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Monitoring of cyanobacterial blooms and assessing polymer-enhanced micro- and ultrafiltration for microcystin removal in an Italian drinking water treatment plant

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Abstract

The water intake of a drinking water treatment plant (DWTP) in Central Italy was monitored over six bloom seasons for cyanotoxin severity, which supplies drinking water from an oligo-mesotrophic lake with microcystin levels up to 10.3 µg/L. The historical data showed that the water temperature did not show extreme/large seasonal variation and it was not correlated either with cyanobacterial growth or microcystin concentration. Among all parameters, the cyanobacteria growth was negatively correlated with humidity and manganese and positively correlated with atmospheric temperature. No significant correlation was found between microcystin concentration and the climatic parameters. Polymer(chitosan)-enhanced microfiltration (PEMF) and ultrafiltration (PEUF) were further tested as an alternative microcystin removal approach from dense cyanobacteria-rich flows. The dominant cyanobacteria in the water intake, *Planktothrix rubescens*, was isolated and enriched to simulate cyanobacterial blooms in the lake. The PEMF and PEUF were separately applied to enriched *P. rubescens* culture (PC) (microcystin = 1.236 µg/L) as well as to the sand filter backwash water (SFBW) of the DWTP where microcystin concentration was higher than 12 µg/L. The overall microcystin removal rates from the final effluent of PC (always <0.15 µg/L) were between 90.1-94.7% and 89.5-95.4% using 4 and 20 mg chitosan/L, respectively. Meanwhile, after the PEMF and PEUF of SFBW, the final effluent contained only 0.099 and 0.057 µg microcystin/L with an overall removal >99%. The presented results are the first from the application of chitosan to remove *P. rubescens* as well as the implementation of PEMF and PEUF on SFBW to remove cyanobacterial cells and associated toxins.

Keywords: chitosan; cyanobacterial blooms; drinking water treatment; microcystin; *Planktothrix rubescens*; polymer enhanced ultrafiltration

1. Introduction

Harmful algal blooms (HABs) are becoming an aquatic stressor of great concern since the anthropogenic perturbations (e.g. climate change and eutrophication) increase continuously in coastal areas (Griffith and Gobler, 2020). Simply, biotoxins that are produced by many HAB species, called phycotoxins, can bioaccumulate in the tissues of bivalve shellfish in time and, when consumed by humans, can cause severe/fatal shellfish poisoning syndromes. Phycotoxins are also a serious threat to marine organisms including marine mammals, fish, sea birds, invertebrates, etc. (Huisman et al., 2018). Furthermore, the presence of toxins from cyanoHABs in drinking water reservoirs may represent serious health risks for the human population (Almuhtaram et al., 2018). The World Health Organization (WHO) set a guideline limit of 1 µg/L for the presence of microcystin-LR, one of the most harmful cyanotoxins known in drinking and recreational waters that inhibits protein phosphatase and causes liver failure and hepatic haemorrhage (WHO, 2020). Moreover, dissolved microcystin can be persistent in water for a long period without any change in its chemical structure, which makes it hard to degrade in natural ecosystems (He et al., 2021).

Planktothrix rubescens is one of the most frequently-detected cyanobacteria species in Europe with microcystins being the most frequent cyanotoxins in freshwaters (Bogialli et al., 2013). *P. rubescens* is a red-colored, filamentous cyanobacterium with gas vesicles, which can adjust its buoyancy and position in the water column to obtain its preferred low light environment. This metalimnic species can adapt to low underwater light climate, and usually forms deep-water maxima in the stratified lakes of temperate latitudes (Gallina et al., 2017). This has implications for planning monitoring activities, especially in the case of drinking water supplies, with offtakes typically at depths of some meters (Manganelli et al., 2016).

The over-enrichment of nutrients in watersheds is one of the main drivers of cyanoHABs. Additional drivers include stratification and associated changes in temperature, light as well as changes in the frequency and timing of extreme weather events (Bertani et al., 2017). While forecasting the effects of climate change is a challenge, the high probability that future climatic conditions will favor bloom

formation poses an added challenge to develop effective mitigation strategies that consider both nutrient and climate drivers (Gobler, 2020). At this point, understanding long-term trends is important to inform forecasting efforts and to reduce uncertainty estimates while developing effective water and cyanoHAB management strategies and ensuring water safety (Urquhart et al., 2017).

In principle, drinking water treatment removes cyanobacteria in the final effluent without compromising cell integrity in order to simultaneously eliminate intracellular and extracellular toxins (Merel et al., 2013). However, when dense cyanobacterial colonies enter a drinking water treatment plant (DWTP), they can block filters and accumulate in these units due to the particulate load on the flocculation and clarification that can further cause the free toxin to pass through the system (Ghernaout et al., 2010). In fact, algal cell accumulation (over 1 x 10^5 cells/mL) in clarifier sludge and filter backwash samples were reported (Almuhtaram et al., 2018) with various cyanotoxin species at varying concentrations.

Conventional coagulation processes are effective to remove cyanobacteria cells but do not remove cyanotoxins efficiently, especially extracellular ones (Sorlini et al., 2013). Most of the DWTPs use conventional adsorbents such as powdered activated carbon (PAC) (Dixon et al., 2011) or coagulants such as polyaluminum chloride (PACl) (Sun et al., 2013) that both achieved high removal efficiencies for cyanobacteria cells, and even microcystins (Ho et al., 2011). However, in order to obtain complete cell removal, high dosages of coagulants must be added, which in return increase operational costs, produce high volumes of inorganic metallic sludges. Hence, the application of organic polymers as coagulants may be a more feasible and sustainable solution. Chitosan is considered as a new generation coagulant (Jin et al., 2017) since it is abundant, non-toxic, biodegradable, renewable, and ecologically sustainable as well as highly effective in removing algae from water (Xu et al., 2013). Chitosan can be easily derived from shellfish and crustaceans through alkaline deacetylation of chitin (Mucci et al., 2020). Its characteristics such as efficient charge neutralization and bridging effects give good coagulation/flocculation properties (Yang et al., 2016), thus it has been widely used in water and wastewater treatment in recent years (Miranda et al., 2017).

Coagulation and sedimentation processes are typically followed by rapid sand filtration in conventional DWTPs. No remarkable impact has been reported for the sand filter process to remove cyanobacteria and/or cyanotoxins (He et al., 2016). Moreover, the major concern for filtration underlies the possible disruption of cells that accumulate in the filter media, and the subsequent release of toxins via the effluent and/or backwashing. Almuhtaram et al. (2018) reported that over 70% of filter backwash samples contained cyanobacteria cell concentration greater than that of raw water, an order of magnitude higher than any other processes in DWTPs. Most of the time, sand filter backwash water (SFBW) is directly discharged or subjected to a sedimentation basin to recycle the water to the plant (Shafiquzzaman et al., 2018), which may not remove cyanotoxins.

Membrane processes (e.g., microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) are particularly efficient to remove cyanobacteria and cyanotoxins (Merel et al., 2013). Specifically, UF was reported to remove intracellular cyanotoxins higher than 98% and NF to remove extracellular toxins in the range of 96-99% (Gijsbertsen-Abrahamse et al., 2006). However, direct membrane application to high algae concentration brings challenges such as fouling due to algal cells and algae-derived organic matters (Qu et al., 2012) and/or cell rejection that causes an increase in extracellular toxins in the permeate due to cell breakage during filtration (Liu et al., 2017). Herein, the use of hybrid systems with enhanced coagulation has a high potential to remove both intra- and extracellular cyanotoxins and to reduce membrane fouling (Dixon et al., 2011; Yan et al., 2017). Polymer-enhanced ultrafiltration (PEUF) is effective and even sustainable in this sense, while it helps to remove foulants before filtration. In comparison to other membrane technologies, PEUF has the capability of separating low molecular weight compounds and a high permeability for solvent transport at relatively low transmembrane pressures, which is desirable from an energy and capital cost perspective (Huang and Feng, 2019).

A multi-barrier approach by developing a monitoring system to facilitate effective treatment is therefore highly applicable to minimize the effects of cyanotoxins in water bodies (He et al., 2016). This study reports the characteristics of cyanobacterial bloom and microcystin production through

127 monitoring six bloom seasons in the water intake of a DWTP in Central Italy that supplies drinking
128 water from an oligo-mesotrophic lake. Whereas the plant can efficiently remove cyanotoxins from
129 the final effluent in the current configuration even in the bloom periods, we proposed PEUF and
130 polymer-enhanced MF (PEMF) as alternative and more sustainable treatment strategies that can also
131 be applied in case of potential toxin accumulation in treatment units. Although chitosan-aided
132 coagulation/flocculation has been reported in a number of studies, to the best of our knowledge, no
133 study has evaluated the removal of *P. rubescens* cells via chitosan. Moreover, we reported the
134 findings obtained from both PEMF and PEUF on SFBW for the first time. Hence, the aims of this
135 study can be listed as follow: i) to monitor and assess critical cyanobacterial blooms and microcystin
136 variations in the DWTP of Lake Castreccioni; ii) to determine any correlation between cyanobacterial
137 bloom/cyanotoxin production and environmental parameters; iii) to evaluate any potential cyanotoxin
138 accumulation within the DWTP even in the absence of a bloom event; iv) to test and optimize PEUF
139 to remove cyanobacterial cells and microcystin in comparison to PEMF; v) to perform PEUF and
140 PEMF configurations to SFBW to mitigate the release cyanotoxins via these flows into water bodies.

141 ## 2. Materials and methods

142 ### 2.1.Site and plant description

143 Lake Castreccioni is in the municipality of Cingoli in the Marche Region, Italy, located across the
144 Musone River near Monte San Vicino, about 70 km far from the coast. The biggest artificial lake in
145 the region, Castreccioni covers about 2.4 km^2 and reaches depths of 16.8 m. The volume of the lake
146 at the maximum altitude is approximately 50 million cubic meters. The DWTP (managed by
147 Acquambiente Marche S.r.l.) is in the district of Castreccioni and responsible for drinking water for
148 the member municipalities as shown in the **e-Supplementary file**. The plant supplies water for a total
149 of about 65,000 inhabitants in the winter and about 95,000 in the summer with a maximum capacity
150 of 500 L/s. The process steps include pre-disinfection with ozone (pre-ozonation), clariflocculation,
151 sand filtration, ozonation, activated carbon filtration, accumulation tanks, and final disinfection with
152 chlorine dioxide.

