



Cyanobacteria: A Propollution Indicator For Environmental Hazards

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Abstract:

Harmful algal blooms (HABs) are an emerging serious environmental issue but that act as the most appropriate indicator for propollution activities. Reason for the concern is mostly due to variety of toxins made by Cynobacterial species present in bloom, and formation of bloom is due to high concentration of P, N and other micronutrition. Besides human health hazards cyanobacteria also pose economic hazard in terms of cell fish poisoning and also disturb ecosystem balance. Formation of HABs is dependent on variety of abiotic factors. High phosphorus availability is a defining and limiting factor that controls bloom formation. Many global studies have linked nutrient loading with bloom formation. Each HAB event has some regional specificity that is to be studied in detail in order to forecast and manage HAB. HAB forecasting can be a good way to predict and thus control factors that contribute to the formation of HAB. There are studies where forecasting tools have successfully helped in managing phosphorus and in turn HAB. Many studies have used remote sensing as a tool to create a monitoring and forecasting model. Remote sensing based analysis of Normalized Difference Vegetation Index (NDVI) is a good way to monitor and predict bloom formation.

Key Words:

Harmful Algal Blooms, Toxicity, Nutrient Loading, Remote Sensing, NDVI, Modeling

Harmful Algal Blooms: An introduction

During early 1990s, UNESCO launched a HAB programs. According to Clement and Lembey in 1994, HAB were increased as microalgae in marine and brackish waters. Hence mass death of fish with toxins and changers in ecosystems noticeable, and scientist consider that harmful and negative effect on public health and epidemic health issue. In 1999, Avaria *et al* studied that

HABs took several days and months to coverage surface water, where a combination of events of different biological, physical, and/or chemical factors play favourable condition.

During end of summer of 1989 in England, more than 450 000 population supplied with bloom contaminated water with *Microcystis* of blue-green alga that potential with high toxicity. The impact of contaminated water deceptive under deaths of 20 lambs; 15 dogs dying and one adjoining farm. These observation noticeable on other place of United Kingdom reservoirs, lakes and closed recreational water region. (Anglian water services report, 1990).

What promotes HAB formation?

Cloern *et al.*, 2010 stated that for preparation of spatial and temporal scale single model, physical features required where inconsistency of biological life within a regional perspective along with indigenous communities viz., primitive to higher; planktons to human. This model preparation entails habitat connectivity, environmental change, climatic change and other large scale influenced driven change. Moreover Imai *et al.*, 2006 studied that although levels of devastation experienced by coastal communities during HAB events might not approximate those of many natural disasters, and also Jin *et al.*, 2008; Dyson and Huppert, 2010 described that local seafood industries had greater economic losses with risk of public health. (Van Dolah *et al.*, 2001). Some researchers correlated impact of ecosystem functioning, community development with HABs that continuous or permanent effects with compound over time (Sekula-Wood *et al.*, 2009, 2011; Paerl *et al.*, 2011; Montie *et al.*, 2012).

The role of natural variability be teased apart from human disturbance requires understanding the ecological role of HABs and its pattern of prominence in phytoplankton communities that the role of natural inconsistency be harassed of from human disturbance (Hallegraeff, 1993, 2010)

Andrew D., 2012, studied there is strong association among concentration of total phosphorus and biovolume of cyanobacteria along with soaking relationship of total nitrogen. Cyanobacterial biovolume shows significant relationship with low nitrogen condition and total phosphorus. Meanwhile Stumpf *et al* in 2012, showed that dissolved reactive or total phosphorus explicated the inter-annual changeability in blooms where in 2014, Obenour *et al* also determined that load of total phosphorus associated with severity of bloom formation that predictable with some forecasting model with impact of HABs.

Toxic cyanobacteria in HAB:

Certain group of scientist viz., Sivonen et al., 1989; Vezie et al., 1998; Bolch *et al.*, 1997 separately reported that dominancy of Cynobacterial populations by diversify species or may be a single species. However, it may not be toxic among them even within a single-species bloom or sometimes it may be a mixture of toxic and non-toxic strains in contaminated area. They also determined wide variety of strains with specific genetic subgroup within a particular species. Moreover, tens or hundreds of strains with different subspecies studied by researchers (Table-1). From time to time by more than three orders of magnitude, certain strains are much more toxic and harmful than others.

Table-1: Toxic cyanobacteria species and their geographical distribution

Toxic species	Cyanotoxin	Location	Reference(s)
<i>Anabaena flos-aquae</i>	Microcystins	Canada	Krishnamurthy et al., 1989; Harada et al., 1991
<i>Anabaena ?</i>	Microcystins	Denmark	Henriksen et al., 1996b
<i>Anabaena spp.</i>	Microcystins	Egypt	Yanni and Carmichael, 1997
<i>Anabaena spp. (flos-aquae, lemmermannii, circinalis)</i>	Microcystins	Finland	Sivonen et al., 1990b; 1992a
<i>Anabaena circinalis</i>	Microcystins	France	Vezie et al., 1998
<i>Anabaena flos-aquae</i>	Microcystins	Norway	Sivonen et al., 1992a
<i>Microcystis aeruginosa</i>	Microcystins	Worldwide	Several; see Rinehart et al., 1994 for a summary
<i>M. viridis</i>	Microcystins	Japan	Kusumi et al., 1987; Watanabe et al., 1986
<i>M. botrys</i>	Microcystins	Denmark	Henriksen et al., 1996b
<i>Planktothrix agardhii</i>	Microcystins	China	Ueno et al., 1996a
<i>P. agardhii</i>	Microcystins	Denmark	Henriksen et al., 1996b
<i>P. mougeotii</i>	Microcystins	Denmark	Henriksen et al., 1996b
<i>P. agardhii</i>	Microcystins	Finland	Sivonen, 1990b; Luukkainen et al., 1993
<i>P. agardhii</i>	Microcystins	Norway	Krishnamurthy et al., 1989; Meriluoto et al., 1989
<i>Oscillatoria limosa</i>	Microcystins	Switzerland	Mez et al., 1996
<i>Nostoc sp.</i>	Microcystins	Finland	Sivonen et al., 1990a, 1992b
<i>Nostoc sp.</i>	Microcystins	England	Beattie et al., 1998
<i>Anabaenopsis millerii</i>	Microcystins	Greece	Lanaras and Cook, 1994
<i>Haphalosiphon hibernicus</i> (soil isolate)	Microcystins	USA	Prinsep et al., 1992
<i>Nodularia spumigena</i>	Nodularins	Australia	Baker and Humpage, 1994; Jones et al., 1994
<i>N. spumigena</i>	Nodularins	Baltic Sea	Sivonen et al., 1989b

