Research Article

SOHAG JOURNAL OF SCIENCES

https://doi.org/10.21608/sjsci.2023.209977.1079

Detection of the Cyanobacterial Neurotoxin β-N-methylamino-L-alanine (BMAA) in Water and Tilapia Fish of a Tropical Fishpond in Egypt

Zakaria A. Mohamed[1*], Vitor Vasconcelos[2,3], Hanan Badawye[1]

[1] *Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt.*
[2] *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*
[3] *Department of Biology, Faculty of Sciences, University of Porto, Rua Do Campo Alegre, 4169-007 Porto, Portugal*
**Email: zakaria.attia@science.sohag.edu.eg*

Received: 9th May 2023 **Revised:** 29th May 2023 **Accepted:** 31st May 2023
Published online: 1st June 2023

Abstract: β-N-Methylamino-L-alanine (BMAA) is a neurotoxic non-protein amino acid and causes neurodegenerative diseases. While the presence of BMAA toxin in phytoplankton communities and its bioaccumulation in aquatic food animals has been demonstrated worldwide, the detection of this toxin has not yet been explored in Egyptian freshwaters. Therefore, the present study determined BMAA levels in phytoplankton samples and edible muscles of tilapia fish sampled from an Egyptian fishpond. BMAA concentrations in phytoplankton samples were higher in summer (227.66 µg L^{-1}) than in winter (0.15µg L^{-1}), in association with the dominance of cyanobacteria in this fishpond. BMAA was detected in edible fish tissues at higher levels in summer (73.2 µg g^{-1}) than in winter (1.16 µg g^{-1}). Compared to the guideline values (GVs) derived during the present study (9.1 and 2.3 µg g^{-1} for adults and children, respectively), BMAA levels detected in edible fish tissues during summer exceeded these GVs by a factor of 8 for adults and 32 for children, but it was below these limits in winter. This may represent a health risk to humans through fish consumption. Therefore, the study suggests monitoring toxic cyanobacteria and their cyanotoxins in fishponds and aquacultures. Additionally, fish edible tissues should be tested for cyanotoxins before marketing.
Keywords: Aquaculture, cyanotoxins, seafood, food poisoning

1. Introduction

Cyanobacteria are prokaryotic autotrophic microorganisms that contribute largely to oxygen production in the Earth's atmosphere [1]. They constitute the base of the food web as primary producers in aquatic ecosystems [2]. However, cyanobacteria can form massive growth (i.e., blooms) under the conditions of high temperatures (25°C or above) and light intensity as well as increased nutrient concentrations (particularly, phosphorous and nitrogen) [3,4]. In fishponds, the liberation of nutrients from fish feed into the water increases the growth of cyanobacteria, resulting in hypoxia and death of fish [5]. Nevertheless, cyanobacterial blooms are of great ecological significance due to their production of toxic secondary metabolites called cyanotoxins, which adversely affect animals, plants, and human health [6-10]. Cyanotoxins are commonly classified into four classes according to their toxicological target: neurotoxins (anatoxins, saxitoxins, and β-Methylamino-L-Alanine–BMAA), hepatotoxins (cylindrospermopsins and microcystins), and dermatoxins (e.g., lipopolysaccharide and lyngbyatoxins) [11].

Fish can be exposed to cyanobacteria and cyanotoxins both actively and passively. Actively, while feeding on harmful cyanobacterial cells, and passively, when the gill epithelium comes into contact with dissolved toxins in the water [12]. These toxins can bioaccumulate in aquatic animals (e.g., fish). Since the majority of cyanotoxins are extremely stable and their toxicity is not decreased by cooking [13,14], humans may potentially be exposed to them by eating fish that have accumulated the toxins in their edible tissues.

Among cyanotoxins, BMAA is produced by cyanobacteria, diatoms, and dinoflagellates [15]. BMAA is a polar basic amino acid (M.W.=118.1Da) with distinct pKa values (2.1, 6.48, and 9.70, respectively) for the carboxyl group, main amino group, and secondary amino group [16]. Neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS), Parkinsonism Dementia Complex of Guam (ALS/PDC), and Alzheimer's disease (AD) have been linked to this neurotoxin, which has been found to cross the blood-brain barrier and incorporate into brain proteins, causing misfolding and aggregation [17-22]. This sparked worries about human exposure to this toxin via eating contaminated aquatic animals used as food e.g., fish [23].

Nile tilapia is one of the most cultured fish and is widely consumed by people worldwide including Egypt. Tilapia fish may endure in lakes and aquacultures plagued with toxic cyanobacteria and consume a significant portion of their cells in its diet [24]. Previous studies reported the accumulation of cyanotoxins: microcystins [25] and cylindrospermopsin [24] toxins in the edible muscles of tilapia fish caught from fish farms containing cyanobacterial blooms. However, no study has been made on the detection of BMAA toxin in Egyptian freshwaters yet. Therefore, this study aimed to investigate BMAA toxin in water and edible tissues of tilapia fish of a tropical Egyptian fishpond with a history of toxic cyanobacterial blooms. The study also evaluated the potential risk to human health from BMAA amounts found in this fish's edible tissues.