153 **2.2.Water intake monitoring**

154 The water intake of the DWTP in Castreccioni was systematically monitored over six bloom seasons
155 from January 2014 to November 2019. The water level of the lake varied between 332-343 m sea
156 level during the monitoring period (**e-Supplementary file**). The physical-chemical parameters were
157 monitored at monthly intervals; while total coliform, *E. coli*, cyanobacteria, and microcystin were
158 measured 4-5 times a month. All physical-chemical parameters were measured according to the
159 Standard Methods of the American Public Health Association (APHA, 2012). *E. coli* and total
160 coliforms were determined using the membrane filtration method explained in Standard Methods
161 9221B (APHA, 2012). Cyanobacteria was measured according to a protocol described elsewhere
162 (Lucentini and Ottaviani, 2011). Briefly, 1 L water sample taken from the water intake was fixed in
163 Lugol's solution and an aliquot of 25 mL was sedimented and observed after 24 h in the Utermöhl
164 chamber under an inverted microscope for the quantitative determination of the cyanobacteria
165 species. Cell dimensions were calculated using a transmitted light optical microscope with an
166 immersion objective. Microcystin analysis was done via enzyme-linked immunosorbent assay
167 (ELISA) as described in **Section 2.6**. The historical meteorological data of Cingoli were obtained as
168 the average monthly data from a national website for the monitoring period.

169 **2.3.Cyanobacteria isolation and cultivation**

170 The isolation of single filaments of the cyanobacteria *P. rubescens* was made from raw water taken
171 from the water intake of the Castreccioni DWTP on 15 November 2019 (water temperature = 10 °C,
172 pH = 7.9, turbidity = 0.23 NTU), following the capillary pipette method (Hoshaw and Rosowski,
173 1973) under an inverted microscope (Zeiss Axiovert 135) equipped with phase contrast, at 200x
174 magnification. After an initial growth in microplates, cells were cultured at 21 ± 0.1 °C under a 12:12
175 h L:D photoperiod and an irradiance di 90-100 µmol $m^{-2}s^{-1}$, in BG11 medium. For the molecular
176 identification, genomic DNA was extracted from the cells collected by centrifugation at 4,000×g for
177 20 min from a 30 mL monoclonal culture of *P. rubescens* (strain PRMC1119) in logarithmic growth
178 phase using the CTAB (N-cetyl-N,N,N-trimethylammoniumbromide) method (Richards et al., 2001).

Extracted DNA was amplified by Polymerase Chain Reaction (PCR) using a SimpliAmpTM Thermal Cycler with the primers CYA371F and CYA783R (Janse et al., 2003) targeting the 16S rRNA gene. The PCR conditions were 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 sec, annealing at 60 °C for 45 sec, and elongation at 72 °C for 1 min, followed by a further elongation at 72 °C for 5 min. PCR products were visualized and quantified in 1.5% agarose gels stained with GelRedTM (Biotium, Hayward, CA, USA) using Low DNA Mass Ladder (Invitrogen, Carlsbad, CA, USA) as a reference, and visualized under UV light. PCR products with expected lengths and yields were purified and directly Sanger-sequenced by Macrogen Europe (Amsterdam, The Netherlands). The sequence was compared to the NCBI database by BLAST search with default settings (see **e-Supplementary file**). The culture was harvested at the final cell yield up to 10^5 cells/mL and further diluted to 10^4 cells/mL with the lake water of Castreccioni to simulate dense cyanobacteria period according to the historical data. The lake water was pre-filtered through a 0.45 µm fiber membrane to remove any background algae before dilution.

2.4.Sampling and characterization for PEMF and PEUF experiments

The raw water sample was taken from the water intake of the Castreccioni DWTP. Based on the information gathered from the water utility, the current treatment configuration is highly effective to remove cyanobacteria and cyanotoxins in the final effluent. In addition, due to the accumulation of cyanobacteria and microcystin in time, the SFBW was also sampled and characterized together with the raw lake water for further bench-scale tests. Physical and chemical characterization of the water intake and SFBW are given in **Table 1**. All analyses were done according to the Standard Methods (APHA, 2012). pH, EC, and DO were measured on-site using a multi-parameter meter of pH/ISE/EC/DO, HI9829 (Hanna Instruments, US).

Table 1. Characteristics of the water intake and sand filter backwash water (SFBW) in the Castreccioni DWTP during the sampling for PEUF tests (average values from duplicate measurements).

Parameter	Unit	Water intake	SFBW
pH	-	7.3	7.8
EC	$\mu s/cm^2$	367	485
Alkalinity	$mgCaCO_3/L$	184	185
DO	mg/L	7.6	7.3
Chemical oxygen demand	mg/L	73	124
Total suspended solids	mg/L	5	60
Total dissolved solids	mg/L	680	520
Total phosphorus	mg/L	<0.1	0.3
Nitrate	mg/L	2.89	<0.1
Sulphate	mg/L	57.8	55.4
Chloride	mg/L	18.4	20.7
Sodium	mg/L	15.6	16.5
Potassium	mg/L	2.4	2.6
Magnesium	mg/L	14.3	14.6
Calcium	mg/L	96.8	99.2
Iron	mg/L	0.044	0.169
Copper	mg/L	0.044	0.123
Manganese	mg/L	0.005	0.034
Aluminium	mg/L	0.044	0.202
Zinc	mg/L	0.113	0.558
Barium	mg/L	0.124	0.069
Lead	mg/L	<0.01	<0.01
Chromium	mg/L	<0.001	<0.001
Nickel	mg/L	<0.001	<0.001
Cadmium	mg/L	<0.0004	<0.0004

2.5.PEMF and PEUF experiments

Chitosan powder from crab shells was obtained from Sigma–Aldrich Company Ltd. and used as the coagulant in the experiments. A chitosan solution of 1 mg/mL was prepared according to the protocol described elsewhere (Divakaran and Pillai, 2002). The experimental setup is depicted in the **e-Supplementary file**. The operating parameters (i.e. coagulant dose, rapid mixing velocity, and time) for the PEMF and PEUF experiments were initially optimized in 28 experimental systems. Briefly, chitosan was added to 500 mL of *P.rubescens* culture (10^4 cells/mL) at the concentrations of 1, 2, 3,

4, 5, 10, and 20 mg/L, and different rapid mixing conditions were tested for each concentration as follows: 100 rpm at 3 min, 150 rpm at 2 min, 200 rpm at 1.5 min and 250 rpm at 1.2 min. Then, the suspensions were slowly mixed at the velocity of 40 rpm for 20 min and finally left for sedimentation for 30 min. The supernatants were carefully collected and subjected to MF (fiber membrane filters - 0.45 µm) and UF (Merck Millipore Amicon^{TM} stirred cells – 10 kDa) separately. The clarification efficiency (CE) of the supernatants after coagulation, MF, and UF were further determined via the measurement of the optical density at 750 nm (OD_{750}) with an ONDA UV-20 spectrophotometer. The CE was then calculated by CE = (1 – ODs/ODf) x 100% (Xu et al., 2013), where ODf is the OD of the feed sample, ODs is the OD of the supernatant after coagulation-flocculation-sedimentation, MF, or UF. Further, the PEMF and PEUF experiments were conducted at the pre-determined optimal conditions using two different cases: cyanobacterial culture (10^4 cells/mL) and SFBW. In addition to the chitosan dose of 4 mg/L, a high concentration of 20 mg/L was also tested to evaluate any possible enhanced removals of cyanobacteria cells and microcystin. In parallel, the effectiveness of the PEMF/PEUF was experimentally compared to that of conventionally used MF and UF, where the *P. rubescens* culture and SFBW were directly filtered without prior coagulation. After each test, the samples were collected for microcystin analysis. The samples after PEUF and UF were also checked to ensure any microbiological contamination in the final effluents using the membrane filtration method. All experiments were carried out in duplicate and mean values are presented.

2.6.Microcystin analysis

Water samples of 10 mL were conditioned at room temperature, mixed by vortex for 60 s, and subjected to ultrasonication (180 W, 40 kHz DU-65, Argolab, Carpi, Italy) for 30 min for cell lysing (Foss and Aubel, 2015). The ELISA was performed by the commercial Microcystins-ADDA-Enzyme-Linked Immunosorbent Assay (Abraxis, Product No. 520011, Lot.No. 19GO113) following the manufacturer's instructions. All reagents, such as microtiter plate (12 x 8 strips) coated with an analog of microcystins conjugated to protein, ready-made Standards solutions at 0, 0.15, 0.40, 1.0, 2.0, 5.0 µg/L, Control at 0.75 ± 0.185 µg/L, Sample Diluent, Antibody solution, Anti-sheep-HRP

conjugate solution, Wash buffer (5x) concentrate, a substrate solution (color solution), and Stop solution, were provided by the manufacturer. The absorbances were read at 450 nm in a Multiskan FC, Labsystems microtiter plate reader (Thermofisher). Microcystin concentrations were calculated by interpolation using the standard curve constructed by plotting the %B/B0 for each standard versus the corresponding microcystins concentration (0, 0.15, 0.40, 1.0, 2.0, 5.0 µg/L). The coefficient of variations (CVs) for standards was always < 10%; for samples always < 15%. Recovery for control standard (QCS, 0.75± 0.185 µg/L), were always between 75% and 125%. A final estimate for microcystins content was obtained and calculated as the mean of at least two subsamples (i.e. two replicates per subsample).

2.7. Statistical analysis

Correlations between environmental parameters and cyanobacterial blooms/microcystin concentrations during the monitoring period were determined by Pearson's correlation method. The significant differences were determined at the $p < 0.001$, $p < 0.05$, and $p < 0.1$ level of significance. Principal component analysis (PCA) was applied to investigate the contribution of each considered variable. All analyses and visualizations were done in R ver. 3.6.3.

