



Detection of microcystin-LR in water supply at one of the Egyptian water treatment plants with potential use of a novel absorbent material for its removal

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ARTICLE INFO

Article History

Received:

Accepted:

Available online:

Keywords:

Cyanobacterial blooms

Microcystin LR

Ismailia Canal

Bioassay

Pottery

ABSTRACT

The occurrence of heavy cyanobacterial blooms in drinking water supplies has become a worldwide problem. About 60-75% of these blooms are toxic and associated with mortality and illness in animals and humans. The present study aims to evaluate the concentration of microcystin-LR in the drinking water supply (Ismailia Canal) and treated drinking water at Port Said Governorate, Egypt, using HPLC and mouse bioassay. Their toxicological effect on the liver and kidney of experimental animals was detected. A treatment approach for such toxic substances was assessed by using a novel material (Egyptian pottery) as absorbent.

Results revealed that in the water supply, the higher concentrations of microcystin-LR (12.28 and 9.8 µg/l) were recorded in winter and summer, respectively. In drinking water, microcystin-LR was not detected during the period of study, except in January (0.67µg/l). Although the concentration of microcystin-LR in the water supply and drinking water was lower than that of LD50, severe alterations were detected in the liver and kidney of a mouse which was particularly associated with samples contained appropriate concentration of microcystin-LR. When Egyptian pottery was tested as an absorbent material for microcystin-LR, it did not only have the ability to completely eliminate microcystin-LR residues with efficiency of 100%, but also completely removed the bad odor of water in 24h. Therefore, the current study presents a low cost effective method that could be applied to eliminate microcystin-LR in treatment strategies of drinking water.

1. INTRODUCTION

The frequent occurrence of cyanobacterial blooms in freshwater lakes and reservoirs has been regarded as a serious global public health problem and a major environmental issue (Ressom, 1994; Blaha *et al.*, 2009). This is because many species of cyanobacteria can produce several toxic metabolites known as cyanotoxins that constitute a serious threat on aquatic organisms, wild life, domestic animals and human (Duy *et al.*, 2000; Malbrouck and Kestemont, 2006).

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ISSN 2156-7530

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Besides posing health hazards, algal cells containing these toxins add bad tastes and odors, which significantly impair drinking water quality (Jüttner and Watson, 2007).

The toxicity of cyanobacterial blooms was originally brought to the attention of scientists through reports of animal poisonings by farmers and veterinarians, with the first reported from Australia in 1878 (Francis, 1878). Afterward, several sick cases appeared worldwide were associated with consumption of water contained bloom of certain cyanobacterial species (Bourke *et al.*, 1983; Falconer, 1989; El Saadi and Cameron, 1993). Cyanotoxins cause abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, blistering at the mouth, and headache.

Microcystins (MCs) are one of cyanobacterial hepatotoxins which cause acute poisonings and have cancer promotion potential by chronic exposure of humans at low microcystin concentrations in drinking water (Ueno *et al.*, 1996; Zhou, 2002). Microcystins are known to be relatively stable compounds that withstand many hours of boiling and may persist for many years when stored dry at room temperature, possibly as a result of their cyclic structure (Jones and Orr, 1994). Jones and Orr (1994) showed that MCs persisted for nine weeks before degradation after an algicide treatment in a recreational lake. Lahti *et al.* (1997) demonstrated that MC-LR was detectable in lake water during decomposition of a *Microcystis* bloom and was present in detectable amounts even weeks after the bloom disappeared. Tsuji *et al.* (1994) showed that MC-LR was very stable because of limited decomposition by exposure with sunlight as compared to MC-RR and MC-YR. This clearly indicates that MCs are not degraded fast under natural conditions and hence need removal strategies that employ traditional as well as modern methods of water treatment.

