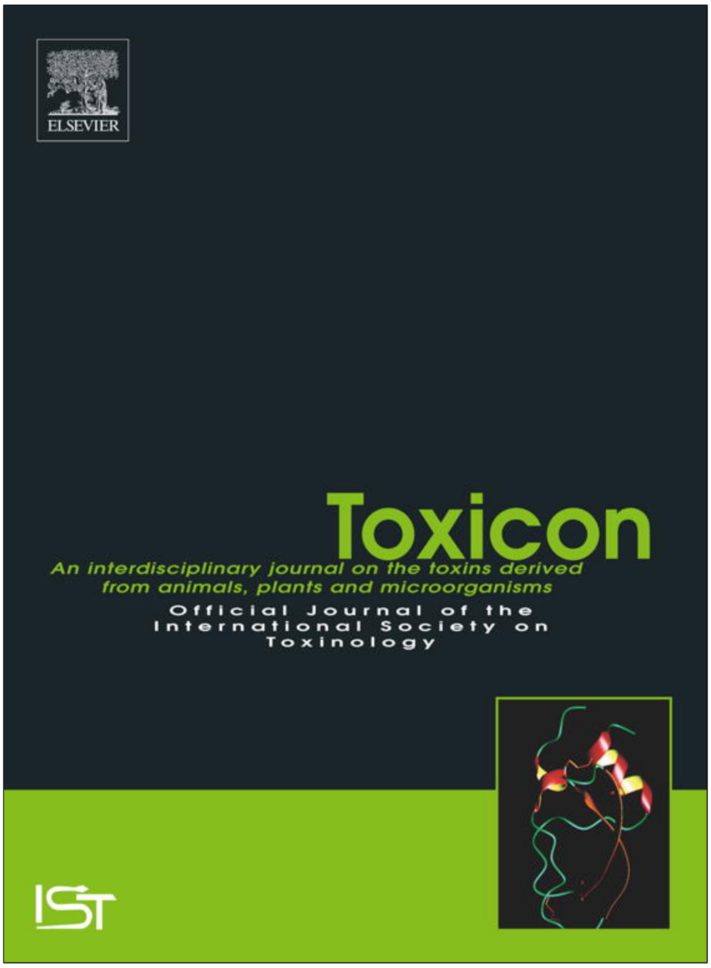

ELSEVIER

Toxicon

An interdisciplinary journal on the toxins derived from animals, plants and microorganisms

Official Journal of the International Society on Toxinology

IST

Toxicon 114 (2016) 75–84

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Cyanobacteria and cyanotoxins are present in drinking water impoundments and groundwater wells in desert environments

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ARTICLE INFO

Article history:
Received 24 October 2015
Received in revised form
11 February 2016
Accepted 17 February 2016
Available online 24 February 2016

Keywords:
Desert
Cyanobacteria
Cyanotoxins
Desalinated water
Sustainable water management
Human health

ABSTRACT

Desert environments and drylands experience a drastic scarcity of water resources. To alleviate dependence on freshwater for drinking water needs, countries have invested in infrastructure development of desalination plants. Collectively, the countries of the Arabian Gulf produce 45% of the world's desalinated water, which is stored in dams, mega-reservoirs and secondary house water tanks to secure drinking water beyond daily needs. Improper storage practices of drinking water in impoundments concomitant with increased temperatures and light penetration may promote the growth of cyanobacteria and accumulation of cyanotoxins. To shed light on this previously unexplored research area in desert environments, we examined drinking and irrigation water of urban and rural environments to determine whether cyanobacteria and cyanotoxins are present, and what are the storage and transportation practices as well as the environmental parameters that best predict their presence. Cyanobacteria were present in 80% of the urban and 33% of the rural water impoundments. Neurotoxins BMAA, DAB and anatoxin-a(*S*) were not detected in any of the water samples, although they have been found to accumulate in the desert soils, which suggests a bioaccumulation potential if they are leached into the aquifer. A toxic BMAA isomer, AEG, was found in 91.7% of rural but none of the urban water samples and correlated with water-truck transportation, light exposure and chloride ions. The hepatotoxic cyanotoxin microcystin-LR was present in the majority of all sampled impoundments, surpassing the WHO provisional guideline of 1 μg/l in 30% of the urban water tanks. Finally, we discuss possible management strategies to improve storage and transportation practices in order to minimize exposure to cyanobacteria and cyanotoxins, and actions to promote sustainable use of limited water resources.

1. Introduction

Arid environments are distinctly characterized by water scarcity. Both natural phenomena and anthropogenic manipulations contribute in making water a limiting natural resource (MEA, 2005; UNCCD, 2012). High temperatures and low precipitation accelerate evaporation rates of surface water, thus reducing groundwater recharge and pushing fauna and flora to the limits of biological existence (Warren-Rhodes et al., 2006). Additionally, improper management of groundwater reservoirs contributes to the fast rate of water depletion and its enrichment with salts, which further poses managerial problems on the agricultural and farming sectors as they rely on groundwater to irrigate crops and water livestock (FAO, 1994, 2008). Soil salinization leads to agricultural unsuitability of the land and reduction of crop yields, posing a threat to food security (Al-Rawahy et al., 2010).

For all the above reasons an increased dependence on marine water desalination is observed. In the Gulf State of Qatar, water demand and thus desalinated water production has increased from 96 Million Imperial Gallons per Day (MIGD) in the year 2000 to 325 MIGD in the year 2011 (QGSDP, 2011). Households in urban environments of Qatar are serviced with drinking water originating from five Multi Stage Flash Distillation (MSF) desalination plants, two Reverse Osmosis (RO) plants and one Multi-Effect Distillation (MED) plant (Dawoud and Al Mulla, 2012). An additional RO plant

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http://dx.doi.org/10.1016/j.toxicon.2016.02.016
0041-0101/

has been commissioned to further accommodate the increasing water demand of an exponentially growing population (4.5% average annual growth in 2014; WB, 2015). The dramatic rate of population increase is currently outpacing the rate of water delivery infrastructure development thereby increasing the reliance on water storage systems.

