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Rates of Humic Substance Photosensitized Degradation of Microcystin-LR in Natural Waters

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In the course of the eutrophication of most inland waters, the abundance of cyanobacteria has increased, and consequences of raised cyanotoxin levels gave rise to numerous studies on their occurrence, ecological impacts, and toxicological effects. One of the most potent cyanotoxin, microcystin-LR, has tumor-promoting properties; thus, knowledge of the persistance of the toxin in natural waters is of high interest. The environmental fate of dissolved microcystin is still poorly understood. Microbial degradation is efficient in most inocula but is achieved only after a lag phase of several days to weeks. Under field conditions, the toxin can be alternatively degraded or transformed by indirect photolysis in the presence of humic substances or algal pigments. In the present study, we determined the rates of degradation of microcystin-LR by natural sunlight in the presence of fulvic acids and natural dissolved organic matter. Microcystin-LR was not degraded by sunlight alone but in the presence of photosensitizers. First-order rate constants depended on the optical density of experimental solutions and showed a saturation effect at higher concentrations of fulvic acids. In filtrates of surface waters, first-order degradation rates depended linearly on the absorbance of respective waters at $\lambda = 350$ nm. Compared to solutions of purified fulvic acids, photolyis in natural waters amounted to only one-third at a given optical density. Therefore, rates of photosensitized degradation in natural waters were rather low, and estimates of in situ half-life times were about 90−120 days per meter depth of the water column. Although photosensitized degradation of microcystin occurs in all surface water bodies immediately following their release, it will be of significance only in very shallow water bodies.

Introduction

Some of the most potent cyanobacterial toxins are microcystins (Mcyst, Figure 1), cyclic heptapeptides that occur in more than 50 structural variants (*1*) mainly differing in two variable amino acids and with varying toxicity. The moiety essential for the toxicity is an unusual amino acid [(2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,4,6-trimethyl-10-phenyldeca-4,6-dienoic acid; ADDA] with two conjugated double bonds in a hydrophobic side chain (*1*).

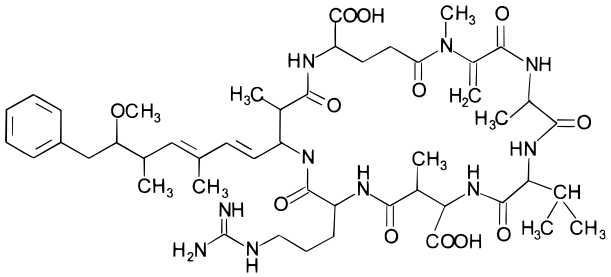

FIGURE 1. Molecular structure of microcystin-LR.

Mcysts are released into the surrounding water mainly through cell lysis, which occurs, for instance, after viral infection or photodamage in surface blooms (*2, 3*). Released, dissolved toxins are of special concern in the production of drinking water since their removal from raw water requires strong oxidants at high concentrations (*4*) and the uptake of low doses of Mcyst is supposed to promote primary liver cancer (*5, 6*), for instance. Clear safety levels of maximum tolerable concentration are still under discussion (*7*), but the World Health Organization recently proposed a maximum tolerable concentration of Mcyst in drinking water of 1 μg of toxin L^{-1} (*8*).

To date, little is known about the persistance of dissolved Mcyst under field conditions. Microbial degradation has been found to be a likely elimination pathway of the toxins (*9*−*11*), but quantitative results were so far mainly obtained under laboratory conditions and should be extrapolated to lakes only with care. However, in most studies a certain recalcitrancy of Mcyst to bacterial degradation was evident: although the toxins were degraded by most bacterial consortia, degradation set on after a lag phase of several days or weeks (*12, 13*).

In a previous study, we qualitatively reported on the indirect photolysis of Mcyst in the presence of humic substances (*14*). No difference in degradation efficiency has been found among three structural variants (LR, YR, and RR). Nonetheless, with the experimental setup of the previous study, a quantification of degradation rates could not be performed in a manner that would allow the estimation of the elimination of the toxins under field conditions. In the present paper, we present the results of a series of experiments that focused on the quantification of rates of photosensitized degradation of Mcyst-LR by solar radiation in natural waters.

Experimental Section

Light Measurements. Global radiation was quantified continuously with a pyranometer equipped with a glass calotte at the institute's landing stage on Lake Müggelsee. Readings of integrated radiation energy (E_{GR} in kJ m^{-2} h^{-1}, $\lambda = 280$−2900 nm) were made every full hour and automatically stored on a PC.

