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Bioaccumulation of cyanotoxins in terrestrial plants and crops

Abstract

The Anthropocene activities not only affects the global climate but also the ubiquitous cyanobacterial lifecycle. With the increased runoffs of biofertilizers causing eutrophication in fragile aquatic ecosystems and water reservoirs that functions for fresh water and irrigation. Eutrophication leads to increase cyanobacterial growth and its harmful cyanotoxins. This combined with the rising scarcity of safe irrigation water for crops increases the risk of cyanotoxin exposure for humans, animals and plants. Better understanding regarding the cyanobacterial lifecycle and degradation of its cyanotoxins is key for enabling safe utilization of irrigation water, thus securing a growing demand on food production. This report reviews the possible event of cyanotoxin bioaccumulation in crops and the potential risk in food production and lowered yields.

Keywords

Crop irrigation; bioaccumulation; cyanotoxins; terrestrial plant; soil

Introduction

Cyanobacteria, commonly described as "blue-green algae", can be found as free-living or symbionts, making them a bacterial species group found globally and in several diverse aquatic niches, e.g. freshwater, brackish, marine and terrestrial areas (Xie *et al*., 2013). The gram-negative prokaryotes have fossil records dating them to originate from more than 3,5 billion years ago, making them one of the first bacteria genera's in the history of Earth. Their ability to perform oxygenic photosynthesis lead to the first oxygen produced, thus creating a primitive atmosphere and enabling the dawn for life on Earth (Osswald *et al*., 2007). For cyanobacteria growth to occur, bioavailable nutrients and specific environmental factors are required. Limiting growth factors are mainly lack of sufficient essential nutrients (C, P, N, K, S etc) and photovoltaic radiation (sunlight), but also factors such as water temperature and salinity (Markou *et al*., 2014). Harmful algae blooming (HAB), refers to the event when cyanobacteria are subject of lysis, thus releasing their contents and spreading cyanotoxins. Most of these toxins, if not all, are believed having the potential of causing toxic reactions in other organisms. Due to climate change and the overall rise of the global temperature which functions as a driver causing a prolonged season for HAB (Corbel *et al*., 2014) (Manning & Nobles, 2017).

Since cyanobacteria can be found most everywhere in various aquatic niches, it also increases the risk for human and animal contact with cyanotoxins. It's estimated that at least 30 bacteria genera have the potential of causing the death of fish (Manning & Nobles, 2017). Numerous of the cyanotoxins, when ingested, or made contact with, affects several human and animal organs, such as liver, skin, nervous systems etc and in some cases they may have a lethal outcome (Corbel *et al.*, 2014).

It has also been discovered that many of these cyanotoxins can bind covalently and/or accumulate through e.g. protein incorporation in organisms, not just for animals and humans, but also numerous members of the Poaceae family such as *Triticum aestivum* (wheat), *Zea mays*

(corn) and *Oryza sativa* (rice) (Contardo-Jara *et al*., 2018; Machado *et al*., 2017). This raises a concern that terrestrially grown produce, irrigated with water contaminated with cyanotoxins, might induce toxic accumulation effects consisting of various cyanotoxins in food consumers.

The purpose of this report is to shed light upon the possibility of the potential bioaccumulative effects of cyanotoxins that may arise in terrestrially grown crops and other vascular plants if irrigated with cyanotoxin-contaminated water.

Material and Method

Peer reviewed articles and reviews from established and highly cited scientific papers.