<i>N. spumigena</i>	Nodularins	New Zealand	Carmichael et al., 1988a; Rinehart et al., 1988
<i>Aphanizomenon ovalisporum</i>	Cylindrospermopsin	Israel	Banker et al., 1997
<i>Cylindrospermopsis raciborskii</i>	Cylindrospermopsin	Australia	Hawkins et al., 1985; 1997
<i>C. raciborskii</i>	Cylindrospermopsin	Hungary	Torokne, 1997
<i>Umezakia natans</i>	Cylindrospermopsin	Japan	Harada et al., 1994
<i>Anabaena flos-aquae</i>	Anatoxin-a	Canada	Carmichael et al., 1975; Devlin et al., 1977
<i>Anabaena spp.</i>	Anatoxin-a	Finland	Sivonen et al., 1989a
<i>Anabaena blooms</i>	Anatoxin-a	Germany	Bumke-Vogt, 1998
<i>Anabaena sp.</i>	Anatoxin-a	Ireland	James et al., 1997
<i>Anabaena sp.</i>	Anatoxin-a (Minor amounts)	Japan	Park et al., 1993a
<i>Anabaena planctonica bloom</i>	Anatoxin-a	Italy	Bruno et al., 1994
<i>Aphanizomenon sp.</i>	Anatoxin-a	Finland	Sivonen et al., 1989a
<i>Aphanizomenon blooms</i>	Anatoxin-a	Germany	Bumke-Vogt, 1998
<i>Cylindrospermum sp.</i>	Anatoxin-a	Finland	Sivonen et al., 1989a
<i>Microcystis sp.</i>	Anatoxin-a (Minor amounts)	Japan	Park et al., 1993a
<i>Oscillatoria sp. benthic</i>	Anatoxin-a	Scotland	Edwards et al., 1992
<i>Oscillatoria sp. ?</i>	Anatoxin-a	Ireland	James et al., 1997
<i>Planktothrix sp.</i>	Anatoxin-a	Finland	Sivonen et al., 1989a
<i>Planktothrix formosa</i>	Homoanatoxin-a	Norway	Skulberg et al., 1992
<i>Anabaena flos-aquae</i>	Anatoxin-a (S)	Canada	Matsunaga et al., 1989; Mahmood and Carmichael, 1987
<i>A. lemmermannii</i>	Anatoxin-a (S)	Denmark	Henriksen et al., 1997; Onodera et al., 1997a
<i>Anabaena circinalis</i>	Saxitoxins	Australia	Humpage et al., 1994; Negri et al., 1995; 1997
<i>Aphanizomenon flos-aquae</i>	Saxitoxins	USA	Jackim and Gentile, 1968; Ikawa et al., 1982; Mahmood and Carmichael, 1986
<i>Cylindrospermopsis raciborskii</i>	Saxitoxins	Brazil	Lagos et al., 1997
<i>Lyngbya wollei</i>	Saxitoxins	USA	Carmichael et al., 1997; Onodera et al., 1997b

Adapted from: Bartram J, Chorus I, editors. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. CRC Press; 1999 Feb

Managing HABs – Prediction tools:

Application of remote sensing in reduction of 11,000 metric ton blooms during the year 1972, Great Lakes Water Quality Agreement (GLWQA) reported. For this support, determination of target algal blooms forecasting models can support. In 2012 GLWQA studies shown update to the targets for phosphorus reductions in order to reduce the incidence of extreme HABs in the western basin. Phosphorus based models are critical to determining targets for phosphorus reduction. Climatological models, such as Stumpf et al in 2012 provide a key component of a multi-model strategy to increase management confidence in the robustness of phosphorus reduction scenarios.

In year 2004 Darecki and Stramski developed MODIS or SeaWiFS to overestimate chlorophyll concentration in the Baltic Sea which can be determine upto 150-200% even in nonbloom conditions whereas the algorithm was parameterized and a CPC-specific inherent optical properties and coefficient.

Dynamics of photosynthetic organisms:

A group of scientists viz., Paerl and Huisman, 2008; O'Neil et al., 2012; Paerl and Paul, 2012; Hense et al., 2013 reported frequently that there is a broad consensus that global warming will intensify cyanobacterial blooms worldwide. Eigemann et al., 2018 reported that preferences of different species of cyanobacteria that co-occur in blooms are species-specific regarding temperature and irradiance, whereas Lehtimäki et al., 1997 and Mazur-Marzec et al., 2005 reported salinity play vital role and Olli et al., in 2015 reported that nutrient acquisition also play equivalent significance in the Cynobacterial bloom.

Lan E. I. et al in 2016 studied cyanobacteria offer distinct advantages over both plants and green algae. Not only they are more efficient at solar energy capture than plants, converting as much as 9% of the solar energy into biomass compared to only 0.5-3% for higher plants. Furthermore, some core metabolic pathways in cyanobacteria behave differently than in heterotrophic organisms or are missing some enzymatic steps. McNeely et al reported in 2010 that cyanobacteria do not have a traditional TCA cycle and are lacking α -ketoglutarate dehydrogenase. As a consequence, in cyanobacteria the TCA cycle functions as a bifurcated pathway for production of biomass precursors rather than a complete cycle.

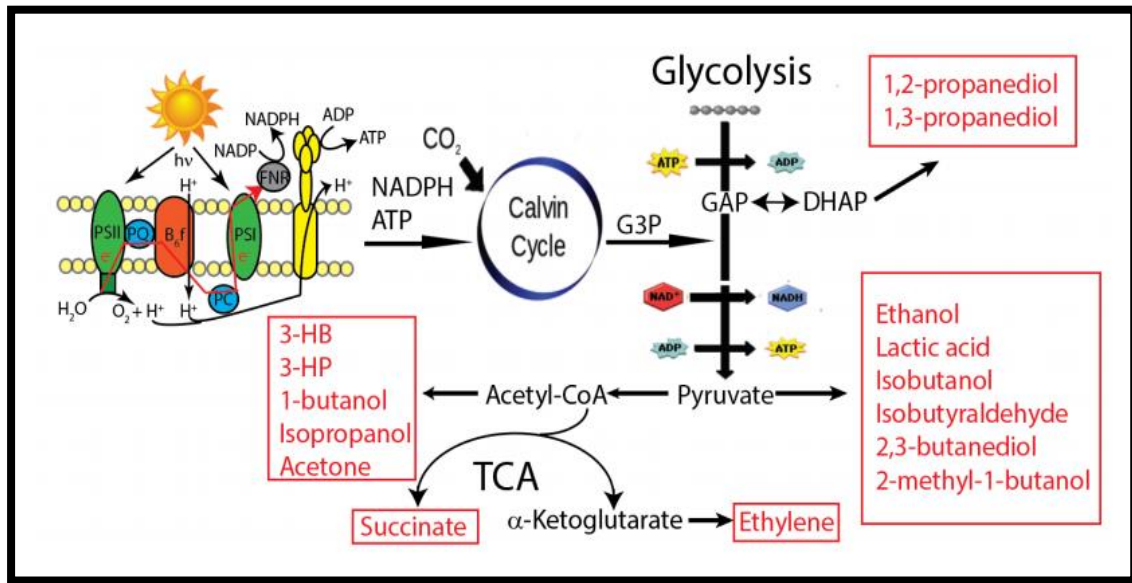


Fig.1: Overview of Photosynthetic metabolism and green chemicals production in cyanobacteria.

In year 2016 Lan et al stated that Energy and reducing equivalents are generated by photosynthetic and respiratory complexes in the thylakoid membrane. ATP, NADPH, and CO₂ feed into the Calvin-Benson cycle and glycolysis which can be further applicable to target chemicals production in cyanobacteria either through native metabolism or engineering.

According to McNeely et al in 2010, the TCA cycle in cyanobacteria is under-utilized compared to that in heterotrophic organisms but is activated in response to certain growth conditions. Increased succinate production via the oxidative TCA cycle branch was engineered by introducing α -ketoglutarate decarboxylase and succinic semialdehyde dehydrogenase.

