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Article:

Removal efficiency of microcystins in river water using activated charcoal

Wafaa Kh. Kelini [1*], Manal A. M. Mahmoud[2], Zakaria M. Zaky [3], Hosnia S. Abdel-Mohsein [2]

[1] *Department of Animal, Poultry and Environment Hygiene, Faculty of Veterinary Medicine, Sohag University.*

[2] *Department of Animal, Poultry Hygiene and Environmental Sanitation, Faculty of Veterinary Medicine, Assiut University.*

[3] *Department of Forensic Medicine and Veterinary Toxicology, Faculty of Veterinary Medicine, Assiut University.*

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Abstract

Microcystins (MCs) are hepatopancereatic toxins released from several cyanobacteria and all living organism can be exposed to the toxic effects of MCs mainly through consumption of contaminated surface water. Although the Nile River is the main source of fresh drinking water supply in Egypt, it is exposed to different anthropogenic pollution sources. Therefore, a total of 16 water samples were collected along river Nile in Sohag Governorate and examined for MCs (MC-LR, MC-RR and MC-YR) using ultra-high performance liquid chromatography (UPLC). In addition, the removal efficiency of MCs in both acidified and neutral water by using activated charcoal (AC) was examined. The results revealed the presence of MCs in all water samples were collected and 25 % of the samples had concentration exceeded the WHO guideline level. Furthermore, the high efficiency of activated charcoal was observed for removal of MCs reaching 89% in the acidified water (pH 4 ± 0.3) and 92.53 % in the neutral water (pH 7 ± 0.3). Extensive monitoring should be paid to the presence of MCs contamination source unless a potential public health hazard could be observed in Egypt due to utilization of river water.

Keywords: *Microcystins, River water, Removal, Activated charcoal.*

Introduction

Microcystins are a group of hepatopancreatic toxins which are produced by several bloom forming cyanobacterial species in water and known for their toxic effects on aquatic organisms and humans (Juliette et al., 2009). More than one hundred different variants of microcystins possess a great threat to animals and humans, due to their potential carcinogenicity through the inhibition of protein phosphatase which lead to various cellular responses such as reduced DNA repair, apoptosis and tumor promotion (Buratti et al., 2017, Khomutovska et al., 2020).

Microcystins were identified and measured in different water sources all over the world. In Egypt, MCs were detected from some drinking water treatment plants at concentrations ranging from9.08 to 12.28µg/l and 0.7 to 341 µg /L according to Fedekar et al., (2015) and Mohamed, (2016), respectively. However, Masango et al., (2010) and Lee and Son, (2019) detected from rivers in South Africa and South Korea MCs concentrations ranged from 49.41- 103.16 µg /l and 1–7.2 µg/l, respectively.

Cyanobacteria secrete toxins at all growth stages and these toxins generally remain in the cell and known as intracellular toxin, until aging or stress so the toxins are released and become extracellular toxin. The half-life of the toxin is 4-14 days in surface water depending on the

IJVMS [*] ***Corresponding author:*** *Wafaa Kh. Kelini, Email: Wafaakhalagallah46@gmail.com, Address: Animal, Poultry and Environment Hygiene, Faculty of Veterinary Medicine, Sohag University 82524, Egypt..*

content of natural organic matter, the degree of sun light and presence of bacteria. Toxin production of cyanobacteria in water has been recently shown to be influenced by a variety of environmental factors including light (Walsby and Schanz, 2002), nutrients (Cantin et al., 2011), temperature (Cuypers et al., 2011) and depth (Holland and Kinnear, 2013). In tropical areas high temperatures and nutrient abundance enhance the cyanobacterial growth and microcystins production (Codd et al., 2016, Buratti et al., 2017).

It was confirmed that about 80% of the daily consumed microcystins came from ingestion of contaminated water and the remaining 20% came from consumption of contaminated food and inhalation (Dietrich and Hoeger, 2005). Moreover, human can be exposed to MCs through various routes as dermal, respiratory, body contact (during recreational activities), hemodialysis (He et al., 2016; Ibelings et al., 2016).

