Water Air Soil Pollut (2024) 235:72
https://doi.org/10.1007/s11270-023-06861-0

Occurrence of β-N-Methylamino-L-Alanine (BMAA) Toxin in irrigation Water and Field Vegetable Plants and Assessing Its Potential Risk to Human Health

Zakaria A. Mohamed · Rehab O. Elnour · Saad Alamri · Mohamed Hashem · Ali M. Alshehri · Alexandre Campos · Vitor Vasconcelos · Hanan Badawye

Received: 8 July 2023 / Accepted: 26 December 2023 / Published online: 9 January 2024

Abstract This study investigates the presence of the neurotoxin β-N-methylamino-L-alanine (BMAA) and its cyanobacterial producers in irrigation water as wells its potential accumulation in nine commonly consumed vegetables from Egyptian farmlands. The HPLC–MS/MS analysis revealed that phytoplankton samples contained higher concentrations of free BMAA (0.6–11.4 μg L^{-1}) than protein-bound form (0.01–3.3 μg L^{-1}), in association with the abundance of dominant cyanobacteria in irrigation water sites. Extracellular dissolved BMAA was also detected in cell-free irrigation water, but with very low concentrations (0.1–0.2 μg L^{-1}). Meanwhile, BMAA was also detected in a protein-bound form (0.05–7.7 μg g^{-1} fresh weight) in most vegetable plants, with highest levels obtained in zucchini fruits followed by watercress levels, tomato fruits, green pepper fruits, radish leaves, and pea fruits. The BMAA concentrations accumulated in these vegetables correlated with BMAA concentrations detected in relevant irrigation water sites.

Hence, the presence of BMAA in vegetables could pose a risk to human through eating contaminated edible pant parts. The study recommends ongoing monitoring of BMAA and other cyanotoxins in irrigation waters and edible plants in order to safeguard the public from exposure to such serious toxins.

Keywords Cyanotoxins · Fruity plants · Health hazard · Leafy plants · Surface water

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-023-06861-0.

Z. A. Mohamed (✉) · H. Badawye
Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt
e-mail: mzakaria_99@yahoo.com

R. O. Elnour
Biology Department, Faculty of Sciences and Arts, Dahran Al-Janoub, King Khalid University, Abha, Saudi Arabia

S. Alamri · M. Hashem · A. M. Alshehri
Department of Biology, College of Science, King Khalid University, 61413 Abha, Saudi Arabia

A. Campos · V. Vasconcelos
CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros Do Porto de Leixões, Av. General Norton de Matos, S/N, 4450-208 Matosinhos, Portugal

V. Vasconcelos
Departament of Biology, Faculty of Sciences, University of Porto, Rua Do Campo Alegre, 4169-007 Porto, Portugal

1 Introduction

The incidence and intensity of cyanobacterial blooms have increased recently as a result of climate change and the enrichment of surface waters with anthropogenic nutrients (Mohamed & Al-Shehri, 2010; Scholz et al., 2017; Zamora-Barrios et al., 2019). Several species of cyanobacteria are able to produce harmful

bioactive substances known as cyanotoxins, such as neurotoxins, hepatotoxins, and dermatoxins (Meriluoto et al., 2017; Mohamed, 2016). Therefore, such harmful microorganisms could have adverse effects on environmental health including death of aquatic animals, poisonous seafood, economic impacts, losses to aquaculture enterprises, and long-term ecosystem changes (Buratti et al., 2017). Also, the toxins of these microorganisms deteriorate the drinking water quality, causing human health disorders.

Harmful effects of cyanobacteria would also include human illness from direct consumption of contaminated drinking water (Mohamed et al., 2015) or indirect exposure via consumption of organisms (e.g., food animals and plants) that accumulate the toxins in their bodies (Mohamed et al., 2020, 2022). Among these toxins, the non-proteinogenic amino acid β-N-methylamino-L-alanine (BMAA) has most recently attracted the interest of researchers due to mounting evidence linking exposure to it to the development of progressive neurodegenerative diseases like the amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) (Cox et al., 2016; Koksharova & Safronova, 2022). The neurotoxicity of BMAA is due to its ability to replace the protein amino acid "serine" in protein synthesis in neurons (i.e., amino acid mimicry), leading to protein misfolding and aggregation (the hallmark of Alzheimer's disease) (Korn et al., 2020; Song et al., 2017).

Cyanobacteria are the primary source of BMAA in the aquatic environment (Cox et al., 2005; Main et al., 2017; Reveillon et al., 2016; Violi et al., 2019a). However, a few species of marine diatoms and dinoflagellates have been documented to produce this toxin (Jiang et al., 2014; Main et al., 2017; Reveillon et al., 2016; Violi et al., 2019b).

These phytoplankton groups would be a major contributor to the intake of BMAA toxin into aquatic food webs, with possible human exposure, as they constitute a sizeable fraction of the primary producers in aquatic environments (Brand et al., 2010; Mondo et al., 2012; Esterhuizen-Londt & Pflugmacher, 2019).

Furthermore, this toxin may transfer from aquatic to terrestrial environments by irrigation with contaminated water or the use of cyanobacterial blooms as a biofertilizer, which could expose agricultural plants to cyanotoxins (Contardo-Jara et al., 2014; Weralupitiya et al., 2022). Because of its hydrophilic nature and capacity to bind with protein, BMAA toxin can bioaccumulate in plant tissues (Esterhuizen-Londt & Pflugmacher, 2019; Murch et al., 2004).

The uptake and accumulation of cyanotoxins in agricultural plants have been widely investigated for cylindrospermopsins and microcystins (Weralupitiya et al., 2022), but a few studies demonstrated the transfer of BMAA toxin into terrestrial plants via irrigation water.

These studies reported the accumulation of BMAA in cereal and silage plants including wheat (*Triticum aestivum*) (Contardo-Jara et al., 2014, 2018) and alfalfa *Medicago sativa* (Samardzic et al., 2021). Other studies also documented the accumulation of BMAA in vegetable plants including watercress (*Nasturtium officinale*) and wild carrot (*Daucus carota*) (Niyonzima, 2010), Chinese cabbage (Li et al., 2019), *Lactuca sativa*, and *Allium fistulosum* (Esterhuizen-Londt & Pflugmacher, 2019). The accumulation of BMAA toxin in vegetable plants may pose a risk to human health upon consumption of contaminated edible plant parts.

Actually, the majority of prior studies on the transfer of BMAA from water to terrestrial plants was carried out in pots under laboratory circumstances or even in the field under preset experimental conditions (Weralupitiya et al., 2022). This urges the necessity to provide evidence of BMAA bioaccumulation in plants grown naturally in agricultural lands irrigated with contaminated water.

The Nile River and irrigation canals branched from it might be an ideal place to conduct such field studies. These irrigation water sources are eutrophic and frequently experience toxic cyanobacterial blooms because of rising anthropogenic activity over the past few decades (Mohamed, 2016; Mohamed et al., 2015). Other cyanotoxins including MCs and CYN have been extensively researched in Egypt's irrigation water sources and the Nile River (Mohamed, 2016); however, the presence of the BMAA toxin in these waters has not yet been investigated.

Therefore, the current study examined for the first time the existence of BMAA toxin and its cyanobacterial producers in irrigation water in Egypt as a representative of tropical countries. Meanwhile, the accumulation of BMAA toxin was investigated for the first time in vegetable plants grown naturally in farmlands irrigated with water containing cyanobacterial blooms. The possible health risk associated

with consuming contaminated vegetable plants was also evaluated using the data of BMAA concentrations detected in edible parts of these plants.

2 Materials and Methods

2.1 Sampling

Water samples were collected from 22 different sites in irrigation canals branching and receiving water from the Nile River. These irrigation canals are located at Sohag province, southern Egypt (Fig. 1). The names of irrigation canals used in this study have been replaced by codes and numbers (S-1:S-22) in the accordance with the national safety information law. Water samples were taken at these sites using sterilized polyethylene bottles at a depth of around 0.3 m during summer 2021 and winter 2022. To identify and count the phytoplankton species, 500 mL of irrigation water was fixed and stored in 1% Lugol's solution. Meanwhile, edible portions (i.e., leaves or fruits) of nine vegetable plants including green pepper (*Capsicum annuum*), lettuce (*Lactuca sativa*), mulukhiyah (*Corchorus olitorius*), pea (*Pisum sativum*), radish (*Raphanus sativus*), spinach (*Spinacia oleracea*), tomato (*Solanum lycopersicum*), watercress (*Nasturtium officinale*), and zucchini (*Cucurbita pepo*) were gathered (after harvest) from farmlands near these canals, where their waters were used to irrigate these plants.

