

Removal of cyanotoxins in drinking water using ozone and ozone-hydrogen peroxide (peroxone)

Guhankumar Ponnusamy, Lijo Francis, Kavithaa Loganathan, Oluwaseun O. Ogunbiyi, Saad Jasim and Jayaprakash Saththasivam

ABSTRACT

Presence of cyanotoxins in drinking water poses a great risk to public health. Elevated levels of cyanotoxins in drinking water can lead to acute gastroenteritis, liver diseases, and neurotoxicity. In this study, drinking water samples were collected across the eastern part of Qatar and screened using a rapid assay to detect the presence of microcystins and nodularins. The results showed that the toxin concentrations in all the water samples were below the WHO prescribed limit of 1 µg/L. Considering a worst-case scenario, toxin removal efficiencies were evaluated using ozone and ozone-hydrogen peroxide by spiking drinking water samples with microcystin-LR (MC-LR) at different oxidant dosages, toxin concentrations, water temperatures, and total organic carbon. It was found that peroxone-treated water samples have better MC-LR removal efficiency than molecular ozone at lower oxidant dosages. Nevertheless, at higher oxidant dosages, both ozonation and peroxone oxidation methods showed a similar removal efficiency. The experimental results also clearly indicated that variation in water temperature between 22 °C and 35 °C has minimal effect on the removal efficiency in both the treatment methods. It was also confirmed that the presence of organic carbon has a more profound detrimental impact than water temperature for toxin removal.

Key words | advanced oxidation process, cyanotoxin, drinking water, enzyme-linked immunosorbent assay (ELISA), microcystin-LR, ozonation

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INTRODUCTION

The presence of harmful cyanotoxins such as hepatotoxins and neurotoxins in drinking water is a serious concern for human health. Microcystins (MCs) are one of the common yet highly potent hepatotoxins that are found in water bodies during algal blooms. MCs are monocyclic heptapeptides that are produced by species like *Microcystis aeruginosa*, *Microcystis* spp., *Anabaena*, *Planktothrix*, *Nostoc*, *Oscillatoria*, and *Anabaenopsis* (Van der Merwe 2014). In a recent study from Ethiopia (Tilahun *et al.* 2019), very high concentrations of MCs in the drinking water source was detected. Exposure to MCs via skin contact, inhalation, or ingestion of toxin-contaminated water sources can lead to breathing problems, nausea, diarrhea, skin

irritation, and even acute liver damage at a high level of exposure (Hunter 1998). The cyclic structure of MCs renders them chemically stable in water and heat resistant (Lawton & Robertson 1999), hence boiling of toxin-contaminated water prior to drinking is ineffective in degrading MCs. In addition, MCs are difficult to physically detect in water due to their colorless, odorless, and tasteless characteristics.

Complete removal of cyanotoxin could be a challenging process for conventional water treatment plants as there is a possibility of dissolved extracellular toxin passing through the treatment process (Kull *et al.* 2006). The potential regrowth of toxin-producing cyanobacteria in distribution pipelines and storage tanks also cannot be ruled out. One

of the effective methods to remove cyanotoxins from drinking water is by using ozone (O₃) and ozone-hydrogen peroxide (peroxone). Ozone has an oxidation potential of 2.07 V and is widely used as an effective oxidant and disinfectant in water treatment industries (Jasim & Saththasivam 2017). The efficiency of ozone treatment process largely depends on the source water pH, contact time, and other constituents present in water such as dissolved organic carbon, iron, and manganese. The oxidation strength of a conventional ozonation system can be further enhanced when hydrogen peroxide is used in conjunction with ozone to promote the formation of free hydroxyl radicals ($\cdot\text{OH}$), which is a superior oxidant when compared to molecular ozone alone (Bourgin *et al.* 2017). Several studies were conducted in the past to evaluate the efficiency of ozone and peroxone in removing cyanotoxins from various water sources. Rositano *et al.* (1998) reported that 0.2 mg/L of ozone with a mere contact time of 15 seconds was sufficient to remove 1 mg/L of microcystin-LR (MC-LR). Similar removal efficiency was observed when peroxone at a H₂O₂/O₃ ratio of 0.5 was used. An ozonation study conducted by Hall *et al.* (2000) to evaluate the removal efficiency of MC-LR in river water reported that 2 mg/L ozone dosage was sufficient to remove both the intra- and extracellular toxin. Another study that evaluated the efficiency of ozonation in removing MC-LR from lake water indicated that approximately 95% of oxidation efficiency can be achieved at ozone dosage of 0.25 mg/L (Rodríguez *et al.* 2007). Al Momani *et al.* (2008) claimed that ozone dosage of 2.4 mg/L was required to completely degrade 5 mg/L of MC-LR. At a lower MC-LR concentration of 1 mg/L, an ozone dosage of 0.6 mg/L was required to eliminate the toxin within 90 seconds of reaction time. For peroxone-related experiments at initial ozone and MC-LR concentrations of 0.1 mg/L and 1 mg/L, respectively, the study reported that the toxin removal efficiency improved from 65% to 100% when H₂O₂ concentration was increased from 0.001 to 0.01 mg/L. A study by Miao *et al.* (2010) showed that at a high initial MC-LR concentration of 50 mg/L, the toxin removal efficiency increased from 29.5% to 92.1% when ozone dosage to MC-LR ratio was increased from 1 to 6, respectively.

