Cylyndrospermopsis raciborskii*, and *Lungbya wollei* [29]. They block voltage-gated sodium channels (LD_{50} (i.p): 08-10 μg/kg) and causing paralysis by disrupting the integral membrane protein of neurons. The SXTs i.e, Saxitoxins also entitled as (PSP) Paralytic Shellfish Poisoning. SXTs or PSPs enter humans via aerosol either through anthropogenic/ natural sources [30].

Table 1: Cyanobacterial Toxicity and its mode of action from Fresh and Brackish water

Toxin	Molecular structure	Sources	Modes (S) of Toxicity	Reference(s)
Nodularia	Cyclic pentapeptide	*Nodularin*	Hepatotoxic tumor promoters, PPase Inhibitors	[31]
Microcystin	Cyclic heptapeptide	*Microcystis, Anabaena, Nostoc, Oscillatoria*	Hepatotoxic tumor promoters, PPase Inhibitors	[32]
Anatoxin-a	Secondary amine alkaloid	*Anabaena*	Neurotoxic, Depolarizing Neuromuscular block	[33,34]
Anatoxin-a(s)	Guanidium methyl phosphate ester	*Anabaena*	Neurotoxic, Cholinesterase Inhibitors	[35]
Saxitoxin	Alkaloid	*Aphanizomenon*	Neurotoxic, Sodium channel blockers	[36,37]
LPS	Lipopolysaccharides	*Microcystis, Oscillatoria*	Toxic shock, gastroenteritis, Inflammations	[38]

LPS: Lipopolysaccharide (Irritant toxin)

Recent studies proved that the lipopolysaccharide from filamentous cyanobacteria involved in causing various health issues such as irritations on the skin, hypersensitivity, respiration dysfunction, gastric disorder, fever, etc. [39]. The LPS toxins are emitted by *Microcystis* and *Oscillatoria* that triggers chemokines and eicosanoids that invigorate aggravation in neurons by emitting distinct proteins [40].

Action mechanism of Cyanobacterial toxicity

The huge fragment of the toxic peptides from the MCs and NOD are aquaphobic and depend on ATP as a carrier [41]. In Rat liver, bile acid acts as a carrier for poisonous proteins that are constrained to organs and impact their cell layers. The toxic cells from different strains are lysed within the digestive tract and permeate into the bloodstream. Afterward, it is being brought to hepatocytes, which quell the protein phosphatase movement assistant leads to the excessive phosphorylation within cells that destroy the hepatic cells [42].

The microcystin and Nodularins are two distinct inhibitors of catalytic protein phosphatase (PPase) enzyme with subunit 1 and 2a (PP1 and PP2A) containing exclusive substrate binding ability in liver cells [43]. These proteins correlated with PK (protein kinase) in the regulation of several phosphorous groups in protein. When focusing on Neurotoxin, the alkaloid neurotoxin anatoxin-a (antx-a) is a potent postsynaptic depolarizing neuromuscular blocking agent. Depending on the pathogenicity of a species, and the number of poisonous substances instigates immediate death. This antx-a poisoning progression causes muscle fasciculation, decreased movement, abdominal breathing, and sudden death. No known therapy exists for antx-a, although respiratory support might allow sufficient time for detoxification and recovery of respiration control.

Saxitoxin are also called paralytic shellfish poison (PSPs) which are produced by species of the genera *Aphanizomenon*, *Anabaena*, *Lyngbya*, and *Cylindrospermopsis*. This Saxitoxin block neuronal transmission when bonded the voltage gated Na+ channel nerve cells [44]. These potent voltage-gated sodium channel antagonist can cause numbness, paralysis and even get to the mammals through respiratory block, channel opening and sodium channel blockers causes muscle paralysis and death by respiratory arrest. This saxitoxin transformation in shellfish update carried out through epimerization, decarbonylation reductive elimination.

In the year august 2013, fifteen livestock died around 2 fishponds in Kentucky. And within the same year, the dog became fatally ill after swimming in clear Lake California, 4 weeks later, the dog with clinical signs was observed. The samples from the water were collected and tested. It showed protein phosphatase inhibition activity, i.e.PP1. The subsequent evaluation was performed using liquid chromatography-mass spectrometry (LC-MS/MS) which detected microcystin LR, - RR, LA, LF but failed to detect YR [45].