2.2 2.2. Environmental and Phytoplankton Analyses

Physical properties of irrigation waters, including temperature, pH, electric conductivity (EC), and total dissolved salts (TDS), were determined in situ at a depth of 10–20 cm from the surface using multiparametric probe (HI 991300 pH/EC/TDS Temperature, HANNA, Italy). The multiparameter meter used in this study is waterproof portable logging meter with microprocessor-based multi-sensor probe that monitors up to 12 different water quality parameters. Concentrations of nutrients including nitrate-NO_3, ammonia-NH4, orthophosphate-PO_4, and dissolved organic matter-DOC were assessed in

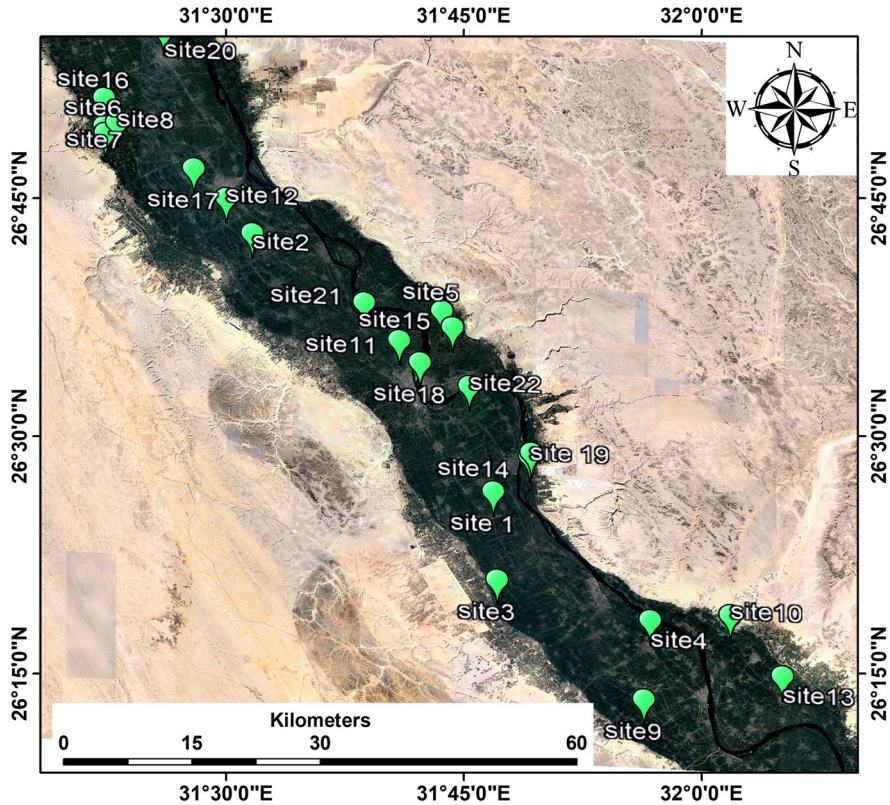

Fig. 1 Geographical location of the 22 sampling sites monitored in the present study

pre-filtered water (via Whatman GF/C Microfiber Glass Filter, Binder free) according to the standard methods (APHA (American Public Health Association). , 1995). The phytoplankton cell density was determined using a Sedgwick–Rafter counting chamber under a compound light microscope and expressed as cells per liter (APHA (American Public Health Association). , 1995). Phytoplankton species were identified morphologically based on pertinent taxonomic keys (Komárek & Anagnostidis, 2005; Komarek & Komarkova, 2003).

2.3 Extraction of BMAA in Phytoplankton and Water Samples

Free BMAA and protein-bound BMAA were extracted from phytoplankton cells following the procedure outlined in Jiang et al. (2014) with a minor modification. Briefly, an aliquot (1 L) of phytoplankton samples was filtered through GF/C filters (Whatman GF/C Microfiber Glass Filter, Binder free), and the filters with retained cells were extracted in 15 ml of 20% methanol (methanol/water, v/v) overnight at room temperature. The filter paper was then squeezed to release toxins and attached cells, and the extract was sonicated for 3 min (70% efficiency) in an ice water bath. The extract was centrifuged for 5 min (4100 × *g*, 4 °C). The pellet was discarded, while the supernatant was combined with two volumes of cold acetone. The mixture allowed to precipitate overnight at − 20 °C and was centrifuged again (10,000 × *g*, 4 °C, 10 min). The resultant pellet containing the protein-bound BMAA fraction and the supernatant containing the free-BMAA fraction wer separated into different new tubes. The protein pellet was hydrolyzed in 1.5 ml of 6 mol L^{-1} HCl for 20 h at 110 °C and centrifuged for 5 min (10,000 × *g*,4 °C) to facilitate the release of protein-bound BMAA. The resultant supernatant containing bound BMAA was exposed to sterilized air to evaporate the organic solvent. The concentration of BMAA was then determined in the residual aqueous fraction. Extracellular dissolved BMAA was determined directly in the filtrate of the same phytoplankton samples without any further process. All samples were stored at − 20 °C until use for LC–MS/MS analysis.

2.4 2.4 Extraction of BMAA Accumulated in Vegetable Plants

Leaves and fruits of vegetable plants were washed with tap and distilled water to remove any potential contaminants that may have stuck to their surfaces. Clean leaves and fruits were ground with 20 ml of 20% methanol, and the mixture was sonicated for 3 min at 70% efficiency in an ice water bath to avoid protein degradation. The extracts were then centrifuged (4100 × *g* 4 °C) for 5 min. The pellet was discarded. The free and bound BMAA in the supernatant were separated by the same approach as was utilized to isolate BMAA from phytoplankton samples.

2.5 2.5 BMAA Analysis by LC–MS/MS

BMAA in phytoplankton and vegetable extracts was analyzed by liquid chromatography-ion trap tandem mass spectrometer (LC–MS/MS) with a Thermo Ultimate 3000 HPLC (Thermo Fisher Scientific, Bremen, Germany) coupled with an AB-Sciex Qtrap 4500 mass spectrometer (AB Sciex Pte. Ltd, Singapore) with an electrospray ionization source according to the method described in Wang et al. (2021). Briefly, BMAA toxin was separated on a SeQuant® ZIC-HILIC column (150 mm × 2.1 mm, 5 µm) maintained at 30 ℃ using a mobile phase of deionized water (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid. The gradient was run from 95 to 60% solvent B over 19 min, decreased to 40% B at 25 min, increased to 95% B at 27.01 min, and held for 2.99 min before re-equilibration for the next run. The injection volume was 5 µL, and the flow rate was 350 µL min^{-1}. The following mass spectrometry settings were made: nitrogen was used for the nebulizer and curtain gases with curtain gas pressure of 40 psi, spray voltage of 5500 V, source temperature of 350 °C, source gas 1 and 2 at a pressure of 55 psi and 50 psi, respectively, and collision cell entrance potential of 10 V. The selective reaction monitoring (SRM) mode was used to quantify BMAA with five transitions *m/z* 119 > 102, 101, 88, 56, and 44 (CE: 13, 11, 17, 24, and 24 eV, respectively). BMAA was quantified an error range of 10–15% using the 119 > 88 and confirmed by the absence of response at the *m/z* 119 > 101.The limit of detection (LOD) for BMAA was 0.01 µg g^{-1} for phytoplankton samples

and 0.015 µg g^{-1} for vegetable extracts. All chemicals such as HCl (37%) and solvents (methanol and acetonitrile, HPLC grade) were purchased from Sigma-Aldrich, Egypt. β-N-methylamino-l-alanine hydrochloride (BMAA, 98%) and 2,4-diaminobutyric acid dihydrochloride (DAB, ≥ 98%) were also obtained from Sigma-Aldrich and used as standard and internal standard, respectively. The percentage of recovery of BMAA was 88%. The comprehensive flowchart of the experimental design of toxin evaluation in irrigation water and vegetables, along with an assessment of its possible health risk, is shown in Fig. 2.

2.6 2.6 Statistical analysis

Differences in cyanobacterial abundance, environmental variables, and BMAA concentrations in phytoplankton samples and vegetables were analyzed using ANOVA ($P < 0.05$) with SPSS17 software for windows. The correlation between cyanobacterial abundance and BMAA concentrations in phytoplankton and vegetables were tested using spearman rank correlation coefficients.

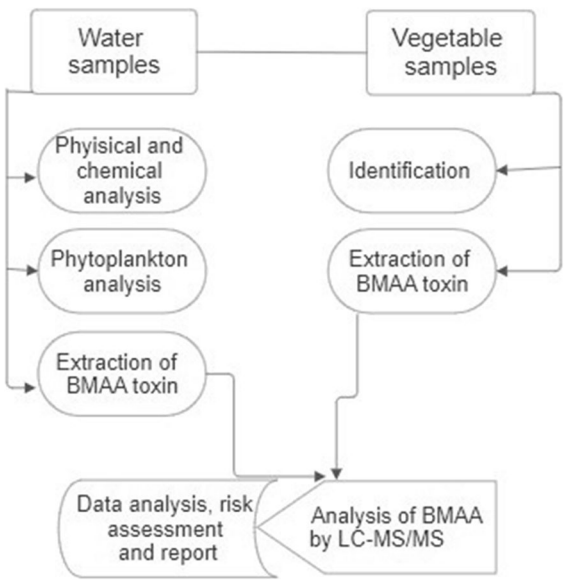

Fig. 2 Flowchart of the experimental methodology applied in the present study

3 Results

3.1 3.1. Environmental Factors and Dominance of BMAA-Producing Cyanobacteria

Table 1 summarizes the data of the physico-chemical characteristics of irrigation waters. The results showed no difference in temperature, or pH between the study sites ($P > 0.05$). On the other hand, there were significant differences ($P < 0.01$) between the sites in nutrient concentrations such as NO_3 TDS, DOM, NH_4, and PO_4. However, these parameters showed significant variation ($P < 0.05$) between summer and winter seasons. The temperature of irrigation waters at all sites ranged from15°C in winter to 32 °C in summer. The pH of irrigation waters was slightly alkaline (7.1–8.2). TDS had a range of 176–446 mg L^{-1}, which was higher in summer than winter. Nutrient concentrations also varied markedly between the two seasons ($P < 0.05$). In winter, irrigation waters had low concentrations of NO_3 (0.06–1.3 mg L^{-1}), NH_4 (0.008–0.7 mg L^{-1}), PO_4 (0.001–0.2 mg L^{-1}), and DOM (3.9–65.7 mg L^{-1}). These nutrients, on the other hand, showed higher concentrations in summer with a range of 0.8–12.5 mg L^{-1} for NO_3, 0.0–1.2 mg L^{-1} for NH_4, 0.003–2.5 mg L^{-1} for PO_4, and 0.3–160 mg L^{-1} for DOM (Table 1).

