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# Beyond cyanotoxins: increased *Legionella*, antibiotic resistance genes in western Lake Erie water and disinfection-byproducts in their finished water

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**Background:** Western Lake Erie is suffering from harmful cyanobacterial blooms, primarily toxic *Microcystis* spp., affecting the ecosystem, water safety, and the regional economy. Continued bloom occurrence has raised concerns about public health implications. However, there has been no investigation regarding the potential increase of *Legionella* and antibiotic resistance genes in source water, and disinfection byproducts in municipal treated drinking water caused by these bloom events.

**Methods:** Over 2 years, source water (total  $n = 118$ ) and finished water (total  $n = 118$ ) samples were collected from drinking water plants situated in western Lake Erie (bloom site) and central Lake Erie (control site). Bloom-related parameters were determined, such as microcystin (MC), toxic *Microcystis*, total organic carbon, N, and P. Disinfection byproducts (DBPs) [total trihalomethanes (THMs) and haloacetic acids (HAAs)] were assessed in finished water. Genetic markers for *Legionella*, antibiotic resistance genes, and mobile genetic elements were quantified in source and finished waters.

**Results:** Significantly higher levels of MC-producing *Microcystis* were observed in the western Lake Erie site compared to the control site. Analysis of DBPs revealed significantly elevated THMs concentrations at the bloom site, while HAAs concentrations remained similar between the two sites. *Legionella* spp. levels were significantly higher in the bloom site, showing a significant relationship with total cyanobacteria. Abundance of ARGs (*tetQ* and *sul1*) and mobile genetic elements (MGEs) were also significantly higher at the bloom site.

**Discussion:** Although overall abundance decreased in finished water, relative abundance of ARGs and MGE among total bacteria increased after treatment, particularly at the bloom site. The findings underscore the need for ongoing efforts to mitigate bloom frequency and intensity in the lake. Moreover, optimizing water treatment processes during bloom episodes is crucial to maintain water quality. The associations observed between bloom conditions, ARGs, and *Legionella*, necessitate future investigations into the potential enhancement of antibiotic-resistant bacteria and *Legionella* spp. due to blooms, both in lake environments and drinking water distribution systems.

## KEYWORDS

trihalomethane, Lake Erie, haloacetic acids, microcystin, *Microcystis*, mobile genetic elements, antibiotic resistance, *Legionella*

## Introduction

Changes in the global climate have been linked to increasing eutrophication and an increase in frequency, duration and intensity of harmful algae blooms (Glibert, 2020). Among these blooms, freshwater blooms are dominated by cyanobacterial blooms, in which most are associated with the production of cyanotoxins (Loftin et al., 2016). These blooms, pose health risks by compromising drinking water sources, food production, aquatic ecosystems, and recreational waters (Brooks et al., 2016; Lee J. et al., 2017; Mrdjen et al., 2018). Microcystins (MCs), prevalent cyanotoxins, are commonly detected in freshwater used for drinking water supplies, irrigation, recreation, and fish farms (Loftin et al., 2016; Lee S. et al., 2017; Mrdjen et al., 2018). Epidemiological and animal studies have linked exposures to bloom-affected water to an increased frequency of non-alcoholic liver diseases, liver cancer, neurodegenerative diseases, gut microbiome disturbance, and more progressed liver cancer (Zhang et al., 2015; Lee et al., 2019, 2020, 2022; Gorham et al., 2020).

Lake Erie in the United States has witnessed recurring severe cyanobacteria blooms, particularly the massive 2011 event spanning 5,000 km<sup>2</sup> (Stumpf et al., 2016; Zhang et al., 2017; Jankowiak et al., 2019). Elevated MCs exceeding 1 µg/L in finished drinking water in 2013–2014 prompted “Do Not Drink” advisories (Stumpf et al., 2016). Effective drinking water treatment is pivotal for safeguarding public health from cyanotoxins, necessitating robust strategies for MCs and cyanotoxin removal in bloom-impacted source water beyond source protection measures. While MCs are effectively removed using disinfection with free chlorine or ozone followed by additional activated carbon treatment (Cheung et al., 2013; Gonsior et al., 2019) in most cases, little is known about disinfection by-products (DBPs) that can form via chlorination in the Lake Erie region from MCs and other organics associated with blooms. Previous studies have demonstrated that bloom conditions are associated with an increase in DBP pre-cursors and DBPs (Zamyadi et al., 2012; Zong et al., 2015; Foreman et al., 2021). Disinfectants and chlorine react easily with all types of organic matter, including dissolved and algal organic matter to produce genotoxic and carcinogenic DBPs including trihalomethanes (THMs) and haloacetic acids (HAAs) (Liu et al., 2018). Therefore, conventional water treatment using activated carbon can be useful for removing cyanotoxins, and dissolved and algal organic compounds (Ho et al., 2011; U.S. EPA, 2016; Cermakova et al., 2017).

While activated carbon and rest procedures reduce unwanted contaminants, biofilms can become established in the system and contribute to promoting antibiotic resistance genes (ARGs) (Guo et al., 2018). Specifically, activated carbon filtration has been linked to increasing the abundance of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) (Xu et al., 2016, 2020a). Furthermore, recent studies report that cyanobacteria, especially *Microcystis*, harbor significant amounts of ARGs and may play a significant role as a reservoir and source for ARGs in bloom seasons

whereby cyanobacterial blooms promote conjugative transfer of ARGs (Wang et al., 2020, 2021). More studies recently reported that cyanobacteria may promote the spread of ARGs in bacteria due to the significant contribution of mobile genetic elements (MGEs) located in genera, such as *Microcystis* (Volk and Lee, 2022). The interest in *Legionella* spp. is heightened in bloom-affected source waters as biofilm-loving *Legionella* spp. including *L. pneumophila* have long been known to grow when cyanobacteria densities are great and biofilm masses exist (Tison et al., 1980; Fliermans et al., 1981; Pope et al., 1982; Declerck, 2010; Cai et al., 2014). Additionally, biofilms can support the growth and persistence of *Legionella* spp. in drinking water systems (Berjeaud et al., 2016) including where activated carbon is used (Li et al., 2017).

While drinking water treatment plants (DWTPs) in bloom-affected areas give necessary and considerable attention to removing cyanotoxins and cyanobacteria during bloom seasons, there are emerging issues not yet studied in the Lake Erie region. We hypothesize that water from heavy bloom areas have (1) higher DBP levels in finished water due to increased interactions between bloom-associated organic matter and chlorine during treatment, (2) higher ARG from using activated carbon to absorb toxins as well as from the higher abundance of *Microcystis* in the source water, and (3) bloom-related aggregated biomass provides a niche for promoting *Legionella* in the lake water and is thus positively associated with *Microcystis* in Lake Erie. In the present study, we investigated two water treatment plants in the Lake Erie region (western Lake Erie for greater bloom frequency and intensity, and central Lake Erie for low bloom frequency and intensity) for 2 years. Specifically, we (1) examined the dynamics of *Microcystis* and MCs in the source water, (2) determined the association between cyanobacteria and *Legionella* in the source water, and (3) monitored both DBPs and ARGs in their finished water. The study outcome provides more holistic understanding about the water quality issues beyond bloom toxicity and cyanotoxin where cyanobacterial blooms are chronically prevalent.

## Materials and methods

### Study sites and water samples

Two drinking water treatment plants in Ohio, United States, were selected based on annual cyanobacterial bloom intensity. The first was the Collins Park Water Treatment Plant in Toledo, which is a historically high-bloom area in western Lake Erie (Ohio Department Health et al., 2020) and the second was the City of Painesville Water Treatment Plant, a low- or no-bloom area in central Lake Erie (Supplementary Figure S1). Water samples were collected once weekly during the summer and fall (May–November) of 2013 and 2014 from the Toledo and Painesville sites, totaling 58 and 60 samples, respectively. Samples were collected from the source water (sampled at the drinking water intakes within Lake Erie) and from the finished

drinking water at each plant. Collections were done in sterile bottles and shipped in a cooler on ice. For toxin measurements, amber glass vials were used. The samples reached The Ohio State University (Columbus, Ohio, United States) for laboratory analysis within 15 h of sample collection. In the lab, the collected source water (200 mL) was filtered using the polycarbonate membrane (0.45  $\mu\text{m}$  pore size, Millipore, Burlington, MA, United States) for molecular analyses, and filters were kept at  $-20^{\circ}\text{C}$  and further analyses were performed as soon as possible.

## Water quality parameters

Conventional water quality parameters (water temperature, turbidity, hardness, and pH) and total chlorine in finished water were measured routinely on-site according to the Standard Methods (Rice et al., 2012) by the two water treatment plants and the data were obtained. Chlorophyll-*a* and phycocyanin were measured using the AquaFluor<sup>®</sup> Handheld fluorometer (Turner Designs, California, United States). Total phosphorus (TP) and soluble reactive phosphorus were measured using the USEPA-accepted Method 8190 (Hach PhosVer 3 with Acid Persulfate Digestion). Total nitrogen (TN) in the higher range (0.23–13.50 mg/L  $\text{NO}_3^-$ -N) and the lower range (0.01–0.5 mg/L  $\text{NO}_3^-$ -N) were analyzed using Method 10206 (Hach dimethylphenol method) and Method 8192, respectively. Total organic carbon (TOC) was analyzed at Alloway Laboratory (Marion, Ohio, United States) using the USEPA 451.3 (U.S. EPA, 2005).

## MC measurement

Total MCs in water samples were measured using enzyme-linked immunosorbent assay (ELISA) kits (Abraxis, Warminster, PA, United States) with MC-LR (0.15–5  $\mu\text{g/L}$ ) as a working standard. The absorbance was read at 450 nm on the Dynex MRX microplate reader (Dynex Technologies, Inc., Chantilly, VA, United States). The detection range of the assay is 0.15–5.0 ng/mL with a detection limit of 0.10 ng/mL. All the ELISA assays were performed in triplicate.