Microcystins were recognized for the first time in the serum (0.228 ng/ml MC-LR) of a chronically exposed human population (China) together with indication of hepatocellular damage (Deng, 2004). The extreme cases of human poisonings were manifested in Caruaru, Brazil in 1996 where 116 of 131 patients showed visual disturbances, nausea, vomiting and muscle weakness following routine dialysis with contaminated water then developed acute liver failure and more than 60 hemodialysis patients died from these symptoms (Catherine et al., 2016). In addition, microcystins are reported to be globally toxic to domestic and wild animals, which reported to cause pet and cattle mortalities in both more and less economically developed nations (Briand et al., 2003, Qin et al., 2010). Furthermore, food crops, vegetables and plants irrigated using water contaminated with MCs are negatively affected by MCs which affect their quality and can cause a reduction in their yields (Drobac et al., 2017). MCs can also cause oxidative stress and negatively influence photosynthetic activities in plants (Bittencourt-Oliveira et al., 2016). Plants in general are capable of accumulating MCs in their tissues as a result of their low concentration absorption capability (Yin et al., 2005). Therefore, consuming these contaminated crop yields and plants are considered a risk factor for human health. MCs cause illness and even death in aquatic life, wild animals, livestock, and even human beings (Cai et al., 2019). They represent great threats to ecological health due to their adverse effects on metabolism of freshwater mussels (Gelinas et al., 2014).

The Nile River in Egypt is the main source of freshwater for drinking water supply; additionally, the Nile River is exposed to pollution by many sources. Furthermore, the conventional water treatment methods including coagulation, flocculation, sedimentation and filtration are ineffective in removing MCs (Newcombe and Nicholson, 2004). Activated carbon either powdered or granular is one of the most commonly filter material applied for MCs removal (Hassan and Youssef, 2014) due to its following advantages: extended surface area (Donati et al., 1994), microporous structure, high adsorption capacity and economic feasibility (Hassan and Youssef, 2014).

The aim of the present study was to use activated charcoal as an adsorbent material for removal of the biological toxin produced by cyanobacteria to preserves the human and livestock health.

Materials and methods:

Sample collection

A total of 16 river water samples were collected from different locations around Sohag Governorate at June and September 2022. The samples were collected near to the sources of possible organic contaminants. Each sample was collected in amber glass bottle. The bottles were rinsed first with methanol followed by distilled water. At sampling sites, each bottle was washed several times by the water from each site before collecting 500 ml of water sample. Samples were transported in portable ice box to the laboratory and stored at -20 o C to prevent cyanotoxin degradation (Justin et al., 2019).

Microcystin Extraction from water samples

Preparation of water samples and extraction of microcystin were performed according to Elbert et al., (2012) with some modifications. Each sample (500 ml) was filtered by using Glass microfiber filter. Then 5 ml methanol and 4 ml of 10% trifluroacetic acid were added to the water sample to adjust the pH at 3. Both filter and filtrates were used for detection of intracellular and extracellular microcystins, respectively.

Extracellular microcystin:

The extracellular MCs were extracted using solid phase extraction (SPE) according to Chorus and Welker (1999) with some modifications. C18 cartridge was preconditioned by washing with 10 ml methanol followed by 10 ml distilled water. The filtrate was passed through the cartridge at a rate of 15 ml/minute continuously (without allowing the cartridge to dry).

Once the entire sample had passed through the cartilage, the cartridge was washed with 10 ml of 10%, then 20% and 30% methanol. The three washed solution were discarded. Air was allowed to pass through the cartridge then elution was performed using 3 ml of acidified methanol using 0.1% v/v TFA, this step was repeated twice. The elutes were evaporated under 45oC using rotatory evaporator and reconstituted by 200 µl of 75 % methanol followed by shaking using vortex mixer, the reconstitution step was repeated 3 times. The 600 µl solution of reconstitution step were filtered and injected in ultra-high performance liquid chromatography (UPLC).

Intracellular microcystins:

The intracellular MCs were extracted using liquid phase extraction according to Rocio A-R et al., (2005). The GF/C filters (containing the cyanobacterial cells) were weighed before drying in hot air oven, till obtaining 2 successive stable weights. The filters were frozen and thawed for destruction of cyanobacterial cells and release of intracellular microcystins (3 freezing and thawing cycles). The filter was dissolved in 20 ml methanol and centrifuged at 300 rpm for one hour & sonicated for 15 minutes. The procedure was repeated three times. The 60 ml methanol containing the destructed filter of each sample were filtered and evaporated using rotatory evaporator and reconstituted by 200 µl of 75 % methanol. A total of 600 µl solution of reconstitution step were filtered and injected in UPLC.

