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Author manuscript

Environ Sci Technol. Author manuscript; available in PMC 2020 March 23.

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Published in final edited form as:

Environ Sci Technol. 2018 November 20; 52(22): 13068–13076. doi:10.1021/acs.est.8b02191.

Biological Weighting Functions for Evaluating the Role of Sunlight-Induced Inactivation of Coliphages at Selected Beaches and Nearby Tributaries.

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Abstract

Coliphages can indicate contamination of recreational waters and previous studies show that sunlight is important in altering densities of coliphages, other indicator microorganisms, and pathogens in aquatic environments. Here, we report on laboratory studies of light-induced inactivation of two coliphage groups-male-specific (F+) and somatic coliphage-under various conditions in phosphate-buffered water (PBW). Strains isolated from wastewater treatment facilities and laboratory strains (MS2 and phiX174 coliphages) were evaluated. Inactivation rates were determined in a series of irradiations using simulated solar radiation passed through light filters that blocked different parts of the ultraviolet spectral region. Inactivation rates and spectral irradiance from these experiments were then analyzed to develop biological weighting functions (BWFs) for the light-induced inactivation. BWFs were used to model the inactivation of coliphages over a range of conditions in aquatic environments that included two beach sites in Lake Michigan and one in Lake Erie. For example, modeled effects of sunlight attenuation, using UV absorption data from the three Great Lakes beach sites, inferred that direct photoinactivation rate constants, averaged over a one-meter water column in swimmable areas, were reduced 2- to 5-fold, compared to near-surface rate constants.

Introduction

Pathogens are infectious microbes such as bacteria or viruses which can cause disease. Swimming and other recreational activities in water contaminated with pathogens can make people ill.(1,2) Viruses are thought to be responsible for most gastrointestinal (GI) illnesses contracted in recreational waters impacted by human fecal contamination;(1,3,4) representative human sewage-borne viruses include enteroviruses, noroviruses, and adenoviruses.

Certain coliphages have been proposed as indicators in recreational waters of the presence of fecal contamination which can include enteric pathogens. Coliphages, which are viruses or bacteriophages that infect *Escherichia coli*, have different shapes, sizes and genome

The authors declare no competing financial interest.

organization. Some coliphage groups have characteristics similar to enteric viruses; for example, male-specific RNA coliphages are similar in size and genome characteristics in that both are single-stranded RNA nonenveloped viruses, and their persistence in environmental waters is very similar.(5,6) Coliphages commonly model survival of enteric viruses in the environment(6,7) and are often more abundant. Their assays are also simple and inexpensive, compared to direct detection of human viral agents.

Inactivation processes that reduce concentrations of coliphage in recreational waters are poorly understood and, as with FIB indicators, differences in inactivation rates caused by various environmental stressors can complicate coliphage use in monitoring microbial water quality. Studies of sunlight inactivation of bacteria in natural waters date back to the 1800s(8) and show that photoinactivation occurs via a variety of pathways.(9) Inactivation of FIB and other indicators upon exposure to solar ultraviolet (UV) radiation is a key determinant of their densities in aquatic environments.(6,7,9–28) For example, sensitivity analysis using three-dimensional hydrodynamic and transport models indicates that, compared to all other loss processes, solar inactivation has the greatest impact on loss rates of *E. coli* at near-shore locations in southern Lake Michigan.(25) A recent review by Nelson et al. provides a wealth of information about sunlight-mediated inactivation of health-related microorganisms in natural and engineered systems.(29)

Two main mechanisms account for photoinactivation in natural waters: direct (endogenous) and indirect (exogenous). Endogenous inactivation can occur by direct damage to microbe nucleic acids from solar UV–B radiation (280–315 nm) or photo-oxidative damage to DNA and other cellular components sensitized by endogenous chromophores (light-absorbing molecules located in the cell). The exogenous pathway involves molecular oxygen. Other indirect pathways involving exogenous photosensitizers in natural waters are available, (21,23,26,30–32) but are not addressed here.

Empirical relationships are needed to quantify the effectiveness, or “weight”, of UV at causing inactivation of microorganisms in relation to wavelength. One approach to developing such relationships is to expose the organisms to narrow bands of radiation, that is, monochromatic light. The other is to use polychromatic light with filters to modify the radiation by blocking parts of the UV. This study used the polychromatic approach. The inactivating effects of light observed can be described in terms of biological weighting functions (BWFs) which quantify the “weight” of UV at causing inactivation relative to wavelength.(11,33,34) Pioneering studies in the use of biological weighting functions(11,34) to model photoinactivation rate constants of coliphages were presented by Fisher et al.(35) and Nguyen et al.(27) Their results are compared to ours later in this manuscript.

