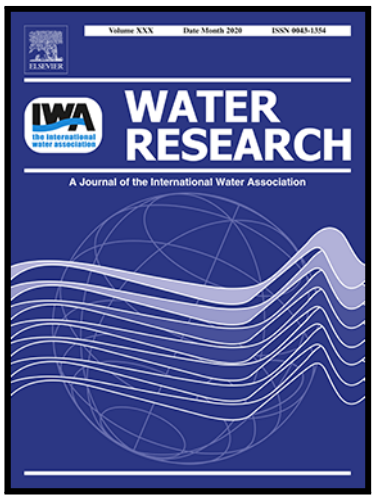
Journal Pre-proof



Cyanobacterial blooms in surface waters – nature-based solutions, cyanotoxins and their biotransformation products

Alba Martinez i Quer , Yrsa Larsson , Anders Johansen , Carlos A. Arias , Pedro N. Carvalho

PII: S0043-1354(24)00023-X
DOI: https://doi.org/10.1016/j.watres.2024.121122
Reference: WR 121122

To appear in: *Water Research*

Received date: 5 September 2023
Revised date: 18 November 2023
Accepted date: 7 January 2024

Please cite this article as: Alba Martinez i Quer , Yrsa Larsson , Anders Johansen , Carlos A. Arias , Pedro N. Carvalho , Cyanobacterial blooms in surface waters – nature-based solutions, cyanotoxins and their biotransformation products, *Water Research* (2024), doi: https://doi.org/10.1016/j.watres.2024.121122

Highlights (revised):

- Cyanobacterial harmful blooms will become more frequent and intense in the future.
- Microbial degradation is the primary mechanism in NBS for cyanotoxin removal.
- Literature on 39 cyanotoxin TPs was compiled, new chemical structures were added.
- Fate of cyanotoxin TPs in the environment is not addressed in the literature.
- The information gathered aims to aid the formulation of new cyanotoxin regulations.

Cyanobacterial blooms in surface waters – nature-based solutions, cyanotoxins and their biotransformation products.

Alba Martinez i Quer[a,*], Yrsa Larsson[a], Anders Johansen[a,b], Carlos A. Arias[b,c], Pedro N. Carvalho[a,b]

[a]Department of Environmental Science, Aarhus University, Frederiksborgsvej 399, Roskilde 4000, Denmark

[b]WATEC - Centre for Water Technology, Aarhus University, Ny Munkegade 120, 8000 Aarhus C, Denmark

[c]Department of Biology, Aarhus University, Ny Munkegade 114-116, Aarhus C 8000, Denmark

*Corresponding author: alma@envs.au.dk

Abstract:

Cyanobacterial blooms are expected to become more frequent and severe in surface water reservoirs due to climate change and ecosystem degradation. It is an emerging challenge that especially countries relying on surface water supplies will face. Nature-based solutions (NBS) like constructed wetlands and biofilters can be used for cyanotoxin remediation. Both technologies are reviewed and critically assessed for different types of water resources. The available information on cyanotoxins (bio)transformation products (TPs) is reviewed to point out the potential research gaps and to disclose the most reliable enzymatic degradation pathways. Knowledge gaps were found, such as information on the performance of the revised NBS in pilot and full scales, the removal processes covering different cyanotoxins (besides the most widely studied microcystin-LR), and the difficulties for real-world implementation of technologies proposed in the literature. Also, most studies focus on bacterial degradation processes while fungi have been completely overlooked. This review also presents an up-to-date overview of the transformation of cyanotoxins, where degradation product data was compiled in a unified library of 22 metabolites for microcystins (MCs), 7 for cylindrospermopsin (CYN) and 10 for nodularin (NOD), most of them reported only in a single study. Major gaps are the lack of environmentally relevant studies with TPs in pilot and full-scale scale treatment systems, information on TP's toxicity, as well as limited knowledge of environmentally relevant

degradation pathways. NBS have the potential to mitigate cyanotoxins in recreational and irrigation waters, enabling the water-energy-food nexus and avoiding the degradability of the ecosystems.

Keywords:
Microcystin, Cylindrospermopsin, Nodularin, Constructed Wetlands, Biofilters, Cyanotoxins metabolites

Background

Mismanagement of nutrients and water bodies has intensified events of cyanobacterial harmful blooms (CyanoHAB), globally. This may happen directly, through the eutrophication of water bodies, increasing the amount of dissolved mineral nutrients (Anderson et al., 2002) and indirectly through climate change (Taranu et al., 2015). The increasing frequency and intensity of extreme weather events, coupled with the periodic optimal conditions for cyanobacterial proliferation (Anderson et al., 2002; Taranu et al., 2015) in surface waters can increase future CyanoHABs.

The present review provides an overview and a critical discussion of the state-of-the-art of application of nature-based solutions (NBS) to manage this increasing CyanoHAB problem. It includes a) a brief description of the most common types of cyanotoxins (microcystins (MCs), cylindrospermopsins (CYN), nodularins (NOD), anatoxins (ATX) and saxitoxins (STX)) in surface freshwaters, b) updates on NBS for remediation of cyanotoxins in surface waters by plants and microorganisms, and c) for the first time, a critical overview on the knowledge on environmentally relevant biotransformation products (TPs) and processes (TPs formed during cyanotoxin biodegradation processes).

Previous reviews mainly address the main causes of CyanoHAB occurrence; however, our review focuses on solutions already implemented and potential NBS, addressing future sustainable managing strategies for CyanoHAB remediation. NBS may consist of a stand-alone solution or a combination of solutions. In the present review, constructed wetlands (CWs) and biofilters (bacterial filters) are considered the most relevant NBS in this respect. In order to develop suitable CyanoHAB management, it is crucial to know the potential biodegradation

products of the cyanotoxins with these technologies to evaluate the potential production of toxic by-products, as well as how to operate the technologies as effectively as possible.

1. Cyanotoxins in the environment - why do they matter?

Cyanobacteria are photosynthetic phytoplankton procaryotes living in freshwater and marine water ecosystems and they proliferate when the optimal environmental conditions are present. Such cyanobacterial blooms are of major concern for water quality (Paerl and Otten, 2013) due to 1) disturbing the ecosystem by creating hypoxia conditions when they die and degrade (Rabalais et al., 2010), 2) the production of geosmin and 2-methylisoborneol which spoils water due to foul odour and taste, affecting drinking water quality (Jüttner and Watson, 2007), 3) producing toxic secondary metabolites, cyanotoxins, which upon ingestion might result in fatal liver, digestive and neurologic disease, as well as cause dermal disease (Merel et al., 2013).

MCs

MCs are cyclic heptapeptides. The amino acid ADDA is a characteristic moiety that is shared with nodularins. The ADDA moiety is crucial for the molecules' activity (Song et al., 2006). More than 250 different variants of MCs have been described and many toxigenic cyanobacterial strains simultaneously produce numerous MCs variants (Puddick et al., 2014). MCs are the most common globally occurring cyanotoxin (Pelaez et al., 2010) and are generally soluble and stable in water (Harada, 1996).

The main cyanobacterial species responsible for producing MCs is *Microcystis aeruginosa*. However, a number of other freshwater cyanobacteria belonging to the *Anabaena/Dolichospermum*, *Nostoc*, *Oscillatoria*, and *Planktothrix* genera are capable of biosynthesising them (De Figueiredo et al., 2004).

The major toxic action mechanism of MCs involves the inhibition of protein phosphatases 1, 2A and 5 in hepatocytes, eventually leading to liver damage (WHO, 2020a). In addition, MCs may potentially promote liver tumour formation. The International Agency on Cancer Research has concluded that MC-LR is possibly carcinogenic (Group 2B) to humans (International Agency for Research on Cancer, 2010). MC-LR (with leucine in position two and arginine in position four) is

the most prevalent of the MCs, the most studied and among the most toxic variants (Szlag et al., 2015).

NODs

NODs are cyclic pentapeptides and structurally very similar to MCs (Rinehart et al., 1988). They also contain an ADDA moiety responsible for their toxicity (Namikoshi et al., 1994). In total, ten different varieties of NODs have been detected (Chorus and Welker, 2021). NODs are soluble and highly stable in water (Harada, 1996). NODs are frequently produced by *Nodularia spumigena* communities in temperate and subtropical environments with recurrent blooms and often prevailing in the Baltic Sea (Chorus and Welker, 2021). NOD toxicity is similar to MCs.

CYNs

CYN are alkaloids that contain a tricyclic guanidino moiety (Ohtani et al., 1992). Four different variants have been characterized (Norris et al., 1999; Banker et al., 2000; Wimmer et al., 2014). CYN is highly water soluble and stable concerning a wide range of pH and temperatures (Chiswell et al., 1999). They are mainly produced by species in the order Nostocales such as *Raphidiopsis (Cylindrospermopsis) raciborskii* (Hawkins et al., 1985) and Oscillatoriales such as benthic *Microseira (Lyngbya) wollei* (Seifert et al., 2007). Their occurrence is mainly restricted to Australia, the Mediterranean region, Brazil and some temperate regions of North America and Europe.

The harmful effect of CYN depends on the concentration; at low concentrations, CYN displays protein synthesis inhibition. At higher concentrations, CYN appears to affect cytochrome P450 potentially inducing stress (WHO, 2020b).

ATXs

ATX are secondary amine alkaloids. Anatoxin-a (ATX-A), the most studied, was first isolated from a strain of *Dolichospermum* (*Anabaena*) *flosaquae* (Devlin et al., 1977). It is produced mainly by a variety of strains within Nostocales, such as *Chrysosporum (Aphanizomenon)*

ovalisporum and Oscillatoriales, all species from the genus *Blennothrix*. Their distribution is worldwide, including temperate, tropical and cold climates (Fristachi et al., 2008).
Anatoxin is mainly a neurotoxin, acting as a potent pre- and postsynaptic depolarizing agent, although the cardiovascular system can also be impacted (WHO, 2020c).

STXs

STXs are a family of 57 alkaloid analogues which are also known as paralytic shellfish toxins because they were first identified from human poisoning events caused by ingestion of molluscs (Wiese et al., 2010). They are produced by marine microalgae dinoflagellates from *Alexandrium*, *Gymnodinium* and *Pyrodinium* genera, besides freshwater cyanobacteria belonging to the Nostocales group such as *Dolichospermum (Anabaena) circinalis* (Humpage et al., 1994) and Oscillatoriales such as *Planktothrix* or *Microseira (Lyngbya) wollei* (Carmichael et al., 1997)**.** Their distribution is ubiquitous in the Arctic (Kleinteich et al., 2013), New Zealand (Smith et al., 2011), Canada and Europe (Wörmer et al., 2011; Lajeunesse et al., 2012).
The toxicity is due to sodium-channel blocking in neuronal cells and cardiac cells. Hence, it is a neurotoxin that restricts the normal functioning of the mussels – respiratory and cardiovascular (WHO, 2020d).

2. CyanoHAB - history and future perspectives

Researchers suspect that freshwater CyanoHABs have occurred since prehistorical times (Braun and Pfeiffer, 2002; Koenigswald et al., 2004). The first toxicologic report on eutrophicated poisonous water bodies was made by George Francis in 1878, suspecting that *Nodularia spumigena*, overgrowing an Australian lake, contained a toxin that led to the death of livestock (Francis, 1878). Since then, a rapidly growing tendency in CyanoHAB has been reported around the world (Taranu et al., 2015).
This has been pushed by a combination of environmental conditions, caused by climate change promoting cyanobacterial growth. As surface waters are expected to be exploited more often for crop irrigation, it is crucial to have clear water-management policies with respect to cyanotoxin contamination. In the last decades, excessive withdrawal of groundwater has led to

groundwater depletion in several countries, creating pressure on surface freshwater resources in temperate climates (Green et al., 2011). Considering that about 70% of global groundwater reservoirs are already used for crop irrigation (Cai and Rosegrant, 2002), future scenarios must increase the utilization of surface freshwater resources. The World Health Organization (WHO) has issued guidelines for monitoring cyanotoxins (ATX-A, CYN, MC and STX), only referring to drinking (WHO, 2022) and recreational waters (Chorus and Welker, 2021). Institutions and governments often neglect to formulate strategies for addressing future scenarios. A notable gap in regulation persists, particularly in areas like crop irrigation, presenting an issue that has yet to be resolved.

3. Treatment solutions to manage CyanoHAB

4.1 Historical perspective and conventional treatment approaches

Historically, the prevention of CyanoHAB was attempted by limiting phosphorous and nitrogen discharge (Ryther and Dunstan, 1971; Brattberg, 1986). Nonetheless, those regulations are still in place but seldom prevent CyanoHABs. To this end, NBS can be used in prevention strategies to reduce phosphorous, and nitrogen loads into surface waters (Cooper et al., 2020). However, challenges such as land use or highly variable hydraulic and nutrient loads can hinder the designs. Alternatively, algicides have been applied as a rapid and cheap emergency tool for eliminating CyanoHAB. Despite its cost-effectiveness, environmental concerns are raised due to the chemical nature of one of the most used algicides, copper sulphate, which may have a negative long-lasting ecological impact when accumulating in lake sediments (Prepas and Murphy, 1988). Other common practices are artificial/mechanical mixing and circulation by pumping either air or water into the water body using aeration systems, mechanical destratification devices or water mixers (Visser et al., 2016). They have high operational costs and are not always considered effective (Chorus and Welker, 2021).

In terms of recreational activities, WHO recommends exposure limits (for a 15 kg child) of 24 µg/L for MCs, 6 µg/L for CYN, 60 µg/L for ATX and 30 µg/L for STX (WHO, 2020a, 2020b, 2020c, 2020d). In the European Union, there is no consensus on regulations for recreational activities regarding the occurrence of CyanoHAB in surface waters (Chorus and Welker, 2021). This lack of consensus and legislation reflects a broader lack of strategy to cope with these challenges.

