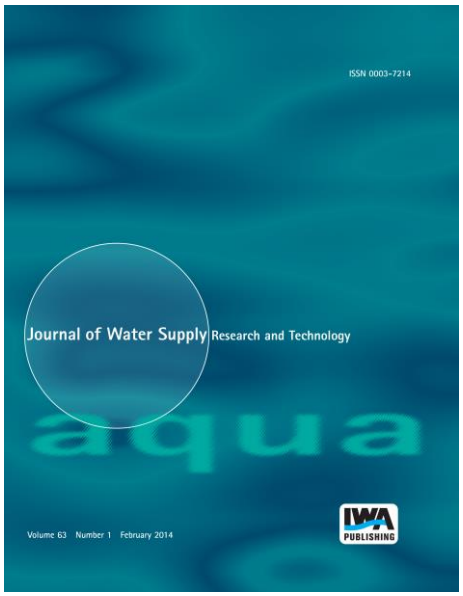

Occurrence of toxic cyanobacteria and microcystin toxin in domestic water storage reservoirs, Egypt

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ABSTRACT

Residents in many developing countries store treated drinking water in tanks or reservoirs because of intermittent and infrequent water supplies. Many studies have focused on bacterial contamination of domestic reservoir waters, the cyanobacterial and algal contamination is largely unexplored. Therefore, the present study investigates toxic cyanobacteria and their microcystin (MC) toxins in some domestic water storage reservoirs in Egypt as an example for developing countries. Three phytoplankton groups including cyanobacteria, green algae and diatoms were found in domestic reservoirs. Among these species, the toxic cyanobacterium *Microcystis aeruginosa* had the highest cell density during warm months (4.2–5.92 cells $\times 10^6$ L^{-1}). This cell density increased along the time, indicating that environmental conditions in these reservoirs promoted the proliferation of this species. Intra- and extracellular MCs were also detected in reservoir waters at concentrations of 3.5–40 and 1–7.6 μg L^{-1}, respectively, exceeding the WHO guideline limit of 1 μg L^{-1} for these toxins in drinking water. Heterotrophic bacteria were found in association with cyanobacteria in reservoir waters. The study suggests that treated-water storage reservoirs should be monitored for the presence of toxic cyanobacteria to protect the public from exposure to their potent toxins.

Key words | cyanobacteria, domestic reservoirs, drinking water, hygienic risk, microcystins

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INTRODUCTION

In developing countries, the intermittent and infrequent water supply necessitates the need to store water in tanks or reservoirs for drinking and other purposes (Chia *et al.* 2013). In Egypt, most of these reservoirs are found above buildings and houses and may therefore be exposed to microbial contamination by receiving contaminated water from drinking water treatment plants or through wind carrying the spores and akinetes of microorganisms, which may germinate and grow under suitable conditions (Codony *et al.* 2003). However, the latter contamination source is less important as it occurs only when the reservoirs are left open, and this can be easily manipulated and overcome. The breakthrough of microorganisms into storage reservoirs from drinking water treatment plants is of particular concern, as it indicates the ineffectiveness of conventional treatment methods for microbial removal. Cyanobacteria are one of the pathogenic agents recognized in water (WHO 1996). The presence of cyanobacterial cells in drinking water reservoirs is of particular concern for human health, due to the ability of some species to produce taste and odorous substances and potent cyanotoxins including neurotoxins, hepatotoxins and skin irritant toxins (Codd *et al.* 2005). Cyanotoxins are produced by both freshwater and marine cyanobacteria (Mohamed & Al-Shehri 2015). Microcystins (MCs) are the most common cyanobacterial hepatotoxins in freshwaters worldwide with increasing health implications, and hence the World Health Organization (WHO 1998) has established a provisional guideline

doi: 10.2166/aqua.2016.115

limit in drinking water of 1 μg L^{-1} for the most toxic variant, MC-LR. MCs damage the liver through altering the cytoskeletal architecture of the hepatocytes (Humpage & Falconer 1999; Metcalf & Codd 2004). They have also been reported as tumor promoters in liver and colon (Ito *et al.* 1997; Humpage *et al.* 2000). Once cyanobacterial cells enter the reservoir water they can grow autotrophically or heterotrophically, and they proliferate, forming films on the water surface or mats attached to the inner sidewalls of reservoirs (Momba & Kaleni 2002; Jagals *et al.* 2003; Fosso-Kankeu *et al.* 2008). These outgrowths of cyanobacteria may stimulate the growth of heterotrophic bacteria in the reservoir water through using photosynthetic products as a carbon source (Cole *et al.* 2014). Furthermore, cyanotoxins can be released into the water upon cyanobacterial cell lysis as a result of senescence (Hitzfeld *et al.* 2000; Schmidt *et al.* 2002) or by mechanical pressure due to pumping of the water through a distribution system (Hitzfeld *et al.* 2000; Mohamed & Al Shehri 2007).