It has to be emphasized that most of the reported MC-LR removal studies using ozone and peroxone were

conducted at a very high MC-LR concentration (over 1 mg/L). The presence of such a high toxin level in water is rare, even during the bloom period. Turner *et al.* (2018) reported that 122 of the total 137 water samples analyzed for MCs in cyanobacterial blooms from freshwater bodies was less than 50 µg/L. A summary by the Michigan Department of Environmental Quality (MDEQ) that reviewed several studies concluded that MCs' level in the 232 Michigan inland lakes from 2002 to 2012 did not exceed 4 µg/L (Birbeck *et al.* 2019). Another study reported that MC-LR in the range of 0.7–1.3309 µg/L was detected in the water storage tanks of the urban area of Qatar (Chatziefthimiou *et al.* 2016). As higher toxin concentration in water requires higher oxidant dosage for complete removal, it is important to conduct experimental studies at an appropriate toxin concentration in order to accurately determine the oxidant dosing range. All the experiments in this study were conducted at lower initial MC-LR concentrations, ranging between 3 µg/L and 50 µg/L. This range was chosen to approximate the concentration of MC-LR that could exist in treated water. Munoz *et al.* (2019) treated real surface water and observed a drop in cyanotoxin removal rate due to the presence of natural organics when compared with toxins spiked in deionized water. In addition, to the best of our knowledge, there are very limited experimental studies that investigate the combined effect of high water temperature and dissolved organic carbon in drinking water using ozone and peroxone. This specific scenario is particularly important for a hot and arid country like Qatar, where the water temperature can easily go beyond 35 °C during the summer months. The following questions were the key motives behind this study: (i) What is the MC-LR removal efficiency at varying ozone dosages and initial toxin concentrations? (ii) What is the effectiveness of MC-LR removal with peroxone process? (iii) How is MC-LR removal efficiency affected by water temperature and total organic carbon (TOC) considering the high water temperature during the summer season in Qatar? The first part of the study involved the collection of drinking water samples from various residential, public, and commercial sites in the eastern part of Qatar. The water samples were tested for basic water qualities such as pH, conductivity, TOC, and algal cells. In addition, the samples were also screened to assess and quantify the presence of specific

cyanotoxins, namely, microcystin and nodularin. A qualitative toxin-screening tool, enzyme-linked immunosorbent assay (ELISA), which detects the presence of toxins based on the Adda moiety, was used (Loganathan 2016). The second part of the study evaluated the effect of initial toxin concentrations, oxidant dosages, water temperature, and quality on the removal efficiency of MC-LR using ozone and peroxone.

MATERIALS AND METHODS

Drinking water sampling

Drinking water samples were collected from residential villas, public mosques, and commercial sites (shopping malls, cafeterias, and public water dispenser units) located in the eastern part of Qatar. Figure 1 shows the sampling

locations that include major areas such as Al Wakrah, Industrial Area, Abu Hamour, Al Sadd, Ar-Rayyan, and Al-Gharrafa. Water samples were collected in amber bottles and were kept at 4 °C during transportation. All the samples were collected and analyzed within 24 hours of collection. Conductivity, pH, and turbidity of the water samples were measured using portable meters (HACH CDC401, HACH PHC201, and HACH 2100Q, USA). The TOC content and chlorophyll cell counts in the water samples were measured using TOC analyzer (SHIMADZU TOC-L, Japan) and flow cytometer (BD ACCURI C6, USA).

Enzyme-linked immunosorbent assay (ELISA)

ELISA method was adopted for the rapid detection of toxins in the drinking water samples (Agrawal *et al.* 2012). The Microcystins-Adda ELISA Microtiter Plate was purchased



Figure 1 | Locations of the drinking water samples collected in the eastern part of Qatar.

from ABRAXIS, USA and the calibration standard solutions (0, 0.15, 0.40, 1.0, 2.0, and 5.0 µg/L) and a positive control sample (0.75 ± 0.185 µg/L) provided along with the kit were used for all measurements. In addition to extracellular toxins, cell-bound toxins were also measured followed by the cell lysis procedure using QuickLyse kit purchased from ABRAXIS, USA (Chorus 2012). All the calibration standards, positive controls used, and the samples were assayed in duplicate, and results were reported as mean values. Toxins that present in the samples will bind to the antibodies developed against Adda moiety which is similarly found in all the MC variants and nodularin. Absorbance was measured at 450 nm using an absorbance microplate reader (BioTek ELx800UV, USA).

Analytical methods

Microcystin-LR (MC-LR – C₄₉H₇₄N₁₀O₁₂) analytical standard purchased from ABRAXIS was used for UHPLC-PDA calibration and quantification. UHPLC (Thermo Scientific Dionex UltiMate 3000, USA) equipped with 1.9 µm; 100 × 2.1 mm C18 UHPLC Column (Sigma Aldrich, Hypersil GOLD™, USA) was used for the calibration and quantification. The UHPLC operating conditions are provided in Table 1.

Based on the calibration points used (0.01–0.5 mg/L), the calculated limit of detection (LOD) was 9.7 µg/L and the limit of quantitation (LOQ) was 29.4 µg/L. The treated water samples after the oxidation experiments were concentrated up to 500 times using a solid-phase extraction (SPE)

instrument (Dionex™ AutoTrace™ 280, USA) prior to UHPLC analyses. Hydrophilic polymer phase SPE cartridge (Sigma Aldrich, Supel™ Select HLB 6 mL/200 mg, USA) was used for concentration. The cartridge was conditioned using 4 mL HPLC grade methanol followed by 8 mL deionized water prior to sample loading, where 500 mL of water sample was passed through the cartridge at a flow rate of 10 mL/min. The cartridge was then dried for 15 min using nitrogen gas and subsequently soaked and eluted with 9 mL of methanol at a flow rate of 1 mL/min. Finally, the eluent was concentrated by evaporating to dryness under a gentle stream of nitrogen gas and re-suspended using 1 mL of 20% methanol prior to UHPLC analysis.