Desalinated water is diverted and stored in mega-water tanks and reservoirs. Main water tanks of residential compounds are directly connected to the water network through pipes (KAHRAMAA, 2015; QSA, 2015). The water then gets distributed to secondary water storage tanks, commonly located on the terrace of each house. In rural environments on the other hand, water is transported to households and farms in water-trucks as there is no direct pipe connection to the water network. The practice of storing water in low-pressure water tanks is a remnant of the British influence in city planning, used as a strategy to combat high peak demand and increase water security (IBP, 2004).

Increased temperatures of the spring and summer months when combined with agricultural and/or industrial discharges lead to eutrophication of water bodies and cyanobacterial bloom formations ensue in arid, temperate and tropical climates alike (Codd et al., 2005; Costa et al., 2006; Mohamed and Al Shehri, 2009; Merel et al., 2013). Cyanobacteria can produce secondary metabolites that negatively affect human and animal health (Banack et al., 2010a; Metcalf and Codd, 2012 and references therein). In our previous work in Qatar we have documented the presence of cyanobacteria and their toxins in terrestrial crusts (Metcalf et al., 2012; Richer et al., 2012); that toxins are persistent in the desert soils (Richer et al., 2015); and that consumption of rain-water accumulated on top of cyanobacterial crusts may cause animal poisonings (Chatziefthimiou et al., 2014).

In this article, we report the findings of our study designed to assess whether humans are directly exposed to cyanotoxins through the consumption of drinking water or indirectly through the consumption of produce irrigated with toxin contaminated water. Our specific questions were: are there cyanobacteria and cyanotoxins in water sources of urban and rural environments? Does the mode of water transportation and storage practices affect the growth of cyanobacteria and production of cyanotoxins? Which environmental parameters best predict the presence of cyanobacteria and cyanotoxins? The specific toxins investigated in this study were the neurotoxins β-N-methylamino-L-alanine (BMAA), and its isomers N-(2-aminoethyl)glycine (AEG), 2,4-diaminobutyric acid (DAB), anatoxin-a(*S*) and the hepatotoxin microcystin-LR.

2. Materials and methods

2.1. Collection of water samples in urban and rural environments

Samples from water tanks of urban compounds were collected between the months of February and April 2014. Our sampling scheme was designed so as to include representative drinking water samples from all major urban cities in Qatar. Seven of the water samples were collected from compounds in the greater Doha area: 1 from Simaisma and 1 from Al Khor located North of Doha, and 1 from Al Wakra, located South of Doha (Fig. 1). Participants were given nitrile gloves, a one-liter sterilized glass bottle and written instructions indicating how to sample water tanks aseptically. Water samples were stored in the dark covered with tin foil to prevent post sampling growth of photosynthetic cyanobacteria.

All rural-environment water samples were collected from the greater area of Al Kharrara in Central Qatar on February 15, 2014 (Fig. 1). A total of 12 samples were obtained from 2 camel farms, 1 agricultural farm, 2 sheep and goat farms and 1 house. In contrast to the urban setting, water from the rural setting served multiple functions including drinking for humans and animals, irrigation, and washing (Table 1 Supplementary Materials). Furthermore, rural water originated from desalination plants and groundwater wells, as compared to the urban compounds receiving water only from desalination plants. At the time of collection, the owners of the water tanks in both urban and rural settings were asked to fill out an Institutional Review Board (IRB) exempt questionnaire pertaining to the source and means of transportation of the water; the appearance and odor of the water; and the material, color, location and internal appearance of the water tank (Table 1 Supplementary Materials).

2.2. Collection of soil profile samples

Soil profile samples were collected on December 4, 2013 from a natural depression in the Al Kharrara area. This site is covered with thin biological desert crust and serves as a long term monitoring station for our research team (Richer et al., 2015). Surface soil samples were collected aseptically from three adjacent plots, followed by 5 cm vertical incremental sampling up to 25 cm depth. Soil profile samples were kept in the dark at room temperature and analyzed for BMAA, AEG and DAB as described below.

2.3. Microscopic observations of water samples

Water sub-samples were fixed using Lugol's Iodine or 20% (v/v) ethanol and were observed with a fluorescence microscope as described in Metcalf et al. (2012). Cyanobacteria were identified to order and genus levels based on taxonomic classification guidelines by Whitton (2002).

2.4. Chemical species analysis

Sub-sample chemical species characterization of all 22 collected water samples was performed using the YSI Professional Plus Multiparameter Instrument with attached probes for pH, ammonium (NH_4^+), chloride (Cl^-), and nitrate (NO_3^-) ions, following manufacturer's instructions (YSI Inc., Ohio, USA).

2.5. Extraction of toxins

Sub-samples of each water sample were aseptically filtered through Whatman Glass Microfiber Filters, GF/C, 47 mm (Sigma Aldrich, Missouri, USA). The filters were then lyophilized in a LabConco FreeZone® Plus™ 4.5 L Cascade Console Freeze Dry System (Labconco, Missouri, USA). Microcystin-LR and anatoxin-a(*S*) were extracted from filters in 100% methanol followed by ultrasonication. A secondary extraction in 70% methanol followed by ultrasonication was also performed as described in detail in Metcalf et al. (2006). To extract BMAA and its isomers AEG and DAB, filters of water samples and soil sub-samples of soil profiles were hydrolyzed in 1 ml of 6 M hydrochloric acid and prepared according to Richer et al. (2015).

