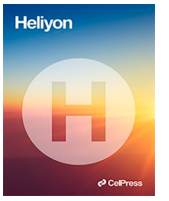
Heliyon 10 (2024) e31350

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

An investigation of cyanobacteria, cyanotoxins and environmental variables in selected drinking water treatment plants in New Jersey

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ARTICLE INFO

Keywords:
Cyanobacteria
Harmful algal blooms
Cyanotoxins
Microcystins
qPCR

ABSTRACT

Harmful Algal Blooms (HAB) have the potential to impact human health primarily through their possible cyanotoxins production. While conventional water treatments can result in the removal of unlysed cyanobacterial cells and low levels of cyanotoxins, during severe HAB events, cyanotoxins can break through and can be present in the treated water due to a lack of adequate toxin treatment. The objectives of this study were to assess the HAB conditions in drinking water sources in New Jersey and investigate relationships between environmental variables and cyanobacterial communities in these drinking water sources. Source water samples were collected monthly from May to October 2019 and analyzed for phytoplankton and cyanobacterial cell densities, microcystins, cylindrospermopsin, *Microcystis* 16S rRNA gene, microcystin-producing *mcyB* gene, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* gene. Water quality parameters included water temperature, pH, dissolved oxygen, specific conductance, fluorescence of phycocyanin and chlorophyll, chlorophyll-a, total suspended solids, total dissolved solids, dissolved organic carbon, total nitrogen, ammonia, and total phosphorus. In addition to source waters, microcystins and cylindrospermopsin were analyzed for treated waters. The results showed all five selected New Jersey source waters had high total phosphorus concentrations that exceeded the established New Jersey Surface Water Quality Standards for lakes and rivers. Commonly found cyanobacteria were identified, such as *Microcystis* and *Dolichospermum*. Site E was the site most susceptible to HABs with significantly greater HAB variables, such as extracted phycocyanin, fluorescence of phycocyanin, cyanobacterial cell density, microcystins, and *Microcystis* 16S rRNA gene. All treated waters were undetected with microcystins, indicating treatment processes were effective at removing toxins from source waters. Results also showed that phycocyanin values had a significantly positive relationship with microcystin concentration, copies of *Microcystis* 16S rRNA and microcystin-producing *mcyB* genes, suggesting these values can be used as a proxy for HAB monitoring. This study suggests that drinking water sources in New Jersey are vulnerable to forthcoming HAB. Monitoring and management of source waters is crucial to help safeguard public health.

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https://doi.org/10.1016/j.heliyon.2024.e31350
Received 16 August 2023; Received in revised form 7 May 2024; Accepted 15 May 2024
Available online 18 May 2024
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1. Introduction

Potable water is vital for human health and survival. The human population is expected to rise to 10 billion by the middle of this century [1]. As human population increases so does the demand for safe drinking water and drinking water sources such as lakes, rivers, and reservoirs [2]. However, in recent years, drinking water resources worldwide are increasingly affected by Harmful Algal Blooms (HAB) [3]. Human health can potentially be jeopardized by toxic algal contamination in drinking water due to cyanotoxin concentrations above established thresholds. For example, microcystins have the potential to cause acute health effects ranging from abdominal pain and headaches to pneumonia and liver disease [4,5]. Previous studies [6] reported that the number of people drinking microcystin-contaminated water was correlated with liver cancer cases in China. Thus, to help meet the demand for safe drinking water, monitoring drinking water sources such as reservoirs, lakes, and rivers is crucial, including HAB water quality parameters [7]. Reservoirs are of special interest to drinking water managers due to their longer water residence times compared to rivers, and water column stratification which help promote HAB events [8,9]. In large quantities, cyanobacteria and the cyanotoxins they produce can have significant negative impacts on water quality, biotic health and economy [10–12].

The overabundance of cyanobacterial biomass and cyanotoxins in source waters are likely to require additional treatment processes in order to supply safe drinking water. However, these treatment processes can be costly, with estimated annual costs of $417,200, and may potentially jeopardize drinking water availability due to its low cyanotoxin removal efficiency as low as 60 % [13–16]. Additional treatment processes such as increases in coagulants, use of chlorine, and management of disinfection by-products, among others will result in large-scale cell lysis, causing high concentrations of cyanotoxins to be released into the water [17–19]. Consequently, cyanotoxins break-through in treated waters have been documented at multiple drinking water facilities in concentrations above which adverse health effects could occur [20,21]. For instance, in 2014, a cyanobacterial bloom near a water intake of a drinking water facility in Toledo, Ohio, led to concentrations of total microcystins in the treated water up to 2.5 μg/L, which affected 500,000 customers [20].

In order to minimize the risk from cyanotoxins, drinking water source management strategies need to incorporate a range of assessments to reduce the probability of cyanobacterial cell lysing and cyanotoxin release [22]. These strategies include but are not limited to dominant cyanobacterial taxa, relevant water treatment system, and cyanotoxin location inside the cell (intracellular) or within the water column (extracellular) [19]. Moreover, cyanobacterial HAB can also affect drinking water quality through odor, taste, and appearance from compounds such as geosmin and 2-methylisoborneol (MIB) [1,17,18]. Conventional water treatment methods such as coagulation, flocculation, sedimentation, and disinfectants, among others, do not completely remove taste and odor compounds [23,24]. Once taste and odor compounds are detected, water is deemed unacceptable by consumers; thus, monitoring of cyanobacterial HAB is considered the most effective approach in managing the taste and odor compounds [24,25].

Potable water regulations are of importance to help safeguard human health against harmful effects of contaminants [26]. The United States Environmental Protection Agency (US EPA) has not imposed a regulation on cyanotoxins for potable waters [26], but instead issued Health Advisories (HA) for cyanotoxins providing concentrations at which detrimental effects on human health are not anticipated [26]. Although no guideline at the federal level, states can implement their own regulations for cyanotoxins in potable waters. New Jersey does not currently have drinking water standards for cyanotoxins; however, there is a pending act under public review, which has an implementation schedule [26]. A draft was developed on drinking water health advisory values for microcystins based on the US EPA's advisory at 0.07 μg/L for people at the age of six and older [27]. Thus, investigating potentially toxin-producing HAB in source and treated waters of New Jersey is of importance since cyanotoxins are unregulated.

Under the current trend of climate change, cyanobacterial HAB events are predicted to increase in frequency, intensity, and duration, posing serious public health risks worldwide [28–33]. Pattern of extreme weather events, such as drought followed by heavy rainfall and flooding can cause excess nutrients and sediment to be transported in runoffs, encouraging algal growth and formation of HAB [34–36]. Moreover, intensifying lake stratification is another documented climate change trend contributing to the formation of HAB [29,37]. Increased thermal stratification and salinization-induced stratification suppress the mixing within the water column, and cause low oxygen in the hypolimnion which, when anoxic, can lead to a complex process where sediment-bound phosphorus is released into the hypolimnion [38,39].

Eutrophication might be further intensified by the current trend of climate change resulting in even greater frequency and magnitude of HAB events, and the negative effects they cause pose a greater risk for drinking water safety [32,40]. To our knowledge, no studies have been published describing the harmful algal blooms, cyanobacteria and cyanotoxin conditions in drinking water sources in New Jersey, which further highlights the need for public knowledge on HAB of New Jersey drinking water sources. The objectives of this study were to (1) assess the water quality conditions in drinking water sources in New Jersey where blooms have recently occurred and identify specific sites of potential concern for HAB, and (2) investigate relationships between environmental parameters and cyanobacterial communities in these drinking water sources. Five drinking water treatment facilities were selected; water quality, HAB parameters and cyanobacterial taxa were examined. Treated water was also tested to determine efficacies of cyanotoxin removal.

