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Monitoring of microcystin-LR concentration in water reservoir

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ABSTRACT

Microcystins (MCs) are a group of cyanotoxins with hepatotoxic effects and can induce liver cancer. Among them, microcystin-LR (MC-LR) is the most toxic with 1 μg/L guideline value for potable waters. Therefore, it is very important to develop analytical methods for the detection and monitoring of MC-LR concentrations in raw water sources for public health protection. For this purpose, water samples were taken from a dam in south of Iran in a period of 2 years. MC-LR was extracted from samples by solid-phase extraction method using a C_{18} cartridge and then measured by reverse phase high performance liquid chromatography after calibration by standard solutions. Total phosphorous concentration and water temperature were measured for determination of environmental conditions in each season. Maximal and minimal concentrations of MC-LR were observed in early autumn (6.2 μg/L) and late winter (0.4 μg/L), respectively. MC-LR existed at all times in water source because of its resistance to natural physiochemical degradation and biodegradation. Thus, raw water source management is necessary to prevent MC-LR secretion for public health protection.

Keywords: Microcystin-LR; Water quality; Water source; Extraction

1. Introduction

Microcystins (MC_s) are a group of cyanotoxins produced by fresh water cyanobacteria, that have a general structure including D-alanine (D-ala), X, D-erythro-β-methylaspartic acid (D-MeAsp), Z, unique C_{20} β-amino acid, (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4 (E), 6(E)-dienoic acid (Adda), D-glutamic acid (D-Glu), and methyldehydroalanine (Mdha). In this structure, X and Z are two variable amino acids [1]. Microcystin-LR (MC-LR) is one of the most toxic MCs which has a leucine and arginine in X and Z positions, respectively [2]. MC-LR has inhibitory effect on protein phosphatase enzymes such as PP1 and PP2A [3]. Also, MC-LR can induce tumor promotion and finally liver cancer, therefore, World Health Organization (WHO) has determined 1 μg/L as guideline value for MC-LR concentration in potable water. Thus, excess MC-LR removal from contaminated waters is required in water treatment train process for public health protection [4]. Measurement and monitoring of MC-LR concentration in water resources is necessary for preparation of required advanced treatment process as a matter of MC-LR removal from water especially when the water source is surface water. Measurement of MC-LR has been performed in many investigations of water bodies, fishes and other aquatic organisms [5–9]. The main aim of this study is the measurement of MC-LR in raw water source in order to determine the concentration profile in depth and time and also in determination of correlation between different seasons, water quality and MC-LR concentration.

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2. Materials and methods

2.1. Sampling

The required number of samples (V = 2 L) were collected bimestrial from Esteghlal dam, in Hormozgan province in Iran (latitude: 27.17 °N, longitude: 57.13°E), during 2 years from March 2014 to March 2016 (Fig. 1). The Esteghlal dam was constructed in 1983. The lake of this dam has problem of algal bloom, because of some environmental factors such as tropical climate and discharge of rich rivers into it. This dam supplies a part of drinking water of Minab city with population of 259,221 in Hormozgan province. All the samples were collected and transferred to the lab by standard method [10]. At each stage of sampling, four samples were always collected from four different depths (surface, 1, 2 and 3 m). The sample's temperatures were measured on site.

2.2. Sample quality measurements

To determine the water quality and measurement of MC-LR content, each sample was neutralized at first. Half of any sample (1 L) was used for measurement of water parameters including TP and *Microcystis* cell count. *Microcystis* cells were counted in samples with high biomass, by gridded Sedgewick-rafter method. For *Microcystis* quantification in low biomass samples, Utermohl counting chamber was used. The other half of the sample (1 L) was used for MC-LR concentration measurements.

2.3. Chlorophyll a analysis

Chlorophyll a concentration was determined in each sample by using Standard Method (method 10200 H) [10]. Briefly, 1 L of each sample was filtrated through 0.45 μm fiberglass filter. Then, the filter was put in a grinder with 3 mL 90% aqueous acetone solution and then grinded. The slurry was transferred to a centrifuge tube and the volume was adjusted to 10 mL by adding 90% aqueous acetone solution and was allowed to remain in a dark place for 2 h in 4°C. After that, the tubes were centrifuged for 20 min at 5,000 RPM. The remained clarified extract was transferred to a clean centrifuge tube. To measure the chlorophyll a concentration, optical density (OD) was read at 750 and 664 nm by spectrophotometer (PerkinElmer, USA). Then, the extract was acidified by adding 0.1 N HCl. Again, OD was read at 665 and 750 nm. The 750 nm OD reading was subtracted from the 664 and 665 nm OD (before and after acidification). Finally, corrected OD was put in the following equation to calculate chlorophyll a concentration.

