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This study presents evidence of nuisance algae commonly found in public beach waters providing UV protection to *E. coli* and *Salmonella*.

Nuisance algae provide UV protection to bacteria present in public beach waters

This study has shown that nuisance filamentous algae species commonly found in surface waters would provide UV protection to bacteria present in same environmental settings. Specifically, *Cladophora* spp., causing undesired water quality issues in Great Lakes region were found to act as a protective habitat for *E. coli* and *Salmonella* against the disinfective properties of natural UV radiation. These findings indicate both organisms might utilize algae as a niche and pose additional public health related risks, as pathogenic bacteria such as *Salmonella* is not routinely sampled for water quality monitoring as often as *E. coli*.

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ARTICLE

Association of nuisance filamentous algae *Cladophora spp.* with *E. coli* and *Salmonella* in public beach waters: Impacts of UV protection on bacterial survival

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This study investigated whether filamentous algae species commonly found in nearshore public beach water systems provide protection from natural UV to bacteria present in the same environmental settings. To test this hypothesis, *Cladophora spp.*, a filamentous nuisance algae group causing undesired water quality in Great Lakes region were selected and its interactions with non-pathogenic indicator organism *Escherichia coli* and a pathogenic strain of *Salmonella enterica* serovar Typhimurium were tested. In laboratory microcosms where lake environment and natural sunlight conditions were simulated, a 7-log removal of *E. coli* was observed in only six hours of exposure to UV with an initial seed concentration of 10^3 CFU/mL. With the presence of algae, the same log removal was achieved in 16 hours. At higher seed concentrations of 10^5 CFU/mL, *E. coli* survived for two days with an extended survival up to 11 days in the presence of *Cladophora spp. S. typhimurium* has shown more resilient survival profiles, with same log removals achieved in 14 and 20 days for low and high seed concentrations respectively, in the absence of algae. *Cladophora spp.* caused extended protection for *S. typhimurium* with much less log reductions reported. Algae-mediated protection from UV irradiation was attributed to certain organic carbon exuded from *Cladophora spp.* In addition, confocal microscopy images confirmed close interaction between bacteria and algae, more prominent with thin filamentous *Cladophora spp*.

Introduction

Nuisance algae growth in many freshwater lakes impairs beach water quality and often causes undesired aesthetics and odor issues. One particular example is *Cladophora spp.,* a filamentous, benthic green alga commonly found in eutrophication dominant marine and freshwater systems. For several decades, *Cladophora spp.* have been documented in Great Lakes region, mainly in nearshore zones of Lakes Erie, Ontario, and Michigan.[1-4] *Cladophora* usually starts growing on rocks and hard surfaces in the nearshore environment, detaching upon maturity and accumulating as floating mats at the water surface, with frequent wash-up ashore in mid- to late summer.[5] Accumulated algae threaten water quality and recreational activities along Lake Erie, Lake Ontario, and the Niagara River that connects them, all crucial components of the region's economic infrastructure.[4]

In addition to hindered recreational experiences and aesthetic concerns, *Cladophora* blooms pose public health associated risks as they may harbor varying bacterial communities. In fact, several studies have documented *Cladophora* mats containing fecal indicator bacteria (FIB), such as *E. coli* and enterococci, as well as human pathogens including *Campylobacter*, *Shigella*, *Salmonella*, and *Clostridium*.[6-9] Recently, several groups reported counts of

FIB exceeding 10^5 colonies per dry weight of algal biomass.[10-14] These observations suggest that *Cladophora* blooms can provide an environment that allows bacteria to proliferate, effectively acting as reservoirs for potentially harmful microorganisms. In fact, there has been some evidence into the role of *Cladophora* in providing a matrix for bacterial attachment and growth and reported elevated populations of indicator bacteria such as *E. coli* and other pathogenic coliform bacteria associated with attached or floating masses of *Cladophora* biomass.[5,7]

These studies suggest that a possible significant portion of this ecological association is from the production of organic photosynthetic exudates from the algae that provide a source of nutrients for the bacterial populations.[15,16] High cell counts of microbes are regularly found as epiphytic populations with *Cladophora* filaments *in situ,*[6,17] which seem to indicate a close physical association that minimizes diffusive transport limitations. Other mechanisms of association, however, such as protection of microbes from ultraviolet spectra in sunlight or increase in local temperatures, may contribute to elevated survival and growth of viable microbial cells.[7,18] The relative contribution of each of these mechanisms, however, has not been thoroughly explored and warrants an investigation into the underlying ecological associative relationships that govern the persistence of microbial populations in benthic algae.

As quite a number of studies identified the presence of both FIB and pathogenic organisms within *Cladophora*, there is also growing interest to investigate the algae-bacteria interactions for public health implications. More studies are needed to understand the effects of environmental conditions on the growth and decay rates of bacteria in association with *Cladophora* and shed light on related risks for both humans and wildlife due to nuisance algae present in freshwater systems.

The overall goal of this study was to examine the association between *Cladophora* and dynamics of bacterial survival. *Cladophora* samples collected from the waterways of the Lake Erie were seeded with varying concentrations of *E. coli* and *S. typhimurium*. Laboratory microcosms were designed to investigate whether *Cladophora* provided protection against UV irradiation under conditions that simulate the natural sunlight. The objective was to determine if nuisance algae present in surface freshwaters would extend the survival of bacterial populations and potentially increase the health risks associated with pathogens. Further water quality and nutrient characterizations were completed to understand the role of organic exudes from algae that would affect bacterial growth. Finally, high resolution confocal microscopy images were taken to corroborate the observed trends with spatial interaction dynamics between algae and bacteria.

Materials and methods

Algae growth and maintenance

Cladophora spp. used in this study were collected from Beaver Island Beach State Park located on Niagara River end of Lake Erie (Grand Island, NY) in August 2011. The algae were cultivated in the laboratory under ambient conditions using a 0.75 m^2 recirculating reactor for benthic algal cultivation following the methods as described in detail elsewhere.[19] Briefly, photosynthetic light was provided by three 150W growth lamps (Type S55, Sunlight Supply Company, Vancouver, WA) providing an average light intensity of 31500 lux, set 0.3 m above the growth substratum. The algae were supplied with a chemical nutrient solution (Miracle Gro, Scotts, Marysville, OH) and Lake Erie Water, at an overall nitrogen loading rate of 0.5 g N m^{-2} d^{-1}.

