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Assessment of microcystin contamination of *Amaranthus hybridus*, *Brassica oleracea*, and *Lactuca sativa* sold in markets: a case study of Zaria, Nigeria

Mathias Ahii Chia · Zinariya Zippora Auta · Akolo Elijah Esson · Abraham G. Yisa · David S. Abolude

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Abstract Microcystins (MCs) are toxic secondary metabolites produced by several cyanobacteria genera that have been implicated in human cancer cases and deaths. Human exposure routes include direct contact with contaminated water and the consumption of contaminated food. The present study investigated the presence of MCs in three commonly consumed vegetables at the point of sale in market places as a means of assessing the direct human health risk of buying vegetables. Overall, 53% of the vegetables obtained from different markets had levels of MCs that were higher than 1.00 μg/g. *Amaranthus hybridus* L. (smooth amaranth) had the highest MC concentration (4.79 μg/g) in samples obtained from Sabon Gari Market, while *Lactuca sativa* L. (garden lettuce) had the lowest concentration (0.17 μg/g) in samples obtained from Dan-Magaji Market. The highest total daily intake (TDI) of MCs by an adult weighing 60 kg was 3.19 μg/kg for *A. hybridus*, 1.41 μg/kg for *Brassica oleracea* L. (cabbage), and 2.94 μg/kg for *L. sativa*. The highest TDI of MCs for a child weighing 25 kg was highest in *A. hybridus* (1.91 μg/kg), followed by *L. sativa* (1.77 μg/kg). These results revealed that the consumption of vegetables sold in markets in Zaria, Nigeria, during the dry season represents a major exposure route to MCs. There is, therefore, an urgent need to develop policies and monitoring strategies to tackle this problem in developing countries.

Keywords Microcystins/secondary metabolites · Bioaccumulation · Food contamination · Public health

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M. A. Chia (✉) · A. E. Esson
Department of Botany, Ahmadu Bello University, Zaria, Nigeria
e-mail: chia28us@yahoo.com

e-mail: amchia@abu.edu.ng

Z. Z. Auta · D. S. Abolude
Department of Biology, Ahmadu Bello University, Zaria, Nigeria

A. G. Yisa
Department of Zoology, Ahmadu Bello University, Zaria, Nigeria

Introduction

Microcystins (MCs) are hepatotoxins found in fresh and brackish waters and are produced by several cyanobacteria genera such as *Microcystis*, *Planktothrix*, *Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Nostoc*, *Anabaenopsis*, *Hapalosiphon*, *Chroococcus*, *Aphanocapsa*, *Pseudanabaena*, and *Phormidium*. They are regarded as the most frequently occurring and widespread cyanotoxins (Mohammed and Al-Shehri 2009) as they account for most of the toxic cyanobacterial blooms in aquatic ecosystems (Bruno et al. 2012; Ettoumi et al. 2011; Lance et al. 2014). There are currently over 200 congeners of MCs that have been described (Steiner et al. 2016).

Microcystins are the most frequently occurring cyanotoxins in freshwater bodies worldwide (Harke et al. 2016). They are released into the environment during normal cell growth, lyses, and senescence of cyanobacteria (Pflugmacher 2004). They are potent inhibitors of protein phosphatase (PP) types PP1 and

PP2A in eukaryotes (Carneiro et al. 2012), which account for their extreme toxicity (Ikehara et al. 2009; Isaacs et al. 2014). MCs affect various organs such as the liver, muscle kidney, intestines, colon, reproductive system, brain, lungs, and heart (Li et al. 2009; Oliveira et al. 2013). Carcinogenic health concerns of exposure to MCs include hepatic cancer, gastrointestinal cancer, and colon tumors (Grosse et al. 2006; Herfindal and Selheim 2006; Svircev et al. 2009).