Fig. 1. Location of Esteghlal dam in Hormozgan province in Iran.

$$\text{Chlorophyll a (mg/m}^3\text{)} = 26.7(664a - 665b) \times V_1/L \times V_2 \quad (1)$$

In this equation, V_1 is extract volume, V_2 is sample volume and L is light path length (cm).

2.4. Total phosphorous analysis

The total phosphorous concentration was determined in each sample by using standard method (method 4500-P) [10]. Briefly, to this purpose, after filtration of samples, 1 mL H_2SO_4 and 5 mL HNO_3 was added to the sample flask for acidic digestion. After cooling, 0.5 mL phenolphthalein indicator was added to the flask and 1 N NaOH was used to appear a faint pink tinge. The volume of the sample adjusted to 100 mL by distillated water and total phosphorous was determined by colorimetric method, using spectrophotometer at 490 nm.

2.5. Solid-phase extraction of MC-LR

For extraction of MC-LR from water samples, C_{18} solid-phase extraction cartridge (Teknokroma, Spain) was used. Extraction steps were as follows: activation of cartridge using methanol and water consecutively, filtration of 1 L of water sample through 0.45 μm fiberglass filter, ferrying the filtered water sample through the cartridge with maximum 10 mL/min flow rate [11]. Then, the cartridge was dried by passing the air through it for 2 min. For the sample clean-up, the cartridge was washed using three solutions of methanol in water (10%, 20% and 30% v/v), consecutively. Then, MC-LR was eluted using 3 mL of trifluoroacetic acid (TFA) solution in methanol. In the last step of extraction, the sample was dried under nitrogen gas and residue was re-dissolved in 100 μL solution of 0.05% v/v TFA in methanol and injected into the high performance liquid chromatography (HPLC) [12]. All the reagents including acetonitrile, methanol and distillated water were of HPLC grade and were purchased from Caledon (Canada). The TFA (HPLC grade) was purchased from Sigma-Aldrich (USA).

2.6. HPLC analysis

Because MC-LR is a hydrophilic molecule, reverse phase liquid chromatography was used for measurement of samples. To this purpose, the C18 HPLC column, ODS A, tracer Excel (25 × 0.46 cm) with 5 μm particle size, was purchased from Teknokroma (Spain). The samples volumes were 100 μL. The calibration curve was achieved using MC-LR standard solutions. Then, the extracted samples were injected into the HPLC. A linear gradient was applied using two acetonitrile solutions (32 and 55% v/v) in water. Mobile phase flow rate was 1 mL/min at the gradient run and temperature was 30°C by direction of 0 min and 100% A, 12 min and 50% A, 15 min and 100% B, 25 min and 100% A, 60 min and 100% B [12].

2.7. Data analysis

All the statistical analyses were done by R software (version 3.4.1) and Microsoft Excel.

3. Results

During the sampling time from March 2014 to March 2016, Esteghlal Dam Lake had a mixture of cyanobacteria species. Dominant species was *Microcystis* and bloom occurred from May to July each year. The mean value of chlorophyll a was 83 ± 18 μg L^{-1} (mean ± SD). During bloom period, *Microcystis* cell population was in the range of 61–82 (×10^6) (surface layer), 1.0–1.7 (×10^6) (3 m depth) in 2014 and 58–81 (×10^6) (surface layer), 1.0–1.5 (×10^6) (3 m depth) in 2015 (Fig. 2). Microcystin-LR was detected throughout the whole year. In surface layer, MC-LR concentration was in the range of 6.2 μg L^{-1} in November 2014 to 1.7 μg L^{-1} in January 2015, and at 3 m depth, it was in the range of 3.8 μg L^{-1} in November 2014 to 0.4 μg L^{-1} in January 2016 (Fig. 3). MC-LR concentration has a significant reverse correlation with *Microcystis* cell numbers ($p < 0.05$). Total phosphorus (TP) concentration in surface was between 37 mg L^{-1} in July 2014 and 7 mg L^{-1} in June 2015 (20.92 ± 9.81) and 13 mg L^{-1} to 0 at 3 m depth (7.07 ± 4.78). Strong correlation was observed between TP concentration and *Microcystis* cell count in all of the four layers of sampling (Fig. 4). Water temperature in this dam was in the range of 2°C in June 2014 to 25°C in July 2015 in surface layer and was between 17°C (July) and 3°C (June) at 3 m depth. Significant correlation between water temperature and *Microcystis* cell count was detected ($p < 0.05$) (Fig. 4) but the linear regression model did not show significant correlation between MC-LR concentration and chlorophyll a ($R^2 = 0.11$). Also simple regression analysis on TP concentration and *Microcystis* cell count showed significant relationship between them ($R^2 = 0.98$, $p < 0.05$). In Fig. 5 the Trends of MC-LR concentration vs. cell counts are shown for the water reservoir at different water level as: surface, 1 m depth, 2 m depth, and 3 m depth.

