

# Endotoxin removal efficiency in conventional drinking water treatment plants, a case study in Egypt

Zakaria A Mohamed<sup>1</sup> , Saad Alamri<sup>2</sup> and Mohamed Hashem<sup>2,3</sup>

<sup>1</sup>Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt

<sup>2</sup>Biological Science Department, College of Science, King Khalid University, PO Box 61413, Abha, Saudi Arabia

<sup>3</sup>Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

The present study determines the endotoxin removal efficiency of drinking water treatment plants (DWTPs) in Egypt, as examples of conventional treatment methods used in developing countries. The total endotoxin in source water (Nile River) of these DWTPs ranged from 57 to 187 EU·mL<sup>-1</sup>, depending on the location of treatment plants. Coagulation/flocculation/sedimentation (C/F/S) after chlorine pre-oxidation removed bound endotoxins by 76.1–85.5%, but caused cell lysis and increased free endotoxins by 28.2–33.3% of those detected in raw waters. Rapid sand filtration had not significant effect on free endotoxins, but reduced bound endotoxins by 23–33.3%. Final chlorine disinfection also reduced bound endotoxins to levels around 1 EU/mL, accompanied by an increase in free endotoxins (37–112 EU·mL<sup>-1</sup>) in finished waters. Simultaneously, final chlorine disinfection removed all heterotrophic bacteria, with low cyanobacterial cell numbers (348–2 450 cells·mL<sup>-1</sup>) detected in finished waters. Overall, conventional treatment processes at these DWTPs could removal substantial amounts of bound endotoxins and bacterial cells, but increase free endotoxins through cell lysis induced by pre-oxidation and final chlorine disinfection. The study suggests that conventional processes at DWTPs should be optimized and upgraded to improve their performance in endotoxin removal and ensure safe distribution of treated water to consumers.

## INTRODUCTION

High nutrient concentrations due to anthropogenic activities lead to eutrophication and formation of harmful cyanobacterial blooms (HCBs) in water sources (O'Neil et al., 2012). HCBs are found in the aquatic environment as complex consortia composed mainly of cyanobacteria with different levels of heterotrophic bacteria (Berg et al., 2009). These blooms constitute a serious environmental and health problem, including oxygen depletion resulting from bacterial decomposition of dying blooms, and leading to suffocation of aquatic animals and fish kills (Paerl and Otten, 2013). Additionally, most species of cyanobacteria produce a wide array of cyanotoxins that are highly detrimental for aquatic and terrestrial animals (Codd et al., 2005), and jeopardize the quality of drinking water if not properly treated (Mohamed et al., 2015).

Endotoxins are among the cyanotoxins that have been associated with adverse health effects, e.g., acute inflammatory gastrointestinal diseases, skin and eye irritation, vomiting, fever and abdominal pain in humans through drinking water or recreational activities (Durai et al., 2015). Indeed, endotoxins are lipopolysaccharides (LPS) constituting the major component of the cell wall of Gram-negative bacteria and some cyanobacteria (Trent et al., 2006) and, therefore, these microorganisms are considered as the main source of endotoxins in the intake water of DWTPs. Generally, endotoxins in water are found in two forms: free endotoxins (i.e., dissolved in cell-free water) and bound endotoxins that are associated with viable bacterial cells and other suspended particles (Zhang et al., 2013).

Endotoxins are released from the cells via cell lysis or during multiplication, and are relatively heat stable (at 121°C for 1 h) (Anderson et al., 2003). These traits pose challenges for endotoxin removal during treatment processes at DWTPs. Although several cyanotoxins are produced by cyanobacteria, most attention has been focused on microcystin and cylindrospermopsin in drinking water. However, the fate of endotoxins in conventional DWTPs is largely unexplored, and no maximum endotoxin limit in drinking water has been established yet.

