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In situ H_2O_2 treatment of blue-green algae contaminated reservoirs causes significant improvement in drinking water treatability

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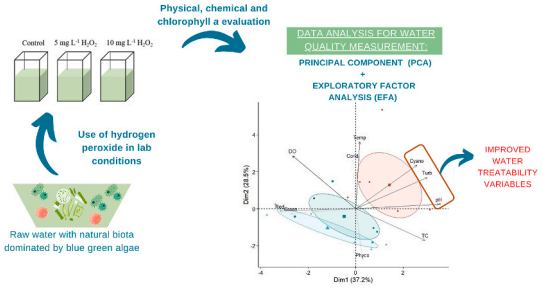
HIGHLIGHTS

- H_2O_2 improved water treatability by reducing turbidity, pH, and cyanobacterial chlorophyll-*a* concentration.
- Exploratory factor analysis (EFA) can be applied to elucidate the individual characteristics of a given reservoir.
- EFAs are able to indicate which variables are important to monitor to determine the effectiveness of *in situ* treatment.
- EFA is a useful tool for monitoring the effects of water treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

The evaluation of water quality improvement brought about by *in situ* treatment of eutrophic water bodies, especially those used for human supply is a challenging task since each water system responds differently. To overcome this challenge, we applied exploratory factor analysis (EFA) to understand the effects of using hydrogen peroxide (H_2O_2) on eutrophic water used as a drinking water supply. This analysis was used to identify the main factors that described the water treatability after exposing blue-green algae (cyanobacteria) contaminated raw water to H_2O_2 at both 5 and 10 mg L^{-1}. Cyanobacterial chlorophyll-*a* was undetectable following the application of both concentrations of H_2O_2 after four days, while not causing relevant changes to green algae and diatoms chlorophyll-*a* concentrations. EFA demonstrated that the main factors affected by both H_2O_2 concentrations were turbidity, pH, and cyanobacterial chlorophyll-*a* concentration, which are important variables for a drinking water treatment plant. The H_2O_2 caused significant improvement in water treatability by decreasing those three variables. Finally, the use of EFA was demonstrated to be a promising tool in identifying which limnological variables are most relevant concerning the efficacy of water treatment, which in turn can make water quality monitoring more efficient and less costly.

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1. Introduction

Eutrophication is a growing concern worldwide (Padedda et al., 2017). It contributes to water quality deterioration and increases harmful algae blooms (HABs) in many lakes and reservoirs (Glibert, 2020). Eutrophication may cause the increase of water turbidity, depletion of dissolved oxygen, and negatively impact fish and other aquatic organisms (Kong et al., 2017; Zi et al., 2018). In addition, the effect of eutrophication can be intensified in tropical regions because of high ambient temperatures and light intensities, contributing to cyanobacterial dominance (Paerl and Huisman, 2008; Haakonsson et al., 2017; Munoz et al., 2019).

Eutrophic waters and HABs present considerable challenges to water treatment plants (WTPs) (Wert et al., 2013, 2014). In addition to the increase of turbidity, cyanobacterial cells may break through to the treated water, accumulate, or lyse in conventional WTP unit processes (coagulation, flocculation, clarification, and filtration), releasing intracellular metabolites and decreasing drinking water safety (Fan et al., 2014; Pestana et al., 2019). There is an urgent need for the development of complementary treatment strategies to help reduce cyanobacterial cells and their metabolites efficiently before raw water enters WTPs.

Hydrogen peroxide (H_2O_2) has received attention as a potential alternative to mitigate HABs *in situ* since it does not leave any chemical residues or disinfection byproducts in the aquatic environment (Santos et al., 2021; Matthijs et al., 2012). Despite this, the initial application may present potential ecotoxicological effects if extreme dosages are applied. Different studies at laboratory and *in-situ* scale (mesocosm) have suggested a concentration of 10 mg/L as the maximum concentration to avoid any negative effects to the phytoplankton and zooplankton community (Matthijs et al., 2012; Weenink et al., 2015, 2022; Santos et al., 2021).

Additionally, natural UV radiation increases the rate of H_2O_2 decomposition, generating reactive oxygen species (ROS) thus increasing the efficiency of organic matter removal (Barrington et al., 2013). Many studies have indicated that cyanobacteria are more sensitive to H_2O_2 than other groups of phytoplankton due to their cellular structure and physiological characteristics, which indicates that the treatment with H_2O_2 can selectively remove cyanobacteria (Matthijs et al., 2012; Sinha et al., 2018; Yang et al., 2018).

The use of H_2O_2 for in-reservoir treatment, in addition to reducing cyanobacteria densities, can also improve other limnological parameters important to water treatment processes such as turbidity, color, dissolved and particulate organic matter, and pH (Menezes et al., 2021; Patki et al., 2021). However, due to the complex nature of monitoring and interpreting the magnitude of H_2O_2 efficiency on parameters important to water treatability only few studies tackled this topic (Varol, 2020). The application of an established statistical tool to normalize the effects of the H_2O_2 treatment on improving the water quality would not only be beneficial for the interpretation of the data but would also simplify comparisons between different treatment strategies and reservoirs.

Multivariate statistics approaches, such as Exploratory Factor Analysis (FEA) and Principal Component Analysis (PCA), can be helpful to interpret environmental systems with many different variables that are intercorrelated. Although those techniques have been frequently used to identify which parameters are important in a water resource system and to develop natural water quality indices (Barros et al., 2020; Tripathi and Singal, 2019; Varol, 2020), to the authors' knowledge no reports on using those tools to assess the water quality improvements after the use of treatment with H_2O_2 exist. This paper aims to apply EFA and PCA to evaluate the effects of H_2O_2 on water quality variables relevant to water treatment plants in a reservoir used for human supply.

2. Materials and methods

2.1. Study area and reservoir water quality

Raw water samples were collected in February and March 2019, from Gavião Reservoir (Fig. 1) in the Northeast of Ceará State (Brazil) at a depth of 90 cm next to the WTP intake (Table and Fig. S1 for average details on physico-chemical and biological parameters of the reservoir). This reservoir has a storage capacity of 33.3 million m^3, a surface area of 5.9 km^2 (COGERH, 2020), which exclusively supplies water to more than 4 million inhabitants in the metropolitan region of Fortaleza (IBGE, 2010). This reservoir, historically, has a high cyanobacterial diversity and is either hypereutrophic or eutrophic with cyanobacterial cell densities as high as 1.16 million cells/mL. Therefore, this reservoir is a suitable model for WTPs and water management agencies worldwide that are challenged with cyanobacterial blooms (COGERH, 2021; Barros et al., 2019, 2020).

2.2. Effect of H_2O_2 on the phytoplankton community

Two jar-test apparatus with six containers each filled with 1.4 L of raw water from Gavião reservoir with a stirring mechanism were used to perform the experiments. A propeller rotation of approximately 70 rpm (velocity gradient $\approx 69.4\ s^{-1}$) was maintained to provide homogeneous suspension. The H_2O_2 concentrations applied to each condition were 5 mg L^{-1} and 10 mg L^{-1}. In addition, the control samples were set up simultaneously using jars with the same raw water but with no H_2O_2 added. The H_2O_2 concentration was monitored daily. On day four the H_2O_2 concentration was below detection limit. Then, samples (5 mL) from each jar were collected (section 2.3.1) and eight days after the application of H_2O_2 new samples were collected again. All the experimental conditions were performed in triplicates.

The experiment was initiated immediately after the raw water collection, and it focused on analyzing the effects of H_2O_2 (5 mg L^{-1} and 10 mg L^{-1}) on the chlorophyll-*a* concentration and photosynthetic yield of the phytoplankton community (cyanobacteria, green algae, and diatoms). Physical-chemical variables important to the water treatment process (pH, temperature, conductivity, turbidity, true color, and dissolved oxygen), and phycocyanin concentration were also analyzed in all samples.

Throughout the treatment, the light source was provided by an LED light. A digital lux meter (YF – 1065 F, Taiwan) was used to measure the light intensity in Lux, and then this measure was converted to photosynthetic photon flux density. Average photosynthetic photon flux density was 10 μmol photons $m^{-2}s^{-1}$ in a light/dark cycle of 12 h:12 h. This intensity was used in other bench-scale studies with cyanobacteria and H_2O_2 (Menezes et al., 2021). This photon flux density is considerably lower compared to the photon flux density that can be expected in the equatorial region (~780 μmol photons $m^{-2}s^{-1}$ - Ritchie, 2010). The lower flux density was chosen to be able to observe the effects of the H_2O_2 on the phytoplankton community in the raw water as higher flux densities made changes so rapid as to be unobservable (data not shown).

2.3. Laboratory analysis

2.3.1. Hydrogen peroxide quantification

The concentration of the H_2O_2 stock solution was determined before the start of the experiment by an iodometric method (Skellon and Wills, 1948). Since the main objective of the present study was to assess the final impact of H_2O_2 on the native phytoplankton and changes in physical-chemical variables used for water treatability assessment, the degradation kinetics of H_2O_2 were not evaluated. Therefore, a semi-quantitative method (Quantofix® Peroxide test strips) was used daily to identify when H_2O_2 reached the limit of detection of this method (<0.5 mg H_2O_2 L^{-1}). Further, this approach was selected as the hydraulic retention time of Gavião Reservoir is 20 days with a constant

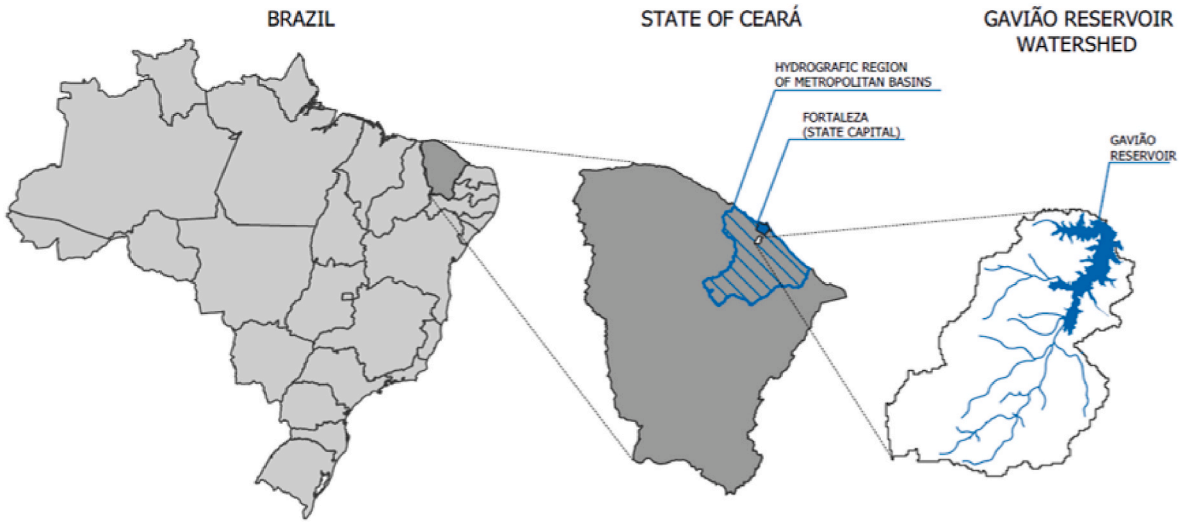

Fig. 1. Location of Gavião reservoir in the metropolitan basin within Ceará State in the Northeast of Brazil.

influx of fresh raw water (Ceará, 2017), which is considered a very short retention time for reservoirs of that size; thus, the results of the oxidation equilibrium would be sufficient information in regard to our determination of the effects of H_2O_2 on the treatability variables of the raw water.