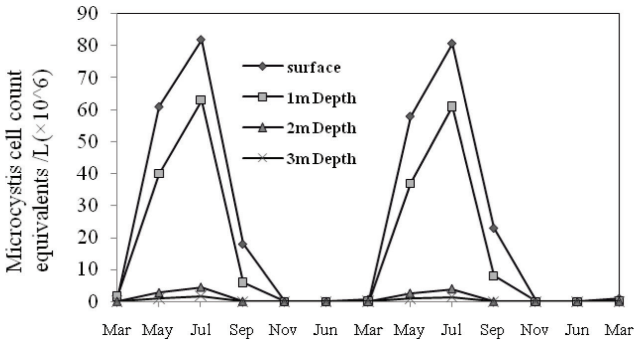

Fig. 2. *Microcystis* cell count at four levels of depth.

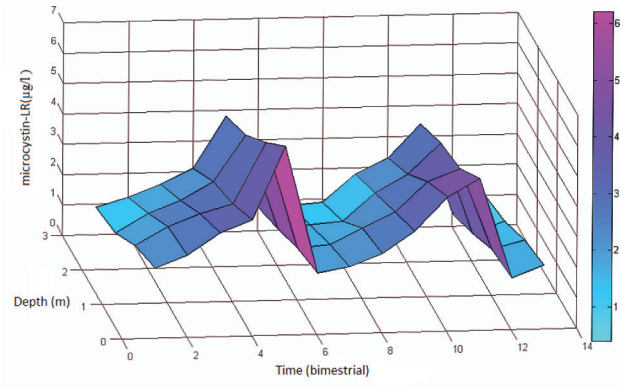

Fig. 3. MC-LR concentration during 2 years (Mar 2014 – Mar 2016).