The Neuro and Hepatotoxicity Consequences on Human and Aquatic Biota

Within the central portion of India, out of fourteen *microcystis*-dominant bloom of cyanobacteria, three MC's were found to be poisonous. [46]. Findings divulged that the dominance of fourteen different genera comprised of distinctive groups of *microcystis* (MC) from the towns of Kundam, Jabalpur, and Shahpura was discerned to be toxic to the crustacean zooplankton "*Moina Macrocopa*" at the concentration level more prominent than 282 μg bloom dry weight/ml. Bloom samples from ponds and lakes of Jabalpur, Dindori, Mandler, Seoni and shahdol districts were gathered and scrutinized for microcystin harmfulness on the premise of an intense poisonous quantity (ca.LD100) i.e 70,100 and 260 mg dry wt/kg of kundam, Jabalpur and Shahpura bloom material. When exposed to Lake Bloom, it appeared a serious necrotic injury within the hepatic tissues.

Subaqueous plant "*Ceratophyllum demersum*" when susceptible to CyanoHABs, showed reduced growth at the dosage of 1.0 μg/L of MC-LR(microcystin) after six weeks, while at a concentration of 5μg/L of MC-LR showed reduced growth after 3 weeks [47]. An invasive organism Zebra mussel (*Dreissena polymorpha*) is subaqueous habitats, originated from Russian lake. They pervade to other countries either by ship/pleasure crafts due to their capacity of clean water impurities and easily take up toxic cyanobacteria. In another way, this has been used as a poultry feed. After the intake of microcystin from *Microcystis aeruginosa,* there will be a loss of net energy balance and their scope of growth was significantly reduced. [48].

Health Consequences of Cyanobacterial Toxins

In addition to human toxicity, aquatic plants, humans and other invertebrates, cyanoHABs can also be toxic to some fishes, by causing liver necrosis, impaired tissues, due to accumulation of toxins (microcystin) in their organs that passes via gills [49]. As proved by UK histopathological investigations of fish deaths during cyanobacterial blooms occurred due to the damage to gills digestive tract and liver. This might be due to the high pH induced by cyanobacterial photosynthesis activity prior to bloom collapses together with the highest level of ammonia arising from the decomposition of the cyanobacteria. However, the microcystin uptake happened through damage of organs and thus led to liver necrosis. Fishes especially phytoplanktivorous species that can be exposed to MC either by feeding or toxins pass through the gills during its breathing.

The Hirakud reservoir in Sambalpur was built fundamentally to generate electricity but it is additionally utilized for the water systems, fisheries, and drinking water which is now completely polluted by manufacturing plants in different wetlands of Sambalpur areas, and its neighboring Jharsuguda area Odisha. Underneath the Hirakud Dam, they found 37 species of cyanobacteria belonging to 17 genera. According to the literature study, seven genera are found to be toxic including *Anabaena*, *Microcystis*, *Nostoc*, *Gloeocapsa*, *Lyngbya*, *Oscillatoria*, and

Phormidium. These cyanotoxins are dangerous to human and animal health who is drinking the water from these lakes. [50,51] Out of 17 genera, the potential toxin-producing genera as said earlier, produces Microcystin, Anatoxin-a. Anatoxin-a(s) and, saxitoxin. Depending upon the effects, they further classified as a hepatotoxin, neurotoxic and Dermatotoxin [52].

Also, more and more attention to be paid for the presence of cyanobacterial toxins in consumer products which include dietary supplements produced from blue-green algae (BGA), fish or seafood [53]. This tends to be more problematic than consuming 1.5 – 2 l/day water, whereas BGA intake would be 20 g [54].

In 1994, at Sweden River, there was heavily populated cyanobacterium *Planktothrix* sp., causes toxicity. The illegitimate usage of river water from a sugar factory from a cross-connection between potable to non-potable river water for a dubious period of hours caused serious health issues. 121 of 304 inhabitants of the village, including animals got affected with hepatotoxicity by microcystin. The samples were tested before and after the incident happened [55]. Neurotoxins that cause Parkinson's, as well as Alzheimer's disease and some, are caused by cyanobacterial species. Alzheimer's disease and Dementia increased drastically in China from 3.7 to 9.2 million in the year 1990 to 2010 [56].

