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Environmental Pollution

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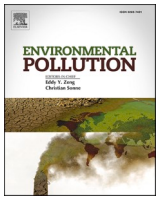
https://doi.org/10.1016/j.envpol.2024.124051

Environmental Pollution 351 (2024) 124051

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Cyanopeptides occurrence and diversity in a Brazilian tropical reservoir: Exploring relationships with water quality☆

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ARTICLE INFO

Keywords:
Harmful algal bloom
Bioactive cyanometabolites
Microcystin-producers

ABSTRACT

Microcystins (MCs) are a class of toxic secondary metabolites produced by some cyanobacteria strains that endanger aquatic and terrestrial organisms in various freshwater systems. Although patterns in MC occurrence are being recognized, divergences in the global data still hamper our ability to predict the toxicity of cyanobacterial blooms. This study aimed (i) to determine the dynamics of MCs and other cyanopeptides in a tropical reservoir, (ii) to investigate the correlation between peptides and potential cyanotoxin producers (iii) identifying the possible abiotic factors that influence the peptides. We analyzed, monthly, eight MC variants (MC-RR, -LA, -LF, -LR, -LW, -YR, [D-Asp3]-RR and [D-Asp3]-LR) and other peptides in 47 water samples collected monthly, all season long, from two sampling sites in a tropical eutrophic freshwater reservoir, in southeastern Brazil. The cyanopeptides were assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The biomass of potential cyanobacterial producers and water quality variables were measured. MCs were detected in both sampling sites year-round; the total MC concentration varied from 0.21 to 4.04 μg L^{-1}, and three MC variants were identified and quantified (MC-RR, [D-Asp3]-RR, -LR). Additionally, we identified 28 compounds belonging to three other cyanopeptide classes: aeruginosin, microginin, and cyanopeptolin. As potential MC producers, *Microcystis* spp. and *Dolichospermum circinalis* were dominant during the study, representing up to 75% of the total phytoplankton. Correlational and redundancy analysis suggested positive effects of dissolved oxygen, nitrate, and total phosphorus on MC and microginins concentration, while water temperature appeared to favor aeruginosins. A comparison between our results and historical data showed a reduction in total phosphorus and cyanobacteria, suggesting increased water quality in the reservoir. However, the current MC concentrations indicate a rise in cyanobacterial toxicity over the last eight years. Moreover, our study underscores the pressing need to explore cyanopeptides other than MCs in tropical aquatic systems.

1. Introduction

Harmful cyanobacteria and their toxins have raised a global environmental challenge for modern society (Chorus and Welker, 2021). Blooms of cyanobacteria in freshwater systems have increased in frequency and intensity with anthropogenic eutrophication, resulting in ecosystem degradation and adverse consequences on human health and global economies (O'Neil et al., 2012; Paerl and Huisman, 2008).

☆ This paper has been recommended for acceptance by Amit Bhatnagar.
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https://doi.org/10.1016/j.envpol.2024.124051
Received 9 October 2023; Received in revised form 19 April 2024; Accepted 24 April 2024
Available online 28 April 2024

Cyanobacteria blooms are often accompanied by the production of a variety of cyanotoxins; one of the most widespread cyanotoxins found in cyanobacterial blooms is hepatotoxic and tumor-promoting microcystins (MCs) (Bouaïcha et al., 2019; Sivonen and Jones, 1999; Svirčev et al., 2019). Various genera of cyanobacteria, such as *Microcystis*, *Planktothrix*, *Nostoc*, and *Dolichospermum* are known to produce MCs (Fastner and Humpage, 2021). However, genus or species may contain both producer (toxigenic) and non-producer strains (Buratti et al., 2017; Harke et al., 2016; Meriluoto et al., 2016; Mowe et al., 2014). In addition, about 300 MC variants have already been recognized (Jones et al., 2020). Many MC variants can be synthesized by one toxigenic strains simultaneously (Puddick et al., 2014), although usually, in most genera, one to three variants tend to be more prevalent than the others (Fastner and Humpage, 2021). Usually reported as total MC concentrations (Sivonen and Jones, 1999), few studies have examined the MC variants on a temporal and spatial scale in tropical aquatic systems, especially in Latin America; the cost of detecting cyanotoxins is expensive, and the necessary equipment for some methods is not typically found in most laboratories in developing and underdeveloped countries (Aguilera et al., 2023). In Latin America, the most frequently MC variants reported are MC-LA, -LR, –RR, -YR, -LF, [D-Asp^3]-RR, [D-Asp^3] -LR and (E)-Dhb^7]- RR, but data from 35% of countries are unavailable (Aguilera et al., 2023). However, the identification of MC variants is an essential consideration since the dominance of one variant over another in a bloom event will influence overall toxicity (Cerasino and Salmaso, 2012; Monchamp et al., 2014).

Recent developments in analytical methods have enabled researchers to detect numerous others cyanopeptide classes, expanding beyond the widely recognized MC class, where, like MCs, the number of amino acids and the order in their composition are highly variable in these compounds, making it possible for several variants to occur in the same class (Janssen, 2019). These newly identified compounds encompass cyanopeptolins, anabaenopeptins, aerucyclamides, aeruginosins, and microginins, among others (Welker and Von Döhren, 2006). These peptides are presumably synthesized by hybrid non-ribosomal peptide synthetase (NRPS) or NRPS/polyketide synthetase (PKS) pathways and present a considerable range of biological activities, many of which are still unknown (Huang and Zimba, 2019). Some studies suggest that cyanopeptides can favor competitive success through allelopathic effects, toxicity, persistence, and 'physiological facilitators' in improving metabolic activities, such as photosynthetic efficiency (Holland and Kinnear, 2013; Omidi et al., 2018). Although we do not yet have a complete understanding of the physiological functions of these compounds, it is essential to highlight that some of them have pharmacological potential, exhibiting antibiotic, immunosuppressive, anticancer, antiviral, and anti-inflammatory activities (Rastogi and Sinha, 2009). In addition, ecotoxicological investigations have hinted that the toxic effect of cyanobacterial extracts cannot be solely ascribed to MCs, emphasizing the importance of studying and considering other bioactive metabolites in risk assessments (Baumann and Jüttner, 2008; Keil et al., 2002; Le Manach et al., 2016; Smutná et al., 2014).

In general, MCs' presence and temporal variation are strongly related to the seasonal succession of cyanobacteria and the waxing and waning of toxic and non-toxic genotypes (Meissner et al., 2013). In addition, environmental factors not only affect the abundance of toxic and non-toxic strains but also influence MC biosynthesis (Bashir et al., 2023; Meissner et al., 2013; Wu et al., 2006). Studies have provided a framework for the typical conditions in which a toxigenic cyanobacterial bloom may occur (e.g., nutrient availability, light availability, warm temperature (Dziallas and Grossart, 2011; Kaebernick et al., 2000; Oh et al., 2000; Puddick et al., 2016). Nertheless, the global data indicates a wide variability in conditions most related to MC occurrence (Aguilera et al., 2023; Buley et al., 2022; González-Piana et al., 2017; Kotak et al., 2000; Wu et al., 2006). However, our understanding of the co-production dynamics of other cyanopeptide classes remains limited (Bober et al., 2023; Tonk et al., 2009). Thus, systematic monitoring is essential to measure the dynamics of MCs and other peptide classes in aquatic environments, providing vital data for public policies and efficient contingency plans during toxic bloom events (Chorus and Welker, 2021).

Thus, this study aimed: (i) to determine the occurrence, concentrations, and dynamics of MC variants (extracellular and intracellular), and the occurrence of other cyanopeptides in the reservoir; (ii) to investigate the correlation between MCs and their variants with potential cyanotoxin producer's; (iii) to identify the possible environmental drivers that influence the occurrence and concentration of each MC variant.

2. Materials and methods

2.1. Study area

Funil Reservoir (40 km^2) is a eutrophic system in Rio de Janeiro state, Brazilian Southeast (22°30′S, 44°45′W; Fig. 1), at 440 m of altitude, in a warm-rainy tropical climate area (Cwa in the Köppen system). Funil Reservoir is the primary water supply source for the Rio de Janeiro metropolitan region. It helps regulate the flow of the Paraíba do Sul River, mitigating the impacts of floods and allowing water transfer to a complex of reservoirs (Soares et al., 2009). The reservoir water is also used in irrigation, pisciculture, and recreation. To date, studies on cyanometabolites in Funil Reservoir have focused mainly on intracellular microcystins; the only study about MCs profile was episodic (Ferrão-Filho et al., 2009; Guedes et al., 2014). In addition, there are no published data on the diversity of cyanometabolites throughout all seasons.

