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# Microcystins in groundwater wells and their accumulation in vegetable plants irrigated with contaminated waters in Saudi Arabia

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ABSTRACT

The present study describes the presence of toxic cyanobacteria and microcystin (MCYST) concentrations in groundwater wells and tissues of vegetable plants irrigated with well waters in Asir region, southwest of Saudi Arabia. The results revealed the presence of cyanobacteria in all groundwater wells with a dominance of *Oscillatoria limentica*. This species was found to produce MCYSTs at a concentration of 336 $\mu g\,g^{-1}$ as determined by enzyme-linked immunosorbent assay (ELISA). HPLC chromatogram for the methanolic extract of this species showed one main peak corresponding to MCYST-YR. MCYSTs were also detected in well waters at concentrations (0.3–1.8 $\mu g\,L^{-1}$), exceeding the WHO guideline level (1 $\mu g\,L^{-1}$) in some wells. All vegetable plants collected during the present study were found to accumulate MCYSTs in their leaves and roots at concentrations ranged from 0.07 to 1.2 $\mu g\,g^{-1}$ fresh weight. The study suggests that ground waters and vegetable plants should be continuously monitored for the presence of MCYSTs to protect the public against the exposure to such potent hepatotoxins.



## 1. Introduction

Groundwater is an important resource which should be protected from contamination. Contamination of groundwater with microorganisms including cyanobacteria can occur from infiltration near rivers, lakes, ponds [1]. Rainfall events can also play a role in the migration of algae and their products into the groundwater [2]. Once algae or algal spores enter groundwater wells, they can find suitable conditions (e.g. sunlight and nutrients in the water) to germinate and grow. The contamination of groundwater with cyanobacteria can pose a serious threat to water quality because many of these cyanobacteria produce toxins posing a severe threat to animals and human [3,4]. Among various toxins produced by cyanobacteria, MCYSTs are one of the predominant notorious types [5,6]. The development of primary liver cancer in China was linked to long-term chronic exposure to these toxins [7]. In addition, animal and human deaths have been occurred upon drinking water contaminated with MCYSTs [8,9].

Besides the effect of MCYSTs on animals and human, irrigation water contaminated with MCYSTs may inhibit germination and plant growth causing crop failures [10]. MCYSTs are potent and specific inhibitors of protein phosphatases 1 and 2A (PP1 and PP2A) in both animals and higher plants [11]. Mcrocystins are known to affect a number of processes in plant tissues, and their presence in water used for irrigation may have considerable impact on the growth and development of crop plants [10,12]. Plants are able to take up cyanobacterial toxins in sufficient concentrations to induce morphological [10,13,14] and physiological changes [15,16] in plants. The exposure of edible crop plants to MCYSTs is also a concern for human health, as the toxins can accumulate in plant tissues [14,17–19] and might be transferred into the food chain.

Most regions of Saudi Arabia are arid or semi-arid and groundwater is main source for spray and surface irrigation. As groundwater wells in Saudi Arabia are usually open, they are exposed to stormwater loadings of nutrients and other contaminants including algal spores and akinetes. Otherwise, these groundwater wells can link rainwater puddles and lakes and thus cyanobacterial toxins can leach from surface water into the groundwater [20,21]. Therefore, the present study has undertaken the investigation of cyanobacteria and their toxins "particularly MCYSTs" in groundwater wells in Asir region, Saudi Arabia. In addition, the study aimed to detect MCYSTs possibly accumulated in edible vegetable plants irrigated with contaminated groundwaters.

## 2. Materials and methods

### 2.1. Collection of groundwater samples and vegetable plants

Ten groundwater wells located in Asir region, southwest of Saudi Arabia (18°10–20′N, 42–43°30′E) were selected for the present

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study. The wells are about 50–80 m in depth and 3 m in diameter, and are usually left open. They lie in proximity to rainwater puddles and ponds, and their waters are used for irrigation of vegetable and crop plants. Groundwater samples were collected from wells using pre-sterilized polyethylene bottles at 0.5 m depth during the period of April–June 2007. At the same time, water samples were also collected from rainwater puddles and ponds close to each well. Subsamples of a known volume of groundwaters were preserved in 1% Lugol's solution for microscopic observations. Meanwhile, specimens of whole vegetable plants including radish, arugula (rocket), lettuce, dill, parsley and cabbage were collected from areas close to these groundwater wells where their waters are used for the irrigation of these plants.

### 2.2. Physico-chemical analysis of groundwater

Temperature, conductivity, total dissolved solids (TDS) and pH were measured in the field using multi-parametric probe (WTW Digit 88 model). Nitrate, ammonia and soluble phosphate concentrations were determined in water samples after filtration according to standard methods [22].

