

# **Evidence-based framework to use *in situ* phycocyanin readings for cyanobacterial risk assessment within drinking water treatment plants**

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## Supplementary materials

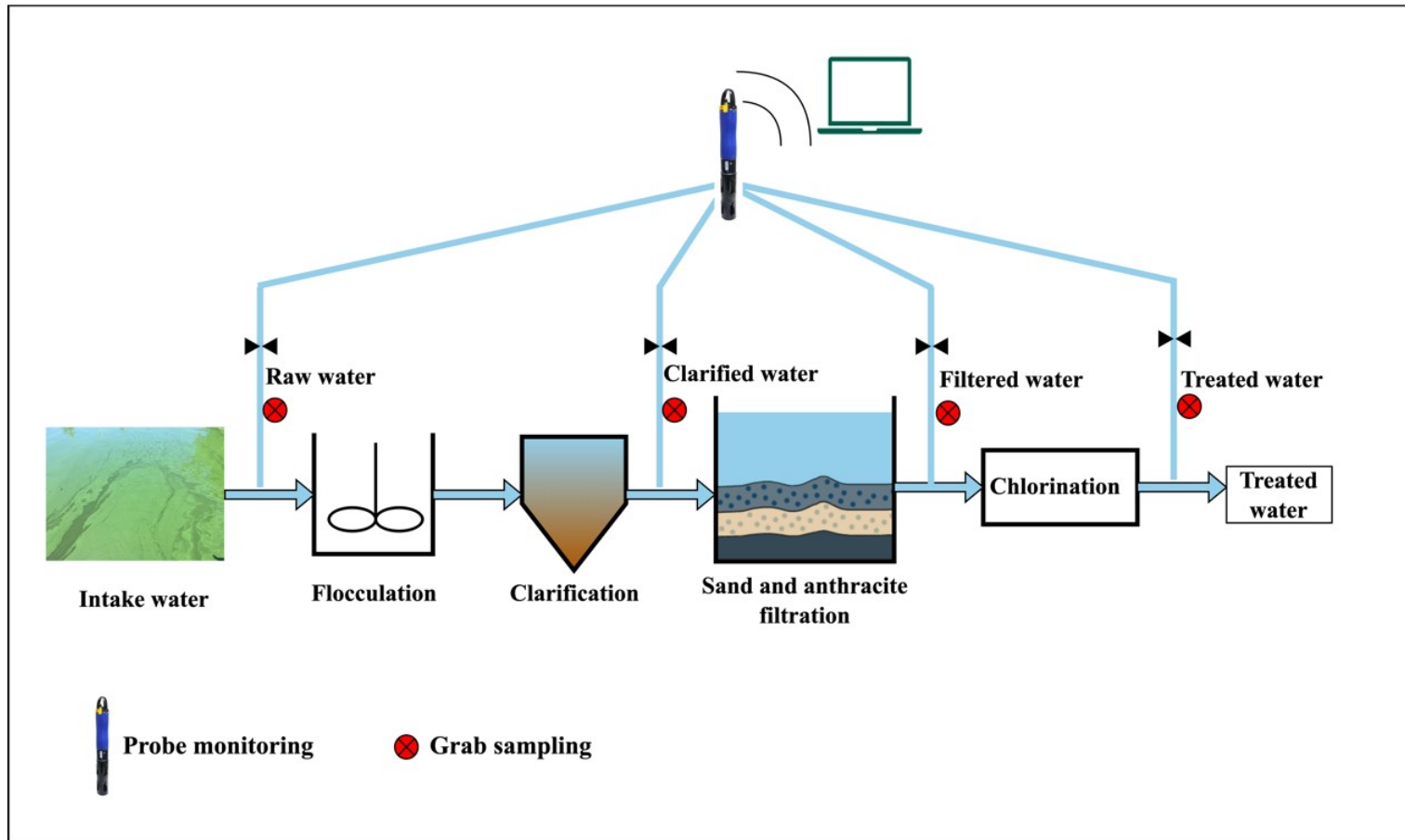


Figure S1 Schematic of the treatment processes of the studied three DWTPs, grab sampling and probe monitoring locations. Note DWTP C also employs pre-chlorination before flocculation