2.2. Sampling and sample analyses

Samples were collected monthly during 2019 from two different sites, both already monitored in previous studies. FL35 is in the main body of the reservoir, in a post-transition region; it has a historical average depth of 36m depth and high chlorophyll concentration during the rainy season. FL50 is located near the dam, with an average depth of 47m and higher chlorophyll concentrations in the dry season (Fig. 1) (Pacheco et al., 2015). Water transparency at each sampling site was estimated by Secchi depth (SD) and euphotic zone (Z_{eu}) as 2.7 times the SD (Cole, 1994). Water temperature (WT, °C), pH, dissolved oxygen concentration (DO, mg L^{-1}), dissolved oxygen saturation (%), conductivity (μS cm^{-1}), turbidity (NTU), were measured with a multiparameter sonde (YSI model 600R). The alkalinity (mg L^{-1} $CaCO_3$) was measured with a portable turbidimeter (HANNA - HI98703). Chlorophyll (μg L^{-1}), cyanotoxins (μg L^{-1}) and nutrients (μg L^{-1}) samples were obtained from an integrated sampling of the euphotic zone (Z_{eu}) when the reservoir was not stratified. During stratified periods, the samples were integrated from the epilimnion. Integrated sampling was carried out using a Van Dorn bottle every 0.5m.; samples were collected and homogenized in a container before being aliquoted for toxins and other analyses. Phytoplankton samples were immediately preserved with Lugol's solution. Cyanobacterial species and other phytoplankton species were enumerated according to the settling technique (Utermöhl, 1958) in random fields (Uehlingher, 1964) using an inverted microscope (Zeiss Oberkochen, Axiovert10, Germany). Biovolume (mm^3 L^{-1}) was estimated by multiplying the density of each species by the average volume of its cells (Hillebrand et al., 1999). Total and cyanobacterial chlorophyll-*a* concentrations (μg L^{-1}) were determined using a PHYTO-PAM phytoplankton analyzer (HeinzWalzGmbH, Effeltrich, Germany), previously calibrated in Chl (MF)-mode according to the manufacturer. Total nitrogen (TN), nitrate (N–NO_3^-); nitrite (N–NO_2-), ammonium (N–NH_4^+), dissolved inorganic nitrogen (DIN) as the sum of nitrate + nitrite + ammonium, total phosphorus (TP), and soluble reactive phosphorus (SRP) concentrations were analyzed by an automated colorimetric flow injection analysis system (FIA) equipped with an autosampler (model FIAlab-2500, FIALab Instruments Inc., Seattle, Washington, USA)

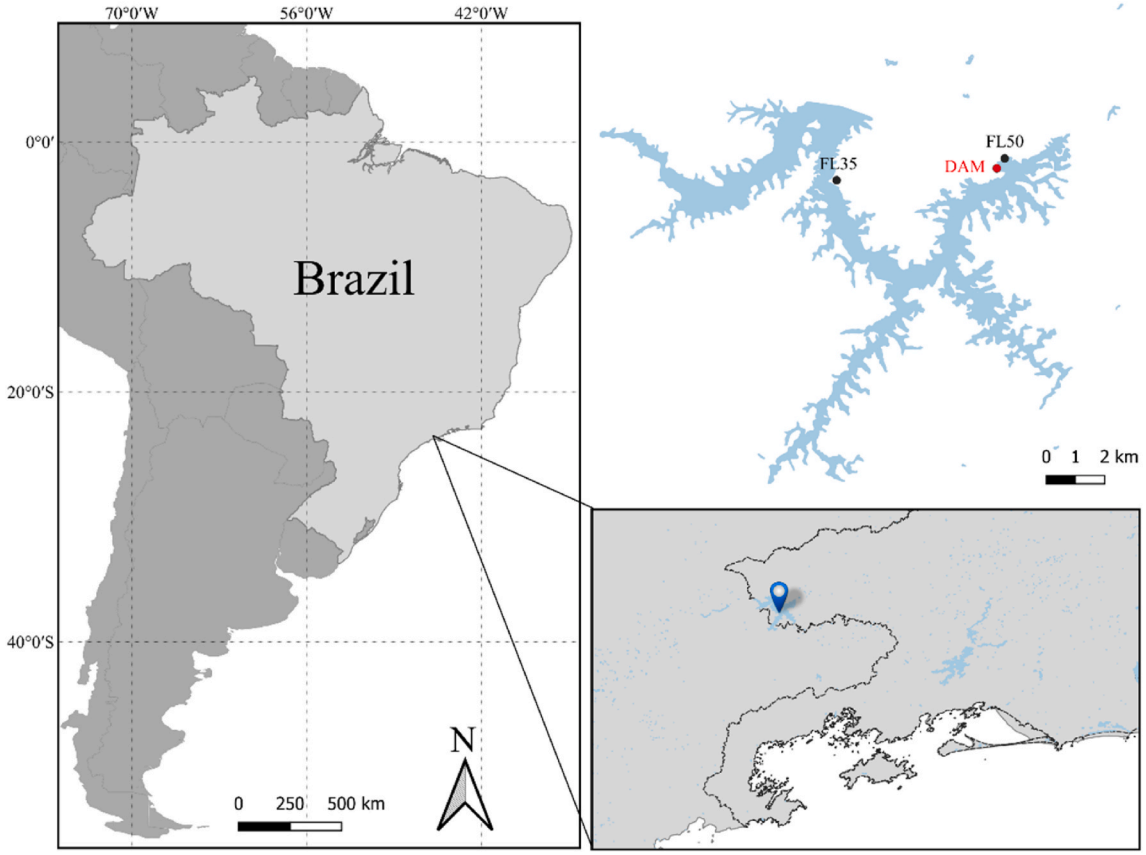

Fig. 1. Map and geographic location of the Funil reservoir showing the two sampling sites: FL35, located in a post-transition region; FL50, located close to the dam.

according to the manufacturer protocols. Samples for dissolved nutrients were filtered through GF-3 filters (Macherey-Nagel). Samples for total nutrients were first digested with potassium persulfate and then analyzed as SRP and nitrate (Gross and Boyd, 1998). Approximately 900 mL of extract samples were filtered through GF-3 filters (Macherey-Nagel) in the laboratory; the filters were used to quantify the intracellular MC and other cyanopeptides. The filtrates (8 mL) were used to analyze the extracellular MC and other cyanopeptide classes. Samples were stored in a freezer (−20 °C) until LC-MS/MS analysis. Sample processing for toxin analysis was performed as described in Arruda et al. (2021). Rainfall data were obtained from the free database of the Instituto Nacional de Meteorologia, Brazil (https://portal.inmet.gov.br, accessed in 04/02/2024). The retention time data (RT) were obtained from the free database of the Operador Nacional do Sistema Elétrico, Brazil (https://www.ons.org.br, accessed in 04/02/2024).

2.3. LC-MS/MS parameters - microcystin quantification

MCs were quantified using a 1260 Infinity chromatographic system consisting of a 1290 VL pump coupled with a 6460 triple quadrupole mass spectrometer (HPLC-QqQ, Agilent Technologies, Santa Clara, USA). The electrospray ionization source (ESI) was utilized in the positive mode at 3500 V. Nitrogen was used as the nebulizer (45 psi) and drying gas (5 mL min^{-1} at 300 °C). A total of 10 μL of each and blank samples were injected. The chromatographic separations were carried out on a Luna C18 column (2) (150 × 2 mm × 3 μm) (Phenomenex, Torrance, CA, USA), performed at a 0.25 mL min^{-1} flow rate at 40 °C following a gradient ratio between phases A and B, with acetonitrile gradients ranging from 25 to 95 %. The mobile phases encompassed Phase A - water containing 0.1% formic acid and Phase B - acetonitrile. The initial composition started at 75% A, followed by a linear gradient reaching 95% B over 10 min. Subsequently, a holding period of 1.5 min at 95% B was observed. The initial composition of 75% A was restored in 0.5 min and maintained for 6 min to permit column re-equilibration before the subsequent injection.

Before sample injection, the needle was washed in the flush port using acetonitrile/water (50:50, v/v) for 5 s. The QqQ instrument functioned in both full scan mode and selected reaction monitoring (SRM) mode, with the selection of specific *m/z* transitions. Monitoring occurred in the positive ion mode for single and double-charged ions. The retention times of eluted peaks were cross-referenced with the MC standards. Characteristic precursor ions in SRM were established as follows: *m/z* 519 (RR), *m/z* 512 ([D-Asp3], RR), *m/z* 995 (LR), *m/z* 981 ([D-Asp3], LR), *m/z* 852.5 (LF), *m/z* 891,5 (LW), *m/z* 910 (LA), *m/z* 1045 (YR). Furthermore, diagnostic MC ions were continuously monitored, specifically 135 *m/z* for Adda and 213 *m/z* for Glu-Mdha.

