doi:10.20944/preprints201907.0064.v1

Article

Microcystins and Daily Sunlight: Predictors of Chronic Liver Disease and Cirrhosis Mortality

Rajesh Melaram[1]

[1] Walden University; rajesh.melaram@waldenu.edu

Abstract: Cyanobacteria (blue-green algae) may rapidly propagate under favorable conditions, forming dense blooms. As water blooms deteriorate, blue-green algae can generate potent toxins, potentially harmful to companion animals, wildlife, and even humans. One widely recognized cyanobacterial toxin is microcystin. This algal toxin has been implicated in surface waters globally, increasing liver cancer and/or disease risk amongst those who depend on sources prone to microcystin contamination. Interestingly, no study looked at weather conditions when connecting liver health outcomes to freshwater cyanotoxins. The purpose of this study was to determine if climate was an important determinant of liver mortality and total microcystins at the ecological level. Secondary data was used to evaluate the proposed hypothesis. Environmental data (CDC WONDER) and toxin data (USEPA) were used in multivariate regression analysis. Mean daily sunlight and total microcystins were significant predictors of age-adjusted chronic liver disease and cirrhosis death rates ($p < 0.05$). Mean annual precipitation ($p = 0.156$) and mean daily max temperature (0.149) were non-significant predictors. This study demonstrated how microcystins in combination with climate may increase liver mortality. The results can prompt others to study environmental exposures of terminal liver diseases, guiding environmental health and the water industry of human survival needs.

Keywords: microcystins; climatic factors; chronic liver disease and cirrhosis; daily sunlight; enzyme-linked immunosorbent assay

Key Contribution: Ecological study indicates chronic liver disease and cirrhosis mortality rates may be induced by microcystins and daily sunlight.

1. Introduction

Microcystins are cyclic heptapeptide structures produced by cyanobacteria in aquatic environments [1,2]. These cyanobacterial toxins may be emitted upon cell lysis [3] or apoptosis [3,4] during algal bloom senescence [5]. *Microcystis* is the main producer of microcystin [2, 6-7], but other toxic cyanobacterial genera can release the biotoxin [6-10]. Such toxins have shown to contaminate water sources used for agriculture, drinking water, and recreation [11,12]. Additionally, microcystin-related mortalities in animals, livestock, and pets have been documented [13,14]. Though rare, the largest episode of human microcystin poisoning occurred in Brazil [15-19], where 52 hemodialysis patients died from a common syndrome known as Caruaru Syndrome [15,16].

Ingestion of contaminated drinking water is the most common route of microcystin exposure [20,21]. The cyanotoxin is transported via the bile acid transport system to mammalian liver [22-25], inactivating protein phosphatases [26]. Consequently, toxin accumulation causes cytoskeletal proteins to become hyperphosphorylated, which can have deleterious effects on the cell, including alterations in hepatocyte structure, degradation of cytoskeleton elements, and cell contacts and hemorrhages to form [27,28]. Liver cancer in hepatocyte culture was found to be initiated by cytokeratin hyperphosphorylation and protein phosphatase inhibition [29,30].

Several epidemiological studies have associated microcystin levels and liver cancer and/or disease [31-36]. Two surveys identified blue-green algal toxins in drinking water sources as a potential risk factor for primary liver cancer [31]. Increased colorectal cancer incidence was related to consumption of microcystin-contaminated pond and river water [32]. A pilot investigation correlated hepatocellular carcinoma risk with surface water proximity [33]. Childhood liver disease was linked to contaminated drinking water in freshwater lakes of Three Gorges Region, China [34]. A county ecological study demonstrated a relationship between cyanobacterial bloom distribution in the contiguous United States and non-alcoholic fatty liver disease [35]. On the contrary, surrogate markers of freshwater cyanoblooms lacked association with liver cancer in Canada [36].

None of the studies evaluated microcystin concentrations in tandem with climatic factors to liver mortality. Global climate change is a key contributor to cyanobacterial expansion worldwide [37]. Many environmental factors influence microcystin production [38,39], such as high temperatures, increased alkalinity, and stagnant waters [40]. Fossil fuel emissions and concomitant air temperatures may enhance algal productivity. Variations in weather patterns, resulting in severe droughts and rainfall, could potentially leach nitrates and phosphates into eutrophic waters. Climatic factors can stir toxic algae blooms while increasing biological oxygen demand in ecosystems [41].

