

Cyanobacteria, The Nebraska Experience

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Nebraska agencies and public health organizations collaboratively addressed cyanobacteria issues for the first time after two dogs died within hours of drinking water from a small private lake south of Omaha on May 4, 2004. A water sample and a necropsy revealed that the dog deaths were due to high concentrations of the cyanobacteria toxin Microcystin LR. Meetings were held between the Nebraska Department of Environmental Quality (NDEQ), Nebraska Health and Human Services (NHHS), Nebraska Game and Parks Commission (NGPC), and the University of Nebraska-Lincoln (UNL). Excellent cooperation and quick action were demonstrated by these agencies in developing unified strategies for cyanobacteria monitoring and public notification within two weeks after the dog deaths occurred (Figure 1). Even with monitoring and notification networks in place, by the end of the 2004 recreation season there were three reported dog deaths, numerous wildlife and livestock deaths, and more than 50 accounts of human skin rashes, lesions, or gastrointestinal illnesses reported at Nebraska lakes.



Figure 1. Signage used to notify the public of unsafe conditions for contact with the water.

Monitoring Cyanobacteria Toxins

Weekly sampling of public lakes for cyanobacteria toxins was initiated during the week of May 17, 2004. Initial monitoring efforts targeted lakes with known or suspected cyanobacteria problems. Citizen complaints were important in providing information on lakes where algae blooms were occurring. Monitoring in 2004 consisted of 671 microcystin samples being collected from 111 different waterbodies, with most of these being sampled only a few times. In 2005, the algae toxin monitoring was combined with the “swimming beach” bacteria network where weekly sampling was conducted. By 2008, with financial assistance from the U.S. Environmental Protection Agency and staff assistance from Nebraska Natural Resources Districts, Nebraska Game and Parks Commission, Nebraska Public Power District, U.S. Army Corp of Engineers, University of Nebraska-Lincoln, and local health agencies, the cyanobacteria monitoring network had expanded to weekly sampling from May through September at 47 lakes and reservoirs, all of which are publicly owned. Concerns and questions on privately owned lakes are addressed through UNL extension programs.

Laboratory Analysis

After sending initial samples to outside laboratories it was soon realized that the cost of laboratory analysis greatly limited the number of samples that could be collected and the time between sample collection and receiving results was not conducive to quick public notification. As such, NDEQ purchased Abraxis LLC Microcystins Enzyme-Linked Immunosorbent Assay (ELISA) laboratory test kits for “in-house” analysis of total microcystins concentrations. ELISA kits provided a low cost, semi-quantitative analytical method for measuring concentrations of total microcystins, the most common toxin released by cyanobacteria. Using ELISA test kits instead of High Performance Liquid Chromatography (HPLC) or Liquid Chromatography/Mass Spectrometry (LC/MS) analyses resulted in an estimated savings of \$77,000, just in 2004. Additionally, analyzing water samples with ELISA kits provided for a quick turnaround time, which allowed weekly updates of lake conditions and public health alerts and advisories prior to each weekend’s recreational activities.

Extent of the Problem

A total of 671 microcystin samples were collected from 111 different lakes in 2004, resulting in health alerts for 26 lakes and health advisories for 69 lakes. A total of 22 of the 26 health alert lakes (84.6%) were located in the eastern one-third of Nebraska. In 2004, triggers for health advisories and health alerts were 2 µg/L and 15 µg/L respectively. In 2005, health advisories were dropped from the protocol and the trigger for health alerts was raised from 15 µg/L to 20 µg/L to align with World Health Organization (WHO) recommendations (WHO 2003). From 2005 through 2008, more than 3,625 samples have been collected on 65 lakes across the state. Of these 65 lakes, 43 (66%) had toxin concentrations greater than the method reporting limit of 0.15 µg/L and 18 lakes (28%) had at least one sample with concentrations above 20 µg/L, which is the trigger for issuing health alerts and posting beaches.

Seasonal and Spatial Variability

NDEQ and other resource agencies had and still have concerns about the spatial variability in cyanobacteria populations in regards to samples being representative and issuing public advisories. NDEQ's current beach sampling protocol relies on a single, mid-beach grab sample to represent the condition and/or quality of an entire beach area. Recognizing the importance of protecting human health and the far-reaching implications of beach closures, NDEQ initiated a beach sampling representativeness study. The objective of this study was to determine if statistically significant differences exist between "single sample" and "multiple sample" beach monitoring approaches for microcystin toxin. To meet this objective, a "multiple sample" monitoring approach was implemented at several lakes where seasonal beach monitoring was already being conducted. Statistical tests indicated microcystin concentrations were distributed similarly at all beach sampling locations and changes to NDEQ single sample beach sampling protocol were not warranted.