2.3.2. Pigment and photosynthetic activity estimation

To quantify chlorophyll-*a* and elucidate the effect of H_2O_2 on the phytoplankton community, the PHYTO-PAM II Phytoplankton Analyzer (Walz, Germany) was used. Chlorophyll-*a* is excited at four different wavelengths making it possible to distinguish phytoplankton groups with different types of light-harvesting pigment antennae (Walz, 2003), estimating the chlorophyll-*a* concentration (μg L^{-1}) from cyanobacteria, green algae, and diatoms. Additionally, using the same equipment, the effective quantum yield of photosynthetic energy conversion in photosystem II (φPSII) was determined at 64 μmol photons. m^{-2} s^{-1} for those three phytoplankton groups considering the following equation (Genty et al., 1989):

$$\varphi PSII = \Delta F / Fm = (Fm - F) / FM \quad (1)$$

where is the maximum fluorescence and is the minimal fluorescence, which is obtained when emitted a low intensity measuring light, which is measured momentarily before the saturation pulse is applied (Figueroa et al., 2017; Houliez et al., 2013; Le Rouzic, 2012). Phycocyanin detection was performed according to Bennet and Bogorad (1973), in which the water sample was concentrated in a centrifuge, the supernatant was removed, and the concentrated sample was frozen and thawed three times to break the cells. Then, sodium azide and distilled water was added and the mixture was again centrifuged. Finally, the sample was read in the spectrophotometer (wavelengths 615 and 652 nm) and the phycocyanin concentration was then calculated.

2.3.3. Physico-chemical analyses

Physical and chemical analyses were performed for all samples to evaluate the effect of H_2O_2 on variables used to assess water treatability: True color (TC) was measured by sample absorbance at 455 nm according to method 2120 C (Eaton et al., 2005). Organic matter was estimated by sample absorbance at 254 nm according to Eaton et al. (2005). To determine turbidity a 2100 P turbidimeter (Hach, USA) was used and analyses were performed according to method 2130-B (Eaton et al., 2005). Sample pH and dissolved oxygen were determined using Model 60 and 55 probes respectively (both YSI, USA). Conductivity and salinity were determined using a 105 A probe (Orion, USA).

2.4. Data analysis

2.4.1. Exploratory factor analysis

An exploratory factor analysis (EFA) was used to evaluate the impact of different H_2O_2 concentrations on latent variables. The latent variables represent the changes of the measured variables (pH, true color, organic matter 254 nm, temperature, turbidity, conductivity, salinity, dissolved oxygen, cyanobacteria, green algae, and diatom chlorophyll estimation). The EFA algorithm diagram is presented in Fig. 2. The EFA was performed using RStudio statistical software applying the library obtained from Revelle (2021). This library contains tools to perform multivariate analysis using factor analysis, principal component analysis, cluster analysis and reliability analysis.

In this step, a Pearson's correlation matrix was created to assess if there were any inappropriate correlations ($r < 0.3$). In the case of inappropriate variables (IV) for the factor analysis, they must be excluded before the analysis is performed (Lu et al., 2016).

ii. Evaluating the measure of sampling adequacy

The Measure of Sampling Adequacy (MSA) is a value used to assess the adequacy of the inter-correlations among other variables in a dataset and was used to select which IV should be excluded. The IV that presented MSA >0.5 were separated from the general dataset. After that, all the possible subsets of 1 to *n* features were obtained, where *n* is the number of IV. Each subset was then removed from the general dataset and the Kaiser-Meyer-Olkin (KMO) was calculated. The KMO is a measure that can vary from 0 to 1 and indicates the degree that each variable in a dataset is predicted without error. Then, the subset removal that generated a dataset with the highest value of KMO was the chosen dataset. This was the variable selection algorithm.

iii. Confirmation of MSA

Bartlett's test of sphericity ($p < 0.05$) and the determinant of the matrix (det>0) were used to confirm the choice of the dataset.

iv. Selection of the EFA number of factors

Parallel analysis was used to preliminarily estimate the number of factors to retain. The first step to determine the number of factors was to simulate a random dataset that has the same range and number of items of the observed values. Then, the eigenvalues were extracted and plotted for both, the simulated and observed data. The eigenvalue is directly

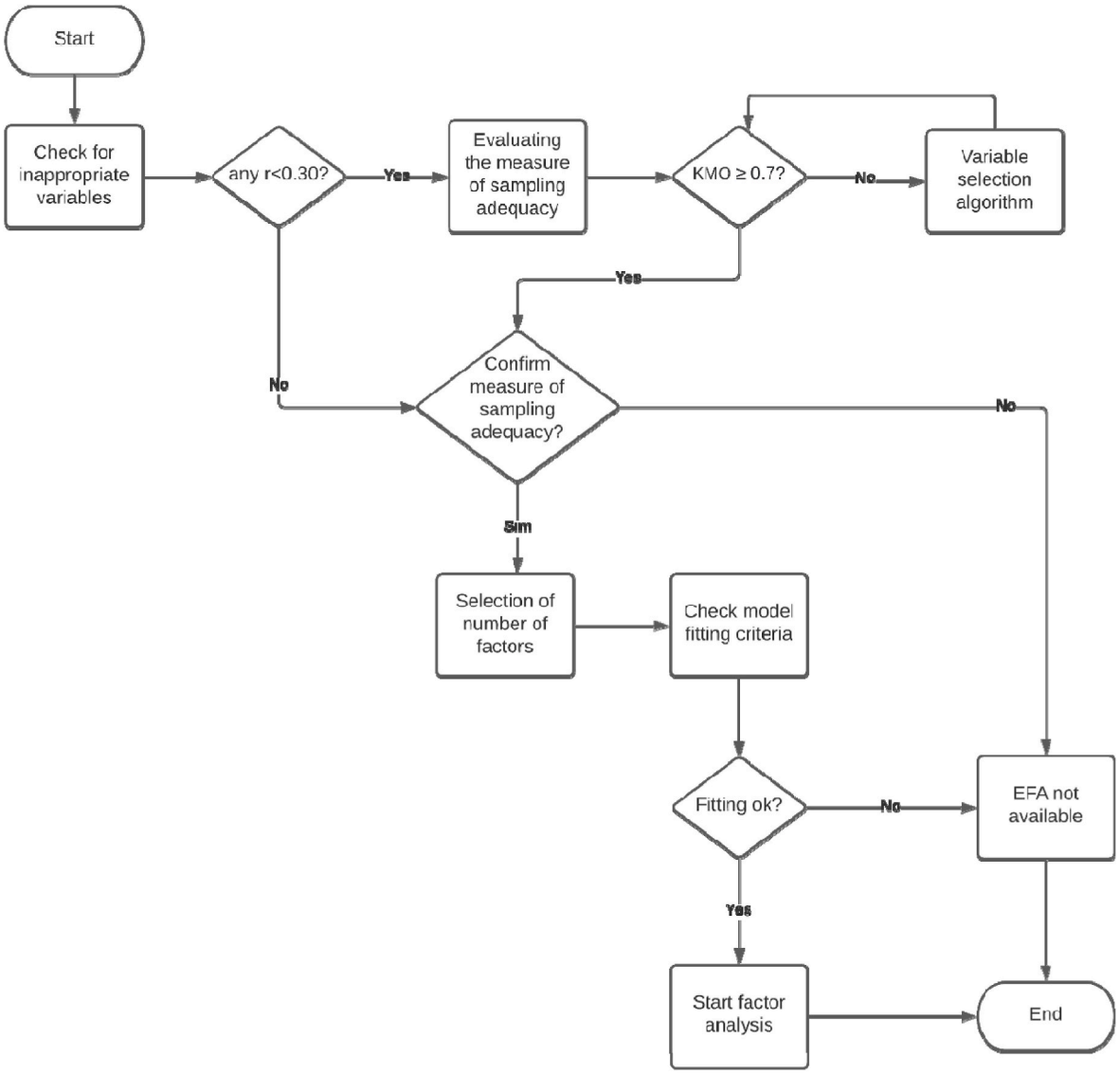

Fig. 2. Diagram presenting the step sequence to perform the factor analysis algorithm.

i. Checking for inappropriate variables

proportional to the variance of the correlation matrix, the higher the eigenvalue, the higher the variance within the correlation matrix (Zanker et al., 2022). The maximum number of factors in which the observed eigenvalues are larger than the simulated eigenvalues is the number of factors to retain. To verify the best fit of the final model, the numbers of factors tested were n-1, n, and n+1, where n is the number of factors obtained after the parallel analysis.

v. Checking the model fitting criteria

First, the orthogonal (varimax) and oblique (promax) rotations were evaluated to find more easily interpretable results. In addition, those rotations were used to confirm, or not, the existence of a correlation for the factors to be used (Revelle, 2021). Then, the Tucker-Lewis index (TLI) and root mean square error of approximation (RMSEA) were calculated. According to Brown (2006) and Preacher et al. (2013), TLI values should be higher than 0.9 or, preferably 0.95, and RMSEA values should be lower than 0.02. The details of the algorithm used to determine the number of factors that should be used on the EFA are available in the supplementary material.

The correlation among each factor was obtained using maximum likelihood (ML) and oblique rotation. Also, communality – which is the variability ratio of each variable that is explained by the four factors – was calculated as an extra fitting criterion. Also, for a variable to continue on the EFA, its communality value must be higher than the specific variance. Watkins (2020) states that communality values above 0.6 are considered robust, which indicates that the variables are well represented by their factors.

2.4.2. *Effect of H_2O_2 on the water quality of the latent variables*

The adjusted model made it possible to evaluate if the effect of the H_2O_2 treatment was significant using the score of each factor. The factor score was obtained with a linear combination where the weights are the factor loading. The factor loading is the correlation coefficient for the variable and factor i.e., it shows the variance explained by the variable on that particular factor.

A non-parametric test was performed to evaluate if the changes in the factors due to the use of H_2O_2 were significant or not. The non-parametric test was the Wilcoxon-rank sum exact with pairwise comparison and p-value adjustment with Bonferroni *post hoc* ($p < 0.05$).

3. Results and discussion

Water quality modeling can present some difficulties, due to non-linear and non-stationary patterns (Rousso et al., 2020). To evaluate patterns and relationships, a high number of observations and variables are needed. Supplementary Table S2 presents a set of limnological variables with their main descriptive statistics to detect the effect of the application of H_2O_2 on water quality.