Calibration standards of non-demethylated MCs were obtained from Abraxis (Eurofins®, Nantes, France) and prepared in 75% methanol. MC quantitation employed calibration curves, with linearity assessed using standard solutions ranging from 0.5 μgL^{-1} to 4 μgL^{-1}. The calibration curves exhibited appropriate linearity for analyte quantification: RR - $R^2 = 0.9963$, with the linear model equation $y = 80584x + 6883.2$; [D-Asp3], RR - $R^2 = 0.9963$, $y = 68531x + 3486.5$; LR - $R^2 = 0.9915$, $y = 201712x - 65176$; [D-Asp^3] LR - $R^2 = 0.9911$, $y = 159474x - 37776$; LF - $R^2 = 0.9396$, $y = 33070x - 29174$; LA - $R^2 = 0.9882$, $y = 134740x - 43306$ and YR - $R^2 = 0.9973$, $y = 187878x - 50933$. Quantification of the demethylated MC structures was performed by relative quantification using the corresponding non-demethylated MC as analytical standards. Samples with low microcystin concentrations was appropriately concentrated within an acceptable range for method from 0.5 μgL^{-1} to 4 μgL^{-1}, a concentration factor was applied during sample processing. The LC-MS method, utilizing specific transitions for cyanotoxins, ensures accurate quantification even at low levels, and the final quantification involved adjusting for the dilution factor to ensure accurate and reliable

results. Data was processed using the Mass Hunter Qualitative Analysis Software and Mass Hunter Quantitative Analysis Software from Agilent Technologies, USA.

2.4. LC-MS/MS parameters - Metabolomics to cyanopeptides

The analysis used an LC-20D Shimadzu Prominence system from Shimadzu (Kyoto, Japan) connected to a quadrupole time-of-flight mass spectrometer, MicroTOF-QII model from Bruker Daltonics (MA, USA). This mass spectrometer featured an electrospray source controlled through a Bruker Compass/HyStar workstation.

Intracellular extract samples and blank samples (100% MeOH) were injected in 10 μL volumes onto a Luna C18 (2) column (150 × 2.1 mm, 2.6 μm) supplied by Phenomenex in Torrance (CA, USA). The mobile phase consisted of high-purity water obtained from a direct-Q8 water purification system from Millipore (Billerica, US) (referred to as A) and acetonitrile from Sigma-Aldrich (referred to as B). Both A and B were augmented with 0.2% formic acid from Sigma-Aldrich. All solvents used in this study were of HPLC grade. Chromatographic separation was conducted at a flow rate of 0.4 mL min^{-1}, employing a linear gradient of solvent B ranging from 5% to 90% over 25 min. The ionization source was configured for positive ionization with a capillary potential of 4000 V. The drying gas (N_2) was set at a temperature of 200 °C with a flow rate of 8 mL min^{-1}, and the nebulizer pressure was maintained at 45 psi. Mass spectra were acquired through electrospray ionization in positive mode across an *m/z* range of 50–1800.

The quadrupole time-of-flight (QTOF) instrument operated in both MS scan mode and auto MS/MS mode, wherein MS/MS experiments were performed on the three most intense ions selected from each MS survey scan. Mass calibration was accomplished using sodium formate from Sigma-Aldrich. High-resolution MS (HRMS) data were processed using Data Analysis 4.4 from Bruker Daltonics in Germany, LC-MS/MS data were converted to mzXML format with DataAnalysis© software and pre-filtered in MSConvert©. The data were entered into the GNPS© platform to generate the molecular network using the Classical Molecular Networking tool. Along with the sample data, data of MS2 internal standard of known compounds were also introduced into the GNPS and seeded into the molecular network (aeruginosin NAL2 *m/z* 587.3571 $[M+H]^+$; guanitoxin *m/z* 253.1051 $[M + H]^+$; cyanopeptolin 1020 *m/z* 1021.5368 $[M+H]^+$; cyanostatin B *m/z* 754.4426 $[M+H]^+$; mycosporins-lysine *m/z* 317.1368 $[M+H]^+$; microginin KR787 *m/z* 788.4016 $[M+H]^+$; namalide B *m/z* 576.3391 $[M+H]^+$; namalide C *m/z* 562.3235 $[M+H]^+$; namalide D *m/z* 560.3561 $[M+H]^+$; nodularin-R *m/z* 825.4490 $[M+H]^+$; Dhb5-nodularin *m/z* 811.4328 $[M+H]^+$ porphyra 334 *m/z* 347.1431 $[M+H]^+$; shizopeptin 791 *m/z* 792.4802 $[M+H]^+$; shinorine *m/z* 333.128 $[M+H]^+$ and spumigin 638 *m/z* 639.3109 $[M+H]^+$). Cytoscape© software was used to visualize molecular networks. The SIRIUS© and ChemCalc© platforms were used to search for prospective molecular formulas. The search for known compounds took place in the databases PubChem©, Chemspider©, Metlin©, NPAtlas©, Dictionary of Natural Products©, GNPS, MetaboScape 4.0 (Bruker Daltonics, Germany) software and CyanoMetDB (JONES et al., 2020). The measurement of cyanopeptides other than MC relied on external calibration using the MC-LR regression model ("calibration curve"), resulting in concentrations expressed as MC-LR equivalents. We recognize the potential for variances between MC-LR and the other cyanopeptides quantified regarding their response factors and any matrix effects associated. This methodology is commonly utilized (Filatova et al., 2021; Natumi et al., 2021; Torres et al., 2023).

2.5. Statistical analysis

Non-metric multidimensional scaling (NMDS) analysis was performed using Canoco® 5.0 software to find patterns in the data matrix. Normality Test (Shapiro-Wilk), Equal Variance Test (Brown-Forsythe), ANOVA (Two-way) and post-hoc test (Holm-Sidak) were performed using Sigmaplot® software to investigate variance between groups besides univariate dimension with possible interaction between the source of variation. Pearson correlation and Redundancy Analyses (RDA) were performed with R Studio® 4.0.2 and Canoco® 5.0 software, respectively.

3. Results

In the NMDS, the two axes of the matrix segregated two groups, months in which MC concentration was below 0.7 μg L^{-1} (Feb–Jul) and months in which MC concentration was above 0.7 μg L^{-1} (Aug–Jan), resulting in 97.05% of cumulative percentage variance of response data (see supplementary material, Fig. S1). The stress obtained for the data was <0.03. From this analysis, we assume two different periods in the reservoir regarding MC, one of lower (Feb–Jul) MC and another of higher MC (Aug–Jan).

3.1. Spatial and temporal microcystin variation

We detected MC in all 47 samples collected during the study. The total MC varied from 0.2 to 4.0 μg L^{-1}, mean of 1.25 μg L^{-1} (Fig. 2). The two-way ANOVA did not show differences between sampling sites ($F_{1,19} = 2.61$; $p > 0.05$), but presented differences regarding MC periods ($F_{1,19} = 15.20$; $p < 0.05$). The total MC concentrations from August to January presented a mean of 1.69 μg L^{-1}; from February to July, the mean was 0.49 μg L^{-1}. The two-way ANOVA did not show an interaction between sampling sites and MC periods ($F_{1,19} = 2.76$; $p > 0.05$).

Three MC variants (MC-RR, [D-Asp^3]-RR, -LR) were quantified in water samples from FL35 and FL50. MC-RR was the most abundant in the intracellular (mean 0.60 μg L^{-1}) and extracellular (mean 0.12 μg L^{-1}) samples, followed by [D-Asp^3]MC-RR (mean 0.15 μg L^{-1} and 0.08 μg L^{-1}) (Fig. 3). MC-LR had the lowest abundance and only appeared in the intracellular samples when the total MC concentration was above 1 μg L^{-1}. In addition to the three quantified MC variants, we also identified MC variants [D-Asp^3]LR, -LY, -LA, and -LF in the intracellular samples but below the quantification limit.

The intracellular MC concentrations were on average 1.11 (±1.28) μg L^{-1} at FL35 and 0.62 (±0.45) μg L^{-1} at FL50; there were no significant differences between sites ($F_{1,19} = 1.14$; $p > 0.05$) (Fig. 3a–c). From Aug–Jan, a mean of 1.4 μg L^{-1} was recorded, representing seven times more intracellular MC than recorded from Feb–Jul ($F_{1,19} = 15.24$; $p < 0.05$), but there was no interaction between sampling sites and periods established in NMDS analysis ($F_{1,19} = 2.79$; $p > 0.05$).

