DETECTION OF CYANOBACTERIAL HARMFUL ALGAE IN DRINKING WATER PLANTS

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ABSTRACT

Presence of microorganisms such as Cyanobacterial harmful algae in drinking water represents a major human health hazard in many parts of the world. Cyanobacterial harmful algae is a group of Blue-green algae Genera that can grow to form thick scum, color the water, produce taste, odor compounds and cyanotoxins. The toxins produced by their cells cause health problems to animals and humans through drinking contaminated water. The main objective of drinking water treatment is to provide microbiologically safe drinking water. The conventional drinking water treatment and disinfection has proved to be one of the major public health advances in modern times. A number of processes; namely water treatment, including coagulation, flocculation, sedimentation, filtration and disinfection influence the quality of drinking water delivered to the customer's tap during transport of treated water via the distribution system. This study was devoted to detect the presence of Cyanobacterial harmful algae in drinking water treatment plants in El-Minufeyia Governorate. Water samples were collected from two main points: (a) outlet of the water treatment plants (b) distribution system at different distances from the water treatment plants. Cyanobacterial harmful algae was concentrated from each water sample by concentration on the mixed-cellulose ester membrane filters (0.45 µm pore size) and detected by the low magnification method. The results showed disappearance of all Cyanobacterial harmful algae species; Oscillatoria Formosa, O. Princes and Microcystis aeruginosa in four of ten stations of drinking water treatment systems that is compatible with the recommended standard by the Egyptian regulation and World Health Organization (WHO) guidelines. A few cells of Microcystis aeruginosa species were found in other stations and ranged between 10 and 29 cells/L. This study suggests using Activated carbon filtration as an additional unit process on a case by case according to the types and numbers of algae presence to ensure a safe drinking water.

Keywords: Cyanobacteria, *Microcystis aeruginosa*, Drinking water and Treatment.

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1. INTRODUCTION

Harmful algae includes different types of algal taxa such as dinoflagellates, diatoms and cyanobacteria. Cyanobacteria are of special concern because of their potential impacts on drinking and recreational waters. Cyanobacteria is one of the earth's most ancient life forms. They are the dominant phytoplankton group in eutrophic freshwater bodies' worldwide (Negri et al. [1]). All Cyanobacteria are photosynthetic and possess chlorophyll *a*. Cyanobacteria, also known as Blue-green algae because the species used for early nomenclature were Blue-green in color.

Morphological diversity of Cyanobacteria ranges from unicells, to small colonies of cells, to simple and branched filamentous forms. Even though there is no nuclear membrane as in bacterial cells. Cell volume ranges from 5 to 50 μm^3, in contrast to 0.01 to 5 μm^3 for bacteria. About one third of all cyanobacteria species are able to fix nitrogen. In most of the cases, nitrogen fixation occurs in specialized cells called heterocysts (Weier et al. [2]).

Cyanobacteria are especially abundant in shallow, warm and nutrient rich water. They can grow to form thick scum that color the water and creating blooms. The blooms decay consumes oxygen, creating hypoxic conditions which result in plant and animal die-off (EPA [3]). Cyanobacterial harmful algae blooms are common events. Nevertheless, they can be sporadic in occurrence and difficult to predict. Even targeting locations that recently have experienced blooms may not mean that blooms will occur in these areas in the future; on the other hand, in developing watersheds blooms may begin to develop in previously "bloom–free" locations (Zurawell et al. [4]).

Cyanobacterial harmful algae defined as a proliferation of microscopic algae that are capable of causing disease or death of humans or beneficial aquatic life through the production of toxins. They produce a variety of taste-and-odor compounds such as jasmine and 2–methylisoborneol, which are not toxic but are a nuisance to the public. Hence, a term for these organisms known as Nuisance algae (Shehata et al [5]).

The most notable feature of Cyanobacterial harmful algae in terms of public health impact is that; some species produce toxic secondary metabolites, known as cyanotoxins according to their exposure to some environmental conditions. The cyanotoxins include neurotoxins (affect the nervous system), hepatotoxins (affect the liver), and dermatoxins (affect the skin) (EPA [3]). Common toxin-producing cyanobacteria and their health effects are listed in Table (1).