Cyanotoxins may end up in our food when cyanotoxin-polluted water is used for irrigation. Plants can sorb cyanotoxins on their surface and/or translocate them to the leaves and stems if absorbed by the roots (Redouane et al., 2023). The cyanotoxins can be further transferred along the food chain through ingestion of cyanotoxin-polluted plant material. Currently, there is no regulation in terms of the concentration of cyanotoxins allowed in food. The health impact of ingesting cyanotoxins via food is unknown and most associated studies are not clear in this respect (Testai et al., 2017), e.g., not considering conjugated metabolite(s) of cyanotoxins. To this end, cyanotoxin-polluted water used for crop irrigation usually goes untreated. This might be blamed on a lack of scientific consensus on adequate treatment technology and a lack of frameworks regulating it.

When surface waters are collected for potable use, most countries stick to WHO recommendation guidelines for MC-LR's highest allowed concentrations of 1 µg/L (WHO, 2020a). The most common drinking water treatment procedures are sand filtration and oxidation. Such treatments are often efficient in removing the cyanobacterial cells but fail to remove the solubilized toxins (Newcombe, 2012). Another classic approach for cyanotoxin removal is the use of an oxidant that interacts chemically with the toxins, being chlorine and ozonation the most common options. Both have proved effective against certain cyanotoxins: MC-LR (Ho et al., 2006b; Onstad et al., 2007), CYN (Senogles et al., 2000; Onstad et al., 2007), ATX-A (Onstad et al., 2007), but failed to eliminate other types of cyanotoxins, such as STX (Orr et al., 2004). Additionally, the formation of by-products remains a concern for both oxidants (Orr et al., 2004; Senogles-Derham et al., 2003).

Regarding the application of biological treatment by NBS, the majority of the available literature is focused on biofilters for drinking water (Kumar et al., 2019), and MCs (Jeon et al., 2023), while the current review focuses on NBS for remediation of surface water used for recreational and crop irrigation purposes.

4.2 Biological treatment and nature-based solutions

Biological prevention for CyanoHAB and cyanotoxins has been a raw remediation approach rather than a water treatment technology. It is usually disregarded as a sole methodology but

rather taken as an auxiliary strategy. However, there is potential for using biological treatment technology.

NBS, also known as eco-technologies, may ensure additional benefits besides the direct control of water pollution, including sustainability and cost-effectiveness (Oral et al., 2021). NBS have been tested for cyanobacterial remediation with the assistance of macrophytes, such as CWs systems (Wang et al., 2018). Other technologies that rely only on the native microbiome's biodegradation capacity such as biofilters and the enhancement of bacterial communities (bioaugmentation), are also considered attractive solutions. Bioaugmentation is defined as the addition of microorganisms or biological agents to an indigenous community to improve degradative capacity. These technologies are often applied in combination, e.g., bioaugmentation combined with CWs (Wang et al., 2018), bioreactors combined with bioaugmentation (Shim et al., 2002), integration of different types of CWs (Fang et al., 2016), or even combining NBS with traditional or advanced methods (e.g., pre-oxidation by potassium permanganate coupled with bioaugmentation sand filters (Bai et al., 2019)).

Phytoremediation, as an NBS itself, or as a mechanism present in all planted NBS, is critical for the water treatment processes. For example, Kucała et al. (2021), reported macrophyte *Lemna trisulca* limits the growth of *D. flos-aquae*, *R. raciborskii*, and *M. aeruginosa* and absorbs their cyanotoxins (ATX-A, CYN and MC-LR). Sorption of 60-90% of the cyanotoxins, and concentration decreases of up to 310 times, were reported. In a separate study also using *L. triscula*, the authors reported the removal of ANATX-A by biodegradation (Kaminski et al., 2014). Another macrophyte, *Cladophora glomerata* (Pflugmacher et al., 2016), removed 95-97% of MC-LR, -RR and -YR and 100% of ATX-A in 7 days, primarily attributed to plant uptake. Overall, it appears, from *in vitro* experiments, that plants alone may remove cyanotoxins and even limit cyano-cell proliferation. However, it is still unclear whether different plants can metabolise the toxins and if so, which enzymatic pathways are involved.

The role of plants in terms of cyanotoxin removal efficiency in CWs has been scarcely studied. Pflugmacher et al. (2001) showed that *P. australis*, a commonly used plant in CWs, can rapidly take up MC-LR and further metabolise it via glutathione S-transferases activity, also validated in

another aquatic macrophyte, *Ceratophyllum demersum* (Pflugmacher et al., 1998). This denotes the double role that plants have in CWs; affecting microbial degradation (Kim et al., 2022), as well as being directly involved in the removal processes (sorption, uptake and metabolisation). However, it is unknown if the removal rates would be significant in a real-world system.
In spite of the detoxification capacity, the toxic effect of MC-LR, has been reported, affecting the growth and photosynthetic activity of *C. demersum*, *Elodea canadensis*, and *Myriophyllum Spicatum* at environmentally relevant concentrations (0.1 – 5.0 µg/L) (Pflugmacher, 2002), as well as in *P. australis* plantlets, but at higher concentrations (≥ 500 µg/L, ≥ 4100 µg/L) (Máthé et al., 2007, 2009). Concerning phytotoxic effects, CYN (≥ 500 µg/L) can also affect *P. australis* plantlets (Beyer et al., 2009). It is important to know the detoxifying capacities of the macrophyte species chosen for the implementation of CWs at full-scale and operational loading rates to ensure the viability and longevity of the CW systems.
Overall, more studies outside of aquaponics/lab settings are needed, for a broader number of macrophytes and cyanotoxins. These studies should also include the complex system dynamics to which macrophytes in CWs are exposed at ambient conditions (e.g., nutrients, other pollutants, water stress). Some plants could display specific cyanotoxin-degrading capabilities that have not yet been discovered, as reported above for MC-LR.

Apart from phytoremediation, microorganisms are known to be an important degradation mechanism in NBS. They ensure systematic biodegradation and facilitate bioavailability of organic pollutants (Cao and Zhang, 2014) as well as nutrient removal (Yan et al., 2019). It is well-known that single isolates, as well as consortia of bacteria, can degrade cyanotoxins mainly in aerobic conditions (Bourne et al., 1996; Martínez-Ruiz et al., 2020), but also in anaerobic conditions (Chen et al., 2010). Nevertheless, cyanotoxin degradation pathways are known only for biodegradation of MC-LR (see Section 4).
Overall, NBS rely on different core mechanisms such as phytoremediation and bacterial biodegradation that are poorly understood. Filling these knowledge gaps is key for optimal system design and operation. In the following sections, two technologies for surface water treatment are discussed in depth: CWs and biofilters (bacterial filters).

4.2.1 Constructed wetlands for the treatment of cyanotoxins

CWs, or treatment wetlands, are engineered systems designed to emphasize specific characteristics of wetland ecosystems for improved treatment capacity (Kadlec and Wallace, 2008). CWs can have different shapes and configurations but are mainly categorized by the type of hydrology/flow direction and type of macrophytes (Vymazal, 2011). CW systems are used broadly for a wide variety of water-treatment types being common technologies for decentralized wastewater treatment (Sundaravadivel and Vigneswaran, 2001). CWs consist of two water pipelines; one that distributes the inflow water in the subsurface or at the surface of the systems and another in the subsurface that collects the outflow water once it is treated. CW key components are macrophytes and the porous media colonized by a naturally developing bacterial community. The following key literature on the technical aspects of CWs is recommended: Kadlec and Wallace (2008) and Vymazal (2007).

In terms of cyanobacterial-polluted water remediation, there are only 6 studies that point out the potential of this technology as a treatment strategy (Table 1). The first study, carried out in 2016, highlights the novelty of applying CWs for cyanobacterial remediation (Fang et al., 2016). Despite the promising results, the detailed mechanisms behind the CW's remediation of cyanotoxins remain Abiotic degradation in CWs has not been specifically tested, but reports suggest that it is of minor grade (Wang et al., 2018).

Table 1: Overview of the different studies regarding CWs to treat cyanobacterial blooms and/or cyanotoxins.

Cyanotoxin	Operational mode	HRT (d)	HLR (cm/d)	Porous Media	Initial concentration (µg/L)	System size	Removal rate	Macrophyte	Bioaugmentation	Ref
MC-LR	Batch (saturated)	5	3.18	Gravel, ceramsite, gravel and iron-carbon, slag.	2-14.5	0.012 m^3	90-95%	*Arundo donax**	NA	(Cheng et al., 2021)
MC-LR	Batch (saturated)	3 and 7	NA	Gravel	3-16	0.012 m^3	100%	*Iris pseudacorus*	Natural community carring *mlrA* gene**	(Wang et al., 2018)
MC-LR	Batch (saturated)	7	0.42	Natural river sediment, sand (1:2)	50	0.008 m^3	99.9%	*Phragmites australis*	NA	(Bavithra et al., 2020)
MC-LR	Floating treatment wetland	NA	NA	Floating mat	0.04-0.06	118, 63 and 178 m^2	NA	Canna, Juncus, Blue Flag Iris, and Agrostis	NA	(Hartshorn et al., 2016)
MC-LR	Batch (saturated)	3	NA	Biochar (0-50%) and gravel	15	0.012 m^3	94-98%	*Arundo donax*	NA	(Cheng et al., 2022)
Microcystis sp., Oscillatoria sp. biomass ***	Vertical Flow	NA	20 - 100	Gravel	Chlorophyll-a (212.2)	2 m^3	98-0%***	*Canna indica*	NA	(Zhong et al., 2018)

* Significant differences in performance between planted/unplanted systems

** Significant differences in performance between bioaugmented/non-bioaugmented systems

***Cyanobacteria or cyanotoxins identification is not reported, efficiency was based on Chlorophyll-a determinations.

Data for cyanotoxin removal in CWs is limited to MC-LR (five studies) and one study assessing the removal of cyanobacterial biomass. Three studies on CW microcosms (in vitro) operated in batch mode (at water-saturated conditions) reported removal rates for MC-LR higher than 90% (Table 1). However, the operational conditions are far from the usual full-scale continuous-mode operation of these systems. The studies operated CWs with comparable retention time (3-7 days) but with different plant species (*Arundo donax* (Cheng et al., 2021)*, Iris pseudacorus* (Wang et al., 2018)*, Phragmites australis* (Bavithra et al., 2020)). Two of these studies compared the effect of the macrophytes, reporting contradictory information: Cheng et al. (2021) found differences between unplanted and planted microcosms, with the planted systems enhancing the removal of MC-LR. Contrary to that, Wang et al. (2018) found that the microcosms planted with *Iris pseudacorus* did not significantly differ from the unplanted microcosms in removing MC-LR. Although the effect of the plant on both studies is significant in terms of nutrient removal, it was not for MC-LR.

Two studies provided knowledge on CyanoHAB removal in larger-scale CWs. Zhong et al. (2018) operated a 2 m^2 vertical flow CW and found that the removal of cyanobacterial biomass (measured as chlorophyll-a) was highly dependent on the hydraulic loading rate (HLR). Their results indicated that at an HLR of 1 m/d no removal was observed, while for an HLR of 0.8-0.2 m/d, the CW could efficiently remove cyanobacterial biomass from the water together with geosmin and β-cyclocitral but failed to report cyanotoxins concentration. It should be noted that this pilot was operated at a much higher HLR than the saturated mesocosms operated in batch mode. However, it is important to mention that they only measured chlorophyll-a as a cyanobacterial mass indicator, which is not a precise parameter for measuring cyanobacterial biomass. According to the other large-scale CW study by Hartshorn et al. (2016), there was no correlation between MC and chlorophyll-a measurements after studying three different floating CWs – floating mats covered by macrophytes - pilot systems (63 – 179 m^2) in wet retention ponds. Despite the interesting dimension of these pilots no quantitative assessment of

cyanobacteria or cyanotoxins was performed, leaving the true potential of vertical flow and floating CWs at full scale open for further exploration.

Another important parameter for CW design is the type of porous media used. The favourite porous media choice is gravel (4 out of the 6 reviewed papers). Cheng et al (2021) compared four different types of porous media in their microcosms (batch mode, water-saturated): ceramsite and iron-carbon materials were more efficient in the removal of MC-LR (and also total phosphorous). Biochar had a similarly good performance as other amended porous media. Moreover, it decreased the extracellular polymeric substances, which the authors argued decreased clogging (Cheng et al., 2022). The significantly higher specific surface area (gravel, biochar, slag) was linked with a higher retention of the cyanotoxins in the media enhancing biotic degradation due to longer exposure time. Overall, porous medium selection has been poorly explored with regard to cyanotoxin removal, even though it influences the overall systems' removal.

In all these studies, an adaptation period with either natural lake water or a rich nutrient solution was used. Generally, it is important to build a system using either a carbon boost for promoting biofilm forming, or a toxin-pre-exposed community to shorten the lag phase of the bacterial toxin-degrading community (Christoffersen et al., 2002).
Bacterial biodegradation is stated as the main removal mechanism occurring within the CWs (4 out 6 studies), while the 2 other studies either did not discuss the removal mechanisms or proposed the combination of multiple factors (biotic and abiotic). However, there has been little to no exploration of the microbial community reported in these studies. Only half of them have conducted 16S amplicon sequencing to analyse the bacterial community. None have explored the fungal communities, plant uptake, or conducted abiotic degradation studies. This lack of comprehensive exploration makes it challenging to disclose the potential biotic removal mechanisms.