### 2.3. Identification of algae and cyanobacteria in groundwater wells

Fixed phytoplankton species were identified according to Prescott [23] and enumerated using 25 mL sedimentation chambers for phytoplankton identification and cell density estimation according to Utermohl [24].

### 2.4. Isolation and culturing of groundwater cyanobacteria

Single species of cyanobacteria were isolated from groundwater samples by streaking-plate method using BG-11 medium [25]. To obtain large biomasses of cyanobacterial species, the single species were grown axenically in 250 mL Erlenmeyer flasks containing 150 mL liquid BG-11 medium at $25 \pm 2\,^{\circ}C$ and irradiance of $24\,\mu mol\,m^{-2}\,s^{-1}$ for 3 weeks.

### 2.5. MCYST analysis in cyanobateria and groundwaters

MCYSTs in pure cultures of isolated species of cyanobacteria and algal samples collected from groundwater wells during the present study, were extracted by homogenizing of a known weight (1 g) of freeze-dried cells in methanol (95%). The homogenates were extracted overnight and for 3 h, and centrifuged at $10{,}000 \times g$ for 10 min. The supernatants of each extract were combined together, and the organic solvent was blown with sterilized air. The aqueous fraction remaining after removing organic solvent was filtered through GF/C filter paper. The filtered fraction was applied directly to preconditioned C18 cartridges for solid phase extraction (SPE) according to Carmichael and An [26]. The toxins were eluted with methanol 80%, evaporated to dryness, reconstituted in 1 mL methanol and applied to SHIMADZU high-performance liquid chromatography (HPLC) with UV photodiode array detector set at 238 nm. The column was a 4.6–150 mm Bondapack C18. The mobile phase was phosphate buffer and methanol (58–42) (pH 2) at room temperature, and flow rate of $1\,mL\,min^{-1}$.

MCYST concentrations in cyanobacterial species and algal samples were also determined in the aqueous fractions of SPE by enzyme-linked immunosorbent assay (ELISA) technique according to the method of Carmichael and An [26] using ENVIROLOGIX, INC., EP 022 kit, which detects total MCYSTs using polyclonal antibodies with a detection limit of $0.03\,mg\,L^{-1}$ Each test was made in triplicate.

Groundwater samples after concentration onto C18 cartridges were analyzed for the presence of extracellular MCYSTs by ELISA according to Carmichael and An [26] using ENVIROLOGIX kit as outlined above. MCYST concentrations were also determined in rainwater puddles and ponds near to the groundwater wells by ELISA as same as groundwater samples.

### 2.6. MCYST analysis in vegetable plants

Portions of roots and leaves of all studied vegetable plants were removed, washed twice with deionized water, dried with clean tissue paper, and accurately weighed. Each root or leaf sample was homogenized in an aliquot of methanol (100%) using an electric homogenizer. The homogenates were extracted and centrifuged ($10{,}000 \times g$, 10 min) twice overnight and for 3 h, respectively. The supernatants of each sample were combined and evaporated to dryness with sterilized air. The extraction of each sample was made in triplicate. The remaining residue was re-suspended in phosphate buffer saline (PBS) and subjected to ELISA for MCYST detection.

### 2.7. Statistical analysis

Differences in algal density, environmental parameters, MCYST concentrations in groundwater wells were compared using one-way ANOVA ($P < 0.05$) using SPSS 9.0 software for Windows. Correlations among algal density, toxin concentrations in well waters, toxin concentrations in rainwater ponds and toxin concentrations in vegetable were measured using Spearman rank correlation coefficients.

## 3. Results

### 3.1. Water quality of groundwater wells

Table 1 presents the mean values of physical and chemical parameters of well waters. The results showed that groundwaters did not show a significant variation in temperature or pH among studied wells ($P > 0.05$). Conductivity and TDS were high and varied significantly among these wells ($P < 0.05$). Groundwater had very high concentrations of nitrate and phosphate with a significant variation among wells ($P < 0.05$).

### 3.2. Phytoplankton and MCYST concentrations in groundwater wells

Cyanonbacteria was the dominant group in Phytoplankton samples collected from groundwater wells in Asir region and represented 88.4% of the total phytoplankton (Table 2). *Oscillatoria limnetica* was the most dominant species among cyanobacteria in most wells. Diatoms and Chlorophyta were also present in the wells but with low densities (8.85%, 3.46%, respectively).

The total density of cyanobacteria and *O. limnetica* correlated significantly with nitrate ($r > 0.8$) and phosphate ($r > 0.72$) concentrations, but not significantly with other environmental variables of groundwater wells ($r < 0.1$). In addition, the total density of cyanobacteria had a marked correlation with the density of *O. limnetica* ($r = 0.7$).