The variation in these environmental factors affected the appearance and abundance of cyanobacteria in irrigation waters. Cyanobacteria dominated phytoplankton populations in irrigation water (71–100%) during summer (Table 2), while they were recorded only at three sites: S-2, S-8, and S-19 during winter, but with low percentages (3.3, 6.2, and 9%, respectively) (Table 3). Contradictory to cyanobacteria, Chlorophyta and diatoms were found with low populations (0–25% and 0–14.5%, respectively) in irrigation canals during summer, but they dominated phytoplankton populations representing 44–98% and 2.3–56%, respectively, in different irrigation canals during winter (Table 2,3).

The results of phytoplankton analysis revealed that14 cyanobacterial species dominated phytoplankton populations and contributed largely to cyanobacterial blooms in irrigation canals during summer (Table 3). Their abundances in irrigation canals correlated positively with temperature ($r = 0.6$–0.7, $P = 0.05$), NO_3 ($r = 0.6$, $P = 0.05$), NH_4 ($r = 0.6$, $P = 0.01$), and PO_4 ($r = 0.5$, $P = 0.03$), and

Table 1 Physico-chemical properties of irrigation water at different sites during the present study. Range of values is within 99% confidence interval

Sites	Temp (°C)		pH		TDS (mg L^{-1})		NO_3^- (mg L^{-1})		NH_4^+ (mg L^{-1})		PO_4^{-3} (mg L^{-1})		DOM (mg L^{-1})	
	Su	Win	Su	Win	Su	Win	Su	Win	Su	Win	Su	Win	Su	Win
S-1	30	15	8.2	7.6	370	397	7.3	0.48	6.3	0.5	2.5	0.1	0.31	0.1
S-2	28	16	7.8	7.9	371	407	2.2	.4	1.09	0.67	0.03	0.015	8.5	3.1
S-3	30	16	7.8	7.7	251	276	1.9	1.2	0.59	0.27	0.06	0.025	20.9	12.7
S-4	26	15	7.6	7.5	347	368	1.7	1.3	0.8	0.49	0.044	0.021	72.03	65.7
S-5	28.5	16	7.1	6.9	473	492	1.3	1	0.05	0.02	0.175	0.098	56.02	40.1
S-6	29	16	6.8	7	272	289	1.6	1.1	0.09	0.06	0.006	0.002	10.02	7.1
S-7	30	15	7.3	7.1	298	315	1.9	1.3	0.95	0.25	0.072	0.014	80.8	70.6
S-8	31	15	7.2	6.8	294	299	2.5	1.2	0.15	0.06	0.008	0.004	18.06	11.4
S-9	31	16	7.4	7.1	289	321	2.1	1.4	0.86	0.39	0.149	0.05	40.3	30.7
S-10	30	17	7.3	7.1	446	461	1.9	1.2	0.13	0.07	0.002	0.001	160.4	40.5
S-11	29	15	6.9	7	267	187	1.1	0.7	0.02	0.07	0.003	0.002	8.01	3.9
S-12	28	14	7.3	7.5	290	214	3.2	1.4	1.15	0.69	1.96	0.813	10.7	5.1
S-13	26	15	7.2	7.1	217	183	1.9	1.2	0.07	0.04	0.005	0.003	60.7	34.2
S-14	26.5	16	6.8	7	199	214	1.1	0.6	0.05	0.023	0.002	0.001	24.01	11.2
S-15	29	16	7.5	7.6	377	262	1.7	1	0.04	0.013	0.165	0.078	50.02	40.1
S-16	26	15	7.1	5.8	187	124	1.4	0.8	0.02	0.008	0.113	0.005	22.9	16.7
S-17	28	16	7.6	7.8	377	332	1.8	0.7	0.26	0.012	0.007	0.002	18.06	8.1
S-18	28	17	8	8.1	192	243	0.8	0.06	0.01	0.093	0.003	0.006	18.85	23
S-19	32	18	7.6	8	356	324	12.5	0.5	5.6	0.3	2.01	0.17	0.1	0.05
S-20	26	16	8.1	7.9	367	388	1.9	0.9	0.148	0.0841	0.05	0.036	24.4	10.4
S-21	29	17	7.8	7.2	248	223	1.8	1.1	0.568	0.068	0.065	0.008	16.8	7.5
S-22	27.5	17	7.7	7.4	248	172	1.3	0.7	0.031	0.024	0.003	0.002	32.5	24.3

Su summer, *Win* winter

negatively with TDS ($r = -0.7$, $P = 0.01$). *Nostoc commune* was an exception, as it had negative correlation with NO_3 ($r = -0.6$, $P = 0.05$). However, some species constituting the blooms were site-specific and found as unique blooms to a certain irrigation canal. For instance, *N. commune* bloom was found only in irrigation water at site 4(S-4). On the other hand, some cyanobacterial blooms consisted of more than one species, for example D. *lemermmanni*, *M. aeruginosa*, and *R. raciborskii* constituted the bloom in site 1 (S-1), while D. *lemermmanni* and *Pl. contorta* constituted the cyanobacterial bloom at site 5(S-5). Moreover, other cyanobacterial species contributed to the blooms in several irrigation canals, including *P. autumnale*, which has appeared at all sites except sites 1, 4, and 19 (Table 2). In winter, cyanobacteria were found only in three sites (S-2, S-8, S-19) and represented only by two species (*Plx. rubescens* and *Synechococcus elongatus)* (Table 3).

3.2 3.2. BMAA Concentrations in Irrigation Waters

Comparison of LC–MS/MS chromatograms presented in Fig. S1 (supplementary materials) clearly revealed that the peaks detected in phytoplankton and vegetable extracts completely matched those of BMAA standard at different transitions *m/z* 119 > 102, 101, 88, 56, and 44, verifying the existence of this toxin in these samples. In the current study, BMAA toxin extracted from phytoplankton cells was found in free and protein-bound forms.

Concentrations of free BMAA (0.6–11.4 µg L^{-1}) were higher than protein-bound BMAA (0.01–3.3 µg L^{-1}) with a ratio ranging from 6:1 to 3:1 for all phytoplankton samples collected from different irrigation water sites (Fig. 3). These concentrations varied significantly ($P < 0.05$) among different sites, and significantly correlated ($r = 0.9$) with the cell density of dominant cyanobacterial species at relevant irrigation water site. Extracellular dissolved BMAA was also

Table 2 Cell density of dominant cyanobacterial species (cells × 10^4 mL^{-1}) and total count of Chlorophyta and diatoms recorded at different sites in irrigation canals during summer 2021. Range of values is within 99% confidence interval

Phytoplankton	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15	S-16	S-17	S-18	S-19	S-20	S-21	S-22
A. platensis	-	0.22	-	-	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	0.5	0.2
Aph. planctonica	-	-	-	-	-	-	-	-	-	-	-	-	8.3	-	-	-	-	-	-	-	-	-
C. stagnale	-	-	-	-	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-	7.2	-	-	-
Ch. minutus	-	-	-	-	-	-	-	-	-	0.4	2.1	-	-	-	-	-	-	-	-	0.43	-	-
D. lemermmanni	5.2	10.6	271	-	38	-	-	7.2	-	-	-	-	-	-	-	-	4.3	0	9.2	-	-	-
M. aeruginosa	1.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	-	-	-
Mr. glauca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.53	-	-	-
N. commune	-	-	-	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. autumnale	-	519	63.7	-	-	94.3	290	138	153.3	125	173.3	484	52.7	5.1	24.3	9.3	10.3	3.4	-	24.5	4.2	2.3
Pl. contorta	-	-	-	-	113.3	-	-	-	-	-	-	-	-	18.3	0	0	6.3	-	-	-	-	-
Plx. agardhii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.53	-	-	0.72
Plx. rubescens	-	-	-	-	-	-	-	-	33.3	-	-	-	-	-	-	-	-	-	-	-	-	11.3
R. raciborskii	1.1	1.51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. elongatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total cyanobacteria	7.85	531.3	334.7	24	151.3	94.3	290	145.2	186.6	125.4	175.4	485.5	61	23.4	24.3	9.3	20.9	3.4	69.16	24.9	10.7	14.52
Total Chlorophyta	4.7	0.3	4.9	4.6	0.6	0.4	-	0.7	5.1	-	2	0.17	2	6	0	2	0.15	-	5	-	1.4	3.1
Total diatoms	0.6	0.25	0.4	0.25	1	0.3	2	-	1.6	0.2	0.15	5	-	-	0.8	-	1	0.1	0.5	-	4	6.3
Total phytoplankton	18.45	532.4	345.3	57.7	267.8	95.7	294	146.6	233.3	126.2	181.8	495.8	63	53.7	25.9	13.3	29.5	3.6	100.9	25.36	27.5	45.34
% Cyanobacteria	71.3	99.9	98.5	91.6	99.4	99.3	99.3	99.5	97.1	99.8	98.8	98.9	96.8	88.8	96.9	84.9	96.1	97.2	94.6	100	80.4	79.3