The microcystins concentrations in the collected samples were measured using UPLC at the Central Lab, Faculty of Veterinary Medicine, Assiut University, Egypt. AC Quity UPLC BEH C18 1.7 µm (WatersTM, Milford, USA) analytical column at 238 nm was used. The flow rate and temperature were 0.4 ml min1 and 35 °C, respectively. The total run time for each injection was 10 min. The sample injection volume was 20 ul.

Characterization of activated charcoal

- pH: Measured using pH meter according to (Ekpete and Horsfall, 2011). Moisture content :Determined using hot air oven according to (Ekpete and Horsfall, 2011).
- Iodine number: Determined according to (Ahmedna et al., 1997).
- Methylene blue removal efficiency: Measured using spectrophotometer according to (Dhuha et al., 2019).
- Removal of MC from the water sample using shaking method: The removal experiment using activated charcoal was performed according to Yasmin et al., (2019) with some modifications.

Adsorption experiment by shaking was conducted in triplicate using neutral water (pH 7 ± 0.3) and the other using acidified water (pH 4 ± 0.3). Four grams of activated charcoal were weighed and placed in a flask and 200 ml of the water sample containing (MC-LR, -RR and -YR at concentration of 7 µg/L) was added and shaking at a rate of 200 rpm for 10 min. The mixture filtered by using double rings filter paper to remove the course particles from the water. The supernatant was filtered with Glass microfiber filter 0.47 to separate the extracellular and intracellular microsystin in water sample.

The filtrate was extracted by solid phase extraction using C18 cartridge (Strata C18-E (55µm, 70A), 500mg/ 6ml, tubes, Reorder part No. 8B-S001-HCH) and the filter was freezed and thawed three times for destruction of cyanobacterial cells and release of intracellular microcystins, centrifugation at 300 rpm for one hour, three times and sonicated for 15 minutes, three times. The elutes of the filtrates and filters were evaporated and reconstituted in 600 µl of 75% methanol which were injected in UPLC to detect the concentration of MCs after the removal process.

Statistical analysis

The obtained data was statistically analyzed with SPSS version 16.0 for Windows. The data are reachable as means and standard deviation. The microcystins levels in the collected samples were compared by One-Way of Variance (ANOVA) and when $P<0.05$, the difference in mean was believed as statistically significant. When the differences between the means were significant the means were separated by Duncan's post hoc test. Differences with $P < 0.05$ or $P < 0.01$ were considered statistically significant.

Results:

The concentrations of MCs (intracellular, extracellular and total) were shown in table 1. The highest concentration of both intracellular and extracellular MCs was found in river water samples collected from Mishta with mean value 1.56± 0.449 and 2.34±0.255, respectively. However, the lowest concentration was reported from Elmaragha river water samples with mean 0.39 ± 0.064 and 0.005 ± 0.0029, respectively

The result concerning the characterization of activated charcoal exhibited in figures 2 and 3. The data presented

in figure 2 revealed the pH value and the moisture content % of the activated charcoal, with mean value 5.37 ± 0.47 and 6.28 ± 0.2, respectively. Moreover, the mean values concerning the iodine number and dye Methylene blue (MB) removal percentage of the examined activated charcoal during the experiment were 9.2 ± 1.1 and 90.95% ± 1.2, respectively (figure 3).

The removal efficiency of total MCs using the activated charcoal from neutral and acidified river water samples were presented in table 4. It was observed that the removal efficiency was 92.5% in neutral water sample while in acidified water samples the efficiency percentage was 89.1%.