We aimed to determine and compare BWFs that quantify wavelength effects on direct photoinactivation of coliphages, and to better understand the role of viral characteristics and environmental changes in their UV sensitivity. BWFs are used in a photobiological model to evaluate effects of changes in location, time, and water depth on direct photoinactivation of these coliphages. Case studies from three Great Lakes beach sites further illustrate uses of the photobiological model. For our experiments, two individual coliphages were selected because of their differences in genome organization and size: phiX174, a circular single

stranded somatic DNA coliphage,(36) and MS2, a linear single-stranded male-specific RNA coliphage like enteroviruses and norovirus.(37) Both have capsids similar in size to enteric viruses such as enteroviruses and norovirus, between 25 and 27 nm in diameter.(38) Results are compared to two native isolates of somatic and male-specific coliphages obtained from effluents of an Ohio wastewater treatment facility.

Experimental Section

Microorganisms and Materials

Purified cultures of MS2 (ATCC 15597-B1) and PhiX174 (ATCC 13706-B1) were obtained from Michigan State University in concentrations of 3×10^9 and 2×10^8 plaque forming unit (PFU)/mL, respectively, and stored at -80°C . Native strains of both somatic and F+ (male-specific) coliphages were isolated from effluents of the Northeastern Ohio wastewater treatment plant (NE OH WWTP) samples, Cleveland, OH during the summer of 2015, using EPA Method 1602.(39) Native coliphage cultures were prepared by propagating the effluent-associated coliphage to a high titer using a serial double agar layer (DAL) procedure (EPA Method 1602). Two mL of effluent sample were inoculated onto the top agar containing *E. coli* CN13 or *E. coli* F_{amp} hosts to grow somatic and F+ coliphage, respectively. After the plaque assays, the coliphages were released from the agar by scraping off the top agar and resuspending them in tryptic soy broth (TSB). The DAL procedure was repeated to determine the coliphage titer on the TSB suspension until the maximum desired concentration was reached. To check results of the coliphage isolation procedure, the somatic native coliphage was run with the F_{amp} host, and the F+ native coliphage was run with the CN13 host. No detectable plaques were found in either case.

Native cultures were stored at -80°C in concentrations of 3×10^{10} and 9.4×10^{11} PFU/mL, respectively. *E. coli* hosts of F_{amp} and CN13 were prepared the day of the experiment, using 10 mL Tryptic Soy Broth, 100 μL of antibiotic (nalidixic acid for CN13/ampicillin for F_{amp}) and 100 μL of corresponding CN13 or F_{amp}, then grown to log phase in a shaking incubator. All other chemicals were used as supplied from Sigma-Aldrich (St. Louis, MO). All aqueous samples were prepared with water purified using a Millipore Milli-Q Gradient A10 system (18.0 M Ω).

Samples for the photoinactivation experiments were prepared in triplicate by diluting cultures of the coliphages by 1000-fold into 20 mL phosphate buffered water (PBW) in 25 mL quartz tubes with screw cap tops.

Coliphage Enumeration

MS2 and NE OH WWTP F+ coliphages, along with phiX174 and NE OH WWTP somatic coliphages, were plated for enumeration using the DAL method (EPA Method 1602). Briefly, the top agar was inoculated with log coliphage host bacteria (F_{amp} or CN12) and the environmental sample containing coliphages. Contents were mixed and added to a plate containing a thin layer of Tryptic Soy Agar, and incubated for 24 ± 2 h. After incubation, circular lysis zones (plaques) were counted and recorded as PFU/mL. Top agar inoculated

with PBW instead of the environmental sample, and plated using the same method, served as a negative control.