Cyanotoxins

	Cyanotoxin class	Genera
Shikimate pathway	Ambigols (3)	*Fischerella*
	Anatoxins and homoanatoxins (3)	*Anabaena*, *Aphanizomenon*, *Blennothrix*, *Cuspidothrix*, *Cylindrospermum*, *Dolichospermum*, *Microcyctis*, *Oscillatoria*, *Phormidium*, *Planktothrix*, *Psudoanabaena*, *Raphidiopsis*
	Cylindrospermopsin (3)	*Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Oscilliatoria*, *Raphidiopsis*, *Umezakia*
	Saxitoxins (>60)	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodosilinea*, *Phormidesmis*, *Phormidium*, *Planktothrix*, *Raphidiopsis*, *Scytonema*
Acetate pathway	Aeruginosins (>15)	*Microcystis*, *Nostoc*, *Oscillatoria*, *Planktothrix*
	Anabaenopeptins (>33)	*Anabaena*, *Aphanizomenon*, *Dolichospermum*, *Planktothrix*, *Microcystis*, *Nodularia*
	Antillatoxin	*Lyngbya*
	β-Methylamino-L-alanine	*Most species*
	Cyanopeptolins (>12)	*Microcystis*, *Planktothrix*
	Hermitamides (2)	*Lyngbya*
	Hormothamnin	*Hormothamnion*
	Jamaicamides (3)	*Lyngbya*
	Laxaphycins (>8)	*Anabaena*
	Lipopolysaccharides	*Most species*
	LyngbyatoxinS (3)	*Lyngbya*, *Moorea*
	Microcystins (>100)	*Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Cyanobium*, *Dolichospermum*, *Fischerella*, *Gloeotrichia*, *Hapalosiphon*, *Leptolyngbya*, *Limnothrix*, *Merismopedia*, *Microcystis*, *Nostoc*, *Oscillitoria*, *Phormidium*, *Planktothrix*, *Raphiodiopsis*, *Synechocystis*, *Synechococcus*, *Trichodesmium*
	Microgenins (>40)	*Microcystis*, *Planktothrix*
	Mueggelone	*Aphanizomenon*
	Nodularins (10)	*Nodularia*
	Oscillamide	*Oscillatoria*, *Planktothrix*
	Oscillapeptins	*Oscillatoria*
	Pahayokolides	*Lyngbya*
	Palytoxin	*Trichodesmium*

Figure 1. Pathways affected by cyanobacterial toxins and genera. Estimated number of congeners in parentheses. Source: Manning & Nobles (2017)

The cyanotoxins presented below have been chosen from a vast number of toxins (Fig. 1), due to their producing species' occurrence in the Baltic Sea but also of their public interest arisen from reports in various news feed and media.

β-Methylamino-L-alanine (BMAA) was chosen to be further investigated due to arisen controversy in media regarding the results obtained in the studies conducted by Cox & Sacks, (2002), Pablo *et al.*, (2009) and later also described by Xie *et al.*, (2013). The result from these research groups suggests a probability that BMAAs similarity of an, for humans, essential amino acid could lead to dietary exposure due to protein-incorporation. BMAA is also

suggested of being able to pass the blood-brain barrier, thus causing misfolding in neural cells leading to several possible degenerative neural diseases.

Anatoxins

These very potent neurotoxins are found in a majority of the cyanobacteria genera. Anatoxin-a is commonly known for being the cause of the death for many pets and livestock, ingesting the toxin during play or by drinking contaminated water. The toxin mimics an essential neurotransmitter, acetylcholine, but is unlike it not degraded, thus causing a non-stop stimulus to the nerve cell ends, leading to paralysis and death (Corbel *et al.,* 2014). Anatoxin-a is an amine that degrades in water into non-toxic components, at slower rate in low pH and at a faster rate in alkaline pH. The bioaccumulation is suggested to be low and the toxin is found to a greater extent in aquatic solutions rather than being significantly adsorbed to particulate material or sediment (Farré, 2017).

Microcystin and nodularin

Cyanobacterial microcystin-LR (MC-LR) and nodularin, are hepatoxins believed to have carcinogenic effects by inducing oxidative stress to cells. The toxins are unable to penetrate animal cell membranes but can enter through uptake via the bile acid system by hepatocytes and cells of the smaller intestine (Corbel *et al.,* 2014). Studies done indicate that MC-LR can accumulate in a wide variety of agricultural plants (Fig. 2), thus posing a risk of toxin exposure to food consumers. It remains however yet to understand and explore exactly how MC-LR can pass through and accumulate in plant cells, whether it be by polarity, diffusion or absorption (Machado et al., 2017). Numerous studies have shown that the physiology and metabolism are affected if plants are exposed to sufficient levels of MC-LR, risking loss of crops as a result from the inhibiting effects of MC-LR resulting in inhibited germination, alteration of chlorophyll, decreased growth and total yield (Manning & Nobles, 2017). Exposing terrestrial plants for nodularin have in studies shown to increase the oxidative stress, lowering the general fitness and reducing growth by increasing the energetic costs of induced stress (Lehtimäki *et al*., 2011).

BMAA bioaccumulation and T. aestivum *– summary of relevant studies*

The experiments and studies featured below were chosen as the crop, *T. aestivum,* is of interest for this report, experiment procedures involving use of soil and irrigation with neurotoxin BMAA and investigation of possible protein-associated bioaccumulation.