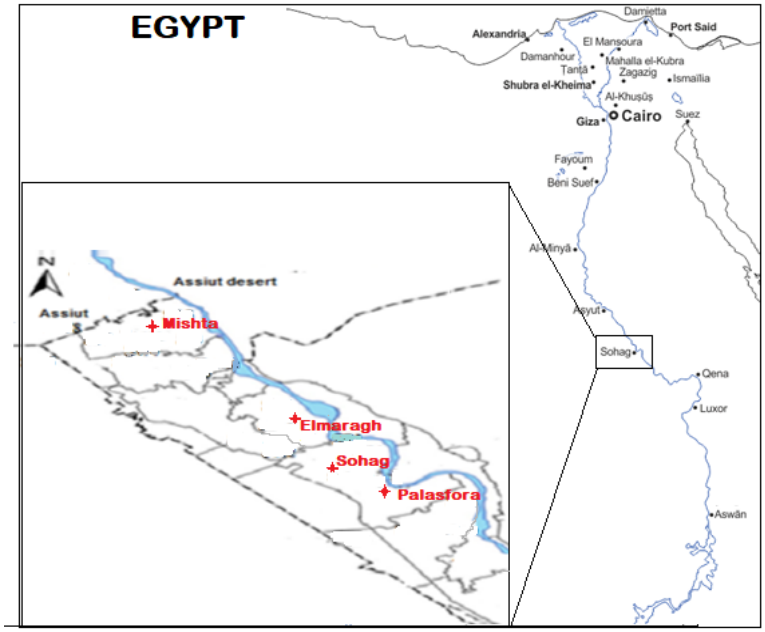

Figure 1. Map of the sampling sites. Palasfora (P1,P2,P3,P4), Sohag (S1,S2,S3,S4), Elmaragha (E1,E2,E3,E4), Mishta (M1,M2,M3,M4).

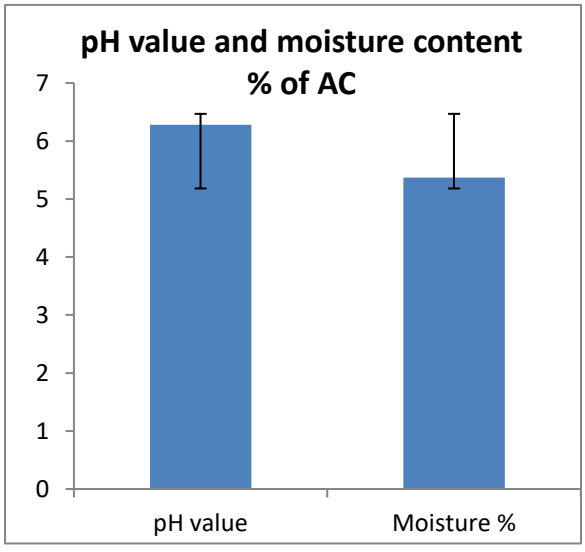

Figure 2. PH and moisture content % of the activated charcoal (AC).

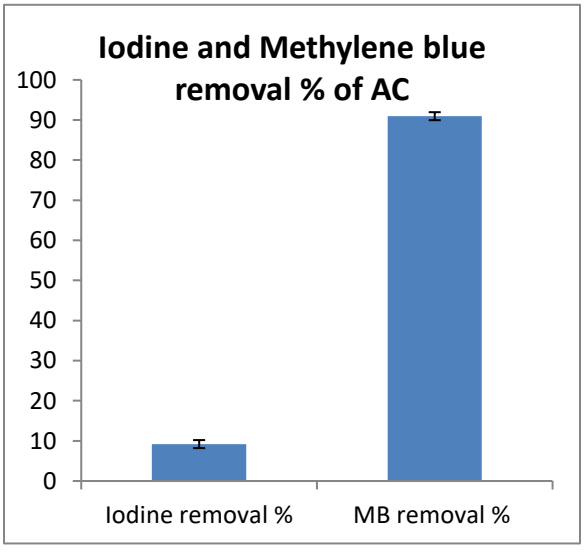

Figure 3. Percentage of iodine removal and methylene blue (MB) removal of activated charcoal (AC).

Table 1. Microcystins concentrations (µg/L) in River water samples from Sohag Governorate

Collection sites	Microcystine in River water		
Palasfora	**Intracellular**	**Extracellular**	**Total**
P1	0.38	0.23	
P2	0.36	0.15	
P3	0.4	0.24	
P4	0.3	0.3	
Mean ± SD	0.36±0.04	0.23±0.06	0.59±0.1
Sohag			
S1	0.39	0.2	
S2	0.33	0.25	
S3	0.3	0.1	
S4	0.38	0.29	
Mean ± SD	0.35± 0.02	0.21±0.08	0.56±0.12
Elmaragha			
E1	0.39	0.005	
E2	0.48	0.008	
E3	0.36	0.001	
E4	0.33	0.006	
Mean ± SD	0.39±0.06	0.005±0.002	0.39±0.06
Mishta			
M1	1.57	2.35	
M2	1.3	2.12	
M3	2.2	2.7	
M4	1.2	2.2	
Mean ± SD	1.6± 0.4	2.34±0.2	3.91±0.7*