Bacterial strains, growth, and preparation

The *E. coli* bacteria used in this study was originally isolated from a swine lagoon located near Western Kentucky University Campus[20] and its identity was confirmed by BOX-PCR analysis as described elsewhere.[21] The *Salmonella* strain (designated as *Salmonella enterica* serovar Typhimurium ST5383) was obtained from the Salmonella Genetic Stock Centre (SGSC) of University of Calgary in Alberta, Canada. Prior to the day of experimentation, both *S. typhimurium* and *E. coli* cells were pre-cultured in Luria-Bertani (LB) broth at 37 ^oC overnight, shaken continuously at 100 rpm (Symphony M5001, VWR, Radnor, PA). On the day of experiment, cells were transferred to fresh LB medium and incubated until cells reached mid-exponential phase (3 and 2.5 hours for *E. coli* and *S. typhimurium,* respectively). Cells were centrifuged at 3600 × *g* force for 15 minutes at 4 ^oC (Model 5804R, Eppendorf, Hamburg, Germany). Waste growth medium was decanted and replaced with 10 mL of pre-sterilized 0.01M KCl solution. Following three repeated washing steps, the final cell pellet was resuspended in 2 mL of 0.01M KCl solution. The concentration of cell stock was determined using a hemocytometer (Burker-Turk, Germany) and visualized under a standard light microscope (Fisher Scientific, Atlanta, GA).

Experimental microcosms

All experiments were conducted in triplicate microcosms each containing 400 mL of filter-sterilized Lake Erie water. Microcosms were placed in a circulated water bath with temperature kept at 21 ^oC and agitated mildly to simulate lake environment. In order to generate similar UV irradiation conditions of the natural sunlight reaching earth surface, a non-germicidal, natural UVA/UVB (90/10) bulb (32W, ReptiSun, Zoo Med, San Luis Obispo, CA) was used for UV exposure analyses. An additional standard

fluorescent bulb (32W Cool White, Philips, Netherlands) was utilized for algal photosynthesis. The light fixtures were placed 12 cm above the microcosms to match natural UV irradiation of Western New York, *i.e.* 0.8 mW/cm^2 on average in summer months, measured and confirmed with a UV light meter (VWR). All light fixtures were scheduled to operate for 14/10 hours of light/dark cycles.

In each batch, three experimental conditions were tested: control (algae only), algae plus bacteria, and bacteria only. For the microcosms containing algae, the amount of algae introduced at the beginning of experiments was kept constant (5.00 ± 0.30 g wet weight, 0.12 ± 0.09 g dry weight), determined gravimetrically by Standard Methods (protocol 2540B).[22] To examine the effect of bacterial concentration, 10^3 and 10^5 cells per mL of *E. coli* and *S. typhimurium* were inoculated separately for each bacteria containing microcosm. In order to determine background survival of non-algae associated bacteria in lake water, separate bacteria only control microcosms were set-up under identical conditions, except exposure to UV irradiation.

Sampling for bacterial survival

Each microcosm was sampled once a day (except the batch inoculated with 10^3 *E. coli* cells per mL which was sampled three times a day due to rapid decline in bacterial count). From each microcosm, 20 mL of sample was collected and filtered through Whatman 4 filter (pore size of 20-25 µm) with successive washing using 20 mL of sterilized deionized (DI) water. The purpose of this initial filtering was to remove algal biomass and elute attached bacteria. Following, the filtrate was serially diluted and passed through 0.44 µm nitrocellulose membranes using a vacuum filtration system. Membranes were aseptically transferred onto selective culture media, *i.e.* modified mTEC and Brilliant Green agar (BD Biosciences, San Jose, CA), for *E. coli* and *S. typhimurium* respectively.[23] All plates were incubated overnight at 37 ºC and colony forming units (CFUs) were enumerated. Initial seed cultures of 10^3 and 10^5 cells per mL of *E. coli* and *S. typhimurium* were also serially diluted and plated on respective agar plates and reported as CFU/mL.

Visualization of algae and bacteria

Plasmid coding for an enhanced green fluorescent protein (GFP) and ampicillin resistance was introduced to same native *E. coli* and *S. typhimurium* strains used in the study via standard electroporation protocols[24], and cultured as described above with the only exception that the growth media was supplemented with 1 mg/L of ampicillin to ensure the selection for cells containing the GFP plasmid.

The interaction of GFP-labeled *E. coli* and *S. typhimurium* with *Cladophora spp.* were visualized by using a Zeiss LSM 710 Axio Observer confocal microscope (Carl Zeiss Microscopy, Germany), under 60× Plan-Apochromat objective, equipped with GFP filter and motorized x,y,z stage. Images were acquired and processed with Zeiss Zen 1 (Carl Zeiss Microscopy) and Fiji[25] image processing software.

Water characterization

Lake water from algae-only microcosms were tested for turbidity, pH, conductivity, dissolved oxygen (DO), hardness, total nitrogen (TN) as nitrate-N, total phosphorus (TP) as phosphate-P, total dissolved solids (TDS), and dissolved organic carbon (DOC) at the end of 5, 10, 15, and 20 days. Water samples from Lake Erie not associated with algae were also tested for reference value comparison. Turbidity measurements were obtained with a nephelometer (TN-100, Oakton Turbidimeter, Vernon Hills, IL). Conductivity, pH, and DO were measured using probes connected to Symphony Meter (B40CID, VWR). Hardness levels were determined by using total hardness test kits (Hach Company, Loveland, CO). Total nitrogen was determined by nitrate test kits (The Nitrate Elimination Company, Lake Linden, MI) and total phosphorus was determined by colorimetric phosphate assay kits (Biovision, Milpitas, CA) following manufacturers' instructions.