The extracellular MC concentration mean was similar in both sites

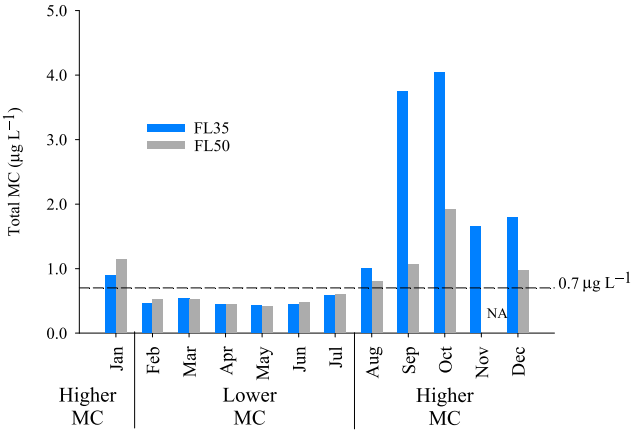

Fig. 2. Total microcystin concentrations in the sampling sites (FL35 and FL50) at Funil Reservoir. The reference dash line defines the months with higher microcystin concentrations (>0.7 μg L^{-1}) and months with lower MC concentration (<0.7 μg L^{-1}). MS – missed sample.

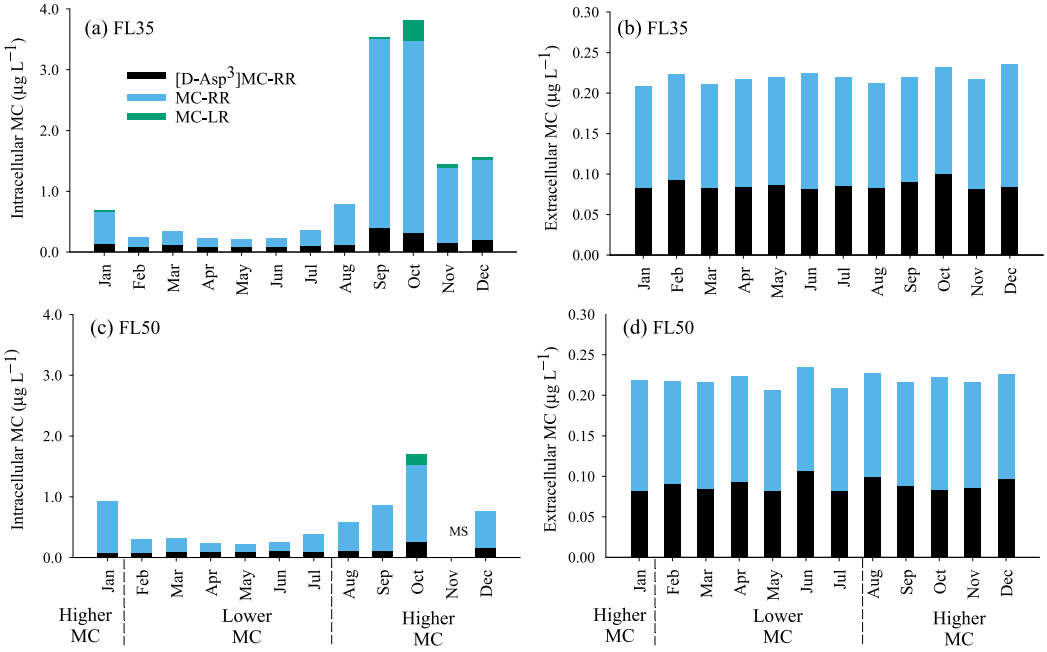

Fig. 3. Concentrations of intracellular and extracellular microcystin variants in FL35 (a, b) and FL50 (c, d) sampling sites at Funil Reservoir. MS – missed sample.

(0.21 ± 0.007 µg L^{-1}) and ranged from 0.20 µg L^{-1} to 0.23 µg L^{-1} throughout the study ($F_{1,19} = 0.0001$; $p > 0.05$) (Fig. 3b–d). The MC-RR variant had 1.5 times higher concentrations than the [D-Asp^3]MC-RR in extracellular samples. There were no differences between MC periods ($F_{1,19} = 0.76$; $p > 0.05$). The MC-RR variant ranged from 0.12 to 0.15 at both sites, while [D-Asp^3]MC-RR ranged from 0.08 to 0.1 µg L^{-1}.

3.2. Other cyanopeptides

The samples were additionally analyzed for oligopeptides other than MCs. We detected and identified 28 compounds belonging to three more cyanopeptide classes only in the intracellular samples: aeruginosin, microginin, and cyanopeptolin. All oligopeptides besides MC, along with their respective *m/z*, intensities, and presence for both sampling sites, are shown in supplementary material (Table S1). The aeruginosin class was the most represented, followed by microginins and cyanopeptolins during the study (Fig. 4). We did not identify differences between sampling site for any oligopeptides besides MC ($p > 0.05$). Regarding the stablish periods of MC (Higher and Lower), just microginins class presented significant variation ($F_{1,19} = 26.7$; $p < 0.05$); where, in period with higher MC period (Aug–Jan) the intracellular microginin was 1.37 (±1) µg L^{-1} and during lower MC period, 0.22 (±0.8) µg L^{-1}.

3.3. Cyanobacteria and potential microcystin producer's

Cyanobacteria dominated the reservoir year-round, representing a mean of 85% (±14%) of the total phytoplankton biovolume, with a mean of 5,5 (±4.6) mm^3. Among cyanobacteria, the potential MC producers represented 73% of the biovolume in FL35 and 65% in FL50. *Dolichospermum circinalis, Microcystis aeruginosa,* and *Microcystis flos-aquae* were the most representative species of potential MC-producer (Fig. 5). *Synechococcus nidulans* represented 0.8% and 0.4% of MC-producer biovolume in FL35 and FL50, respectively.

Statistical analysis did not show spatial variation in cyanobacteria biovolume ($F_{1,20} = 2.11$; $p > 0.05$) and potential MC producers'

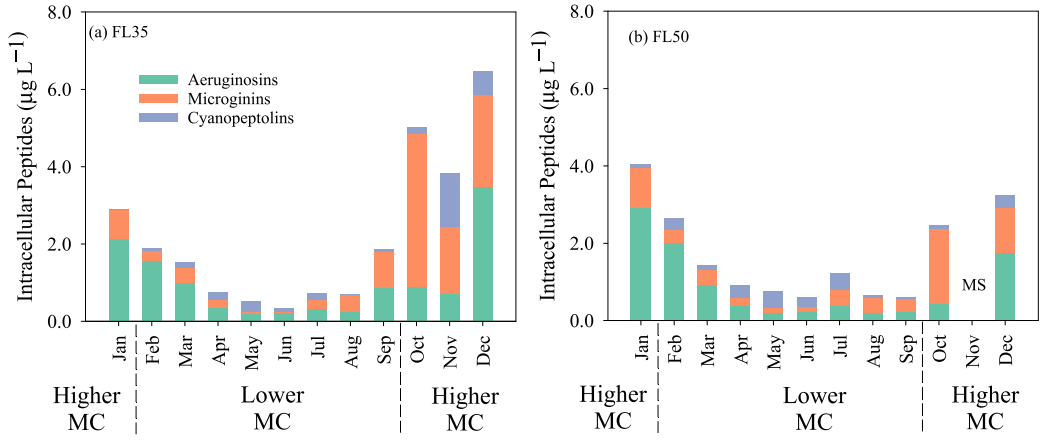

Fig. 4. Concentration of intracellular aeruginosin, microginin and cyanopeptolin classes in FL35 (a) and FL50 (b) sampling sites at Funil Reservoir. MS – missed sample.