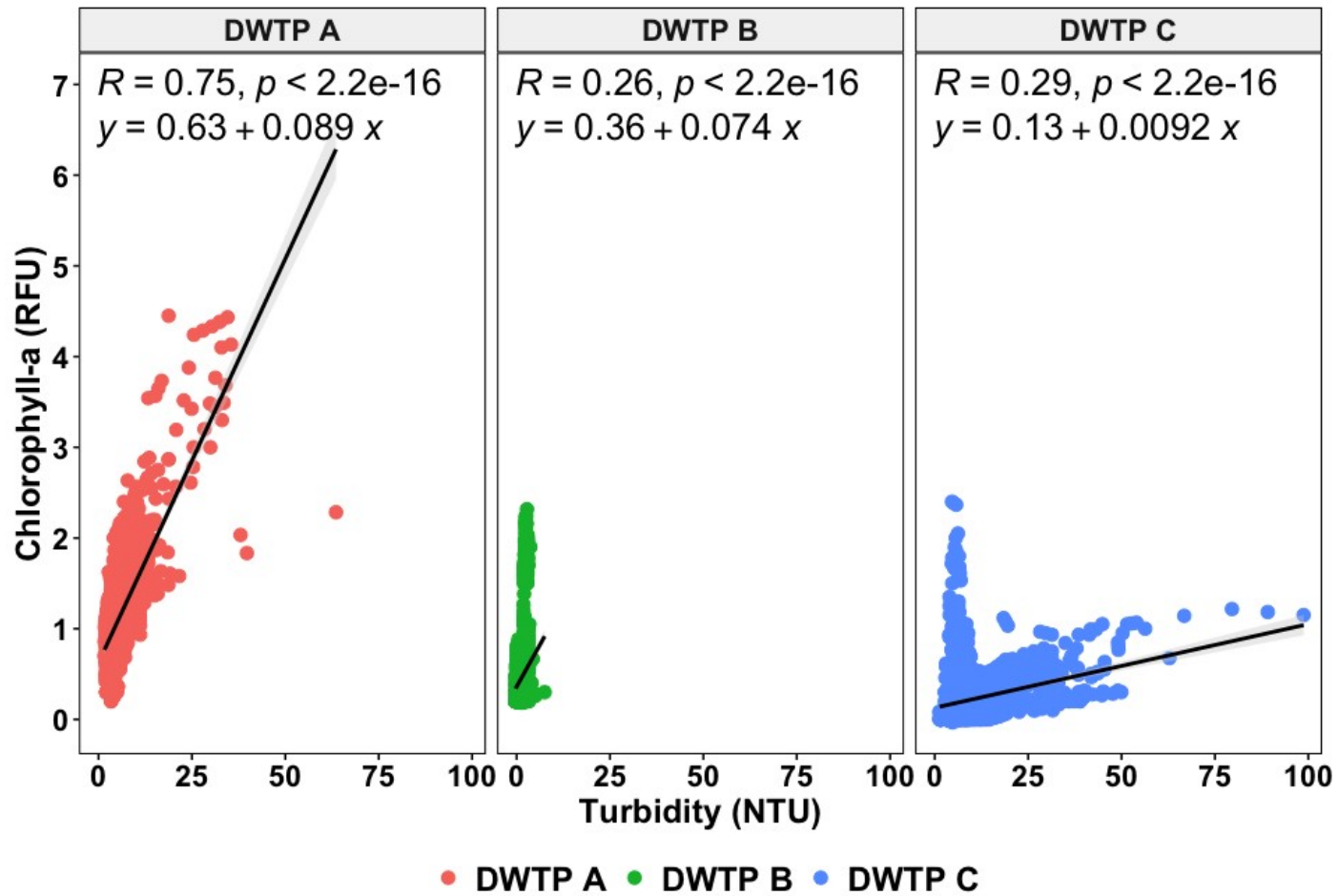
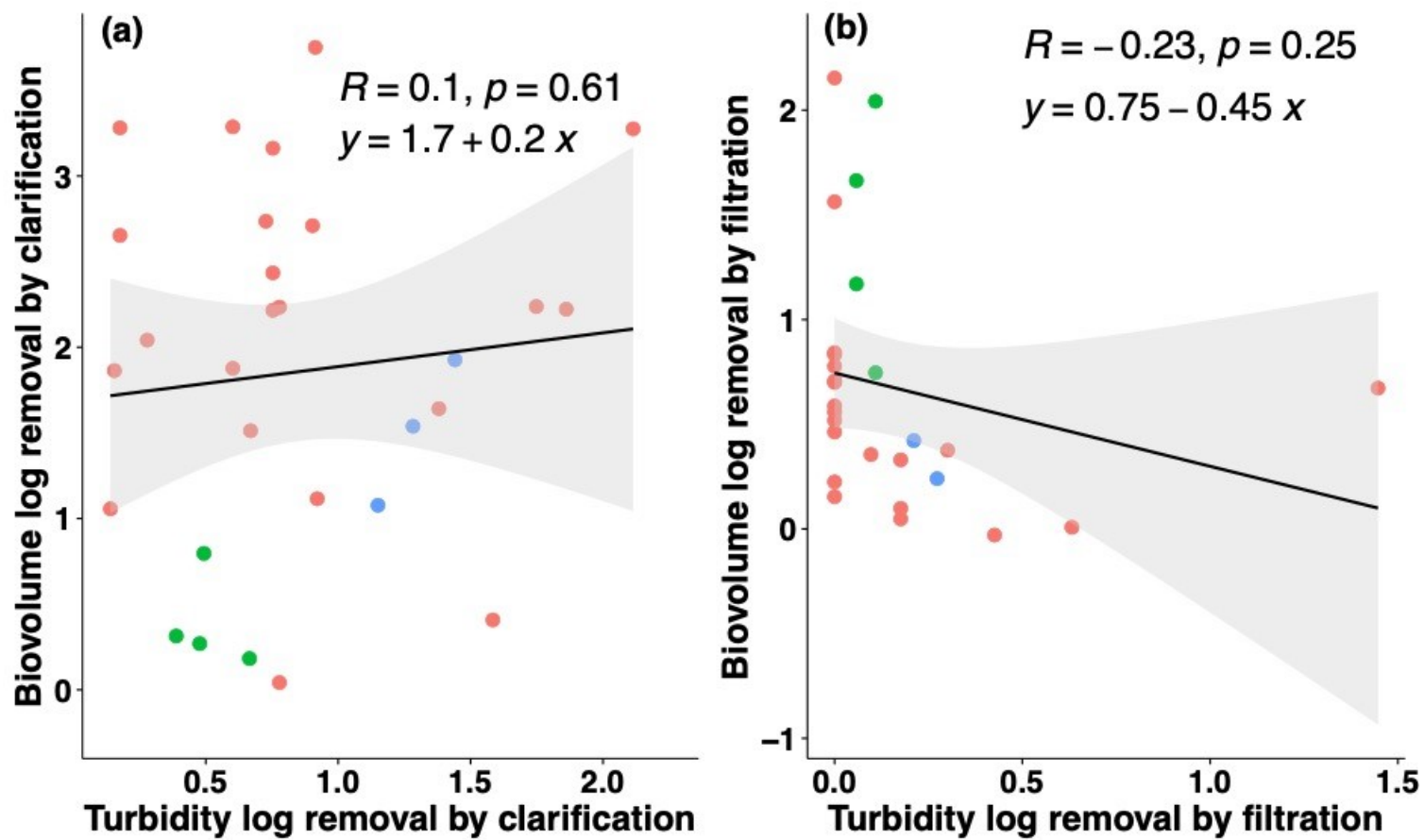


Figure S2 Turbidity (NTU) versus chlorophyll-a (RFU) of the source water of the three studied DWTPs



● DWTP A ● DWTP B ● DWTP C

Figure S3 Log removal of total cyanobacterial cell biovolume ( $\text{mm}^3/\text{L}$ ) versus log removal of turbidity (NTU) by (a) clarification, and (b) filtration