Total dissolved solids were measured according to Standard Methods protocol 2540B with slight modifications.[22] TDS were obtained by passing 60 mL of sample through a vacuum filtration system using 0.44 µm glass microfiber filters (VWR). Following filtration, eluted samples were collected on pre-weighed evaporating dishes. Samples were placed in a drying oven held at 180 °C for 24 hours. Following, dishes were kept in a desiccator for 2 hours and re-weighed. Dissolved organic carbon was measured according to Standard Methods protocol 5310B and adapted according to the Dohrmann Carbon Analyzer (Teledyne Tekmar, Mason, OH) attached to a gas analyzer (PIR-2000, Horiba, Clifton Park, NY).

Results and discussion

Role of algal protection from UV exposure

One of the initial goals of the study was to investigate whether *Cladophora spp.* would protect *E. coli* from natural UV and affect its survival in lake water. In order to test the hypothesis, three sets of microcosms (with individual triplicates) were set-up, where each microcosm contained only algae as control, algae seeded with *E. coli* and only *E. coli* without algal presence (Fig. 1A). For the initial batch, 10^3 CFU/mL *E. coli* was selected as seeding amount to represent often encountered FIB concentrations in Lake Erie and other Great Lakes.[26,27] In microcosms where only *E. coli* was present, a rapid decay was observed corresponding to 7-log removal in only six hours of exposure to UV. However,

in microcosms where algae were present in addition to *E. coli*, the same log removal was achieved in 16 hours of exposure to UV (Fig. 1A). This observation has indicated a clear extension of survival when the *E. coli* was in association with algae. Expectedly, no *E. coli* was observed in algae only control microcosms during the course of the experiment (Fig. 1A).

Upon the conferral of the hypothesis that being in association with algae increase the survival of *E. coli* under UV exposure, the second batch of microcosms were set-up similar to as described above, however seeded with *S. typhimurium* instead of *E. coli* (Fig. 1B). The rationale was to test the same hypothesis with an additional organism, bearing conceded pathogenic characteristics.[28] In order to compare the results, 10^3 CFU/mL of *S. typhimurium* was used as initial seed concentration. As can be seen in Fig. 1B, when only *S. typhimurium* was present, there was an initial rapid decay of 2-log removal by the end of 24-hours of exposure to UV. Later on, approximately 10 CFU/mL of *S. typhimurium* survived for another week of UV exposure till Day 8. This prolonged survival suggested that the *S. typhimurium* might possibly get acclimated to UV. This rather unusual behavior was also observed by others as UVA and photosynthetic radiation may create counter inhibitory effects in bacteria.[1,29,30] At the end of eighth day, approximately another 1.5-log removal was observed followed by a second prolonged survival trend that lasted relatively shorter until Day 12. At that point, remaining *S. typhimurium* concentration was less than 1 CFU/mL, which were completely dead by Day 14 (Fig 1B). Surprisingly, in microcosms where *S. typhimurium* was in association with algae, there was very minimal decay. Specifically, at the end of the experimental duration of 15 days, approximately 200 CFU/mL of *S. typhimurium* remained viable (Fig 1B). This trend was attributed to a stronger association of *S. typhimurium* with *Cladophora spp.* compared to *E. coli* when seeded with lower concentration, *i.e.* 10^3 CFU/mL compared to 10^5 CFU/mL (Fig. 1D).

[INSERT FIGURE 1 HERE]

As presented in Fig. 1A-B, both *E. coli* and *S. typhimurium* survived longer under UV exposure when they were associated with *Cladophora* compared to bacteria only microcosms. In order to investigate whether the observed trend was dependent on initial seed concentration of bacteria, *E. coli* and *S. typhimurium* inoculations were increased to 10^5 CFU/mL for the next two batches (Fig. 1C and Fig. 1D respectively). The rationale for increasing the seed concentration was to investigate the possible effects of extreme events such as sewer overflows, flooding, and wildlife run-off.

Similar to previous batches, in microcosms where only *E. coli* was present, a fast decay rate was observed with more than 4-log removal at the end of first day of UV exposure (Fig. 1C). The remaining *E. coli* died completely by the end of Day 2. The increase in the seed concentration from 10^3 to 10^5 CFU/mL resulted in approximately 40 hours of extended survival in *E. coli* only microcosms. However, *E. coli* in association with algae showed a relatively slower decay rate, where a 5-log removal was achieved in 10 days. At the end of experimental period of 11 days, all *E. coli* had decayed in all microcosms except one in which less than 1 CFU/mL was recorded. As shown in Fig. 1C, algal presence extended the survival of *E. coli* and the effects of increased seed concentration was more evident with respected to prolonged survival of *E. coli*. Under the identical UV exposure conditions, microcosms inoculated with 10^5 CFU/mL of *S. typhimurium* resulted in longer survival durations confirming the effect of seed concentration increase (Fig. 1D). When compared to 10^3 CFU/mL of *S. typhimurium* only microcosms, 3- to 5-log removal was observed in the first couple of days of UV exposure followed by relatively stable survival (similar UV-acclimation effect) until Day 18, followed by complete removal by Day 20 (Fig. 1D). Expectedly, in microcosms where *Cladophora spp.* were inoculated with 10^5 CFU/mL of *S. typhimurium*, the decay rate was decreased compared to *S. typhimurium* only microcosms and less than 3-log removal was observed by the end of Day 4. For the following week of UV exposure, the concentration of recovered *S. typhimurium* was stable until Day 11, when an additional 2-log removal was recorded. In the remaining nine days of UV-exposure, a slight re-growth from 2 to 20 CFU/mL was observed (Fig. 1D). This was rather unexpected, as no re-growth was reported in other microcosms, and pursued with additional experiments and discussed further below.