2.6. Analysis of toxins

Extracted BMAA, AEG and DAB were separated by a Waters Acquity-UHPLC system with a Binary Solvent Manager and a Waters AccQTag Ultra column (part# 186003837, 2.1 × 100 mm) at 55 °C and analyzed by LC-MS/MS (Thermo Scientific Finnigan TSQ Quantum UltraAM, CA, USA) using standard methods and protocols (Banack et al., 2010b, 2012; Richer et al., 2015). Microcystin-LR was assessed using an Acquity ultra performance liquid chromatography system (Waters Corp., MA, USA) with photodiode array (PDA) detection at 238 nm (Meriluoto and Codd, 2005; Metcalf et al.,

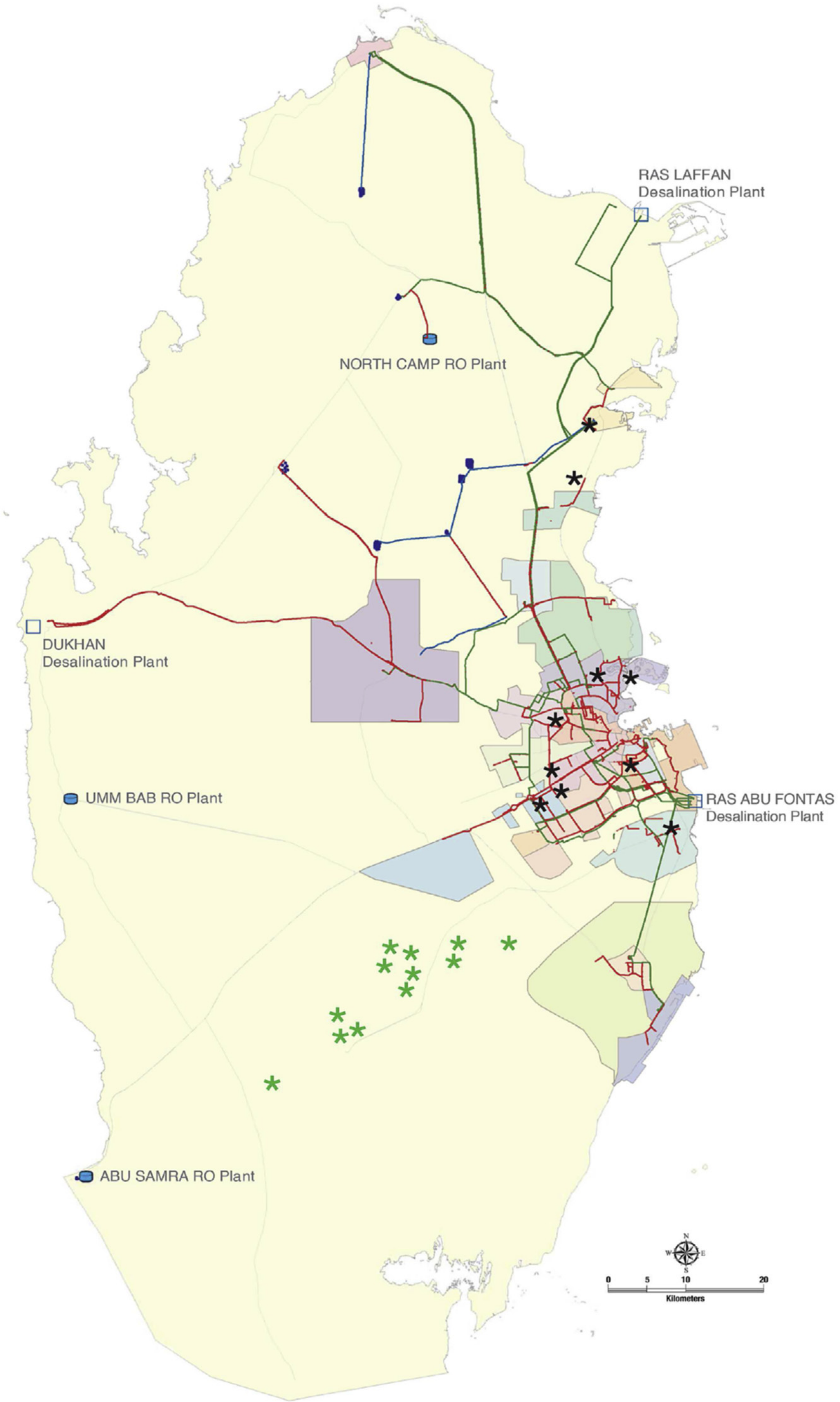

Fig. 1. Map of sampling locations in the State of Qatar superimposed on the water network. Black asterisks denote sampled water tanks in urban environments and green asterisks sampled water impoundments of households and farms in rural environments. Water district areas are color-coded block areas. Blue squares: MSF and MED desalination plants; blue cylinders: RO desalination plants; green lines: trunk/distillate main; blue line: well field main; red lines: rising main; blue circles: potable water well fields (QSA, 2015).

2006). Anatoxin-a(*S*) analysis was performed using a colorimetric acetylcholine esterase inhibition assay adapted from Mahmood and Carmichael (1987).

2.7. *Chlorophyll a extraction and analysis*

Chlorophyll a was extracted from lyophilized filter papers obtained from a known volume of filtrate, as described above, in 90% acetone at concentrations between 0.4 and 1.1 g (dry wt.)/ml, and incubated overnight at 4 °C in the dark (SCOR-UNESCO, 1966). Samples were then centrifuged at maximum speed and 0.25 ml of the supernatant transferred in triplicate to a 96-well microtiter plate. Samples were spectrophotometrically measured at wavelengths of 630, 645, 663 and 750 nm using a PowerWave XS Microplate Spectrophotometer (BioTek®, Vermont, USA). Chlorophyll a concentration in ng/ml of filtrate was calculated as

$11.64(A_{663}) - 2.16(A_{645}) + 0.1(A_{630})$, after subtraction of the absorbance at 750 nm from each of the 630, 645, 663 nm absorbance readings.

2.8. Statistical analysis

For water results, categorical binary data from questionnaire responses (Table 1 Supplementary Materials) and cyanobacterial species composition, as well as numerical results for chemical species analysis (pH, NH_4^+, Cl^- and NO_3^-), toxins and chlorophyll a concentrations were used in the statistical analysis. BMAA, DAB and anatoxin-a(*S*) were below the detection limit in all tested water samples and thus were excluded from the analysis. Microcystin-LR, NO_3 and NH_4 were log-transformed to achieve normal distribution. In more detail, bivariate linear regressions were conducted to evaluate the association between each outcome (cyanobacterial species composition, pH, NH_4^+, Cl^-, NO_3^-, AEG, microcystin-LR and chlorophyll a) and the inputs (water origin, transportation, appearance, odor and tank location, color, appearance). Additionally, non-parametric methods were used to examine the crude relationship between the remaining outcomes (AEG and Cl^-) and the inputs (Motulsky, 2010). The type of non-parametric test used depended on the input's variable type: the Spearman's correlation test for continuous input variables, the Wilcoxon rank-sum test for dichotomous variables, and the Kruskall–Wallis test for multi-categorical input variables. P-values are reported and tested for each association at the level of significance of 0.05. All analyses were performed in STATA/IC version 13.0.

