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The impact of micropollutants on native algae and cyanobacteria communities in ecological filters during drinking water treatment

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Abstract

An attractive alternative for drinking water production is ecological filtration. Previous studies have reported high removal levels of pharmaceutical and personal care products (PPCPs) by this technology. Algae and cyanobacteria play an important role in the biological activity of ecological filters. The aim of this study was to characterize and identify the community of algae and cyanobacteria in relation to its composition, density and biovolume from 22 ecological filters that received spikings of 2 μg L^{-1} PPCPs. For algae and cyanobacteria species, triplicate samples were collected before and 96 hours after each spiking from the interface between the top sand layer of the ecological filters and the supernatant water. Results show that Chlorophyceae and Cyanobacteria were present in high numbers of taxa and abundance. The specie *Lepocinclis* cf. *ovum* (Euglenophyceae) had the highest percentage occurrence/abundance and frequency into the filters, indicating a possible tolerance by *Lepocinclis* cf. *ovum* to the concentration of selected PPCPs. Although the concentration of PPCPs did not affect the treated water quality, they did affect the algae and cyanobacteria community. No differences were detected between filters that received a single PPCP and filters that received a mixture of the six compounds. Also, changes in the composition of algae and cyanobacteria communities were observed before and 96 hours after the spikings.

Keywords: phytoplankton, ecological purification, slow sand filtration, PPCPs, taxonomy, biovolume.

1. Introduction

One of the world's concerns has been about invisible water contamination by micropollutants such as pharmaceuticals and personal care products (PPCPs) (Evgenidou et al., 2015). These chemicals can cause unknown damage to both aquatic biota and humans, exposed to or consuming it, even in very low concentrations (μg to ng L^{-1}) (Daughton and Ternes, 1999; Heberer, 2002; Fent et al., 2006; Matamoros et al., 2009; Kumar et al., 2020). Effluents from wastewater treatment plants (WWTPs) are considered to be the main contributing source of PPCPs to the environment (Chen et al., 2012). This is because conventional WWTPs are designed to remove mainly organic matter, nitrogen and phosphate but not PPCPs, and consequently, they are also found in surface waters at concentrations of ng L^{-1} to μg L^{-1} worldwide (Ebele et al., 2017; Oluwole et al., 2020). Water contaminated by these compounds ends up in drinking water treatment plants.

Advanced oxidation processes (AOPs) such as ozonation, UV-based oxidation, Fenton and Fenton-like methods, electrochemical processes, ultrasonication, photocatalysis, ionizing radiation, and other combined processes have been shown to effectively remove PPCPs. For example, Masud et al. (2020) synthesized reduced graphene oxide with nanoscale zero-valent iron to remove a complex mixture of 12 diverse PPCPs (including antibiotic, anti-inflammatory, anti-seizure, and antidepressant) at 200 μg L^{-1}. Removals of 95–99% were found within 10 min in the presence of H_2O_2, and 82–99% in the absence of H_2O_2 at the end of 30 min. Pai and Wang (2022) investigated the removal of PPCPs through chlorination, UV, UV/Chlorine, and UV/H_2O_2 processes using 2500 ng L^{-1} PPCP-spiked Milli-Q water and finished drinking water. They found UV was not effective to remove the selected PPCPs. But using chlorine or H_2O_2 in combination with UV led to an increased removal of PPCPs (≥ 56.5% for UV/Chlorine and ≥ 27.6% for UV/H_2O_2) within 5 min. Degradation efficiency of 4 mg

L^{-1} of diclofenac in distilled water was found to be 90%–94% by the combination of ozonation with ultrasonication process during 10 min (Fraiese et al., 2019). Methylparaben at 10 mg L^{-1} in pure water was 100% degraded by the combination of sepiolite catalyst and ultrasonic during 30 min (Savun-Hekimoglu and Ince, 2019). In addition, ibuprofen and diclofenac at 10 mg L^{-1} in pure water were degraded 85% and 96%, respectively, using TiO_2 combined with ultrasound in 120 min (Michael et al., 2014).

However, because AOPs are expensive, their application at large-scale is constrained (Xu et al., 2017), especially in low- and middle-income countries (LMICs) such as Brazil. An attractive alternative for drinking water production is ecological filtration (or slow sand filtration-SSF), and previous studies have reported high levels of removal of PPCPs by this technology (Erba et al., 2014; Pompei et al., 2017; Li et al., 2018; Li et al., 2019; Pompei et al., 2019; Xu et al., 2021). For example, Pompei et al. (2019), who evaluated the removal of 2 µg L^{-1} of PPCPs by ecological filtration in tropical climate, reported the efficiency of 81 to 99% for removal of pharmaceuticals whereas the personal care products were removed 70 to 71%. Different from this, Pompei et al. (2017) evaluated the performance of household slow sand filters in temperate climatic conditions and found that diclofenac, naproxen, ibuprofen and methylparaben were totally removed (2 µg L^{-1}) by the filter, while benzophenone-3 and paracetamol had 47.5% and 65.2% averange removal, respectively. In addition, Li et al. (2018) evaluated the removal of 25 µg L^{-1} of target PPCPs by granular activated carbon (GAC) sandwich slow sand filter and conventional SSF. Paracetamol removals in the filter with only sand were between 78-68%, while in the GAC sandwich filter, removals were up to 100%. These studies demonstrate the potential of slow sand filtration in removing PPCPs.

There are significant differences between SSF and conventional rapid sand filtration. SSF does not require chemical coagulation, backwashing, and energy for maintenance and operation, making it a low-cost and zero carbon solution. As the filtration rate in SSF is slow, it provides sufficient time for the development of the biofilm (i.e. schmutzecke) required for the natural and biological purification of water. These advantages make the ecological filter very attractive as a green and sustainable treatment system (Zeeman, 2012). On the other hand, for large flowrates, SSF implementation requires large areas, hence, it is appropriate for household and decentralised water treatment systems (Ngai et al., 2007; Pompei et al., 2017; Liu et al., 2019; Sabogal-Paz et al., 2020). However, SSF is becoming attractive over rapid filtration as it is a low carbon and nature-based solution.

Algae and cyanobacteria play an important role in the biological activity of ecological filters, as they form a "mesh" on the top sand layer of the filter that helps to retain impurities. In addition, during their photosynthetic process, algae are responsible for absorbing carbon dioxide, nitrates, and phosphates and producing oxygen, providing the ideal conditions for microorganism development, as they depend on oxygen for their survival, facilitating the decomposition process (Nakamoto, 2008; Nakamoto, 2014). However, some cyanobacteria are potentially toxic and produce, for example microcystins when found in drinking water supply (Huisman et al., 2018) but together with microalgae they form a successful consortium for water purification in ecological filters.

SSF is used in many parts of the world. For example, it is a common method for water treatment in rural areas in Colombia (Österdahl, 2015). It is applied to water treatment as a single or combined process in Brazil (de Souza et al., 2017); Japan

(Nakamoto, 2008); the Netherlands (Van der Kooij et al., 2018) and UK (Campos et al., 2002).

In terms of filter media, some studies have considered the use of zeolites (Mahlangu et al., 2011) and blast furnace slag (Abdolahnejad, Ebrahimi & Jafari, 2014) in combination with sand media. However, the most common combination used by utilities seems to be activated carbon and sand. Activated carbon is a porous material, so it creates a further environment for developing the biofilm, enhancing the removal mechanisms (Li et al., 2018).

Some previous studies on the removal of phytoplankton by slow sand filters (SSF) conducted in Brazil showed promising results with removals of around 97%, including cyanobacteria (Pereira et al., 2012); also, the removal of *M. aeruginosa* cells and microcystin-LR by a full-scale household slow sand filter (Terin and Sabogal-Paz, 2019). However, Miazek and Brozek-Pluska (2019) described the effect of various PPCPs on growth limitation in microalgae at concentrations of μg L^{-1} to mg L^{-1}in different species of algae and cyanobacteria. Therefore, considering that the affluent water of ecological filters/SSF may be contaminated by emergent chemical compounds such as PPCPs, it is necessary to determine the impact of these micropollutants on the essential micro-community of these filters to understand if treatment performance is affected.

Previous studies exploring the biofilm of SSF, ecological filters or “household biosand filters”, focused mainly on bacteria that are also part of the biofilm (Calvo-bado et al., 2003; Wakelin et al., 2010; Wakelin et al., 2011; Hwang et al., 2014; D'Alessio et al., 2015; Haig et al., 2015; Pompei et al., 2017; Xu et al., 2020; Lamon et al., 2021). However, none of these studies looked at the base of the food chain like algae and cyanobacteria.

In addition, most recent studies use metagenomic methods (Chenet al., 2021), and despite their precision and practicality (i.e., speed in obtaining results), they are expensive. In Brazil, the classic taxonomy is still the only technique used by governmental agencies for monitoring algal and cyanobacterial blooms in reservoirs and lakes used for drinking water (e.g., CETESB, SABESP, and other municipal water utilities) in accordance with ordinance n. 2914/2011 (Brazil, 2011). It is known that Brazilian water utilities cannot invest in metagenomic methods due to scarcity of resources. Therefore, monitoring by metagenomics in practice is not currently feasible for Brazil, and taxonomy seems the more affordable option.

The aim of this study was to characterize and identify the community of algae and cyanobacteria in relation to its composition, density and biovolume from 22 ecological filters that were contaminated by selected PPCPs, using the taxonomy method. The evaluation included the effect that these PPCPs had on algae and cyanobacteria communities developed at the interface between the top layer of the ecological filters (i.e., schmutzdecke) and the supernatant water.

While several studies have looked at changes in algal communities in natural environment, this study seeks to explore whether PPCP contamination can alter the long-term biota in the operation of water treatment systems. To the best of the authors' knowledge, this is the first study to present a list of algae and cyanobacteria species living in SSF treating water contaminated by PPCPs and to describe the impacts of PPCPs on the algae and cyanobacteria community.

2. Materials and Methods

2.1. Study site

The affluent water that was put through the ecological filters (n = 22) was pumped from the Lobo reservoir (22° 10'18,09" S 47°54'5,00" W), located in southeast Brazil. The pilot scale ecological water treatment system was constructed at the reservoir margins, at the University of São Paulo (EESC-USP) facilities i.e., the Water Resources and Environmental Studies Centre (CRHEA in Portuguese).

The reservoir was classified by Calijuri and Tundisi (1990) as oligomesotrophic, and they identified some environmental changes caused by human activities (e.g., deforestation, discharge of domestic sewage and fertilizers).

The influence of meteorological parameters during this study was described by Pompei et al. (2020), who collected meteorological data at the climatological station of CRHEA which follows the rules of the World Meteorological Organization. Their statistical analyses showed that the possible increase in global air temperature may have influenced treatment performance. Other parameters, such as conductivity, average air temperature, and average water temperature had significant and positive correlations with the water quality of the 22 ecological filters.

2.2. Construction and operation of ecological filters

Twenty-two ecological filters were constructed using PVC columns. Each ecological filter had a diameter of 25 cm and height of 72 cm. The filtration rate used for the filters was 3 m^3 m^{-2} day^{-1}. All information regarding the filters' construction and operation, the spikings of the 6 target micropollutants, analytical methodology and

198 removal efficiencies of micropollutants by the filters are described in detail by Pompei et
199 al. (2019).
200 The background concentration of PPCPs in the Lobo reservoir water was also
201 evaluated and presented by Pompei et al. (2019). The name of each filter was determined
202 according to the PPCPs spiked on each one (Table S1 - Supplementary Material).
203 Paracetamol, diclofenac, naproxen, ibuprofen, methylparaben and benzophenone-
204 3 were each spiked at an initial concentration of 2 μg L^{-1} in all 22 filters. This
205 concentration was assumed based on the quantification limit of the compounds (Pompei
206 et al., 2019). The 6 PPCPs used were 99% purity or more, and purchased from Sigma-
207 Aldrich (St. Louis, MO, USA). All chemicals used for PPCPs extraction and detection
208 were obtained as previously reported by Pompei et al. (2019).
209 The 22 filters operated continuously for a duration of 4 months (September to
210 December), with the first month (around 30 days) dedicated to the maturation of the
211 filters. Filter maturation means that the microbial community is established and is reached
212 when 99% removal of *Escherichia coli* (*E. coli*) and total coliforms are observed
213 (D'Alessio et al., 2015). After the maturation period, there were 3 spikings of the target
214 PPCPs with an interval of 15 days between each spiking (November to December). In
215 addition to a control filter, there were triplicates of each filter receiving only paracetamol,
216 diclofenac, naproxen, ibuprofen, methylparaben, benzophenone-3, and the mixture of the
217 6 PPCPs.
218
219 **2.3. Sampling procedure for the abiotic parameters**
220 For algae species identification, water samples (300 mL each) were collected from
221 the interface between the sand bed and supernatant water of each ecological filter, in
222 triplicate before and at 96 hours after each spiking, totalling 396 samples. The collection

time of 96 hours was chosen as it is the standard duration of algae toxicity tests (ABNT, 2011) and the aim was to assess the effect of PPCPs on algae and cyanobacteria species.

