Breakthrough of *Oscillatoria limnetica* and microcystin toxins into drinking water treatment plants – examples from the Nile River, Egypt

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ABSTRACT

The presence of cyanobacteria and their toxins (cyanotoxins) in processed drinking water may pose a health risk to humans and animals. The efficiency of conventional drinking water treatment processes (coagulation, flocculation, rapid sand filtration and disinfection) in removing cyanobacteria and cyanotoxins varies across different countries and depends on the composition of cyanobacteria and cyanotoxins prevailing in the water source. Most treatment studies have primarily been on the removal efficiency for unicellular *Microcystis* spp., with little information about the removal efficiency for filamentous cyanobacteria. This study investigates the efficiency of conventional drinking water treatment processes for the removal of the filamentous cyanobacterium, *Oscillatoria limnetica,* dominating the source water (Nile River) phytoplankton in seven Egyptian drinking water treatment plants (DWTPs). The study was conducted in May 2013. The filamentous *O. limnetica* was present at high cell densities (660–1 877 cells/mL) and produced microcystin (MC) cyanotoxin concentrations of up to 877 $\mu g \cdot g^{-1}$, as determined by enzyme-linked immunosorbent assay (ELISA). Results also showed that conventional treatment methods removed most phytoplankton cells, but were ineffective for complete removal of *O. limnetica*. Furthermore, coagulation led to cell lysis and subsequent microcystin release. Microcystins were not effectively removed and remained at high concentrations (0.37–3.8 $\mu g \cdot L^{-1}$) in final treated water, exceeding the WHO limit of 1 $\mu g \cdot L^{-1}$. This study recommends regular monitoring and proper treatment optimization for removing cyanobacteria and their cyanotoxins in DWTPs using conventional methods.

Keywords: cyanobacteria, removal, conventional drinking water treatment

INTRODUCTION

Frequent occurrence of extensive overgrowth of cyanobacteria in water bodies worldwide has been attributed to human activities (Huber et al., 2012; Mohamed and Al-Shehri 2015). The increasing incidence of harmful blooms of cyanobacteria in drinking water sources is a major environmental and public health concern as many of these cyanobacteria can produce potent toxins called cyanotoxins (Merel et al., 2011). Cyanotoxins have been shown to cause adverse human health effects such as gastroenteritis, skin rashes, liver damage and neurotoxic effects (Chorus and Bartram, 1999).

Microcystins (MCs) are the most common cyanotoxins in freshwater sources worldwide, with more than 100 variants identified thus far (Pantelic et al., 2013). They are produced by some cyanobacteria, such as *Microcystis, Planktothrix, Oscillatoria, Nostoc, Anabaena* and *Gloeotrichia* (Hisbergues et al., 2003). MCs are extremely stable and resistant to heat, hydrolysis and oxidation (de la Cruz et al., 2011). Human poisoning episodes have been reported upon consumption of drinking water contaminated with MCs. For instance, the death of 60 patients in a hemodialysis clinic in Brazil (Pouria et al., 1998), and the high incidences of primary liver cancer in China (Ueno et al., 1996) and Serbia (Svircev et al., 2009) have been linked to drinking water containing MCs. Moreover, Lévesque et al. (2014) documented a relationship between gastrointestinal symptoms and consumption of drinking water from a plant in Canada equipped with a full treatment system but whose source water is highly contaminated with cyanobacteria and MCs. These events highlight the importance of adequate water treatment methods to remove cyanobacteria and their toxins to provide safe drinking water.