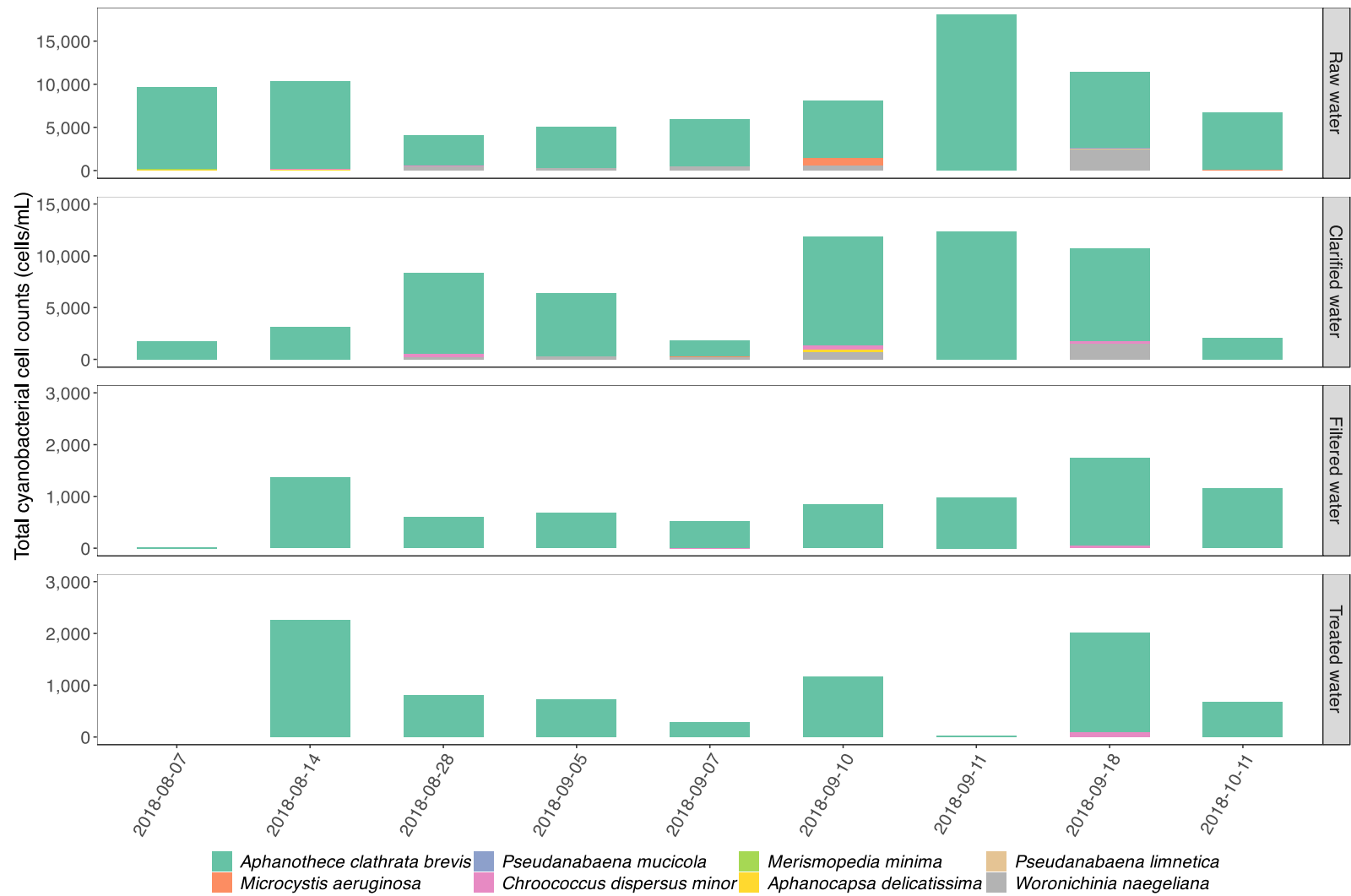


Figure S4 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP B

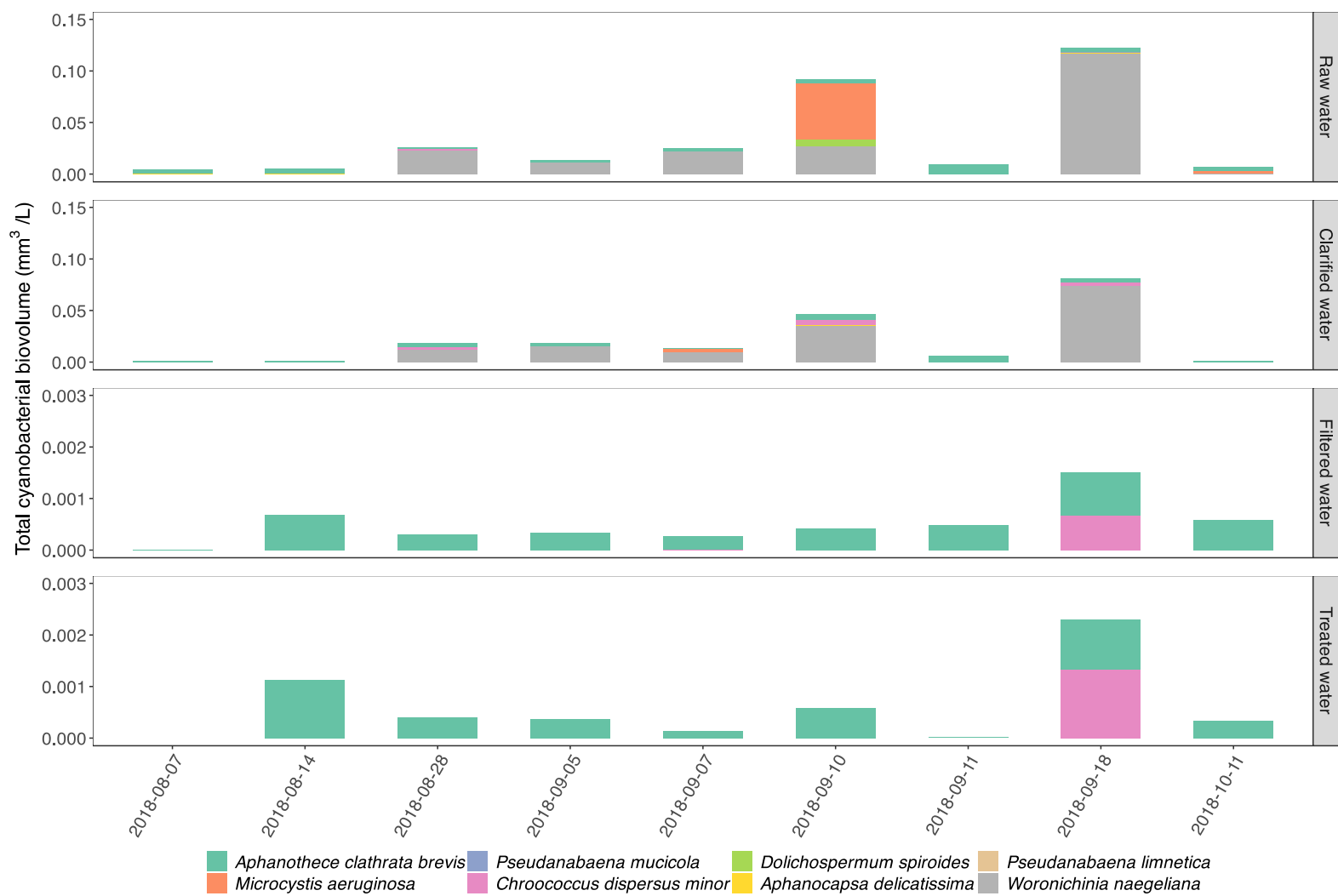


Figure S5 Cyanobacteria speciation evaluated as cell biovolumes (mm<sup>3</sup>/L) in the raw, clarified, filtered, and treated water in DWTP B