Therefore, both cyanobacteria and associated heterotrophic bacteria should be monitored along with endotoxin concentrations during treatment processes, to evaluate their removal and ensure that safe water is distributed to the consumer. The results of previous studies on the efficiency of conventional treatment processes in DWTPs are contradictory. Some studies reported that endotoxins are mainly reduced in the early stages of conventional processes, including coagulation, sedimentation and sand filtration, with little effect from chlorine oxidation (Anderson et al., 2002, 2003; Rapala et al., 2002, 2006; Gehr et al., 2008; Huck et al., 2013; Xue et al., 2019). A recent study by Simazaki et al. (2018) showed that coagulation and sedimentation, followed by rapid sand filtration, at Japanese DWTPs were highly effective to decrease both bound and free endotoxins, with limited decrease in free endotoxins by chlorination and ozonation. It seems that removal efficiency for endotoxins in DWTPs varies according the source waters containing producer microorganisms, and treatment processes. Hence, the aim of this study was to evaluate the performance of endotoxin (bound and free endotoxins) removal from water during conventional drinking water treatment processes in Egypt.

## CORRESPONDENCE

Zakaria A Mohamed

## EMAIL

Zakaria.attia@science.sohag.edu.eg  
mzakaria\_99@yahoo.com

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## MATERIALS AND METHODS

### Water sampling

Three DWTPs in different provinces in Egypt were selected for collection of water samples, considering the location and the extent of exposure of the source water (Nile River) to discharges and pollution (i.e., eutrophication). DWTP1 is located in the Nile Delta (i.e., Lower Egypt) region with daily treatment capacity of 25 000 m<sup>3</sup>, and its intake water is affected by high discharges and heavy cyanobacteria blooms. DWTP 2 has a daily treatment capacity of 650 000 m<sup>3</sup> and is located in Greater Cairo, where intake water is exposed to moderate discharge and high cyanobacterial cell density. DWTP3 has a daily treatment capacity of 25 000 m<sup>3</sup> and is located in Upper Egypt, with intake water characterized by low cyanobacterial biomass (Mohamed et al., 2016). All three DWTPs employ similar conventional processes, including chlorine pre-oxidation, coagulation/flocculation/sedimentation (C/F/S), rapid sand filtration (without biofilters), and final chlorine disinfection. However, chemical doses used differ among these plants depending on the quality of raw water. The alum doses used in the coagulation basins of DWTP1, DWTP2 and DWTP3 were 35, 30 and 30 mg·L<sup>-1</sup>, respectively. The chlorine gas dose amounts were 3.5, 4.5 and 5 mg·L<sup>-1</sup> for the pre-chlorination and 3, 2, 2.5 mg·L<sup>-1</sup>, respectively, for the disinfection of the finished treated water. The contact time with chlorine was 30 min in all DWTPs and final residual chlorine concentrations in finished water of these plants ranged from 1.5–1.7 mg·L<sup>-1</sup> (Table 1).

Water samples were collected in triplicate from the raw water, and the effluent of each process unit, i.e., C/F/S, sand filtration, and finished water after final chlorine disinfection, during August 2020. All water samples were collected in 500 mL amber glass bottles that were previously depyrogenated by heating at 350–400°C for 30 min. All glassware used in the experiments was also depyrogenated as mentioned above. Residual chlorine in water samples was immediately quenched with sodium thiosulfate (0.1 M) during sampling. Bottles were kept in 4°C ice box, transported to the laboratory, and used for the analysis of endotoxin concentrations, HPC and cyanobacterial cell counts within 24 h. Quality parameters of raw and treated water were determined using protocols outlined in Standard Methods (APHA, 2005). The pH and conductivity were measured using multi-parametric probe (HI 991300 pH/EC/TDS Temperature, HANNA, Italy). Turbidity, in nephelometric turbidity units (NTU), was measured using a HACH 2100 turbidity meter. Dissolved organic carbon (DOC) concentrations were determined using organic carbon analyser (Teledyne Tekmar, TOC fusion). UV absorbance at 254 nm wavelength (UV254) was measured using an ULTROSPEC II: UV/Vis spectrophotometer (Model 80-2091-73, Biochrom, UK). DOC and UV254 samples were filtered through Millipore 0.45 µm filters. The specific ultraviolet absorbance (SUVA, in L·mg<sup>-1</sup>·m<sup>-1</sup>) was expressed as a ratio between UV254 and DOC according to the following formula:

$$\text{SUVA} = \text{UV254}/\text{DOC}$$

### Heterotrophic plate count

Heterotrophic plate counts were determined after dilution and incubation on R2A agar plates at 37°C for 48 h according to APHA (1995). The number of heterotrophic bacteria was expressed as CFU·mL<sup>-1</sup>. Total coliform bacteria in water samples were determined by the membrane filter technique and cultured on selective and differential medium (M-endo-LES agar) (APHA, 1995).