However, effective removal of cyanobacteria and MCs by conventional methods differs according to the type and concentrations of chemicals used in the water treatment processes, the concentrations of cyanotoxins entering the treatment process, and the cell density of cyanobacteria (Pantelic et al., 2013). Furthermore, removal efficiency could also depend on the cyanobacterial species prevailing in the intake water of treatment plants (Zamyadi et al., 2013). Most studies on the removal of cyanobacteria at drinking water treatment plants were conducted with *Microcystis*, being the most common species in temperate and tropical freshwater sources (Mohamed and Carmichael, 2000; Zamyadi et al., 2012). Filamentous cyanobacteria, particularly *Oscillatoria* spp., can dominate both benthic and planktonic cyanobacterial populations in water sources, and some of its species can produce MCs (Mohamed et al., 2006). *Oscillatoria limnetica* is dominant in the Nile River phytoplankton (Mohamed et al., 2007), and recently regular field observations revealed heavy growth of this species in the intake area of most drinking water treatment plants constructed on the Nile River (Author's personal observation). To our knowledge, no study has been conducted on the removal of this species by conventional methods in drinking water treatment plants. Therefore, the present study was undertaken to study the removal efficiency for *O. limnetica* during conventional drinking water treatment processes. Simultaneously, microcystin concentrations were monitored in both raw and treated waters to evaluate the release and removal of these toxins during different treatment processes.

MATERIALS AND METHODS

Sample preparation

Water samples were collected from the intake of 7 drinking water treatment plants (DWTPs) along the Nile River, Egypt, and after each step of treatment processes including coagulation/flocculation/sedimentation (C/F/S), rapid sand filtration and final treated water. The DWTPs are located 200–300 m from the banks of Nile. The intakes of DWTPs are 100–120 m offshore and located at 8–10 m depth in the Nile River. The names and locations of DWTPs used in this study have been replaced by numbers in accordance with the national safety information laws. Treatment processes include pre-chlorination (3–5 mg·L⁻¹), coagulation/flocculation with $Al_2(SO_4)_3$, sedimentation (1.5 h), rapid sand filtration and finally chlorine disinfection (2–3 mg·L⁻¹) with a residual concentration of 0.2–0.3 mg·L⁻¹. Water samples were collected within 2–3 h of midday when there had been no rain for one month. Water samples (1 L) were collected in dark glass bottles, kept at 4°C and transported to the laboratory for analyses immediately after sampling. Subsamples of a known volume (500 mL) of water sample were preserved in 1% Lugol's solution for phytoplankton analysis. Other subsamples (250 mL) were filtered through 0.45 μm cellulose filter (Whatman, UK) to analyse both intracellular and extracellular toxins.

Phytoplankton analysis

Phytoplankton in the fixed samples were identified under a light microscope based on taxonomic publications such as Komárek and Anagnostidis (2005). Algal and cyanobacterial cells in raw water and during treatment processes were counted with a hemacytometer and calculated as number of cells per milliliter (cells·mL⁻¹). The removal efficiency of each treatment step was represented as the difference in the cell number between each two consecutive steps divided by the cell number found in the former step.

Microcystin analysis

Intracellular microcystins (cell-bound) were determined by extracting the filters with associated cells in 90% methanol with sonication. The extract was left overnight at room temperature, and then centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was air-dried to evaporate the organic solvent, and the remaining aqueous fraction was kept at −20°C till analysis. Extracellular MCs were determined directly in filtered water. MC concentrations were determined by Microcystin-ADDA ELISA kit purchased from Abraxis (Warminster, PA, USA) according to the method of Carmichael and An (1999). Negative (deionized water) and positive controls (0.75 μg·L⁻¹ MC-LR) were tested by this assay. Analysis was done in triplicate.

Statistical analysis

Differences in cyanobacterial density and MC concentrations in source water and at different treatment stages were compared by one way ANOVA ($P<0.05$) using SPSS 17 software for Windows. Correlations between the variables were calculated using the Spearman correlation test.

