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# The link between microcystin levels in groundwater and surface Nile water, and assessing their potential risk to human health



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# ABSTRACT

Although groundwater is an important source for drinking and irrigation water worldwide, particularly in arid countries, they have been paid little attention to their contamination with microcystins (MCs) compared to surface water. Our study is the fourth one reporting existence of MCs in groundwater due to surface-water and groundwater interaction. Dissolved MCs in groundwater were found with higher concentrations in summer (0.1 to 0.84  $\mu$ g L<sup>-1</sup>) than in winter (0–0.06  $\mu$ g L<sup>-1</sup>), in association with MCs detected in nearby surface Nile water. The chronic daily intake (CDI) of MCs for both adults and children (0–0.003  $\mu$ g kg<sup>-1</sup> body weight d<sup>-1</sup>) in groundwater were lower than the chronic reference dose (RfD, 0.003  $\mu$ g kg<sup>-1</sup> body weight d<sup>-1</sup>) during winter, with hazard quotient less than 1. Conversely, CDI values exceeded the reference dose during summer for both adults (0.005–0.024  $\mu$ g kg<sup>-1</sup> body weight d<sup>-1</sup>) and children (0.012–0.05  $\mu$ g kg<sup>-1</sup> body weight d<sup>-1</sup>), with hazard quotient greater than 1. This indicates that MCs concentrations in these groundwater wells might pose adverse health effects to both adults and children during summer, but not during winter. The study provides evidence for the risk of cyanotoxins in groundwater supplies used for drinking-water should be undertaken when cyanobacteria bloom events are noted in nearby surface waters.

# 1. Introduction

Freshwater sources have been experiencing eutrophication due to industrial and agricultural wastewater discharges that induce the formation of harmful cyanobacterial blooms (HCBs). Most cyanobacteria species produce potent toxins (called cyanotoxins) including hepatotoxins, neurotoxins and skin irritant toxins (Carmichael et al., 2001). These cyanotoxins have serious health risk and even death in animals and humans (Dittmann and Wiegand, 2006). MCs are the most common and widespread hepatotoxins in natural water, with over 270 variants have been identified so far (Bouaicha et al., 2019). MCs can be found in drinking-water (Mohamed and Al-Shehri, 2007) and may cause death from liver hemorrhage or liver failure upon acute exposure to high doses (Pouria et al., 1998). MC toxin was classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC, 2020). Therefore, World Health Organization (WHO) proposed guideline value of 1  $\mu g \, L^{-1}$  for MC-LR in drinking-water (WHO, 2017). Besides drinkingwater, MCs can also deteriorate the irrigation water quality and

negatively affect plant growth and yield, with potential accumulation in edible vegetable plants (McElhiney et al., 2001; Mohamed and Al Shehri, 2009). These accumulated toxins can be transferred to humans via consumption of contaminated edible plant parts (Campos et al., 2021). Although, MCs mainly reside inside cyanobacterial cells (Spoof et al., 2020), they can release to the surrounding water after cell lysis at the end of bloom events or algicide application, leading to high concentrations of extracellular/dissolved MCs (up to 180 µg L<sup>-1</sup>) in natural water (Wijewickrama and Manage, 2019).

Surface water and groundwater systems are connected and interrelated in most landscapes (Liu et al., 2016). Surface water may interact with nearby groundwater through groundwater inflow, seepage loss, or combination of the two processes (Winter et al., 1998). Groundwater inflow occurs when the stage of surface water body is lower than groundwater, while surface water would move towards groundwater if the surface water body stage is higher than groundwater (Abesh, 2019). Such physical interaction between surface water and groundwater could implicate in migration of pollutants across these

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# water resources (Kong et al., 2018).

Several hazardous compounds like pharmaceuticals and pesticides were found to enter groundwater from surface water due to lake-aquifer interaction (Wu et al. 2014; Lin et al., 2015). Most likely, MCs can seep into the groundwater from near lakes containing heavy HCBs (Yang et al., 2016). Lack of photodegradation and low redox conditions in the groundwater make chemicals such as MCs more stable and harder to be degraded (Wu et al., 2014). Therefore, there is a potential risk of MCs exposure for residents using groundwater for drinking and irrigation. While there are a large number of studies on MCs in surface water, information on MC concentration and distribution in groundwater near eutrophic lakes is still limited (Mohamed and Al Shehri, 2009; Tian et al., 2013; Yang et al., 2016). Egypt is an arid country with limited freshwater sources including Nile River and groundwater (Gaber et al., 2020). The major feeding sources of groundwater in Egypt are Nile River water, rainwater seepage and irrigation water. As the groundwater quality is greatly influenced by the type of water that feeds underground reservoirs (Elnashar, 2014), chemical contaminants (e.g., MCs) in surface water can leach into the groundwater and impair its use for drinking or irrigation purposes. Therefore, this study investigates for the first time the presence of MCs in groundwater in Egypt and arid countries in Africa, and to link its existence to nearby surface waters. Furthermore, the data of MCs concentrations obtained from study were used to estimate the potential risk of contaminated groundwater to human health.

#### 2. Materials and methods

#### 2.1. Study area and sampling

Our study was carried out in Sohag province, Upper Egypt, where cyanobacterial blooms and MCs are frequently detected in the Nile River water (Mohamed, 2016). The selected study area, represents a part of the Nile valley extending between latitude  $26^{\circ}05'58''$  to  $27^{\circ}00'00''$ N and longitude  $31^{\circ}10~8''$  to  $32^{\circ}1500''$ E (Fig. 1). Climatologically, the study area belongs to the arid belt of Egypt, where it is characterized by long and hot summer, warm winter, and scarce rainfall. Temperature varies from 49 °C in summer to 11 °C.