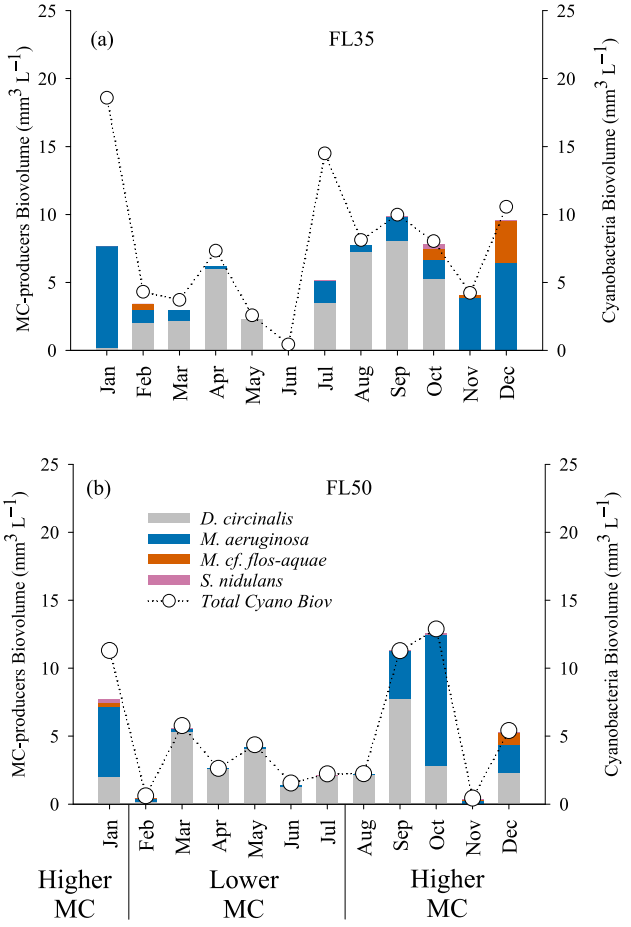

Fig. 5. Cyanobacteria biovolume (dotted line; $mm^3\ L^{-1}$) and biovolume of potential microcystin (MC) producers (bars; $mm^3\ L^{-1}$) in FL35 (a) and FL50 (b) sites during the periods of lower and higher MC concentration at Funil Reservoir.

biovolume ($F_{1,20} = 0.63$; $p > 0.05$), as well as individual biovolume of *D. circinalis* ($F_{1,20} = 0.11$; $P > 0.05$), *M. aeruginosa* ($F_{1,20} = 0.09$; $p > 0.05$), *M. flos-aquae* ($F_{1,20} = 0.98$; $p > 0.05$), and *S. nidulans* ($F_{1,20} = 0.03$; $p > 0.05$). However, when MC concentrations were higher, cyanobacteria presented biovolume two times higher than in months with lower MC concentrations ($F_{1,20} = 11.06$; $p < 0.05$). It was mainly due to the increase of *M. aeruginosa* ($F_{1,20} = 10.83$; $p < 0.05$) and *S. nidulans* ($F_{1,20} = 3.89$; $p < 0.05$), while *D. circinalis* did not present significant variation over the year ($F_{1,20} = 0.23$; $p > 0.05$). *Microcystis* spp. and *S. nidulans* raised their biovolume ~13 times in Aug–Jan.

3.4. Environmental variables

Regarding environmental variables, the Two-way ANOVA was performed considering the factors sampling sites and the two MC periods exhibited previously in NMDS analysis. No significant differences were detected in Z_{eu}, WT, DO, saturation O_2, pH, Conductivity, Turbidity, Alkal, N–NO_3^-, NO_2^-, NH_4^+, DIN, TN, SRP, and TP between sampling sites. Thus, we exhibit the median values of the variables for the two sites in supplementary material (Table S2). The reservoir has a median of 17.8 $\mu g\ L^{-1}$ TP, ranging from 4 to 102 $\mu g\ L^{-1}$, and the TN ranged from 232 to 2807 $\mu g\ L^{-1}$, with a median of 1341 $\mu g\ L^{-1}$. The annual median of total chlorophyll-*a* is 4.8 $\mu g\ L^{-1}$, with the highest value of 29.2 $\mu g\ L^{-1}$ recorded in January. The system was turbid (mean of 13.3 NTU), with the lowest light availability in January ($Z_{eu} = 1.4$ m) and the highest in June ($Z_{eu} = 8.1$ m), following inversely the variation in total chlorophyll-*a*.

Cyanobacteria chlorophyll presented spatial variance; the mean recorded in FL35 was 8.2 $\mu g\ L^{-1}$, while FL50 presented 3.2 $\mu g\ L^{-1}$ ($F_{1,20} = 7.89$; $p < 0.05$). The water column depth also varied according to the sampling sites; FL35 presented a mean depth of 39.3 (±2.9) m, and FL50 had 53.2 (±3.3) m ($F_{1,20} = 129.30$; $p < 0.05$).

During the months with higher MC concentrations (Jan and Aug–Dec), we recorded an accumulated rainfall volume of 142.7 mm and a significant increase in mean values of cyanobacteria chlorophyll (9.9 $\mu g\ L^{-1}$), pH (8.3), turbidity (25.5 NTU) and a slightly higher TP (31.6 $\mu g\ L^{-1}$) and decrease of Z_{eu} (3.0 m). In contrast, months with lower MC concentrations (Feb–Jul) presented 62 mm^3 of accumulated rainfall, a reduction in the mean of cyanobacteria chlorophyll (1.4 $\mu g\ L^{-1}$), with the highest Z_{eu} values (>5 m), and consequently low turbidity (12.4 NTU), neutral pH (7.1), and slightly lower TP (28.7 $\mu g\ L^{-1}$).

3.5. Relationship between cyanopeptides and environmental factors

The total MC, intra-MC, and the variants –RR and [D-Asp^3]-RR were significantly and positively correlated with cyanobacteria chlorophyll, MC producer's biovolume and N–NO_3^- ($p < 0.05$; Fig. 6). Microginins and Aeruginosins were significantly and positively correlated with cyanobacteria chlorophyll, cyanobacteria density, and potentially MC-producers ($p < 0.05$; Fig. 6). TP presented a positive correlation with total MC, intra-MC, MC-LR, and Microginins ($p < 0.05$; Fig. 6); TN was positively correlated with MC-LR and Microginins ($p < 0.05$; Fig. 7); DO showed a positive correlation with total MC, intra-MC, and the variants –RR,-LR, and Microginins ($p < 0.05$; Fig. 6). A negative correlation was observed between Secchi depth, as well retention time and total MC, intra-MC, –RR, and Microginins ($p < 0.05$; Fig. 6); Sill we observed a negative correlation between Secchi depth and Aeruginosins. Extra-MC and Cyanopeptolins had no significant correlation with any of the parameters, but Extra-MC was positively correlated with microginins Cyanobacteria density and MC producer's density showed no significant correlations with MCs.

In the RDA (Fig. 7), we determined the effect of environmental variables on the peptides' classes. The first two axes explained 29.63% of the data variability (axis 1 = 19.93%; axis 2 = 9.7%, Fig. 7). All peptides, except cyanopeptolins, were associated with axis 1. The MC-LR, MC-RR, [D-Asp^3]MC-RR, cyanopeptolins, and microginins were correlated positively with TP, DO, Rain, N–NO_3^-, and TN. The aeruginosin was positively associated with TN, pH, turbidity, and water temperature. On the other hand, all MC variants, cyanopeptolin, and microginin were negatively correlated with Secchi depth, N–NO_2^-, and residence time.

4. Discussion

This study describes microcystin (MCs) and other cyanopeptide classes occurrence and diversity in a Brazilian tropical reservoir. Our analysis identified the MCs, aeruginosins, microginins, and cyanopeptolins in the reservoir year-round. We did not record spatial heterogeneity in MC concentrations and other peptides classes but observed remarkable temporal variation. Three MC variants were quantified in the reservoir: RR, ([D-Asp^3]-RR and -LR. The highest MC and microginin concentrations were recorded between Aug–Jan when we also recorded the increase of rainfall, cyanobacteria chlorophyll by the rise of 9-fold of *M. aeruginosa* and *S. nidulans* species, or 13-fold regarding biovolume. However, during the months with low MC concentrations, *Dolichospermum* was dominant. Cyanobacteria chlorophyll was the biological parameter best correlated and associated positively to the total MC, intracellular MC, the three identified MC variants, microginins, and aeruginosins. At the same time, N–NO_3^- DO and Rain were the chemical parameters best associated positively with the MCs variants and cyanopeptolins and microginins. Besides that, water temperature and turbidity were best associated positively with aeruginosins. The limited

