1 PHOTORELEASE OF MICROCYSTIN-LR FROM RESUSPENDED SEDIMENTS

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13 **ABSTRACT**
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15 A series of ten photolysis experiments was conducted with sediments exposed to
16 *Microcystis* sp. blooms to determine if sunlight is capable of mobilizing the biotoxin
17 microcystin-LR (MC-LR) into the water column. There was a net photorelease of MC-LR in
18 irradiated suspensions in all cases relative to dark controls, ranging from 0.4 - 192 $\mu\text{g L}^{-1} \text{ g}^{-1}$ into
19 the dissolved phase. This should be viewed as a minimum estimate of photorelease due to
20 concurrent photodegradation of dissolved toxin. Dissolved MC-LR concentrations in a sediment
21 suspension increased linearly in the aqueous phase during a six-hour irradiation with simulated
22 sunlight suggesting that longer exposure times produce greater quantities of MC-LR. There was
23 a significant positive correlation between photorelease of toxin and percent organic carbon of the
24 resuspended material, implying that organic-rich sediments yield the greatest photorelease of
25 MC-LR upon exposure to full spectrum sunlight. Samples exposed to photosynthetically active
26 radiation (400 nm – 700 nm) were responsible for less than 2% of the photorelease compared to
27 full spectrum exposures. Model calculations indicate that photochemical processing of bloom
28 impacted sediments could be responsible for as much as 100% of the average standing stock of
29 MC-LR in a freshwater pond located in southeastern North Carolina, where surface water
30 concentrations were also measured. Mass spectrometric analysis revealed a new peak in light
31 exposed flasks that appears to be a photo-induced isomerized product of MC-LR.
32 Photoproduction from resuspended sediments therefore represents a significant but previously
33 unrecognized source of highly toxic MC-LR and photoproducts of unknown toxicity and fate to
34 aquatic ecosystems.

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36 **Keywords:** photochemistry; sediments; sunlight; MC-LR

INTRODUCTION

Cyanobacterial blooms are an increasing environmental concern not only for their unsightly appearance in natural waters but also for the toxins that are produced. One group of toxins created by several cyanobacteria genera are cyclic heptapeptides with over 100 different congeners distinguished primarily by variations around two amino acids (Buratti and Testai, 2015). One of the most toxic and frequently detected compounds is microcystin-LR (MC-LR) with leucine (L) and arginine (R) in variable positions. This toxin is an inhibitor of protein phosphatases, which causes an imbalance in protein phosphorylation that can ultimately lead to serious liver damage (Dawson, 1998).

Blooms of MC-LR-producing cyanobacteria are associated with a variety of freshwater ecosystems, but are also commonly found in low-salinity estuarine environments worldwide (Lehman et al., 2005; Tas et al., 2006). It is well established that microcystins are degraded by microbial processes (Cousins et al., 1996; Dziga et al., 2013) as well as by ultraviolet (UV) and visible light (Fotiou et al., 2013; Welker and Steinberg, 1999, 2000; Wormer et al., 2010) with hydroxyl radical attack and photosensitization as the primary destructive mechanisms. Sorption to sediments is also a major sink for microcystins due to their strong affinity for particles (Liu et al., 2008; Mohamed et al., 2007; Morris et al., 2000). Microcystins enter sediments through a variety of pathways including chemical coagulation and flocculation (Drikas et al., 2001) as well as coprecipitation with clays and other particles (Pan et al., 2006). The sorptive capacity of sediments is typically related to the surface area of sediment and the amount of organic material present with high sorption rates in sediments containing relatively large amounts of organic or clay material (Miller et al., 2001; Wu et al., 2012). Microcystins can be detected in sediments as deep as 30 cm but are primarily found at the surface (Chen et al., 2008; Tsuji et al., 2001).