3. Results

3.1. Monitoring of environmental parameters and observation of cyanobacterial blooms

The physico-chemical parameters measured in the water intake between 2014-2019 are given in the **e-Supplementary file**. The water temperature measurements showed limited mean variations between different years, ranged between 8.4-10.9 °C with a maximum value measured in 2019. The dissolved oxygen (DO) levels showed different patterns during the monitoring period (2014-2019). Whereas the highest average and maximum value were recorded in 2014 (8.6 mg/L and 8.8 mg/L, respectively), a remarkable decrease was detected in the following years with a minimum value of 0.1 mg/L. However, an increase in DO levels was observed in 2019 with an average concentration of 6.7 mg/L. The highest electrical conductivity (EC) values were measured in 2014 with an average concentration of 577 µS/cm and a maximum of 1507 µS/cm. A decreasing pattern was observed in

the following years showing more stable characteristics. The lowest EC value was detected in 2019 as 416 µS/cm. In general, the lake water was found to be slightly alkaline, the average pH was in the range of 7.68 and 7.85. The highest average and maximum pH values were measured in 2016 (7.3 and 8.2, respectively). The average alkalinity was in the range of 242 mg/L – 274 mg/L until 2018, then lower values were detected. The average alkalinity was 183 mg/L in 2019. The average bicarbonate concentration was recorded in the range of 236-260 mg/L during the monitoring period without any significant variation. The average total organic carbon (TOC) levels were mostly under 4 mg/L. However, a high peak was detected in 2019 and the highest concentration was recorded for the monitoring period (8.1 mg/L). Ammonium concentration was steadily below the detection limit (0.1 mg/L) for most of the time except for a sharp increase in January 2019. Thus, it resulted in an increase in the average concentration in 2019. The average nitrate concentration ranged from <5 mg/L to 7.17 mg/L. The highest values were recorded in 2018 whereas the average and maximum concentrations were 7.17 mg/L and 12 mg/L, respectively. The phosphate concentration was measured steadily below 3 mg/L for the entire monitoring period. The highest iron concentration was measured in 2014 (82 µg/L) and there was a tendency towards decreasing for the following years. However, higher concentrations were recorded in 2019 in which the average and maximum values were 27.3 µg/L and 61 µg/L, respectively. Similarly, there was a decreasing trend for manganese, the highest value was measured in 2015 as 136 µg/L and it resulted to record the highest average value (63.5 µg/L). The lower values were recorded in 2019 when the manganese levels ranged from <5 mg/L to 17 mg/L. There was not a significant difference in calcium and magnesium levels in the lake water, the variation between the average and the maximum levels was quite low. The average potassium concentration ranged from 3.07 mg/L to 3.87 mg/L and the highest levels were detected in 2019. There was not a high variation in the average sulfate levels in the last three years of the monitoring period (ranged from 54 mg/L to 58 mg/L). The highest sulfate concentration was observed in 2018. The chloride concentration varied between 21 mg/L and 25 mg/L, showing a constant pattern for the entire monitoring period. Since the average aluminum concentration varied from 33 µg/L to

50 µg/L between 2014 to 2016, the concentrations went up in 2017, and the highest levels were measured in this year whereas the minimum, average, and maximum levels were recorded as 34 mg/L, 83 mg/L, and 183 mg/L, respectively. Zinc concentrations were constantly measured below the detection limits (<0.05 mg/L). The lead concentrations were very low during the monitoring period: it was measured under 2 µg/L during the study period. A tendency towards decreasing was detected for barium and the average concentration ranged from 0.02 mg/L to 0.57 mg/L.

Concentrations of total coliforms ranged from 12 to $5.7x10^3$ cfu/100 mL during the monitoring period. Until 2017, the highest total coliforms concentration was recorded as 3000 cfu/100 mL. Then, there was a sharp increase in the concentration in October 2017 (5700 cfu/100 mL). The highest average value was also detected in this year among the monitoring period (742 cfu/100 mL). Thereafter, the enhancement was observed in the water quality in terms of total coliform bacteria concentration. The lowest average value was measured in 2018 (138.75 cfu/100 mL). As expected, the trend of *E.coli* concentration mimicked that of total coliform bacteria, and the highest value was recorded in 2017 as $1.7x10^3$ cfu/100 mL with an average of 145 cfu/100 mL. In the following years, the average concentrations were quite low and recorded as 5.33 cfu/100 mL and 8 cfu/100 mL for 2018 and 2019, respectively.

The cyanobacteria abundance and microcystin concentrations are presented in **Fig. 1**. In 2014, the abundance of cyanobacteria started to increase in winter and this trend continued until the beginning of spring. The cyanobacteria abundances reached the highest levels in March and April which revealed a bloom. A second and relatively not heavy bloom was observed in July and then the cyanobacteria abundance showed a tendency towards decreasing. In 2015, a sharp increase in the abundance occurred in June which was also the highest abundance detected during the entire monitoring period and a heavy bloom was formed. The abundance went down in the following months except for a slight increase in September. In 2016, the bloom was observed in August and the cyanobacteria abundances were quite low for the rest of the months. In 2017, the bloom was observed early compared to the previous years. The abundances increased significantly in April and May, then

318 decreased in the following seasons. In 2018, the bloom was formed in August and the abundance was
319 lower than that of the previous cases. On the other hand, in 2019, the first bloom was recorded in
320 May and the highest abundance was recorded all year round. A second bloom was observed in early
321 autumn, but the abundance was not as high as the first one.
322 The contribution of *Plantothrix* to total phytoplankton abundance is also given in the **e-**
323 **Supplementary file**. The phytoplankton community was comprised of diatoms, dinoflagellates,
324 golden algae, green algae, and yellow algae. The genus *Planktothrix* constituted the prevalent part of
325 the cyanobacteria (>97% in most cases), mainly represented by *P. rubescens*. During the bloom
326 events especially in the summer period, the contribution decreased to around 70%.
327 The trend of microcystin concentrations in the water intake showed a similar pattern with
328 cyanobacterial growth. As a result of the increase in cyanobacteria abundance, higher microcystin
329 concentrations were detected during bloom formation. The highest microcystin level was detected as
330 10.3 µg/L in July 2015 in the monitoring period. The second peak concentration was recorded in 2014
331 (9.54 µg/L) followed by the levels observed in April and May 2017. After 2017, lower microcystin
332 levels were detected in the water samples during the bloom events.

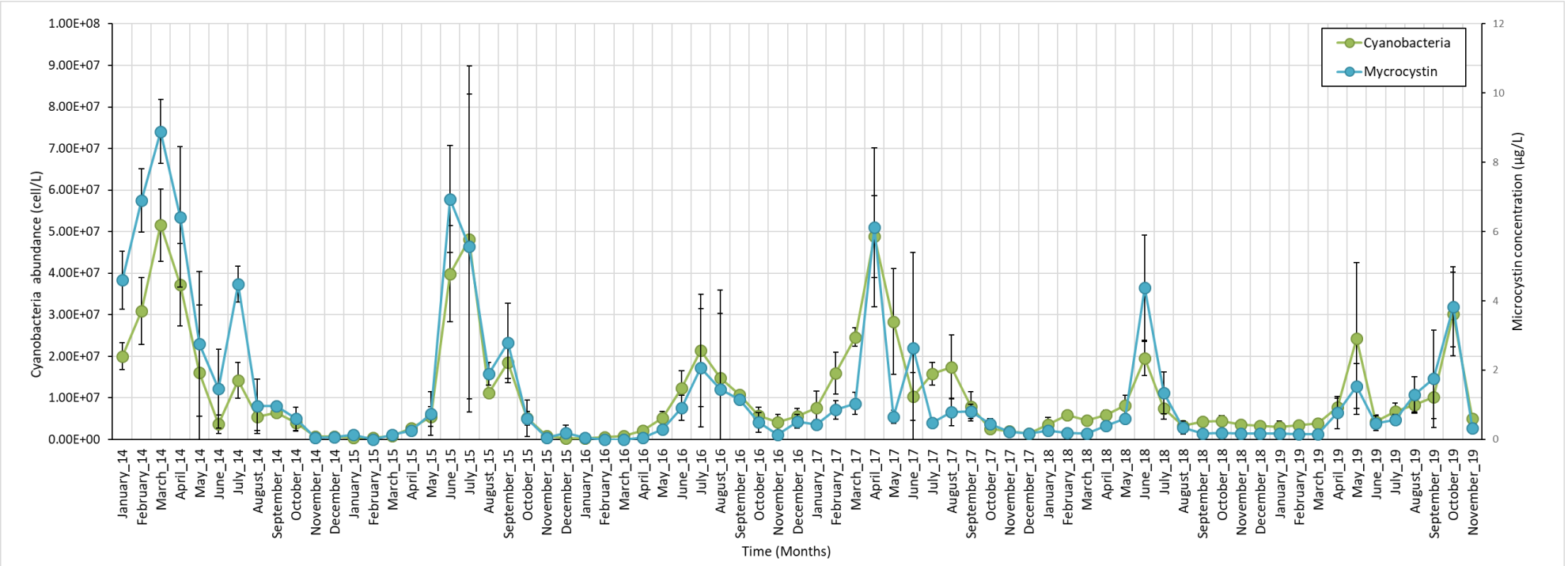

Fig. 1. Monthly data of cyanobacteria abundance and microcystin concentrations in the water intake of Castreccioni DWTP throughout the monitoring period (2014-2019).