The Nile riverbed is sandy in the area of our study (Ghodeif et al., 2016), and the Quaternary aquifer in this area is formed from the Nile alluvial deposits consisting of graded sand and gravel with thin interbeds of clay (Megahed and Farrag, 2019). Surface water samples were collected from five sites in the Nile River at different towns, and groundwater samples were collected from three pumping wells closest to each River site (Fig. 1). Surface and groundwater samples were collected in triplicate using pre-sterilized glass bottles during the period of July–September 2019 (i.e., the time of cyanobacteria dominance). In winter, water samples were collected during the period of January–February 2019, as the conditions are not suitable for cyanobacterial growth and toxin production in surface Nile water. Water samples were kept at 4 °C until analysis.

# 2.2. Physico-chemical analysis

Temperature, pH and conductivity of water samples were measured in situ by multi-parametric probe (HI 991300 pH/EC/TDS/Temperature, HANNA, Italy). Nitrate and soluble phosphate concentrations were determined in filtered water samples according to standard methods (APHA, 1995).

#### 2.3. Identification of cyanobacteria water samples

To investigate cyanobacterial cells in surface and groundwater samples, subsamples of a known volume (500 mL) were preserved in 1% Lugol's solution. Cyanobacterial species in the fixed samples were identified according to Komárek and Anagnostidis (2005), and total cyanobacterial cells were enumerated using Sedgewick–Rafter counting chamber.

#### 2.4. Microcystin analysis in water samples

In our study, total MCs (intra- and extracellular toxins) were analyzed according to water quality monitoring programs proposed by Sangolkar et al. (2006). To determine dissolved MCs (i.e., extracellular MCs) in surface and groundwater samples, an aliquot of water sample



**Fig. 1.** Map showing location of sampling sites in the study area. Violet cones indicate sites of surface Nile water samples (S1-S5); blue circles indicate sites of groundwater samples from wells close to relevant Nile site (i.e., S1G1, S1G2 and S1G3 are sampling sites of groundwater wells related to the Nile River site 1(S1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was passed through GF/C filters to remove suspended solids and cyanobacterial cells. The filtrate was used for determination of dissolved MCs, while the filter with retained cells was used for determination of intracellular MCs. The filtrate (one liter) was applied onto C-18 cartridges to concentrate MCs, and MCs adsorbed on cartilages were then eluted with 80% methanol. The organic solvent was evaporated and the volume of the remaining aqueous solution was adjusted with sterilized distilled water to 1 mL. Dissolved MC concentration was then determined directly in the remaining aqueous fraction by enzyme-linked immunosorbent assay (ELISA) according to Carmichael and An (1999) using available ELISA kits for MCs purchased from Abraxis (Warminster, PA) with a detection limit of 0.15 ng  $mL^{-1}$ . Each test was made in triplicate. MCs within cyanobacterial cells in water samples were determined by extracting filters with retained cells in 80% methanol according to Carmichael and An (1999). Intracellular MCs were then detected in the aqueous fraction remained after evaporation of organic solvent by ELISA as described above. The final concentrations of MCs were calculated in relation to the original volume of water samples.

# 2.5. Risk assessment of microcystins in groundwater

To evaluate the safety of MCs in groundwater and their potential health risks for adults and children, chronic daily intake (CDI) was calculated using the following equation according to Funari and Testai (2008).

$$CDI = \frac{Cwx IR x EFxED}{BWx AT}$$
(1)

where, Cw is the concentration of dissolved MCs in groundwater ( $\mu$ g L<sup>-1</sup>);IR is the ingestion rate of water (2 L/day for adults, and1L/day for children);EF is the exposure frequency, drinking-water (365 days/year); ED is exposure duration (70 years);BW is the average body weight (70 kg for adults, and 15 kg for children); AT is the average time equal to ED multiplied by 365 days/year. The standard values of parameters applied in the above equation were used according to World Health Organization (WHO, 2017).The health risk assessment model recommended by United States Environmental Protection Agency (US EPA) was used to evaluate the non-carcinogenic health risk of MCs in groundwater. The non-carcinogenic risk was described using a hazard quotient (HQ) by the following formula:

$$HQ = \frac{CDI}{RfD}$$
(2)

where, RfD is the US EPA chronic reference dose for MCs (0.003  $\mu$ g kg<sup>-1</sup> body weight (bw)/d; Corbel et al., 2016). The HQ is usually used as a danger indicator: HQ > 1 indicates that the exposure is higher than the RfD, which is dangerous to the human health; HI  $\leq$ 1 indicates that the exposure level is lower than the RfD, which is unlikely to be harmful.

# 2.6. Statistical analysis

Differences in MC concentrations among different sites and groundwater wells were compared using one-way ANOVA (P < 0.05) using SPSS 18.0 software for Windows. Correlations between MC concentrations in surface water and groundwater were tested using Spearman rank correlation coefficients to verify that MCs in groundwater are leached from nearby surface Nile water.

#### 3. Results

# 3.1. Physico-chemical quality of surface and groundwater samples

As shown in Table 1, surface water samples exhibited slightly alkaline pH (7.4–8.7) with values insignificantly (P = 0.1-0.3) varied among different sites at the Nile River. Similarly, all groundwater samples were slightly alkaline (pH 7.3–8.1). Conductivity differed significantly (P =0.001–0. 0.004) between surface (136-220 µs cm<sup>-1</sup>) and groundwater samples (401-924 µs cm<sup>-1</sup>). Nitrate concentrations in surface groundwater samples were 0.3–1.2 mg L<sup>-1</sup> and 7.8–31.2 mg L<sup>-1</sup>, respectively. Phosphate concentrations were higher in surface water (1.4–1.9 mg L<sup>-1</sup>) than groundwater (0.02–0.6 mg L<sup>-1</sup>).