Possible human exposure routes to MCs include drinking of contaminated water; eating of contaminated vegetables, fruits, and aquatic organisms; and inhalation or nasal contact with mucous membrane and skin. The contamination of irrigated vegetables occurs primarily because MCs bioaccumulate in plant tissues (Bittencourt-Oliveira et al. 2016; Cordeiro-Araujo et al. 2016; Peuthert et al. 2015). As a result of these findings coupled with those with other biological (plant and animal) matrices, the World Health Organization (WHO) recommended a maximum total daily intake (TDI) of MCs of 0.04 μg per kilogram of body weight (WHO 2011). Unfortunately, several investigations show that plant tissues accumulate higher concentrations of MCs than the recommended maximum TDI (Hereman and Bittencourt-Oliveira 2012). Vegetables are often eaten raw without much processing, and even after cooking, MCs remain structurally and functionally stable (Bruno et al. 2009; Zhang et al. 2010), thereby demonstrating the need to assess the risk of buying and consuming contaminated vegetables. Most bioaccumulation studies have been carried out under controlled conditions involving irrigation of vegetables (Bittencourt-Oliveira et al. 2015; Cordeiro-Araujo et al. 2016) and irrigated crops in the field under farming conditions (Codd et al. 1999; Crush et al. 2008). However, recent evidence suggests that when the irrigation of the crops with MC-contaminated water is replaced with uncontaminated water, MC depuration occurs (Cordeiro-Araujo et al. 2016). Therefore, assessing the quality of vegetables at the point of sale in different markets is extremely important because it reveals the real and direct risks to which humans are exposed. Unfortunately, this aspect has been poorly reported or assessed all over the world.

Freshwater bodies used for irrigation farming in Zaria have already been reported to be frequently contaminated by MCs (Chia et al. 2009a, b; Chia and Kwaghe 2015). These water bodies serve as a sink for domestic and industrial effluents and as a source of water supply for human consumption and irrigation farming. Irrigation, which is the supply of water for agricultural purpose, is widely practiced in Zaria and has accounted for very high yield and year-round cultivation of crops. Irrigation of vegetables in Zaria and North-Western Nigeria is widely practiced as a result of inadequate rainfall for vegetable production (Tsoho et al. 2012).

This study aimed to assess MC contamination of vegetables sold in different markets in Zaria, Nigeria. The generation of data on the bioaccumulation of MCs in crops will contribute to the development of policies and monitoring strategies for the presence of toxin-producing strains/species of cyanobacteria in water and the contamination of vegetables in several countries. The vegetables selected in this study, namely *Amaranthus hybridus* L., *Brassica oleracea* L., and *Lactuca sativa* L. are widely consumed in raw and cooked forms and form part of several delicacies worldwide.

Materials and methods

Study area, sample collection, and MC analysis

The study was carried out in Zaria (longitude 11° 30′ N and latitude 7° 42′ E), Kaduna State, northwest Nigeria. It has an area of 46,053 km^2 and a population of 6,066,562. It has a northern guinea savannah vegetation and a tropical continental climate comprising well-defined rainy season and dry seasons. The long dry season spell (October to April) necessitates the irrigation of vegetable crops to meet the demand of residents in most of northern and southern Nigeria. Unfortunately, the water used for irrigation is not analyzed for organic and inorganic contaminations before irrigation, which has led to the sales of contaminated vegetables (see Fig. S1 in supplementary materials). In April 2017, samples of *A. hybridus*, *L. sativa*, and *B. oleracea* were purchased from four markets namely Sabon Gari Market, Dan-Magaji Market, Samaru Market, and Giwa Market, and transported to the laboratory on ice in a cooler. Each vegetable was purchased from at least three different vendors per market. A total of 36 samples were collected from the four markets. The locations of the markets in Zaria, Nigeria, are shown in Fig. 1.