Most drinking water plants use coagulation or flocculation, sedimentation,

filtration and disinfection as basic treatment methods. However, these conventional water treatment procedures are in some cases insufficient in the removal of cyanobacterial toxins and need to be optimized for cyanotoxin removal (Falconer and Humpage, 2005; Westrick *et al.*, 2010). Alternative processes, such as granular activated carbon, powdered activated carbon, and membrane filtration have been proven efficient for the removal of microcystins (Svrcek and Smith, 2004). The toxin removal strategy should be planned based on the microcystin or other cyanotoxin composition in the given water bloom. Prevention of bloom formation is naturally the most efficient method for avoiding a potential risk of toxin exposure for water consumers. Besides the chemical and physical methods used, there is a need for simple, low-cost and effective water treatment procedures.

Ismailia Canal is the main source of drinking water supply for a great number of the Egyptian citizens (about 12 million inhabitants). Cyanobacterial blooms were recorded for the first time in Ismailia Canal at drinking water intakes of Port Said treatment plant in 1994 and 1995 during autumn and winter (Amine, 2001). The blooms were associated with unpleasant odors and change in the taste of drinking water. On the other hand, liver and kidney diseases have progressively increased in the recent years in Egypt. Some of these diseases has been linked to drinking water supplies include heavy metal poisoning, bacterial and viral infections. Little researches focused on the relationship between the presence of cyanotoxins in the drinking water and these diseases.

The present study is focusing on assessing the concentration of cyanotoxin (microcystin-LR) in the water supply of water treatment plant at Port Said and treated drinking water, as well as evaluating their pathological effect on liver and kidney of the experimental animals. Also it aimed to investigate the efficiency of novel absorbent

material (Egyptian pottery) in removal of microcystins dissolved in potable water.

2. MATERIAL AND METHODS

2.1 Study area and samples

The present study focused on one of the Egyptian drinking water treatment plant located at Port Said Governorate (Figure 1). The plant has two intakes, the first essential intake comes from Fresh Water Canal branched from Ismailia Canal. The second one is a tributary which comes from west Qantara and collect its water in a reservoir in front of the plant inlet.

Water samples were taken monthly during the period from February 2013 to January 2014, from two intakes; the reservoir (site I) and the canal (site II), in addition to treated drinking water that comes directly from the treatment plant. During months with cyanobacterial bloom in the water intakes, the algae were collected using plankton net (20 μ m mesh size). The samples were stored immediately in a portable refrigerator, transported to the laboratory then immediately frozen at -20 °C.



Fig. 1: Location of drinking water treatment plant at Port Said, Egypt

2.3 Estimation of microcystin-LR by HPLC

For microcystin-LR isolation from *Microcystis* bloom, samples were dried at 50°C overnight. Twenty milligram of dried samples were extracted with 1.5 ml of 5% acetic acid and sonicated for 5 minutes using ultrasonic micro-tip probe of 400 watt. The resulted suspension was centrifuged at 4500rpm for 7 minutes and supernatant was collected for cleanup procedure.

Extracted sample (supernatant) was applied to a C18 solid phase extraction cartridge (strata C18, 500 mg /3 ml, phenomenex), which was previously conditioned with 10 ml methanol and 10 ml of 5% acetic acid. The cartridge was washed using 10, 20 and then 30 % aqueous methanol, respectively. Toxin was eluted with 10 ml methanol HPLC grade. The eluted methanol was evaporated to dryness under reduced pressure (40°C) and

suspended in 200 μ l methanol prior to HPLC analysis (Meriluoto, 1997; Ame *et al.*, 2003).

The HPLC system used for MC-LR determination was Perkin-Elmer, series 200 system (USA), equipped with quaternary pump, UV diode array detector set at 238nm and C18 column chromatography (phenomenex, kintex 5 μ m C18 100A 150x4.6). The isocratic mobile phase 10 mM ammonium acetate:acetonitrile (75:25) was run at flow rate of 1 ml/min. The concentration was calculated using standard curve of microcystin-LR purchased from Biovision INC, USA.