Ozone and peroxone experimental studies

Ozone was produced using an oxygen-fed 4 g/hr corona discharge ozone generator (BMT 802 N, Germany). The high concentration ozone gas was then bubbled and dissolved in a glass reactor that was pre-filled with 1 liter of deionized water. The reactor temperature was maintained at 5 °C using chilled water jackets to maximize ozone dissolution. After 1 hour of bubbling, dissolved ozone stock concentration in the reactor was measured spectrophotometrically (258 nm, ε_{O₃} = 3,000 M⁻¹ cm⁻¹). Once a stable dissolved ozone stock concentration was achieved in the glass chamber, an appropriate volume of the dissolved ozone was transferred to aliquots of drinking water samples as per the required ozone dosages. For peroxone studies, hydrogen peroxide was added and dispersed uniformly prior to ozone dosage. After the addition of oxidants, the water samples were stirred momentarily and allowed to react with the oxidants for 5 minutes. Ozone residuals in the treated water were measured using the indigo-trisulfonate method (Bader & Hoigné 1981) and quenched by purging the samples using nitrogen prior to SPE and UHPLC analyses.

Drinking water samples collected from two of the sampling locations were spiked with MC-LR and used for oxidation efficiency studies. These samples (Sample ID: 15 and 26) were chosen in order to study the effect of low and high TOC on the oxidation efficiency, respectively. The TOC values of the chosen samples were 0.58 and 5.42 mg/L, respectively. The low TOC drinking water samples were used to investigate the effect of different initial MC-LR

Table 1 | UHPLC operating condition

Parameters	Conditions
Solvent A	Acetonitrile (≥99.93% purity, HPLC grade)
Solvent B	Water with 0.05% H ₃ PO ₄
Injection volume (µL)	10
Run time (min)	17
Flow rate (mL/min)	0.3
Temperature (°C)	40
Wavelength (nm)	238
Gradient	0 to 1 min – 85% B, 1 to 10 min – 40% B, 10 to 15 min – 85% B followed by a stable 85% B for 2 min

concentration (3, 5, 20, and 50 µg/L) at a fixed ozone dosage of 0.1 mg/L. This study was then followed by varying the ozone and peroxone dosages from 0.1 mg/L O₃ to 0.75 mg/L O₃ at a fixed MC-LR concentration of 50 µg/L. The hydrogen peroxide dosages for peroxone studies were maintained at H₂O₂/O₃ ratio of 0.25 throughout all the studies. For example, 1 mg/L peroxone dose is equivalent to 1 mg/L of O₃ and 0.25 mg/L of H₂O₂. Additional studies were conducted to investigate the effect of temperature on the oxidant efficiency in the low TOC drinking samples (Sample ID: 26) by conducting the experiments at 22 and 35 °C. The temperature of the water samples was regulated by placing the reactors in a temperature regulated bath. Two different oxidant dosages (0.5 mg/L and 0.75 mg/L) at an initial MC-LR concentration of 50 µg/L were used for this particular study. Another important experimental study that evaluated the combined effect of high DOC and high temperature on the MC-LR removal efficiency was conducted by spiking the drinking water samples (Sample ID: 15 and 26) at 50 µg/L of MC-LR and oxidant dosage of 0.5 mg/L.

RESULTS AND DISCUSSION

Characteristics of the drinking water samples

A total number of 35 samples collected from public and private locations were analyzed in this study. The pH values ranged from 7.12 to 7.75 while the electrical conductivity ranged from 118.6 µS/cm to 240.20 µS/cm (equivalent to total dissolved solids (TDS) levels of 77.1 mg/L–156.1 mg/L). The turbidity of the samples varied between 0.20 NTU and 0.41 NTU. TOC values of several samples exceeded the maximum requirement of 4.0 mg/L set by the local authority, Kahramaa, for water quality in distribution systems

(Al Naamaa 2014). TOC value as high as 6.10 mg/L was recorded for one of the collected water samples. Among the samples collected from the different mosques, there were six samples with higher TOC values than the guideline limit. Out of the six samples collected from the malls, cafeterias, and public water dispensers, four of the samples showed TOC values of 5.4 mg/L or higher. A summary of the measured basic water quality parameters of the samples collected against the local drinking water requirements are provided in Table 2.

Based on the ELISA analyses, it was found that the cyanotoxin concentrations (equivalent of all microcystin variants and nodularin) in the water samples collected from the mosques (Sample ID: 1 to 19) ranged from 0.01 µg/L to 0.45 µg/L. As for the samples collected from residential villas (Sample ID: 20 to 28), toxin levels of a minimum of 0.05 µg/L and a maximum of 0.33 µg/L were detected. A similar range of toxin concentrations were also measured for the samples collected from shopping malls (Sample ID: 29 to 31), cafeterias, and public water dispensing units (Sample ID: 32 to 35) where the levels vary between 0.03 µg/L and 0.28 µg/L. This study confirms that the concentration of the toxins (i.e., all the MC variants and nodularin) in all the drinking water samples were below the 1 µg/L MC-LR limit set by the World Health Organization (WHO) (Roegner *et al.* 2014). It can be confirmed that the drinking water supplies in the above-sampled areas are free from the two potent hepatotoxic cyanotoxins. The overall results for the basic water quality parameters and cyanotoxins are provided in Table 3.