2. Materials and methods

2.1. Sampling

This study was conducted in an earthen fish pond situated in Sohag, southern Egypt (26º 36`N and 31º 43`E). The fishpond is about 3 m in depth and receives water from the Nile River. Water samples were collected in triplicate from three

different sectors in the fishpond by polyethylene bottles at a depth of around 30 cm during September 2021 and January 2022, where cyanobacterial bloom was present and not present, respectively in the pond. Meanwhile, triplicate samples of tilapia fish (*Oreochromis niloticus*) were collected from these fishpond sectors. All samples were taken between mid-morning to early-afternoon.

2.2. Environmental and phytoplankton analyses

Physical characteristics of fishpond waters (temperature, pH, conductivity, and dissolved oxygen-DO) were measured in situ using a multiparametric probe (HI 991300 pH/EC/TDS Temperature, HANNA, Italy) prior to water sampling. The Light intensity was measured at the water surface of fishponds using a Lumen Lux meter and then converted to µmol m^{-2} s^{-1}. Nutrient concentrations including nitrate, ammonia, orthophosphate, and dissolved organic matter were assessed in filtered water samples (through GF/C glass fiber filter) following standard methods described in Mohamed and Bakr [25]. Algal species were identified and enumerated in phytoplankton samples preserved in Lugol's iodine solution Algae and cyanobacteria were identified according to taxonomic keys based on cell or colony morphology [26,27]. Phytoplankton cell density (cells L^{-1}) was determined using a Sedgwick–Rafter counting chamber following the method described by Mohamed and Bakr [25]. The most dominant species were photographed using Digital LCD Binocular Microscope, SKU NM-105-10x (N12 Industrial Park, Bartlett, Boksburg).

2.3. Determination of BMAA in phytoplankton and water samples

Concentrations of BMAA within phytoplankton cells (free BMAA/ bounded BMAA) were determined according to the method described by Jiang et al. [23] with a minor modification. Briefly, one liter of phytoplankton samples was filtered through GF/C filters, and the filters with retained cells were extracted in 15 ml of 20 % methanol (methanol/water, v/v) with sonication for 3 min (70 % efficiency) in an ice water bath. The samples were centrifuged for 5 min (4100 x *g*, 4°C). The pellet was discarded, while the supernatant was mixed with two volumes of cold acetone. After overnight precipitation of the samples (−20°C), the solution was centrifuged again (10,000 x *g*, 4°C, 10 min). The resulting pellet containing the protein-bound BMAA fraction (i.e., bound BMAA) and the supernatant containing the free-BMAA fraction (i.e., free BMAA) were separated into new tubes. To allow the release of protein-bound BMAA, 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 (10,000x *g*,4°C). The resulting supernatant corresponding to bound BMAA was subjected to sterilized air to evaporate the organic solvent. BMAA concentration was then determined in the remaining aqueous fraction by High-Performance Liquid Chromatography HPLC. Extracellular (i.e., dissolved) BMAA was determined in the filtrate of the same phytoplankton samples. All samples were kept at −20°C until used for HPLC analysis.

2.4. Determination of BMAA in edible fish tissues

Tilapia fish caught from the fishpond during the present study were rinsed with tap and distilled water to get rid of any toxins possibly adhered to their surfaces. Edible muscles were excised, weighed (wet weight), and immediately frozen for BMAA determination. Fish muscles were ground in a pestle, extracted with 20 ml of 20% methanol, and then sonicated for 3 minutes at 70% efficiency to lyse them. To avoid protein degradation, samples were placed in an ice water bath during the sonication process. The extracts were then centrifuged (4100 x *g*, 4°C) for 5 min. The pellet was discarded, while the supernatant was used for the determination of free and bound BMAA concentrations.

HPLC analysis

HPLC analysis was performed on an Agilent 1200 HPLC-DAD coupled with a variable wavelength diode-array detector (Cox et al. 2003) (Agilent, USA). Separation was operated on A Zorbax – C18 (250 mm ×4.6 mm, 5µm) column (USA). The analytical column was equilibrated for two minutes and maintained at 25°C. A mobile phase pumped at 1 ml /min was used to elute the analytes from the column. The mobile phase consisted of acetonitrile: sodium acetate 140 mM with 0.5 % Triethyl amine (pH 5.7) (65:35v/v). A full spectral scan was performed from 190 to 450 nm, and the signal was recorded at a wavelength of 270 nm which provided better specificity compared to low-range UV wavelengths. BMAA identification was conducted by matching the peak retention times and spectra with that of pure BMAA standards. A calibration curve for standard BMAA (BMAA Hydrochloride, Sigma-Aldrich) was estimated too. The limits of detection (LOD) and limits of quantification (LOQ) were 10.0 ng, respectively. Additionally, a liquid chromatography-ion trap tandem mass spectrometer (LC-MS/MS) was used for confirmation and accurate quantification of BMAA in the samples following the method described in Faassen et al. [28].