# 3.2. Cyanobacteria and total microcystins in surface and groundwater

Cyanonbacteria were found with high cell densities  $(1.7-3.1 \times 10^5 \text{cells L}^{-1})$  in surface water samples at different Nile sites during summer season (Table 2). The most dominant species in all sites were *Anabaena circinalis, Merismopedia glauca, Microcystis aeruginosa, Oscillatoria limnetica, Oscillatoria tenuis.* On the other hand, in winter season, cyanobacteria were present in surface Nile water with low cell densities  $(0.1-0.4 \times 10^5 \text{cells L}^{-1})$ , and dominated by *O. limnetica.* The total

Table 1

Limnological parameters of the sampling wells from Lake Chaohu watershed in September 2013.

| Samples | Distance from Nile River Site (Km) | Depth (m) | ЕС (µs cm <sup>-1</sup> ) рН |     | $NO_3$ -N (mg L <sup>-1</sup> ) |     | $PO_4$ -P (mg L <sup>-1</sup> ) |      |      |      |
|---------|------------------------------------|-----------|------------------------------|-----|---------------------------------|-----|---------------------------------|------|------|------|
|         |                                    |           | w                            | S   | W                               | S   | W                               | S    | W    | S    |
| SW1     | -                                  | _         | 172                          | 149 | 8.7                             | 8.5 | 1.1                             | 1.8  | 0.02 | 1.9  |
| GW1-S1  | 13.43                              | 42        | 497                          | 487 | 7.9                             | 7.7 | 25.6                            | 3.1  | 0.4  | 0.6  |
| GW2-S1  | 15.2                               | 41        | 516                          | 401 | 7.6                             | 7.5 | 31.2                            | 39   | 0.3  | 0.4  |
| GW3-S1  | 11.37                              | 34.5      | 842                          | 711 | 8                               | 7.9 | 23.4                            | 36   | 0.2  | 0.5  |
| SW2     | -                                  | -         | 201                          | 220 | 8.3                             | 8.1 | 1.2                             | 1.4  | 2.2  | 1.7  |
| GW1-S2  | 5.76                               | 15        | 449                          | 416 | 8                               | 7.9 | 7.8                             | 9.2  | 0.02 | 0.03 |
| GW2-S2  | 1.78                               | 17        | 603                          | 479 | 7.6                             | 7.4 | 11.6                            | 14   | 0.05 | 0.07 |
| GW3-S2  | 35.39                              | 36        | 541                          | 413 | 7.8                             | 7.5 | 9.5                             | 12   | 0.04 | 0.05 |
| SW3     | _                                  | -         | 155                          | 123 | 8.4                             | 8.2 | 1                               | 1.3  | 1.5  | 1.5  |
| GW1-S3  | 28.23                              | 31        | 566                          | 417 | 7.8                             | 7.6 | 19.2                            | 22   | 0.3  | 0.4  |
| GW2-S3  | 29.65                              | 48        | 589                          | 434 | 7.7                             | 7.5 | 18.1                            | 20.1 | 0.3  | 0.6  |
| GW3-S3  | 10.17                              | 36.5      | 519                          | 489 | 7.6                             | 7.3 | 15.3                            | 17.2 | 0.4  | 0.5  |
| SW4     | _                                  | -         | 161                          | 145 | 8.7                             | 7.9 | 0.4                             | 1.7  | 1.7  | 1.6  |
| GW1-S4  | 14.65                              | 31        | 499                          | 487 | 7.4                             | 7.2 | 10.6                            | 14   | 0.08 | 0.1  |
| GW2-S4  | 10.98                              | 11        | 924                          | 911 | 7.9                             | 7.5 | 14.4                            | 17.2 | 0.06 | 0.2  |
| GW3-S4  | 14.92                              | 47.0      | 541                          | 417 | 7.3                             | 7.1 | 17.2                            | 21   | 0.08 | 0.2  |
| SW5     | _                                  | -         | 166                          | 136 | 8.6                             | 8.4 | 0.3                             | 1.6  | 2.1  | 1.4  |
| GW1-S5  | 9.27                               | 36        | 612                          | 489 | 7.6                             | 7.4 | 18.1                            | 20   | 0.2  | 0.5  |
| GW2-S5  | 32.4                               | 45.0      | 806                          | 667 | 7.5                             | 7.3 | 13.5                            | 15.1 | 0.1  | 0.3  |
| GW3-S5  | 11.99                              | 29.5      | 516                          | 419 | 8.1                             | 7.7 | 15.2                            | 18.3 | 0.2  | 0.4  |

EC is electric conductivity; SW1, SW2, SW3, SW4, SW5, surface water samples from Nile sties 1,2,3,4 and 5, respectively. GW1, GW2 and GW3, groundwater samples from wells close to relevant Nile site.

# Table 2

Total cyanobacterial count (cells x10<sup>5</sup> L<sup>-1</sup>) and total microcystin concentrations in surface Nile River sites near groundwater wells in the present study.

| Site | Total count of cyanobacteria    |                                 | Total MCs (µg                   | g L <sup>-1</sup> )              | Dominant species        |  |  |  |
|------|---------------------------------|---------------------------------|---------------------------------|----------------------------------|-------------------------|--|--|--|
| _    | Winter                          | Summer                          | Winter                          | Summer                           | Winter                  | Summer   |  |  |
| SW1  | $\textbf{0.4} \pm \textbf{0.2}$ | $3.1\pm0.8$                     | $2.1\pm0.3$                     | $\textbf{23.2} \pm \textbf{1.1}$ | O. tenuis, O. limnetica | M. aeruginosa, O. tenuis, O. limnetica                             |  |  |
| SW2  | $\textbf{0.3}\pm\textbf{0.3}$   | $\textbf{2.2} \pm \textbf{0.6}$ | $\textbf{0.7}\pm\textbf{0.2}$   | $18.7 \pm 2.3$                   | O. limnetica            | A. circinalis, Mer. glauca, O. limnetica                           |  |  |
| SW3  | $0.1\pm0.2$                     | $1.7\pm0.3$                     | $0.8\pm0.1$                     | $14.6\pm2.4$                     | O. limnetica            | M. aeruginosa, C. minor, O. limnetica                              |  |  |
| SW4  | $\textbf{0.2}\pm\textbf{0.2}$   | $2.3\pm0.5$                     | $\textbf{0.8} \pm \textbf{0.1}$ | $16.8 \pm 2.1$                   | O. limnetica            | M. aeruginosa, Mer. glauca, O. tenuis, O. limnetica                |  |  |
| SW5  | $\textbf{0.1} \pm \textbf{0.1}$ | $\textbf{2.1} \pm \textbf{0.4}$ | $\textbf{0.9}\pm\textbf{0.1}$   | $20.7 \pm 2.7$                   | O. limnetica            | A. circinalis, M. aeruginosa, Mer. glauca, O. tenuis, O. limnetica |  |  |

A = Anabaena, M = Microcystis, Mer = Merismopedia, C = Chroococcus, O = Oscillatoria.