Degradation of MC-LR using ozone and peroxone

The MC-LR removal efficiency at different initial toxin concentrations at a fixed ozone dosage of 0.1 mg/L O₃ is shown

Table 2 | Summary of drinking water quality results against Qatar requirements

Parameters	KAHRAMAA requirements in distribution system	Mosque		Villa		Others	
		Min	Max	Min	Max	Min	Max
pH	6.5–8.5	7.12	7.72	7.26	7.75	7.26	7.69
Conductivity (µS/cm)	150–500	118.6	240.2	167.9	210.9	178.5	235.3
Turbidity (NTU)	<4.0	0.23	0.39	0.24	0.41	0.20	0.35
TOC (mg/L)	<4.0	0.01	5.91	0.03	4.44	0.05	6.12

Table 3 | Water quality results of the collected samples

Sample ID	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Turbidity (NTU)	TOC mg/L	Chlorophyll cell/ μL	Toxin concentration ($\mu\text{g}/\text{L}$)	Location
1	7.12	178.8	0.3	0.92	<1	0.14	Mosque
2	7.25	180.0	0.28	0.32	<1	0.03	Mosque
3	7.62	204.7	0.3	1.23	<1	0.10	Mosque
4	7.58	187.9	0.39	0.97	<1	0.45	Mosque
5	7.49	202.7	0.29	0.83	<1	0.10	Mosque
6	7.36	240.2	0.32	0.63	<1	0.01	Mosque
7	7.19	179.5	0.29	0.76	<1	0.14	Mosque
8	7.51	171.7	0.35	1.75	<1	0.19	Mosque
9	7.61	184.7	0.29	0.01	<1	0.16	Mosque
10	7.33	201.5	0.35	5.09	<1	0.29	Mosque
11	7.28	182.0	0.28	5.91	<1	0.10	Mosque
12	7.54	230.5	0.32	0.68	<1	0.26	Mosque
13	7.71	175.0	0.25	0.17	<1	0.14	Mosque
14	7.69	135.2	0.34	4.86	<1	0.32	Mosque
15	7.72	177.1	0.32	5.42	<1	0.04	Mosque
16	7.49	122.4	0.27	4.47	<1	0.17	Mosque
17	7.52	118.6	0.23	0.61	<1	0.26	Mosque
18	7.48	174.5	0.31	0.42	<1	0.05	Mosque
19	7.52	118.8	0.29	5.37	<1	0.10	Mosque
20	7.40	181.8	0.29	1.12	<1	0.06	Residential villas
21	7.39	180.3	0.35	1.24	<1	0.23	Residential villas
22	7.28	202.0	0.24	1.15	<1	0.10	Residential villas
23	7.42	179.5	0.34	0.03	<1	0.33	Residential villas
24	7.26	178.1	0.32	1.04	<1	0.13	Residential villas
25	7.41	210.9	0.24	0.07	<1	BDL	Residential villas
26	7.74	175.6	0.41	0.58	<1	0.07	Residential villas
27	7.68	177.0	0.33	4.44	<1	0.05	Residential villas
28	7.75	167.9	0.35	0.13	<1	0.28	Residential villas
29	7.53	233.7	0.34	0.05	<1	0.19	Mall
30	7.42	231.4	0.2	6.12	<1	0.19	Mall
31	7.38	179.9	0.35	5.48	<1	0.03	Mall
32	7.69	235.3	0.28	5.58	<1	0.24	Cafeteria
33	7.41	207.6	0.31	5.8	<1	0.28	Water dispenser
34	7.26	208.2	0.31	0.08	<1	0.11	Water dispenser
35	7.37	178.5	0.31	0.11	<1	BDL	Water dispenser

BDL, below detection limit.

in Figure 2. It can be observed that MC-LR removal efficiency decreased from 74.1% to 66.6% as the toxin concentration in the water increased from 3 $\mu\text{g}/\text{L}$ to 50 $\mu\text{g}/\text{L}$. Ozone degrades MC-LR by primarily cleaving the

conjugated diene structure of the Adda group in MC-LR, as shown in Figure 3. The oxidation of the Adda moiety is important as it governs the toxicity of MC-LR (Sharma et al. 2012). Another destruction pathway of MC-LR during

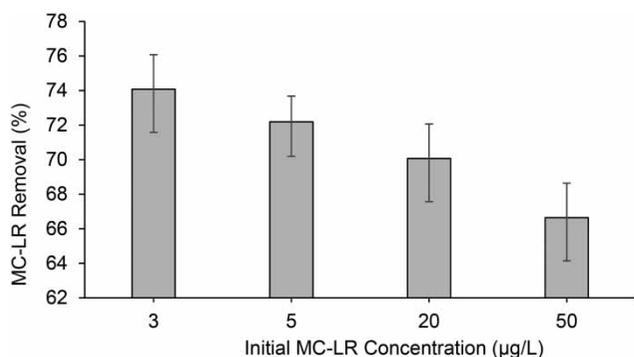


Figure 2 | Removal efficiency at varying initial MC-LR concentrations. Experimental conditions: pH, 7.3; MC-LR concentration, 3, 5, 20, and 50 µg/L; ozone dose, 0.1 mg/L; contact time, 5 min.

