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Occurrence of *Merismopedia minima* in a Drinking Water Treatment Plant in Sohag City and Removal of Their Microcystins by Sediments

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CYANOBACTERIA in drinking water are a major problem that threatens humans, animals and plants, especially if they produce toxins.This study aimed to remove cyanobacterial species, especially *Merismopedia mianima* and their microcystins (MCs) during the treatment process of drinking water. Water samples were collected from raw water, after each process and from the final treated water including coagulation, sedimentation, filtration and disinfection as well as from the final treated during the study period (May–December 2019). In all of these samples, *M. minima* was detected with an average cell count of 80-2200 cells/ mL.This increase in algal count may be due to the warm season when the temperature was favorable for cyanobacterial growth (blooming). Results also showed that traditional treatment methods could remove some cyanobacterial species but are ineffective in for completely removing MCs produced by *M. minima*, which was isolated even from the final stage of drinking water treatment. Furthermore, MCs were determined by High-Performance Liquid Chromatography (HPLC) analysis and the particulate MCs concentration of *M. minima* was found to record 500µg MCs/L (2.27pg/cell). Extracellularly released MCs were also not completely removed and remained at high concentrations of 0.74–1.47µg/L which exceeded the limit proposed by the World Health Organization (WHO= 1µg/L).The toxin concentration was mitigated to levels less than WHO limits when water sediments were used to remove MCs. Therefore, this study recommends using sediments to remove cyanobacteria and their cyanotoxins from water plants, which is an inexpensive method especially for the developing countries.

Keywords: Cyanobacteria, Drinking water treatment plant, Microcystin removal, Nile River sediments.

Introduction

Cyanobacteria (blue-green algae) are photosynthetic prokaryotes found in many environments (Mur et al., 1999). Cyanobacteria are important components of the ecosystem and are at the base of the food chain. As a result of environmental contamination by nitrogen and phosphorus, massive water blooms are dominated by cyanobacteria (Sharma et al., 2011). Besides other adverse ecological factors, prokaryotic cyanobacterial species can produce several hazardous and toxic substances. substances (Huisman et al., 2005; Meriluoto et al., 2017). Most species can produce many potent toxins, such as hepatotoxins, alkaloids neurotoxins and skin irritants dermatotoxins (lipopolysaccharides) (Bláha et al., 2009). Microcystins (MCs) are cyclic heptapeptides enclosing 7 amino acids, with molecular weights ranging between 900 and 1,100Da (Zurawell et al., 2005). Over 50 varied microcystins have been revealed so far, with microcystin-LR is being the most common. Hepatotoxins are poisonous to the liver and can cause liver damage due to the mechanism that they pick up from the intestine, blood, or the liver (Schmidt et al., 2014; Khairy & El-Sheekh, 2019). MC cause liver injury through the inhibition of protein phosphatase, which may lead successively to

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the accumulation of phosphorylated proteins in the liver, cell necrosis, massive hemorrhage and death (Runnegar et al., 1995; Jaeschke, 2015). Furthermore, liver toxins may cause tumor promotion in both the liver and colon (Williamson et al., 2019). When water contaminated by MCs is used for agriculture, applied by either direct spraying or taking them through the roots, hepatotoxins were accumulated in plants, causing a hazardous risk (Spoof et al., 2020). Toxins may affect crops through their effect on either germination or growth rates of some vegetables, such as lettuce or grasses (Freitas et al., 2015). Cyanobacterial toxins (especially MCs) are produced inside the cells (endotoxins). They are not released into the environment until cells lyse due to senescence or degradation resulting from the application of chemicals during the drinking water treatment process (Mohamed et al., 2015). The MCs concentration increases due to the release of the endotoxin as a result of cell damage or degradation. The dissolved toxins are not removed by conventional treatment methods, such as sedimentation, sand filtration and flocculation (Rapala et al., 2002). This study is aimed to investigate the prevalence of toxic cyanobacteria and cyanotoxins in raw and treated waters in a drinking water treatment plant in Sohag City.The adsorption of cyanobacterial hepatotoxins on water sediments and the possibility of using these sediments for reducing the concentration of the toxins during water treatment were also demonstrated

Materials and Methods

Collection of water samples

This study was conducted at a drinking water treatment plant in Sohag Governorate. The name and location of the drinking water treatment plant weren›t mentioned due to compliance with national safety information laws. Water samples from this plant were collected near the points of raw water intake. After each step of the treatment process, coagulation, sedimentation, filtration and disinfection The final treated water was collected by filling a 1L plastic bottle ~50 cm beneath the surface. The raw samples were collected monthly and after each step of the water treatment processes during warm periods (from May to December 2019) where the temperature was suitable for flourishing of cyanobacteria.