2. Methods

2.1. Study sites

Five water treatment plants in New Jersey that pump water from reservoirs or directly from river sources were included in this

study. Due to the sensitivity of identity, the names and specific locations of these treatment plants were not disclosed; instead, sites are referred to as sites A through E. Site A draws its source water from the confluence of Millstone River and Raritan River, serves as a drinking water supply for more than 1 million residents, and produces an average of 500,000 cubic meter per day (CMD) [41]. Site B draws its source water from a reservoir that is fed by the following bodies of water: Ramanessin Brook, Fourth Creek, Bordens Brook, Willow Brook, Hopp Brook, Big Brook, Fulling Mill Brook, Barren Neck Brook, Trout Brook, Yellow Brook, Miry Bog Brook, Mine Brook, Slope Brook, Hockhockson Brook, and Pine Brook [41]. This 8.7 million cubic meter reservoir has a catchment basin of 125.6 km. Site C receives source water for its reservoir from Shark River and Jumping Brook with a catchment area of 41.4 km and a storage capacity of 3.79 million CMD [41]. Sites B and C serve as a drinking water supply to a combined total of 366,000 people [41]. Site D is sourced directly from Pompton River and Passaic River and has an off-stream reservoir with a storage capacity of 11 million cubic meter. It produces an average of 83 million CMD and serves about 1 million people [41]. Site E is sourced from a different section of the Passaic River and serves 2.7 million people [41]. The water from Site E is pumped and collected into three raw water reservoirs before the treatment process and has a combined storage capacity of 10.75 million cubic meter.

2.2. Field sampling

Samples were collected once a month during the HAB season in New Jersey between May and October 2019 from two locations at each treatment plant: source (at 0.5 m water depth in a reservoir/river) and treated waters. For source waters, in-situ water characteristics such as water temperature, pH, dissolved oxygen, and specific conductance were recorded on location using a multiparameter sonde (Yellow Springs Instrument Professional Plus; Yellow Springs, OH). Fluorescence of phycocyanin and fluorescence of chlorophyll were recorded on locations in source waters using a hand-held fluorometer (CyanoFluor, Turner Designs; San Jose, CA). Water samples were collected, stored on ice in coolers (4°C), and transferred to Montclair State University laboratories for further analysis. Parameters analyzed at the laboratory included total suspended solids, total dissolved solids, chlorophyll-a, total nitrogen, total phosphorus, ammonia, non-purgeable dissolved organic carbon, phytoplankton cell density, cyanobacterial cell density, microcystins, cylindrospermopsin, *Microcystis* 16S rRNA gene, microcystin-producing *mcyB* gene, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* gene. Treated water samples were only analyzed for microcystins and cylindrospermopsin. Samples for total suspended solids, total dissolved solids, total nitrogen, ammonia, dissolved organic carbon, phytoplankton and cyanobacterial cell densities were collected in plastic bottles cleaned with phosphorus-free soap, while chlorophyll-a were collected in the same containers and wrapped in foil to protect from light. Microcystin and cylindrospermopsin samples were collected in amber glass containers cleaned with phosphorus-free soap. Treated water samples for cylindrospermopsin analysis were preserved using ascorbic acid and pH was adjusted between 4 and 7. Total phosphorus samples were collected using a plastic bottle cleaned with 1:1 hydrochloric acid and rinsed with deionized water. Polypropylene bottles were washed with phosphorus-free soap and autoclaved for sterilization prior to collecting samples for molecular analysis, including *Microcystis* 16S rRNA gene, microcystin-producing *mcyB* gene, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* gene.

2.3. Phytoplankton identification and enumeration

Phytoplankton water samples were preserved by adding Lugol's iodine solution as soon as they were back in the lab. Samples were kept in a cold room until processing. Phytoplankton samples were identified via sedgwick-rafter chamber under a light microscope with phase contrast using a long-distance working objective using the following taxonomic keys: *Freshwater algae of North America: ecology and classification* [42], *Cyanoprokaryota-1. Teil/Part 1: Chroococcales* [43], and *Cyanoprokaryota-3. Teil/Part 3: Heterocytous Genera* [44]. Samples were homogenized by gently inverting the sample container 25 times. For each replicate, 1 mL of sample was pipetted into the chamber and settled for 15 min before counting. Cells were counted and identified at 400x to the genus level until at least 200 natural units or 40 fields of view were reached. Cells with GALD (greatest axial linear dimension) less than 2 μm were not recorded due to identification difficulty. Zooplankton and nonviable cells were excluded from counts. For quality control, samples were counted in triplicates to account for natural variability.

2.4. Water quality parameters

Total suspended solids and total dissolved solids were analyzed within 24 hours of sample collections according to *Standard Methods for the Examination of Water and Wastewater* (Method 2540D and 2540C). Chlorophyll-a was filtered within 24 hours of sampling. The filters were then stored in a freezer and analyzed within 24 days following the EPA Method 445.0. Total nitrogen, ammonia, and total phosphorus were preserved to pH less than 2 with concentrated sulfuric acid and kept at or below 6 °C. These samples were analyzed within 28 days, where pH was adjusted to 7 with 5.0 N sodium hydroxide solution, and the results were later corrected for dilution. Total nitrogen was determined using the Total Nitrogen by the Persulfate Digestion Test 'N Tube method (Hach Method 10071), Total phosphorus with the Total Phosphorus by the PhosVer® 3 Ascorbic Acid method with Acid Persulfate Digestion (Hach Method 8190), and ammonia with Ammonia Nitrogen by the Salicylate method (Hach Method 8155) using a UV–Vis spectrophotometer (Hach, DR 5000, CO). Dissolved organic carbon was analyzed within 24 hours of collections, and the concentrations were determined using a Total Organic Carbon Analyzer (Shimadzu TOC-L CPH, Columbia, MD). Microcystin and Cylindrospermopsin samples were kept frozen and analyzed using the Microcystins-ADDA ELISA (Microtiter Plate) kit for Microcystins/Nodularins and Cylindrospermopsin ELISA kit (Gold Standard Diagnostics, Warminster, PA) following the manufacturer's instructions within three months. Quantified microcystin concentrations less than 0.15 μg/L, and cylindrospermopsin concentrations less than 0.04 μg/L were

reported as "less than minimal reporting limit" according to the manufacturer's instructions.

2.5. Real-time PCR

Two hundred mL of water samples were filtered within 24 hours with mixed cellulose ester filters (0.45 mm, 47 mm) for *Microcystis* 16S rRNA gene, microcystin-producing *mcyB* gene, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* genetic analyses. The filters were kept in a freezer and analyzed within six months. DNeasy Power Water Kits (Qiagen, Germantown, MD) were used for DNA extractions. The final eluate was 100 mL. The DNA concentrations and purity were checked using a NanoDropTM 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Real-time polymerase chain reaction (qPCR) analysis was performed using StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Known concentrations of recombinant plasmids containing gene targets were used to establish calibration curves against cycle threshold values (C_T). DNA extracts from *Microcystis aeruginosa* UTEX B2662 culture or water samples identified with presence of *Raphidiopsis* were used as template for cloning to establish recombinant plasmids. Detailed process of cloning was described elsewhere [45]. Contents of Master Mix stock included 1X PowerUp SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and 10 mM of forward and reverse primers (Table 1). Programs started at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and polymerization at 60 °C for 1 min. Quality control measures included duplicate reactions for standards and samples, no-template-control, coefficient of determination of calibration curves greater than 0.99 as well as melting curve programs to confirm the specificity of amplifications. Levels of genetic markers were reported in copies/mL of water. The reporting limit for *Microcystis* 16S rRNA gene was 117 copies/mL, 228 copies/mL for microcystin-producing *mcyB* gene, 141 copies/mL for *Raphidiopsis raciborskii*-specific *rpoC1* gene, and 187 copies/mL for cylindrospermopsin-producing *pks* gene.

2.6. Statistical analysis

Descriptive statistics, including mean, median, standard deviation, minimum, maximum, and range, were derived for each water quality and HAB parameters. One-way repeated ANOVA was conducted to test whether significant differences in parameters existed across the 5 study sites. Parameters were log-transformed to meet the assumptions of normality and homogeneity if original data did not meet such assumptions. Tukey's HSD tests were performed for the post hoc analysis to examine whether there was any significant differences in parameters between any two sites. Non-metric multidimensional scaling (NMDS) was applied to analyze and visualize multivariate data for water quality and HAB parameters as well as cyanobacterial taxa data in 2 dimensions. Bray Curtis distance was used to construct matrix of dissimilarities between samples. Spearman's correlation analysis was performed to identify significant relationships between parameters. A significance level of 0.05 was used for hypothesis testing. Half values of the reporting limits were used to replace the data that were below the reporting limits, including microcystin concentration (0.075 μg/L), *Microcystis* 16S rRNA gene (59 copies/mL), and microcystin-producing *mcyB* gene (114 copies/mL). Cylindrospermopsin, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* gene were excluded from statistical analysis because most values of these parameters were at or below the reporting limits. All statistical analyses were completed using R and RStudio [52,53].