The present study was therefore undertaken to evaluate treated-water storage reservoirs in Egypt as an example, for the presence of cyanobacteria and MC toxins. The study also aimed to define the contamination source of these reservoirs with such pathogenic agents. Furthermore, the study aimed to follow the growth of cyanobacteria in these reservoirs along one year, to confirm their proliferation under storage conditions.

MATERIALS AND METHODS

Sampling

Water samples were collected from six different domestic reservoirs (DR) receiving their water from Damietta treatment plant, Damietta city, Egypt. These reservoirs are cylindrical and made of polyethylene with a height of 273 cm and diameter of 216 cm. The reservoirs are untightly covered, leaving a gap between the reservoir and its lid. They also have valves fitted at the outlet of water reservoir (i.e. breathing outlets) that allow the reservoir water to breathe. The water in these reservoirs has a detention time exceeding 3 or 4 weeks. Water samples were collected monthly during the period January–December 2013 using 1 L sterilized glass bottles at 20 cm depth and at the surface of the reservoirs. Each water sample was an integrated sample collected randomly from three different sites in the reservoir. Samples for physico-chemical analysis were filtered through Whatman GF/C fiberglass filters and kept in the freezer until use. Samples for phytoplankton analysis were preserved in Lugol's iodine solution. Water samples for toxin and bacteriological analysis were also collected from reservoir waters by the same method stated above.

Physico-chemical characteristics of reservoir waters

Water temperature, pH, dissolved oxygen (DO), and electric conductivity (EC) were measured *in situ* using a multi-parametric probe (HI 991300 pH/EC/TDS/Temperature, HANNA, Italy). Ammonia, nitrite, nitrate, reactive (Ortho) phosphate, and dissolved organic carbon (DOC) were determined in filtered water samples, while total nitrogen (TN) and total phosphorus (TP) were determined in unfiltered water samples in the laboratory using standard methods according to APHA (2005).

Phytoplankton analysis

Fixed phytoplankton were microscopically identified according to taxonomic keys (Descikachary 1959; Komárek & Anagnostidis 2005). Phytoplankton cells were enumerated using a haemocytometer and expressed in the number of cells per liter. Chlorophyll a as a measure of biomass was determined spectrophotometrically in a methanol extract of a known volume of reservoir waters according to Talling & Driver (1963). Analysis of heterotrophic bacteria was carried out in an aliquot of phytoplankton sample with serial dilution. The heterotrophic plate counts (HPC) expressed as colony-forming units (CFU) at 37 °C were determined using the spread-plate method on petri dishes containing nutrient agar medium (APHA 1995; Briganti & Wacker 1995). Isolated bacteria were identified according to their morphological and biochemical characteristics (Holt *et al.* 1994).

Analysis of MCs

To determine intracellular and extracellular MCs in reservoir waters, subsamples (500 mL) were filtered through

GF/C filters (Whatman, UK) to separate phytoplankton cells. The filtrate was stored in the freezer for the analysis of extracellular (dissolved) MCs. The filters with trapped cells were extracted twice in 80% methanol and centrifuged at 10,000 × g for 10 min. The supernatants were pooled together, and the organic solvent was blown with sterilized air. The aqueous fraction remaining after removing organic solvent was filtered through GF/C filters and kept in the freezer until analysis. Concentrations of extracellular and intracellular MCs were determined by enzyme-linked immunosorbent assay (ELISA) according to the method of Carmichael & An (1999) using the commercial kit, MC-ADDA ELISA kit purchased from Abraxis (Warminster, PA), which detects total MCs using polyclonal antibodies with a detection limit of 0.1 μg L^{-1}. All analyses were done in triplicate.

Statistical analysis

Differences in cyanobacterial cell density, chlorophyll a, MC concentrations and environmental variables in reservoir waters during the present study were compared using one-way analysis of variance (ANOVA) ($P < 0.05$) using SPSS 18.0 software for Windows. Correlations among the above variables were calculated using the Spearman correlation test.