MCYST concentrations within phytoplankton cells showed a significant variation among wells ($P < 0.05$) and associated with the density of *O. limnetica* ($r = 0.85$), where no MCYST level was detected in phytoplankton samples not-containing this species (Table 3). The ELISA results also revealed that only *O. limnetica* can produce MCYSTs ($336\,\mu g\,g^{-1}$) among cyanobacteria species isolated from groundwater wells. In addition, HPLC chromatogram for the methanolic extract of *O. limnetica* showed one main peak corresponding to MCYST-YR ($283\,\mu g\,g^{-1}$) plus minor unidentified

**Table 1**
Physico-chemical properties of groundwater wells in Asir region, Saudi Arabia during the period of May–June (2007).

| Wells | Temperature (°C) | pH | Conductivity ($\mu S\,cm^{-1}$) | TDS ($mg\,L^{-1}$) | $NO_3^-$ ($mg\,L^{-1}$) | $NH_4^+$ ($mg\,L^{-1}$) | $PO_4^{-3}$ ($mg\,L^{-1}$) |
|---|---|---|---|---|---|---|---|
| 1 | 18 | 7.2 | 1861 ± 34 | 949 ± 22 | 13 ± 0.3 | 1.2 ± 0.4 | 6.1 ± 0.6 |
| 2 | 17 | 7.5 | 2331 ± 42 | 1130 ± 16 | 13.6 ± 1.1 | 1.3 ± 0.2 | 5.5 ± 0.3 |
| 3 | 18 | 7.2 | 1310 ± 25 | 604 ± 27 | 14 ± 1.3 | 2.2 ± 0.5 | 4.7 ± 0.7 |
| 4 | 18 | 7.3 | 1418 ± 31 | 827 ± 13 | 19.8 ± 1.1 | 1.3 ± 0.3 | 6.4 ± 0.8 |
| 5 | 16 | 7.5 | 1850 ± 34 | 960 ± 35 | 18.1 ± 0.8 | 1.6 ± 0.4 | 8.1 ± .0.3 |
| 6 | 17 | 7.1 | 1830 ± 42 | 940 ± 33 | 17.1 ± 0.3 | 2.2 ± 0.5 | 7.5 ± 0.5 |
| 7 | 16 | 7.4 | 2300 ± 51 | 1080 ± 41 | 11.2 ± 0.2 | 1.1 ± 0.2 | 5.8 ± 0.3 |
| 8 | 18 | 7.2 | 1360 ± 22 | 620 ± 24 | 11.9 ± 0.1 | 0.9 ± 0.1 | 3.1 ± 0.4 |
| 9 | 17 | 7.3 | 1470 ± 28 | 704 ± 21 | 13.1 ± 0.1 | 1.6 ± 0.3 | 6.5 ± 0.6 |
| 10 | 17 | 7.1 | 1750 ± 26 | 915 ± 31 | 14.8 ± 0.3 | 1.6 ± 0.4 | 4.6 ± 0.4 |

**Table 2**
Density and frequency of occurrence of most common algal species in groundwater wells ($n = 10$) in Abha city, Saudi Arabia during the period of May–June (2007).

| Species | Density ($cells\,L^{-1}$) | | | | Frequency (%) |
|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | SD | |
| Cyanobacteria | | | | | |
| *Chroococcus minor* | $4.5 \times 10^5$ | $7.8 \times 10^5$ | $6.33 \times 10^5$ | 568 | 60 |
| *Gleocapsa* sp. | $0.23 \times 10^5$ | $1.9 \times 10^5$ | $1.12 \times 10^5$ | 330 | 70 |
| *Oscillatoria limnetica* | $68.1 \times 10^5$ | $92.3 \times 10^5$ | $86.4 \times 10^5$ | 2580 | 60 |
| *Spirulina* sp. | $0.07 \times 10^5$ | $0.18 \times 10^5$ | $0.13 \times 10^5$ | 88 | 40 |
| Chlorophyta | | | | | |
| *Chlorella* sp. | $1.9 \times 10^5$ | $4.6 \times 10^5$ | $3.1 \times 10^5$ | 432 | 80 |
| *Coelastrum* sp. | $0.01 \times 10^5$ | $0.05 \times 10^5$ | $0.03 \times 10^5$ | 48 | 60 |
| *Scenedesmus* sp. | $0.2 \times 10^5$ | $0.48 \times 10^5$ | $0.42 \times 10^5$ | 64 | 100 |
| Diatoms | | | | | |
| *Gomphonema* sp. | $0.88 \times 10^5$ | $1 \times 10^5$ | $0.95 \times 10^5$ | 180 | 70 |
| *Nitzschia palea* | $3.7 \times 10^5$ | $5.1 \times 10^5$ | $4.6 \times 10^5$ | 640 | 100 |
| *Synedra ulna* | $2.8 \times 10^5$ | $4.6 \times 10^5$ | $3.3 \times 10^5$ | 560 | 100 |

MCYSTs (Fig. 1). MCYST concentrations in filtered groundwaters varied significantly among the 10 wells surveyed ($P < 0.05$). These concentrations had a weak correlation ($r = 0.3$) with MCYST concentrations within phytoplankton cells collected from groundwater wells. But, they had a strong correlation ($r = 0.9$) with MCYST concentration in pond waters surrounding these wells.