3.1. Effect of H_2O_2 on the phytoplankton community

Although Table S2 shows that the H_2O_2 treatment caused clear changes on part of the limnological variables, drawing conclusions about the improvements on the water treatability and which variables are more important for water quality is challenging due to their large number. For instance, the mean value of true color decreased but pH did not change considerably (Table S2). As for the biological variables, the treatment with both concentrations of H_2O_2 improved water quality by reducing the cyanobacterial chlorophyll to a level below the limit of detection the method (Fig. 3). This improvement can have highly positive effect on WTPs (section 3.2.4), as a reduction of chlorophyll-a, in general, represents a reduction in the biological load of the raw water, which, in turn, means that lower dosages of treatment chemicals can be applied and that filter run times are extended, all of which represents a significant economical and energy saving. Additionally, if the chlorophyll-a is of cyanobacterial origin, drinking water safety is improved, provided the cyanobacterial assemblage in the raw water is non-toxigenic or potentially released cyanotoxins are otherwise mitigated against. On the other hand, both H_2O_2 concentrations (5 and 10 mg L^{-1}) did not present great changes in the chlorophyll estimation from the diatoms and green algae groups (Fig. 3A). Green algae even showed an increase on the 8th day compared to the control condition and became the most dominant phytoplankton after the treatment (Fig. 3B). Additionally, neither diatom and green algae groups presented any change in their photosynthetic activity as cyanobacteria showed in both concentrations and time when comparing to control (Fig. 4).

The results of this experiment were similar to other studies which demonstrated that cyanobacteria are more sensitive to H_2O_2 than green algae and diatoms (Matthijs et al., 2012; Sinha et al., 2018). Cyanobacteria do not produce sufficient enzymes to eliminate reactive oxygen species (ROS) generated after the application of H_2O_2, causing the oxidation of lipids and proteins in the cells, leading to loss of cellular membrane integrity, inactivation of enzymes, and, ultimately, leading to cell death (Sinha et al., 2018). The main enzymes responsible for ROS degradation are ascorbate peroxidase, not present in cyanobacteria cells, and the haem peroxidase family found only in some cyanobacteria (Bernroitner et al., 2009). Furthermore, Matthijs et al. (2012), proposed that the apparent high sensitivity of cyanobacteria to H_2O_2 could be related to the Mehler reaction. The Mehler reaction is a photosynthetic electron transfer that produces H_2O_2 in eukaryotic phytoplankton and higher plants when exposed to high light intensities. This reaction is suppressed in cyanobacterial cells by flavoproteins without further formation of ROS, suggesting that cyanobacteria are not adapted to these higher levels of oxidative stress and lacking the mechanisms to deal with the presence of these radicals.

Even though chlorophyll *a* concentration and photosynthetic yield are important to characterize water quality, they provided only isolated and individual conclusions and do not consider possible interactions between all the variables. Thus, a broad interpretation of the H_2O_2 impact on water treatability is desirable. Therefore, exploratory factor analysis was performed also considering pH, true color, organic matter (254 nm), conductivity, temperature, dissolved oxygen and phycocyanin concentration.

3.2. Factor analysis modeling

Although in some cases not all limnological variables monitored are used during the modeling process, it is important to maintain, at least preliminarily, the largest dimensionality possible to verify which variables are more relevant to the studied system. The level of correlation on the database was verified using the Pearson's correlation matrix (presented on the supplementary data). Since there were inappropriate correlations ($r < 0.3$) in Fig. S2, the dataset was not suitable for the EFA. Therefore, a process to adapt the database was performed before starting the EFA.

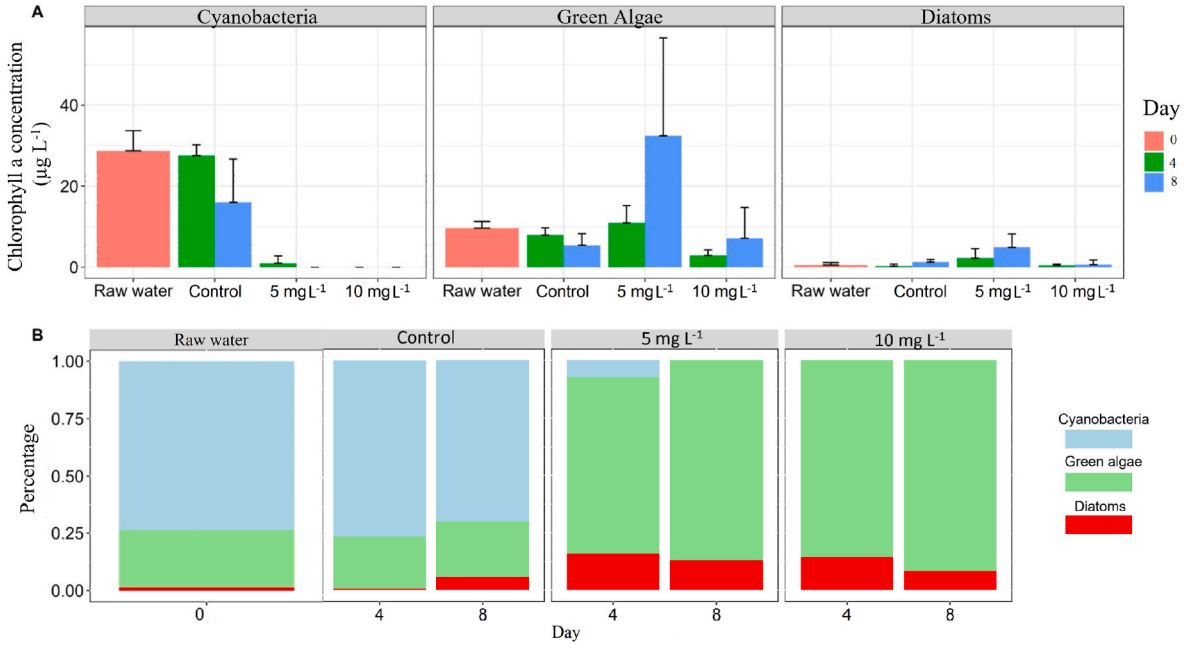

Fig. 3. Effect of H_2O_2 (5 and 10 mg L^{-1}) on the phytoplankton of Gavião reservoir compared to the raw water and the control condition (no H_2O_2) over 4 and 8 days after the application of the H_2O_2. (A) Shows the variation of estimated chlorophyll-*a* concentration due to the H_2O_2 application for each group of phytoplankton. The error bars represent the standard deviation (n = 3). (B) Shows the relative percentage of chlorophyll *a* from each phytoplankton group of the total chlorophyll *a*.

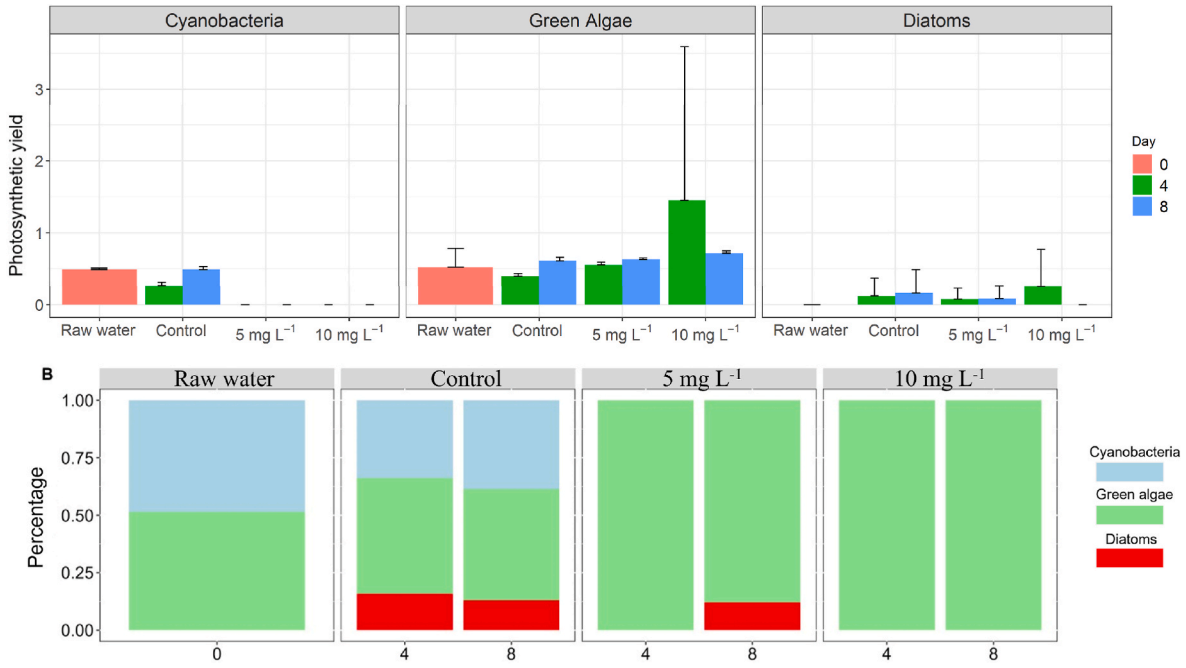

Fig. 4. Quantum yield of the photosystem II estimating the photosynthetic activity of major phytoplankton groups in the different H_2O_2 concentrations over time: (A) represents the variation of photosynthetic yield due to the H_2O_2 application for each group of phytoplankton. (B) Shows the relative percentage of the photosynthetic yield from each phytoplankton group of the total. The error bars of each mean in (A) represent the standard deviation (n = 3).

