Chapter 14: Causes, Mitigation, and Prevention Workgroup Posters

Application of immobilized titanium dioxide photocatalysis for the treatment of microcystin–LR

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Introduction

This research is currently focused on the development of efficient water treatment processes for the hepatotoxin, microcystin–LR (MC–LR). Both conventional and advanced oxidation technologies have been tested against this cyanotoxin. Pilot plant studies have shown that conventional treatment processes such as coagulation, flocculation, and sedimentation result in increased levels of soluble toxin. Chlorination, activated carbon adsorption, and chemical oxidation [ozonation, Fenton reagent (FR)] have been used for the inactivation, physical removal, and degradation of the toxin, respectively. A promising chemical oxidation technology for the treatment of MC–LR is titanium dioxide (TiO_2) photocatalysis. This emerging "green" technology efficiently performs water purification and disinfection. Due to high catalytic surface area and minimized mass transfer limitations, TiO_2 in suspension exhibits high photocatalytic activity.

However, nanosize TiO_2 particles are difficult to handle and remove after their application in MC–LR treatment.

Hypotheses

In this study, immobilized TiO_2 photocatalysis was utilized as a proposed alternative to suspended TiO_2 treatment for MC–LR. Immobilized TiO_2 has higher mass transfer limitations than the suspended TiO_2. Also, fewer active catalytic sites are available to the contaminant resulting in reduced photocatalytic activity. Thus, it is necessary to immobilize TiO_2 particles onto substrates and enhance the photocatalytic and structural properties of TiO_2 material. We are developing novel methods for fabricating porous and non–porous photocatalytic films that possess high photocatalytic activity for a given TiO_2 mass.

Methods

Highly active photocatalytic films were prepared with two methods resulting into porous and non–porous films on glass and stainless steel substrate, respectively. The non–porous films are synthesized by a modified sol–gel method employing a mixture of titania sol and colloidal TiO_2 particles to immobilize commercially available TiO_2 powder nanoparticles. The porous films are prepared with an acetic acid based sol–gel method modified with surfactant templates.

Results

Control experiments demonstrated the high stability of MC–LR. The observed degradation in all the experiments with the films is due to the photocatalytic properties of the titanium dioxide. Both dark reaction and direct photolysis of MC–LR showed no obvious degradation.

Conclusion

The study showed that immobilized titanium dioxide photocatalysis could effectively destroy MC–LR in water, at concentrations up to 5000 ppb.

Environmental conditions, cyanobacteria and microcystin concentrations in potable water supply reservoirs in North Carolina, U.S.A.

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Introduction

Run–of–river impoundments or reservoirs, the "lakes" of the Southeast, provide potable water supplies and recreational value for rapidly urbanizing areas. Cyanobacteria blooms and the potential for cyanotoxin contamination of water supplies have not been well studied in these turbid systems. The objective of this ongoing research (summers, 2002–current) is to characterize environmental conditions and cyanobacteria composition, abundance, and microcystin concentrations in potable water supply reservoirs of different age in North Carolina. The reservoirs selected for this work provide potable water to two million people in the western and central areas of the state.

Materials and Methods

Physical/chemical environmental conditions have been assessed using a YSI multiprobe water quality system (model 566MPS). Nutrient concentrations (TP, SRP, TN, NO_3^-+NO_2^-, NH_4^+) and chlorophyll *a* concentrations have been analyzed by the state–certified CAAE water quality laboratory. Cyanobacteria assemblages have been identified and quantified following current keys of Komárek and colleagues (phase contrast microscopy, 600x), supplemented by molecular probes where available. Total microcystins (free and cell–bound fractions) in raw and finished water have been quantified using enzyme–linked immunosorbent assays (ELISA, Envirologix, Inc.), confirmed by high–performance liquid chromatography with mass spectroscopy (LCMS). Here, nutrient and microcystin concentrations were compared in reservoirs of two age groupings: newer reservoirs (20–30 years post–fill, n = 5) and older reservoirs (60–85 years post–fill, n = 6). Differences in the two age groupings in selected physical, chemical, and biological factors were determined by non–parametric one–way ANOVA (Mann–Whitney–Wilcoxon test; SAS Institute, Inc. 1999; $\alpha = 0.05$). Linear regressions were also conducted to examine relationships between biological parameters and physical/chemical factors.