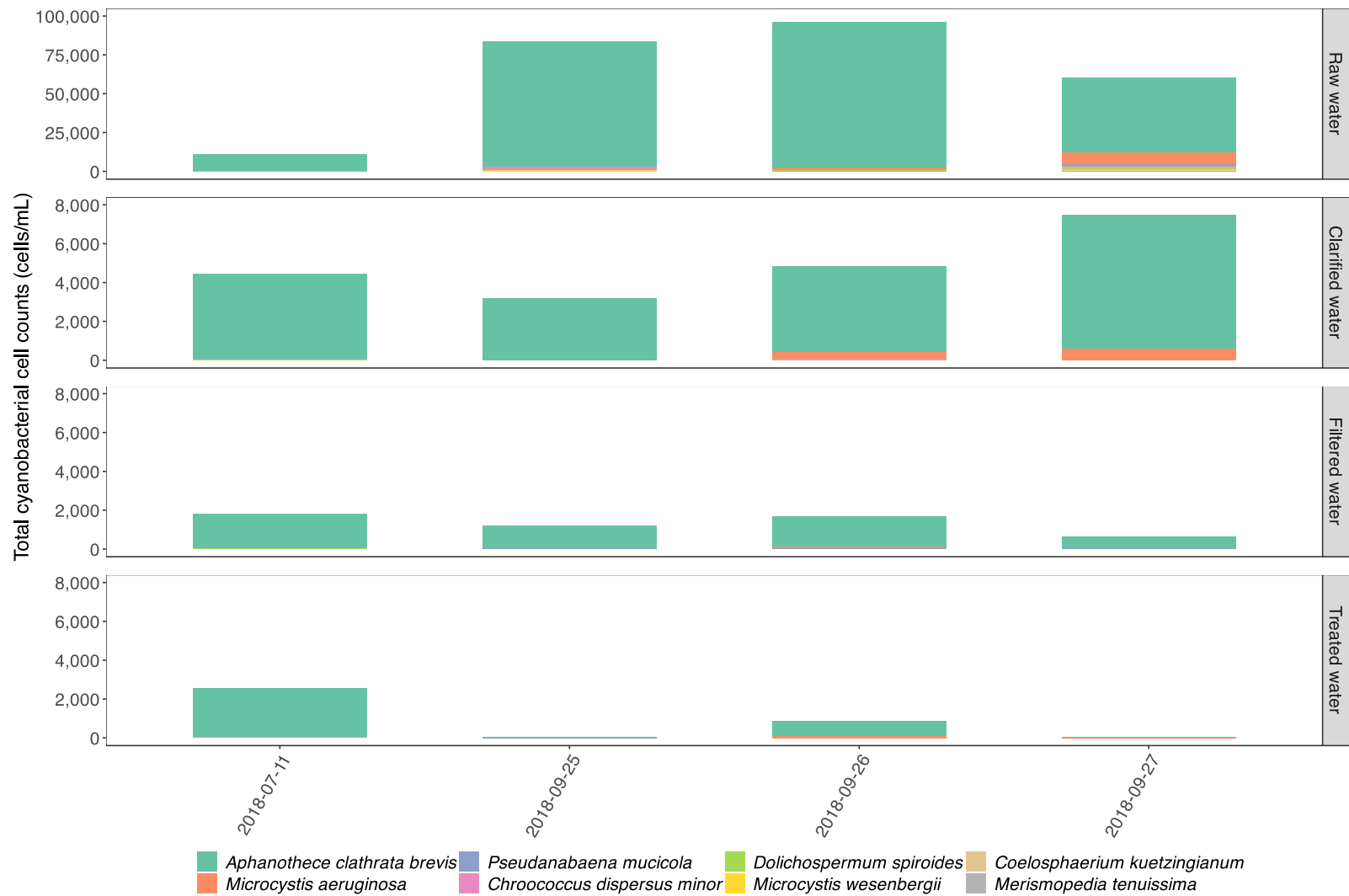


Figure S6 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP C

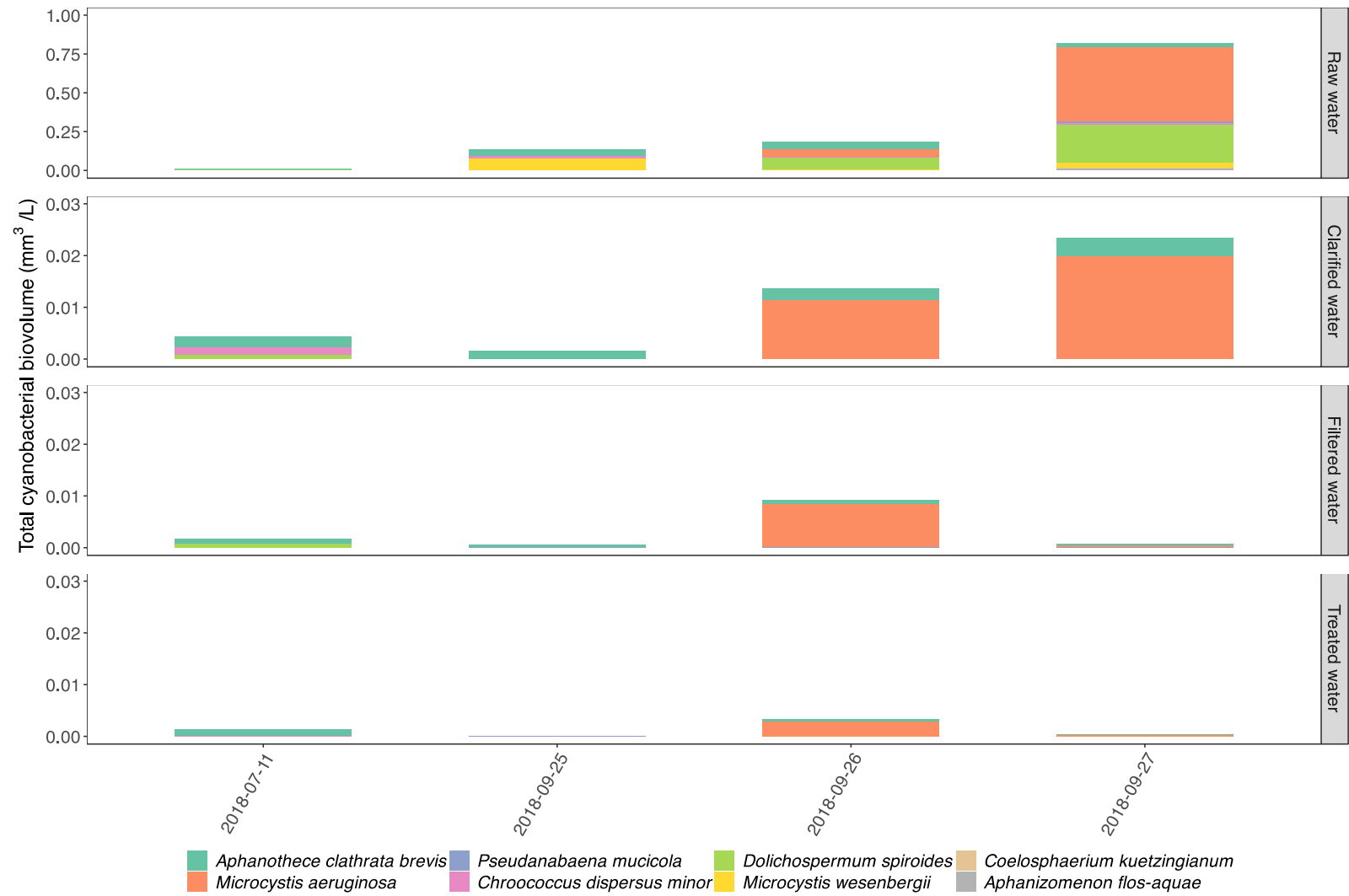


Figure S7 Cyanobacteria speciation evaluated as cell biovolumes (mm<sup>3</sup>/L) in the raw, clarified, filtered, and treated water in DWTP C