## Disinfection by-products

DBPs (TTHMs and HAA5) in finished water were quantified with gas chromatography–mass spectrometry (GC–MS) using the USEPA-524.2 method.<sup>1</sup> TTHMs included bromoform ( $\text{CHBr}_3$ ), chloroform ( $\text{CHCl}_3$ ), bromodichloromethane ( $\text{CHCl}_2\text{Br}$ ), chlorodibromomethane ( $\text{CHClBr}_2$ ). HAA5 included monochloroacetic acid ( $\text{CH}_2\text{ClCOOH}$ , MCAA), dichloroacetic acid ( $\text{CHCl}_2\text{COOH}$ , DCAA), trichloroacetic acid ( $\text{CCl}_3\text{COOH}$ , TCAA), monobromoacetic acid ( $\text{CH}_2\text{BrCOOH}$ , MBAA) and dibromoacetic acid ( $\text{CHBr}_2\text{COOH}$ , DBAA). DBPs were quantified at Alloway Laboratory (Marion, Ohio, United States).

## Quantification of total and toxic *Microcystis* abundance

We quantified concentrations of total and MC-producing *Microcystis* by targeting the *Microcystis* phycocyanin intergenic spacer (PC-IGS) and microcystin synthetase gene B (*mcyB*), using published approaches with slight modifications (Klase et al., 2019). For *Microcystis* PC-IGS, the PCR mixture (20  $\mu\text{L}$ ) in duplicate contained TaqMan universal PCR master mix (10  $\mu\text{L}$ ) (Life Technologies, United States), each primer (188F/254R, 300 nM), PC-IGS probe (100 nM), additional  $\text{MgCl}_2$  (1.25 mM) and DNA template (2  $\mu\text{L}$ ). The PCR profile followed an initial cycle of  $50^{\circ}\text{C}$  for 2 min,  $95^{\circ}\text{C}$  for 10 min, 50 cycles at  $95^{\circ}\text{C}$  for 30 s,  $56^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 30 s. For *Microcystis mcyB*, the PCR protocol was identical to that for PC-IGS, except we used 900 nM of each *mcyB*-specific-primer (30F/108R) and 250 nm of *mcyB* probe. The standard working curve was generated for each assay according to the standard *Microcystis* PC-IGS and *mcyB* DNA standard (pGEMT-PC-IGS and *mcyB*), respectively. The PC-IGS probe had a 5' end of the fluorescent reporter, 6FAM, and a 3' end of the non-fluorescent quencher (NFQ) that was attached with a minor groove binder (MGB) moiety (MGB-NFQ). In contrast, the *mcyB* probe contained a 5' end of the fluorescent reporter, instead. We estimated the proportion of the potentially MC-producing genotype in the *Microcystis* population according to the ratio between *Microcystis mcyB* and PC-IGS in percentage unit (%).

## Droplet digital PCR for total bacteria, ARGs and *Legionella*

We targeted three antibiotic resistance genes (*tetQ* for tetracycline resistance, *sul1* for sulfonamide resistance, and *bla*<sub>KPC</sub> for carbapenem resistance), total bacteria (targeting 16S rRNA gene), *Legionella* species (targeting the 5S rRNA gene), as well as a mobile genetic element [MGE, class 1 integron-integrase gene (*intI1*)] (Krøjgaard et al., 2011; Jia et al., 2014; Yin et al., 2017; Gupta et al., 2019; Klase et al., 2019) using the QX200 droplet digital PCR system (ddPCR, Bio-Rad, Hercules, CA, United States). For quantification of total bacteria, *tetQ*, *sul1*, and *intI1*, the ddPCR mixture (20  $\mu\text{L}$ ) contained 2X EvaGreen supermix (Bio-Rad), 200 nM of each primer, DNA template, and RNase-/DNase free PCR water. To quantify KPC and *Legionella*, the ddPCR mixture (20  $\mu\text{L}$ ) contained 2X supermix for probes, 200 nM of each primer, 200 nM of the probe, DNA template, and RNase-/DNase free PCR water. With 20  $\mu\text{L}$  of PCR mixture, droplets were generated using the Droplet generator (Bio-Rad) with droplet generation oil. PCR was performed using a thermal cycler (C1000 touch thermal cycler, Bio-Rad, Hercules, CA, United States), following previous studies (Yin et al., 2017; Gupta et al., 2019; Klase et al., 2019). After the PCR reaction, concentrations of targeted genes were analyzed using the Droplet Reader and QuantaSoft software (Bio-Rad Hercules, CA, United States).

## Statistical analysis

Data were explored using scatterplots, time-series plots, box plots and bar charts. Means and standard deviations (SD) were calculated

<sup>1</sup> <https://www.epa.gov/esam/epa-method-5242-measurement-purgeable-organic-compounds-water-capillary-column-gas>

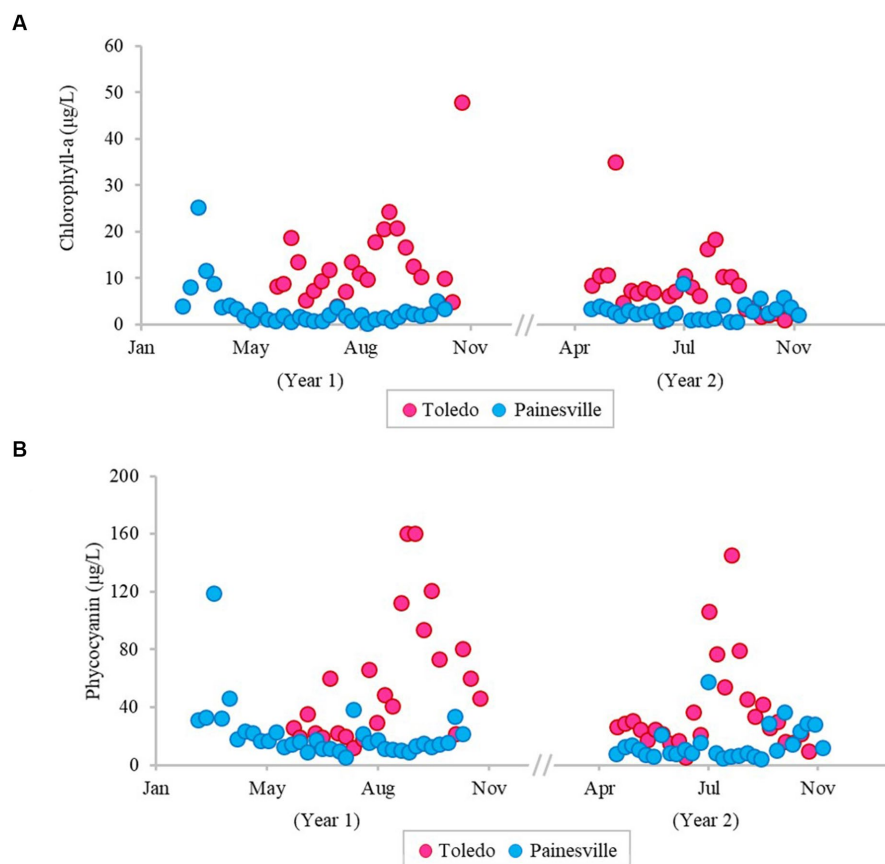


FIGURE 1

Temporal changes of concentration ( $\mu\text{g/L}$ ) of bloom-related parameters, chlorophyll-*a* (A) and phycocyanin (B), in source water from the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue).

and data are presented as the mean  $\pm$  SD. A one-way analysis of variance (ANOVA) was used for the spatial difference in the variables with a significance level set at  $p < 0.05$ . After ANOVA, Tukey Honest Significant Difference tests were used to determine if the means were different ( $p < 0.05$ ) between the groups. All analyses were conducted using SPSS 24.0 (SPSS, Chicago, IL, United States).

## Results

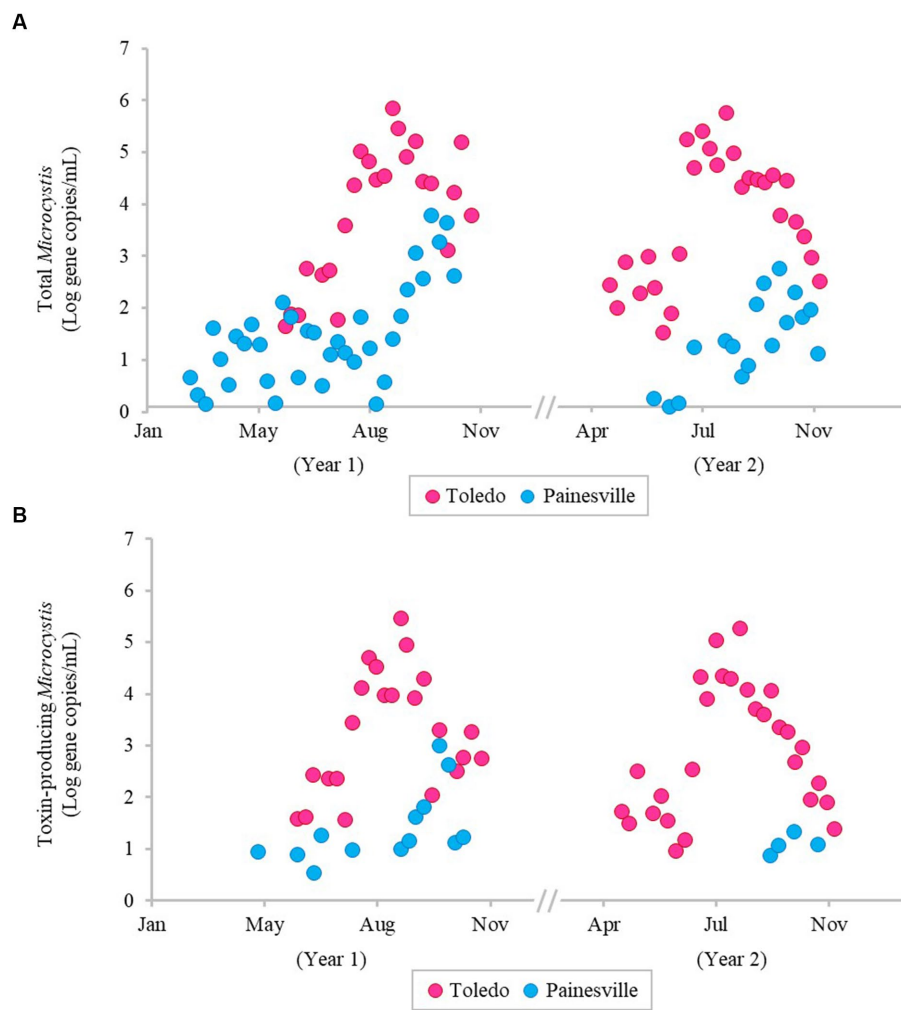
### Source water

The severity of blooms in the source water from western (Toledo) and central (Painesville) Lake Erie was examined (Figures 1–3) as were source water chemical parameters and water temperatures. The mean concentrations of chlorophyll-*a* and phycocyanin in the source water from western Lake Erie (bloom site) were  $8.32 \mu\text{g/L}$  and  $37.4 \mu\text{g/L}$ , respectively (Figure 1). The mean concentrations of chlorophyll-*a* and phycocyanin in the source water from central Lake Erie (control site) were  $2.92 \mu\text{g/L}$  and  $14.8 \mu\text{g/L}$ , respectively (Figure 1). The mean concentrations of two bloom indicators in the bloom site were significantly higher than that from the control site ( $p < 0.05$ ). Similarly, significantly higher concentrations of total *Microcystis* (PC-IGS gene) and toxin-producing *Microcystis* (*mcyB* gene) were observed in the bloom site source water ( $p < 0.05$ ) (Figure 2).