Special studies were initiated in 2005 to better identify causes and ecological consequences of cyanobacteria blooms and evaluate seasonal variability. NDEQ contracted with the Center for Advanced Land Management Information Technologies at UNL to conduct a cyanobacteria remote sensing project. Preliminary remote sensing data indicated that algae succession in lakes varied significantly throughout the year, even among lakes located in close proximity to one another. The project also documented a significant amount of spatial variability within individual lakes (Figure 2).

The NDEQ toxic algae monitoring network specifically analyzes for the microcystin toxin. While this is the most common toxin found in Nebraska waters, it is realized there are other toxins of concern. In addition, within the microcystin toxin, there are 71 different variants presently identified. While the ABRAXIS ELISA test analyzes the combined total of variants, some literature suggests only the LR is significant and some criteria are based solely on this. In an effort to further understand this somewhat complex group of toxins and to help determine the significance of these other components, additional analysis was performed. During 2006 through 2008, samples from 5 sites at 3 lakes were split and also analyzed for these additional fractions by the UNL Water Science Lab using liquid chromatography mass spectrometry. The results of this study are still pending.

Health Alerts and Health Advisories

A weekly routine has been established in which water samples are collected and delivered to the

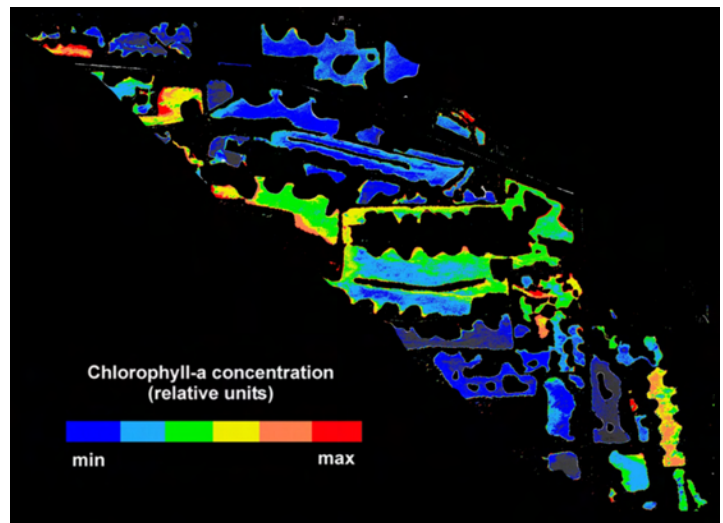


Figure 2. Processed aerial image of a chain of lakes in Nebraska showing spatial variation in chlorophyll within lakes and between lakes in close proximity (May 24, 2006).

laboratory on Monday and Tuesday, processed using freeze-thaw methods on Wednesday, and analyzed on Thursday. Sample results are reported on Thursday, and by Friday morning, NDEQ website information is updated and if necessary, warning signs are posted at lakes.

Because of its initial unfamiliarity with cyanobacteria issues, Nebraska chose to err on the side of safety by selecting a conservative approach for protecting public health, which included measuring worst-case total microcystins conditions. One of the reasons for selecting the Abraxis total microcystins ELISA kit was that it measures all microcystin variants, not just the LR variant which is used as the basis for WHO action level guidelines. Also, the freeze/thaw process prior to analysis to lyse the cyanobacteria cells simulates the exposure risk that might exist from ingesting cells and having them lyse and release toxins in the stomach. These procedures provided additional public safety factors in case grab samples failed to measure the highest total microcystins concentrations in a lake.

In 1998, WHO recommended that Microcystin LR concentrations of 20 µg/L or higher should trigger further action for recreational uses. Nebraska chose an initial action level of 15 µg/L of total microcystins for issuing health alerts in 2004, but was changed it to 20 µg/L in 2005. Lakes placed on health alert status remain so until the total microcystins concentration falls below 20 µg/L for two consecutive weeks.

Methods used to notify the public of potential health hazards from cyanobacteria include; the development of a fact sheet about cyanobacteria; weekly updates of total microcystins sampling results and health alerts on the NDEQ web site; emails to interested agencies and organizations; news releases and interviews with newspapers, radio, and TV stations; and posting of warning signs at lake beaches and boat ramps.