Results

These potable water supply reservoirs are eutrophic, with high nutrient levels, high turbidity, and low alkalinity. High precipitation in turbid, well–flushed systems is not conducive to cyanobacteria blooms, and two of three summers were above–average in precipitation, yet cyanobacteria comprised 60–95% (usually more than 75%) of the total phytoplankton cell number each summer, with densities as high as ~400,000 cells mL–1. Common toxigenic cyanobacteria included Anabaena circinalis, Anabaena flos–aquae, Aphanizomenon flos–aquae, Microcystis aeruginosa, Cylindrospermopsis raciborskii; blooms of Lyngbya wollei and Planktothrix cf agardhii also occurred. Microcystins were detected in most samples, at low concentrations (less than 1 µg L–1). Older reservoirs had significantly higher NO3–+NO2–, TP, and microcystin concentrations, and significantly lower TN:TP ratios than newer reservoirs. Total microcystin levels were positively correlated with TP and TN concentrations in newer reservoirs, and with TP in older reservoirs, indicating potential importance of both TN and TP in blooms.

Conclusion

North Carolina potable water supply reservoirs in both age groups are impacted by nutrient over–enrichment and cyanobacteria blooms, including toxigenic species that may adversely impact the utility of these systems for potable water supplies and recreational activities as the watersheds become increasingly urbanized with associated increases in nutrient inputs.

Removal of microcystins using portable water purification systems

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Introduction

Blooms of blue–green algae occurring in lakes and rivers are frequently toxic, producing a range of cyclic peptides known as microcystins. Extensive studies on toxicity and several toxicoses have led to a WHO guide line of 1 μg per litre in drinking water supply. Consequently there has been a large amount of research on the efficiency of water treatment processes to remove algal cells and toxins along with a range of management strategies.

Over recent years a number of portable water purification systems have been developed to meet the needs of recreational, military and emergency use. However, limited research on domestic and field purification devices has shown that few systems are able to remove microcystins to below the recommended level. As many lakes produce blooms, and an alternative water source may not be available, it is important to examine the performance of the portable purification systems.

In our study two systems were investigated, an MSR®Miniworks™ unit and a First Need® Deluxe from General Ecology both of which are claimed to exceed EPA requirements for removal of bacteria and protozoa. Both systems were evaluated for the removal of dissolved toxins and cyanobacterial cells.

Methods

Both filters were used and cleaned according to manufacturer's instructions. Micrcystin–LR (MC–LR) and the cell free extract of *M. aeruginosa* PCC 7820 which contained MC–LR, –LY, –LW and –LF were prepared as previously described (Lawton *et al.* 1995) and dissolved in tap and lake water to give realistic concentrations (50 μg L^{-1}) . Microcystins were extracted from water samples using solid phase extraction and analysed by HPLC as described by Lawton, *et al*. 1994. Removal of *Microcystis* cells was quantified using a particle analyser.

Results

Virgin filters removed 100% of MC–LR at a concentration of 50 μg l^{-1} from distilled water and lake water. Experiments were repeated where the water samples were spiked with the extract which contained MC–LR, -LY, –LW and –LF. Although systems performed well, the MSR no longer removed 100% MC–LR. Performance after cleaning was assessed and the MSR unit deteriorated, removing only 66.2–88.8 % of microcystin variants compared to 100% removal by the First Need unit.

The ability to remove whole cells was investigated, along with determination of intra– and extra–cellular microcystin concentrations. Both filters removed 100 % of cells but high concentrations of the microcystin variants were detected in the MSR filtered water, indicating that cells had been damaged and toxins released.

Conclusion

This short study demonstrated that the First Need filter was highly effective at removing both dissolved microcystins and cells, whereas the performance of the MSR filter rapidly declined, despite the fact that only 6 L of lake water had been passed though it and would therefore not be recommended such an application. More research is needed on continued use of the First Need filter to ensure that performance is maintained and there is no release of microcystins from trapped cells.

References

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Multiple Scenarios for Fisheries to Increase Potentially Toxin Producing Cyanobacteria Populations in Selected Oregon Lakes

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Introduction

The dominance of cyanobacteria, many of which produce toxins, in lakes is often associated with external loads of phosphorus from activities in the watersheds. However, we have identified multiple pathways in selected Oregon lakes whereby fisheries management activities play a crucial role in promoting cyanobacteria populations.