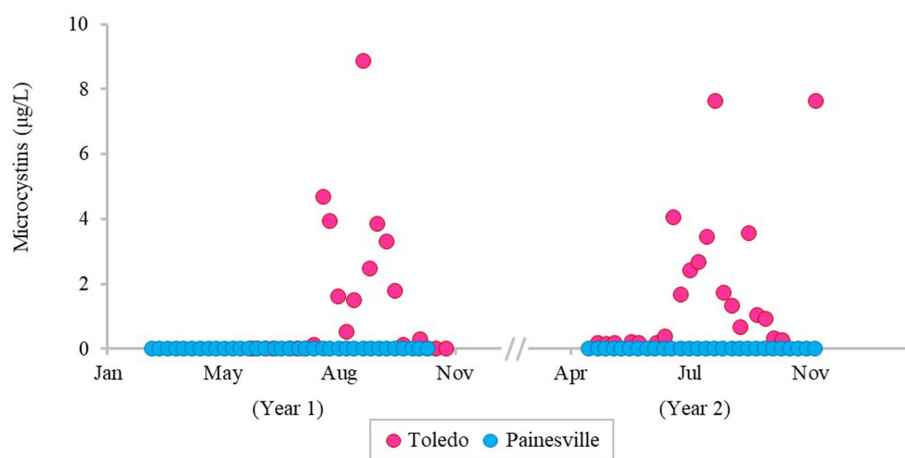
Specifically, the mean concentration of the PC-IGS gene (gene copies/mL) was  $5.57 \times 10^4$  in Toledo source water (bloom site) vs.  $8.79 \times 10^1$  in the Painesville source water (control site). The mean concentration of the *mcyB* gene (gene copies/mL) was  $1.48 \times 10^4$  in the bloom site vs.  $1.32 \times 10^1$  in the control site. In regard to the cyanotoxin measurement, the mean concentration of microcystin in the bloom site source water was  $1.65 \mu\text{g/L}$  which was also higher than the control site (not detected) (Figure 3). These results show that the intensity and frequency of blooms at the bloom site (Toledo) was obviously higher than the control site (Painesville).

Among water chemistry parameters, concentrations were consistently higher at the bloom sites; whereby mean total phosphorus levels for the two-year study period were  $140 \mu\text{g/L}$  and  $90 \mu\text{g/L}$  for the Toledo and Painesville DWTP source water samples. The mean total organic carbon was  $0.36 \text{ mg/L}$  (Toledo) and  $0.24 \text{ mg/L}$  (Painesville) and the mean total nitrate was  $0.64 \text{ mg/L}$  and  $0.33 \text{ mg/L}$  in the Toledo and Painesville source water samples, respectively. In addition to chemistry parameters, the mean temperatures of source water samples were  $19.6^\circ\text{C}$  and  $16.1^\circ\text{C}$  for Toledo and Painesville, respectively.

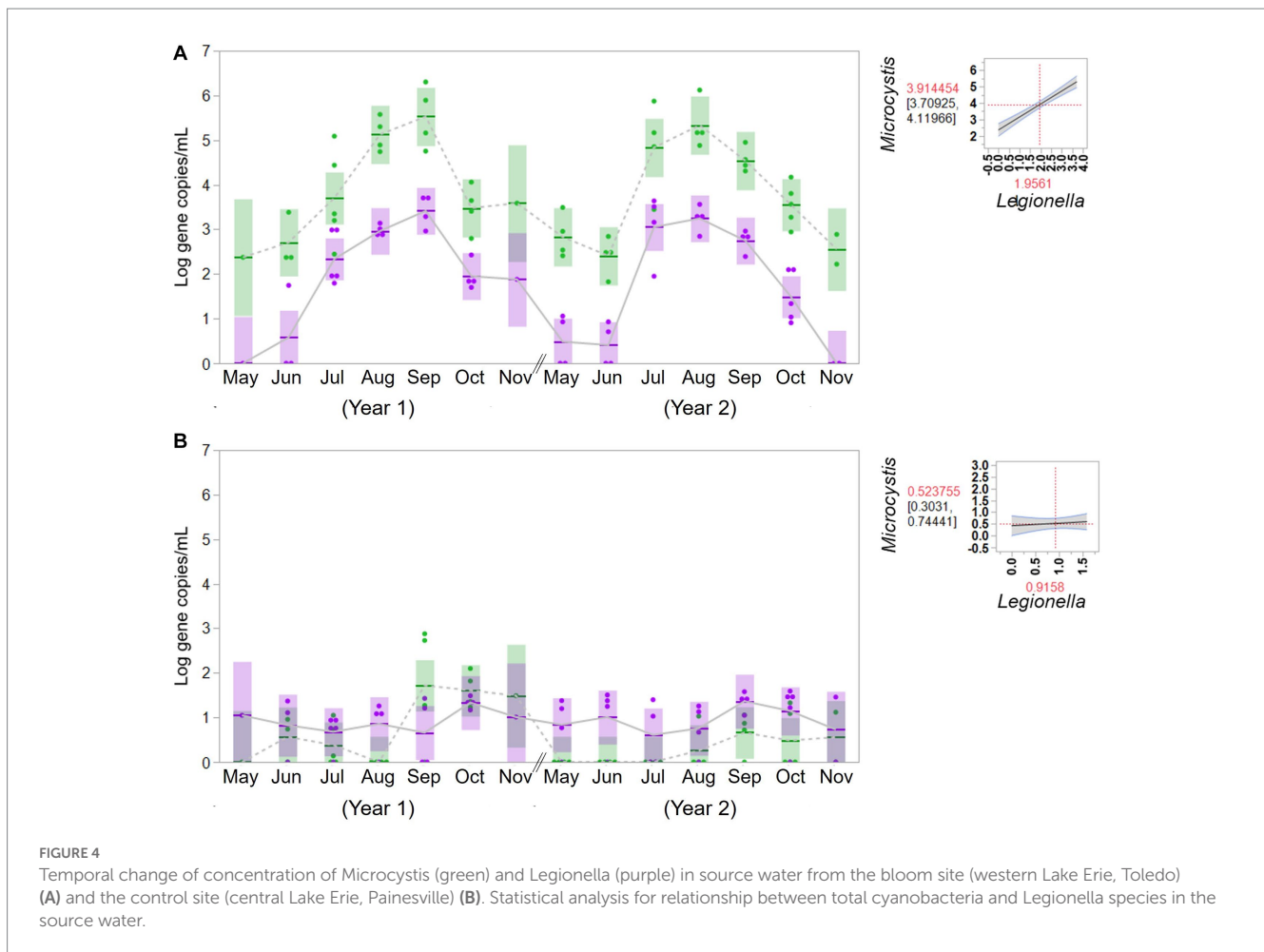
To evaluate the potential association between cyanobacteria and *Legionella*, the concentrations of *Legionella* were examined in the source water from the western Lake Erie and the control site for 2 years. The concentrations of *Legionella* (gene copies/mL) were  $2.49 \times 10^2$  (ranging from  $5.10 \times 10^0$  to  $5.09 \times 10^3$ ) in the bloom source water and  $1.63 \times 10^2$  (ranging from  $4.66 \times 10^1$  to



**FIGURE 2**  
Temporal changes of population of total *Microcystis* (A) and toxin-producing *Microcystis* (B) in source water from the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue).



**FIGURE 3**  
Temporal changes of concentration of microcystin in source water from the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue).



$3.85 \times 10^2$ ) in the control source water. The associations between cyanobacteria and *Legionella* in the source water are summarized in Figure 4. An apparent significant relationship was observed in western Lake Erie between the concentration of the molecular markers for total cyanobacteria and *Legionella* spp. ( $F = 89.82$ ,  $p = 0.001$ ), but there was no relationship observed between the markers for total cyanobacteria and *Legionella* spp. observed in source water samples from the non-bloom site in Painesville ( $F = 0.31$ ,  $p = 0.5814$ ).