[INSERT FIGURE 2 HERE]

Considering the fact that the microcosms were exposed to UV irradiation similar to natural sunlight, any possible background growth of bacteria would be harder to detect due to disinfectant properties of natural UV.[31-34] Therefore, four additional microcosms were set-up and separately seeded with 10^3 and 10^5 CFU/mL of *S. typhimurium* and *E. coli.* The microcosms were kept at identical conditions except they were exposed to ambient indoor light conditions rather than UV irradiation. No algae were added to microcosms. Without algal presence and UV irradiation, both bacteria showed similar survival profiles with less then 2-log reduction at the end of 20 days in lake water (Fig. 2). It is important to highlight that these decay rates were similar at both 10^3 and 10^5 CFU/mL inoculations, suggesting that the effects of initial seed concentration was only evident if the bacteria were associated with algae (Fig 1). Another noticeable difference was *E. coli* showed slightly more perseverance in survival compared to *S. typhimurium*, especially during the first week in lake water (Fig. 2). Comparable results were obtained by other groups and *E.*

coli was reported to persist longer than *S. typhimurium* in similar laboratory conditions.[35]

At the end of the first phase of the study, *Cladophora spp.* were clearly shown to provide protection from UV to both *E. coli* and *S. typhimurium* at low and high initial seed concentrations of 10^3 and 10^5 CFU/mL (Fig 1). In the presence of algae, *S. typhimurium* not only showed better survival but also acclimation to UV as indicated by relatively stable and slower decay rates compared to *E. coli* (Fig. 1). Despite some variations that existed among microcosms, without algal presence and UV irradiation, there was no major background growth of bacteria kept in lake water under same experimental conditions tested and discussed earlier (Fig. 2).

Role of nutrients and water quality characteristics

Dissolved organic matter (DOM) under UV irradiation has been shown to impact bacterial survival in surface water systems.[36-40] As the contribution of algal primary production to DOM pool in the form DOC is highly variable,[36] it was essential to determine whether *Cladophora spp.* would exude any organic material to and/or modulate the availability of macronutrients in microcosms. Although a comprehensive documentation of photochemical transformation of organic matter into low-molecular weight compounds would be out of the scope and studied well in detail by others[41-46], major water quality characteristics (TDS, DO, turbidity, pH, hardness, and conductivity), and nutrients (DOC, TN, and TP) that would possibly impact survival of the bacteria in association with algae were determined and reported in Table 1.

[INSERT TABLE 1 HERE]

Initial nutrient and water quality characterization was conducted with samples collected from Lake Erie that were used in all experimental microcosms in order to generate the background profile and reference values for comparison. As can be seen in Table 1, all Lake Erie water quality parameter values obtained were similar to those of (east basin-nearshore) continuously monitored by U.S. EPA and other agencies.[47] Amounts of TDS and DOC were approximately 300 and 6 mg/L respectively and comparable to long-term water quality monitoring and modeling reports on Lake Erie.[48,49] The amount of TP was below detection limit (typically 0.2-0.8 μM) and the amount of TN was 9.7 μM (typically 12-16 μM).[50] The background profile of Lake Erie water quality and nutrient characterization was linked to data presented in Fig. 2 and corroborated with no major re-growth of bacteria under the experimental conditions tested. Consequently, to determine any possible organic exude and/or nutrient uptake by algae, the next set of samples were collected from algae-only microcosms kept under UV exposure (as discussed in previous section and presented in Fig. 1) on days 5, 10, 15, and 20. This sampling scheme represented the association of *Chladophora spp.* with bacteria during the full course of the study (Table 1).

With respect to water quality parameters, measured values in algae associated microcosms were generally in agreement with reference samples from Lake Erie. Total dissolved solids concentrations increased from 200 to 320 mg/L in days 5 and 10, followed by fluctuating concentrations measured at 243 and 265 mg/L in days 15 and 20, respectively. Compared to Lake Erie reference TDS concentration of 300 mg/L, it was safe to conclude that there were no major changes in TDS balance due to algal presence. Similarly, no noticeable changes were reported in hardness and conductivity compared to typical lake water (Table 1). There was a slight increase in pH from 8.2 to 9.9-10.6 ranges, attributed to the inorganic carbon uptake and photosynthetic activity by algae.[51,52] This is not uncommon due to natural diurnal cycle of photosynthesis and reported in *Cladophora* previously.[53] Minor increases in DO from 9.6 mg/L to 9.8-10.5 mg/L levels also confirmed the observed trend with indication of photosynthetic activity. Natural filamentous and dense morphology of *Cladophora spp.* increased the measured turbidity of ambient lake water from 0.02 to 135-187 NTU levels. Due to an incidental agitation issue in the one of the microcosms sampled on Day 10, an increased mean turbidity of 483 NTU was observed creating a temporary outlier, which was resolved by Day 15 and Day 20 (Table 1).

In terms of nutrient characterization, the amount of TN decreased in the algae-containing microcosms to as low as 1.9 μM compared to ambient lake water reference values as discussed above and presented in Table 1. This was rather expected, as the only available nitrogen source for algae was present in lake water since no additional nitrogen was supplied to microcosms. Although a similar trend was expected for TP, it was below detection levels for multiple trials in reference lake water samples. Nevertheless, based on approximate TP levels reported in Lake Erie basin (as given above) the concentration of TP was generally in agreement, and decreased slightly from Day 5 to Day 20 all within experimental variation (Table 1).

In this set of the experiments, it was observed that DOC concentrations substantially increased from 5.7 mg/L to 24 mg/L between reference lake water samples and samples from the microcosms in which *Cladophora* was present for 5 days. Following a continuous gradual increase, approximately 33, 38, and 39 mg/L of DOC was recorded in samples collected on days 10, 15, and 20 respectively (Table 1). This clear trend confirmed that dissolved organic carbon was exuded from algae into the microcosms and became available to bacteria. As discussed in earlier sections, the availability of additional DOC might have resulted in the slight re-growth of *S. typhimurium* as shown in Fig. 1D.

Although bacterial re-growth was not observed in other microcosms, the extended survival of both *E. coli* and *S. typhimurium* when associated with algae might be attributed to available DOC in addition to protection from UV exposure. A recent study reported evidential results as beaches with higher organic matter had higher concentrations of bacteria compared to beaches with low organic matter levels.[54] Parallel results extensively confirmed *Cladophora spp.* providing a niche by minimizing the effects of environmental stress on enteric bacteria found in freshwater systems.[6,11,12,14,26,35]

Extent of algae-bacteria association

The final goal of the study was to investigate the structural and spatial interactions of bacteria with algae in order to complement the observed trends in bacterial survival under UV exposure when algae was present. For this purpose, the exact strains of *E. coli* and *S. typhimurium* were GFP-labeled and subjected to identical conditions (UV exposure and algal presence) as described in earlier sections and visualized with confocal microscopy (Fig. 3).