Physicochemical analyses of the collected water samples

Physicochemical analyses of the collected water samples were performed. Water temperature, electric conductivity and pH were measured at the same time of sampling using a multiparametric probe HI7698194-0. Dissolved oxygen (DO) was measured using a Lutron Oxygen Meter Model-PO2-250 and light intensity was measured by lux meter instruments. Water samples (1L) were filtered through Whatman, GF/C cellulose filter for ammonium, a soluble phosphate and nitrate content estimation and analyzed in the laboratory according to APHA (2017).

Isolation and identification of algal species.

Water samples (~500mL) were collected in dark glass bottles and immediately transported to the laboratory for analyses immediately after sampling and preserved in 1% Lugol's solution for cell counting. Algal counts were performed using a hemocytometer or a Sedgwick–-Rafter counting chamber and an Olympus binocular microscope. The cell number was calculated per liter of original lake water (Komarek & Hindak, 1988; Komarek & Kling, 1991; Komarek & Anagostidis, 1999). Single species of cyanobacteria was isolated from the water samples by successive streaking-plate method or single-cell method (Stanier & Cohen-Bazire, 1977). Cultures of isolated algal species were grown in liquid BG-11 medium and irradiance light of about ~24µmol/m^2/s at 16h: 8h light dark cycles at 25ºC (Stanier & Cohen-Bazire, 1977).

Determination of chlorophyll–a (Chl-a) concentration in collected water samples

To determine the chlorophyll content, water samples (~250mL) were filtered through a 0.45µm cellulose filter (Whatman, GF/C).The chlorophyll content was extracted from algal biomass in these filters with90% methanol and then determined spectrophotometrically according to the method of Anderson & Banse (1965) and the results were expressed as µg/L.

Microcystin determination

Microcystins within phytoplankton cells (endotoxins) and cell-free water were determined by filtering ~250mL water samples through GF/C filters and then extracted from algal cells in these filters with using 80% methanol. Microcystins concentrations in the filter extracts (intracellular) and the filtrate (extracellular) were detected by enzyme-linked immunosorbent assay (ELISA)

according to Carmichael & An (1999) by using commercial ELISA kits purchased from Abraxis (54 Steamwhistle Drive Warminster, PA 18974). MCs were extracted from M. minima cells by homogenizing 1 g dried biomass in methanol (80%) at room temperature while stirring. The procedure was repeated thrice followed by centrifugation at 6000 × g for 10min and the supernatants of each extract were combined. The organic solvent was blown with sterilized air and the aqueous fraction remaining after removing the organic solvent was filtered through a GF/C filter paper (Mohamed & Al Shehri, 2009). The filtered fraction was applied directly to a preconditioned C18cartridge (Waters Corporation, USA) for solid-phase extraction according to according to the method described by Filatova et al. (2020). The toxins were applied to Agilent-1200 high-performance liquid chromatography (HPLC) with an ultraviolet photodiode array detector set at 238nm (Mohamed et al., 2020). Chromatographic separation was performed on a Zorbax Eclipse-C18 (150mm× 4.6mm, 5µm) column (USA) in a run with 60% solution A [100% (v/v) methanol] and 40% (v/v) solution B [0.05% (v/v) aqueous trifluoroacetic acid (TFA)]. The detector resolution was set at 1.2m for 30min and data were acquired from 200 to 300nm. The flow rate was set at 1mL/ min and the column temperature was maintained at 30◦C. (Forensic M◦edicine and Clinical Toxicology Department, Faculty of Medicine, Sohag University, Sohag) (Mohamed et al., 2020).

Determination of cyanotoxins from sediments

MCs in sediments were extracted with TFA/ methanol (Babica et al.,2006; Abbas et al., 2020). An extraction solvent solution (~100mL) was added to 10g dried sediments of Nile River sediment samples and the samples were shaken (at 1200rpm) for 24h in an shaking. After removing the organic solvent, the MCs concentration of the filtrate was determined using ELISA kits as mentioned above.

Statistical analysis

Variance in cell number, environmental parameters, and MC concentrations in the collected water samples was compared using a one-way analysis of variance(one-way ANOVA test) at ($P< 0.05$) using SPSS 17 software for Windows (2009). Spearman correlations among cell number, particulate toxins, and dissolved toxins were assessed. All the experiments were carried out in triplicates and the resulted data were expressed as means ± Standard deviation (SD).