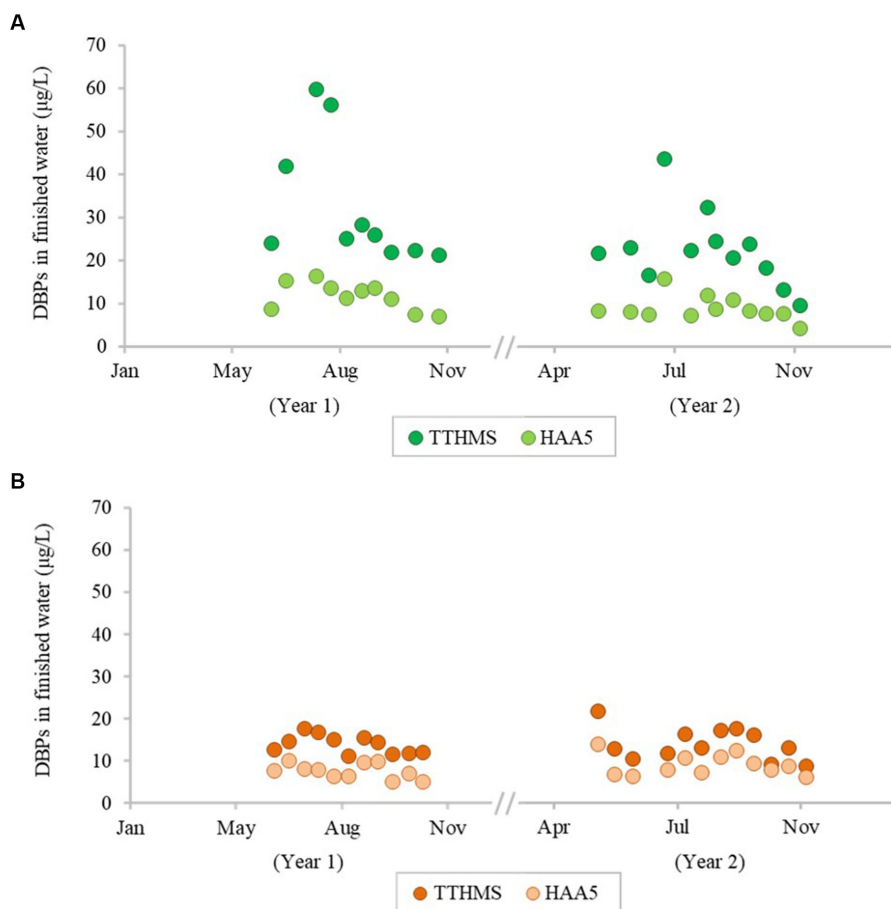
## Finished water

The concentrations of DBPs (TTHMs and HAA5) in the finished water from the bloom vs. control sites are summarized in Figure 5. The mean concentrations of TTHMs were  $22.43 \mu\text{g/L}$  from the bloom site and  $14.03 \mu\text{g/L}$  from the control site, and the mean TTHMs concentration from the bloom site was significantly higher than the control site ( $p < 0.05$ ). The mean concentrations of HAA5 were  $8.86 \mu\text{g/L}$  from the bloom site and  $9.06 \mu\text{g/L}$  from the control site, and the mean HAA5 concentrations between the two locations were not significantly different ( $p > 0.05$ ). Figure 6 shows that TOC levels in finished water were not statistically different between the bloom vs. control sites ( $p > 0.05$ ). The mean

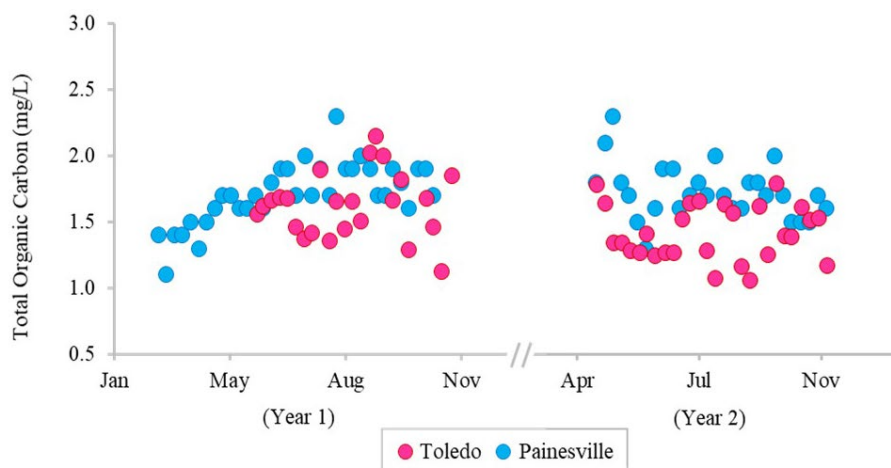
concentrations of TOC were  $1.42 \text{ mg/L}$  from the bloom site and  $1.72 \text{ mg/L}$  from the control site. Furthermore, microcystin was not detected in finished water samples from either location.

Concentrations of ARGs [tetracycline (*tetQ*), sulfonamide (*sul1*), carbapenem (*bla*<sub>KPC</sub>) resistance genes] were quantified in source and finished waters. The concentrations of all the ARGs were significantly lower ( $p < 0.05$ ) in the finished water vs. the source water at bloom and control locations (Figure 7). In comparing the mean concentrations (gene copies/100 mL) of ARGs in the finished water, the Toledo finished water mean ARG concentrations were  $3.70 \times 10^2$  (*tetQ*),  $3.26 \times 10^2$  (*sul1*), and  $1.91 \times 10^2$  (*bla*<sub>KPC</sub>). The mean concentrations (gene copies/100 mL) of ARGs in the finished water from Painesville WTP were  $2.43 \times 10^2$  (*tetQ*),  $4.31 \times 10^2$  (*sul1*), and  $4.69 \times 10^2$  (*bla*<sub>KPC</sub>).

Concentrations of MGE (class 1 integron-integrase gene [*int11*]) and the total bacteria gene (16S rRNA) were also significantly reduced by drinking water treatment. After water treatment processing, the mean concentrations of the MGE (gene copies/100 mL of *int11*) in the finished water were  $4.19 \times 10^0$  in Toledo and  $0.82 \times 10^0$  in Painesville which represented significant ( $p < 0.01$ ) reductions from their source water (Figure 8). Similarly, concentrations of the total bacterial gene in the finished water of the bloom and control sites ( $4.56 \times 10^3$  in Toledo,  $3.55 \times 10^4$  in Painesville) were significantly lower ( $p < 0.01$ ) than in their



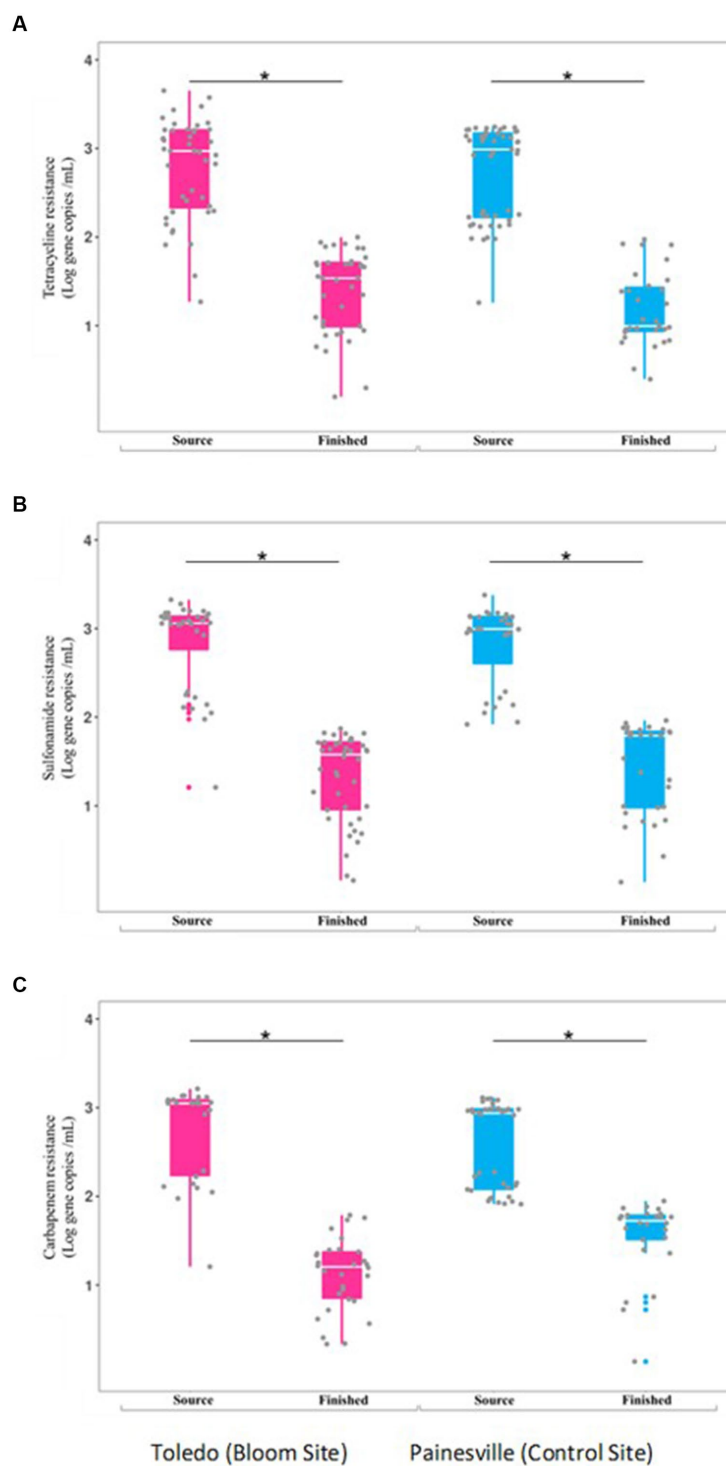
**FIGURE 5**  
Temporal change of concentration of disinfection by-products in finished water from the bloom site (A) and the control site (B).



**FIGURE 6**  
Temporal change of concentration of total organic carbon in the finished water of the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue).

source water ( $6.48 \times 10^8$  in Toledo,  $9.00 \times 10^9$  in Painesville). While drinking water treatment resulted in significantly lower concentrations of total bacteria, ARGs, and MGE, the relative

abundance of ARGs among total bacteria (16S rRNA) increased significantly ( $p < 0.05$ ) at both the bloom and control sites (Figure 9).



**FIGURE 7** Concentrations of antibiotic resistance genes [tetQ (A), sull (B), and blak PC (C)] in source and finished waters from the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue). \* $p < 0.05$ .