341 ### 3.2. Correlation of environmental parameters to cyanobacterial blooms

342 #### 3.2.1. Water quality changes and correlations with cyanobacteria and microcystin variations

343 To determine the relationship between the cyanobacterial growth, microcystin concentration, and
344 major environmental parameters, the Pearson correlation was calculated and the results are displayed
345 in **Fig. 2A**. As expected, a strong positive correlation was found between total coliform and *E.coli*
346 concentrations ($p < 0.001$) as well as cyanobacterial growth and microcystin concentration
347 ($p < 0.001$). Since the variation in the water temperature was quite small during the study period, it is
348 apparent from the correlogram that very few parameters were negatively correlated with temperature
349 (alkalinity ($p < 0.1$), pH ($p < 0.1$), and iron ($p < 0.05$)). Neither coliform bacteria nor *P. rubescence*
350 was correlated with water temperature ($p>0.1$). Whereas pH was negatively correlated with *E. coli*
351 concentration ($p < 0.1$), there was a strong correlation between pH and total coliform bacteria levels
352 ($p < 0.001$). The parameters, calcium, and sodium, showed a similar profile in the correlation analysis,
353 they had a strong negative correlation with magnesium and alkalinity. Within all physico-chemical
354 parameters, cyanobacteria and microcystin were only significantly correlated with manganese.
355 **Fig. 2B** provides an overview of the contribution of the parameters influencing the water quality in
356 the water intake of Lake Castreccioni DWTP. The results revealed that the main drivers shaping the
357 water quality throughout the monitoring period were microcystin and cyanobacteria concentrations
358 as well as calcium. On the other hand, EC, nitrate, TOC, and water temperature were the least
359 effective parameters showing the lowest contribution within the analysed parameters in the water
360 samples.
361

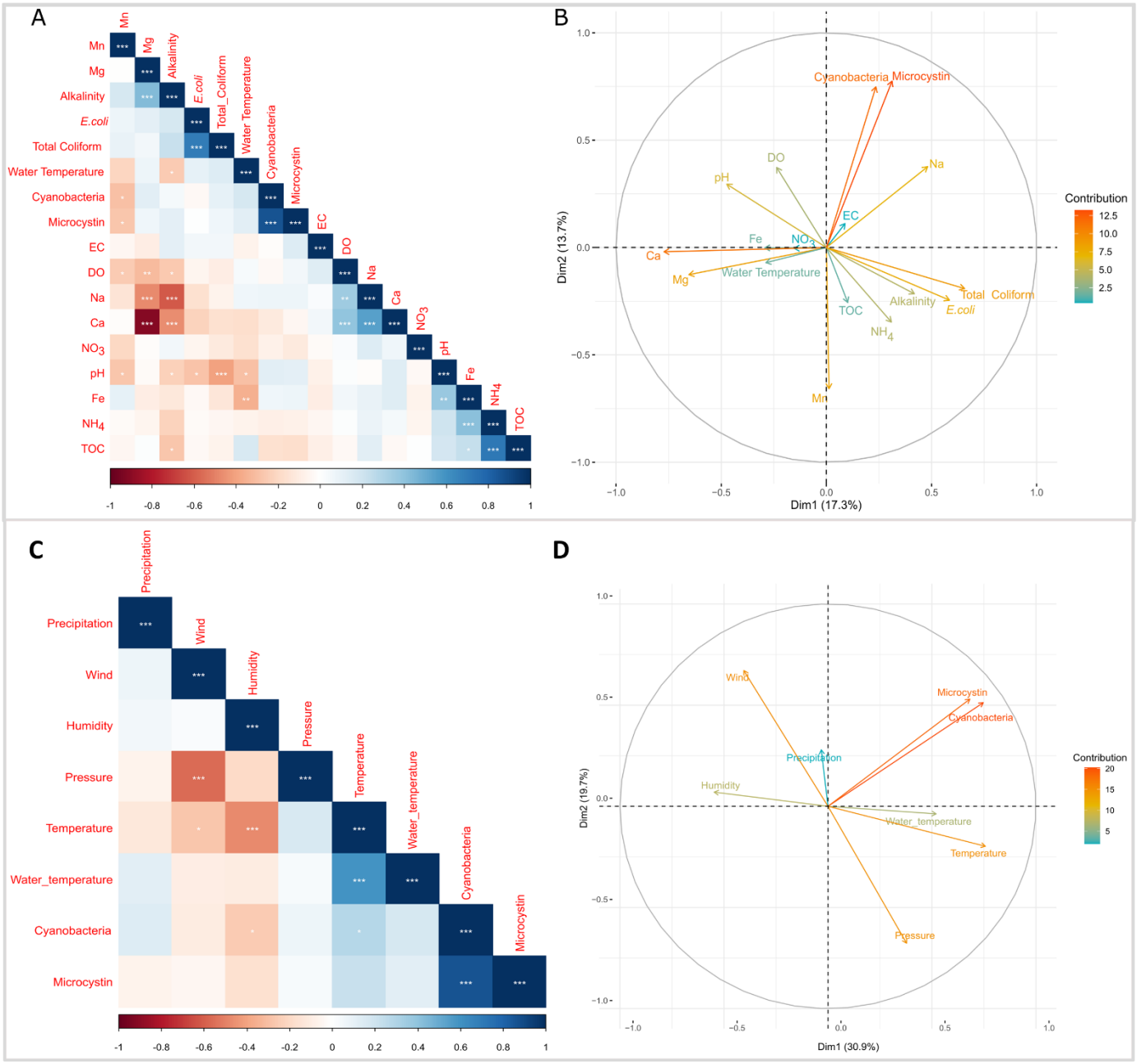

Fig. 2. A) Pearson correlation between water quality parameters and cyanobacteria and microcystin occurrence throughout the monitoring period (2014-2019). Positive significant correlations are highlighted in blue while negative significant correlations are highlighted in red. Lower significant positive and negative correlations are highlighted in lighter and lightest blue and red colors, in proportion to the correlation coefficient (*$p < 0.1$, **$p < 0.05$, ***$p < 0.001$). **B)** Variable-factor map. **C)** Pearson correlation between climatic parameters and cyanobacteria and microcystin occurrence throughout the monitoring period (2014-2019). **D)** Variable factor map.

3.2.2. Climatic conditions and correlations with cyanobacteria and microcystin variations

Meteorological factors have a key role in bloom formation. The effects of climatic variables on cyanobacterial growth and microcystin production were evaluated and the correlation analyses are shown in **Fig. 2C**. Although the atmospheric temperature showed significant changes among seasons, the water temperature did not differ significantly during the study period. Among all parameters, the cyanobacterial growth was only significantly negatively correlated with humidity ($p < 0.1$). Besides, there was a significant positive correlation between cyanobacterial growth and atmospheric temperature ($p < 0.1$). No significant correlation was found between microcystin concentration and the climatic parameters. The contributions of the climatic parameters on cyanobacteria abundance were shown in the variable factor map (**Fig. 2D**). The precipitation had the lowest contribution to the cyanobacterial growth. On the other hand, pressure and wind were not likely to be correlated with cyanobacterial abundance.

3.3. Existing treatment configuration to remove microcystin

The sample taken from the water intake of the plant for the experimental tests contained 0.099 µg/L microcystins since the sampling was done in a non-bloom period. In the meantime, the SFBW contained 12.32 µg microcystin/L, which highlighted that the sand filter unit is one of the main accumulation points for cyanotoxins in the treatment line, in particular microcystin. On the other hand, the microcystin concentration in the effluent of the sand filter was measured as 0.115 µg/L, which indicated that there was no re-release of microcystin in the effluent. In fact, considering the following treatment units in the plant, microcystin concentration in the final effluent is always much lower than the required limits (<1 µg/L). Consequently, we considered the SFBW as the most critical flow in the plant in terms of microcystin release into the environment, as well as in any other similar DWTPs, since these (waste)waters are often discharged without any treatment.

3.4. Alternative mitigation strategies to remove *P. rubescens* and microcystin using PEMF and PEUF

The optimal coagulation condition in PEMF and PEUF tests was found at the chitosan dose of 4 mg/L and at the rapid mixing velocity at 200 rpm for 1.5 min (data not shown). The effect of two chitosan doses, optimal (4 mg/L) and extra high dosage (20 mg/L), on microcystin removal was further tested in pre-determined coagulation-flocculation conditions and subjected to MF and UF. **Fig. 3** shows the results obtained from *P. rubescens* culture (PC) and SFBW with respect to microcystin concentration (**Fig. 3A, B**) and removal rate (**Fig. 3C**). PC had an initial microcystin concentration of 1.236 µg/L and decreased to 0.562 µg/L (54.3% removal) after the coagulation-flocculation at 4 mg/L of chitosan. After the MF and UF, the effluent has 0.065 µg/L (94.7% removal) and 0.122 µg/L (90.1% removal) of microcystin, respectively. Differently, 20 mg/L chitosan was achieved to obtain 0.073 µg/L (94.1% removal) of microcystin after the coagulation-flocculation. Following the MF and UF, the microcystin concentrations in the effluents were detected as 0.056 µg/L (overall 95.4% removal) and 0.13 µg/L (overall 89.5% removal), respectively. Meanwhile, the direct MF and UF of the PC (without prior coagulation step) resulted in the remaining microcystin concentrations of 0.364 µg/L (70.5%) and 0.125 µg/L (89.9% removal), respectively. The initial concentration of microcystin in the SFBW was measured as 12.32 µg/L, which is much greater than the PC. With the aid of 4 mg/L of chitosan, the effluent after coagulation-flocculation still contained 10.583 µg/L of microcystin (14.1% removal). On the other hand, further MF and UF application ended up with microcystin concentration of 0.555 µg/L (overall 95.5% removal) and 0.106 µg/L (overall 99.1% removal), respectively. There was a significant contribution of the addition of 20 mg/L of chitosan compared to 4 mg/L, where the remaining concentration was 2.44 µg/L of microcystin (80.2% removal) after the coagulation-flocculation. After the MF and UF, there was only 0.099 µg/L (overall 99.2% removal) and 0.057 µg/L microcystin (overall 99.5% removal) in the final effluent, respectively, where the highest removal efficiencies were achieved. At the same time, the direct MF and UF of SFBW resulted in 0.842 µg/L (93.1% removal) and 0.119 µg/L of microcystin (99% removal) in the effluent, respectively.