Results and Discussion

Microscopic examination of the water sample from the final treated water during the study period revealed that *M. minima* were a dominant cyanobacterium with a cell count ranged of 80-2200 cells/mL as shown in Table 1. Other phytoplankton taxa from Chlorophyta and diatoms were also recorded (Table 1). Water treatment processes also differed in their efficiency to remove phytoplankton species. They could remove all species from Chlorophyta and diatoms from raw water but could not remove *M. minima* which was also detected even after the final drinking water treatment processes in the plant. Moreover, *M. minima* was found to produce hepatotoxins MCs (as revealed by the following HPLC analysis results) so its presence in drinking water sources might represent a serious risk to human health. This toxin should be removed by different treatment processes in drinking water treatment plants. In this study, the most observed green algae and diatom cells were removed during different drinking water treatment processes though they were not toxic, but in the same time *M. minima* cells and their toxins were not completely removed from the final treated water. This study was consistent with Hitzfeld et al. (2000) who reported that drinking water treatment processes are not able to completely removed Microcystin. In this study, the total number of cyanobacterial cells before the coagulation treatment was greater than their count after coagulation indicating that coagulation could effectively removes cyanobacterial cells from water. Chl-a concentration had a positive correlation with the total number of algal flora in water samples ($r= 0.507$, $P= 0.10$). The highest concentrations of chlorophyll-a water samples were obtained in October in water collected after each step of the treatment process concomitant with the highest cell number of cyanobacteria in this month (Fig. 1.). The analysis of the physicochemical properties showed that most drinking water treatment parameters underwent monthly variation ($P< 0.05$) in relation to the water treatment processes. The temperature of the drinking water treatment plant ranged from 20^0C in December to 40^0C in August (Fig. 2). The temperature was strongly correlated with the total count of planktonic cyanobacterium *M. minima* ($r= 0.8$). Thus, the high temperature was an important factor that induces an increase in the density of some cyanobacteria in drinking

water treatment plants (Mohamed, 2016). The pH did not change markedly during the study months (P> 0.05) and it was generally shown to be neutral (7.2-7.6) (Fig. 2A&B). Light intensity at the water surface ranged between 1220 and 1423µmol/m^2/S. DOconcentrations in waters were ranged from 4 to 14.8mg/L (Fig. 3). Besides physical parameters, nutrient concentrations varied among different months (P< 0.05). In this study the most important nutrient such as nitrate, ammonia and soluble phosphorus have been investigated, which favor the dominance of *M. minima* and trigger their toxin production in the studied drinking water treatment plant (Mohamed. 2016). Nitrate concentrations increased from May to August with the highest level recorded in October during all treatment processes and decreased during winter months (3mg/L in raw water and 2mg/L in the final treated water (Fig. 4B). Similarly, soluble phosphates were determined in all treatment processes to be at high concentrations, particularly during warm months (6-10µg/L) (Fig. 5B). Phosphate levels correlated positively with the number of dominant algal flora (r= 0.643) and Chl-a concentrations (r= 0.943). Intracellular MC was detected by ELISA in the final treated water. A significant variation in MC content was detected during the study period (P< 0.05). In October, the MC value was the highest in the final treated water (2.68 ± 0.01µg/L) exceeding the WHO limit of 1µg/L (WHO, 2004). This value was associated markedly with the densities of *M. minima* (P< 0.05). However, after passing the final treated water on water sediments, the MC concentration became 0.82201µg/L, which did notexceed the WHO limit (1µg/L). The lowest concentration was recorded in December (1.8 ± 0.05µg/L) when extracellular MC concentrations increased greatly after flocculation in most treated drinking water studied samples compared to their concentrations in the source water (Tables 2, 3). The extracellular MC (dissolved MC) concentration was lower than the particulate concentrations ranging between 0.74 and 1.47µg/L in the water source of the final treated water (Tables 2, 3). Which could pose a risk to humans and animals. These results were compatible with previous studies that reported the inefficiency of some drinking water treatment processes in removing MCs (Zamyadi et al., 2012).The efficiency of removing MCs by chlorination depends on several parameters, including the different types of chlorine compounds, the concentration used, pH and contact time (De La Cruz et al., 2011). For instance, a chlorine dose of 2.8 mg/L for a contact time of 30min was sufficient to cause 99% destruction of Microcystin-LR MC-LR (Tsuji et al., 1997). The MC concentration from *M. minima* isolated from the final treated drinking water plant during the study was analyzed by HPLC and was found contained 500µg MCs/L (2.27pg/cell), which also exceeded the limit of WHO (1µg/L) (Fig. 6). Its presence in drinking water sources may represent a risk to human health. This toxin should be removed through different treatment processes of drinking water treatment in the treatment plants. The most effective step in removing MC from drinking water was passing the water over some sediments after the final treatment process. Due to its high adsorption capacity, sediments which were found to reduced dissolved MCs by 59.6% and intracellular MC by 30% compared to MC concentrations in the final treated water (Tables 2 and 3). These results agreed with Tomasz et al. (2005) due to its high adsorption capacity which reduced dissolved MCs by 59.6% and intracellular MC by 30% compared to MC concentrations in the final treated water (Tables 2 and 3). These results agreed with Tomasz et al. (2005).

