SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 2, pp. 349–355, 2006

Printed in the USA
0730-7268/06 $12.00 + .00

SEASONAL VARIATIONS IN THE CONCENTRATION OF MICROCYSTIN-LR IN TWO LAKES IN WESTERN TEXAS, USA

MADHAVI BILLAM, LILI TANG, QINGSONG CAI, SANDEEP MUKHI, HONGXIA GUAN, PIWEN WANG, ZEMIN WANG, CHRISTOPHER W. THEODORAKIS, RONALD J. KENDALL, and JIA-SHENG WANG*
Department of Environmental Toxicology and The Institute of Environmental and Human Health, Texas Tech University, Lubbock, Texas 79409, USA

(*Received* 3 *March* 2005; *Accepted* 5 *August* 2005)

Abstract—Seasonal variations in the concentration of microcystin-LR (MCLR) in Buffalo Springs Lake (BSL) and Lake Ransom Canyon (LRC; both locations in Lubbock, TX, USA) were monitored from 2003 to 2004. In BSL, the average concentrations of MCLR were 1.78 ± 1.43 μg/L (mean ± SD; range, 0.177–4.914 μg/L) in spring, 0.41 ± 0.096 μg/L (range, 0.191–0.502 μg/L) in summer, 0.46 ± 0.41 μg/L (range, 0.205–1.598 μg/L) in fall, and 1.04 ± 0.71 μg/L (range, 0.096–2.428 μg/L) in winter. In LRC, the corresponding concentrations were 1.08 ± 1.29 μg/L (range, 0.2–5.83 μg/L) in spring, 0.83 ± 0.46 μg/L (range, 0.315–1.671 μg/L) in summer, 0.44 ± 0.03 μg/L (range, 0.368–0.555 μg/L) in fall, and 0.78 ± 0.52 μg/L (range, 0.225–2.130 μg/L) in winter. In both lakes, the seasonal fluctuation of MCLR concentrations correlated positively with dissolved oxygen and negatively with temperature and pH.

Keywords—Cyanotoxins Microcystin-LR Seasonal variations Enzyme-linked immunosorbent assay Protein phosphatase inhibition assay

INTRODUCTION

Contamination of cyanobacterial toxins in freshwater sources is a worldwide phenomenon. With increasing amounts of agricultural activities, growing population, and increasing industrialization, eutrophication is an unavoidable outcome in most freshwater lakes [1]. Eutrophication favors algal bloom formation and production of cyanobacterial toxins, such as microcystins (MCs). Microcystins are cyanotoxins that are detectable in freshwater bodies from most European and Asian nations as well as North America [2].

Microcystins are hepatotoxins produced primarily by the freshwater cyanobacterium *Microcystis aeruginosa*. They are monocyclic heptapeptides with approximately 60 structural variants formed by the variation in seven amino acids, primarily the L-amino acids at position 2, 3, 4, or 7 [3], which are responsible for their toxicity [4]. Microcystin-LR (MCLR), the most common and representative MC, contains the amino acids leucine and arginine. Its toxicity has been investigated in fish [5], birds, and mammals [6]. Exposure to MCLR resulted in progressive degeneration of the liver in salmon smolts (Net Pen Liver Disease) in coastal waters of British Columbia, Canada, and Washington, USA [7], as well as livestock poisoning and death [8–11]. In humans, exposure resulted in gastroenteritis and dermal contact irritations [12]. Human exposure to MCLR is primarily through ingestion of contaminated drinking water and by recreational contact with contaminated water [2], consumption of fish or blue-green algae products from contaminated water [13], or through accidental use of MCLR-contaminated water, as reported in Caruaru, Brazil, where renal dialysis patients exposed to MCLR had liver failure initially and then death (Caruaru syndrome) [14–16]. Recognizing its potential health effects, the World Health Organization (WHO) has set a provisional guideline of 1 μg/L for MCLR in freshwater [13].

Because widespread bloom formations and fish kills have been reported in water bodies of Texas, USA, during the past few years [17], the present study was performed to monitor MCLR concentrations during the period from 2003 to 2004 in two recreational lakes in western Texas, Buffalo Springs Lake (BSL) and Lake Ransom Canyon (LRC), which are quite similar in their geographic location but different in their dimensions, flow properties, and inhabitation. The purpose of the present study was to determine the levels and temporal fluctuations of MCLR and the relationship between MCLR concentration and a range of physical and chemical parameters.

MATERIALS AND METHODS

Materials

Purified protein phosphatase 2A (PP2A) enzyme was obtained from Upstate Cell Signaling Solutions (Lake Placid, NY, USA). The MCLR (purity, 98%) was obtained from Axorra LLC (San Diego, CA, USA). The 6,8-difluo-4-methylumbelliferyl phosphate (DiFMUP) was purchased from Molecular Probes (Eugene, OR, USA). The Quantiplate Microcystin Plate Kit was obtained from Envirologix (Portland, ME, USA). All other chemicals and reagents were obtained from Sigma (St. Louis, MO, USA) unless specified.