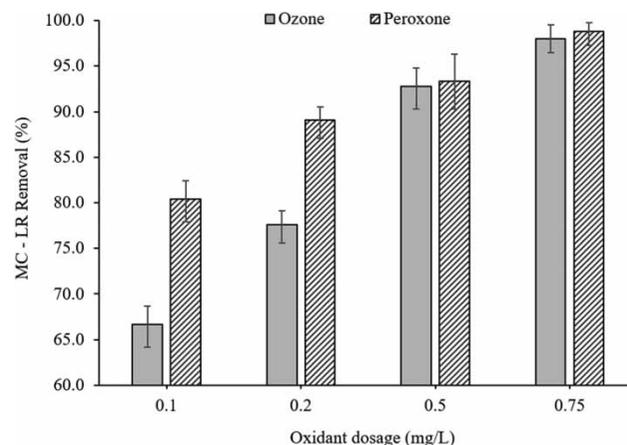


Figure 4 | Removal efficiency of MC-LR at different ozone and peroxone dosages. Experimental conditions: pH, 7.28; MC-LR concentration, 50 µg/L; oxidant dosage, 0.1, 0.2, 0.5, and 0.75 mg/L; contact time, 5 min.

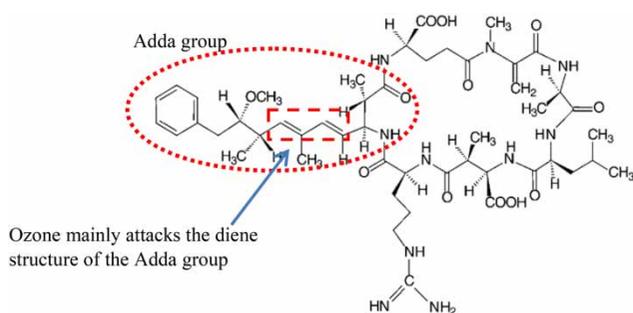


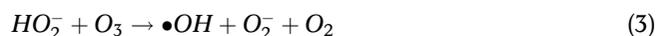
Figure 3 | Ozone oxidation of Adda group of MC-LR (figure adapted from Newcombe & Nicholson 2004).

ozonation is by fragmenting its Mdha-Ala peptide bond (Miao *et al.* 2010). Apart from the initial toxin concentration of 3 µg/L, ozone dosage of 0.1 mg/L was insufficient to oxidize the other higher MC-LR concentrations (e.g., 5, 20, and 50 µg/L) below the WHO guideline of 1 µg/L. The presence of other competing compounds such as organic carbon in the drinking water sample used in this study could contribute to the additional ozone demand and hence lead to the poor toxin removal efficiency at such a low ozone dosage of 0.1 mg/L. Similar observations were made by Rositano *et al.* (1998), who reported that 220 µg/L of pure MC-LR was degraded beyond the detection limit using ozone dosage of 0.2 mg/L while ozone dosage as high as 1.0 mg/L was required to remove a similar amount of toxin from an algal extract solution.

Additional studies were carried out to determine the required oxidant dosage for the removal of other higher initial MC-LR concentrations below the WHO limit. Figure 4 shows the MC-LR removal efficiency using ozone and peroxone at varying dosages from 0.1 mg/L to 0.75 mg/L. It can be seen

that the toxin degradation efficiency improved from 66.6% to over 98% when the ozone dosage was increased from 0.1 mg/L to 0.75 mg/L. As explained earlier, the oxidation of double bonds and peptide rings by ozone and hydroxyl radical leads to degradation of MC-LR. These results show that a minimum of 0.75 mg/L O₃ is required to remove the MC-LR in the tested water sample below the safe limit of 1 µg/L. The trend of our result is in agreement with another reported study that claimed that 0.5 mg/L of ozone was sufficient to completely degrade 10 µg/L MC-LR within a short contact time (Hoger *et al.* 2002).

It can be seen from Figure 4 that peroxone has better removal efficiency when compared to conventional ozonation at lower oxidant dosage. The addition of hydrogen peroxide accelerates the oxidation of MC-LR due to the formation of •OH, which is a stronger oxidant than ozone (Rodríguez *et al.* (2007). The following reactions (Equation (1)) to (Equation (3)) explain the ozone decomposition and •OH production and the overall reaction in a peroxone system is provided in Equation (4) (Deng & Zhao 2015):



The overall reaction of peroxone system is indicated below:



At a low peroxone dosage of 0.1 mg/L, the addition of hydrogen peroxide improved the MC-LR degradation efficiency to 80.4% when compared to a mere 66.6% of removal using conventional ozonation. A similar trend was also observed for 0.2 mg/L oxidant dosages where peroxone had better MC-LR removal efficiency than ozone. It is worth noting that the pH and TOC of the drinking water samples (Sample 1D: 26) used for this particular experiment was 7.74 and 0.58 mg/L, respectively. As ozone decomposes to form $\cdot\text{OH}$ at alkaline pH, the availability of molecular ozone for toxin oxidation is limited. In addition, the oxidation potential of ozone in alkaline water is lower than 2.07 V (under acidic conditions). These conditions could lead to lower oxidation efficiency of MC-LR. Although $\cdot\text{OH}$, a superior oxidant than molecular ozone, is expected to improve the toxin degradation via the indirect pathway at alkaline pH, the presence of other competing organics and scavengers such as organic carbon and bicarbonates could further contribute to the lower MC-LR removal efficiency. On the other hand, the addition of hydrogen peroxide in the peroxone process accelerates ozone decomposition rate promoting the formation of $\cdot\text{OH}$. The higher yield of $\cdot\text{OH}$ with a stronger oxidation potential of 2.8 V leads to better MC-LR removal efficiency even at lower oxidant dose. At higher dosage (e.g., 0.5 and 0.75 mg/L), it can be observed from Figure 4 that MC-LR removal efficiencies for both ozone and peroxone were more or less identical. A maximum removal efficiency of 98% and 98.8% was achieved for ozone and peroxone at oxidant dosage of 0.75 mg/L. These results are in agreement with the findings of Rositano *et al.* (1998), who reported that at higher oxidant dosages, both ozone and peroxone exhibited similar MC-LR removal efficiency.