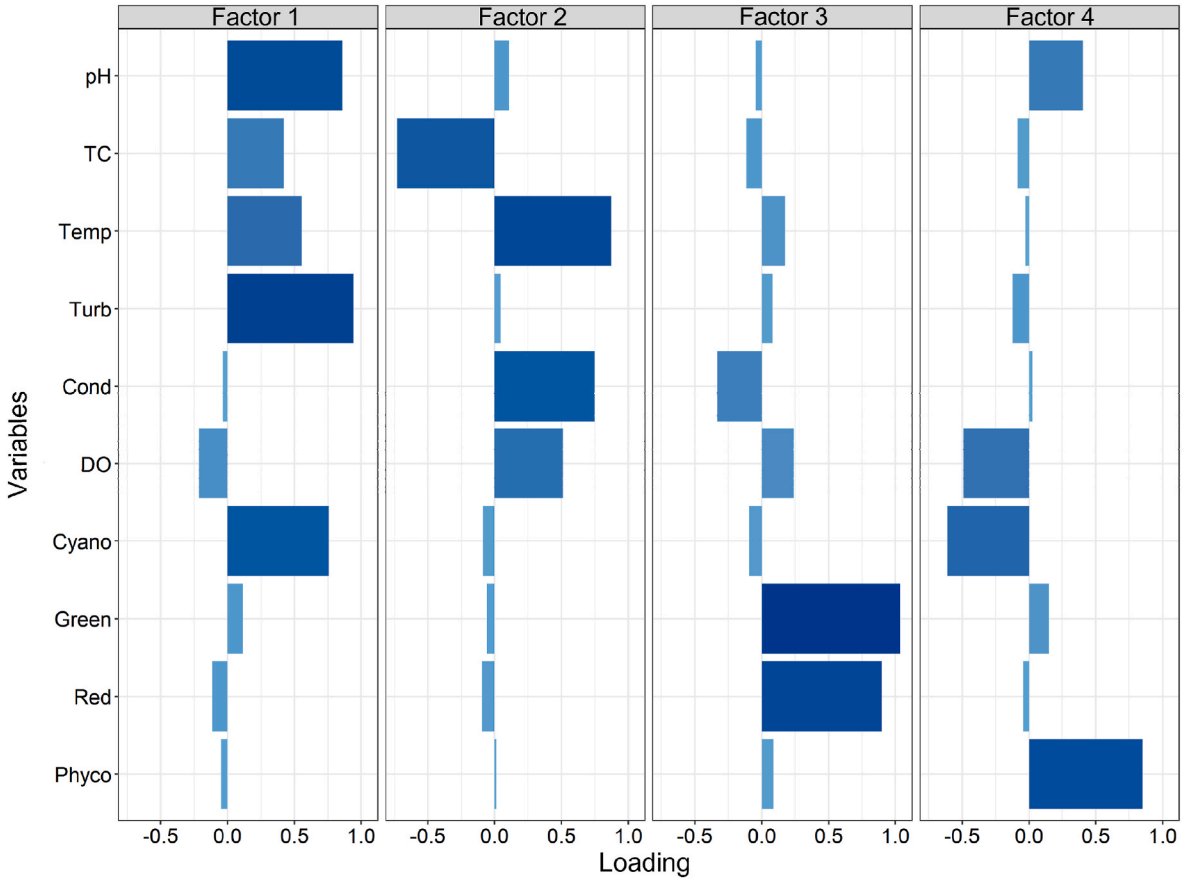

Fig. 5. Factor loading to analyze the correlation coefficient for the variable and the factor. The darker the bars, the higher is the correlation value. The variables chosen are represented by the darker bars (Loading >0.5). TC – True Color; Temp – Temperature; Turb – Turbidity; Cond – Conductivity; DO – Dissolved oxygen; Cyano – Cyanobacteria chlorophyll-*a* concentration; Green – Green algae chlorophyll-*a* concentration; Red – Diatom chlorophyll-*a* concentration; Phyco – Phycocyanin concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2.1. Adequacy of the dataset

This step was performed to extract the inappropriate variables (IV) for the EFA from the database. To select the IV, measure of sampling adequacy (MSA) was applied to the dataset, and variables with MSA<0.5 were considered inappropriate. Supplementary Fig. S3A shows that true color, organic matter 254 nm, temperature, turbidity, salinity, and phycocyanin presented MSA<0.5. Then, the variable selection algorithm was applied to the dataset and showed that the removal of organic matter 254 nm and salinity allowed for the best value of KMO (KMO = 0.7). The variable selection algorithm also improved the MSA values of the remaining variables (Fig. S3B). Besides the KMO criteria, Bartlett's sphericity test (354, gl, $p < 0.001$) and the matrix determinant (3.3981×10^{-5}) confirmed the adequacy of the remaining dataset, which indicates that the EFA could be performed (Brown, 2006; Reyment and Jvreskog, 1996).

3.2.2. – number of factors definition

Parallel analysis was performed to determine the preliminary number of factors for the EFA. This is a crucial step because extracting too many factors can reduce the quality of the analysis due to analyzing factors that are not relevant for that water treatability analysis. On the other hand, extracting too few factors can remove a factor that represents the system (Watkins, 2020).

Supplementary Fig. S4 shows the result of the parallel analysis, indicating that the best number of factors was three. However, the model fitting evaluation using the Tucker-Lewis index (TLI) showed that four factors had a better fit for the model. Therefore, to reduce over- or underestimation of the number of factors, models containing two, three, and four factors were adjusted based on TLI (>0.95) and RMSE (<0.02) values. The model using four factors presented a better fit.

3.2.3. – Grouping the variables into factors

After deciding the number of factors, the factor loading was calculated to define the variables that would be contained in each factor (factor loading>0.5). Fig. 5 shows that the variables that were similar and better explained the variance on factor 1 were: Turbidity, pH, and cyanobacteria chlorophyll-*a* concentration. Factor 2 were: Temperature, conductivity, true color, and dissolved oxygen; factor 3: green algae and diatoms Chlorophyll-*a* concentrations; factor 4: phycocyanin concentration.

Supplementary Fig. S5 presents the relevant correlations among each factor. Factor 1 is negatively correlated to factor 3 (−0.5), indicating that when turbidity, pH, and cyanobacteria chlorophyll-*a* concentration decrease, the green algae and diatom chlorophyll groups would increase. Factor 2 is negatively correlated to factor 4 (−0.4), suggesting that when there is a decrease in temperature, conductivity, and true color, phycocyanin concentration increases. The temperature seemed to be relevant for the experiment probably because the temperature influences the decomposition rate of H_2O_2. Arvin and Pedersen (2015) found that when the temperature increases, so does the H_2O_2 decomposition rate. However, the negative correlation of Factor 2 with Factor 4 should be further analyzed in a larger scale study (mesocosm), since the microcosm scale created a unique scenario and it appears that, in this case, the phycocyanin pigment would not be a good proxy for estimating viable cyanobacteria cells.

Then the communality was calculated to evaluate the model fitting. Supplementary Fig. S6 shows that all ten variables presented communality above 0.5 and that all variables of Factor 1 present high values of communality. The same trend can be observed for Factors 2, 3, and 4. It is important to highlight that even though conductivity presents the lowest value of communality from all the variables, it is still higher than its specific variance (represented by the darker grey in Fig. S6). Therefore, it is possible to infer that the four chosen factors well represented the original space of the ten variables, allowing a clear evaluation of the H_2O_2 effects on the water quality with a smaller number of variables.

3.2.4. – Effect of H_2O_2 on the factors

The factor analysis result (Fig. 6) showed that the H_2O_2 application promoted significant impact only in factors 1 and 4. For factor 1 (pH, chlorophyll-*a* concentration of cyanobacteria, and turbidity) the concentration of 5 and 10 mg L^{-1} H_2O_2 caused a similarly significant impact. As for factor 4, only the concentration of 10 mg L^{-1} caused a significant increase in phycocyanin concentration. Factors 2 and 3 (temperature, conductivity, true color, dissolved oxygen, and green algae and diatoms chlorophyll-a concentrations) were not significantly affected by the application of H_2O_2.

In general, the grouping of the variables within factors can provide a better understanding of the ecosystem dynamics and describe how they interact with each other. For instance, it is important to notice that factor 1 is formed by three variables essential for water treatment processes: turbidity, pH, and the presence of cyanobacteria (estimated by cyanobacteria chlorophyll-*a* concentration). Observing how the application of H_2O_2 significantly decreased the factor 1 score (Fig. 6), the model shows that the use of H_2O_2 improved water quality and treatability, since removing turbidity and cyanobacteria are the main goals (Clemente et al., 2020) and can directly affect the performance of a WTP (Zhang et al., 2021).

The EFA modeling confirmed that cyanobacteria were highly impacted by the application of H_2O_2. It is important to notice how the factor analysis grouped turbidity and cyanobacterial chlorophyll-*a* on the same factor, which may be because cyanobacteria can cause more turbidity per unit of phosphorus than eukaryotic phytoplankton (Scheffer et al., 1997). In addition, pH is an essential variable for the coagulation process in a WTP (Lv et al., 2018), Tables S1 and S2 shows that the value for the pH after the use of 10 mg L^{-1} H_2O_2 lowered from 9.15 (raw water) to an average of 8.37 on day eight. A decrease in pH due to H_2O_2 application was also observed by Fan et al. (2019), where the pH reached its lowest value (≅ 8) on day six.

Fig. 6 also shows how factors 2 and 3 did not significantly change after the application of H_2O_2. The tendency of variables in factor 2 (temperature, conductivity, DO, and TC) to remain unchanged was also observed in other studies using H_2O_2 treatment (Santos et al., 2021; Fan et al., 2019; Aguilera et al., 2014). The fact that variables in factor 3 (green algae and diatoms chlorophyll-*a* concentration) did not change consists of one of the principal advantages of the H_2O_2 application over other *in situ* treatments such as copper sulfate, is that it does not significantly harm non-target organisms such as green algae and diatoms (Yang et al., 2018). Additionally, since H_2O_2 did not appear to be harmful to the green algae and diatoms groups, it indicates that the dosage used in the present study could be further tested directly into the reservoir, for instance. On the negative side, however, if the application of H_2O_2 promotes the suppression of cyanobacteria, there could be significant growth of green algae and diatoms which in turn could burden the WTP by increasing the natural organic matter load arriving at the head of the WTP, increasing the need for coagulants and oxidants, and reducing filter run times (Dai et al., 2020; Pestana et al., 2019).

Even though factor 4 presented a significant increase (Fig. 6) with the use of 10 mg L^{-1} of H_2O_2, it does not mean that the phycocyanin concentration increased. Phycocyanin concentration decreased after the use of H_2O_2, but only on day eight (Table S2) suggesting that the H_2O_2 took longer to degrade phycocyanin. This trend was not observed by Yang et al. (2018), where the phycocyanin consistently decreased only one day after the H_2O_2 application, probably because the phycocyanin concentration in the water of the present study was much lower (7.47 μg L^{-1}) than in Yang et al. (2018) study's (300 μg L^{-1}). Phycocyanin concentration may not be suitable as a variable to evaluate the short-term efficiency of H_2O_2 application where the phycocyanin concentrations are low.

Also, PCA was performed to compare results with the ones obtained from EFA. PCA explained nearly 66% of the data variation and showed a clear division into two clusters: control and H_2O_2 application data (Fig. 7). There is an intersection between the 5 and 10 mg L^{-1} H_2O_2 data,

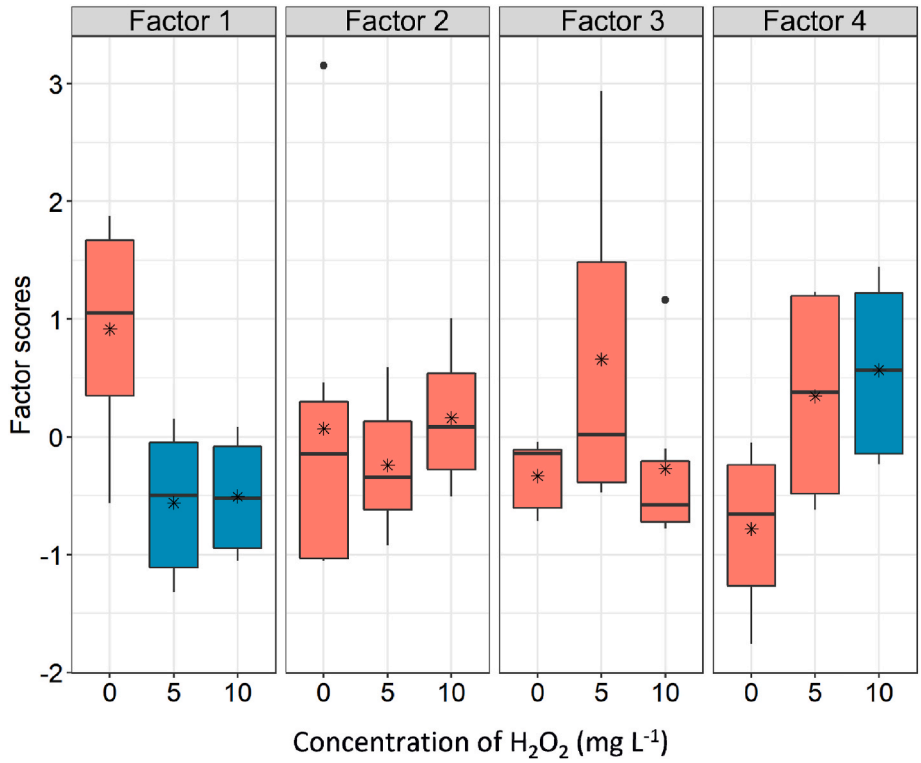

Fig. 6. Effect of the different concentrations of H_2O_2 on the four factors analyzed. Different color shows a significant difference ($p < 0.05$) and the asterisks represent the mean of the values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