Hypotheses

The proliferation of cyanobacteria in freshwater environments is aided by increased availability of phosphorus. Phosphorus availability is increased by alteration of native food–webs, thus changing the trophic dynamics of nutrient cycling in lakes. Fisheries management can alter food webs numerous ways.

Methods

Several Oregon lakes were investigated through the use of paleolimnological techniques. Sediment cores were dated using ^{210}Pb and changes in water quality and cyanobacterial populations were examined using akinetes (resting cells) preserved in the sediments.

Results

Fish populations were shown to alter trophic structure and thus increase phosphorus availability in several ways. In Diamond Lake, the inadvertent introduction of a minnow (tui chub, *Gila bicolor*) native to an adjoining basin led to increased fish biomass and translocation of nutrients from the shallow waters to the pelagic zone. Diamond Lake was treated with rotenone in 1954, thus eliminating all fish and resulting in an immediate drop in cyanobacteria populations. The cyanobacteria densities in Diamond Lake have increased in recent years with the reintroduction of the tui chub and leading to lake closures. In Odell Lake, the State intentionally introduced kokanee (land–locked sockeye salmon, *Oncorhynuchus nerka*) to enhance a native salmonid fishery. The kokanee became the dominant fish and increased nutrient cycling by consuming large quantities of zooplankton in the metalimnion and recycling nutrients back into the photic zone. The native salmonids occupy the hypolimnion during the summer, providing relatively little opportunity for returning phosphorus back to the photic zone in the summer. Devils Lake is a shallow coastal lake with a native salmonid fishery and introduced centrarchids. In an effort to reduce the abundant macrophyte growth throughout much of the lake, the local lake district (with permission of the State) introduced triploid grass carp (*Ctenopharyngoden idella*). The stocking was successful and the grass carp eventually ate all submerged macrophytes in the lake. Once the macrophytes were eliminated, cyanobacterial populations increased dramatically most likely because of increased light availability, reduced competition for nutrients, and increased nutrient supply associated with disturbance of the sediment by the grass carp. Crane Prairie Reservoir is a shallow impoundment in central Oregon and historically was a rainbow trout fishery. It currently has five introduced, non–native fish species and is experiencing major blooms of cyanobacteria.

Conclusions

Fish management activities can increase the likelihood of cyanobacterial blooms through a variety of mechanisms involving translocation of nutrients, increased retention of nutrients in the photic zone, substrate disturbance, and reduction of large cladoceran zooplankton.

Removal of the cyanobacterial toxin microcystin–LR by biofiltration

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Introduction

The occurrence and persistence of the cyanobacterial toxin, microcystin–LR, in natural waters has been reported worldwide, and its risk to public health and animals has been associated with water consumption. The effects of this toxin on humans and animals include total failure of respiratory system, hepatocyte necrosis and tumor promotion in the liver. Conventional water treatment processes such as coagulation, flocculation and filtration, have failed to remove algal toxins to recommended levels required by the World Health Organization (WHO). However, there has been reported effective biological degradation of microcystin–LR in field and laboratory studies using water samples from lakes where cyanobacterial blooms have historically occurred.

Hypothesis

Given that biological degradation of microcystin–LR is very effective, and that filters in water treatment plants can successfully remove naturally organic matter (NOM) via biofiltration, it is expected that microcystin–LR can be degraded by biologically active filters. Biological filters are established in water treatment plants when ozonation is introduced as a disinfectant. Following the approval by the US Environmental Protection Agency legislation to reduce Disinfection By–Products (DBP's) in potable waters, biological filters have become an important water treatment unit to meet the newly established DBP's standards.

Methods

Bench–scale microcystin biodegradation tests were carried out using an enrichment bacterial culture from Lake Mead, Nevada. Three bioreactors were incubated at room temperature for 7 days in the dark to avoid phototrophic growth. In each reactor containing 100 ml of Errington & Powell's medium, 32 mg/L of microcistin–LR was added. In order to evaluate the biodegradability of microcystin in the presence of different amounts of carbon, the amounts of glucose, citric acid, L–glutamic acid and succinic acid from the aforementioned medium were varied to obtain bioreactors containing 100%, 50 % and 0% additional carbon. Sub–samples from the reactors were taken daily for microcystin analysis and evaluation of its degradation rates.