### Total count of cyanobacteria

To investigate cyanobacterial cells in water samples, subsamples of a known volume (500 mL) were preserved in 1% Lugol's solution. Cyanobacterial species in the fixed samples were then identified according to Komárek and Anagnostidis (2005), and total cyanobacterial cells were counted using a Sedgewick–Rafter counting chamber.

### Endotoxin analysis

Limulus amoebocyte lysate (LAL) assay can detect both free endotoxin dissolved in water as well as bound endotoxins associated with bacterial and cyanobacterial cell walls (Ohkouchi et al., 2007). Therefore, an aliquot of water samples was centrifuged for 10 min at 10 000 g to determine free endotoxins in the supernatants. Water samples without centrifugation were used for determination of total endotoxin. Bound endotoxins were estimated by subtracting the free-endotoxins from the total endotoxins. To confirm that bound endotoxins detected in finished treated water were related to cyanobacteria-bound endotoxins only, the pellet of centrifuged finished treated water was washed with depyrogenated water and passed through 2 µm membrane filters to remove particle-attached endotoxins and endotoxin aggregates with diameter of < 2 µm. This was carried out based on the finding of Zhang et al. (2013) that bound endotoxins could be related to cell-bound endotoxins, endotoxin aggregates and particle-attached endotoxins, and endotoxin aggregates and particles are in the range of  $d < 2$  µm. Endotoxin concentrations were determined using the Limulus amoebocyte lysate (LAL) gelation clot technique according to WHO (2016) and the instructions of the manufacturer (ICN pharmaceuticals Inc., Costa Mesa, CA). The test is considered valid when the lowest concentration of the standard solutions shows a negative result. The activity was expressed at the lowest dilution needed to form a solid gel. The endotoxin concentration (EU·mL<sup>-1</sup>) was calculated by multiplying the lysate sensitivity (antilog 10 of the average log value of endotoxin endpoint) by endpoint dilution of the sample.

### Statistical analysis

Differences in endotoxin concentrations, HPC and cyanobacterial cell density in raw water, and different treatment processes, were compared using one-way ANOVA ( $P < 0.05$ ) using SPSS 18.0 software for Windows. Correlations between endotoxin concentrations and HPC and cyanobacterial cell density in water samples were measured using Spearman rank correlation coefficients.

## RESULTS AND DISCUSSION

### Water quality

The physico-chemical properties of raw and treated water at the three DWTPs are shown in Table 1. The pH was slightly alkaline and did not vary significantly between raw and treated water ( $P < 0.05$ ). Treated water of all conventional DWTPs are under the permissible limit for human health (Turbidity < 1 NTU; WHO, 2017). The DOC concentrations of treated water samples were also under the suggested limit (DOC < 2 mg·L<sup>-1</sup>; Edzwald and Tobiasson, 2011). Treated water of all DWTPs had SUVA values less than 2 L·mg<sup>-1</sup>·m<sup>-1</sup>, indicating that the DOC of the water is mainly composed of a high fraction of non-humic matter, with a low UV254 (Edzwald and Tobiasson, 2011). Residual chlorine values of treated water samples from DWTPs ranged from 1.5 to 1.7 mg·L<sup>-1</sup>, which is within the permissible limit (< 5 mg·L<sup>-1</sup>; WHO, 2004) but higher than minimum free chlorine residual level (0.2 mg·L<sup>-1</sup>; WHO, 2004). In general, the studied conventional DWTPs were effective in removal of carbon DOC and UV254.