Bioaugmentation has been applied to one out of six studies, which makes it difficult to state whether it is effective. This microcosms study claimed the best removal rates for the lowest retention time (3 days) (Wang et al., 2018). Nevertheless, a study within the same retention time using biochar-amended CWs without bioaugmentation resulted in only a slightly lower biodegradation rate (Cheng et al., 2022). Wang et al (2018), besides using CWs, also enriched the CWs porous media with a mixed culture taken from a previously exposed lake, attempting bioaugmentation. They claimed that the community was stabilised, but they stopped the experiment after 7 days. More information is needed to know whether, after a period of toxin-free media, the indigenous bacterial community would irreversibly outcompete the bioaugmented community.

Nothing has been reported in terms of mineralization/biotransformation processes in CWs. It is unknown if cyanotoxins are simply removed by sorption or if biodegraded, and by which mechanisms. Knowledge of total biodegradation of cyanotoxins is limited to studies with degrading strains (more details in Section 4).

Overall, CWs seem to be a suitable and promising technology, but the operational parameters for the optimal functioning of the systems to treat cyanotoxins are still not clear.

4.2.2 Biofilters for the treatment of cyanotoxins

Biofilter technology employs a porous medium with microorganisms, which physically retains, adsorb (depending on compounds and filter conditions), and enzymatically degrade contaminants. CyanoHAB treatment in biofilters is an underexplored field and few efforts have been made to remediate MC-LR in drinking water by biofilters. Kumar et al. (2019), Jeon et al. (2023) and the book chapter 10.2.3 (Chorus and Welker, 2021) offer an overview of cyanotoxin treatment in drinking water using biofilters. In the present review, the authors broaden the water usage to include other water applications, such as irrigation (Table 2).

Table 2: Overview of the different published studies regarding biofilters treating cyanobacterial blooms and/or cyanotoxins.

Cyanotoxin	Usage of treated water	HRT (EBCT) / HLR	Porous media	Initial concentration (µg/L)	Removal rate	Bioaugmentation	Bioaugmentation improvement	Ref
MC-LR	Drinking	20 min HRT	Raw sand, manganese dioxide coated sand and sugar-coated sand	5-50	40-96%	*Arthrobacter ramosus* (NRRL B-3159), *Bacillus sp.* (NRRL B-14393) and *Sphingomonas sp.* (NRRL B-59555	10-15%*	(Kumar et al., 2020c)
MC-LR	Drinking	60 L/day HLR	Sand	5.55	75-76%	No	No	(Terin and Sabogal-Paz, 2019)
MC-LR and cyanobacterial biomass	Drinking	20 min and 10 min EBCT	GAC and sand columns	5	87-100%	No	No	(Jeon et al., 2020)
MC-LR and cyanobacterial biomass	Drinking	0.52 m/h HLR	Sand	50	30-100%	*Arthrobacter ramosus* and *Bacillus sp.*	19.5%*	(Kumar et al., 2020b)
MC-LR	Drinking	64 min HRT	Sand	50	87.9-94.2%	*Arthrobacter, Bacillus, Sphingomonas* and native strains *Pseudomonas fagi* and *Chryseobacterium sp.*	0-38%*	(Kumar et al., 2020a)
Dha7, MC-LR, MC-LA	Drinking	15 min	Sand and GAC	5	100%	No	No	(Wang et al.,

		EBCT						2007)
MC-LR, MC-LA	Drinking	7.5-30 min EBCT	Sand	20-25	>95%	No	No	(Ho et al., 2006a)
MC-LR	Drinking	30 h EBCT	Sand	50	>80%	*Sphingomonas sp.*	No	(Bourne et al., 2006)
CYN surrogate	Drinking	1.2 min EBCT GAC cap	Sand with GAC cap	500-100000	0-40%	No	No	(Crowe et al., 2022)
MC-LR	Drinking	7.5 min HRT	GAC/PAC/Anthracite	12	100%	NA	-10%	(Drogui et al., 2012)
MC-LR, CYN	Irrigation	10 d HRT	Sand	2-4, 0.5	90%	NA	NA	(Wanielista, 2009)

* Significant improvement of the removal in bioaugmented samples vs. non-bioaugmented samples.

Eleven papers on CyanoHAB biofiltration were found (Table 2), and most of them were concerned with MC-LR (10 out of 11). One study also assessed MC-LA (Wang et al., 2007) and only two included CYN and a CYN surrogate (Wanielista, 2009; Crowe et al., 2022). In addition, only one paper focused on irrigation water (Morón-López and Molina, 2020), all the other papers concerned drinking water. The first study was published in 2002 (Grützmacher et al., 2002), indicating an earlier interest in this field in comparison with the CWs studies. Some of the studies are difficult to compare due to their variability in the filter residence time of the toxin; varying from minutes (Jeon et al., 2020) to several hours (Wanielista, 2009). Also, the initial cyanotoxin concentrations used were ranging from 1 to 50 $\mu g \cdot L^{-1}$, besides one study that used even higher concentrations of a surrogate (Crowe et al., 2022). Also, all the studies were in microcosm or mesocosm scales, apart from two pilot-scale studies (Wanielista, 2009; Crowe et al., 2022).

Sorption, as well as biodegradation, are highly dependent on the chemical structure of each toxin and the type of porous medium material. Even for the same group of molecules, e.g., MC,

MC-LR and MC-LA display different sorption behaviour in Granular Activated Carbon (GAC) biofilters (Wang et al., 2007). Some of the studies explored the dominant removal mechanisms of biofilters: sorption vs. biodegradation. Results are not conclusive, and they are highly dependent on the type of porous media used. For most studies that use GAC as porous media (4 out of 11), sorption is hypothesised as being the main removal mechanism (Wang et al., 2007; Drogui et al., 2012; Crowe et al., 2022), while in one study, biodegradation is the dominant mechanism (Jeon et al., 2020). When comparing studies that used sand as a porous media (7 out of 11 studies), 6 articles reported biodegradation as the main removal process (Bourne et al., 2006; Ho et al., 2006a; Wanielista, 2009; Terin and Sabogal-Paz, 2019; Kumar et al., 2020c, 2020b). Only one article reported sorption as the main removal route (Kumar et al., 2020a). However, in that study, there is a lack of an "abiotic" control which makes it hard to state the effect of the sorption without considering biodegradation. Using GAC as porous media seems to deliver higher removal percentages within a shorter contact time. Nonetheless, when designing a GAC filter, some considerations need to be made, not least the high cost of GAC relative to other filtering materials (Chys et al., 2015). Knowledge of the cyanotoxin concentrations in the inlet water is crucial for estimating the amount of GAC needed and, consequently, its costs.

In full-scale biofilter systems, the inflow content consists of a complex aqueous phase with cyanotoxins and additional natural carbon and nitrogen sources. Cyanobacterial debris together with other organic compounds from the water sources will be present. Hence, 3 out of 11 studies have researched the influence of additional carbon sources on the cyanotoxin removal rate (Wang et al., 2007; Jeon et al., 2020; Kumar et al., 2020b). They all concluded that external carbon substrates compete with the cyanotoxins and hinder their removal, either by competing for sorption sites, pore blockage or enzymatic active sites. They attributed the removal of MC-LR to a secondary carbon source (co-metabolism), rather than a specific substrate for the degrading strains. To tackle this, the authors proposed either a higher contact time (Jeon et al., 2020), the use of GAC as a filter media (Wang et al., 2007; Jeon et al., 2020) or a bioaugmentation strategy (Kumar et al., 2020b). It is important to note that in all three studies, even though removal was hindered by the additional carbon substrate, they obtained outlet

concentrations below the WHO guidelines (1 µg/L) (WHO, 2020a). When operating a biofilter, this has to be taken into consideration and the nutrient composition must be monitored accordingly.

Biofilter studies are often linked with bioaugmentation strategies; 5 out of 11 (Table 2). Bioaugmentation may significantly improve MC-LR removal in biofilters as observed in 3 out of 5 studies (Kumar et al., 2020, ; Kumar, Kaur Brar, et al., 2020a). On the other side, two studies reported no difference in removal rates of the toxin in comparison to the naturally occurring biofilm. In one study, bioaugmentation did not improve the removal rates but it lowered the lag phase and toxicity of by-products found (Bourne et al., 2006), and in another study, bioaugmented systems performed with 10% less removal, compared to abiotic systems, due to a decrease of the sorption capacity in the porous medium (Drogui et al., 2012).

A main challenge of bioaugmentation is the long-term survival of added degrader bacteria, e.g., during periods of low concentration of the toxin. Considering that the availability of organic carbon in the environment may shift dynamically, it is suggested that other organisms may outcompete or predate the added bacteria. Also, Kumar et al (2020a) continuously added cells of a bioaugmented strain prior to a bioaugmentation experiment, with no change in biodegradation activity during 56 days. Nevertheless, the precise role of the bioaugmented bacteria, in the degradation process, was not clear as the dynamics of the structural community (including degrading genes) were not studied. Therefore, the removal enhancement could be attributed to sequential exposure to the toxin, which may induce the native bacterial community to perform more efficiently. In another study, the bioaugmentation was monitored only for 11 days, finding improvements in MC-LR removal by up to 20% with respect to the non-bioaugmented filters (Kumar et al., 2020b). It might be possible to continuously introduce a bioaugmented degrader bacterium into a native biofilter microbiome rather than performing a one-time spiking. Besides economic challenges for real-world implementation, monitoring the survival of the bioaugmented bacteria within the community is necessary to assess the effectiveness of the strategy.

In the studies shown in Table 2, most of them confirm that biodegradation is the main removal mechanism. 3 out of 11 studies applied biomolecular techniques to measure the gene cluster *mlrA-D,* which is the only known degradation pathway for any cyanotoxin (for more detailed information read Section 4). In all three studies, the presence of the *mlrA* gene correlated with MC-LR removal (Bourne et al., 2006; Ho et al., 2006a; Jeon et al., 2020). However, Bourne et al. (2006) also detected biodegradation occurring in the non-bioaugmented biofilters without the detection of *mlrA*. Other studies suggest that there are alternative degradation pathways for MC-LR (Lezcano et al., 2016). Efforts must be made in order to disclose alternative degradation pathways, as this would constitute a quick and cost-effective approach to monitor the cyanotoxin degradation potential and performance of biofilter microbiomes.

4. Biotransformation of cyanotoxins processes and products

TPs derived from the biodegradation of cyanotoxins need to be taken into consideration when treating cyanobacteria-contaminated water as they may retain some toxicity or even be more toxic than the parent compounds. For instance, STX- studies have reported increased toxicity of its TPs compared to the parent compound (Kotaki, 1989; Kayal et al., 2008).

Two types of transformation processes are expected in NBS: abiotic transformation (photodegradation, hydrolysis) and/or biotic transformation (bacterial, fungal, and plant-mediated). In this section, due to the lack of information on NBS systems, the focus was placed on biotic transformation and expanded by reviewing the state of the art of cyanotoxin TPs formed in any biological system (Table 3-5).

In total, 39 biogenic TPs cyanotoxins have been reported (Table 3-5). Twenty-two out of 39 are TPs derivate from Microcystis (MC-TP1-22), 7 TPs from CYN (CYN-TP1-7) and 10 TPs from NOD (NOD-TP1-10). The TP corresponding to the ADDA amino acid (MC-TP22/NOD-TP5) is a common TP originating from MCs and NOD.

MCs

MCs, especially MC-LR, are among the cyanotoxins for which additional information on TPs can be found in the literature (Table 3). A total of 22 TPs have been reported for MCs, 13 TPs reported for MC-LR (MC-TP1-13), 3 TPs for [Dha7]MC-LR (MC-TP14-16), 1 TP for MC-RR (MC-

TP17), and 3 TPs for MC-LF (MC-TP18-20). One TP, the tetrapeptide (MC-TP21), is shared among MC-LR and MC-RR and another TP, the adda amino acid (MC-TP22), commonly derived from the MCs, is also shared with NOD.

The first study on the biotransformation of cyanotoxins dates back to 1996 by Bourne et al. (1996). This study describes the MC-LR bacterial degradation route, leading to the best-characterised enzymatic pathway for MC-LR and any other existing cyanotoxin. Bourne et al. (1996) described *Sphingomonas sp.* strain as capable of producing an enzyme named Microcystinase able to hydrolyse the Adda-Arg bond of MC-LR into a linearised molecule (MC-TP1), which is further transformed into a tetrapeptide (MC-TP21). The gene cluster responsible for transcribing those enzymes was identified as *mlrA-D* and codifies for three hydrolytic enzymes and one oligopeptide transporter (Bourne et al., 2001). Metabolites resulting from the *mlrA-D* gene cluster have been found consistently by several authors: linearised forms (MC-TP1) (Bourne et al., 1996; Edwards et al., 2008; Okano et al., 2009; Zhang et al., 2011; Yang et al., 2014, 2020; Ding et al., 2018; Wei et al., 2023), (MC-TP17) (Yang et al., 2014) and tetrapeptide (MC-TP21) (Bourne et al., 1996; Yang et al., 2014, 2020; Ding et al., 2018; Wei et al., 2023). Several intermediates (TP2-TP10) have been reported in different studies. More recently, a further step in the biodegradation route was proposed, suggesting that some bacteria can fully mineralize MC-LR through the aromatic compound phenylacetic acid metabolism (MC-TP11) (Yang et al., 2020; Wei et al., 2023).

On the other hand, Zhang et al. (2011) and Okano et al. (2009) studying single-strain degradation products, could find the linearised MC-LR (MC-TP1) but did not find the rest of the products. The same situation happened to Edwards et al. (2008) using natural lake consortia instead of single-strains for their study. While Okano et al. (2009) amplified the *Mlr* gene cluster, the other two studies did not investigate the presence or expression of *mlrA* gene. This lack of information makes it challenging to conclusively state that the genetic pool was not present in their samples.