Study site

The chosen study sites, as shown in Figure 1, were the LRC and the BSL. The BSL is a major recreational lake near Lubbock, Texas, USA. It has a water area of 91.06 ha and is located 8.05 km east of Lubbock (at 33°32′N, 101°42′W) on the North Fork of Double Mountain Fork of Brazos River (Texas Parks and Wildlife Department, Austin, TX, USA). It has a maximum depth of 15.85 m and a mean depth of 4.57 m, with poor water

* To whom correspondence may be addressed (js.wang@ttu.edu).

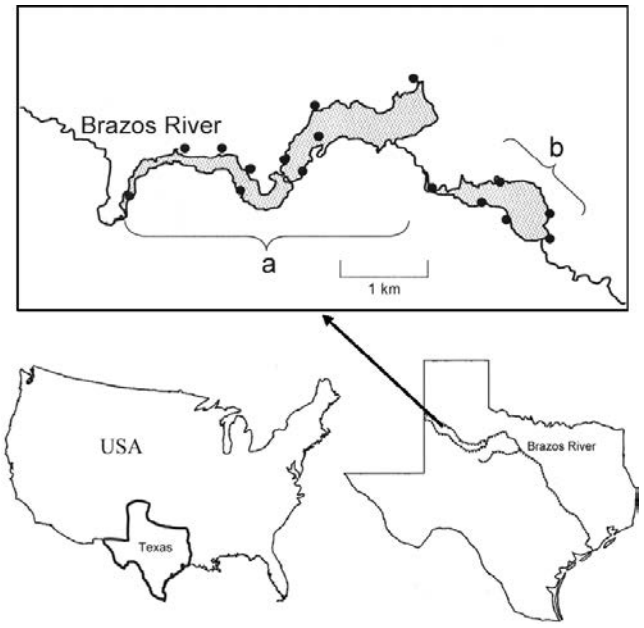

Fig. 1. Sampling points (●) in west Texas, USA, freshwater bodies. Buffalo Springs Lake (Lubbock, TX, USA) (a). Lake Ransom Canyon (Lubbock, TX, USA) (b).

quality and a visibility of 0.3 to 0.6 m. Ten sampling points were identified at equal distances based on a geographic map to represent the water area of the lake. Samples were collected in duplicate from each point to monitor changes in MCLR concentrations. The LRC is located downstream of the BSL (Fig. 1) [18] and has an elevation of 944.9 m and a water area of 28.25 ha. Six sampling points were identified to represent the lake, and duplicate samples were collected at each sampling site to monitor MCLR concentrations.

Sampling

Sampling was conducted in these two lakes from 2003 to 2004 at four different times of the year representing different seasons. Sampling was always conducted during the morning hours, generally between 9 AM and noon. The sampling points were fixed throughout the study year. One liter of water was collected at each point from the surface at a depth of 1 to 2 m using Nalgene® sampling bottles (Nalge Nunc, Rochester, NY, USA). Physical and chemical parameters were measured at each sampling.

Water parameters

Physical and chemical parameters, including temperature (°C), dissolved oxygen (DO; mg/L), salinity (ppt), and conductivity (μS/cm), were determined by YSI meter (Yellow Springs, OH, USA), and pH was determined by pH meter (Oakton Instruments, Vernon Hills, IL, USA). Determination of ammonia nitrogen (NH_3-N) was done by Nesslers method using a Hach® spectrophotometer (model DR/2010; Loveland, CO, USA). Analysis of reactive phosphorus in the water samples was conducted using the PhosVer3 method with a Hach spectrophotometer (model DR/2010; accepted by the U.S. Environmental Protection Agency [U.S. EPA] for reporting wastewater analysis–equivalent to U.S. EPA method 365.2 and standard method 4500-PE [19]) according to the manufacturer's protocol (Hach method 8048).

Sample preparation

Water samples were aliquoted immediately for NH_3-N and reactive phosphorus analysis and for sample preparation for enzyme-linked immunosorbent assay (ELISA) and protein phosphatase (PP) inhibition assays. Sample preparation for ELISA and PP inhibition assay involved repeated freeze–thaw cycles (twice) of a 10 ml sample, followed by sonication in a Branson 1510 Sonicator (Danbury, CT, USA) and centrifugation at 3,000 rpm for 10 min. The supernatant was collected, and the centrifugate was homogenized, sonicated for 10 min, and then centrifuged. This supernatant was pooled to produce a crude and clear extract that was immediately used in the assays.

Enzyme-linked immunosorbent assay

Following the manufacturer's protocol (EnviroLogix, Portland, ME, USA), 125 μl of blocking buffer were added into each antibody-coated well, and then 20 μl of water sample, standard solution, or distilled water blank were added, mixed, and incubated for 30 min at 37°C. One-hundred microliters of MCLR–enzyme conjugate were added and incubated for another 30 min to compete with MCLR-binding sites of antibody. The plate was emptied and washed four times with washing buffer containing phosphate-buffered saline–Tween 20 (pH 7.4; EnviroLogix) to remove all nonspecific binding. One-hundred microliters of substrate was added into each well and incubated for 30 min. The reaction was terminated by adding 100 μl of a stop solution (1 M HCl). The plate was then read at 450 nm with a reference wavelength of 630 nm in a microplate reader (Bio-Tek, Winooski, VT, USA). A semilog standard calibration curve was used to calculate the concentration of MCLR in water samples, with a detection limit of 0.15 μg/L.