TABLE 1. Algal cell count (cells/mL) found in water samples during the study period (May–December 2019)

Algal flora	Cell count(cell/mL)
M. minima	80-2200
Chlorella vulgaris	8-30
Chlorococcum sp.	2-12
Scenedesmus sp.	2-20
Navicula sp.	1-5
Nitzschia sp.	2-6
Cosmarium sp.	2-5
Staurastrum sp.	3-7

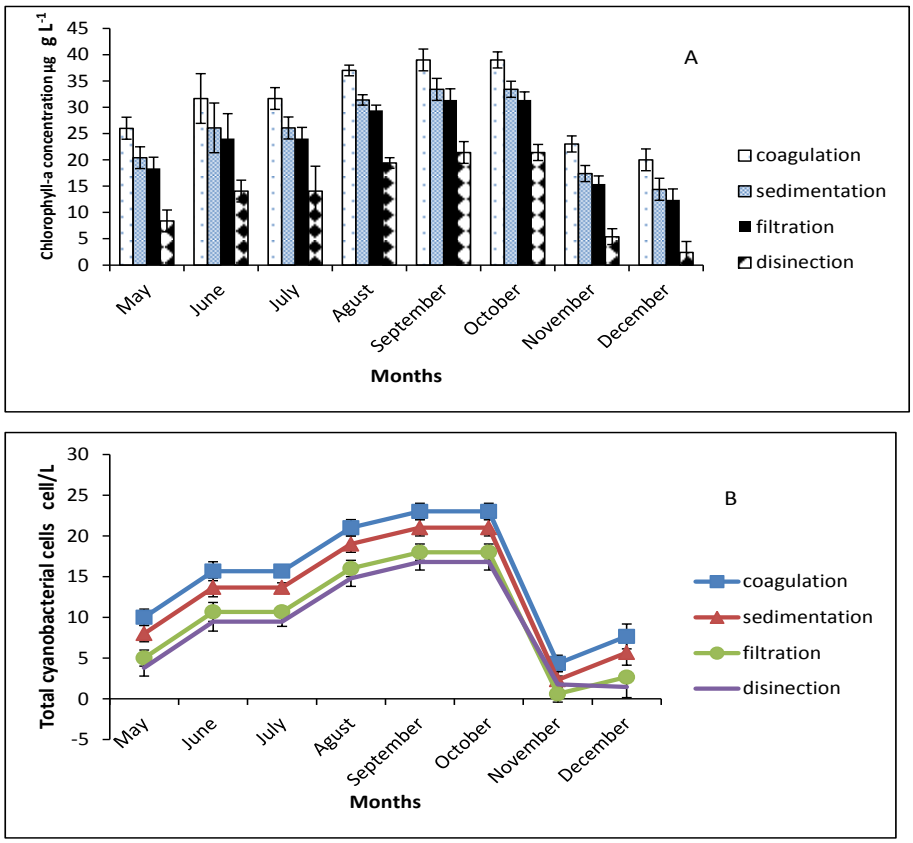

Fig. 1. Monthly variation in Chl-a concentration (µg /L) (A), and total cyanobacterial cell count (cells/L) (B) in the drinking water treatment plant in relation to the water treatment processes during the study period (May–December 2019)