Table 1: Common toxin-producing cyanobacteria and their health effects

Most common Cyanobacteria producing toxin	Cyanotoxin	Primary organ affected	Health Effects
Microcystis *Anabaena* *Planktothrix* *Anabaenopsis* *Aphanizomenon*	Microcystin-LR	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion
Cylindrospermopsis *Aphanizomenon* *Anabaena* *Lyngbya* *Rhaphidiopsis* *Umezakia*	Cylindrospermopsin	Liver	
Anabaena *Planktothrix* *Aphanizomenon* *Cylindrospermopsis* *Oscillator*	Anatoxin-a	Nervous System	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death

Source: Environmental Protection Agency (EPA [3]) after modification.

Neurotoxins and peptide hepatotoxins were first characterized from the unicellular species, *Microcystis aeruginosa*. It is the most common toxic cyanobacterium in eutrophic freshwater may give rise to potential health hazards to consumers (Oberholster et al. [6]). The World Health Organization (WHO) released a provisional guideline of 1 μg/L for microcystin-LR in drinking-water based on water consumption of 2 L/day in 1998 [7].

Conventional water treatment facilities can remove the algal cells but it can't remove potentially harmful cyanobacterial metabolites like cyanotoxins, taste and odor compounds (Shehata et al. [5]). Slow sand filtration has proven very effective in removing microcystins, as cyanobacterial cells are retained and dissolved toxin is degraded in the uppermost substrate layers (Grützmacher et al. [8]). Different cyanotoxins react differently to chlorination. While chlorination is an effective treatment for destroying microcystins and its effectiveness is dependent on the pH (EPA [3]).

The proliferation of Cyanobacterial harmful algae in drinking water sources is problematic for water authorities as they can interfere with water treatment processes (Daly et al. [9]).

2. MATERIALS AND METHODS

The methodology for the detection of Cyanobacterial harmful algae in drinking water consists of three stages: (i) sample collection (ii) concentrated and (iii) detection of organisms. In this research, water samples were collected from water treatment plants fed raw water from the River Nile during spring 2013 where Cyanobacteria dominate in warm and calm weather. The monitoring study was carried out in the El Minufeyia Governorate including five provinces, Ashmoon, El Bagour, Minouf, Shibeen El Koom and Sadat. From each province, two water treatment stations were evaluated for its water quality in the outlet and the distribution system. Water samples of 10 liter (L) were collected from each station and samples looked for the presence of the Cyanobacterial harmful algae.

2.1 Physicochemical Analysis of Water

The following parameters were measured in all water samples in outlets and distribution system of water treatment plants: Hydrogen Ion Concentration (pH), Turbidity, Total Dissolved Solids (TDS) and Residual chlorine concentration according to the standard methods (APHA [10]).

2.2 Cyanobacterial harmful algae concentration

In each water sample, Cyanobacterial harmful algae was concentrated through mixed-cellulose ester membrane according to standard methods (APHA [10]). The samples were filtered through a mixed-cellulose ester membrane (0.45µm pore size, 142 mm diameter, Millipore) in vacuum. The Cyanobacterial harmful algae, that might be present on the surface of the membrane filter after sample filtration, were collected by soaking and re-suspending in 20ml of distilled water. The samples were stained using Lugol's Iodine solution, then Tri-replicates sub-samples of 1ml were put in a Rafter cell for microscopic examination.

2.3 Microscopic Examination

Cyanobacterial harmful algae was examined and identified according to standard methods (APHA [10]).

3. RESULTS AND DISCUSSION

The provision of safe drinking water plays an important role in preventing the incidence of many water transmitted diseases. Harmful algae, especially those belonging to Cyanobacteria (Blue-green algae) are of interest to water treatment authorities because of their production of taste, odor compounds and cyanotoxins according to their exposure to some environmental conditions. Also, they interfere with certain water treatment processes for drinking water production.

Cyanobacterial harmful algae does not proliferate within the human body after uptake and cause illness in healthy persons. In contrast to pathogenic bacteria, they proliferate only in the aquatic environment before intake and cause harmful through generations of tastes and odors or discoloration of drinking-water supplies (WHO [7]).

The results of the physical - chemical analysis, including pH, Turbidity, Total Dissolved Solids (TDS) and Residual chlorine of the ten stations are shown in Table (2). It is clear that, the results of the physical-chemical analysis of the potable water produced by the different water treatment systems under investigation establish that; all values of the various parameters are within permissible limits of the Egyptian regulation [11] and WHO guidelines [7]. Our results agree with Shehata et al. [5] And Donia [12].