Protein phosphatase inhibition assay

The PP inhibition assay was modified from procedures described by Bouaicha et al. [20]. The principle of the assay is that PP2A specifically removes phosphate groups from a fluorescent substrate, DiFMUP, to produce the 6,8-difluo-4-methylumbelliferyl (DiFMU) that was measured by the fluorometer. The MCLR specifically inhibits PP2A; therefore, the production of DiFMU was reduced, which was correlated with the concentrations of MCLR presented. Two-hundred microliters of sample solution or MCLR standards were buffered with 50 μl of 5× Tris-HCl buffer (200 mM Tris-HCl [Sigma], 170 mM $MgCl_2$, 20 mM ethylenediaminetetra-acetic acid [Sigma], and 20 mM dithiothreitol [Sigma]) in a 1.5-ml microcentrifuge tube, then mixed with 25 μl of 120 mU/ml of PP2A enzyme solutions made in 1× Tris-HCl buffer supplemented with 0.5 mg/ml of bovine serum albumin (Sigma). After incubation at 37°C for 5 min, 25 μl of DiFMUP substrate in 1× Tris-HCl was added and incubated at 37°C for 1 h. The production of DiFMU was measured in an *F* max fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation/emission wavelengths of 355/460 nm. All analysis was done in quadruplicate. The standard inhibition curve generated with different concentrations of MCLR was used to determine the level of MCLR in water samples, with a detection limit of 5.0 ng/L.

Statistical analysis

Results are expressed as the mean ± standard deviation of the data taken during each sampling occasion from the sam-

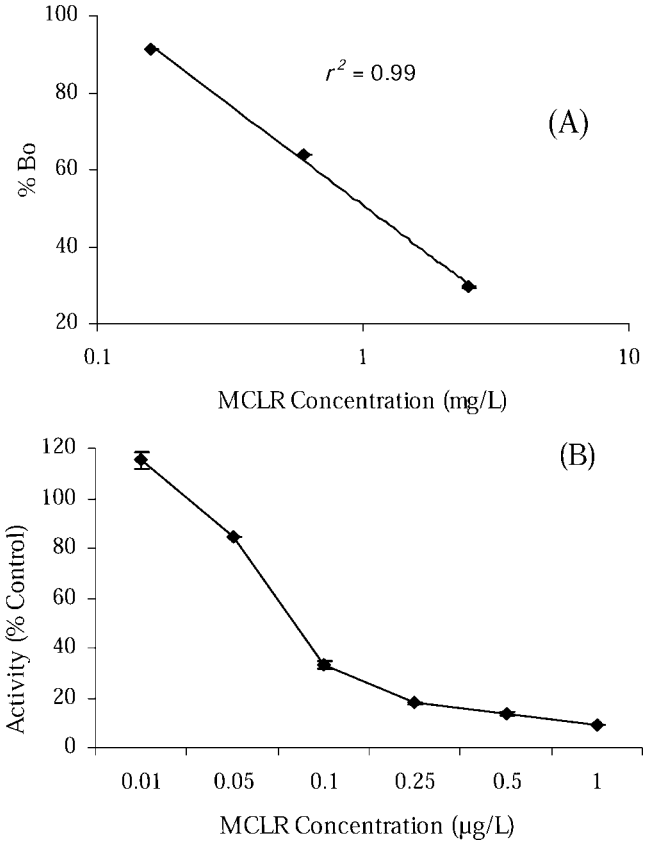

Fig. 2. Validation of microcystin-LR (MCLR) detection methods. (**A**) Semilog plot of enzyme-linked immunosorbent assay standard curve. (**B**) Standard curve of protein phosphatase inhibition assay. The % blank optical density (%Bo) = (OD of sample or calibrator/OD of negative control) × 100, where Bo is bound, %Bo is % antibody bound, and OD is the optical density.