For soil profile results, data trends were visually evaluated for each toxin (AEG, BMAA, and DAB) and the depth of the soil horizon. Each of the outcomes was log-transformed to statistically prove that the data followed a normal distribution. A series of linear bivariate and multivariate regressions were conducted to evaluate the crude and the adjusted associations between the different outcomes and soil horizon depth, controlling for the presence of the remaining soil toxins in the latter. P-values were reported and tested for each association at the level of significance of 0.05. All statistical analyses were performed using IBM SPSS Statistics 22.

3. Results

Rural and urban environments exhibited distinct patterns in terms of tank and water use characteristics (Table 1). All of the urban tanks are supplied with drinking water treated in a desalination plant and are directly connected to the water network. In rural environments one third of the water supply comes from groundwater wells and is destined for irrigation and washing purposes. Desalinated drinking water to the remaining water tanks is transported by truck from the reservoirs. Sixty percent of the urban tanks and all of the rural water impoundments were directly exposed to the sunlight and the majority of the water tanks in both environments were covered (urban 100%, rural 91.7%). The material of the tank was very diverse in both settings including plastic, metal, and fiberglass. The appearance of water within the tanks was uniformly clear in both settings and only 20% of the urban tanks emanated an odor.

Cyanobacterial growth was visible on the inside walls of 40% of the urban tanks and 66.7% of the rural tanks (Table 1). In some cases, the presence of cyanobacterial growth could not be assessed due to an obstructed view, as is the case for groundwater wells, although the soil surrounding the well-pump was covered by active green cyanobacterial crusts. Chlorophyll a, used as a proxy for cyanobacterial growth, was observed in all water impoundments with the exception of one urban tank, and attained higher concentration ranges of 0.112–8.047 ng/ml in the urban environment than in the rural environments (0.119–0.991 ng/ml). Microscopic observation of the water sub-samples revealed the presence of known toxin producing Cyanobacteria of the order *Chroococcales* and the genus *Microcoleus* in 80% of the urban tanks and in 33% of the rural ones (Cox et al., 2005; Metcalf et al., 2015). The protozoan parasite *Cryptosporidium* known to cause recreational water illness (RWI) and food poisoning was found in one sub-sample of water in the rural setting (Table 1 Supplementary Materials; Cantey et al., 2012). This water was primarily used for washing.

The BMAA isomer AEG was found in 11 out of 12 (91.7%) of the water sub-samples of the rural impoundments and in none of the urban sub-samples (Table 1). Besides the rural location (Wilcoxon rank-sum test, p-value = 0.0001), presence of AEG was also correlated with statistical significance to water-truck transportation (Kruskall–Wallis test, p-value = 0.0005), tank exposure to direct sunlight (Wilcoxon rank-sum test, p-value = 0.0454), and to the concentration of chloride ions in the water (Spearman's correlation test, p-value = 0.0072; Table 2). The neurotoxins BMAA, DAB (structural isomers of AEG) and anatoxin-a(*S*) were not found in any of the tanks sampled in this study. The hepatotoxin microcystin-LR was found in 83.3% of the rural water samples, and in all of the urban samples. In 3 of the urban water tanks the concentration of microcystin-LR surpassed the World Health Organization (WHO) provisional guideline of 1 μg/l and was significantly and positively correlated to tank material (fiberglass; p-value = 0.035) and odor in the water (p-value = 0.027; Tables 2 and 1 Supplementary Materials; WHO, 2011a). The pH of all water impoundments fell within the WHO provisional guideline for drinking water (6.5–8.5; WHO, 2011a). The concentration range of ammonium (NH_4^+), chloride (Cl^-), and nitrate (NO_3^-) ions was higher in the rural setting and correlated significantly with characteristics associated to groundwater wells, no transportation and no tank. In 2 of the groundwater wells the Cl^- concentration was about 2.5 times higher than the Food and Agriculture Organization (FAO) threshold for irrigation water that will cause severe restriction of crop growth and health (FAO, 1994).

The BMAA isomer AEG was detected at all but one depth (0–5 cm) in the sub-samples of the soil profile and there was no statistical significance relative to depth (Tables 3 and 4). BMAA and DAB were detected in all soil profile samples, decreasing with depth. LogBMAA was significantly correlated with the presence of both AEG (p-value = 0.034) and DAB (p-value < 0.001), but not with depth. LogDAB was correlated with soil profile depth (p-value = 0.007) and BMAA (p-value = 0.029), yet not with AEG.

4. Discussion

4.1. Cyanobacteria and cyanotoxins in drinking and irrigation water and risk assessment

Cyanobacteria and cyanotoxins are present in water impoundments and groundwater wells destined for drinking and irrigation purposes in the State of Qatar. On a global scale, there is a rich body of literature with respect to the presence of cyanobacteria and toxins in aquatic environments and in water treatment plants with a particular focus on the hepatotoxins microcystin-LR and cylindrospermopsin, and the neurotoxin anatoxin-a (Carmichael et al., 2001; Metcalf and Codd, 2012). Physiologically, in cyanobacteria, it is speculated that toxins may act as intracellular chelators of iron, or regulators of cellular nitrogen assimilation according to nitrogen availability and light intensity (Utkilen and Gjolme, 1995; Downing et al., 2011). From an ecological standpoint, cyanobacterial toxins are thought to serve as attractants of bacterial heterotrophs inhabiting the same niche to cooperate on the cycling of nutrients (Paerl and Millie, 1996). It is also speculated that cyanotoxins act as

Table 1
Compiled and averaged results from questionnaires, microscopy, toxin and chemical analyses of water samples.