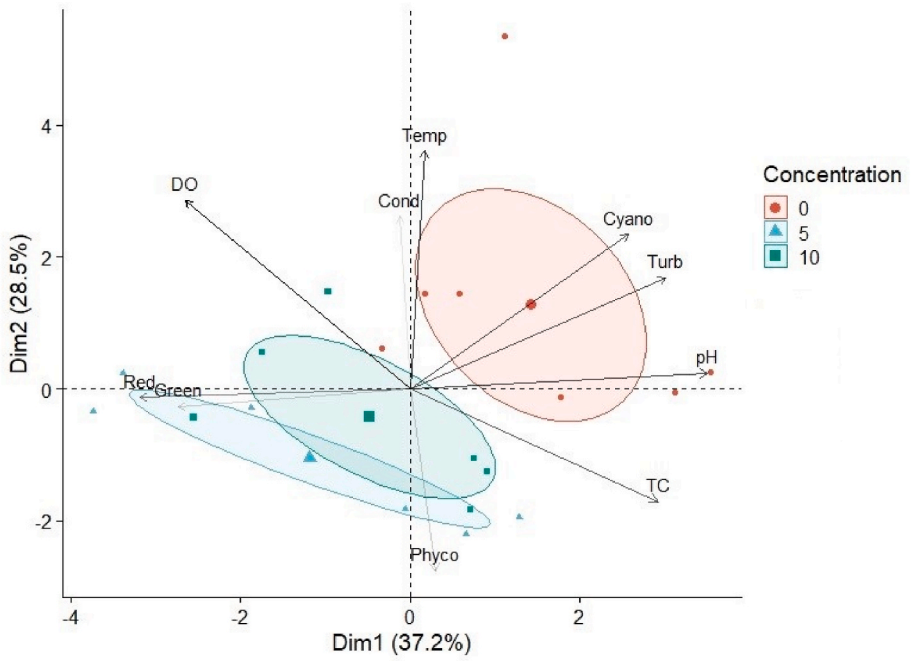

Fig. 7. Correlation values among the variables. The two dimensions observed on the axis explain approximately 66% of the data variation. Positively correlated variables are grouped together, and negatively correlated variables are positioned in opposite quadrants. Orthogonal variables mean no correlation. The color of the vector changes according to its value of squared cosine (that varies from 0 to 1), the closer the value is to 1 (represented by darker lines) means that the vector is more relevant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

indicating no clear difference between the impacts of the two H_2O_2 concentrations. It is important to observe how the three variables that form Factor 1 (pH, turbidity, and cyanobacteria chlorophyll-*a* concentration) are pointing in the same direction on the PCA graph (Fig. 7), indicating that they are positively correlated. In addition, diatom and green algae chlorophyll-*a* (Factor 3) are in the opposite quadrants from the variables that form Factor 1, indicating that those variables are negatively correlated.

It is important to observe also in Fig. 7 that the variables of phycocyanin, conductivity, and diatom and green algae chlorophyll-*a* presented the lowest values of squared cosine. This indicates that they are less representative of the changes observed in the factor map and, for the studied environmental system, those variables are less relevant for the water quality evaluation.

With the EFA and with PCA it was possible to understand the impacts of the H_2O_2 application on the water quality variables and how they

interact with each other. Since cyanobacteria blooms are a challenge for WTPs worldwide, the methodology applied in the present study can be a useful tool to evaluate and improve water treatment technologies. This is especially true, as the proposed techniques not only consider direct cyanobacterial variables, but also other ones that are crucial in determining water treatability of raw waters from different reservoirs.

4. Conclusions

The selection of a treatment strategy for water treatment must act simultaneously on different variables. Thus, some technologies can have isolated effective on results controlling cyanobacteria or reducing turbidity, but an important factor for any strategy to be considered suitable for water treatment is to control all treatability variables simultaneously.

The current study used Gavião reservoir as a model representing potable water reservoirs that are challenged with cyanobacterial blooms globally. This problem affects the water treatment and supply of many WTPs and needs to be better understood. The methods applied in the present study showed that the H_2O_2 had positive effect on the water treatability considering important parameters for a WTP (Factor 1: Turbidity, cyanobacterial chlorophyll, and pH). Another advantage of the use of H_2O_2 that was confirmed is that it is selective in the control of cyanobacteria without causing an excessive increase of the green algal and diatom chlorophyll.

Another important use of the methods applied in the present study is the evaluation of the most efficient and most suitable treatment strategy for a reservoir. For instance, we tested two concentrations of H_2O_2 (5 and 10 mg L^{-1}), and they displayed similar effectiveness. Therefore, the determining factor in selecting which concentration to apply may be the cost involved. Further studies of this subject matter would benefit from a cost-benefit analysis to determine the most economical approach while still ensuring treatment targets.

Therefore, based on the present study we recommend EFA to be used on further studies as a tool to determine the efficacy of a water treatment strategy and to identify the main factors that influence water treatability, since EFA effectively highlighted those main factors and how the H_2O_2 positively contributed to improving them. We also recommend including important variables on further studies with H_2O_2 and other similar oxidants such as cost, residence time of the reservoir and the impact of the presence of natural UV radiation.

Credit author statement

Maria Aparecida Melo Rocha: Investigation, Writing – Original Draft, Data Curation; Jessica da Silva Melo: Investigation; Carlos J. Pestana: Writing – Review and Editing; Linda A. Lawton: Writing – Review and Editing, Funding acquisition; Allan Clemente de Souza: Formal Analysis, Visualization, Data Curation; Allan Amorim Santos: Investigation, Writing – Review and Editing; José Capelo-Neto: Conceptualization, Supervision, Project administration, Writing – Review and Editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.138895.

References

Aguilera, A., Echenique, R., Giannuzzi, L., 2014. Application of hydrogen peroxide to the control of eutrophic lake systems in laboratory assays 2657–2675. https://doi.org/10.3390/toxins6092657.

Arvin, E., Pedersen, L.-F., 2015. Hydrogen peroxide decomposition kinetics in aquaculture water. Aquacult. Eng. 64, 1–7. https://doi.org/10.1016/j.aquaeng.2014.12.004.

Barrington, D.J., Reichwaldt, E.S., Ghadouani, A., 2013. The use of hydrogen peroxide to remove cyanobacteria and microcystins from waste stabilization ponds and hypereutrophic systems. Ecol. Eng. 50, 86–94. https://doi.org/10.1016/j.ecoleng.2012.04.024.

Barros, M.U.G., Leitaõ, J.I.R., Aranha, T.R.B.T., Simsek, S., Buley, R.P., Fernandez-Figueroa, E.G., Gladfelter, M.F., Wilson, A.E., Capelo-Neto, J., 2020. Icyano: a cyanobacterial bloom vulnerability index for drinking water treatment plants. Water Sci. Technol. Water Supply 20, 3517–3530. https://doi.org/10.2166/ws.2020.239.

Barros, M.U.G., Wilson, A.E., Leitão, J.I.R., Pereira, S.P., Buley, R.P., Fernandez-Figueroa, E.G., Capelo-Neto, J., 2019. Environmental factors associated with toxic cyanobacterial blooms across 20 drinking water reservoirs in a semi-arid region of Brazil. Harmful Algae 86, 128–137. https://doi.org/10.1016/j.hal.2019.05.006.

Bennet, Bogorad, 1973. Complementary chromatic adaptation in a filamentous blue-green alga. JCB (J. Cell Biol.) 58, 419–435. https://doi.org/10.1083/jcb.58.2.419.

Bernroitner, M., Zamocky, M., Furtmüller, P.G., Peschek, G.A., Obinger, C., 2009. Occurrence, phylogeny, structure, and function of catalases and peroxidases in cyanobacteria. J. Exp. Bot. 60, 423–440. https://doi.org/10.1093/jxb/ern309.

Brown, T.A., 2006. Confirmatory Factor Analysis for Applied Research. The Guilford Press, New York.

Ceará, 2017. Fundação Cearense de meteorologia e recursos hídricos: Inventário ambiental do açude Gavião [WWW Document]. URL. http://cdn.funceme.br/hidro-ce/uploads/documentos/inventarios/IVA-Gavi%C3%A3o_PSH.pdf. (Accessed 16 April 2023). accessed.

Companhia de Gestão de Recursos Hídricos (COGERH), 2020. Portal Hidrológico do Ceará [WWW Document]. URL. http://www.hidro.ce.gov.br/hidro-ce-zend/app/pagina/show/162. (Accessed 14 April 2020).

Companhia de Gestão de Recursos Hídricos (COGERH), 2021. Portal Hidrológico do Ceará [WWW Document]. URL. http://www.hidro.ce.gov.br/hidro-ce-zend/acude/eutrofizacao. (Accessed 20 February 2022).

Clemente, A., Wilson, A., Oliveira, S., Menezes, I., Gois, A., Capelo-Neto, J., 2020. The role of hydraulic conditions of coagulation and flocculation on the damage of cyanobacteria. Sci. Total Environ. 740 https://doi.org/10.1016/j.scitotenv.2020.139737.

Dai, R., Xiong, Y., Ma, Y., Tang, T., 2020. Algae removal performance of UV-radiation-enhanced coagulation for two representative algal species. Sci. Total Environ. 745 https://doi.org/10.1016/j.scitotenv.2020.141013.

Eaton, A.D., Association, A.P.H., Association, A.W.W., Federation, W.E., 2005. Standard Methods for the Examination of Water and Wastewater (Washington, D.C. APHA-AWWA-WEF).

Fan, F., Shi, X., Zhang, M., Liu, C., Chen, K., 2019. Comparison of algal harvest and hydrogen peroxide treatment in mitigating cyanobacterial blooms via an in situ mesocosm experiment. Sci. Total Environ. 694, 1–8. https://doi.org/10.1016/j.scitotenv.2019.133721.

Fan, J., Hobson, P., Ho, L., Daly, R., Brookes, J., 2014. The effects of various control and water treatment processes on the membrane integrity and toxin fate of cyanobacteria. J. Hazard Mater. 264, 313–322. https://doi.org/10.1016/j.jhazmat.2013.10.059.