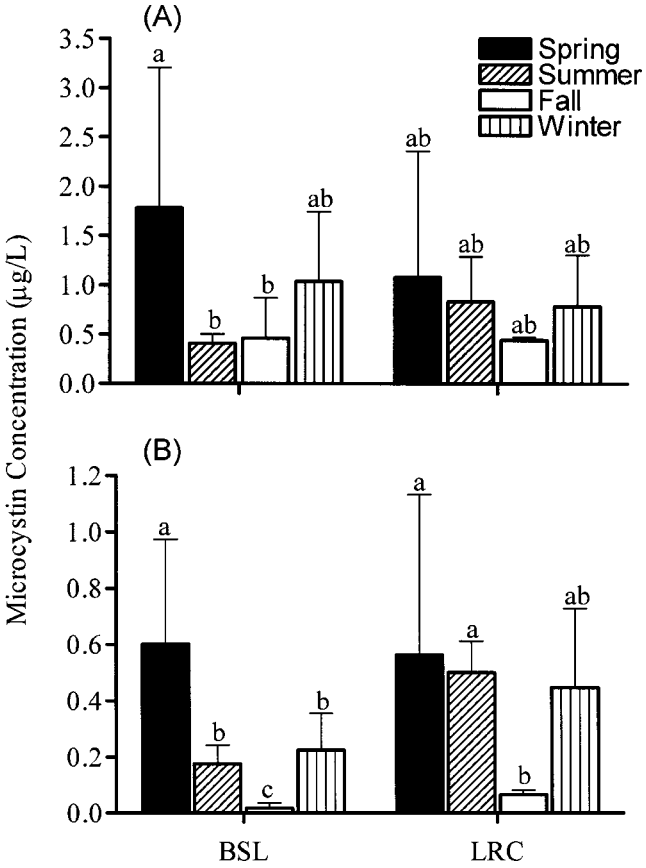

Fig. 3. Seasonal fluctuations of microcystin-LR during the period from 2003 to 2004 in Buffalo Springs Lake and Lake Ransom Canyon (both in Lubbock, TX, USA) as determined by (**A**) enzyme-linked immunosorbent assay and (**B**) protein phosphatase inhibition assay. Significant differences ($p < 0.05$) between the seasons in a lake were determined by Tukey's honestly significantly difference test. Adjacent letters indicate significant differences in pairwise comparison (c = significantly different from a and b; b = significantly different from a; ab = not significant).

pling stations in each lake. Probit analysis was used for determination of the median inhibitory concentration of MCLR to inhibit PP2A activity and to calculate the concentration of MCLR in water samples for the PP inhibition assay according to the standard curve generated. For ELISA, concentrations of MCLR in water samples were calculated from a regression equation of the standard curve. Comparisons of MCLR concentrations over different seasons were conducted by analysis of variance; when significant, post hoc tests (Tukey's honestly significantly difference) were conducted. Correlation and linear-regression analyses were conducted to determine the relationship of MCLR concentrations between PP inhibition assay and ELISA. All significance was set at $\alpha = 0.05$. All analysis was performed using SPSS software (Ver 12.0; SPSS, Chicago, IL, USA).

RESULTS

Development and validation of methods

Both the ELISA and PP inhibition assay were established, optimized, and validated with spiked and original water samples. The standard curve with antibody-based ELISA showed a linear correlation with various concentrations of MCLR (Fig. 2A). The inhibitory effect of MCLR on phosphatase activity was dose-dependent, with curves fitting the enzymatic sigmoid model (Fig. 2B). Spiking of water samples with MCLR before assay yielded 90% recovery.

Seasonal variations of MCLR concentrations in BSL

As shown in Figure 3, results from two assays indicated that 100% of the water samples collected from BSL in a whole-year period had detectable concentrations of MCLR. Great seasonal variations in MCLR concentrations were found. The maximum concentration of MCLR detected was in spring samples, and concentrations of MCLR as determined by ELISA were 1.78 ± 1.426 µg/L (range, 0.177–4.914 µg/L) (Fig. 3A), and 7 of 10 sites sampled (70%) had MCLR concentrations greater than 1.0 µg/L. The average MCLR concentration in water samples collected during the summer season was 0.407 ± 0.096 µg/L (range, 0.191–0.502 µg/L); none of the sites had concentrations greater than 1.0 µg/L. The average MCLR concentration in water samples collected during fall season was 0.463 ± 0.409 µg/L (range, 0.205–1.598 µg/L), and 1 of 10 sites had a MCLR concentration greater than 1.0 µg/L. The average MCLR concentration in water samples collected during the winter season was 1.036 ± 0.708 µg/L (range, 0.096–2.428 µg/L), and 4 of 10 sites (40%) sampled during this season had MCLR concentrations greater than 1.0 µg/L. In comparison, the MCLR concentrations in spring water samples were significantly higher than MCLR concentrations in summer and fall samples ($p < 0.05$). No statistical significance

was found for MCLR concentrations between spring and winter seasons.

Seasonal variations of MCLR concentrations in LRC

The MCLR concentrations, as detected by ELISA, in water samples collected during the spring season from LRC were 1.08 ± 1.55 μg/L (range, 0.2–5.83 μg/L) (Fig. 3A); two of six sampling points had MCLR concentrations greater than 1.0 μg/L. Average MCLR concentration in water samples collected during the summer season was 0.83 ± 0.45 μg/L (range, 0.315–1.671 μg/L), and one of six sampling sites had MCLR concentration greater than 1.0 μg/L. Concentration of MCLR in water samples collected during the fall season was 0.44 ± 0.06 μg/L (range, 0.368–0.555 μg/L); none of the sampling sites had MCLR concentrations greater than 1.0 μg/L. Concentration of MCLR in water samples collected during winter season was 0.782 ± 0.53 μg/L with a range of 0.225–2.130 μg/L, and one of six sampling sites had MCLR concentrations greater than 1.0 μg/L. Although the MCLR concentrations in spring water samples were higher than those in samples collected from other seasons, no statistical differences were found in the MCLR concentrations of LRC among seasons.