	Tank and water characteristics/Sampled environment			Urban (%)[a]	Rural (%)[b]
1	Use of water	a	Drinking	100	50
		b	Washing	0	25
		c	Irrigation	0	25
2	Source of water	a	Desalination plant	100	66.7
		b	Groundwater well	0	33.3
3	Water transported	a	In a truck	0	75
		b	Through a pipe	100	0
		c	Not transported	0	25
4	Location of tank	a	Under the sun	60	100
		b	In the shade	40	0
5	Material of tank	a	Metal	10	41.7
		b	Plastic	50	25
		c	Fiberglass	30	8.3
		d	Concrete	10	0
		e	Stone	0	0
		f	No tank	0	25
6	Color of tank	a	White	90	41.7
		b	Black	0	0
		c	Yellow	0	0
		d	Blue	0	8.3
		e	Gray	10	25
		f	No tank	0	25
7	Is the tank	a	Covered	100	91.7
		b	Uncovered	0	8.3
8	Appearance of water	a	Clear	100	100
		b	Greenish	0	0
		c	Gray	0	0
		d	Black	0	0
9	Does the water have odor	a	Yes	20	0
		b	No	80	100
10	Cyanobacterial growth	a	Yes	40	66.7
		b	No	50	0
		c	Obstructed view	10	33.3
11	Chlorophyll a ([range] ng/ml)			90 (0.112–8.047)	100 (0.36–1.835)
12	Cyanobacterial species observed under the microscope			80 (Chr[c], Mic[d])	50 (Chr, Mic, Crypto[e])
13	Toxins[f]	a	AEG ([range] ng/ml)	0 (ND[g])	91.7 (0.001–0.0035)
		b	BMAA ([range] ng/ml)	0 (ND[h])	0 (ND)
		c	DAB ([range] ng/ml)	0 (ND[i])	0 (ND)
		d	Anatoxin-a(*S*) ([range] ng/ml)	0 (ND[j])	0 (ND)
		e	Microcystin-LR[k] ([range] μg/l)	100 (0.6628–1.3309)	83.3 (ND[l] – 0.8902)
14	pH[m]			7.96–8.1	8.01–8.23
15	(NH_4^+)[n] ([range] (mg/l)			100 (0.01–0.53)	100 (0.02–3.25)
16	(Cl^-)[o/p] ([range] (mg/l)			100 (2.41–12.98)	100 (2.03–902.72)
17	(NO_3^-)[q] ([range] (mg/l)			100 (0.18–0.78)	100 (0.06–7.64)

[a] Ten water tanks were sampled in the Urban environment.
[b] Twelve water tanks were sampled in the Rural environment.
[c] *Chroococcales*.
[d] *Microcoleus*.
[e] *Cryptosporidium*.
[f] There are no WHO provisional guidelines for AEG, BMAA, DAB and Anatoxin-a(*S*).
[g] Not Detected Limit for AEG is $\leq$ 0.018 ng/ml.
[h] Not Detected Limit for BMAA is $\leq$ 0.018 ng/ml.
[i] Not Detected Limit for DAB is $\leq$ 0.070 ng/ml.
[j] Not Detected Limit for Anatoxin-a(*S*) is $\leq$ 3 ng/ml.
[k] WHO provisional guideline for Microcystin-LR is 1 μg/l.
[l] Not Detected Limit for Microcystin LR is ND $\leq$ 102 μg/l.
[m] WHO provisional guideline for pH in drinking water is 6.5–8.5. The Qatar General Electricity and Water Corporation (KAHRAMAA) uses the same guideline.
[n] There is no WHO provisional guideline for NH_4^+ in drinking water. KAHRAMAA has a concentration threshold for Ammonia at 0.5 mg/l.
[o] There is no WHO provisional guideline for Cl^- in drinking water. KAHRAMAA's concentration threshold is 80 mg/l.
[p] FAO provisional guidelines for Cl^- in irrigation water are: <143 mg/l concentration that will not cause restriction to growth and health and >355 mg/l concentration that will cause severe restriction to crop growth and health.
[q] WHO provisional guideline for NO_3^- in drinking water is 50 mg/l. Safe Drinking Water Act requires US EPA maximum contaminant level goals (MCLG) for NO_3^- at 10 mg/l and KAHRAMAA has adopted this value.

repellents to zooplanktonic predators and other cyanobacteria competing for the same resources (Berry et al., 2008). In aquatic ecosystems, increased temperature, light and nutrient availability of the spring and summer months lead to exponential growth of cyanobacteria and bloom formation. Bioaccumulation of toxins has been documented in the marine food chain, in terrestrial food chains and toxins have been detected in dietary supplements and food intended for human consumption (Cox et al., 2003; Mohamed and Al Shehri, 2009; Jonasson et al., 2010; Field et al., 2013; Masseret et al., 2013; Mondo et al., 2014; Banack et al., 2014, 2015). Human exposure to toxins can occur through dietary ingestion of supplements or species in the terrestrial and marine food chains, but also through inhalation of aerosolized bloom material in water droplets and by drinking toxin-contaminated water (Stommel et al., 2013). The cyanotoxins microcystins, nodularins, cylindrospermopsins, anatoxin-a, and anatoxin a(*S*), are known to

Table 2
Bivariate statistical analysis predicting associations of sampled environment, tank and water characteristics to toxins and nutrients. Only significant correlations are presented.

Sampled environment	Tank and water characteristics	Toxins & chemical species	β	95% CI[a]		P-value	β_0	R^2
				Lower	Upper			
Urban	Tank material – Fiberglass	LogMic[b]	0.261	0.021	0.500	0.035	−0.249	0.279
	Odor in the tank – Yes	LogMic	0.292	0.038	0.547	0.027	−0.187	0.244
Urban/Rural	Color of tank – White[c]	Cl^-	NA[d]	NA	NA	0.0433[e]	NA	NA
Rural	Rural location	AEG	NA[d]	NA	NA	0.0001[f]	NA	NA
		LogNH_4^+	1.714	0.253	3.176	0.024	−3.420	0.230
	Groundwater well	LogNH_4^+	3.811	2.600	5.022	0.000	−3.178	0.683
		Cl^-	NA	NA	NA	0.0064[f]	NA	NA
		LogNO_3^-	3.188	2.424	3.953	0.000	−1.498	0.791
	Water transported by truck	AEG	NA	NA	NA	0.0005[e]	NA	NA
		Cl^-	NA	NA	NA	0.0179[e]	NA	NA
	No transportation (groundwater well)	LogNH_4^+	2.876	0.983	4.769	0.005	−2.425	0.498
		LogNO_3^-	3.103	1.696	4.511	0.000	−1.494	0.540
	Tank located under the sun[g]	AEG	NA	NA	NA	0.0454[f]	NA	NA
	Tank material – No Tank	LogNH_4^+	3.212	0.944	5.479	0.008	−2.760	0.436
		LogNO_3^-	3.301	1.746	4.857	0.000	−1.692	0.561
	Color of tank – Blue	LogNH_4^+	4.413	2.037	6.788	0.001	−3.234	0.692
		LogNO_3^-	3.394	1.888	4.900	0.000	−1.463	0.795
		pH	0.135	0.019	0.251	0.025	8.045	0.273
	Color of Tank – No Tank	LogNH_4^+	3.685	2.225	5.145	0.000	−3.234	0.692
		LogNO_3^-	3.071	2.146	3.998	0.000	−1.463	0.795
	Cyanobacterial growth – obstructed view	LogNH_4^+	2.962	1.232	4.691	0.002	−3.054	0.534
		LogNO_3^-	2.263	1.218	3.307	0.000	−1.075	0.719
	Chemical species – Chloride	AEG	NA	NA	NA	0.0072[h]	NA	NA