The uppercase letter in the name of cyanobacteria refers to the genus name as follows: *A*, *Arthrospira*; *Aph*, *Aphanocapsa*; *C*, *Cylindrospermum*; *Ch*, *Chroococcus*; *D*, *Dolichospermum*; *M*, *Microcystis*; *Mr*, *Merismopedia*; *N*, *Nostoc*; *O*, *Oscillatoria*; *P*, *Phormidium*; *Pl*, *Planktolyngbya*; *Plx*, *Planktothrix*; *R*, *Raphidiopsis*; *S*, *Synechocystis*

Table 3 Cell density of dominant cyanobacterial species (cells × 10^4 mL^{-1}) and total count of Chlorophyta and diatoms recorded at different sites in irrigation canals during winter 2022. Range of values is within 99% confidence interval

Phytoplankton	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15	S-16	S-17	S-18	S-19	S-20	S-21	S-22
Plx. rubescens	-	4.3	-	-	-	-	-	6.7	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-
S. elongatus	-	0.23	-	-	-	-	-	0.45	-	-	-	-	-	-	-	-	-	-	0.16	-	-	-
Total cyanobacteria	-	4.6	-	-	-	-	-	7.2	-	-	-	-	-	-	-	-	-	-	0.26	-	-	-
Total Chlorophyta	20	37	17	67	50	30	32	40	29	54	42	38	77	87	10	46	39	0.5	21	38	83	9
Total diatoms	21	7	22	3	1.3	4	3.9	5	6	8	7	7.7	1	2.1	1	1.9	4.8	0.3	2.6	5.8	2	1.4
Total phytoplankton	41	48.6	39	70	51.3	34	35.9	52.2	35	62	49	45.7	78	89.1	11	47.9	43.8	0.8	23.9	43.8	85	10.4
% cyanobacteria	0	3.3	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	6.15	0	0	0

The uppercase letter in the name of cyanobacteria refers to the genus name as follows: *Plx*, *Planktothrix*; *S*, *Synechocystis*

detected in cell-free irrigation water, but with very low concentrations (0.1–0.2 μg L^{-1}) (Fig. 3). These extracellular BMAA concentrations varied significantly among irrigation water sites ($P < 0.05$), associating with concentrations of free BMAA detected in phytoplankton cells ($r = 0.9$). On the other hand, irrigation water samples at sites not-containing cyanobacteria during winter (i.e., S-2, S-8, S-19) did not have any detectable levels of BMAA. Nevertheless, low concentrations of free (0.2–1.3 μg L^{-1}), bound (0.06–0.17 μg L^{-1}), and dissolved (0.04–0.1 μg L^{-1}) BMAA were found in sites containing only the cyanobacteria, *Plx. rubescens* and *S. elongatus* during winter, indicating the involvement of these species in the production of BMAA toxin in irrigation water.

3.3 3.3. BMAA Concentrations in Vegetable Plants

The data in Table 4 showed the accumulation of BMAA toxin in edible parts (leaves or fruits) of 9 vegetable plants (green pepper, Lettuce, mulukhiyah, pea, radish, spinach, tomato, watercress, and zucchini) collected from farmlands irrigated with water containing BMAA-producing cyanobacteria. Results showed that BMAA was only found in protein-bound form in all vegetable species, except for zucchini, which had small amounts of free BMAA besides the bound form. BMAA concentrations in edible parts of the same plant varied considerably ($P < 0.05$) between the collection sites (i.e., the irrigation water source), and this variation was correlated with the amount of free and dissolved BMAA found in the irrigation water ($r = 0.8$–0.9). For instance, watercress leaves from farmland that had been irrigated with water from site 18 containing high levels of free (2.6 μg L^{-1}) and dissolved (0.2 μg L^{-1}) BMAA had a high concentration of BMAA (6.7 μg g^{-1} fresh weight), while watercress leaves from farmland irrigated with water from site 8 containing low levels of free and dissolved BMAA (0.86 and 0.16 μg L^{-1}, respectively) had lower concentrations of BMAA (3.9 μg g^{-1} fresh weight).

Similar results were observed with tomato fruits; the amount of BMAA that accumulated in the fruits increased (from 0.34 to 5.9 μg g^{-1} fresh weight) in proportion to the amounts of free (1–2.7 μg L^{-1}) and dissolved (0.08–0.16 μg L^{-1}) BMAA that were found in the relevant irrigation water sites (Table 4 and Fig. 2).

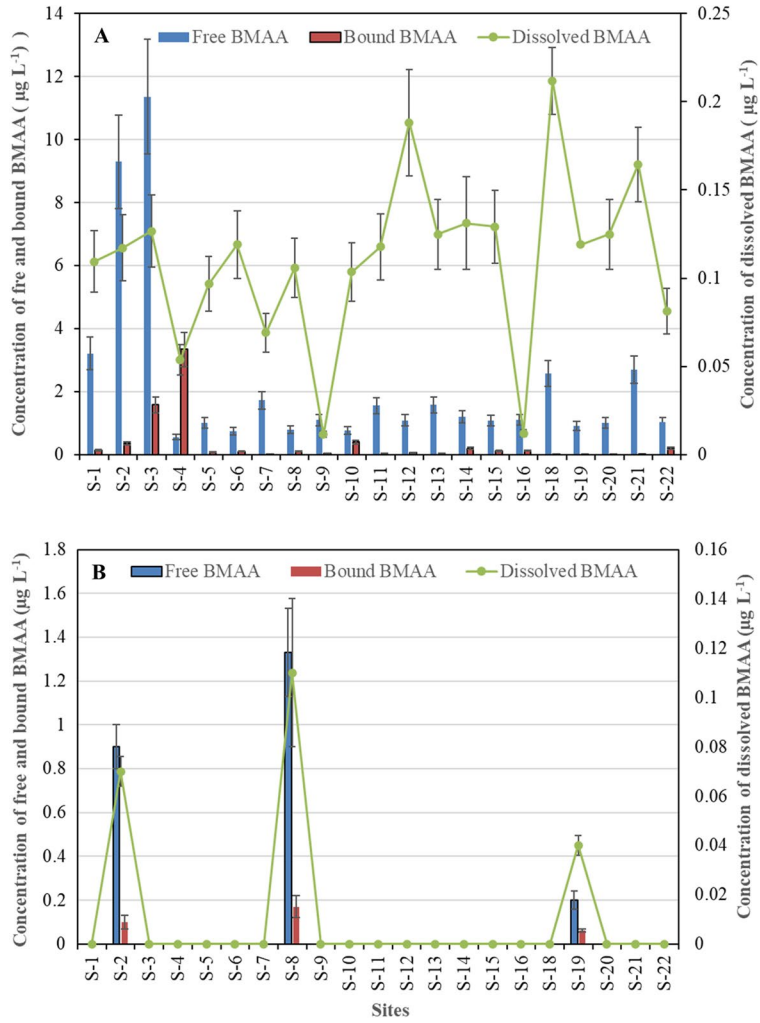

Fig. 3 Concentrations of free and protein-bound BMAA in phytoplankton samples (µg L^{-1}) and dissolved BMAA in cell-free irrigation water (µg L^{-1}) at different sites surveyed during the present study. Values are expressed as mean ± SD. Difference is significant at $P < 0.05$

What is noteworthy here in the present study is the absence of BMAA toxin in spinach and lettuce plants (vegetables growing during winter in Egypt), in concomitance with the absence of BMAA toxin in irrigation waters at relevant sites (S-5 and S-11, respectively) due to the nonexistence of cyanobacteria during winter (Table 3 and Table 4). Nonetheless, when BMAA toxin was detected in some irrigation water sources such as S-19 and S-8 (0.2 and 1.33 µg L^{-1}, respectively) during winter, vegetable plants such as pea and zucchini receiving water from these sites were found to accumulate this toxin (0.05 and µg g^{-1} fresh weight, respectively) in their fruits.