[INSERT FIGURE 3 HERE]

Close-up micrographs of confocal imaging have shown that both bacteria were found to be more associated with thin filamentous *Cladophora spp.* such as *Cladophora liniformis* (Fig. 3A-B) rather than thicker filamentous species such as *Cladophora fracta* (Fig. 3C) compared to random dispersion in bulk fluid (Fig 3 A-C). The composite image shown in Fig. 3B also indicated a biofilm type structure which might cause the increased interaction. This was rather anecdotal as it was not fully clear whether the biofilm was formed by bacteria or *Cladophora liniformis*.

Finally, higher magnification micrographs were taken to visualize if either bacteria would be able to penetrate into and/or be internalized by algae as a protection mechanism against UV. As clearly seen in Fig. 3C at high resolution, and in much greater detail in Fig. 3D, the bacteria were not able to penetrate nor were taken up by algae, ruling out the possibility. Nevertheless, there was convincing evidence that thin filamentous *Cladophora spp.* were hosting more bacteria and showed increased association, which likely contributed to protection of bacteria from UV exposure.

Conclusions

In this study, the association of *Cladophora spp.* with FIB, specifically *E. coli* was investigated to determine whether this nuisance algae commonly found in Lake Erie would become a barrier against the natural UV irradiation. The results indicated that *E. coli* in fact survived for extended periods in lake water when associated with algae, confirming that *Cladophora spp.* act as a protective host from the disinfectant properties of UV against small sized bacteria.

Under identical tested conditions, *S. typhimurium* was also found to survive for much longer periods under UV exposure if associated with algae. Differing from *E. coli*, certain re-growth of *S. typhimurium* was observed suggesting that *Cladophora spp.* not only protects from UV but also may serve as a reservoir for this resilient pathogen.

Although *S. typhimurium* is not among the indicator organisms routinely sampled for water quality monitoring purposes by public authorities, it is often encountered in Great Lakes region. Considering the findings of this study, certain public health related risks might increase upon exposure to *Cladophora spp.*, especially for swimmers, children, and immunocompromised individuals. Finally, more risk assessment studies are recommended for pathogens like *S. typhimurium* in addition to routine FIB monitored in Lake Erie and other Great Lakes. Nuisance algae growth in many freshwater lakes impairs beach water quality and often causes undesired aesthetics and odor issues.

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Notes

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References

1. R. P. Herbst, *American Midland Naturalist*, 1969, 90-98.
2. M. T. Auer, L. M. Tomlinson, S. N. Higgins, S. Y. Malkin, E. T. Howell and H. A. Bootsma, *Journal of Great Lakes Research*, 2010, **36**, 248-255.
3. M. T. Auer, J. M. Graham, L. E. Graham and J. A. Kranzfelder, in *Periphyton of freshwater ecosystems*, Springer, Editon edn., 1983, pp. 135-145.
4. S. N. Higgins, E. Todd Howell, R. E. Hecky, S. J. Guildford and R. E. Smith, *Journal of Great Lakes Research*, 2005, **31**, 547-563.
5. S. N. Higgins, S. Y. Malkin, E. Todd Howell, S. J. Guildford, L. Campbell, V. Hiriart□Baer and R. E. Hecky, *Journal of Phycology*, 2008, **44**, 839-854.