2.4 Mouse bioassay estimation of microcystin-LR

About 200 ml of water samples were taken and concentrated to 10 ml by rotary evaporator, then used in a mouse bioassay.

2.4.1 Toxicity experiment

Mouse bioassay was performed on male albino Swiss mice weighting 20 ± 2 g. Animals were obtained from the animal house, Faculty of Science, Port Said University, Egypt. The animals were housed in cages under proper environmental conditions and received human care in compliance with the guidelines of Scientific Ethical Committee, Faculty of Science, Port Said University. The animals were acclimatized to laboratory conditions at temperature of $25 \pm 2^\circ\text{C}$ for a week. They were fed on a commercial pellet diet and had free access to water throughout the experimental period. Mice were divided into three groups of 5 mice each. Group I served as a vehicle control which received distilled water. Other groups were accessed to water supply source (sites I and II) and drinking water. Mice were observed 48 hours and death time was recorded for each group. Potency of toxicity was expressed as mouse unite (MU) according to Lehane *et al.* (2000).

2.4.2 Histopathological examination

To evaluate the acute effect of microcystin-LR concentration present in

samples, the survived mice were accessed to samples for a weak. By the end of the mouse bioassay experiment, the remained mice in each group were sacrificed. Liver and kidney from each animal were excised. Organs were initially fixed in neutral buffered formalin for 24 h at 4°C . The organs were immediately dehydrated in a graded series of ethanol, immersed in xylene and embedded in paraffin. Sections of $5\ \mu\text{m}$ were then mounted and stained with the haematoxylin and eosin (Humason, 1972). All tissues were examined microscopically, and their histological abnormalities were recorded.

2.5 Removal of microcystin-LR

Handmade Egyptian pottery was used as adsorbent material in the removal of microcystin-LR (Figure 2). It was made of soil of the riverbank and purchased from local market. The pottery was grinded by grinder (RM200) to obtain fine particles ($150\text{--}200\ \mu\text{m}$). Bloomed water was taken from intakes of water treatment plant to test the removal of cyanobacterial toxin as well as its taste, odor and color. Bloomed water was passed through $0.22\ \mu\text{m}$ membrane filter to remove algae and other particles.



Fig. 2: Handmade Egyptian pottery

To maximize microcystin-LR removal, the conditions of relevant factors (i.e., adsorbent quantity and contact time) were optimized. Grinded pottery was added to filtrate in ratio of 1:2 (W/V) then shaken. PH was adjusted at 6 and the temperature at 25° , with change in contact time (1, 2, 3, 6 and 24 hours). The concentration of

microcystin-LR was measured by HPLC at the initial of the treatment and after each contact time. The removal efficiency (E) of microcystin-LR by the adsorbent was calculated as follow:

$$E = \frac{C_0 - C_e}{C_0} \times 100\% \quad (\text{Asada } et al, 2002)$$

Where; C_0 and C_e are the initial and equilibrium concentrations of microcystin-LR ($\mu\text{g/l}$), respectively.

The toxin, microcystin-LR, was obtained from Biovision INC, American company. The standard microcystin-LR solution was prepared by adding 100 μg microcystin-LR to 1 ml distilled water at a final concentration of 100 $\mu\text{g/ml}$. A control was set up by adding 10 $\mu\text{g/l}$ of standard microcystin-LR to 10 ml distilled water and measured by HPLC.

3. RESULTS

3.1 Estimation of microcystin-LR by HPLC

Sharp peak of the reference microcystin-LR was observed after 6.9 minutes of retention time. MC-LR in positive samples were displayed at the same retention time. As shown in Figure 3, site I contained approximately high levels of microcystin-LR during December (12.28 $\mu\text{g/l}$) and August (9.08 $\mu\text{g/l}$) while site II contained small concentration in August. Otherwise, no microcystin-LR was detected during the rest of the year. Drinking water contained low value (0.67 $\mu\text{g/l}$) in January.