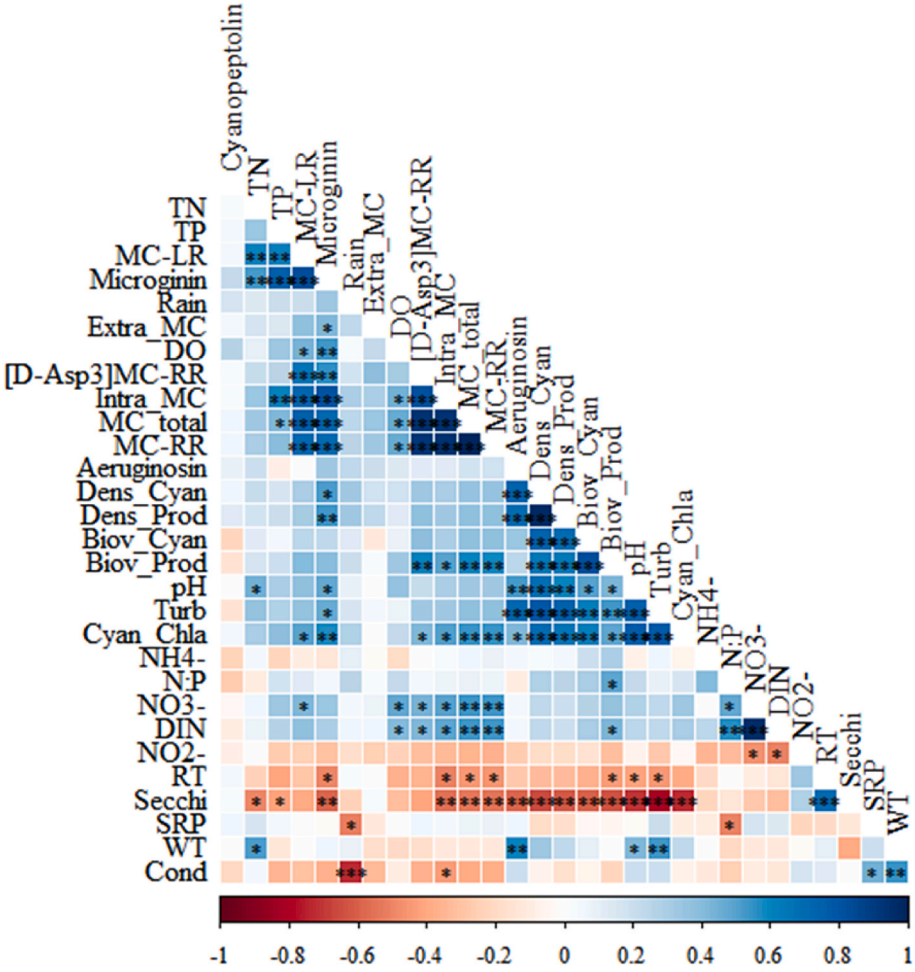

Fig. 6. Pearson correlation analysis among microcystins and environmental variables. Biov_Cyan – cyanobacteria biovolume; Biov_Prod – microcystins producer's biovolume; Cyan_Chla – cyanobacteria chlorophyll-a; Dens_Cyan – cyanobacteria density; Dens_Prod – microcystins producer's density; WT – water temperature; DO – dissolved oxygen; pH – hydrogen potential; Cond conductivity; Secchi depth; RT – residence time; TP total phosphorus; TN total nitrogen; N–NO_3^- nitrate; N–NO_2^- nitrite and N–NH_4^+ ammonium, DIN dissolved inorganic nitrogen, SRP soluble reactive phosphorus: Rain – rainfall; MC_Total – total microcystins; Intra_MC – intracellular MC; Extra_MC – extracellular MC; Aeruginosins – aeruginosins; Cyanopep – cyanopeptolins; Microgin – microginins. The color variation between blue and red is associated with the analysis correlation coefficient, ranging from +1 to −1. Asterisks indicate a significant correlation between two variables (*P < 0.05, **P < 0.01, ***P < 0.001). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significance within the other 19 variables tested in this study comes as no surprise, considering our limited understanding of the function of most cyanobacterial secondary metabolites, including MC, and the factors responsible for their production (Bashir et al., 2023; Henao et al., 2019; Ibelings et al., 2021; Neilan et al., 2013; Sivonen, 2009).

4.1. Microcystins

A recent study about cyanobacterial monitoring and assessment in Latin America showed the scarcity of data on toxic blooms in the region, reflecting not only the precariousness of current environmental policies in this continent part but also the limited technical-scientific resources (Aguilera et al., 2023). In this way, we furthered our knowledge of tropical reservoirs by monitoring MC and other cyanopeptides for a year in Funil Reservoir (Brazil).

Historically, we have observed an increase in intracellular MC concentrations in the studied reservoir; the mean intra-MC concentration recorded in the rainy season in this study (1.8 μg L^{-1}) was five times more than recorded in 2011/12 (0.2 μg L^{-1}) in the same season (Guedes et al., 2014). However, even recording this sharp increase in MC concentration in Funil Reservoir, this value remains below the concentrations recorded in the studies carried out in several reservoirs in Latin America (see supplementary material, Table S3). Although no recent study are mapping the measures that influenced water quality improvement in the Funil Reservoir in the last decade, we hypothesize, that the Vegetal Recomposition Program started in 1994, managed by Furnas Centrais Elétricas S.A (Santos-Neves et al., 2023) has promoted such effects. This program aims to reduce erosion processes on the reservoir's banks and improve the surrounding environmental conditions, which were already highly degraded before the construction of the reservoir due to the irrational exploitation of extensive low-quality livestock farming, reducing diffuse pollution. In parallel, another essential factor, is the increase in the sewage collection rate in the Vale do Paraíba River Basin, which was 57.3% in 2010, when the rate began to be evaluated, reaching 92.1% in 2020 (data available https://datasan-ibre.fgv.br/). These actions have also played a significant role in enhancing water quality in the Funil Reservoir. They apparently resulted in a 56% reduction in TP concentration, decreasing from 40.8 μg L^{-1} to 17.8 μg L^{-1}, and in Chl-*a* concentration by 63%, dropping from 13.1 μg L^{-1} to 4.8 μg L^{-1} over the past 17 years (Rangel et al., 2012). Considering that the proliferation of potentially toxic cyanobacteria is the main consequence of eutrophication (Paerl et al., 2011), reducing the external nutrients load in the reservoir is the most efficient way to neutralize the cause of the problem (Huisman et al., 2018; Paerl et al.,

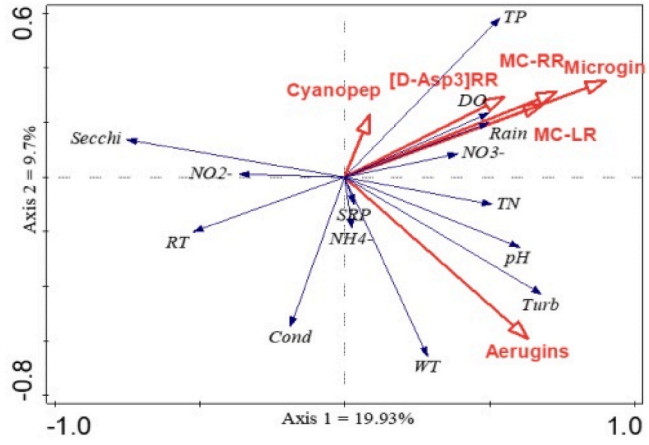

Fig. 7. Ordination diagram of redundancy analysis (RDA) encompassing microcystin (MC) variants and environmental variables. WT – water temperature; Z_{eu} – euphotic zone; DO – dissolved oxygen; pH – hydrogen potential; RT – residence time, N–NO_3- nitrate; N–NO_2- nitrite; N–NH_{4+} ammonium; SRP soluble reactive phosphorus; Rain – rainfall; Turb – Turbidity; MC-RR, MC-LR and [D-Asp^3]MC-RR – microcystin variants; Aerugins – aeruginosins; Cyanopep – cyanopeptolins; Microgin – microginins.

2016).

The statistical analysis of MC concentrations allowed us to recognize a pattern of MC variation, grouping months with lower MC concentrations, with a mean of 0.48 ± 0.04 μg L^{-1} (Feb–Jul), 4.2 times lower than months with higher MC concentrations, that reached a mean of 1.7 ± 1.1 μg L^{-1} (Aug–Jan); the last one coinciding with the period of increased of rainfall explicitly observed for the year in which the study was carried out. It was evident that the months with a high concentration of MC coincide with the period of high rainfall (Aug–Jan) but not necessarily with the tropical rainy season (Oct–Mar); this is due to the anticipation of rainfall in the study year due to the El Niño phenomenon. The observed MCs concentration pattern is corroborated by the Pearson correlation results, where the retention time (RT) in the reservoir presented a negative correlation with MCs (Total, Intra and –RR); once the RT during Oct–Mar is greater than Aug–Jan. Similarly to this temporal pattern, the mean of total MC recorded in the rainy season for six Brazilian reservoirs was 4.7–15.4 times higher than in the dry season (Fonseca et al., 2015). The highest MC concentrations in an Argentinian reservoir during the rainy season were also observed (Amé et al., 2003; Ruiz et al., 2013). Unfortunately, there is not a clear relationship between rain and MC once the increase and decrease of rainfall can change several water conditions and influence directly or indirectly many variables in aquatic systems (e.g., nutrient load, salinity, turbidity, depth, mixed layer, sediment re-suspension, turbulence) (Reichwaldt and Ghadouani, 2012). Contrary, the highest MC concentration was found in months with low precipitation (León and Peñuela, 2019); the authors argue that rainfall has dilution effects on nutrients, affecting cyanobacteria and MC concentration. These differences may be associated with several reasons, such as the type of the reservoir (whether it is a damming of a river or it was already a lake), the primary source of pollution (whether it is diffuse, direct contribution by the river), the type of occupation of the drainage basin, or even the size of the drainage basin.