6. R. L. Whitman, D. A. Shively, H. Pawlik, M. B. Nevers and M. N. Byappanahalli, *Applied and Environmental Microbiology*, 2003, **69**, 4714-4719.
7. S. Ishii, T. Yan, D. A. Shively, M. N. Byappanahalli, R. L. Whitman and M. J. Sadowsky, *Applied and environmental microbiology*, 2006, **72**, 4545-4553.
8. E. T. Englebert, C. McDermott and G. T. Kleinheinz, *Science of the Total Environment*, 2008, **404**, 10-17.
9. G. Kleinheinz, A. Coenan, T. Zehms, J. Preedit, M.-C. Leewis, D. Becker and C. McDermott, *Lake and Reservoir Management*, 2009, **25**, 149-154.
10. B. D. Badgley, F. I. Thomas and V. J. Harwood, *Environmental microbiology*, 2011, **13**, 932-942.
11. M. N. Byappanahalli, R. L. Whitman, D. A. Shively, J. Ferguson, S. Ishii and M. J. Sadowsky, *Water research*, 2007, **41**, 3649-3654.
12. O. A. Olapade, M. M. Depas, E. T. Jensen and S. L. McLellan, *Applied and environmental microbiology*, 2006, **72**, 1932-1938.
13. A. Vanden Heuvel, C. McDermott, R. Pillsbury, T. Sandrin, J. Kinzelman, J. Ferguson, M. Sadowsky, M. Byappanahalli, R. Whitman and G. T. Kleinheinz, *Journal of environmental quality*, 2010, **39**, 333-344.
14. W. B. Ksoll, S. Ishii, M. J. Sadowsky and R. E. Hicks, *Applied and environmental microbiology*, 2007, **73**, 3771-3778.
15. N. Malinsky-Rushansky and C. Legrand, *Oceanographic Literature Review*, 1996, **43**.
16. I. Valiela, J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh and K. Foreman, *Limnology and Oceanography*, 1997, **42**, 1105-1118.
17. M. N. Byappanahalli, D. A. Shively, M. B. Nevers, M. J. Sadowsky and R. L. Whitman, *FEMS Microbiology Ecology*, 2003, **46**, 203-211.
18. R. Santas, A. Korda, C. Lianou and P. Santas, *Marine Biology*, 1998, **131**, 153-162.
19. W. W. Mulbry and A. C. Wilkie, *Journal of Applied Phycology*, 2001, **13**, 301-306.
20. C. H. Bolster, K. L. Cook, I. M. Marcus, B. Z. Haznedaroglu and S. L. Walker, *Environmental science & technology*, 2010, **44**, 5008-5014.
21. P. E. Dombek, L. K. Johnson, S. T. Zimmerley and M. J. Sadowsky, *Applied and Environmental Microbiology*, 2000, **66**, 2572-2577.
22. *Standard methods for the examination of water and wastewater*, 22 edn., APHA, WEF, AWWA, 2012.
23. M. J. Zimbro, Power, D.A., Miller, S.M., Wilson, G.E., Johnson, J.A. , *Difco & BBL Manual of Microbiological Culture Media*, 2 edn., Becton, Dickinson and Company, Sparks, MD, 2009.
24. J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular cloning*, Cold spring harbor laboratory press New York, 1989.
25. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld and B. Schmid, *Nature methods*, 2012, **9**, 676-682.
26. M. Verhougstraete, M. Byappanahalli, J. Rose and R. Whitman, 2010.
27. S. K. Haack, L. R. Fogarty, E. A. Stelzer, L. M. Fuller, A. K. Brennan, N. M. Isaacs and H. E. Johnson, *Environmental science & technology*, 2013.
28. R. Curtis, Ingraham, J.L., Lin, E.C.C., Low, K.B., Magasanik, B., Reznikoff, W.S. Riley, M., Schaechter, Umbarger, H.E., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, American Society for Microbiology.
29. G. J. Herndl, *The effects of ozone depletion on aquatic ecosystems*, 1997, 143-154.
30. J. Medina□Sánchez, M. Villar□Argaiz and P. Carrillo, *Freshwater Biology*, 2002, **47**, 2191-2204.
31. M. Wegelin, S. Canonica, K. Mechsner, T. Fleischmann, F. Pesaro and A. Metzler, *Aqua*, 1994, **43**, 154-169.
32. J. Lonnen, S. Kilvington, S. Kehoe, F. Al-Touati and K. McGuigan, *Water research*, 2005, **39**, 877-883.
33. E. Ubomba-Jaswa, C. Navntoft, M. I. Polo-López, P. Fernandez-Ibáñez and K. G. McGuigan, *Photochemical & Photobiological Sciences*, 2009, **8**, 587-595.
34. F. Sciacca, J. A. Rengifo-Herrera, J. Wéthé and C. Pulgarin, *Solar Energy*, 2011, **85**, 1399-1408.
35. E. T. Englebert, C. McDermott and G. T. Kleinheinz, *Journal of Great Lakes Research*, 2008, **34**, 377-382.
36. L. J. Tranvik and S. Bertilsson, *Ecology Letters*, 2001, **4**, 458-463.
37. A. M. Anesio, W. Granéli, G. R. Aiken, D. J. Kieber and K. Mopper, *Applied and environmental microbiology*, 2005, **71**, 6267-6275.
38. M. J. Pullin, S. Bertilsson, J. V. Goldstone and B. M. Voelker, *Limnology and oceanography*, 2004, **49**, 2011-2022.
39. M. Abboudi, W. Jeffrey, J.-F. Ghiglione, M. Pujo-Pay, L. Oriol, R. Sempere, B. Charrière and F. Joux, *Microbial ecology*, 2008, **55**, 344-357.
40. A. S. Gong, C. A. Lanzl, D. M. Cwiertny and S. L. Walker, *Environmental science & technology*, 2011, **46**, 241-249.
41. R. G. Wetzel, P. G. Hatcher and T. S. Bianchi, *Limnology and Oceanography*, 1995, **40**, 1369-1380.
42. O. C. Zafiriou, J. Joussot-Dubien, R. G. Zepp and R. G. Zika, *Environmental science & technology*, 1984, **18**, 358A-371A.
43. S. Bertilsson and L. J. Tranvik, *Limnology and Oceanography*, 2000, **45**, 753-762.
44. A. M. Shiller, S. Duan, P. van Erp and T. S. Bianchi, *Limnol. Oceanogr*, 2006, **51**, 1716-1728.
45. M. J. Lindell, W. Granéli and L. J. Tranvik, *Limnology and Oceanography*, 1995, **40**, 195-199.
46. E. Kaiser and G. J. Herndl, *Applied and environmental microbiology*, 1997, **63**, 4026-4031.
47. U. S. EPA, *Lake Erie Lakewide Management Plans and Reports*, http://www.epa.gov/lakeerie/, Accessed November 2013, 2013.
48. S. C. Chapra, A. Dove and G. J. Warren, *Journal of Great Lakes Research*, 2012, **38**, 550-560.
49. C. E. Binding, J. H. Jerome, R. P. Bukata and W. G. Booty, *Remote Sensing of Environment*, 2008, **112**, 1702-1711.
50. M. Charlton, *Status of Nutrients in the Lake Erie Basin*, 2010.
51. M. Granbom and M. Pedersen, *Hydrobiologia*, 1999, **399**, 349-354.
52. J. R. Andria, J. L. Perez-Llorens and J. J. Vergara, *Planta*, 1999, **208**, 564-573.
53. K. S. Choo, P. Snoeijs and M. Pedersén, *Journal of phycology*, 2002, **38**, 493-502.

54. M. Garrido-Pérez, E. Anfuso, A. Acevedo and J. Perales-Vargas-Machuca, *International journal of hygiene and environmental health*, 2008, **211**, 510-517.