Table 2: Results of physicochemical parameters

Cities	Stations	Sampling point	PH	Turbidity	TDS	Residual Chlorine
Shibeen Elkoom province	Shibeen Elkoom Station	Outlet	7.9	0.17	179	1.1
		Distribution system	7.66	0.39	206	0.9
	Meet Mousa Station	Outlet	7.87	0.32	200	2.1
		Distribution system	7.81	0.54	220	1.6
El Bagour province	Bir Shams Station	Outlet	8.04	0.28	203	1.9
		Distribution system	8.1	0.4	201	0.9
	Shoubra Zanga Station	Outlet	7.86	0.42	211	1.3
		Distribution system	7.96	0.16	349	0.1
Minouf province	Minouf Station	Outlet	7.82	0.27	207	2
		Distribution system	7.8	0.25	205	1.6
	Bahwash Station	Outlet	7.8	0.88	207	1.4
		Distribution system	7.8	0.83	210	1.2
Sadat province	Sadat Station	Outlet	8	0.48	212	1.1
		Distribution system	7.7	0.73	364	0.9
	Mansheyat El-Nour Station	Outlet	8.1	0.87	223	0.6
		Distribution system	7.9	2.5	227	0.1
Ashmoon province	Ashmoon Station	Outlet	7.79	0.93	203	2
		Distribution system	7.90	0.92	306	0.7
	Firruaneyyia Station	Outlet	7.82	0.51	279	2.5
		Distribution system	7.61	0.42	205	1.5
Regulation No. 458, year 2007 for drinking water		6.5-8.5	-	1	1000	-

However, El-Manawy and Amin [13] studied the factors associated with Cyanobacteria and they found Higher turbidity, COD, EC, TDS, Ammonium, low winter temperature and the nutrient status especially nitrogen forms indicated the presence harmful algae blooms. It is known that water temperature affects the growth of harmful algae reproduction. As these algae is known to thrive during warm, windless days of late summer and fall when water can stagnate (Mohamed et al. [14]. This result explains the presence of their numbers in the distribution system during spring time where this study was carried out and contradicts with El-Manawy and Amin [13].

Cyanobacterial harmful algae constitutes a group of organisms, which widespread success in aquatic systems cannot be explained by any single factor. It has been suggested that several factors synergistically enhance the growth of cyanobacteria compared with other phytoplankton at certain times (Hyenstrand [15]).

Carmichael and Falconer [16] stated that; species within certain Genera of Cyanobacteria namely; *Anabaena*, *Hapalosiphon*, *Microcystis*, *Nostoc* and *Oscillatoria* produce cyclic peptide hepatotoxins called microcystins that cause liver damage in both mammals and fish thus constitutes a risk for human being.

In Egypt; the harmful algae species recorded were *oscillatory Formosa* and *O. Princes* and *Microcystis aeruginosa* (Marzouk et al. [17] and El-Manawy and Amin [13]).

During this study; no species of the mentioned previous species were recorded except *Microcystis aeruginosa*. The results of *Microcystis aeruginosa* species recorded during this study showed that; its cells were different and variable between the ten stations as the following:

In the Shibeen Elkoom province: two stations were evaluated: Shibeen El Koom Station and Meet Mousa Station. Although the Outlet of Shibeen El Koom Station did not give any count of *Microcystis aeruginosa*, the distribution system of the Shibeen El Koom Station had (22 cells/L) of *Microcystis aeruginosa* cells. In addition, the outlet of the Meet Mousa station and the distribution System of Meet Mousa Station gave (12 & 18 cells/L) of *Microcystis aeruginosa* cells (Table 3).