Another single-strain study, using an aquatic fungus, found a detoxifying pathway that could be responsible for MC-LR removal in fungi: glutathione S-transferase (MC-TP13) (Balsano et al., 2017). The same pathway has been found in aquatic plants (Pflugmacher et al., 1998, 2001).

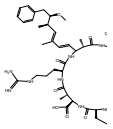
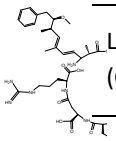
Indicating that might be a common MC-LR detoxifying mechanism in higher organisms, more research needs to be done to affirm that.

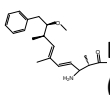
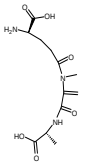
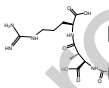
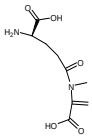
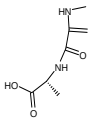
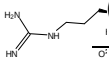
For other MC varieties, MC-RR was found to follow a similar pathway as for MC-LR, the linearised congener has been detected (MC-TP17), while the tetrapeptide (MC-TP21) and ADDA (MC-TP22) are common (Yang et al., 2014).

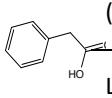
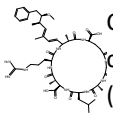
Dziga et al. (2017) reported TPs of dmMC-LR using unknown lake consortia in Poland. They found a common intermediate, the tetrapeptide (MC-TP14) but they suggested that potentially the biotransformation happens while the molecule remains with its cycle structure, rather than undergoing a previous linearisation. In the study by Dziga et al. (2017), *Mlr* gene homologues were not found in the isolated bacteria from the different water bodies. They suggested that the elimination mechanisms were not related to the *mlrA-D* gene cluster. In addition, Edwards et al (2008) studied MC-LF degradation in different natural lake consortia and identified diverse intermediate products (MC-TP18-20); different from those for the standard MC degradation pathway.

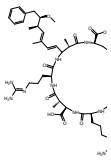
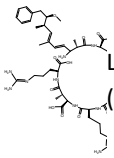
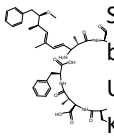
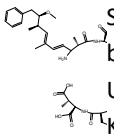
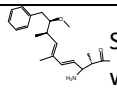
It is expected that in natural environments, MCs are submitted to different metabolic routes than in in vitro studies. Often, in vitro studies use concentrations higher than those typically observed in the environment (mg/L instead of µg/L) and pure toxin instead of mixing it with the cyanobacterial biomass, which would be equally available in a natural bloom. As a result, the microorganisms in the environment will be exposed to a range of different metabolic challenges. Natural blooms often contain more than one type of MC (Chen et al., 2010), and in a natural environment, MCs will compete for enzymatic catalytic sites, likely undergoing similar degradation pathways (Yang et al., 2014). Notably, 14 of the 22 MC TPs (MC-TP3-7 and MC-TP12-20) have been reported by one single study, and more studies are needed for broader confirmation. Our knowledge is still far from being environmentally relevant and requires further investigation.

Table 3: Overview of the different known MC-LR TPs.

Compound ID	Parents	Other ID	PubChem CID *	Chemical formula	Monoisotopic mass [Reported m/z]	Chemical Structure	Natural strain origin	Degradation strain	Reference
MC-LR	MC-LR		44543 4	$C_{49}H_{74}N_{10}O_{12}$	994.5488 [995.5590 [M+H]+]				
MC-TP1**	MC-LR	Linearised MC-LR / acyclo MC-LR [NH2–Adda–Glu–Mdha–Ala–Leu–MeAsp–Arg–OH]	139585701	$C_{49}H_{76}N_{10}O_{13}$	1012.5593 [1013.5638 [M+H]+]		Surface waters	*Sphingomonas sp.* ACM-3962	(Bourne et al., 1996)
							Hongfeng Lake (China)	*Sphingopyxis sp.* C-1	(Okano et al., 2009)
							Six water bodies in United Kingdom	Unknown, lake consortia	(Edwards et al., 2008)
							Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Yang et al., 2020)
							Lake Taihu (China)	*Bordetella sp*. MC-LTH1	(Yang et al., 2014)
							Lake Dianchi (China)	*Ralstonia solanacearum*	(Zhang et al., 2011)
							Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Wei et al., 2023)
							Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP2	MC-LR	Dehydration of linearised form		$C_{49}H_{74}N_{10}O_{12}$	994.54877 [995.8 [M+H	n.a.	Lake Dianchi (China)	*Ralstonia solanacearum*	(Zhang et al., 2011)

				]+]				
MC-TP3	MC-LR	Adda-Glu-Mdha [Adda-Glu-Mdha-H]		$C_{29}H_{41}N_3O_7$	543.2945 [544.3400 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP4	MC-LR	Glu-Mdha-Ala [Glu-Mdha-Ala-H]		$C_{12}H_{19}N_3O_6$	301.1274 [302.1354 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP5	MC-LR	Leu-MeAsp-Arg [Leu-MeAsp-Arg-H]		$C_{17}H_{32}N_6O_6$	416.2383 [417.2458 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP6	MC-LR	Glu-Mdha [Glu-Mdha-H]		$C_9H_{14}N_2O_5$	230.0902 [231.1057 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP7	MC-LR	Mdha-Ala [Mdha-Ala-H]		$C_7H_{12}N_2O_3$	172.0845 [173.0925 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
MC-TP8	MC-LR	MeAsp-Arg [MeAsp-Arg-H]		$C_{11}H_{21}N_5O_5$	303.1543 [304.1619 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
							Hongfeng Lake (China)	*Sphingopyxis sp.* C-1	(Okano et al., 2009)
MC-TP9	MC-LR	Leu [Leu-H]	6106	$C_6H_{13}NO_2$	131.0946 [132.1023 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
							Hongfeng Lake (China)	*Sphingopyxis sp.* C-1	(Okano et al., 2009)
MC-	MC-LR	Arg		C_6H_{15}	174.1		Lake Taihu	*Sphingop*	(Ding et al.,

TP10		[Arg-H]		N_4O_2	117 [175.1202 [M+H]+] [175.5 [M+H]+]		(China)	*yxis sp.* m6	2018)
							Hongfeng Lake (China)	*Sphingopyxis sp.* C-1	(Okano et al., 2009)
MC-TP11	MC-LR	Phenylacetic acid (PAA)	999	$C_8H_8O_2$	136.0524 [136.090 [M+H]+]		Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Yang et al., 2020)
							Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Wei et al., 2023)
MC-TP12	MC-LR	MC-LR-CYS MC-LR-Cysteine conjugate	100798779	$C_{52}H_{81}N_{11}O_{14}S$	1115.5685 [1116 [M+H]+]	n.a.	Culture collection (aquatic fungi)	*Mucor hiemalis* (fungi)	(Esterhuizen-Londt et al., 2017)
MC-TP13	MC-LR	MC-LR-GSH MC-LR-Glutathione conjugate	101091619	$C_{59}H_{91}N_{13}O_{18}S$	1301.6326 [652 [M+2H]2+]		Culture collection (aquatic fungi)	*Mucor hiemalis* (fungi)	(Esterhuizen-Londt et al., 2017)
dmMC-LR	dmMC-LR	dmMC-LR [Adda-Glu-Mdha-Ala-Leu-Asp-Arg]	101642889	$C_{48}H_{72}N_{10}O_{12}$	980.5331 [981.5 [M+H]+]	n.a.			
MC-TP14	dmMC-LR	Product A Tetrapeptide [NH2-Adda-Glu-Mdha-Ala-OH]		$C_{32}H_{46}N_4O_8$	614.3316 [615.4 [M+H]+]	n.a.	Twenty-one freshwater bodies (Poland)	Unknown, lake consortia	(Dziga et al., 2017)
MC-TP15	dmMC-LR	Product C [Adda-Glu-Mdha-Ala-Leu-Asp-Arg]		n.a.	n.a. [954.5 [M+H]+]	n.a.	Twenty-one freshwater bodies (Poland)	Unknown, lake consortia	(Dziga et al., 2017)
MC-TP16	dmMC-LR	Product D [Adda-Glu-		n.a.	n.a. [920.	n.a.	Twenty-one freshwater	Unknown, lake	(Dziga et al., 2017)

		Mdha-Ala-Leu-Asp-Arg]			5 [M+H]+]	bodies (Poland)	consortia	
MC-RR	MC-RR		643835 7	$C_{49}H_{75}N_{13}O_{12}$	1037.5658 [1038.5709 [M+H]+]			
MC-TP17**		Linearised MC-RR*** [NH2–Adda–Glu–Mdha–Ala–Arg–MeAsp–Arg–OH]		$C_{49}H_{77}N_{13}O_{13}$	1055.5763 [1056.4970 (M+H2O+H]+]	 Lake Taihu (China)	*Bordetella sp.* MC-LTH1	(Yang et al., 2014)
MC-LF	MC-LF		167605 63	$C_{52}H_{71}N_7O_{12}$	985.5161 [986 [M+H]+]			
MC-TP18	MC-LF	MC-LFa / linearised MC-LF*** [NH2-Adda-Glu-Mdha-Ala-Leu-MeAsp-Phe-OH]		$C_{52}H_{73}N_7O_{13}$	1003.6267 [1004 [M+H]+]	 Six water bodies in United Kingdom	Unknown, lake consortia	(Edwards et al., 2008)
MC-TP19	MC-LF	MC-LFb Loss of H2O		$C_{52}H_{71}N_7O_{11}$	969.5212 [968 [M+H]+]	n.a. Six water bodies in United Kingdom	Unknown, lake consortia	(Edwards et al., 2008)
MC-TP20	MC-LF	MC-LFc*** [NH2-Adda-Glu-Mdha-Ala-Leu-MeAsp-OH)]		$C_{43}H_{64}N_6O_{12}$	856.4582 [856 [M+H]+]	 Six water bodies in United Kingdom	Unknown, lake consortia	(Edwards et al., 2008)
MC-TP21**	MCs (MC-LR, MC-RR)	Tetrapeptide [NH2–Adda–		$C_{32}H_{46}N_4O_8$	614.3316 [615.	 Surface waters	*Sphingomonas sp*. ACM-	(Bourne et al., 1996)

		Glu–Mdha–Ala-OH]			3394 [M+H]+]		3962	
						Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
						Lake Taihu (China)	*Bordetella sp.* MC-LTH1	(Yang et al., 2014)
						Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Yang et al., 2020)
						Lake Taihu (China)	*Sphingopyxis sp*. YF1	(Wei et al., 2023)
MC-TP22/NOD-TP5	MCs (MC-LR, MC-RR)	Adda [Adda-H]	142 052 64	$C_{20}H_{29}NO_3$	331.2147 [332.2215 [M+H]+]	Lake Taihu (China)	*Bordetella sp.* MC-LTH1	(Yang et al., 2014)
						Lake Taihu (China)	*Sphingopyxis sp.* m6	(Ding et al., 2018)
						Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Yang et al., 2020)
						Lake Taihu (China)	*Sphingopyxis sp.* YF1	(Wei et al., 2023)

* Unequivocal ID on Pubchem verified against the CyanoMetDB.
** The symbol indicates that this TP is part of the *mlrA-D* gene cluster degradation pathway.
*** Neither a unique chemical identifier nor a chemical structure was provided by the authors
n.a. Information not available in the original reference or not possible to calculate/deduce from the original reference

CYNs

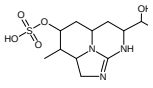
In one single report on CYN TPs (Table 4), Martínez-Ruíz et al. (2020) found the same seven TPs (CYN-TP1-7) in cultures of four strains of manganese oxidising bacteria; *Pseudomonas* sp. OF001, *Ideonella* sp. A288, *Ideonella* sp. A226 and strain A210 (Table 3). Some of the TPs found in this study (namely CYN-TP5-7) have also been observed in other CYN photodegradation study (Fotiou et al., 2015). They found that uracil moiety is the most susceptible molecule target. It was also proposed that the catalyst of the degradation was unspecific reactions of reactive manganese species formed by the bacteria. To this end, the TPs showed reduced toxicity to hepatocytes (Martínez-Ruiz et al., 2020).

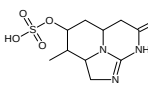
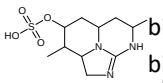
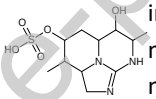
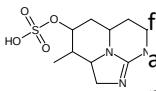
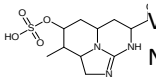
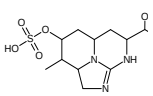
To date, no report of biodegradation of CYNs, or formation of related TPs, in natural waters (even after pre-exposure to the toxin) has been reported (Wormer et al., 2008; Klitzke et al., 2010). Also, there is no evidence of enzymatic pathway or gene activity linked to CYN degradation.

NODs

NOD degradation by microbial activity has been demonstrated in marine and freshwater ecosystems (Edwards et al., 2008; Mazur-Marzec et al., 2009). Two studies report NODs intermediate TPs (Table 5), Edwards et al. (2008) (NOD-TP1, NOD-TP6-8) worked with natural communities of Scottish lakes, while Mazur-Marzec et al. (2009) worked with a natural sediment consortium (NOD-TP1, NOD-TP2-5, NOD-TP9-10). Both studies have found the linearised form of NOD-R (NOD-TP1) and demethylation of NOD-R (NOD-TP6). However, demethylated NOD was found in the abiotic control samples too (Mazur-Marzec et al., 2009), which indicate that it was a parent compound itself, proposing NOD-TP9 and NOD-TP10 as for the biotransformation of [D-Asp^1]NOD-R. Edward et al. (2008) found that all the intermediate products contained the Adda moiety, which probably still conferred them toxic activity. However, none of the studies carried out toxicology tests with the observed TPs. Mazur-Marzec et al. (2009) also found the Adda moiety alone (NOD-TP5/MC-TP22) as well as a tetrapeptide that has lost the Adda moiety (NOD-TP2). No evidence of enzymatic pathways or gene activity linked to NODs has been reported.