Comparisons of MCLR concentrations between lakes

The averaged annual MCLR concentrations, detected by both assays, in water samples collected from BSL were significantly higher than those from LRC ($p = 0.036$). No statistically significant difference were found in MCLR concentrations of water samples collected from both lakes in the spring season, although the average MCLR concentration was 1.65-fold higher in BSL than in LRC. However, the MCLR concentrations were significantly higher in BSL than in LRC in the winter ($p < 0.018$) season. No statistical significance was found between two lakes for the summer and fall seasons.

Correlations between ELISA and PP inhibition assay in detection of MCLR

Results detected by the PP inhibition assay from water samples of both lakes are shown in Figure 3B. Although the seasonal variation of MCLR concentrations was similar to that detected by ELISA, the absolute MCLR concentrations detected by the PP inhibition assay were far less than the concentrations detected by ELISA (Fig. 3A). Twofold differences were found across most of the samples. Correlation analysis of the combined samples from both lakes, as shown in Figure 4A, demonstrated high correlation between these two assays ($r = 0.82$, $p < 0.01$). Correlation analysis of samples collected from the individual lake—either BSL ($r = 0.847$, $p < 0.01$), as shown in Figure 4B, or LRC ($r = 0.923$, $p < 0.01$), as shown in Figure 4C—also revealed high correlation between both assays.

Correlation between selected physical and chemical parameters and MCLR concentrations

Means and standard deviations for selected parameters of both lakes are shown in Table 1. The levels of ammonia nitrogen ($p < 0.05$), DO ($p < 0.01$), and total reactive phosphorus ($p < 0.01$) and the conductivity ($p < 0.01$) were significantly different between the two study lakes. Correlation analysis between these physical and chemical parameters and detected MCLR concentrations was conducted. Pearson correlation coefficients (r) are shown in Table 2. For combined data from both lakes, the pH ($p < 0.01$) and temperature (p

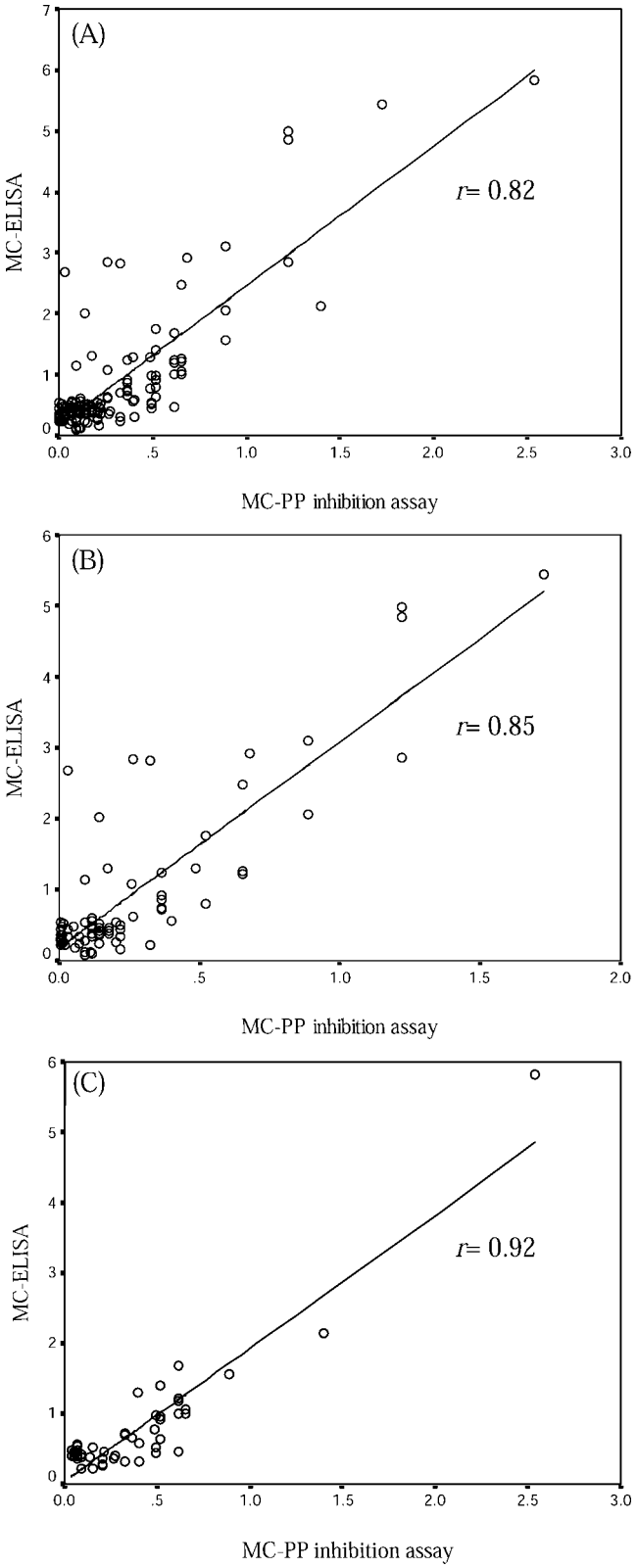

Fig. 4. Relationship between microcystin-LR (MCLR) concentration in lakes during the study period as determined by enzyme-linked immunosorbent assay (ELISA) and protein phosphatase (PP) inhibition assay. (**A**) MCLR concentration in both lakes taken together. (**B**) MCLR concentration in the Buffalo Springs Lake (Lubbock, TX, USA). (**C**) MCLR concentration in the Lake Ransom Canyon (Lubbock, TX, USA). Linear-regression plots are shown. The Pearson correlation coefficient is indicated by *r*. All correlations were significant at the 0.01 level (two-tailed).