[a] CI: Confidence Interval.
[b] LogMic: Log Microcystin-LR.
[c] 90% of Urban water tanks and 41.7% of the Rural water impoundments were white.
[d] Not Applicable: non-parametric regression was used in the case of Cl^- and AEG.
[e] Kruskall-Wallis test.
[f] Wilcoxon rank-sum test.
[g] 50% of Urban water tanks and all of the Rural water impoundments were located under the sun.
[h] Spearman's correlation test.

Table 3
Toxin analysis of soil profile samples.

Depth (cm)	AEG (ng/g)	BMAA (ng/g)	DAB (ng/g)
Surface	0.7335[a]	0.5046	101.32
0–5	ND[b]	1.3597	187.39
5–10	0.6085	0.7537	101.67
10–15	0.4921	0.3558	49.42
15–20	1.4863	0.3707	53.12
20–25	1.5849	0.4301	22.03

[a] Toxin concentration for AEG, BMAA and DAB are averages of triplicate analysis.
[b] Not Detected Limit for AEG is ≤ 0.018 ng/ml.

produce liver damage, promote tumors, and cause paralysis (Carmichael et al., 2001; Cox et al., 2003; Metcalf and Codd, 2012; Banack et al., 2015). In a recently published study, Cox et al. (2016), showed that chronic ingestion of BMAA in vervets (*Chlorocebus sabaeus*) causes the development of neurofibrillary tangles and sparse β-amyloid deposits in the brain, hallmarks of the Guamanian neurodegenerative disease Amytotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC), which contains clinical elements of ALS, Alzheimer's and Parkinson's.

Antithetically to aquatic environments, toxin research in terrestrial and arid environments is not as advanced and thus environmental conditions that promote exponential cyanobacterial growth and toxin accumulation as well as human exposure routes are not exhaustively characterized. Furthermore, at present cyanotoxin-testing is not generally mandatory with respect to water quality, nor do WHO provisional guidelines exist for the toxins studied in this article, with the exception of microcystin-LR. Although acutely toxic in animal test systems, with an LD_{50} of 25–150 µg/kg in mice via intraperitoneal injection (Fawell et al., 1994), the WHO Guideline Value (GV) for microcystin-LR in drinking water of 1 µg/l is based on long term toxicological studies for life time protection (WHO, 2011a). This is due to the potential action of microcystins acting as liver tumor promoters and a 60 Kg person drinking 2 L of water per day and obtaining 80% of their microcystin through said water. In Qatar, a person is more than likely to drink 2 L of water per day, and exceed that volume especially in the summer months due to the extreme heat conditions of the desert climate (temperature maxima can reach up to 50 °C; MOE, 2014). Although the drinking water intake of the population is not reported as a separate figure in the water consumption per capita report by KAHRAMAA, the Qatar General Electricity and Water Corporation, which does increase in the summer months, the guidelines for workers to prevent heat stress advise intake of 0.25 L of water per hour (KAHRAMAA, 2014a; SCHQ, 2014). This amounts to 4 L in an 8-h shift bringing the concentration of

Table 4
Statistical analysis predicting associations between toxins and soil depth.

Toxin tested	Variable	β	95% CI[a]		P-value
			Lower	Upper	
LogAEG	Soil depth	0	−0.33	0.033	0.99
	BMAA	650.44	−49.588	1350.476	0.066
	DAB	−6.193	−12.807	0.421	0.064
LogBMAA	Soil depth	0.004	−0.009	0.017	0.507
	AEG	98.377	8.631	188.123	0.034
	DAB	3.626	2.036	5.217	0
LogDAB	Soil depth	−0.022	−0.038	−0.007	0.007
	AEG	−112.885	−238.826	13.057	0.075
	BMAA	268.882	32.646	505.118	0.029

[a] CI: Confidence Interval.

microcystin-LR to double (4 μg/day) the WHO GV for lifetime protection. If drinking water consumption volumes of the general population mirror those of the workers, and if their body weight is 60 Kg, based on the results of the present study all consumers of the water sources we have tested are exposed to microcystin-LR exceeding the WHO GV (range 2.6–5.3 μg/day; Table 1 Supplementary Materials). Therefore, the WHO GV for microcystin-LR would probably act as a good surrogate for lifetime protection from microcystin-LR. To the best of our knowledge, the incidence of liver tumors in the population has not yet been determined for the State of Qatar, information that may provide further indication of microcystin-LR exposure (SCHQ, 2013).

Studies conducted in arid environments of Egypt and the Kingdom of Saudi Arabia, and semi-arid environments in Brazil, corroborate our results as they have found toxin-producing cyanobacteria and microcystins in recreational, drinking and irrigation water contained in dams and tanks, in groundwater wells, hot springs and rainwater pools (Costa et al., 2006; Mohamed and Al Shehri, 2007, 2009; Mohamed, 2008; Chatziefthimiou et al., 2014). In recent years evidence is amassing that a potential route of exposure to toxins in arid environments is through inhalation of desert dust (Bradley and Mash, 2009; Cox et al., 2009; Genitsaris et al., 2011; Bradley et al., 2013). Cyanobacteria and cyanotoxins of surface crusts become airborne by the high winds of desert storms and persistent inhalation has been implicated with the onset of neurological disorders such as ALS as was the case of military personnel deployed in the Iraqi war of 1991–1992 (Horner et al., 2003; Cox et al., 2009).