RESULTS

Physico-chemical characteristics of domestic reservoir waters

Physico-chemical parameters of domestic reservoir waters are presented in Table 1. During the sampling period, water temperature varied significantly ($P < 0.05$) with the maximum value (32 °C) obtained in July and minimum value (17 °C) in January. The domestic reservoir waters were slightly alkaline (7.8–8.3). No significant difference in DO was observed during the study period ($P > 0.05$). EC, biological oxygen demand (BOD) and DOC showed marked and significant variation between study months in all domestic water reservoirs ($P > 0.05$). Nutrient concentrations, particularly, nitrogen and phosphorus, in domestic reservoir waters changed significantly along the study period ($P < 0.05$). NH_4 concentrations were very low (0.01–0.05 mg L^{-1}) in DR, and NO_2 was not detectable during the whole study period. On the other hand, NO_3 (0.18–0.31 mg L^{-1}) and Total-N (0.5–0.9 mg L^{-1}) concentrations were considerably high in all domestic reservoir water (Table 1). Soluble PO_4 concentrations were very low (1–8 μg L^{-1}) in all DR, but total phosphorus concentrations were high (0.5–0.82 mg/L). Additionally, total nitrogen to total phosphorus (TN/TP) ratios were low (0.8–2.1) and varied markedly during the study period ($P < 0.05$). Iron concentrations were detected at very low concentrations (1–10 μg L^{-1}) in domestic reservoir waters (Table 1). It is noticed in the present study that all physico-chemical parameters did not differ significantly ($P > 0.05$) among different DR surveyed. Therefore, the data of these parameters were presented as a range of values, not for each reservoir.

Cyanobacteria and MCs in DR

Phytoplankton community in domestic reservoir waters consisted only of three groups including cyanobacteria, green algae and diatoms. The dominance of these algae varied seasonally, whereby green algae dominated the community during winter months, and cyanobacteria dominated during warm months (April–September 2013). Cyanobacterial species in DR consisted of two species only, namely, *M. aeruginosa* and *G. sanguinea* (Table 2). However, *M. aeruginosa* was found in high numbers (4.6–5.5 × 10^6 cells L^{-1}) constituting the largest proportion (>95%) of cyanobacterial population in these reservoirs along the study period. The cell density of *M. aeruginosa* did not show significant difference ($P > 0.05$) among the DR studied. Conversely, *M. aeruginosa* cell density changed markedly ($P < 0.05$) among the sampling months. It has positive correlation with temperature, NO_3, PO_4, and iron concentrations ($r = 0.6$–0.8), and negative correlation with TN/TP ratio ($r = -0.7$). Chlorophyll a concentrations in domestic reservoir waters were high (0.7–6.6 μg L^{-1}) during warm months (Table 2), correlated with the cell density of *M. aeruginosa* ($r = 0.85$), and behaved in the same manner as *M. aeruginosa* towards environmental factors. The results of toxins analysis for MCs in domestic reservoir waters showed that all these reservoirs contained intracellular MCs at concentrations ranging from 3.5 to 40 μg L^{-1} (Figure 1). These concentrations varied significantly among the different

Table 1 | Physico-chemical properties of domestic water reservoirs of Damietta water treatment plant

Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Temp (°C)	18–19	19–20	22–24	21–26	25–29	27–29	28–31	30–32	30–31	23–25	23–24	19–20
pH	7.2–7.3	7.5–7.7	7.2–7.5	7–7.5	7–7.4	7.4–7.9	7.6–8	7.3–7.6	7.3–7.4	7.4–7.5	7–7.1	7–7.1
EC ($\mu S\ cm^{-1}$)	320–335	340–347	350–357	352–367	366–371	372–377	380–388	359–366	353–365	348–350	340–345	328–335
DO ($mg\ L^{-1}$)	8.8–8.9	8.9–9	9–9.1	9–9.3	9.5–9.8	8.5–8.8	8.7–8.9	8.8–8.9	9–9.3	8.6–8.9	9–9.3	8.4–8.8
BOD ($mg\ L^{-1}$)	1.9–2.4	2.2–2.3	1.9–2.4	2.1–2.2	1.8–2.4	2–2.4	2.2–2.3	2.3–2.4	1.8–2.4	1.9–2.4	1.7–2.3	1.8–2.2
DOC ($mg\ L^{-1}$)	2.3–2.6	2.1–2.4	1.8–2.2	2.3–2.4	1.7–1.9	1.8–2.3	2–2.4	1.9–2.2	1.8–1.9	2.5–2.8	2.4–2.7	2–2.1
PO_4 ($\mu g\ L^{-1}$)	6–7	1	1–1.2	1.1–1.4	1.5–2	2.2–2.6	4–6	6.6–8	1.5–2	2.2–3	1–1.5	2–3
NO_3 ($mg\ L^{-1}$)	0.3–0.31	0.23–0.25	0.2–0.22	0.2–0.21	0.21–0.22	0.2–0.21	0.22–0.24	0.18–0.2	0.2–0.23	0.22–0.24	0.24–0.26	0.26–0.29
NO_2 ($mg\ L^{-1}$)	0	0	0	0	0	0	0	0	0	0	0	0
NH_4 ($\mu g\ L^{-1}$)	18–20	10–11	0	0	0	45–50	18–20	20–21	0	0	0	0
Iron ($\mu g\ L^{-1}$)	0–2	1–4.5	1–3	3–6	5–8	4–7	6–8	8–10	6–9	4–7	2–5	1–3
TP ($mg\ L^{-1}$)	0.4–0.7	0.3–0.5	0.3–0.5	0.6–0.8	0.3–0.5	0.5–0.7	0.4–0.6	0.5–0.7	0.4–0.6	0.4–0.6	0.4–0.6	0.5–0.6
TN ($mg\ L^{-1}$)	0.8–1	0.8–0.9	0.8–0.9	0.7–0.8	0.7–0.8	0.7–0.9	0.7–0.9	0.7–0.8	0.5–0.7	0.6–0.7	0.7–0.8	0.7–0.9
TN/TP ratio	1.6–2.4	1.7–2.1	1.8–2.2	0.9–1.1	1.7–2	1.3–1.6	1.1–1.3	1.3–1.5	0.8–1	1–1.4	1.1–1.5	1.3–1.6

These parameters did not significantly vary among six DR studied ($P > 0.05$). Therefore, the data are presented as a range of values, not for each reservoir.

Table 2 | Cell concentrations (cells $\times 10^6$ L^{-1}) of cyanobacterial and algal species in domestic water storage reservoirs