This may be the first attempt to predict age-adjusted chronic liver disease and cirrhosis mortality rates based on microcystin levels and climate exposure variables. The aim of the study was to assess whether the ecologic association between liver mortality and total microcystins is dependent on climatic factors.

2. Results

2.1. Total Microcystins and Climatic Factors

2.1.1 Census Region

Table 1 displays a summary of mean total microcystins and climatic factors by census region in 2007. Mean total microcystins was highest in the Midwest, with a concentration of 1.89 µg/L. The South and West had comparable mean total microcystins of 1.02 µg/L and 1.10 µg/L, respectively. The lowest mean total microcystins was in the Northeast, at 0.302 µg/L. Mean daily max temperature ranged between 56.71 in the Midwest to 69.54 in the South. Mean daily precipitation ranged from 1.62 mm in the West to 2.96 mm in the Northeast. Mean daily sunlight ranged between 16502.70 KJ/m^2 in the South and 17216.87 KJ/m^2 in the West.

Table 1. Summary of mean total microcystins above 0.10 µg/L and mean climatic factors by census region in 2007.

Census Region	Mean Total Microcystins (µg/L)	Microcystin WHO Category	Mean Daily Max Temperature (F)	Mean Daily Precipitation (mm)	Mean Daily Sunlight (KJ/m^2)
South	1.02	1	69.54	2.64	16502.70
Northeast	0.302	1	57.99	2.96	15575.05
Midwest	1.89	1	56.71	2.59	15097.44
West	1.10	1	63.85	1.62	17216.87

WHO = World Health Organization. Category 1 = < 10 µg/L. F = Fahrenheit, mm = millimeters, KJ/m^2 = Kilojoule/square meter

2.1.2 State

Mean total microcystins and mean climatic factors by state in 2007 are depicted in Table 2. The mean total microcystins for the 43 states was 0.865 µg/L. The lowest mean total microcystins was 0.20 µg/L (Missouri), and the highest mean total microcystins was 18.18 µg/L (North Dakota). 41 of 43 states (95.35%) had a microcystin WHO category of 1, while 2 states had a microcystin WHO category of 2, comprising the remaining 4.65%. For climatic factors, mean daily max temperature was 69.64 F, mean daily precipitation was 2.64, and mean daily sunlight was 16502.70 KJ/m².

Table 2. Summary of mean total microcystins above 0.10 μg/L and mean climatic factors by state in 2007.

State	Mean Total Microcystins (μg/L)	Microcystin WHO Category	Mean Daily Max Temperature (F)	Mean Daily Precipitation (mm)	Mean Daily Sunlight (KJ/m²)
Alabama	0.33	1	76.7	2.43	17761.61
Arizona	0.885	1	72.72	0.85	19804.18
Arkansas	1.00	1	73.29	3.19	16681.82
California	0.22	1	69.8	0.99	19698.04
Colorado	2.73	1	56.59	1.36	17497.51
Connecticut	0.343	1	57.61	3.11	15452.60
Delaware	0.58	1	63.74	2.48	16249.63
Florida	1.62	1	81.11	3.09	18945.54
Georgia	0.31	1	76.64	2.47	18231.50
Idaho	3.04	1	54.14	1.35	16188.47
Illinois	1.47	1	64.0	2.56	15591.87
Indiana	0.55	1	63.26	2.93	15603.23
Iowa	0.69	1	59.11	2.82	15311.84
Kansas	0.98	1	66.5	2.57	16770.71
Kentucky	0.76	1	68.36	2.89	16220.59
Louisiana	0.631	1	77.68	3.71	17654.09
Maine	0.845	1	48.95	3.25	14242.49
Maryland	0.267	1	63.6	2.48	16034.71
Massachusetts	0.903	1	55.62	3.06	15315.42
Michigan	1.26	1	54.77	2.09	14985.34
Minnesota	1.79	1	53.2	1.83	14622.10
Mississippi	0.465	1	76.79	2.87	17554.24
Missouri	0.20	1	66.58	2.92	15957.14
Montana	1.27	1	54.0	1.30	15080.89
Nebraska	4.52	1	61.93	2.11	16054.05
Nevada	0.53	1	60.96	0.54	18346.94
New Jersey	0.703	1	61.32	3.25	15758.56
New York	0.593	1	53.46	3.06	14393.31
North Carolina	0.266	1	70.8	2.34	17402.86
North Dakota	18.18	2	53.65	1.40	14816.28
Ohio	13.91	2	61.55	2.75	15197.93
Oklahoma	1.03	1	70.87	3.09	16921.44
Oregon	1.18	1	56.68	1.84	16404.71
Pennsylvania	1.17	1	57.74	2.91	14594.54
Rhode Island	0.26	1	58.37	2.81	15697.50
South Dakota	2.53	1	58.8	1.61	15374.59
Tennessee	0.75	1	71.32	2.40	16648.09
Texas	2.48	1	76.91	2.52	17999.03
Utah	6.94	1	59.63	0.85	17701.46
Virginia	0.691	1	66.69	2.35	16634.94
Washington	1.14	1	54.98	2.39	14629.55