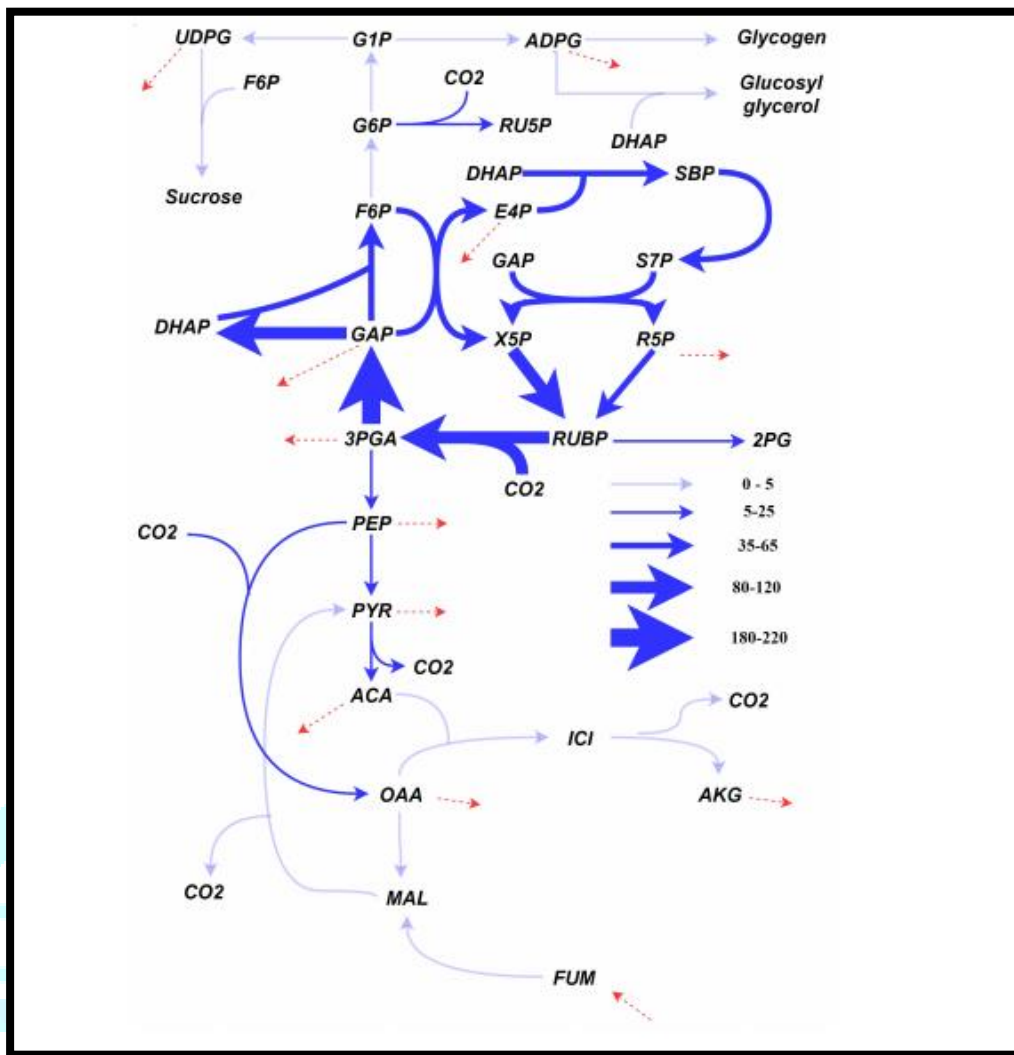


Fig.2: A generalized flux map for cyanobacterial photoautotrophic metabolism.

Cyanobacterial Health Hazard:

The World Health Organization (WHO) has established two guidance levels for recreational water. Two alert levels according to cyanobacterial growth in sources of drinking water supply have also been suggested in Australia. As per studies by Bartram *et al.* in 1999, the thresholds for Alert Level 1 are 2,000 cyanobacterial cells/ml or 1 $\mu\text{g/L}$ chlorophyll *a* or a biovolume of 0.2 mm^3/L , and the thresholds for Alert Level 2 are 100,000 cyanobacterial cells/ml or 50 $\mu\text{g/L}$ chlorophyll *a* or a biovolume of 10 mm^3/L . In Korea in 1997, an “alert system for algal bloom” was established by the Ministry of Environment, in which the Caution, Warning, and Outbreak Levels are determined by cyanobacterial cell densities of 500, 5,000, and 1,000,000 cells/ml and chlorophyll *A* of 15, 25, and 100 $\mu\text{g/L}$, respectively. According to Ahn *et al.*, 2003, all of these alert levels were developed on the basis of the assumption that the principal bloom-formers are cyanobacteria, and that they are toxic.

Awareness of toxic cyanobacterial blooms and scums in freshwaters, and of health hazards which they can present, is long established and a pronounced history of animals and fish deaths as well as outbreaks of human illness and poisonings are present. The extreme cases of human poisonings were manifested in the death of more than 60 hemodialysis patients in Caruaru, Brazil in 1996 and in the incidences of primary liver cancer in China. Freshwater cyanobacteria are reported to produce hepatotoxins, microcystins and nodularins more potently than other toxins and, that is why, most research on cyanobacterial toxins has been centered on these toxins and their producer cells in freshwaters. Microcystins and nodularins cause severe disruption of liver architecture and function and induce clinical signs such as weakness, recumbency, pallor, vomiting and diarrhea and death occurs due to pooling of blood in the liver. These hepatotoxins irreversibly inhibit protein phosphatase PP1 and PP2 A and can have diverse inhibitory effects at genetic, developmental, metabolic, and physiological levels, including and beyond liver function. The high susceptibility of liver cells to damage by microcystins in vitro and in vivo is accounted for by the active uptake of the toxins by bile acid transport system.

	1800–1900	1901–10	1911–20	1921–30	1931–40	1941–50	1951–60	1961–70	1971–80	1981–90	1991–2000	2001–10	Total
Animals													
Number of incidents	10	–	8	11	12	27	25	24	45	70	79	95	406
Location													
– Australia/New Zealand	2	–	–	–	–	1	1	3	6	2	12	4	31
– Canada	–	–	3	1	5	14	12	10	17	3	–	1	66
– Europe	4	–	2	2	1	2	5	3	5	33	26	12	95
– Rest of World	–	–	–	–	–	2	1	1	3	2	6	7	22
– South Africa	–	–	–	1	–	1	–	–	4	3	13	2	24
– United States	4	–	3	7	6	7	6	7	10	27	22	69	168
Species affected ^a													
– Birds	1	–	1	1	4	16	5	3	–	11	8	12	62
– Dogs	1	–	–	1	–	5	7	5	7	21	31	72	150
– Livestock & poultry	7	–	6	8	5	13	15	13	17	27	24	7	142
– Fish & aquatic organisms	4	–	2	2	3	5	8	5	20	20	19	13	101
– Other mammals	–	–	–	1	1	6	3	–	2	8	5	6	32
– Other	–	–	–	1	1	–	–	–	2	1	1	3	9
Humans													
Number of incidents	3	–	–	2	8	9	9	12	13	18	16	25	115
Location													
– Australia	1	–	–	–	–	–	–	–	1	2	5	–	9
– Canada	–	–	–	–	–	–	4	1	–	–	–	–	5
– Europe	2	–	–	–	–	–	1	–	1	13	6	4	27
– Rest of World	–	–	–	–	–	–	1	11	2	2	4	4	24
– United States	–	–	–	2	8	9	3	–	9	1	1	17	50
Exposure mode													
– Drinking/domestic use	1	–	–	2	–	–	1	11	3	6	3	–	27
– Recreational activities	1	–	–	–	7	9	8	1	8	6	6	19	65
– Haemodialysis	–	–	–	–	–	–	–	–	1	–	1	1	3
– Occupational	–	–	–	–	–	–	–	–	–	2	1	2	5
– Other ^b	1	–	–	–	1	–	–	–	1	4	5	3	15