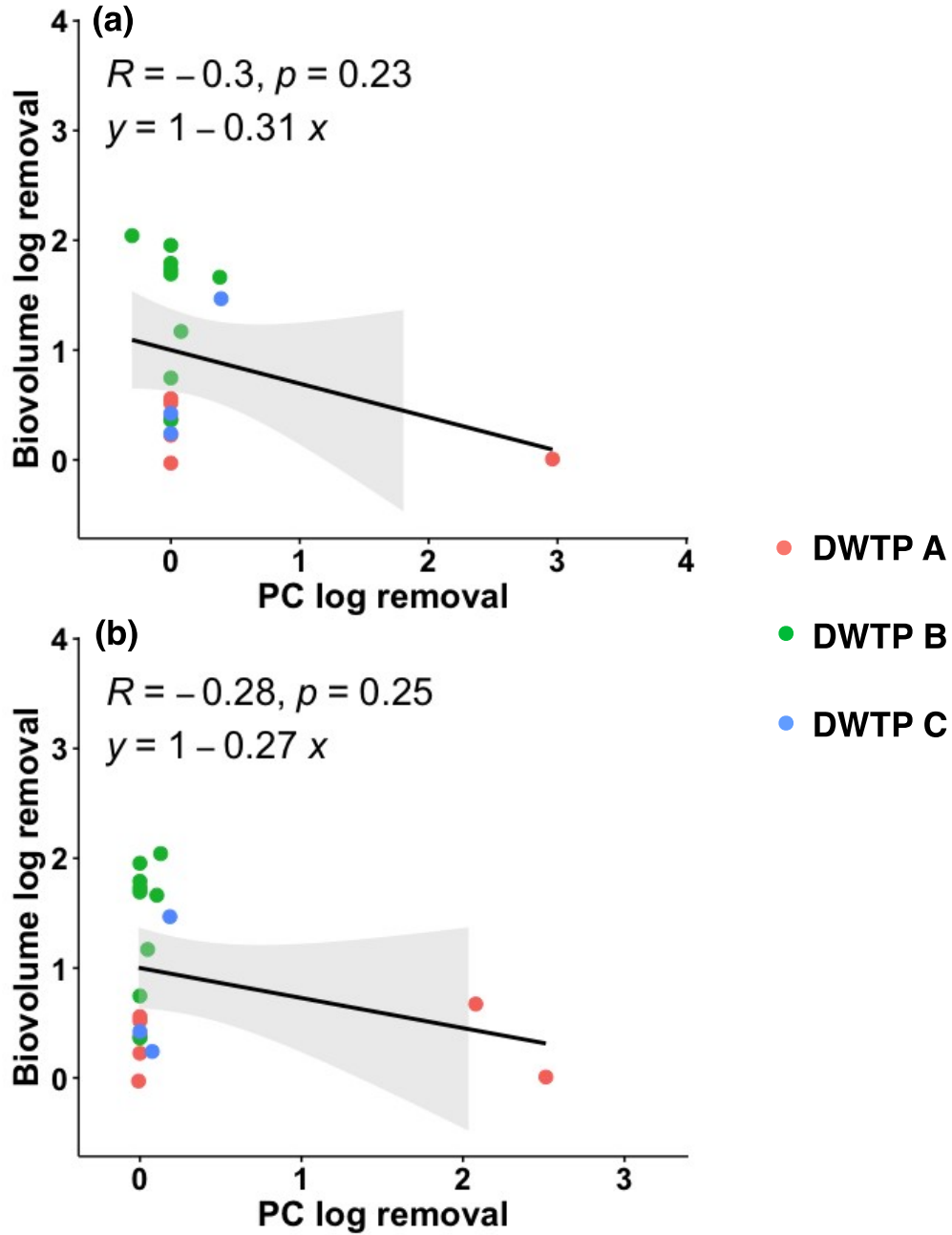


Figure S8 The filtration log removal efficacies evaluated by cell biovolumes versus evaluated by (a) 2-hour moving average of the phycocyanin readings, (b) daily mean phycocyanin readings. Paired data are from the samples of all DWTPs

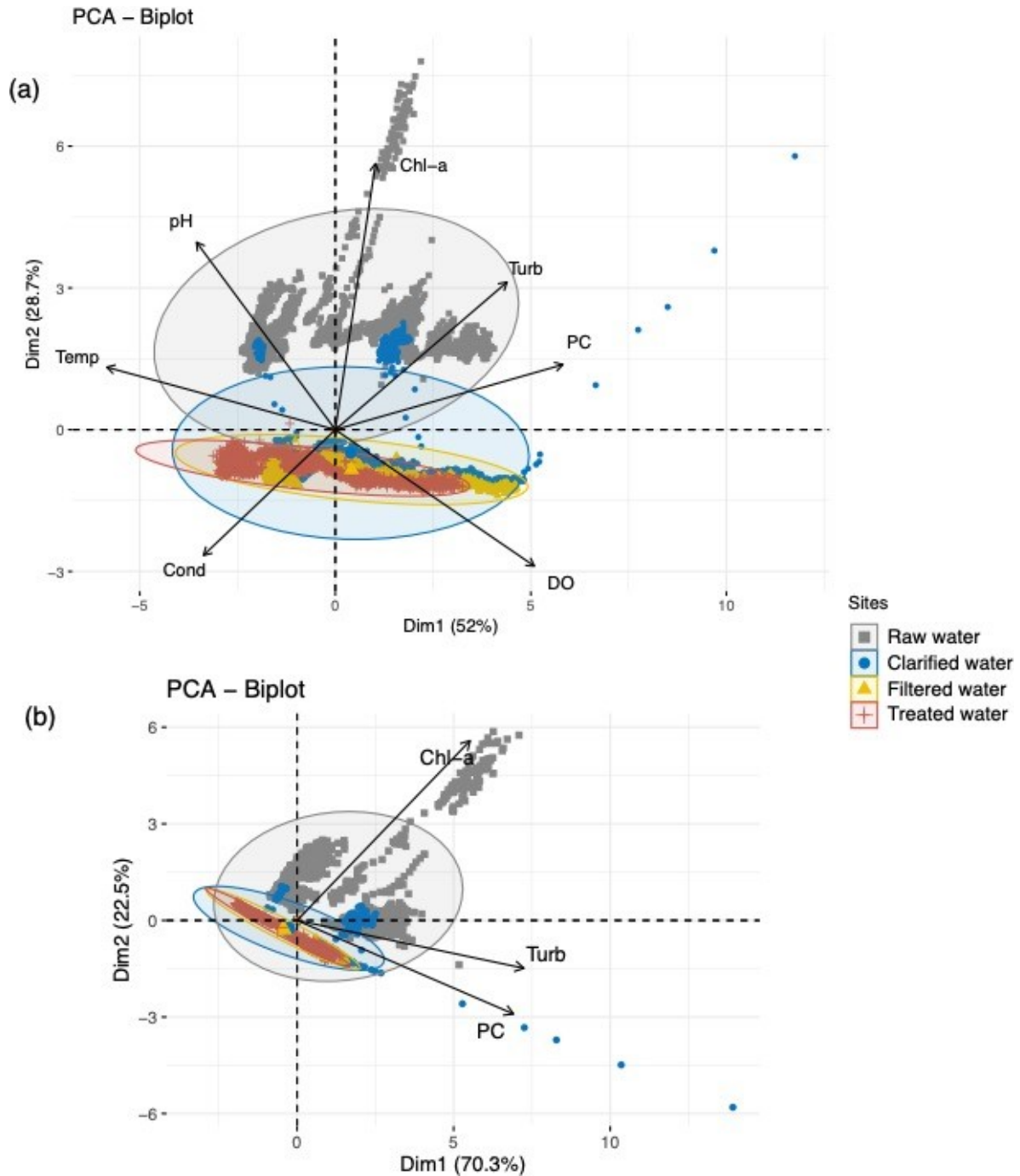


Figure S9 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP B. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a