West Virginia	1.7	1	62.37	2.85	15243.78
Wisconsin	0.735	1	54.3	2.35	14883.03

WHO = World Health Organization. Category 1 = < 10 μg/L. Category 2 = F = Fahrenheit, mm = millimeters, KJ/m^2 = Kilojoule/square meter

2.2. Regression Models

Multiple linear regression was run to assess the predictive function of climatic factors and total microcystins on age-adjusted chronic liver disease and cirrhosis death rates. All predictors were initially incorporated into the model. Results for the A positive association was observed between total micocystins, climate exposure variables, and liver mortality (R = 0.726). Approximately 46.4% (R^2 = 0.464) of variance in age-adjusted chronic liver disease was explained by the predictors. It partially supported the hypothesis that climatic factors in concurrence with total microcystins predict liver mortality (Table 3). The stepwise method was selected to determine which explanatory variables fitted the regression model. In Table 3, the final model revealed a positive correlation among mean daily sunlight, total microcystins, and age-adjusted chronic liver disease and cirrhosis death rates (R = 0.676 and R^2 = 0.423). Mean daily max temperature and mean daily precipitation were not statistically significant predictors ($p > 0.05$) (Table 4).

Table 3. Multivariable regressions of exposure correlates of liver mortality rates in the U.S.

Model	R	R^2	F-change
Enter	0.726	0.464	0.000117
Stepwise	0.676	0.423	0.009

Table 4. Coefficients of predictors of liver mortality

Variables	Standardized Coefficients Beta	Significance
Total Microcystins	0.365	0.009
Daily Sunlight	0.621	0.000044
Daily Max Temperature	-0.290	0.149
Daily Annual Precipitation	-0.188	0.156

3. Discussion

This was perhaps the first investigation to consider the role of climate exposure variables in conjunction with microcystin concentrations relative to liver disease-associated mortality. The results highlighted a potential correlation between total microcystins, mean daily sunlight, and age-adjusted chronic liver disease and cirrhosis death rates. Warming climate is expected to promote cyanobloom formation worldwide [42]. Since warm temperatures increase microcystin production in waterbodies [40] and earlier work has identified the cyanotoxins in areas of increased liver cancer/disease prevalence [31,32], then a possibility exists that both factors co-exist to impact health. However, more research on microcystins and climatic variables is needed to justify this proposition.

The study findings reflect and extend upon others in reference to microcystins and fatal liver disease. Liver cancers were attributed to drinking water sources tainted with microcystin [31-33]. Enzyme-linked immunosorbent assay (ELISA) was used to quantify total microcystins in these studies. This study integrated accessible USEPA ELISA data rather than using individually performed toxin measurements. Cyanotoxin analysis can often pose challenges (i.e., concentrated samples, interference, etc.). Hence, the study offers a feasible method to analyze potential relationships between microcystins and liver mortality. Additionally, coverage of cyanobacterial

doi:10.20944/preprints201907.0064.v1

bloom contamination was connected to non-alcoholic liver disease mortality [35]. This study was similar in that liver mortality correlated to microcystin polluted waters. The difference was the contribution of weather conditions in the assessment.