In addition to algae species, other water quality parameters were monitored on a weekly basis. Total phosphorus (TP) and total Kjeldahl nitrogen (TN) were monitored according to APHA (1995). The pH was monitored using a pHmeter B374 - Micronal; Dissolved oxygen (DO) using an Oximeter YSI; water temperature (Temp) was monitored using an Orion - model 145.

Chlorophyll-*a* (Chl-*a*) was extracted according to Nusch (1980), using glass microfiber filters (0.45 μm – Macherey-Nagel, Germany) and acetone PA (Merck - Darmstadt, Germany). After extraction, the samples remained in the dark for a minimum of 14 hours and then were analysed by spectrophotometry (UV- spectrophotometer 600, Femto), at 665 nm and 750 nm wavelengths. The concentration of Chl-*a* was calculated according to the equation described in Lorenzen (1967).

2.4. Qualitative analysis of algae and cyanobacteria community

For taxonomic analysis, phytoplankton samples were collected with a 20 μm-mesh plankton net on the subsurface (i.e., schmutzdecke) of each filter (n= 22). With samples taken before and 96 hours after spiking, this totalled 132 samples. The collected material was preserved in 4–5% formaldehyde solution formalin (37%, Carl Roth, Germany). About 20 individuals from each taxon were evaluated morphologically and morphometrically by a Zeiss Axioplan 2 imaging microscope. Specialized bibliographies were used, including floras and revisions, as described in Table S2. After taxonomic analysis, they were deposited in the liquid collection of algae at the Herbarium of the Botany Institute (São Paulo) called "Maria Eneida P.K. Fidalgo". The samples from the

spikings gave rise to a composite sample which was included with voucher numbers from SP469.577 to SP469.608.

2.5. Quantitative analysis of the algae and cyanobacteria community

Phytoplankton sample collection for quantitative analysis was conducted by drawing one of the quadrants from the area of each filter (i.e., 0.1963 m^2). A glass bottle (100 mL) was submerged into the quadrant and fixed with 1% acetic lugol solution (Sigma-Aldrich - St. Louis, MO, USA) in a 1:100 ratio, totalling 132 samples.

Quantitative analyses were performed according to Utermöhl (1958) using an inverted microscope Zeiss Axiovert 25 in 400 times magnification. The sedimentation time of samples was 3 hours per centimetre of chamber height (Lund et al., 1958). Two sedimentation chambers were used (2 and 10 mL), depending on the phytoplankton density concentration of each sample.

The results of the phytoplankton counting were carried out according to Bicudo, (1990), and were expressed in density (organism mL^{-1}) and calculated according to Weber (1973). More detailed information is at Supplementary Material (Section 1.1).

2.5.1. Biovolume of the phytoplanktonic community

The cell volume for each species was calculated based on geometric models according to Hillebrand et al. (1999), Wetzel and Likens (2000), Sun and Liu (2003), and Fonseca et al. (2014).

The biovolume (mm^3 L^{-1}) was estimated by multiplying the densities of each species by the average volume of each cell. The value obtained in μm^3 mL^{-1} was transformed into mm^3 L^{-1} by dividing this value by 10^6.

More detailed information about other parameters calculated as Richness (R) and the Shannon and Weaver's (1963) Diversity Index (H') are in the Supplementary Material (Section 1.1).

To calculate the density and the biovolume of algae and cyanobacteria in each filter, an average for these values of each triplicate filter was found. This was assumed based on the statistical analyses carried out by Pompei et al. (2019) which showed no significant difference between the triplicate filters.

2.5.2. Descriptor and abundance species of algae and cyanobacteria community

The criterion for selecting the descriptor species was applied to the biovolume results. Descriptors considered the taxa that contributed greater than 1-2% of the total biovolume obtained and that together added up to 80% of the total biovolume. Abundant species had a higher occurrence than the mean total number of individuals of the sample (Lobo and Leighton, 1986).

2.6. Statistical analysis

A cluster analysis was carried out with the identified species, generating a species similarity dendrogram by pairing species and Jaccared index with the calculation of the cophenetic coefficient. The principal coordinate analysis (PCoA) (Valentin, 2000) was used to determine the variability of abiotic data in relation to contamination (temporal) and filters (spatial). The covariance matrix was used, i.e., the data transformed by the range of variation "ranging" ([x-xmin)/(xmax-xmin)]).

The PCoA was first performed using biovolume matrices of all species of algae and cyanobacteria identified in the 3 spikings, and after the selection of significant species ($r \geq 0.5$), another PCoA was generated.

The evaluation of the relationship between abiotic and biotic (species of algae and cyanobacteria) parameters was carried out by Canonical Correspondence Analysis (CCA) of the biovolume matrices of the total species of algae and cyanobacteria from the spikings carried out and 6 environmental parameters from ecological filters.

The CCA was performed from covariance matrices, with transformation of the abiotic data by the amplitude of variation "ranging" (xxmin)/(xmax-xmin)]) and of the biotic data by [log (x + 1)].

To test the level of significance of the first 2 axes, the Monte Carlo test (999 permutations, $p \leq 0.05$), which determines if there is a probability that the eigenvalues have a random distribution, was used.

The data were analysed by multivariate statistical analysis using PC-ORD version 6.0 for Windows (Mccune and Mefford, 2011). Parameters with significant correlation were those that presented $r > 0.5$ with axes 1 and 2 of sorting.

3. Results and discussion

3.1. Abiotic parameters

The water quality parameters such as temperature, DO, TN, TP (Table S1) monitored across all filters during the study period are described in detail in Section 2.1 of the Supplementary Material.

Compared with the control filter (0.04 mg L^{-1} before and 0.07 mg L^{-1} 96 hours after the spiking), the filters that received PPCPs and had higher TP values were those with methylparaben (0.07 mg L^{-1} before and 0.10 mg L^{-1} 96 hours after spiking) and ibuprofen (0.10 mg L^{-1} before and 0.06 mg L^{-1} 96 hours after spiking) (Table S3). However, these different TP concentrations had no significant increase in the mean values of the evaluated nutrients (TP and TN). Although higher TP and TN values are associated

with algal blooms, other conditions are required for this, e.g. temperature, luminosity, system residence time, and others (Bouvy et al., 2000). In addition, the values of a Chl-*a* analysis for the filters that received spikings of PPCPs and the control filter were not different (Table S3).

3.2. Species of algae and cyanobacteria in the filters

During the PPCP spikings (n = 3) in the ecological filters (n = 22), 156 taxa were identified and distributed in 9 taxonomic groups (Table S4 and Table S5). Chlorophyceae and Cyanobacteria were the groups that presented the highest number of taxa, with 58 and 37 respectively. These taxonomic groups are mentioned as the most representative classes in relation to the rate richness in shallow waters and classified as eutrophic (Sant'Anna et al., 2006; Tucci et al., 2006).

Brook (1984) who evaluated the bottom-living algal flora of SSF beds in waterworks in England described 3 taxonomic groups: Bacillariophyceae, Chlorophyceae and Cyanobacteria (66, 9, and 2 species respectively). In contrast with the findings of our study, the author observed a dominance of diatoms in the filters, which may be related to filter bed clogging (Henderson et al., 2008; Joh et al., 2011). Bernhardt (1984) also reported a reduction in the filters' run time from 30 to 8h in a water treatment works (WTW) in Germany, caused by a bloom of *Melosira*.

Also contrary to our study, Varesche and Di Bernardo (1998) evaluated the interference of algae on 2 pilot scale SSFs and reported the dominance of the diatom *Aulacoseira italica* (Ehrenberg) Simonsen in the influent water of the filters. The difference between our study and theirs may be due to the inlet water quality, as they collected more than 20 years ago. Varesche and Di Bernardo (1998) used a flow-splitting box with triangular weirs before the SSF, while in our study the water supplied to the

346 filters was pumped from the Lobo's reservoir to a constant level box that supplied the
347 filters. The classes or species that dominate in the filters is directly related to the native
348 flora of the water body that supplies the treatment system. This was also previously
349 reported by some authors from the UK (Casterlin and Reynolds, 1977; Benson-Evans et
350 al., 1999; Henderson et al., 2008). Interestingly, Varesche and Di Bernardo (1998)
351 associated the occurrence and the dominance of this species with headloss in the sand
352 bed, especially at the top of the sand. However, headloss was not monitored in our present
353 work.

354 The various descriptions available for the schmutzdecke observed in SSF indicate
355 that its characteristics vary significantly from one place to another and seasonally
356 (Campos et al., 2002). Therefore, this may explain the differences between the values
357 presented in Table 1. For example, Bowles et al. (1983) found that the diatoms *Melosira*
358 sp., *Navicula* sp. and *Nitzschia acicularis* (Kützing) W.Smith were the predominant
359 species during winter. However, a thick green carpet of the filamentous seaweed *Zygnema*
360 sp. had developed at a thickness of approximately 2 mm with increasing temperature and
361 solar radiation in the spring.

362 According to Haig et al. (2015) the compositions of microbial communities of
363 SSF are significantly different depending on the state (operational or drained), age of the
364 filter, sample location, month of sample collection, and the distances of the tributary and
365 effluent from the tubes and the depths at which the samples were taken. In our study, it
366 was observed that, over the operation time of the filters, a thick layer of *Spirogyra* sp.
367 (Fig. S1), a filamentous algae type, developed on the top of the sand layer, including the
368 interface between the wall and sand bed of the filters. This finding is in agreement with
369 Campos et al. (2006) who observed that the structure of the schmutzecke in full-scale SSF
370 consisted mainly of filamentous algae.

Considering the general average of algae species richness (from all spikings), the richness increased as the operating time increased. The smallest numbers were observed in the 1st spiking and the highest during the 3rd spiking (40 and 49 species richness, respectively) (Table S6). Brook (1984) also observed that the species richness increased with operating time of SSF beds in an English WTW, although they observed differences in the abundance of the species that appeared more frequently in those filter beds when operated for a longer time.

The sampling before the 2nd spiking shows higher average value of species richness (52 species), against 38 and 44 of the 1st and 3rd spikings, respectively (Table S6). Despite the spikings with PPCPs of on average 2 µg L^{-1}, there was no reduction or increase in developing algae and cyanobacteria taxa in ecological filters. After the spikings, an average richness was observed of 43 taxa for 1st spiking, 41 taxa for 2nd spiking and 54 taxa for 3rd spiking (Table S6). The concentrations of all PPCPs after filtration were on average 0.01- 0.02 µg L^{-1} and more details can be found in Pompei et al. (2019).

The control filter (no PPCPs) presented a smaller richness of species when compared to the filters that received PPCPs. Thus, the presence of PPCPs seemed to increase species richness in the ecological filters, probably because the PPCPs increase the carbon source for microalgae grow. For example, the literature describes that the presence of paracetamol and diclofenac improves the growth of *Chlorella* strains up to 43% (Escapa et al., 2017).

Although the time factor (age of the filters) is being considered, when comparing the control filter, which also suffered the influence of time, we observed that the species richness remained higher in the contaminated filters after the 3rd spiking.

The cluster analysis (Fig. 1) grouped by species similarity identified in each filter showed the grouping of the 3 groups (divided into the 3 spikings), but the control filters (before and after each spiking) did not group, showing that there was a similarity between the species identified in the control filters that was not related to the effect of the operating time of the filters, but to the presence of PPCPs. The co-expressed coefficient of dendrogram analyses was 0.846.

At the 40% similarity level, two large groups of species were formed, one referring to the 1st spiking and the other containing the 2nd and 3rd spikings. Also, with 50% similarity, 2 distinct groups were formed (2nd and 3rd spikings). This shows that the first spiking differed by 60% from 2nd and 3rd in relation to the identified species, and 2nd spiking differed by 50% from 3rd due to the increase in operating time. In relation to the addition of PPCPs, it was observed the formation of large groups which refers to the different collection times of each spiking (before and after spikings).