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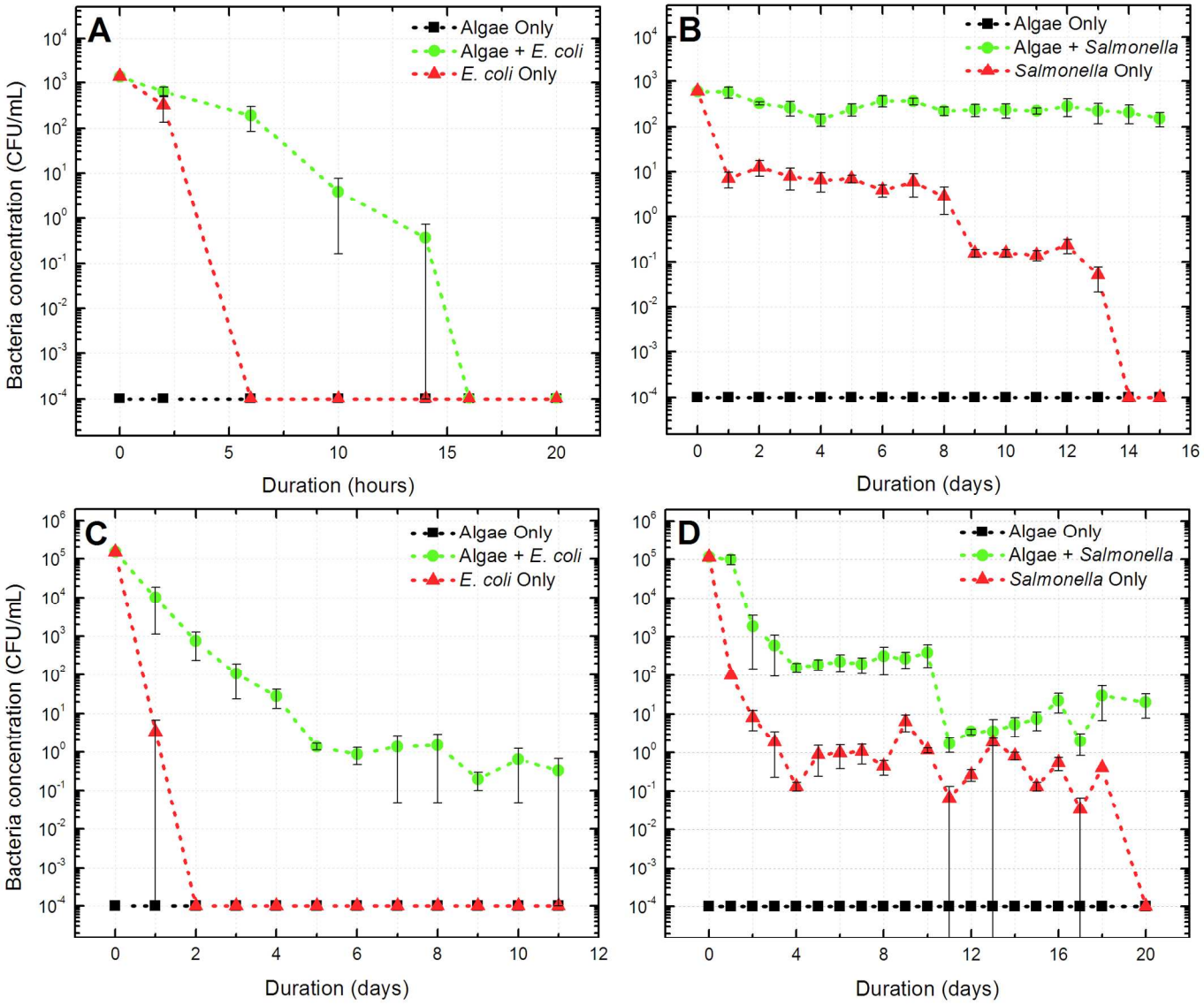

Fig. 1 Concentration of bacteria in microcosms exposed to UV irradiation with respect to time. Data points represent mean log CFU reduction per mL. Circles denote algae-association and triangles denote non-association. Squares denote only algae microcosms. (A) *E. coli* with an initial seed concentration of 10^3 CFU/mL; (B) *S. typhimurium* with an initial seed concentration of 10^3 CFU/mL; (C) *E. coli* with an initial seed concentration of 10^5 CFU/mL; (D) *S. typhimurium* with an initial seed concentration of 10^5 CFU/mL. All conditions were tested in triplicate and error bars represent standard deviation.

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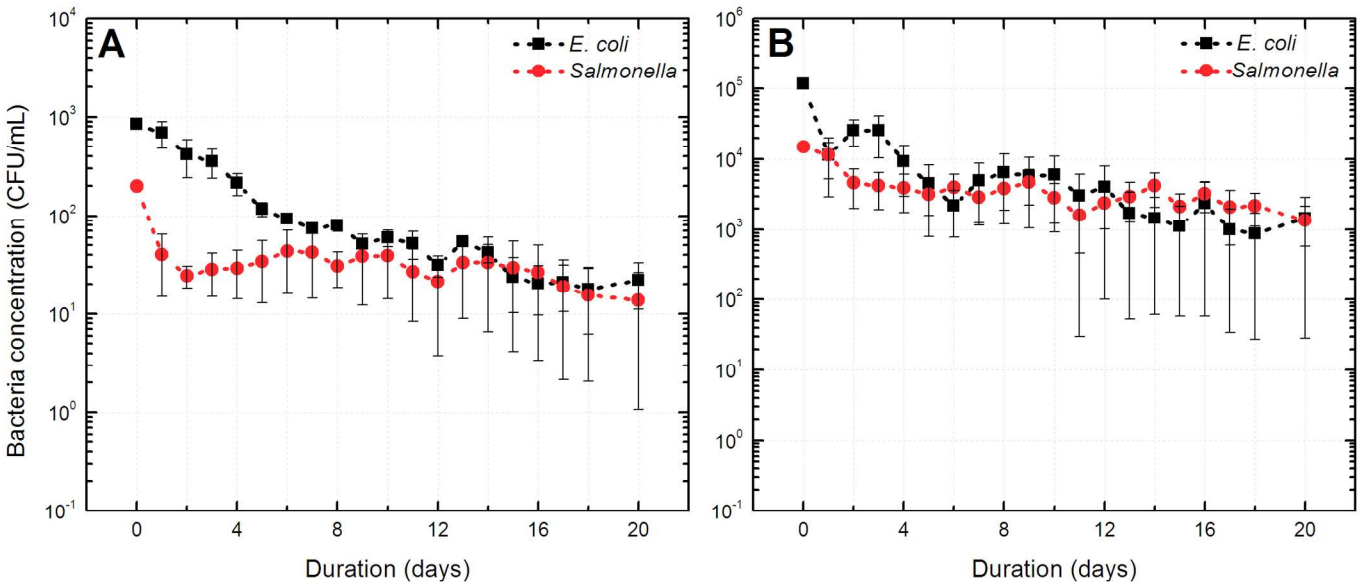

Fig. 2 Concentration of bacteria in lake water microcosms not exposed to UV irradiation with respect to time. Data points represent mean log CFU reduction per mL. Squares denote *E. coli*, and circles denote *S. typhimurium.* (A) *E. coli* and *S. typhimurium* with an initial seed concentration of 10^3 CFU/mL; (B) *E. coli* and *S. typhimurium* with an initial seed concentration of 10^5 CFU/mL. All conditions tested in duplicate and error bars represent standard deviation.