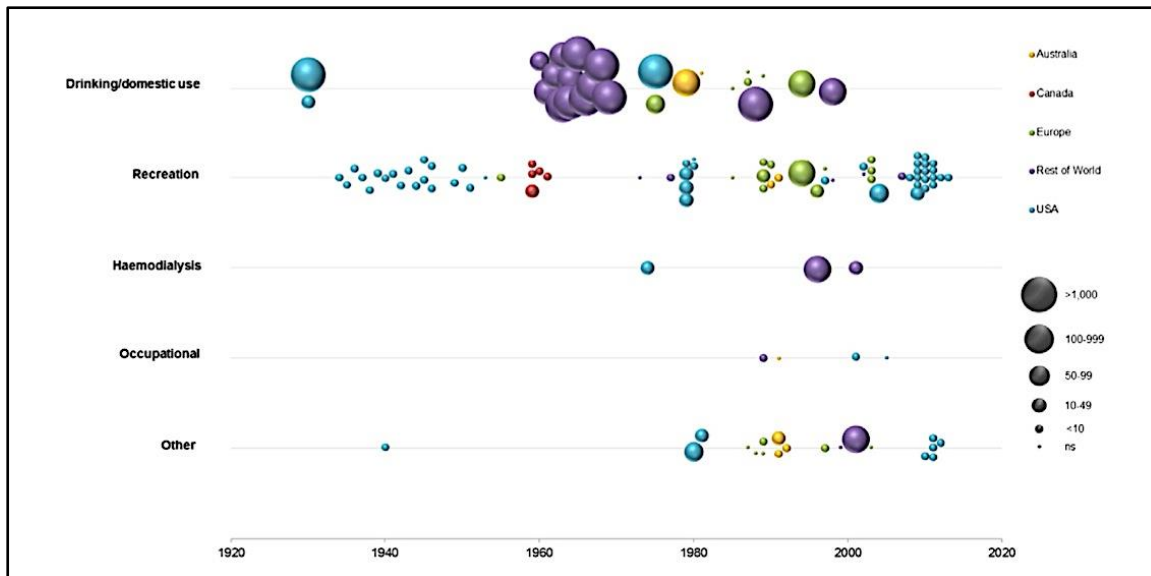
Table 2: Summary of global health issues reported on account of cyanobacteria

(Adapted from: Wood, 2016)

HABs – A global concern ?

According to Beeton in year 2002, biogeochemical processes controlling the global increase in HABs are the topic of extensive ongoing research, much of the debate has centered on HABs in marine ecosystems, and similar stressors apply in freshwater systems. Meanwhile, Hallegraef studied in 2003, Four main hypotheses for the apparent increase have emerged: increased scientific awareness of toxic species, increased anthropogenic nutrient loading, increased

frequency and magnitude of extreme climatic events, and increased exposure to invasive species. In year 2008, Perovich *et al.* stated that Understanding the role of each of these mechanisms in explaining global HAB trends is an ongoing area of research.



(Adapted from: Wood, 2016)

Fig. 2 Publications showing growth in reporting human health hazards

There are numerous examples worldwide of increases in HABs linked to increased nutrient loading, a few of which are studied by Schindler, 1977 and Burkholder 2002, the strong relationships have been shown for many years in freshwater ecosystems between phosphorus loadings and harmful cyanobacteria blooms, increasing linkages between nutrient loading and estuarine/coastal marine HABs have more recently been recognized by Anderson *et al.*, 2002. For example, in the Gulf of Mexico, the sedimentary record of concentration of potentially toxic diatoms, *Pseudo-nitzschia* spp., increased in parallel to increased nitrate loading over the past several decades said Parsons *et al.* in 2002.

According to Anderson *et al.* in 2002, the relationship between alteration in nutrient composition and the development of HABs is supported by examples in freshwaters, estuaries and marine coastal waters worldwide. Off the coast of Germany, time series analysis of nutrient concentrations over several decades showed that a 4-fold increase in the N:Si ratio coincided with decreased abundance of diatoms and an increase in *Phaeocystis* blooms, while in nearly 4-decade time series from Narragansett Bay shows a relationship between increases in the N:Si ratio and a proportional increase in flagellates. Decreases in N:P ratios due to phosphorus loading have sometimes been related to increased abundance of certain harmful dinoflagellate species.

In year 1997 Hodgkiss and Ho as well in 2001 Hodgkiss noticed that in Tolo Harbor, Hong Kong, where phosphorus loading increased due to human population growth in the late 1980s, a shift from diatoms to dinoflagellates was observed, coincident with a decrease in the ambient N:P ratio from ca. 20:1 to <10:1. On shorter time scales, in Tunisian aquaculture lagoons, blooms of toxic dinoflagellates have been shown to develop when the N:P ratio drops in autumn and along the eastern seaboard of the US, outbreaks of the toxic dinoflagellates, *Pfiesteria piscicida* and *P. shumwayae*, have been associated with low N:P ratios from high phosphate loading by effluent spills from concentrated animal operations. Blooms of *Karenia brevis* on the western Florida shelf are also found in waters with lower dissolved inorganic N:P ratios than in water directly to the south with higher N:P ratios, where diatoms tend to be more prevalent.

According to Cloern in 2001, the relationships between nutrient loading and algal proliferations are complicated by shifts in food webs, habitat changes, climate changes and other system alterations that affect the extent to which a given species may accumulate, which is known as typically a “phase II” model of eutrophication. As per his remarkable conclusion, increased nutrient enrichment can lead to a shift in plankton community composition, which in turn can affect predator-prey relationships, further altering the transfer of nutrients not only, incoming nutrients may be regenerated, recycled, or removed in space and time from the set of conditions that would otherwise support blooms furthermore these links are frequently difficult to establish.

Aguilera-Belmonte *et al* in 2011 studied that historically, in Chile the main toxins have been found (Paralytic Shellfish Poisoning, PSP, Diarrhetic Shellfish Poisoning, DSP, and Amnesic Shellfish Poisoning, ASP) associated with HAB events. However, the only cases of severe poisoning and death have been generated in the south of the country by the dinoflagellate *Alexandrium catenella* (associated with the production of saxitoxins causing PSP). This species belongs to the complex *Alexandrium tamarense/ catenella/fundyense* (complex *tamarense*) defined by its morphological attributes.

Factors that lead to HAB formation:

Vila *et al.*, 2001 noted that the positive relationship of temperature with the higher abundance of phytoplankton, the easy obtaining and recording from the first events Vidal *et al.*, 2012 had constituted the temperature as the factor of greatest knowledge and perhaps the most important in the study of HABs. Stumpf, R.P studied in 2012 that general increases in phytoplankton biomass in frequency and duration of algal blooms have been associated with an overall increase in nutrient inputs, which found similarly in lake Chaohu, in eastern China where eutrophication

due to the increase in population, economic activity, and wastewater generated, GDP increased along with waste increase in catchment area. The average nutrients concentration exceeded cyanobacteria growth requirements, although nutrient enrichment is a prerequisite to bloom formation, the role of nutrient concentrations in controlling floating algae bloom dynamics might be limited due to elevated concentrations and low inter-annual variation.

Paerl, H.W. et al stated in 2009, Temperature play crucial role in growth of blooms such as *Microcystis aeruginosa* shown to grow and enter the water column which resulting in close correlation between algal recruitment and cumulative temperature, cyanobacteria exhibit optimal growth rates at higher temperatures and compete more effectively with diatoms, chlorophytes, and cryptophytes.

Guzmán et al., 2015 studied stratification (caused by various mechanisms) seems to be a preponderant factor to trigger HAB events, especially in the ability to influence the decrease of the mixing and generate possible particle retention zone which lead to regulate the vertical and horizontal distribution of *A. catenella* blooms, which affect the distribution of their possible triggering factors (e.g., temperature, salinity) and drive (or inhibit) the transport of harmful species to the photic layer.

Clement, 2002 shown, the evidence those seems to point out that climatic disturbances and interactions lead to trigger events of blooms of *A. catenella* and *Pseudo-nitzschia spp.* They have been related from local factors like atmospheric disturbances in winter. As other agents that initiate or support the maintenance of a harmful bloom can be considered biological factors such as resistance cysts, changes in the structure of the phytoplankton community (phytoplankton taxocenosis), and chemical factors such as nutrients or ocean acidification.