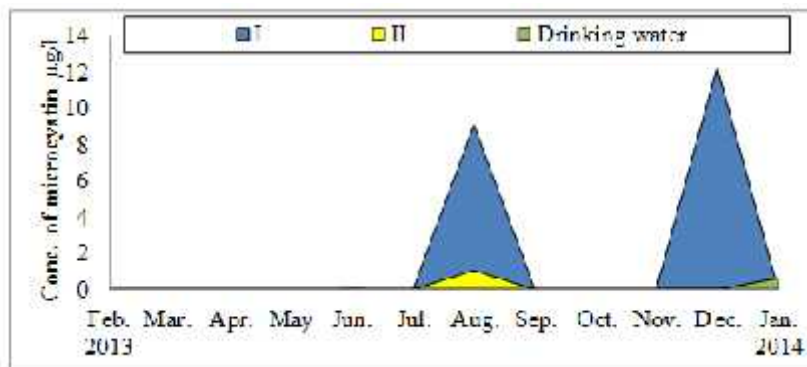


Fig. 3: concentration of microcystin-LR in water supply (sites I and II) and drinking water during the study period

3.2 Mouse bioassays estimation of microcystin-LR

3.2.1 Toxicity experiment

The toxicity effect of microcystin-LR occurred in water supply and drinking water on mice is represented in Figure 4. Samples collected from site I exhibited high toxicity during the period November-January, with the highest toxicity effect (0.69 MU) in January. Site

II showed toxicity effect with lower value (0.31 MU) in November. Otherwise, little or even no toxicity symptoms were observed during the rest of the year. Drinking water showed low toxicity effect in only three months, August, November and January, giving the maximum value (0.34 MU) in January.

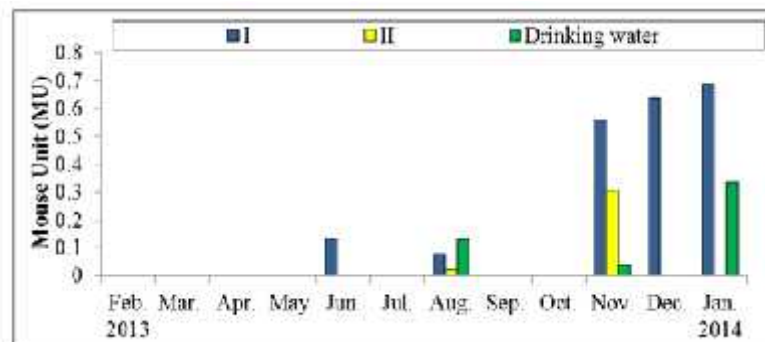


Fig. 4: Toxicity effect of water supply (sites I and I) and drinking water on mice

3.2.2 Histopathology examination

Figure 5 shows the effect of water supply and drinking water on the liver of mice. The control group showed liver with

normal central vein (cv) (Fig. 5a). Liver of mice accessed with water supply, showed severe diffuse congestion (c), mild vacuolar degeneration, hyperplasia of hepatic areas

and necrosis normal minimal vocal (n) during the whole study period (Figs, 5b-e). On the other hand, mice accessed with treated drinking water showed diffuse congestion, hydropic degeneration and

necrosis minimal vocal during winter, summer and autumn (Figs. 5f, g and i), while it showed normal central vein in spring (Fig. 5h).