### 3.3. MCYST concentrations in vegetable plants

All vegetable plants collected from farms used groundwater for irrigation in Asir region were found to accumulate MCYSTs in their leaves and roots. The total MCYST concentrations in plants correlated positively with their concentrations in well waters ($r = 0.92$). On the other hand, MCYST concentrations varied significantly among vegetable plants ($P < 0.05$). The highest concentration of MCYSTs was detected in radish ($1.2\,\mu g\,g^{-1}$ fresh weight) and the lowest was in cabbage ($0.07\,\mu g\,g^{-1}$ fresh weight) (Fig. 2). Moreover, MCYST concentrations differed significantly ($P < 0.05$) between leaves and roots of vegetable plants. Collectively, roots were found to accumulate higher toxin concentrations than leaves (Fig. 3). However, dill leaves accumulated greater toxins than roots as an exception.

**Table 3**
Microcystin concentrations in phytoplankton samples ($\mu g\,g^{-1}$ dry weight) collected from groundwater wells, and in rainwater ponds ($\mu g\,L^{-1}$ pond water) found near to the wells.

| Wells | Algal samples ($\mu g\,g^{-1}$) | Rainwater ponds ($\mu g\,L^{-1}$) |
|---|---|---|
| 1 | 1100 ± 78 | 2.3 ± 0.25 |
| 2 | 820 ± 53 | 1.6 ± 0.3 |
| 3 | 350 ± 22 | 1.1 ± 0.14 |
| 4 | 260 ± 31 | 0.8 ± 0.1 |
| 5 | 310 ± 43 | 0.65 ± 0.08 |
| 6 | 740 ± 36 | 0.72 ± 0.06 |
| 7 | ND | 1.4 ± 0.15 |
| 8 | ND | 1.9 ± 0.33 |
| 9 | ND | 1.2 ± 0.27 |
| 10 | ND | 0.85 ± 0.11 |

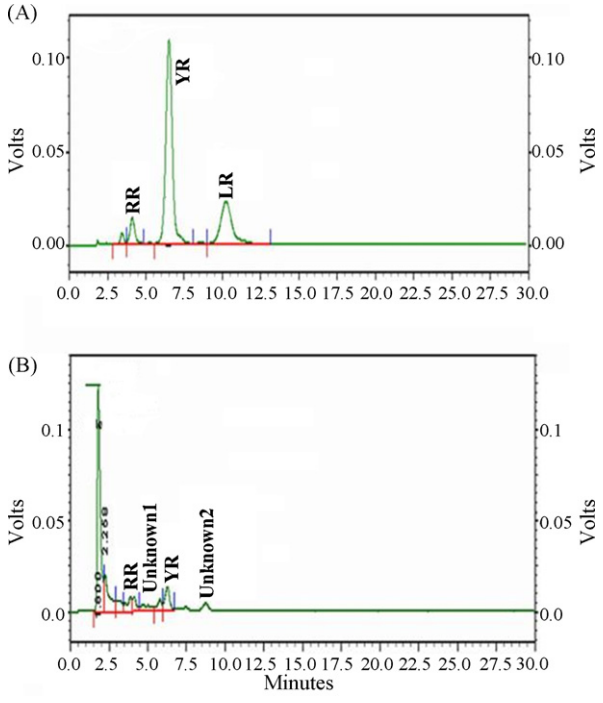


**Fig. 1.** HPLC chromatogram of standard microcystins (A) and of the methanolic extract of *oscillatoria limnetica* collected from groundwater wells during the present study (B).