Researcher like Uribe et al., 1995; Arriagada et al., 2003; Vidal et al., 2012 Studies infer that, for a wide spatial scale, the periods where the highest values of PSP are recorded, are related to certain environmental conditions that allow differences in the composition, distribution and abundance of phytoplankton, going from a predominance of diatoms (in qualitative terms) to a predominance of dinoflagellates. These mechanisms are manifested due to the constant competition for resources, where in the presence of an environment without nutrient limitations and high availability of silicon, diatoms tend to predominate (M. Vergara, pers. comm.). They live and reproduce quickly but have low efficiency of resource use and when there are not enough, they begin to lose competitiveness and longer-lived species such as dinoflagellates appear which tolerate lower concentrations of certain nutrients better (Fig.3).

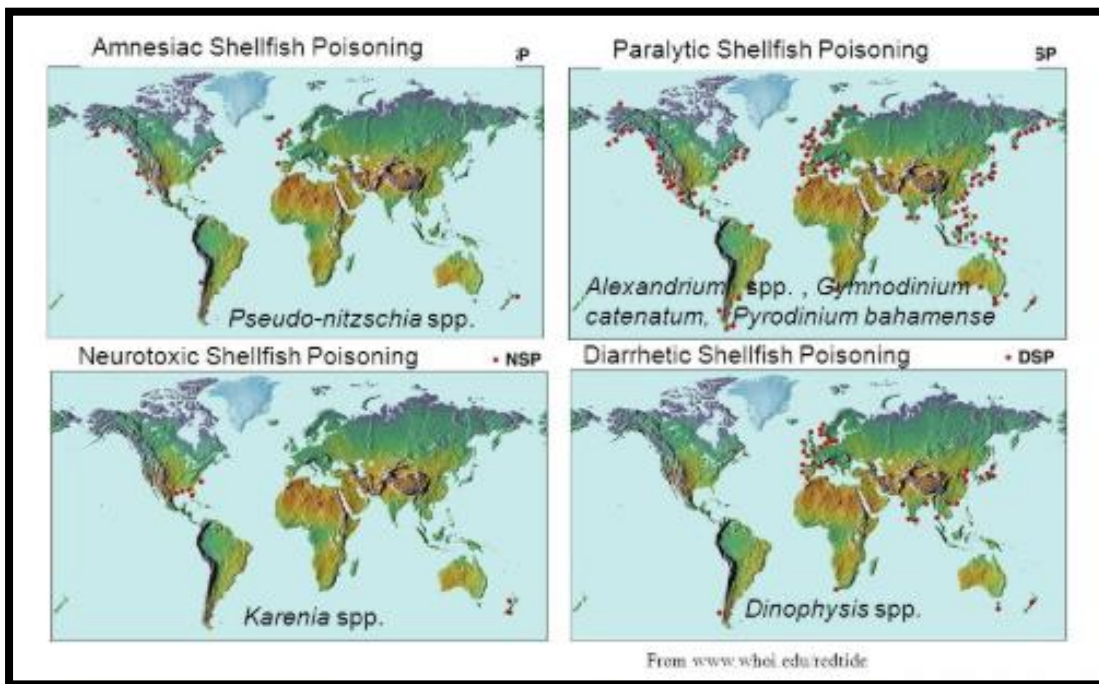


Fig.3 Distribution of some major HAB toxic events world wide

Use of remote sensing for the study of toxic cyanobacteria:

Unprecedented access to environmental and satellite datasets have presented many opportunities for aquatic ecological research and remote sensing alternatives to current monitoring systems. Available satellite data together with empirical and semi-analytical techniques can be used to develop algal detection remote sensing models. Stumpf et al., observed in 2009 Some HABs evaluated with remote sensing HAB Species (Table-3). However optical in situ Shellfish toxin Other major HABs not clearly monitored with remote sensing

Table-3: Some HABs Evaluated with Remote Sensing

HAB Species	Region	Sensing Type	Impact
<i>Pseudo-nitzschia spp.</i>	Upwelling region	SST, chlorophyll	ASP, variable
<i>Karenia brevis</i>	Gulf of Mexico	Chlorophyll, optical ratio, absorption spectra	NSP, respiratory, fish toxin

<i>Karenia mikimotoi</i>	Coastal ocean (Hong Kong, Ireland, New Zealand)	SST, chlorophyll	NSP
<i>Gymnodinium catenatum</i>	Estuaries, coastal ocean, upwelling	SST, chlorophyll	PSP
<i>Alexandrium spp</i>	Coastal ocean (Gulf of Maine, Gulf of Alaska)	SST	PSP
<i>Gonyaulax</i>	Upwelling region	Chlorophyll, possible UV absorption	Fish toxin
<i>Cochlodinium</i>	Coastal ocean (British Columbia, Korea)	SST, color	Shellfish toxin
<i>Nodularia, Microcystis</i>	Enclosed Brackish	Color	Hepatotoxin
Other major HABS not clearly monitored with remote sensing			
<i>Dinophysis</i>	Ireland, Portugal Norway	Maybe SST, However optical in situ	Shellfish toxin

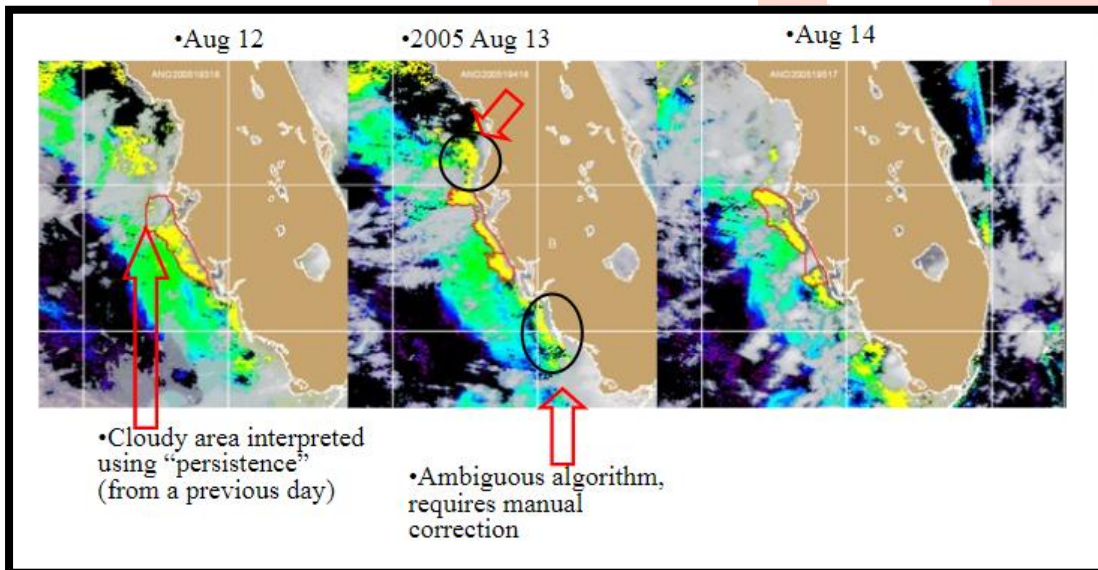


Fig.4 spectral use in 30 year time series studies of occurrence of HABS in Gulf of Mexico

The NDVI have been used widely to examine the relation between spectral variability and the vegetation vigor or growth rate. It is also useful to determine the production of green vegetation as well as detect vegetation changes. Chouhan R and Rao N in 2011 studied that in the Indian remote sensing agency have two types of generic sensors which employed for the collection of information, named passive remote sensing and active remote sensing. This passive remote

sensing is used for gathering the satellite information where the sun is the source of energy. It consists of an array of small sensors or detectors, which is used to record the amount of electromagnetic radiation that is reflected or emitted from the Earth's surface. They also stated that, in case of active remote sensing, the remote sensing system propagates its own electro-magnetic radiation and measures the intensity of the return signal. For the feature extraction, mostly passive remote sensing is applied and the active remote sensing is used for the radar imaging system.