(*) Means that the detected concentration of MCs was higher than the maximum limit established by WHO (1 µg/l) (WHO, 2017). S, P, E and M are the codes of the sampling locations where S1, S2, S3, S4 (Sohag), P1,P2,P3,P4 (Palasfora), E1, E2, E3, E4 (Elmaragha) and M1, M2, M3, M4 (Mishta).

Table 2. Removal efficiency % of MCs using Activated Charcoal (AC) from neutral river water

Material	Intracellular mcs	Extracellular mcs	Total
Activated charcoal	99.9	87.38	89.13
	91.9	93.68	95.93
	95.9	90.53	92.53
	95.9±4	90.53±3.15	92.53±3.40

Table 3. Removal efficiency % of MCs using Activated Charcoal (AC) from acidified water

Material	Intracellular	Extracellular	Total
Activated charcoal	79.31	95.71	89.5
	70.41	98.81	89.11
	74.86	97.26	88.71
	74.86±4.45	97.26±1.55	89.11±0.40

Table 4.Removal efficiency of total MCs from acidified and neutral water

Material	Acidified	Neutral
Activated charcoal	89.5	89.13
	89.11	95.93
	88.71	92.53
	89.11±0.40	92.53±3.40

Discussion:

Microcystins have become one of the greatest water pollution problems for public health worldwide, as they blamed for water quality and treatment problems and have a lot of side effects on animals and human health. Exposure of MCs is mainly through drinking contaminated water (Dietrich and Hoeger, 2005).

Concentration of MCs in the collected river water samples: The detected concentration of total MCs in river water ranged from 0.39 to 3.93 (1.37 ±1.7) µg/L depending upon variation in sampling location characteristics, environmental factors and concentration of nutritional factors. The concentration of total MCs from 25% of the collected samples (Mishta) was higher than the provisional guideline established by WHO (1µg/l).

Highest level of total MCs concentration (3.93µg/L) was from Mishta village which was nearly four folds higher than the WHO guideline because it was near to Mishta's abattoir at a distance of 8 meters and subjected to contamination from public human activities.

Our results concerning the concentration of the total MCs in the collected river water samples from Sohag governorate agreed with those recorded by Kann (2007) and Lee and Son, (2019) who found that MCs concentrations in Klamath River and Han River (South Korea) were 0.32 µg/L and 1–7.2 µg/L, respectively.

Our results were lower than those found by Mohamed (2016) who revealed that, in Egypt, MCs were detected in the source water of some drinking water treatment plants at concentrations ranging from 0.7 to 341 µg /L in summer season and also they are lower than those revealed by Masango et al., (2010) who found that there was an increase of microcystins from an average of 49.41 µg /L in February during summer blooming of the cyanobacteria, to 103.16 µg /L in June in the dam water found in the Kruger National Park.

Our findings concerning the MCs concentration in the collected river water samples were higher than the concentration found in the samples collected from the Nile River water at Sohag City, Egypt and the Huai River Basin of China where the concentration of microcystins was56.1-87.1 ng/L with maximum value of 0.4-0.78 µg/L and 0.741 ± 0.623 µg/L with maximum of 1.846 µg/L, respectively. (Mohamed and Carmichael, 2000, Dajun et al., 2013)

Removal efficiency experiment: In the present study, the pH value of the activated charcoal and the result was (6.28 ± 0.2 largely agreed with those (pH 7) found by Ekpete and Horsfall (2011). However, our results are lower than the results of Ariany et al., (2018) who they measured that pH values of activated carbon derived from bio-char waste of bio-oil pyrolysis were in the range of 9.56-10.5.

The moisture content of the activated charcoal was 5.37% ± 0.47which was lower than those found by Ekpete and Horsfall (2011) who demonstrated that the moisture contents of Fluted Activated Carbon (FAC) and Commercial Activated Carbon (CAC) were 19.50 ± 0.02 and 16.67 ± 0.07, respectively.