Bioassay for the Detection of Cyanotoxins

There are numerous natural approaches (Table 2) established to spot toxins generated by cyanobacteria based on their interactions, immunological response, and several enzymatic actions.

Bioassay using Microbes

Toxicity can be detected by the use of microbes helps in detecting low amounts with in short time, though this idea might not be very suitable for detecting toxicities presented by cyanobacteria. Bioassays using bacterial community for detection of cyanobacterial toxins have been confined only to the detection of toxic extracts from cyanobacteria and not to pure toxins such as microcystin-LR.

Upon tested various solvents for the strains of *Cylindrospermum majus*, *Oscillatoria*, *Calothrix gracilis*, and *Nostoc*, n-hexane solvent restrained these microbial clusters at the concentration of 100 μg/ml, and when experienced distinctive extracts for the cultures such as *Pseudanabaena catenata*, *Anabaena variabilis*, and *Gloeocapsa caldariorum*, menthanol extract repressed the microbial growth of *Bacillus subtilis* with the concentration extend from 1.4 to 70 μg/ml. [57]. The *Limnothrix redekei* also inhibits the growth of *Staphylococcus aureus* and *Micrococcus flavus* with the concentration of 50/100 μg of the isolated substances per paper disk. [58]. The *Pseudomonas* (50 μg/ml) were possibly involved in the degradation of MC-LR. The use of bacterial strains, however, needs further investigations [59,60].

Bioassay Using Invertebrate Animals

Daphnid bioassay used to look at the harmfulness of MC and NOD. The harmfulness finding of microcystin at the dose adapted from 16.4μg/g body weight to 1226μg/g body weight [61]. Daphnid bioassays are not well suitable for diagnosing a lesser quantity of toxic peptide. The eggs of *Artemia salina* are commercially accessible and are viable for years beneath subzero temperatures. Nevertheless, the toxicity towards all divergences of neural toxin (anatoxins, saxitoxins), liver toxin (nodularin, microcystin) as well as protease inhibitors, have not been flaunted towards *Artemia* bioassay (brine shrimp eggs) which restricts the usage of this particular bioassay [62].

The mosquito (larvae and adult) has too found to be as conceivable bioassay approaches against cyanotoxins [63]. Larvae of *Aedes aegyptii* have been found to be influenced by neural toxins (anatoxin-a) and hepatotoxins (*Microcystis* strains coupled with *Oscillatoria* strains). Distinct concentrations related to 0.5 to 17.5 mg of desiccated cyanobacteria/mL were exploited in assays. The toxicity of these strains was evident only after 48 hrs of incubation period and the strains killed all larvae efficiently ($P<0.05$/ $P<0.001$).

Adults of *Culex pipens* were found to be sensitive towards MC-LR when injected. Due to the difficulties of handling this organism, both mosquitoes were relatively sensitive, but have not been widely adopted.

Similarly, [64] adult house flies (*Musca* sp.) with the concentration 0.5 and 3.7 mg/kg, diamond-back/Cabbage moth (*Plutella* sp.) 1.0μg cm^2, and cotton leafworm (*Spodoptera* sp.) of 4.7 and 13.1 mg/kg concentration, were found sensitive when administered with purified toxins via treated leaf towards MC-LR. Positive results were observed when compared with other toxicity results. The handling of flies is strenuous and needed microinjection that is difficult to administer [65].

Except for the handling difficulties of an insect, fruit fly (*Drosophila melanogaster*), can detect microcystins successfully in bloom samples and can be maintained in the laboratory, with no requirement of special equipment. The toxin (LC_{50} 0.8 d.w /mL ± 0.3 scale of 95% confidence interval) can be administered orally to a 24 (hour) pre-starved fruit fly by spotting a sample along with sucrose on filter discs [66]. *Drosophila* has been utilized to experiment with the dietary ingestion of BMAA toxin (β-N-Methyl amino-L-Alanine) which is exuded by the "*Nostoc* sp" provokes brain malfunction in aged adult flies and in females, it revealed diminished fertility. No efficacious clambering actions were scrutinized in flies when nourished with 8 or 10 mM of BMAA toxin [67].