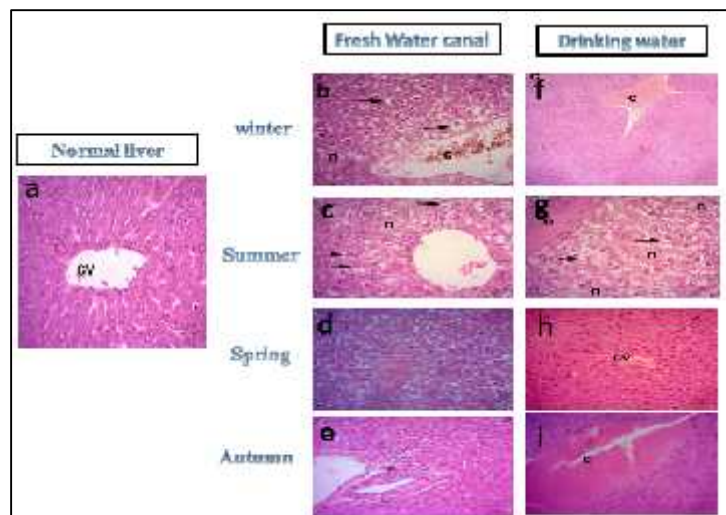


Fig. 5: Structural features of liver examined by light microscopy at X 400 for mice accessed with water supply and treated drinking water at Port Said during study period (Note: all sections were stained with H&E) .

Figure 6 shows the effect of water supply and drinking water on the kidney of mice. Control group showed kidney with normal glomeruli (g) and renal tubules (Fig. 6a). During winter, summer and autumn, kidney of mice accessed with water supply showed severe congestion (c), diffuse degeneration, cystic dilatation of renal tubules, necrosis of tubular epicellial, degeneration of tubular epithelium, peritubular edema and renal casts inside their

lumen and thickening of kidney capsule (Figs. 6 b, c and e) while mice accessed with treated drinking water showed little symptoms, such as, diffuse congestion and degeneration, periglomerular edema and hyaline cast (Figs. 6 f, g and i).

In spring, kidney of mice in all groups showed normal glomeruli and renal tubules, while it showed normal central vein in spring (Figs. 6: d and h).

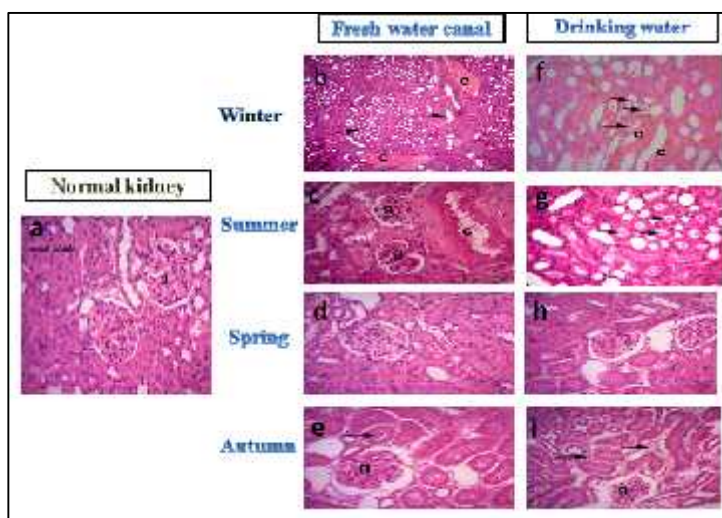


Fig. 6: Structural features of kidney examined by light microscopy at X 400 for mice accessed with water supply and treated drinking water at Port Said during study period (Note: all sections were stained with H&E).

3.3 Removal of microcystin-LR

As shown in Table 1 and Figure 7, Egyptian pottery decreased the concentration of microcystin-LR after one hour from 0.585 µg/ml to 0.119 µg/ml. After 6 hours,

removal efficacy reached to 91.9% and the toxin was completely removed after 24 hours. Besides, the bad odor and color were completely absent.