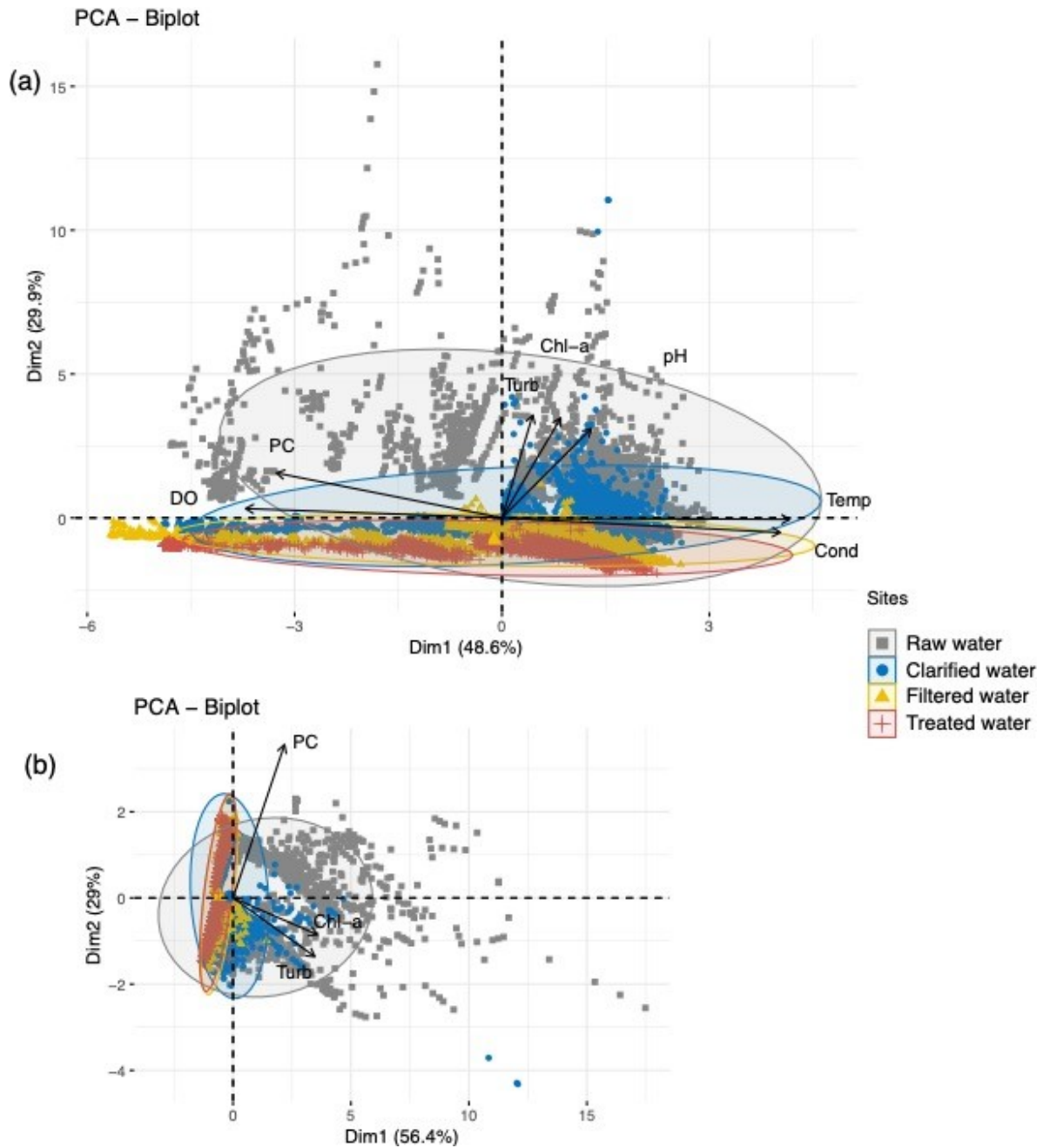


Figure S10 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP C. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a

Table S1 Species found in the source water of three studies DWTPs, arranged in descending order based on biovolumes (mm<sup>3</sup>/L)

DWTP A	DWTP B	DWTP C
<i>Dolichospermum spiroides</i>		
<i>Microcystis aeruginosa</i>		
<i>Microcystis wesenbergii</i>		
<i>Dolichospermum circinalis</i>	<i>Woronichinia naegeliana</i>	<i>Microcystis aeruginosa</i>
<i>Aphanothece clathrata brevis</i>	<i>Microcystis aeruginosa</i>	<i>Dolichospermum spiroides</i>
<i>Chroococcus dispersus minor</i>	<i>Aphanothece clathrata brevis</i>	<i>Aphanothece clathrata brevis</i>
<i>Pseudanabaena mucicola</i>	<i>Dolichospermum spiroides</i>	<i>Microcystis wesenbergii</i>
<i>Merismopedia punctata</i>	<i>Chroococcus dispersus minor</i>	<i>Chroococcus dispersus minor</i>
<i>Dolichospermum solitaria planctonica</i>	<i>Pseudanabaena limnetica</i>	<i>Coelosphaerium kuetzingianum</i>
<i>Chroococcus minimus</i>	<i>Pseudanabaena mucicola</i>	<i>Pseudanabaena mucicola</i>
<i>Aphanizomenon flos-aquae</i>	<i>Aphanocapsa delicatissima</i>	<i>Aphanizomenon flos-aquae</i>
<i>Aphanocapsa delicatissima</i>	<i>Gloeotrichia echinulata</i>	<i>Dolichospermum flos-aquae</i>
<i>Aphanizomenon issatschenkoi</i>	<i>Aphanizomenon gracile</i>	<i>Merismopedia punctata</i>
<i>Pseudanabaena limnetica</i>	<i>Dolichospermum solitaria planctonica</i>	<i>Merismopedia tenuissima</i>
<i>Planktolyngbya sp cf</i>	<i>Planktolyngbya limnetica</i>	<i>Aphanocapsa delicatissima</i>
<i>Planktolyngbya limnetica</i>	<i>Aphanothece smithii</i>	<i>Pseudanabaena limnetica</i>
<i>Aphanocapsa holsatica</i>	<i>Aphanocapsa planctonica</i>	<i>Aphanocapsa holsatica</i>
<i>Chroococcus prescottii</i>	<i>Merismopedia minima</i>	<i>Cyanodictyon imperfectum</i>
<i>Aphanocapsa planctonica</i>	<i>Aphanocapsa holsatica</i>	
<i>Merismopedia tenuissima</i>		
<i>Merismopedia minima</i>		
<i>Spirulina laxissima</i>		