**Table 1.** The quality of raw and finished treated water at the time of sampling. Each value is the mean of 9 results. Raw water was sampled before chlorination. Finished water samples were taken after chlorination. The free chlorine residual had been quenched with sodium sulphite.

Parameter	DWTP1		DWTP2		DWTP3	
	Raw water	Treated water	Raw water	Treated water	Raw water	Treated water
pH	8.3±0.1	7.9±0.2	7.8±0.2	7.8±0.2	8.1±0.1	7.7±0.1
Turbidity (NTU)	10.8±2	0.8±0.1	11.4±3	0.7±0.1	9.1±2	0.8±0.2
Conductivity (µS·cm <sup>-1</sup> )	678±31	523±23	542±21	497±19	734±34	573±27
DOC (mg·L <sup>-1</sup> )	4.1±0.7	3.1±0.4	3.2±0.5	2.1±0.3	4.6±0.9	3.6±0.8
UV254 (cm <sup>-1</sup> )	0.18±0.03	0.12±0.02	0.1±0.02	0.05±0.01	0.16±0.04	0.09±0.02
SUVA (L·mg <sup>-1</sup> ·m <sup>-1</sup> )	4.4±0.5	3.8±0.7	3.1±0.7	2.3±0.6	3.5±0.08	2.5±0.5
Residual chlorine (mg·L <sup>-1</sup> )	-	1.5±0.3	-	1.7±0.4	-	1.5±0.2
Dominant cyanobacteria	<i>Microcystis aeruginosa</i> <i>Merismopedia incerta</i> <i>Planktothrix agardhii</i>		<i>Anabaena flos aquae</i> <i>Merismopedia elegans</i> <i>Microcystis aeruginosa</i> <i>Oscillatoria limnetica</i>		<i>Merismopedia elegans</i> <i>Microcystis aeruginosa</i> <i>Oscillatoria limnetica</i>	

### Endotoxins in raw water

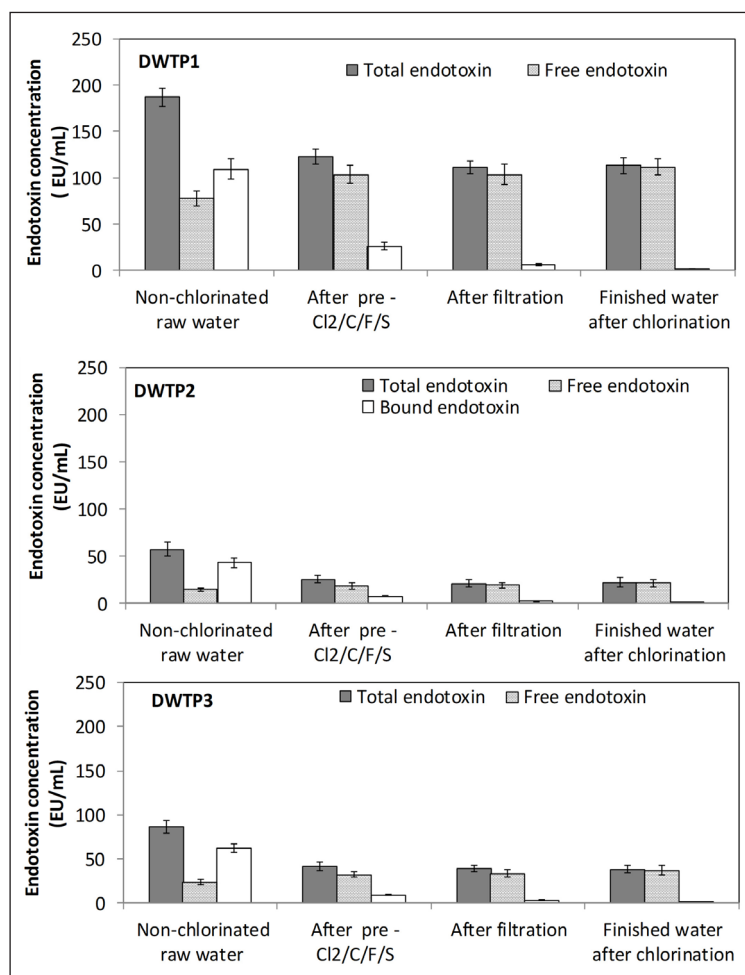
Endotoxin concentrations in the water samples collected at the three DWTPs are presented in Fig. 1. The total endotoxins in raw waters of these DWTPs ranged from 57 to 187 EU·mL<sup>-1</sup>, with 14–78 EU·mL<sup>-1</sup> of free-endotoxin and 43–109 EU·mL<sup>-1</sup> of bound endotoxin (Fig. 1). These concentrations differed significantly among the three DWTPs ( $P < 0.05$ ), and were within the range (9–118 EU·mL<sup>-1</sup>) of endotoxin levels reported in raw waters of DWTPs worldwide (Rapala et al., 2002; Zhang et al., 2013; Simazaki et al., 2018). Our results also showed that total endotoxin measured in the raw water of DWTP 1 (187 EU·mL<sup>-1</sup>), located in the Nile delta, was higher than its concentrations in the raw waters of DWTP2 in the greater Cairo region (57 EU·mL<sup>-1</sup>) and DWTP3 located in Upper Egypt (86 EU·mL<sup>-1</sup>). This may be due to high artificial/natural wastewater discharges into the Nile River which causes the water quality to deteriorate downstream and reach alarming levels in the Nile Delta (Abdel-Dayem, 2011). This pollution has resulted in increased nutrient concentrations, which promote harmful cyanobacterial blooms and the growth of associating heterotrophic bacteria (Mohamed et al., 2015), the main producers of endotoxins in water.