3. Results

3.1. Water quality and cyanobacterial taxa at five study sites

Site A: HAB water quality parameters, including phytoplankton and cyanobacterial cell densities, microcystins, cylindrospermopsin, *Microcystis* 16S rRNA gene, microcystin-producing *mcyB* gene, *Raphidiopsis raciborskii*-specific *rpoC1* gene, and cylindrospermopsin-producing *pks* gene, were evaluated for the HAB condition. Table 2 lists descriptive statistics, including mean, median, standard deviation, min, max, and range, for each of the 5 study sites. Results showed that there were low cyanobacterial and phytoplankton cell densities (4.5 ± 11.0 and 115.7 ± 62.8 cells/mL, respectively), and undetected cyanotoxins at Site A and the treated water samples within the duration of the study. Overall, this site had the lowest cyanobacterial cell density documented among all sites (0 in all months except June at 27 cells/mL). Only *Pseudanabaena* and *Raphidiopsis* were identified in June. The lowest median copy number of the *Microcystis* 16S rRNA gene among all sites at 1425 copies/mL was seen (minimum of 364 in September and

Table 1
Primers selected for qPCR quantifications in the analysis of water samples collected from five New Jersey drinking water treatment facilities.

Target Genes	Primer/Probe	Sequence	Size (bp)	References
Microcystis 16S rRNA	Micr184F	GCCGCRAGGTGAAAMCTAA	247	[46,47]
	Micr431R	AATCCAAARACCTTCCTCCC		
Microcystin *mcy*B	mcyB#04F	TGTGGAGTCTATTTATCCTCTTTCC	95	[21,47]
	mcyB#04R	GAGTTTGACTACAATAAATCCCTGAAT		
Raphidiopsis raciiborski rpoC1	Cyl2	GGCATTCCTAGTTATATTGCCATACTA	308	[47,48]
	Cyl4	GCCCGTTTTTGTCCCTTTGCTGC		
Cylindrospermopsin *pks*	m4	GAAGCTCTGGAATCCGGTAA	422	[47,49,50,51]
	k18	CCTCGCACATAGCCATTTGC		

Table 2
Harmful Algal Bloom data of the drinking water sources in New Jersey, including fluorescence of phycocyanin (PC_RFU, RFU), fluorescence of chlorophyll (Chl_RFU, RFU), extracted phycocyanin (PC, μg/L), extracted chlorophyll-a (Chl-a, μg/L), phytoplankton cell density (Phyto, cells/mL), cyanobacterial cell density (Cyano, cells/mL), microcystins (MIC, μg/L), *Microcystis* 16S rRNA gene (MCS, copies/mL), microcystin-producing *mcyB* gene (MCN, copies/mL), cylindrospermopsin (CY, μg/L), *Raphidiopsis raciborskii*-specific *rpoC1* gene (CYS, copies/mL), and cylindrospermopsin-producing *pks* gene (CYN, copies/mL).

	Site A			Site B			Site C			Site D			Site E		
	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
PC_RFU	32.6	108.06	65.61	387.11	1234.5	853.23	65.27	1812.2	384.95	14.51	405.93	87.37	855.38	3983.3	3704.2
Chl_RFU	147.71	1011.9	279.67	1257.7	6361.8	4241.25	298.66	11051	2030.1	207.89	2359.3	392.47	903.28	2675.9	1880.3
PC	65.84	67.28	66.47	72.6	88.74	81.48	66.46	99.75	72.56	65.5	72.95	66.89	81.52	141.11	135.8
Chl-a	2	8	4.7	5.7	14.6	13.1	3.1	26.1	7.65	1.2	11.5	2.65	10.6	51.4	46.25
Phyto	37	218	102	378	5111	804.5	262	9065	1684	17	851	236.5	2862	11903	3785.5
Cyano	0	27	0	0	4131	398	0	8844	268.5	0	259	56	1603	11807	3687
MIC	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	0.18	<0.15	0.26	8.39	5.31
MCS	364	5572	1425	571	6018	3039	<117	336,077	64,277	<117	7555	5116	794,435	7,076,480	3,648,401
MCN	<228	922	511	<228	2606	988	<228	13,583	6347	<228	1285	808	82,437	8,660,900	2,723,562
CY	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.09	<0.05
CYS	<141	2033	1131	<141	1627	941	<141	292	141	<141	211	211	<141	2794	958
CYN	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187	<187

Table 3
Water quality data of the selected drinking water sources in New Jersey, including maximum (Max), minimum (Min) and medium (Med) of conductivity (Cond, μS/cm), dissolved oxygen (DO, mg/L), pH, temperature (T, °C), total suspended solids (TSS, mg/L), total dissolved solids (TDS, mg/L), dissolved organic carbon (DOC, mg/L), ammonia (NH_3, mg/L), total nitrogen (TN, mg/L), and total phosphorus (TP, mg/L).

	Site A			Site B			Site C			Site D			Site E		
	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
Cond	261.40	362.80	313.55	252.10	299.20	281.00	–	–	–	375.00	609.00	492.55	363.40	424.10	405.65
DO	6.27	9.70	7.33	6.32	9.71	8.05	–	–	–	2.80	7.65	5.22	2.85	8.55	6.77
pH	7.35	7.63	7.54	6.57	8.64	7.19	6.23	7.12	6.33	7.07	7.72	7.30	7.32	9.32	8.41
T	15.00	25.50	21.10	15.50	27.80	22.20	17.00	23.80	20.85	14.70	24.50	20.55	15.10	28.20	22.95
TSS	2.60	43.17	7.30	1.80	3.10	2.60	4.20	8.50	4.85	1.60	11.40	3.80	2.50	12.77	7.14
TDS	88.00	194.00	115.50	91.00	166.00	134.00	4.00	188.00	127.50	73.00	267.00	236.50	78.00	253.00	138.50
DOC	3.34	17.84	7.30	4.02	13.95	8.20	11.51	13.94	12.41	4.00	20.74	8.71	5.10	20.14	11.45
NH_3	0.01	0.11	0.05	0.01	0.48	0.06	0.03	0.19	0.11	0.06	0.36	0.13	0.01	0.10	0.04
TN	0.85	1.56	1.26	0.35	1.30	0.75	0.35	1.31	0.73	1.50	2.85	1.86	0.34	1.35	0.90
TP	0.15	0.60	0.22	0.13	0.25	0.15	0.13	0.57	0.20	0.27	0.39	0.33	0.22	0.40	0.31

-, not tested.

maximum of 5572 copies/mL in October). All microcystin concentrations were found to be below the minimal reporting limit. Results from molecular analysis supported the observations of low microcystin concentrations with microcystin-producing *mcyB* gene showing the lowest median copy number among all sites at 494 copies/mL (the lowest was undetected in August and highest was 922 copies/mL in July). *Raphidiopsis* was detected at this site, however cylindrospermopsin concentrations for all samples were below the minimal reporting limit. As for *Raphidiopsis* and cylindrospermopsin-producing genes, low copies of *Raphidiopsis raciborskii*-specific *rpoC1* and cylindrospermopsin-producing *pks* genes were observed. The concentrations for *rpoC1* ranged from undetected in May, June, July, and October to 2033 and 228 copies/mL in September and August, respectively. Levels of *pks* remained undetected throughout the course of study.

Descriptive statistics for water quality and nutrients parameters are shown in Table 3. With regards to the nutrient water quality, site A had elevated nutrient concentrations. This site had the second lowest median ammonia concentration among all sites at 0.05 mg/L (minimum of 0.01 in June to maximum of 0.11 mg/L in August). Total nitrogen concentrations ranged from 0.85 in October to 1.56 mg/L in June and had the second highest median total nitrogen concentration among all sites at 1.26 mg/L. Total phosphorus concentrations were the lowest at 0.15 mg/L in September and highest at 0.60 mg/L in July (median 0.22 mg/L), and all concentrations documented were above the threshold for rivers (equal to or less than 0.1 mg/L) under the New Jersey Surface Water Quality Standards (NJSWQS). This waterbody had the highest median total suspended solid concentration of all sites at 7.3 mg/L, ranging from 2.6 in October to 43.17 mg/L in July, which was above the threshold of 40 mg/L under NJSWQS and indicated water was not clear. Dissolved organic carbon concentrations were lowest at 3.3 in June and highest at 17.8 mg/L in October. The lowest median dissolved organic carbon value of all sites was recorded in Site A at 7.3 mg/L.