Sediments containing MC-LR can be naturally resuspended by wind, waves, and water currents as well as by anthropogenic activities including dredging, bow waves, propeller wash and fishing activities. Once sediments are resuspended they may be exposed to sunlight for extended periods as they enter the photic zone. This is significant because earlier research has definitively demonstrated that exposure of sediments to sunlight dramatically impacts a variety of important biogeochemical processes including trace metal mobilization, nutrient generation and organic matter cycling (Kieber et al., 2006; Mayer et al., 2006; Southwell et al., 2010). In an engineered experiment, Munusamy et al. (2012) demonstrated that sediment- water interactions were also important in the biogeochemical cycling of MC-LR in natural waters (Munusamy et al., 2012). The authors suggested that photodegradation of dissolved toxin was almost complete after exposure to high energy 254 nm light for 60 min in the presence of sediments with bound toxin.

The goal of the present study was to explore the role of sunlight exposure on natural sediments recently impacted by blooms of *Microcystis sp.* that are resuspended into the photic zone under environmentally relevant conditions. We hypothesize that exposure to sunlight will engender release of the toxin from the sediment to the aqueous phase and that this release will be dominated by abiotic processes. The importance of photochemical processes was evaluated by examining changes in dissolved MC-LR in laboratory created sediment suspensions in the presence and absence of simulated sunlight. The goal of these controlled photolysis studies is to determine if photorelease of MC-LR occurs with sediment collected from geographically diverse locations in the eastern and midwestern United States. The significance of photochemical processes to the structure and abundance of sediment-bound MC-LR was also evaluated. This information better clarifies the role of light and sediment resuspension on the biogeochemistry

and cycling of MC-LR in sensitive aquatic ecosystems under environmentally realistic conditions.

METHODS

Study Sites and Sediment Sampling

Surface sediments were collected in Wilmington, North Carolina in a subdivision retention pond (Porter's Neck; PN) along with locations along the Cape Fear River (Burgaw) and Lake Tabor, NC. Samples were also collected in a pond in Dacula, GA (Atkinson Pond), Lake Erie, OH and Lake Winnebago, WI. Collection sites were chosen based on locations where visible blooms of *Microcystis sp.* were observed. Table 1 contains pertinent sample information including sampling date, location and the concentration of MC-LR in non-size fractionated sediment extracts. In order to keep the integrity of the surface suspendable material intact, the top 1 cm of sediment at each site was collected by scraping with a plastic spatula and placed in either a Kapak or Ziploc bag that was sealed and stored at 4°C for no more than 2 months after collection.

Sediment resuspension and irradiation

All glassware used in sediment resuspension and irradiation experiments was combusted for at least 4 h at 450°C to remove organic impurities. In order to generate the sediment resuspension approximately 10-20 g of homogenized sediment samples was added to a 2 L Nalgene fluorinated polyethylene carboy filled with deionized water (Milli-Q, Waters, $\geq 18 \text{ M}\Omega \text{ cm}^{-1}$). The fine fraction of the resuspension was isolated by shaking the carboy for 1 minute after which the mixture sat undisturbed for 17.5 min allowing sediment particles larger than 10-20 μm

(calculated using Stokes' law) to settle to the bottom. The suspended material remaining in the top 20 cm containing fine particles, referred to as size fractionated sediment, was chosen for these experiments to ensure environmental relevance of results, as this fraction is more susceptible to resuspension and also remains resuspended over greater temporal and spatial scales relative to the coarse fraction.