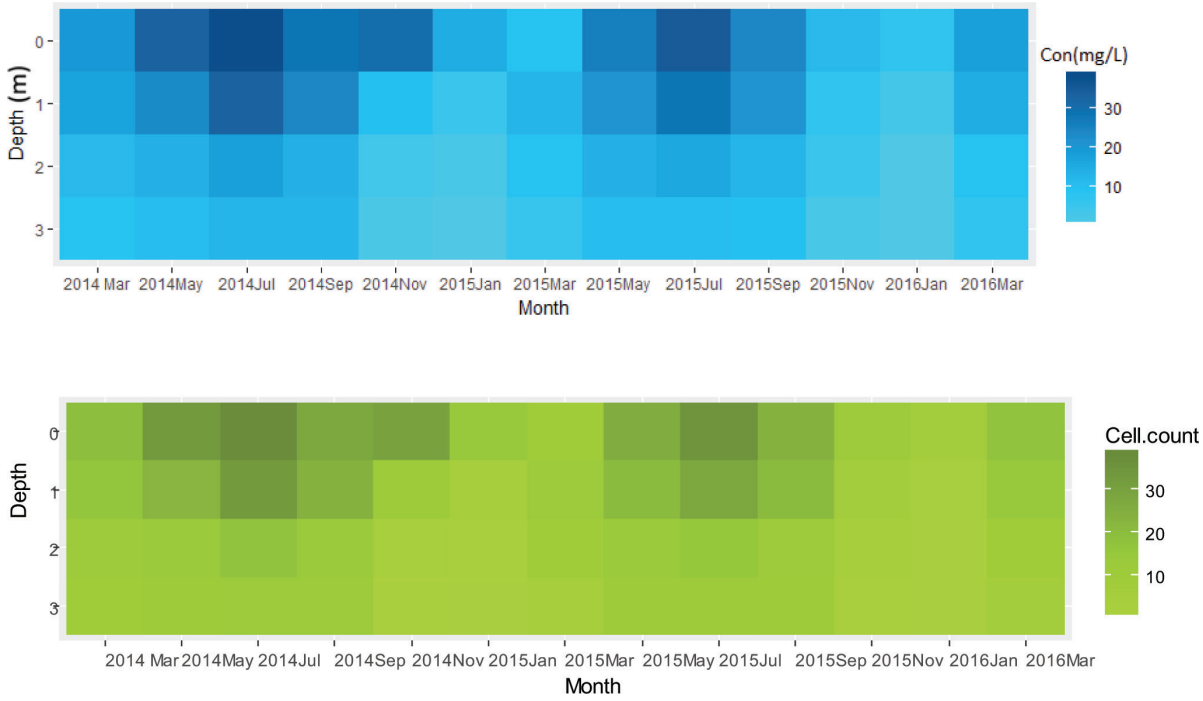

Fig. 4. Trends TP concentration (blue) and *Microcystis* cell count (green) at four levels of depth.

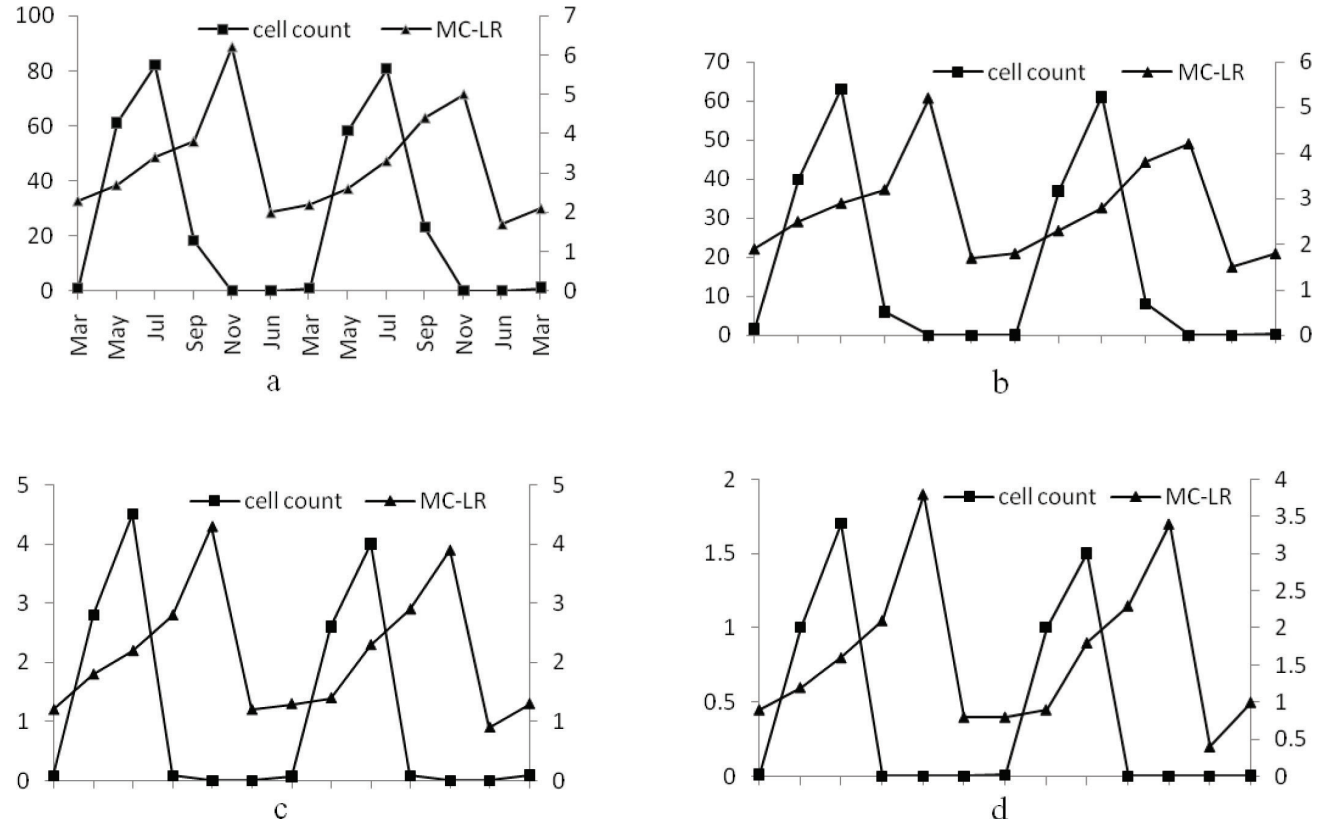

Fig. 5. Trends of MC-LR concentration vs. cell count ((a) surface, (b) 1 m depth, (c) 2 m depth, (d) 3 m depth), MC-LR: μg L^{-1} (right Y axis), cell count: ×10^6 cell L^{-1} (left Y axis).