Site B: Similar to Site A, low cyanobacterial and phytoplankton cell densities (970.3 ± 1569.4 and 1536.3 ± 1819.5 cells/mL, respectively), and undetected cyanotoxins were reported in Site B and treated water samples during the study period. Cyanobacterial cell density reported in Site B was the second highest median among all sites at 398 cells/mL (ranging from 0 in June to 4131 cells/mL in September). Phytoplankton enumeration identified the presence of *Aphanizomenon*, *Chroococcus*, *Planktolyngbya*, *Pseudanabaena*, *Raphidiopsis*, *Synechococcus*, and *Phormidium*. *Microcystis* 16S rRNA gene showed the second lowest median copy number at 3039 copies/mL (minimum of 571 in May to maximum of 6018 copies/mL in October). The low cyanobacterial cell density reported was also supported by low concentrations of microcystins and molecular markers. This site had undetected microcystin concentrations for all samples. Microcystin-producing *mcyB* gene copies ranged from undetected in May to 2606 copies/mL in June (median 771 copies/mL). As for *Raphidiopsis raciborskii*-specific *rpoC1* and cylindrospermopsin-producing *pks* genes, low copies were observed. The lowest *rpoC1* value was undetected in May, June, August, and October, and the highest was 1627 copies/mL in September. The cylindrospermopsin-producing gene was not detected in any Site B water sample.

In regard to the nutrient water quality, site B had excess nutrients. Ammonia concentrations ranged from 0.01 in June and July to 0.48 mg/L in October, total nitrogen had the second lowest median concentration among all sites at 0.75 mg/L (minimum of 0.35 in July to maximum of 1.30 mg/L in August). Total phosphorus had the lowest median concentration at 0.15 mg/L (0.13 in June to 0.25 mg/L in May); however, these values were above the NJSWQS total phosphorus threshold for lakes (equal to or less than 0.05 mg/L). This site was characterized by clear water, with the lowest median total suspended solids concentration among all sites at 2.6 mg/L (minimum of 1.8 in May to maximum of 3.1 mg/L in September). Dissolved organic carbon concentrations peaked at 14.0 mg/L in October, increasing from 4.0 in July (median 8.2 mg/L).

Site C: Cyanobacterial cell density was low but phytoplankton cell density was higher (1744.3 ± 3502.5 and 3393.0 ± 3709.6 cells/mL, respectively), and toxins were undetected in Site C and treated water samples, however cyanobacteria taxa were documented, including *Aphanizomenon*, *Chroococcus*, *Dolichospermum*, *Microcystis*, *Planktolyngbya*, *Raphidiopsis*, *Synechococcus*, and *Phormidium*. Cyanobacterial cell density was low at this site and ranged from 0 in June to 8844 cells/mL in October (median 269 cells/mL) while all microcystin concentrations recorded for this site were undetected. Molecular results showed *Microcystis* 16S rRNA and microcystin-producing *mcyB* genes with the second highest median copy number among all sites at 38,138 copies/mL (minimum of undetected in May to maximum of 336,077 copies/mL in August) and 3436 copies/mL (ranging from undetected to 13,583 copies/mL in July), respectively. In contrast, *Raphidiopsis raciborskii*-specific *rpoC1* gene was only detected in August at 292 copies/mL. Although *Raphidiopsis* was present, all of the samples tested in this site had undetected cylindrospermopsin concentrations.

Nutrient water quality at this site suggested presence of substantial amount of nutrients. Among the five sites, ammonia had the second highest median concentration at 0.11 mg/L, with the lowest value of 0.03 in August and highest value of 0.19 mg/L in June. Total nitrogen had the lowest reported median concentration among all sites at 0.73 mg/L (ranging from 0.35 in October to 1.31 mg/L in May). While total phosphorus had the second lowest median concentration at 0.20 mg/L (minimum of 0.13 in August to maximum of 0.57 mg/L in May), all of the samples were above the NJSWQS threshold of 0.1 mg/L. This site had the lowest concentrations of total suspended solids at 4.2 in June to the highest concentrations at 8.5 mg/L in August (median 4.8 mg/L). However, the highest median dissolved organic carbon concentration among the five study sites was reported at Site C at 12.4 mg/L (ranging from 11.5 in June to 13.9 mg/L in September).

Site D: The results showed that Site D had low cyanobacterial and phytoplankton cell densities (103.8 ± 122.6 and 304.2 ± 291.4 cells/mL, respectively), and undetected cyanotoxins at both Site D and treated water samples within the duration of the study. Cyanobacterial cell density was low, with Site D having the second lowest median concentration among all sites at 56 cells/mL (minimum of 0 in June and August to maximum of 259 cells/mL in October) (Fig. 1, Table 3). *Aphanizomenon*, *Chroococcus*, *Pseudanabaena*, and *Raphidiopsis* were found at this site. *Microcystis* 16S rRNA gene ranged from undetected in May, June, and September to 7555 copies/mL in July (median 5116 copies/mL). The low cyanobacterial cell density was supported by low toxin and molecular results (Fig. 2). This site had microcystin concentrations that ranged from undetected in May, July, August, September, and October to 0.18 μg/L in June. None of the treated water was detected with microcystin. Two out of the six months were detected with microcystin-

producing *mcyB* gene (330 and 1285 copies/mL in July and August, respectively), while the others remained undetected. *Raphidiopsis raciborskii*-specific *rpoC1* gene was only detected in September at 211 copies/mL. While *Raphidiopsis* was observed at this site, cylindrospermopsin was undetected in the source and treated waters.

Nutrient water quality in Site D was elevated with nutrients being the highest among all sites; The highest median ammonia, total nitrogen, and total phosphorus concentrations among all sites were reported at 0.13 mg/L (ranging from 0.12 in July to 0.36 mg/L in August), 1.85 mg/L (ranging from 1.50 in July to 2.85 mg/L in September), and 0.33 mg/L (ranging from 0.27 in August to 0.39 mg/L in June), respectively. All total phosphorus concentrations recorded at this site exceeded the NJSWQS thresholds of 0.1 mg/L. The second highest median total suspended solid concentration among all sites was reported at 3.8 mg/L (ranging from 1.6 in October to

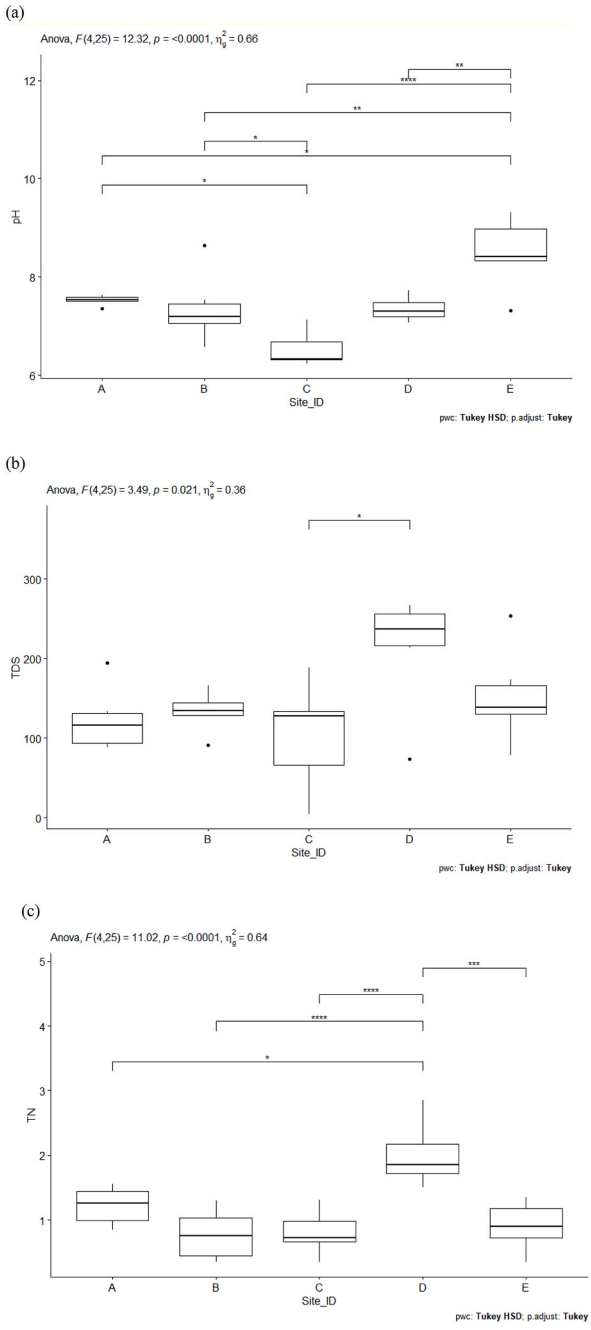

Fig. 1. ANOVA analysis showing existence of significant differences in water quality parameters among 5 drinking water resources. Parameters including (a) pH; (b) total dissolved oxygen (TDS, mg/L); and (c) total nitrogen (TN, mg/L). Significance levels including *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