Toxin Migration

In 2006, NDEQ conducted a study to evaluate the presence of the microcystin toxin in fish tissue and liver samples from three impoundments heavily impacted by cyanobacteria. The three impoundments consisted of one oxbow lake, one sandpit lake, and one recreational reservoir. Fish species sampled included White Crappie, Largemouth Bass, and Channel Catfish. Forty-six samples were collected for microcystin analysis using ELISA methodologies adapted by An and Carmicheal (1994) and Carmicheal and An (1999). Results of the analysis revealed eight of the 46 samples exhibited microcystin concentrations greater than the detection limit of 0.175 µg/L. All detectable concentrations, which ranged from 0.20 µg/L to 0.32 µg/L, were collected from the sandpit lake and were from four liver and four tissue samples. Detectable concentrations of the microcystin toxin were found in all species sampled. At this time there is very little guidance on what levels of the microcystin toxin are acceptable in fish tissue for safe consumption.

NDEQ initiated a toxin migration study in 2006 to investigate potential impacts to groundwater quality. Shallow wells (2-12 feet deep) were installed in sand dominated soils, along predicted groundwater flow paths at Fremont State Lake #20, located 35 miles northwest of Omaha. These wells were sampled monthly year-round from May 2006 to October 2008. The investigation indicated that the toxin will flow with lake water into groundwater at significant concentrations relative to the WHO drinking water standard of 1 µg/L. An algal bloom occurred in May of 2007 with Microcystin concentrations in the lake measured at 95.69 µg/L and a filtered concentration of 24.29 µg/L. In June of 2007, toxin concentrations were mea-

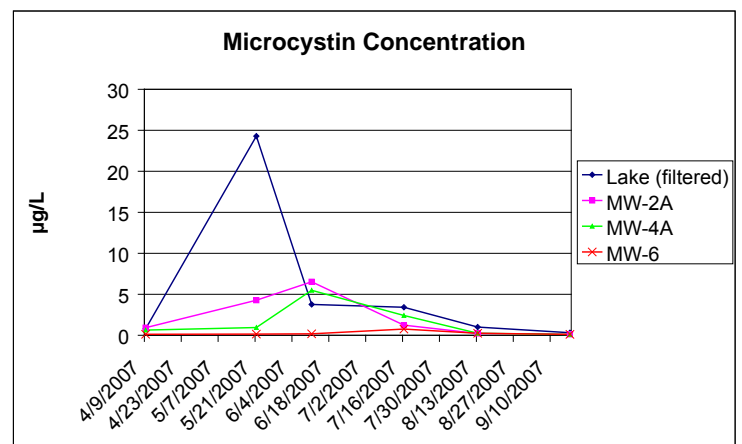


Figure 3. Total microcystin concentration measured in Fremont Lake #20 (filtered sample) and in shallow groundwater wells located 19 feet (MW-2A), 130 feet (MW-4A), and 250 feet (MW-6A) from the lake.

sured in groundwater at 5.49 µg/L as far as 130 feet from the lake's edge, and a 0.76 µg/L microcystin concentration was detected 250 feet from the lake's edge in July of 2007 (Figure 3).

Management and Treatment

In 2006, the NGPC coordinated an experimental algaecide treatment project on Pawnee Reservoir, located near Lincoln, NE. The treatment project was conducted to evaluate the effectiveness of early season algaecide treatments in areas of the reservoir where blue greens were starting to form. Pawnee Reservoir has had a history of severe blooms in May and officials were hopeful that the early treatment would reduce bloom magnitude and duration. The treated area encompassed approximately 25 percent of the 740 acre reservoir. While the reservoir did experience a decrease in chlorophyll and microcystin concentrations shortly after the treatment, the improvements lasted only a couple of weeks. It was determined that treating the early stages of a bloom in a larger reservoir can be difficult given the rapid expansion of blooms. While treatment of the entire reservoir would have increased effectiveness, the cost of doing so was prohibitive.

In 2007, the NDEQ, NGPC, and UNL conducted an alum treatment project on Fremont Lake #20. Fremont Lake #20 consists of 50 surface acres and is part of a chain of sandpit lakes collectively known as the Fremont State Lakes. Phosphorus inactivation was determined to be the best option for treatment given it's a "closed" system that lacks external nutrient loading. Prior to the project, summer epilimnetic total phosphorus and chlorophyll averaged 110 µg/L and 82 µg/L respectively. The lake system was dominated by the cyanobacteria genera *Oscillatoria* which resulted in routine beach postings due to high concentrations of microcystin toxin. From June of 2004 through September 2007, 68 of the 209 (33 percent) of the algae toxin samples collected from Fremont Lake #20 exceeded beach posting criterion of 20 µg/L. This resulted in the beach being closed for 36 weeks making this one of the most impacted public lakes in the state for blue green algae toxins. The alum project, which was conducted in October, 2007 produced immediate results. Average summer epilimnetic phosphorus was reduced from 110 µg/L to 21 µg/L, chlorophyll was reduced from 82 µg/L to 8 µg/L, and microcystin concentrations were reduced from an average of 21 µg/L to concentrations below the reporting limit of 0.15 µg/L (Figure 4). There were no beach postings in 2008 as the maximum microcystin concentration reported that year was 0.23 µg/L.