Solar spectral irradiance was recorded with a spectroradiometer (LI-1800UW, LI-COR, Lincoln, USA) calibrated for measurements in air. Spectral irradiance was recorded every 20 min (Figure 2) and UV radiation ($\lambda = 300$−400 nm) was calculated as integrated radiation power P_{UV} (W m^{-2}). UV radiation data were used to calculate cumulated radiation energy ($E_{UV} = P_{UV}t$, in kJ m^{-2}) and compared to measurements of global radiation during the same time intervals. A value of 300 nm was the lowest detectable wavelength of the spectroradiometer, thus UV−B radiation was partly cut off

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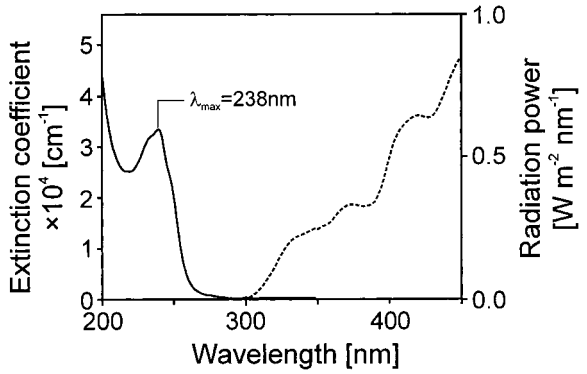

FIGURE 2. Molar, decadic extinction spectrum of microcystin-LR in water (full line, left scale) and mid-European summer solar spectrum (dashed line, right scale).

in the recordings. However, at mid-European latitudes, UV−B radiation of $\lambda < 300$ nm is negligible. The wavelength interval of $\lambda = 300-400$ nm was arbitrary with regard to the mechanisms likely involved in the observed processes and was chosen according to the conventional definition of solar UV radiation ($\lambda = 280-400$ nm). During the experiments with natural sunlight, the actual irradiation could not be controlled. Hourly UV radiation data calculated as described above correlated linearly to global radiation ($R^2 = 0.72$). In the following, incident UV radiation energy refers to the value calculated from this relationship.

Experimental Solutions. Microcystins were isolated from cyanobacterial bloom material kindly provided by Gary Jones, CSIRO. Extraction and purification (*9*) was performed at the CSIRO Laboratory, Department of Land and Water, in Griffith, Australia. Purity of Mcyst-LR was higher than 90%. The absorbance spectrum of Mcyst-LR is given in Figure 2. Concentrations of Mcyst in experimental solutions were chosen allowing an HPLC analysis without further processing of the samples in a concentration range of 5−55 μg L^{-1}.

Concentrated and desalted fulvic acids originated from a water treatment plant in Fuhrberg, Germany (FuFA; *15*). In experiments with FuFA, ammonium acetate buffer in a final concentration of 4 mM was added to the solutions.

In two experiments, indirect photolytical degradation of Mcyst in natural waters was recorded. Samples were taken from lakes, ponds, and rivers near Berlin and filtered through cellulose−acetate filters (0.2 μm, Sartorius). Concentration of dissolved organic carbon (DOC) in the water samples was measured on a total carbon analyzer (TOC-5000, Shimadzu). UV absorption spectra of the samples were obtained on a UV−Vis scanning spectrophotometer (UV-2101 PC, Shimadzu). Since all humic solutions had similar featureless absorption spectra with exponentially increasing absorbance toward shorter wavelengths the absobance at $\lambda = 350$ nm (A_{350}), converted to absorptivity ($a_{350} = A_{350} \times 2.303/l$, in m^{-1}) was taken as a measure of optical density in the UV range. As a further characteristic, the slope (S, in nm^{-1}) of ln-transformed absorbance spectra ($\lambda = 300-450$) was calculated (*16*, *17*).

Exposure and Sampling. Solutions of Mcyst in natural waters and solutions of FuFA were prepared freshly and filtered directly in the experimental tubes. In one set of experiments performed at the Bundesanstalt für Materialforschung (BAM), Berlin, the exposure was done under artificial light; another set of experiments was performed at the IGB on the shore of Lake Müggelsee, exposing the samples to natural sunlight.