Table S2 Log removal of the cyanobacterial species by clarification, filtration, and post-chlorination of DWTP A. The species were arranged in descending order based on their cell biovolume (mm<sup>3</sup>/L). Legend: NaN—impossible values resulting from division by zero, NA—not available

Species	Clarification		Filtration		Post-chlorination	
	mean	SD	mean	SD	mean	SD
<i>Dolichospermum spiroides</i>	2.6	0.6	-0.3	0.3	NaN	NA
<i>Microcystis aeruginosa</i>	3.0	0.7	0.6	NA	0.0	NA
<i>Microcystis wesenbergii</i>	NaN	NA	NaN	NA	NaN	NA
<i>Dolichospermum circinalis</i>	NaN	NA	NaN	NA	NaN	NA
<i>Aphanothece clathrata brevis</i>	1.2	0.5	0.2	0.3	0.0	0.2
<i>Chroococcus dispersus minor</i>	0.6	NA	NaN	NA	NaN	NA
<i>Pseudanabaena mucicola</i>	1.0	1.1	-0.1	0.6	-0.1	0.3
<i>Merismopedia punctata</i>	NaN	NA	NaN	NA	NaN	NA

Table S3 Log removal of the cyanobacterial species by clarification, filtration, and post-chlorination of DWTP B. The species were arranged in descending order based on their cell biovolume (mm<sup>3</sup>/L). Legend: NaN—impossible values resulting from division by zero, NA—not available

Species	Clarification		Filtration		Post-chlorination	
	mean	SD	mean	SD	mean	SD
<i>Woronichinia naegeliana</i>	0.1	0.2	NaN	NA	NaN	NA
<i>Microcystis aeruginosa</i>	NaN	NA	NaN	NA	NaN	NA
<i>Aphanothece clathrata brevis</i>	0.2	0.4	0.9	0.5	0.2	0.6
<i>Dolichospermum spiroides</i>	NaN	NA	NaN	NA	NaN	NA
<i>Chroococcus dispersus minor</i>	NaN	NA	NaN	NA	NaN	NA
<i>Pseudanabaena limnetica</i>	NaN	NA	NaN	NA	NaN	NA
<i>Pseudanabaena mucicola</i>	NaN	NA	NaN	NA	NaN	NA
<i>Aphanocapsa delicatissima</i>	NaN	NA	NaN	NA	NaN	NA

Table S4 Log removal of the cyanobacterial species by clarification, filtration, and post-chlorination of DWTP C. The species were arranged in descending order based on their cell biovolume (mm<sup>3</sup>/L). Legend: NaN—impossible values resulting from division by zero, NA—not available

Species	Clarification		Filtration		Post-chlorination	
	mean	SD	mean	SD	mean	SD
<i>Microcystis aeruginosa</i>	1.0	0.5	0.9	1.1	0.3	0.2
<i>Dolichospermum spiroides</i>	0.7	NA	0.1	NA	NaN	NA
<i>Aphanothece clathrata brevis</i>	1.0	0.5	0.6	0.3	1.0	1.1
<i>Microcystis wesenbergii</i>	NaN	NA	NaN	NA	NaN	NA
<i>Chroococcus dispersus minor</i>	NaN	NA	NaN	NA	NaN	NA
<i>Coelosphaerium kuetzingianum</i>	NaN	NA	NaN	NA	NaN	NA
<i>Pseudanabaena mucicola</i>	NaN	NA	NaN	NA	NaN	NA
<i>Aphanizomenon flos-aquae</i>	NaN	NA	NaN	NA	NaN	NA

Table S5 Physio-chemical variables for the raw, clarified, filtered, and treated water of the studied three DWTPs

	Parameters	Units	Raw Water			Clarified Water			Filtered Water			Treated Water			
			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
DWTP A	PC	RFU	1.1	0.0	38.2	0.1	0.0	10.1	0.0	0.0	0.1	0.0	0.0	0.1	
	Chl-a	RFU	1.3	0.2	4.5	0.1	0.0	2.8	0.0	0.0	0.3	0.0	0.0	0.3	
	DO	mg/L	5.8	0.5	14.5	7.1	5.0	13.4	6.6	3.0	11.3	7.3	5.7	8.6	
	TDS	mg/L	91.0	84.0	106.0	106.8	86.3	132.9	107.6	98.5	133.2	116.9	102.0	149.7	
	Temp	°C	25.7	23.0	28.2	26.4	23.2	28.7	27.6	23.1	33.0	26.1	23.1	28.0	
	Turb	NTU	7.3	1.6	63.6	0.6	0.0	25.2	0.7	0.0	77.3	0.4	0.0	4.7	
	Cond	µS/cm	141.8	132.0	165.5	168.6	135.3	210.4	173.8	153.0	226.0	183.9	153.0	237.3	
	pH			7.3	6.5	9.4	6.6	6.2	9.3	6.5	6.2	7.1	6.9	6.4	8.1
	DWTP B	PC	RFU	0.1	0.0	0.4	0.1	0.0	0.4	0.1	0.0	0.4	0.1	0.0	0.3
Chl-a		RFU	0.5	0.2	2.3	0.0	0.0	0.5	0.0	0.0	0.1	0.0	0.0	0.1	
DO		mg/L	6.9	5.0	10.8	8.2	5.8	11.8	8.5	6.3	11.8	8.1	6.7	11.3	
TDS		mg/L	110.3	98.8	126.7	119.8	105.0	135.2	125.0	115.0	139.3	153.4	140.9	167.0	
Temp		°C	22.8	12.8	27.8	21.0	9.6	26.1	20.6	9.5	26.1	21.9	12.4	26.5	
Turb		NTU	1.3	0.0	7.5	0.4	0.0	20.2	0.2	0.0	1.0	0.2	0.0	2.0	
Cond		µS/cm	163.5	141.8	175.5	169.2	143.4	182.4	175.1	146.2	205.0	221.6	182.0	243.1	
pH			7.1	6.1	8.1	5.5	4.6	7.9	5.3	4.6	6.5	6.4	5.4	7.9	
DWTP C	PC	RFU	0.0	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1	
	Chl-a	RFU	0.2	0.0	2.4	0.0	0.0	1.1	0.0	0.0	0.2	0.0	0.1	0.1	
	DO	mg/L	8.5	3.9	11.1	8.6	5.0	11.2	8.9	5.1	13.7	8.6	11.2	11.2	
	TDS	mg/L	153.6	145.0	165.0	155.0	146.3	166.0	156.1	148.0	166.0	156.6	166.0	166.0	
	Temp	°C	22.4	12.0	26.4	21.9	11.3	26.0	22.1	11.7	26.0	21.6	25.6	25.6	
	Turb	NTU	9.7	1.3	98.8	3.0	0.0	78.3	1.0	0.0	15.9	0.5	8.1	8.1	
	Cond	µS/cm	224.6	172.3	253.0	224.5	173.3	252.0	226.7	176.7	253.8	225.4	252.0	252.0	
	pH			8.4	7.8	8.9	8.2	7.7	8.6	7.9	7.5	8.2	7.7	7.9	7.9