Vegetable samples (leaves) were repeatedly rinsed with deionized distilled water to remove debris and

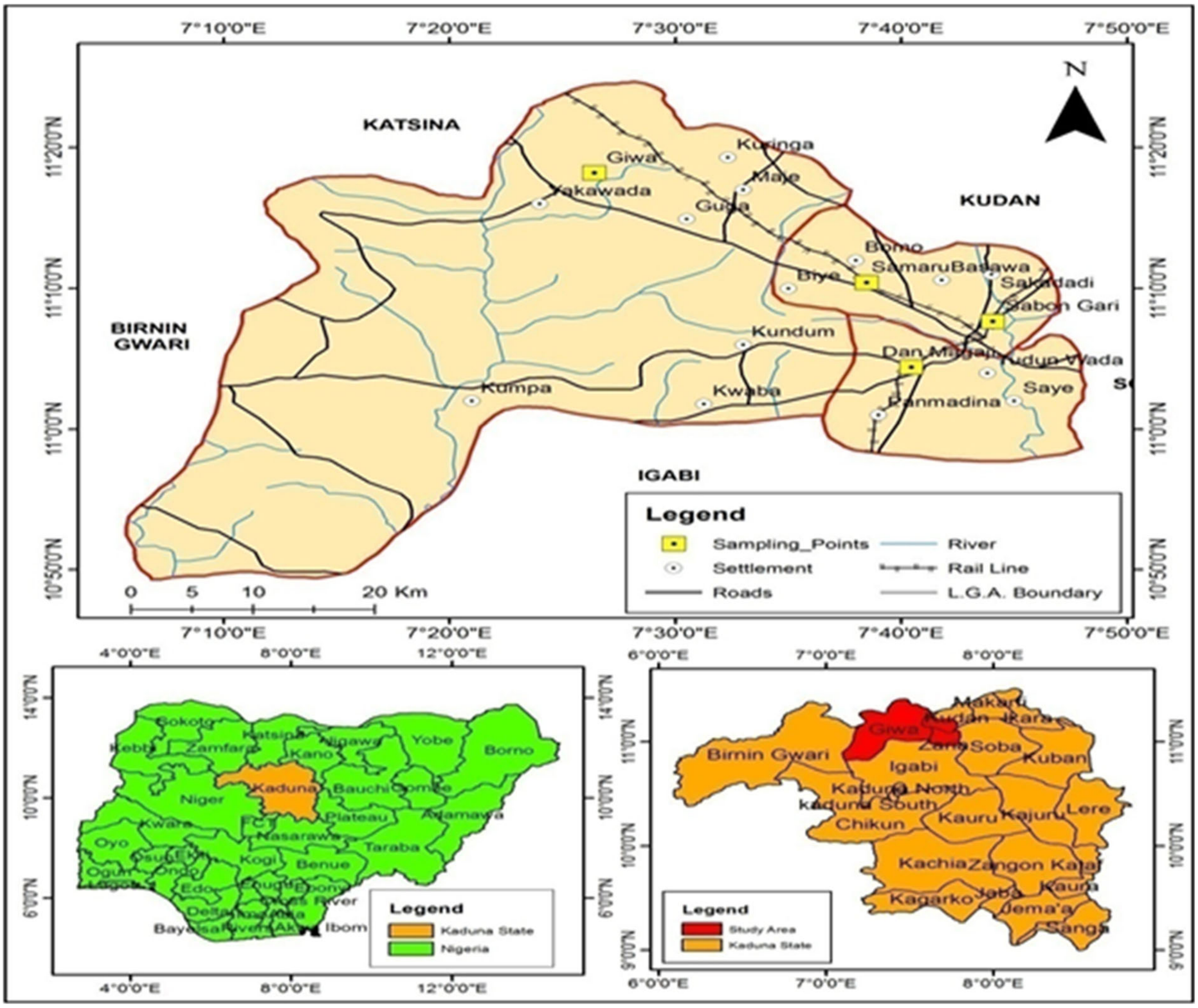

Fig. 1 Map of Zaria showing the location of the different markets from which vegetables were purchased (adapted from Administrative Map of Kaduna State, 2015)