The microcystin–degrading enrichment culture was then used to inoculate two bench–scale biofilters operating with typical design parameters of a drinking water treatment facility. The filters were packed with variable amounts of silica sand (effective size 0.51 mm) and anthracite (effective size 0.9 mm) to provide different empty bed contact times (EBCT). After a biofilm was established on the surface of the filter media, the filters were fed in continuous mode at hydraulic loading rate of 2.5 m/h. Dechlorinated tap water containing readily biodegradable organic matter (i.e. acetate and formate), bentonite and the toxin were added tothe filters. Acetate and formate are typical by–products of the ozonation of natural organic matter (NOM) and they are present in the influent water to the filtration units. Bentonite was used to simulate the particulate matter in surface water. Microcystin–LR concentrations varying from 10–130 g/L were added in the influent water. This range corresponds to dissolved microcystin–LR levels detected in lake waters during algal blooms. The concentration of microcystin–LR in the effluent was monitored every 12 hours and determined by Enzyme Linked Immunosorbent Assay (ELISA).

Results

The results of the biodegradation tests revealed a reduction of approximately 67% in the bioreactor to which no additional carbon source was added. Lower reductions were obtained in the experiments with carbon source addition. Therefore, it appears that microcystin itself can be used as a carbon source by the enrichment bacterial culture. These results are encouraging because concentrations of acetate and formate, byproducts of NOM ozonation, in drinking waters are low. Therefore, the potential to remove microcystin via biofiltration is high.

Water quality and cyanobacterial management in the Ocklawaha Chain–of–Lakes, Florida

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Introduction

The Ocklawaha Chain–of–Lakes are large, shallow water bodies located in central Florida. These surface waters are naturally productive. However, water quality has been severely degraded by nutrient loading, primarily from large agricultural operations. Water quality in the lakes ranges from mesotrophic to hypereutrophic, and the lakes have experienced prolonged severe cyanobacterial blooms.

Restoration program

The program to manage water quality and cyanobacteria in the lakes includes purchase and restoration of wetland habitat in former agricultural areas to reduce external phosphorus loading, operation of a marsh flow–way to remove particulate phosphorus from lake water, harvesting of gizzard shad to reduce recycling of stored phosphorus, and re–establishment of desirable aquatic vegetation.

Lake responses

There are strong relationships between external phosphorus loading, phosphorus concentrations, and cyanobacterial biovolume in the Ocklawaha Chain–of–Lakes. Following external phosphorus load reduction and shad harvesting, Lake Griffin has seen substantial improvements in water quality, including decreases in phosphorus and chlorophyll concentrations, and increases in transparency. Cyanobacterial biovolume has also decreased, and there have been changes in the composition, including a decrease in dominance by *Cylindrospermopsis*. The phytoplankton community has shifted from year–round cyanobacterial dominance to cyanobacterial dominance only during the warm season.

Conclusion

Data indicate that meeting phosphorus targets for the lakes will significantly improve water quality. Cyanobacteria likely will remain seasonally dominant even if phosphorus reduction goals are achieved, although at substantially lower biovolume.

A shift in phytoplankton dominance from cyanobacteria to chlorophytes following algaecide applications

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Introduction

Cyanobacteria can form massive blooms in nutrient enriched environments. Some species are capable of producing hepatotoxins that are harmful to both humans and animals following ingestion. Chemical algaecides are frequently used to control these blooms in order to minimize their impact to both humans and wildlife. In this study, we compared copper sulfate with sodium carbonate peroxyhdrate (PAK–27™) on natural cyanobacterial populations. Studies have shown that copper does not readily dissipate from the environment and can thus accumulate over time to levels considered lethal to other organisms – especially invertebrates and fishes. PAK–27™ was developed to be an environmentally friendly algaecide that specifically targets cyanobacteria and decomposes within several weeks into water and oxygen. For this study, three levels of algaecide (0.15, 1.5, and 5.0 ppm; either copper sulfate or PAK–27™) were used to treat a cyanobacterial bloom (dominated by *Anabaena circinalis*, *Microcystis aeruginosa*, and *Planktolyngbya limnetica)* cultured in 4L microcosms. The treatments were monitored over time, to evaluate changes in species abundance and composition.

Hypotheses

Both algaecides would be effective in controlling algal blooms. There would be treatment (algaecide) level–specific community responses due to chemical applications, with most of the changes in phytoplankton community composition occurring at lower levels.