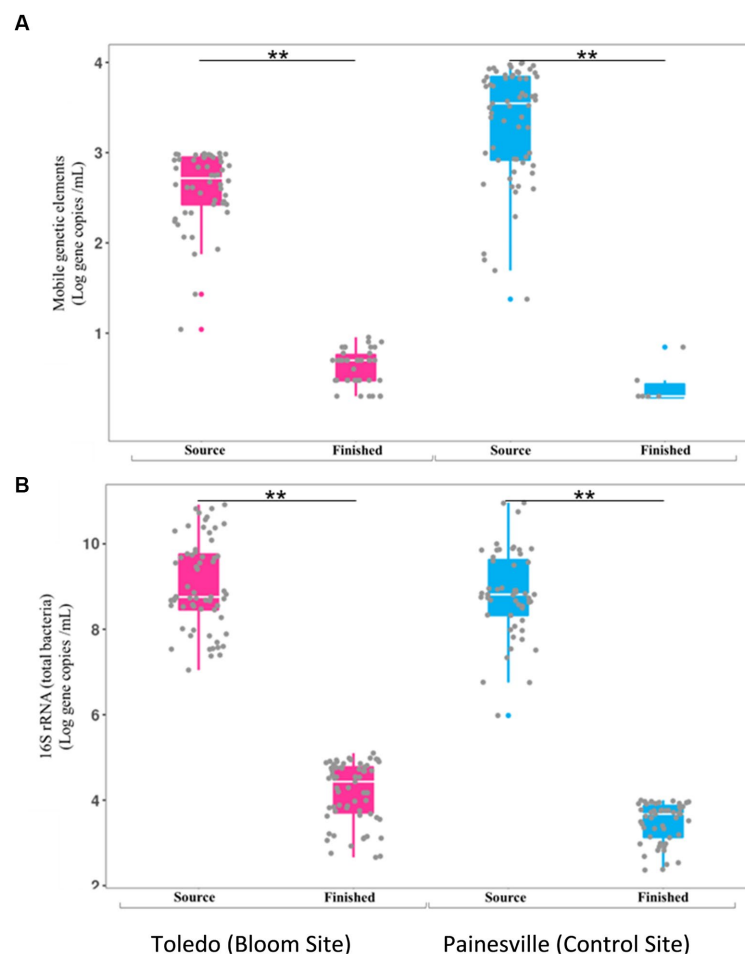
## Discussion

### Bloom conditions at the western Lake Erie site

Two drinking water treatment plants, drawing from Lake Erie as their source water, were chosen in Toledo (a high-bloom region in

western Lake Erie) and Painesville (a low- or no-bloom area in central Lake Erie) to examine water quality challenges beyond cyanotoxins when utilizing bloom-impacted source water. In assessing blooms, with an emphasis on cyanobacteria, multiple water quality parameters were employed to evaluate source and finished water quality. Upon characterizing bloom conditions, as expected, there were significant differences in chlorophyll-*a*, phycocyanin, total *Microcystis*,





**FIGURE 8**  
Concentrations of mobile genetic elements [MGES (A)] and 16S rRNA [total bacteria (B)] in source and finished waters from the bloom site (Toledo) (dark pink) and the control site (Painesville) (blue). \*\* $p < 0.01$ .

MC-producing *Microcystis*, and MC between the two locations. These differences were supported by the underlying water chemistry and temperatures enabling greater primary productivity by cyanobacteria as warmer water temperatures and higher concentrations of nutrients (P and N) are linked to cyanobacteria bloom formation, particularly when water temperatures approach or exceeds 20°C (Paerl and Huisman, 2008; Stumpf et al., 2012).

As predicted, bloom conditions manifested in source water during the warm months, mainly from July, August and September at the Toledo water intake (bloom site). Figures 1–4 show corroboration between bloom indicators (chlorophyll-*a* and phycocyanin), *Microcystis* densities, and ultimately cyanotoxins. Three of the source water MC results from Toledo exceeded the provisional 2015 Ohio contact recreation advisory threshold (6 µg/L) and one sample exceeded the revised and current (post-2019) Ohio and U.S. EPA contact recreation advisory threshold (8 µg/L standard) (Kasich et al., 2015; U.S. EPA, 2019). Notably, although MC levels were elevated in Toledo's source water, they remained undetectable in finished water samples. Of note, the *Microcystis* concentrations indicated by PC-IGS genetic marker measurement in the source water in Toledo were similar to those in a Japanese Lake Mikata where *Microcystis* blooms occurred (Yoshida et al., 2007).

## Elevated DBP formation potential and DBPs from bloom conditions

Once cyanobacterial blooms occur, blooms can impair surface water supplies used for drinking water. Blooms are associated with increases in TOC, turbidity, taste and odor compounds, and precursors for DBP formation (Nguyen et al., 2005). As hypothesized, TOC and turbidity levels were elevated in the bloom site source water relative to the control site (Painesville). Algal-derived organic carbon can be a significant source of DBP precursors for drinking water treatment facilities (Nguyen et al., 2005).

While DBP precursors can be elevated in source water, the presence of DBPs in finished drinking water varies according to the source water characteristics (e.g., temperature, pH, natural organic matter, etc.) and the processes used in water treatment. TOC concentrations are often used to predict DBP formation because TOC is a precursor to DBP formation. In this study, we investigated not only temporal variation in TOC (DBP formation precursor), but also the concentration of two commonly regulated DBPs, TTHMs and HAA5, in drinking water. TTHMs and HAA5, considered potentially carcinogenic, are the most important groups of DBPs (Rodriguez et al., 2004). The primary drinking water regulations for the

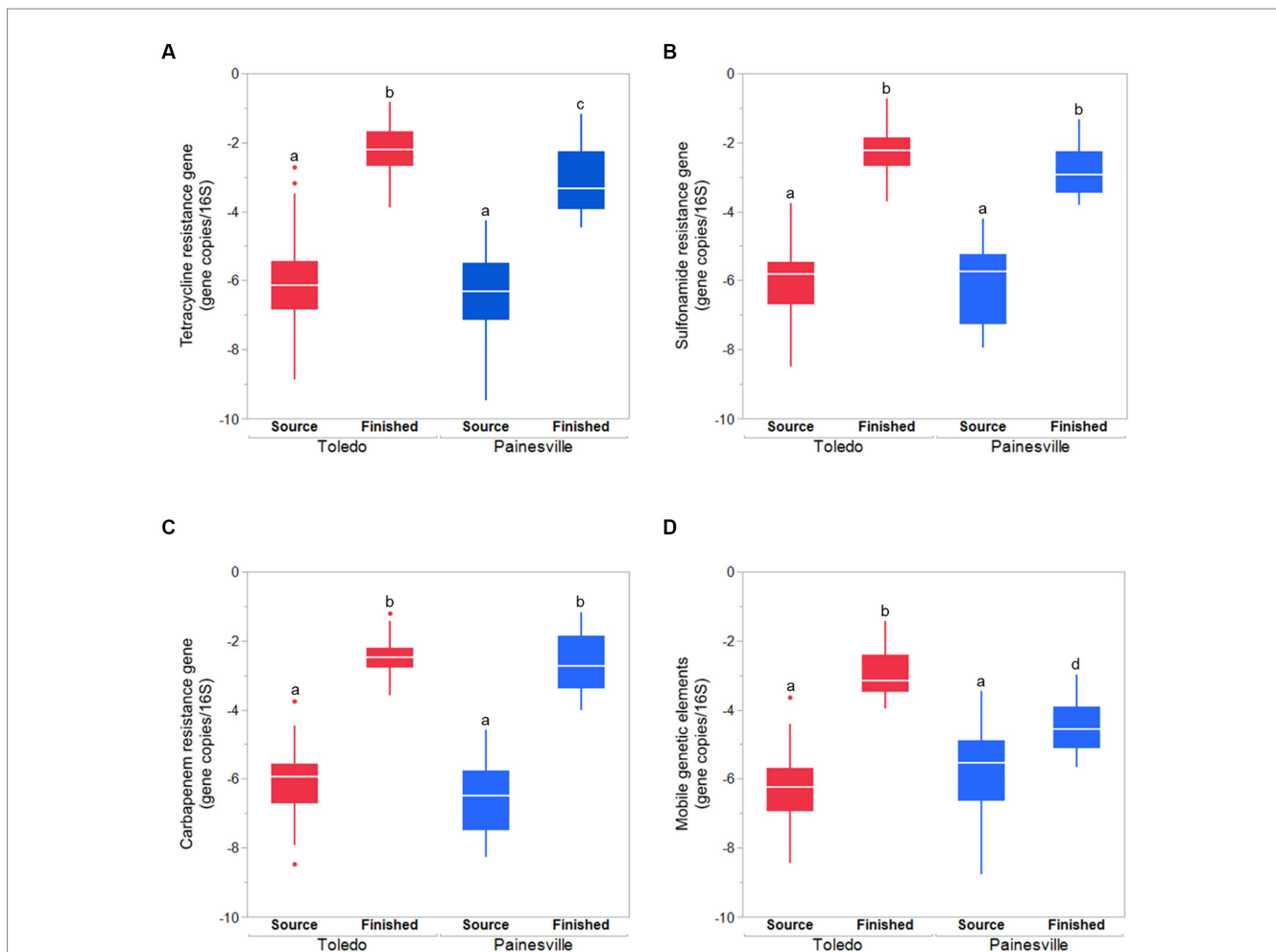


FIGURE 9

The relative abundances (gene copies/16S rRNA gene) of ARGs [tetracycline (A), sulfonamide (B), carbapenem resistance genes (C)] and mobile genetic element (D) in source water and finished water from the bloom site (Toledo) and the control site (Painesville). <sup>a</sup>Represents a significant difference from "a" ( $p < 0.01$ ). <sup>c</sup>Represents a significant difference from "a" ( $p < 0.01$ ) and from "b" ( $p < 0.05$ ).

U.S. mandates that the maximum acceptable levels of DBPs are 80  $\mu\text{g/L}$  for TTHMs and 60  $\mu\text{g/L}$  for HAA5 (U.S. EPA, 2023). Our results show that TOC concentrations in Toledo source water were higher than Painesville source water, and it is likely that the increase in TOC is related to cyanobacteria densities (Cory et al., 2016). As TOC levels were higher in the source water at Toledo, so were TTHMs and HAA5 in the finished water relative to the Painesville finished water. While DBPs were quantifiable, none of the samples exceeded primary standards set by the U.S. EPA, indicating that the current TOC removal processes at the two DWTPs can effectively control common DBPs.

## Relationship between *Legionella* and *Microcystis* in bloom site source water

Beyond altering source and finished water quality, cyanobacteria blooms in western Lake Erie occur in the context of a complex microbial ecosystem and can alter microbial communities.