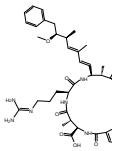
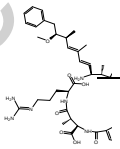
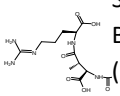
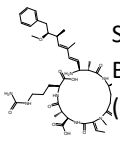
Table 4: Overview of the different known CYN TPs.

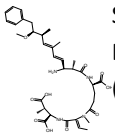
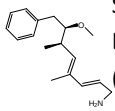
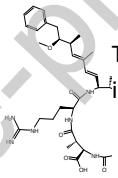
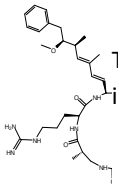
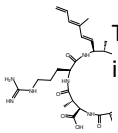
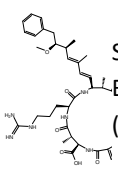
Compound ID	Parents	Other ID	PubChem CID*	Chemical formula	Monoisotopic mass [Reported m/z]	Chemical Structure	Natural strain origin	Degradation strain	Reference
CYN	CYN		115005	$C_{15}H_{21}N_5O_7S$	415.1162 [416.1234 [M+H]+]				

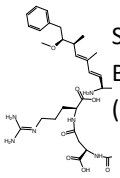
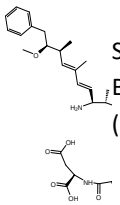
CYN-TP1	CYN	TP290	$C_{10}H_{15}N_3O_5S$	289.0732 [290.0804 [M+H]+]		*Pseudomonas sp.* OF001 isolated from effluent of a fixed-bed biofilm bioreactor, others from iron manganese-depositing biofilm in a freshwater pond in the Lower Oder Valley National Park, Germany	Manganese-Oxidising Bacteria: *Pseudomonas sp.* OF001, *Comamonadaceae* bacterium A210, *Ideonella sp.* A226, and *Ideonella sp.* A288 (Martínez-Ruiz et al., 2020)
CYN-TP2	CYN	TP292a	$C_{10}H_{17}N_3O_5S$	291.0889 [292.0961 [M+H]+]	n.a.		
CYN-TP3	CYN	TP292b	$C_{10}H_{17}N_3O_5S$	291.0889 [292.0961 [M+H]+]			
CYN-TP4	CYN	TP308	$C_{10}H_{17}N_3O_6S$	307.0838 [308.0910 [M+H]+]			
CYN-TP5	CYN	TP320	$C_{11}H_{17}N_3O_6S$	319.0838 [320.0909 [M+H]+]			
CYN-TP6	CYN	TP347	$C_{12}H_{18}N_4O_6S$	346.0945 [347.1019 [M+H]+]			
CYN-TP7	CYN	TP448	$C_{15}H_{21}N_5O_9S$	447.1060 [4481.135 [M+H]+]			

* Unequivocal ID on Pubchem verified against the CyanoMetDB.

Table 5: Overview of the different known NOD TPs.

Compound ID	Parents	Other ID [Amino acid sequence]	PubChem CID*	Chemical formula	Monoisotopic mass [Reported m/z]	Chemical Structure	Natural strain origin	Degradation strain	Reference
NOD	NOD-R		14217092	C41H60N8O10	824.4432 [825 [M+H]+]				
NOD-TP1	NOD-R	Linearised NOD [NH2–Adda–Glu–Mdhb–MeAsp–Arg–OH]	139584373	$C_{41}H_{62}N_8O_{11}$	842.4538 [843 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)
		NODa					Three water bodies in United Kingdom	Unknown, freshwater consortia	(Edwards et al., 2008)
NOD-TP2	NOD-R	Tetrapeptide ** [H2N-Glu-Mdhb-MeAsp-Arg-OH]		$C_{21}H_{35}N_7O_9$	529.2496 [530 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)
NOD-TP3	NOD-R	Linearised NOD Missing the N-terminals ** [H-Adda-Glu-Mdhb-		$C_{41}H_{61}N_7O_{12}$	843.4378 [844 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)

		MeAsp-Cit-CONH2]						
NOD-TP4	NOD-R	Tetrapeptide ** [H2N-Adda-Glu-Mdhb-MeAsp-OH]		$C_{35}H_{50}N_4O_{10}$	686.3527 [687 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)
NOD-TP5/MC-TP22	NOD-R	Adda [Adda-H]	1420 5264	$C_{20}H_{29}NO_3$	331.2147 [663 [2M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)
NOD-TP6	NOD-R	NODb / Demethylated NOD-R "*on the methylde hyrobutyrine*"		$C_{40}H_{58}N_8O_{10}$	810.4276 [811 [M+H]+]		Three water bodies in United Kingdom	Unknown, freshwater consortia	(Edwards et al., 2008)
NOD-TP7	NOD-R	NODc (cyclic, decarboxylation)		$C_{40}H_{60}N_8O_8$	780.4534 [781 [M+H]+]		Three water bodies in United Kingdom	Unknown, freshwater consortia	(Edwards et al., 2008)
NOD-TP8	NOD-R	NODd (modification in Adda)		$C_{32}H_{48}N_8O_9$	688.3544 [689 [M+H]+]		Three water bodies in United Kingdom	Unknown, freshwater consortia	(Edwards et al., 2008)
[D-Asp^1]NOD-R	[D-Asp^1] NOD-R	Demethylated NOD-R	1005 6208	$C_{40}H_{58}N_8O_{10}$	810.4276 [811 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)

NOD-TP9	[D-Asp^1] NOD-R	Linearised [D-Asp]NOD	$C_{40}H_{60}N_8O_{11}$	828.4382 [829 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)
NOD-TP10	[D-Asp^1] NOD-R	Tetrapeptide [H2N-Adda-Glu-Mdhb-Asp-OH]	$C_{34}H_{48}N_4O_{10}$	672.3370 [673 [M+H]+]		Sediment of brackish Baltic Sea waters (Poland)	Unknown, sediment consortia	(Mazur-Marzec et al., 2009)

* Unequivocal ID on Pubchem verified against the CyanoMetDB.
** Structure not available in the original publication, presently proposed based on the descriptions of the publication.

ATXs

To the best of the author's knowledge, only one study from Rapala et al. (1994) reported degradation by different types of inoculum. However, no information on TPs is available, besides those due to abiotic processes (dihydro- and epoxy-derivatives) (James et al., 2005). Both TPs are also part of *de novo* biosynthesis toxin pathway (Heath et al., 2014). Thus, the hypothetical enzymatic route for biodegradation of ATXs is unknown.

STXs

The biodegradability of STXs in natural waters is controversial: some studies pointed out non-biodegradability in two water bodies (Ho et al., 2012b), while the same authors reported biodegradability in another water body (Ho et al., 2012a). *In vitro* studies found that *Pseudomonas sp.* and *Vibrio sp.* isolated from marine environments were able to transform five less toxic variants (gonyautoxins) into more toxic variants (STXs) (Kotaki, 1989). Kayal et al. (2008) also found that STX variants doubly-sulphated C-toxins were bioconverted to gonyautoxins and finally to STX by biofilter bacteria of two water treatment plants, also increasing its toxicity. Interestingly, the processes are reported as conversion and not necessarily TPs from their biodegradation activity. Large gaps exist in the biodegradation potential and pathways for these toxins.

Overall, TPs information and their metabolic pathways are scarce, mostly in terms of environmentally relevant studies. Moreover, none of the past studies clarified if any of the identified TPs could potentially be persistent in the environment, and consequently be a potential concern for water management. It is remarkable that only 2 TPs (of the total 39 TPs) are a result of fungal transformation (Esterhuizen-Londt et al., 2017). In the ambient and in technical systems, fungi are highly ubiquitous sharing the ecological niches with bacteria. Most probably they also contribute to the degradation of the toxins, in the same way they contribute to the degradation of other organic pollutants (e.g. ibuprofen and diclofenac in CWs (Hu et al., 2021) or alkylpyrazines in biofilters (Araya et al., 2022)). There is a clear bias in terms of the available information towards studies on bacterial degradation processes.
Clearly, more research combining transcriptomics and non-target screening of metabolites in natural environments is needed to shed light on this field. As access to increasingly more potent high-resolution mass spectrometry is available, such future studies will help to close this gap.

5. Conclusions and future perspectives

The European Green Deal is seriously promoting the implementation of NBS for adapting rural and urban areas to climate change scenarios. This is done by integrating them into the water-energy-food nexus, thus trying to stop the notorious ecosystem deterioration happening due to anthropogenic effects. The authors believe that this topic will be of high importance in the future of water management and NBS implementations. Hence, the scope of this paper is to highlight the most important knowledge gaps and list the common ground that has been established after decades of research.

The attempt to control N and P input into our water bodies is far from sufficient to prevent CyanoHAB. Even though most of the present review covers mitigation measures, one cannot neglect all the necessary preventive work. Stringent regulations on nutrient discharge, as well as more precise fertilization strategies in the agriculture sector, are needed together with a responsible water extraction plan. NBS can be used both as a prevention strategy to control N

and P discharge into our water reservoirs and/or as a treatment system for the CyanoHAB and cyanotoxins. Also, a need for a unified regulation in terms of what is considered a cyanotoxin critical concentration in waters for bathing or irrigation should be implemented worldwide, as per MC-LR in drinking water. The health risks linked to the consumption of MC-accumulating crops have been increasing worldwide (Redouane et al., 2023), both in CyanoHAB-occurring regions but also in importing regions.

When it comes to delivering solutions, besides effective, they need to be cost-effective. In the case of CyanoHAB remediation strategies, they will only be implemented if the endpoint water quality is regulated to meet ecosystem or human health protection, and if they are affordable (Caron et al., 2016). For water usage, such as recreational or for irrigation, the operating budget is often much lower than for drinking water supply, hindering even more the implementation of treatment technology.

NBS and cyanotoxin remediation is a fast-developing research field. While some studies pointed out the capacity of some plants, micro- or mesocosms, or some specific bacterial strains to detoxify MC-LR, a lack of full-scale NBS studies makes it difficult to roll out the different solutions to the field - mechanistic understanding of removal processes on NBS is missing. Bacterial biodegradation is the most studied removal process, but the related enzymatic pathways are poorly understood. Full-scale studies or mesocosms studies should be used to reveal the environmentally relevant pathways, as well as to boost them in terms of design and operation.

In addition, most of the existing knowledge is about MCs, and mainly MC-LR. Certainly, MC-LR is one of the most ubiquitous and toxic cyanotoxins (Szlag et al., 2015), but climate change is shifting the cyanobacteria distribution (e.g. CYN normally restricted to tropical climates is occurring more often in other locations) (Padisák, 1997). Therefore, there is an urgent need to study other types of toxins to be ready for future scenarios. Ultimately, one should not forget that cyanotoxins occur in blooms, meaning that treatment dynamics are quite different in real conditions from studies with single compounds. When treating surface waters, other pollutants and carbon sources will be present, which might affect the performance of NBS for treating the

targeted cyanotoxins. It is hypothesized that cyanotoxins may not be a specific substrate for the degrading community; but rather an opportunistic carbon and energy source that they encounter once the bloom happens (co-metabolism). Therefore, in conditions where enhanced available carbon sources are present, cyanotoxin biodegradation will probably decrease (Jones et al., 1994).

Studies with cyanotoxins and CWs have been restricted to measurements of inlet and outlet concentrations. More effort should be invested in terms of understanding which processes are driving the degradation of cyanotoxins inside the CWs. In terms of basic design parameters, this review highlights that plant effect on MC-LR removal seems to be species-dependant. Operational hydraulic conditions are hard to compare as different studies quantify the CyanoHAB using different parameters (cyanotoxin concentration or chlorophyll-a). Also, most of the systems are operated in batch conditions which is far from full-scale operation. In terms of porous media, most of the systems are operated using gravel so little comparisons among different porous media or even engineered media have been made. Also, many of the studies proclaimed bacterial degradation as the main removal mechanism, but there is little or no research evidence to support this.

The biofilter studies reviewed were mainly lab-scale studies focused on drinking water, which often fell into methodologies far from the field, such as an axenic single-strain membrane biofilm reactor operated at sterile conditions, or bioaugmentation using pure strains. Studies with GAC filters were the closest to full-scale. When it comes to delivering realistic solutions, althought biofiltration is effective against CyanoHAB, it failed to be cost-effective (Caron et al., 2016).

A complementary research area for both NBS has been the cyclic and long-term persistence of bioaugmentation strategies. While Wang et al. (2018) postulated that CWs can maintain the growth and activity of the bioaugmented degrading bacteria, different studies in biofilters stated that other environmental factors would outweigh the bioaugmentation influence (Leviram et al., 2023). More consistent research with bioaugmentation needs to be carried out

using biomolecular techniques, gathering data for longer periods, and performing cycles of exposure, as it will be closer to the environmental situation.

TPs are a relatively new issue in the assessment of pollutant transformation. They are important to understand removal/degradation pathways and potentially support technological improvements, but they are also relevant to clarify which are the true endpoints of the treatment. In the present review, for the first time, three tables comprising all the published TPs from biological degradation processes for different cyanotoxins were included. While most of the TPs have been detected in single-strain *in-vitro* studies, fewer are found in studies using mixed cultures in treatment systems. There is a clear lack of environmentally relevant studies with TPs in pilot and full-scale scale treatment systems. Most of the TP's toxicity is unknown and all the relevant environmental degradation pathways for the toxins are also unknown, except for the *Mlr* gene pathway known for degrader strains but not reported for any NBS treatment system.