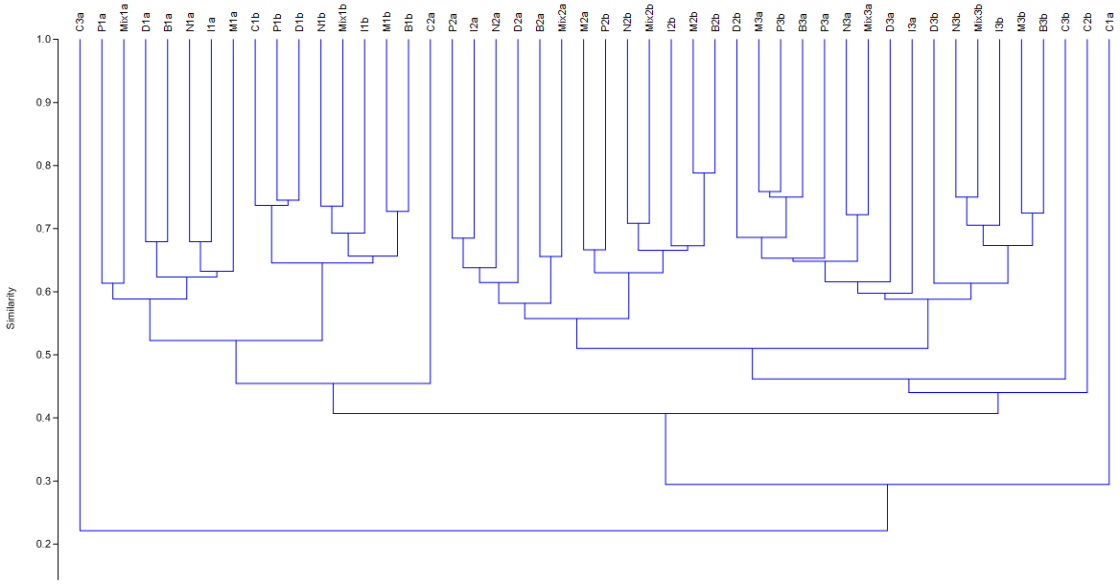


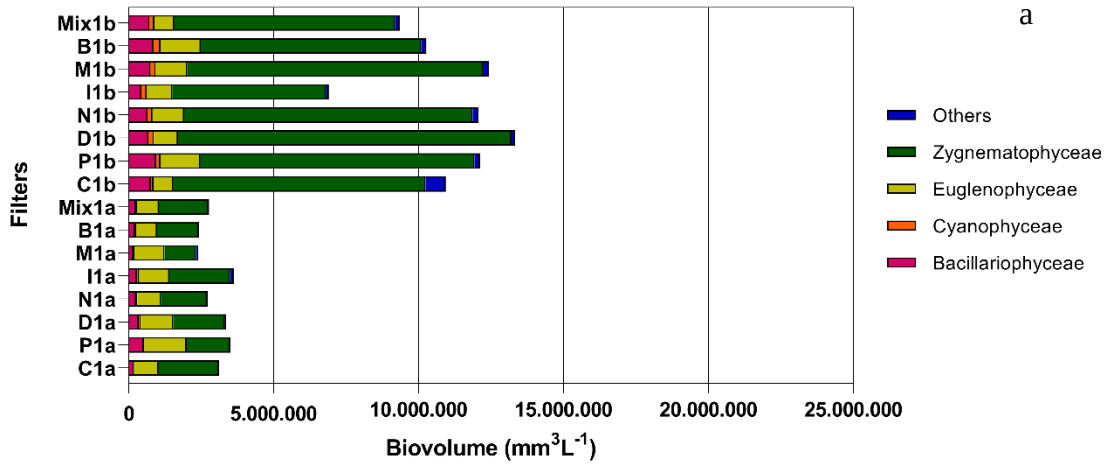
Figure 1: Dendrogram of similarity of species identified in each ecological filter (C= control; P= paracetamol, D= diclofenac; N= naproxen; I= ibuprofen; M= methylparaben;

411 B= benzophenone-3; Mix= PPCP mixture) in each spiking (1= first; 2= second; 3= third),
412 and in each defined sampling time (a= before spiking; b= 96 hours after spiking).
413
414 Although the study by Pompei et al. (2019) reported a background presence of
415 PPCPs at the Lobo reservoir, the concentrations were low for pharmaceuticals (on average
416 0.01 µg L^{-1}) but high for personal care products (on average 86 µg L^{-1} as a high
417 concentration of methylparaben was detected in one single day), which are smaller than
418 the spiking value of 2 µg L^{-1} into the filters, which have a much smaller effective volume
419 of water compared to the Lobo reservoir. This may have affected the results in relation to
420 food source or species formation into the filters.
421
422 **3.3. Quantitative analyses of phytoplankton**
423 A variation on biovolume of algae and cyanobacteria was observed during the
424 whole experiment (1st, 2nd, and 3rd spikings), and between collection times (a = before
425 spiking, b = 96 hours after spiking).
426 On the 1st spiking, the groups with the highest biovolume values were in the
427 following order: Euglenophyceae>Cyanobacteria>Bacillariophyta>Cryptophyceae. On
428 the 2nd and 3rd spikings, some of the groups were similar to the first but in the following
429 order: Bacillariophyta>Cyanobacteria>Euglenophyceae>Zygnemaphyceae, (Fig. 2).
430 On the 2nd spiking the Euglenophyceae biovolume reduced in all filters (Fig. 2b),
431 and there was also a reduction in the control filter, showing that this was not associated
432 with the presence of PPCPs. This may have been caused only by a change in the
433 community composition. A reduction of the total biovolume 96 hours after the 2nd spiking
434 was observed, for example the biovolume sum of all classes on the control filter varied
435 from 2,612,593.29 to 147,527.60 mm^3 L^{-1}.

Among the biovolume identified on the filters, individuals of the classes Euglenophyceae and Zygnemaphyceae, in particular *Lepocincles* cf *ovum* and *Spirogyra* sp. respectively, had notably high biovolumes. Another genus that occurred with frequency in this study was *Aulacoseira*. This genus had a high biovolume, as already described by the authors Varesche and Di Bernardo (1998). Although these authors used another method of counting and presented values in a different way (1 ASU represents 400 μm^2, one filament was equivalent to 4.4 ASU), their work describes how the presence of *Aulacoseira* can increase in total biovolume.

In Fig. 2, 'Others' represents the biovolume sum of the classes Chlorophyceae, Chrysophyceae, Dinophyceae, Xanthophyceae, Zygnemaphyceae for the 1st spiking, and the sum of Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae, Xanthophyceae for the 2nd and 3rd spikings.

A high biovolume of Zygnemaphyceae was observed 96 hours after the ibuprofen spiking (20,684,314.25 $mm^3\ L^{-1}$) (Fig. 2c), during the 3rd spiking, caused by *Spirogyra* sp., a filamentous Zygnemaphyceae. The same occurred on the 2nd and 3rd spikings for ibuprofen, indicating that this pharmaceutical may have been toxic to other species and favoured the predominance of *Spirogyra* sp.. Madikizela and Ncube (2021), who evaluated the occurrence and ecotoxicological risk assessment of non-steroidal anti-inflammatory drugs in South African, describe that the ibuprofen seems to be the one with the high ecotoxicological risks than other non-steroidal anti-inflammatory drugs (NSAIDs) for algae.



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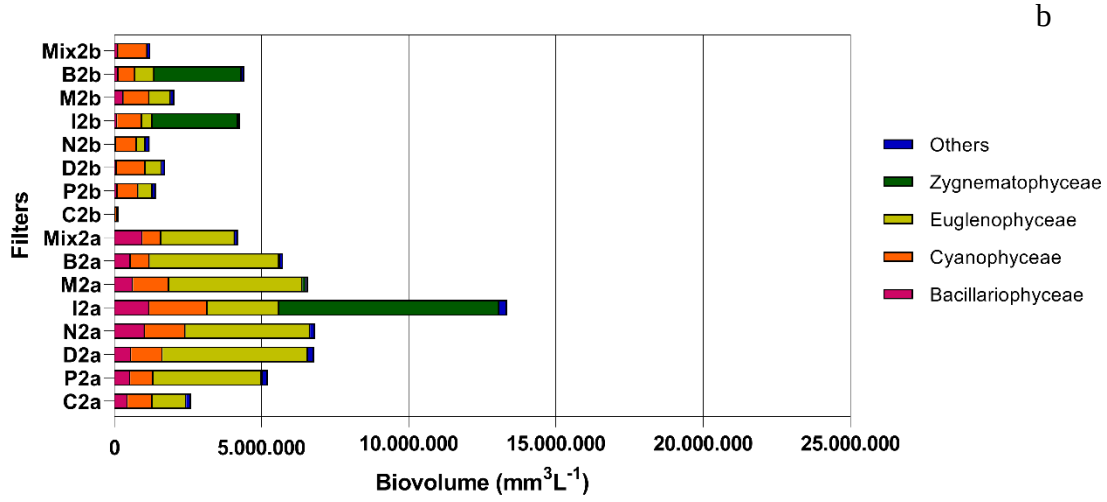

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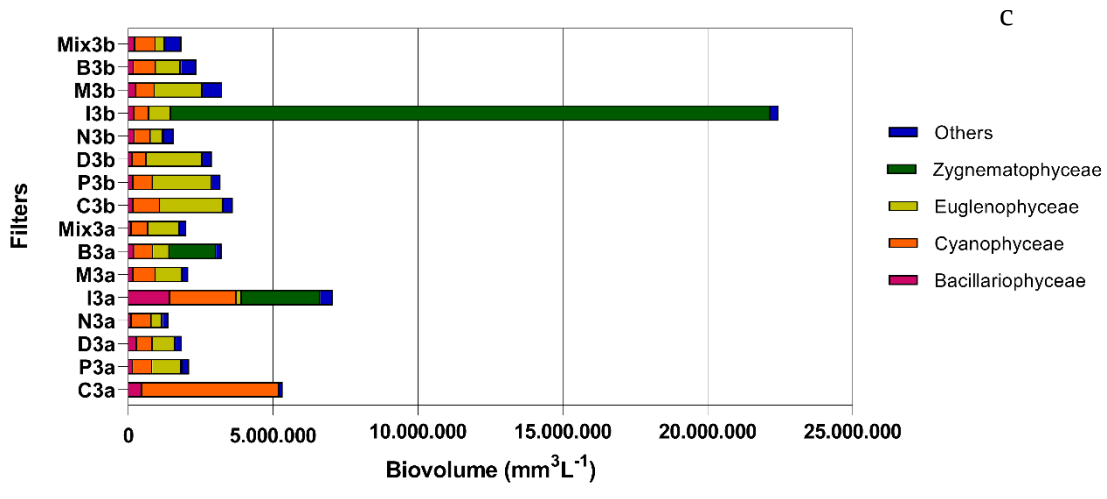


459

460 **Figure 2:** Biovolume (mm^3 L^{-1}) of algae and cyanobacteria in each filter (C= control; P=