Richardson, 1996; Park and Ruddick, 2007 observed that remote sensing can provide regular, synoptic coverage of algal blooms over large areas for regional monitoring programs at resolutions unattainable by field measurements. Several satellites scan entire regions of the earth with frequent revisit times. Satellite measurement is especially useful for bloom monitoring because of the unique spectral absorbance/reflectance characteristics of photosynthetic pigments like chl-a.

S. L. Ozesmi in 2002, Based on various remote sensing data types, many methods for delineating water bodies have been described, particularly those in floodplains and in coastal areas. Wetland vegetation also provides a natural barrier to fast moving water and therefore aids in flood speed reduction. The visual interpretation of wetlands from maps, aerial photography, and hard copy of satellite images have been used extensively for characteristics like water quality, turbidity and chlorophyll contents can also be determined using optical remote sensing techniques but are more complicated to assess.

Unprecedented access to environmental and satellite datasets has presented many opportunities for aquatic ecological research and remote sensing alternatives to current monitoring systems. This review will look at the historical application of satellite imagery to monitor phytoplankton and harmful algal blooms in coastal and inland waters. The intent of this study is to provide a background on available satellite data and the empirical and semi-analytical techniques used to develop algal detection remote sensing models.

Brown et al. studied in year 2008, Several satellites at varying degrees of spatial, spectral and temporal resolution have been used for measuring algae propagation and distribution. Table 1 shows a summary of past and currently operational sensors. Satellite models have proven especially useful at detecting algal blooms based on the spectral characteristics of photosynthetic pigments. Chl-a has two absorbance peaks near 433 nm (blue) and 686 nm (red), a reflectance maximum near 550 nm (green) and a reflectance peak around 690-700 nm in the visible portion

of the electromagnetic spectrum. The relationship of light reflected or absorbed at specific wavelengths (λ) in the visible spectrum can be used to estimate chl-a concentrations using satellite bio-optical algorithms.

Microcystin toxins and *Microcystis* species:

Chorus et al., 2000 reported that in the presence of full sunlight, microcystins undergo photochemical breakdown, but this varies by microcystin congener. The presence of water-soluble cell pigments, in particular phycobiliproteins, enhances this breakdown. Breakdown can occur in as few as two weeks to longer than six weeks, depending on the concentration of pigment and the intensity of the light.

Kosakowska et al., 2007 studied that under the right conditions of pH, nutrient availability, light, and temperature, cyanobacteria can reproduce quickly forming a bloom. Although they also studies of the impact of environmental factors on cyanotoxin production are ongoing, nutrient (N, P and trace metals) supply rates, light, temperature, oxidative stressors, interactions with other biota (viruses, bacteria and animal grazers), and most likely, the combined effects of these factors are all involved Fulvic and humic acids reportedly encourage cyanobacteria growth.

Microcystins are produced by several cyanobacterial species, including *Anabaena*, *Fischerella*, *Gloeotrichia*, *Nodularia*, *Nostoc*, *Oscillatoria*, members of *Microcystis*, and *Planktothrix*. Microcystins are the most common cyanotoxin found worldwide and have been reported in surface waters in most of the U.S. and Europe. Dry-weight concentrations of microcystins in surface freshwater cyanobacterial blooms or surface freshwater samples reported worldwide between 1985 and 1996 ranged from 1 to 7,300 $\mu\text{g/g}$. Graham et al., 2012 studied that water concentrations of extracellular plus intracellular microcystins ranged from 0.04 to 25,000 $\mu\text{g/L}$. The concentration of extracellular microcystins ranged from 0.02 to a high of 1,800 $\mu\text{g/L}$ reported following treatment of a large cyanobacteria bloom with algaecide and the U.S. Geological Survey (USGS) reported a concentration of 150,000 $\mu\text{g/L}$ total microcystins, in a lake in Kansas.

In year 2016, Matthew J. Harke reviewed regarding the toxic, bloom-forming cyanobacterium, *Microcystis*, with a specific focus on its geographic distribution, toxins, genomics, phylogeny, and ecology. A global analysis found documentation suggesting geographic expansion of *Microcystis*, with recorded blooms in at least 108 countries, 79 of which have also reported the hepatotoxin microcystin (Fig.5).

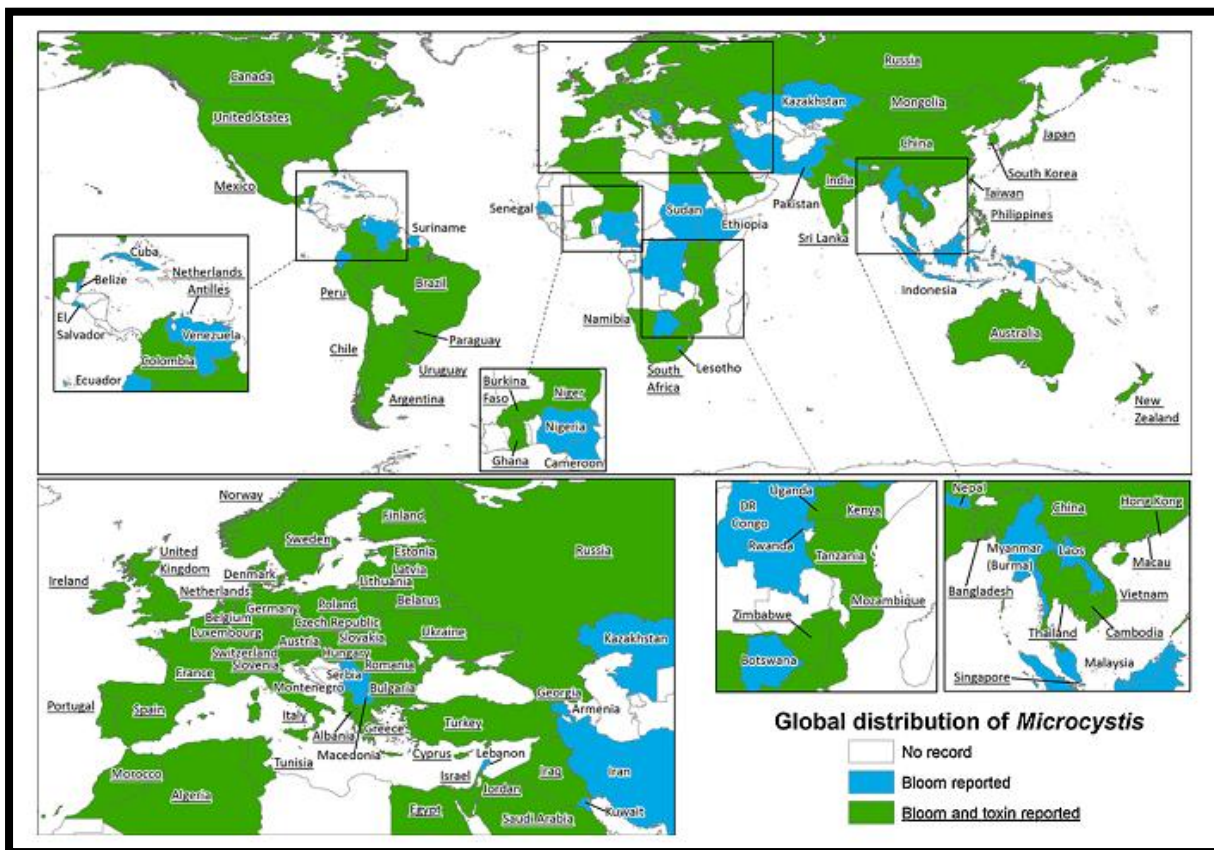


Fig. 5 The global occurrence of *Microcystis* blooms and microcystin as determined through literature searches for records of *Microcystis* blooms from 257 countries and territories during the year 2016.