2.5. Risk assessment and estimation of guideline value of BMAA

The noncarcinogenic human health risk of BMAA was assessed using the reference dose (RfD, 40µg $^{-1}$ kg^{-1} d^{-1}) of this toxin, which was previously calculated by Wu et al. [29] for risk assessment of BMAA toxin in aquatic products to human health in China according to the following equation:

$$RfD = \frac{NOAEL}{UF} \quad (1)$$

Where NOAEL (no observed adverse effect level) is the highest dose that would not cause harmful health effects, which was determined to be 40 mg/kg bw/d according to the toxicity test data for male cynomolgus monkeys published in Karlsson et al. [30]; UF is the uncertainty factor, with its value set as 1000. Therefore, this RfD was used by Wu et al. [29] to estimate the guideline value (GV) of this toxin in aquaculture products according to the following equation:

$$GV = \frac{RfD\ x\ BW\ x\ AF}{FIR} \quad (2)$$

where GV is the recommended limit for BMAA (µg g^{-1}), AF is the empirical coefficient, with a value set as 0.30, indicating that 30% of the total daily intake of BMAA in humans comes from aquatic products, BW is the average body weight (60 kg for adults and 15 kg for children) and FIR is the food intake rate, specifically the daily amount of aquatic product intake by human (100 g d^{-1}).

Here in our study, we applied the same equation of Wu et al. [29], but we modified AF and FIR values as these parameters may vary according to the country's traditions for the consumption of each comestible (e.g., fish). For African and Middle east countries, FIR is 100 g of fish per day. If fish constitutes 30% of human food in these countries and assuming the oral bioavailability of BMAA is 80% as reported by Duncan et al. [31], the total daily intake of BMAA in humans comes from fish would be 0.38 (i.e., AF= 0.3÷0.8).

Statistical analysis

Variations in cyanobacterial densities, environmental parameters, and BMAA concentrations in water samples and fish tissues were estimated using ANOVA ($P < 0.05$) with SPSS17 software for Windows. The correlation between cyanobacterial density and concentrations of BMAA in phytoplankton and fish tissues was evaluated using Spearman rank correlation coefficients.

3. Results

3.1. Quality of fishpond water

Table 1 presents the data on the physicochemical properties of the fishpond water obtained during the present study. The results revealed a significant difference in the temperature of fishpond water between summer and winter ($P < 0.05$). It ranged from 30°C to 31°C in summer, and from 15°C to 18°C in winter. Light intensity at the water surface of this fishpond was higher in summer (2.2-3.4 mmol photons $m^{-2}s^{-1}$) than in winter (0.42-0.7mmol photons $m^{-2}s^{-1}$). The conductivity of fishpond water also showed higher values in summer (0.22-0.6 mmos cm^{-1}) than in winter (0.4–1.6 mmos cm^{-1}). The pH of fishpond water was slightly alkaline (7.1–8.4) and it did not significantly change ($P>0.05$) between summer and winter seasons. Concentrations of nutrients (NO_3, NH_4, PO_4 dissolved organic matter) in the fishpond water changed markedly ($P<0.05$) between the two seasons, showing higher values in summer than in winter (Table 1).

Table 1 Limnological characteristics of fishpond water during the present

Variables	Winter	Summer
Temperature(°C)	15-18	30-31
PH	7.4-8	7.1-8.4
Conductivity (mS cm^{-1})	0.22-0.6	0.4-1.6
Light intensity (m mol photons m^{-2} s^{-1})	0.42-0.7	2.2-3.4
DO (mg L^{-1})	7.8-12	7-11
NO_3 (mg L^{-1})	0.45-0.5	4-13
NH_4 (mg L^{-1})	0.25-2.4	7-25
PO_4 (mg L^{-1})	0.14-0.36	2-25
Dissolved organic matter (mg L^{-1})	0.05-0.06	0.1-0.34

3.2. Phytoplankton composition in fishpond water

The results showed that cyanobacteria were the dominant group in phytoplankton samples collected from the studied fishpond during the summer season representing 99.4% of the total phytoplankton (Fig.1).

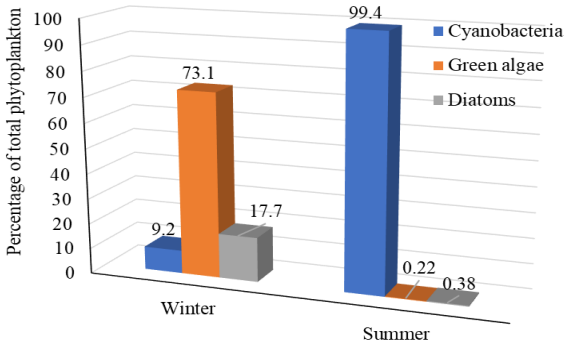

Fig. 1 Percentage distribution of phytoplankton groups in the Sohag fishpond during the present study.

Cyanobacterial populations in this fishpond were dominated by potentially toxic species including *Dolichospermum flos-aquae*, *Merismopedia glauca*, *Microcystis aeruginosa*, *Planktolyngbya contorta*, *Raphidiopsis raciborskii*, *Synechococcus elongatus* (Table 2 and Fig.2).