Artificial light for the former experiments was provided by a temperature-controlled incubator equipped with a set of four different types of fluorescent tubes emitting light with a quality and intensity similar to natural sunlight in the range of $\lambda = 300-400$ nm (Global-UV-Testgerät UV200, Weiss Umwelttechnik). For longer wavelengths, light intensities in the incubator were considerably lower when compared to natural sunlight. The temperature during the experiments was set at 30 ± 1 °C.

Experimental solutions were exposed in quartz tubes (200 mm, 38 mm i.d.) sealed with polyethylene stoppers. All glassware was acid washed (10% HCl) and autoclaved prior to experiments. Subsamples were taken with a polyethylene syringe fitted to PTFE tubing. This setup allowed the mixing as well as the aeration of the tube content in regular intervals, thus avoiding oxygen depletion. In all experiments, two kinds of controls were run in parallel. The "light control" was Mcyst-LR in Milli-Q water without humic substances, and the "dark control" was an unirradiated treatment containing humic substances.

At the IGB, the samples were exposed to sunlight at the shore of Lake Müggelsee in immediate proximity to the site of light measurement at an angle of 30° to the ground in July and August 1998. The temperature could not be controlled during the experiments, and in order to avoid high temperatures, the tubes were externally cooled by rinsing with chilled Milli-Q water. The duration of exposure varied from 8 to 9 h in experiments with FuFA as the photosensitizer to several days in experiments with natural waters.

Sample Analysis. Subsamples (1 mL) taken from the exposed solutions were filled directly in HPLC vials and analyzed immediately or kept at 4 °C in the dark for less than 2 days. The HPLC system consisted of a Waters 717plus autosampler, a 616 pump, a 600S controller, and a 996 photodiode array detector (PDA). HPLC analysis of Mcyst was slightly modified from Jones et al. (*9*) with a gradient from 22% to 37% acetonitrile (4 mM ammonium acetate) within 5 min followed by 10 min of 37% acetonitrile isocraticically, applied to a LiChrospher column (RP18 endcapped, 250 × 4 mm, Merck) at a flow rate of 1 mL min^{-1}. Absorbance data were collected at a rate of 5 spectra s^{-1} and a resolution of 3.6 nm, allowing a quantification at a level of >0.5 ng on column, i.e., of concentrations >2.5 μg L^{-1}. The system was calibrated with Mcyst-LR obtained from Calbiochem (La Jolla, USA).

Data Processing. All Mcyst data obtained were series of concentrations. Assuming a first-order kinetic of the degradation with respect to Mcyst-LR rate constants were calculated by fitting a linear regression to ln-transformed concentrations versus cummulated incident radiation energy. The slope of the fitted line was then taken as energy-related rate constant, $-k_E$ in (kJ m^{-2})$^{-1}$. This was given preference to a time-based rate constant for the fluctuating irradiance on some days. All rate constants were tested for significance by Students t-test (H_0: $-k_E = 0$, $t_{(\alpha,2,\nu)}$).

Results

In all treatments combining humic substances and irradiation, the concentration of Mcyst significantly declined, whereas humic substances or irradiation alone had no significant effect on Mcyst concentrations. During the exposition to sunlight for several days a slight decline of Mcyst in Milli-Q water was observed. Regression analysis, however, revealed the degradation rate as insignificant ($P > 0.05$). Thus, direct photolysis and adsorption to dissolved organic matter could be excluded as mechanisms responsible for the observed decline in toxin concentrations. Reinjection of selected samples 24 h and 5 days, respectively, after exposure proved the loss to be irreversible. For neither treatment in which a loss of Mcyst could be observed, new peaks were detected in HPLC chromatograms, not even when a high initial concentration of 0.5 mg of Mcyst L^{-1} was applied. In the following, the decline in Mcyst concentration will be referred to as degradation, although the molecular mech-