In the present study, total endotoxin concentration in raw waters of Egyptian DWTPs is significantly correlated with HPC ( $r = 0.9$ ) and total cyanobacteria cell count ( $r = 0.99$ ). The higher HPC and total cyanobacteria cell count, the higher endotoxin concentrations (Figs 1, 2). Moreover, our results revealed a strong linear positive correlation between the number of heterotrophic bacteria and total cyanobacteria ( $r = 0.8$ ) in source waters of DWTPs. This confirms the fact that cyanobacterial blooms stimulate the growth of heterotrophic bacteria through providing attachment sites and nutrients (Eiler and Bertilsson, 2004), and thereby contribute largely to endotoxin levels in the aquatic environment. In this context, Rapala et al. (2002) detected high concentrations of endotoxins (356 EU·mL<sup>-1</sup>) in DWTP raw waters experiencing cyanobacteria blooms in Finland. Zhang et al. (2013) recorded relatively low endotoxin concentrations (41 EU·mL<sup>-1</sup>) in source water of a DWTP in Beijing, compared to its concentration in source water of DWTPs in in Wuhan (86–101 EU·mL<sup>-1</sup>). The authors attributed the low endotoxin levels in Beijing water to low cell density of cyanobacteria and associated bacteria, which were inhibited by the lower temperature at this city. Ohkouchi et al. (2007) also reported that the fluctuation in endotoxin concentrations in Yodo River water (311–2 430 EU·mL<sup>-1</sup>) was related to the cell density of endotoxin-producing *Synechococcus* sp. Recently, Simazaki et al. (2018) found that the highest total endotoxin (236.7 EU·mL<sup>-1</sup>) was observed in DWTP source water containing cyanobacterial blooms dominated by *Microcystis* spp. and *Anabaena* spp.