RESULTS

Microscopic examination of the source water (Nile River in Egypt) of the seven DWTPs in May 2013 revealed a dominant cyanobacterium, *O. limnetica* (660–1 877 cells·mL⁻¹) (Table 1). Other phytoplankton taxa of green algae and diatoms at low levels were also found in the source water of these DWTPs (Table 1). The different treatment processes also revealed differential removal of phytoplankton species. Coagulation/flocculation/sedimentation (C/F/S) removed all green algal cells and about 92–95% of diatoms from the raw water, but was ineffective for the removal of all cyanobacterial species. While all cells of *Gomphosphaera lacustris* and *Merismopedia elegans* were removed during C/F/S, the cells of *O. limnetica* were not completely removed. Furthermore, the removal efficiency for *O. limnetica* by C/F/S varied significantly ($P < 0.05$) among DWTPs surveyed (5.6–86.4 %). Similarly, rapid sand filtration could not effectively (25–34%) remove the *O. limnetica* cells that remained after C/F/S. In disinfected final treated water, the cell density of *O. limnetica* cells was reduced by 25–66.7%.

The results of microcystin analysis by ELISA showed that intracellular MCs (cell-bound MCs) in the raw water varied significantly among DWTPs ($P < 0.05$). The highest concentration was detected in the raw water of DWTP 7 (14.1 μg·L⁻¹), while the lowest concentration was in DWTP 4 (0.66 μg·L⁻¹).

TABLE 1
Cell density (cells·mL⁻¹) of phytoplankton present in the source water of drinking water treatment plants during the study period

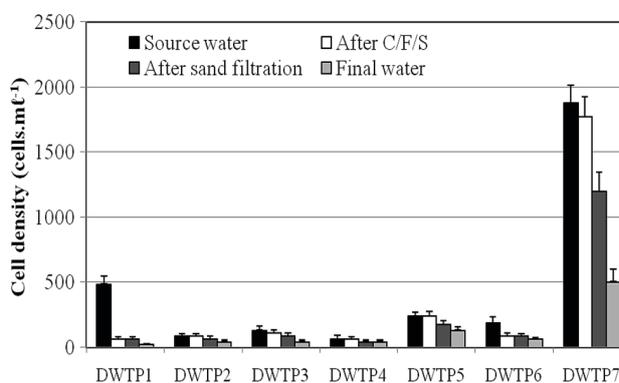| Species	Cell density
Gomphosphaera lacustris.	86–980
Merismopedia elegans	160–1 470
Oscillatoria limnetica	66–1 877
Cyclotella sp	20–650
Nitzschia sp	60–1 020
Synedra sp.	30–310
Pediastrum sp.	40–160
Scendesmus sp.	30–230
Chlorella sp.	50–410
Ankistrodesmus sp	20–330

Figure 1
Cell density of Oscillatoria limnetica *(cells·mL⁻¹) in source and treated waters at different water treatment plants in Egypt*

These concentrations of cell-bound MCs associated markedly with the cell density of *O. limnetica* rather than that of other cyanobacterial species present in the samples, indicating that these toxins were mainly produced by this species. In this context, dried material of *O. limnetica* cells isolated from the raw water of DWTP 7 during the present study was analysed and found to contain 877 µg MCs·g⁻¹ dry weight (7.5 pg·cell⁻¹). On the other hand, the concentrations of intracellular MCs decreased sharply along the different treatment stages, reaching its lowest values in the final water of all DWTPs (Fig. 2). Conversely, extracellular MCs (dissolved MCs) were detected at lower concentrations (0.05–0.8 µg·L⁻¹) in the source water of all DWTPs (Table 2). But, these concentrations increased dramatically in the clarified water after C/F/S process (0.3–4.2 µg·L⁻¹) and sand filtration (0.41–4.4 µg·L⁻¹), and remained without significant change in the final water (0.37–3.8 µg·L⁻¹) after chlorine disinfection.