other particles on them prior to analysis. The leaves of the vegetables were cut with a razor blade into smaller pieces, weighed to 2 g, and transferred into well-labeled sample bottles and preserved at – 40 °C until MC analysis. Subsequently, the samples were homogenized using ceramic mortar and piston in 80% methanol (v/v) to obtain a homogenous matrix (Diez- Quijada et al. 2018; Lefebvre 2013). The choice of 80% methanol was to dissolve the intracellular toxins (Turner et al. 2017). The homogenate was centrifuged at 2000×*g* for 20 min at room temperature and the supernatant extracted. The procedure was repeated three times to ensure maximum extraction of MCs. The total MC content of the extract was determined using the Abraxis enzyme-linked immunosorbent assay (ELISA) kit per the manufacturer's instructions. The absorbance of the color reaction of the assay was measured with a Bio-Rad iMark™ Microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). MC concentrations were presented in micrograms per gram.

Bioaccumulation of MCs in the vegetables by a child and an adult

TDI of MCs in vegetables by a child and an adult was carried out by assuming that 40 g of each vegetable is consumed per day by an adult weighing 60 kg and 10 g of each vegetable is consumed per day by a child weighing 25 kg (Cordeiro-Araujo et al. 2016).

$$\mathrm{TDI}_{\mathrm{vegetable}} = {}^{\mathrm{MCs}_{40\ \mathrm{g}^{-1} or\ 10\ \mathrm{g}^{-1}}}/_{BM_{\mathrm{adult\ or\ child}}} \quad (1)$$

where $\mathrm{MCs}_{40\ \mathrm{g}^{-1}\ \mathrm{or}\ 10\ \mathrm{g}^{-1}}$ is the amount of MCs accumulated per 40 g or 10 g of vegetable and $BM_{\mathrm{adult\ or\ child}}$ is the body mass of an adult (60 kg) or a child (25 kg).

Data analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) at 5% significant level. Where significant differences were observed, the means were separated using Fisher's least significant difference (LSD) post hoc test.

Results

Levels of MCs in vegetables and total daily intake of MCs from contaminated vegetables obtained from different markets

Overall, 53% of the vegetable samples had MC concentrations that were higher than 1.00 μg/g (Table 1). *A. hybridus* had the highest MC concentration (4.79 μg/g), followed by *L. sativa* (4.41 μg/g) and *B. oleracea* (2.11 μg/g) in Sabon Gari Market (Table 1). Similar to the results obtained in Sabon Gari Market, *A. hybridus* had the highest MC concentration, while *L. sativa* had the lowest (0.17 μg/g) in Dan-Magaji Market. *A. hybridus* had the highest concentration of MCs, while in Giwa and Samaru markets, *L. sativa* had the highest concentration. In Samaru Market, the highest concentration of MCs was detected in *L. sativa* (2.09 μg/g), followed by *A. hybridus* (1.38 μg/g) and *B. oleracea* (0.62 μg/g). *L. sativa* (2.98 μg/g) also contained the highest levels of MCs in Giwa Market, followed by *B. oleracea* (0.95 μg/g) and *A. hybridus* (0.82 μg/g) (Table 1).

The TDI of MCs by an adult (TDIA) and a child (TDIC) revealed that the vegetables obtained from Sabon Gari Market generally had the highest values (Table 1). For example, TDIA and TDIC of *A. hybridus* were 3.19 μg/kg and 1.19 μg/kg, respectively, in Sabon Gari Market, whereas the values in Dan-Magaji (TDIA = 1.43 μg/kg, TDIC = 0.86 μg/kg), Samaru (TDIA = 0.92 μg/kg, TDIC = 0.55 μg/kg), and Giwa (TDIA = 1.43 μg/kg, TDIC = 0.86 μg/kg) markets were lower. The lowest TDIA and TDIC of MCs were 0.41 μg/kg and 0.25 μg/kg, respectively, for *B. oleracea* obtained from Samaru Market, and 0.11 μg/kg and 0.07 μg/kg, respectively, for *L. sativa* obtained from Dan-Magaji Market. Regardless of the market or vegetable type assessed, the TDIA and TDIC values were higher than the 0.04-μg/kg limit set by the WHO.