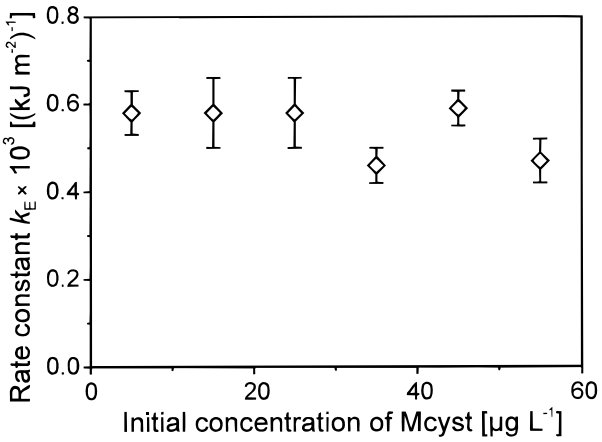

FIGURE 3. Energy-related rate constants of the photosensitized degradation of microcystin-LR in relation to initial concentrations of microcystin-LR. Exposure was done in solution of fulvic acids at a concentration of 4.7 mg of DOC L^{-1} or with a_{350} = 10.1 m^{-1}, respectively. Error bars represent ± SE.

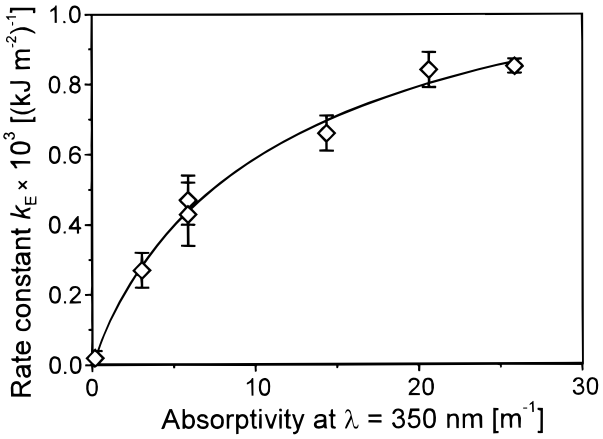

FIGURE 4. Energy-related rate constants of the photosensitized degradation of microcystin-LR in relation to the optical density of solutions of fulvic acids. Initial concentration of microcystin-LR was 35 µg L^{-1}. Error bars represent ± SE.

anism is not discovered yet and most likely the loss does not reflect a complete mineralization. Nonetheless, there is a wealth of evidence that the decline in Mcyst concentration reflects a proportional decline of toxicity, which is the main topic of the present paper.

Photosensitized Degradation in Solutions of Fulvic Acids. Varying initial concentrations of Mcyst had no effect on degradation rates (Figure 3), thus proving the assumption of a first-order kinetic. $[Mcyst]_{init}$ ranged from 5 to 55 µg L^{-1}, spanning the range of potentially occurring concentrations in natural waters. Though irradiation with artificial light gave a similar form of result, the rate constants acounted for only about 50% of those achieved in sunlight. Similar results in experiments with natural waters and possible reasons for this effect will be discussed below.

Incident daily UV radiation energy accounted for about 10^3 kJ m^{-2} in the summer months (June−September). Thus, about 50% of the initial Mcyst could be degraded per day. This value, however, was strictly related to the actual dimensions of experimental vessels and actual concentrations of FuFA.

Variation of the concentration of FuFA from 1.5 to 12.0 mg of DOC L^{-1} or a_{350} = 3.1−25.9 m^{-1}, respectively, resulted in increasing degradation rates with increasing optical density (Figure 4). The relationship of k_E to [FuFA] was a saturation function with a strong increase in k_E for a_{350} = 0−15 m^{-1}. For

TABLE 1. Properties of Filtrates of Natural Waters and a Solution of Fulvic Acids (FuFA)[a]

	DOC (mg L^{-1})	pH	a_{350}, initial (m^{-1})	S, initial	a_{350}, end (m^{-1})	S, end
Milli-Q	0.1	7.3	0.2	0.000	0.2	0.000
FuFA	5.0	7.1	10.7	−0.019	nd[b]	nd
lake 1	2.9	8.1	4.8	−0.016	2.4	−0.014
lake 2	3.4	7.8	4.8	−0.017	1.7	−0.017
lake 3	6.9	7.6	7.4	−0.014	2.5	−0.015
lake 4	8.3	7.5	3.5	−0.013	3.3	−0.010
lake 5	5.2	7.5	6.1	−0.017	2.8	−0.018
pond 1	8.7	7.7	10.0	−0.018	8.9	−0.019
pond 2	15.4	7.6	7.8	−0.017	3.5	−0.014
pond 3	17.3	7.4	15.1	−0.018	6.9	−0.015
pond 4	11.5	7.6	17.0	−0.014	10.0	−0.015
pond 5	17.2	7.5	22.3	−0.013	8.4	−0.015
river 1	3.8	7.6	7.0	−0.014	2.3	−0.009
river 2	3.9	7.5	8.5	−0.014	5.2	−0.009
river 3	4.8	7.9	6.8	−0.016	2.0	−0.017