4 Discussion

4.1 3.1. Cyanobacteria and BMAA Concentrations in Irrigation Waters

In the present study, irrigation water was characterized by considerable levels of nutrients (NO_3, PO_4 and DOC) as well as suitable temperature (> 25 °C) and pH (> 7) that promoted the growth of cyanobacterial species (Reynolds, 2006) in these irrigation canals during summer. In previous study by Hai et al. (2019), cyanobacteria were found to dominate phytoplankton populations in various Chinese lakes when NO_3 and PO_4 concentrations were in the range of 0.29–1.92 and

Table 4 BMAA concentrations (μg g^{-1} fresh weight) measured in vegetable plants collected from farmlands irrigated with cyanobacteria-containing water in Sohag, Egypt, during winter and summer 2022

Plants	Site of collection	Season of collection	BMAA concentration (μg g^{-1} FW)	
			Free	Bound
Green pepper	Site 1	Summer	ND	1.1 ± 0.4
	Site 7	Summer	ND	1.5 ± 0.4
	Site 21	Summer	ND	1.4 ± 0.3
Lettuce	Site11	Winter	ND	ND
Mulukhiyah	Site 2	Summer	ND	0.35 ± 0.07
	Site 3	Summer	ND	0.7 ± 0.2
	Site 4	Summer	ND	0.4 ± 0.06
Pea	Site 19	Winter	ND	0.05 ± 0.008
Radish leaves	Site9	Summer	ND	0.83 ± 0.2
	Site19	Summer	ND	0.61 ± 0.1
Radish roots	Site9	Summer	ND	0.31 ± 0.07
	Site19	Summer	ND	0.2 ± 0.05
Spinach	Site 5	Winter	ND	ND
Tomato	Site 14	Summer	ND	0.93 ± 0.1
	Site 21	Summer	ND	5.9 ± 1.1
	Site 22	Summer	ND	0.34 ± 0.06
Watercress	Site 8	Summer	ND	1.9 ± 0.4
	Site 11	Summer	ND	5.1 ± 1.2
	Site 12	Summer	ND	4.7 ± 0.6
	site 13	Summer	ND	4.98 ± 1.1
	Site 18	Summer	ND	6.9 ± 1.3
Spinach	Site 5	Winter	ND	ND
Zucchini	Site 8	Winter	1.23 ± 0.2	7.7 ± 1.3

0.02–1.05 mg L^{-1}. The NO_3 and PO_4 concentrations found in in our irrigation water canals are within these ranges. In our study, cyanobacterial blooms covering irrigation water sources during summer were replaced by Chlorophyta and diatoms in winter, concurrent with the decline in water temperature. This shift in phytoplankton composition appears to be related to water temperature and supports the findings of numerous earlier field studies stating that water temperature is a key factor for the growth rate and proliferation of phytoplankton in freshwater environments (Huang et al., 2018). In this regard, it has been noted that high water temperature favors cyanobacteria by maximizing their growth rates over other phytoplankton in aquatic habitats where nitrogen and phosphorus are non-limiting (Lürling et al., 2018). Other research showed that the two main determinants of the biomass and abundance of phytoplankton groups in water bodies are temperature and nutrients, especially nitrogen and phosphorus (Lv et al., 2014; O'Neil et al., 2012). However, several cyanobacterial species exhibit a distinct competition advantage over other eukaryotic algae (i.e., with eukaryotic nucleus such as green algae, dinoflagellates, and diatoms) during the high-temperature period, and when water temperature drops, water bodies experience the dominance of Chlorophyta and diatom species (Grime & Pierce, 2012; Lv et al., 2014). This suggests that cyanobacterial blooms in water sources would spread more widely, last longer, and be more intense in the future as a result of climate change brought on by rising water temperatures and high nutrient concentrations (i.e., eutrophication) (Elliott, 2012). Strictly speaking, cyanobacteria benefit largely from human impact through the interaction of rising temperature and increased nutrient loading. Hence, reduction of nutrient loading into aquatic ecosystems is the most practical and realistic approach for controlling cyanobacterial blooms.

The BMAA toxin was found with high concentrations in phytoplankton samples gathered from various irrigation water sites in the current study. This is the first report of the presence of BMAA toxin in water sources in Egypt. These concentrations correlated with the abundance of cyanobacteria rather than green algae and diatoms found in phytoplankton samples. This was manifested by the presence of BMAA toxin in phytoplankton samples collected from the irrigation sites containing cyanobacteria (S-2, S-8, and S-19) during winter, and the absence of the toxin in the remaining sites, which lacked cyanobacteria but had a sizable biomass of green algae and diatoms at that time. This finding supports the hypothesis that Cyanobacteria are the main BMAA producers (Lopicic et al., 2022, and the references therein). Other researchers, however, reported the capability of some species of dinoflagellates and diatoms of BMAA production (Jiang et al., 2014; Main et al., 2017; Reveillon et al., 2016; Violi et al., 2019a, 2019b).

Here in our study, despite our phytoplankton samples contained diatoms, they did not show any detectable levels of BMAA. This may indicate that the diatom species in found in phytoplankton samples are unable to produce this toxin. In this respect, it has been stated that not all algal species are toxic, but there exist genetic variants that do not produce BMAA toxin (Violi et al., 2019a, 2019b). Specifically, the presence of BMAA in phytoplankton samples was associated with one or more of 14 cyanobacterial species that dominated the phytoplankton populations in the irrigation canals surveyed during the current study (Table 2). This supports the likelihood that these cyanobacterial species are involved in BMAA production. Five of these species (*Aph. planctonica*, *Ch. minutus*, *D. lemmermanni*, *N. commune*, and *O. tenuis*) were previously isolated from the same irrigation canals and have been reported as BMAA producers in a parallel study by our team (under publication). Other 5 cyanobacterial species including *Merismopedia glauca*, *Microcystis aeruginosa*, *Planktolyngbya contorta*, *Raphidiopsis raciborskii*, and *Synechococcus elongatus* were recently linked to BMAA production in a tropical Egyptian fishpond (Mohamed et al., 2023). Additionally, *Dolichospermum planctonica*, *M. aeruginosa*, *Merismopedia* sp., and *Raphidiopsis raciborskii* from Australian lakes (Cox et al., 2005; Main et al., 2017, Violi et al., 2019a, 2019b), *Pl. agardhii* from British and Dutch waterbodies (Faassen et al., 2009a, 2009b; Metcalf, et al., 2008), and *Synechococcus elongatus* from Portuguese and French waters (Cervantes Cianca et al., 2012; Réveillon et al., 2014) were all implicated in BMAA production. Furthermore, BMAA has also been found in food supplements made of biomass of *Arthrospira platensis* (McCarron et al., 2014) or *A. fusiformis* (Lage et al., 2015). These findings support the hypothesis that BMAA can be produced by diverse taxa belonging to all five known morphological sections of cyanobacteria (Cox et al., 2005; Lopicic et al., 2022; Nunes-Costa et al., 2020).

In our study, both free and protein-binding forms of the BMAA toxin were found in phytoplankton samples, with larger quantities of the free form than the bound one. Our results are thus consistent with earlier studies, which found that phytoplankton samples had a higher proportion of free BMAA than protein-bound BMAA (Faassen et al., 2009a, 2009b, 2016; Blaszczyk et al., 2021). The finding of high concentrations of free BMAA in phytoplankton samples is particularly worrisome because BMAA can release more readily from cells into the water during natural senescence or the use of algaecides. This raises the toxin burden in irrigation water (i.e., extracellular dissolved toxin), which can then transfer to plants and have a negative impact on crop productivity and quality (Contardo-Jara et al., 2014, 2018; Li et al., 2019). Therefore, determining this free fraction of BMMA is crucial when cyanotoxins are being monitored in irrigation and drinking waters. In the present study, the quantities of free BMAA found in phytoplankton samples collected from irrigation waters (0.6–11.4 μg L^{-1}) are within the range found in other investigations of freshwater bodies.

According to these studies, BMAA concentrations in phytoplankton samples from freshwater lakes ranged from 0.11–0.3 μg L^{-1} in Canada (Roy-Lachapelle et al., 2015; Vo Duy et al., 2019) to 25.3 μg L^{-1} in the USA (Al-Sammak et al., 2014). In contrast, neither free nor protein-bound BMAA was found in phytoplankton samples from freshwater lakes dominated by cyanobacteria in China (Fan et al., 2015). Additionally, a recent study conducted by Abbes et al. (2022) found no BMAA in any water sample of lakes and reservoirs plagued with cyanobacterial blooms in Brazil, Canada, France, Mexico, and the UK. This discrepancy may be due to the

different cell densities of BMAA-producing organisms in the environment as well as the diverse capacity of species/strains to synthesize BMMA (Lance et al., 2018).

4.2 3.2. Accumulation of BMAA Toxin in Vegetable Plants

Water used for irrigation that contains BMAA may help the toxin bioaccumulate in plants and their edible parts (e.g., fruits, seeds, leaves, roots). Therefore, plants irrigated with BMAA-containing water could provide an additional route (besides drinking water) via consumption of contaminated edible plant parts. In the current study, it was found that 9 vegetable plants (green pepper, lettuce, mulukhiyah, pea, radish, spinach, tomato, and watercress) harvested from farmlands where water contaminated with BMAA was used for irrigation accumulated BMAA in their edible parts (leaves and fruits) at levels ranging from 0.05 to 6.7 μg g^{-1} fresh weight. This clearly indicates that these plants have taken up BMAA toxin from these irrigation waters.