There were several limitations inherent in the study. First, it was an ecological analysis, so the hypothesized relationship is relevant to populations as opposed to individuals. That is, one is unable to assume that an individual succumbs to liver disease in the wake of microcystin exposure. Second, confounding bias resulted from omitted liver mortality risk factors. Failure to account for recognized attributes can either increase or decrease the effect of the exposure variable. The inclusion of cigarette smoking and alcohol consumption could have strengthened the study. Furthermore, the data in the study were obsolete. Data on microcystins is limited and restricted. Thus, it was imperative to maintain consistency in using data on pertinent variables which aligned with the USEPA data.

In conclusion, total microcystins and mean daily sunlight correlated with age-adjusted chronic liver disease and cirrhosis death rates. The explained causal effect does not imply causation. Whether microcystin toxicity and climate affect liver disease mortality merits further exploration. Future work should assess environmental and lifestyle factors of chronic liver disease and cirrhosis, including hepatotoxins. This may aid public health and water municipalities in attaining human necessities.

4. Materials and Methods

Secondary data on total microcystins was collected from the 2007 United States Environmental Protection Agency (USEPA) National Lakes Assessment. Total microcystins was determined by the enzyme-linked immunosorbent assay (ELISA) method (Abraxis, LLC, Warminster, PA). The limit of detection was 0.10 µg/L. 7 states (Alaska, Hawaii, New Hampshire, New Mexico, South Carolina, Vermont, Wyoming) were excluded from the analysis due to non-detectable levels or absence in the original dataset. Detectable levels were averaged for repeated measurements and combined with individual ones to create a composite average.

Environmental data on annual precipitation, average daily max temperature, daily precipitation, and daily sunlight, derived from the North America Land Data Assimilation System (NLDAS) (1979-2011), was gathered from the Centers for Disease Control and Prevention Wide-ranging Online Data for Epidemiologic Research (CDC WONDER). Data were obtained for the year 2007 to coincide with total microcystins.

The Underlying Cause of Death database was utilized to retrieve age-adjusted chronic liver disease and cirrhosis death rates of the continental United States for the 2003-2007 period. The International Classification of Disease, Tenth Revision (ICD-10) 113 Cause List was used to examine records of age-adjusted chronic liver disease and cirrhosis death rates (K70, K73-K74). All ages, genders, origins, and races were selected in the demographics of age-adjusted chronic liver disease and cirrhosis death rates.

Statistical Package for the Social Sciences (SPSS) version 25, was employed to conduct multivariate analyses. Normality was achieved by log-transforming (base 10) all variables in the analysis. Further examination identified extraneous variables within the dataset. Removal of the outliers resulted in a total of 35 states in the final analysis (Table A1). Statistical significance was determined if $p < 0.05$. Descriptives were grouped by census region and state. Inferential statistics were applied to aggregated national data.

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

Appendix A

Table A1. Summary of mean total microcystins, average age-adjusted chronic liver disease and cirrhosis death rates, mean daily max temperature, and mean daily precipitation.

State	Mean Total Microcystins (µg/L)	Age-Adjusted Chronic Liver Disease and Cirrhosis Death Rates Per 100,000 (2003-2007)	Mean Daily Max Temperature (F)	Mean Daily Precipitation (mm	Mean Daily Sunlight (KJ/m^2)
Alabama	0.33	9.6	76.7	2.43	17761.61
Arizona	0.885	11.9	72.72	0.85	19804.18
Arkansas	1.00	8.0	73.29	3.19	16681.82
California	0.22	11.2	69.8	0.99	19698.04
Colorado	2.73	9.9	56.59	1.36	17497.51
Connecticut	0.343	7.5	57.61	3.11	15452.60
Delaware	0.58	8.6	63.74	2.48	16249.63
Florida	1.62	10.5	81.11	3.09	18945.54
Georgia	0.31	8.0	76.64	2.47	18231.50
Idaho	3.04	9.1	54.14	1.35	16188.47
Illinois	1.47	8.2	64.0	2.56	15591.87
Indiana	0.55	7.6	63.26	2.93	15603.23
Iowa	0.69	6.2	59.11	2.82	15311.84
Kansas	0.98	7.4	66.5	2.57	16770.71
Kentucky	0.76	8.3	68.36	2.89	16220.59
Louisiana	0.631	7.9	77.68	3.71	17654.09
Maine	0.845	8.4	48.95	3.25	14242.49
Maryland	0.267	7.5	63.6	2.48	16034.71
Massachusetts	0.903	7.8	55.62	3.06	15315.42
Michigan	1.26	9.4	54.77	2.09	14985.34
Minnesota	1.79	6.4	53.2	1.83	14622.10
Mississippi	0.465	8.7	76.79	2.87	17554.24