Bioassay Using Vertebrate Animals

Mouse bioassay is the most preferred bioassay for testing microcystins. The total toxicity caused by cyanobacteria can be estimated in drinking water supplies using mouse bioassays. Swiss Albino Mouse (Male) is the commonly used strains for

testing Cyanobacterial toxins (oval dose LD_{50} used 5000μg toxin /kg body weight) [68]. The major drawback in using mouse assay is the need for an animal house facility for rearing the animals for routine experiments, ethical clearance and microcystin-LR may mask other symptoms.

A desert locust (*Schistocerca gregaria*) based bioassay are easy to handle and samples can be administered by injecting low volumes (10μl). The use of locusts is very simple, ethically acceptable, broad-specificity functional bioassay, for the monitoring of saxitoxins and other paralytic shellfish toxins. [69].

Bioassay Using Cell Cultures

For hepatotoxicity testing, the fish cell line is most preferable [86]. Use of hepatocellular carcinoma of the topminnow *poeciliopsis lucida* (PLHC-1) and Rainbow trout *Oncorhynchus mykiss* (RTG-2 fibroblast) was chosen for treating hepatotoxins produced by Microcystin which was isolated from bloom. These cell line techniques drastically reduce the size of the cell leads to apoptosis. Whereas neurotoxin, neuroblastoma cell line bioassay was used to test saxitoxin [70,71].

For neurotoxins assays, a neuro receptor-binding assay was developed earlier for saxitoxins, a neuroblastoma cell line method for sodium (Na) channel blocking activity has also become an advanced technique for the analysis of neurotoxins [72]. Again, the use of cell cultures for toxicity needs further experiments before a universal cell line can be adopted for all known cyanotoxins in freshwaters. [73-75].

Bioassay Using Plants And Plant Extracts

Microcystins produced by cyanobacteria exhibit secondary metabolites shows algicidal or herbicidal properties. Bioassay using *Anacystis*, *Phormidium*, *Plectonema* and *Chlorella* has been used to investigate algicidal effects posed by *Oscillatoria*. Little work has, however, been done on establishing a simple, cost effective and sensitive plant based bioassay for the detection of cyanotoxins in drinking water.

The effect of a microcystin-LR extract on the growth of *Lepidium sativum* over 6 days. Exposure to 10 μg L-1 microcystin-LR concentration resulted in a significant decrease in root and leaf lengths as well as fresh weights of seedlings when compared to the controls. The use of this bioassay needs vast exploration. Pollen germination was inhibited by cylindrospermopsin between 5 and 1000 μg ml−1. The inhibition of tobacco pollen germination may be amenable for development as a bioassay for cylindrospermopsin, although this would require a pre-concentration step for the monitoring of environmental samples. [76,77].

Enzyme Linked Immunosorbent Assay (ELISA)

This bioassay was sensitive to sub-nanogram levels (1 ng/ml) of nodularin and microcystin. The method has also been used successfully for quantitation of microcystins in environmental samples that identifies approximately 0.2 μg/l and 0.25μg/g [78]. The novel ELISA technology used to detect free saxitoxin on the basis of non-covalent immobilization technique which is demonstrated on polyclonal rabbit anti saxitoxin antibody and compared with conventional ELISA of saxitoxin using saxitoxin bovine serum albumin conjugate as the coating antigen. This technique has restriction usage because of antibodies against all possible variants of hepatotoxins and anatoxins are still not available. Moreover, the ELISA kits and consumables are far more expensive than any other bioassay system [79].

Treatment for the Removal Of Toxin Producing Cyanobacteria

There are distinct approaches that can be adopted to treat contaminated water. At first, colonies of cyanoHABs/ cyanotoxins have to be confiscated carefully from fresh and brackish water without affecting the cells. Since the toxicity within the cell may be detonated in the water. Methodology for removing toxic cells, such as settling, coagulation, filtration, and flocculation can be performed as mentioned in Table 3 [80]. The Coagulation method/flocculation method along with alum is acknowledged around the world. The procedures might not give a satisfactory result [81].

When compared to ultrasonic removal, Ultrafiltration technique is most reliable for the removal of Microcystin which remove more than 99.99% [82]. Microcystins can be directly removed with the help of naturally existing chlorine using electrochemical method. In-situ electro generator active chlorine from chlorine in water completely remove the microcystin. [83]. Chlorine treated water was not toxic to Mouse as shown by the histological examination except when treated *Cylindrospermopsis*.