Photoinactivation Experiments

Photoinactivation experiments were conducted by irradiating the quartz tubes containing coliphage suspensions (initial concentrations shown in Supporting Information (SI) Table S1) in a Solar Light LS-1000 solar simulator equipped with a 1 kW xenon-arc lamp and UV-optical filters, with 50% cutoffs at 280, 295, 305, 320, and 345 nm that modified UV exposure during a series of experiments (see SI Figure S1 for UV transmission of the filters). Source of the filters was Newport Corp, Irvine, CA. Each organism/filter combination (4×5) was run in triplicate, for a total of 60 experiments. The 1.5 atm filter was not used to increase the irradiance at wavelengths <300 nm to facilitate measurements in this important spectral region. Spectral irradiance of the filtered light was measured using an Optronics OL 756 spectroradiometer, calibrated using an OL 756–150 Dual Calibration Source. Samples of each coliphage were prepared in PBW (pH 7.0) at concentrations shown in SI Table S1; the coliphage suspensions were added to 18 mm OD quartz tubes for each set of irradiations. The quartz tubes were kept at 20 °C by immersion in a thermostated water bath during the irradiations.

Sunlight studies at Athens GA were performed in the same manner as indoor experiments, with the exception of cutoff filters, as no modulation of the light was necessary. We chose days where there was minimal cloud cover ($<10\%$) and performed irradiations in 4 h increments for each coliphage (solar noon ± 2 h). Spectral irradiance of natural sunlight was measured from 240 to 800 nm using an Optronics OL 756 spectroradiometer. Since the coliphages were irradiated on separate days, we normalized all inactivation data to a single date for modeling calculations and comparison.

Modeling Procedures

Photoinactivation of viruses and bacteria can be modeled using kinetic and spectroscopic properties derived from laboratory and field studies. Water samples used for the depth studies were collected at three Great Lake recreational areas including Edgewater Beach on Lake Erie near Cleveland, OH; Grant Park on western Lake Michigan near South Milwaukee, WI; and Washington Park on southern Lake Michigan at Michigan City, IN. Methods used for obtaining the UV–visible absorption spectra of the samples have been described elsewhere.(40) We followed procedures described previously for using weighted irradiances to model photoreactions as a function of time and location.(33,40,41) These techniques modeled photoinactivation of bacterial indicators(28) and viruses.(27,32) The inactivating effects of light can be described in terms of BWFs, which are the photoinactivation rate constants related to the irradiance ($\text{W cm}^{-2} \text{ nm}^{-1}$) at wavelength λ . Spectral BWFs for a given virus or bacterium were determined by an approach that involves irradiation by a polychromatic light source with UV cutoff filters in place. To summarize, the rate constant of inactivation near the water surface is given by the following:

$$k_i = \int E_0(\lambda) \text{BWF}_\lambda d\lambda \quad (1)$$

where $E_0(\lambda)$ is the underwater scalar irradiance near the surface at wavelength λ ; BWF_λ is the biological weighting function (units of $\text{cm}^2 \text{J}^{-1}$) for the photoinactivation at wavelength λ ; and the integration is across the range of photobiologically effective wavelengths. Irradiance at the water surface can be simulated with models such as the Tropospheric Ultraviolet–Visible (TUV, version 5.3) model.(42)

Results and Discussion

Biological Weighting Functions (BWFs)

Kinetic studies conducted with each coliphage showed that photoinactivation could be described by first-order rate expressions, with up to 7 orders of magnitude decrease in enumerated coliphage densities. Thus, rate constants were independent of coliphage concentration (Figure 1). There was little evidence that deviation from first-order kinetics was caused by photorepair. This was expected with MS2 and phiX174 because they either do not undergo photorepair (MS2) or exhibit only minimal photorepair capability (phiX174). (38) Our results further show that coliphages isolated from the NE OH WWTP did not photorepair under these conditions. Therefore, photoinactivation data were represented by first-order rate constants (k_i, sec^{-1}) computed for each data set (Table 1). Our results agree with earlier results(26,32) that photoinactivation kinetics of two species of coliphages (MS2 and PhiX174) can be described by first-order rate expressions. We further show that photoinactivation of two coliphage isolates from the effluent of a wastewater treatment plant can also be described by first-order rate expressions that apply up to 7 orders of magnitude change in density.

We did not purify the stock samples of MS2 and phiX174 using the precipitation–centrifugation procedures described by others to remove any residual broth.(7,35) Adventitious impurities can affect photoreactions by acting as sensitizers,(7) but the rate constants of such indirect photoreactions are generally reduced by dilution. Direct photoreaction rate constants, by comparison, are little affected by dilution. Thus, we minimized impurity sensitization by diluting the stocks used here by 1000-fold. First order rate constants for the coliphage photoreactions in these diluted systems were not changed upon further dilution, indicating that broth-derived impurities did not affect our kinetic results (Figure 1B).