Methods

A total of 65 microcosms (4L cubitainers) were used during this investigation. Chemical treatments were implemented during a bloom period at peak densities, and consisted of three dose levels of sodium carbonate peroxyhdrate or copper sulfate (0.15 ppm, 1.5 ppm, and 5.0 ppm; n=10). Population densities were monitored on Day–1, Day–5, and Day–15. Plankton counts were performed using a Sedgwick–Rafter counting cell according to APHA methods. During incubation, all microcosms were situated within the lake proper to insure natural temperature and light regimes.

Results & Conclusion

The experiment involved microcosms inoculated with pond water (late September – October) that was experiencing a cyanobacterial bloom comprised primarily of *Anabaena circinalis* (up to 6,000 units mL^{-1}), *Microcystis aeruginosa* (100 units mL^{-1}), and *Planktolyngbya limnetica* (200 units mL^{-1}). Copper treatments were more effective than PAK–27™ on all three cyanobacterial species by Day–15, as illustrated by a two–fold decrease in *A. circinalis*, and greater than a 10–fold decrease in both *M. aeruginosa*, and *P. limnetica*. In contrast, no significant declines were observed in microcosms treated with PAK–27™. One of the more interesting findings from this study was the dominance of green algae following chemical removal of cyanobacteria. In this case, for low and moderate copper, the removal of cyanobacteria resulted in significant increases in *Staurastrum sp.* (up to 500 units mL^{-1}), *Eudorina elegans* (up to 7,000 units mL^{-1}), and *Scenedesmus sp*. (up to 6,000 units mL^{-1}). This shift in community composition, from cyanobacteria to green algae following chemical treatments, may indicate a competitive exclusion of green algae by cyanobacteria. Moreover, this response indicates that cyanobacteria are less tolerant to copper relative to green algae. Nevertheless, high copper levels (at 5 ppm) resulted in substantial declines in all algal taxonomic groups.

Ultrasonically–induced degradation of microcystin LR and RR: Identification of byproducts and effect of environmental factors

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Introduction

Microcystins (MCs) are a family of strongly hepatotoxic peptides produced by different species of cyanobacteria commonly found in lakes, water reservoirs, and recreational facilities. The increased eutrophication of fresh water supplies has led to the increase in the incidence of cyanobacteria blooms and concerns over the public health implications of these toxins in the water supply. Conventional water treatment methods are poor at removing low concentrations of the cyanotoxins, and specialized treatment is usually necessary for treatment of contaminated water.

Hypotheses

Advanced oxidation technologies (AOTs) employ photochemical and radical processes for the oxidation of pollutants. While AOTs have shown tremendous promise for the remediation a variety of anthropogenic pollutants, there has been a limited number of reports on the remediation of naturally occur toxins by AOTs. Unlike AOTs involving photochemical process, ultrasonic irradiation can be used for slurries and turbid solutions such as those encountered during cyanobacterial blooms. Ultrasonic treatment is a reagent–free process and does not produce disinfection by–products. We hypothesize that ultrasonic irradiation can be used to effectively destroy MCs.

Methods

Purification of MC–LR from a laboratory culture, the ultrasonic irradiation, HPLC, and LC–MS procedures are available from the literature. Microcystin–RR was purchased from ALEXIS.

Results

Ultrasonic irradiation leads to the rapid degradation of MC–LR and dramatically reduces the PP1 toxicity of the treated solution. Hydroxyl radical is responsible for a significant fraction of the observed degradation, but other processes (hydrolysis/pyrolysis) are also important. The decomposition products of the ultrasonic destruction of microcystin–LR and microcystin–RR were analyzed by liquid chromatography–mass spectrometry (LC–MS) and the mechanisms of degradation involve oxidation of the Adda moiety. The effects of pH, Fe^{2+} and H_2O_2 on the ultrasonic degradation were investigated.

Conclusions

The major by–products of ultrasonically induced degradation of MCs result from hydroxyl radical attack on the benzene ring of Adda, substitution and cleavage the Adda conjugated diene structure. The initial rate of MC degradation is strongly pH dependent, in a manner mirrored by the pH dependence of toxin hydrophobicity. While hydroperoxide and organic peroxides are formed during ultrasonically induced irradiation of MCs, addition of Fe^{2+} effectively destroys the peroxides and promotes further oxidation of the MCs. These findings suggest that ultrasonically induced irradiation may be a suitable method for the treatment and detoxification of MCs in drinking water and in response to bioterrorism.