Risk factors of exposure to MCs

Besides the physical visits to vegetable farms that supply markets in Zaria, Nigeria, traders were interviewed to obtain data on the source, method of cultivation, and availability as risk factors of exposure to MCs. This showed that 100% of vegetables analyzed in this study were cultivated via irrigation (Table 2). Seventy-two percent of the vegetables analyzed were obtained from large commercial farms, 25% from small farms, and 3% from small household farms. Sixty-one percent of traders agreed that water used for irrigation was obtained from the river, 19% from a river or borehole, 8% from a borehole or well, 6% from a well or river, and 3% from a well and also 3% from a borehole (Table 2). Fifty-eight percent of the water was colored while 25% was slightly colored and 17% was colorless.

As a result of the method of cultivation, vegetables were available, leading to high sales/purchases. Fifty percent of the respondents agreed that the availability of vegetables was not seasonal, while 50% disagreed (Table 3). However, 53% agreed that the rainy season had more supply of vegetables compared with the dry season. Forty-two percent agreed that both seasons had high sales, while 5% went for the dry season. Due to availability also, 50% of traders purchase these vegetables in large quantity while 50% in small quantity. Forty-two percent buy after 2 to 3 days while 17% buy after 3 days or weekly; 5% buy weekly while 25% buy daily. Fifty percent of these vegetables are purchased from middlemen or third parties, while 28% of traders get vegetables themselves without the help of middlemen and 22% get it themselves and from middlemen (Table 3). Eighty-three percent agreed that the volume of sales was large, while 17% was small. Forty-four percent of sales were not seasonal, while 39% were seasonal, and 17% was not sure whether sales were seasonal or not. Fifty-six percent of sales were in the rainy seasons, while 14% were in the dry season, and

Table 1 Microcystin contamination of *Amaranthus hybridus*, *Brassica oleracea*, and *Lactuca sativa* obtained from different market places in Zaria, Nigeria

Market	Vegetable	MC (μg/g)	TDIA (μg/kg)	TDIC (μg/kg)
Sabon Gari	*Amaranthus hybridus*	4.79 ± 2.61	3.19 ± 1.74	1.91 ± 1.04
	Brassica oleracea	2.11 ± 1.06	1.41 ± 0.71	0.85 ± 0.42
	Lactuca sativa	4.41 ± 1.10	2.94 ± 0.73	1.77 ± 0.44
Dan-Magaji	*Amaranthus hybridus*	2.14 ± 1.15	1.43 ± 0.76	0.86 ± 0.46
	Brassica oleracea	0.64 ± 0.30	0.43 ± 0.20	0.25 ± 0.12
	Lactuca sativa	0.17 ± 0.17	0.11 ± 0.11	0.07 ± 0.07
Samaru	*Amaranthus hybridus*	1.38 ± 0.28	0.92 ± 0.19	0.55 ± 0.11
	Brassica oleracea	0.62 ± 0.08	0.41 ± 0.05	0.25 ± 0.03
	Lactuca sativa	2.09 ± 0.75	1.39 ± 0.50	0.84 ± 0.30
Giwa	*Amaranthus hybridus*	0.82 ± 0.25	0.54 ± 0.17	0.33 ± 0.10
	Brassica oleracea	0.95 ± 0.39	0.63 ± 0.26	0.38 ± 0.15
	Lactuca sativa	2.98 ± 0.50	1.99 ± 0.33	1.19 ± 0.20

MC, total microcystin concentrations (μg/g) in vegetable tissues; *TDIA*, total daily intake of microcystins (μg/kg) by an adult; *TDIC*, total daily intake of microcystins (μg/kg) by a child. Values are mean plus and minus standard deviation for $n = 3$

30% agreed that sales were high in both the rainy and dry seasons. Forty percent of the vegetables sold were leafy vegetables, 22% were fruit vegetables, 13% were root vegetables, 8% were seed vegetables, and 17% were spices (Table 3). From the above responses gathered from the traders, the risk factors were as follows: inferior irrigation methods and facilities, contaminated water from rivers, and lack of irrigation monitoring and regulation by concerned agencies.