Besides the temporal pattern in MC concentration, we also recognized no spatial variation in the reservoir. Spatial and temporal fluctuation in MC concentrations, MC variants, and other peptides also occur mainly due to the seasonal succession of cyanobacteria (Meissner et al., 2013). This succession primarily results from changes in genotype composition of toxic versus non-toxic strains and low versus high biomass of MC producers (Meissner et al., 2013; Sabart et al., 2010; Wu et al., 2006). In the Funil Reservoir, for example, several *Microcystis* genotypes with a wide proportion variation among potentially toxic cells have already been recorded (Guedes et al., 2014). Nevertheless, even though changes in the proportion of MC-producer cells must be considered, sometimes they cannot be fully explained by temporal or spatial variation in MC (Guedes et al., 2014; Sabart et al., 2010). The selection process between MC-producer and non-MC-producer genotypes is relative and likely influenced by numerous local environmental factors and processes (Sabart et al., 2010). Spatial variation in MC concentration is associated with different environmental conditions between different reservoir areas, especially in large reservoirs and those with complex morphology (Chung et al., 2014; Michalak et al., 2013). However, as observed in our study, homogeneity in the concentration of MC across the reservoir is not uncommon, as observed in two South African reservoirs (Conradie and Barnard, 2012). However, it is essential to recognize such patterns of spatial variation, especially in multiple-use aquatic systems, such as the Funil Reservoir. Another survey using water collected in a branch of the same reservoir (Aug/2019) recorded different MC variant profiles in the intra and extracellular portion (-RR, -LR, -YR, FR, WR, [D-Asp3]-RR). Moreover, different abundances with similar MC-producer species composition to our data were recorded (Arruda et al., 2021). It is suggested that the spatial variation of MC may occur in Funil Reservoir. However, we indicate new studies covering more sampling sites in the system for better comprehension.

On the other hand, extracellular MC showed no significant spatial or temporal variation in this study. Even in the months with high intra-MC concentrations, the extra-MC remained at about 0.21 μg L^{-1}. In natural populations, as cyanobacteria cells enter the stationary phase, the increased rate of cell death may raise the extra-MC fraction (Rohrlack and Hyenstrand, 2007). However, the MC concentration dissolved in water depends on dilution, adsorption, and degradation (Welker and Jones, 2001). Since, in our study, the time with higher intra-MC overlaps with the rainy season, we suppose the increase in the water column by Paraíba do Sul River contribution and the local rainfall could dilute the extra-MC. Water input can also bring considerable suspended organic matter, contributing significantly to MC adsorption (Lahti, 1997; Liu et al., 2008; Morris et al., 2000). An alternative hypothesis for the constant values of extra-MC could be that these toxins accumulated in the sediment due to senescence and the potential settling of intact cells within the water column (Wörmer et al., 2011). Thus, more studies aiming to evaluate the dynamic of MC fate are necessary to understand the extra-MC dynamic in Funil Reservoir.

4.2. Other cyanopeptides

In this study, we have also considered exploring other oligopeptides besides the MCs. Our finds consist the first report of the occurrence of a variety of peptides beyond MC in Funil Reservoir, providing unprecedented data for Brazilian aquatic systems. Three other peptides classes, in addition to the MC, were found in intracellular samples: aeruginosin, cyanopeptolin, and microginin. These compounds were quantified based on MC-LR equivalents and presented as a total per class (Fig. 4). Among them, only microginin presented temporal pattern. Concomitantly with the period of higher MC concentrations (Aug–Jan), the microginins showed an increase of up to 6-fold compared to the period of lower MC concentrations. Co-production of microginins and microcystins has already described for Brazilian strains of *Microcystis* (Carneiro et al., 2012). In *M. aeruginosa* culture was observed a similar relative concentrations of all cyanopeptides across different peptide classes (Natumi and Janssen, 2020). However, in our study, only microginins show linearity with MC concentration, including extracellular MC, which in turn does not show a significant correlation with any other parameter evaluated. (Fig. 6). Temporal variation in cyanopeptides beyond MC has already been described in freshwater reservoirs in the United Kingdom (Filatova et al., 2021) and in six eutrophic lakes in the USA (Beversdorf et al., 2017). Temporal and spatial variation in cyanopeptides were

identified in Lake Vegoritis, Greece (Zervou et al., 2021). Nonetheless, we did not observe significative spatial variation for the three quantified classes, evaluated as a total concentration. However, it was possible to identify some compounds present at only one of the sampling sites during the study (e.g., Aeruginosin 618 in FL50, Aeruginosin 602 in FL35 sampling sites). Other cyanopeptides compounds may also vary over the months (see supplementary material, Table S1). The Aeruginosin class was the most abundant in our study concerning the number of compounds, as well as in their concentration in Jan, Feb, Mar, and Dec compared to Total MC. Aeruginosins have been shown inhibit human serine proteases related to blood clotting at IC_{50} values varying from 4 to 93 μg L^{-1} (Hanessian et al., 2006; Kohler et al., 2014). Additionally, in toxicity tests with crude extracts of cyanobacterial biomass and fractions from the Funil Reservoir, Aeruginosin 298A (*m/z* 605.36) showed similar biological activity scores to MC-RR (Medice and Arruda, 2024 - under review).

As a less abundant class, considering the number of compounds, cyanopeptolins presented only two compounds. Meanwhile, we found these compounds in 80% of our samples. Cyanopeptolins are recognized for inhibiting proteases associated with metabolism and blood coagulation (Fujii et al., 2002; Janssen, 2019). Compounds of this class have been identified in a lower abundance in samples collected from six eutrophic Wisconsin lakes (USA), in a mean of 50% (Beversdorf et al., 2017). It suggests the distinctive ecological and environmental attributes of tropical freshwater ecosystems can influence the production and prevalence of these cyanometabolites. This holds great significance because even though they are not currently classified as toxins, aeruginosins, cyanopeptolin, and microginins could potentially contribute to the overall toxicity of cyanobacterial blooms (Beversdorf et al., 2017; Chorus, 2001; Jacinavicius et al., 2023; Janssen, 2019). In addition, the simultaneous production of several cyanopeptides alongside MC underscores the importance of providing reference standards to promote further research on their presence in blooms, persistence, and potential toxicity (Natumi and Janssen, 2020).

4.3. Cyanobacteria and the potential MC producers

Cyanobacteria still dominate the reservoir, representing 85% of the total phytoplankton community, even with 7.6-fold reductions in TP and 4-fold in DIN, and a slight 1.2-fold increase in SRP in the rainy season over the last eight years (Guedes et al., 2014). Potential MC producers were also highly representative, 72% among cyanobacteria throughout the study in the reservoir. However, the population of potential MC producers tends to increase even more in the months of high rainfall, reaching 84% of the relative abundance among cyanobacteria. Regarding species, we observed an increase in *Microcystis* spp. biovolume in the rainy period. The previous literature pointed out, for temperate regions, that the high availability of light provided by longer spring/summer days and water column stratification promoted due to the high temperatures also may favor *Microcystis* (Dokulil and Teubner, 2000; Ibelings et al., 2021; Paerl and Huisman, 2008; Reynolds et al., 1994). However, the water temperature average observed in the dry season months was 1 °C warmer than recorded in the rainy months but considered quite high in both periods (25 °C ±1); in the dry season, the reservoir showed lower depth and absence of thermal stratification during the study (unpublished data). Reduction of the mixing zone has been pointed out as an essential key factor in promoting *M. aeruginosa* in Funil Reservoir (Soares et al., 2009) and other tropical reservoirs (Li et al., 2023).