Cultural eutrophication of three midwest urban reservoirs: The role of nitrogen limitation in determining phytoplankton community structure

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The cultural eutrophication of three Midwest urban reservoirs (Ford Lake, MI; Belleville Lake, MI; and Eagle Creek Reservoir, IN) has resulted in impaired water quality. Nutrient loading to these reservoirs has resulted in the formation of nuisance algal blooms, including possible toxin–producing and/or taste and odor causing, heterocyst–forming blue–green genera such as *Anabaena*, *Aphanizomenon*, and *Cylindrospermopsis*. Analysis of monthly nutrient concentrations (Total P, NO_3^-, NH_4^+) taken from 1998 – 2000 for two southeastern Michigan reservoirs, Ford Lake and Bellville Lake, and weekly nutrient data taken from 1976 – 1996 and bi–weekly data collected in 2003 for Eagle Creek Reservoir, Indiana showed consistent annual trends of NO_3^- + NH_4^+ depletion and P abundance from mid– to late summer, suggesting that phytoplankton growth became seasonally N–limited in these reservoirs. Data from the three reservoirs showed that low N–to–P ratios correlated more strongly with phytoplankton standing stock than N or P alone. Data from Eagle Creek Reservoir showed that low N–to–P ratios preceded an increase in heterocystous *Anabaena* and *Aphanizomenon* concentrations. In 2005, a combination bioassay and high resolution sampling study on Eagle Creek Reservoir began to determine if nuisance algal blooms of these heterocystous blue–green algae were preceded by transition from nitrogen rich (P–limited) to nitrogen poor (N–limited) growth conditions.

Cyanobacteria in eutrophied fresh to brackish lakes in Barataria estuary, Louisiana

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Introduction

Lakes Cataouatche, Salvador and des Allemands in upper Barataria Bay estuary, LA were historically oligotrophic to hypereutrophic fresh and brackish waters. Mississippi River diverted water into Cataouatche and Salvador since late 2003 changes salinity and introduces high nutrient loads. Lac des Allemands does not directly receive diverted water, but receives high nutrient loads from adjacent lands.

Hypotheses

We examined cyanobacteria to detect increases in the extent and duration of cyanobacterial blooms and HABs in response to nutrient additions, particularly the high nitrogen load from the Mississippi River.

Methods

We conduct monthly and biweekly surveys for epifluorescent counts of HABs, HPLC pigments, chlorophyll biomass, and associated water quality. 10-L microcosm experiments of nutrient additions and mixtures of Mississippi River water with lake water have been used to examine nutrient limitation shifts in community structure, and stimulation of potentially toxic HABs. Limited toxin analyses have been conducted.

Results

Common cyanobacteria (and their toxins) include *Anabaena* cf. *circinalis* (saxitoxins and microcystins), *Microcystis* spp. (microcystins), *Cylindrospermopsis raciborskii* (anatoxin), *Anabaenopsis elenkinii*, *Planktonlyngbya* spp., *Raphidiopsis curvata*, and other *Anabaena* spp. (hepatotoxins and/or neurotoxins). In Lakes Cataouatche and Salvador, where diatoms generally dominated phytoplankton communities, cyanobacteria were mostly detected in late spring and summer. The highest concentrations of *A. circinalis* in August had significant increase from 6×10^6 cells L^{-1} in 2003 to 1.1×10^7 in 2004, whereas *R. curvata* remained at the same level of 5×10^5 cells L^{-1}. HPLC pigment analyses and chemical taxonomy results suggest an increase in overall chlorophyll *a* reaching over 70 μg L^{-1} in Lake Salvador in 2003 and 180 μg L^{-1} in spring 2004. *Microcystis* sp. and *Anabaena* spp. were the main contributors to these elevated chlorophyll *a* levels. Lac Des Allemands had higher levels of chlorophyll *a* in comparison to the other lakes due to high abundances of *Anabaena* spp and *Microcystis* sp.. The phytoplankton community was generally dominated by *A. circinalis* during most annual cycles (3.9×10^4 - 6.0×10^7 cells L^{-1}) as indicated by good correlation between its biomass and chlorophyll data. *A. circinalis* counts were high in 2003-2004 in Lac des Allemands, while other cyanobacteria such as *Cylindrospermopsis* spp., *Raphidiopsis* spp. and *Aphanizomenon* sp. increased in 2004 compared to 2003. Dense cyanobacterial blooms occurred in all three lakes in spring and early summer of 2005. Microcosm experiments indicate that phytoplankton growth in the lakes is potentially N limited most of the time. Nutrient additions stimulated the growth of most potentially harmful cyanobacteria. Microcystins were detected in Lake Salvador in April 2005 at levels of 0.3-0.6 μg (protein phosphates inhibition assay; G. Boyer, SUNY-Syracuse, unpubl. data).