	Phytoplankton	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Reservoir 1	*Gloeocapsa sanguinea*	0	0	0	1.18	1.50	0.70	0.52	0.70	1.12	0	0	**0**
	Microcystis aeruginosa	0	0	0	46	49.8	54.3	48.1	46.78	55.2	0	0	**0**
	Total cyanobacteria	**0**	**0**	**0**	**47.18**	**51.3**	**55**	**48.62**	**47.48**	**56.32**	**0**	**0**	**0**
	Chlorella sp.	4	2	6	2	2	0	0	3	6	5	5	**5**
	Pediastrum simplex	8	6	8	9	6	0	0	0	0	0	0	**0**
	Scenedesmus dimorphus	6	7	7	8	2	0	0	0	0	0	0	**0**
	Navicula radiasa	0	0	0	0	0	10	8	2	0	0	0	**2**
	Nitzchia palea	0	0	10	8	4	0	0	0	2	4	20	**0**
	Synedra acus	10	11	0	0	0	5	5	0	9	9	9	**10**
	Total phytoplankton	**28**	**26**	**31**	**74.18**	**65.3**	**70**	**61.62**	**52.48**	**73.32**	**69.9**	**34**	**17**
	Chl.a (μg L^{-1})	0.8	0.7	0.9	2.1	4.9	3.8	3.8	1.5	3.7	1.9	1	0.5
Reservoir 2	*Gloeocapsa sanguinea*	0	0	0	1.	1.58	0.78	0.54	0.74	1.14	0	0	0
	Microcystis aeruginosa	0	0	0	46	52.1	56.7	50.4	48.83	57.2	0	0	0
	Total cyanobacteria	**0**	**0**	**0**	**47**	**53.68**	**57.48**	50.94	**49.57**	**58.34**	**0**	**0**	**0**
	Scenedesmus dimorphus	5.5	6.5	5.5	6.5	2.5	0	0	0	0	0	0	0
	Pediastrum simplex	7.2	5.4	7.3	8.1	5.7	0	0	0	0	0	0	0
	Navicula radiasa	0	0	0	0	0	11.2	9.5	3.7	0	0	0	**3.1**
	Nitzchia palea	0	0	11.6	8.9	5.8	0	0	0	3.8	5.1	19.3	**0**
	Total phytoplankton	**12.7**	**11.9**	**24.5**	**70.68**	**67.6**	**68.68**	**60.44**	**53.27**	**62.14**	**55.8**	**19.3**	**3.1**
	Chl.a (μg L^{-1})	0.4	0.4	0.8	1.8	4.8	3.7	3.6	2.7	3.3	1	0.5	0.1
Reservoir 3	*Gloeocapsa sanguinea*	0	0	0	1.5	1.9	0.9	0.7	0.9	1.3	0	0	0
	Microcystis aeruginosa	0	0	0	50.3	56.2	61	54.7	51.25	56.05	0	0	0
	Total cyanobacteria	**0**	**0**	**0**	**51.8**	**58.1**	**61.9**	**55.4**	**52.15**	**57.35**	**0**	**0**	**0**
	Chlorella sp.	5.3	2	6.3	2.2	2.4	0	0	3.1	6.2	5.2	5.1	**5.3**
	Pediastrum simplex	7.9	5.5	7.7	8.5	5.2	0	0	0	0	0	0	0
	Nitzchia palea	0	0	12.6	9.9	6.8	0	0	0	4.8	6.1	18.3	0
	Synedra acus	11.2	12.3	0	0	0	5.2	5.8	0	9.7	9.8	9.2	10.3
	Total phytoplankton	**24.4**	**19.8**	**26.6**	**72.4**	**72.5**	**67.1**	**61.2**	**55.25**	**78.05**	**74.1**	**32.6**	**15.6**
	Chl.a (μg L^{-1})	0.6	0.6	0.8	1.9	6.6	3.4	3.9	3.8	4.1	2.1	0.8	0.4
Reservoir 4	*Gloeocapsa sanguinea*	0	0	0	0.8	1.58	0.78	0.54	0.74	1.14	0	0	0
	Microcystis aeruginosa	0	0	0	46	52.1	56.7	50.4	48.83	57.2	0	0	0
	Total cyanobacteria	**0**	**0**	**0**	**46.8**	**53.68**	**57.48**	**50.94**	**49.57**	**58.34**	**0**	**0**	**0**
	Chlorella sp.	6.1	4.2	8.3	4.1	4.5	0	0	5.4	8.2	8.1	8.1	8.2
	Nitzchia palea	0	0	13.6	7.9	7.8	0	0	0	5.8	7.1	17.3	0
	Total phytoplankton	**6.1**	**4.2**	**8.3**	**59.18**	**65.98**	**57.48**	**50.94**	**54.97**	**72.34**	**63.3**	**25.3**	**8.3**
	Chl.a (μg L^{-1})	0.2	0.1	0.2	1.1	4.2	2.9	4.4	3.4	3.6	1.8	0.6	**0.3**
Reservoir 5	*Gloeocapsa sanguinea*	0	0	0	2.18	2.58	1.78	1.54	1.74	2.14	0	0	**0**
	Microcystis aeruginosa	0	0	0	43	48.1	52.7	43.4	41.83	47.2	0	0	**0**
	Total cyanobacteria	**0**	**0**	**0**	**45.18**	**50.68**	**54.48**	**44.94**	**43.57**	**49.34**	**0**	**0**	**0**
	Chlorella sp.	4	2	6	2	2	0	0	3	6	5	5	**5**
	Scenedesmus dimorphus	5.5	6.5	5.5	6.5	2.5	0	0	0	0	0	0	**0**
	Navicula radiasa	0	0	0	0	0	11.2	9.5	3.7	0	0	0	**3.1**
	Nitzchia palea	0	0	13.6	7.9	7.8	0	0	0	5.8	7.1	17.3	**0**
	Total phytoplankton	**9.5**	**8.5**	**25.1**	**61.58**	**62.98**	**65.68**	**54.44**	**50.27**	**61.14**	**55**	**22.3**	**8.3**
	Chl.a (μg L^{-1})	0.2	0.3	0.7	1.5	3.8	2.3	2.7	3.1	3.4	0.9	0.4	0.25
Reservoir 6	*Gloeocapsa sanguinea*	0	0	0	2.18	2.58	1.78	1.54	1.74	2.14	0	0	**0**
	Microcystis aeruginosa	0	0	0	48	54.1	58.7	52.4	50.83	59.2	0	0	**0**
	Total cyanobacteria	**0**	**0**	**0**	**50.18**	**56.68**	**60.48**	**53.94**	**52.57**	**61.34**	**0**	**0**	**0**
	Chlorella sp.	3.5	2.1	5.6	2.2	2.3	0	0	3.5	5.7	5.5	5	**5**
	Navicula radiasa	0	0	0	0	0	11.6	8.5	4.7	0	0	0	**4.1**
	Total phytoplankton	**3.5**	**2.1**	**5.6**	**52.38**	**58.98**	**72.08**	**62.44**	**60.77**	**67.04**	**59.2**	**5**	**9.2**
	Chl.a (μg L^{-1})	0.1	0.3	0.1	0.7	2.7	4.1	4.4	4.4	3.6	1.3	0.1	**0.3**