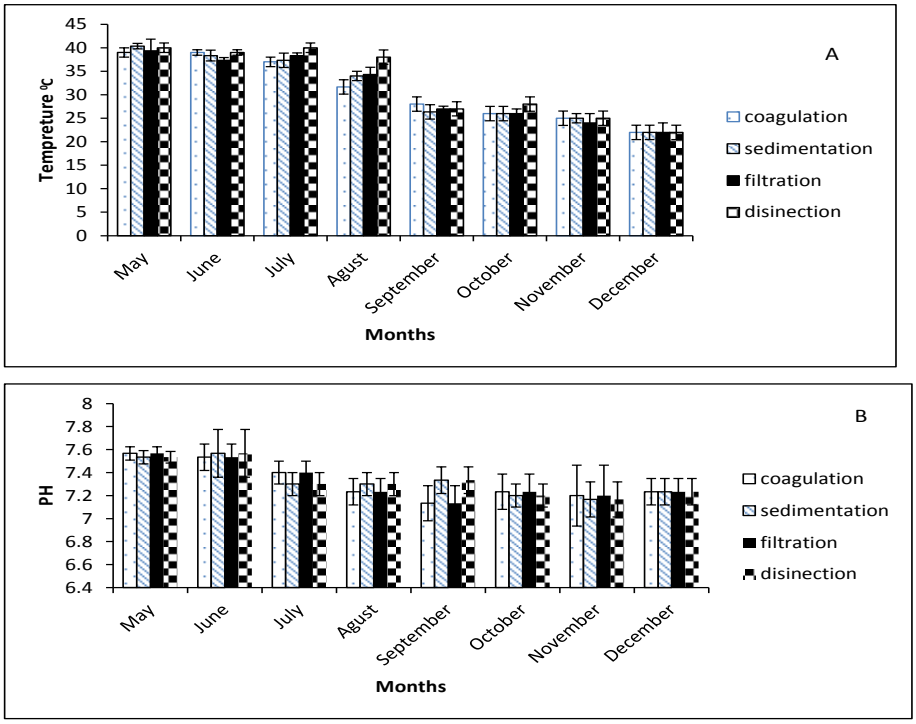

Fig. 2. Monthly variation of temperatures (^{0}C) (A) and pH (B) of the drinking water samples in relation to the water treatment processes during the study period (May–December 2019)

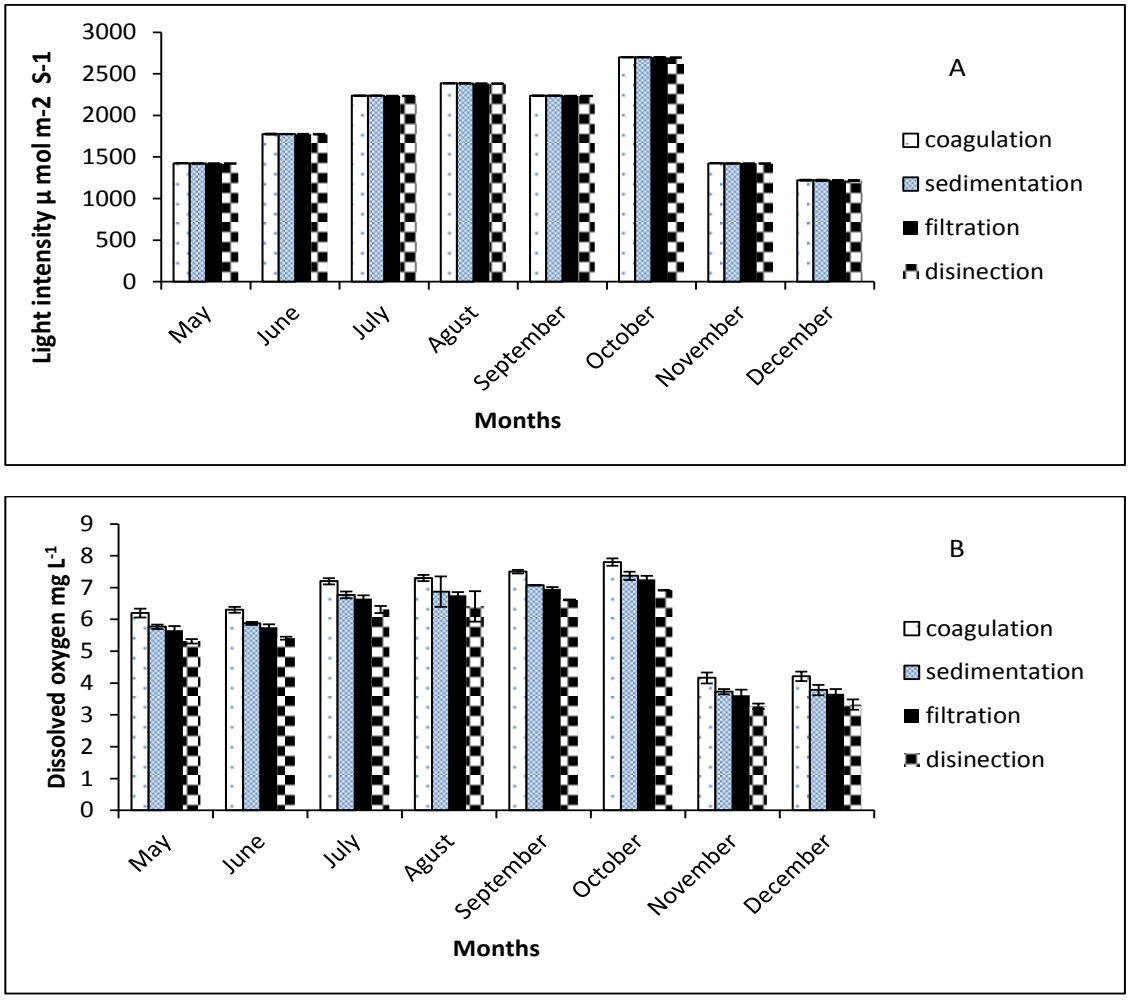

Fig. 3. Monthly variation of light intensity (µmol/m2/S) (A) and DO (mg /L) (B) in the drinking water treatment plant in relation to the water treatment processes during the study period (May–December 2019)