In our study, the quantities of BMAA detected in edible parts of a similar plant varied significantly between collecting sites (i.e., farmlands), in association with free and extracellular dissolved BMAA concentrations in these sites. This agrees with the results of Niyonzima (2010) reporting that the uptake and bioaccumulation of BMAA in *N. officinale* and *D. carota* was dependent on BMAA concentration in irrigation water. In contrast, no measurable levels of BMAA were found in vegetable plants (spinach and lettuce) that had received water from irrigation water sites (S-5 and S-11) that were known to be devoid of BMAA toxin and its cyanobacterial producers during winter. Meanwhile, significant proportions of BMAA were detected (0.05 and 1.23 μg g^{-1} fresh weight, respectively) in the tissues of the winter crops (pea and zucchini) irrigated with water from sites 19 and 8 that contained this toxin during winter. This finding proves that the irrigation water is the main source of BMAA in vegetable plants.

In comparison, watercress leaves contained the highest BMAA concentration (6.7 μg g^{-1} FW) among the leafy vegetable plants, followed by radish leaves (0.83 μg g^{-1} FW) and mulukhiyah leaves (0.7 μg g^{-1} FW). For fruity vegetable plants, zucchini fruits accumulated higher amounts of BMAA (7.7 μg g^{-1} FW) followed by tomato (5.9 μg g^{-1} FW), green pepper (1.4 μg g^{-1} FW), and pea (0.05 μg g^{-1} FW). The variation in the quantities of BMMA found in edible parts of different plants may be due to the varied plant species with varying capacity for toxin accumulation in their parts (Weralupitiya et al., 2022).

The uptake of BMAA has been demonstrated in earlier studies in some vegetable plants, including watercress (*Nasturtium officinale*) and wild carrot (*Daucus carota*) (Niyonzima, 2010), Lettuce (*Lactuca sativa*) and onion (*Allium fistulosum*) (Esterhuizen-Londt & Pflugmacher, 2019), Chinese cabbage (Li et al., 2019), and radish leaves (4.7 μg g^{-1}) (Ewert, 2021). However, direct comparisons of BMAA concentrations accumulated in plants between previous reports, and the results of our study are difficult due to differences in reported units (dry and fresh weight, respectively) and plant types. Another difference is that most of previous studies were conducted in laboratory experiments and hydroponics compared to our study carried out in farmlands under field circumstances. In the soil-based system, BMAA can bind to soil particles, which would reduce the potential for plants to uptake BMAA from the soil leading to low accumulation of this toxin in plant parts (Contardo-Jara et al., 2018; Esterhuizen-Londt & Pflugmacher, 2019).

The obvious likeness between the current study and earlier ones is that the protein-bound fraction of BMAA accumulated in edible parts of vegetable plants was more than the free form (Esterhuizen-Londt & Pflugmacher, 2019; Li et al., 2019; Niyonzima, 2010).

This can be explained by the hydrophilic nature of BMAA, which enables it to enter plants through their vascular system and transport to other parts in xylem sap (Näsholm et al., 2009), where free BMAA quickly binds to proteins to form protein-bound BMAA.

However, the detection of small amounts of free BMAA in zucchini fruits as an exception may be explained by the fact that BMAA binds to receptor molecules until saturation and the remaining unbound BMAA molecules remains in the free form, as suggested by Li et al. (2019) for the presence of free BMAA in Chinese cabbage.

Noticeably, the vegetable plants surveyed during the present study appeared healthy (data not shown),

despite we did not investigate the morphological alterations in these plants because that was not the purpose of our study. However, other studies found no morphological changes in plants that were either acutely (Niyonzima, 2010) or chronically (Contardo-Jara et al., 2018; Esterhuizen-Londt & Pflugmacher, 2019) exposed to the BMAA toxin. Nevertheless, further research is needed to determine the effects of BMAA toxin at environmentally relevant concentrations (i.e., in irrigation water) on the morphology and ultrastructure of these plants using scanning electron microscopy of the full-surface metal coating.

4.3 3.3. Risk Assessment

Taken that all vegetable plants surveyed in the present study are utilized for human consumption, the presence of BMAA in their edible parts (e.g., leaves and fruits) may pose a risk to human health, even the toxin is found in protein-bound form. In this regard, it has been proposed that protein-bound BMAA may serve as an endogenous reservoir that, during protein metabolism, gradually releases the toxin in the free form into the cerebral tissues (Murch et al., 2004). There is currently no recommended value for BMAA in food or water international organizations (such as the WHO). However, Wu et al. (2019) derived a guideline value (7.2 and 1.8 μg g^{-1} DW for adults, children, respectively) to assess the noncarcinogenic health risk of BMAA in aquatic products based on NOAEL (no observed adverse effect level) value of 40 mg kg^{-1} bw d^{-1} from the toxicity test data of BMAA for male cynomolgus monkeys published in Karlsson et al. (2013) and an uncertainty factor (UF) set as 1000. Those authors estimated a reference dose-RfD of BMAA (40 μg kg^{-1} d^{-1}) by dividing NOAEL by UF and used this RfD to derive guideline value (GV) according to the following equation:

$$GV = \frac{RfDxBWxAF}{FIR} \quad (1)$$

Here in our study, we applied this Eq. (1), but AF and FIR values were modified based on the Egyptian national food consumption conditions, where these parameters may vary according to the country traditions for the consumption of each comestible (bread, vegetable, fruit …etc.).

where GV is the recommended limit for BMAA (μg g^{-1}), AF is the empirical coefficient, with value set as 0.38, assuming that vegetables constitute 30% of human food, and the oral bioavailability of BMAA is 80% as reported by Duncan et al. (1992) (i.e., $AF = 0.3 \div 0.8$).

FIR is the food intake rate, specifically the daily amount of vegetable consumed by human (100 and 200 g d^{-1} for children and adults, respectively). For African and Middle East countries, FIR is 200 g for adults and 100 g for children of vegetables per day. BW is the average body weight (60 kg for adults and 15 kg for children). By applying the equation above, GV of BMAA for adults with 60 kg body weight would be 4.5 μg g^{-1} and 2.2 μg g^{-1} for children with 15 kg body weight. BMAA concentrations found in watercress leaves (4.7–6.9 μg g^{-1} FW) and tomato (5.9 μg g^{-1} FW) zucchini (7.7 μg g^{-1} FW) fruits in our study exceeded these proposed GV values for both adults and children, while BMAA concentrations in other vegetables were below these GVs (Table 4). This suggests that long-term consumption of watercress, tomato, and zucchini with toxin levels surpassing the proposed GV may threaten human health. On the other hand, although BMAA levels in green pepper, lettuce, mulukhiyah, pea, radish, and spinach did not go above the suggested GVs, these levels could rise with an increase in the BMAA concentration in irrigation water. Therefore, these vegetable plants should be regularly monitored for the presence of BMAA toxin before delivering to the market.

5 Conclusions

The present study demonstrated the presence of BMAA toxin and its cyanobacterial producers in irrigation water sources in Egypt. A probable transfer of BMAA from irrigation water to vegetable plants and accumulation in their edible parts was also reported in the study.

The concentrations of BMAA in irrigation water mainly linked to the cell density of the most dominant cyanobacterial species. Furthermore, BMAA was also found in protein-bound form in leaves edible portions of vegetable plants collected from farmlands utilizing BMAA-containing irrigation water. The highest BMAA concentrations were detected in watercress leaves and zucchini and

tomato fruits, whereas the lowest levels were found in radish leaves and pea fruits.

The BMAA concentrations in watercress leaves, and zucchini and tomato fruits exceeded the proposed GVs for children and adults (2.2 and 4.5 μg g^{-1}, respectively) of this toxin, which were derived during our study based on the Egyptian national food consumption conditions and the reference dose-RfD estimated by Wu et al. (2019) for noncarcinogenic health risk assessment of BMAA. The study suggests that irrigation water sources worldwide should be shielded from urban and agricultural runoff to limit the proliferation of toxin-producing cyanobacteria. In addition, it is important to constantly check for the presence of cyanotoxins in irrigation water and crop plants to protect the public from exposure to dangerous toxins through consuming food.

Author Contribution All authors contributed to the study design. HB and ZM collected samples and cultured cyanobacterial species. RE, SA, and MH made LC–MS/MS analysis and interpreted the related data. AC and VV made data analysis. The first draft of the manuscript was written by ZM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors would like to thank the Deanship of Scientific Research, King Khalid University for funding this work through research groups program under grant number R.G.P. 2/75/44. The authors declare that funding received from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 823860 were used in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Consent for Publication All authors have consented to publish this manuscript.

Competing Interests The authors declare no competing interests.

References

Abbes, S., Vo Duy, S., Munoz, G., Dinh, Q. T., Simon, D. F., Husk, B., Baulch, H. M., Vinçon-Leite, B., Fortin, N., & Greer, C. W. (2022). Occurrence of BMAA isomers in bloom-impacted lakes and reservoirs of Brazil, Canada, France, Mexico, and the United Kingdom. *Toxins, 14*, 251. https://doi.org/10.3390/toxins1404025

Al-Sammak, M. A., Hoagland, K. D., Cassada, D., & Snow, D. D. (2014). Co-occurrence of the cyanotoxins BMAA, DABA and anatoxin-a in Nebraska reservoirs, fish, and aquatic plants. *Toxins, 6*, 488–508. https://doi.org/10.3390/toxins6020488

APHA (American Public Health Association). (1995). *Standard methods for the examination of water and wastewater* (19th ed.). APHA.