461 paracetamol, D= diclofenac; N= naproxen; I= ibuprofen; M= methylparaben; B=

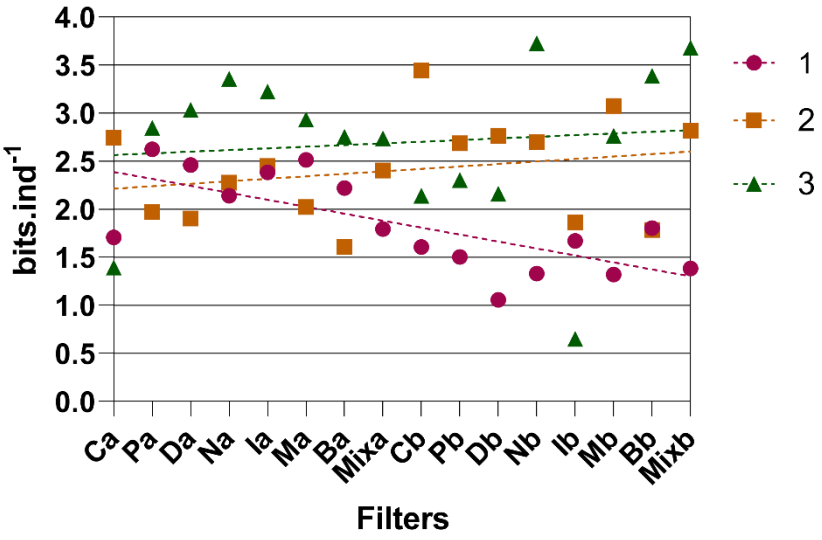
462 benzophenone-3; Mix= PPCP mixture) during sampling times (a= before spiking; b= 96
463 hours after spiking) and spikings (1= first; 2= second; 3= third).
464
465 It was observed that the Bacillariophyta group had a reduced contribution to the
466 community throughout the addition of PPCPs, indicating a possible sensitivity to the
467 presence of the PPCPs. Varesche and Di Bernardo (1998) described some genera of
468 diatom that were dominant during colonization of the medium (e.g., *Cymbella, Eunotia,*
469 *Gomphonema, Aulacoseira italica, Neidium, Surirella*) but these genera were not
470 dominant in our study. In a way, this was beneficial for the success of our filters operation
471 as some diatoms, specially *Aulacoseira italica*, were reported to clog SSF (Varesche and
472 Di Bernardo, 1998).
473 Based on the biovolume results, 9 taxa were classified as descriptors before PPCP
474 addition, and 8 taxa after 96 hours, for both 1st and 2nd spikings (Tables S6 and S7). The
475 species that contributed most in biovolume for both spikings was *Lepocinclis* cf. *ovum*
476 (Ehrenberg) Lemmermann. According to Varesche and Di Bernardo (1998) species such
477 as *Lepocinclis* cf. *ovum* only appeared after the establishment of the pioneer community
478 and the consequent modification of the ecosystem, attesting the stabilization of the
479 ecological filters.
480 On the 3rd spiking, 14 taxa were classified as descriptors before the addition of
481 PPCPs, and 9 taxa after (Table S8), suggesting again an effect from the PPCPs. Together
482 they represented > 90% of the total biovolume. As on previous spikings, on the 3rd
483 spiking, *Lepocinclis* cf. *ovum* was the species with major contribution in biovolume. In
484 addition, the diatom *Aulacoseira granulata* (Ehrenberg) Simonsen was classified as a
485 descriptor species in all spikings, varying from 0.5 to 6.2% in contribution.

Among the classes of cyanobacteria, the significant ones were *Chroococcus minutus* (Keissler) Lemmermann (0.8 to 21%), *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L.Hoffmann & Komárek (0.6 to 43.1%) and *Microcystis aeruginosa* (Kützing) Kützing (0.7 to 22.3%), with these being descriptors for all spikings. According to literature, blooms of *Microcystis aeruginosa* can be potentially toxic due to their release of microcystins. Aside from toxicity to humans, microcynstins can cause damage to surface waters by contaminating biota (Carmichael et al., 2001; Azevedo et al., 2002, de Figueiredo et al., 2004; Buratti et al., 2017; Hinojosa et al., 2019). However, there are some studies demonstrating the potential of SSF in removing microcystins during treatment (Pereira et al., 2012; Terin and Sabogal-Paz, 2019), indicating that when some of these toxic species are present in the biofilm, the filtration system itself is capable of providing safe water.

Diversity index

The values of diversity index were based on biovolume, and varied from H'=0.65 bits ind^{-1} to H'=3.72 bits ind^{-1}. The median value was H'= 2.39 bits ind^{-1}, considering all spikings and different sampling times (Fig. 3).

Despite the values of species diversity dropping after a PPCP spiking (from 3.72 to 0.65 bits ind^{-1}, and on average of 2.39 bits ind^{-1}), this difference was not considered significant (p = 0.72) in the diversity index of the species identified in the filters after each spiking.



507

508 **Figure 3:** Values of diversity index (H') (bits ind^{-1}) calculated based on biovolume in
509 each filter (C = control; P = paracetamol, D = diclofenac; N = naproxen; I = ibuprofen;
510 M = methylparaben; B = benzophenone-3; Mix = PPCP mixture) for sampling times (a =
511 before spiking; b = 96 hours after spiking) and spikings (1= first; 2= second; 3= third).

512

513 The highest value of diversity index (H'=3.72 bits ind^{-1}) and the lower (H'= 0.65
514 bits ind^{-1}) occurred 96 hours after the 3rd spiking, as the highest value was detected in the
515 filter with naproxen and the lowest value in the filter with ibuprofen, suggesting that
516 ibuprofen was more toxic to the community than naproxen, which did not restrict species
517 diversity. This is in agreement with the study carried out by Madikizela and Ncube,
518 (2021), who claim that ibuprofen have highest ecotoxicological risk than naproxen,
519 diclofenac and ketoprofen.

520 As the operating time of the ecological filters increased with the spiking, the
521 average diversity index (1.84 bits ind^{-1} on 1st spiking; 2.40 bits ind^{-1} on 2nd spikings, and
522 2.69 bits ind^{-1} on 3rd spiking) also increased. These values show and reinforce the
523 previously discussed fact that the community of algae and cyanobacteria developed

increased in number and diversity of individuals, and showed changes in composition over the time of operation of the ecological filters.

Statistical analysis

The 1st ACoP was generated considering the 3 spikings (Fig. 4). The control filter was included for this analysis. The analyses show 65.4 % of the articular variability of the data in their 1st two components (axis 1 – 55.06% and 2 – 10.41%) (Fig. 4 and Table S9).

On the positive side of axis 1, the filter species are grouped on the 1st spiking (dark blue dots). These are associated with the largest biovolumes of the species with a positive correlation with the values of axis 1 (Table S9), which included 30 species, 27 with $r >$ 0.5. The species that presented highest correlation values biovolume of the species were *Dictyosphaerium pulchellum* H.C.Wood (r= 0.835), *Desmodesmus intermedius* (Chodat) E.Hegewald (r= 0.791), and *Cryptomonas obovata* Skuja (r= 0.772).

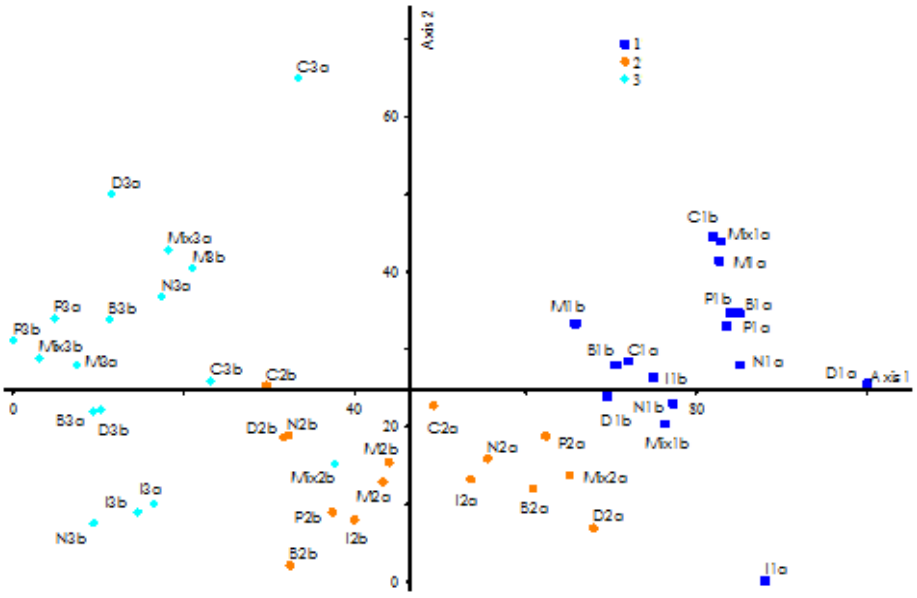


Figure 4: Principal Coordinate Analysis (PCoAs) of significant species for each filter (C= control; P= paracetamol, D= diclofenac; N= naproxen; I= ibuprofen; M=

541 methylparaben; B= benzophenone-3; Mix= PPCP mixture) in for spikings (1= first; 2=
542 second; 3= third), and sampling times (a= before spiking; b= 96 hours after spiking).
543
544 The filter species grouped during the 3rd spiking (light blue) are on the negative
545 side of axis 1. The species *Coenocystis quadriguloides* Fott and *Eutetramorus fottii*
546 (Hindák) Komárek have the highest negative correlation values of significant species (*r*
547 = -0.85). The filter species grouped during the 2nd spiking (orange dots) are on the
548 negative side of axis 2. The control filter species before the 3rd spiking dispersed from the
549 other filter species on the same spike, indicating that the biovolume of the species found
550 in this filter was not similar with the others, just as happened in the filter before the
551 application of ibuprofen in the 1st spiking (Fig. 4 and Table S9).
552 The PCoAs generated for each spiking (n=3) are shown in Fig. 5 and data for
553 correlations with species is presented in Tables S10 to S12. On the 1st spiking (Fig. 5a
554 and Table S10), the analysis shows 58.76% of the variability of the articulations of the
555 data in their 1st two components (axis 1 – 43.00% and 2 – 15.76%). The grouping of the
556 filter species before spiking is on the positive side of axis 1. The species *Dolichospermum*
557 *planctonicum* (*r*= 0.707), *Eutetramorus tetrasporus* Komárek (*r*= 0.602) and
558 *Planktolyngbya* sp.1 (*r*= 0.754) are common among these filters. The control filter (C1a)
559 before the 1st spike was not grouped with the other filters, showing a different composition
560 of significant species.
561 There is a similarity between the control filter (C1a) and those that received
562 isolated paracetamol (P1a) and diclofenac (D1a) on the negative side of axis 1, where the
563 filters were ordered 96 hours after spiking. In addition, there was similarity between the
564 filters that received the mix of selected PPCPs and the filters that received personal care

565 products (i.e., methylparaben - M1a and benzophenone-3 - B1a), indicating similar
566 effects for microalgae community in the presence of the same classes of compounds.
567 Overall, for the 1st spike, the presence of the mixture of PPCPs and diclofenac
568 and paracetamol alone may cause differences in the composition of the descriptor species
569 as these were grouped with the control filter. This is supported by Miazek and Brozek-
570 Pluska (2019) who described a rather moderate inhibitory activity towards growth of
571 green microalgal strains in mg L^{-1}. It was also reported that paracetamol and diclofenac
572 are a possible carbon source for *Chlorella* strains with growth improvement up to 43%
573 (Escapa et al., 2017). However, the presence of naproxen (N1), and ibuprofen (I1), added
574 separately, caused a difference in the composition species of these filters, indicating an
575 impact of these compounds on the algae community established in the filters. Madikizela
576 and Ncube, (2021) reported that ibuprofen in surface water posed low to high
577 environmental risks to algae, and ibuprofen seems to be the one with the highest
578 ecotoxicological risk than other NSAIDs, indicating that they could be toxic for algae
579 community depending on the dosage.
580 Despite not being able to find studies that used similar concentrations as our work,
581 our results agree well with studies using higher concentrations of pharmaceuticals. For
582 example, Miazek and Brozek-Pluska (2019) describe how ibuprofen can inhibit the
583 growth of various green microalgae (e.g., *Chlorella vulgaris* Beijerinck, *Chlorella* sp.
584 cells, *Desmodesmus subspicatus* (Chodat) E.Hegewald & A.W.F.Schmidt in
585 E.Hegewald), besides being responsible for 50% inhibition in photosynthetic activity
586 (Escher et al., 2005) and 50% inhibition in growth (Cleuvers, 2004) – all these effects in
587 g L^{-1}. Also, Ding et al. (2017) found that the growth of the diatom *Navicula* sp. in the
588 presence of ibuprofen in mg L^{-1} could be completely suppressed in 2-10 days. For
589 naproxen, it was reported that it can cause inhibited growth of 50% when present in mg

590 L^{-1} in *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák (Villain et al., 2016; Isidori
591 et al., 2005), *Chlorella vulgaris or Ankistrodesmus falcatus* (Corda) Ralfs (El-Bassat et
592 al., 2012) and *Desmodesmus subspicatus* (Cleuvers, 2004), among other effects described
593 by Miazek and Brozek-Pluska, (2019).

594 In our study, the presence of personal care products added separately to each filter
595 caused a difference in the distribution of descriptor species between them, but this
596 difference was different from the other contaminants. The literature describes how the
597 personal care product methylparaben can cause inhibition of *P. subcapitata* growth at 35
598 mg L^{-1} (Tamura et al., 2013). Ecotoxicological studies and risk assessment involving
599 microorganisms and algae show that the effects of PPCPs at ng to µg L^{-1} levels are related
600 to growth and/or development inhibition, while the lethality is reached when PPCPs are
601 in the order of mg to g L^{-1} (Yamamoto et al., 2011; Ramaswamy et al., 2011; Derisso et
602 al., 2020).