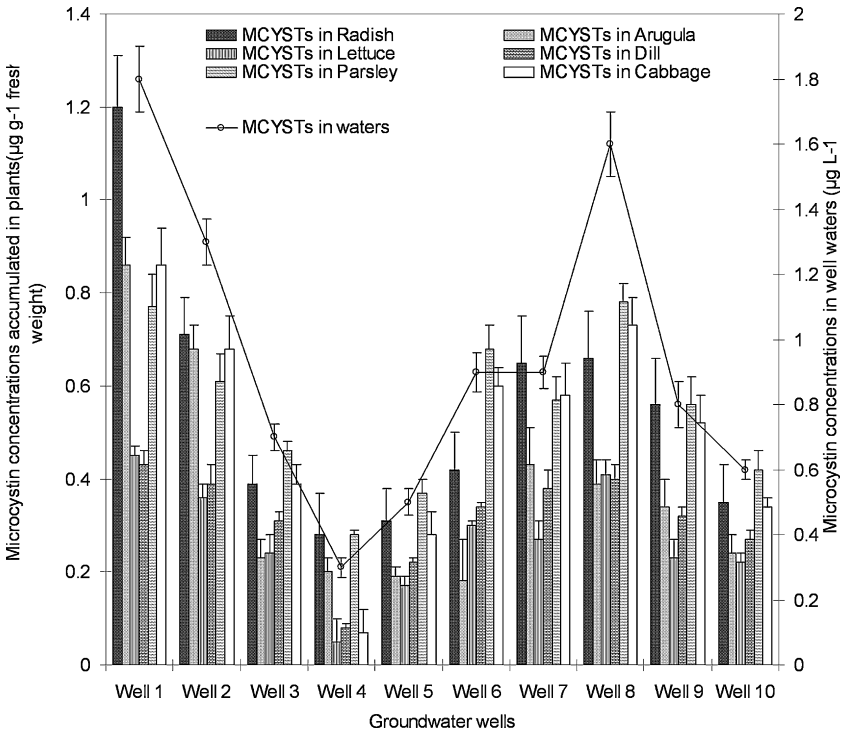


**Fig. 2.** Microcystin concentrations ($\mu g\,L^{-1}$) in well waters and in vegetable plants ($\mu g\,g^{-1}$ fresh weight) collected from farms using these groundwaters for irrigation.

## 4. Discussion

### 4.1. Toxic cyanobacteria and MCYSTs in groundwater wells

The conditions which favor the growth of cyanobacteria and lead to blooms in water bodies are nutrient enrichment (largely phosphorus but also nitrogen), warm temperatures (15–30 °C), pH between 6 and 9 and calm stable water conditions [27,28]. These conditions are often caused by human actions and activities by discharge of nutrients from sources of urban and agricultural pollution. Groundwater wells surveyed during the present study, were characterized by high concentrations for TDS, nitrate and phosphate, indicating that these well waters are highly eutrophic. The high nutrient concentrations along with suitable light, temperature (>15 °C) and pH (>6) favored the growth of cyanobacteria in Asir groundwater wells. Previously, White et al. [29] reported that Lake Elphinstone (Australia) experienced recurrent toxic cyanobacteria when nitrogen and phosphorus concentrations reached 3 and 0.2 $mg\,L^{-1}$, respectively. Nitrogen and phosphorus concentrations detected in Asir wells were higher than these levels. This may be due to the exposure of these wells to agricultural drainage. The invasion of cyanobacteria into open groundwater wells may be occurred by wind or rainwater carrying cyanobacterial akinetes and spores [2,30].

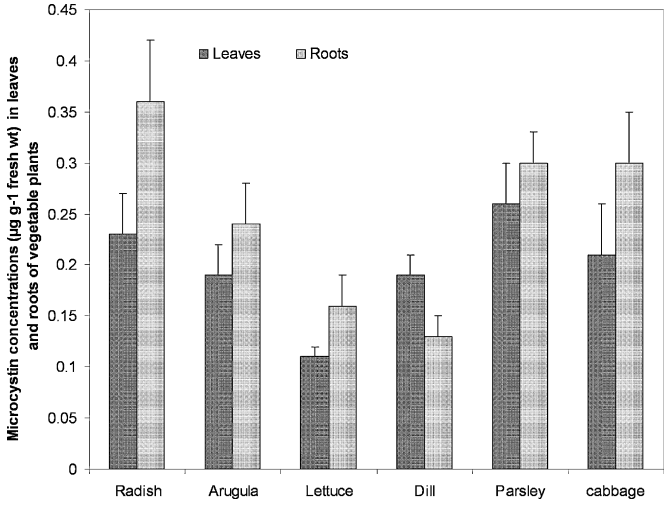


**Fig. 3.** Relative proportions of microcystin concentrations ($\mu g\,g^{-1}$ fresh weight) in the roots and leaves of vegetable plants collected during the present study.

MCYST concentrations in phytoplankton samples were statistically correlated only with the abundance of *O. limnetica*. Consistently, ELISA and HPLC analysis for the extracts of isolated species showed that only *O. limnetica* can produce MCYSTs with a profile of MCYST-YR as a main toxin plus minor unidentified MCYSTs. To our knowledge, this study is the first to report that *O. limnetica* can produce MCYSTs. However, previous studies reported the MCYST production in other species of *Oscillatoria* [31–33]. In addition, the absence of MCYSTs in phytoplankton samples not-containing *O. limnetica* confirms that MCYST production is confined only to this species. However, the cell-free waters of wells not containing such a MCYST producing species contained MCYST concentrations ranged from 0.6 to 1.6 $\mu g\,L^{-1}$. These MCYST concentrations in well waters were also correlated significantly with MCYST concentrations in rainwater ponds and puddles surrounding these wells. This may point to the leakage of MCYSTs from rainwater ponds into groundwater wells. Thus, MCYST concentrations detected in grounderwater well containing *O. limnetica* is the sum of MCYSTs released from cyanobacterial cells upon lysis and MCYSTs leaked from surrounding ponds. Groundwater can link lakes across the landscape [1], and thus MCYSTs in contaminated lakes can migrate into groundwater [20]. The contamination of groundwater wells with MCYSTs from lakes waters were earlier reported in Italy [21], Latvia [20] and China [7]. In addition, Chen et al. [34] demonstrated that MCYSTs have high mobility in soil with low clay content. Therefore, they can leach easily from lakes into groundwater.