Missouri	0.20	7.0	66.58	2.92	15957.14
Montana	1.27	11.0	54.0	1.30	15080.89
Nevada	0.53	11.1	60.96	0.54	18346.94
New Jersey	0.703	7.6	61.32	3.25	15758.56
New York	0.593	6.3	53.46	3.06	14393.31
North Carolina	0.266	8.7	70.8	2.34	17402.86
Oklahoma	1.03	11.2	70.87	3.09	16921.44
Oregon	1.18	10.3	56.68	1.84	16404.71
Pennsylvania	1.17	7.6	57.74	2.91	14594.54
Rhode Island	0.26	9.4	58.37	2.81	15697.50
South Dakota	2.53	10.7	58.8	1.61	15374.59
Tennessee	0.75	10.0	71.32	2.40	16648.09
Texas	2.48	11.4	76.91	2.52	17999.03
Virginia	0.691	7.4	66.69	2.35	16634.94
Washington	1.14	9.0	54.98	2.39	14629.55

References

1. Blàha, L.; Babica, P, Maršàlek, B. Toxicity produced in cyanobacterial water blooms – toxicity and risks. *Interdiscip Toxicol* **2009**, 2, 36-41. doi: 10.2478/v10102-009-0006-2
2. Sivonen, K., Jones, G. Cyanobacterial toxins. In *Toxic Cyanobacteria in Water: A Guide to Public Health Significance, Monitoring and Management*; Chorus, I., Bertram, J., Eds.; The World Health Organization: London, UK, 1999; pp. 41-111.
3. Environmental Protection Agency (EPA). *Water Treatability Database*; EPA: Washington D.C., 2007.
4. Lone, Y., Koiri, R.K., Bhide, M. An overview of the toxic effect of potential human carcinogen microcystin-LR on testis. *Toxicol Rep* **2015**, 2, 289-296. doi: 10.1016/j.toxrep.2015.01.008
5. Schmidt, J.R., Wilhelm, S.W., Boyer, G.L. The fate of microcystins in the environment and challenges for monitoring. *Toxins* **2014**, 6, 3354-3387. doi: 10.3390/toxins6123354
6. Carmichael, W.W. Cyanobacteria secondary metabolites – the cyanotoxins. *J Appl Bacteriol* **1992**, 72, 445-459. doi: 10.1111/j.1365-2672.1992.tb01858.x
7. Pineda-Mendoza, R.M., Zúñiga, Martínez-Jerónimo, F. Microcystin production in M*icrocystis aeruginosa*: effect of type of strain, environmental factors, nutrient concentrations, and N:P ratio on *mcyA* gene expression. Aquat Ecol **2016**, 50, 103-119. doi: 10.1007/s10452-015-9559-7
8. Codd, G., Bell, S., Kaya, K., Ward, C., Beattie, K., Metcalf, J. Cyanobacterial toxins, exposure routes and human health. Eur J Phycol **1999**, 34, 405-415. doi: 10.1080/09670269910001736462
9. Pflugmacher, S., Codd, G.A., Steinberg, C. Effects of the cyanobacterial toxin microcystin-LR on detoxication enzymes in aquatic plants. Environ Toxicol **1999**, 14, 111-115. doi: 10.1002/(SICI)1522-7278(199902)14:1<111::AID-TOX14>3.0.CO;2-3
10. Pflugmacher, S., Wiegand, C. Metabolism of microcystin-LR in aquatic organism. In *Cyanotoxins*; Chorus, I. Springer, Berlin; pp. 257-260.
11. Saqrane, S., Oudra, B. CyanoHab occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins **2009**, 1, 113-122. doi: 10.3390/toxins1020113
12. Hilborn, E.D., Beasley, V.R. One health and cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. Toxins **2015**, 7, 1374-1395. doi: 10.3390/toxins7041374
13. Carmichael, W.W., Boyer, G.L. Health impacts from cyanobacteria harmful algae blooms: implications for the North American Great Lakes. Harmful Algae **2016**, 54, 194-212. doi: 10.1016/j.hal.2016.02.002
14. Backer, L.C., Miller, M. Sentinel animals in a one health approach to harmful cyanobacterial and algal blooms. *Vet Sci* **2016**, 3, 8. doi: 10.3390/vetsci3020008
15. Azevedo, S.M., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., Eaglesham, G.K. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. Toxicology **2002**, 181-182, 441-446. doi: 10.1016/S0300-483X(02)00491-2
16. Carmichael, W.W., Azevedo, S.M., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ Health Perspect **2001**, 109, 663-668. doi: 10.1289/ehp.01109663
17. Hitzfield, B.C., Höger, S.J., Dietrich, D.R. Cyanobacterial toxins: removal during drinking water treatment, and human risk assessment. Environ Health Perspect **2000**, 108, 113-122. doi: 10.1289/ehp.00108s1113
18. Jochimsen, E.M., Carmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E., Antunes, M.B., de Melo Filho, D.A., Lyra, T.M., Barreto, V.S., Azevedo, S.M., Jarvis, W.R. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. N Engl J Med **1998**, 338, 873-878. doi: 10.1056/NEJM199803263381304
19. Pouria, S., de Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.T., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H., Codd, G.A. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet **1998**, 352, 21-26. doi: 10.1016/S0140-6736(97)12285-1
20. Greer, B., Meneely, J.P., Elliot, C.T. Uptake and accumulation of microcystin-LR based on exposure through drinking water: an animal model assessing the human health risk. Sci Rep **2018**, 8. doi: 10.1038/s41598-018-23312-7
21. Zanchett, G., Oliveira-Filho, E.C. Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects. Toxins **2013**, 5, 1896-1917. doi: 10.3390/toxins5101896