One type of change was the observed apparent positive relationship between *Legionella* spp. abundance and *Microcystis* in the source water for the Toledo DWTP (bloom site), which was

present in both study years, but non-existent in the source water of the Painesville DWTP that served as a control site. Recent research from western Lake Erie (near Toledo [bloom site]) focusing on bacterial diversity from non-cyanobacteria showed clear differential responses among non-cyanobacteria to cyanobacteria abundance (estimated by chlorophyll-*a*) (Berry et al., 2017). Among bacteria that are linked to an increase in abundance during bloom conditions, there is evidence that the density of *Legionella* is correlated with *Microcystis* and eutrophication (Numberger et al., 2022). When looking at similar warm season water samples from three inland Ohio lakes, which like Western Lake Erie exhibit eutrophic conditions, Lee et al. (2016) documented *Legionella* spp. and the Legionellaceae as being among the most abundant and ubiquitous bacteria present in Ohio inland lakes.

While *Legionella* species are common in various natural and human-made aquatic environments, some species, mostly *L. pneumophila*, may cause legionellosis, which is a serious pulmonary infection established in persons following the inhalation of particles of contaminated aerosolized water. While there is some evidence that certain cyanobacteria can stimulate the growth of *Legionella* (Świątecki and Zdanowski, 2007; Bergeron et al., 2015), there are few studies primarily focusing on *Legionella* and cyanobacteria densities. Tison

et al. (1980) suggested an intimate association between *Legionella* spp. and cyanobacteria (*Fisherella*). Their observation indicated that *Legionella* could use algal extracellular products as its carbon and energy sources (Tison et al., 1980; Berendt, 1981). A previous study also reported that symbiotic interactions between *Legionella* and cyanobacteria may help the colonization of aquatic environments (Carvalho et al., 2007). Several factors (e.g., temperature, pH, and concentrations of nutrients and ions) and possibly products produced by other non-cyanobacterial bacteria, such as earlier arriving Betaproteobacteriales (van der Kooij et al., 2018), which are also abundant in Lake Erie during blooms (Berry et al., 2017), may all contribute to *Legionella* growth in aquatic environments as part of an ecological succession linked to bloom conditions of certain cyanobacteria species, like *Microcystis*.

While the potential for an ongoing increased global incidence of legionellosis due to a warmer climate has been described (Walker, 2018), the role of environmental waters warrants additional study. In Ohio, the incidence rate of legionellosis has consistently been 1.9 to 2.6 times greater than the U.S. average in recent years (Han, 2021). Recently, aerosols associated with roadway exposures have been considered as part of the increased incidence (Han, 2021), but of particular interest here is how anticipated future increases in eutrophic waters experiencing further eutrophication and warming globally may contribute to increased aerosol-related *Legionella* exposures, which could occur from boat wake (water skiing), fountains, etc. Beyond warmer water, there may be possible synergisms occurring in warming eutrophic waters as high concentrations of phosphates can enable substantial growth of cyanobacteria and increased turbidity and/or aggregated biofilm materials which can prompt or enhance the growth of *Legionella* and other Gammaproteobacteria (Cai et al., 2013; Lee et al., 2016; Srivastava et al., 2016). While the Gammaproteobacteria include many biofilm formers that may benefit from increased turbidity, these bacteria are also associated with an abundance of antibiotic resistance genes (Zhang et al., 2021).

## Relative abundance of ARGs increases in finished water from Lake Erie

Adding to the complexity of bloom ecology beyond *Legionella* densities are other broader emergent concerns regarding the establishment, maintenance, or promotion of antibiotic resistance bacteria (ARB) and ARGs in the source water (Volk and Lee, 2022), finished water (Li et al., 2016), and the distribution system (Xi et al., 2009; Xu et al., 2016). When the source water is impacted by bloom conditions, a convergence of numerous selective pressures emerge in the microbial community, including interspecies competition in the source water coupled with impacts from the water treatment processes, such as activated carbon use and disinfection. Xu et al. (2020b) demonstrated that when MCs were present an increase in ARGs was observed in the DWTP.

In this study, while hypothesized there would be differences in the densities of MGEs and ARGs in the source water samples from bloom and control sites, no differences were observed for MGEs or the ARGs for tetracycline, sulfonamide, and carbapenem resistance (Figure 7). At both study locations, the DWTP processes significantly reduced the amount of MGEs and ARGs from source water to finished water. The differences that emerged were specific to relative abundances of ARGs and MGEs. At both DWTPs, while reduced in overall density, the total bacterial density was reduced even more

greatly by the treatment processes. In comparison of the two DWTPs, the MGEs increased in relative abundance more in the Toledo DWTP than the Painesville DWTP.

Previous studies have revealed that commonly used disinfection technology can enrich ARB and spread ARGs (Shi et al., 2013). Water processing, including filtration and chlorination, remove most bacteria; however, extracellular stress can promote the replication of plasmids in bacteria. For example, chlorination might increase the copy number of plasmids in the surviving bacteria cells, resulting in the higher relative abundance of ARGs in treated water (Shi et al., 2013). While densities of ARGs and MGE were higher in the source water than the finished water, a limitation of this study was that it did not study the water distribution system, which can have higher levels of bacteria than the finished water due to regrowth potential of bacteria in the distribution system (Xi et al., 2009). If the source water selected for greater survival of biofilm formers preferring higher phosphate levels, corrosion control measures for the distribution system using phosphate may play a role in the regrowth of antibiotic resistant bacteria (Kappell et al., 2019; Kimbell et al., 2020). As expected, the finished water which included corrosion control had higher total P levels than the source water at the bloom site and control site (Supplementary Table S1).

## Future research needs

The main scope of the present study was source water and finished water in Lake Erie region, thus tap samples or samples from within the distribution system were not included in this study, but we suggest that future study includes examining the distribution systems of bloom-impacted community water systems since it can contribute great knowledge regarding the public health implications of biofilm formation, ARGs, MGEs, and potential bacterial regrowth. This study adds to the body of evidence that some efforts should be made to monitor ARG concentrations before, during, and after drinking water treatments. As part of monitoring for ARGs and *Legionella*, methods reliant on culturable *Legionella* or other bacteria (e.g., *Pseudomonas* spp., *Mycobacterium* spp.) may provide benefits, but may underreport densities of some bacteria resistant to disinfection when hosted inside free-living Amoeba, which warrants a need to use a PCR-based approach (Calvo et al., 2013).

Complicating future studies on ARGs and MGEs in finished water and distribution systems are not only disinfectants but also disinfection byproducts. While this study illustrated that chlorine-related DBPs (THMs and HAA5) were elevated during bloom conditions, yet meeting regulatory thresholds, we suggest to characterize other DBPs that are likely elevated in cyanobacteria-impacted waters. Since there is considerable evidence that THMs and HAAs are linked to ARGs in the finished water and distribution system (Lv et al., 2014; Li and Gu, 2019; Zhang et al., 2023), we recommend to assess unregulated N-DBPs which likely are less abundant than THMs and HAAs, but are more common in bloom-impacted waters, and able to elicit greater cytotoxicity (Fang et al., 2010; Liu et al., 2020) in a future study. In addition, emerging literature indicates that future studies on DBPs ought to consider a broader range of DBPs for also assessing potential human health risks (Li and Mitch, 2018; Kali et al., 2021). Thus, in bloom-impacted waters there would likely be benefits from assessing N-DBPs which are associated with the chlorination of *Microcystis aeruginosa* (Fang et al., 2010).

These same N-DBPs may also be of interest for improving understanding of antibiotic resistance phenomena in treated waters.

## Conclusion

In this study, a compelling and statistically significant correlation emerged between *Legionella* and cyanobacteria within the water of the bloom site. Increasing levels of *Microcystis* were also associated with disinfection byproducts (THMs and HAA5) in the water of the bloom site, but did not exceed primary regulatory standards. At both the bloom site and control site, the DWTPs reduced the density of ARGs and MGEs; however, their relative abundance increased in finished water. This study not only fills existing gaps in the understanding of cyanobacteria and *Legionella* ecology but also underscores several pivotal areas of needed future research for aquatic environments with human exposure potential. Furthermore, our findings indicate a potential health risk of *Legionella*-related disease in proximity to areas impacted by blooms.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

## Author contributions

JL: conceptualization, funding acquisition, methodology, supervision, and writing—original draft. SL: formal analysis, methodology, visualization, writing—review and editing. CH: formal analysis, methodology, visualization, and writing—review and editing. JM: methodology, writing—review and editing. All authors contributed to the article and approved the submitted version.