### Endotoxin removal in water treatment processes

Figure 1 depicts changes in concentrations of free, bound and total endotoxins during different conventional treatment processes at the three DWTPs. C/F/S preceded by chlorine pre-oxidation caused a reduction in total endotoxin concentrations by 34%, 45%, and 42% of total endotoxins present in raw waters of DWTP1, DWTP2 and DWTP3, respectively. Meanwhile, free endotoxins exhibited remarkable levels in the clarifier water of all three DWTPs by 33%, 28%, 33%, respectively (Fig. 1). Bound endotoxins, on the other hand, were reduced by 76%, 83%, and 86%, during these treatment processes at the DWTPs studied. Our results are not in line with those obtained by Zhang et al. (2013), who found that coagulation/sedimentation processes at DWTPs in Beijing, China, can remove 52% of free endotoxins and 72% of bound-endotoxins, nor with the results of Simazaki et al. (2018) who reported a decrease in free and bound endotoxins by 86.5% and 60.8%, respectively, during coagulation and sedimentation at DWTPs in Japan. The discrepancy between our results and those of Zhang et al. (2013) and Simazaki et al. (2018) is due to the application of chlorine pre-oxidation in Egyptian DWTPs, but not in Chinese or Japanese DWTPs, which caused lysis of bacterial and cyanobacterial cells leading to toxin release and increased free endotoxins in the clarifier water. However, our findings are in accordance with the data of Massoudinejad et al. (2017), who demonstrated that pre-chlorination induced cell lysis and resulted in an increase in the concentration of total endotoxins by 6–7%. Mohamed et al. (2015) also found that chlorine pre-oxidation during the coagulation process led to cell lysis and release of microcystin toxin in the clarifier water at Egyptian DWTPs. Therefore, our study demonstrates that coagulation/flocculation/sedimentation processes are effective for removing bound endotoxins only. The decrease in the concentration of bound endotoxins during coagulation/flocculation/sedimentation processes was concomitant with the decrease in the cell number of heterotrophic bacterial and cyanobacterial cells (Fig. 2). This reflects the efficiency of these treatment processes for removing heterotrophic bacteria and cyanobacterial cells. This is in agreement with previous studies demonstrating that bound endotoxins are mainly correlated with heterotrophic bacteria and cyanobacteria in water (Zhang et al., 2013; Simazaki et al., 2018).

The present study also showed that after rapid sand filtration total endotoxins slightly decreased by 10%, 18%, and 5% of the levels found in the clarifier water of the three DWTPs, respectively (Fig. 1). Our results also demonstrated that rapid sand filtration was effective for bound endotoxin removal at all DWTPs, by lower percentages (23%, 27%, and 33 %, respectively), with no significant



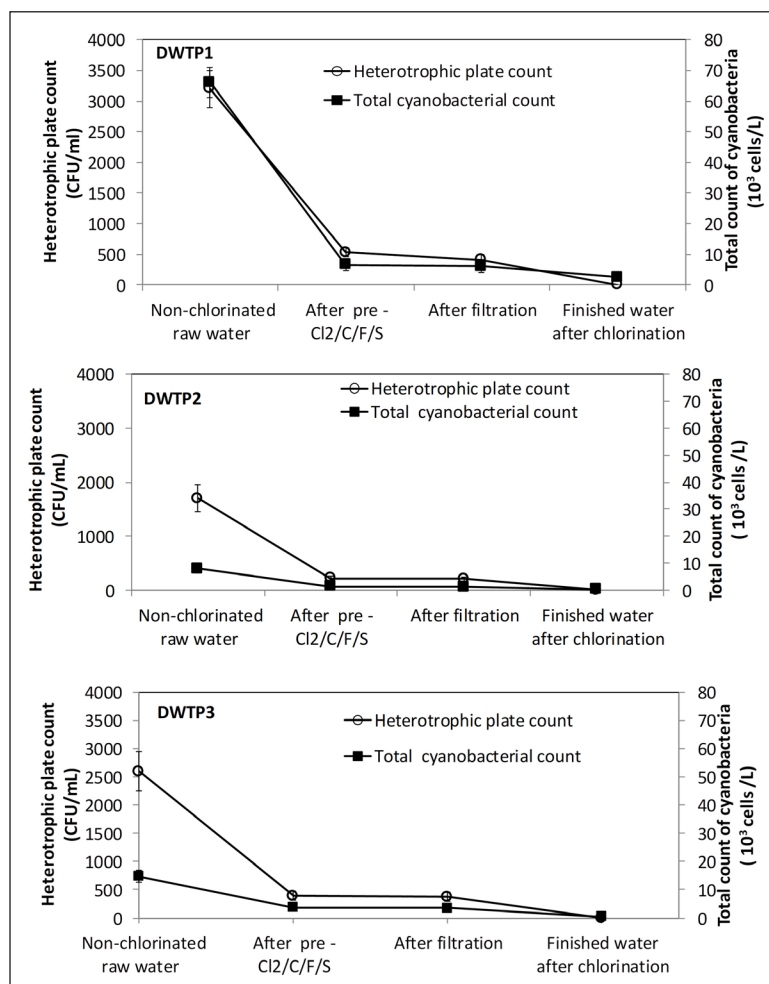
**Figure 1.** Endotoxin concentrations in water samples collected at three drinking water treatment plants in Egypt. Each value is the mean  $\pm$  SD ( $n = 9$ ).