The effect of water temperature on the MC-LR oxidation efficiency using ozone and peroxone is shown in Figure 5. The result shows that the toxin removal efficiency using ozone was unaffected when the water temperature was increased from 22 °C to 35 °C. As it is often speculated that ozone is inefficient at elevated water temperature due to high off-gas, poor solubility, and short half-life, it is important to understand the degradation kinetics of MC-LR is enhanced at high temperature if compared with ozone half-life. For the peroxone treatment, there is an improvement of 5% in the toxin removal efficacy at a dosage of

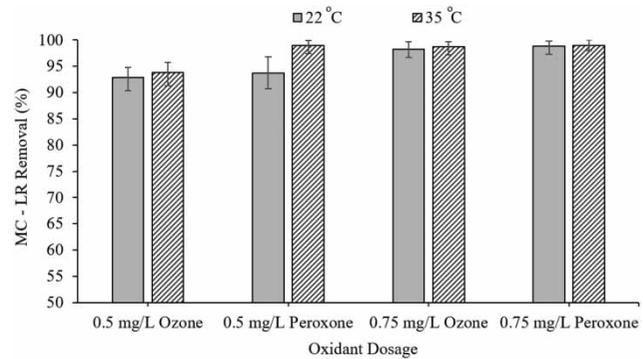


Figure 5 | MC-LR removal at different water temperatures. Experimental conditions: pH, 7.32; MC-LR concentration, 50 µg/L; oxidant dosage, 0.5 and 0.75 mg/L; contact time, 5 min. Temperature was maintained using a water bath.

0.5 mg/L while the removal efficiency remained the same at 0.75 mg/L peroxone dosage. In summary, the increase in water temperature either maintains or improves the MC-LR removal efficiency in all the case studies.

Figure 6 shows the MC-LR removal efficiency at two different temperatures and TOCs at an oxidant dosage of 0.5 mg/L and initial MC-LR concentration of 50 µg/L. It is obvious from Figure 6 that TOC content in the drinking water samples used in this study plays a detrimental role in the oxidation of MC-LR. The oxidation efficiency of the ozone and peroxone dropped drastically from 92.8% and 93.8% to 67.6% and 66.4%, respectively, due to the difference in the TOC of the two different samples. A similar response was observed when the oxidation experiments were carried out at a higher water temperature for the two drinking water samples. Data shown in the graph were provided with error bars and are statistically significant. This

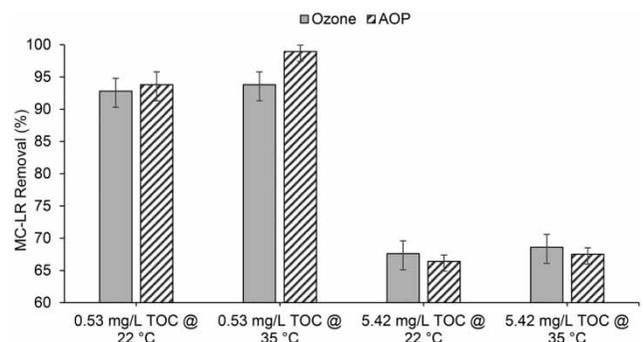


Figure 6 | MC-LR removal at different DOC and temperature. Experimental conditions: pH, 7.35; MC-LR concentration, 50 µg/L; oxidant dosage, 0.5 mg/L; contact time, 5 min. Temperature was maintained using water baths at 22 °C and 35 °C.

study clearly shows that organic carbon content in the water plays a more critical role than water temperature during the oxidation of MC-LR. The competitive reaction between the organic material and MC-LR increased the oxidant demand of the water and hence led to the poor toxin removal efficiency (Al Momani *et al.* 2008). Table 4 summarizes the results obtained in this research against published data related to the removal of MC-LR using ozone and peroxone.

Further evaluation of the oxidation by-products and understanding the toxicity of the transformation products using advanced characterization tools such as LC-QTOF MS would be necessary (Leon *et al.* 2019). This helps in

ensuring clean and safe water, which is of utmost importance to public health. Especially in the developing countries, providing contamination-free drinking water despite the global risks such as water scarcity, rising temperature, and harmful cyanobacteria bloom is of serious concern. The presence of cyanotoxin in drinking water is a major threat to humans as it could lead to various health issues such as gastroenteritis, liver damage, and neurotoxicity. Hence, monitoring of cyanotoxins in drinking water at appropriate intervals using rapid screening tests such as ELISA is important to ensure a safe supply of drinking water. In addition, one of the feasible ways of degrading cyanotoxins is by using ozone and other advanced oxidation