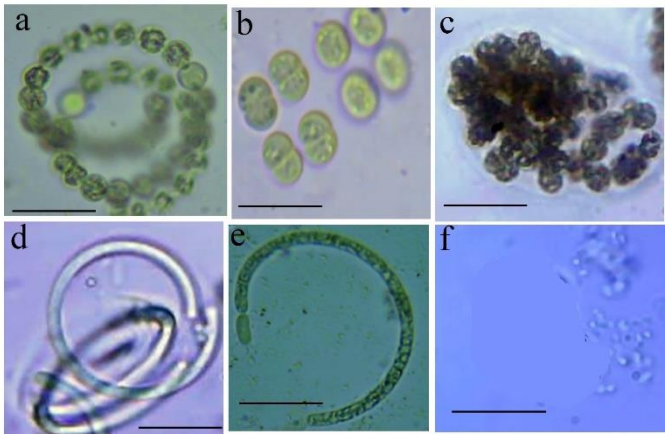

Fig. 2. Light microscope photographs of dominant cyanobacteria recorded in the studied fishpond during present study:(a) *Dolichospermum flos-aquae*, (b) *Merismopedia glauca*, (c) *Microcystis aeruginosa*, (d) *Planktolyngbya contorta*, (e) *Raphidiopsis raciborskii*, and (f) *Synechococcus elongatus*. Scale bar =10µm.

The most abundant cyanobacterial species were *Dolichospermum flos-aquae* (2760000 cells L^{-1}) and *Planktolyngbya contorta* (2260000 cells L^{-1}). The highest cell density of cyanobacteria in the fishpond during summer correlated positively with temperature (r= 0.9), light intensity (r= 0.85), conductivity (r= 0.8), NO_3 (r=0.9), NH_4 (r=0.9) and PO_4 (r= 0.8) concentrations (Table 1). Green algae and diatoms were also found in the fishpond during summer, but with low cell densities (0.22%, and 0.38%, respectively) (Fig.1). On the other hand, cyanobacteria showed lower abundance during the winter season (9.2%) and was represented only by two species (*Dolichospermum flos-aquae* and *Synechococcus elongatus)* (Table 2). However, phytoplankton samples collected from the fishpond during winter were dominated by diatoms and green algae (73.1%, and 17.7%, respectively).

Table 2. Cell density (cells L^{-1}) of common cyanobacteria recorded in the studied fishpond during winter and summer seasons

Species	Cell density	
	Winter	Summer
Dolichospermum flos-aquae	1000±577	2760000 ±249800
Merismopedia glauca	-	256000 ± 24440
Microcystis aeruginosa	-	960000±64000
Planktolyngbya contorta	-	2260000±233524
Raphidiopsis raciborskii	-	216000±18000
Synechococcus elongatus	1600±924	140000±8327

3.3. BMAA concentrations in fishpond water

In our study, BMAA toxin was detected by HPLC and confirmed by LC-MS/MS in phytoplankton samples in two forms: free and protein-bound BMAA (Fig.3).

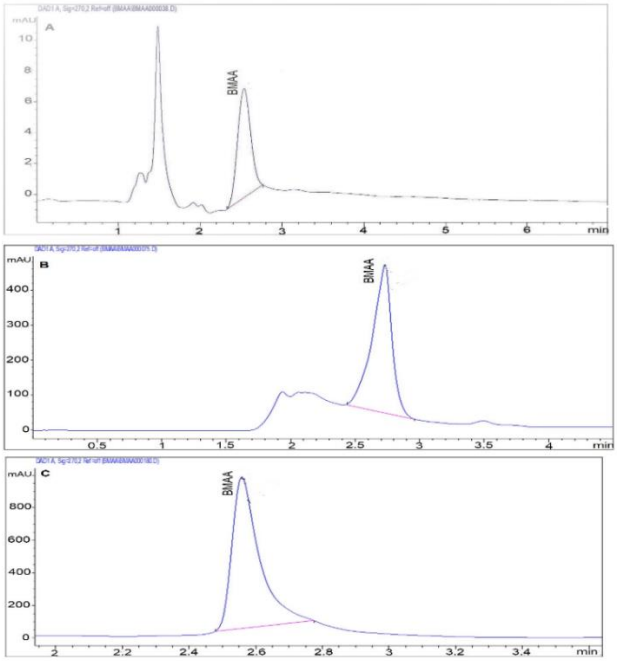

Fig. 3. HPLC chromatograms of BMAA: (a) standard, (b) free BMAA, (c) protein-bound BMAA in phytoplankton samples collected from the fishpond during the present study.

BMAA concentrations within phytoplankton cells showed a significant variation ($P < 0.05$) between winter and summer seasons and correlated markedly with the total cell density of cyanobacteria (r=0.85) rather than green algae and diatoms (r=0.2). The highest concentrations of total BMAA were obtained in phytoplankton samples collected during summer (227.66 µg L^{-1}), while very low concentrations were recorded in phytoplankton samples collected in winter (0.1 µg L^{-1}).