Following sediment resuspension, 70 mL aliquots were siphoned into combusted 100 mL quartz tubes with an additional aliquot set aside as initial untreated samples. Four quartz tubes were wrapped completely in aluminum foil and acted as controls. The remaining four tubes were irradiated with a solar simulator (Spectral Energy solar simulator LH lamp housing with a 1000 W Xe arc lamp) with a LPS 256 SM power supply for 6 h unless noted otherwise. A sun lens diffuser and an AM1 filter were used to remove wavelengths below 290 nm that are not present in the solar spectrum. Irradiance measurements in our laboratory demonstrate that the solar simulator spectrum closely mimics the measured mid-summer solar spectrum at 40 °N latitude. Six hours was chosen for the length of most experiments because it represents approximately 1 full day of mid summer sunlight exposure at this latitude. Long pass cutoff filters were used in selected experiments to isolate photosynthetically active radiation (PAR; 400-700 nm) by eliminating UV wavelengths. Quartz tubes were placed on a Gyrotory® shaker at approximately 110 rpm during the duration of the experiment to ensure the sediment remained in suspension. The effect of irradiation was evaluated by comparing changes in toxin concentrations between irradiated flasks and dark controls. The concentration difference was normalized to the dry weight of total suspended material (TSS) in each experiment to yield units of $\mu\text{g g}^{-1} \text{L}^{-1}$ present in the 70 mL irradiation flasks. Ten resuspension experiments were conducted with sediments impacted by *Microcystis* sp. collected from 5 sites.

Another series of controlled photolysis experiments was conducted with sediments impacted by blooms of *Microcystis* sp. to better understand which parameters were most important in controlling photorelease of the toxin. The first series explored the influence of exposure time by irradiating a suspension of Burgaw sediment. Aqueous phase samples were collected at 0, 1, 3, and 6 h and the concentration of MC-LR in the aqueous phase normalized to the mass of dry resuspended sediment. The effect of irradiation wavelength on photochemical production of MC-LR was also evaluated with size-fractionated sediments collected from PN 2. This has important ramifications with respect to the depth at which photorelease of toxin from resuspended sediments occurs. In the optically thick, highly turbid waters of the Cape Fear River in southeastern North Carolina, for example, the depth to which PAR penetrates is approximately 1.3 m whereas the penetration of high energy UV light ($\lambda < 400$ nm) is typically < 0.30 m. One portion was exposed to full spectrum sunlight while an analogous portion was irradiated for 6 h using only photosynthetically active radiation (PAR). The concentration of MC-LR in each aliquot was measured initially and after 6 h.

Dissolved phase extractions

Sediment resuspension samples were filtered (0.2 μ m polysulfone Supor; Gelman Sciences) and the dissolved microcystin-LR extracted using a C-18 SPE reverse phase cartridge (Agela, 500 mg bed volume, 50 μ m particle size). Each cartridge was conditioned with 6 mL methanol and equilibrated with 10 mL deionized water after which the 70 mL sample was loaded onto the cartridge. All glassware that came into contact with the filtered sample was also rinsed and the washings applied to the cartridge. After sample loading the cartridge was rinsed with 6 mL deionized water followed by 5 mL of 20% methanol:80% deionized water. MC-LR was then

eluted off the column with 6 mL of 100% methanol into a 10 mL round bottom flask. Each round bottom flask was dried under vacuum to approximately 0.5 mL and quantitatively transferred to a Teflon lined screw cap vial. Samples were dried under a gentle stream of pre-purified nitrogen gas and no heat. The content of each vial was evaporated to dryness and brought up in 600 μ L of 70% methanol:30% deionized water (Hyenstrand et al. 2001). Samples were sonicated for 30 sec and stored at -20 C^o until further analysis.

Sediment extractions for MC-LR were performed following the methodology developed by Chen et al. (2006). Briefly, 25 mL of a solution of 0.1M EDTA, 0.1M Na₄P₂O₇, and 0.1% v/v TFA was added to approximately 2 g of freeze dried sediment in a 40 mL screw cap vial. Each vial was sonicated and the supernatant drawn up through a Pasteur pipette and applied to a conditioned C18 SPE column. Sediment extract samples followed the same treatment as dissolved extractions once applied to the C18 column. The amount of MC-LR extracted from size-fractionated sediments in all cases was below the detection limit. The volume of quartz tubes utilized (100 mL maximum capacity) limited the quantity of resuspended sediment (0.004 g – 0.053 g) that could be extracted. This amount of sediment resuspended was chosen to keep within an environmentally relevant range for TSS.