[a] Optical properties (a_{350} and S) were recorded before and after exposure to simulated sunlight for 72 h at an intensity of 36 W m^{-2} s^{-1}. Natural waters in the table originated from water bodies near Berlin, Germany, and are represented by filled squares in Figure 5. [b] nd, not determined.

a_{350} > 15 m^{-1}, increases in k_E in relation to the optical density were much lower. We plotted the data of Figure 3 in a double-reciprocal plot to obtain characteristic values of the saturation relationship. A linear function fitted to the transformed data had a coefficient of determination of R^2 > 0.99, and by extrapolation, Y- and X-axis intercepts were determined. By re-transformation of these values, the maximum rate constant, $k_{E,max}$, and the optical density at which $0.5k_{E,max}$ is reached, $a_{350,max/2}$, were obtained. The respective values were $k_{E,max}$ = 0.012 kJ^{-1} and $a_{350,max/2}$ = 10.6 m^{-1}, which corresponds to a $[FuFA]_{max/2}$ = 5.0 mg of DOC L^{-1}.

Degradation Rates in Natural Waters. From preliminary experiments, we knew that photosensitized degradation is slower in natural waters as compared to solutions of purified fulvic acids. Therefore, the duration of experiments with natural waters was about 10 times the duration of experiments with FuFA. Furthermore, HPLC analysis in natural waters is hinderted due to humic and other substances, which are more hydrophobic than fulvic acids and therefore can produce interfering peaks in chromatograms under the actual analytical conditions. A further purification of the samples was not possible for technical reasons, and thus, the sensitivity of HPLC analysis had to be considered lower. Sample pH ranged from pH 7.4 to pH 8.1, and DOC concentrations ranged from 2.9 to 17.3 mg of DOC L^{-1}. Optical density correlated to DOC concentration, but coefficient of determination accounted for only R^2 = 0.67; DOC concentrations higher than 10 mg L^{-1} especially showed broad residual scatter.

During exposure, the optical density of the natural waters decreased considerably (Table 1). a_{350} declined to 51 ± 21% of the initial a_{350} on average. In individual waters, however, a_{350} at the end of exposure ranged from 30 to 95% as compared to the initial values. S values did not change significantly (P > 0.05, paired sample t-test), and mean values of S were −0.016 ± 0.002 and −0.015 ± 0.003 before and after exposure, respectively.

The concentration of Mcyst declined significantly ($P \ll$ 0.001) in all natural waters but not in Milli-Q water, and rate constants significantly correlated to the initial optical density of the respective waters (Figure 5). In the experiment with natural sunlight, optical densities were distributed in a range of a_{350} = 1.9−7.4 m^{-1} with one exception (a_{350} = 22.7, shaded circle). Because of the strong unevenness in this distribution, the outlying value was not included in the regression analysis. In the experiment with artificial sunlight, the distribution of

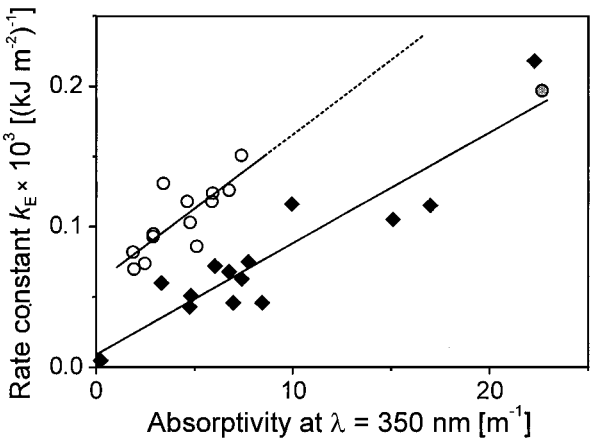

FIGURE 5. Energy-related rate constants of the photosensitized degradation of microcystin-LR in relation to the optical density of filtrates of natural waters. Samples were exposed to natural sunlight (open circles) or simulated sunlight (filled squares). Initial concentration of microcystin-LR was 35 μg L^{-1}.