Once MCYSTs enter groundwater, they will degrade their quality for drinking and recreational purposes particularly if their con-

centrations exceed WHO guideline value of 1 $\mu g\,L^{-1}$ for drinking water [35]. Unfortunately, MCYST concentrations (1.3–1.8 $\mu g\,L^{-1}$) detected by ELISA in some groundwater wells (3 out of 10 wells) during the present study did exceed this level. Although MCYST concentrations (0.3–0.9 $\mu g\,L^{-1}$) in the remaining wells did not surpass the WHO guideline level for drinking water, they exceeded the chronic exposure limit (0.1 $\mu g\,L^{-1}$) for tumor promotion [36]. These toxins can persist for long time (100 days) in groundwater [37] compared to 4 weeks in surface waters [38], because of lack of bacteria in groundwaters.

### 4.2. Accumulation of MCYSTs in vegetable plants

Besides the direct ingestion of MCYSTs via drinking, irrigation with water contaminated with MCYSTs may inhibit the growth of plants [10], with a potential accumulation of these toxins in their edible parts [14,19]. Therefore edible plants exposed to MCYSTs via irrigation may provide an additional route for human intoxication [39]. During the present study, seven vegetable plants collected from farms used MCYST-contaminated groundwater for irrigation, were found to accumulate MCYSTs in their tissues at levels ranging from 0.05 to 1.2 $\mu g\,g^{-1}$ fresh weight. MCYST concentrations accumulated in plant tissues differed significantly among the seven plants. The toxin concentrations accumulated in plants correlated positively with MCYST concentrations in groundwater wells. These results are concomitant with those obtained by Chen et al. [16] showing that the concentration of MCYSTs accumulated in rape and rice plants increased in accordance with the exposure toxin concentration. On the other hand, all plants surveyed during the present study almost looked healthy (data not shown). This may be attributed to that MCYST concentrations in groundwater wells (0.3–1.8 $\mu g\,L^{-1}$) are not enough to inhibit the growth or make any morphological and physiological changes in these plants. All previous studies reported growth inhibition and morpho-physiological changes in terrestrial plants upon exposure to MCYSTs at concentrations more than 10 $\mu g\,L^{-1}$ [4]. MCYST concentrations detected in vegetable plants during the present study, can be compared to those obtained in rape and rice plants (0.65, 0.005 $\mu g\,g^{-1}$ fresh weight, respectively) [16], salad lettuce (2.5 $\mu g\,g^{-1}$ dry weight) [39], and in broccoli and mustard (2.4, 2.6 $\mu g\,g^{-1}$ fresh weight, respectively) [19]. On the other hand, the results of the present study interestingly showed that except drill plant, the roots of all vegetable plants accumulated much more toxin than leaves. Previously, MCYSTs were detected solely in the roots of broccoli and mustard plants exposed to 10 $\mu g\,L^{-1}$ MCYSTs [19]. The latter authors lessened the importance of the presence of MCYSTs in broccoli and mustard plants because the roots of these plants are not usually consumed by human. Similarly, the roots of dill, arugula, lettuce, parsley and cabbage which found to accumulate high amount of toxins during the present study are not consumed by human. However, the radish roots contained the highest amount of MCYSTs are commonly and largely consumed by human. As these vegetable plants are usually eaten freshly, the radish roots and dill, arugula, lettuce, parsley and cabbage leaves containing MCYSTs will pose a threat to human health. Although MCYST concentrations detected in plants of edible leaves (0.11–0.26 $\mu g\,g^{-1}$ fresh weight) and plants of edible roots (0.36 $\mu g\,g^{-1}$ fresh weight) did not exceed the WHO guideline of MCYSTs (1 $\mu g\,L^{-1}$) [35], they may surpass this level based on the amount of these plants consumed by human. Assuming that the average portion of these plants eaten by a person is about 200–300 g; at the levels found in the edible leaves and roots in our study (0.11–36 $\mu g\,g^{-1}$ fresh weight), a 100-g serving would contain from 11 to 36 $\mu g$ MCYSTs, or about 5–18 times the recommended daily MCYST intake from drinking water.