Liquid chromatography-mass spectrometry

Samples containing the sediment or aqueous extracts were analyzed on an Agilent 1290 LC coupled to a Bruker Amazon SL ion trap mass spectrometer. The column used was a reverse-phase Agilent Zorbac C18 column (2.0 x 50 mm; 1.8 μ m) with a flow rate of 0.2 mL/min. The mobile phases were (A) 100% HPLC grade water and 0.1% formic acid and (B) 100% HPLC grade methanol and 0.1% formic acid with a 10 μ L injection volume. The initial solvent

conditions were 55% B from 0-2 minutes, then B was increased to 99% from 2-17 min with re-equilibration to 55% B from 17 to 24 minutes. The total analysis time was 24 min. The mass spectrometer source was operated in positive mode with a capillary voltage of -4500V. Ultrahigh purity nitrogen was used as dry gas at a flow rate of 8 L/min. The mass spectrometer was scanned from 50-2200 u at a speed of 8,100 m/z per second. Samples collected from LT, LW and TO were analyzed under a modified scan setting on the mass spectrometer. For these samples the mass spectrometer was held fixed at 995 m/z for the duration of the analytical run. The MC-LR peak was quantified by integrating the M+1 ion at 955.5 m/z and comparing to an external calibration curve treated under the same conditions as the unknown sample. The detection limit of the analysis is 50 pg of MC-LR on the chromatographic column which is the minimum mass of toxin that can be analyzed by the detector. This analytical detection limit corresponds to 42 ng L⁻¹ of MC-LR in the original 70 mL irradiation experiment.

Percent Organic Carbon (%OC)

Freeze dried sediment samples collected on 0.7 µm glass fiber filters were wrapped in Al foil for percent organic carbon analysis. Filters were exposed to fuming HCl for 24 h to remove any inorganic carbon after which they were re-foiled and placed in drying oven for 24 h. Once dried, filter papers were divided into eight equal pieces, placed in tin capsules (9 x 10 mm) and combusted at 1000 °C in a CE Elantech NC2100 elemental analyzer (CE Elantech, Lakewood, NJ). After combustion, gas products were carried by helium flow through a GC column and detected by a thermal conductivity detector.

Statistical treatment

Normalized photochemical release of MC-LR was calculated as the difference between light and dark treatments divided by the mass of size fractionated sediment resuspended as determined by separate analyses of TSS in each experiment. All uncertainties are reported as ± 1 standard deviation (SD). Differences in means between experimental treatments in Table 1 were analyzed using the t-test. For the time course experiment the flux of MC-LR was calculated using linear regression of TSS-normalized concentrations versus time, using concentrations from individual flasks. The significance of correlations in the time course experiments and photoreleased, TSS-normalized MC-LR vs. %OC was evaluated using the non-parametric Spearman rank correlation procedure.

RESULTS

Photochemical release of microcystin-LR from resuspended sediments

MC-LR concentrations in bulk sediment samples ranged from below the detection limit to $0.09 \mu\text{g g}^{-1}$ while surface water concentrations measured at the time of sediment collection ranged from $1.1 - 8.3 \mu\text{g L}^{-1}$ (Table 1). In all cases the dissolved concentration of MC-LR (normalized to mass of dry suspended sediment) was higher in light-exposed samples compared to dark controls (Table 1). There was a great deal of variability in net photorelease with a range of $0.4 \mu\text{g g}^{-1} \text{L}^{-1} - 192 \mu\text{g g}^{-1} \text{L}^{-1}$ and an average photorelease of $28 \mu\text{g g}^{-1} \text{L}^{-1}$. This variability was not driven by differences in bulk sediment-bound toxin concentrations as no pattern was observed between photoreleased and bulk sediment toxin concentrations. For example, the highest sediment concentration found in Porters Neck 3 ($0.09 \mu\text{g g}^{-1}$) had a lower than average release of $8.6 \mu\text{g g}^{-1} \text{L}^{-1}$ whereas the highest release at Porters Neck 5 ($192 \mu\text{g g}^{-1} \text{L}^{-1}$) was accompanied by a relatively low sediment bound toxin concentration of $0.011 \mu\text{g g}^{-1}$.