Each Value is the mean  $\pm$  SD (n = 3).



Fig. 2. Microcystin concentrations ( $\mu g L^{-1}$ ) in surface water and nearby groundwater wells at different sites of the Nile River.

cyanobacterial cell density in surface Nile water showed positive correlations with nitrate (r = 0.85), phosphate (r = 0.8), pH (r = 0.4) and conductivity (r = 0.15). Total MCs (i.e., intracellular plus extracellular dissolved MCs) in surface Nile water were detected with high concentrations in summer (15-23 µg L<sup>-1</sup>) and low concentrations in winter (0.7–2.1 µg L<sup>-1</sup>) (Table 2). These toxin concentrations correlated significantly with the total cyanobacterial cell density (r = 0.83) (Table 2). The results also showed significant correlations between MC concentrations and pH (r = 0.4), conductivity (r = 0.15), phosphate (r =0.5), and nitrate (r = 0.7). No cells of either cyanobacteria or other phytoplankton species were observed in all groundwater wells investigated (therefore data not shown). Hence, intracellular MCs were not accounted in groundwater, and MCs detected in groundwater samples were considered as extracellular dissolved toxins.

#### 3.3. Concentrations of dissolved microcystin in surface and groundwater

Concentrations of dissolved MCs (i.e., extracellular MCs released from cells into the surrounding water) in surface water varied significantly (P = 0.006) among the five Nile sampling sites (Fig. 2). These concentrations were lower in winter (0.03–0.06  $\mu$ g L<sup>-1</sup>) than in summer (1.1–1.9  $\mu$ g L<sup>-1</sup>), in association with total MCs detected at these sites (r = 0.9) (Table 2, Fig. 2). Dissolved MCs in surface Nile water also had positive correlations with pH (r = 0.8), conductivity (r = 0.1), phosphate (r = 0.6), and nitrate (r = 0.7). Dissolved MCs in groundwater wells were positively correlated (r = 0.8) with those detected in the nearby surface Nile water site (Fig. 2). This is manifested by the increase of dissolved MCs in groundwater (0.1–1.6  $\mu$ g L<sup>-1</sup>) when concentrations (1.1 to 1.9  $\mu$ g L<sup>-1</sup>) were high in surface Nile water in summer, and their decrease in winter (0–0.03  $\mu$ g L<sup>-1</sup>), coincident with their low concentrations  $(0.03-0.06 \ \mu g \ L^{-1})$  in Nile water (Table 2, Fig. 2). Additionally, our results revealed that concentrations of dissolved MCs in groundwater showed negative correlation (r = -0.97 to -0.89) with the depth of wells (Fig. 3). For instance, for groundwater wells near Nile site 4, the highest MC concentration (0.48  $\mu$ g L<sup>-1</sup>) was obtained in GW1-S4 well with a depth of 11 m, while the lowest (0.16  $\mu$ g L<sup>-1</sup>) was detected in GW3-S1 with a depth of 47 m. Furthermore, the results of present study

also showed a negative correlation between MCs concentrations in groundwater and the distance of the wells from the Nile River shore (r = -0.6 to -0.8) (Fig. 4). On the other hand, MCs in groundwaters showed weak correlations with pH (r = -0.4), conductivity (r = -0.2), phosphate (r = -0.01), and nitrate (r = 0.4).

#### 4. Discussion

# 4.1. Physico-chemical quality and cyanobacteria in surface and groundwater samples

The present study clearly revealed that surface Nile water and nearby groundwaters.

were slightly alkaline (pH 7.3-8.7), within the permissible limit of 6.5 to 8.5 drinking-water by WHO (2011). Conductivity of surface and groundwater samples was also close to the acceptable limit for drinkingwater (500  $\mu$ s cm<sup>-1</sup>). Nitrate concentrations in surface Nile water  $(0.3-1.2 \text{ mg L}^{-1})$  were below the WHO limit for drinking-water (10 mg  $L^{-1}$ ), but they exceeded this limit in groundwaters (7.8–31.2 mg  $L^{-1}$ ). Phosphate concentrations in surface and groundwater exceeded the WHO drinking-water limit (0.01 mg  $L^{-1}$ ). These conditions along with high temperatures might lead to the presence of cyanobacteria with high cell densities in surface water samples at different Nile sites during summer season. These results agree with the findings and facts deduced by several authors who stated that freshwater bodies enriched with high nutrients exhibit a shift towards the dominance of cyanobacteria when other factors such as high temperatures (15-30 °C), pH (6-9), calm stable water conditions and salinity of not more than 4 ppt are prevalent in the environment (Mohamed et al., 2015; Okello et al., 2010). Similar to the cell density of cyanobacteria, total MC concentrations in surface Nile water also correlated with physico-chemical parameters. These results are thus in agreement with those obtained by Oliver and Ganf (2000), which reported that high MC concentrations contained in cyanobacterial blooms were related to the abundance of nutrients and favorable conditions for cyanobacterial growth. In our study, cyanobacterial populations in surface Nile water were dominated by A. circinalis, Mer. glauca, M. aeruginosa, O. limnetica and O. tenuis. These



Fig. 3. The relationship between the depth of wells and concentrations of dissolved microcystins in groundwater at the study area.