603 During the 2nd spiking (Fig. 5b), the PCoAs showed 50.9 % of variability in the
604 data conjunction of the 1st two components (axis 1 – 34.94% and 2 – 15.99%) – Table
605 S10. The filter species before 2nd spiking were grouped on the positive side of axis 1, and
606 the filter species 96 hours after the spiking were grouped on the negative side of axis 1,
607 just as for the 1st spiking. Among the filter groupings before the 2nd spiking (axis 1), the
608 species with the highest correlation values were *Aulacoseira* sp.2 (r = 0.813) and
609 *Aulacoseira* sp.3 (r = 0.776), both belonging to the Bacillariophyta class.

610 The control filter before spikings was not grouped with the other filters, showing
611 a lack of similarity with the others in terms of species composition and, consequently,
612 confirming that the presence of PPCPs changed the composition of algae and
613 cyanobacteria communities in the filters after 96 hours of exposure. Interestingly, these

614 changes in the algae and cyanobacteria communities did not affect the filtered water
615 quality (Pompei et al., 2019).

616 The samples collected 96 hours after the 2nd spiking are represented on the
617 negative side of axis 1 (Fig. 5b). A grouping of filters with a spiking of benzophenone-3
618 (B2) and ibuprofen (I2), and those filters with the addition of naproxen (N2) and the
619 mixture of PPCPs (Mix2) is observed, showing that there was a similarity in the
620 composition of the community. The control filter (C2) samples are not grouped with the
621 other filters (Fig. 5b), demonstrating again the difference in composition between algae
622 species from the control filter and the spiked filters with individual compounds or a
623 mixture of them.

624 The PCoAs of the 3rd spiking (Fig. 5c) showed 51.02% of the variability of data
625 articulation in the 1st two components (axis 1 – 39.89% and axis 2 – 20.13%). The 1st two
626 axes were statistically significant ($p < 0.05$) (Table S12).

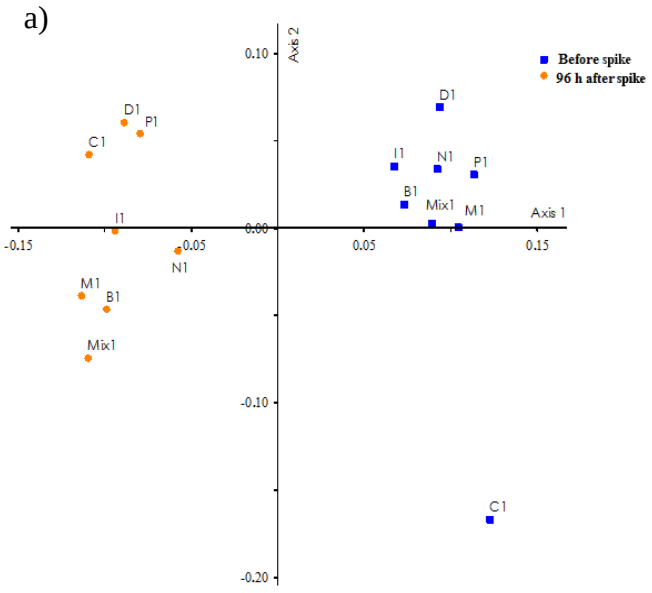
627 Filter samples before the 3rd spiking are grouped on the negative side of axis 2
628 (Fig. 3c and Table S12); and the species with the higher values of negative correlation are
629 associated with the biovolumes of species *Chroococcus minor* (r= -0.706), *Gomphonema*
630 sp*1*, *Oedogonium* sp., *Ochromonas ovalis* Doflein (r= -0.559 for each) and *Aphanocapsa*
631 *holsatica* (Lemmermann) G.Cronberg & Komárek (r= -0.554). This confirms that these
632 species, which developed in greater quantity (biovolume) in the micro-environment, were
633 more adaptable.

634 As occurred during spikings 1 and 2, on the 3rd spiking, the control filter (C3) was
635 not grouped with the others and was associated with the negative side of axis 1 (Fig. 3c),
636 showing a different composition of algae and cyanobacteria communities between some
637 of the filters that received PPCPs and the control filter. The species present in the control

filter probably did not adapt or had factors such as growth and development hampered by the presence of PPCPs, even though they were present in low concentrations.

The species with the highest correlation value in the PCoA for the 3rd spiking was *Chroococcus minor* (r= 0.872 on axis 1, r= -0.706 on axis 2). The filter samples collected 96 hours before the spiking were grouped on the positive side of axis 1, and on the positive side of axis 2, having the greatest weight in the axis ordering due to the biovolume of the species on filters with ibuprofen (I3), naproxen (N3) and mixture of PPCPs (Mix3). It is also observed that the filters which had the addition of personal care products (B3 and M3) were close to each other (Fig. 5c) at 96 hours after the spiking, as it was the case in the 1st spiking (Fig. 5a). This indicates that compounds of the same class caused similar reactions on the composition of species of algae and cyanobacteria in our study.

a)



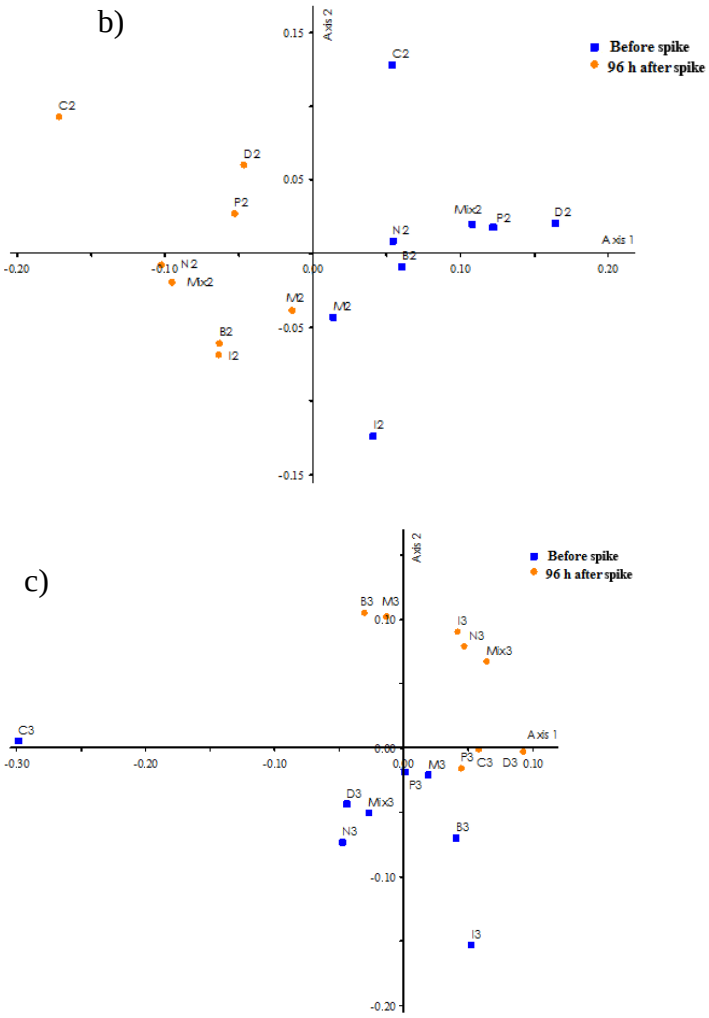


Figure 5: Principal Coordinate Analysis (PCoA) using significant species in each filter (C= control; P= paracetamol, D= diclofenac; N= naproxen; I= ibuprofen; M= methylparaben; B= benzophenone-3; Mix= PPCP mixture) for spikings (1= first; 2= second; 3= third), and sampling times.

3.4. Integrated analysis of biotic and abiotic parameters based on biovolume

The eigenvalues for axis 1 ($\lambda = 0.29$) and 2 ($\lambda = 0.09$) together explained 40.7% of all the data variability. The Monte Carlo test revealed that the first two axes were statistically significant ($p < 0.05$). Species-environment correlations were high for axis 1 ($r = 0.90$) and 2 ($r = 0.88$) and significant for the two axes of the CCA ($p = 0.001$),

663 indicating a strong relationship between the distribution of all environmental parameters
664 and the descriptor species (Table S13).

665 The intra-set correlations and the canonical coefficient (Table S14) indicate that
666 TN and DO were the parameters of greatest influence in the ordering of the positive side
667 of axis 1 (grouping of 1st spiking), with r = 0.82 and r = 0.56, respectively. Also, it is
668 observed that the filter samples were grouped according to before and after spiking (Fig.
669 6). These groups also included the control filter. Associated with the positive side of the
670 axis, the highest values of biovolumes were for species *Desmodesmus intermedius* (r =
671 0.88), *Dictyosphaerium pulchellum* (r = 0.70) and *Cryptomonas obovata* (r = 0.70).

672 The samples associated with *Pseudodidymocystis fina* (Komárek) E.Hegewald
673 & Deason (r = -0.72) and with the values of Chl-*a* (r = -0.76) (Fig. 6; Tables S13 and
674 S14) from the filters during the 3rd spiking with some from the 2nd spiking (B2b and P2b)
675 are on the negative side of axis 1, indicating the influence that Chl-*a* parameter had on
676 the ordering of the axis, and consequently, on the predominance of the species described
677 in the period. On the positive side of axis 2 in the CCA (Fig. 6), the ordering of the filter
678 samples in the 2nd spiking stands out, where the TP presented the greatest weight in the
679 axis ordering (r = 0.54) (Table S13). Chlorophyceae *Monoraphidium minutum* (Nägeli)
680 Komárková-Legnerová (r = 0.61), *Chlorella minutissima* (r = 0.55) and *Monoraphidium*
681 *irregulare* (G.M.Smith) Komárková-Legnerová (r = 0.54) had the higher values of
682 correlations on the axes and, therefore, better representation on the negative side of the
683 axis (Table S14).On the negative side of axis 2, Temp presented the greatest weight in
684 the axis ordering (r = -0.72), with some samples before the 3rd spiking, including the
685 control filter (C3a) and filter with paracetamol (P3a) (Fig. 6).

686 Different species, in most cases Chlorophyceae, were those that showed higher
687 values of correlation with the axes (Table S14), and this agrees well with other studies in

the case of bacteria. According to Haig et al. (2015), compositions of microbial communities in SSF are different depending on the state (operational or drained), age of the filter, sample location, month of sample collection, distances from the tributary and effluent, and depths at which the items were collected. In our study, the operation time of filters influenced the grouping of species in relation to the biovolume, as each spiking was grouped in a well-defined group (1 group for each spike), as can be seen in Fig. 4 and Fig. 6.

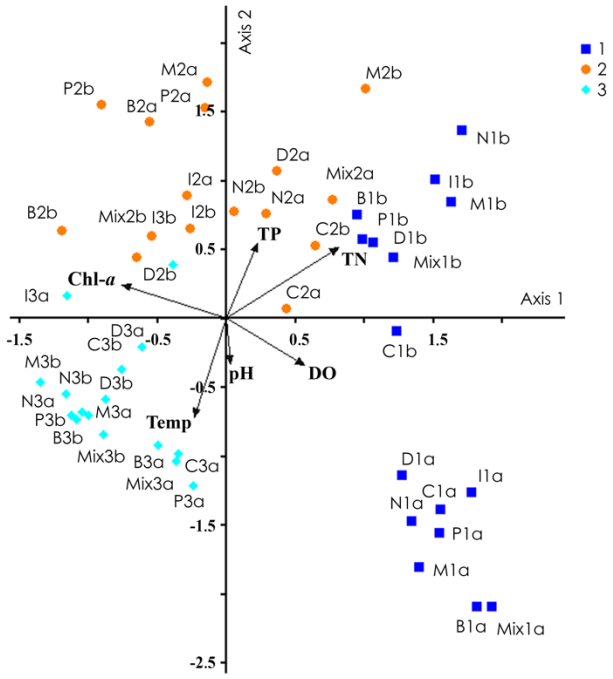


Figure 6: CCA biplot for each filter (C= control; P= paracetamol, D= diclofenac; N= naproxen; I= ibuprofen; M= methylparaben; B= benzophenone-3; Mix= PPCP mixture) during spikings (1= first; 2= second; 3= third) and sampling times (a= before spiking; b= 96 hours after spiking).

4. Conclusion

The studied ecological filters presented a diverse flora of algae and cyanobacteria, with Chlorophyceae and Cyanobacteria having high numbers of taxa and abundance. Although low, the concentration of PPCPs (2µg L^{-1}) affected the studied communities.

However, there was no difference between filters that received a single PPCP and between filters that received a mixture of the 6 compounds.