22. Eriksson, J.E., Grönberg, L., Nygård, S., Slotte, J.P., Meriluoto, J.A. Hepatocellular uptake of ^{3}H-dihydromicrocystin-LR, a cyclic peptide toxin. Biochim Biophys Acta **1990**, 1025, 60-66. doi: 10.1016/0005-2736(90)90190-Y
23. Runnegar, M., Berndt, N., Kaplowitz, N. Microcystin uptake and inhibition of protein phosphatases: effects of chemoprotectants and self-inhibition in relation to known hepatic transporters. Toxicol Appl Pharmacol **1995**, 134, 264-272. doi: 10.1006/taap.1995.1192
24. Falconer, I.R. *Cyanobacterial toxins of drinking water supplies*, 1st ed. Taylor & Francis: London, UK, 2004; pp. 1-296.
25. Pearson, L., Mihali, T., Moffitt, M., Kellman, R., Neilan B. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Mar Drugs **2010**, 8, 1650-1680. doi: 10.3390/md8051650
26. Erikkson, J.E., Toivola, D., Meriluoto, J.A., Karaki, H., Han, Y.G., Hartshorne, D. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem Biophys Res Commun **1990**, 173, 1347-1353. doi: 10.1016/S0006-291X(05)80936-2
27. Honkanen, R.E., Zwiller, J., Moore, R.E., Daily, S., Khatra, B.S., Dukelow, M., Boynton, A.L. Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases. J Biol Chem **1990**, 265, 19401-19404.
28. MacKintosh, C., Beattie, K.A., Klumpp, S., Cohen, P., Codd, G.A. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett **1990**, 264, 187-192. doi: 10.1016/0014-5793(90)80245-E
29. Ohta, T., Nishiwaki, R. Yatsunami, J., Komori, A., Suganuma, M., Fujiki, H. Hyperphosphorylation of cytokeratins 8 and 18 by microcystin-LR, a new liver tumor promoter, in primary cultured rat hepatocytes. Carcinogenesis **1992**, 13, 2443-2447. doi: 10.1093/carcin/13.12.2443
30. Xing, Y., Xu, Y., Chen, Y., Jeffrey, P.D., Chao, Y., Lin, Z., Li, Z., Strack, S., Stock, J.B., Shi, Y. Structure of protein phosphatase 2A core enzyme bound to tumor-inducing toxins. Cell **2006**, 127, 341-353. doi: 10.1016/j.cell.2006.09.025
31. Ueno, Y., Nagata, S., Tsutsumi, T., Hasegawa, A., Watanabe, M.F., Park, H.D., Chen, G.C., Chen, G., Yu, S.Z. Detection of microcystins, 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 **1996**, 17, 1317-1321. doi: 10.1093/carcin/17.6.1317
32. Zhou, L., Yu, H., Chen, K. Relationship between microcystin in drinking water and colorectal cancer. Biomed Environ Sci **2002**, 15, 166-171.