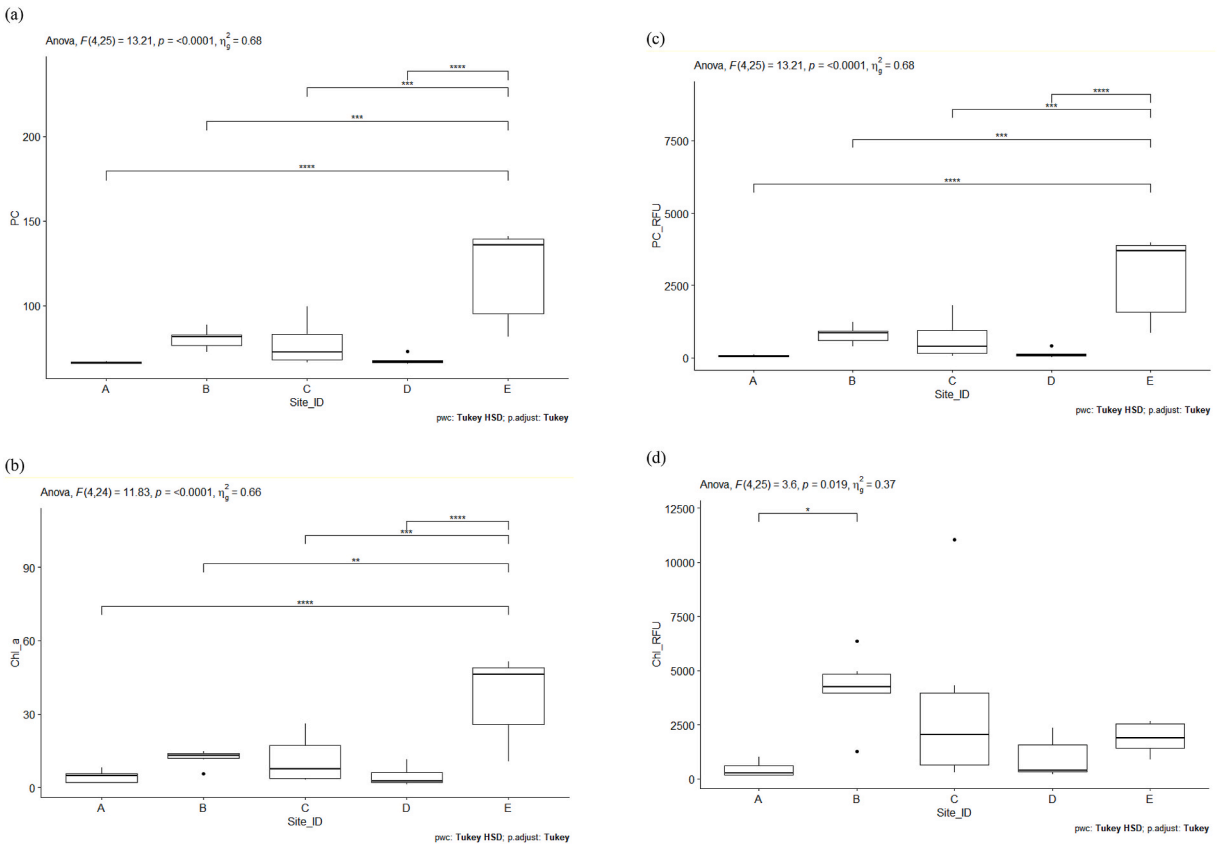

Fig. 2. ANOVA analysis showing existence of significant differences in HAB parameters among 5 drinking water resources. Parameters including (a) extracted phycocyanin (PC, μg/L); (b) extracted chlorophyll-a (Chl-a, μg/L); (c) fluorescence of phycocyanin (PC-RFU, RFU); (d) fluorescence of chlorophyll (Chl-RFU, RFU); (e) phytoplankton cell density (Phyto, cells/mL); (f) cyanobacterial cell density (log_Cyano, log (cells/mL)); (g) microcystins (log_MIC, log (μg/L)); and (h) *Microycstis* 16S gene (log_*Microcystis*_16S, log (copies/mL)). Significance levels including *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

11.4 mg/L in May. Dissolved organic carbon concentrations were second lowest among all sites with a median of 8.71 mg/L, with the lowest value at 4.0 in May and the highest value at 20.7 mg/L in October.

Site E: Among all sites surveyed, the only site that exhibited HAB issues, specifically elevated microcystins levels, was Site E. The cell densities for cyanobacteria and phytoplankton were 4861.3 ± 3692.6 and 5206.8 ± 3468.9 cells/mL, respectively. Identified cyanobacterial taxa included *Aphanizomenon*, *Chroococcus*, *Dolichospermum*, *Microcystis*, *Pseudanabaena*, and *Phormidium*. The highest median cyanobacterial cell density was documented at this site at 3687 cells/mL (ranging from 1603 in August to 11,807 cells/mL in September), which was below the 20,000 cells/mL HAB Watch Alert Level enforced during the study period by New Jersey Department of Environmental Protection (NJDEP) [41]. These results were supported by *Microcystis* 16S rRNA with the highest median copy number among all sites at 3,648,401 copies/mL (lowest at 794,435 in May to highest at 7,076,480 copies/mL in July.

Presence of microcystins was documented at all months at this site, with a median of 5.31 μg/L. Treated water samples were not detected with microcystins. Monthly microcystin concentrations for the source waters were from 0.26, 4.80, 3.32, 5.81, 8.39 μg/L, and 6.80 μg/L from May to October, respectively. Molecular analyses showed comparable cyanobacterial cell density and toxin results with the highest median microcystin-producing *mcyB* gene copy number at 2,723,562 copies/mL (minimum of 82,437 in May and maximum of 8,660,900 copies/mL in September. Cylindrospermopsin was only detected in Site E at 0.09 μg/L in July and was undetected in treated waters. *Raphidiopsis raciborskii*-specific *rpoC1* gene were detected in August to October, ranging from 680 copies/mL in August to 2794 copies/mL in September.