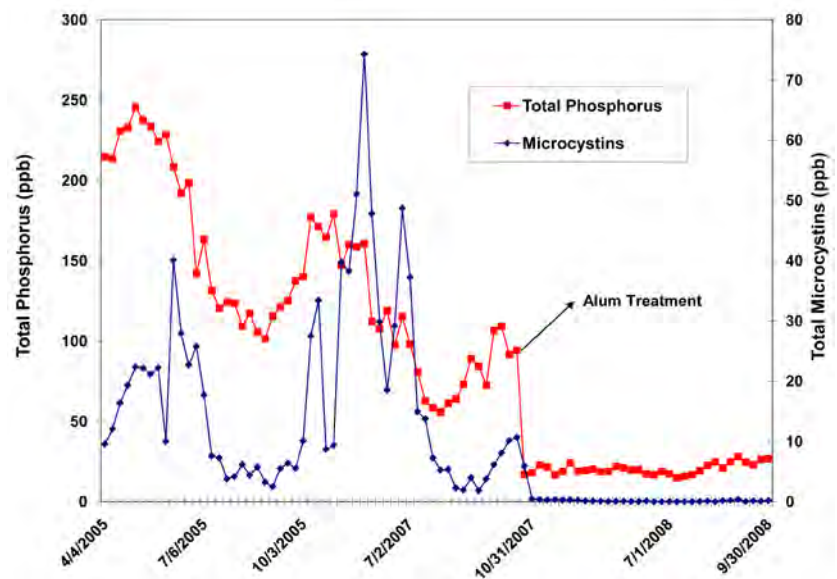


Figure 4. Pre- (2005, 2007) and post (2008) alum treatment total phosphorus and microcystin toxin concentrations in Fremont Lake #20 near Fremont, Nebraska

Summary

Nebraska continues to promote activities that reduce nutrient loading to lakes. While Nebraska's Surface Water Quality Standards do not allow point source discharges to lakes, nutrient loading from nonpoint sources are a concern. Nebraska's Nonpoint Source Management Program has been successfully reducing nutrient loading in impaired watersheds through coordinated efforts between local, state, and federal agencies and watershed stakeholders. While significant progress has been made in many watersheds, the reservoirs response to these reductions will be a much longer process than in closed systems where alum can be applied. Additionally, watershed treatment addresses the external nutrient load which is only part of the picture. Many of Nebraska's reservoirs and sandpit lakes were constructed in the 1950s

and 1960s and have significant internal nutrient loads. Addressing internal sources of nutrients is a difficult and costly task but a critical component in achieving measurable nutrient reductions and shifting algal communities to a more desirable or at least more tolerable state. In many cases, expectations in water quality improvement can exceed realistic outcomes, particularly if a holistic approach to nutrient management is not taken.

References

An, J-S. and Carmichael, W.W. (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicon* 32: 1495-1507

Carmichael, W.W. and An, J-S (1999) Using an enzyme linked immunosorbant assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. *Natural Toxins*. 7:377-385.

NDEQ. (2008). Beach sampling representativeness study for microcystin and E.coli bacteria. Greg Michl (ed.). Surface Water Unit, Nebraska Department of Environmental Quality. Lincoln, NE

Walker, S., J.C. Lund, D.G. Schumacher, P.A. Brakhage, B.C. McManus, J.D. Miller, M.M. Augustine, J.J. Carney, R.S. Holland, K.D. Hoagland, J.C. Holz, T.M. Barrow, D.C. Rundquist, A.A. Gitelson, (2007). Chapter 6: Nebraska Experience. In Proceedings H. Kenneth Hudnell (ed.), Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. *Advances in Experimental Medicine & Biology*, i-x.

WHO (2003). *Algae and cyanobacteria in Coastal and Estuarine Waters: Guidelines for Safe Recreational Water Environments – Vol. 1 Coastal and Fresh Waters*. Geneva, Switzerland: World Health Organization 128-35.