First-order rate constants for the four coliphages we studied increased substantially at wavelengths $<320 \text{ nm}$. (Figure 1): for example, reductions of the filter cutoff from 320 to 295 nm led to more than two-orders of magnitude increases in the rate constants. BWFs were computed using irradiance data and first-order rate constants observed with each of the five cutoff filters in place (Table 1). An experimental decay rate for Somatic coliphage under the 345 nm cutoff filter could not be determined.

Satisfactory fits that produce BWFs could be developed using exponential equations of the general form suggested by Rundel,(34) Cullen and Neale,(11) and Neale:(33)

$$BWF_\lambda = C \times e^{(a\lambda + b)} \quad (2)$$

where λ is the wavelength in nanometers, a and b are fitted parameters, and C is a scaling factor (arbitrarily set to 1) with units of $\text{cm}^2 \text{J}^{-1}$ where J is energy, measured in joules. As previously shown by Cullen and Neale,(11) including the scaling factor and integrating the product of BWF_λ and irradiance ($\text{W cm}^{-2} \text{nm}^{-1}$ or $\text{J s}^{-1} \text{cm}^{-2} \text{nm}^{-1}$) over wavelength (eq 1) produces the desired units of s^{-1} for the first-order rate constant. Since this form led to acceptable fits to observed decay rates, other mathematical functions were not investigated. To compute an estimated BWF, the Microsoft Excel “solver” package, implementing a generalized reduced gradient (GRG) algorithm, was used to find fitting coefficients that minimized the sum of squared errors of mean experimentally measured log10 decay rates versus modeled log10 decay rates across the five UV-cutoff filters. Each modeled decay rate was computed by summing $\text{BWF}_\lambda \times \text{irradiance}$ over λ from 280 to 800 nm. Confidence intervals for BWF curves (SI Figure S3) were determined using a Monte Carlo simulation of the effect of variability in experimentally measured decay rates (Table 1) on estimates of the a and b parameters in eq 2, as described in the SI.

Comparison of BWFs for the two species of coliphages and the somatic and F+ communities in our study showed that the shortest wavelengths of sunlight—UV-B radiation (280 nm–315 nm)—predominantly influenced their direct photoinactivation. MS2 had the lowest photoreactivity, while phiX174 had the highest; these observations are consistent with recent studies of coliphage photoreactivity.(26–28,31,32) The two coliphage strains isolated from NE OH WWTP exhibited intermediate photoreactivity, with the somatic strain being more photoreactive. When BWF_λ values for each coliphage were normalized to its BWF at 300 nm (Figure 2), we observed that, except for phiX174 > 310 nm, normalized BWFs were similar. This could be related to findings that inactivation kinetics often parallel the genome size and light absorption cross-section of viruses.(15,38) The normalized BWF_λ values for the native strains of somatic coliphages isolated from effluents of the Northeastern Ohio (NE OH WWTP) samples are compared to irradiance for mid-July at Washington Park Beach on Lake Michigan near Michigan City, IN in Figure 3. The BWF_λ increases significantly with shorter wavelengths in UV-B, while the irradiance decreases sharply. The product of the irradiance and normalized BWF values is referred to as the weighted irradiance for photoinactivation. For all phages included here, integration of the weighted irradiance over all solar wavelengths is greatest in the UV-B, and increases in the order of phage photoreactivity in sunlight: MS2 < NE Ohio F+ < NE Ohio somatic < phiX174. These results indicate that phage photoinactivation should be quite sensitive to environmental factors that influence underwater UV-B radiation.

Earlier studies using the viral genome characteristic to evaluate theoretical resistance of viruses to UV inactivation hypothesized that viruses with large genomes are more susceptible to UV inactivation than those with very small genomes.(15,40,43) Recent studies in the germicidal spectral region are consistent with this hypothesis and our study supports it in the solar spectral region. For example, the genome of phiX174 is larger than that of MS2, and phiX174 photolysis rate constants at 254 nm are larger than for MS2. Qiao et al.(2018) reported that photolysis rate constants observed for phiX174 with 254 nm radiation were 0.062 (Region A) and 0.074 (Region B), and for MS2 with 254 nm radiation were 0.011 (Region A) and 0.024 (Region B).(44) The rate ratio of phiX174 to MS2 in these Regions ranged from 3.1 to 5.6. The rate ratio estimated in this study under July sunlight at noon at

Washington Park, Lake Michigan was much higher, 9.2. The higher ratio is attributable in part to the spectral overlap of the BWFs for the coliphages with solar spectral irradiance. Photoinactivation of phiX174 in sunlight occurs at longer wavelengths than for MS2, as shown in Figure 2. Solar spectral irradiance in the UV-B sharply increases with increasing wavelength so the shift to longer wavelengths in the BWFs of phiX174, compared to MS2, results in a significant increase in the ratio of their solar rate constants, compared to the germicidal spectral region.