DISCUSSION

This study reports the presence of the filamentous cyanobacterium, *O. limnetica,* as the dominant species in the source water (Nile River) of 7 Egyptian DWTPs, with cell densities not exceeding the WHO alert level 1 (2 000 cells·mL⁻¹) proposed for drinking water monitoring programmes (Chorus and Bartram, 1999). However, this species was found to produce MCs, and its presence in drinking water sources may therefore pose a risk to human health. The *O. limnetica* strain isolated from groundwater wells in Saudi Arabia (Mohamed and Al-Shehri 2009) has previously been reported to produce microcystins. Cyanotoxins can enter a DWTP in two forms, either as intracellular toxins (inside cyanobacterial cells) or as extracellular (outside the cells) toxins. These toxins should be removed through different treatment processes adopted in DWTPs. In this study, although conventional methods used in DWTPs removed all the cells of green algae and most cyanobacterial species, they were ineffective for the complete removal of *O. limnetica* cells. The results of this study are thus in accordance with the study of Kommineni et al. (2009) conducted in 8 DWTPs in the USA, which showed selective removal of cyanobacterial species, where *Oscillatoria* sp., *Pseudanabaena* sp., and *Lyngbya* sp. were the least effectively removed species. Similarly, Ewerts et al. (2013) reported the removal of all cyanobacteria except *Oscillatoria* sp. during coagulation, flocculation and sand filtration. Zamyadi et al. (2013) provided evidence that the efficiency of conventional treatment methods in removing cyanobacterial cells differs among genera. While *Anabaena* and *Microcystis* were removed effectively, *Aphanizomenon* cells were not effectively coagulated and not fully retained on the filter, and passed into chlorinated water at high cell densities (Zamyadi et al., 2013). The discrepancy in the removal efficiency for different genera of cyanobacteria by conventional methods used in DWTPs may be due to specific characteristics, including cell morphology, surface charge and cell density (Henderson et al., 2008). These characteristics influence the mechanism of destabilization of cyanobacterial cells during flocculation (Ewerts et al., 2013). Bernhardt and Clasen (1991) reported that the coagulation of smooth and more or less spherical algal cells occurs largely by charge neutralization, while filamentous algae could be dealt with by sweep coagulation, which requires higher coagulant dosage for precipitation of the metal salts and entrapment of filamentous cyanobacteria and algae on the sweep flocs. Conversely, high doses of coagulant can affect the coagulant charge neutralization mechanism. Exceeding the

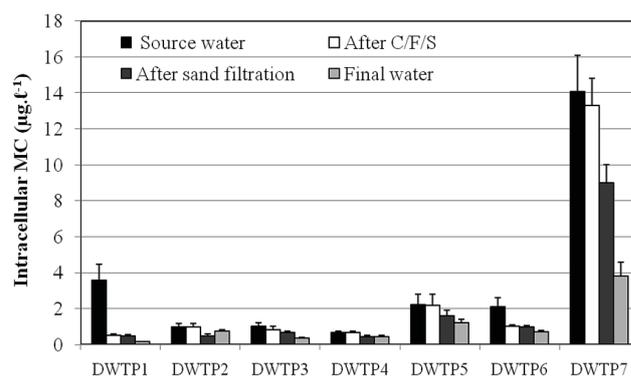|	Table 2 Concentrations of extracellular microcystins (µg·L⁻¹) in source and treated waters at different water treatment plants in Egypt			
Treatment plants	**Microcystin concentrations (µg·L⁻¹)**			
	Source water	**After C/F/S**	**After sand filtration**	**Final water**
DWTP1	0.1±0.02	0.3±0.07	0.5±0.08	0.38±0.04
DWTP2	0.07±0.003	0.45±0.1	0.6±0.07	0.53±0.05
DWTP3	0.06±0.005	0.35±0.08	0.41±0.05	0.37±0.04
DWTP4	0.05±0.003	0.51±0.07	0.53±0.09	0.42±0.06
DWTP5	0.6±0.1	1.5±0.2	1.4±0.2	1.2±0.2
DWTP6	0.2±0.05	0.36±0.07	0.43±0.07	0.4±0.07
DWTP7	0.8±0.09	4.2±0.8	4.4±0.8	3.79±0.6

Figure 2
Concentrations of intracellular microcystins (µg·L-1) in source and treated waters at different water treatment plants in Egypt

water coagulant demand can result in an over-abundance of coagulant positive charges relative to negative charges, thereby destabilizing the particle (Liu and Chin, 2009).