In 2002, the Monitoring and Event Response to Harmful Algal Blooms in the Lower Great Lakes (MERHAB-LGL) project evaluated the occurrence and distribution of cyanobacterial toxins in the lower Great Lakes region. According to Burns in 2008, the most frequently observed cyanobacteria were *Microcystis* (43.1%), *Cylindrospermopsis* (39.5%), and *Anabaena* spp (28.7%) of 167 surface water samples taken from 75 waterbodies, 88 samples were positive for cyanotoxins among them *Microcystin* was the most commonly found cyanotoxin in water samples collected, occurring in 87 water samples.

Boyer stated in 2007 Analysis for total microcystins was performed using Protein Phosphatase Inhibition Assay (PIIA). *Microcystins* were detected in at least 65% of the samples, mostly in Lake Erie, Lake Ontario, and Lake Champlain. The National Oceanic and Atmospheric Administration (NOAA) Center of Excellence for Great Lakes and Human Health (CEGLHH) continues to monitor the Great Lakes and regularly samples algal blooms for *microcystin* in response to bloom events.

Some findings of Makarewicz et al., in 2006 that study of the Great Lakes found high levels of cyanobacteria during the month of August, those Microcystin-LR was analyzed by PPIA (limit of detection of 0.003 µg/L) and was detected at levels of 0.084 µg/L in the near shore and 0.076 µg/L in the bays and rivers.

In 2007 US government under the National Lakes Assessment (NLA) conducted the first-ever national probability-based survey of the nation's lakes, ponds and reservoirs. This baseline study of the condition of the nation's lakes provided estimates of the condition of natural and man-made freshwater lakes, ponds, and reservoirs greater than 10 acres and at least one meter deep. The NLA measured microcystins using Enzyme Linked Immunosorbent Assays (ELISA) with a detection limit of 0.1 µg/L as well as cyanobacterial cell counts and chlorophyll-a concentrations, which were indicators of the presence of cyanobacterial toxins.

As per a 2008 study by Funari and Testai, microcystins are relatively stable and resistant to chemical hydrolysis or oxidation at or near neutral pH. Elevated or low pH or temperatures above 30°C may cause slow hydrolysis whereas microcystin is not destroyed by boiling but in natural waters kept in the dark, microcystins have been observed to persist for 21 days to 2-3 months in solution and up to 6 months in dry scum.

Genome level study of Microcystis:

Kaneko et al., in 2007 sequenced the first *Microcystis* genome from the toxic isolate *Microcystis aeruginosa* NIES-843 which had been followed shortly that of *M. aeruginosa* PCC 7806 in 2008 through Frangeul et al. In late of 2015, only one *M. aeruginosa* genomes had been closed, however, the number of draft genomes has subsequently increased, as strains isolated from diverse locations have been sequenced. To date, 15 draft or closed genomes are available, sequenced from strains isolated in Japan by team of scientist Kaneko et al., 2007; Okano et al., 2015, from Netherlands 2008 Frangeul et al., from China Yang et al., in year 2015, and from Brazil Fiore et al. in year 2013 accompany a collection of draft sequences from Humbert et al. in 2013 for isolates from Canada, the Central African Republic, France, the United States, South Africa, Australia, and Thailand.

Genomes range in size from 4.26 Mbp (*M. aeruginosa* PCC 9806) to 5.84 Mbp (*M. aeruginosa* NIES 843). Previous studies have highlighted genetic diversity between species of *Microcystis*, while Harke et al., in year 2012 studied between the potentially toxic *M. aeruginosa* and nontoxic *Microcystis wesenbergii*, and sequencing of such species may

reveal important insight into the divergent ecological strategies that may exist between strains, potentially driven by each strain's unique flexible genes.

In year 2015, Otten et al. studied the use of targeted genomics (e.g., PCR/QPCR, amplicon and shotgun sequencing) for detection, quantification, and phylogenetic analysis of *Microcystis* in the environment has rapidly expanded in recent years. The most frequent targets of these techniques include the microcystin synthetase gene operon, cyanobacterial and *Microcystis*-specific 16S rRNA or c-phycoerythrin photopigment genes *cpcBA* along with the genes involved in nutrient transportation and metabolism.

Similarly, Baxa et al., 2010 and Wood et al., 2011 developed such tools to bolstered the ability to identify organism(s) responsible for toxin production, even in mixed phytoplankton communities. With the advent of high-throughput DNA sequencing, it is now tenable to compare microbial genomes in silico. The average nucleotide identity (ANI) of conserved genes from two strains of bacteria has been demonstrated to be as robust as DDH for delineating species when using a cut-off for delineation of 95–96% identity or greater. According to Goris et al in 2007, this metric is also slowly replacing the use of 16S rRNA comparisons to infer phylogeny because it is based on a larger sample of genetic information (Table-2).

Recent studies as per Kim et al., 2014 suggest that when using 16S rRNA gene sequences to infer phylogeny, the cut-off to distinguish one species from another should be raised from 97% to 98.7% or greater. However, Luc Cornet et al, 2018 studied phylogenomic tree of Cyanobacteria which is based on the largest supermatrix (in terms of conserved positions) to date (64 non-contaminated and complete reference strains; >170 000 unambiguously aligned amino-acid positions). It is congruent with other recent cyanobacterial phylogenies that to root the tree on the *Gloeobacter* species (clade G), which may following the practice of many recent cyanobacterial phylogenies.

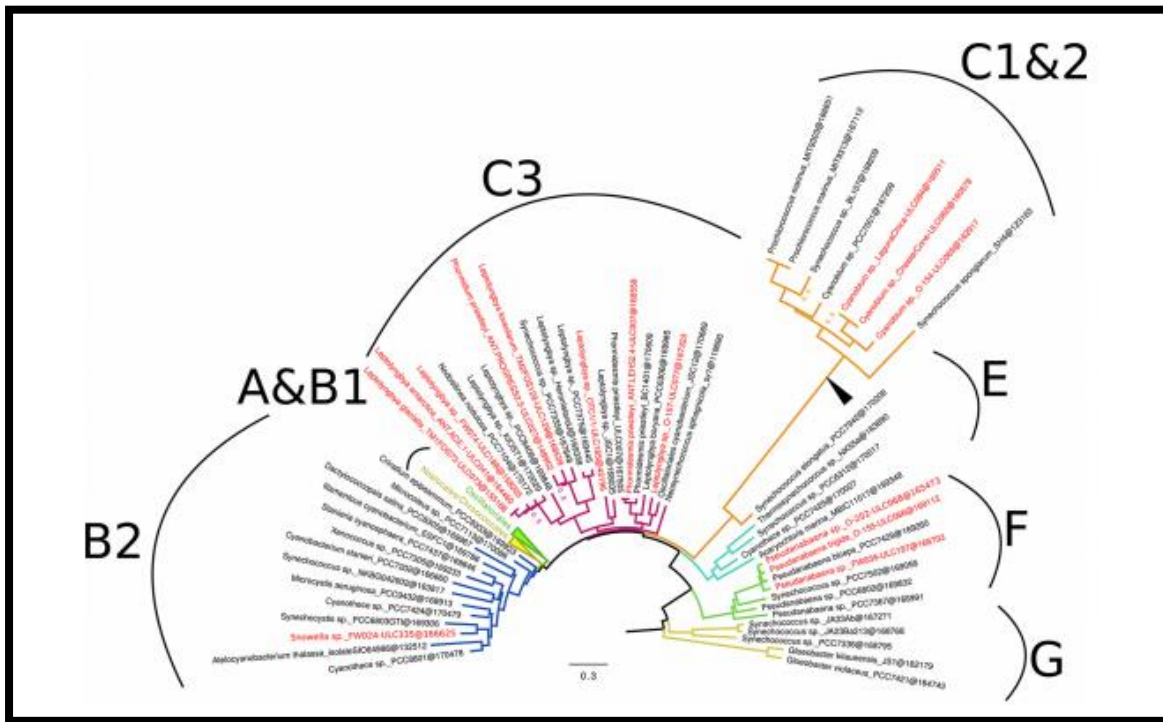


Fig.6 Phylogenomic tree of 64 broadly sampled Cyanobacteria showing the phylogenetic position of the 15 cyanobacterial genome bins. The Bayesian tree was inferred under the CAT+G4 model from a supermatrix made of 675 genes (79 organisms 170 983 amino acid positions). Cyanobacterial clades (see Table 1) were named according to Shih et al.