There is a need to include the role of fungi in the degradation of cyanotoxins as well as their presence in NBS systems. In nature, biodegradation of organic pollutants happens via a combination of different organisms, leading to the total transformation of the cyanotoxins.

To conclude, each of the three NBS themes discussed in this review paper will benefit from each other to advance the development of safe technologies. Several of the identified knowledge gaps can be dealt with by employing advanced biomolecular techniques. Next Generation Sequencing can elucidate microbial dynamics, while high-resolution mass spectrometry and non-target approaches can identify TPs and their biodegradation pathways. Complex analyses are required for complex systems. Thus, the requirements for reliable monitoring of CyanoHABs treatment by NBS should provide removal rates and emission thresholds that comply with current legislation. More information will enable policymakers to deal with the lack of legislation that irrigation and recreational water bodies suffer.

Acknowledgements

This work was supported by the Independent Research Fund Denmark – project WETCYANO, and the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 823860.

6. Bibliography

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25, 704–726. https://doi.org/10.1007/BF02804901

Araya, B., Diaz, C., Martín, J.S., Vergara-Fernández, A., Aroca, G., Scott, F., 2022. Biodegradation of 2,5-dimethylpyrazine in gas and liquid phase by the fungus Fusarium solani. J. Chem. Technol. Biotechnol. 97, 1408–1415. https://doi.org/10.1002/jctb.6903

Bai, Y., Hu, W., Jian, Z., Qi, W., Chang, Y., Huo, Y., Liao, K., Qu, J., 2019. Combining KMnO4 pre-oxidation and bioaugmented sand filtration to simultaneously treat cyanobacterial bloom lake water and released Mn(II). Sep. Purif. Technol. 228, 115765. https://doi.org/10.1016/J.SEPPUR.2019.115765

Balsano, E., Esterhuizen-Londt, M., Hoque, E., Lima, S.P., 2017. Responses of the antioxidative and biotransformation enzymes in the aquatic fungus Mucor hiemalis exposed to cyanotoxins. Biotechnol. Lett. 39, 1201–1209. https://doi.org/10.1007/s10529-017-2348-8

Banker, R., Carmeli, S., Werman, M., Teltsch, B., Porat, R., Sukenik, A., 2010. Uracil Moiety is Required for Toxicity of the Cyanobacterial Hepatotoxin Cylindrospermopsin. http://dx.doi.org/10.1080/009841001459432 62, 281–288. https://doi.org/10.1080/009841001459432

Banker, R., Teltsch, B., Sukenik, A., Carmeli, S., 2000. 7-epicylindrospermopsin, a toxic minor metabolite of the cyanobacterium Aphanizomenon ovalisporum from Lake Kinneret, Israel. J. Nat. Prod. 63, 387–389. https://doi.org/10.1021/np990498m

Bavithra, G., Azevedo, J., Oliveira, F., Morais, J., Pinto, E., Ferreira, I.M.P.L.V.O., Vasconcelos, V., Campos, A., Almeida, C.M.R., 2020. Assessment of constructedwetlands' potential for the removal of cyanobacteria and microcystins (MC-LR). Water (Switzerland) 12. https://doi.org/10.3390/w12010010

Beyer, D., Surányi, G., Vasas, G., Roszik, J., Erdodi, F., M-Hamvas, M., Bácsi, I., Bátori, R., Serfozo, Z., Szigeti, Z.M., Vereb, G., Demeter, Z., Gonda, S., Máthé, C., 2009. Cylindrospermopsin induces alterations of root histology and microtubule organization in common reed (Phragmites australis) plantlets cultured in vitro. Toxicon 54, 440–449. https://doi.org/10.1016/J.TOXICON.2009.05.008

Bourne, D.G., Blakeley, R.L., Riddles, P., Jones, G.J., 2006. Biodegradation of the cyanobacterial toxin microcystin LR in natural water and biologically active slow sand filters. Water Res. 40, 1294–1302. https://doi.org/10.1016/j.watres.2006.01.022

Bourne, D.G., Jones, G.J., Blakeley, R.L., Jones, A., Negri, A.P., Riddles, P., 1996. Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. Appl. Environ. Microbiol. 62, 4086–4094. https://doi.org/10.1128/aem.62.11.4086-4094.1996

Bourne, D.G., Riddles, P., Jones, G.J., Smith, W., Blakeley, R.L., 2001. Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environ. Toxicol. 16, 523–534. https://doi.org/10.1002/tox.10013

Brattberg, G., 1986. Decreased phosphorus loading changes phytoplankton composition and biomass in the Stockholm Archipelago. Vatten 42, 141–153.

Braun, A., Pfeiffer, T., 2002. Cyanobacterial blooms as the cause of a Pleistocene large mammal assemblage. Paleobiology 28, 139–154. https://doi.org/10.1666/0094-8373(2002)028<0139:cbatco>2.0.co;2

Cai, X., Rosegrant, M.W., 2002. Global Water Demand and Supply Projections: Part 1. A Modeling Approach. Water Int. 27, 159–169. https://doi.org/10.1080/02508060208686989

Cao, W., Zhang, Y., 2014. Removal of nitrogen (N) from hypereutrophic waters by ecological floating beds (EFBs) with various substrates. Ecol. Eng. 62, 148–152. https://doi.org/10.1016/J.ECOLENG.2013.10.018

Carmichael, W.W., Evans, W.R., Yin, Q.Q., Bell, P., Moczydlowski, E., 1997. Evidence for paralytic shellfish poisons in the freshwater cyanobacterium Lyngbya wollei (Farlow ex Gomont) comb. nov. Appl. Environ. Microbiol. 63, 3104–3110.

https://doi.org/10.1128/aem.63.8.3104-3110.1997

Caron, D.A., Echt, M., Folsom, J., Seubert, E.L., Tatters, A.O., Gellene, A.G., 2016. Managing Algal Blooms at a Botanical Garden Lake : A Success Story 17–21.

Chen, J., Hu, L. Bin, Zhou, W., Yan, S.H., Yang, J.D., Xue, Y.F., Shi, Z.Q., 2010. Degradation of Microcystin-LR and RR by a Stenotrophomonas sp. Strain EMS Isolated from Lake Taihu, China. Int. J. Mol. Sci. 2010, Vol. 11, Pages 896-911 11, 896–911. https://doi.org/10.3390/IJMS11030896

Chen, X., Yang, X., Yang, L., Xiao, B., Wu, X., Wang, J., Wan, H., 2010. An effective pathway for the removal of microcystin LR via anoxic biodegradation in lake sediments. Water Res. 44, 1884–1892. https://doi.org/10.1016/J.WATRES.2009.11.025

Cheng, R., Hou, S., Wang, J., Zhu, H., Shutes, B., Yan, B., 2022. Biochar-amended constructed wetlands for eutrophication control and microcystin (MC-LR) removal. Chemosphere 295, 133830. https://doi.org/10.1016/j.chemosphere.2022.133830

Cheng, R., Zhu, H., Shutes, B., Yan, B., 2021. Treatment of microcystin (MC-LR) and nutrients in eutrophic water by constructed wetlands: Performance and microbial community. Chemosphere 263, 128139. https://doi.org/10.1016/j.chemosphere.2020.128139

Chiswell, R.K., Shaw, G.R., Eaglesham, G., Smith, M.J., Norris, R.L., Seawright, A.A., Moore, M.R., 1999. Stability of Cylindrospermopsin, the Toxin from the Cyanobacterium, Cylindrospermopsis raciborskii: Effect of pH, Temperature, and Sunlight on Decomposition, Environ Toxicol. https://doi.org/10.1002/(SICI)1522-7278(199902)14:1

Chorus, I., Welker, M., 2021. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management., Taylor & Francis. Taylor & Francis. https://doi.org/10.1201/9781003081449-5

Christoffersen, K., Lyck, S., Winding, A., 2002. Microbial activity and bacterial community structure during degradation of microcystins. Aquat. Microb. Ecol. 27, 125–136. https://doi.org/10.3354/ame027125

Chys, M., Declerck, W., Audenaert, W.T.M., Van Hulle, S.W.H., 2015. UV/H2O2, O3 and (photo-) Fenton as treatment prior to granular activated carbon filtration of biologically stabilized landfill leachate. J. Chem. Technol. Biotechnol. 90, 525–533.

https://doi.org/10.1002/jctb.4344

Cooper, R.J., Hawkins, E., Locke, J., Thomas, T., Tosney, J., 2020. Assessing the environmental and economic efficacy of two integrated constructed wetlands at mitigating eutrophication risk from sewage effluent. Water Environ. J. 34, 669–678. https://doi.org/10.1111/WEJ.12605

Crowe, G.T., Almuhtaram, H., Andrews, R.C., McKie, M.J., 2022. Granular activated carbon caps - A potential treatment barrier for drinking water cyanotoxins. J. Water Process Eng. 49, 102977. https://doi.org/10.1016/J.JWPE.2022.102977

De Figueiredo, D.R., Azeiteiro, U.M., Esteves, S.M., Gonçalves, F.J.M., Pereira, M.J., 2004. Microcystin-producing blooms—a serious global public health issue. Ecotoxicol. Environ. Saf. 59, 151–163. https://doi.org/10.1016/J.ECOENV.2004.04.006

Devlin, J.P., Edwards, O.E., Gorham, P.R., Hunter, N.R., Pike, R.K., Stavric, B., 1977. Anatoxin- a , a toxic alkaloid from Anabaena flos-aquae NRC-44h. Can. J. Chem. 55, 1367–1371. https://doi.org/10.1139/v77-189

Ding, Q., Liu, K., Xu, K., Sun, R., Zhang, J., Yin, L., Pu, Y., 2018. Further Understanding of Degradation Pathways of Microcystin-LR by an Indigenous Sphingopyxis sp. in Environmentally Relevant Pollution Concentrations. Toxins 2018, Vol. 10, Page 536 10, 536. https://doi.org/10.3390/TOXINS10120536

Drogui, P., Daghrir, R., Simard, M.C., Sauvageau, C., Blais, J.F., 2012. Removal of microcystin-LR from spiked water using either activated carbon or anthracite as filter material. Environ. Technol. 33, 381–391. https://doi.org/10.1080/09593330.2011.575186

Dziga, D., Maksylewicz, A., Maroszek, M., Budzyńska, A., Napiorkowska-Krzebietke, A., Toporowska, M., Grabowska, M., Kozak, A., Rosińska, J., Meriluoto, J., 2017. The biodegradation of microcystins in temperate freshwater bodies with previous cyanobacterial history. Ecotoxicol. Environ. Saf. 145, 420–430. https://doi.org/10.1016/j.ecoenv.2017.07.046

Edwards, C., Graham, D., Fowler, N., Lawton, L.A., 2008. Biodegradation of microcystins and nodularin in freshwaters. Chemosphere 73, 1315–1321. https://doi.org/10.1016/j.chemosphere.2008.07.015

Esterhuizen-Londt, M., Hertel, S., Pflugmacher, S., 2017. Uptake and biotransformation of pure commercial microcystin-LR versus microcystin-LR from a natural cyanobacterial bloom extract in the aquatic fungus Mucor hiemalis. Biotechnol. Lett. 39, 1537–1545. https://doi.org/10.1007/s10529-017-2378-2

Fang, T., Bao, S., Sima, X., Jiang, H., Zhu, W., Tang, W., 2016. Study on the application of integrated eco-engineering in purifying eutrophic river waters. Ecol. Eng. 94, 320–328. https://doi.org/10.1016/j.ecoleng.2016.06.003

Fotiou, T., Triantis, T., Kaloudis, T., Hiskia, A., 2015. Photocatalytic degradation of cylindrospermopsin under UV-A, solar and visible light using TiO2. Mineralization and intermediate products. Chemosphere 119, S89–S94. https://doi.org/10.1016/j.chemosphere.2014.04.045

Francis, G., 1878. Poisonous australian lake. Nature 18, 11–12.

Fristachi, A., Sinclair, J.L., Hall, S., Berkman, J.A.H., Boyer, G., Burkholder, J., Burns, J., Carmichael, W., Dufour, A., Frazier, W., Morton, S.L., O'Brien, E., Walker, S., 2008. Occurrence of cyanobacterial harmful algal blooms workgroup report. Adv. Exp. Med. Biol. https://doi.org/10.1007/978-0-387-75865-7_3

Green, T.R., Taniguchi, M., Kooi, H., Gurdak, J.J., Allen, D.M., Hiscock, K.M., Treidel, H., Aureli, A., 2011. Beneath the surface of global change: Impacts of climate change on groundwater. J. Hydrol. https://doi.org/10.1016/j.jhydrol.2011.05.002

Grützmacher, G., Böttcher, G., Chorus, I., Bartel, H., 2002. Removal of microcystins by slow sand filtration. Environ. Toxicol. 17, 386–394. https://doi.org/10.1002/tox.10062

Harada, KI, 1996. Chemistry and detection of microcystins, in: Watanabe, M., Harada, K-i, Carmichael, W. (Eds.), Toxic Microcystis. Boca Raton (FL): CRC Press, pp. 103–148.