Factors controlling MC-LR release

There was a significant positive correlation (Spearman $\rho = 1.00$; $p = 0.05$) between irradiation time and the normalized concentration of dissolved MC-LR (Figure 1). There was also a significant positive correlation ($\rho = 1.00$; $p = 0.05$) between net normalized photorelease of MC-LR ($\mu\text{g L}^{-1} \text{g}^{-1}$) and percent organic carbon content in size-fractionated sediment suspensions collected from Porters Neck samples 1-4 (Figure 2). This suggests longer irradiation times and sediments with higher concentrations of organic carbon (20% - 40%) lead to a higher photorelease of MC-LR when exposed to sunlight.

Effect of irradiation wavelength on MC-LR photorelease

There was a significant increase in the dissolved concentration of MC-LR ($28 \mu\text{g L}^{-1} \text{g}^{-1}$) in the sample exposed to full spectrum light (Figure 3) similar to what was observed in the irradiated sediments samples presented in Table 1. The photorelease of MC-LR in the sample exposed to photosynthetically active radiation only was significantly less ($p < 0.01$) ($0.6 \mu\text{g L}^{-1} \text{g}^{-1}$) suggesting that UV radiation (290 nm - 400 nm) is responsible for the majority of MC-LR photorelease from this sediment and that MC-LR photorelease comes primarily from an abiotic source.

DISCUSSION

Results from this study suggest that release of MC-LR from irradiation of resuspended sediments impacted by *Microcystis* sp. is a significant and previously unrecognized source of dissolved toxin to surface waters. Such exposure results from natural or anthropogenic

resuspension by shipping operations, dredging, wind-driven waves or weather events in lakes, rivers, and estuaries that can initiate remobilization of MC-LR. Results of this study are significant and timely as recent studies have suggested that climate change and rising CO₂ levels may enhance cyanobacterial blooms thereby magnifying the potential for photorelease of the toxin (Visser et al., 2016). Current mitigation strategies aimed at controlling these blooms will also most likely be impacted by global climate change (Paerl, 2016) therefore it is essential that researchers and resource managers understand all the processes involved in their biogeochemical cycling including release of the toxin from sediment resuspension.

The net MC-LR produced from sediments upon light exposure presented in Table 1 should be viewed as minimum estimate of photorelease as there was most likely also concurrent photodegradation of dissolved toxin (Welker and Steinberg, 1999, 2000; Wormer et al., 2010). Wormer et al. (2010) found approximately 30% of the initial dissolved toxin was degraded after 5 days of full solar exposure at the water surface, while after 22 days only 21% of the toxin remained intact. Welker and Steinberg (1999) found much higher rates of MC-LR photodegradation in the presence of humic substances, where 45% of the toxin was lost after only 8 h.

The positive correlation between irradiation time and the normalized concentration of dissolved MC-LR presented in Figure 1 suggests that longer exposure times will result in greater release of the toxin. Southwell et al. (2010) found similar results in an earlier study examining nutrient photorelease from size-fractionated resuspended tidal creek sediments. The authors reported a strong positive correlation between dissolved organic nitrogen (DON), NH₄⁺, and PO₄³⁻ concentrations and irradiation time suggesting that nutrient concentration, in addition to MC-LR, have a larger photorelease with longer irradiation times (Southwell et al. 2010).

In an earlier study examining the role of sediment composition on dissolved organic carbon (DOC) photorelease from resuspended sediments, Kieber et al. (2006) found that photoproduction increased in direct proportion to the %OC of added sediment similar to what was observed for MC-LR. In a related study with a wider sediment compositional range, the photorelease of DOC relative to the initial sedimentary %OC was nonlinear (approximately logarithmic between 1.8 and 45% OC) with significant variability below 10% OC (Helms et al., 2014). Helms et al. (2014) suggested that for a given light exposure and particle load the photolytic flux of DOC from resuspended sediments is controlled largely by the source and diagenetic state of the organic material present. Some fraction of dissolved toxin released during our sediment resuspensions may result from cell lysing however given the high sorptive capacity of organic rich sediments for MC-LR and the demonstrated photoreactivity of these sediments (Kieber et al. 2006; Helms et al. 2014), it is also likely that the mechanism of photorelease of the toxin is associated with conversion of sedimentary POC to DOC.