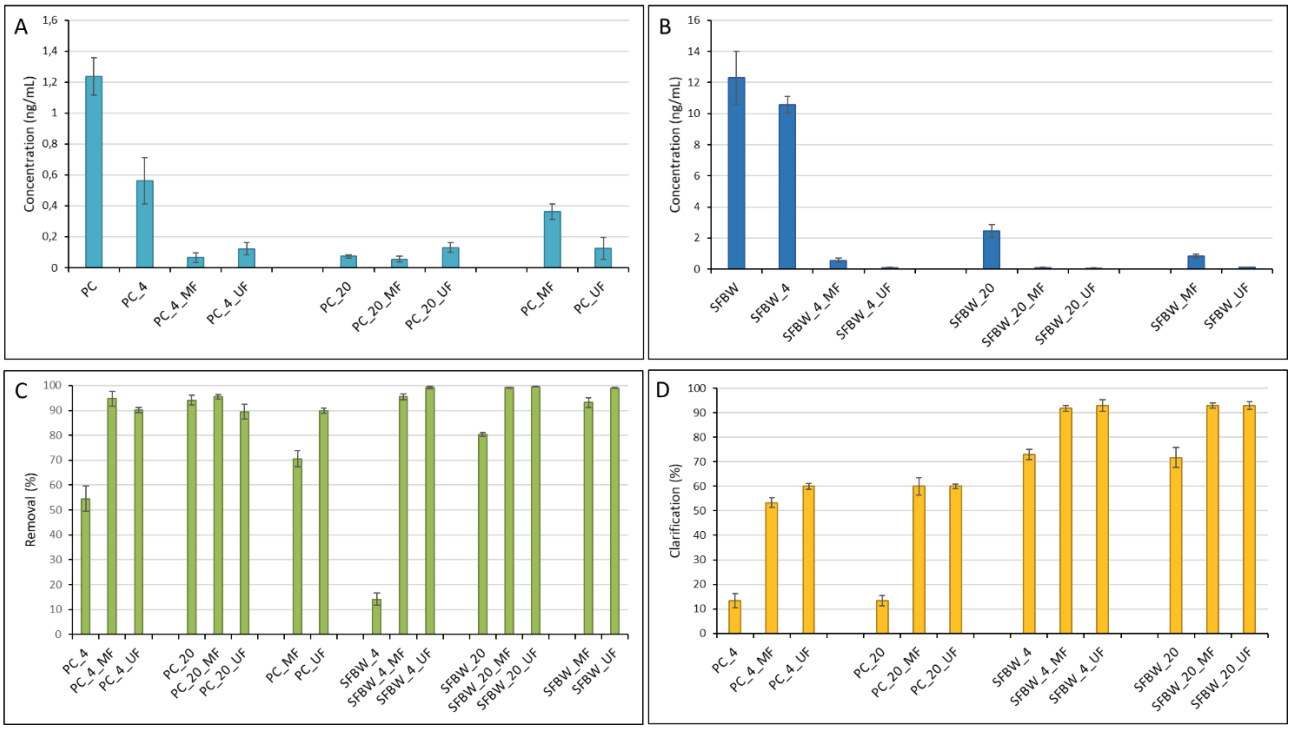

Fig. 3. Microcystin removal and clarification efficiencies after each process in PEMF and PEUF tests with the addition of 4 and 20 mg/L chitosan. **A)** Microcystin concentrations in pure culture (PC) after chitosan-enhanced coagulation-flocculation, MF, and UF. **B)** Microcystin concentrations in sand filter backwash water (SFBW) after chitosan-enhanced coagulation-flocculation, MF, and UF. **C)** Overall microcystin removal efficiencies of PC and SFBW samples. **D)** Clarification efficiencies of PC and SFBW samples.

As shown in **Fig. 3D**, the clarification efficiencies of the PEMF and PEUF tests confirmed the microcystin removals in the experiments. Chitosan-aided coagulation-flocculation had approximately 13.3% clarification efficiency in PC samples at both concentrations, and it increased to 53.3% and 60% after MF at the concentrations of 4 and 20 mg/L, respectively. There was not a remarkable contribution of UF to the clarification, since the efficiency was measured as 60% in both flows. Similarly, there was not a difference in the clarification efficiency between 4 and 20 mg/L of chitosan in the SFBW samples after the coagulation-flocculation process, calculated as 72.9% and 71.8%, respectively. When MF and UF were applied to SFBW samples after the coagulation-flocculation, the maximum clarification efficiencies were obtained as 91.8% and 92.9%, respectively.

436 **4. Discussion**

437 According to the historical data, the cyanobacteria proliferated multiple times throughout the year in
438 Lake Castreccioni reaching a bloom usually in spring-summer periods. Furthermore, there were some
439 periods when the microcystin concentrations reached up to 10 µg/L with respect to the dense
440 cyanobacteria occurrence and the average concentration ranged from 0.6 µg/L to 3.81 µg/L, which
441 often exceeded the limit set by WHO (1 µg/L). Our results are in line with the previous study carried
442 out by Manganelli and co-authors (2016), where *P. rubescens* was found to be dominant in another
443 meso-eutrophic lake in Central Italy (Vico Lake) with 0.5-5 µg/L of microcystin in the drinking water
444 collection point. The authors also found no relevant correlation between *P. rubescens* density nor
445 toxicity and total phosphorus, phosphate, and inorganic nitrogen. In another Italian drinking water
446 basin, high levels of cyanobacteria (up to 160 × 10^6 cells/L) and microcystin (28.4 µg/L) were
447 recorded over 28 months of monitoring, which was mostly represented by *P. rubescens* (Bogialli et
448 al., 2013). A discharge of microcystin-contaminated freshwater into a coastal environment primarily
449 originated from *P. rubescens*, has also been reported in the Adriatic coast, Italy (Rita et al., 2014). It
450 is among the predominant cyanobacterial species in many Italian regions (Funari et al., 2017). *P.*
451 *rubescens* produces mainly variants of the hepatotoxin microcystin, known to be associated with
452 oncological and endocrinological risks. At high levels of exposure (representative of acute
453 intoxication), microcystin produces a cascade of events (e.g. cytoskeleton alterations, lipid
454 peroxidation, oxidative stress, apoptosis), while at low doses (typical of long-term exposure),
455 phosphatases inhibition induces cellular proliferation and hepatic hypertrophy (Funari et al., 2008).
456 Lake Castreccioni is characterized by relatively low nutrient values (oligo-mesotrophic lake), which
457 is typical for most Italian lakes (Rogora et al., 2018). DO is one of the key parameter indications for
458 a healthy aquatic ecosystem and 4 to 6 mg/L of DO indicates a good water quality (Avvannavar and
459 Shrihari, 2008). The oxygen levels in Lake Castreccioni also pointed a healthy ecosystem. According
460 to the data obtained in our study during the monitoring period, there were not any notable changes
461 either in phosphorous nor in nitrate concentration (except in several cases) in the water intake which

462 resulted to have no significant interaction between the cyanobacterial growth as well as microcystin
463 concentration and nutrient concentration. Carvalho et al. (2011) also found that the nutrient
464 concentrations were not the key drivers for cyanobacteria growth. Our results also reflect those of
465 Zamyadi et al. (2012) who could not determine any correlation between the physico-chemical
466 parameters such as pH, turbidity, dissolved oxygen, conductivity, and microcystin concentration.
467 Like other organisms, cyanobacteria also require trace metals for their growth especially as cofactors
468 in electron transfer apparatus (e.g. cytochromes, plastocyanin) in photosynthesis, and a sufficient
469 amount of trace metals are necessary. Despite manganese is required for the metabolic functions in
470 the organism, it is mostly found at high levels in aquatic environments and is not considered as a
471 limiting factor (Facey et al., 2019). Contrarily, high levels can be toxic for cyanobacteria which results
472 in a reduction of growth rates (Rueter and Petersen, 1987). In fact, we found a significant negative
473 correlation between cyanobacteria abundance and manganese concentration. In accordance with our
474 findings, Giles et al. (2016) also recorded an increased manganese concentration during the bloom
475 collapse period. Although the blooms were correlated with atmospheric temperature in our study, we
476 could not determine any correlation between the algal bloom and water temperature.
477 *P. rubescens* is stenoterm species, its optimal growth temperature is 12 °C (Walsby et al., 2005), and
478 Lake Castreccioni provides a favorable temperature for its growth. Many factors have a role in the
479 phytoplankton succession such as ecological requirements and competition between species (Hadas
480 et al., 2002) and *P. rubescens* is superior in competition with other phytoplankton species thanks to
481 regulating its buoyancy (Akçaalan et al., 2014). When the environmental conditions are not
482 convenient for their growth, they can migrate to the appropriate depth and benefit from the conditions
483 and proliferate. A strong correlation between the cyanobacteria abundance and microcystin
484 concentration was inherently found in our study, which is also introduced by the WHO. Since the
485 toxin analysis is quite costly and cannot be performed regularly, the WHO proposed two threshold
486 levels for cyanobacteria density (Merel et al., 2013). Our correlation analysis also revealed that within
487 all climatic parameters, cyanobacteria density was only in relation to humidity and atmospheric

488 temperature. In a study carried out by Zhang et al. (2016), significant correlations were found for
489 algal bloom area, wind speed, and air pressure. On the other hand, similar to our study, they could
490 not determine any correlation between algal growth and precipitation, also the main contributors in
491 the lake were atmospheric temperature, air pressure, and wind speed.
492 There are many studies (summarized in the **e-Supplementary file)** on the removal of various
493 cyanobacteria species and/or cyanotoxins via coagulation using chitosan (Pei et al., 2014; Xu et al.,
494 2013) as well as other coagulants (Sun et al., 2013). Whereas this approach is common, with and
495 without membrane processes, it was mainly used to remove *M. aeruginosa* and not *P. rubescens*.
496 Moreover, depending on the quantity and quality of the cyanotoxins, membrane technologies have
497 been reported with high removal rates on cyanobacteria and/or cyanotoxins (Campinas and Rosa,
498 2010; Gijsbertsen-Abrahamse et al., 2006; Sorlini et al., 2013). The most common type is UF, while
499 MF was not often applied and compared to UF, as we demonstrated in our study. Finally, either
500 enriched pure culture or real lake water was used in these studies and SFBW was not tested so far.
501 Sorlini et al. (2013) obtained high removals of cyanobacteria (*Anabaena lemmermannii*) (98%) and
502 total algae (99%) using a pilot-scale MF and further reported severe fouling that caused a rapid
503 increase in the transmembrane pressure. In addition, UF has also been used to treat SFBW to enable
504 safe reuse in a DWTP (Reissmann and Uhl, 2006). Regarding membrane processes, such as UF that
505 shows superb separation properties while treating dense-cyanobacteria water of lakes or reservoirs,
506 they still face challenges due to membrane fouling and poor rejection of soluble organics (Liu et al.,
507 2017). A combination of coagulation and membrane processes (e.g. PEMF, PEUF) can effectively
508 remove cyanobacteria and associated cyanotoxins as proposed and confirmed with the results of this
509 study while alleviating the fouling on the membranes (Yan et al., 2017). At this point, the
510 determination of proper coagulant dosage is an important factor controlling membrane fouling in the
511 membrane process of algal-rich water as highlighted by Park and colleagues (2019) that tested the
512 combination of PACl-coagulation and MF.