Table S6 Alert level frames, standards and guideline values of countries and regions for cyanobacteria and cyanotoxin in drinking water supplies

Cell counts and/or cell biovolumes guidelines	Cyanotoxin guidelines (value in µg/L)	References
<b>WHO</b>		
Vigilance Level in raw water: ≥ 10 colony/mL or ≥ 50 filaments/mL Alert level 1 in raw water: ≥ 0.3 mm <sup>3</sup> /L total CB biovolume Alert Level 2 in raw water: ≥ 4 mm <sup>3</sup> /L total CB biovolume	Alert level 1 in raw water: Microcystin: 1, or Cylindrospermopsin: 0.7, or Anatoxin: 3, or Saxitoxins: 0.3 Alert level 2 in raw water: Microcystin: 12, or Cylindrospermopsin: 3, or Anatoxin: 30, or Saxitoxins: 3	<a href="#">(Chorus et al., 2021b)</a>
<b>USEPA</b>		
Not specified	Microcystin: Bottle-fed infants and pre-school children: 0.3 School-age children and adults: 1.6 Cylindrospermopsin: Bottle-fed infants and pre-school children: 0.7 School-age children and adults: 3.0	<a href="#">(United States Environmental Protection Agency (USEPA), 2015)</a>
<b>Ohio EPA</b>		
Not specified	Microcystins: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6 Cylindrospermopsin: Do not drink for children under 6 and sensitive populations: 0.7 Do not drink for children 6 and older and adults: 3.0 Anatoxin-a: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6 Saxitoxins: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6	<a href="#">(Ohio EPA, 2022)</a>

Cell counts and/or cell biovolume guidelines	Cyanotoxin guidelines (value in µg/L)	References
<b>Australia—NHMRC</b>		
Initial notification in raw water: ≥ 4500 cells/mL (0.2 mm <sup>3</sup> /L) <i>Cylindrospermopsis raciborskii</i> or ≥ 2000 cells/mL (0.2 mm <sup>3</sup> /L) <i>Microcystis aeruginosa</i> or ≥ 12,000 cells/ mL (2.7 mm <sup>3</sup> /L) <i>Nodularia spumigena</i> or ≥ 6000 cells/ mL (1.5 mm <sup>3</sup> /L) <i>Dolichospermum circinalis</i>	Cylindrospermopsin: 1 Microcystin: 1.3 Saxitoxins: 3 Nodularin: 1.3	<a href="#">(NHMRC, 2022)</a>
Alert in raw water: ≥ 15,500 cells/mL (0.6 mm <sup>3</sup> /L) <i>Cylindrospermopsis raciborskii</i> or ≥ 6500 cells/mL (0.6 mm <sup>3</sup> /L) <i>Microcystis aeruginosa</i> or ≥ 40,000 cells/ mL (9.1 mm <sup>3</sup> /L) <i>Nodularia spumigena</i> or ≥ 20,000 cells/ mL (5 mm <sup>3</sup> /L) <i>Dolichospermum circinalis</i>		
<b>New Zealand Ministry of Health</b>		
Vigilance Level in raw water: ≥ 50 cells/mL (excluding picocyanobacteria).		<a href="#">(Ministry of Health, 2020; Ministry of Health, 2022)</a>
Alert level 1 in raw water: ≥ 75 cells/mL for potential cylindrospermopsin producers or ≥ 100 cells/mL for potential microcystin / nodularin producers or ≥ 300 cells/mL for potential saxitoxin producers or ≥ 750 cells/mL for potential anatoxin producers		
Alert Level 2 (toxin concentrations exceed their maximum acceptable value): Microcystin: 1, Cylindrospermopsin: 0.8, Anatoxins: 6, Saxitoxins: 3, Nodularin: 1		
<b>Canada Health Canada</b>		
Not specified	Microcystin in drinking water: 1.5	<a href="#">(Health Canada, 2021)</a>
<b>MELCC Province of Quebec, Canada</b>		
Water intake alert level: ≥ 10,000 cells/mL cyanobacteria Water body alert level: ≥ 20,000 cells/mL cyanobacteria	Microcystin in drinking water: 1.5	<a href="#">(Ellis, 2009; Government of Québec, 2022)</a>

Table S7 Comparison of equivalent phycocyanin RFU to WHO alert levels

R <sup>2</sup> value	Equation Y: biovolume (mm <sup>3</sup> /L) X: phycocyanin (RFU)	Equivalent RFU to WHO 2021 Alert levels		Probe model	Scale of study (dominant species)	Reference
		Alert level 1 ≥ 0.3 mm <sup>3</sup> /L	Alert level 2 ≥ 4 mm <sup>3</sup> /L			
R <sup>2</sup> = 0.53	Y = 3.8X + 0.0057	0.1 RFU	1.1 RFU	YSI EXO2	Field ( <i>Dolichospermum spiroides</i> )	( <a href="#">Ma et al., 2023</a> )
R <sup>2</sup> = 0.46	Y = 1.16X - 1.78	1.8 RFU	5.0 RFU	YSI 6600	Field ( <i>Microcystis</i> spp.)	( <a href="#">McQuaid et al., 2011</a> )
R <sup>2</sup> ≥ 0.94	Y = 1.1X Y = 3.8X	0.3 RFU 0.1 RFU	3.6 RFU 1.1 RFU	YSI EXO2	Lab (2 strains of <i>M. aeruginosa</i> )	( <a href="#">Choo et al., 2018</a> )
R <sup>2</sup> = 0.73	Y = 0.0564X - 0.0025	5.4 RFU	70.9 RFU	YSI EXO2	Field ( <i>Microcystis</i> sp.)	( <a href="#">Almuhtaram et al., 2018</a> )
R <sup>2</sup> = 0.999	Y = 0.5X	0.6 RFU	8.0 RFU	YSI 6600	Lab ( <i>M. aeruginosa</i> )	( <a href="#">Zamyadi et al., 2012</a> )
R <sup>2</sup> = 0.77 R <sup>2</sup> = 0.72 R <sup>2</sup> = 0.19	Log Y = 2.0446 log X - 0.3592 Log Y = 1.8458 log X - 0.2318 Log Y = 0.6743 log X + 0.3804	0.8 RFU 0.7 RFU 0.1 RFU	3.0 RFU 2.8 RFU 2.1 RFU	YSI EXO2	3 field locations (mixed species)	( <a href="#">Bowling et al., 2016</a> )
R <sup>2</sup> = 0.70	Y = 0.068X	4.4 RFU	58.8 RFU	YSI EXO2	Field ( <i>Pseudanabaena</i> sp.)	( <a href="#">Zamyadi et al., 2016</a> )
0.71 ≤ R <sup>2</sup> ≤ 0.81	No equation provided	NA	NA	YSI EXO2	Lab (4 species)	( <a href="#">Bertone et al., 2019</a> )

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