Site E nutrient water quality exhibited considerable amount of nutrients. Of all sites documented, site E had greater HAB issues with the highest values of HAB water quality parameters. Seasonal trends indicated that nutrients were high in all seasons. This site had the lowest median ammonia concentration at 0.04 mg/L (minimum of 0.01 in August and maximum of September to 0.10 mg/L in July). Total nitrogen concentrations ranged from 0.34 in May to 1.35 mg/L in September (median 0.90 mg/L), while the second highest median total phosphorus concentration among all sites was reported at 0.31 mg/L (lowest at 0.22 in October and highest at 0.40 mg/L in May). Total phosphorus concentrations exceeded the NJSWQS thresholds for all month at this site. The highest median total suspended solid concentration among all sites was reported at 7.1 mg/L (minimum of 2.5 in May and maximum of 12.77 mg/L in

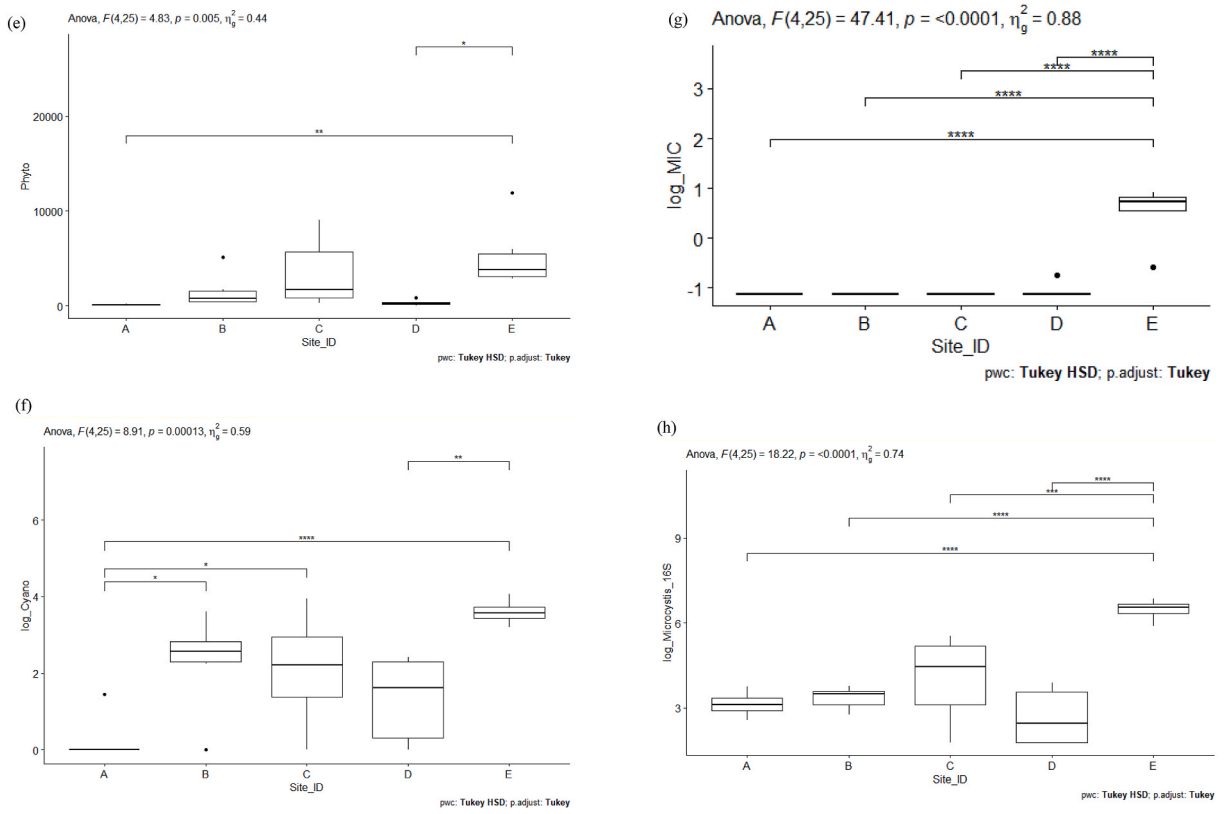

Fig. 2. (*continued*).

September), This site had the second highest dissolved organic carbon median concentration at 11.5 mg/L (ranging from 5.1 in July to 20.1 mg/L in October).

3.2. Comparison of source water quality

One-way repeated ANOVA was conducted to discern whether significant differences in parameters existed across the 5 study sites. Raw data were log-transformed to meet the assumptions of normality and homogeneity. The results showed that there were significant differences ($p < 0.05$) in water quality and nutrient parameters across the 5 study sites, including pH (Fig. 1a), total dissolved solids (Fig. 1b), and total nitrogen (Fig. 1c). Tukey's HSD post hoc analyses identified that pH at Site E was significantly greater than the rest of the sites ($p < 0.05$ Fig. 1a). Total dissolved solid was significantly greater at Site D than Site C ($p < 0.05$, Fig. 1b). Site D had significant greater total nitrogen than the other sites ($p < 0.05$, Fig. 1c).

HAB parameters, including extracted phycocyanin (Fig. 2a), extracted chlorophyll-a (Fig. 2b), fluorescence of phycocyanin (Fig. 2c), fluorescence of chlorophyll (Fig. 2d), phytoplankton cell density (Fig. 2e), cyanobacterial cell density (Fig. 2f), microcystins (Fig. 2g), and *Microcystis* 16S rRNA gene (Fig. 2h) were also reported showing significant differences ($p < 0.05$) across all study sites. Extracted phycocyanin (Fig. 2a), extracted chlorophyll-a (Fig. 2b), fluorescence of phycocyanin (Fig. 2c), microcystins (Fig. 2g), and *Microcystis* 16S gene (Fig. 2h) at Site E were significantly greater than the other sites ($p < 0.05$). Fluorescence of chlorophyll at Site B was significantly greater than Site A ($p < 0.05$, Fig. 2d). Phytoplankton cell density at Site E was significantly greater than Site A or Site D ($p < 0.05$, Fig. 2e). Cyanobacterial cell density was significantly greater at Site E than sites A or D ($p < 0.05$, Fig. 2f).

3.3. Impact of environmental conditions on HAB

Non-metric multidimensional scaling (NMDS) analysis reduced the multivariate dataset into 2 dimensions with a stress value of 0.099 (Fig. 3). The NMDS plot illustrated clustering of Site E separating from the rest of the study sites and month of sampling; however, no distinct seasonalityof distribution of cyanobacterial communities was identified. *Chroococcus*, *Dolichospermum*, *Microcystis*, and *Pseudanabaena* were identified to significantly contribute the distribution patterns of cyanobacterial communities of each sampling event ($p < 0.05$). Water quality and HAB parameters, including pH, total suspended solids, extracted phycocyanin, fluorescence of phycocyanin, extracted chlorophyll-a, phytoplankton and cyanobacterial cell densities, microcystins, *Microcystis* 16S rRNA gene, showed strong correlations of *Dolichospermum* and *Microcystis* at Site E.

Spearman's correlation analysis identified significant relationships between water quality and HAB parameters (Fig. 4). HAB

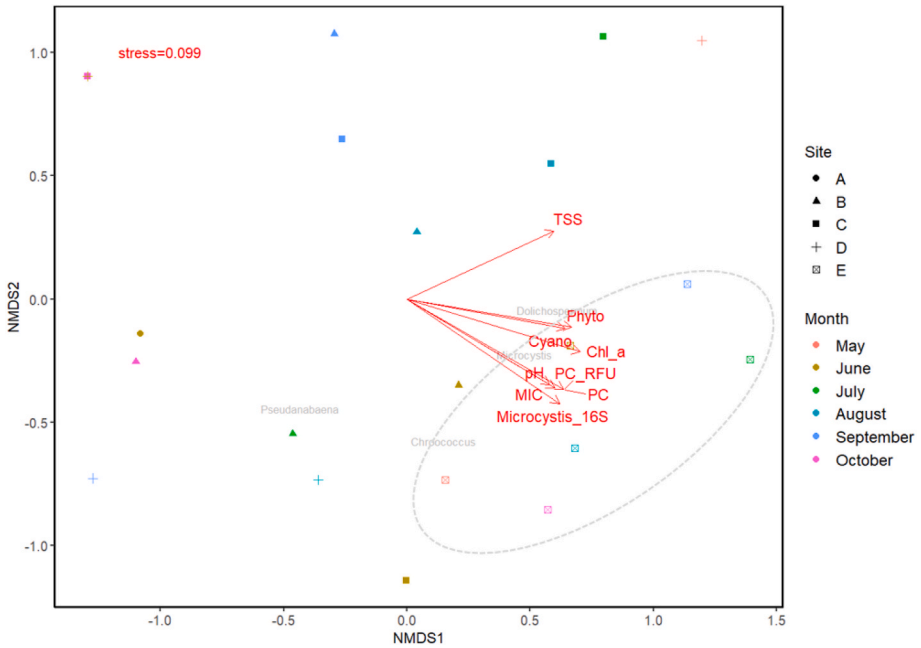

Fig. 3. Non-metric multidimensional scaling (NMDS) plot of cyanobacterial communities and environmental parameters among 5 drinking water sources. Shapes of the points indicate different study sites, while color of the points show different month of sampling. Only significantly correlated parameters are shown in red ($p < 0.05$), including pH, total suspended solids (TSS), phytoplankton cell density (Phyto), cyanobacterial cell density (Cyano), extracted chlorophyll-a (Chl_a), extracted phycocyanin (PC), fluorescence of phycocyanin (PC_RFU), microcystins (MIC), *Microcystis* 16S gene (Microcystis_16S). Cyanobacterial communities from different months for Site E are clustered compared to the rest of study sites and month of sampling. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

parameters, including extracted phycocyanin, fluorescence of phycocyanin, extracted chlorophyll-a, phytoplankton and cyanobacterial cell densities, microcystins, and *Microcystis* 16S rRNA gene, were all significantly and positively correlated ($p < 0.05$). Total nitrogen was significantly and positively correlated with conductivity and total dissolved solids, while negatively correlated with fluorescence of chlorophyll ($p < 0.05$). Total phosphorus had significantly positive relationship with total suspended solids (TSS) ($p < 0.05$).