Fig. 4. The relationship between the distance of the wells from the Nile River shore and concentrations of dissolved microcystins in groundwater at the study area.

species were previously identified in the same sites and found to produce MCs (Mohamed and Al-Shehri, 2010; Mohamed, 2016). Our results also revealed a significant correlation between total MC concentrations and the cell density of these species in surface Nile water. These toxin concentrations (14.6–23.2  $\mu$ g L<sup>-1</sup>) were higher in summer than winter  $(0.7-2.1 \ \mu g \ L^{-1})$ . This was due to the presence of cyanobacteria in surface Nile water with low cell densities (dominated by O. limnetica) in winter. Low cell concentrations of cyanobacteria are due to low temperature (<15 °C) in winter, which is below the optimal temperature (20-3 °C)) for the growth of many species (Lurling et al., 2013). Nevertheless, Oscillatoria can survive low temperature up to 7 °C (Chu et al., 2007). Such linear relationship between MC production and the cell density of dominant cyanobacterial species was previously observed in the Nile delta (Mohamed et al., 2015). On the other hand, our results demonstrated that all groundwater samples did not contain any cells of cyanobacteria or other phytoplankton species. This is in concordance with the results of Tian et al. (2013) that revealed the absence of phytoplankton in groundwater wells near Shaying River, northern China. This is reasonable finding, because such photosynthetic organisms cannot survive under dark condition in groundwater wells.

#### 4.2. Concentrations of dissolved microcystin in surface and groundwater

Dissolved MCs were detected in surface Nile water at all sites during the present study with higher concentrations in summer than winter. These toxin concentrations were associating with total MCs recorded in water samples of these sites. The high concentrations of dissolved MCs obtained during summer in the present study can be compared with those concentrations detected previously in the Nile Delta water  $(1.2-4.5 \ \mu g \ L^{-1})$  (Mohamed et al., 2015). Generally, MCs are well known to be contained within the cells and only released into surrounding water by cell lysis during senescence at the end of bloom or application of chemicals (Tsuji et al., 2001). In our study, the high concentrations of dissolved MCs recorded in surface water samples could be attributed to the natural cell lysis during senescence, as no algicide application is practiced in this area of Nile River.

The results of statistical analysis of correlation revealed a clear significant association between dissolved MCs in groundwater and nearby surface Nile waters. These results are consistent with those of other studies elsewhere in the world (Mohamed and Al Shehri, 2009; Tian et al., 2013; Yang et al., 2016). Concentrations of dissolved MCs in our groundwater wells (0.1–1.6  $\mu$ g L<sup>-1</sup>) can be compared with those found in groundwater wells (0.3–1.8 µg L<sup>-1</sup>) in Saudi Arabia (Mohamed and Al Shehri, 2009) and in groundwater wells (>0.1–1.1  $\mu$ g L<sup>-1</sup>) near Lake Chaohu, China (Yang et al., 2016). However, our concentrations are slightly lower than concentration detected in groundwater (0.02-0.45  $\mu$ g L<sup>-1</sup>) near Shaying River in China (Tian et al., 2013). The discrepancy in MC concentrations in groundwater between different studies may be due to the difference in lakebed and aquifer material that is the key parameter for lake-groundwater interaction and controls the movement of contaminates in lake water to groundwater (El-Zehairy et al., 2018). In this respect, we would refer to that the carboxyl groups in MC toxin, upon deprotonation, could bind with the clay mineral by ligand exchange and cation bridge (Pochodylo et al., 2016). This way, MCs can be adsorbed onto lake and aquifer sediments, and the strength of this sorption increases with the increase of the clay content in these sediments (Chen et al., 2006; Xue et al., 2020). Eynard et al. (2000) also reported that soil could not protect groundwater from MCs occurring in the rivers and lakes of Riga. The Nile riverbed is sandy in the area of our study (Sohag region) (Ghodeif et al., 2016), and the Quaternary aquifer in this area is mainly formed of sands, gravels with very low content of clay minerals (El Tahlawi et al., 2008). These geological characteristics facilitate the passage of MCs downward to the groundwater system. Previously, it has been found that groundwater wells close to the Nile River showed high concentrations of contaminants as a result of the seepage water from the Nile and return flow from irrigation to the aquifer (Abdelshafy et al., 2019). Collectively, taken that no rainfall is accounted in our study area, the Nile River is the only source of surface water, and the irrigation system relies on using groundwater rather than surface water, MCs detected in groundwater could be leached out from Surface Nile water.

Our study also demonstrated that concentrations of dissolved MCs in groundwater decreased with the well depth. This contradicts the results of Tian et al. (2013) and Yang et al. (2016), which demonstrated that well depth did not significantly affect MC concentrations in groundwater. This inconsistency between our results and Tian's and Yang's ones may be due to the difference of soil texture between such geologically different regions (i.e., Egypt & China). Previously, Townsend and Young (1995) reported that downward-moving contaminants would be decreased with increasing thickness of clay in the subsurface above the well screen. Therefore, the higher concentrations of MCs in shallower wells compared to lower concentrations in deeper wells could be due to the low clay in the unsaturated zone and the high clay in saturated zone, which retarded the downward transport of MCs. This supports the hypothesis that shallow groundwater is more susceptible to contaminants than deeper water (Nemcic-Jurec and Jazbec, 2017).

Furthermore, MCs concentrations in groundwater correlated negatively with the distance of the wells from the Nile River shore. These results agree with those obtained by Tian et al. (2013) and Yang et al. (2016), who observed marked association between MC concentration in the groundwater and the distance of the well from the lake shore, with high MCs detected in wells close to the lake shore and low MCs in the farther wells. This reflects that the MCs contamination of groundwater in this region is a point source of pollution (i.e., Nile River water). This finding also emerges the importance of drilling groundwater wells at a safe distance from an existing surface-water source.