Hartshorn, N., Marimon, Z., Xuan, Z., Cormier, J., Chang, N. Bin, Wanielista, M., 2016. Complex interactions among nutrients, chlorophyll-a, and microcystins in three stormwater wet detention basins with floating treatment wetlands. Chemosphere 144, 408–419. https://doi.org/10.1016/j.chemosphere.2015.08.023

Hawkins, P.R., Runnegar, M.T.C., Jackson, A.R.B., Falconer, I.R., 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) Cylindrospermopsis raciborskii

(Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl. Environ. Microbiol. 50, 1292–1295. https://doi.org/10.1128/aem.50.5.1292-1295.1985

Heath, M.W., Wood, S.A., Barbieri, R.F., Young, R.G., Ryan, K.G., 2014. Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a production by Phormidium autumnale. Toxicon 92, 179–185. https://doi.org/10.1016/J.TOXICON.2014.10.014

Ho, L., Meyn, T., Keegan, A., Hoefel, D., Brookes, J., Saint, C.P., Newcombe, G., 2006a. Bacterial degradation of microcystin toxins within a biologically active sand filter. Water Res. 40, 768–774. https://doi.org/10.1016/j.watres.2005.12.009

Ho, L., Onstad, G., Gunten, U. Von, Rinck-Pfeiffer, S., Craig, K., Newcombe, G., 2006b. Differences in the chlorine reactivity of four microcystin analogues. Water Res. 40, 1200–1209. https://doi.org/10.1016/J.WATRES.2006.01.030

Ho, L., Tang, T., Hoefel, D., Vigneswaran, B., 2012a. Determination of rate constants and half-lives for the simultaneous biodegradation of several cyanobacterial metabolites in Australian source waters. Water Res. 46, 5735–5746. https://doi.org/10.1016/J.WATRES.2012.08.003

Ho, L., Tang, T., Monis, P.T., Hoefel, D., 2012b. Biodegradation of multiple cyanobacterial metabolites in drinking water supplies. Chemosphere 87, 1149–1154. https://doi.org/10.1016/J.CHEMOSPHERE.2012.02.020

Hu, B., Hu, S., Chen, Z., Vymazal, J., 2021. Employ of arbuscular mycorrhizal fungi for pharmaceuticals ibuprofen and diclofenac removal in mesocosm-scale constructed wetlands. J. Hazard. Mater. 409, 124524. https://doi.org/10.1016/J.JHAZMAT.2020.124524

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16, 471–483. https://doi.org/10.1038/s41579-018-0040-1

Humpage, A.R., Rositano, J., Bretag, A.H., Brown, R., Baker, P.D., Nicholson, B.C., Steffensen, D.A., 1994. Paralytic shellfish poisons from australian cyanobacterial blooms. Mar. Freshw. Res. 45, 761–771. https://doi.org/10.1071/MF9940761

International Agency for Research on Cancer, 2010. Ingested nitrate and nitrite, and cyanobacterial peptide toxins., IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. IARC Press, International Agency for Research on Cancer, Lyon.

James, K.J., Crowley, J., Hamilton, B., Lehane, M., Skulberg, O., Furey, A., 2005. Anatoxins and degradation products, determined using hybrid quadrupole time-of-flight and quadrupole ion-trap mass spectrometry: forensic investigations of cyanobacterial neurotoxin poisoning. Rapid Commun. Mass Spectrom. 19, 1167–1175. https://doi.org/10.1002/RCM.1894

Jeon, Y., Baranwal, P., Li, L., Piezer, K., Seo, Y., 2023. Review: Current understanding on biological filtration for the removal of microcystins. Chemosphere 313, 137160. https://doi.org/10.1016/j.chemosphere.2022.137160

Jeon, Y., Li, L., Calvillo, J., Ryu, H., Santo Domingo, J.W., Choi, O., Brown, J., Seo, Y., 2020. Impact of algal organic matter on the performance, cyanotoxin removal, and biofilms of biologically-active filtration systems. Water Res. 184, 116120. https://doi.org/10.1016/j.watres.2020.116120

Jones, G.J., Bourne, D.G., Blakeley, R.L., Doelle, H., 1994. Degradation of the cyanobacterial hepatotoxin microcystin by aquatic bacteria. Nat. Toxins 2, 228–235. https://doi.org/10.1002/NT.2620020412

Jüttner, F., Watson, S.B., 2007. Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Appl. Environ. Microbiol. 73, 4395–4406. https://doi.org/10.1128/AEM.02250-06

Kadlec, R.H., Wallace, S., 2008. Treatment Wetlands, Treatment Wetlands. CRC Press. https://doi.org/10.1201/9781420012514

Kaminski, A., Bober, B., Chrapusta, E., Bialczyk, J., 2014. Phytoremediation of anatoxin-a by aquatic macrophyte Lemna trisulca L. Chemosphere 112, 305–310. https://doi.org/10.1016/J.CHEMOSPHERE.2014.04.064

Kayal, N., Newcombe, G., Ho, L., 2008. Investigating the fate of saxitoxins in biologically active water treatment plant filters. Environ. Toxicol. 23, 751–755. https://doi.org/10.1002/TOX.20384

Kim, S., Kang, H., Megonigal, J.P., McCormick, M., 2022. Microbial Activity and Diversity Vary with Plant Diversity and Biomass in Wetland Ecosystems. Estuaries and Coasts 45, 1434–1444. https://doi.org/10.1007/s12237-021-01015-z

Kleinteich, J., Wood, S.A., Puddick, J., Schleheck, D., Küpper, F.C., Dietrich, D., 2013. Potent toxins in Arctic environments - Presence of saxitoxins and an unusual microcystin variant in Arctic freshwater ecosystems. Chem. Biol. Interact. 206, 423–431. https://doi.org/10.1016/j.cbi.2013.04.011

Klitzke, S., Apelt, S., Weiler, C., Fastner, J., Chorus, I., 2010. Retention and degradation of the cyanobacterial toxin cylindrospermopsin in sediments - The role of sediment preconditioning and DOM composition. Toxicon 55, 999–1007. https://doi.org/10.1016/j.toxicon.2009.06.036

Koenigswald, W. v., Braun, A., Pfeiffer, T., 2004. Cyanobacteria and seasonal death: A new taphonomic model for the Eocene Messel lake. Paläontologische Zeitschrift 78, 417–424. https://doi.org/10.1007/bf03009232

Kotaki, Y., 1989. Screening of bacteria which convert gonyautoxin 2, 3 to saxitoxin. Nippon Suisan Gakkaishi 55.

Kumar, P., Hegde, K., Brar, S.K., Cledon, M., Kermanshahi-pour, A., 2019. Potential of biological approaches for cyanotoxin removal from drinking water: A review. Ecotoxicol. Environ. Saf. 172, 488–503. https://doi.org/10.1016/j.ecoenv.2019.01.066

Kumar, P., Hegde, K., Brar, S.K., Cledon, M., Kermanshahi-pour, A., Roy-Lachapelle, A., Sauvé, S., Galvez-Cloutier, R., 2020a. Co-culturing of native bacteria from drinking water treatment plant with known degraders to accelerate microcystin-LR removal using biofilter. Chem. Eng. J. 383, 123090. https://doi.org/10.1016/j.cej.2019.123090

Kumar, P., Kaur Brar, S., Surampalli, R.Y., 2020b. Ozonation in Tandem with Biosand Filtration to Remove Microcystin-LR. J. Environ. Eng. 146, 04020124. https://doi.org/10.1061/(asce)ee.1943-7870.0001801

Kumar, P., Rehab, H., Hegde, K., Brar, S.K., Cledon, M., Kermanshahi-pour, A., Vo Duy, S., Sauvé, S., Surampalli, R.Y., 2020c. Physical and biological removal of Microcystin-LR and other water contaminants in a biofilter using Manganese Dioxide coated sand and Graphene

sand composites. Sci. Total Environ. 703, 135052. https://doi.org/10.1016/j.scitotenv.2019.135052

Lajeunesse, A., Segura, P.A., Gélinas, M., Hudon, C., Thomas, K., Quilliam, M.A., Gagnon, C., 2012. Detection and confirmation of saxitoxin analogues in freshwater benthic Lyngbya wollei algae collected in the St. Lawrence River (Canada) by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1219, 93–103. https://doi.org/10.1016/j.chroma.2011.10.092

Leviram, I., Gross, A., Lintern, A., Obayomi, O., Chalifa-Caspi, V., Gillor, O., Henry, R., Schang, C., Herzberg, M., Mccarthy, D.T., 2023. Engineering a biofilters microbiome with activated carbon and bioaugmentation to improve stormwater micropollutant removal. J. Pre-proof. https://doi.org/10.1016/j.eti.2023.103338

Lezcano, M.Á., Morón-López, J., Agha, R., López-Heras, I., Nozal, L., Quesada, A., El-Shehawy, R., 2016. Presence or absence of mlr genes and nutrient concentrations co-determine the microcystin biodegradation efficiency of a natural bacterial community. Toxins (Basel). 8, 318. https://doi.org/10.3390/toxins8110318

Martínez-Ruiz, E.B., Cooper, M., Fastner, J., Szewzyk, U., 2020. Manganese-oxidizing bacteria isolated from natural and technical systems remove cylindrospermopsin. Chemosphere 238, 124625. https://doi.org/10.1016/j.chemosphere.2019.124625

Máthé, C., Beyer, D., Erdodi, F., Serfozo, Z., Székvölgyi, L., Vasas, G., M-Hamvas, M., Jámbrik, K., Gonda, S., Kiss, A., Szigeti, Z.M., Surányi, G., 2009. Microcystin-LR induces abnormal root development by altering microtubule organization in tissue-cultured common reed (Phragmites australis) plantlets. Aquat. Toxicol. 92, 122–130. https://doi.org/10.1016/J.AQUATOX.2009.02.005

Máthé, C., M-Hamvas, M., Vasas, G., Surányi, G., Bácsi, I., Beyer, D., Tóth, S., Tímár, M., Borbély, G., 2007. Microcystin-LR, a cyanobacterial toxin, induces growth inhibition and histological alterations in common reed (Phragmites australis) plants regenerated from embryogenic calli. New Phytol. 176, 824–835. https://doi.org/10.1111/J.1469-8137.2007.02230.X

Mazur-Marzec, H., Toruńska, A., Błońska, M.J., Moskot, M., Pliński, M., Jakóbkiewicz-Banecka, J., Wegrzyn, G., 2009. Biodegradation of nodularin and effects of the toxin on bacterial

isolates from the Gulf of Gdańsk. Water Res. 43, 2801–2810. https://doi.org/10.1016/j.watres.2009.03.042

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ. Int. 59, 303–327. https://doi.org/10.1016/j.envint.2013.06.013

Morón-López, J., Molina, S., 2020. Optimization of Recycled-Membrane Biofilm Reactor (R-MBfR) as a sustainable biological treatment for microcystins removal. Biochem. Eng. J. 153. https://doi.org/10.1016/j.bej.2019.107422

Namikoshi, M., Choi, B.W., Sakai, R., Sun, F., Rinehart, K.L., Carmichael, W.W., Evans, W.R., Cruz, P., Munro, M.H.G., Blunt, J.W., 1994. New Nodularins: A General Method for Structure Assignment. J. Org. Chem. 59, 2349–2357. https://doi.org/10.1021/jo00088a014

Newcombe, G., 2012. International Guidance Manual for the Management of Toxic Cyanobacteria. Water Intell. Online 11. https://doi.org/10.2166/9781780401355

Norris, R.L., Eaglesham, G.K., Pierens, G., Shaw, G.R., Smith, M.J., Chiswell, R.K., Seawright, A.A., Moore, M.R., 1999. Deoxycylindrospermopsin, an analog of cylindrospermopsin from cylindrospermopsis raciborskii. Environ. Toxicol. 14, 163–165. https://doi.org/10.1002/(SICI)1522-7278(199902)14:1<163::AID-TOX21>3.0.CO;2-V

Ohtani, I., Moore, R.E., Runnegar, M.T.C., 1992. Cylindrospermopsin: A Potent Hepatotoxin from the Blue-Green Alga Cylindrospermopsis raciborskii. J. Am. Chem. Soc. 114, 7941–7942. https://doi.org/10.1021/ja00046a067

Okano, K., Shimizu, K., Kawauchi, Y., Maseda, H., Utsumi, M., Zhang, Z., Neilan, B.A., Sugiura, N., 2009. Characteristics of a Microcystin-Degrading Bacterium under Alkaline Environmental Conditions. J. Toxicol. 2009, 1–8. https://doi.org/10.1155/2009/954291

Onstad, G.D., Strauch, S., Meriluoto, J., Codd, G.A., Von Gunten, U., 2007. Selective oxidation of key functional groups in cyanotoxins during drinking water ozonation. Environ. Sci. Technol. 41, 4397–4404. https://doi.org/10.1021/es0625327

Oral, H.V., Radinja, M., Rizzo, A., Kearney, K., Andersen, T.R., Krzeminski, P., Buttiglieri, G., Ayral-Cinar, D., Comas, J., Gajewska, M., Hartl, M., Finger, D.C., Kazak, J.K., Mattila, H., Vieira, P., Piro, P., Palermo, S.A., Turco, M., Pirouz, B., Stefanakis, A., Regelsberger, M.,

Ursino, N., Carvalho, P.N., 2021. Management of urban waters with nature-based solutions in circular cities—exemplified through seven urban circularity challenges. Water (Switzerland) 13, 3334. https://doi.org/10.3390/w13233334

Orr, P.T., Jones, G.J., Hamilton, G.R., 2004. Removal of saxitoxins from drinking water by granular activated carbon, ozone and hydrogen peroxide - Implications for compliance with the Australian drinking water guidelines. Water Res. 38, 4455–4461. https://doi.org/10.1016/j.watres.2004.08.024

Padisák, J., 1997. Cylindrospermopsis raciborskii (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. Arch. Für Hydrobiol. Suppl. Monogr. Beitrage.