In Qatar, drinking water production and treatment is heavily regulated and water quality is constantly monitored. In cases where there are no WHO provisional guidelines (NH_4^+, Cl^-), KAHRAMAA has set stringent concentration limits thus effectively removing ions and metals that pose health risks and which may be metabolic stimulants of cyanobacteria, possibly leading to their enrichment and toxin accumulation (KAHRAMAA, 2014b). Indeed, our results from tested drinking water in urban environments suggest that the presence of microcystin-LR is influenced by the water storage process, the tank material, and that pH, NH_4^+, Cl^-, NO_3^- are all within the permissible concentration limits set by the regulatory company (Tables 1 and 2). The cyanotoxins BMAA, AEG, DAB and anatoxin-a(*S*) were not detected in any of the sampled water in urban environments.

To the best of our knowledge, no water quality testing is in effect nor any regulations exist for water stored in main or secondary water tanks within the urban compound boundary, for groundwater wells or for water transported in trucks and stored in tanks in rural environments. The cyanobacterially-produced BMAA isomer AEG was present in all but one of the rural water samples and was significantly correlated with chloride ions that surpassed the FAO warning concentration (>355 mg/l) by about 2.5 times, potentially causing severe restrictions to crop health and yield (Tables 1 and 2; FAO, 1994). In an independent study on the physicochemical characteristics of groundwater in Qatar, it was observed that Cl^- and fluoride (F^-) were above drinking water standards and F^- and molybdenum (Mo) were above the regulatory concentrations for irrigation water (Kuiper et al., 2015).

Fluoride occurs naturally in soils and waters, and it is also anthropogenically emitted from aluminum smelting facilities (WHO, 1970; Tjahyono et al., 2011; Richer, 2015). Fluoride has been shown to have deleterious effects on the skeletal system, kidneys and thyroid and the US Department of Agriculture has recognized that airborne fluorides cause the most damage to livestock than any other airborne pollutant (Azar et al., 1961; WHO, 1970; USDOA, 1972). The heavy metal Mo has been shown to have negative effects on human reproduction, yet for some cyanobacteria it serves as an essential co-factor in the nitrogenase enzymatic machinery used to fix atmospheric nitrogen (Howarth et al., 1988; WHO, 2011b).

Many water storage tanks have as part of their design a partially covered hole near the top lid, which serves as an overflow control. Exchange of air and dust particles as well as light penetration occurs through that hole allowing for recruitment of atmospheric microbes attached to particulates and for photosynthesis to take place. Nitrogen fixation and carbon dioxide fixation through photosynthesis, allow metabolic independence and enable cyanobacteria to be potent eco-strategists and pioneer species in otherwise nutrient-limited water bodies and dryland soil surfaces (Powell et al., 2015). In the absence of growth controls exerted on cyanobacteria through e.g. Mo bioavailability, light and nutrients, and the lack of disturbance by water-mixing, cyanobacteria can attach to the internal walls of water tanks and proliferate. Our finding that AEG correlates significantly with tank exposure to the sun may then be explained by the increased activity of cyanobacterial-toxin-producers found in these water impoundments (Table 2). Finally, as the cyanotoxins BMAA, AEG and DAB are persistent on surface desert crusts and as deep as 25 cm in the soil horizon, a bioaccumulation potential exists if they are leached into groundwater (Tables 3 and 4; Richer et al., 2015).

4.2. *Sustainable use of water in arid environments: implications and suggested measures*

Qatar is a hyper-arid desert with an Aridity Index (AI) of less than 0.05, annual precipitation average of 76.4 mm and average evapotranspiration rate of 6 mm per day (UNESCO, 1979; MEA, 2005; MOE, 2014). The Falkenmark Water Stress Index for Qatar is 0.2, which is defined as beyond the water barrier of manageable capacity (Falkenmark and Widstrand, 1992). The Water Poverty Index (WPI), which takes into consideration water resource availability, access, capacity, use and environmental factors that affect water quality, was 57.2 for Qatar, as compared to 78 for Finland, the richest country in terms of WPI (Lawrence et al., 2002). The global Water Quality Index (WATQI), which is part of the 2008 Environmental Performance Index (EPI) and is indicative of ecosystem impacts on water quality, for Qatar was 39.89 out of a 100 (Srebotnjak et al., 2012). Taken together, these indices lead to the conclusion that water is not only scarce, but also not sustainably managed. The current state of affairs and future directions for an overall sustainable development in Qatar are comprehensively reviewed in Richer (2014).

To combat scarcity of freshwater, the government owned KAHRAMAA and the private Qatar Electricity and Water Company (QEWC) have collectively set in place 8 desalination plants that use MSF, RO and MED technologies, another MSF is about to start production, and another which is designed to use the less-energy-costly process of reverse osmosis, has been recently commissioned to a private company (Dawoud and Al Mulla, 2012; KAHRAMAA, 2015, QEWC, 2015). Reduced production costs and more energy efficient technologies make water desalination an appealing solution to water scarcity, not only in arid environments, but also in temperate climates and sub-tropical drylands that have historically relied on diversion of freshwater supplies for drinking water and are currently experiencing drought (Ghaffour et al., 2013). Although seemingly a beneficial solution, water desalination is associated with three intrinsic problems. First, the ecological impacts on marine life of the highly saline, alkaline and hot brine end product of desalination that is released back into the sea are not well understood, thus more environmental impact assessments are required (WHO, 2007; Dawoud and Al Mulla, 2012). Furthermore, fish larvae get entrained in the plant's seawater intake and fish get impinged on screens that prevent their entry to the plant (Miri and Chouikhi, 2005). Although the scale of this phenomenon may be small, increased seawater temperature caused by the brine effluents combined with the release of nutrients such as phosphorus and nitrogen from the decomposing fish may be taken up by aquatic cyanobacteria, which at high densities can lead to localized bloom formations (WHO, 2007).