In our study, free BMAA showed higher concentrations (180.5µg L^{-1}) than protein-bound form (47.16µg L^{-1}) (Fig.4). Concentrations of dissolved BMAA in cell-free fishpond water ranged from 0.1µg L^{-1} in winter to 1.34 µg L^{-1} in summer (Fig.4). These concentrations had a strong correlation with BMAA concentrations within phytoplankton cells (r=0.9) and the cell density of cyanobacteria (r=0.8) recorded in the relevant samples.

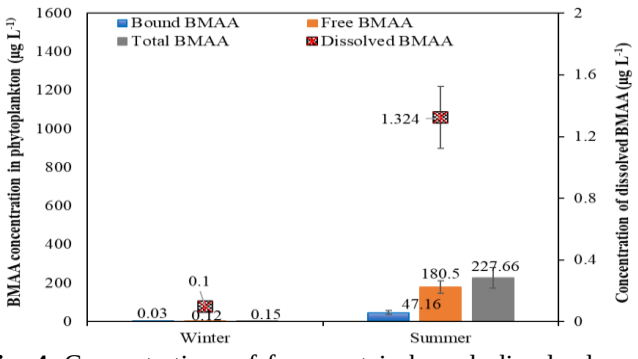

Fig. 4. Concentrations of free, protein-bound, dissolved, and total BMAA (µg L^{-1}) in phytoplankton samples in phytoplankton samples collected from the fishpond during the present study.

3.4. BMAA concentrations in tilapia fish tissues

The results of HPLC analysis showed that edible tissues (i.e., muscles) of Tilapia fish caught from the fishpond during summer contained higher amounts of free BMAA (65.1µg g^{-1} FW) than protein-bound BMAA (8.14µg g^{-1} FW) (Fig.5). Additionally, free and bound BMAA was also found in the edible tissues of fish collected during winter, but at a lesser concentration (1.07, 0.087 µg g^{-1} FW, respectively). Statistically, these BMAA concentrations had a strong correlation with their concentrations in phytoplankton cells (r=9) and the cell density of cyanobacteria (r=0.85) investigated in the fishpond.

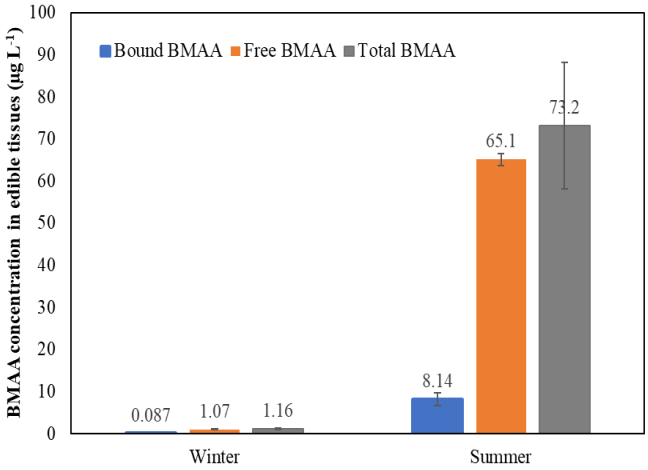

Fig. 5. Concentrations of free, protein-bound, and total BMAA (µg L^{-1}) in edible tissues of tilapia fish collected from the fishpond during the present study.

4. Discussion

4.1. BMAA concentrations in fishpond water

The conditions favoring the growth of cyanobacteria in water sources include warm temperatures (15–30°C), pH6-9, nutrient enrichment (particularly, nitrogen and phosphorus), and calm stable water conditions [32,33]. These conditions occurred in the fishpond surveyed during the present study and caused the formation of cyanobacterial blooms in summer. The BMAA toxin was detected at high concentrations in phytoplankton samples from the fishpond which was mainly dominated by cyanobacterial species: *Dolichospermum flos-aquae*, *Merismopedia glauca*, *Microcystis aeruginosa*, *Planktolyngbya contorta*, *Raphidiopsis raciborskii*, *Synechococcus elongatus*) during summer. Interestingly, BMAA toxin was also detected but with low concentrations in phytoplankton samples collected from the fishpond during winter when cyanobacteria accounted only for 9.2% of the total phytoplankton and represented only by *Dolichospermum flos-aquae* and *Synechococcus elongatus.* This verifies the involvement of these cyanobacterial species in BMAA production. These results support the previously presented hypothesis that BMAA can be produced by diverse species belonging to all five known groups of cyanobacteria [34]. Recently, Błaszczyk et al. [35] detected BMAA toxin in environmental samples, dominated by cyanobacteria of the genera *Dolichospermum*, *Planktothrix*, and *Limnothrix.* However, further studies are required to verify the production of BMAA toxin in pure cultures of these species.

In the present study, both free and protein-bound of the BMAA toxin were detected in phytoplankton samples, but the free form was found in higher concentrations. These results are thus in line with previous studies that reported both free and

protein-bound BMAA fractions in phytoplankton samples [35-37].