In earlier studies examining the impact of irradiation wavelength on DOC production from resuspended sediments, the high-energy UV wavelengths were primarily responsible for DOC photorelease (Kieber et al. 2006; Mayer et al. 2006). The amount of DOC production in organic-rich Cape Fear River estuary sediment from PAR was approximately half of that produced under full spectrum light exposure. Mayer et al. (2006) suggested that half the total observed particulate organic carbon POC loss during long term incubations (33 h) of Atchafalaya River sediments occurred with visible light while the remaining half occurred by UV light (Mayer et al. 2006).

The influence of light exposure on the fate of MC-LR in aqueous environmental systems is important not only for its impact on the photorelease from sediments but also on the

fate of the toxin once it enters the water column. After observing photodegradation of aqueous MC-LR in autoclaved reservoir water irradiated with natural light at various depths and wavelengths (full spectrum, PAR, UV-A, and UV-B), Wormer et al. (2010) created a photodegradation model based on the percent of MC-LR degraded over time. In a mixed water column the half-life of dissolved MC-LR at the surface ranged from 6.3 – 11.5 d whereas in deeper water (6 m – 10 m) where PAR is the dominant radiation, half-lives of dissolved MC-LR were significantly longer (13.5 – 52 d). This suggests that although photoproduction after exposure to PAR presented in Figure 3 is smaller in magnitude it may still play a role in the biogeochemical cycling of MC-LR because of the potentially longer half-life of the photoreleased toxin.

Environmental Relevance

Measurements of photochemical production of MC-LR from resuspended sediments can be used to estimate the quantitative importance of photorelease as a source of toxin to freshwater ecosystems. Porters Neck pond was used for this calculation because it is the location with the most extensive measurements of the concentrations of extracellular MC-LR in the current study (Table 1). The surface water concentration of MC-LR in Porters Neck pond ranged from a low of $1.1 \mu\text{g L}^{-1}$ to a high of $8.3 \mu\text{g L}^{-1}$ with an average concentration of $3.5 \mu\text{g L}^{-1}$ ($n=5$). The volume of the pond is 3.5×10^7 L determined from its approximate dimensions (1 m deep x 100 m wide x 350 m long). The standing stock of MC-LR determined from the average surface water concentration multiplied by the total volume of the pond is 122 g.

The concentration of MC-LR photoreleased from resuspended sediments into the pond was determined from the normalized concentration of photoreleased MC-LR for PN5 ($192 \mu\text{g L}^{-1}$

g⁻¹) multiplied by the mass of sediment in this sample (9.2×10^{-3} g). The increase in dissolved MC-LR in PN5 light flasks should be viewed as a minimum estimate of photorelease because of concurrent photodegradation and microbial degradation which are also most likely occurring. The amount of MC-LR supplied to the pond by sediment resuspension was determined by taking the photoreleased concentration for PN5 ($1.77 \mu\text{g L}^{-1}$) multiplied by the total volume of the pond (3.5×10^7 L). Results of these calculations suggest that photochemical processing of *Microcystis* sp. impacted sediments resuspended into the photic zone can replace approximately half the 122 g average standing stock in the pond after 6 hours of sunlight exposure with over 100% replaced in instances where ambient MC-LR concentrations were on the lower end of the range ($1.1 \mu\text{g L}^{-1}$). This calculation should be viewed with some caution since it does not account for particle light scattering, advection and settling however, it is an important exercise in that it demonstrates the potential importance of this process in the aquatic environment.