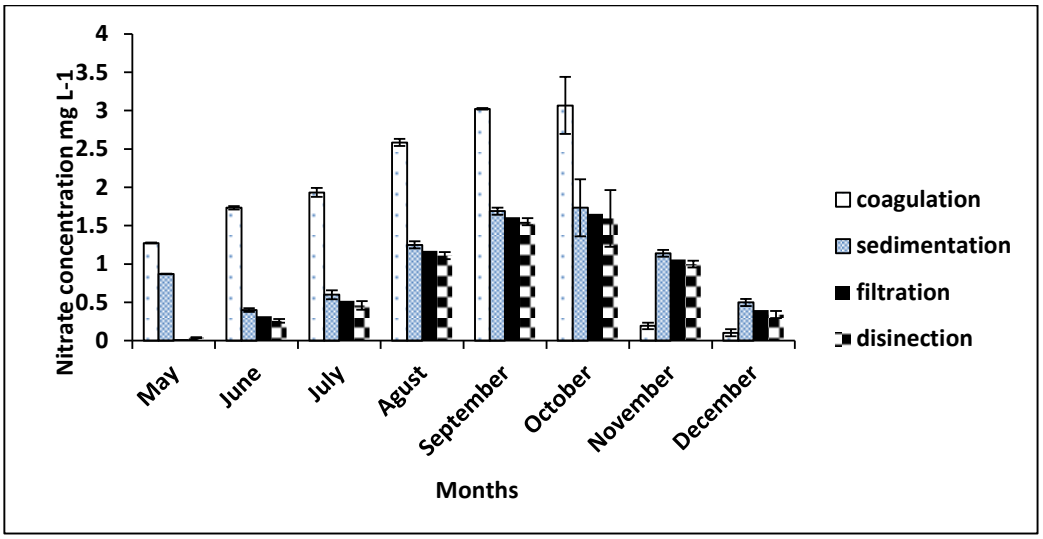

Fig. 4. Monthly variation of nitrate concentration (mg /L) in the drinking water treatment plant in relation to the water treatment processes during the study period (May–December 2019)