Each value is the mean of three readings.

reservoirs during the study period, and are associated with the cell density of *M. aeruginosa* ($r = 0.6$). The lowest concentration was recorded in April, and the highest was in August and September (Figure 1). Extracellular (dissolved) MCs were also detected in the cell-free water of all DR at high concentrations (1–7.6 μg L^{-1}) (Figure 2). They had weak correlations with *Microcystis* cell density and intracellular MCs ($r = 0.1$–0.2). Dissolved MCs were not detected at the lower detection limit of ELISA kit (0.03 ppb) during the period January–March 2013 and October–December 2013 (therefore data not shown). In addition to cyanobacteria, the results revealed the presence of heterotrophic bacteria in the water of all DR studied. The total HPC levels in DR varied significantly during the sampling period ($P < 0.05$), and correlated with the cell density of *M. aeruginosa* ($r = 0.6$) and DOC concentrations ($r = 0.7$). However, HPC values did not significantly vary between the months of a season ($P > 0.05$). Therefore, the data of HPC were presented per season (Table 3). The highest HPC was obtained during summer months (105 CFU mL^{-1}), and the lowest was in spring months (41 CFU mL^{-1}).

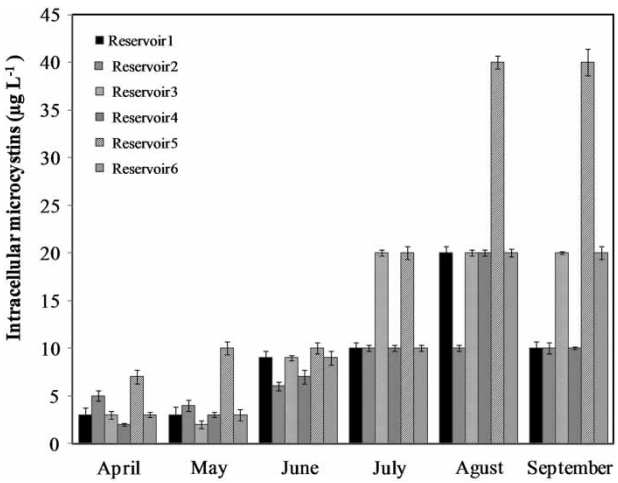

Figure 1 | Concentrations of intracellular MCs (μg L^{-1}) in domestic water storage reservoirs.

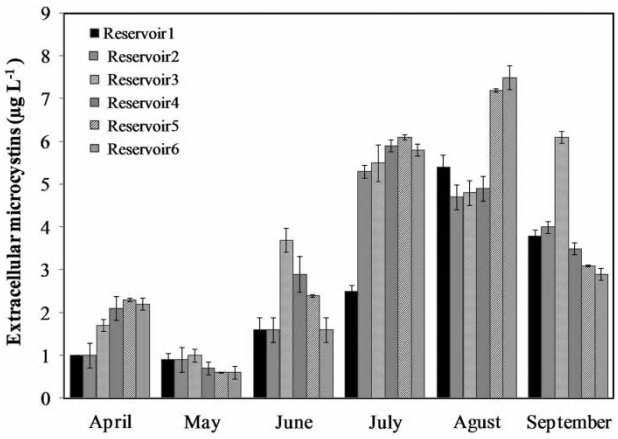

Figure 2 | Concentrations of extracellular MCs in (μg L^{-1}) in domestic water storage reservoirs.