Table 2 Source and method of cultivation of vegetables. Values are in percentage (%) distribution

S/no.	Parameter	Description	Distribution (%)
1.	Source of vegetables	Household farms	3
		Small farms	25
		Large farms	72
2.	Method of watering	Normal	
		Irrigation	100
3.	Source of water	Borehole	3
		Well	3
		River	61
		Borehole/well	8
		Borehole/river	19
		Well/river	6
4.	Color of water used	Non-colored	17
		Not too colored	25
		Colored	58

Discussion

Mean concentration of MCs in vegetables

Leaves of vegetable crops form an integral part of the human diet, which means substances that contaminate them directly threaten human health. Irrigation of plants with MC-contaminated water is an important pathway of human exposure to these bioactive metabolites (Bittencourt-Oliveira et al. 2016; Chen et al. 2004). In the present study, high concentrations of MCs were detected in the leaves of *A. hybridus*, *L. sativa*, and *B. oleracea*. This is not surprising as the leaves of irrigated vegetables tend to retain higher concentrations of MCs than other plant parts. For example, the leaves of *B. oleracea*, *Brassica juncea*, and *Salix alba* (Kittler et al. 2012); *Lycopersicon esculentum* (Gutierrez – Praena et al. 2013); and *Brassica rapa chinensis* and *Cucurbita pepo* (Peuthert et al. 2015) have been shown to bioaccumulate higher amounts of MCs than other organs. This could be related to higher evapotranspiration rate taking place at the leave surfaces compared with other organs that are not directly exposed to solar irradiation (Constable and Rawson 1980).

Table 3 Availability, purchases, and sales of vegetables. Values are in percentage (%) distribution

S/N	Title	Parameters	Percentage (%) distribution
1.	Availability of vegetable	Not seasonal	50
		Seasonal	50
2.	Season of abundance	Rainy	53
		Dry	5
		Both	42
3.	Method of purchase	Self	28
		Third party	50
		Both	22
4.	Frequency of purchase	Daily	25
		Daily/after 2 days	11
		After 2/3 days	42
		After 3 days/weekly	17
		Weekly	5
5.	Amount purchase	Small	50
		Large	50
6.	Type of vegetable sold by trader	Spices	17
		Seed vegetable	8
		Root vegetable	13
		Fruit vegetable	22
		Leafy vegetable	40
7.	Location of purchase	Same location	8
		Different location	56
		Not always	36
8.	Volume of sales	Small	17
		Large	83
9.	Seasonality of sales	Not seasonal	44
		Not sure	17
		Seasonal	39
10.	Season with highest sales	Rainy	56
		Dry	14
		Both	30

The differences observed in the MC content of the investigated vegetables are not surprising as plants have different physiological, biochemical, and morphological adaptations that determine the rate at which they uptake and bioaccumulate MCs (Bittencourt-Oliveira et al. 2016; Cordeiro-Araujo et al. 2016). Pindihama and Gitari (2017) found that *A. hybridus* generally bioaccumulated MCs at a faster rate than *Ludwigia adscendens* when both plants were exposed to aqueous extracts containing cyanotoxins. Similar to our results, Mohammed and Al-Shehri (2009) observed that the levels of MCs in *A. hybridus* were higher than those detected in other vegetables from irrigation farms. The authors recorded concentrations as high as 70–1200 μg/kg fw of MCs. In terms of morphological variations, the size of leaves influences the rate of MC uptake and bioaccumulation in spray-irrigated vegetables (Crush et al. 2008). Plants with larger leaf surface areas tend to retain and uptake more MCs than those with smaller surface areas. Crush et al. (2008) demonstrated that higher concentrations of MCs were detected in *L. sativa* and *Trifolium repens* with broader leaves than in *Brassica napus* and *Lolium perenne* with smaller leaves. Plants also differ in the rates at which they can excrete or depurate MCs and chemically modify and/or detoxify MCs with antioxidant enzymes (Cordeiro-Araujo et al. 2016). Furthermore, the differences in MC concentration of vegetables obtained from the different markets might be because they were sourced from different farms. Depending on the location, the farms are irrigated with water from different aquatic ecosystems with different MC concentrations. Higher MCs in samples collected from Sabo-Gari Market could be attributed to a higher concentration of MCs in its irrigated water relative to other locations. The dry season during which irrigation farming takes place in Zaria, Nigeria, is characterized by reduced water dilution and increased eutrophication of lakes and reservoirs used for irrigation. The eutrophication of these aquatic ecosystems leads to the excessive proliferation of cyanobacteria and production of cyanotoxins (Chia et al. 2009a; Chia and Kwaghe 2015).