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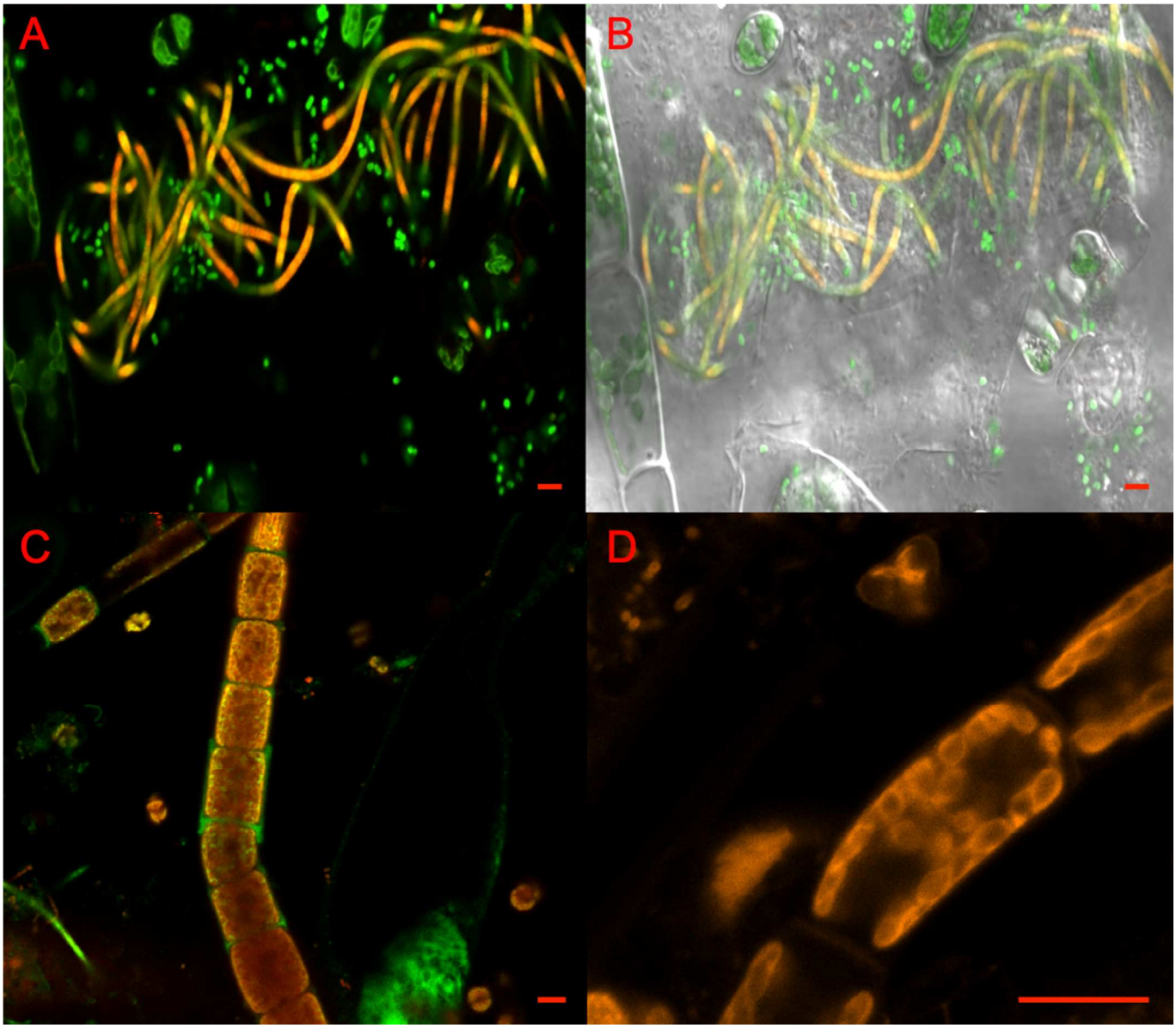

Fig. 3 Confocal microscopy images showing the association of GFP-labeled *E. coli* and *S. typhimurium* with algal filaments. (A) More *E. coli* cells attached to thin filamentous *Cladophora liniformis* than bulk fluid; (B) Composite image of (A) clearly showing no bacteria was attached to thicker filamentous *Cladophora fracta.*; (C) Similar to (A) and (B) no *S. typhimurium* was observed to attach thicker filaments; (D) A close micrograph of *Cladophora* showing no bacteria was internalized or penetrated into algal tissue. Scale bars denote 5 μm.

Table 1. Nutrient concentrations and water quality characteristics of non-algae associated Lake Erie Water compared to algae associated Lake Water during the course of the study.

Parameter (unit)	LW	Algae + LW (Day 5)	Algae + LW (Day 10)	Algae + LW (Day 15)	Algae + LW (Day 20)
TDS (mg/L)	296.92 ± 4.91	200.00 ± 6.12	320.00 ± 8.17	243.08 ± 4.21	264.62 ± 3.85
DOC (mg/L)	5.67 ± 1.40	24.03 ± 4.08	32.93 ± 0.54	37.71 ± 2.89	39.14 ± 4.16
TN (μM)	9.70 ± 0.00	5.82 ± 1.94	1.94 ± 0.00	4.53 ± 2.96	3.88 ± 0.00
TP (μM)	BDL	1.32 ± 0.47	0.23 ± 0.29	0.54 ± 0.51	0.49 ± 0.1
DO (mg/L)	9.56	9.75	10.50	10.40	9.92
Turbidity (NTU)	0.02	177	483	135	187
pH	8.23	9.91	10.59	10.60	10.40
Hardness (ppm as $CaCO_3$)	200	225	200	200	200
Conductivity (μS/cm)	257	224	243	255	258

LW: Lake Erie Water; DOC: Dissolved organic carbon; TDS: Total dissolved solids; TN: Total nitrogen; TP: Total phosphorus; BDL: Below detection level