## 5. Conclusions

The results of present study revealed that groundwater wells in Asir region, Saudi Arabia have high conductivity and nutrient concentrations (nitrate and phosphate); and these factors promoted the growth and proliferation of algae and cyanobacteria in such open wells. *O. limnetica* isolated from groundwater wells was the only species that can MCYSTs. MCYSTs were also detected in well waters at concentrations exceeded the WHO acceptable level in some wells. In addition, MCYSTs were detected in wells not-containing the toxin producing species, indicating the contamination of well waters with MCYSTs through leaching from other water sources e.g. rainwater puddles and ponds near these wells. On the other hand, six vegetable plants collected from farms irrigated with MCYST-contaminated groundwaters were found to accumulate these toxins in their edible organs at concentrations can exceed the acceptable level based on the amount of plant tissues consumed by human. As groundwaters are the main sources for the irrigation in such semi-arid and arid countries e.g. Saudi Arabia, they should be avoided from the urban and agricultural discharge to prevent the growth of toxic cyanobacteria. In addition, the artificial puddles and ponds made for storing rainwater have to be dug far from the wells to protect them against the leakage of MCYSTs from contaminated ponds. In addition, groundwater wells and vegetable plants should be continuously monitored for the presence of MCYSTs to protect the public against the exposure to such potent hepatotoxins.

## References

[1] W.K. Dodds, Freshwater Ecology. Concepts and Environmental Applications, Academic Press, San Diego, USA, 2002.
[2] I.E. Dubovik, Migration of aerophytic algae and their colonization on different substrata, Int. J. Algae 4 (2002) 48–55.
[3] W.W. Carmichael, Cyanobacteria secondary metabolites: the cyanotoxins, J. Appl. Bacteriol. 72 (1992) 445–459.
[4] E. Dittmann, C. Wiegand, Cyanobacterial toxins-occurrence, biosynthesis and impact on human affairs, Mol. Nutr. Food Res. 50 (2006) 7–11.
[5] W.W. Carmichael, The toxins of cyanobacteria, Sci. Am. 270 (1994) 78–86.
[6] G.A. Codd, L.F. Morrison, J.S. Metcalf, Cyanobacterial toxins: risk management for health protection, Toxicol. Appl. Pharm. 203 (2005) 264–272.
[7] Y. Ueno, S. Nagata, T. Tsutsumi, A. Hasegawa, M.F. Watanabe, H.D. Park, G.C. Chen, G. Chen, S.Z. Yu, Detection of MCYSTs, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay, Carcinogenesis 17 (1996) 1317–1321.
[8] W.W. Carmichael, I.R. Falconer, Diseases related to freshwater blue-green algal toxins and control measures, in: I.R. Falconer (Ed.), Algal Toxins in Seafood and Drinking Water, Academic Press, New York, 1993, pp. 187–209.
[9] E.M. Jochimsen, W.W. Carmichael, J.S. An, D.M. Cardo, S.T. Cookson, C.E.M. Holmes, M.B. Antunes, D.A. de Melo-Filho, T.M. Lyra, V.S.T. Barreto, S.M.F.O. Azevedo, W.R. Jarvis, Liver failure and death after exposure to MCYSTs at a hemodialysis center in Brazil, New Engl. J. Med. 338 (1998) 873–878.
[10] S. Pflugmacher, K. Jung, L. Lundvall, S. Neumann, A. Peuthert, Effects of cyanobacterial toxins and cyanobacterial cell free crude extract on germination on alfalfa (*Medicago sativa*) and induction of oxidative stress, Environ. Toxicol. Chem. 25 (2006) 2381–2387.
[11] C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, G.A. Codd, Cyanobacterial MCYST-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants., FEBS Lett. 264 (1990) 187–192.
[12] C. Wiegand, S. Pflugmacher, Ecotoxicological effects of selected cyanobacterial secondary metabolites. A short review, Toxicol. Appl. Pharmacol. 203 (2005) 201–218.
[13] P. Kos, G. Gorzo, G. Suranyi, G. Borbely, Simple and efficient method for isolation and measurement of cyanobacterial hepatotoxins by plant tests (*Sinapis alba* L.), Anal. Biochem. 225 (1995) 49–53.
[14] J. McElhiney, L.A. Lawton, C. Leifert, Investigations into the inhibitory effects of MCYSTs on plant growth, and the toxicity of plant tissues following exposure, Toxicon 39 (2001) 1411–1420.
[15] M.M. Gehringer, K.S. Downs, T.G. Downing, R.J. Naude, E.G. Shephard, An investigation into the effect of selenium supplementation on MCYST hepatotoxicity, Toxicon 41 (2003) 451–458.
[16] J. Chen, L. Song, J. Dai, N. Gan, Z. Liu, Effects of MCYSTs on the growth and the activity of superoxide dismutase and peroxidase of rape (*Brassica napus* L.) and rice (*Oryza sativa* L.), Toxicon 43 (2004) 393–400.
[17] K. Kurki-Helasmo, J. Meriluoto, MCYST uptake inhibits growth and protein phosphatase activity in mustard (*Sinapis alba* L.) Seedlings, Toxicon 36 (1998) 1921–1926.