Forty-six percentage of toxicity deduction was perceived with heat-treated *L. rhamnosus* strain GG for microcystin-LR as well as the *Bifidobacterium* sp at 0.5 μg/ml intensity at 35 °C after 7 h of incubation [84,85]. The activated carbon is the another method for the potential removal of toxin from water source [86] which showed total elimination of cyanobacteria under low concentration of hepatotoxins through the combined action of pre ozonation and absorption of powdered activated carbon. Wood based carbon absorb more Microcystin than the coconut-based carbon [87].

Bioassay methods are mandatory to keep the toxin level below the safe level guidelines proposed by WHO. However, analytical techniques such as reverse phase HPLC and MALDI- TOF are necessary to identify and quantify the cyanotoxins in the water bodies [88]. Once the toxin identified using the above techniques, the appropriate bioassay can be chosen based on the biological activity of toxins as well as the facilities available.

Table 2: The Overall View of Bioassay Methods and Its Reliability

Methods	Toxins	Cost	Remarks	Reference
Bioassay using Vertebrate				
Mouse	M,N,A,A(s),C,S	Medium	Require permission for license	[89]
Bioassay using Invertebrates				
Brine Shrimp	M,N	Medium	Availability of commercial kit and Expensive	[90]
Daphnid	M,N	High	Culturing techniques are labor intensive	[91]
Thamnotox	M,A,C	Medium	Availability of Commercial kit. Need full assessment for cyanotoxin evolution	[92]
Mosquito	M	Medium	Handling is difficult.	[93]
Fruit fly	M,N	Low	Culturing is easy	[94]
Locust	S	Low	Easy Handling	[95]
House fly	S	Low	Administer of toxin is difficult	[96]
Bacterial				
Microtox	M,N	Low	No connections.	[97]
Serratia sp	M,S	Medium	Poor Connections.	[98]
Biochemical Assay	M,N		Sensitivity is high.	[99]
PPase inhibition				
1. Radioactive		Low	Require special Facilities.	[100]
2. Calorimetric		Low	Enzyme need to be purified.	[101]
AChE	A(s)	Low	Only available alternative bioassay for A(s) May react with OP3 Pesticides	[102]
ELISA Technique			Very Sensitive	[103]
1.Polyclonal	M,N	Low	Variant may vary for the reaction	[104]
2. Monoclonal	M,N	Low	Variant may vary for the reaction	[105]
3. Polyclonal	S	Low	Cross reactivity may vary and does not detect C-toxin	[106]
Mammalian Cell Line Culture				
1.Hepatocyte	M,N	Medium	Sensitive but Rapid bioassay.	[107]
2.V79 fibroblast	M	High	Some false Negative results observed.	[108]
3.Neuroblastoma	S	High	Careful standardization needed.	[109]

AChE: Aetylacholinesterase; M: Microcystin; N: Nodularin; A: Anatoxin A(s)/(s); C: Cylindrospermopsin; S: Saxitoxin

Table 3: Treatment measures

S.No.	Traditional method	Modern method
1.	Screening	Bioassay Method
2.	Settling	Removal of Dissolved Toxin
3.	Filtration	Ultrafiltration, Nano filtration, reverse osmosis Fact sheet
4.	Disinfection steps	Ozonization, UV, chlorine dioxide

The toxin detection is equally important in treated waters for monitoring purposes [110]. Focused screening is presently well built up as a fruitful hit era methodology. With focused screening, it ought to moreover be conceivable to utilize a measure that's more fitting, instead of one that works well on an expansive scale [111]. Daphnids and Shrimps are the two excellent models used in bioassays because of their sensitivity for the changes in water chemistry and are inexpensive and simple to cultivate in an aquarium.

Biochemical tests and ELISA methods are more precise and useful for waterworks and most of the toxin forms can be identified in raw as well as treated water in very short time. However, such methods have limited implications when the compounds other than toxins (microcystins) are present such as protease inhibitors along with the toxins [112].

Though, every method has its own limitation, a combination of bioassays can be adopted in cases where more than one type of toxin is suspected, or where one technique is not sufficient to identify all the variants. So many methods are adopted worldwide for the removal of toxins in raw water. Since most the methods discussed effectively remove toxins from the raw water, techniques in which no external chemical is added to the water, should be adopted.