4. Discussion

Cyanobacteria were found at the source waters of all five drinking water facilities selected for this study. *Microcystis* was the most dominant cyanobacteria taxon. Previous studies have shown contradicting results regarding the positive correlations between *Microcystis* and cyanotoxin concentrations since toxin-producing genes can be present or absent in *Microcystis* [46,54–56,57–60]. Furthermore, even when toxin-producing genes are present, toxins might not be produced, which is believed to possibly be controlled by environmental factors [46,61,62,57–60]. Previous studies such as [60] reported that high *Microcystis* density does not necessarily indicate high toxicity in water. Thus, quantifying *Microcystis* 16S rRNA and microcystin-producing genes can be alternative parameters that provide closer estimations of the potential toxicity in waters and a valuable tool with increased sensitivity for monitoring blooms [63,46,64–66]. Results from this study also confirmed that higher *Microcystis* 16S rRNA and microcystin-producing gene copies were associated with higher microcystin concentrations as well as two major toxin-producing taxa at Site E, including *Microcystis* and *Dolichospermum*. Higher microcystin concentrations with increasing copies of *Microcystis* 16S rRNA and microcystin-producing *mcyB* genes were also reported elsewhere [63,46,67–69]. Our results aligned with published literature [70–72]. Furthermore, our results suggest that microcystin-producing gene was more sensitive and can be detected at a lower level compared with microcystin. Thus, the genetic marker can be used for early detection of potentially toxic HAB before microcystin accumulates to a detectable concentration using current available quantification methods. However, while significant correlations were found between cyanobacterial cell density and genetic markers, it should be noted that definite conversions between the two cannot be realistically achieved. For instance, variations in estimating cell counts by microscopic techniques, copies of 16S rRNA gene in natural population, amplification of 16S rRNA gene by choice of primers, and other operational factors, such as sample handling and DNA extraction, could all lead to discrepancies in determining cyanobacterial cell density solely based on genetic markers [73,74].

Phycocyanin is the primary photosynthetic pigment found mainly in freshwater cyanobacteria and has been widely used as a proxy to estimate cyanobacterial biomass and HAB in freshwater in-situ [72,75–79]. In our study sites, fluorescence of phycocyanin values were significantly correlated with various cyanobacterial parameters including microcystin concentrations, copies of *Microcystis* 16S rRNA and microcystin-producing genes. These results support the concept that fluorescence of phycocyanin is a good proxy to estimate

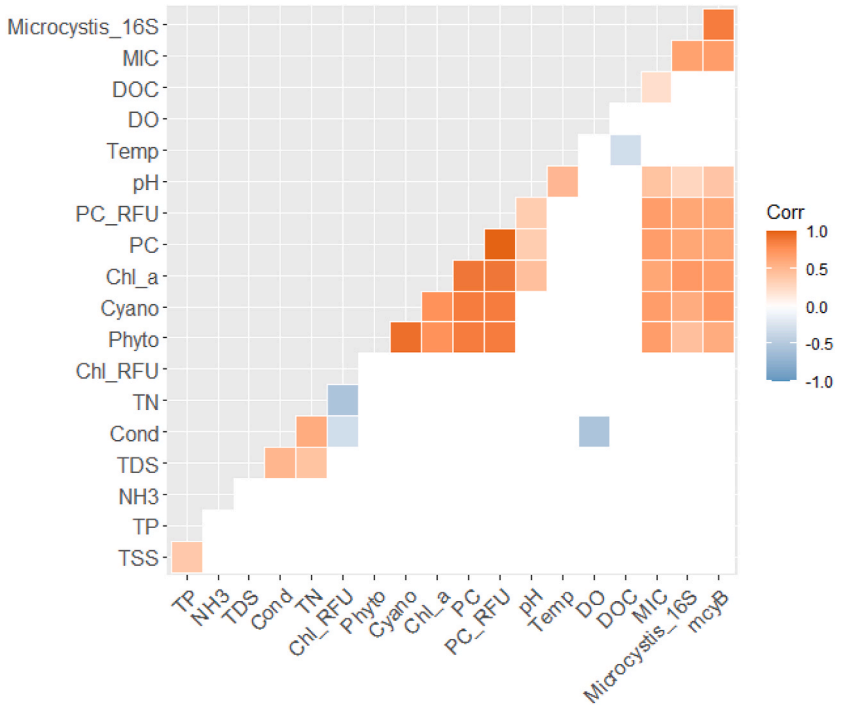

Fig. 4. Correlogram showing Spearman's correlation results among 5 drinking water sources. Only parameters showing significant correlations ($p < 0.05$) were highlighted. Conductivity (Cond), dissolved oxygen (DO), pH, temperature (Temp), total suspended solids (TSS), total dissolved solids (TDS), dissolved organic carbon (DOC), ammonia (NH3), total nitrogen (TN), total phosphorus (TP), fluorescence of phycocyanin (PC_RFU), fluorescence of chlorophyll (Chl_RFU), extracted chlorophyll-a (Chl_a), phytoplankton cell density (Phyto), cyanobacterial cell density (Cyano), microcystins (MIC), *Microcystis* 16S rRNA gene (Microcystis_16S), and microcystin-producing *mcyB* gene (mcyB).

HAB conditions at our study sites. However, it is important to consider that phycocyanin has limitations since certain environmental conditions and pigment content within cells can interfere with the in-situ measurement of phycocyanin fluorescence and thus, these interferences need to be accounted for [80–82]. The same phycocyanin pigments can result in different RFU measurements at different temperatures and light conditions [82,83]. For example, light intensity influences cyanobacteria fluorescence emission by light harvesting regulation processes, thus darker environments have higher production rates per unit volume than brighter ones [83,84]. This suggests that cyanobacteria that occupy the top of the water column during the day would have a reduction in pigment production and less fluorescence emitted by each cell due to intense light availability [82,85]. The effects of light stress on phytoplankton cellular fluorescence have been documented to be correlated to contractions and movement of chloroplasts associated with photosynthesis photoinhibition and fluctuations in cellular fluorescence [86]. In contrast, cyanobacteria located at the bottom of the water column with reduced light might increase their phycocyanin pigment production to have a greater ability to process light and lead to an increase in their phycocyanin fluorescence per cell [82]. Furthermore, fluorescence intensity has been documented to decrease as water temperature increases [87,88]. Similarly, previous studies [89] have concluded that organic matter fluorescence intensity decreased with increased temperature. Thus, the effects of water temperature on phycocyanin fluorescence and field measurements are a subject that should be further studied. While fluorescence of phycocyanin has been reported as a good real-time proxy for cyanobacterial biomass, it should not be used as a HAB indicator exclusively.