The coagulation process in DWTPs should be adjusted/optimized according to the type and morphology of algal and cyanobacterial species found in the source water. Furthermore, coagulation/flocculation is not only ineffective for cell removal but also can induce cell lysis and toxin release (Pietsch et al., 2002; Zamyadi et al., 2012). This was also observed in the Egyptian DWTPs, where the extracellular MCs concentrations increased greatly after flocculation in most DWTPs studied, compared to their concentrations in the source water. Additionally, the cell lysis and MC release in the Egyptian DWTPs during coagulation/flocculation process may be due to chlorine pre-oxidation used in these treatment plants, as demonstrated by many authors (Pietsch et al., 2002). On the other hand, the high concentrations of extracellular MCs during C/F/S process may be due to the release of these toxins from cells contained in the sludge as they can break down rapidly (Mohamed et al., 2015). Rapid sand filtration did not effectively remove either *O.limnetica* cells or MCs, but slightly increased dissolved MC concentrations at all DWTPs studied. Our results are thus in agreement with previous studies reporting that rapid sand filtration is ineffective for the removal of cyanobacterial cells or extracellular cyanotoxins (Ho et al., 2006; Mohamed et al., 2015). Rapid sand filtration can lead to increased dissolved MCs in water due to cell lysis caused by shear stress, inadequate backwashing or cell ageing (Pietsch et

163

al., 2002; Zamyadi et al., 2012). MCs may then concentrate in the filter backwash water. Therefore, the filter backwash water should not be returned to the head of the plants for re-use without treatment. Furthermore, chlorine, used as a disinfectant in the final treatment step in Egyptian DWTPs, was ineffective for complete cell removal of *O. limnetica* (25–67%) and did not fully degrade MCs. Consequently, MC concentrations in the final treated water in DWTPs 5 and 7 were high (1.3 and 3.8 µg·L^{-1}, respectively) and exceeded the WHO provisional guideline limit (1 µg·L^{-1} MC-LR) for these toxins in drinking water (WHO, 1998). These results are in agreement with previous studies reporting the inefficiency of chlorination in destroying MCs (Zamyadi et al., 2012). The efficiency of MC degradation by chlorination depends on a number of parameters including the type of chlorine compound, the concentration used, pH and contact time (De la Cruz et al., 2011). For instance, a chlorine dose of 2.8 mg·L^{-1} for a contact time of 30 min was sufficient to cause 99% destruction of MC-LR (Tsuji et al., 1997). On the other hand, the inability of chlorination to remove *O. limnetica* cells may be explained by the fact that the susceptibility of cyanobacterial cells to oxidants is influenced by the species present, the physiological state of the cells, and oxidation conditions (Pietsch et al., 2002; Zamyadi et al., 2012).

In conclusion, this study showed that conventional water treatment processes, such as pre-oxidation, coagulation, sedimentation, sand filtration, and chlorination, in Egyptian DWTPs were ineffective in the elimination of all cyanobacterial cells and/or extracellular MC toxins. These toxins were found in final treated water in two Egyptian DWTPs at concentrations exceeding the WHO guideline limit proposed for these toxins in drinking water (1 µg·L^{-1} MC-LR). The efficiency of cell removal can be influenced by the cyanobacterial species, and *O. limnetica* was the least effectively removed species in these treatment systems. The presence of toxic *O. limnetica* and/or its MC toxins in the final drinking water poses a risk to human and animal health. Therefore, DWTPs using such conventional treatment methods in Egypt and other countries necessitates alternative treatment approaches to remove cyanobacterial cells and their toxins. The pre-oxidation step which causes cell lysis and toxin release must be eliminated during harmful bloom events of cyanobacteria. The application of activated carbon simultaneously with clarification or in percolation units has been shown to be efficient in removing extracellular cyanotoxins (Merel et al., 2013). Finally, regular monitoring of cyanobacteria and their cyanotoxins in the source and clarified waters of such DWTPs is necessary for proper treatment optimization.

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