DISCUSSION

The results of the present study clearly demonstrated the presence of cyanobacterial cells and MC toxins in DR receiving water from Damietta treatment plant. The cyanobacteria found in these reservoirs were constricted only to the same species (*M. aeruginosa* and *Gloeocapsa sanguinea*) detected during the same study period in the finished drinking water of Damietta treatment plant by our research team (Mohamed *et al.* 2015). This indicates that the water received from Damietta treatment plant is the only contamination source of these reservoirs with cyanobacteria. This is in accordance with a previous study made by Chia *et al.* (2013), reporting the dominance of *M. aeruginosa* in storage water tanks which received their water from a treatment plant in Zaria, Nigeria. *Microcystis* was also detected in high proportions in household storage-water containers in Limpopo Province in South Africa (Fosso-Kankeu *et al.* 2008). Other toxic cyanobacteria such as *Caltothrix parietina* were also investigated in treated-water storage reservoirs in Saudi Arabia (Mohamed & Al Shehri 2007). *Oscillatoria limnetica* was also detected in both raw and final treated waters of eight Egyptian drinking water treatment plants (Mohamed 2015). Additionally, *M. aeruginosa* in DR during the present study showed an increase in the cell numbers about 50–60 times higher than that recorded by Mohamed *et al.* (2015) in water received from the relevant treatment plant (1.9–13.5×10^3 cells L^{-1}). This increase in the cell density of *M. aeruginosa* indicates that the conditions detected in reservoir waters including temperature, pH, and nutrients, particularly N and P in the reservoirs, were suitable for its proliferation. This finding agrees with previous studies reporting that cyanobacteria can proliferate

Table 3 | Heterotrophic bacterial levels (CFU ml^{-1}) in domestic reservoir waters, Damietta, Egypt

	Heterotrophic plate count (CFU mL^{-1})				
Reservoir no.	**Winter**	**Spring**	**Summer**	**Autumn**	**Dominant bacteria**
1	41–43	74–76	95–97	71–73	*Aeromonas* sp., *Bacillus* sp. *Corynebacterium* sp., *Proteus* sp.
2	43–44	47–75	98–99	73–75	
3	41–43	75–77	90–94	75–76	
4	44–46	77–79	101–105	74–78	
5	46–48	78–82	95–98	70–74	
6	44–45	71–75	92–96	71–72	

HPC values did not significantly vary between months of a season ($P > 0.05$). Therefore, the data of HPC were presented per season.

at warm temperatures (15–30 °C), pH between 6 and 9 (Sotero-Santos *et al.* 2006), phosphorus (10 μg L^{-1}) (WHO 1999) and nitrogen (100 μg L^{-1}) (Rusin *et al.* 2000). The toxins were detected in domestic reservoir waters in two forms, cell-bound (intracellular) and dissolved (extracellular) forms. As the cyanobacteria detected in reservoir waters were found not to produce nodularin when analyzed by high-performance liquid chromatography (HPLC) during an earlier study (Al-Raghi 2015), the toxins detected by ELISA could be related only to MCs. Intracellular MCs were found in high concentrations in reservoir waters, with significant difference along the study period. This variation associated with the cell density of *M. aeruginosa*, confirming the linear relationship between MC production and the cell density of toxin producer documented by many studies (Petr *et al.* 2006; Wu *et al.* 2006; Mohamed & Al-Shehri 2009). These results also support the hypothesis that the favorable conditions for cyanobacterial growth also increase MC production in these organisms (Oliver & Ganf 2000). In the present study, temperature, NO_3, PO_4 and iron concentrations were found to be the key factors promoting the growth of *M. aeruginosa* and MC production in DR. The cell density of *M. aeruginosa* and concentration of intracellular MCs increased with the increase of water temperature from 21 °C in April to 32 °C in August. This is in accordance with the finding of O'Neil *et al.* (2012), stating that high temperatures not only favor the dominance of cyanobacteria, but also promote MC production and result in an increase in their concentration. Regarding nutrient concentrations, the peaks of *M. aeruginosa* and MCs associated with the highest concentrations of NO_3 and PO_4 during warm months, where the temperature was suitable for the growth of cyanobacteria. These results agree with the previous studies reporting that phosphorus and nitrogen induce *Microcystis* cell abundances and MC production (Vezie *et al.* 2002; Xu *et al.* 2010). However, the high cyanobacterial cell abundance and MC concentrations in reservoir waters were correlated to low TN/TP ratio. This coincides with the results of many studies around the world, reporting that low TN/TP ratio (<29) favors cyanobacterial growth (Smith 1983; Jacoby *et al.* 2015) and increase cellular MC content (Harris *et al.* 2014). Additionally, the abundance of *M. aeruginosa* and intracellular MC levels were correlated with iron, though it was found in very low concentrations in reservoir waters. Our results are thus consistent with earlier studies stating that cyanobacterial growth (Lee *et al.* 2011; Sinang *et al.* 2015) and MC production (O'Neil *et al.* 2012) are induced at low iron concentrations. It has been reported that iron plays an essential role in many metabolic pathways including MC biosynthesis in cyanobacteria (Wang *et al.* 2010). Cyanobacteria can change their cellular iron requirements, and increase the ability to utilize iron at low concentrations, through the synthesis of siderophores (Lee *et al.* 2011). Although we did not determine the light intensity on the surface of reservoir water, cyanobacteria have grown autotrophically by gaining light through a gap between the reservoir and its lid. Cyanobacteria have been proven to use far-red light through the synthesis of chlorophyll *d* and chlorophyll *f*, which allow cells to grow and maintain a high growth rate under low light conditions (Gan & Bryant 2015).