513 Our results remarked that there was an apparent difference in the microcystin concentrations in both
514 effluents, PC, and SFBW, after the coagulation at 4 and 20 mg chitosan/L. Although 4 mg chitosan/L
515 was comparatively more efficient to remove microcystin in PC, a high concentration of microcystin,
516 as well as the more complex nature of SFBW, required a higher dosage of chitosan. After the
517 coagulation-flocculation-clarification steps, 20 mg chitosan/L achieved relatively 0.73 and 4.69-fold
518 higher microcystin removal rates in PC and SFBW compared to 4 mg/L, respectively. The difference
519 in the increment of removal efficiency between these two water matrices can be due to the better
520 formation of flocs via the suspended matter in SFBW. In the study of Jin et al. (2017), chitosan
521 quaternary ammonium salt (HTTC) was used to coagulate *Microcystis aeruginosa* cells in different
522 coagulation conditions. The authors found complete removal of algal cells at the HTCC dosage of
523 1.5 mg/L, rapid mixing for 0.5 min at 5.04 g, and slow mixing for 30 min at 0.20 g. On the contrary,
524 chitosan was found to be effective in the removal of *Microcystis* spp. but not *Cylindrospermopsis*
525 spp. (Miranda et al., 2017). This finding points out the importance of algal species and the selection
526 of relevant coagulants.
527 Furthermore, there was not a remarkable difference between PEMF and PEUF in terms of overall
528 microcystin removal and clarification efficiencies. In all conditions, the microcystin concentration in
529 the final effluents was < 0.15 µg/L. A slight increase in microcystin concentration was even observed
530 in the final PEUF effluents in PC samples. This can be contributed to high transmembrane pressure
531 that may have caused a slight release of microcystin (Gijsbertsen-Abrahamse et al., 2006).
532 Meanwhile, direct MF and UF applications without prior coagulation resulted in poorer removal
533 efficiencies. Although PEMF and PEUF resulted in similar removal efficiencies, the superiority of
534 UF over MF in terms of microbial elimination should also be noted. PAC-aided coagulation and UF
535 were investigated by (Dixon et al., 2011) with above 90% removal of intracellular and extracellular
536 cyanobacterial toxins. The authors highlighted the importance of PAC-coagulation on cell removal
537 and improving the flux of the UF. Similarly, up to 94% of microcystin was effectively removed when
538 the coagulation/flocculation and PAC systems were combined with UF membranes, while

coagulation/flocculation alone could not lead to an appreciable removal of microcystin (Şengül et al., 2018). Eventually, the adaptation and optimization of such hybrid systems are crucial since their performance is governed by multiple mechanisms.

Although polymer-enhanced membrane treatment is a promising approach for the removal of cyanobacteria and cyanotoxins from drinking water, most studies on PEUF were carried out on a small scale to date. The biggest obstacle in membrane-based systems is high capital expenditure (CAPEX) as well as a high operational expenditure (OPEX) for membrane replacements due to fouling. For instance, a pilot test was conducted by Lermontov et al. (2014) to make a comparative cost assessment of conventional water treatment (with coagulation, flocculation, settling, filtration steps followed by chlorine for disinfection) and UF unit. Even though UF comprises a higher CAPEX, its net present value shows a lower cost than the conventional option. When considering the land required and its cost for each solution, the UF solution indicates an equivalent or even more financially feasible option, since the UF-based DWTP requires eight times less land than a conventional DWTP. Hybrid systems that include polymer-aided coagulation/flocculation before UF can ease the membrane fouling and thus help to decrease OPEX in the long term. However, water-soluble polymers added to the feed solution to carry out PEUF can often cause severe foulant in some cases (Huang and Feng, 2019). From the coagulant point of view, the unit price of chitosan is still much higher than that of traditional inorganic flocculants (Yang et al., 2016), but its environmental benefits can encompass the high operational cost. Farid et al. (2013) indicated that flocculant chitosan for water treatment on the industrial scale can be provided with the price of about $1.5/kg, while calculated the cost of coagulation/flocculation process of microalgae around $0.0246/(kg dry biomass) including (nano)chitosan and acetic acid. Hence, novel and simplified techniques can be developed to improve the functionality and to decrease the cost of chitosan-based flocculants (Yang et al., 2016).

5. Conclusion

564 The historical data of the water intake supplied from Lake Castreccioni revealed unconventional
565 water quality (manganese) and climatic (humidity and atmospheric temperature) correlations with the
566 dense cyanobacteria periods while no correlation was found with conventional indicators such as
567 nutrients and water temperature. This can lead to alternative choices of parameters for future decision
568 support systems to control cyanotoxins in the drinking water supply chain and put different
569 management/treatment options in place if needed. In addition to the occurrence of microcystin in the
570 water intake of the DWTP, SFBW was found to be another critical flow (microcysin > 12 µg/L). Such
571 intermediate waste flows during drinking water treatment often exhibit a high level of cyanotoxin
572 accumulation and therefore should not be neglected and thoroughly treated before being discharged
573 into water bodies or recirculated within the plant. The overall PEMF and PEUF test results enabled
574 to reach the concentration of microcystin for national drinking water standards and also indicated
575 great removal efficiencies (>99%) to eliminate microcystin in the final effluent of the SFBW.
576 Although PEMF can be applied for water flows with low concentrations of microcystin, PEUF can
577 be more effective at higher concentrations and/or on more complex water matrices like SFBW. In
578 addition, higher chitosan dosage (i.e. 20 mg/L) performed better in terms of microcystin removal in
579 both water flows, while it did not have a major effect on the overall removal performance of the
580 further membrane units. Thus, the required chitosan dosage may also vary with respect to water
581 matrix and/or initial microcystin concentration. The superior removal efficiency of PEUF systems as
582 well as their help to alleviate membrane fouling highlights the cruciality of multiple barrier
583 approaches for removing cyanotoxins from drinking water. However, further research is needed to
584 test bench- and/or pilot-scale PEUF units in continuous operation and to understand the relationship
585 between cyanobacterial removal and possible re-release of cyanotoxins as well as the fouling
586 mechanism of membranes before these results can be generalized.

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ENVIRONMENTAL POLLUTION

E-SUPPLEMENTARY FILE

Monitoring of cyanobacterial blooms and assessing polymer-enhanced micro- and ultrafiltration for microcystin removal in an Italian drinking water treatment plant

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Fig. S1. Lake Castreccioni, Cingoli (Marche Region, Italy).

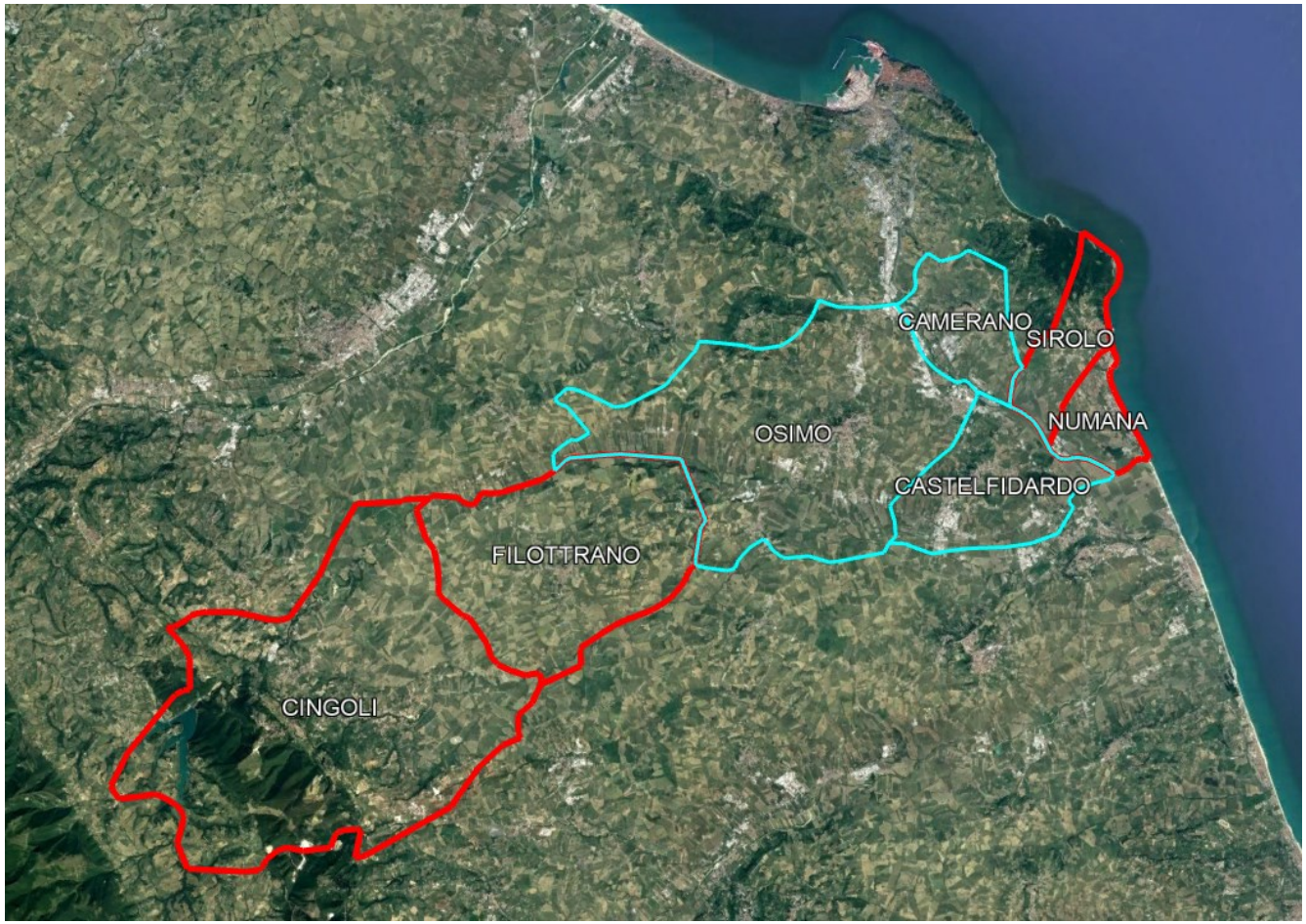

Fig. S2. Basin of the municipalities through the Castreccioni pipeline.