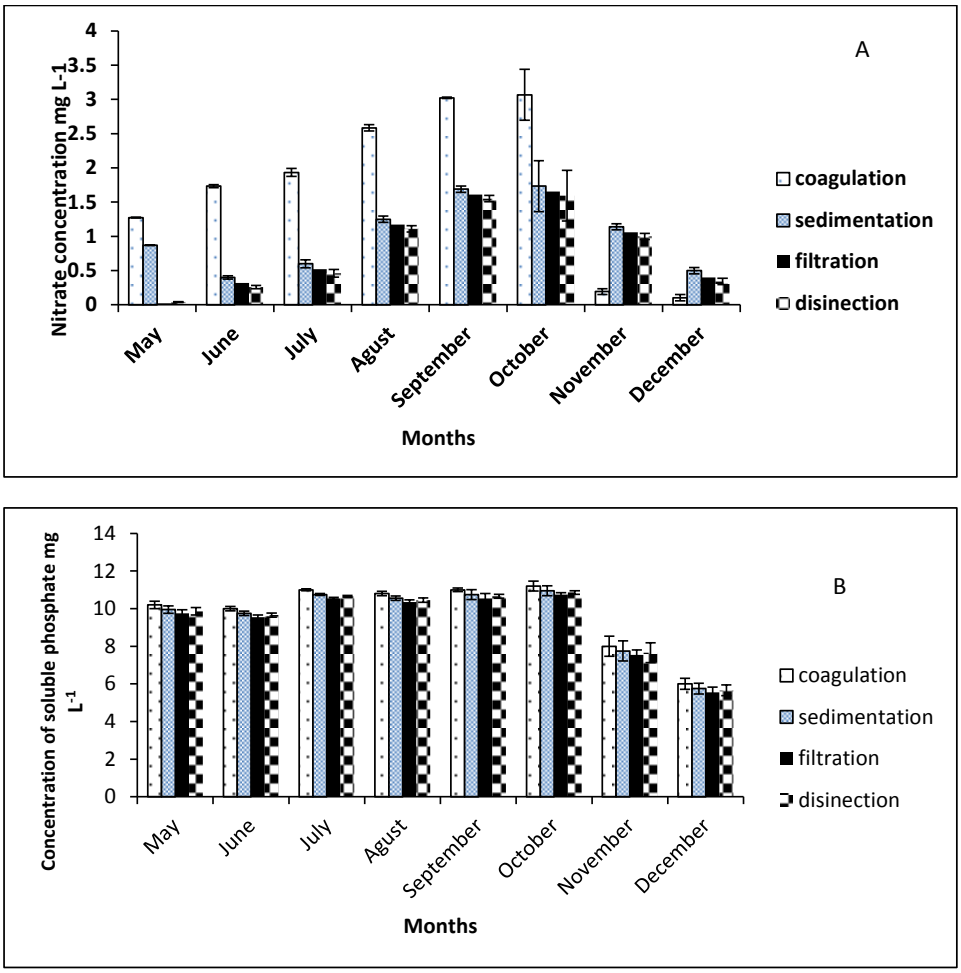

Fig. 5. Monthly variation in ammonia concentration (mg /L) (A), and soluble phosphate concentration (µg /L) (B), in the drinking water treatment in relation to the water treatment processes plant during the study period (May–December 2019)

TABLE 2. Dissolved micricystin concentration (µg/L in the final treated water samples before and after sediments application during) (May–December 2019)

Months	MCs (µg/L) in the final treated water	MCs (µg/L) in the final treated water after sediments application
May	1.47 ± 0.008	0.492 ± 0.09
June	1.51 ± 0.005	0.532 ± 0.07
July	1.52 ± 0.006	0.542 ± 0.9
August	1.54 ± 0.1	0.562 ± 0.7
September	1.59 ± 0.09	0.612 ± 0.1
October	1.62 ± 0.97	0.642 ± 0.03
November	1.04 ± 0.04	0.062 ± 0.04
December	0.74 ± 0.06	0.023 ± 0.06

TABLE 3. Particulate MCs concentrations (µg/L) in the final treated water samples before and after sediments application during May–December 2019

Months	Particulate MC concentration (µg/L) in the final treated water	Particulate MCs concentration (µg/L) after sediments application
May	2.53 ± 0.09	0.732 ± 0.1
June	2.57 ± 0.07	0.756 ± 0.04
July	2.58 ± 0.06	0.762 ± 0.05
August	2.6 ± 0.09	0.774 ± 0.07
September	2.65 ± 0.1	0.804 ± 0.06
October	2.68 ± 0.01	0.822 ± 0.07
November	2.1 ± 0.05	0.474 ± 0.08
December	1.8 ± 0.05	0.294 ± 0.05