Whether or not all *Microcystis* morpho-species should be placed within a single *Microcystis aeruginosa* complex could be construed as a purely esoteric question, but doing so could provide additional applied benefits for the scientific, research, and managerial communities. For example, it would simplify the task of microscopic identification and enumeration for public health purposes and remove much of the subjectivity inherent to each taxonomist. More importantly, such a unification of *Microcystis* morpho-species would also counter the widespread belief that certain cyanobacterial species are universally toxic or nontoxic.

Table-2: Major Scientific Contributions in the field of Cyanotoxins

Year	Author	Contribution
1883	Arthur, JC	Study of bloom forming poisonous algal species

1887	Arthur, JC	Study of bloom forming poisonous algal species
1903	Nelson, NPB	Primary study of bloom forming algae
1925	Wilmot Enterprise	Livestock poisoning due to lake water
1925	Wilmot Enterprise	Farm cattle deaths due to lake water
1927	Woodcock, EF	Livestock poisoning in Michigan
1931	Tisdale, ES	Connection of water supply quality and intestinal disorders
1931	Utermohl, H	Method for the quantification of cyanobacterial cells
1934	Fitch, CP	Review of toxic water effect on domestic animals
1939	Deem, AW	Toxic effect of bloom algae on laboratory rabbits
1940	Durrell, LW	Laboratory study of bloom algal species toxicity
1943	Quin, AH	Effect of bloom water on domestic animals like sheep
1947	Brandenburg, TO	Effect of water toxicity on livestock animals
1948	Mackenthun, KM	Total fish mortality in lake due to poisoning
1952	Ingram, WM	Species diversity of toxic algal species
1953	Rose, ET	Study of toxic algal blooms in the lakes of Iowa state
1953	Gerhardt, RW	Possible use of blue-green algae toxicity as mosquito control
1954	Rose, ET	Warning of possible human health hazard due to algal blooms
1959	Banner, AH	Toxicity of a fish is connected to an algal species
1960	Palmer, CM	Review of algal growth in South Central USA lakes and rivers
1961	Phinney, HK	Study of eutrophication issue at Klamath Lake
1964	Olson, TA	Effect of blue-green algae on fishes and birds
1966	Maloney, TE	Estimation of toxin Lethal Dose in mice
1971	Moiikeha, SN	Study of chemical properties of toxin by chromatography
1975	Hindman, SH	Pyrogenic reactions at a dialysis center traced to cyanobacteria
1976	Lippy, EC	62 percent users of Sewickley water facility reported to be sick
1977	Barker, RJ	Bloom algae were suggested toxic to honey bee
1977	Stanier, RY	Reviewed taxonomy of cyanobacteria
1978	Solomon, AE	Cutaneous inflammation reported using purified toxin
1979	Rippka, R	Revised taxonomy of cyanobacteria
1981	Juday, RE	Cattle and dog death due to <i>Anabaena flos-aquae</i> bloom
1981	Sasner, JJ	Fish toxicity study of Aphanotoxin from <i>Aphanizomenon</i>
1981	Billings, WH	Three outbreaks connected to <i>Anabaena</i> bloom in lakes
1981	Carmichael, WW	Hemagglutination for detection of freshwater cyanobacteria
1982	Runnegar, MTC	Explained mechanism of in vitro hepatic toxicity of microcystin
1985	Hawkins, PR	Hepatotoxicity in mice was reported for <i>Cylindrospermopsis</i>
1986	Carmichael, WW	Toxicity of <i>Microcystis</i> bloom from Lake Erie
1986	Falconer, IR	Provided evidence of liver damage by cyanotoxins
1987	Galey, FD	Outbreak of <i>Microcystis aeruginosa</i> toxicity in dairy cows
1988	Lung, W	Mathematical modeling of predicting harmful algal bloom
1988	Mahmood, NA	Neurotoxic study of <i>Anabaena flos-aquae</i> toxin outbreak in dogs
1988	Anagnostidis, K	Classification scheme of cyanobacteria
1989	Cook, WO	Study of anti-cholinesterase activity of algal bloom toxin
1990	Honkanen, RE	Proved inhibition of liver Protein phosphatase enzyme by toxins
1992	Namikoshi, M	Identification of nine new variants of Microcystin-LR
1993	DeVries, SE	Chemical studies on purified Microcystin toxins
1994	Toivola, DM	Identified hepatic enzyme target of toxins

1995	Butler, MJ	Microcystin toxicity in lobsters and sponges of Florida Bay
1995	Namikoshi, M	New method of assigning structure to new Microcystins
1996	Nagai, H	Chemical study on marine red algae toxin in Hawaii
1996	Ueno, Y	Connection of primary liver cancer cases in China with toxins
1996	Falconer, IR	Connected liver tumor progression with cyanotoxins
1997	Carmichael, WW	Study of paralytic shellfish poisoning by cyanobacteria
1998	Richardson, LL	Black band disease of corals is connected to cyanobacteria
1999	Senogles, P	Study on the effectiveness of chlorination in removing toxin
2000	Metcalf, JS	Use of polyclonal antibodies for ELISA detection of toxins
2001	Karner, DA	Microcystin detection in raw and treated drinking water
2001	Zimba, PV	Confirmation of microcystin as agent of pond catfish death
2002	Baker, JA	Use of PCR for toxin gene detection
2003	Schrader, KK	Study of natural algicides to control cyanobacteria
2003	Zimba, PV	Study of Microcystin variants in aquaculture ponds
2005	MacElhiney, J	HPLC based detection of toxins
2007	Ahn, C	Use of phycocyanin as alert system for harmful blooms
2007	Kaneko, T	Sequenced complete genome of <i>Microcystis aeruginosa</i>
2009	Valerio, E	Multiplex PCR based detection of toxin producing genes
2013	Pedro, O	Quatification of toxin using Real-Time PCR
2016	Neumann, AC	Gold nanoparticle based immunodetection of toxins
2016	Balest, L	Very low level detection of toxins using HPLC-MS
2017	Zhang, F	Toxin tracking in Lake by remote sensing
2017	Vidal, F	Report of liver failure due to recreational exposure of bloom
2017	Walls, JT	Explained role of temperature in toxin release
2017	Parulekar, NN	Study of bloom community by 16S rDNA amplicons
2018	Ansari, RR	A new method of studying bloom by volunteering pilots

While acute toxicity is the most obvious problem in cyanobacterial poisoning, a long-term risk may also be present. Short exposures to toxins may result in long-term injury and chronic low-level exposure may cause adverse health effects. Animal experiments have shown chronic liver injury from continuing oral exposure to microcystins. But presence of cyanobacteria lead to indication of high pollution level that make the as best indicator organisms for propollution.

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