In addition, the effect of time (duration of filtrations) influenced the density and, consequently, the biovolume; richness, descriptor species and the composition of the algae and cyanobacteria community present in ecological filters in general. The collection time (before and 96 hours after the spikings) also influenced the communities' composition.

The species *Aulacoseira granulata, Chroococcus minutus, Dolichospermum planctonicum* and *Microcystis aeruginosa* were considered common descriptors for all samples in all spikings, indicating that they could be resistant to the 2µg L^{-1} of PPCPs added into the filters and to the PPCPs background concentration found in the raw water. *Lepocinclis* cf. *ovum* was the most abundant species on the filters, indicating it may have a tolerance to the concentration of selected PPCPs on filters.

Both qualitative and quantitative analyses of microalgae were performed by a microscope combined with manual discrimination, which may be affected by subjectivity. Therefore, it is recommended that a quantitative method, such as a molecular tool, be used to confirm the accuracy of the microscope method. This will support the use of the microscope method, which is a low-cost method compared to molecular tools, as a monitoring tool in water treatment plants in LMICs.

Despite observing that the low concentration of PPCPs did not affect the treated water quality, the fact that PPCPs impacted the algae and cyanobacteria communities indicates that the presence of these micropollutants in the aquatic environment can modify their structure and potentially pose a risk to the biotreatment performance, which requires further investigation.

The study demonstrates the capacity of SSF to produce high water quality, including the removal of bacteria and pharmaceuticals. This efficiency is attributed to the physical (i.e. fine sand) and biological mechanisms operating within the filters. This is a great benefit offered by SSF, in addition to being a low carbon and nature-based solution.

For future research, it is recommended that other emerging contaminants (e.g. estrogens, PFOS/PFAS) and their effect on other microorganisms (e.g. protists present in the biofilm) are evaluated.

Declaration of Competing Interests

No conflict of interest declared.

5. References

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Supplementary material

1. Material and Methods

1.1. Quantitative analysis of the algae and cyanobacteria community

The phytoplankton counting was carried out in horizontal and/or vertical transects and the counting limit was established through the species-rarefying curve, obtained from new species added with the number of fields counted, and until reaching up to 100 individuals of the most abundant or common species (Bicudo, 1990).

In the case of cyanobacteria or microalgae bloom, a count of 100 individuals of the second most abundant species was performed. Each cell, colony, cenobium and filament were considered as an individual. The results were expressed in density (organism mL^{-1}) and calculated according to Weber (1973):

$$\text{Organisms} = \left(\frac{n}{sc}\right).\left(\frac{1}{h}\right).(F) \quad (1)$$

where: n = number of individuals actually counted; s = field area in mm^2 at 40 times magnification; c = number of fields counted; h = height of the sedimentation chamber in mm; F = correction factor for milliliter (10^3 mm^3 mL^{-1}).

The Richness (R) was considered as the total number of the taxa found per sample. From the density results (organism mL^{-1}) and biovolume (μm^3 mL^{-1}) from the algae and cyanobacteria community, the Shannon and Weaver's (1963) Diversity Index (H') was calculated (bits ind^{-1}/bits μm^3), according to Eq. 2:

$$H' = -\sum_{i=1}^{n}(pi * \ln pi) \quad (2)$$

where: pi = ni/n; ni = total number of individuals from each taxon in the sample; n = total number of individuals in the sample.

2. Results and Discussions

2.1. Abiotic parameters

The mean temperature values were close to the control and mix filter values (around 22 °C) comparing samples collected before and after 96 hours of spiking the filters with single PPCP (around 21 °C). The DO varied from 6.5 to 6.7 mg L^{-1} in all filters during the studied period (Table S1).

The mean TN values were about to 0.4 - 0.5 mg L^{-1} in all filters during the study period, with a standard deviation (SD) of ± 0.5, while the TP concentration varied from 0.04 to 0.10 mg L^{-1}, with SD values around 0.03.

The filters with the highest TP values were those with methylparaben (0.07 mg L^{-1} before and 0.10 mg L^{-1} 96 hours after the spike) and ibuprofen (0.10 mg L^{-1} before and 0.06 mg L^{-1} 96 hours after spike), while the control filter had a mean value of 0.04 and 0.07 mg L^{-1} of TP. Filters that received PPCPs had higher TP values when compared with the control filter, except for paracetamol (0.05 mg L^{-1} for both, before and 96 hours after spiking).

Assessing the nutrients availability on water is directly related with the phytoplankton community, which can present accelerated growth (cyanobacteria and algae blooms). However, other conditions are required for bloom events, such as water temperature, luminosity, system residence time, photoperiod, wind activity, as well as the presence of zooplankton, that feed on algae (Reynolds, 1980; Padisák, 1997; Benson-Evans et al., 1999; Bouvy et al., 2000).

As with TP, Chl-*a* concentration also showed variability between samplings and between filters. The filter with paracetamol (P) had a higher concentration of Chl-*a* (21.53 µg L^{-1}), and the control filter (C) had the lowest concentration detected, followed by the filter with a mix of PPCPs (Mix) (10.00 µg L^{-1} and 10.45 µg L^{-1}, respectively).

Table S1: Description of PPCP applied in each ecological filter.

Filter number	Each added PPCP	Namely
FEco1	no adding of PPCP	C
FEco 2 to 4	Paracetamol	P
FEco 5 to 7	Diclofenac	D
FEco 8 to 10	Naproxen	N
FEco 11 to 13	Ibuprofen	I
FEco 14 to 16	Methylparaben	M
FEco 17 to 19	Benzophenone-3	B
FEco 20 to 22	Mixture of the 6 PPCPs	Mix
Contaminations	**Acronyms**	
First spiking event	1	
Second spiking event	2	
Third spiking event	3	
Before spiking event	a	
After 96 hours of spiking event	b	

1146

1147 **Table S2:** Description of used bibliography for the system of classification adopted and

1148 for taxonomy identification of genus and species.

System of classification adopted	
Class Chlorophyta	Round, (1971)
Class Bacillariophyceae, Fragilariophyceae and Coscinodiscophyceae	Round et al., (1990)
Class Cyanobacteria	Komárek and Anagnostidis (1989, 1998 and 2005); Hoffmam et al., (2005).
Other Classes	van den Hoek et al., (1995).
For Taxonomic identification	
For all green algae	Komárek and Fott (1983); Sant'Anna (1984); Comas (1996); Godinho et al., (2010); Rodrigues et at., (2010); Rosini et al., (2012 and 2013a); Ramos et al., (2012).
For Euglenophyceae	Tell and Conforti (1986); Conforti (1994);
For Cryptophyceae	Castro et al., (1991).
For Cyanobacteria	Komárková-Legnerová and Cronberg (1994); Azevedo et al., (1996); Azevedo and Sant'Anna, (1999, 2003); Komárek and Azevedo, (2000); Rosini et al., (2013b); Sant'Anna et al., (2004).

1149

Table S3.: Mean value and Standard Deviation (SD) of Temperature (Temp) (°C), Dissolved Oxygen (DO) (mg L^{-1}), pH, Total Nitrogen (TN) (mg L^{-1}), Total Phosphorus (TP) (mg L^{-1}), and Chlorophyll-*a* (Chl-*a*) (µg L^{-1}) for the composite sample before and after the 3 spiking events. The filters were named according to the spiked PPCP: control filter (C), paracetamol (P), diclofenac (D), naproxen (N), ibuprofen (I), methylparaben (M), benzophenone-3 (B), and a mix of all PPCPs (Mix). The nomenclature "a" and "b" refers to before and 96 hours after the spike event. Values are presented with ± SD.

	Filters															
	C		P		D		N		I		M		B		Mix	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Temp	22.60±0.79	21.53±1.46	22.26±0.86	21.26±1.47	22.21±0.93	21.22±1.53	22.44±0.96	21.14±1,45	22.39±0.94	21.18±1.45	22.47±1.16	21.13±1.28	22.46±1.23	21.19±1.33	22.68±1.09	22.68±1.40
DO	6.80±0.54	6.78±0.94	6.61±0.97	6.58±0.46	6.73±0.68	6.55±0.34	6.65±0.78	6.58±0.49	6.84±0.55	6,71±0,53	6.73±0.59	6.43±0.44	6.70±0.72	6.35±0.30	6.95±0.36	6.64±0.55
pH	6.68±0.31	6.50±0.12	6.68±0.21	6.61±0.06	6,75±0.14	6.61±0.08	6.65±0.08	6.59±0.05	6.72±0.06	6.70±0.10	6.68±0.07	6.60±0.07	6.58±0.06	6.55±0.09	6.62±0.09	6.62±0.06
TN	0.51±0.24	0.57±0.21	0.51±0.27	0.49±0.30	0.49±0.20	0.37±0.19	0.49±0.25	0.62±0.51	0.48±0.23	0.61±0.28	0.49±0.28	0.68±0.49	0.49±0.20	0.32±0.26	0.62±0.37	0.38±0.28
TP	0.04±0.01	0.07±0.06	0.05±0.03	0.05±0.03	0.08±0.02	0.05±0.04	0.04±0.02	0.07±0.06	0.10±0.04	0.06±0.01	0.07±0.04	0.10±0.12	0.06±0.04	0.06±0.05	0.07±0.06	0.07±0.07
Chl-*a*	9.65±1.09	10.35±5.25	10.36±2.61	32.71±40.13	11.49±2.61	12.00±8.12	14.33±9.24	12.51±5.09	18.10±11.53	15.77±9.07	17.11±10.53	11.03±6.36	13.15±8.88	11.20±7.02	10.56±5.81	10.35±5.04

Table S4: Taxonomic groups registered in SSF compared with the present study with ecological filters.

Taxonomic grups	Brook (1984) Nº of taxons	Brook (1984) %	This study Nº of taxons	This study %
Chlorophyceae	9	11%	58	37%
Cyanobacteria	2	3%	37	23%
Zygnemaphyceae	-	-	18	11%
Bacillariophyceae	68	86%	17	11%
Cryptophyceae	-	-	7	4%
Chrysophyceae	-	-	6	4%
Euglenophyceae	-	-	6	4%
Xanthophyceae	-	-	5	3%
Dinophyceae	-	-	2	1%

Table S5.: Taxa recorded in ecological filters during the studied period.

Cyanobacteria
Aphanocapsa cf. *conferta* (West & G.S.West) Komárková-Legnerová & Cronberg
Aphanocapsa delicatissima West & G.S.West
Aphanocapsa elachista West & G.S.West
Aphanocapsa holsatica (Lemmermann) G.Cronberg & Komárek
Aphanocapsa incerta (Lemmermann) G.Cronberg & Komárek
Aphanocapsa sp.1
Aphanothece sp. 1
Aphanothece sp. 2
Aphanothece sp. 3
Calothrix sp.
Chroococcus limneticus Lemmermann
Chroococcus minor (Kutzing) Nageli
Chroococcus minutus (Kutzing) Nageli
Coelosphaerium kuetzingianum Nägeli
Coelosphaerium minutissimum Lemmermann
Cyanodictium sp.
Dolichospermum circinale (rabenhorst ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek
Dolichospermum planctonicum (Brunnthaler) Wacklin, L.Hoffmann & Komárek
Geitlerinema sp. 1
Geitlerinema sp. 2
Gloeotrichia sp.
Merismopedia sp. 1
Merismopedia sp. 2
Merismopedia sp. 3
Microcystis aeruginosa (Kützing) Kützing
Microcystis protocystis W.B.Crow
Phormidium sp.1
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg
Planktolyngbya sp. 1
Planktolyngbya sp. 2

Planktothrix sp. 1
Pseudanabaena limnetica (Lemmermann) Komárek
Pseudanabaena mucicola (Naumann & Huber-Pestalozzi) Schwabe
Pseudanabaena sp. 1
Pseudanabaena sp. 2
Radiocystis fernandoi Komárek & Komárková-Legnerová
Synechocystis aquatilis Sauvageau

Bacillariophyceae

Achnanthidium minutissimum (Kützing) Czarnecki
Aulacoseira granulata (Ehrenberg) Ralfs
Aulacoseira sp. 1
Aulacoseira sp. 2
Aulacoseira sp. 3
Cyclotella meneghiniana Kützin
Discostella stelligera (Cleve & Grunow) Houk & Klee
Encyonema cf. *minutum* (Hilse ex Rabenh.) D.G.Mann
Eunotia camelus Ehrenberg
Fragilaria sp.
Gomphonema gracile Ehremberg
Gomphonema sp. 1
Gomphonema sp. 2
Melosira sp.
Navicula sp.
Pennales sp.
Surirella sp.