Modeling Potential Photoinactivation

As part of modeling efforts, we compared our results for MS2 and phiX174 to previous kinetic studies of these viruses. Detailed studies of Love et al.,(7) Fisher et al.,(35) and Nguyen et al.(27) of the endogenous inactivation rate of MS2 were particularly useful. Studies of Mattle et al.(31)and Kohn et al.(26) were also helpful because they included comparisons of the endogenous and exogenous inactivation kinetics of MS2 and phiX174 that are difficult to find. The BWF approach used here involves use of polychromatic radiation with a series of cutoff filters. The BWFs are more realistic and can be acquired much more rapidly than with monochromatic continuous irradiation. Only Nelson's group used the BWF approach used here to evaluate photoinactivation of MS2 (Fisher et al, 2011, Nguyen et al., 2014; Silverman et al, 2015). Others such as Love et al. (2010) and Kohn and Nelson (2007) provided useful data on MS2, phiX174 and other coliphages with a solar simulator or natural sunlight, but did not include the wavelength information needed to understand how solar disinfection of viruses varies with changing environmental conditions, such as evaluating changes in depth dependence resulting from changes in colored organic matter in water bodies, responses to ozone depletion, and other processes. Previous work using the BWF approach only focused on one coliphage, MS2, whereas this work provided additional results on phiX174 and two native isolates of somatic and male specific coliphages obtained from a wastewater treatment facility in the Great Lakes area. Although Fisher et al.(35)and Nguyen et al.(27)used the BWF approach, BWFs for MS2 derived from their study and ours differed somewhat in spectral distribution (Figure 4A), including a peak at about 380 nm that seemed particularly inconsistent with ours. In a paper by Nguyen et al. (27) the authors stated that the BWFs reported by Fisher et al.(35) needed to be re-examined. A subset of results of these studies is compared to our results in Figure 4B: our MS2 results, both modeled and observed in sunlight, agreed within a factor of 2 to those reported by Nguyen et al.(27) The modeled results of Love et al.,(7) Fisher et al.,(35) and Silverman et al.(32) were computed using BWFs from Fisher et al. in Figure 4a (red curve) and spectral irradiance measured by spectroradiometers or estimated by models. In addition, BWFs and modeled results for phiX174 and the native coliphages are presented elsewhere in this study. As expected, comparisons to results obtained with solar simulators varied. Solar simulators can approximate the spectra of solar irradiance, but there is no lamp/filter combination that exactly reproduces solar irradiance. Although kinetic results for MS2 from Mattle et al. (31)agreed closely with modeled and observed sunlight results obtained by Nguyen et al. (27) and with us, rate constants from Love et al.(7) were much larger. On the other hand, our modeled results for phiX174 rate constants in sunlight were about a factor of 2 larger than those observed by Mattle et al.(31) using a solar simulator. The results confirm that both our research as well as previous studies by those mentioned here have provided data and

procedures that can be used to effectively model inactivation of coliphages in aquatic environments, using biological weighting functions.

Our computations of the dependence of potential somatic coliphage photoinactivation on time and location (Figure 5) also agreed reasonably well with computed seasonal variations of MS2 by Nguyen et al.,(27) although MS2 is a male-specific RNA coliphage that is inactivated by somewhat shorter UV-B wavelengths than the somatic phage isolated from the NE OH WWTP. To better illustrate variability with latitude, our results were normalized to the maximum rate constant for all computations (mid-day at latitude 20°N during midsummer). The computed rate constants in Figure 5 are relative to rate constants at mid-day and midsummer and apply only to shallow depths.