33. Fleming, L.E., Rivero, C., Burns, J., Williams, C., Bean, J.A., Shea, K.A., Stinn, J. Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. Harmful Algae, **2002**, 1, 157-168. doi: 10.1016/S1568-9883(02)00026-4
34. Li, Y., Chen, J., Zhao, Q., Pu, C., Qiu, Z., Zhang, R., Shu, W. A cross-sectional investigation of chronic exposure to microcystin in relationship to childhood liver damage in the Three Gorges Reservoir Region, China. Environ Health Perspect **2011**, 119, 1483-1488. doi: 10.1289/ehp.1002412
35. Zhang, F., Lee, J., Liang, S., Shum, C.K. Cyanobacterial blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. Environ Health **2015**, 14, 1-11. doi: 10.1186/s12940-015-0026-7
36. Labine, M.A., Green, C., Mak, G., Xue, L., Nowatzki, J., Griffith, J., Minuk, G.Y. The geographic distribution of liver cancer in Canada does not associate with cyanobacterial toxin exposure. Int J Environ Res Public Health **2015**, 12, 15143-15153. doi: 10.3390/ijerph121214969
37. Rastogi, R.P., Madamwar, D., Incharoensakdi, A. Bloom dynamics of cyanobacteria and their toxins: environmental health impacts and mitigation strategies. Front Microbiol **2015**, 6. doi: 10.3389/fmicb.2015.01254
38. Joung, S.H., Oh, H.M., Ko, S.R., Ahn, C.Y. Correlations between environmental factors and toxic and non-toxic *Microcystis* dynamics during bloom in Daechung Reservoir, Korea. Harmful Algae **2011**, 10, 188-193. doi: 10.1016/j.hal.2010.09.005
39. Neilan, B.A., Pearson, L.A., Muenchhoff, J., Moffitt, M.C., Dittmann, E. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environ Microbiol **2013**, 15, 1239-1253. doi: 10.1111/j.1462-2920.2012.02729.x

40. Sharma, N.K., Choudhary, K.K., Bajpai, R., Rai, A.K. Freshwater cyanobacterial (blue-green algae) blooms: causes, consequences and control. In *Impact, Monitoring and Management of Environmental Pollution*; El-Nemr, A. Nova Science Publishers, Hauppauge, New York, pp. 74-89.
41. Whitehead, P.G., Wilby, R.L., Battarbee, R. W., Kernan, M., Wade, A.J. A review of the potential impacts of climate change on surface water quality. *Hydrol Sci* **2009**, 54, 101-123. doi: 10.1623/hysj.54.1.101
42. de Souza, M.,S., Muelbert, J.H., Costa, L.D.F., Klering, E.V., Yunes, J.S. Environmental variability and cyanobacterial blooms in a subtropical coastal lagoon: searching for a sign of climate change effects. Front Microbiol **2018**, 9. doi: 10.3389/fmicb.2018.01727