#### 4.3. Potential risk of MCs in groundwater

The results of present study showed that concentrations of dissolved MCs in groundwater were below WHO limit (1  $\mu$ g L<sup>-1</sup>), with higher concentrations (0.1–0.84  $\mu$ g L<sup>-1</sup>) detected during summer season than in winter (0–0.06  $\mu$ g L<sup>-1</sup>). Taken that groundwater is used in the study area for drinking without treatment, these low MC concentrations might still represent a risk to human health through chronic daily exposure in drinking-water. Compared to USEPA chronic reference dose (RfD, 0.003  $\mu$ g kg<sup>-1</sup> bw d<sup>-1</sup>; Corbel et al., 2016), and the WHO TDI (0.04  $\mu$ g kg<sup>-1</sup> bw), which have been widely used for guidelines, our CDI values for both the adults and children exceeded these limits in summer (Table 3). Fortunately, these CDI values (0-0.003) did not surpass the acceptable limits during winter either for adults or children. Applying hazard quotient (HQ) for determining the MCs human health hazard, all HQ values for adults (0-0.67) and children (0-1) through exposure to MCcontaminated are lower than 1 (Table 3). This indicates that MC concentrations in our groundwater wells might not pose adverse health effects to adults or children during winter. Conversely, HQs of MCs in these groundwater wells were greater than 1 during summer for both adults (1.7-6.7) and children (4-16.7). This reflects that the probability for adverse health effects associated with exposure to such potent toxins in groundwater is high. Previous studies have reported that the CDI of MCs in groundwater exceeded WHO TDI value and cause potential

#### Table 3

Hazard quotient summary for an adult and a baby consuming raw water.

health risk on population who drink groundwater directly (Tian et al., 2013; Yang et al., 2016). Additionally, taken that groundwater is being used for irrigation at the Nile region, high concentrations of MCs in groundwater detected in our study during summer would affect plant growth and/or accumulate in edible vegetables, similar to cases reported elsewhere for plants irrigated with MC-containing water (Mohamed and Al Shehri, 2009; Corbel et al., 2016). Therefore, besides the exposure to MCs via drinking-water, the presence of MCs in groundwater would add another potential exposure route to human health through consumption of edible vegetables contaminated with these toxins. However, MC concentrations potentially accumulating in edible parts of vegetable plants irrigated with groundwater in the study area should be determined in a further study in order to assess the risk to human health via the food ingestion route.

# 5. Conclusions

The present study clearly revealed the presence of MCs in groundwater used by residents for drinking and irrigation purposes. MC concentrations in groundwater had a direct link with those concentrations in surface Nile water in the study area. MC levels in groundwater negatively correlated with the distance from the Nile River shore and the well depth. Our study, therefore, suggests that groundwater wells in the Nile valley should be drilled as far away as possible from the Nile shore, and to be deep enough to avoid MC contamination by water draining from the surface water. The results also showed that MC concentrations detected in groundwater during winter were below WHO limit for MC in drinking-water, while they surpassed the WHO limit in summer, with hazard quotient greater than 1. This indicates that MCs in groundwater at the study area represents a high risk to human health. As it is used for drinking and irrigation purposes without treatment, particularly in rural communities, groundwater close to surface water worldwide should be monitored regularly for the presence of MCs and other cyanotoxins.

#### Author contribution

ZA collected samples from surface and groundwater, determined physicochemical properties of water samples. MH and SA determined microcystin concentration in water samples. ZM tabulated data, and was a major contributor in writing the manuscript. All authors participated in analysis and interpretation of the data, read and approved the final manuscript.

| Groundwater wells | Potential risk for adults |        |        |        | Potential risk for children |        |        |        |  |
|-------------------|---------------------------|--------|--------|--------|-----------------------------|--------|--------|--------|--|
|                   | CDI                       |        | HQ     | HQ     |                             | CDI    |        | HQ     |  |
|                   | Winter                    | Summer | Winter | Summer | Winter                      | Summer | Winter | Summer |  |
| GW1-S1            | 0.002                     | 0.017  | 0.67   | 5.67   | 0.003                       | 0.04   | 1.33   | 13.33  |  |
| GW2-S1            | 0.0014                    | 0.019  | 0.47   | 6.33   | 0.003                       | 0.04   | 1      | 13.33  |  |
| GW3-S1            | 0.001                     | 0.024  | 0.33   | 8      | 0.002                       | 0.06   | 0.67   | 20     |  |
| GW1-S2            | 0.0006                    | 0.019  | 0.2    | 6.34   | 0.001                       | 0.05   | 0.33   | 16.67  |  |
| GW2-S2            | 0                         | 0.013  | 0      | 4.34   | 0                           | 0.03   | 0      | 10     |  |
| GW2-S2            | 0.0003                    | 0.018  | 0.1    | 6      | 0.001                       | 0.04   | 0.33   | 13.33  |  |
| GW1-S3            | 0.0009                    | 0.008  | 0.3    | 2.67   | 0.002                       | 0.018  | 0.67   | 6      |  |
| GW2-S3            | 0                         | 0.005  | 0      | 1.67   | 0                           | 0.012  | 0      | 4      |  |
| GW3-S3            | 0.0006                    | 0.007  | 0.2    | 2.33   | 0.001                       | 0.02   | 0.33   | 6.67   |  |
| GW1-S4            | 0.0009                    | 0.006  | 0.3    | 4.67   | 0.002                       | 0.014  | 0.67   | 4.67   |  |
| GW2-S4            | 0.0003                    | 0.014  | 0.1    | 2      | 0.001                       | 0.032  | 0.33   | 10.67  |  |
| GW3-S4            | 0                         | 0.005  | 0      | 1.67   | 0                           | 0.01   | 0      | 3.33   |  |
| GW1-S5            | 0.0003                    | 0.013  | 0.1    | 4.33   | 0.001                       | 0.03   | 0.33   | 10     |  |
| GW2-S5            | 0                         | 0.008  | 0      | 2.67   | 0                           | 0.02   | 0      | 6.67   |  |
| GW3-S5            | 0.0003                    | 0.02   | 0.1    | 6.67   | 0.001                       | 0.04   | 0.33   | 13.33  |  |

 $CDI = Chronic daily intake (0.003 \ \mu g \ kg^{-1} body \ weight); HG = Hazard quotient.$ 

# **Declaration of Competing Interest**

We the authors of the manuscript "entitled' The link between microcystin levels in groundwater and surface Nile water, and assessing their potential risk to human health; declare that there is no any kind of conflict of interest.