The experiments performed by Contardo-Jara *et al*., 2014, studied *T. aestivum* that was irrigated from seed to shoot with water containing free BMAA 100–1000 µg/L for 28 days. 100 µg/L was considered to represent a potential worst case of HAB. Sprouting was conducted for 4 days on paper tissues laid on Petri dishes and sprayed with water containing free BMAA. The later sprouted seedlings remaining growth was conducted in potting soil for the remaining 24 days. Irrigation with water containing free BMAA was resumed 7 days after planting, to ensure the study of uptake and subsequent allocation of BMAA. Samples were taken in germination state and root and shoots taken frequently over the course of the experiment.

In conclusion the experiment showed that irrigation with water containing free BMAA can transfer BMAA from water to crop. Compared with other experiments that have been conducted in different aquatic, marine and terrestrial for BMAA distribution in plant and animal species, this experiment only found BMAA in the form of a protein-associated incorporation.

In 2018, Contardo-Jara *et al.* redesigned their previous experiment to study the acute BMAA exposure effects on the germination and development by irrigating seeds and seedlings with 10

Accumulation of MC-LR in several edible plant species and the daily consumption calculated based on the concentration reported in plant tissues.

Plant species	Concentration of exposure (µg/L)	Exposure time (days)	Analyzed organ	Concentration reported in plant tissues (ng/g F.W)	Daily consumption (µg/kg BW)[a]	Reference
Brassica napus	24	10	Extract of plant (excluding roots)	2.61	0.01	Chen et al., 2004
	120			8.32	0.02	
	600			123.57	**0.31**	
	3000			651	**1.63**	
Cicer arietinum	5	1	Shoots	≈10	≈0.03	Peuthert et al., 2007
Glycine max	5	1	Shoots	≈17	**≈0.04**	Peuthert et al., 2007
Lactuca sativa	2	15	Leaf	≈ 33	0.02–**0.09**[b]	Bittencourt-Oliveira et al., 2016
	5			≈ 103		
	10			≈ 143		
Lactuca sativa	5	1	Leaf	≈ 1.30[c]	0.02[b]	Cordeiro-Araújo et al., 2016
		4		≈ 1.59[c]	0.03[b]	
		7		≈ 2.05[c]	0.03[b]	
	10	1		≈ 2.94[c]	**0.05**[b]	
		4		≈ 3.83[c]	**0.06**[b]	
		7		≈ 4.04[c]	**0.07**[b]	
Lens culinarian	5	1	Shoots	≈20	**≈0.05**	Peuthert et al., 2007
Lycopersicon esculentum	100	7	Green Fruits	≈5	≈0.01	Gutiérrez-Praena et al., 2014
			Mature Fruits	≈10	≈0.03	
	5	90	Leaves	n.d	–	Corbel et al., 2016
			Roots	≈ 4.5	≈0.01	
	20		Leaves	≈ 0.29	≈0.00	
			Roots	≈ 4.8	≈0.01	
	50		Leaves	≈ 0.33	≈0.00	
			Roots	≈ 5.7	≈0.01	
	100		Leaves	≈0.55	≈0.00	
			Roots	≈8.1	≈0.02	
Malus pumila	30	14	Shoots	14.76	**0.04**	Chen et al., 2010
	300			43.94	**0.11**	
	3000			510.23	**1.28**	
Medicago sativa	5	1	Shoots	≈27	**≈0.07**	Peuthert et al., 2007
Oryza sativa	120	10	Extract of plant (excluding roots)	2.94	0.01	Chen et al., 2004
	600			5.12	0.01	
	3000			5.40	0.01	
Pisum sativum	5	1	Shoots	≈ 18	**≈ 0.05**	Peuthert et al., 2007
Phaseolus vulgaris	5	1	Shoots	≈ 38	**≈ 0.10**	Peuthert et al., 2007
Vigna radiata green	5	1	Shoots	≈ 18	**≈ 0.05**	Peuthert et al., 2007
Vigna radiata red	5	1	Shoots	≈ 4	≈ 0.01	Peuthert et al., 2007
Triticum aestivum	5	1	Shoots	≈ 28	**≈ 0.07**	Peuthert et al., 2007
Zea mays	5	1	Shoots	≈ 40	**≈ 0.10**	Peuthert et al., 2007

Daily consumption values highlighted in bold indicate TDI values higher than those recommended by WHO. n.d., non-detectable.
[a] The daily consumption of MC was calculated assuming that a person of 60 kg consumes 150 g of the vegetable species per day.
[b] The daily consumption of MC was calculated by the authors assuming that a person of 60 kg consumes 40 g of lettuce leaves per day.
[c] Value expressed in µg per 40 g of lettuce leaves.