Table 1. Physical and chemical parameters of water samples from west Texas, USA, lakes[a]

Parameters	Levels (mean ± standard deviation)[b] BSL	LRC
Ammonia nitrogen (mg/L)	0.22 ± 0.15 (0.02–0.59)*	0.19 ± 0.14 (0.04–0.5)
pH	7.52 ± 1.20 (5.38–8.6)	7.33 ± 1.59 (5.03–8.6)
Temperature (°C)	17.71 ± 7.91 (3.70–27.4)	18.04 ± 8.41 (4.9–28.1)
Dissolved oxygen (mg/L)	7.82 ± 1.94 (3.34–11.82)**	8.84 ± 1.77 (5.4–11.34)
Salinity (ppt)	0.92 ± 0.25 (0.60–1.7)	1.00 ± 0.18 (0.8–1.3)
Total reactive phosphorus (mg/L)	0.30 ± 0.16 (0.02–0.7)**	0.48 ± 0.18 (0.2–1.06)
Conductivity (μS/cm)	1,573 ± 216 (1,218–2,369)**	1,721 ± 192 (1,422–1,956)

[a] BSL = Buffalo Springs Lake (Lubbock, TX, USA); LRC = Lake Ransom Canyon (Lubbock, TX, USA).
[b] Values in parentheses represent the range, * $p < 0.05$; ** $p < 0.01$ compared to the levels of LRC.

< 0.05) of the water samples had a significant negative correlation with MCLR concentrations, whereas DO showed a significant positive influence ($p < 0.01$). Although other parameters did fluctuate over the seasons, no significant correlations with MCLR concentrations were found. As shown in Table 2, similar correlations also were found with MCLR concentrations in water samples collected from BSL, but not in those from LRC.

DISCUSSION

All the samples from both study lakes contained detectable concentrations of MCLR. The highest MCLR concentrations in both lakes were found in water samples collected in the spring, with 70% of BSL samples and 33% of LRC samples showing concentrations greater than 1 μg/L, the proposed WHO guideline for drinking-water sources [13]. Contamination with MCLR was higher in these water bodies than those published previously for other U.S. freshwater resources. Fifty-three of 92 surface-water samples collected in Minnesota, USA [21], and 25 of 102 samples in Wisconsin, USA [22], contained hepatotoxic cyanotoxins. Concentrations as high as 2.7 μg/L of MCLR in water samples collected from the Sassafras River was observed in Maryland, USA. Approximately 87% of water samples collected from Wisconsin in 1993 was contaminated with MCLR, with the highest concentration up to 200 μg/L [23]. In a study conducted from June 1996 to January 1998 in 24 public water systems of the United States and Canada, 80% of the water samples tested positive for MCLR. Of these, 4.3% had concentrations beyond the WHO guideline of 1.0 μg/L. Johnston and Jacoby [24] have reported MCLR concentrations from 0.19 to 3.8 μg/L in Lake Sammamish, western Washington, USA. Microcystin-LR also was reported in water samples collected from Lakes Onondaga, Oneida, and Champlain in New York, USA (G.L. Boyer et al., Second Annual Onondaga Lake Conference, Syracuse, NY, USA, November 20, 2000), and from lakes in Wisconsin and Florida, USA [25]. A variety of climactic and ecological factors may contribute to the higher concentrations of MCLR in BSL and LRC compared to those reported in previous studies, with the most obvious difference being that these two western Texas lakes are in areas that are more arid than those in the previous studies. Because this is, to our knowledge, the first report of MC concentrations in water bodies from arid or semiarid ecosystems in North America, more studies are needed to determine if climate and landscape play a part in determining the concentration of MCs in natural surface waters.

Table 2. Correlation of different physical and chemical parameters on microcystin-LR production[a]

Parameters	Pearson correlation coefficient (r) BSL	LRC	BSL + LRC
Dissolved oxygen	0.399**	0.056	0.270**
Temperature	−0.298**	−0.073	−0.223*
pH	−0.511**	−0.239	−0.390**
Conductivity	−0.13	0.166	−0.064
Salinity	−0.03	0.029	−0.26
Ammonia nitrogen	−0.019	−0.075	−0.029
Total reactive phosphorus	−0.128	−0.092	−0.131

[a] BSL = Buffalo Springs Lake (Lubbock, TX, USA); LRC = Lake Ransom Canyon (Lubbock, TX, USA). * $p < 0.05$; ** $p < 0.01$ (two-tailed).