Table 1: Microcystin-LR concentration and removal efficiency during 24h using the Egyptian pottery

Contact Time (h)	Microcystin-LR conc. (µg/ml)	Removal efficiency (%)
Zero time	0.585	zero
1	0.119	79
2	0.071	87.8
3	0.050	91.4
6	0.049	91.6
24	ND	100

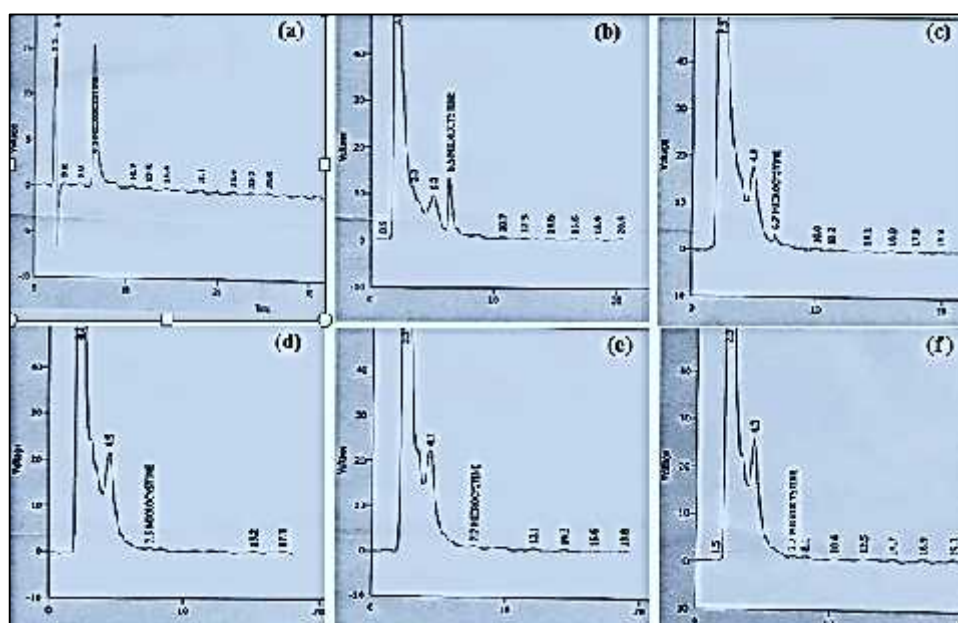


Fig. 7: HPLC chromatograms of microcystin-LR concentrations using pottery. (a) separation of standard microcystin-LR after 6.5 min, (b-f) initial concentration of microcystin-LR and after 1, 2, 3 and 6 hours

4. DISSCUSSION

Freshwater blooms of toxic cyanobacteria have been reported worldwide (Sivonen, 1996). There have been many reports of the intoxication of birds, fish and other animals by cyanobacterial toxins (Krienitz *et al.*, 2003). The present study focused on a cyanotoxin, microcystin-LR, in fresh water supply of drinking water treatment plant and drinking water at Port Said, Egypt. The present results revealed that in the water supply, the higher concentrations of microcystin-LR (12.28 and 9.8 µg/l) were recorded in winter and summer, respectively. This was

synchronized with the presence of blooms of *Microcystis aeruginosa*, *Oscillatoria princips* and *O. brevis* in water supply during these seasons (unpublished data). This is in accordance with that reported by several researches on Ismailia Canal. Amine (2001) studied the presence of cyanotoxins in Ismailia Canal and detected the occurrence of cyanobacterial bloom in winter (November-February) which consisted from the toxic species, *Oscillatoria princips* and *O. brevis*. Marrez (2010) recorded the presence of cyanobacterial bloom in Ismailia Canal in winter and spring. When he examined the toxicity effect of these blooms

on *Artemia* and mice, he observed the presence of toxicity effect exclusively in winter of the two years, 2008 and 2009.

On the other hand, microcystin-LR was not detected in drinking water during the period of study, except in January ($0.67\mu\text{g/l}$). The concentration of microcystin-LR in drinking water for human as prescribed by the world health organization (WHO) is $1\mu\text{g/l}$ (WHO, 1999). However, Ueno *et al* (1996) proposed a value of $0.01\mu\text{g/l}$, based on a possible correlation of primary liver cancer in certain areas of China with the presence of microcystins in water of ponds, rivers and shallow wells. This means that the concentration of microcystin-LR in Port Said drinking water is compatible with the world health organization.