Secondly, desalinated water is diverted and stored in mega-water reservoirs as a measure to secure water supply beyond daily use, which if not properly mixed may serve as breeding grounds for cyanobacterial bloom formations as is observed in dams of other arid countries (Mohamed and Al Shehri, 2007). Management and treatment options for cyanobacterial blooms in waterbodies are extensively reviewed in Merel et al. (2013) and include retention and degradation based techniques. Additional toxin exposure prevention measures include shading of water tanks, use of tank materials that are not conducive to cyanobacterial attachment and growth as well as modification of water transportation practices. Also, home filtration devices can be used to efficiently remove microcystin-LR from potable water, yet further research is required to assess whether neurotoxins can be successfully removed by this method (Pawlowicz et al., 2006).

Finally, of particular concern to Qatar is the loss of the produced desalinated water (110 million m^3) destined for human use through leakage into the aquifer (QGSDP, 2011). This exacerbates an existing problem in Qatar, that of unsustainable water use in agricultural practices. Water security is linked to food security through irrigation, water availability and quality. Although 95% of the groundwater abstracted is used for growing crops, this produce meets only 10% of the country's food supply needs, while the rest is imported with an enormous carbon footprint from countries as geographically distant as Chile, the USA and Australia (FAO, 2008; QNFSP, 2012). Furthermore, due to improper management and over-use for agriculture, the groundwater supply has become enriched with salts leading to soil salinization and agricultural unsuitability (Table 1 Supplementary Materials; FAO, 2008). This type of land degradation is a driver to desertification (UNCCD, 2012). If more sustainable practices were to be employed, such as drip irrigation, or adoption of hydroponic and aquaponic agricultural processes where the soil matrix dependency has been removed and water is recycled, then groundwater from wells could be used for drinking water to alleviate the demand for desalination (Tyson et al., 2011; Sardare and Admane, 2013).

To address issues of sustainability, the Qatar National Food Security Programme (QNFSP) was established in 2008, with the aim to reduce Qatar's dependence on imported goods through a comprehensive plan of investments on economic sectors of renewable energy, desalination and water management, agricultural production and food processing and transport (QNFSP, 2012). Furthermore, Treated Sewage Effluent (TSE) is currently used for ornamental plant irrigation in highway corridors, private-company initiatives have implemented the use of TSE in toilets, and plans exist for the production of fecal pellets from TSE which can be used as fertilizer in agriculture (Darwish et al., 2015). Artificial recharge of groundwater may be an added positive measure to increase water security, yet more environmental impact assessments are needed before plan implementation (Kimrey, 1989; Darwish et al., 2015). Finally, in 2004 the Qatar National Biodiversity Strategy and Action Plan was prepared in an effort to proactively conserve biodiversity (QNBSAP, 2004). Between the years 2003 and 2015, there was a 17% increase of land covered by protected areas. As biodiversity conservation is one of the components of WPI, this increase in protected areas is a positive step toward improvement of water quality (Lawrence et al., 2002; UNEP, 2003; CBD, 2015).

4.3. Future directions

We have determined that cyanotoxin-producing species are present in water impoundments in both urban and rural environments; that cyanotoxins accumulate in both drinking and irrigation water as well as in the soil horizon; and that the mode of transportation and tank material correlate with toxin accumulation. Although a pilot study, the findings warrant more extensive research efforts to determine cyanobacterial and toxin bioaccumulation hot spots in the water treatment process from desalination to the tap. Specifically, samples should be taken of seawater abstracted and used in the desalination plant as well as brine that is disposed of and returned to the sea. Drinking water in water treatment plants, storage reservoirs and main water storage tanks in compounds should be consistently sampled and tested.

Sewage effluents and wastewater should also be sampled at point sources and all throughout their purification process. The resulting TSE is used in Qatar to irrigate plants in parks and landscaped corridors of highways. Analysis of this water along with that of groundwater well samples will allow us to accurately determine the cyanotoxin bioaccumulation potential in ornamental plants and in crops destined for human and animal consumption.

Finally, a more elaborate scheme that covers the whole geographical area and extends over a year will enable us to obtain a better resolved picture of the spatio-temporal and diurnal variation of toxin accumulation, when combined with microbial metagenomic analysis, water chemistry and environmental parameters will provide a better understanding of cyanobacterial abundance and the environmental factors that control it.

Collectively, elucidation of these missing links will help in taking appropriate actions to limit human exposure to cyanobacteria and cyanotoxins, and in making managerial decisions that best preserve and conserve this natural resource and to develop a sustainable local economy as is envisioned in the QNFSP 2008 and Qatar National Vision (QNV) 2030 (QGSDP, 2008, 2011).

5. Conclusion

A general lack of knowledge concerning cyanotoxins is seen throughout the medical community and the general public. Despite the dangers associated with the presence of cyanotoxins in most water sources in arid and semi-arid regions, no management action has yet been considered. The greater awareness concerning cyanotoxins has been at a scientific level, but has not been reflected at a national government level in terms of the development of provisional guidelines for BMAA, DAB, AEG, anatoxin-a(*S*) and their inclusion in standard water testing, monitoring programs, legislation or implementation of environmental impact assessments. While the dangers of cyanobacterial toxins associated with blooms are a global issue and are being addressed nationally or regionally (Donohue and Orme-Zavaleta, 2008), arid countries where cyanobacteria often exhibit ecological dominance may face an additional challenge. Countries with adequate water supplies can turn to alternative sources in the event of a toxic bloom while arid countries may be limited in the availability of alternative sources of water, thus placing this population at greater risk.

Acknowledgments

This publication was made possible by NPRP grant 4-775-1-116 from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of the authors. We wish to acknowledge that the graphical abstract of this manuscript was created by Ms. Jenine Davidson. We wish to thank Mr. Nasser Bin Marzook and Mr. Mohammed Al-Kubaisi for their valuable help in collecting water samples in the rural environment and Dr. Laith Abu Raddad for his guidance in the statistical analysis and the services provided by the Biostatistics, Epidemiology, and Biomathematics Research Core at Weill Cornell Medicine-Qatar.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.toxicon.2016.02.016.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2016.02.016.

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