Conclusion

The Davis Pond Mississippi River diversion is not yet fully operational, but limited outflows are affecting the salinity and nutrient regimes in Barataria estuary. Nutrient inputs, either from periodic diverted Mississippi River water or adjacent areas, have increased the growth of harmful bacteria and changed the phytoplankton community in three upper basin lakes. Little is known about trophic transfer of cyanobacterial toxins, but recent studies indicate there may be both environmental effects and human health concerns.

Chemical characterization of the algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginosa*

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Introduction

The algistatic properties of barley straw (*Hordeum vulgare*) have been observed in laboratory studies and *in situ.* Laboratory studies have produced inhibition as well as stimulation of growth in freshwater and marine species. While taxa known to be inhibited have increased, comparatively little has been done to isolate and classify the compound(s) responsible for the algistatic effect.

Hypotheses

The aim of this study was to confirm and characterize the nature of the toxic component(s) in decomposing barley straw that inhibit the growth of *Microcystis aeruginosa*.

Methods

A microplate assay system was developed using *M. aeruginosa* to help isolate and identify the inhibitory components of barley straw extract. *M. aeruginosa* was selected for the bioassay as it has been consistently inhibited by barley straw extract in studies conducted in our laboratory and by others. The 24–well plate assay utilizes *in vivo* fluorescence monitoring with a TECAN GENios plate reader for determination of chlorophyll–a levels in each 2 mL culture.

Results

Fractionation and partial characterization of inhibitory extracts prepared using several different procedures suggests the inhibitors are polyphenolics with molecular weights between 1000 and 3000. Percolation of the aqueous extract through a Polyamide CC6 resin or through various MW cutoff filters resulted in the loss of algistatic activity, which confirms this assertion. Hydrolysis of the extract resulted in little change in the activity profile. Fractionation by HPLC methods yielded a highly potent multi–compound fraction, which is algicidal at 353 mg L^{-1} and algistatic between 11.1 – 3.53 mg L^{-1}.

Conclusion

The consistent inhibition of *M. aeruginosa* observed by ourselves and several other investigators suggests that the algistatic components of barley straw may be useful in the management of *M. aeruginosa in situ*. In the Chesapeake Bay region recent closures of public beaches in response to *M. aeruginosa* blooms and the presence of microcystin in livers of great blue herons in a recent mortality event emphasizes the need for management methods.

Invertebrate herbivores induce saxitoxin production in *Lyngbya wollei*

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Introduction

Most studies of freshwater benthic algal communities have attributed changes in community composition to anthropogenic eutrophication, even though selective herbivory can influence community structure by removing palatable species. The benthic community of Lake Guntersville, AL, USA is dominated by the cyanobacterium *Lyngbya (Plectonema) wollei*, but also includes the green alga *Rhizoclonium hieroglyphicum* and a variety of invertebrate herbivores, such as snails (*Pleurocera annuliferum*) and amphipods (*Hyalella azteca*). The dominance of *L. wollei* in this community may be reinforced by the production of chemical defenses, including saxitoxin (STX).

Hypotheses

1. *L. wollei* is less palatable to snail and amphipod grazers than *R. hieroglyphicum*.
2. Herbivores decrease the growth rates of monocultures of *L. wollei*.
3. Herbivores induce the production of STX.
4. Increased STX concentrations are correlated with decreased *L. wollei* growth rates.

Methods

We used artificial foods to test the palatability of these primary producers to an herbivorous snail, *Pleurocera annuliferum*, and to an omnivorous amphipod, *Hyalella azteca*. For amphipods, we also examined the palatability of crude extracts of *L. wollei*, pure STX, and *L. wollei* sheath material. We grew 1 g monocultures of *L. wollei* to test whether snails, amphipods, and mechanical damage induce STX production. We grew 1, 2, and 3 g of *L. wollei* and *R. hieroglyphicum* in a response surface design to examine how snail herbivory and potential competitors impact STX production.