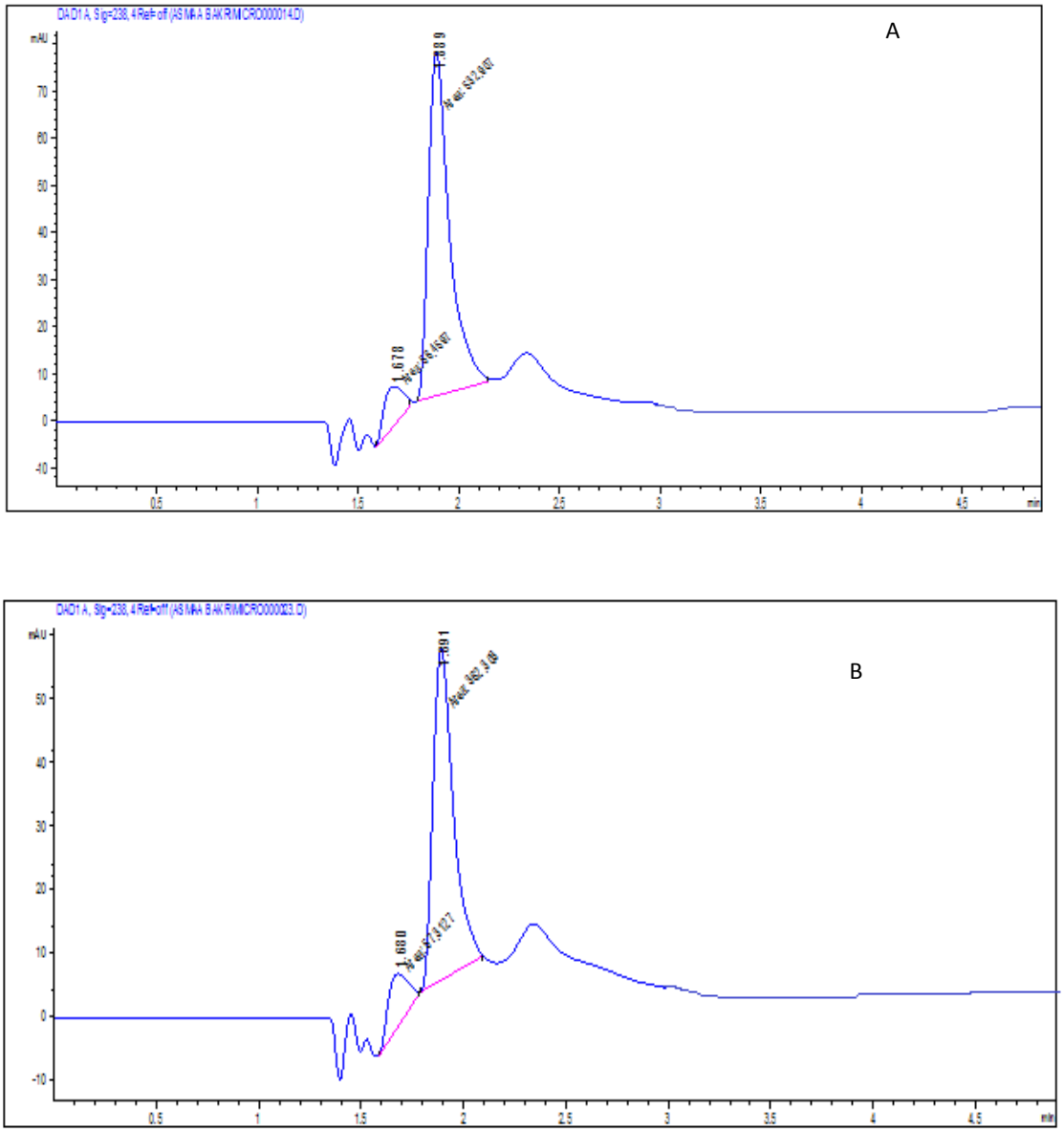

Fig. 6. HPLC profile of standard (MCs) and MCs produced from *M. minima* isolated from the final treated water in relation to the water treatment processes during the study period (May–December 2019)

Conclusions

This study showed that conventional drinking water treatment processes, filtration and disinfection in an Egyptian drinking water treatment plant are not completely efficient in removing all cyanobacterial cells and/or their extracellular MC toxins. MC concentrations in the final treated water from the drinking water treatment plant exceeded the WHO limit. The presence of toxic *M. minima* and/or its MC toxins in the final drinking water poses a risk to human and animal health. Therefore, there is a need to search for alternative treatment approaches to remove cyanobacterial cells and their toxins. The disinfection step, which causes cell death and toxin release, must be eliminated during the harmful bloom of cyanobacteria. Therefore, this study recommends using sediments to remove cyanobacteria and their cyanotoxins from water plants by using an inexpensive method, especially for the developing countries.

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وجود طحلب الميريزموبيديا مينيما في محطة معالجة مياه الشرب بمدينة سوهاج وإزالة سم الميكروسيستين بواسطة الرواسب

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وجود طحلب الميريزموبيديا مينيما في محطة معالجة مياه الشرب بمدينة سوهاج وإزالة ميكروسيستين بواسطة الرمال. تعد البكتيريا الزرقاء في مياه الشرب المعالجة مشكلة كبيرة تهدد الإنسان والحيوان والنبات، خاصةً إذا كانت تنتج السموم. هدفت هذه الدراسة إلى إزالة الميريزموبيديا مينيما من محطة معالجة مياه الشرب بمدينة سوهاج وإزالة الميكروسيستين بواسطة الرمال. العينة المأخوذة من المياه المعالجة النهائية التي تم جمعها خلال فترة الدراسة (مايو - ديسمبر 2019) تحتوي على عدد خلايا من الميريزموبيديا مينيما يتراوح ما بين 80- 2200 خلية/ ملى، وقد ترجع ازدهار نمو البكتيريا الزرقاء والزياده في عدد الطحالب إلى ارتفاع درجة الحرارة. وأظهرت النتائج أيضًا أن طرق المعالجة التقليدية يمكن أن تزيل بعض أنواع من الطحالب الخضراء والدياتومات وانواع من البكتيريا الزرقاء ولكنها غير فعالة في الإزالة الكاملة لسم الميكروسيستين المنتج بواسطتها. بواسطه HPLC كان تركيز سم الميكروسسيستن داخل خلايا الميريزموبيديا 500 ميكروجرام لكل لتر وكان ايضا تركيز السم خارج الخلايا 740.و -1.47 ميكروغرام/ لتر) وهذه النسبة عاليه عن الحد المقترح بواسطه منظمة الصحه العالمية. واصبح تركيز السموم أقل من المقترح بواسطة منظمة الصحة العالمية (1 ميكروغرام/ لتر) عند استخدام رواسب المياه لإزالة هذا السم لذلك، توصي هذه الدراسة بأن تستخدم البلدان النامية الرمل لإزالة الميكروسيستين وهي غير مكلفة وامنة.