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#### References

- Abdelshafy, M., Saber, M., Abdelhaleem, A., Abdelrazek, S.M., Seleem, E.M., 2019. Hydrogeochemical processes and evaluation of groundwater aquifer at Sohag city, Egypt. Sci. Afr. 6, e00196 https://doi.org/10.1016/j.sciaf.2019.e00196.
- Abesh, B.F., 2019. Modeling and Understanding Groundwater Contamination Caused by Cyanotoxins from Harmful Algal Blooms in Lake Erie. Master's thesis. Bowling Green State University. http://rave.ohiolink.edu/etdc/view?acc\_num=bgsu1562953 927561716.
- APHA (American Public Health Association), 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. APHA, Washington, DC.
- Bouaicha, N., Miles, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins 11, 714. https://doi.org/10.3390/toxins11120714.
- Campos, A., Redouane, E.M., Freitas, M., Amaral, S., Azevedo, T., Loss, L., Máthé, C., Mohamed, Z.A., Oudra, B., Vasconcelos, V., 2021. Impacts of Microcystins on Morphological and Physiological Parameters of Agricultural Plants: A Review. Plants 10, 639. https://doi.org/10.3390/plants10040639.
- Carmichael, W.W., An, J., 1999. Using of enzyme linked immunosobent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of MCYST and nodularin. J. Nat. Toxins 7, 377–385. https://doi.org/10.1016/S0003-2670(02) 00588-3.
- Carmichael, W.W., Azevedo, S.M.F.O., An, J., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109, 663–668. https://doi.org/10.1289/ehp.01109663.
- Chen, W., Song, L.R., Gan, N.Q., Li, L., 2006. Sorption, degradation and mobility of microcystins in Chinese agriculture soils: risk assessment for groundwater protection. Environ. Pollut. 144, 752–758. https://doi.org/10.1016/j. envpol.2006.02.023.
- Chu, Z., Jin, X., Iwami, N., et al., 2007. The effect of temperature on growth characteristics and competitions of *Microcystis aeruginosa* and *Oscillatoria mougeotii* in a shallow, eutrophic lake simulator system. Hydrobiol. 581, 217–223. https://doi. org/10.1007/s10750-006-0506-4.
- Corbel, S., Mougin, C., Nélieu, S., Delarue, G., Bouaïcha, N., 2016. Evaluation of the transfer and the accumulation of microcystins in tomato (solanum Lycopersicum cultivar Micro tom) tissues using a cyanobacterial extract containing microcystins and the radiolabeled microcystin-LR (14 C-MC-LR). Sci. Total Environ. 54 (1052–1058), 1058. https://doi.org/10.1016/j.scitotenv.2015.10.004.
- Dittmann, E., Wiegand, C., 2006. Cyanobacterial toxins-occurrence, biosynthesis and impact on human affairs. Mol. Nutr. Food Res. 50, 7–17. https://doi.org/10.1002/ mnfr.200500162.
- El Tahlawi, M.R., Farrag, A.A., Ahmed, S.S., 2008. Groundwater of Egypt, an environmental overview. Environ. Geol. 55, 639–652. https://doi.org/10.1007/ s00254-007-1014-1.
- Elnashar, W.Y., 2014. Groundwater Management in Egypt. IOSR-J.M.C.E. 11, 69-78. DOI:https://doi.org/10.9790/1684-11446978.
- El-Zehairy, A.A., Lubczynski, M.W., Gurwin, J., 2018. Interactions of artificial lakes with groundwater applying an integrated MODFLOW solution. Hydrogeol. J. 26, 109–132.
- Eynard, F., Mez, K., Walther, J.L., 2000. Risk of cyanobacterial toxins in Riga waters (Latvia). Water Res. 34, 2979–2988. https://doi.org/10.1016/S0043-1354(00) 00042-7.
- Funari, E., Testai, E., 2008. Human health risk assessment related to cyanotoxins exposure. Crit. Rev.Toxicol. 38, 97–125. https://doi.org/10.1080/ 10408440701749454.
- Gaber, A., Mohamed, A.K., Elgalladi Abdelkareem, M.A., Beshr, A.M., Koch, M., 2020. Mapping the groundwater potentiality of West Qena area, Egypt, using integrated remote sensing and hydro-geophysical techniques. Remote Sens. 12, 1559. https:// doi.org/10.3390/rs12101559.
- Ghodeif, K., Grischek, T., Bartak, R., Wahaab, R., Herlitzius, J., 2016. Potential of river bank filtration (RBF) in Egypt. Environ. Earth Sci. 75, 1–13. https://doi.org/ 10.1007/s12665-016-5454-3.
- IARC, 2020. Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. World Health Organization; International Agency for Research on Cancer, Lyon, France.
- Komárek, J., Anagnostidís, K., 2005. Cyanoprokaryota, Süßwasserflora von Mitteleuropa, Gustav Fischer, Jena Stuttgart Lübeck Ulm.