a_{350} was more even, but here also a main cluster was around $a_{350} = 8$ m^{-1}. These waters represent lakes and rivers, whereas the highly humic waters were derived from small ponds with high allochtonous input of organic matter. Regression analyses revealed good linear relationships between k_E and a_{350} with $R^2 = 0.64$ ($P = 0.0011$) for the experiment with natural sunlight and $R^2 = 0.84$ ($P < 0.0001$) for the experiment with artificial light. The residual scatter, however, was much higher than in experiments with FuFA. The regression lines calculated for the particular experiments differed both in slope and in elevation, with lower rates at the same optical density under artificial light as compared to sunlight ($P < 0.05$, $t_{\alpha(2),\nu}$).

Discussion

The results of our experiments clearly demonstrate both the stability of pure Mcyst under sunlight irradiation and the potential of humic substances to mediate the photolytical degradation of the toxin. Furthermore , the dark controls showed that Mcyst did not undergo dark reactions with humic substances. Coagulation−flocculation experiments with alum (*18*) in several natural waters led to similar results (unpublished data). The molecular mechanisms of the degradation (transformation) process are not known yet, but as discussed earlier (*14*), it is evident that the conjugated double bond in the ADDA moiety is involved in the process. We could not evaluate the toxicity of irradiated Mcyst solutions, e.g., by mouse bioassay, for technical and legal reasons, but the reduction of toxicity proportional to the decline in Mcyst-LR is nonetheless to be expected since the conjugated system in the ADDA moiety is crucial for the toxicity (*1*) and any derivatization at this site results in proportional reduction in toxicity (*19*).

Photosensitized transformation of Mcyst in the presence of algal pigments resulted in $E \rightarrow Z$ transformation at one of the double bonds (*20*). The same transformation was observed when the toxin was irradiated with UV light of $\lambda =$ 254 nm (*21*). However, in both studies the *Z* isomers had similar UV spectra as compared to *E* isomers and were thus detectable by PDA detection. In our study no new peaks were encountered in HPLC chromatograms, so we conclude that the conjugated system is destroyed by derivatization of at least one of the double bonds. By direct photolysis, this is achieved through the formation of tricyclo-ADDA-Mcyst (*21*), which is undetectable by PDA. Photocatalytic degradation in TiO_2 suspension leads to the dihydroxylation of one of the double bonds, which is followed by a cleavage of part of the ADDA side chain (*19*).

In natural waters, transformation of trace pollutants is known to be achieved by the formation of various reactive species (*22*) and/or electronic energy transfer (*23*). Nitrate-induced hydroxy radicals can effectively oxidize pollutants in natural waters (*24*), but they likely were not the oxidizing agent in our study. The linear relationship of degradation rates to optical densities in natural waters indicates that the absorption of photons by DOM is crucial; since natural DOM is an effective scavenger of hydroxyl radicals, any nitrate-induced degradation should be slower in highly humic waters. Hydrogen peroxide is formed in natural waters in dependence of DOM concentration (*25*), but even at doses of 10 mg L^{-1}, H_2O_2 did not oxidize Mcyst (*26*). Concentrations of H_2O_2 in natural waters are about 2 orders of magnitude lower (*25*).

On the other hand, the conjugated double bond at the ADDA moiety not only is mainly responsible for the toxicity of the molecule but also is likely the most sensitive part of the molecule with regard to transformation by electronic energy transfer (*20*, *23*) and singlet oxygen oxigenation (*27*). Further studies should be focused on these mechanisms.

When comparing rate constants in natural waters and FuFA solutions for equal a_{350}, the latter ones were about a factor of 3−4 higher, i.e., FuFA had a considerably higher net quantum yield with respect to the degradation of Mcyst. Although we do not know which structures in DOM are essential for the degradation of Mcyst, most likely reactive chromophores are less abundant in the photochemically aged DOM of surface waters than in the photonaive fulvic acids. FuFA were isolated from groundwater, thus they had not been submitted to photochemical transformation before. Among natural waters, net quantum yields were more evenly as expressed by the linear relationship of k_E to a_{350}. The residual scatter, however, indicates that the respective source of inividual DOM influenced its reactivity. This is also underlined by the remarkable differences in photobleaching. Nonetheless, the variability of a_{350} explains the variability of k_E to high percentages. For estimates of the photosensitized degradation of Mcyst in surface waters, the relationship will be assumed as linear in the range of a_{350} representing lake and river waters.