Figure 2. Microcystin-LR can accumulate in a wide variety of agricultural plants. Source: Machado et al., (2017)

µg/L BMAA water (representing a mild HAB) for five days. They also added an investigation of BMAA bioaccumulation in *T. aestivum* when exposed long-term (10 µg/L BMAA water) during its entire lifecycle i.e. seed to seedbearing, a total growth time of 205 days. The crops where grown in soil in a simulated environment with regulated light-dark exposure and wind stimuli to enable pollination and fruit bearing. The results from the experiments found no morphologic changes due to either acute nor long-term exposure of BMAA. In the long-term

effect study, protein-associated BMAA was found in equal amounts of roots and stems. The seeds produced by these plants displayed a 10-fold higher concentration of protein-associated BMAA in comparison to their roots and shoots.

BMAA have in earlier studies, using *in vivo* mouse models and ^{14}C-labelled BMAA, been found incorporated in neural tissues and therefore lead to the belief of causing misfolding in proteins associated with plausible neurodegenerative diseases such as dementia, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Parkinsonism dementia complex (PD) (Xie *et al*., 2013).

The studies regarding BMAA as a possible cause for neurodegenerative disease was first described by Spencer et al., in 1987. The research was then carried on and made famous by Cox & Sacks in 2002, who hypnotised a possible linkage of BMAA dietary exposure and ALS. These hypotheses have led to a speculation of a possible paradigm shift in how neurodegenerative diseases arise, seeing that approximately 5–10 % of e.g. ALS, PD, AD and dementia are deemed to be inherited genetic mutations (Holtcamp, 2012).

Their research is however deemed to be highly disputable. Several attempts in replicating Cox's studies have been done by various other groups, none of whom have been able in succeeding. The most extensive and elaborated study conducted, to this day, was done in 2016 in which they found no free or protein bound BMAA in AD confirmed patients (n = 20) (Meneely et al., 2016). In their research they also attempt to explain underlying potential causes to the suggested misidentification of BMAA in earlier studies.

Meneely *et al*., (2016) points out that the animal models used are not compliant with the observations done in the case studies. The animal models received high doses in a short period of time, whereas the cases studies showed that exposure to BMAA was at a constant low concentration over a longer period of time. They conclude that their study seriously contests and questions the results found by Cox (2002), whether BMAA is at all one of the underlying factors in the development of spontaneous neurodegenerative diseases or not.

Discussion

The ongoing climate change due to Anthropocene activities affects the ubiquitous cyanobacterial lifecycle, leading to more cyanotoxins in circulation thus risking toxic exposure for humans, animals and plants. Better understanding regarding the cyanobacterial life cycle and degradation of its cyanotoxins is thus key for enabling utilization of irrigation water. Should it be that cyanotoxins fully degrade between the season of HAB periods, water for irrigation could be extracted from surface water before HABs occurs and at lower depths when HAB occurs, to avoid spread of cyanotoxins. If, however, cyanotoxins are stable and occur in the sediment-water interface, there's a potential risk that the irrigation water taken from lower depths might contain these regardless of the time of year. The same can be said of surface water, depending on cyanotoxins densities.

The aspects that cyanotoxins might degrade on terrestrial surface and/or be digested by other microbes must also be taken under consideration. The studies in this report suggest that

cyanotoxins can enter plants vascular systems, thus enabling incorporation and accumulation in crops but also as free-floating in water compartments of plants vascular system.

Conclusion

There's today no tolerable daily intake rate (TDI) set for all the various cyanotoxins, making it difficult to establish recommendations of food intake, to avoid possible health risks due to long-term bioaccumulation effects from consuming food products containing cyanotoxins. The same TDI for cyanotoxins should also be established for consumption of drinking water. UNICEF and WHO is currently evaluating cyanotoxin and establishing possible health effects they might contribute to (UNICEF & WHO, 2017).

This report has attempted to summarise the most recent and up-to-date research from various unrelated research groups, regarding possible cyanotoxin bioaccumulation in terrestrial crops when irrigated with cyanotoxin polluted water. Mutual for all research groups reviewed for this report, is that they all advise extensive risk assessment before undertaking irrigation with cyanotoxin contaminated water.

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