Table 3: Results of *Microcystis aeruginosa*

Provinces	Stations	Sampling point	*Microcystis aeruginosa* (Cells/L)
Shibeen Elkoom province	Shibeen Elkoom Station	Outlet	0
		Distribution system	22
	Meet Mousa Station	Outlet	12
		Distribution system	18
El Bagour province	Bir Shams Station	Outlet	0
		Distribution system	12
	Shoubra Zanga Station	Outlet	0
		Distribution system	0
Minouf province	Minouf Station	Outlet	0
		Distribution system	0
	Bahwash Station	Outlet	29
		Distribution system	10
Sadat province	Sadat Station	Outlet	0
		Distribution system	0
	Mansheyat El-Nour Station	Outlet	0
		Distribution system	0
Ashmoon province	Ashmoon Station	Outlet	28
		Distribution system	11
	Firruaneyyia Station	Outlet	13
		Distribution system	7

In the El Balfour province, two stations were evaluated: Bir Shams Station and Shoubra Zanga Station. The distribution system of the Bir Shams Station had (12 cells/L) of *Microcystis aeruginosa* cells. While the Outlet of Bir Shams Station, the outlet and the distribution system of Shoubra Zanga Station did not give any count of *Microcystis aeruginosa* cells.

In Minouf province, the evaluated stations where Main Minouf Station and Bahwash Station. The outlet and the distribution system of Main Minouf Station did not give any count of *Microcystis aeruginosa* cells. While the outlet of Bahwash Station had the highest numbers of *Microcystis aeruginosa* cells (29 cells/L), contradict to the distribution system of Bahwash Station, which had the lowest numbers of *Microcystis aeruginosa* cells (10 cells/L). In Sadat province, two stations were evaluated: Main Sadat Station and Mansheyat El-Nour Station. The results indicated that the numbers of *Microcystis aeruginosa* cells disappeared completely in Sadat province drinking water treatment stations.

Ashmoon province was the last station and including two stations: Main Ashmoon Station and Firruaneyyia Station. The cells *Microcystis aeruginosa* were recorded in the outlet and distribution system in two stations of Ashmoon province and ranged between (7 & 28 cells/L) in the distribution system of Firruaneyyia Station and the outlet of Ash Moon Station respectively (Table 3).

El-Sebaie et al. [18] in his study of water treatment plant assessment at Talkha power plant recorded 15.6 org. /L of Blue-green algae in drinking water.

The quality of water delivered to the customers depend on (i) its initial chemical and physical composition, (ii) the proper choice of purification technology, (iii) technical conditions of water storage tanks and pipe network as well as (iv) hydraulic condition and exploitative manner of the water distribution system. Thus, water distribution system acts as large-scale chemical and biological reactors and sometimes, due to improper design or operation, can greatly modify the quality of water e.g. Long retention times which lead to water aging, reduced disinfectant residual and formation of disinfection byproducts, bacterial growth, appearance of taste and odor and so on.

Most of these microorganisms are totally dependent on light as a source of energy for growth and for this reason; their presence in drinking water systems is expected as an anecdotal fact and may be attributed to treatment limitations (Francesc et al. [19]).

Two different hypotheses on the origin of algae in drinking water can be considered. Firstly, it can be assumed that water treatments are not 100% effective in removing algae. Abed El Rahman [20] said that; Clarifiers algae fluctuates between 85% and 99% in an average 92 %. For this reason some of them are capable of penetrating in the drinking water systems. Since algae can travel all the way from origin to the end point, the levels detected in tap water are exclusively from algae not removed by treatment.

Secondly, some of these algae could proliferate or have the ability to grow in the system in the dark, using the ability of some Genera to develop heterotrophic metabolisms. These microscopic algae, having a relatively active role and could become members of the normal flora of drinking water systems (Neilson and Lewis [21]). In most pipe surfaces, the development of bio-films containing algae and bacteria has been proven. In this case, it is logical to consider the possibility of algae re-growth, probably into the bio-films (Allen et al. [22]). So; their presence is not affected by the seasonal changes occurring in the surface waters used for supply (Francesc et al. [19]).

In this study, the presence of *Microcystis aeruginosa* cells in outlets of drinking water samples may be attributed to failure of sand filters. While their presence in the distribution system may be attributed to aging of some networks and pipes, leakage to sewer systems, deterioration of municipal and buildings' water reservoirs and the intakes of these plants exist on small canals where the lowest water level is affected by the winter closure period. Also; No existence of cleaning valves at the end of pipes that results in the increase of precipitation and the increase in the probability of pollution; in addition to the operation and maintenance is done by unqualified trainees (Donia [12]). This study agrees with Amer [23] where attributed presence of pathogenic organisms in outlets of drinking water samples to failure of sand filter stage and in the distribution system to cross connections between drinking water and sewer lines, backflows, breakthroughs in drinking water, wastewater treatment plant operations, leaking pipes, valves, joints and seals as well as contamination of the tap by the final users.