Table 4 | Comparison study of the MC-LR removal using ozone and peroxone

Oxidant	Oxidant dose (mg/L)	Initial MC-LR Conc. (mg/L)	Treatment conditions	Removal efficiency (%)	Reference
Ozone	0.2	0.166	Medium: Pure microcystin in water; Reaction time: 4 min	100	Rositano <i>et al.</i> (1998)
Ozone	0.2	0.22	Medium: Microcystin in algal extract water; Reaction time: 4 min	100	Rositano <i>et al.</i> (1998)
Ozone	2.0	0.0035–0.0086	Medium: Raw lowland river water	100	Hall <i>et al.</i> (2000)
Ozone	0.25	1.0	Medium: Lake water; pH = 8; DOC = 3.6 mg/L	95	Rodríguez <i>et al.</i> (2007)
Ozone	0.6	1.0	Medium: Water spiked with 2 mg/LNOM (humic acid); pH = 7; Temperature = 20 °C; Reaction time = 90 s	75	Al Momani <i>et al.</i> (2008)
Ozone	0.1	0.003	Medium: Purified toxin spiked in drinking water; pH = 7.74; Reaction time = 5 min; Temperature = 25 °C; DOC = 0.58 mg/L	74.1	Current study
Ozone	0.1	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.74; Reaction time = 5 min; Temperature = 25 °C; DOC = 0.58 mg/L	66.6	Current study
Peroxone	0.1 O ₃ + 0.025 H ₂ O ₂	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.74; Reaction time = 5 min; Temperature = 25 °C; DOC = 0.58 mg/L	80.4	Current study
Ozone	0.75	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.74; Reaction time = 5 min; Temperature = 25 °C; DOC = 0.58 mg/L	98	Current study
Peroxone	0.75 O ₃ + 0.188 H ₂ O ₂	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.74; Reaction time = 5 min; Temperature = 25 °C; DOC = 0.58 mg/L	98.8	Current study
Ozone	0.5	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.72; Reaction time = 5 min; Temperature = 25 °C; DOC = 5.42 mg/L	67.6	Current study
Peroxone	0.50 O ₃ + 0.125 H ₂ O ₂	0.05	Medium: Purified toxin spiked in drinking water; pH = 7.72; Reaction time = 5 min; Temperature = 25 °C; DOC = 5.42 mg/L	66.4	Current study

processes such as peroxone. Considering the negative impacts of algal bloom and based on the source of water, finished drinking water from conventional treatment plants can be treated further using peroxone, an advanced oxidation process to ensure it is cyanotoxin-free before supply.

CONCLUSION

The cyanotoxin analyses of our study using ELISA test kits on the 35 drinking water samples that were collected from various public locations such as malls, cafeterias, and private residential villas showed that the toxin levels in all the samples were below the WHO limit. Our experimental results using ozone and peroxone to remove MC-LR in drinking water showed that oxidant dosages are governed by the initial toxin concentrations and organic carbon content in water samples. Higher TOC values led to poor removal of MC-LR due to competition kinetics between the toxin and organic carbon during the oxidation process. As toxin concentration in real cases exists at extremely low concentration, an ozone dose of 0.75 mg/L would be sufficient to degrade MC-LR. Although ozone and peroxone are capable of removing MC-LR below the regulation limit, the formation of by-products during the oxidation process is a concern and requires a human risk assessment. In addition, the presence of bromide in drinking water is also a major concern as it could lead to the formation of bromate, a suspected human carcinogen.

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REFERENCES

Agrawal, M., Yadav, S., Patel, C., Raipuria, N. & Agrawal, M. K. 2012 Bioassay methods to identify the presence of cyanotoxins in drinking water supplies and their removal

- strategies. *European Journal of Experimental Biology* **2** (2), 321–336.
- Al Momani, F., Smith, D. W. & Gamal El-Din, M. 2008 Degradation of cyanobacteria toxin by advanced oxidation processes. *Journal of Hazardous Materials* **150** (2), 238–249. <https://doi.org/10.1016/j.jhazmat.2007.04.087>.
- Al Naamaa, A. R. 2014 *Overview on Kahraamaa Drinking Water Quality Requirements*. KAHRAMAA Publications, Qatar.
- Bader, H. & Hoigné, J. 1981 Determination of ozone in water by the indigo method. *Water Research* **15** (4), 449–456. [https://doi.org/10.1016/0043-1354\(81\)90054-3](https://doi.org/10.1016/0043-1354(81)90054-3).
- Birbeck, J. A., Westrick, J. A., O'Neill, G. M., Spies, B. & Szlag, D. C. 2019 Comparative analysis of microcystin prevalence in Michigan lakes by online concentration LC/MS/MS and ELISA. *Toxins* **11** (1), 13. <https://doi.org/10.3390/toxins11010013>.
- Bourgin, M., Borowska, E., Helbing, J., Hollender, J., Kaiser, H.-P., Kienle, C., McArdell, C. S., Simon, E. & von Gunten, U. 2017 Effect of operational and water quality parameters on conventional ozonation and the advanced oxidation process O₃/H₂O₂: kinetics of micropollutant abatement, transformation product and bromate formation in a surface water. *Water Research* **122**, 234–245. <https://doi.org/10.1016/j.watres.2017.05.018>.
- Chatziefthimiou, A. D., Metcalf, J. S., Glover, W. B., Banack, S. A., Dargham, S. R. & Richer, R. A. 2016 Cyanobacteria and cyanotoxins are present in drinking water impoundments and groundwater wells in desert environments. *Toxicon* **114**, 75–84. <https://doi.org/http://dx.doi.org/10.1016/j.toxicon.2016.02.016>.
- Chorus, I. 2012 *Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries*. Text 63/2012. Federal Environment Agency, Germany.
- Deng, Y. & Zhao, R. 2015 Advanced oxidation processes (AOPs) in wastewater treatment. *Current Pollution Reports* **1** (3), 167–176. <https://doi.org/10.1007/s40726-015-0015-z>.
- Hall, T., Hart, J., Croll, B. & Gregory, R. 2000 Laboratory-scale investigations of algal toxin removal by water treatment. *Water and Environment Journal* **14** (2), 143–149. <https://doi.org/10.1111/j.1747-6593.2000.tb00241.x>.
- Hoger, S., Dietrich, D. & Hitzfeld, B. 2002 Effect of ozonation in drinking water treatment on the removal of cyanobacterial toxins. *Environmental Health Perspectives* **110**, 1127–1132.
- Hunter, P. R. 1998 Cyanobacterial toxins and human health. *Journal of Applied Microbiology* **84** (S1), 35S–40S. <https://doi.org/10.1046/j.1365-2672.1998.0840s135S.x>.
- Jasim, S. Y. & Saththasivam, J. 2017 Advanced oxidation processes to remove cyanotoxins in water. *Desalination* **406**, 83–87. <https://doi.org/10.1016/j.desal.2016.06.051>.
- Kull, T. P. J., Sjövall, O. T., Tammenkoski, M. K., Backlund, P. H. & Meriluoto, J. A. O. 2006 Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: influence of natural organic matter. *Environmental Science & Technology* **40** (5), 1504–1510. <https://doi.org/10.1021/es051729g>.