effect on free endotoxins. This is in agreement with the results of Simazaki et al. (2018), which revealed rapid sand filtration was effective for removing bound endotoxins rather than free ones. This could be explained by the fact that bound endotoxins that are usually associated with large particles such as heterotrophic bacterial and cyanobacterial cells would be efficiently retained by the sand filter, whereas free endotoxins with low molecular size (10–20 KDa) and dissolved in water would pass easily through the sand filter (Simazaki et al., 2018). Earlier studies reported that rapid sand filtration does not remove dissolved matter (e.g., cyanotoxins), but also can promote the increase of dissolved MC concentration in the water due to cell lysis caused by shear stress, inadequate backwashing, or cell ageing (Pantelic et al., 2013; Mohamed et al., 2015).

The results also revealed that concentrations of total endotoxins in finished treated water, after final chlorine disinfection (i.e., post-chlorination), did not change significantly ( $P < 0.05$ ) from those of the previous step (sand filtration) at all three DWTPs (Fig. 1). However, bound endotoxins declined dramatically post-chlorination, reaching levels below 1 EU·mL<sup>-1</sup> in the finished water. Such a decrease in bound endotoxins post-chlorination was accompanied by increased concentrations of free endotoxins, which remained at levels of 112, 21 and 37 EU·mL<sup>-1</sup>, respectively, in finished waters of the three DWTPs (Fig. 1). Simultaneously, post-chlorination also caused a reduction in the cell number of heterotrophic bacteria and cyanobacteria, with no cells of heterotrophic bacteria observed in finished water (Fig. 2). But, cyanobacteria were recorded with varying cell numbers (348–2 450 cells·mL<sup>-1</sup>) in the finished water of the three DWTPs (Fig. 2). This indicates that cyanobacteria are more resistant to

chlorine oxidation than HPCs, which may be attributed to the protection provided by their mucilaginous sheath (Fan et al., 2016).

Additionally, cyanobacterial species that remained (i.e., were not removed) in the finished water varied among the three DWTPs. They include *Microcystis aeruginosa* in DWTP1, *Anabaena flos aquae* and *Microcystis aeruginosa* in DWTP2, and *Oscillatoria limnetica* in DWTP3. These results, therefore, indicate that final chlorine disinfection with doses used in Egyptian DWTPs (Table 1) could not remove free endotoxins, but rather increased their concentrations through induction of cell lysis leading to the release of bound endotoxins from bacterial and cyanobacterial cells. This reflects that concentrations of free endotoxins increased at the expense of bound endotoxins in finished water after final chlorine disinfection in the studied DWTPs. Similarly, Zhang et al. (2013) demonstrated that total endotoxins in finished water at DWTPs in China increased slightly after final chlorine disinfection, with significant decrease in bound endotoxins and release of free endotoxins. Huang et al. (2011) also found that chlorination at 10 mg·L<sup>-1</sup> after 30 min contact time could not effectively reduce endotoxin concentrations in effluent of a wastewater treatment plant, and significantly increased free endotoxin concentrations under conditions of high dose (50 mg·L<sup>-1</sup>). In this respect, Gehr et al. (2008) suggested that chlorine may have little or no effect on endotoxin levels. Anderson et al. (2003b) found that chlorination at 100 mg/L free chlorine residual could induce an incredibly slow inactivation rate for endotoxins. Simazaki et al. (2018) also observed that final chlorine disinfection at DWTPs in Japan is more effective in reducing bound endotoxins rather than free endotoxins. Recently, Xue et al. (2019) found that chlorine alone slightly increased the endotoxin activity of LPS.