The presence of *M. aeruginosa* in these reservoirs with high cell numbers ($4.6–5.5 \times 10^6$ cells L^{-1}) exceeding the WHO limit (2,000 cells mL^{-1}) could represent a risk to the

health of consumers, as this cyanobacterium was found to produce MC toxins (Mohamed *et al.* 2015). Furthermore, extracellular MCs were also detected in reservoir waters in high concentrations (1–7.6 μg L^{-1}), surpassing the WHO acceptable limit of MC-LR in drinking water (1 μg L^{-1}). These concentrations of extracellular MCs in some reservoirs showed a slight non-significant decrease compared to those detected in water (1.1–7.4 μg L^{-1}) received from Damietta treatment plant (Mohamed *et al.* 2015). The slight decrease in MC concentrations could be due to their degradation by solar photolysis and/or bacteria, while the increased concentrations occurred by releasing from the cells of *M. aeruginosa*. It is well known that MCs are produced and remain within the cells during the growth and stationary phase, but they are released into the water during cell death, senescence or any sudden stress conditions (Tsuji *et al.* 2001; Merel *et al.* 2013). Furthermore, cyanobacterial cells present in reservoir waters pass through distribution tubes to the public. During distribution, the cells may adhere or attach to the surface of pipelines or householding filters and thereby will be lysed by aging and release the toxins into the water. This will increase the concentration of dissolved MCs in the drinking water consumed by humans. Additionally, heterotrophic bacteria were detected in these domestic reservoir waters with counts higher than European guidelines (100 CFU mL^{-1}) for drinking water standards (European Union 1998). As these domestic reservoir waters are disinfected, the chlorine residue in reservoir water was insufficient to kill all heterotrophic bacteria. In this respect, Ollos *et al.* (1998) stated that the concentration of free chlorine should be more than 0.5 mg L^{-1} to reduce bacterial growth. Otherwise, these heterotrophic bacteria could be associated with extracellular mucus zone of *Microcystis* (Tuomainen *et al.* 2006), which provides protection against disinfectants and offers particle surfaces to which the heterotrophic bacteria can attach (Maruyama *et al.* 2003; Eiler *et al.* 2006). Moreover, these bacteria can use the carbon fixed by cyanobacteria, particularly released during growth in the phycosphere zone and grow forming biofilms (Cai *et al.* 2014). The most predominant heterotrophic bacterial strains isolated from DR during the present study represented the genera *Aeromonas, Bacillus*, *Corynebacterium and Proteus*, which were previously isolated from the Nile river raw water of Damietta treatment plant (Al-Raghi 2015). This implies that the Nile River water is the original source of the contamination of these DR with heterotrophic bacteria. Previously, these genera were investigated in treated and untreated drinking waters in other countries (Allen *et al.* 2004; Pavlov *et al.* 2004; Jeenaa *et al.* 2006).

CONCLUSIONS

Our results indicate that domestic reservoir waters in Damietta (Egypt) are contaminated with toxic cyanobacteria. The contamination source is most likely the finished treated water received from the relevant treatment plant. The environmental conditions in these reservoirs favored the proliferation of toxic *M. aeruginosa*, with highest cell density obtained during warm months. Both intracellular and extracellular MCs were also detected in reservoir waters at concentrations exceeding the WHO guideline limit for these toxins in drinking water. The presence of cyanobacteria and MCs in domestic water storage reservoirs deteriorates the water quality and constitutes health hazards to households. Furthermore, the occurrence of heterotrophic bacteria associating with cyanobacteria in these reservoirs may also affect the water quality and form biofilms, which provide protection of the organisms inside them from the effects of disinfectants (Berry *et al.* 2006). Therefore, domestic water storage reservoirs should be regularly monitored for the presence of toxic cyanobacteria and their cyanotoxins. The households and consumers should be educated on the physical appearance of water and the actions to be taken during biofilm and scum occurrence. It is also recommended that the amount of chlorine added to domestic reservoir waters to be adjusted to a level that will protect water quality during storage.

The authors declare that there is no conflict of interest

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First received 28 November 2015; accepted in revised form 12 March 2016. Available online 13 April 2016