[18] S. Pflugmacher, G.A. Codd, C.E.W. Steinberg, Effects of the cyanobacterial toxin MCYST-LR on detoxication enzymes in aquatic plants, Environ. Toxicol. 14 (1999) 111–115.
[19] S. Jarvenpaa, C. Lundberg-Niinisto, L. Spoof, O. Sjo vallb, E. Tyystja rvic, J. Meriluoto, Effects of MCYSTs on broccoli and mustard, and analysis of accumulated toxin by liquid chromatography–mass spectrometry, Toxicon 49 (2007) 865–874.
[20] F. Eynard, K. Mez, J.L. Walther, Risk of cyanobacterial toxins in Riga waters (*Latvia*), Water Res. 34 (2000) 2979–2988.
[21] V. Messineo, D. Mattei, S. Melchiorre, G. Salvatore, S. Bogialli, R. Salzano, R. Mazza, G. Capelli, M. Bruno, MCYST diversity in a *Planktothrix rubescens* population from Lake Albano (Central Italy), Toxicon 48 (2006) 160–174.
[22] APHA (American Public Health Association), Standard Methods for the Examination of Water and Wastewater, 19th ed., APHA, Washington, DC, 1995.
[23] G.W. Prescott, How to know the freshwater phytoplankton, 3rd ed., Cambridge University press, 1978.
[24] H. Utermohl, Zur Vervollkommung der quantitativen phytoplankton, Methodik (1958).
[25] R.Y. Stanier, R. Kunisawa, M. Mandel, G. Cohen-Bazire, Purification and properties of unicellular blue-green algae (order Chroociccales), Bacteriol. Rev. 35 (1971) 171–205.
[26] W.W. Carmichael, J. An, 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 (1999) 377–385.
[27] L.R. Mur, O.M. Skulberg, H. Utkilen, Cyanobacteria in the environment, in: I. Chorus, J. Bartram (Eds.), Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management, E&FN Spon, London, 1999, pp. 15–37.
[28] R.B. Sotero-Santos, C.R.D. Silva, N.F. Verani, K.O. Nonka, O. Rocha, Toxicity of a cyanobacteria bloom in Barra Bonita reservoir (Middle Tiete River, Sao Paulo Brazil), Ecotoxicol. Environ. Safe. 64 (2006) 163–170.
[29] S.H. White, L.D. Fabbro, L.J. Duivenvoorden, Changes in canoprokaryote populations, microcystismorphology, and MCYST concentrations in Lake Elphinstone (Central Queensland Austuralia), Environ. Toxicol. 18 (2003) 403–412.
[30] Z.A. Mohamed, A.M. Al Shehri, Cyanobacteria and their toxins in treated-water storage reservoirs in Abha city, Saudi Arabia, Toxicon 50 (2007) 75–84.
[31] B.C. Hitzfeld, S.J. Hoger, D.R. Dietrich, Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment, Environ. Health Perspect. 108 (2000) 113–122.
[32] Z.A. Mohamed, Allelopathic activity of *Spirogyra* sp.: stimulating bloom formation and toxin production by *Oscillatoria agardhii* in some irrigation canals, Egypt, J. Plankton Res. 24 (2002) 137–141.
[33] Z.A. Mohamed, Toxic cyanobacteria and cyanotoxins in public hot springs in Saudi Arabia, Toxicon 51 (2008) 17–27.
[34] W. Chen, L. Song, N. Gan, L. Li, Sorption, degradation and mobility of MCYSTs in Chinese agriculture soils: risk assessment for groundwater protection, Environ. Pollut. 144 (2006) 752–758.
[35] WHO, Guidelines for Drinking-Water Quality, third ed., World Health Organization, Geneva, 2004, p. 195.
[36] S.J. Hoeger, G. Shaw, B.C. Hitzfeld, D.R. Dietrich, Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants, Toxicol. Appl. Pharmacol. 203 (2005) 231–242.
[37] T. Holst, N.O.G. Jørgensen, C. Jørgensen, A. Johansen, Degradation of MCYST in sediments at oxic and anoxic, denitrifying conditions, Water Res. 37 (2003) 4748–4760.
[38] H. Ishii, M. Nishijima, T. Abe, Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium, Water Res. 38 (2004) 2667–2676.
[39] G.A. Codd, J.S. Metcalf, K.A. Beattie, Retention of *Microcystis aeruginosa* and MCYST by salad lettuce (*Lactuca sativa*) after spray irrigation with water containing cyanobacteria, Toxicon 37 (1999) 1181–1185.