Furthermore, the moisture content of activated charcoal were lower than the moisture content of the activated carbon measured by Ariany et al., (2018) where it was 0 – 13.06 %. According to Ariany et al., (2018), the lower the moisture content of the adsorbent the greater the adsorption capacity.

The percent of iodine removal (PIR) of the AC was 9.2% ± 1.19. A higher degree of iodine adsorption indicates a higher surface area and largely microporous structure (Gergova et al., 1993).

The percentage of methylene blue (MB) removal using activated charcoal was 90.95% ± 1.19 which was closely agree with the results of Ariany et al., (2018) as dye removal efficiency was ranged from 96.75 – 98.19%. Our results regarding dye removal using activated charcoal were higher than those found by Zahanggir et al., (2022) who revealed that the percentage of dye removal with charcoal was 42.46 ± 1.4 to 59.96 ± 1.1.

The adsorption capacity of activated charcoal increased with increasing the percentage of methylene blue removal so that, depending on our results concerning MB removal activated charcoal is a good adsorbent material.

The results showed that the adsorption capacity of activated charcoal for MCs from the acidified water and neutral water were up to 89.5% (89.11 ± 0.40) and 95.9% (92.53 ± 3.40), respectively. Moreover, the activated charcoal is an efficient adsorbent material for removal of microcystins from water samples with non-significance differences between acidified and neutral water samples.

Our results concerning the efficiency of activated charcoal for MCs removal were closely related to those found by Wael et al., (2015) who collected samples from raw water, treated water, and distribution system of different DWTPs from the Nile River and the branches in Dakhalia Governorate.

Conclusion:

Microcystins were detected intracellularly and extracellularly in all collected river water samples from Sohag and about 25% of the samples had concentrations higher than the maximum limit of WHO (1 µg /l) (WHO, 2017), may result from high anthropogenic activity. Removal of MCs from river water using

activated charcoal as an adsorbent material showed high efficiency for both neutral and acidified water which could preserve public and livestock health from high potential health hazard of cyanobacterial toxins.

Conflict of interest:

The authors declare that there was no conflict of interest for publication of an article. We stated that the manuscript has been read and approved by all authors; also we have no financial fund of support.

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Authors' contribution:

Concept, idea and proposal: Hosnia S. Abdel-Mohsein, Manal A. M. Mahmoud and Zakaria M. Zaky.Web research and data collection: Wafaa Kh. Kelini. Methodology: Hosnia S. Abdel-Mohsein, Manal A. M. Mahmoud and Zakaria M. Zaky.Data analysis and statistics: Wafaa Kh. Kelini Draft writing: Wafaa Kh. Kelini. Revision and editing: Hosnia S. Abdel-Mohsein, Manal A. M. Mahmoud and Zakaria M. Zaky. Paper submission: Wafaa Kh. Kelini.

References:

1. Ahmedna M., Johns M. M., Clarke S. J., Marshall W. E. and Rao R. M., (1997). Potential of Agricultural By-Product-Based Activated Carbons for Use in Raw Sugar Decolourisation, J Sci Food Agric, 75, 117-124.

2. Ariany Z.Ghina H. F., and Amelia S.R., (2018). The potential of activated carbon derived from bio-char waste of bio-oil pyrolysis as adsorbent , MATEC Web of Conferences 154, 01029.

3. Bittencourt-Oliveira Mdo C., Cordeiro-Araujo M. K., Chia M. A., Arruda-Neto J. D., de Oliveira E. T., dos Santos F., (2016). Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners. Ecotoxicol Environ Saf, 128, 83-90.

4. Briand J.F., Jacquet S., Bernard C., Humbert J.F., (2003). Health hazards for terrestrial vertebrates from toxic Cyanobacteria in surface water ecosystems. Vet. Res. 34 (4), 361–377.

5. Buratti F. M., Manganelli M., Vichi S., Stefanelli M., Scardala S., Testai E., Funari E., (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Arch Toxicol. 91(3):1049-1130.

6. Cai W., Zhao Z., Li D., Lei Z., Zhang Z., Lee D. J., (2019). Algae granulation for nutrients uptake and algae harvesting during wastewater treatment. Chemosphere 214, 55-59.