The highest concentration of total BMAA detected in phytoplankton samples collected from the fishpond reached 227.66 µg L^{-1} (corresponding to 42.8µg g^{-1} dry mass). These values are several orders of magnitude higher than BMAA concentrations measured in cyanobacteria-rich phytoplankton samples (0.001- 0.015µg BMAA/g dry mass) from the Baltic Sea [38], Canada (2 µg L^{-1}) [39] and South Africa (0.25 µg g^{-1}) [40], but they can be compared to those recorded in the Netherlands [42 µg g^{-1}) [36], and USA (39.6 µg L^{-1}) [41] On the other hand, our concentrations are lower to BMAA concentrations detected in phytoplankton samples in UK (276 µg g^{-1}) [42]. Such discrepancy may be due to the difference in the cell density of BMAA-producers in the environment and the varying capacity of BMAA production in different species and strains of producers [43].

4.2. Accumulation of BMAA in edible fish tissues

The results showed that tilapia fish caught from the fishpond during the present study accumulated BMAA toxin in edible tissues (i.e., muscles). The results revealed a substantial accumulation of BMAA in edible fish muscles during summer, along with a high cell density of harmful cyanobacteria and high amounts of BMAA in phytoplankton samples collected in the fishpond. The detection of high concentrations of BMAA in fish tissues indicates that this toxin has been taken by tilapia fish in a dissolved form (i.e., found in cell-free fishpond water) through gills by direct diffusion or the digestive tract. Since the concentration of dissolved BMAA detected in fishpond water during our study was very low, the high toxin amount in fish was unlikely to happen via gills by direct diffusion. Alternatively, BMAA uptake most likely occurred via oral ingestion of toxin-containing phytoplankton, as Tilapi is considered an herbivorous fish, because of its feeding habit as a phytoplanktivorous filter feeder [44]. Once entering the digestive tract, BMAA may be transported to other tissues by the hemolymph [45]. A similar mechanism has been proposed for tilapia fish exposed to MC-producing cyanobacteria and microcystins (MC) [25]. In our study, edible tissue (i.e., muscles) of tilapia fish contained higher amounts of free BMAA (171.2 µg g^{-1} F.W) than protein-bound BMAA (21.4 µg g^{-1} F.W). This is in accord with the findings of Al-Sammak et al. [41] who found that some fish species such as Shed, Wiper, and Bluegil accumulated higher amounts of free BMAA than bound ones. Also, high levels of free BMAA were detected in mussels and scallops bought from grocery stores in Sweden [46]. The presence of bound BMAA in edible fish tissues may increase the burden of this toxin for consumers, as it can be released in the human digestive system [45, 47]. Overall, the concentration of total BMAA found in edible tissues of tilapia fish during the present study (192.6 µg g^{-1} F.W) is higher than BMAA concentrations reported for fish (35.91 µg/g DW) in Lake Taihu, China [48] and for mussels and oyster (1.6-9.7 µg g^{-1} DW) in Mediterranean sites [37,49,50]. Whereas our BMAA concentrations are considered low compared to those detected in puffer fish (1907 µg g^{-1} FW) and white perch (1806 µg g^{-1} FW) collected in South Florida, USA [51,52].

4.3. Potential health hazards of BMAA toxin in fish tissues

Recognizing that BMAA is chemically stable that is difficult to break down [29] and 80% of orally ingested BMAA are absorbed from the gut into the bloodstream [31] and then crosses the blood-brain barrier by large neutral amino acid carriers [31], its accumulation in fish tissues poses a risk to human health when eating such contaminated fish [53,54]. In our study, we used the data of BMAA concentrations detected in fish edible tissues as a data source to evaluate the extent to which these toxin concentrations could pose a risk to humans eating contaminated fish.

Based on Egyptian conditions of fish consumption, the GVs for the BMAA proposed and calculated by Eq. (2) in this study were 9.1 µg g^{-1} for adults and 2.3µg g^{-1} for children.

These GV values of consumption of fish contaminated with BMAA can be compared with those (7.2 and 1.8 µg g^{-1} for adults and children, respectively) reported by Wu et al. [29] during the evaluation of the health risk of BMAA in edible tissues of freshwater aquaculture products including mollusks and fish Chinese aquaculture ponds. Compared to these GVs, BMAA concentrations detected in edible fish muscles during summer (73.2 µg g^{-1}) in our study surpassed these GVs by a factor of 8 for adults and 32 for children. On the other hand, BMAA concentrations detected in fish muscles during winter (1.16 µg g^{-1}) were below these GVs. Our study, therefore, reflects that tilapia fish collected in summer during the prevalence of cyanobacterial blooms in the fishpond is not safe for human consumption, while fish collected during winter is safe and might not constitute a severe health risk.

5. Conclusions and recommendations

The present study demonstrated the occurrence of toxic cyanobacterial blooms in an Egyptian fishpond used as an aquaculture of tilapia fish. Cyanobacteria dominated phytoplankton populations in this fishpond during summer, but not in winter. As a result, there were higher concentrations of free and bound BMAA toxin in fishpond waters in the summer and lower concentrations in the winter. The study also demonstrated that tilapia fish grown in this fishpond can accumulate this toxin in their edible tissues. Concentrations of BMAA in fish edible tissues were higher in summer and lower in winter, in association with its concentrations within phytoplankton cells and the cell density of cyanobacteria in the fishpond water.