Great seasonal variations in MCLR concentrations were found in both lakes with the highest MCLR concentrations in the spring season. The seasonal fluctuation of MCLR concentrations could be attributed to many factors. The occurrence of cyanobacteria was suggested to be dependent on different abiotic factors, such as temperature, light intensity, pH, turbulence, nutrients, and DO; however, production of MCLR was believed to be regulated by both abiotic and biotic factors [2]. The major MCLR-producing cyanobacteria, *Microcystis* spp., are less tolerant to cool temperature and shorter day length compared with other species [26]. In the present study, we found that the MCLR concentrations were negatively correlated with temperature (Table 2). Several previous studies reported that production of MCLR was maximal at a temperature range of 15 to 20°C and was minimal when temperature increased to 30°C or decreased to less than 10°C [27]. The present results were consistent with these reports. The highest MCLR concentrations were detected in the spring, when the average local temperature was 15.6°C. The lower MCLR concentration was found in the fall, when average local temperature reached 26.5°C. The lowest MCLR concentrations were found in winter, when average local temperature fell to less than 6°C. However, two other studies reported that the maximal production of MCLR was at 22 to 25°C [24,27]. The difference between studies may be caused by the variants of cyanobacterial strain in studied geographic areas, because certain differential strains could develop tolerance to different temperatures [26].

In addition to temperature, the present study found that other physical and chemical parameters, such as pH and DO in water samples, also were correlated with MCLR concentrations in both lakes (Table 2), which is in agreement with previously published reports [28,29]. Significant differences in levels of ammonia nitrogen, DO, total reactive phosphorus, and conductivity also were found in the two lakes (Table 1),

which may explain the difference in MCLR concentrations in these two lakes (Fig. 3). A previous study found that *M. aeruginosa* growth was influenced by levels of ammonia nitrogen, DO, and total reactive phosphorus and that high levels of nitrogen and phosphorus favor cyanobacterial bloom formation [30]. How these factors influence the production of MCLR, however, remains unclear. Some studies show that high levels of nitrogen and phosphorus increase the amount of toxin production [2,24,30,31]. Other studies show that a high nitrogen to phosphorus ratio increases MC concentration [28], whereas Utkilen and Gjolme [32] observed that limited conditions of nitrogen and phosphorus did not have any effect on toxin content in *M. aeruginosa*.

Many methods, including chemical, biological, and immunochemical assays, are available to measure cyanotoxins in water samples [6]. High-performance liquid chromatography (HPLC)–ultraviolet or liquid chromatography/mass spectrometry are the most common chemical analytical methods that can identify different types of cyanotoxins, including MCs. However, higher costs for sample extraction and cleanup limit this practice in field studies. The higher detection limit and the time involved also are disadvantages. Immunological methods, such as ELISA, can provide fast detection of many samples in field studies. Biochemical methods, such as the PP inhibition assay, are more sensitive than HPLC or ELISA, as demonstrated by the limit of detection for the PP inhibition assay (~5.0 ng/L) and the limit of detection for ELISA (0.15 μg/L). In the present study, we used both ELISA and PP inhibition assay to measure water samples collected from both lakes. Although differences were found in absolute values of MCLR (Fig. 3), Pearson correlation analysis (Fig. 4) confirmed that MCLR concentrations measured by the two assays were highly correlated.

In conclusion, the potent cyanotoxin MCLR was present in detectable amounts in water bodies of western Texas. The MCLR concentrations are greater in water samples collected in the spring, when it is a peak time for recreational activities.

Acknowledgement—The present study was supported by the National Institute of Health grant CA94683 and by the research contract DAAD 13-02-C0070 from Soldiers Biological Chemical Command, U.S. Army, Department of Defense. We would like to acknowledge the help of Todd Anderson, Christopher McKean, and Fujun Liu during the study and manuscript preparation.