**Figure 2.** Heterotrophic plate count and cyanobacterial total count in the water samples collected at the three drinking water treatment plants in Egypt. Each value is the mean  $\pm$  SD ( $n = 9$ ).

What is noteworthy in our study is that although final chlorine disinfection caused complete removal of heterotrophic bacteria (i.e., no HPC in finished water), levels of bound endotoxins in finished water of the three DWTPs were around 1 EU·mL<sup>-1</sup>. Given that cyanobacterial cells were still present (i.e., not completely removed by final chlorine disinfection) in finished water, such bound endotoxins could be related to cyanobacteria-bound endotoxins, in addition to endotoxin aggregates and particle-attached endotoxins. However, since the pellet of centrifuged finished water was washed with sterile distilled water and passed through 2  $\mu$ m membrane filters to remove particle-attached endotoxins and endotoxin aggregates with diameter of < 2  $\mu$ m, bound endotoxins detected in finished water in our study were comprised of cyanobacteria-bound endotoxins only. Concurrently, cyanobacterial cells investigated in the finished water were confined to specific species and varied among the three DWTPs: namely, *Microcystis aeruginosa* in DWTP1, *Anabaena flos aquae* and *Microcystis aeruginosa* in DWTP2, and *Oscillatoria limnetica* in DWTP3. These results imply that these cyanobacterial species could be the source of bound endotoxins detected in the finished water of DWTPs. These cyanobacterial species have been associated with endotoxin production in earlier studies (Ohkouchi et al., 2007; Mohamed and Al-Shehri, 2007; Bernardová et al., 2008; Zhang et al., 2019). These findings confirm the results of Ohkouchi et al. (2015) suggesting that cyanobacteria are major contributors to the increase in endotoxins in water sources and treatment processes. However, further studies are needed to isolate and characterize endotoxins produced by these cyanobacterial strains and evaluate their inflammatory potency in water supply systems.

## CONCLUSIONS

The present study profiled endotoxin concentrations, along with heterotrophic bacteria and cyanobacteria, in source water, and their fate during conventional treatment processes at three DWTPs in Egypt. Overall, conventional treatment processes (chlorine pre-oxidation, coagulation/flocculation/sedimentation, rapid sand filtration, and final chlorine disinfection) at the three DWTPs effectively removed almost all heterotrophic bacteria and 95.6–96.3% of cyanobacterial cells. These treatment processes also removed 98.5–98.8% of bound endotoxins, while they were not efficient in removing free endotoxins, but instead led to an increase in their concentration in treated water by 43–55% through inducing cell lysis and toxin release. Our study verified that chlorine pre-oxidation (i.e., prior C/F/S) causes cell lysis and toxin release, and thereby contributed largely to increased free endotoxins in finished water of DWTPs. Hence, we suggest that the pre-chlorination step should be avoided in DWTPs. Furthermore, our results confirmed the contribution of cyanobacteria to endotoxins in water sources. Endotoxin risks occurred following exposure to drinking water via bioaerosol inhalation from showers and humidifiers, and these hazards could be triggered when endotoxin activity of drinking water in humidifiers was > 1 000 EU·mL<sup>-1</sup> (Anderson et al., 2007; Zhang et al., 2019). The presence of endotoxins in bioaerosols can induce an inflammatory response in the lung-blood barrier, which made it more permeable (Tabrizi et al., 2010), and may result in a variety of acute and chronic clinical respiratory effects including cough, wheezing, dyspnea, upper airways irritation, asthmatic symptoms,

and chronic bronchitis) to some gastrointestinal disorders (Searl and Crawford, 2012; Farokhi et al., 2018). Therefore, there is an urgent need for regular monitoring of endotoxins along with cyanobacteria and associated heterotrophic bacteria in the intake raw water of conventional DWTPs worldwide, including Egypt. Moreover, conventional treatment processes at DWTPs should be optimized and upgraded to improve their performance in endotoxin removal.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## ORCIDS

Zakaria A Mohamed

<https://orcid.org/0000-0001-6378-1914>

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