Figueroa, F.L., Álvarez-Gómez, F., del Rosal, Y., Celis-Plá, P.S.M., González, G., Hernández, M., Korbee, N., 2017. In situ photosynthetic yields of cave photoautotrophic biofilms using two different Pulse Amplitude Modulated fluorometers. Algal Res. 22, 104–115. https://doi.org/10.1016/j.algal.2016.12.012.

Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron- transfer and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990 (1), 87e92.

Glibert, P.M., 2020. Harmful algae at the complex nexus of eutrophication and climate change. Harmful Algae 91. https://doi.org/10.1016/j.hal.2019.03.001.

Haakonsson, S., Rodríguez-Gallego, L., Somma, A., Bonilla, S., 2017. Temperature and precipitation shape the distribution of harmful cyanobacteria in subtropical lotic and lentic ecosystems. Sci. Total Environ. 609, 1132–1139. https://doi.org/10.1016/j.scitotenv.2017.07.067.

Houliez, E., Lizon, F., Artigas, L.F., Lefebvre, S., Schmitt, F.G., 2013. Spatio-temporal variability of phytoplankton photosynthetic activity in a macrotidal ecosystem (the Strait of Dover, eastern English Channel). Estuar. Coast Shelf Sci. 129, 37–48. https://doi.org/10.1016/j.ecss.2013.06.009.

Instituto Brasileiro de Geografia e Estatística (IBGE), 2010. Censo Demográfico.

Kong, X., Sun, Y., Su, R., Shi, X., 2017. Real-time eutrophication status evaluation of coastal waters using support vector machine with grid search algorithm. Mar. Pollut. Bull. 119, 307–319. https://doi.org/10.1016/j.marpolbul.2017.04.022.

Le Rouzic, B., 2012. Changes in photosynthetic yield (Fv/Fm) responses of salt-marsh microalgal communities along an osmotic gradient (Mont-Saint-Michel Bay, France). Estuar. Coast Shelf Sci. 115, 326–333. https://doi.org/10.1016/j.ecss.2012.09.012.

Lu, F., Chen, Z., Liu, W., Shao, H., 2016. Modeling chlorophyll-a concentrations using an artificial neural network for precisely eco-restoring lake basin. Ecol. Eng. 95, 422–429. https://doi.org/10.1016/j.ecoleng.2016.06.072.

Lv, M., Zhang, Z., Zeng, J., Liu, J., Sun, M., Yadav, R.S., Feng, Y., 2018. Roles of magnetic particles in magnetic seeding coagulation-flocculation process for surface water treatment. https://doi.org/10.1016/j.seppur.2018.11.011.

Matthijs, H.C.P., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J., 2012. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. Water Res. 46, 1460–1472. https://doi.org/10.1016/j.watres.2011.11.016.

Menezes, I., Maxwell-McQueeney, D., Capelo-Neto, J., Pestana, C.J., Edwards, C., Lawton, L.A., 2021. Oxidative stress in the cyanobacterium Microcystis aeruginosa PCC 7813: comparison of different analytical cell stress detection assays. Chemosphere 269. https://doi.org/10.1016/j.chemosphere.2020.128766.

Munoz, M., Nieto-Sandoval, J., Cirés, S., de Pedro, Z.M., Quesada, A., Casas, J.A., 2019. Degradation of widespread cyanotoxins with high impact in drinking water (microcystins, cylindrospermopsin, anatoxin-a and saxitoxin) by CWPO. Water Res. 163 https://doi.org/10.1016/j.watres.2019.114853.

Padedda, B.M., Sechi, N., Lai, G.G., Mariani, M.A., Pulina, S., Sarria, M., Satta, C.T., Virdis, T., Buscarinu, P., Lugliè, A., 2017. Consequences of eutrophication in the management of water resources in Mediterranean reservoirs: a case study of Lake Cedrino (Sardinia, Italy). Glob. Ecol. Conserv. 12, 21–35. https://doi.org/10.1016/j.gecco.2017.08.004.

Paerl, H.W., Huisman, J., 2008. CLIMATE: blooms like it hot. Science 320, 57–58. https://doi.org/10.1126/science.1155398.

Patki, V.K., Jahagirdar, S., Patil, Y.M., Karale, R., Nadagouda, A., 2021. Prediction of water quality in municipal distribution system. Mater. Today: Proc. https://doi.org/10.1016/j.matpr.2021.02.826.

Pestana, C.J., Capelo-Neto, J., Lawton, L., Oliveira, S., Carloto, I., Linhares, H.P., 2019. The effect of water treatment unit processes on cyanobacterial trichome integrity. Sci. Total Environ. 659, 1403–1414. https://doi.org/10.1016/j.scitotenv.2018.12.337.

Preacher, K.J., Zhang, G., Kim, C., Mels, G., 2013. Choosing the optimal number of factors in exploratory factor analysis: a model selection perspective. Multivariate Behav. Res. 48 (1), 28–56. https://doi.org/10.1080/00273171.2012.710386.PMID:26789208.

Revelle, W., 2021. Psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.1.6. https://CRAN.R-project.org/package=psych.

Reyment, R.A., Jvreskog, K.G., 1996. Applied Factor Analysis in the Natural Sciences. Cambridge University Press.

Ritchie, R.J., 2010. Modelling photosynthetic photon flux density and maximum potential gross photosynthesis. Photosynthetica 48 (4), 596–609.

Rousso, B.Z., Bertone, E., Stewart, R., Hamilton, D.P., 2020. A systematic literature review of forecasting and predictive models for cyanobacteria blooms in freshwater lakes. Water Res. 182, 115959 https://doi.org/10.1016/j.watres.2020.115959.

Santos, A.A., Guedes, D.O., Barros, M.U.G., Oliveira, S., Pacheco, A.B.F., Azevedo, S.M.F.O., Magalhães, V.F., Pestana, C.J., Edwards, C., Lawton, L.A., Capelo-Neto, J., 2021. Effect of hydrogen peroxide on natural phytoplankton and bacterioplankton in a drinking water reservoir: mesocosm-scale study. Water Res. 197 https://doi.org/10.1016/j.watres.2021.117069.

Scheffer, M., Rinaldi, S., Gragnani, A., Mur, L.R., Van Nes, E.H., 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78, 272–282.

Sinha, A.K., Eggleton, M.A., Lochmann, R.T., 2018. An environmentally friendly approach for mitigating cyanobacterial bloom and their toxins in hypereutrophic ponds: potentiality of a newly developed granular hydrogen peroxide-based compound. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.05.023.

Skellon, J.H., Wills, E.D., 1948. Iodimetric methods of estimating peroxidic oxygen. Analyst 73, 78. https://doi.org/10.1039/an9487300078.

Tripathi, M., Singal, S.K., 2019. Allocation of weights using factor analysis for development of a novel water quality index. Ecotoxicol. Environ. Saf. 183 https://doi.org/10.1016/j.ecoenv.2019.109510.

Varol, M., 2020. Spatio-temporal changes in surface water quality and sediment phosphorus content of a large reservoir in Turkey. Environ. Pollut. 259 https://doi.org/10.1016/j.envpol.2019.113860.

Walz, 2003. System Component and Principles of Operation, second ed. Phytoplankton Analyzer, Germany.

Watkins, M.W., 2020. In: A Step-by-step Guide to Exploratory Factor Analysis with R and RStudio. Routledge. https://doi.org/10.4324/9781003120001.

Weenink, E.F.J., Kraak, M.H.S., van Teulingen, C., Kuijt, S., van Herk, M.J., Sigon, C.A.M., Piel, T., Sandrini, G., Leon-Grooters, M., de Baat, M.L., Huisman, J., Visser, P.M., 2022. Sensitivity of phytoplankton, zooplankton and macroinvertebrates to hydrogen peroxide treatments of cyanobacterial blooms. Water Res. 225, 119169 https://doi.org/10.1016/j.watres.2022.119169.

Weenink, E.F.J., Luimstra, V.M., Schuurmans, J.M., van Herk, M.J., Visser, P.M., Matthijs, H.C.P., 2015. Combatting cyanobacteria with hydrogen peroxide: a laboratory study on the consequences for phytoplankton community and diversity. Front. Microbiol. 6, 1–15. https://doi.org/10.3389/fmicb.2015.00714.

Wert, E.C., Dong, M.M., Rosario-Ortiz, F.L., 2013. Using digital flow cytometry to assess the degradation of three cyanobacteria species after oxidation processes. Water Res. 47, 3752–3761. https://doi.org/10.1016/j.watres.2013.04.038.

Wert, E.C., Korak, J.A., Trenholm, R.A., Rosario-Ortiz, F.L., 2014. Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species. Water Res. 52, 251–259. https://doi.org/10.1016/j.watres.2013.11.001.

Yang, Z., Buley, R.P., Fernandez-Figueroa, E.G., Barros, M.U.G., Rajendran, S., Wilson, A.E., 2018. Hydrogen peroxide treatment promotes chlorophytes over toxic cyanobacteria in a hyper-eutrophic aquaculture pond. Environ. Pollut. 240, 590–598. https://doi.org/10.1016/j.envpol.2018.05.012.

Zanker, J., Blackwell, T., Patel, S., Duchowny, K., Brennan-Olsen, S., Cummings, S.R., Evans, W.J., Orwoll, E.S., Scott, D., Vogrin, S., Duque, G., Cawthon, P.M., 2022. Factor analysis to determine relative contributions of strength, physical performance, body composition and muscle mass to disability and mobility disability outcomes in older men. Exp. Gerontol. 161, 111714 https://doi.org/10.1016/j.exger.2022.111714.

Zhang, Y., Yao, X., Wu, Q., Huang, Y., Zhou, Z., Yang, J., Liu, X., 2021. Turbidity prediction of lake-type raw water using random forest model based on meteorological data: a case study of Tai lake, China. J. Environ. Manag. 290 https://doi.org/10.1016/j.jenvman.2021.112657.

Zi, J., Pan, X., MacIsaac, H.J., Yang, J., Xu, R., Chen, S., Chang, X., 2018. Cyanobacteria blooms induce embryonic heart failure in an endangered fish species. Aquat. Toxicol. 194, 78–85. https://doi.org/10.1016/j.aquatox.2017.11.007.

In Situ H_2O_2 treatment of blue-green algae contaminated reservoirs causes significant improvement in drinking water treatability

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SUPPLEMENTARY MATERIAL

1 Physico-chemical and biological raw water characteristics of Gavião reservoir

Table S1: Water quality data of Gavião reservoir (Ceará, 2023). Where TP is total phosphorus concentration and TN is total nitrogen concentration.