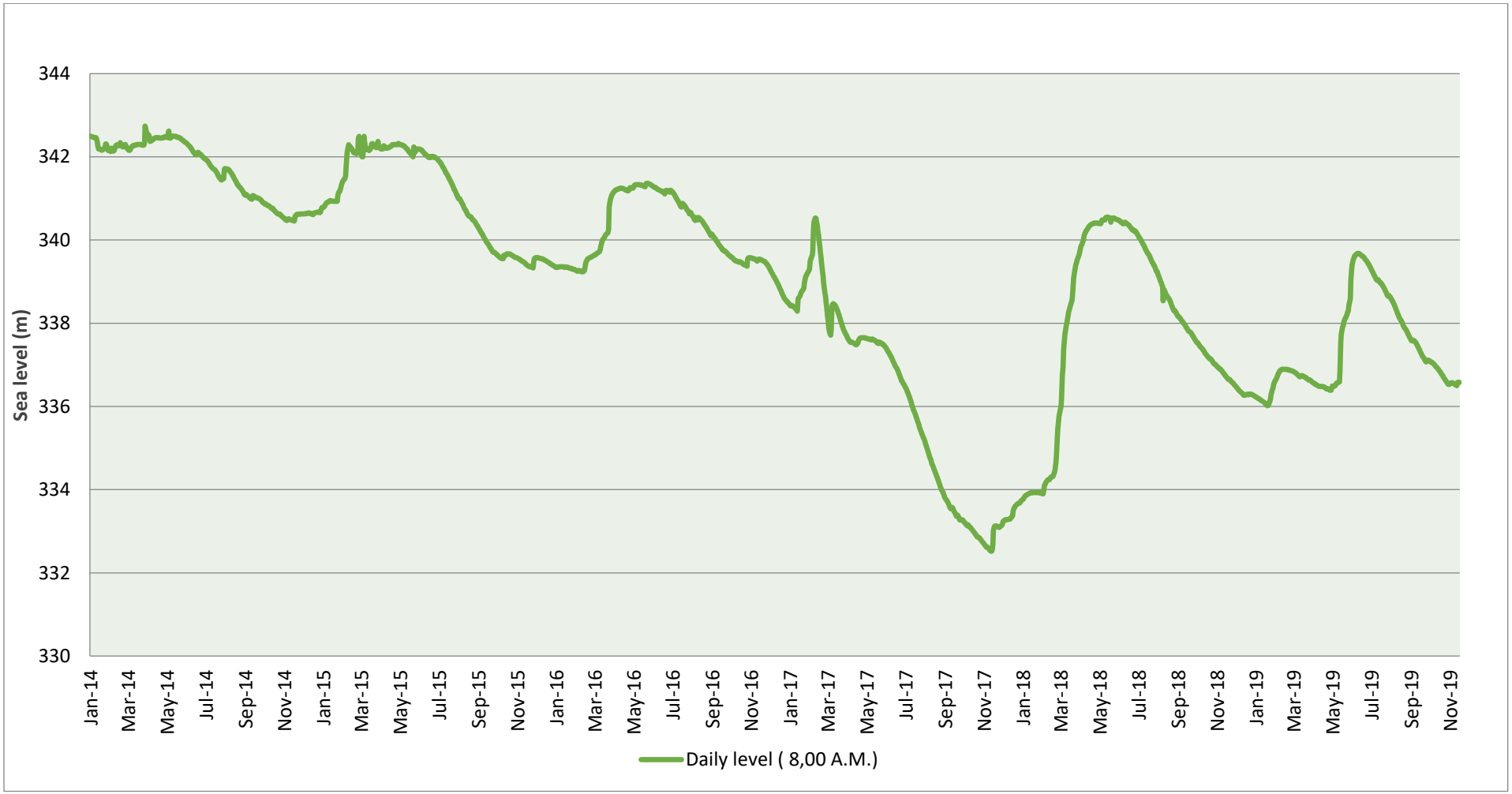

Fig. S3. Water level in Lake Castreccioni during the monitoring period.

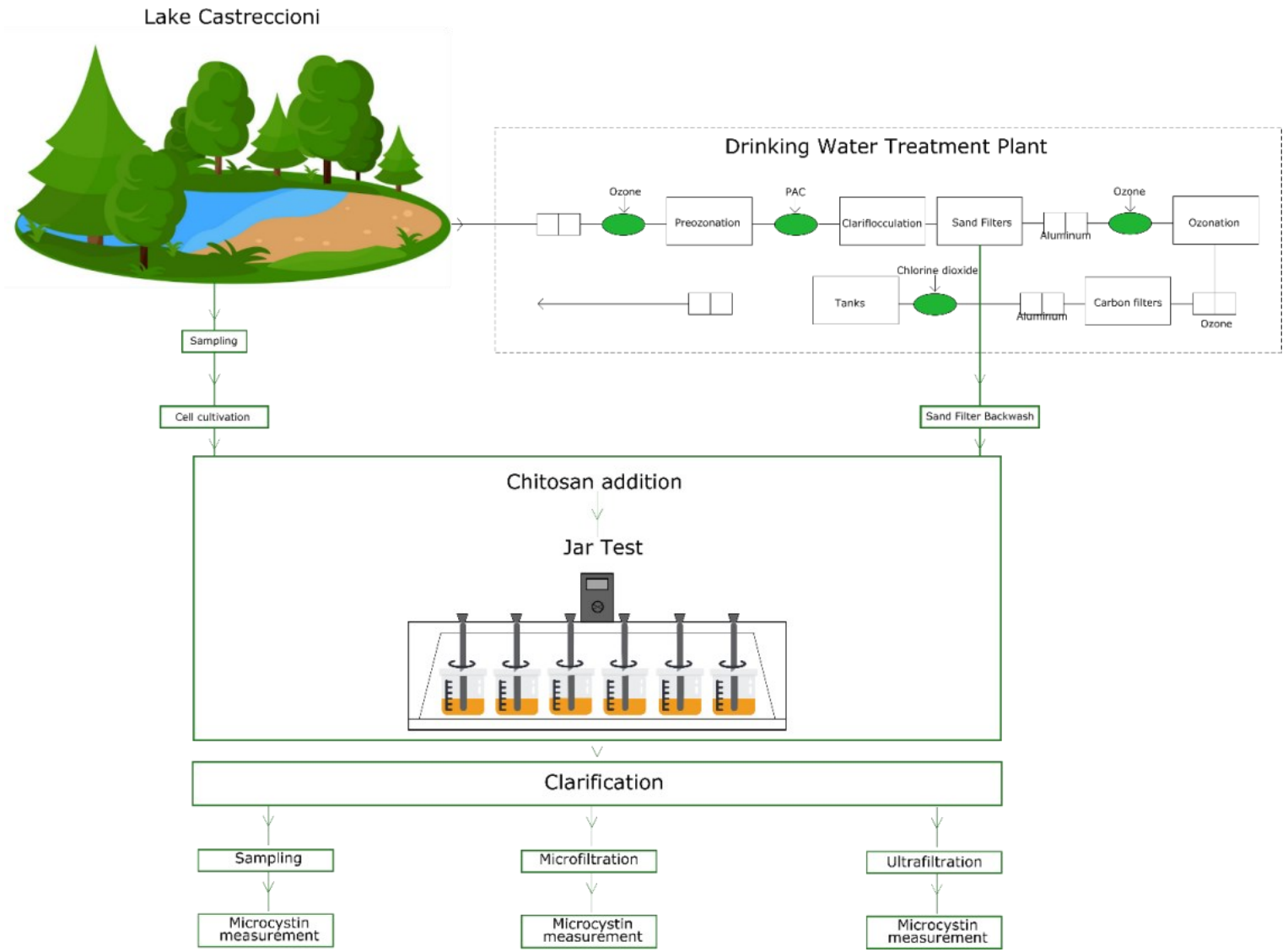

Fig. S4. Experimental design and scheme in PEUF and PEMF tests.