While nitrogen is an essential macronutrient needed for cyanobacterial growth, helps regulate metabolic activity, and promotes cyanobacterial growth, phytoplankton and cyanobacteria can differ in their preference and reactions to various forms and levels of nitrogen [57]. In this study, no significant correlations between total nitrogen or ammonia and primary HAB parameters were identified, such as cyanobacterial cell counts, microcystins, or *Microcystis* 16S rRNA gene. The non-nitrogen fixing cyanobacteria mostly take up and use nitrate, ammonia, and urea, with ammonia being the most favorable nitrogen source since ammonia assimilation is less energetically costly than nitrate reduction and nitrogen fixation [90–93]. On the other hand, ammonia has toxic effects on cyanobacteria at higher concentrations, pH values, and light intensity [94–96]. Ammonia toxicity can suppress the enzyme activity, photosynthesis, and growth of cyanobacteria [97–100]. Previous studies have reported that chlorophyll concentrations in freshwaters are inversely correlated to ammonia and have concluded that ammonia could be a factor that limits phytoplankton growth [101]. Temporal analyses suggested that low ammonia concentrations and high phytoplankton and cyanobacteria presence is observed during the growing seasons [57,102]. Certain cyanobacteria taxa, such as the non-N-fixing *Microcystis*, have also been reported to be efficient at taking up ammonia during the summer and have higher competitiveness for ammonia nitrogen than other phytoplankton and thus ammonia concentrations could influence cyanobacterial dominance [57,103]. Thus, no clear relationships between forms of nitrogen and HAB parameters may be found. The lack of significant correlations between nutrients and cyanobacterial cell density in

the study could also be attributed to variations in source water hydrology. For example, higher TN or TP were found in Site A or D, where source water samples were collected near confluence of two streams instead of reservoirs. The cyanobacterial cell density was lower in these stream systems, which explains why there was not straight positive correlations like other studies focusing merely on reservoirs or lakes. The only significant relationship in this study was between total nitrogen and fluorescence of chlorophyll with negative correlations, which requires further investigations to clarify this relationship.

All drinking water treatment sources had total phosphorus concentrations above the threshold for the New Jersey Surface Water Quality Standards (NJSWQS,< 0.05 mg/L for lakes and <0.1 mg/L for rivers). The availability of phosphorus can lead to an increase in cyanobacterial growth and ultimately have detrimental effects on the surrounding biota and water quality [104]. Phosphorus concentrations thus should be reduced to achieve target concentrations at the study sites. Total phosphorus was identified with significantly positive correlations with total suspended solids in this study, suggesting potential input of total phosphorus from adjacent overland runoffs, particularly during storm events [34–36]. Moreover, HAB intensity and frequency in these sites could be further exacerbated by climate change. Factors such as increased water stratification and warmer temperatures can lead to increased cyanobacterial population growth and their dominance in the phytoplankton assemblages, which can be worsened when combined with nutrient enrichment [105,106]. Specifically, thermal stratification favors cyanobacteria with some mobility that can change their positions within the water column. Those cyanobacteria are able to move downward in the water column to be closer to the nutrient-rich deeper water while other phytoplankton taxa are without access to the nutrients [105,107,108]. In contrast, mixing events during spring and fall turnovers can disrupt cyanobacteria scums, and transport cyanobacterial cells to the profundal zone where no sunlight is available. Additionally, the pressure from increasing depths can cause cyanobacteria's gas vesicles to collapse [109,110]; cells are unable to control buoyancy and sink to the bottom where light and temperature conditions negatively affect their survival [111–114]. It has been hypothesized that one of the main causes for increases in cyanobacterial blooms is increased stratification influenced by climate change [105,115]. With impending climate change affecting multiple drivers of HAB formation, all sites present in this study are vulnerable for future eutrophication and HAB events [116].

Among all five study sites, Site E is of particular concern since it exhibited a prevalence of HAB and impaired water quality conditions throughout the duration of the study. Overall, this was the only site with significant microcystin concentrations during all months, with some microcystin above the 8 μg/L USEPA recreational water Health Advisories. However, toxin concentrations in the treated waters were below the Health Advisories. This suggests that Site E is equipped to effectively remove cyanobacteria and microcystins from the source waters. Common processes that water treatment plants employ to remove cyanotoxins include activated carbon, nanofiltration, and oxidants such as chlorine, potassium permanganate, or ozone [117]. While the microcystin levels in the treated water were below drinking water health advisory guidelines at Site E, this site is still with a greatest risk of future HAB events and ultimately greater treatment costs associated with cyanobacteria and cyanotoxin removal, given the elevated cyanobacterial cell densities and microcystin concentrations among the five tested drinking water sources. Future monitoring plans should be focused on Site E. Additionally, while the other four drinking water sources did not have detectable cylindrospermopsin-producing gene present, cylindrospermopsin was detected in Site E despite very low concentrations. However, our results also showed that cylindrospermopsin was effectively reduced to acceptable drinking water levels after treatment. Similarly, during a four-year sampling period in the neighboring state of New York, cylindrospermopsin was rarely detected in natural waterbodies [118]. The authors []reported cylindrospermopsin was detected in five eutrophic lakes in New York with concentrations ranging from 0.1 μg/L to 0.25 μg/L.

HAB events when dominated by cyanobacteria bring additional issues for water treatment plants. *Microcystis* is the taxa known to form surface scums which can be further concentrated by wind [1,119]. The specialized adaptations (i.e., akinetes, buoyancy, nitrogen-fixation, luxury phosphorus uptake and storage) that cyanobacteria possess related to nutrient stress aid in their survival and dominance within their communities and create further difficulties in managing *Microcystis* for drinking water facilities [105]. *Microcystis* is a cyanobacterial taxon known to dominate eutrophic lakes with sufficient stratification which aids in their buoyancy regulation throughout the water column [120,121]. Water column stability allows for cyanobacteria to either grow somewhere in the middle with favorable conditions or move towards the surface to access light and carbon dioxide [120]. These varying depth positions can impact drinking water facilities by having to adjust the intake depth if a bloom is occurring to avoid excess cyanobacterial cells [19,122]. Furthermore, adjusting intake depth is not practical for all drinking water treatment facilities since most have a fixed intake depth. For instance, a study [118] concluded that the 1-m depth sampling method for analyzing cylindrospermopsin was biased since the cylindrospermopsin-producing *Raphidiopsis raciborskii* blooms are dispersed throughout the water column [118,123]. Thus, future cylindrospermopsin sampling should consider that *R. raciborskii* population peaks in stratified lakes occur at various depths within the epilimnion [123]. Similarly, dispersal and toxin concerns apply to other cyanobacteria taxa in addition to *Microcystis* and *Raphidiopsis* and toxins with these adaptations. However, research on diurnal vertical buoyancy patterns of cyanobacteria and impacts on water quality and treatment is limited and should be further examined [19,124]. Overall, the best strategy on HAB management is to focus on minimizing algae abundance at the source water before initiating the treatment process since conventional treatment processes (coagulation, flocculation, sedimentation, and filtration) have been reported as limited to remove all algal cells, cyanotoxins and the treatment by-products [19,125–127]. Large-scale solutions to manage HAB in source water include catchment-scale nutrient management plans, investment in sewage system improvement, and political changes related to nutrient loads [1]. Due to the presence of possibly toxic cyanobacteria and future susceptibility of all drinking water sources to HAB events, routine monitoring and management are suggested to reduce the risk of cyanotoxin contamination. Routine monitoring would provide early bloom detection, lower treatment costs, and lower public health risk to the community.

5. Conclusions

In summary, commonly found cyanobacteria were identified across the study sites, such as *Microcystis* and *Dolichospermum*. Site E was the most vulnerable site since it had significant microcystins concentrations at all months. This site is at greatest risk for future HAB blooms and greater treatment costs. Our results suggest that phycocyanin values could be used as a proxy for HAB monitoring since they were positively correlated with microcystin concentrations, *Microcystis* 16S rRNA and microcystin-producing genes. Cyanotoxins in treated waters were undetected. These findings suggest that HAB monitoring and management are necessary for safe and cost-effective drinking water treatment and delivery to the public. Additional studies in drinking water reservoirs should investigate other toxins such as saxitoxins in order to assess the risk of its exposure to the general public.

Data availability statement

The data of this manuscript is available at Montclair State University Digital Commons (https://digitalcommons.montclair.edu/etd/1282/).

CRediT authorship contribution statement

Tsung-Ta David Hsu: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Yaritza Acosta Caraballo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Meiyin Wu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thank you to the reviewers and manuscript editor of the journal for taking the time and effort to review this manuscript. This project was done in collaboration with the selected New Jersey drinking water treatment plants. The project was partially funded by the National Science and Technology Council (Taiwan), National Cheng Kung University (Taiwan) and Montclair State University's New Jersey Center for Water Science and Technology (NJ, USA).

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