Date	Chlorophyll a (µg/L)	Cyanobacteria (cells/mL)	TP (mg/L)	TN (mg/L)	Secchi depth (m)
2/1/2015	22.43	65317	0.096	0.963	0.8
5/1/2015	25.42	278373	0.22	1.043	0.95
8/1/2015	31.93	2549422	0.05	0.661	1.1
11/1/2015	32.75	441526	0.054	1.647	1
2/1/2016	41.12	234691	0.113	0.236	0.9
5/1/2016	92.92	23845	0.091	1.059	NA
8/1/2016	64.93	66094	0.061	1.985	0.7
11/1/2016	55.11	86052	0.031	1.475	0.75
2/1/2017	68.46	81386	0.201	2.411	0.8
5/1/2017	36.46	98542	0.274	0.799	0.8
8/1/2017	70.06	574540	0.178	1.329	0.6
11/1/2017	91.63	86831	0.107	1.988	0.8
2/1/2018	65.03	151628	0.098	1.775	0.7
5/1/2018	42.96	7604	0.089	1.275	0.7
8/1/2018	48.44	189982	0.126	1.363	1
11/1/2018	66.96	180022	0.066	1.725	0.9
2/1/2019	69.72	88904	0.065	1.338	0.8
5/1/2019	35.24	221592	0.083	0.975	0.5
8/1/2019	40.6	494149	0.065	1.25	0.9
11/1/2019	65.36	445783	0.079	1.39	0.9
5/19/2020	43.38	855	0.042	1.05	1
8/18/2020	47.25	683.131	0.05	1.28	1
11/17/2020	72.89	677.821	0.047	1.55	0.8
2/2/2021	48.42	1422.893	0.051	1.038	0.8
5/5/2021	43.82	397.312	0.073	1.26	0.8
8/3/2021	37.07	252.191	0.055	0.82	0.8

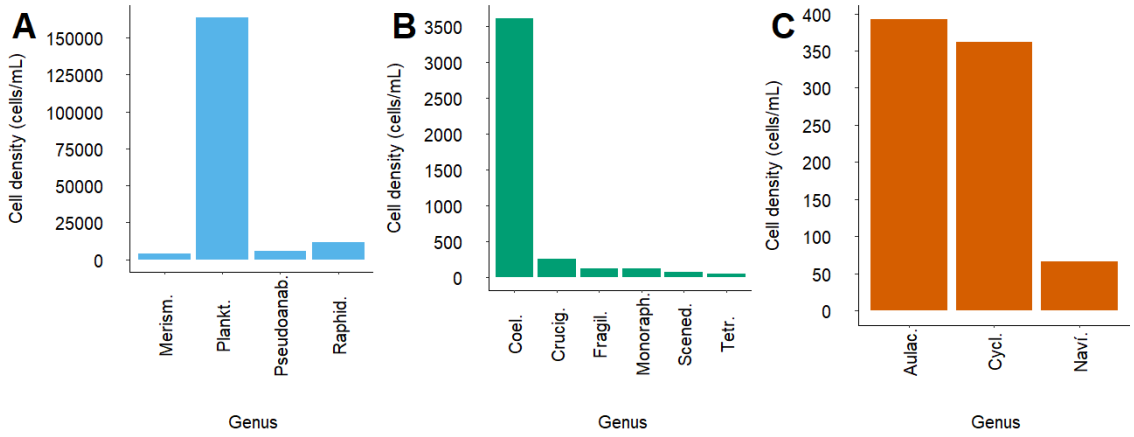

Figure S1 – Cell density of cyanobacteria (A), green algae (B), and diatoms (C) in the raw water used to perform the microcosm experiment. Merism. – Merismopedia sp.; Plankt. – Planktothrix sp.; Pseudoanab. – Pseudoanabaena sp.; Raphid. – Raphidiopsis sp.; Coel. – Coelastrum microporum; Crucig. – Crucigenia sp.; Fragil. – Fragilaria sp.; Monoraph. – Monoraphidium contortum; Scened. – Scenedesmus sp.; Tetr. – Tetraedron minimum; Aulac. – Aulacoseira granulatta; Cycl. – Cyclotella sp.; Navi. – Navícula sp.

2 General effects of H_2O_2 on water quality variables

Table S2: Descriptive statistics for the investigated limnological variables, used as water treatability parameters, after the H_2O_2 treatment (n=3).

Parameter	Day	H_2O_2 dosage	Minimum	Mean	Maximum	Standard deviation
pH	0 (All)	Raw water	9.12	9.15	9.2	0.05
	4	Control	8.63	8.70	8.75	0.06
		5	8.58	8.64	8.71	0.07
		10	8.60	8.61	8.63	0.02
	8	Control	8.47	8.47	8.48	0.01
		5	8.14	8.24	8.33	0.10
		10	8.32	8.37	8.44	0.06
True Color (uH)	0 (All)	Raw water	21.19	22.64	25.53	2.51
	4	Control	55.94	55.94	55.94	0.00
		5	38.57	42.91	47.25	4.34
		10	38.57	41.46	42.91	2.51
	8	Control	34.22	41.46	47.25	6.63
		5	29.88	31.33	34.22	2.51
		10	25.53	26.98	29.88	2.51
Organic matter 254 nm (mg L^{-1})	0 (All)	Raw water	18.8	20.87	23.6	2.47
	4	Control	20.36	25.87	28.78	4.77
		5	27.56	27.75	27.75	0.17
		10	27.56	27.64	27.68	0.06
	8	Control	24.46	25.57	26.35	0.99
		5	24.79	24.91	25.13	0.19

		10	24.46	25.24	26.01	0.78
Temperature (°C)	0 (All)	Raw water	30.8	31.27	31.6	0.41
	4	Control	26.30	26.43	26.50	0.12
		5	25.50	25.67	25.80	0.15
		10	25.70	26.00	26.20	0.26
	8	Control	26.40	26.60	26.70	0.17
		5	26.50	26.53	26.60	0.06
		10	26.40	26.53	26.70	0.15
Turbidity (NTU)	0 (All)	Raw water	11	11.17	11.4	0.21
	4	Control	5.60	9.87	13.20	3.89
		5	5.66	5.87	6.14	0.25
		10	5.23	5.44	5.74	0.27
	8	Control	4.49	6.76	8.15	1.98
		5	3.42	4.29	4.98	0.80
		10	3.16	3.81	4.78	0.86
Conductivity ($\mu S\ cm^{-1}$)	0 (All)	Raw water	565	568.33	571	3.06
	4	Control	537.00	539.33	543.00	3.21
		5	528.00	536.33	545.00	8.50
		10	542.00	547.67	556.00	7.37
	8	Control	539.00	547.33	552.00	7.23
		5	533.00	536.67	541.00	4.04
		10	543.00	556.00	568.00	12.53
Salinity (%)	0 (All)	Raw water	0.2	0.23	0.3	0.06
	4	Control	0.20	0.23	0.30	0.06
		5	0.20	0.27	0.30	0.06
		10	0.30	0.30	0.30	0.00
	8	Control	0.20	0.27	0.30	0.06
		5	0.20	0.23	0.30	0.06
		10	0.30	0.30	0.30	0.00
Dissolved oxygen ($mg\ L^{-1}$)	0 (All)	Raw water	7.7	7.99	8.2	0.26
	4	Control	4.50	4.58	4.72	0.12
		5	4.50	4.64	4.78	0.14
		10	4.39	4.61	4.78	0.20
	8	Control	7.02	7.12	7.21	0.10
		5	6.90	7.18	7.34	0.24
		10	6.61	6.84	7.14	0.27
Chlorophyll of Cyanobacteria ($\mu g\ L^{-1}$)	0 (All)	Raw water	23.45	28.73	33.32	4.97
	4	Control	24.87	27.60	30.10	2.62
		5	0.00	1.05	3.14	1.81
		10	0.00	0.00	0.00	0.00
	8	Control	8.12	20.55	27.68	10.81
		5	0.00	0.00	0.00	0.00
		10	0.00	0.00	0.00	0.00
	0 (All)	Raw water	8.68	9.69	11.57	1.63
	4	Control	6.20	8.01	9.66	1.74
		5	8.44	11.02	15.89	4.22

Chlorophyll of Green Algae (µg L^{-1})		10	1.55	2.99	3.84	1.26
	8	Control	1.64	3.17	4.27	1.37
		5	4.86	41.43	78.41	36.78
		10	1.59	17.87	39.88	19.78
Chlorophyll of Diatom (µg L^{-1})	0 (All)	Raw water	0.46	0.84	1.22	0.54
	4	Control	0.00	0.30	0.91	0.53
		5	1.00	2.30	4.90	2.25
		10	0.16	0.51	0.77	0.31
	8	Control	2.51	2.90	3.42	0.47
		5	1.44	7.53	10.87	5.28
		10	0.72	2.65	5.29	2.37
Phycocyanin (µg L^{-1})	0 (All)	Raw water	-	7.47	-	-
	4	Control	6.48	7.47	7.98	0.86
		5	8.91	10.98	12.25	1.80
		10	9.88	10.63	11.12	0.66
	8	Control	6.27	6.80	7.27	0.50
		5	7.07	7.79	8.33	0.65
		10	6.88	8.15	9.91	1.57

Comments on table S2:

The increase in true color and organic matter in the control samples is likely due to extracellular organic matter (EOM) released by the cyanobacteria (and other phytoplankton) in the raw water samples. Characteristically for cyanobacteria, a large fraction of the released EOM consists of humic acid which is known to contribute to true color in water samples (Liang et al., 2021).

The decrease in the DO could be accounted for by consumption by other (non-cyanobacterial) bacterioplankton in the raw water samples.

Increased phycocyanin concentrations were observed for both H_2O_2 concentrations compared to the control samples. This observation can readily be explained by an enhanced extraction yield from the cyanobacterial cells previously damaged by the H_2O_2 dose and its resistance to oxidative stress. Thus, explaining why no cyanobacteria chlorophyll-a was detected (indicating the removal of cyanobacteria) but yet increased concentrations of phycocyanin were detected. Recent studies have conclusively shown that phycocyanin is markedly more resistant to oxidative stress than chlorophyll-a is (Hong et al., 2020).

3. Dataset verification for the exploratory factor analysis results

Figure S2 – Correlation matrix that points to the presence of inappropriate correlations for the EFA. In the main diagonal, it is presented the histogram and the graph of the probability density function. Above the main diagonal, the Pearson correlations, the type of correlation ("inappropriate", highlighted in red, or "appropriate" in black) and its p-value. Below the main diagonal are the scatter plot and smoothed trend line.