Chlorophyceae

Actinastrum sp.
Ankistrodesmus fusiformis Corda
Ankistrodesmus gracilis (Reinsch) Korshikov
Ankyra sp.
Botryococcus sp.
Bulbochaete sp.
Carteria sp. 1
Carteria sp. 2
Chlamydomonas sp. 2
Chlamydomonas sp. 3
Chlamydomonas sp. 4
Chlamydomonas gloeopara Rodhe & Skuja
Chlamydomonas planctogloea Skuja
Chlamydomonas sp. 1
Chlorella minutissima Fott & Nováková
Chlorella vulgaris Beverinck (Beijerinck)
Coelastrum microporum Nägeli
Coenocystis planktonica Korshikov
Coenocystis quadriguloides Fott
Desmodesmus abundans (Kirchner) E.Hegewald
Desmodesmus brasiliensis (Bohlin) E.Hegewald
Desmodesmus communis (E.Hegewald) E.Hegewald
Desmodesmus intermedius (Chodat) E.Hegewald
Dictyosphaerium ehrembergianum Nageli

Dictyosphaerium pulchellum H.C.Wood
Elakatothrix gelatinosa Wille
Eutetramorus fotti (Hindák) Komárek
Eutetramorus planctonicus (Korshikov) Bourrelly
Eutetramorus tetrasporus Komárek
Golenkinia sp.
Kirchneriella lunaris (Kirchner) Mobius
Kirchneriella rosellata Hindák
Monoraphidium arcuatum (Korshikov) Hindák
Monoraphidium caribeum Hindák
Monoraphidium contortum (Thuret) Komárková-Legnerová
Monoraphidium irregulare (G.M.Smith) Komárková-Legnerová
Monoraphidium komarkovae Nygaard
Monoraphidium minutum (Nägeli) Komárková-Legnerová
Monoraphidium tortile (West & G.S.West) Komárková-Legnerová
Oedogonium sp.
Oocystis lacustris Chodat
Oocystis marssoni Lemmermann
Oocystis sp.1
Pseudodidymocystis fina (Komárek) E.Hegewald & Deason
Pseudodidymocystis planctonica (Korshikov) E. Hegewald & Deason
Radiococcus hindakii (J.Komárek) I.Kostikov, T.Darienko, A.Lukesová, & L.Hoffmann
Radiococcus planktonicus J.W.G.Lund
Scenedesmus bijugus (Turpin) Lagerheim
Scenedesmus caudato-aculeolatus Chodat
Scenedesmus cf. *quadriculata* (Turpin) Brébisson
Scenedesmus opoliensis P.G.Richter
Scenedesmus sp. 1
Scenedesmus sp. 2
Sphaerocystis sp.
Stauridium tetras (Ehrenberg) E.Hegewald
Tetradesmus lunatus Korshikov
Tetrastrum heteracanthum (Nordstedt) Chodat
Tetrastrum komarekii Hindák

Chrysophyceae

Chromulina elegans Doflein
Chromulina sp. 1
Mallomonas sp. 1
Mallomonas sp. 2
Mallomonas sp. 3
Ochromonas ovalis Doflein

Cryptophyceae

Cryptomonas brasiliensis A.Castro, C.Bicudo & D.Bicudo
Cryptomonas curvata Ehremberg
Cryptomonas erosa Ehremberg
Cryptomonas marssonii Skuja
Cryptomonas obovata Skuja
Cryptomonas tetrapyrenoidosa Skuja
Rhodomonas lacustris Pascher & Ruttner

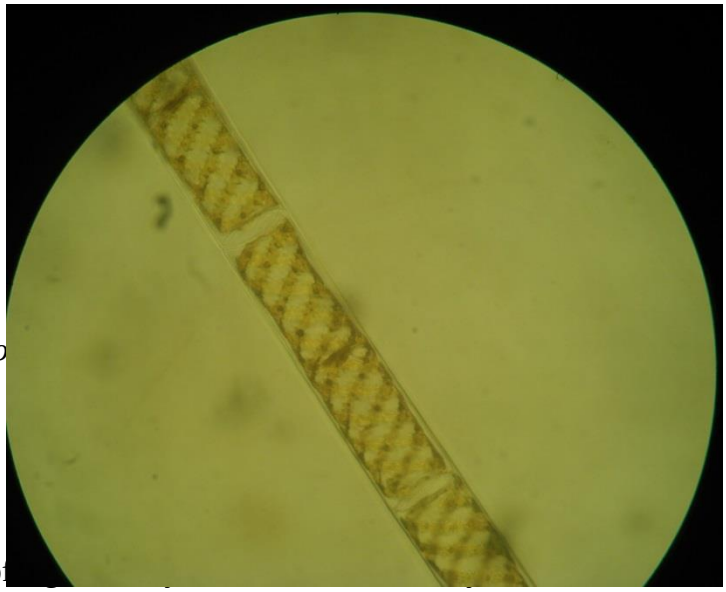
Dinophyceae

Gymnodinium sp.
Peridinium sp.
Euglenophyceae
Lepocinclis cf. *ovum* (Ehrenberg) Lemmermann 1901
Phacus curvicauda Svirenko
Phacus tortus (Lemmermann) Skvortzov
Trachelomonas sp. 1
Trachelomonas volvocina (Ehrenberg) Ehrenberg
Trachelomonas volvocinopsis Svirenko
Xanthophyceae
Characiopsis sp. 1
Characiopsis sp. 2
Isthmochlorom lobulatum (Nägeli) Skuja
Tetraediella spinigera Skuja
Tetraplektron torsum (W.B.Turner) Dedusenko-Shchegoleva
Zygnemaphyceae
Actinathaenium sp.
Arthrodesmus sp.
Closterium sp.1
Cosmarium humile Nordstedt ex De Toni
Cosmarium sp. 3
Cosmarium sp. 4
Cosmarium sp. 5
Cosmarium sp.1
Cosmarium sp. 2
Mougeotia sp.
Pleurataenium sp. 1
Spirogyra sp.
Staurastrum rotula Nordstedt
Staurastrum sp. 1
Staurastrum apical view sp. 1
Staurastrum apical view sp. 2
Staurodesmus sp.
Staurodesmus triangularis (Lagerheim) Teiling

Table S6: Richness (R) of taxa (species) identified in each filter (22) during the spike events (3), as the first letter of each filter indicates the type of PPCP applied – Table S1), followed by the respective time of sampling (a= before spike; b= 96 hours after spike).

	Richness (number of species)		
Filters	**1st spike**	**2nd spike**	**3rd spike**
Ca	21	30	14
Pa	41	56	49
Da	45	62	43
Na	41	54	43
Ia	46	65	57
Ma	41	56	46

Ba	42	50	53
Mixa	27	49	48
Cb	37	25	36
Pb	43	47	56
Db	43	43	62
Nb	45	41	58
Ib	41	42	58
Mb	45	46	60
Bb	48	44	57
Mixb	45	40	52



ro

of

contribution in biovolume (acronyms according to Table S1), 1st spike event.

	%							
Descriptors species (before)	**C1a**	**P1a**	**D1a**	**N1a**	**I1a**	**M1a**	**B1a**	**Mix1a**
Aulacoseira granulata	0.0	3.6	0.9	1.2	0.9	0.5	2.2	0.5
Aulacoseira sp. 2	2.8	3.3	1.8	1.1	1.1	1.5	0.9	1.4
Aulacoseira sp. 3	0.0	2.1	1.3	1.1	0.0	1.6	2.8	0.5
Chroococcus minutus	0.8	2.2	2.8	2.3	2.3	3.1	2.3	3.1
Cyclotela meneghinuana	0.9	4.3	5.0	4.9	3.1	2.4	1.5	5.6
Dolichospermum planctonicum	13.7	14.6	17.6	20.2	15.1	29.6	20.2	23.5
Lepocinclis cf. *ovum*	66.0	42.4	51.9	58.4	57.8	45.0	58.3	61.5
Microcystis aeruginosa	11.6	21.6	11.7	6.0	8.7	6.2	4.5	0.0
Microcystis protocystis	0.9	1.0	0.3	0.2	1.4	2.4	2.0	0.2
Descriptors species (96 hours after)	**C1b**	**P1b**	**D1b**	**N1b**	**I1b**	**M1b**	**B1b**	**Mix1b**
Aulacoseira granulata	3.0	4.1	2.7	2.3	1.5	3.1	2.1	1.3
Aulacoseira sp. 2	1.9	1.2	1.0	0.8	1.3	1.0	1.2	1.8
Aulacoseira sp. 3	2.2	1.3	0.0	1.1	0.6	0.7	1.8	0.5
Chroococcus minutus	2.2	4.4	2.5	2.7	4.9	3.1	4.3	3.3
Cryptomonas tetrapyrenoidosa	0.5	0.8	1.2	0.8	1.2	1.1	2.0	1.6
Dolichospermum planctonicum	3.3	5.7	2.6	3.0	5.0	2.2	3.9	2.3
Lepocinclis cf. *ovum*	77.0	78.0	86.2	82.4	76.5	82.2	74.0	81.4

Microcystis aeruginosa	0.0	0.0	0.0	2.6	0.0	2.7	3.8	1.4

1180

1181 **Table S8:** Descriptor's species of algae and cyanobacteria community based on % of

1182 contribution in biovolume (acronyms according to Table S1), 2nd spike event.

	%							
Descriptors species (before)	**C2a**	**P2a**	**D2a**	**N2a**	**I2a**	**M2a**	**B2a**	**Mix2a**
Aulacoseira granulata	6.2	2.8	2.2	5.6	2.3	3.0	2.7	8.6
Aulacoseira sp. 2	5.8	3.6	2.5	3.7	2.4	3.9	3.6	7.1
Aulacoseira sp. 3	0.0	1.0	2.0	3.4	1.5	1.4	1.7	3.3
Chroococcus minutus	9.6	3.9	3.0	5.0	3.6	3.8	4.1	3.8
Dolichospermum planktonicum	16.1	5.6	5.1	11.0	5.4	5.9	5.6	6.6
Gomphonema gracile	0.0	1.6	1.1	1.3	2.0	1.0	1.2	2.9
Lepocinclis cf. *ovum*	44.0	70.5	72.4	61.8	18.3	68.7	76.7	59.1
Microcystis aeruginosa	0.0	4.8	0.7	1.7	3.2	6,0	0.0	2.3
Spirogyra sp.	0.0	0.0	0.0	0.0	55.3	0,0	0.0	0.0
Descriptors species (96 hours after)	**C2b**	**P2b**	**D2b**	**N2b**	**I2b**	**M2b**	**B2b**	**Mix2b**
Aulacoseira granulata	0.0	2.8	0.7	1.3	0.3	1.7	0.8	3.8
Aulacoseira sp. 2	20.6	1.2	1.4	0.6	0.9	3.4	0.6	2.0
Chroococcus minutus	13.9	9.5	8.9	12.1	3.1	7.1	2.6	8.1
Dolichospermum planktonicum	21.6	37.5	36.2	43.1	12.0	26.4	9.1	45.5
Gomphonema gracile	0.0	1.5	0.6	1.0	0.3	4.9	1.3	1.2
Lepocinclis cf. *ovum*	0.0	33.6	31.9	24.7	8.4	35.5	14.8	0.0
Microcystis aeruginosa	0.0	0.0	7.0	0.0	2.8	5.2	0.0	22.3
Spirogyra sp.	0.0	0.0	0.0	0.0	67.4	0.0	66.9	0.0

1183

1184 **Table S9:** Descriptor's species of algae and cyanobacteria community based on % of

1185 contribution in biovolume (acronyms according to Table S1), 3rd spike event.