- Kong, F., Song, J., Zhang, Y., Fu, G., Cheng, D., Zhang, G., Xue, Y., 2018. Surface Water-Groundwater Interaction in the Guanzhong Section of the Weihe River Basin (China).
- Lin, Y.C., Lai, W.W.P., Tung, H.H., Lin, A.Y.C., 2015. Occurrence of pharmaceuticals, hormones, and perfluorinated compounds in groundwater in Taiwan. Environ. Monit. Assess. 187, 256. https://doi.org/10.1007/s10661-015-4497-3.
- Liu, C., Liu, J., Wang, X.-S., Zheng, C., 2016. Analysis of groundwater-lake interaction by distributed temperature sensing in Badain Jaran Desert, Northwest China. Hydrol. Process. 30, 1330–1341. https://doi.org/10.1002/hyp.10705.
- Lurling, M., Eshetu, F., Faassen, E.J., Kosten, S., Huszar, V.L.M., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshw. Biol. 58, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x.
- McElhiney, J., Lawton, L.A., Leifert, C., 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. Toxicon 39, 1411–1420. https://doi.org/10.1016/S0041-0101(01)00100-3.
- Megahed, H.A., Farrag, A.E.H.A., 2019. Groundwater potentiality and evaluation in the Egyptian Nile Valley: case study from Assiut governorate using hydrochemical, bacteriological approach, and GIS techniques. Bull. Natl. Res. Cent. 43, 48. https:// doi.org/10.1186/s42269-019-0091-0.
- Mohamed, Z.A., 2016. Harmful cyanobacteria and their cyanotoxins in Egyptian fresh waters – state of knowledge and research needs. Afr. J. Aquat. Sci. 41, 361–368. https://doi.org/10.2989/16085914.2016.1219313.
- Mohamed, Z.A., Al Shehri, A.M., 2009. Microcystins in groundwater wells and their accumulation in vegetable plants irrigated with contaminated waters in Saudi Arabia. J. Hazard. Mater. 172, 310–315. https://doi.org/10.1016/j. jhazmat.2009.07.010.
- Mohamed, Z.A., Al-Shehri, A.M., 2007. Cyanobacteria and their toxins in treated-water storage reservoirs in Abha city, Saudi Arabia. Toxicon 50, 75-84. Doi:https://doi. org/10.1016/j.toxicon.2007.02.021.
- Mohamed, Z.A., Al-Shehri, A.M., 2010. Differential responses of epiphytic and planktonic toxic cyanobacteria to the allelopathic substances of the submerged macrophyte *Stratiotes aloides*. Int. Rev. Hydrobiol. 95, 224–234. https://doi.org/10.1002/ iroh.200911219.
- Mohamed, Z.A., Deyab, M.A., Abou-Dobara, M.I., Kamel, A., El-Raghi, W.M., 2015. Occurrence of cyanobacteria and microcystin toxins in raw and treated waters of the Nile River, Egypt: implication for water treatment and human health. Environ. Sci. Pollut. Res. 22, 11716–11727. https://doi.org/10.1007/s11356-015-4420-z.
- Okello, W., Portmann, C., Erhard, M., Gademann, K., Kurmayer, R., 2010. Occurrence of microcystin-producing cyanobacteria in Ugandan freshwater habitats. Environ. Toxicol. 25, 367–380. https://doi.org/10.1002/tox.20522.
- Oliver, R.L., Ganf, G.G., 2000. Freshwater blooms. In: Whitton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria. Kluwer Academic Publishers, The Netherlands, pp. 149–194.
- Pochodylo, A.L., Aoki, T.G., Aristilde, L., 2016. Adsorption mechanisms of microcystin variant conformations at water-mineral interfaces: a molecular modeling investigation. J. Colloid Interface Sci. 480, 166–174. https://doi.org/10.1016/j. jcis.2016.07.016.
- Pouria, S., de Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.T.S., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H., Codd, G.A., 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet 352, 21–26. https://doi.org/ 10.1016/s0140-6736(97)12285-1.
- Sangolkar, L.N., Maske, S.S., Chakrabarti, T., 2006. Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria. Water Res. 40, 3485–3496. https://doi.org/10.1016/j.watres.2006.08.010.
- Spoof, L., Jaakkola, S., Važić, T., Häggqvist, K., Terhi Kirkkala, T., Ventelä, A., Kirkkala, T., Svirčev, Z., Meriluoto, J., 2020. Elimination of cyanobacteria and microcystins in irrigation water—effects of hydrogen peroxide treatment. Environ. Sci. Pollut. Res. 27, 8638–8652. https://doi.org/10.1007/s11356-019-07476-x.
- Tian, D., Zheng, W., Wei, X., Sun, X., Liu, L., Chen, X., Wang, X., 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. https://doi.org/ 10.1016/j.chemosphere.2013.01.051.
- Townsend, M.A., Young, D.P., 1995. Factors affecting nitrate concentrations in ground water in Stafford County, Kansas. Curr. Res. Kansas Geol. 238, 1–9. Corpus ID: 36169602.
- Tsuji, K., Masui, H., Uemura, H., Mori, Y., Harada, K., 2001. Analysis of microcystins in sediments using MMPB method. Toxicon 39, 687–692. https://doi.org/10.1016/ S0041-0101(00)00196-3.
- WHO, 2011. Guidelines for Drinking-Water Quality. Recommendations, 4th edn. World Health Organization, Geneva.
- WHO, 2017. Guidelines for Drinking-Water Quality, 4th Edition, Incorporating the 1st Addendum. World Health Organization, Geneva.
- Wijewickrama, M.M., Manage, P.M., 2019. Accumulation of Microcystin-LR in Grains of Two Rice Varieties (Oryza sativa L.) and a Leafy Vegetable, Ipomoea aquatica. Toxins 11 (8), 432. https://doi.org/10.3390/toxins11080432.
- Winter, T.C., Harvey, J.W., Franke, O.L., Alley, W.M., 1998. Ground Water and Surface Water: A Single Resource. U.S. Geological Survey, Denver, Colo.
- Xue, Q., Steinman, A.D., Xie, L., Yao, L., Su, X., Cao, Q., Zhao, Y., Cai, Y., 2020. Seasonal variation and potential risk assessment of microcystins in the sediments of Lake

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Taihu, China. Environ. Pollut. 259, 113884. https://doi.org/10.1016/j. envpol.2019.113884.

Wu, B., Zheng, Y., Tian, Y., Wu, X., Yao, Y., Han, F., Liu, J., Zheng, C., 2014. Systematic assessment of the uncertainty in integrated surface water-groundwater modeling

based on the probabilistic collocation method. Water Resour. Res. 50, 5848–5865. https://doi.org/10.1002/2014WR015366.

Yang, Z., Kong, F., Zhang, M., 2016. Groundwater contamination by microcystin from toxic cyanobacteria blooms in Lake Chaohu, China. Environ. Monit. Assess. 188, 280. https://doi.org/10.1007/s10661-016-5289-0.