BMAA levels detected in edible tissues of tilapia fish collected during winter were lower than the derived GVs (9.1 and 2.3µg g^{-1} for adults and children, respectively) proposed safe for human consumption. Conversely, concentrations of this toxin detected in edible tissues of fish collected in summer exceeded these GV values by several folds. This suggests that BMAA in edible tissues of tilapia fish from this pond could constitute a potential risk to the health of humans who consume such contaminated fish. Therefore, aquacultures and fishponds should be monitored for the presence of toxic cyanobacteria and their cyanotoxins, particularly the regions expected to be impacted by climate change, which would favor the occurrence of cyanobacterial blooms (even in winter) and help to spread some toxin-producing species to latitudes outside of their usual range. Additionally, strict restrictions must be implemented in aquaculture systems, and cyanotoxins should be tested in edible fish tissues taken from eutrophic waters before being sold in order to protect people from exposure to such potent toxins.

Acknowledgments

This work was supported financially by the Marie Skodowska-Curie grant agreement No. 823860 from the Horizon 2020 research and innovation program of the European Union.

References

[1] B. Rasmussen, I. R. Fletcher, J.J. Brocks, M.R. Kilburn, *Nature*, 455 (2008)1101–1104.

[2] L. Naselli-Flores, J. Padisák, *Hydrobiologia*, (2022). https://doi.org/10.1007/s10750-022-04795-y.

[3] T. Boopathi, Ki, J. S, *Toxins (Basel)*, 6(2014) 1951-1978.

[4] S. Merel, D. Walker, R. Chicana, S. Snyder, E. Baurès, O. Thomas, *Environment International*,59 (2013) 303-327.

[5] J. Martins, V. Vasconcelos, *Journal of Toxicology and Environmental Health B*, 12(2009) 65–82.

[6] H. Sun, K. Lü, EJ. Minter, Y. Chen, Z. Yang, DJ. Montagnes, *Journal of Hazardous Materials*, 221-222(2012) 213–219.

[7] HW. Paerl, WS. Gardner, KE. Havens, AR. Joyner,MJ. McCarthy, SE. Newell, B. Qin, JT. Scott, *Harmful Algae*, 54(2016) 213–222.

[8] J. Huisman, GA. Codd, H.W. Paerl, BW. Ibelings, J.M. Verspagen, P.M. Visser, *Nature Reviews Microbiology*, 16(2018) 471–483.

[9] S.M. Gene, RS. Shahmohamadloo, X. Ortiz, RS. Prosser, Environmental Toxicology and Chemistry, 38(2019) 2137–2144.

[10] R.S. Shahmohamadloo, DG. Poirier, XO. Almirall, SP. Bhavsar, PK. Sibley, *Ecotoxicology and Environmental Safety*, 188 (2020) 109945.

[11] I. Sanseverino, D. Conduto, L. Pozzoli, S. Dobricic, T. Lettieri, in: Algal bloom and its economic impact. Report No. EUR 27905 EN, (2016) p.52; doi:10.2788/660478.

[12] Z. Svirčev, J. Lujić, Z. Marinović, D. Drobac, N. Tokodi, B. Stojiljković, J. Meriluoto, *J Environ Sci Health, Part C*, 33(2015)125–167.

[13] J. Dyble, D. Gossiaux, P. Landrum, DR. Kashian, S. Pothoven, *Mar Drugs*, 9(2011)2553–2571.

[14] D. Zhang, P. Xie, J. Chen, *Bull Environ Contam Toxicol*, 84(2010) 202–207.

[15] Koksharova, O.A.; Safronova, *Toxins* 14 (2022), 539. https://doi.org/10.3390/ toxins14080539

[16] Norden, In: Analysis, occurrence, and toxicity of BMAA, A risk for the consumer? Nordic Council of Ministers, Copenhagen, (2007), pp. 128. 10.6027/TN2007-561.

[17] W.B. Glover, DC. Mash, S.J. Murch, *Amino Acids*, 46(2014) 2553–2559.

[18] X. Xie, M. Basile, DC. Mash, *Neuroreport*, 24(2013) 779–784.

[19] B.J. Main, CJ. Italiano, K.J. Rodgers, *Amino Acids*, 50(2017) 397-407.

[20] R.A. Dunlop, PA. Cox, SA. Banack, KJ. Rodgers, *PLoS One*, 8(2013) e75376.

[21] P.A. Cox, D.A. Davis, D.C. Mash, J.S. Metcalf, S.A. Banack, *Proceedings of the Royal Society B: Biological Sciences* 283 (2016) 20152397.

[22] D. Lobner, P.M. Piana, A.K. Salous, R.W. Peoples, *Neurobiology of Disease*, 25(2007) 360–366.

[23] L. Jiang, N. Kiselova, J. Rosén & L. Ilag, *Scientific Reports* 4(2014) 6931.