Biological sand filtration and river bank filtration are some of the methods which not only effectively remove cyanotoxins

Table 4: Physicochemical method for the detection of toxicity from cyanobacterial species

Methodology	Personal cost	Remarks	Reference
Microystins and Nodularin			
HPLC- PDA	Low	UV spectra can give tentative id	[113]
LC/MS	Medium/low	A number of different interface; mass confirmation; can have PDA	[114]
TLC	Medium	Qualitative; requires standards and further confirmation of toxins	[115]
MMPB	Medium	Detection by GC-MS or LC-MS detects total microcystin/ Nodularin	[116]
MALDI	Medium/low	Initially poor but recent developments have improved accuracy	[117]
CE-MS	Medium	Requires further development but has future promise	[118]
NMR	Medium/high	Can characterize cyanotoxins; needs mg quantities and expert interpretation	[119]
Anatoxin a, Homoanatoxin a			
HPLC-PDA	Low	Characteristic UV-spectra	[120]
GC-MS	Low	Characteristic ion spectra	[121]
GC –ECD	Low	Requires sample cleanup	[122]
LC/MS	Medium/low	Sensitive and specific	[123]
Anatoxin a(s)			
HPLC	Low	Very poor chromophore, not suitable for routine detection.	[124]
Cylindrospermopsin			
HPLC-PDA	Low	Lack of available standards; give characteristic UV spectra.	[125] [126]
Saxitoxin			
HPLC-PRE	High	Preinoculum derivatization; poor stability of derivatives.	[127]
HPLC-POST	Medium	Three solvent systems required to analyze for all variants	[128]
LC/MS	Medium/low	Best method for all variants but equipment cost can be prohibited	[129,130]
CE-MS	Medium	Poor detection, need further development	[131]

and other toxic substances from water, they are also cheap as well as environment friendly methods [132]. It should be however, noted that some biological control program should be introduced to the water reservoir, so that toxic cyanobacterial blooms can be controlled and the aquatic ecology can be maintained. Various studies showed that some species of aquatic grazers consume toxic cyanobacteria without getting affected by it.

Another approach for biological control of toxic cyanobacteria may be the application of allelopathic interactions between a toxic and non-toxic cyanobacteria. Algicidal compounds from cyanobacteria such as one from *Oscillatorialaete-virens*, whose algicide effectively eliminates and detoxicify *Microcystis* blooms; yet lack presence of any type of toxic metabolite [133]. It can be introduced to the water reservoir. However, strains should be introduced only after proper screening for non-production of other toxic metabolites. Genetically modified strains, which lack toxin-producing genes, may provide a better solution in this regard. The biological control of toxic cyanobacterial bloom will not only provide support to the waterworks, but will help in protecting the environment too.

Analysis of Physicochemical Parameters for Cyanotoxins

Physicochemical analysis of Cyanotoxins is recommended to check cyanobacterial species composition and to determine the existence and quantification of the toxins in a sample. Rapid screening for the large quantities of samples also necessary for the frequent monitoring of water where the toxin is well entrenched. The analytical methods are required to utilize the properties like the molecular weight of a toxin, reactivity held with a functional group of a molecule and chromophores. The analytical method is also necessary to identify the cyanotoxins detection. The suitable methods are adopted and discussed in the Table 4.

CONCLUSIONS

CyanoHABs are the growing concern in surface water, which instigates health-associated stake among human, aquatic, domestic, and wild animals. In order to eradicate these threats, numerous methods are espoused globally for the exclusion of toxic compounds from fresh and brackish water. In order to understand the pathogenicity of a toxin, some of the physicochemical techniques, biochemical assays, and ELISA techniques were adopted. This helps to determine and quantify cyanotoxins which cause neural dysfunction, liver disorders, stomach and skin related issues. For the removal of contaminants from surface water, some of the traditional methods like filtration, screening, and settling are wildly adopted. However, strategies towards effective removal of cyanobacterial bloom have not been substantiated in field environs. So, several biological control programs like aquatic grazers that consume cyanotoxin, and allelopathy interactions among toxic and non-toxic cyanobacteria, which are properly screened can be established in the water reservoirs along with the traditional treatment. So that the cyanotoxins can be diminished/removed completely in the aquatic ecology and maintained further. GMS (Genetically Modify Strains might provide better treatment in this regard. Despite every methodology, there will be some kind of limitations. So, the combination of various bioassay treatments can be adopted for the diversified toxins.

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