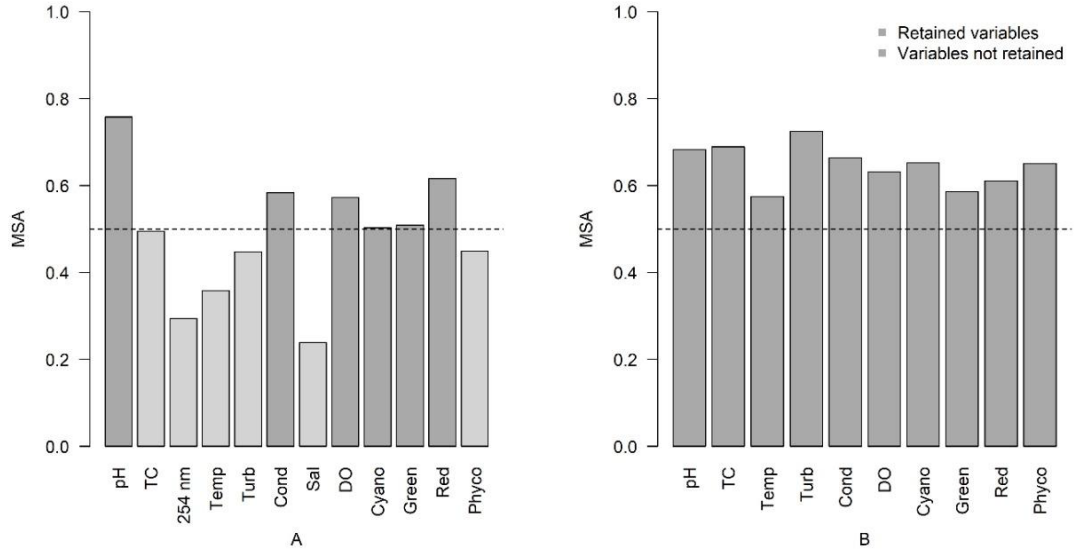

Figure S3 – Measure of Sampling Adequacy (MSA) for the variable dataset. (A) and (B) present the MSA values before and after the variable selection algorithm, respectively. Variables that presented MSA<0.5 were considered as inappropriate (highlighted in light gray), the dotted line represents this threshold. The variables 254 nm and Salinity were removed. TC – True Color; 254 nm – Organic matter 254 nm; Temp – Temperature; Turb – Turbidity; Cond – Conductivity; Sal – Salinity; DO – Dissolved oxygen; Cyano – Cyanobacteria chlorophyll-*a* concentration; Green – Green algae chlorophyll-*a* concentration; Red – Diatoms chlorophyll-*a* concentration; Phyco – Phycocyanin concentration.

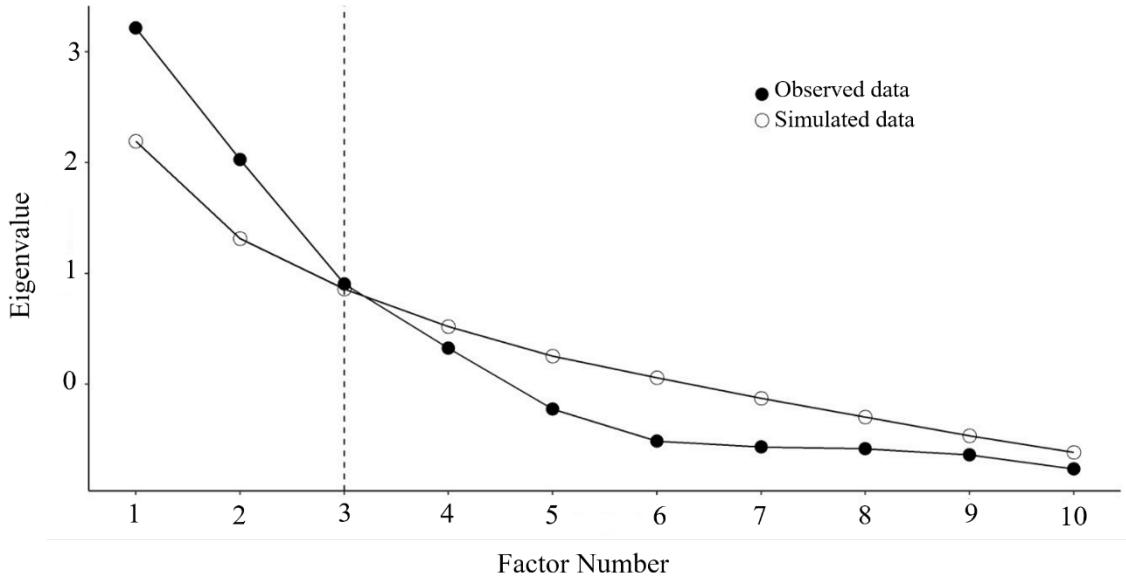

Figure S4 - Eigenvalue plot result of the parallel analysis comparing the observed and simulated data.

3. Definition of the variables into factors

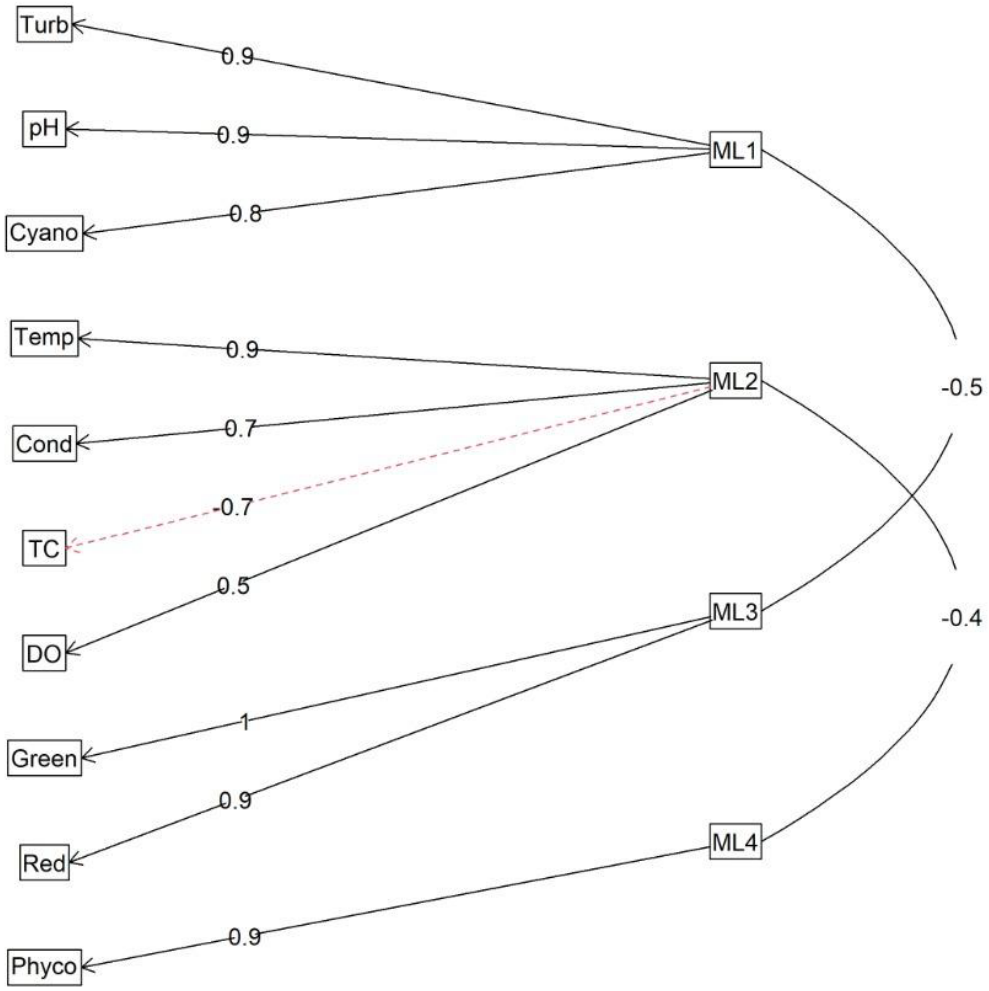

Figure S5 – Correlation for the factors obtained on the factor analysis using maximum likelihood (ML) and oblique rotation (promax). The numbers between the variables and the factor number (ML1, ML2, ML3, and ML4) are the factor loading of each variable. The numbers between the factors are the calculated correlation. TC – True Color; 254 nm – Organic matter 254 nm; Temp – Temperature; Turb – Turbidity; Cond – Conductivity; Sal – Salinity; DO – Dissolved oxygen; Cyano – Cyanobacteria chlorophyll-*a* concentration; Green – Green algae chlorophyll-*a* concentration; Red – Diatom chlorophyll-*a* concentration; Phyco – Phycocyanin concentration.

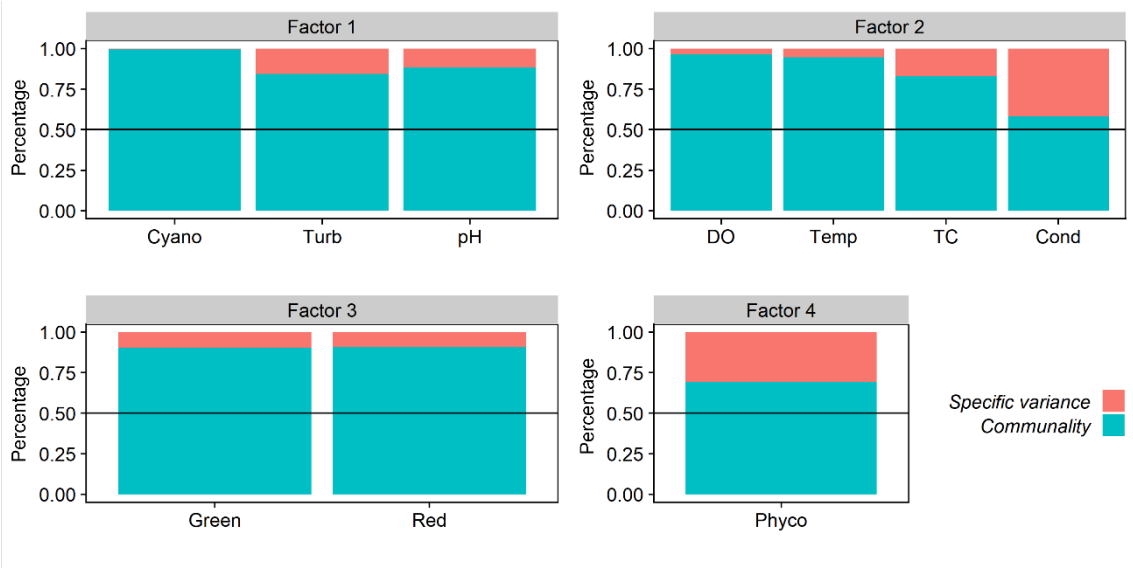

Figure S6 – Communality of the variables grouped according to each of their corresponding factors. The line on the 0.5 value was adopted as an extra criterion for the representativity quality of the variable on the factor analysis. TC – True Color; 254 nm – Organic matter 254 nm; Temp – Temperature; Turb – Turbidity; Cond – Conductivity; Sal – Salinity; DO – Dissolved oxygen; Cyano – Cyanobacteria chlorophyll-*a* concentration; Green – Green algae chlorophyll-*a* concentration; Red – Diatom chlorophyll-*a* concentration; Phyco – Phycocyanin concentration.

4. References

Ceará, 2023. Companhia de Gestão dos Recursos Hídricos: Monitoramento Quantitativo e Qualitativo dos Recursos Hídricos. Available online: http://www.hidro.ce.gov.br (accessed on 02 May 2023).

Liang, H., Huang, X., Wang, H., Xu, W., Shi, B., 2021. The role of extracellular organic matter on the cyanobacteria ultrafiltration process. Journal of Environmental Sciences 10, 12-20.

Hong, J.-E., Lim, J.-H., Kim, T.-Y., Jang, H.-Y., Oh, B.-H., Chung, B.-G., Lee, S.-Y., 2020. Photo-oxidative protection of chlorophyll-a in c-phycocyanin aqueous medium. Antioxidants 9(12), 1235.