Results

Both snails and amphipods preferred *R. hieroglyphicum* over *L. wollei*. *L. wollei* crude extracts and pure STX stimulated amphipod feeding; amphipods were deterred by *L. wollei* sheath material. In monocultures, snail herbivory generated strong compensatory growth, while amphipod herbivory decreased *L. wollei* growth rates. Snail herbivory induced high concentrations of STX, but amphipod herbivory did not. In the response surface design, with low N:P ratios, increased STX concentrations were correlated with decreased relative growth rates, suggesting a cost of STX production. However, no trade–offs were observed in monocultures, with higher N:P ratios.

Conclusions

Our results indicate that invertebrate herbivores can strongly influence the composition of freshwater benthic algal communities and the production of cyanobacterial secondary metabolites. Since previous reports of STX production in *L. wollei* have documented a high variability in toxicity among locations, efforts to reduce toxicity should not only focus on reducing nutrient availability, but should also consider interactive effects of palatability, herbivory and competition. Trade–offs between *L. wollei* growth rates and saxitoxin production may depend on the relative supply of nitrogen and phosphorus. The dominance of *L. wollei* in aquatic communities may be maintained by both induced chemical defenses and strong compensatory growth.

A comparison of cyanotoxin release following bloom treatments with copper sulfate or sodium carbonate peroxyhdrate

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Introduction

Nuisance and harmful algal blooms are often controlled by the use of chemical treatments. However, many cyanobacteria retain cyanotoxins within their cell structure, and upon cell lyses (cell death) will release these toxins into the water column. These secondary compounds can be potentially harmful to humans who drink these waters, either from contaminated drinking water reservoirs or from recreational activities (e.g., swimming, water skiing) that promote water ingestion. For example, in the 1970's approximately 150 people (mostly children) in Palm Island, Australia were hospitalized with severe hepatoenteritis and kidney failure. This outbreak was attributed to a cyanotoxin, cylindrospermopsin, which had accumulated in drinking waters following the reservoir's treatment with copper sulfate. In this study, we evaluated both cell-bound and soluble (released following cell lyses) microcystin concentrations, following chemical treatment (with copper sulfate, or sodium carbonate peroxyhydrate; PAK-27™) of a cyanobacterial bloom dominated by *Microcystis aeruginosa*.

Hypotheses

The application of algaecides will increase the level of soluble (free) microcystins and decrease the level of cell bound microcystins. The degree of algaecide treatment will influence the how much cyanotoxin will be released following treatment.

Methods

Bloom samples (composed primarily of *Anabaena* and *Microcystis*) were incubated in 4 L microcosms. The microcosms were dosed with varying levels of algaecides (low ~0.15 ppm, moderate ~1.5 ppm, and high ~5.0 ppm), of either $CuSO_4$ or PAK-27™. All microcosms were situated within the lake to insure natural temperature and light regimes. Water samples were collected (100 mL) initially (Day-1), and 10-, 20-, and 30-days post treatment. Samples were analyzed for microcystin-LR using ELISA (Enzyme-Linked ImmunoSorbent Assay) molecular techniques. The water samples were filtered to separate cell-bound and soluble microcystin. The soluble fraction was concentrated to 2.0 mL using solid phase extraction techniques (C-18 silica cartridges; Waters Sep-Pak Plus). The cell-bound fractions, collected on glass fiber filters, were extracted in 80% methanol. Chlorophyll-*a* levels were determined spectrophotometrically.

Results and Conclusion

In this study, we observed significant declines in cell-bound microcystin-LR by Day-10 (by as much as 0.8 μg L^{-1}) and continued through Day-30 (up to 1.8 μg L^{-1}) in copper-treated microcosms. There were no reductions in cell-bound microcystin-LR observed in PAK-27™ treated microcosms. The declines in cell-bound toxins observed in this study indicate that copper sulfate chemically disrupted cyanobacterial cells, thereby releasing toxins into the water column. This release of toxin was observed in the soluble microcystin-LR fraction by Day-20 (by as much as 1.3 μg L^{-1}) for both PAK-27™ and copper treatments. When considering that the World Health Organization (WHO) placed a provisional guideline of 1.0 μg L^{-1} for potable water, it is critical that cyanobacterial blooms be approached with caution when applying chemical treatments. In this study, for example, the increase of soluble toxin was nearly double the recommended level by the WHO.