Blaszczyk, A., Siedlecka-Kroplewska, K., Wozniak, M., & Mazur-Marzec, H. (2021). Presence of ß-N-methylamino-L-alanine in cyanobacteria and aquatic organisms from waters of Northern Poland. *BMAA Toxicity Studies. Toxicon, 194*, 90. https://doi.org/10.1016/j.toxicon.2021.02.007

Brand, L. E., Pablo, J., Compton, A., Hammerschlag, N., & Mash, D. C. (2010). Cyanobacterial blooms and the occurrence of the neurotoxin beta-N- methylamino-L-alanine (BMAA) in South Florida aquatic food webs. *Harmful Algae, 9*, 620–635. https://doi.org/10.1016/j.hal.2010.05.002

Buratti, F. M., Manganell, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., & Funari, E.l. (2017). Cyanotoxins: Producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology, 91*, 1049–1130. https://doi.org/10.1007/s00204-016-1913-6

Cervantes Cianca, R. C., Baptista, M. S., Lopes, V. R., & Vasconcelos, V. M. (2012). The non-protein amino acid ß-N-methylamino-L-alanine in Portuguese cyanobacterial isolates. *Amino Acids, 42*, 2473–2479. https://doi.org/10.1007/s00726-011-1057-1

Contardo-Jara, V., Schwanemann, T., & Pflugmacher, S. (2014). Uptake of a cyanotoxin, β–N-methylamino-L-alanine, by wheat (Triticum aestivum). *Ecotoxicology and Environmental Safety, 104*, 1271e31. https://doi.org/10.1016/j.ecoenv.2014.01.039

Contardo-Jara, V., Schwanemann, T., Esterhuizen-Londt, M., & Pflugmacher, S. (2018).

Cox, P. A., Banack, S. A., Murch, S. J., Rasmussen, U., Tien, G., Bidigare, R. R., Metcalf, J. S., Morrison, L. F., Codd, G. A., & Bergman, B. (2005). Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. *PNAS, 102*, 5074–5078. https://doi.org/10.1073/pnas.050152610

Cox, P. A., Davis, D. A., Mash, D. C., Metcalf, J. S., & Banack, S. A. (2016). Dietary exposure to an environmental toxin triggers neurofibrillary tangles and amyloid deposits in the brain. *Proceedings of the Biological Sciences, 283*, 1823. https://doi.org/10.1098/rspb.2015.2397

Duncan, M. W., Markey, S. P., Weick, B. G., Pearson, P. G., Ziffer, H., Hu, Y., & Kopin, I. J. (1992). 2-Amino-3-(methylamino)propanoic acid (BMAA) bioavailability in

the primate. *Neurobiology of Aging, 13*, 333–337. https://doi.org/10.1016/0197-4580(92)90047-2

Elliott, J. A. (2012). Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. *Water Research, 46*, 1364–1371. https://doi.org/10.1016/j.watres.2011.12.018

Esterhuizen-Londt, M., & Pflugmacher, S. (2019). Vegetables cultivated with exposure to pure and naturally occurring β-N-methylamino-Lalanine (BMAA) via irrigation. *Environmental Research, 169*, 357–361. https://doi.org/10.1016/j.envres.2018.11.030

Ewert, A. C. (2021). Accumulation of cyanobacteria toxins in lettuce and radishes exposed to water and aerosols from a low-toxin lake. Master's Theses and Capstones. 1457. https://scholars.unh.edu/thesis/1457

Faassen, E. J., Gillissen, F., Zweers, H. A., & Lürling, M. (2009a). Determination of the neurotoxins BMAA (ß-N-methylamino-L-alanine) and DAB (alpha-, gamma-diaminobutyric acid) by LC-MSMS in Dutch urban waters with cyanobacterial blooms. *Amyotrophic Lateral Sclerosis, 2*, 79–84. https://doi.org/10.3109/17482960903272967

Faassen, E. J., Gillissen, F., Zweers, H. A. J., & Lurling, M. (2009b). Determination of the neurotoxins Bmaa (β-N-methylamino-L-alanine) and Dab (α-, γ-diaminobutyric acid) by LC-MSMS in Dutch urban waters with cyanobacterial blooms. *Amyotrophic Lateral Sclerosis, 10*(2), 79–84. https://doi.org/10.3109/17482960903272967

Faassen, E., Antoniou, M., Beekman-Lukassen, W., Blahova, L., Chernova, E., Christophoridis, C., Combes, A., Edwards, C., Fastner, J., & Harmsen, J. (2016). A collaborative evaluation of LC-MS/MS based methods for BMAA analysis: Soluble bound BMAA found to be an important fraction. *Marine Drugs, 14*, 45. https://doi.org/10.3390/md14030045

Fan, H., Qiu, J., Fan, L., & Li, A. (2015). Effects of growth conditions on the production of neurotoxin 2,4-diaminobutyric acid (Dab) in Microcystis aeruginosa and its universal presence in diverse cyanobacteria isolated from freshwater in China. *Environmental Science and Pollution Research, 22*, 5943–5951. https://doi.org/10.1007/s11356-014-3766-y

Grime, J.P., & Pierce S. (2012) . The evolutionary strategies that shape ecosystems. Wiley-Blackwell, Chichester, UK.

Hai, X., Jie, C., Guangwei, Z., Boqiang, Q., & Yunlin, Z. (2019). Effects of nitrogen and phosphorus nutrient levels on the formation of cyanobacteria dominance. *Lake Science, 31*, 1239–1247. https://doi.org/10.18307/2019.0518

Huang, G., Wang, X., Chen, Y., Xu, L., & Xu, D. (2018). Seasonal succession of phytoplankton functional groups in a reservoir in central China. *Fundamental and Applied Limnology, 192*, 1–14. https://doi.org/10.3390/w12041167

Jiang, L., Kiselova, N., Rosén, J., & Ilag, L. L. (2014). Quantification of neurotoxin BMAA (β-N-methylamino-L-alanine) in seafood from Swedish markets. *Scientific Reports, 4*, 06931. https://doi.org/10.1038/srep06931

Karlsson, O., Kultima, K., & Wadensten, H. (2013). Neurotoxin-induced neuropeptide perturbations in striatum of neonatal rats. *Journal of Proteome Research, 12*, 1678e1690. https://doi.org/10.1021/pr3010265

Koksharova, O. A., & Safronova, N. A. (2022). Non-proteinogenic amino acid β-N-methylamino-L alanine (BMAA): Bioactivity and ecological significance. *Toxins, 14*, 539. https://doi.org/10.3390/toxins14080539

Komárek, J., & Anagnostidis. K. (2005). Cyanoprokaryota. 2. Teil: Oscillatoriales. Süßwasserflora von Mitteleuropa; Band 19/2. Elsevier, Műnchen 759.

Komarek, J., & Komarkova, J. (2003). Phenotype diversity of the cyanoprocaryotic genus Cylindrospermopsis (Nostocales); review 2002. *Czech Phycology,* 3,1–30. https://fottea.czechphycology.cz › fot › 2003/01

Korn, A., Höfling, C., Zeitschel, U., Krueger, M., Roßner, S., & Huster, D. (2020). Incorporation of the nonproteinogenic amino acid β-methylamino-alanine affects amyloid β fibril properties and toxicity. *ACS Chemical Neuroscience, 11*, 1038–1047. https://doi.org/10.1021/acschemneuro.9b00660

Lage, S., Burian, A., Rasmussen, U., Costa, P. R., Annadotter, H., Godhe, A., & Rydberg, S. (2015). BMAA extraction of cyanobacteria samples: Which method to choose? *Environmental Science and Pollution Research, 23*, 338–350. https://doi.org/10.1007/s11356-015-5266-0

Lance, E., Arnich, N., Maignien, T., & Biré, R. (2018). Occurrence of β-N-methylamino-L-alanine (BMAA) and isomers in aquatic environments and aquatic food sources for humans. *Toxins, 10*, 83. https://doi.org/10.3390/toxins10020083

Li, B., Yu, S., Li, G., Chen, X., Huang, M., Liao, X., Li, H., Hu, F., & Wu, J. (2019). Transfer of a cyanobacterial neurotoxin, β-methylamino-L-alanine from soil to crop and its bioaccumulation in Chinese cabbage. *Chemosphere, 219*, 997–1001. https://doi.org/10.1016/j.chemosphere.2018.12.104

Lopicic, S., Svirčev, Z., Palanački Malešević, T., Kopitović, A., Ivanovska, A., & Meriluoto, J. (2022). Environmental neurotoxin β-*N*-methylamino-L-alanine (BMAA) as a widely occurring putative pathogenic factor in neurodegenerative diseases. *Microorganisms, 10*, 2418. https://doi.org/10.3390/microorganisms10122418

Lürling, M., Mello, M. M. E., Van Oosterhout, F., de SenerpontDomis, L., & Marinho, M. M. (2018). Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. *Frontiers in Microbiology, 13*(9), 1851. https://doi.org/10.3389/fmicb.2018.01851

Lv, H., Yang, J., Liu, L., Yu, X., Yu, Z., Chiang, P.-C., & Jun, Y. (2014). Temperature and nutrients are significant drivers of seasonal shift in phytoplankton community from a drinking water reservoir, subtropical China. *Environmental Science and Pollution Research, 21*, 5917–5928. https://doi.org/10.1007/s11356-014-2534-3