REFERENCES

1. Tusseau-Vuillemin MH. 2001. Do food processing industries contribute to the eutrophication of aquatic systems? *Ecotoxicol Environ Saf* 50:143–152.
2. Chorus I, Bartram J. 1999. *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring, and Management.* E&FN Spon, London, UK.
3. Carmichael WW. 1986. Algal toxins. In Callow JA, ed, *Advances in Botanical Research,* Vol 12. Academic, London, UK, pp 47–101.
4. Dawson RM. 1998. The toxicology of microcystins—Review article. *Toxicon* 36:953–962.
5. Fischer WJ, Hitzfeld BC, Tencalla F, Eriksson JE, Mikhailov A, Dietrich DR. 2000. MCLR toxicodynamics, induced pathology, and immunohistochemical localization in livers of blue-green algae exposed rainbow trout (*Oncorhynchus mykiss*). *Toxicol Sci* 54:365–373.
6. Matsunaga H, Harada KI, Senma M, Ito Y, Yasuda N, Ushida S, Kimura Y. 1999. Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: Sudden appearance of toxic cyanobacteria. *Nat Toxins* 7:81–84.
7. Anderson RJ, Luu HA, Chen DZ, Holmes CF, Kent M, LeBlanc M, Taylor FJ, Williams DE. 1993. Chemical and biological evidence links microcystins to salmon 'netpen liver disease.' *Toxicon* 31:1315–1323.
8. Zin LL, Edwards WC. 1979. Toxicity of blue-green algae in livestock. *Bovine Pract* 14:151–153.
9. Galey FD, Beasley VR, Carmichael WW, Kleppe G, Hooser SB, Haschek WM. 1987. Blue-green algae (*Microcystis aeruginosa*) hepatotoxicosis in dairy cows. *Am J Vet Res* 48:1415–1419.
10. Carmicheal WW, Schwartz LD. 1994. Prevention of livestock death from blue-green algae poisoning. *U.S. Department of Agriculture Farmers' Bulletin* 2275:1–12.
11. Puschner B, Galey FD, Johnson B, Dickie CW, Vondy M, Francis T, Holstege DM. 1998. Blue-green algae toxicosis in cattle. *J Am Vet Med Assoc* 213:1605–1607.
12. Rao PV, Gupta N, Bhaskar AS, Jayaraj R. 2002. Toxins and bioactive compounds from cyanobacteria and their implications on human health. *J Environ Biol* 23:215–224.
13. World Health Organization. 1998. Cyanobacterial toxins: Microcystin-LR. In *Guidelines for Drinking Water Quality,* 2nd ed, Addendum to Vol 2. Health Criteria and Other Supporting Information. Geneva, Switzerland, pp 95–110.
14. Pouria S, de Andrade A, Barbosa J, Cavalcanti RL, Barreto VT, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA. 1998. Fatal microcystin intoxication in hemodialysis unit in Caruaru, Brazil. *Lancet* 352:21–26.
15. Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CE, Antunes MB, de Melo Filho DA, Lyra TM, Barreto VS, Azevedo SM, Jarvis WR. 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *N Engl J Med* 338:873–878.
16. Carmichael WW, Azevedo SM, An JS, Molica RJ, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK. 2001. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. *Environ Health Perspect* 109:663–668.
17. Smith VH. 2001. Blue-green algae in eutrophic fresh waters. *Lakeline* Spring:34–37.
18. Tyler R, Barnett DE, Barkley RR, Anderson PC, Odintz MF. 1996. *The New Handbook of Texas*. Texas State Historical Association, Austin, TX, USA.
19. U.S. Environmental Protection Agency. 1983. Methods for the Chemical Analysis of Water and Wastes. EPA-600/4-79/020. Washington, DC.
20. Bouaicha N, Maatouk I, Vincent G, Levi Y. 2002. A colorimetric and fluorometric microplate assay for the detection of microcystin-LR in drinking water without preconcentration. *Food Chem Toxicol* 40:1677–1683.
21. Olson TA. 1960. Water poisoning—A study of poisonous algae blooms in Minnesota. *Am J Public Health* 50:883–884.
22. Repavich WM, Sonzogni WC, Standridge JH, Wedepohl RE, Meisner LF. 1990. Cyanobacteria (blue-green algae) in Wisconsin waters: Acute and chronic toxicity. *Water Res* 24:225–231.
23. McDermott CM, Feola R, Plude J. 1995. Detection of cyanobacterial toxins (microcystins) in waters of northeastern Wisconsin by a new immunoassay technique. *Toxicon* 33:1433–1442.
24. Johnston BR, Jacoby JM. 2003. Cyanobacterial toxicity and migration in a mesotrophic lake in western Washington, USA. *Hydrobiologia* 495:79–91.
25. Williams CD, Chapman A, Carmichael W, Pawlowicz M, Burns J. 2000. Cyanotoxic algal blooms in Florida's lakes, rivers, and tidal river estuaries. *Proceedings*, Florida Lake Management Society, 2000 Annual Conference, Duck Key, FL, USA, May 22–25, pp 26–27.
26. Orr PT, Jones GJ, Douglas GB. 2004. Response of cultured *M. aeruginosa* from the Swan River, Australia, to elevated salt concentration and consequences for bloom and toxin management in estuaries. *Mar Freshw Res* 55:277–283.
27. Codd GA, Poon GK. 1988. Cyanobacterial toxins. *Proc Phytochem Soc Eur* 28:283–296.
28. Van der Westhuizen AJ, Eloff JN. 1985. Effect of temperature and light on the toxicity and growth of blue-green alga *M. aeruginosa* (UV-006). *Planta* 163:55–59.
29. Richard JW, Peter GT. 1990. Environmental factors affecting the production of peptide toxins in floating scums of cyanobacterium *M. aeruginosa* in hypertrophic African reservoir. *Environ Sci Technol* 24:1413–1418.
30. Cheng K, Zhao YJ, Xu M, Yin J, Xiong Q, Zhou JC, Wang YZ, Zhang W, Wang JW, Liu ZY, Wang FR. 2002. An ecological study

on dynamics of toxic *Microcystis* blooms in a eutrophic park pond, Wuhan, China. *Progress in Natural Science* 12:30–35.
31. Kotak BG, Lam AK-Y, Prepas EE, Hrudey SE. 2000. Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes. *Can J Fish Aquat Sci* 57:1584–1593.
32. Utkilen H, Gjolme N. 1995. Iron-stimulated toxin production in *Microcystis aeruginosa*. *Appl Environ Microbiol* 61:797–800.