Paerl, H.W., Otten, T.G., 2013. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. Microb. Ecol. 65, 995–1010. https://doi.org/10.1007/s00248-012-0159-y

Pelaez, M, Antoniou, M G, He, X, Dionysiou, D D, De La Cruz, A A, Tsimeli, K, Triantis, T, Hiskia, A, Kaloudis, T, Williams, C, Aubel, M, Chapman, A, Foss, A, Khan, U, O'shea, K E, Westrick, J, Pelaez, Miguel, Antoniou, Maria G, He, Xuexiang, Dionysiou, Dionysios D, De La Cruz, Armah A, Tsimeli, Katerina, Triantis, Theodoros, Hiskia, Anastasia, Kaloudis, Triantafyllos, Williams, Christopher, Aubel, Mark, Chapman, Andrew, Foss, Amanda, Khan, Urooj, O'shea, Kevin E, Westrick, Judy, 2010. Sources and Occurrence of Cyanotoxins Worldwide 101–127. https://doi.org/10.1007/978-90-481-3509-7_6

Pflugmacher, S., 2002. Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems. Environ. Toxicol. 17, 407–413. https://doi.org/10.1002/TOX.10071

Pflugmacher, S., Kwon, K.-S.S., Baik, S., Kim, S., Kühn, S., Esterhuizen-Londt, M., 2016. Physiological responses of Cladophora glomerata to cyanotoxins: a potential new phytoremediation species for the Green Liver Systems. Toxicol. Environ. Chem. 98, 241–259. https://doi.org/10.1080/02772248.2015.1119835

Pflugmacher, S., Wiegand, C., Beattie, K.A., Krause, E., Steinberg, C.E.W., Codd, G.A., 2001. Uptake, effects and metabolism of cyanobacterial toxins in the emergent reed plant Phragmites australis (CAV.) Trin. Ex Steud. Environ. Toxicol. Chem. 20, 846–852.

https://doi.org/10.1002/etc.5620200421

Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K.A., Krause, E., Codd, G.A., Steinberg, C.E.W., 1998. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR: the first step of detoxication. Biochim. Biophys. Acta - Gen. Subj. 1425, 527–533. https://doi.org/10.1016/S0304-4165(98)00107-X

Prepas, E.E., Murphy, T.P., 1988. Sediment-water interactions in farm dugouts previously treated with copper sulfate. Lake Reserv. Manag. 4, 161–168. https://doi.org/10.1080/07438148809354391

Puddick, J., Prinsep, M.R., Wood, S.A., Kaufononga, S.A.F., Cary, S.C., Hamilton, D.P., 2014. High levels of structural diversity observed in microcystins from microcystis CAWBG11 and characterization of six new microcystin congeners. Mar. Drugs 12, 5372–5395. https://doi.org/10.3390/md12115372

Rabalais, N.N., Díaz, R.J., Levin, L.A., Turner, R.E., Gilbert, D., Zhang, J., 2010. Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7, 585–619. https://doi.org/10.5194/bg-7-585-2010

Rapala, J., Lahti, K., Sivonen, K., Niemelä, S.I., 1994. Biodegradability and adsorption on lake sediments of cyanobacterial hepatotoxins and anatoxin-a. Lett. Appl. Microbiol. 19, 423–428. https://doi.org/10.1111/J.1472-765X.1994.TB00972.X

Redouane, E.M., Tazart, Z., Lahrouni, M., Mugani, R., Elgadi, S., Zine, H., Zerrifi, S.E.A., Haida, M., Martins, J.C., Campos, A., Oufdou, K., Vasconcelos, V., Oudra, B., 2023. Health risk assessment of lake water contaminated with microcystins for fruit crop irrigation and farm animal drinking. Environ. Sci. Pollut. Res. 30, 80234–80244. https://doi.org/10.1007/s11356-023-27914-1

Rinehart, K.L., Harada, K.I., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M.H.G., Blunt, J.W., Mulligan, P.E., Beasley, V.R., Dahlem, A.M., Carmichael, W.W., 1988. Nodularin, Microcystin, and the Configuration of Adda. J. Am. Chem. Soc. 110, 8557–8558. https://doi.org/10.1021/ja00233a049

Ryther, J.H., Dunstan, W.M., 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science (80-.). 171, 1008–1013.

https://doi.org/10.1126/science.171.3975.1008

Seifert, M., McGregor, G., Eaglesham, G., Wickramasinghe, W., Shaw, G., 2007. First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, Lyngbya wollei (Farlow ex Gomont) Speziale and Dyck. Harmful Algae 6, 73–80. https://doi.org/10.1016/j.hal.2006.07.001

Senogles-Derham, P.J., Seawright, A., Shaw, G., Wickramisingh, W., Shahin, M., 2003. Toxicological aspects of treatment to remove cyanobacterial toxins from drinking water determined using the heterozygous P53 transgenic mouse model. Toxicon 41, 979–988. https://doi.org/10.1016/S0041-0101(03)00070-9

Senogles, P., Shaw, G., Smith, M., Norris, R., Chiswell, R., Mueller, J., Sadler, R., Eaglesham, G., 2000. Degradation of the cyanobacterial toxin cylindrospermopsin, from Cylindrospermopsis raciborskii, by chlorination. Toxicon 38, 1203–1213. https://doi.org/10.1016/S0041-0101(99)00210-X

Shim, H., Shin, E.B., Yang, S.T., 2002. A continuous fibrous-bed bioreactor for BTEX biodegradation by a co-culture of Pseudomonas putida and Pseudomonas fluorescens. Adv. Environ. Res. https://doi.org/10.1016/S1093-0191(01)00132-0

Smith, F.M.J., Wood, S.A., van Ginkel, R., Broady, P.A., Gaw, S., 2011. First report of saxitoxin production by a species of the freshwater benthic cyanobacterium, Scytonema Agardh. Toxicon 57, 566–573. https://doi.org/10.1016/j.toxicon.2010.12.020

Song, W., De La Cruz, A.A., Rein, K., O'Shea, K.E., 2006. Ultrasonically induced degradation of microcystin-LR and -RR: Identification of products, effect of pH, formation and destruction of peroxides. Environ. Sci. Technol. 40, 3941–3946. https://doi.org/10.1021/es0521730

Sundaravadivel, M., Vigneswaran, S., 2001. Constructed wetlands for wastewater treatment. Crit. Rev. Environ. Sci. Technol. https://doi.org/10.1080/20016491089253

Szlag, D.C., Sinclair, J.L., Southwell, B., Westrick, J.A., 2015. Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants. Toxins 2015, Vol. 7, Pages 2198-2220 7, 2198–2220. https://doi.org/10.3390/TOXINS7062198

Taranu, Z.E., Gregory-Eaves, I., Leavitt, P.R., Bunting, L., Buchaca, T., Catalan, J., Domaizon, I.,

Guilizzoni, P., Lami, A., Mcgowan, S., Moorhouse, H., Morabito, G., Pick, F.R., Stevenson, M.A., Thompson, P.L., Vinebrooke, R.D., 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecol. Lett. 18, 375–384. https://doi.org/10.1111/ele.12420

Terin, U.C., Sabogal-Paz, L.P., 2019. Microcystis aeruginosa and microcystin-LR removal by household slow sand filters operating in continuous and intermittent flows. Water Res. 150, 29–39. https://doi.org/10.1016/j.watres.2018.11.055

Testai, E., Buratti, F.M., Funari, E., Manganelli, M., Vichi, S., Arnich, N., Biré, R., Fessard, V., Sialehaamoa, A., 2017. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. EFSA Support. Publ. 13, 998E. https://doi.org/10.2903/sp.efsa.2016.en-998

Visser, P.M., Ibelings, B.W., Bormans, M., Huisman, J., 2016. Artificial mixing to control cyanobacterial blooms: a review. Aquat. Ecol. 50, 423–441. https://doi.org/10.1007/S10452-015-9537-0/FIGURES/3

Vymazal, J., 2011. Constructed wetlands for wastewater treatment: Five decades of experience. Environ. Sci. Technol. 45, 61–69. https://doi.org/10.1021/es101403q

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 380, 48–65. https://doi.org/10.1016/J.SCITOTENV.2006.09.014

Wang, H., Ho, L., Lewis, D.M., Brookes, J.D., Newcombe, G., 2007. Discriminating and assessing adsorption and biodegradation removal mechanisms during granular activated carbon filtration of microcystin toxins. Water Res. 41, 4262–4270. https://doi.org/10.1016/J.WATRES.2007.05.057

Wang, R., Tai, Y., Wan, X., Ruan, W., Man, Y., Wang, J., Yang, Y.Y., Yang, Y.Y., 2018. Enhanced removal of Microcystis bloom and microcystin-LR using microcosm constructed wetlands with bioaugmentation of degrading bacteria. Chemosphere 210, 29–37. https://doi.org/10.1016/j.chemosphere.2018.06.140

Wanielista, M., 2009. Stormwater Irrigation Volume III : Transport of the Cyanotoxin Microcystin in Groundwater Beneath Stormwater Ponds: results of soil column experiments. BMP Trains Res. Publ. III.

Wei, J., Pengji, Z., Zhang, J., Peng, T., Luo, J., Yang, F., 2023. Biodegradation of MC-LR and its key bioactive moiety Adda by Sphingopyxis sp. YF1: Comprehensive elucidation of the mechanisms and pathways. Water Res. 229, 119397. https://doi.org/10.1016/j.watres.2022.119397

WHO, 2022. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda, Resuscitation. Geneva. Licence: CC BY-NC-SA 3.0 IGO

WHO, 2020a. Cyanobacterial toxins: microcystins. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. Geneva.(WHO/HEP/ECH/WSH/2020.6).Licence: CC BY-NC- SA 3.0 IGO

WHO, 2020b. Cyanobacterial toxins: cylindrospermopsins. Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. Geneva. (WHO/HEP/ECH/WSH/2020.4).Licence: CC BY-NC-SA 3.0 IGO.

WHO, 2020c. Cyanobacterial toxins: anatoxin-a and analogues. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments. Geneva. (WHO/HEP/ECH/WSH/2020.1).Licence: CC BY-NC-SA 3.0 IGO.

WHO, 2020d. Cyanobacterial toxins: saxitoxins.Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. Geneva. (WHO/HEP/ECH/WSH/2020.8).Licence: CC BY-NC-SA 3.0 IGO.

Wiese, M., D'Agostino, P.M., Mihali, T.K., Moffitt, M.C., Neilan, B.A., 2010. Neurotoxic alkaloids: Saxitoxin and its analogs. Mar. Drugs. https://doi.org/10.3390/md8072185

Wimmer, K.M., Strangman, W.K., Wright, J.L.C., 2014. 7-Deoxy-desulfo-cylindrospermopsin and 7-deoxy-desulfo-12-acetylcylindrospermopsin: Two new cylindrospermopsin analogs isolated from a Thai strain of Cylindrospermopsis raciborskii. Harmful Algae 37, 203–206. https://doi.org/10.1016/j.hal.2014.06.006

Wörmer, L., Cirés, S., Agha, R., Verdugo, M., de Hoyos, C., Quesada, A., 2011. First detection of cyanobacterial PSP (paralytic shellfish poisoning) toxins in Spanish freshwaters. Toxicon 57, 918–921. https://doi.org/10.1016/j.toxicon.2011.02.022

Wormer, L., Cirés, S., Carrasco, D., Quesada, A., 2008. Cylindrospermopsin is not degraded by

co-occurring natural bacterial communities during a 40-day study. Harmful Algae 7, 206–213. https://doi.org/10.1016/j.hal.2007.07.004

Yan, Y., Fu, D., Shi, J., 2019. Screening and Immobilizing the Denitrifying Microbes in Sediment for Bioremediation. Water 2019, Vol. 11, Page 614 11, 614. https://doi.org/10.3390/W11030614

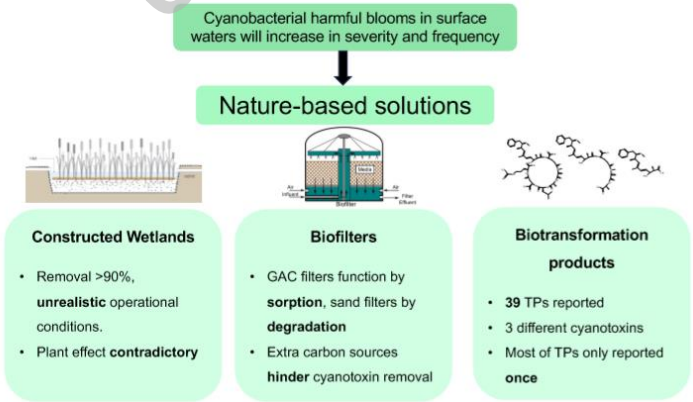
Yang, F., Huang, F., Feng, H., Wei, J., Massey, I.Y., Liang, G., Zhang, F., Yin, L., Kacew, S., Zhang, X., Pu, Y., 2020. A complete route for biodegradation of potentially carcinogenic cyanotoxin microcystin-LR in a novel indigenous bacterium. Water Res. 174, 115638. https://doi.org/10.1016/j.watres.2020.115638

Yang, F., Zhou, Y., Sun, R., Wei, H., Li, Y., Yin, L., Pu, Y., 2014. Biodegradation of microcystin-LR and-RR by a novel microcystin-degrading bacterium isolated from Lake Taihu. Biodegradation 25, 447–457. https://doi.org/10.1007/S10532-013-9673-Y/FIGURES/5

Zhang, M.L., Yan, H., Pan, G., 2011. Microbial degradation of microcystin-LR by Ralstonia solanacearum. Environ. Technol. 32, 1779–1787. https://doi.org/10.1080/09593330.2011.556148

Zhong, F., Liu, W., Lv, M., Deng, Z., Wu, J., Cheng, S., Ji, H., 2018. The use of vertical flow constructed wetlands for the treatment of hyper-eutrophic water bodies with dense cyanobacterial blooms. Water Sci. Technol. 77, 1186–1195. https://doi.org/10.2166/wst.2017.635

Graphical abstract



Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Pedro Nieves Carvalho reports financial support was provided by Independent Research Fund Denmark. Pedro Nieves Carvalho, Carlos Alberto Arias reports financial support was provided by European Commission Marie Sklodowska-Curie Actions.