Main, B. J., Italiano, C. J., & Rodgers, K. J. (2017). Investigation of the interaction of β-methylamino-L-alanine with eukaryotic and prokaryotic proteins. *Amino Acids, 50*, 397–407. https://doi.org/10.1007/s00726-017-2525-z

McCarron, P., Logan, A. C., Giddings, S. D., & Quilliam, M. A. (2014). Analysis of β-N-methylamino-L-alanine (BMAA) in spirulina-containing supplements by liquid chromatography-tandem mass spectrometry. *Aquatic Biosystems, 10*, 5. https://doi.org/10.1186/2046-9063-10-5

Meriluoto, J., Spoof, L., & Codd, G.A. (2017). Handbook of cyanobacterial monitoring and cyanotoxin analysis; Wiley: Chichester, UK,2017 548

Metcalf, J. S., Banack, S. A., Lindsay, J., Morrison, L. F., Cox, P. A., & Codd, G. A. (2008). Co-occurrence of ß-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990–2004. *Environmental Microbiology, 10*(3), 702–708. https://doi.org/10.1111/j.1462-2920.2007.01492.x

Mohamed, Z. A. (2016). Harmful cyanobacteria and their cyanotoxins in Egyptian fresh waters–state of knowledge and research needs. *African Journal of Aquatic Science, 4*, 361–368. https://doi.org/10.2989/16085914.2016.1219313

Mohamed, Z. A., & Al-Shehri, A. M. (2010). Microcystin production in epiphytic cyanobacteria on submerged macrophytes. *Toxicon, 55*, 1346–1352. https://doi.org/10.1016/j.toxicon.2010.02.007

Mohamed, Z. A., Deyab, M. A., Abou-Dobara, M. I., Kamel, A., & El-Raghi, W. M. (2015). Occurrence of cyanobacteria and microcystin toxins in raw and treated waters of the Nile River, Egypt: Implication for water treatment and human health. *Environmental Science and Pollution Research, 22*, 11716–11727. https://doi.org/10.1007/s11356-015-4420-z

Mohamed, Z., Ahmed, Z., Bakr, A., Hashem, M., & Alamri, S. (2020). Detection of free and bound microcystins in tilapia fish from Egyptian fishpond farms and its related public health risk assessment. *Journal of Consumer Protection and Food Safety, 15*, 37–47. https://doi.org/10.1007/s00003-019-01254-0

Mohamed, Z., Bakr, A., Campos, A., Vasconcelos, V., & Nasr, S.A.-M. (2022). The growth inhibition and microcystin accumulation in bush beans (Phaselous vulgaris L.) plant irrigated with water containing toxic Chroococcus minutus. *Agricultural Water Management, 261*, 10738. https://doi.org/10.1016/j.agwat.2021.107381

Mohamed, Z. A., Vasconcelos, V., & Badawye, H. (2023). Detection of the cyanobacterial neurotoxin β-N-methylamino-L-alanine (BMAA) in water and tilapia fish of a tropical fishpond in Egypt. *Sohag Journal of Science, 8*, 323–328. https://doi.org/10.21608/sjsci.2023.209977.1079

Mondo, K., Hammerschlag, N., Basile, M., Pablo, J., Banack, S. A., & Mash, D. C. (2012). Cyanobacterial neurotoxin beta-N-methylamino-L-alanine (BMAA) in shark fins. *Marine Drugs, 10*, 509–520. https://doi.org/10.3390/md10020509

Murch, S. J., Cox, P. A., & Banack, S. A. (2004). A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. *PNAS, 101*, 12228–12231. https://doi.org/10.1073/pnas.0404926101

Näsholm, T., Kielland, K., & Ganeteg, U. (2009). Uptake of organic nitrogen by plants. *New Phytology, 182*, 31–48. https://doi.org/10.1111/j.1469-8137.2008.02751.x

Niyonzima, F. N. (2010). *Bioaccumulation and ecotoxicology of β-methylamino-L-alanine (BMAA) in model crop plants (Master thesis)*. Nelson Mandela Metropolitan University.

Nunes-Costa, D., Magalhães, J. D., G Fernandes, M., Cardoso, S. M., & Empadinhas, N. (2020). Microbial BMAA and the pathway for Parkinson's disease neurodegeneration. *Frontiers in Aging Neuroscience, 12*, 26. https://doi.org/10.3389/fnagi.2020.00026

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae, 14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Réveillon, D., Abadie, E., Séchet, V., Brient, L., Savar, V., Bardouil, M., Hess, P., & Amzil, Z. (2014). Beta-N-methylamino-L-alanine: LC-MS/MS optimization, screening of cyanobacterial strains and occurrence in shellfish from Thau, a French Mediterranean lagoon. *Marine Drugs, 12*, 5441–5467. https://doi.org/10.3390/md12115441

Reveillon, D., Sechet, V., Hess, P., & Amzil, Z. (2016). Systematic detection of BMAA (beta-N-methylamino- L -alanine) and DAB (2,4-diaminobutyric acid) in mollusks collected in shellfish production areas along the French coasts. *Toxicon, 110*, 35–46. https://doi.org/10.1016/j.toxicon.2015.11.011

Reynolds, CS (2006). The ecology of phytoplankton. Cambridge Press.535

Roy-Lachapelle, A., Solliec, M., & Sauvé, S. (2015). Determination of BMAA and three alkaloid cyanotoxins in lake water using dansyl chloride derivatization and high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry, 407*, 5487–5501. https://doi.org/10.1007/s00216-015-8722-2

Samardzic, K., Steele, J. R., Violi, J. P., Colville, A., Mitrovic, S. M., & Rodgers, K. J. (2021). Toxicity and bioaccumulation of two non-proteinamino acids synthesized by cyanobacteria, beta-N-methylamino-L-alanine (BMAA) and 2,4-diaminobutyric acid (DAB), on a crop plant. *Ecotoxicology and Environmental Safety, 208*, 111515. https://doi.org/10.1016/j.ecoenv.2020.111515

Scholz, S. N., Esterhuizen-Londt, M., & Pflugmacher, S. (2017). Rise of toxic cyanobacterial blooms in temperate freshwater lakes: Causes, correlations and possible countermeasures. *Toxicology and Environmental Chemistry, 99*, 543–577. https://doi.org/10.1080/02772248.2016.1269332

Song, Y., Zhou, H., Vo, M. N., Shi, Y., Nawaz, M. H., Vargas-Rodriguez, O., Diedrich, J. K., Yates, J. R., Kishi, S., Musier-Forsyth, K., & Schimmel, P. (2017). Double mimicry evades tRNA synthetase editing by toxic vegetable-sourced non-proteinogenic amino acid. *Nature Communications, 8*, 1–8. https://doi.org/10.1038/s41467-017-02201-z

Violi, J. P., Facey, J. A., Mitrovic, S. M., Colville, A., & Rodgers, K. J. (2019a). Production of βmethylamino-L-alanine (BMAA) and its isomers by freshwater diatoms. *Toxins, 11*, 512. https://doi.org/10.3390/toxins11090512

Violi, J. P., Mitrovic, S. M., Colville, A., Main, B. J., & Rodgers, K. J. (2019b). Prevalence of β-methylamino-L-alanine (BMAA) and its isomers in freshwater cyanobacteria isolated from eastern Australia. *Ecotoxicology and Environmental Safety, 172*, 72–81. https://doi.org/10.1016/j.ecoenv.2019.01.046

Vo Duy, S., Munoz, G., Dinh, Q. T., Do, D. T., Simon, D. F., & Sauvé, S. (2019). Analysis of the neurotoxin β-N-methylamino-L-alanine (BMAA) and isomers in surface water by FMOC derivatization liquid chromatography

high resolution mass spectrometry. *PLoS ONE, 14*, e0220698. https://doi.org/10.1371/journal.pone.0220698

Wang, C., Yan, C., Qiu, J., Liu, C., Yan, Y., Ji, Y., Wang, G., Chen, H., Li, Y., & Li, A. (2021). Food web biomagnification of the neurotoxin β-N-methylamino-L-Alanine IN a diatom-dominated marine ecosystem in China. *Journal of Hazardous Materials, 404*, 124217. https://doi.org/10.1016/j.jhazmat.2020.124217

Weralupitiya, C., Wanigatunge, R. P., Gunawardana, D., Vithanage, M., & Magana-Arachchi, D. (2022). Cyanotoxins uptake and accumulation in crops: Phytotoxicity and implications on human health. *Toxicon, 2022*(211), 21–35. https://doi.org/10.1016/j.toxicon.2022.03.003

Wu, X., Wu, H., Gu, X., Zhang, R., Ye, J., & Sheng, Q. (2019). Biomagnification characteristics and health risk assessment of the neurotoxin BMAA in freshwater aquaculture products of Taihu Lake Basin, China. *Chemosphere, 229*, 332–340. https://doi.org/10.1016/j.chemosphere.2019.04.210

Zamora-Barrios, C. A., Nandini, S., & Sarma, S. (2019). Bioaccumulation of microcystins in seston, zooplankton and fish: A case study in Lake Zumpango, Mexico. *Environmental Pollution, 249*, 267–276. https://doi.org/10.1016/j.envpol.2019.03.029