## References

- Berendt, R. F. (1981). Influence of blue-green algae (cyanobacteria) on survival of *Legionella pneumophila* in aerosols. *Infect. Immun.* 32, 690–692. doi: 10.1128/iai.32.2.690-692.1981
- Bergeron, S., Boopathy, R., Nathaniel, R., Corbin, A., and LaFleur, G. (2015). Presence of antibiotic resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. *Int. Biodeterior. Biodegrad.* 102, 370–374. doi: 10.1016/j.ibiod.2015.04.017
- Berjeaud, J. M., Chevalier, S., Schlusshuber, M., Portier, E., Loiseau, C., Aucher, W., et al. (2016). *Legionella pneumophila*: the paradox of a highly sensitive opportunistic waterborne pathogen able to persist in the environment. *Front. Microbiol.* 7:486. doi: 10.3389/fmicb.2016.00486
- Berry, M. A., Davis, T. W., Cory, R. M., Duhaime, M. B., Johengen, T. H., Kling, G. W., et al. (2017). Cyanobacterial harmful algal blooms are a biological disturbance to Western Lake Erie bacterial communities. *Environ. Microbiol.* 19, 1149–1162. doi: 10.1111/1462-2920.13640
- Brooks, B. W., Lazorchak, J. M., Howard, M. D., Johnson, M. V. V., Morton, S. L., Perkins, D. A., et al. (2016). Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environ. Toxicol. Chem.* 35, 6–13. doi: 10.1002/etc.3220
- Cai, H., Jiang, H., Krumholz, L. R., and Yang, Z. (2014). Bacterial community composition of size-fractionated aggregates within the phycosphere of cyanobacterial blooms in a eutrophic freshwater lake. *PLoS One* 9:e102879. doi: 10.1371/journal.pone.0102879
- Cai, H. Y., Yan, Z. S., Wang, A. J., Krumholz, L. R., and Jiang, H. L. (2013). Analysis of the attached microbial community on mucilaginous cyanobacterial aggregates in the eutrophic Lake Taihu reveals the importance of planctomycetes. *Microb. Ecol.* 66, 73–83. doi: 10.1007/s00248-013-0224-1
- Calvo, L., Gregorio, I., García, A., Fernández, M. T., Goñi, P., Clavel, A., et al. (2013). A new pentaplex-nested PCR to detect five pathogenic bacteria in free living amoebae. *Wat. Res.* 47, 493–502. doi: 10.1016/j.watres.2012.09.039
- Carvalho, F. R., Vazoller, R. F., Foronda, A. S., and Pellizari, V. H. (2007). Phylogenetic study of legionella species in pristine and polluted aquatic samples from a tropical Atlantic forest ecosystem. *Curr. Microbiol.* 55, 288–293. doi: 10.1007/s00284-006-0589-1
- Cermakova, L., Kopecka, I., Pivokonsky, M., Pivokonska, L., and Janda, V. (2017). Removal of cyanobacterial amino acids in water treatment by activated carbon adsorption. *Sep. Purif. Technol.* 173, 330–338. doi: 10.1016/j.seppur.2016.09.043
- Cheung, M. Y., Liang, S., and Lee, J. (2013). Toxin-producing cyanobacteria in freshwater: a review of their problems, impact on drinking water safety, and efforts for protecting public health. *J. Microbiol.* 51, 1–10. doi: 10.1007/s12275-013-2549-3
- Cory, R. M., Davis, T. W., Dick, G. J., Johengen, T., Deneff, V. J., Berry, M. A., et al. (2016). Seasonal dynamics in dissolved organic matter, hydrogen peroxide, and cyanobacterial blooms in Lake Erie. *Front. Mar. Sci.* 3:54. doi: 10.3389/fmars.2016.00054
- Declerck, P. (2010). Biofilms: the environmental playground of *Legionella pneumophila*. *Environ. Microbiol.* 12, 557–566. doi: 10.1111/j.1462-2920.2009.02025.x
- Fang, J., Ma, J., Yang, X., and Shang, C. (2010). Formation of carbonaceous and nitrogenous disinfection by-products from the chlorination of *Microcystis aeruginosa*. *Wat. Res.* 44, 1934–1940. doi: 10.1016/j.watres.2009.11.046

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1233327/full#supplementary-material>

- Fliermans, C. B., Cherry, W. B., Orrison, L. H., Smith, S. J., Tison, D. L., and Pope, D. H. (1981). Ecological distribution of *Legionella pneumophila*. *Appl. Environ. Microbiol.* 41, 9–16. doi: 10.1128/aem.41.1.9-16.1981
- Foreman, K., Vacs Renwick, D., McCabe, M., Cadwallader, A., Holsinger, H., Kormondy, C., et al. (2021). Effects of harmful algal blooms on regulated disinfection byproducts: findings from five utility case studies. *AWWA Wat. Sci.* 3:e1223. doi: 10.1002/aww2.1223
- Glibert, P. M. (2020). Harmful algae at the complex nexus of eutrophication and climate change. *Harmful Algae* 91:101583. doi: 10.1016/j.hal.2019.03.001
- Gonsior, M., Powers, L. C., Williams, E., Place, A., Chen, F., Ruf, A., et al. (2019). The chemodiversity of algal dissolved organic matter from lysed *Microcystis aeruginosa* cells and its ability to form disinfection by-products during chlorination. *Wat. Res.* 155, 300–309. doi: 10.1016/j.watres.2019.02.030
- Gorham, T., Root, E., Jia, Y., Shum, C. K., and Lee, J. (2020). Relationship between cyanobacterial bloom impacted drinking water sources and hepatocellular carcinoma incidence rates. *Harmful Algae* 95:101801. doi: 10.1016/j.hal.2020.101801
- Guo, X. P., Yang, Y., Lu, D. P., Niu, Z. S., Feng, J. N., Chen, Y. R., et al. (2018). Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze estuary. *Wat. Res.* 129, 277–286. doi: 10.1016/j.watres.2017.11.029
- Gupta, M., Lee, S., Bisesi, M., and Lee, J. (2019). Indoor microbiome and antibiotic resistance on floor surfaces: an exploratory study in three different building types. *Int. J. Environ. Res. Public Health* 16:4160. doi: 10.3390/ijerph16214160
- Han, X. Y. (2021). Effects of climate changes and road exposure on the rapidly rising legionellosis incidence rates in the United States. *Plos one* 16:e0250364. doi: 10.1371/journal.pone.0250364
- Ho, L., Lambling, P., Bustamante, H., Duker, P., and Newcombe, G. (2011). Application of powdered activated carbon for the adsorption of cylindrospermopsin and microcystin toxins from drinking water supplies. *Wat. Res.* 45, 2954–2964. doi: 10.1016/j.watres.2011.03.014
- Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B. J., Ladds, M., and Gobler, C. J. (2019). Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. *Limnol. Oceanogr.* 64, 1347–1370. doi: 10.1002/lno.11120
- Jia, S., He, X., Bu, Y., Shi, P., Miao, Y., Zhou, H., et al. (2014). Environmental fate of tetracycline resistance genes originating from swine feedlots in river water. *J. Environ. Sci. Health* 49, 624–631. doi: 10.1080/03601234.2014.911594
- Kali, S., Khan, M., Ghaffar, M. S., Rasheed, S., Waseem, A., Iqbal, M. M., et al. (2021). Occurrence, influencing factors, toxicity, regulations, and abatement approaches for disinfection by-products in chlorinated drinking water: a comprehensive review. *Environ. Poll.* 281:116950. doi: 10.1016/j.envpol.2021.116950
- Kappell, A. D., Harrison, K. R., and McNamara, P. J. (2019). Effects of zinc orthophosphate on the antibiotic resistant bacterial community of a source water used for drinking water treatment. *Environ. Sci. Water Res. Technol.* 5, 1523–1534. doi: 10.1039/C9EW00374F
- Kasich, J. R., Taylor, M., Butler, C. W., Zehring, J., and Hodges, R. (2015). State of Ohio harmful algal bloom response strategy for recreational waters. Available at: <https://www.epa.gov/sites/default/files/2016-12/documents/draft-hh-rec-ambient-water-swimming-document.pdf>.
- Kimbell, L. K., Wang, Y., and McNamara, P. J. (2020). The impact of metal pipe materials, corrosion products, and corrosion inhibitors on antibiotic resistance in drinking water distribution systems. *Appl. Microbiol. Biotech.* 104, 7673–7688. doi: 10.1007/s00253-020-10777-8
- Klase, G., Lee, S., Liang, S., Kim, J., Zo, Y. G., and Lee, J. (2019). The microbiome and antibiotic resistance in integrated fishfarm water: implications of environmental public health. *Sci. Total Environ.* 649, 1491–1501. doi: 10.1016/j.scitotenv.2018.08.288
- Krojsgaard, L. H., Krogfelt, K. A., Albrechtsen, H. J., and Uldum, S. A. (2011). Detection of legionella by quantitative-polymerase chain reaction (qPCR) for monitoring and risk assessment. *BMC Microbiol.* 11, 254–257. doi: 10.1186/1471-2180-11-254
- Lee, S., Jiang, X., Manubolu, M., Riedl, K., Ludsin, S. A., Martin, J. F., et al. (2017). Fresh produce and their soils accumulate cyanotoxins from irrigation water: implications for public health and food security. *Food Res. Int.* 102, 234–245. doi: 10.1016/j.foodres.2017.09.079
- Lee, S., Kim, J., Choi, B., Kim, G., and Lee, J. (2019). Harmful algal blooms and non-alcoholic liver diseases: focusing on the areas near the four major rivers in South Korea. *J. Environ. Sci. Health Part C* 37, 356–370. doi: 10.1080/10590501.2019.1674600
- Lee, C. S., Kim, M., Lee, C., Yu, Z., and Lee, J. (2016). The microbiota of recreational freshwaters and the implications for environmental and public health. *Front. Microbiol.* 7:1826. doi: 10.3389/fmicb.2016.01826
- Lee, J., Lee, S., and Jiang, X. (2017). Cyanobacterial toxins in freshwater and food: important sources of exposure to humans. *Annu. Rev. Food Sci. Technol.* 8, 281–304. doi: 10.1146/annurev-food-030216-030116
- Lee, J., Lee, S., Mayta, A., Mrdjen, I., Weghorst, C., and Knobloch, K. (2020). Microcystin toxin-mediated tumor promotion and toxicity lead to shifts in mouse gut microbiome. *Ectotoxicol. Environ. Saf.* 206:111204. doi: 10.1016/j.ecoenv.2020.111204
- Lee, S., Choi, B., Kim, S., Kim, J., Kang, D., and Lee, J. (2022). Relationship Between Freshwater Harmful Algal Blooms and Neurodegenerative Disease Incidence Rates in South Korea. *Environmental Health* 21, 1–11. doi: 10.1186/s12940-022-00935-y
- Li, D., and Gu, A. Z. (2019). Antimicrobial resistance: a new threat from disinfection byproducts and disinfection of drinking water? *Curr. Opin. Environ. Sci. Health* 7, 83–91. doi: 10.1016/j.coesh.2018.12.003
- Li, X. F., and Mitch, W. A. (2018). Drinking water disinfection byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. *Environ. Sci. Technol.* 52, 1681–1689. doi: 10.1021/acs.est.7b05440
- Li, Q., Yu, S., Li, L., Liu, G., Gu, Z., Liu, M., et al. (2017). Microbial communities shaped by treatment processes in a drinking water treatment plant and their contribution and threat to drinking water safety. *Front. Microbiol.* 8:2465. doi: 10.3389/fmicb.2017.02465
- Li, D., Zeng, S., He, M., and Gu, A. Z. (2016). Water disinfection byproducts induce antibiotic resistance-role of environmental pollutants in resistance phenomena. *Environ. Sci. Technol.* 50, 3193–3201. doi: 10.1021/acs.est.5b05113
- Liu, C., Ersan, M. S., Plewa, M. J., Amy, G., and Karanfil, T. (2018). Formation of regulated and unregulated disinfection byproducts during chlorination of algal organic matter extracted from freshwater and marine algae. *Wat. Res.* 142, 313–324. doi: 10.1016/j.watres.2018.05.051
- Liu, C., Ersan, M. S., Wagner, E., Plewa, M. J., Amy, G., and Karanfil, T. (2020). Toxicity of chlorinated algal-impacted waters: formation of disinfection byproducts vs. reduction of cyanotoxins. *Wat. Res.* 184:116145. doi: 10.1016/j.watres.2020.116145
- Loftin, K. A., Graham, J. L., Hilborn, E. D., Lehmann, S. C., Meyer, M. T., Dietze, J. E., et al. (2016). Cyanotoxins in inland lakes of the United States: occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. *Harmful Algae* 56, 77–90. doi: 10.1016/j.hal.2016.04.001
- Lv, L., Jiang, T., Zhang, S., and Yu, X. (2014). Exposure to mutagenic disinfection byproducts leads to increase of antibiotic resistance in *Pseudomonas aeruginosa*. *Environ. Sci. Technol.* 48, 8188–8195. doi: 10.1021/es501646n
- Mrdjen, I., Fennessy, S., Schaal, A., Dennis, R., Slonczewski, J. L., Lee, S., et al. (2018). Tile drainage and anthropogenic land use contribute to harmful algal blooms and microbiota shifts in inland water bodies. *Environ. Sci. Technol.* 52, 8215–8223. doi: 10.1021/acs.est.8b03269
- Nguyen, M. L., Westerhoff, P., Baker, L., Hu, Q., Esparza-Soto, M., and Sommerfeld, M. (2005). Characteristics and reactivity of algae-produced dissolved organic carbon. *J. Environ. Eng.* 131, 1574–1582. doi: 10.1061/(ASCE)0733-9372(2005)131:11(1574)
- Numberger, D., Zoccarato, L., Woodhouse, J., Ganzert, L., Sauer, S., Márquez, J. R. G., et al. (2022). Urbanization promotes specific bacteria in freshwater microbiomes including potential pathogens. *Sci. Total Environ.* 845:157321. doi: 10.1016/j.scitotenv.2022.157321
- Ohio Department of Health, Ohio Environmental Protection Agency & Ohio Department of Natural Resources. (2020). State of Ohio harmful algal bloom response strategy for recreational waters. Available at: <https://epa.ohio.gov/static/Portals/35/hab/HABResponseStrategy.pdf>.
- Paerl, H. W., and Huisman, J. (2008). Blooms like it hot. *Science* 320, 57–58. doi: 10.1126/science.1155398
- Pope, D. H., Soracco, R. J., Gill, H. K., and Fliermans, C. B. (1982). Growth of *Legionella pneumophila* in two-membered cultures with green algae and cyanobacteria. *Curr. Microbiol.* 7, 319–321. doi: 10.1007/BF01566871
- Rice, E. W., and Bridgewater, L. American Public Health Association (Eds) (2012). *Standard methods for the examination of water and wastewater (vol. 10)*. Washington, DC: American Public Health Association.
- Rodriguez, M. J., Sérodes, J. B., and Levallois, P. (2004). Behavior of trihalomethanes and haloacetic acids in a drinking water distribution system. *Wat. Res.* 38, 4367–4382. doi: 10.1016/j.watres.2004.08.018
- Shi, P., Jia, S., Zhang, X. X., Zhang, T., Cheng, S., and Li, A. (2013). Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. *Wat. Res.* 47, 111–120. doi: 10.1016/j.watres.2012.09.046
- Srivastava, A., Ko, S. R., Ahn, C. Y., Oh, H. M., Ravi, A. K., and Asthana, R. K. (2016). Microcystin biosynthesis and mcyA expression in geographically distinct *Microcystis* strains under different nitrogen, phosphorus, and boron regimes. *Biomed. Res. Int.* 2016:5985987. doi: 10.1155/2016/5985987
- Stumpf, R. P., Johnson, L. T., Wynne, T. T., and Baker, D. B. (2016). Forecasting annual cyanobacterial bloom biomass to inform management decisions in Lake Erie. *J. Gt. Lakes Res.* 42, 1174–1183. doi: 10.1016/j.jglr.2016.08.006
- Stumpf, R. P., Wynne, T. T., Baker, D. B., and Fahnenstiel, G. L. (2012). Interannual variability of cyanobacterial blooms in Lake Erie. *PLoS One* 7:e42444. doi: 10.1371/journal.pone.0042444
- Świątecki, A., and Zdanowski, B. (2007). The seasonal dynamics of organic matter remineralization by bacterial consortia in the heated Konin lakes. *Fish. Aquatic Life* 15, 309–320.
- Tison, D. L., Pope, D. H., Cherry, W. B., and Fliermans, C. B. (1980). Growth of *Legionella pneumophila* in association with blue-green algae (cyanobacteria). *Appl. Environ. Microbiol.* 39, 456–459. doi: 10.1128/aem.39.2.456-459.1980