Total daily intake of MCs by humans (adult/child) and risk factors

The uptake and bioaccumulation of MCs by edible plants have significant implications for public health as these metabolites can negatively affect the normal functioning of various organs of the human body such as the liver by inhibiting protein phosphatases (McElhiney et al. 2001). The concentration of MCs in vegetable tissues is usually directly proportional to TDI by humans. Therefore, the higher the concentration of MCs in vegetables in the present study, the higher the TDI by adults and children. The TDIA and TDIC values were above the recommended daily intake limit of 0.04 μg per kg body weight per day by WHO. This has grievous consequences on human health, particularly in children and the aged. According to Wood et al. (2004), the tolerable daily intake of MC-LR set by WHO is a recommended limit for healthy adults, implying that children, elderly, and sensitive/unhealthy individuals are at a higher risk of suffering from the adverse effects of MC ingestion. The risk is higher when exposure is consistent and frequent over a long period. According to Zhang et al. (2010), chronic toxic effects from exposure through food are possible, especially if it is long-term and frequent exposure. This is a serious problem because the investigated vegetables form an integral part of the daily diet of individuals in this part of the world. Unfortunately, diagnosing MC-related symptoms in patients in this part of the world will be nearly impossible as they are yet to be incorporated into the common causes of ailments.

The human health risks from TDI of MCs are plant-specific (Cordeiro-Araujo et al. 2016) and proportional to degree and rate of bioaccumulation (Crush et al. 2008), and the amount and frequency of consumption of vegetables as indicated in the current results. The present study revealed that frequently consuming *A. hybridus* poses more risk than consuming *L. sativa* and *B. oleracea*, as the former had the highest concentration of MCs throughout the study. Our data also revealed that a higher proportion of the population buys at least one of these vegetables once per week. This demonstrates a chronic human exposure problem. The chronic exposure problem is further complicated by the fact that these vegetables are either eaten raw or cooked, and cooking increases the risk of MC exposure because the heat increases the extraction of the MCs (Zhang et al. 2010). Furthermore, several studies have shown that MC-LR is resistant to heat, being stable at temperatures as high as 300 °C (Bruno et al. 2012; Dietrich and Hoeger 2005; Harada et al. 1996).

The results of the present study highlight a growing and relatively unmonitored problem in developing countries that needs urgent attention. While the technical know-how may not be there, governments of developing countries must develop policies and management programs that will aid the control of the risk factors identified in the present study such as avoiding the use of waters containing blooms of cyanobacteria for irrigation, monitoring of water used for irrigation for cyanotoxin contamination, monitoring of vegetables sold to vendors for toxin contamination, and improved general cultural practices to ensure the protection of public health. The establishment of monitoring programs will ensure that the risks identified in the present study are minimized or eliminated. This will ensure that the source of vegetables and quality of water used for irrigation are safe for human consumption.

Conclusion

We confirm that the vegetables sold in market places in Zaria, Nigeria, contain MCs at concentrations exceeding the WHO limit.

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