4. Discussion

Many investigations were performed on MCs' concentrations in water reservoirs that were often focused on the relationship between cyanobacteria and the overall concentration of all types of MCs [13,14]. In addition, MC-LR is recognized by the WHO as one of the most toxic types of MC in potable waters, so, this study was focused on relation among *Microcystis* and MC-LR concentration profile in time and depth.

The results of this study indicate that the high water temperature and nutrients enrichment are two important factors in cyanobacteria growth, in accordance with other studies [15–19]. Consequently, this phenomenon can increase potential of MCs secretion in water resources.

Several investigations emphasized that appropriate conditions such as sunlight and maximum level of nutrients for *Microcystis* cell growth exist in the top layer of water [20]. Besides, *Microcystis* has gas vacuoles that help the cells float at the surface (depending on water viscosity). So, the majority of *Microcystis* cells exist in the top layer of water (Fig. 2).

Unlike several studies that mentioned strong correlation between *Microcystis* cell population and MCs' concentrations [16,21], strong reverse correlation was found between them in this study (R^2 = 0.78) (Fig. 4). It can be described that MC-LR is an intracellular kind of MC and is resistant to biodegradation in natural waters [22]. So, MC-LR presence in water bodies is related to algal cell distraction rate. Therefore, at early autumn, enhanced distraction rate of *Microcystis* because of the decrease in temperature can increase MC-LR concentration strongly. During autumn and winter, water dilution because of precipitation and runoff inlet can decrease MC-LR concentration, but also can distribute MC-LR in depth by mixing effect. So, even during winter at 3 m depth without any *Microcystis* cells, MC-LR is present. As reported by Kankaanpää et al. [9], MC-LR is detectable even at 5 m depth.

Various investigations have reported several significant changes in MCs' concentrations during the course of a year [19]. But, this study shows that there is little change in measured MC-LR level in depth and time except during seasonal change from summer to autumn when an intense increase in MC-LR concentration is detectable (Fig. 2). WHO indicated the correlation between chlorophyll a concentration and MCs' concentrations [23] but, as mentioned by Gobler et al. [19] this correlation is very weak. We found in this study that the correlation between MC-LR and chlorophyll a concentration is indeed very weak. But, as we found there is strong reverse correlation between MC-LR concentration and *Microcystis* cell count so that always the peak concentration of MC-LR occur almost 2 months after peak of cell count of *Microcystis*. Thus, *Microcystis* cell count can be used for prediction MC-LR concentration and it can be suggested that *Microcystis* cell count be replaced with chlorophyll a for the risk assessment of exposure to MC-LR. As mentioned in results, there is strong relationship between TP concentration and *Microcystis* cell count. Amano et al. [24] mentioned, *Microcystis* cells have more susceptibility to phosphorous

absorption and cell proliferation in comparison of other type of algal cell like diatoms [24]. Therefore, by increasing TP we expect more *Microcystis* cell count, following more MC-LR concentration.

MC-LR has several health outcomes, so, in the case that MC-LR concentration is more than the guideline values in potable water in the entire duration of the year, water resource protection plans are necessary to control *Microcystis* cell growth.

5. Conclusion

MC-LR has a seasonal trend in water reservoirs. Maximum concentration of this cyanotoxin occurs in early autumn because of *Microcystis* cells distraction and minimum concentration occurs in late winter as a consequence of dilution. MC-LR is detectable in water bodies even at a depth of 3 m and it exists for the entire period of the year because of resistance to biodegradation. Therefore, water protection plans are necessary for surface water in order to control *Microcystis* cells growth and maintain MC-LR levels below the acceptable value for public health protection. Maximum rate of MC-LR secretion will be determined in future studies for identifying corresponding species of cyanobacteria that produce majority of MC-LR in surface waters.

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