- U.S. EPA (2005). Method 415.3 - measurement of total organic carbon, dissolved organic carbon and specific UV absorbance at 254nm in source water and drinking water. Available at: [https://cfpub.epa.gov/si/si\\_public\\_record\\_report.cfm?Lab=NERL&dirEntryId=103917](https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=103917).
- U.S. EPA (2016). Water treatment optimization for cyanotoxins. Available at: <https://www.epa.gov/ground-water-and-drinkingwater/water-treatment-optimization-cyanotoxins-document>.
- U.S. EPA (2019). Recommended human health recreational ambient water quality criteria or swimming advisories for microcystins and cylindrospermopsin. Available at: <https://www.epa.gov/sites/default/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf>.
- U.S. EPA (2023). National primary drinking water regulations. Available at: <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations>.
- van der Kooij, D., Veenendaal, H. R., Italiaander, R., van der Mark, E. J., and Dignum, M. (2018). Primary colonizing Betaproteobacteriales play a key role in the growth of *Legionella pneumophila* in biofilms on surfaces exposed to drinking water treated by slow sand filtration. *Appl. Environ. Microbiol.* 84, e01732–e01718. doi: 10.1128/AEM.01732-18
- Volk, A., Lee, J. (2022). Cyanobacterial blooms: A player in the freshwater environmental resistome with public health relevance?. *Environmental Research* 114612. doi: 10.1016/j.envres.2022.114612
- Walker, J. T. (2018). The influence of climate change on waterborne disease and legionella: a review. *Perspect. Public Health* 138, 282–286. doi: 10.1177/1757913918791198
- Wang, Z., Chen, Q., Zhang, J., Guan, T., Chen, Y., and Shi, W. (2020). Critical roles of cyanobacteria as reservoir and source for antibiotic resistance genes. *Environ. Int.* 144:106034. doi: 10.1016/j.envint.2020.106034
- Wang, Z., Chen, Q., Zhang, J., Yan, H., Chen, Y., Chen, C., et al. (2021). High prevalence of unstable antibiotic heteroresistance in cyanobacteria causes resistance underestimation. *Wat. Res.* 202:117430. doi: 10.1016/j.watres.2021.117430
- Xi, C., Zhang, Y., Marrs, C. F., Ye, W., Simon, C., Foxman, B., et al. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl. Environ. Microbiol.* 75, 5714–5718. doi: 10.1128/AEM.00382-09
- Xu, L., Campos, L. C., Canales, M., and Ciric, L. (2020a). Drinking water biofiltration: behaviour of antibiotic resistance genes and the association with bacterial community. *Wat. Res.* 182:115954. doi: 10.1016/j.watres.2020.115954
- Xu, L., Ouyang, W., Qian, Y., Su, C., Su, J., and Chen, H. (2016). High-throughput profiling of antibiotic resistance genes in drinking water treatment plants and distribution systems. *Environ. Poll.* 213, 119–126. doi: 10.1016/j.envpol.2016.02.013
- Xu, L., Zhou, Z., Zhu, L., Han, Y., Lin, Z., Feng, W., et al. (2020b). Antibiotic resistance genes and microcystins in a drinking water treatment plant. *Environ. Pollut. B.* 258:113718. doi: 10.1016/j.envpol.2019.113718
- Yin, Y., Gu, J., Wang, X., Song, W., Zhang, K., Sun, W., et al. (2017). Effects of copper addition on copper resistance, antibiotic resistance genes, and intl1 during swine manure composting. *Front. Microbiol.* 8:344. doi: 10.3389/fmicb.2017.00344
- Yoshida, M. T., Yoshida, T., Takashima, Y., Hosoda, N., and Hiroishi, S. (2007). Dynamics of microcystin-producing and non-microcystin-producing Microcystis populations is correlated with nitrate concentration in a Japanese Lake. *FEMS Microbiol. Lett.* 266, 49–53. doi: 10.1111/j.1574-6968.2006.00496.x
- Zamyadi, A., Ho, L., Newcombe, G., Bustamante, H., and Prévost, M. (2012). Fate of toxic cyanobacterial cells and disinfection by-products formation after chlorination. *Wat. Res.* 46, 1524–1535. doi: 10.1016/j.watres.2011.06.029
- Zhang, F., Hu, C., Shum, C. K., Liang, S., and Lee, J. (2017). Satellite remote sensing of drinking water intakes in Lake Erie for cyanobacteria population using two MODIS-based indicators as a potential tool for toxin tracking. *Front. Mar. Sci.* 4:124. doi: 10.3389/fmars.2017.00124
- Zhang, F., Lee, J., Liang, S., and Shum, C. K. (2015). Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. *Environ. Health* 14:41. doi: 10.1186/s12940-015-0026-7
- Zhang, M., Zhang, L., Lin, K., Wang, Y., Xu, S., Bai, M., et al. (2023). Influence of chlorinated disinfection by-products on transmission of antibiotic resistance genes in biofilms and water of a simulated drinking water distribution system. *Environ. Eng. Res.* 28:210617. doi: 10.4491/eer.2021.617
- Zhang, Q., Zhang, Z., Lu, T., Yu, Y., Penuelas, J., Zhu, Y. G., et al. (2021). Gammaproteobacteria, a core taxon in the guts of soil fauna, are potential responders to environmental concentrations of soil pollutants. *Microbiome* 9, 1–17. doi: 10.1186/s40168-021-01150-6
- Zong, W., Sun, F., Pei, H., Hu, W., and Pei, R. (2015). Microcystin-associated disinfection by-products: the real and non-negligible risk to drinking water subject to chlorination. *Chem. Eng. J.* 279, 498–506. doi: 10.1016/j.cej.2015.05.048