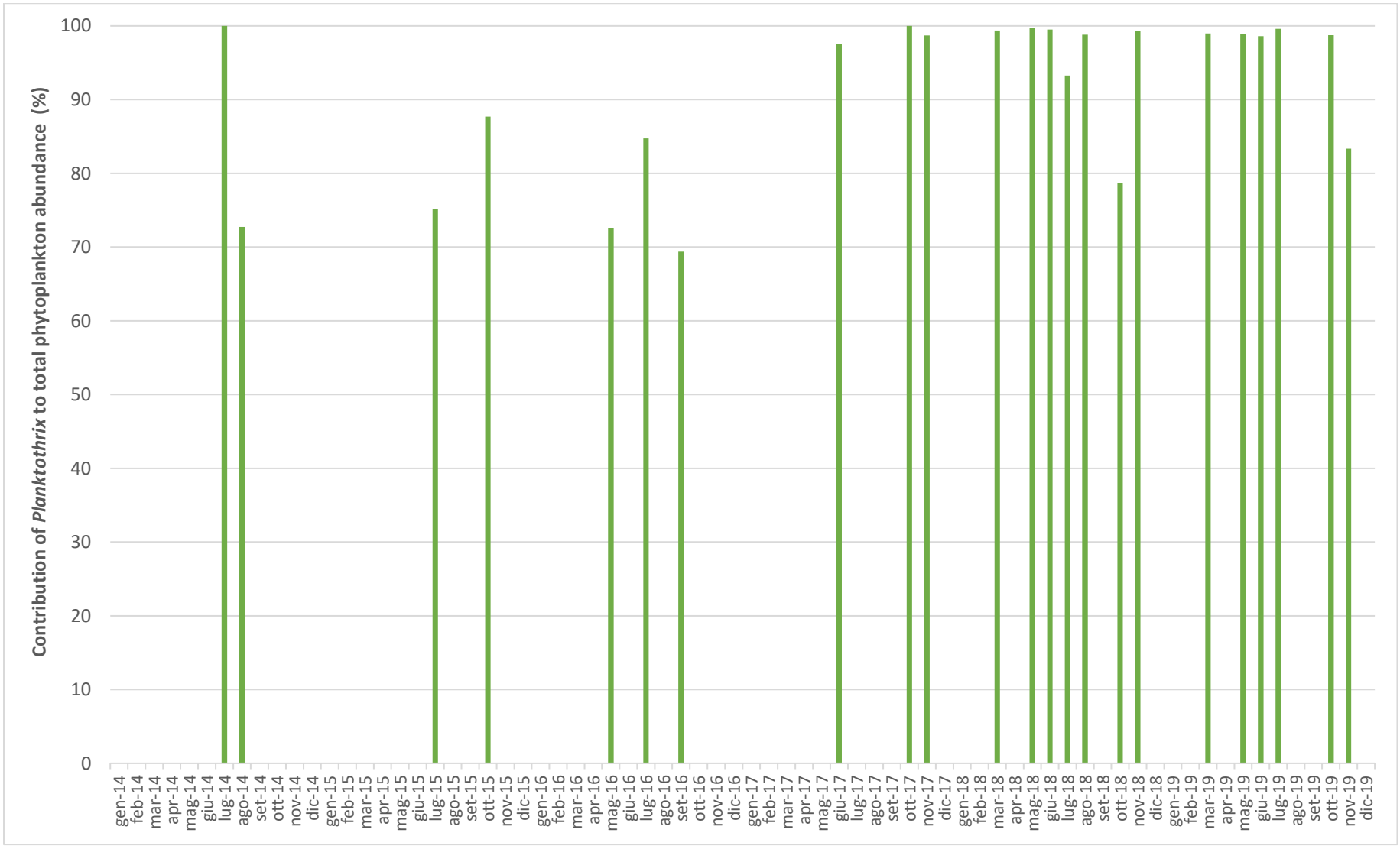

Fig. S5 The contribution of *Planktothrix* to total phytoplankton abundance in the water intake of Castreccioni DWTP.

Table S1. Blast result of *Planktothrix rubescens* PRMC1119 (GenBank Accession number MW785248) from this study showing the first best matches.

Scientific Name	Cover%	Identity%	Accession number
uncultured *Planktothrix* sp.	100	100.00	HG973449.1
uncultured cyanobacterium	100	100.00	HQ386321.1
Planktothrix rubescens CCAP 1459/14	100	100.00	FJ184422.1
Planktothrix rubescens 1NA15S1	100	100.00	DQ264184.1

Table S2. Temporal variations of biotic and abiotic characteristics of in the water intake of Castreccioni DWTP.

	2014			2015			2016			2017			2018			2019		
	Ave	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**
DO (mg/L)	8.62	8.6	8.8	1.28	0.1	5.1	4.65	0.1	7	1.73	0.1	5.1	3.62	1.4	4.8	6.66	4.9	7.4
EC (µS cm^{-1})	576.58	465	1507	508.92	466	553	485.08	469	505	488.75	451	548	499.67	475	523	485.09	416	548
Water Temperature (°C)	8.42	7	10	8.58	6	11	9.33	7	12	9.96	1	12	8.62	6.2	10.4	10.87	6.4	16.2
pH	7.68	7.3	8	7.71	7.4	8	7.81	7.5	8.2	7.69	7.3	8.1	7.72	7.5	8.1	7.85	7.5	8.1
Alkalinity (mg/L)	259	250	268	255	250	260	249.08	241	255	273.75	260	285	241.67	235	245	182.73	180	190
HCO_3(mg/L)	260.92	250	270	248.17	225	270	235.83	230	260	241.25	230	270	242.42	223	290	237.27	230	250
TOC (mg/L)	3.08	<3.00	3.49	<3.00	<3.00	<3.00	3.09	<3.00	3.6	3.38	<3.00	4	3.65	<3.00	5.27	3.88	<3.00	8.1
NH_4(mg/L)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.11	<0.10	0.15	<0.10	<0.10	<0.10	7.55	<0.10	82
NO_3(mg/L)	<5.00	<5.00	6	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	5.42	<5.00	10	7.17	<5.00	12	5.09	<5.00	6
PO_4(mg/L)	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00	<3.00
Fe (µg/L)	28.92	<20.00	82	24.67	<20.00	41	25.17	<20.00	46	22.33	<20.00	41	22.25	<20.00	33	27.27	<20.00	61
Mn(µg/L)	11.5	<5.00	83	63.5	<5.00	136	33.33	<5.00	134	27	<5.00	89	9.67	<5.00	28	6.55	<5.00	17
Ca(mg/L)	73.5	69	78	75.5	70	81	62.78	53	74.3	51.92	38	64	74.75	73	79	73.45	70	77
Mg(mg/L)	8.5	7.6	9.4	8.8	8.5	9.1	8.13	7.1	9.6	7.59	5.2	15	9.13	8.8	9.6	9.45	8.5	10
Na(mg/L)	15.25	14	16.5	13.55	11	16.1	9.54	0.17	13	11.75	8	17	16.25	13	19	19.73	17	23
K(mg/L)	3.75	3.7	3.8	3.33	3.1	3.55	3.07	2.6	3.7	2.56	1.1	3.7	3.23	2.7	3.7	3.87	3.8	3.9
SO_4(mg/L)	49.5	48	51	47.5	45	50	52.58	51	55	54.07	50	61	57.75	53	65	55.91	54	61
Cl (mg/L)	21	21	21	21.5	21	22	23	22	24	24.88	23.5	26	22	20	24	22.55	22	24
Al (µg/L)	33	20	42	49.75	28	70	43.75	20	67	83.17	34	183	45.33	20	100	25.45	20	36
Zn (mg/L)	<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	<0.05	<0.05	<0.05	<0.05	<0.05
Pb (µg /L)	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Ba (mg/L)	0.14	0.13	0.14	0.57	0.13	1	0.55	0.1	1	0.16	0.1	0.64	0.02	0.01	0.16	0.04	0.01	0.12
Total Coliforms *(cfu/100 mL)*	577.75	30	3000	797.5	37	3000	358.5	24	3000	742	26	5700	138.75	12	850	265	28	1000
E.coli (cfu/100 mL)	98.17	<1.00	350	4.42	<1.00	16	4.08	<1.00	30	145.42	<1.00	1700	5.33	<1.00	28	8	<1.00	19

Cyanobacteria (cell/L)	19808907	832300	60402960	16716090	207580	106509000	9717563	297540	57066880	20033925	906311	53891584	6526061	2522640	25729062	14350671	482735	58309544
Microcystin (µg/L)	3.81	0	9.54	2.22	0	10.3	0.9	0	5.85	2.26	0.15	9.3	0.57	0.15	2.96	1.41	0.15	5

Table S3. A comparison of related studies to remove cyanobacteria cells and associated toxins.

Flow	Coagulant/adsorbent	Membrane	Cyanobacteria type (overall removal)	Cyanotoxin type (overall removal)	Removal note	Reference
Enriched pure culture	Chitosan	-	*Chlorella sorokiniana* (99%)	n.a*	Clarification via optical density	(Xu et al., 2013)
Enriched pure culture	PACl	-	*Microcystis aeruginosa* (up to 94.3%)	n.a*	Removal via Chlorophyll-a concentrations	(Sun et al., 2013)
Enriched pure culture	Chitosan	-	*M. aeruginosa* (99%)	n.a*	Cell count via microscope	(Pei et al., 2014)
Enriched pure culture	Chitosan quaternary ammonium	-	*M. aeruginosa* (93.6%)	n.a*	Removal via Chlorophyll-a concentrations	(Zhu et al., 2016)
Enriched pure culture	Chitosan quaternary ammonium	-	*M. aeruginosa* (up to 100%)	n.a*	Removal via Chlorophyll-a concentrations	(Jin et al., 2017)
Lake water from bloom event	Chitosan	-	*M. aeruginosa* (n.a)*	n.a*	Removal via Chlorophyll-a concentrations	(Lürling et al., 2017)
Lake water from bloom event	PAC and chitosan	-	*M. aeruginosa* and *P. agardhii* ((up to 99%))	n.a*	Removal via Chlorophyll-a concentrations	(de Magalhães et al., 2017)
Lake water from bloom event	PAC and chitosan	-	*Cylindrospermopsis* and *Microcystis*	n.a*	Removal via Chlorophyll-a concentrations	(Miranda et al., 2017)
Enriched pure culture	Chitosan	-	*M. aeruginosa* (n.a)*	Microcystin (n.a)*	-	(Mucci et al., 2020)
Enriched pure culture, lake water from bloom event	-	UF, NF	*Planktothrix agardhii* (n.a)*	Microcystin (96-98%)	-	(Gijsbertsen-Abrahamse et al., 2006)

Enriched pure culture	-	UF	*M. aeruginosa* (100%)	Microcystin (60-80%)	Removal via Chlorophyll-a concentrations	(Campinas and Rosa, 2010)
Lake water from bloom event	-	MF	*Anabaena lemmermannii* (98-100%)	Microcystin (100%)	Cell count via microscope	(Sorlini et al., 2013)
Enriched pure culture	-	UF	*M. aeruginosa* (n.a)*	Microcystin (up to 57%)	Removal via cellular organic matter	(Liu et al., 2017)
Lake water from bloom event	Aluminium chlorohydrate and PAC	UF	*A. circinalis* (100%) and *M. flos-aquae* (100%)	Saxitoxin (90%) and microcystin (92%)	Count via Sedgewick–Rafter chamber	(Dixon et al., 2011)
Artificially contaminated deionized water	*Moringa oleifera* seeds	UF	*Microcystis* sp. (20-91%)	n.a*	Cell count via microscope	(Nishi et al., 2012)
Enriched pure culture	PAC	UF	*M. Aeruginosa* (n.a)*	Microcystin (94%)	-	(Şengül et al., 2018)
Enriched pure culture	Al, permanganate, manganese oxide	UF	*M. aeruginosa* (n.a)*	n.a*	-	(Yan et al., 2017)
Lake water from bloom event	PACl	MF	*Microcystis sp.* (n.a)*	n.a*	-	(Park et al., 2019)
Enriched pure culture and SFBW	Chitosan	MF, UF	*Planktothrix rubescens* (up to 92.9%)	Microcystin (up to >99%)	Clarification via optical density	This study

* n.a: not available

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