The ramifications of MC-LR photoproduction from impacted sediments may go well beyond increasing concentrations of the toxin in the affected watershed. This is because once in the dissolved state MC-LR can be photo transformed into various products of unknown toxicities and fate. The structure of MC-LR has a unique side chain attached to the cyclic peptide which is a C20 linear amino acid (3-methyl-9-methoxy-2,6,8-trimethyl-10-phenyl-4(E),6(E)-dienoic acid) often abbreviated as Adda (Tsuji et al., 1994). The Adda group is non-toxic by itself but is essential for the expression of microcystin toxicity (Botes et al., 1985; Dawson, 1998). The Adda side chain has configuration of 4(E) and 6(E) and it has been observed to photo-isomerize (Liu et al., 2003). Structures I and II (Figure 4) appear to be photo-induced isomerized products of MC-LR. Both have the same protonated molecular ion (995 m/z), which would be the case for an

isomerization. Peak I was present in the dark control but increased in the light exposed sample.

Structure II was not present in the dark control but did appear in the light-treated sample.

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Figure captions

Figure 1. Normalized concentration ($\mu\text{g L}^{-1} \text{ g}^{-1}$) of dissolved MC-LR from Burgaw sediment in the presence of simulated sunlight as a function of irradiation time (n=1)

Figure 2. Difference in light exposed and dark control normalized concentration ($\Delta (\text{L} - \text{D}) \text{ MC-LR } \mu\text{g L}^{-1} \text{ g}^{-1}$) as a function of the % organic carbon of the resuspended sediments (n=4).

Figure 3. Difference between light exposed and dark controls ($\Delta \text{L-D MC-LR } \mu\text{g L}^{-1} \text{ g}^{-1}$) of size-fractionated Porters Neck #2 sediment exposed to full spectrum sunlight and PAR only wavelengths (n=4).

Figure 4. Total ion chromatogram of photolyzed Porters Neck Pond 5 (PN5) and dark control illustrating photoproducts. Structure I and II are tentatively identified as photo-induced isomers of MC-LR given the same protonated molecular ion (995 m/z).

Figure 1.

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380 Figure 2.

Figure 3.

Figure 4.

Table 1. Location and date of sediment collection for irradiation experiments. Photorelease reported as the difference in light and dark incubations respectively Δ (L – D) of the average normalized-concentration of MC-LR ($\mu\text{g g}^{-1} \text{ L}^{-1}$) using size-fractionated sediments from all sample sites. Error represents $\pm$ one standard deviation. Photorelease values in bold significant at $p < 0.05$ NA = not analyzed, ND = none detected.

Sampling Site	Date Collected	Latitude (Dec Deg)	Longitude (Dec Deg)	Surface Water Concentration ($\mu\text{g L}^{-1}$) (n=1)	Size- fractionated Δ (L – D) MC-LR ($\mu\text{g L}^{-1} \text{ g}^{-1}$) (n=4)	Bulk Sediment Concentration ($\mu\text{g g}^{-1}$)
Burgaw	July 27, 2011	34.59889	-77.87528	NA	4.0	0.08 ± 0.02 (n=4)
Atkinson Pond	August 07, 2013	33.93864	-83.83183	6.9	0.4	ND
Porters Neck 1	August 14, 2013	34.28052	-77.76676	8.3	3.0	$0.06 \pm .003$ (n=4)
Porters Neck 2	August 17, 2013	34.28052	-77.76676	5.5	11.7	0.08 ± 0.01 (n=4)
Porters Neck 3	August 22, 2013	34.28052	-77.76676	1.1	8.6	0.09 ± 0.003 (n=4)
Porters Neck 4	August 26, 2013	34.28052	-77.76676	1.1	8.3	$0.02 \pm .001$ (n=4)
Porters Neck 5	September 23, 2013	34.28052	-77.76676	1.4	192	0.01 (n=1)
Lake Tabor	June 26, 2014	34.165476	-78.852165	NA	1.8	0.004 (n=1)
Lake Winnebago	August 11, 2014	44.009080	-88.416170	NA	26.4	NA
Lake Erie	September 03, 2014	41.718801	-83.457291	NA	29.1	NA

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