	%							
Descriptors species (berofe)	**C3a**	**P3a**	**D3a**	**N3a**	**I3a**	**M3a**	**B3a**	**Mix3a**
Aphanocapsa holsatica	75.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aulacoseira granulata	0.0	0.7	1.9	0.0	6.0	2.2	1.9	0.0
Aulacoseira sp.2	0.0	1.9	1.9	0.3	9.5	0.9	0.8	2.1
Aulacoseira sp.3	5.6	0.0	1.5	2.0	0.0	0.0	0.0	1.2
Chroococcus minutus	11.1	18.9	15.0	16.8	9.8	20.5	9.1	12.2
Coenocystis quadriguloides	0.5	1.4	1.7	2.7	0.5	2.0	0.8	1.2
Cryptomonas marssonii	0.0	1.7	2.3	2.9	0.6	1.4	1.1	2.2
Cyclotela meneghiniana	3.5	4.2	10.4	4.2	2.2	4.4	2.6	2.1
Dolichospermum planktonicum	0.0	6.3	9.0	12.3	1.9	7.5	4.0	7.2
Lepocinclis cf. *ovum*	0.0	48.0	41.7	25.7	2.4	44.3	17.4	52.9
Microcystis aeruginosa	0.0	0.0	0.0	18.0	15.4	2.5	3.4	6.1
Microcystis protocystis	2.4	1.7	2.3	0.4	1.8	3.0	1.8	1.7
Radiococcus fotti	0.0	4.4	5.0	4.1	2.4	2.0	1.3	3.8
Spirogyra sp.	0.0	0.0	0.0	0.0	38.0	0.0	49.3	0.0

Descriptors species (96 hours after)	C3b	P3b	D3b	N3b	I3b	M3b	B3b	Mix3b
Aulacoseira granulata	4.3	2.0	2.3	1.8	0.0	1.5	1.4	2.6
Chroococcus minutus	4.4	8.4	8.7	16.4	1.0	13.3	13.5	21.0
Cryptomonas tetrapyrenoidosa	2.2	1.4	1.9	4.8	0.1	3.9	2.5	5.2
Cyclotela meneghiniana	0.0	2.1	1.4	5.8	0.4	3.1	3.4	5.9
Dolichospermum planktonicum	5.2	3.5	5.6	10.4	0.6	4.2	8.2	10.6
Lepocinclis cf. *ovum*	60.7	63.7	66.5	26.6	3.4	50.6	36.3	16.6
Microcystis aeruginosa	14.1	6.0	0.0	0.0	0.0	0.0	0.0	0.0
Radiococcus fotti	4.7	4.4	4.9	9.6	0.6	11.3	12.3	14.6
Spirogyra sp.	0.0	0.0	0.0	0.0	92.1	0.0	0.0	0.0

Table S10: Pearson correlation coefficient between the significant species identified in the 22 ecological filters including control filter, in the three spike events, in the first two ordering axes (n = 48).

	Correlation	
Taxa	**Axis 1**	**Axis 2**
Aphanocapsa cf. *conferta*	0.568	0.022
Aphanocapsa elachista	-0.524	0.011
Aphanocapsa sp.1	-0.596	0.182
Aphanothece sp. 2	-0.658	0.112
Aulacoseira sp. 2	0.539	0.387
Chlorella minutíssima	-0.551	-0.046
Chromulina sp. 1	-0.593	-0.048
Closterium sp.1	0.692	-0.075
Coelasphaerium minutissimum	-0.726	0.159
Coenocystis quadriguloides	-0.854	0.041
Cryptomonas obovata	0.722	0.236
Desmodesmus brasiliensis	-0.729	-0.274
Desmodesmus intermedius	0.791	0.206
Dictyosphaerium pulchellum	0.835	0.068
Eutetramorus fotti	-0.854	0.033
Eutetramorus planctonicus	-0.682	0.005
Fragilaria sp.	0.408	-0.530
Geitlerinema sp. 1	-0.770	-0.056
Gomphonema gracile	0.374	-0.559
Kirchneriella rosellata	-0.777	0.346
Monoraphidium arcuatum	-0.629	-0.304
Monoraphidium irregulare	-0.415	-0.576
Monoraphidium minutum	-0.636	-0.100
Peridinium sp.	0.537	-0.024
Planktolyngbya limnetica	-0.688	-0.397
Planktolyngbya sp. 1	0.519	0.136
Pseudodidymocystis fina	-0.750	-0.035
Radiococcus planktonicus	-0.683	0.156
Synechocystis aquatilis	-0.589	-0.013
Trachelomonas volvocinopsis	0.537	0.165

Percentage of axle explanability	55.060	10.411

Table S11: Pearson correlation coefficient between the significant species identified in the 22 ecological filters including control filter, in the 1st spike event, in the first two ordering axes (n = 16).

	Correlation	
Taxa	**Axis 1**	**Axis 2**
Aphanocapsa delicatissima	- 0.560	0.566
Aphanocapsa incerta	- 0.741	0.329
Aphanothece sp.1	- 0.792	0.357
Aulacoseira granulata	- 0.601	0.663
Aulacoseira sp.2	- 0.737	- 0.172
Chlorella minutíssima	- 0.797	- 0.234
Chlorella vulgaris	- 0.194	0.695
Chroococcus minor	- 0.687	0.197
Chroococcus minutus	- 0.929	0.189
Closterium sp.1	0.113	0.507
Cryptomonas brasiliensis	- 0.389	0.700
Cryptomonas erosa	- 0.681	0.375
Cryptomonas marssonii	- 0.495	0.693
Cryptomonas obovata	0.431	0.708
Cryptomonas tetrapyrenoidosa	- 0.982	- 0.053
Dictyosphaerium ehrembergianum	- 0.826	0.199
Dictyosphaerium pulchellum	- 0.633	0.480
Dolichospermum planctonicum	0.707	0.406
Eutetramorus tetrasporus	0.602	0.445
Gymnodinium sp.	- 0.740	- 0.257
Kirchneriella lunaris	- 0.217	- 0.627
Lepocinclis cf. *ovum*	- 0.947	- 0.045
Mallomonas sp.2	- 0.770	0.067
Melosira sp.	- 0.699	0.057
Monoraphidium arcuatum	- 0.637	- 0.106
Monoraphidium contortum	- 0.251	0.590
Monoraphidium irregulare	- 0.572	- 0.317
Monoraphidium komarkovae	- 0.677	0.218
Monoraphidium minutum	- 0.979	- 0.036
Monoraphidium tortile	- 0.850	- 0.014
Ochromonas ovalis	- 0.540	- 0.439
Oocystis sp.1	- 0.537	- 0.442
Peridinium sp.	- 0.548	0.287
Planktolyngbya sp. 1	0.754	0.375
Pleurataenium sp. 1	0.329	- 0.743
Pseudanabaena mucicola	- 0.726	0.520
Percentage of axle explanability	43.005	15.760

Table S12: Pearson correlation coefficient between the significant species identified in the 22 ecological filters including control filter, in the 2nd spike event, in the first two ordering axes (n = 16).

	Correlation	
Taxa	**Axis 1**	**Axis 2**
Achnanthidium minutissimum	0.424	- 0.691
Aphanocapsa delicatissima	0.650	0.013
Aphanocapsa incerta	0.568	- 0.122
Aulacoseira sp.2	0.813	- 0.106
Aulacoseira granulata	0.720	- 0.351
Aulacoseira sp.3	0.776	- 0.313
Bulbochaete sp.	- 0.342	- 0.691
Characiopsis sp. 1	- 0.758	- 0.423
Chlamydomonas sp. 1	- 0.006	- 0.686
Chlorella minutissima	- 0.702	- 0.190
Chlorella vulgaris	- 0.163	- 0.588
Chroococcus minor	0.375	- 0.748
Chroococcus minutus	0.697	- 0.367
Closterium sp.1	0.746	0.006
Coenocystis quadriguloides	- 0.715	- 0.334
Cosmarium humile	0.117	- 0.531
Cryptomonas brasiliensis	0.653	- 0.092
Cryptomonas erosa	0.733	0.511
Cryptomonas tetrapyrenoidosa	0.546	0.002
Desmodesmus brasiliensis	- 0.173	- 0.525
Desmodesmus communis	0.578	0.124
Dictyosphaerium pulchellum	0.752	0.247
Discostella stelligera	- 0.133	- 0.584
Eutetramorus planctonicus	0.126	- 0.579
Fragilaria sp.	0.371	- 0.710
Geitlerinema sp. 1	- 0.752	0.113
Gomphonema gracile	0.398	- 0.728
Gomphonema sp. 1	0.117	- 0.531
Lepocinclis cf. *ovum*	0.695	- 0.224
Mallomonas sp.1	0.606	0.122
Melosira sp.	0.569	- 0.222
Mougeotia sp.	0.262	- 0.509
Navicula sp.	0.603	0.118
Oedogonium sp.	0.587	- 0.111
Oocystis sp.1	0.652	- 0.205
Phormidium sp.1	0.741	- 0.321
Pseudanabaena mucicola	0.706	- 0.193
Radiococcus planktonicus	0.235	0.569
Radiocystis fernandoi	0.509	- 0.011
Spirogyra sp.	- 0.150	- 0.679
Staurastrum sp. 1	0.114	- 0.525
Synechocystis aquatilis	- 0.728	- 0.315
Percentage of axle explanability	34.941	15.991

Table S13: Pearson correlation coefficient between the significant species identified in the 22 ecological filters including control filter, in the 3rd spike event, in the first two ordering axes (n = 16).

	Correlation	
Taxa	**Axis 1**	**Axis 2**
Aphanocapsa delicatissima	0.886	0.166
Aphanocapsa elachista	0.360	0.565
Aphanocapsa holsatica	-0.763	-0.554
Aphanocapsa sp.1	0.150	0.746
Aphanothece sp. 1	0.280	0.588
Aphanothece sp. 2	0.463	0.120
Aulacoseira granulata	0.569	-0.018
Aulacoseira sp.2	0.881	-0.106
Aulacoseira sp.3	-0.756	0.076
Characiopsis sp. 1	-0.619	0.435
Chlamydomonas sp. 2	0.224	0.697
Chlorella vulgaris	0.884	-0.018
Chromulina sp.1	0.480	-0.444
Chromulina sp.2	0.561	0.137
Chroococcus limneticus	0.507	0.008
Chroococcus minor	0.872	-0.706
Chrysophyceae não identificada	0.156	-0.559
Cryptomonas brasiliensis	-0.197	0.627
Cryptomonas marssonii	0.844	0.818
Cryptomonas tetrapyrenoidosa	0.565	0.018
Cyanophyceae filamentosa não identificada	0.061	0.043
Desmodesmus brasiliensis	0.915	0.131
Dictyosphaerium ehrembergianum	0.030	0.521
Discostella stelligera	0.511	- 0.060
Dolichospermum planctonicum	0.879	0.750
Euglenophyta não identificada	0.868	0.559
Eutetramorus fottii	0.881	0.111
Fragilaria sp.	0.548	-0.187
Geitlerinema sp. 1	0.525	0.649
Geitlerinema sp. 2	0.240	0.042
Gomphonema sp.1	0.156	-0.559
Mallomonas sp.3	0.029	0.522
Melosira sp.	0.884	0.013
Microcystis aeruginosa	0.211	-0.007
Monoraphidium arcuatum	0.760	-0.220
Monoraphidium contortum	0.531	-0.170
Monoraphidium irregulare	0.675	0.101
Monoraphidium tortile	0.837	0.082
Ochromonas ovalis	0.156	-0.559
Oedogonium sp.	0.156	-0.559
Oocystis sp.1	0.074	0.675
Pseudanabaena mucicola	-0.544	0.772
Pseudodidymocystis planctonica	0.166	0.851
Radiococcus planktonicus	0.933	0.056
Rhodomonas lacustris	0.285	0.044

Synechocystis aquatilis	0.444	0.549
Tetraediella spinigera	0.156	0.559
Tetrastrum komarekii	0.080	0.517
Percentage of axle explanability	30.891	20.138

Table S14: Synthesis of the results from Canonical Correspondence Analysis (ACC) carried out from 6 environmental parameters and 37 significant species (n = 48).

	Axis 1	**Axis 2**
Eigenvalues (λ)	0.290	0.090
Percentage of explained variance (%)	30.700	10.000
Percentage Accumulated Variance	30.700	40.800
Correlation of Pearson (specie-environment)	0.900	0.880
Monte Carlo test (p) Eigenvalues	0.001	0.001
Monte Carlo test (p) correlation of specie-environment	0.001	0.001

Table S15: Canonical coefficient and "intra-set" correlations of the six environmental parameters with axes 1 and 2 of the ACC, performed with the 37 significant species of ecological filters (n = 48).

	Canonical Coefficient		**Correlation coefficient "intra-set"**	
Parameters	**Axis 1**	**Axis 2**	**Axis 1**	**Axis 2**
Temperature (Temp)	-0.234	-0.725	-0.212	-0.643
Dissolved Oxigen (DO)	0.564	-0.339	0.511	-0.301
pH	0.031	-0.335	0.028	-0.297
Total Nitrogen (TN)	0.817	0.511	0.741	0.453
Total Phosphurus (TP)	0.228	0.541	0.206	0.479
Chlorophyll-*a* (Chl-*a*)	-0.762	0.244	-0.691	0.216

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