7. Cantin A., Beisner B. E., Gunn J. M., Prairie Y. T., Winter J. G., (2011). Effects of thermocline deepening on lake plankton communities. Can. J. Fish. Aquat. Sci. 68, 260-276.

8. Catherine A., Bernard C., Spoof L., Bruno M., (2016). Microcystins and nodularins. In: Meriluoto J, Spoof L, Codd GA (eds) Handbook of cyanobacterial monitoring and cyanotoxin analysis. Wiley, Chichester, pp 107–126.

9. Chorus and Welker (1999), Toxic Cyanobacteria in Water - A guide to their public health consequences, monitoring and management.

10. Codd G. A., Meriluoto J., Metcalf J. S., (2016). Introduction. In: Meriluoto J, Spoof L, Codd GA (eds) Handbook of cyanobacterial monitoring and cyanotoxin analysis. Wiley, Hoboken, pp 1–8.

11. Cuypers Y., Vinçon-Leite B., Groleau A., Tassin B., Humbert J. F., (2011). Impact of internal waves on the spatial distribution of Planktothrix rubescens (cyanobacteria) in an alpine lake. ISME J. 5, 580-589.

12. Dajun T., Weiwei Z., Xiao W., Xin S., Li L, Xin C., Hao Z., Yin Z., Hanyi C., Hongmei Z., Xia W., Rongjie Z., Songhui J., Yuxin Z., Gonghuan Y., Weidong Q., (2013). Dissolved microcystins in surface and ground waters in regions with high cancer incidence in the Huai River Basin of China, Chemosphere 91. 1064–1071.

13. DENG D.G., (2004). Ecological studies on the effects of eutrophicatio on plankton communities in a large shallow lake, Lake Chaohu. PhD thes Chinese Academy of Science. 137 pp.

14. Dietrich D, Hoeger S., (2005). Guidance values for microcystins in water and cyanobacterial supplement products (blue green algal supplements): a reasonable or misguided approach? Toxicological and Applied Pharmacology 203: 273–289.

15. Donati C., Drikas M., Hayes R., Newcombe G., (1994). Microcystin-LR adsorption by powdered activated carbon, Water Res. 28, 1735–1742.

16. Drobac D., Tokodi N., Kiprovski B., Malencic D., Vazic T., Nybom S., Meriluoto J., Svircev Z., (2017). Microcystin accumulation and potential effects on antioxidant capacity of leaves and fruits of Capsicum annuum. Journal of Toxicology and Environmental Health-Part a-Current Issues, 80, 145-154.

17. Ekpete O.A. and Horsfall M. JNR, (2011). Preparation and Characterization of Activated Carbon derived from Fluted Pumpkin Stem Waste (Telfairia occidentalis Hook F), Research Journal of Chemical Sciences Vol. 1(3).

18. Elbert A. M., Titus A. M. M.,* and Bhekie B. M., (2012). Quantitative Variations of Intracellular Microcystin-LR, -RR and -YR in Samples Collected from Four Locations in Hartbeespoort Dam in North West Province (South Africa) During the 2010/2011 Summer Season, Int J Environ Res Public Health. 9(10): 3484–3505.

19. Fedekar F. M., Shymaa M. S.1*, Yusuf Y. S.2, Amina A. D.3 and Adel A. M.4, (2015). 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. International Journal of Environmental Science And Engineering (Ijese). 6:75 - 84 (2015).

20. Gelinas M., Fortier M., Lajeunesse A., Fournier M., Gagnon C., Barnabe S., Gagne F., (2014). Responses of freshwater mussel (Elliptio complanata) hemocytes exposed in vitro to crude extracts of Microcystis aeruginosa and Lyngbya wollei. Ecotoxicology 23, 260–266.

21. Gergova K., Petrov N., Eser S., (1993). A comparison of adsorptionvcharacteristics of various activated carbons. J Chem Technol Biotechnol 56 77-82.

22. Hassan A. F., Youssef A. M., (2014). Preparation and characterization of microporous NaOH activated carbons from hydrofluoric acid leached rice husk and its application for lead(II) adsorption. Carbon Letters 15(1): 57–66.

23. He X., Liu Y. L., Conklin A., Westrick J., Weavers L. K., Dionysiou D. D., Lenhart J. J., Mouser P. J., Szlag D., Walker H. W., (2016). Toxic cyanobacteria and drinking water: Impacts, detection, and treatment. Harmful Algae. 54:174-193.