Estimated effects of changes in atmospheric ozone on coliphage photoinactivation rates are illustrated in SI Figure S4. Weighted UV irradiances computed with the different BWFs of coliphages included in our study show coliphages respond similarly to changes in atmospheric ozone. A widely used measure of this dependence on ozone is the radiation amplification factor (RAF), which is defined by a power function:(42)

$$(UV_{int})_2/(UV_{int})_1 = [C(O_3)_1/(O_3)_2]^{RAF} \quad (3)$$

where $(UV_{int})_2$ and $(UV_{int})_1$ are the UV exposures corresponding to total ozone amounts $(O_3)_1$ and $(O_3)_2$, respectively. Our calculations indicate that photoinactivation of the MS2 coliphage is the most sensitive to ozone changes of coliphages studied here, with a RAF of ~0.5. By comparison, DNA damage computed using the standard DNA action spectrum of Setlow (Figure 2) has a RAF of ~2.2.

As part of modeling we also examined the depth dependence of direct coliphage inactivation in selected recreational waters of the Great Lakes. Irradiance as a function of depth was computed using Beer–Lambert’s law:

$$E_0(z, \lambda) = E_0(z, \lambda)e^{-K_d(\lambda)Z} \quad (4)$$

where $E_0(z, \lambda)$ is the irradiance at depth z and wavelength λ ($W\ cm^{-2}\ nm^{-1}$), and assuming the diffuse attenuation coefficient for downward irradiance (K_d) is close in value to that of absorption coefficients for the Great Lakes beach or tributary water collected at sites of interest. Available data for the UV-B radiation in some freshwater lakes and the coastal ocean indicate that its attenuation (measured as diffuse attenuation coefficients) is close in magnitude to absorption coefficients.(40,45) Publications show that suspended particulate matter can account for a significant fraction of UV absorbance (even at 305 nm) in the Great Lakes,(46) estuaries,(47) and other water bodies. Moreover, K_d depends not only on the light path length, but also on the extent of scattering. All these effects tend to make K_d higher than absorption coefficients. Inactivation rate constants were computed using eq 1. Results of modeling studies during July 2015 for swim areas of beach sites located at Washington Park (Michigan City, IN) illustrate our findings that there are significant differences in depth dependence of the four coliphage preparations examined in this work (Figure 6); also, see results shown in SI Figures S5 and S6 for beach sites located at Grant Park (South

Milwaukee, WI) and Edgewater Beach (Cleveland, OH). Comparisons of surface rates versus the average rate over a one-meter water column of each beach site indicated that light attenuation reduced direct photoinactivation most at Edgewater Beach (5.2-fold), followed by Grant Park (2.7-fold), then Washington Park (1.8-fold) (Figure 7). Modeling results further show that coliphages are strongly protected from photoinactivation at all tributary sites we examined, with the greatest protection in the tributary to Edgewater Beach, followed by tributaries to Grant Park and Washington Park, respectively; ratios of surface versus average in a one-meter water column were: Edgewater Beach, 25-fold; Grant Park, 18-fold; and Washington Park, 16-fold (Figure 7). The tributaries had much higher concentrations of chromophoric dissolved organic matter (CDOM) and, thus, much higher UV-B protective light absorption than beach waters.(40) This is consistent with other recent studies that found storm-related runoff from tributaries can increase the persistence (thus exposure densities) of viruses in near-coastal waters by protecting them from disinfection by sunlight.(40)

Additional work should be conducted to determine the nature and proportions of the WWTP male-specific (F+) isolates and how they were affected by photoinactivation. For example, Cole et al. (2003) developed techniques for analyzing subgroups and suggested this could be useful source-specific information.(48) RNA and DNA content could help explain observed differences in photoinactivation of the coliphages in this study. Other potential mechanisms for photoinactivation in recreational waters should be studied in the future. Photoinactivation sensitized by exogenous chromophores such as chromophoric dissolved organic matter(31,32) in recreational waters should be further quantified and results compared to the endogenous pathway studies here. Results from such studies could also be applied to improve the design and modeling of drinking water treatment processes that rely on solar disinfection.(49–51)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's (U.S. EPA) peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use by the U.S. EPA. We gratefully acknowledge the editorial assistance of F. Rauschenberg in preparation of this paper and the assistance of J. Kinzelman (City of Racine Health Department, Racine, WI), M. Citriglia (Northeast Ohio Regional Sewer District, Cuyahoga Heights, OH), and Fu-Chih Hsu (Scientific Methods, Inc.) with water sampling at the Great Lakes beach and tributary sites.

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Figure 1.