The relationship of k_E to a_{350} was nonlinear in experiments with FuFA. The saturation function could be due to an increasing competition for photoreactants of quenching structures of the fulvic acids with increasing concentration of FuFA. In this case, however, a similar effect should have resulted in different rate constants in experiments where Mcyst-LR concentrations were varied for an order of magnitude. Another possibility is that the photolysis became light limited with increasing optical density. As the FuFA concentration increases, so did the fraction of photochemically active light absorbed. Starting with short wavelength UV, the proportion of particular wavelength band that is absorbed reaches unity, and at high FuFA concentrations a further increase can only result in the additional absorption of photons relatively low in energy.

Degradation rates in both natural waters and FuFA solution were significantly lower in the experiments with artificial light as compared to rates recorded for sunlight with the former measuring about one-half of the latter ones. One reason simply may be technical since spectral irradiance could not be measured with a single instrument, and spectral irradiance in the incubator could be overestimated due to a lacking instrument comparison (*28*). Partly, the difference could be attributed to differences in light quality between natural sunlight and the artificial light in the incubator. When the degradation rates were calculated based on light energy in a wavelength band of $\lambda = 300-450$ nm instead of conventional UV light ($\lambda = 300-400$ nm), the differences were less marked (data not shown). Thus, sunlight of $\lambda > 400$ nm likely is also active in the degradation process.

Estimation of In Situ Degradation of Mcyst. Aquatic photochemical processes involving natural DOM are highly wavelength dependent. First, incident sunlight radiation is distributed unevenly in the wavelength band that is most active with increasing photon fluxes from high energy to low energy wavelength bands. Second, sunlight is absorbed unevenly by natural DOM with increasing absorbance toward shorter wavelength, and the quantum yields of photochemical processes involving DOM are often strongly wavelength dependent with high values in the UV−B that tail down to zero in the UV−A or visible light range (*29*). On the other hand, the UV−B radiation is absorbed within the topmost layers of the water column in even moderately humic waters (*30*, *31*), and the solar spectrum shifts to longer wavelength with increasing depth. Therefore, photochemical processes in natural waters show a strong vertical gradient (*32*−*34*) that has to be considered when rates of photolysis were to be estimated for whole water bodies.

A depth integrated exposure was impeded by the wave-action in wind-exposed Lake Müggelsee, which would not allow an exposure in defined near-surface water depths and by the limited number of quartz tubes. Instead, the photolytical degradation was tested in a number of waters, and the obtained results were used to indirectly estimate in situ degradation rates of Mcyst-LR.

As mentioned in the Results, the k_E were calculated strictly in relation to the actual dimensions of the experimental vessels. Assuming all photoreactive light is absorbed in in a layer of similar depth (0.038 m), the degradation rate will be $k_{E,max}$ within this layer. Assuming a similar $a_{350,max/2}$ for solutions of FuFA and DOM, a $k_{E,max}$ of 0.34×10^{-3} $(kJ\ m^{-2})^{-1}$ was calulated. With a daily incident UV radiation of 960 kJ m^{-2} in Berlin in the summer on a cloudless day, about 28% of Mcyst-LR is degraded per day in the considered water layer. Averaged for a water column of 1 m depth, this account for 1.1%/day, i.e., a half-life time of $T_{1/2} = 63$ days/m depth. Since exposure was done in air, the rates obtained in our experiments have to be considered as overestimations as compared to rates in a water body due to internal refraction and reflection of sunlight in experimental vessels (*22*, *35*). The correction factor is not clearly defined, but an enhancement of photolytical degradation due to the lens effect by a factor of 1.5−2 is in agreement with most studies. Thus, for sunlight intensity and spectra comparable to mid-European values, the half-life time of Mcyst-LR is estimated to about 90−120 days/m water column in surface waters with humic substance photosensitized degradation as the sole degradation pathway. A half-life time of that length is rather long as compared to degradation by microbial activity. In deeper water bodies, the half-life of the toxin would exceed the duration of a growing season and, therefore, be insignificant for its elimination. On the other hand, all studies on microbial degradation of Mcyst imply that for a period of several days to weeks virtually no degradation occurs (*9*, *11*), whereas the main degradation in terms of declines in absolute concentrations by photolysis is to be expected to occur directly following the release of the toxin.

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