24. Holland A., Kinnear S., (2013). Interpreting the possible ecological role(s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? Mar. Drugs 11, 2239-2258.

25. Ibelings B. W., Bormans M., Fastner J., Visser P. M., (2016). CYANOCOST special issue on cyanobacterial blooms: synopsis—a critical review of the management options for their prevention, control and mitigation. Aquat Ecol 50:595–605.

26. Juliette L. Smith, Gregory L. Boyer, (2009). Standardization of microcystin extraction from fish tissues: A novel internal standard as a surrogate for polar and non-polar variants, Toxicon 53, 238–245.

27. Justin D. Chaffin, Erica L. Fox, Callie A. Nauman and Kristen N. Slodysko, (2019). The ability of household pitcher-style water purifiers to remove microcystins depends on filtration rate and activated carbon source, The Authors, water supply 19-1-2019.

28. Kann J., (2007). Toxic Cyanobacteria Results for Copco/Iron Gate Reservoirs: September 18-19. AQUATIC ECOSYSTEM SCIENCES LLC: Ashland, OR.

29. Lee E.H., Son A., (2019). Fluorescence resonance energy transfer based quantum dot- Aptasensor for the selective detection of microcystin-LR in eutrophic water, Chem. Eng. J. 359, 1493–1501.

30. Masango M. G., Myburgh J. G., Labuschagne L., Govender D., Bengis R. G., Naicker D., (2010): Assessment of Microcystins bloom toxicity associated with wildlife mortality in the Kruger National Park, South Africa. - Journal of Wildlife Diseases 46: 95-102.

31. Mohamed Z. A, CarmichaelW. W., (2000) Seasonal variation inmicrocystin levels of River Nilewater at Sohag city, Egypt. Ann Limnol 36:227– 234

32. Mohamed Z. A., (2016), Harmful cyanobacteria and their cyanotoxins in Egyptian fresh waters – state of knowledge and research needs, African Journal of Aquatic Science, 41(4): 361–368.

33. Nataliia Khomutovska, Małgorzata Sandzewicz, Łukasz Łach and Małgorzata Suska-Malawska. (2020). Limited Microcystin, Anatoxin and Cylindrospermopsin Production by Cyanobacteria from Microbial Mats in Cold Deserts, Toxins 12(4):244,

34. Newcombe G., Nicholson B., (2004). Water treatment options for dissolved cyanotoxins. Journal of Water Supply: Research and Technology-Aqua 53: 227–239.

35. Qin B., Zhu G., Gao G., Zhang Y., Li W., Paerl H.W., Carmichael W.W. (2010). A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. J. Environ. Manage, 45, 105–112.

36. Rocio A-R, Angeline T., Frank M. B., Frances R.P., Jeromy H-vie, Lioudmila S., (2005). Pressurized Liquid Extraction of Toxins fromCyanobacterial Cells, Environmental Toxicology, 2005.

37. Wael M. I., Emad H. S., Yahia A. and Abdel-Hamid M., (2015). Monitoring and removal of cyanobacterial toxins from drinking water by algal-activated carbon Toxicology and Industrial Health 2016, 32(10) 1752–1762.

38. Walsby A. E., Schanz F., (2002). Light-dependent growth rate determines changes in the population of Planktothrix rubescens over the annual cycle in Lake Zürich, Switzerland. New Phytol. 154, 671-687.

39. World Health Organization (WHO), (2017). Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum; WHO: Geneva, Switzerland.

40. Yasmin R., Kiran A. and Muhammad K., (2019). Removal of microcystin-LR from aqueous solution using Moringa oleifera Lam. Seeds, water science and technology, 79.1, Water Science & Technology | 79.1 | 2019.

41. Yin L., Huang J., Huang W., Li D., Wang G., Liu Y., (2005). Microcystin-RR-induced accumulation of reactive oxygen species and alteration of antioxidant systems in tobacco BY-2 cells. Toxicon, 46, 507-512.

42. Zahanggir A., Niamul B., Sayera K., (2022). Statistical optimization of Methylene Blue dye removal from a synthetic textile wastewater using indigenous adsorbents, Environmental and Sustainability Indicators 14, 100176.