[24] Z. Mohamed, Z. Ahmed, A. Bakr., M. Hashem, S. Alamri. *Journal of Consumer Protection and Food Safety*,15(2020)37–47.

[25] ZA. Mohamed, A. Bakr, *Environmental Science and Pollution Research*, 25(2018) 36287–36297.

[26] J. Komarek, J. Komarkova, *Czech Phycology*, 3(2003)1–30.

[27] J. Komárek, K. Anagnostidis, In: *Cyanoprokaryota*, Band 19/2. Elsevier, Műnchen, (2005).

[28] E.J. Faassen, F. Gillissen, M. Lurling, *PLoS ONE*, 7(2012) e36667.

[29] X. Wu, H. Wu, X. Gu, R. Zhang, J. Ye, Q. Sheng, *Chemosphere*, 229(2019)332-340.

30. O. Karlsson, K. Kultima, H. Wadensten, *Journal of Proteome Research*, 12(2013)1678-1690.

[31] M.W. Duncan, S.P. Markey, B.G. Weick, P.G. Pearson, H. Ziffer, Y. Hu, I.J. Kopin, *Neurobiology of Aging*,13(1992)333–337.

[32] R.B. Sotero-Santos, C.R.D. Silva, N.F. Verani, K.O. Nonka, O. Rocha, Ecotoxicology and Environmental Safety, 64 (2006)163-170.

[33] ZA. Mohamed, AM. Al-Shehri, *FEMS Microbiology Ecology*, 69(2009) 98–105.

[34] P.A. Cox, S.A. Banack, S.J. Murch, U. Rasmussen, G. Tien, R.R. Bidigare, J.S. Metcalf, L.F. Morrison, G.A. Cod, B. Bergman, *PNAS*, 102(2005)5074–5078.

[35] *A.* Blaszczyk, K. Siedlecka-Kroplewska, M. Wozniak and H. Mazur-Marzec, *Toxicon* 194(2021) 90-97.

[36] E.J. Faassen, F. Gillissen, H.A.J. Zweers, M. Lurling, *Amyotrophic Lateral Sclerosis*, 10(2009)79–84.

[37] D. Reveillon, E. Abadie, V. Sechet, E. Masseret, P. Hess, Z. Amzil, *Marine Environmental Research*, 110(2015)8–18.

[38] S. Jonasson, J. Eriksson, L. Berntzon, Z. Spácil, L.L. Ilag, L.O. Ronnevi, U. Rasmussen B. Bergman, *Proceedings of the National Academy of Sciences of the United States of America* ,107(2010) 9252–9257.

[39] E. Pip, K. Munford, L. Bowman, Environmental Pollution 5 (2016)110-118.

[40] L.L. Scott, S. Downing, R.R. Phelan, T.G. Downing, *Toxicon*, 87(2014)1–5.

[41] M.A. Al-Sammak, K.D. Hoagland, D. Cassada, *Toxins*, 6 (2014)488–508.

[42] J.S. Metcalf, S.A. Banack, J. Lindsay, L.F. Morrison, P.A. Cox, G.A. Codd, Environmental Microbiology, 10 (2008)702–708.

[43] E. Lance, N. Arnich, T. Maignien, R. Biré, *Toxins*, *5*(2018)191.

[44] D. Zhang, P. Xie, Y. Liu, T. Qiu, *Science of The Total Environment* 407(2009) 2191–2199.

[45] A. Lepoutre, EJ. Faassen, AJ. Zweers, M. Lürling, A. Geffar, E. Lance, *Toxins*,12(2020) 61.

[46] M. L. Salomonsson, E. Fredriksson, A. Alfjorden, M. Hedeland, U. Bondesson, *Toxicology Reports*, 2(2015)1473–1481.

[47] S.J. Murch, P.A. Cox, S.A. Banack, *Proceedings of the Nationl Academy of Sciences of the United States of Americ*, 101(2004)12228–12231.

[48] Y.Q. Chen, X. Chen, X. Wang, X. Liao, L. Jiang, L. Yang, *Science of the Total Environment*, 468(2014) 457–463.

[49] EMasseret, S. Banack, F. Boumédiène, E. Abadie, L. Brient, F. Pernet, *PloS One*, 12(2013)8-83406.

[50] Reveillon, V. Sechet, P. Hess, Z. Amzil, *Toxicon*, 110(2016)35–46.

[51] LE Brand, J. Pablo, A. Compton, N. Hammerschlag, D.C. Mash, *Harmful Algae*, 9(2010)620-635.

[52] J.Regueiro, N. Negreira, A. Carreira-Casais, C. Pérez-Lamela, J. Simal-Gándara, *Food Research International*, 1(2017)1–13.

[53 A.S. Chiu, M.M. Gehringer, N. Braidy, G.J. Guillemin, J.H. Welch, B.A. *Scientific Reports*,1482 (2013)1-5.

[54] H. Yin, S. Yu, C.I. Hsu, J. Liu, A. Acab, R. Wu, A. Tao, B.J. hiang, J.H. Weiss, *Experimental Neurology*, 261(2014)1-9.