The LD50 of microcystin-LR i.p. or i.v. in mice and rats is in the range 36-122 $\mu\text{g/kg}$ (Stoner *et al.*, 1989). This indicates that the concentration of microcystin-LR in water supply and drinking water was lower than that of LD50. This appeared in the low values of the recorded mouse units that did not exceed 0.69 MU.

Illnesses caused by cyanobacterial toxins to humans fall into three categories; gastroenteritis and related diseases, allergic and irritation reaction, and liver diseases (Codd and Bell, 1996). Microcystins have also been implicated as tumor-promoting substances (Bell and Codd, 1996; Zegura *et al.*, 2003). Microcystin targets the liver causing cytoskeletal damage, necrosis and pooling of blood in the liver, with a consequent large increase in liver weight. Membrane blabbing and blistering of hepatocytes in vitro has been observed (Runnegar *et al.*, 1991)

In the present study, microcystin-LR intoxication on mice appeared in histological investigation during the period with massive cyanobacterial blooms in both water supply and drinking water. The toxicity effect on liver was observed in the form of severe congestion, vacuolar degeneration and hyperplasia of hepatic areas and necrosis in liver hepatocytes. Several damages in kidney were observed; such as cystic

dilatation of renal tubules, renal casts inside their lumenth, thickening of kidney capsule, degeneration of tubular epithelium and peritubular edema. The present result is in consistence with findings of Nishiwaki-Matsushima *et al.* (1992) who recorded some toxicity remarks for microcystin-LR in mice such as hepatocytes shrink and liver damage. Yoo (1995) discussed that hepatotoxin in freshwater blooms of cyanobacteria were more commonly found than neurotoxic blooms. However, all symptoms recorded in liver and kidney during the period of experiment were reversible degenerated changes.

Microcystin-LR is a stable cyclic structure of toxic heptapeptide that represents many challenges to conventional water treatment facilities which have limited capability for the removal of microcystins (Himberg *et al.*, 1989). Significant advances in water treatment technologies over the last two decades provided solutions for efficient removal of these toxins (Lawton and Robertson, 1999). However, these facilities are expensive to implement and maintain, and their efficiency may decrease under different conditions.

In the present study, Egyptian pottery (earthenware) was tested as an alternative material to remove microcystin-LR during the drinking water treatment process. Egyptian pottery did not only have the ability to completely eliminate microcystin-LR residues with efficiency of 100% but also completely removed the bad odor of water in 24hr. Since Egyptian pottery is made of soil of the river bank, so these results was evidenced by the finding of Pendleton *et al.* (2001) who proposed the importance of soil (river bank filtration) in the adsorption of toxins. There were no sufficient published results available in evaluation of bank filtration with respect to cyanotoxin removal. Chorus *et al.* (1993) recorded that bank filtration will be a highly promising method to avoid contamination with cyanobacterial cells as well as dissolved toxins. This expectation is supported by the favorable results which demonstrated good

performance of experimental soil and sediment columns for cell and toxin removal by Lahti *et al.* (1997). The present study confirmed the ability of river bank, which usually used in made of earthenware, in removing microcystin-LR, in addition to bad odor and color caused by cyanobacterial bloom. This method is considered a low cost effective method for degradation of microcystins in water.

Conclusively, the current study indicated that although the concentration levels of microcystin-LR in Port Said drinking water are compatible with the world health organization (WHO), little changes in some physico-chemical parameters of drinking water resources may maximize these levels by stimulating harmful algal blooms. Finally, the current study presents a cost effective method that could be applied to eliminate microcystin-LR in treatment strategies of drinking water.

5. ACKNOWLEDGMENTS

This study was funded and sponsored by Academy of Scientific Research and Technology, Egypt as a part of M.Sc. scholarship.

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