(A) First-order rate constants ($k_i \times 10^3, s^{-1}$) for photoinactivation observed with radiation in a solar simulator filtered by various UV-cutoff filters (see the Experimental Section and the Supporting Information): blue circles, phiX174; green circles, NE OH somatic; red circles, NE OH F+; black circles, MS2; (B) First order rate constants with 305 nm cutoff filter for the photoinactivation of phiX174 in samples used in this study. There was no statistically significant change in the first order rate constants with dilution by PBW, indicating that residual broth was not sensitizing phage photoinactivation.

Figure 2. Biological weighting functions for photoinactivation of coliphages normalized to unity at 300 nm, compared to the normalized DNA action spectrum (Setlow 1974).(52) Plotted values are for MS2 (black line), NE OH F+ (red), NE OH somatic (green), phiX174 (blue) and Setlow DNA action spectrum (cyan).

Figure 3. Comparison of the BWF (black line, normalized to its value at 300 nm) for somatic coliphages isolated from a Northeast Ohio wastewater treatment plant and near-surface solar spectral irradiance during mid-July at Washington Park Beach (red line). Weighted irradiance (dashed green line, normalized to its maximum value) peaked at about 308 nm.

Figure 4.

Comparison of inactivation rate constants for MS2: A) BWFs for MS2 photoinactivation determined by Fisher et al.(35) (red line) and this study (black line); B) photo inactivation rate constants (k_i) for MS2 estimated using BWF_λ and solar spectral irradiance (black bars) and observed (red bars) in either solar simulator or natural sunlight as noted above. Data are derived from Love et al.,(7) Fisher et al.,(35) Silverman et al.,(32) Nguyen et al.,(27) and this study.

Figure 5. Potential latitudinal variation in diurnal photoinactivation rate constants for somatic coliphage isolated from a Northeast Ohio wastewater treatment plant. Latitude 20°N (black); latitude 40° N (red); latitude 60° N (blue).

Figure 6.

Simulated depth dependence for direct photoinactivation of coliphages at mid-day, July 15, 2015 in beach water located in Washington Park (Michigan City, IN). MS2 (filled black circles); NE OH F+ (red); NE OH somatic (green); phiX174 (blue). Dashed lines are 95% confidence intervals based on a Monte Carlo simulation. Average $k_i \text{ (s}^{-1}\text{)}$ over a one-meter water column: MS2 (0.139×10^{-4}); NE Ohio F+ (0.408×10^{-4}); NE Ohio Somatic (0.846×10^{-4}); phiX174 (1.33×10^{-4}).

Figure 7. Simulated depth dependence of coliphage photoinactivation in beach waters and their tributaries in the Great Lakes. Bars represent ratios of photoinactivation rates at the surface to rates averaged over a one-meter deep water column: A) ratios in swimmable areas of beaches located at Edgewater Park in Lake Erie near Cleveland OH (red); Grant Park (South Milwaukee, WI) on Lake Michigan (green), and Washington Park (Michigan City, IN) on Lake Michigan (blue). B) ratios in tributaries of beaches located at Edgewater Park (red); Grant Park (green); and Washington Park (blue). The much larger bars for the tributaries indicates the higher levels of UV protection provided to coliphages and other microorganisms in these systems.(40)

Table 1.

Experimentally-Determined Mean Decay Rate Constants (k_i , sec^{-1}) for Coliphage Using Five Different UV-Cutoff Filters^a

cutoff filter					
coliphage	280	295	305	320	345
MS2	7.262×10^{-04} (2.366×10^{-05})	3.908×10^{-04} (1.883×10^{-05})	3.191×10^{-04} (1.376×10^{-05})	5.093×10^{-06} (1.604×10^{-07})	1.713×10^{-06} (5.973×10^{-07})
phiX174	2.135×10^{-03} (1.955×10^{-04})	1.194×10^{-03} (6.293×10^{-05})	7.098×10^{-04} (1.543×10^{-05})	1.701×10^{-04} (6.067×10^{-06})	1.616×10^{-05} (1.744×10^{-06})
NE OH somatic	2.091×10^{-03} (1.288×10^{-04})	1.211×10^{-03} (2.064×10^{-05})	4.112×10^{-04} (1.662×10^{-05})	8.243×10^{-05} (1.398×10^{-05})	
NE OH F+	1.454×10^{-03} (5.640×10^{-05})	6.757×10^{-04} (6.177×10^{-05})	3.648×10^{-04} (4.999×10^{-06})	3.248×10^{-05} (2.226×10^{-06})	4.667×10^{-06} (2.566×10^{-06})

^aStandard deviations given in parentheses. These values are based on three experimental replicates for each organism/cutoff filter combination.