- Lawton, L. A. & Robertson, P. K. J. 1999 Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. *Chemical Society Reviews* **28** (4), 217–224. <https://doi.org/10.1039/a805416i>.
- Leon, C., Boix, C., Beltran, E., Peneula, G., Lopez, F., Sancho, J. & Hernandez, F. 2019 Study of cyanotoxin degradation and evaluation of their transformation products in surface waters by LC-QTOF MS. *Chemosphere* **229**, 538–548. <https://doi.org/10.1016/j.chemosphere.2019.04.219>.
- Loganathan, K. 2016 Ozone-based advanced oxidation processes for the removal of harmful algal bloom (HAB) toxins: a review. *Desalination and Water Treatment* **59**, 65–71. <https://doi.org/10.5004/dwt.2016.0346>.
- Miao, H. F., Qin, F., Tao, G. J., Tao, W. Y. & Ruan, W. Q. 2010 Detoxification and degradation of microcystin-LR and -RR by ozonation. *Chemosphere* **79** (4), 355–361. <https://doi.org/10.1016/j.chemosphere.2010.02.024>.
- Munoz, M., Sandoval, J., Cires, S., Pedro, Z., Quesada, A. & Casas, J. 2019 Degradation of widespread cyanotoxins with high impact in drinking water (microcystins, cylindrospermopsin, anatoxin-a and saxitoxin) by CWPO. *Water Research* **163**, 114853. <https://doi.org/10.1016/j.watres.2019.114853>.
- Newcombe, G. & Nicholson, B. 2004 Water treatment options for dissolved cyanotoxins. *Journal of Water Supply: Research and Technology-Aqua* **53** (4), 227–239. <https://doi.org/10.2166/aqua.2004.0019>.
- Rodríguez, E., Onstad, G. D., Kull, T. P. J., Metcalf, J. S., Acero, J. L. & Von Gunten, U. 2007 Oxidative elimination of cyanotoxins: comparison of ozone, chlorine, chlorine dioxide and permanganate. *Water Research* **41** (15), 3381–3393. <https://doi.org/10.1016/j.watres.2007.03.033>.
- Roegner, A. F., Brena, B., González-Sapienza, G. & Puschner, B. 2014 Microcystins in potable surface waters: toxic effects and removal strategies. *Journal of Applied Toxicology* **34** (5), 441–457. <https://doi.org/10.1002/jat.2920>.
- Rositano, J., Nicholson, B. C. & Pieronne, P. 1998 Destruction of cyanobacterial toxins by ozone. *Ozone: Science and Engineering* **20** (3), 223–238. <https://doi.org/10.1080/01919519808547273>.
- Sharma, V. K., Triantis, T. M., Antoniou, M. G., He, X., Pelaez, M., Han, C., Song, W., O'Shea, K. E., de la Cruz, A. A., Kaloudis, T., Hiskia, A. & Dionysiou, D. D. 2012 Destruction of microcystins by conventional and advanced oxidation processes: a review. *Separation and Purification Technology* **91**, 3–17. <https://doi.org/10.1016/j.seppur.2012.02.018>.
- Tilahun, S., Kifle, D., Zewde, T. W., Johansen, J. A., Demissie, T. B. & Hansen, J. H. 2019 Temporal dynamics of intra-and extra-cellular microcystins concentrations in Koka reservoir (Ethiopia): implications for public health risk. *Toxicon* **168**, 83–92. <https://doi.org/10.1016/j.toxicon.2019.06.217>.
- Turner, A. D., Dhanji-Rapkova, M., O'Neill, A., Coates, L., Lewis, A. & Lewis, K. 2018 Analysis of microcystins in cyanobacterial blooms from freshwater bodies in England. *Toxins* **10**, 1. <https://doi.org/10.3390/toxins10010039>.
- Van der Merwe, D. 2014 Freshwater cyanotoxins. In: *Biomarkers in Toxicology* (R. Gupta ed.). Elsevier, Amsterdam, The Netherlands, pp. 539–548.

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