The presence of *Microcystis aeruginosa* cells in potable water were not in compliance with the Egyptian Decree of Minister of Health No. (108/1995) that said that; Blue-green algae must not be detectable when microscopic analysis of samples is conducted (WHO [24]).

During this study; it was noticed that *Microcystis aeruginosa* cells disappeared completely from the outlet and inlet of drinking water treatment plants in Sadat Province. This result can be explained due to the sedimentation and filtration processes (El-Manawy and Amin [13]).

The addition of oxidants such as chlorine, ozone or permanganate to untreated water prior to coagulation has been shown to increase the removal of algae. These oxidants alter the surface charge of algae, thus improving their removal during coagulation (Chen and Yeh [25]). *Microcystis aeruginosa* cell density of 300,000 cells/ml and a chlorine dose of 12 mg/L. Chlorine caused losses in greater than 98% cells within the first minute of contact while the pH was 7.6. The use of chlorine as a pretreatment step to conventional water treatment should be avoided when a bloom of toxic *Microcystis* is present in the source water as chlorine causes intact cells to lyse releasing microcystin into solution and produce tri-halomethanes during water treatment (Hussein et al. [26]).

The degradation of microcystin by chlorine was found to be dependent upon the pH, chlorine exposure with half-lives ranging from 162 min at pH 9 to 12.4 min at pH 6 and the presence of cyanobacterial cells (Daly et al. [9]). Most of the common microcystin variants are well removed also by activated carbon in conventional water treatment in addition to taste and odor compounds through its adsorption (Cook and Newcombe [27] and Ahn et al. [28]).

Many water utilities use chlorine residual to inactivate potential pathogenic organisms and preserve water quality during distribution. Thus, controlling the residual chlorine concentration in drinking water is a very important aspect, since the decrease of chlorine (concentration below the minimal level) may cause secondary development of microorganisms and excessive chlorine concentration may cause formation of dangerous disinfection by-products. Disinfectant dose, contact time, residual disinfectant concentration at the end of the contact time, pH, and temperature are commonly used to monitor the performance of disinfection processes. The most critical conditions for disinfection processes are low temperatures and high turbidity in the water to be treated (Amer [23]).

Drinking water should not contain microorganisms, parasites or substances that might represent a potential hazard to human health and it must meet the minimal requirements stipulated in regulation concerning the quality parameters of potable water (microbiological and chemical indicators). So; drinking water operators can undertake different management strategies during Cyanobacterial harmful algal blooms through changing sources and adjusting the intake depth to avoid drawing contaminated water and cells in the treatment plants.

It is expected that the demand for drinking water in Egypt will increase dramatically in the future. Although many utilities in Egypt use underground water supplies, many also use surface water and that proportion is expected to increase in many areas. Increasing population has placed increased demand on surface water supplies, either through their anthropogenic inputs or through their increased demand for drinking water. Also, it is expected that the risk of Cyanobacterial harmful algae to drinking and recreational waters will increase with increased stress and demand on those systems (Glibert and Burkholder [29]). River Nile is considered the main water source of drinking water in Egypt and prevention of contamination, especially in rural areas will enhance the efficiency of drinking water treatment facilities for Cyanobacterial harmful algae removal.

4. CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that water treatment plants are not 100% effective in removing Cyanobacterial harmful algae. For this reason some of them are capable of penetrating in the drinking water systems. They can travel all the way from origin to the end point and the levels detected in tap water are exclusively from Cyanobacterial harmful algae not removed by treatment. The presence of species like *Microcystis aeruginosa* in drinking water treatment plants during this study may be due to its small size and structure of cysts that make it may be resistant to chlorine disinfection. The implementation of new technologies as such Activated carbon filtration as an additional unit process to meet current and proposed regulations, in case of Cyanobacterial harmful algal blooms are of great necessary to produce biologically safe drinking water. The drinking water treatment plants must modify their treatment method according to the numbers and types of algae present. This study is recommended by replacement of depreciated plants and networks in different provinces and the establishment of a database for the water type that must be purified and treated for the production of healthy water and therefore identification of the optimum treatment technique. Also, continuous monitoring and maintenance of the different units of drinking water treatment plants. Finally, proper selection of drinking water treatment plants is of prime importance in the provision of safe drinking water.

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