Besides that, in our study, the high *Microcystis* spp. biomass overlapped with the period of high MC concentration in the reservoir; high MC concentration has already been found in Funil Reservoir during the *M. aeruginosa* bloom (Ferrão-Filho et al., 2009). *Microcystis* strains capable of producing MC are frequently found in stratified lakes and reservoirs (Davis et al., 2009). It could be linked with the temperature, which can cause upregulation of the *mcyB* gene in *M. aeruginosa*, which is responsible, in part, for MC concentration (Kim et al., 2005; Scherer et al., 2017). In turn, *D. circinalis*, although present year-round in the reservoir during the study, was more representative in the dry season, not necessarily due to increased biomass, but because *Microcystis* reduced in density. Although *Dolichospermum* spp. are commonly associated with blooms and are considered potential MC producers (Dreher et al., 2019; Ekman-Ekebom et al., 1992), a survey from Funil Reservoir did not identify *mcyE* for *Dolichospermum* (*Anabaena*) during rainy season but recorded a high proportion of *Microcystis* toxic genotypes when *Dolichospermum* was not dominant (Guedes et al., 2014). While this data does not rule out that *Dolichospermum* was an MC producer through our study, it does suggest that the leading MC producers in the reservoir in the rainy season were *Microcystis* spp.

4.4. Relationship between microcystins and other peptides class, biological and chemical factors

The ubiquity of MC has led several studies to assess the effects of environmental factors on toxic bloom, but observations across the world indicate high variability in the documented conditions related to MC occurrence and cell content (Billam et al., 2006; Duong et al., 2013; Dziallas and Grossart, 2011; González-Piana et al., 2017; Pimentel and Giani, 2014; Rinta-Kanto et al., 2009; Tao et al., 2012).

In our study, just cyanobacteria chlorophyll and potential MC producers biovolume positively correlated with MCs (variants, total and intra-MC; Fig. 6), and the correlation coefficient varied. In contrast, a study with global data showed that chlorophyll-a had the most significant positive correlation slope ($r = 0.45$) to total MC among several parameters (Buley et al., 2022). Here, we found strong correlations. MC producer biovolume presented $r = 0.59$ with total MC, followed by the cyanobacteria chlorophyll with a correlation coefficient 0.55. Differently, the microginins and aeruginosins presented great correlation (>0.6) with cyanobacteria density, MC-producers density, and cyanobacteria chlorophyll. In general, the lack of correlation between some cyanobacteria biomass proxies and MC can be assigned to the variation in the relative genotype dominance (toxic and non-toxic) and the abundance and diversity of cyanobacteria, has been observed in Funil Reservoir and other systems across the world (Conradie and Barnard, 2012; Davis et al., 2009; Guedes et al., 2014; Li et al., 2017, 2023; Martins and Vasconcelos, 2011; Rinta-Kanto et al., 2009; Wu et al., 2008). This may be the reason for the absence of a significant correlation between MC producer density and MCs in this study. On the other hand, the correlation observed among microginins, aeruginosins concentration, and biomass proxies, specifically cell density, has been observed in experimental studies for peptides and *M. aeruginosa*, where the concentration of total cyanopeptides were directly correlated with cell abundance and the cyanopeptide production per cell (Natumi and Janssen, 2020).

We also observed other significant correlations between MC and environmental variables. Secchi depth was the only variable in this study to present a negative correlation with MCs, microginins, and aeruginosins; the total-MC, intra-MC, and MC-RR had similar coefficients to those recorded for tropical systems ($r = -0.55$) and higher when compared with North temperate and Southern temperate regions ($r = -0.16$; $r = -0.06$) (Buley et al., 2022). However, there are no data from field monitoring studies for other classes of peptides. The reduction of Secchi depth occurred in the rainy season, parallel to the rise of *Microcystis* spp. and the increase of MC in Funil Reservoir. The Secchi reduction can equate to very different ecological stressors or processes in freshwater systems (Swift et al., 2006). However, in the Funil Reservoir, we suggest the Secchi reduction occurs mainly due to the increase of *Microcystis* biomass, favored by rainfall, as discussed before. The link between Secchi and rain was evidenced by RDA (Fig. 7), showing a negative trend and a positive relationship between rainfall and MC variants and other peptides classes. Rainfall also presented a negative association with residence time of reservoir; considering that the

residence time in the Funil Reservoir is directly related to the management of the reservoir's gates, it is expected that during the rainy season, the water residence time will be shorter than in the dry season. Thus, the negative association between residence time and cyanopeptides, evidenced in the RDA, is an indirect relationship. In addition, considering the high turbidity recorded in the rainy season, we cannot ignore that a significant amount of suspended organic matter and other suspended solids brought by rainfall can increase the turbidity, and consequently, reducing the light availability may favoring potential toxic *Microcystis* spp.; some studies suggest the selection of toxic-genotypes of *Microcystis* and other MC-producers under unfavorable environmental conditions (Briand et al., 2009, 2008; Kardinaal et al., 2007; Martins and Vasconcelos, 2011).

On the other hand, DO presented a positive correlation with total MC ($r = 0.46$), intra-MC ($r = 0.44$), MC-RR (r = 0.47), -LR ($r = 0.43$), and microginins ($r = 0.47$). Although surveys show a negative correlation between DO and MCs for temperate regions, we have observed the opposite in tropical areas (Billam et al., 2006; Buley et al., 2022; Hartnell et al., 2020; Te and Gin, 2011). This positive correlation may be related to biomass growth and consequent increases in primary productivity and not a direct connection with MC and other peptides. Regarding nutrients, TN has been associated with cyanobacterial bloom formations (Paerl et al., 2001; Paerl and Otten, 2013) and has even been identified as the most crucial driver of MC concentration in some systems (Giani et al., 2005; Paerl and Otten, 2013). Despite that, a recent meta-analysis showed a positive correlation between TN and MC for tropical regions ($r = 0.30$) and a negative in the temperate areas ($r = -0.03$) (Buley et al., 2022). However, our data showed significant correlation between TN, MC-LR, and microginins. The effects of nitrogen on peptides production apparently are species dependent, once lowering nitrogen decreased cyanopeptide production in *Dolichospermum flos-aquae*, but the relative abundance of individual cyanopeptides remained stable; while cyanopeptide production in *M. aeruginosa* was not affected (Natumi and Janssen, 2020). On the other hand, among the nitrogen forms, N–NO_3- presented a positive correlation with total-MC ($r = 0.53$), intra-MC ($r = 0.49$), and all variants (r ranged from 0.43 to 0.59). Although field studies in the tropical regions and north temperate areas show this correlation tends to be negative (r = −0.31; r = −0.11), in southern temperate regions, a positive but weak correlation has been recorded ($r = 0.06$) (Buley et al., 2022). Since MC is a nitrogen-rich metabolite, a mesocosm study verified that an increase in N–NO_3- and urea in a hypertrophic lake total MC concentration increases by up to 13-fold (Donald et al., 2013, 2011). Furthermore, a laboratory study demonstrated that N–NO_3^- supply increases MC cell quotas in the *M. aeruginosa* strain, with the MC-RR being the most significantly affected (Van de Waal et al., 2009). This would explain the significant correlation between MC-RR and N–NO_3^- found in our study ($r = 0.59$). The RDA performed here suggested positive and synergic effects of N–NO_3^- and rainfall on MC-LR and [D-Asp^3]MC-RR (Fig. 7). Still, the absence of correlation between N–NO_3^- and MC producer biovolume may suggest the increase of MC is not directly related to the cyanobacteria biomass growth but due to the rise of MC biosynthesis. Studies evaluating the effects of nitrate on *Microcystis aeruginosa* showed that, *in situ*, when surface nitrate concentrations increased, there was a rise in the relative abundance of the *mcyA* (Yoshida et al., 2007). Another study performed in the laboratory showed that the increase in nitrate concentration stimulated the biomass, intracellular MC, and extracellular MC genes (Yang et al., 2023). Regarding N–NH_4^+, it has been negatively correlated with MC in studies from tropical areas ($r = -0.16$) (Buley et al., 2022), but no significant relationship with MC was identified in our research. Similarly, a survey performed in three lakes in Canada showed that N–NH_4^+ did not present a correlation with MC, although it is considered necessary in structuring cyanobacterial communities (Monchamp et al., 2014); the authors assigned the absence of correlation to the low and constant N–NH_4^+ concentrations in the studied systems. However, in Funil Reservoir N–NH_4^+ presented significant variation. Their concentration cannot be considered low during the study (supplementary material, Table S2). N–NO_2^- also did not correlate with MCs, but in the RDA, it was negatively associated with MC-RR and [D-Asp^3]-RR, going against the global trend, which has shown a positive relationship with MCs (Buley et al., 2022).

Our data still showed a positive correlation between TP, total MC, intra-MC, and microginins, in line with the literature (Buley et al., 2022; Kotak et al., 2000; Rinta-Kanto et al., 2009), but we did not observe any correlation between the TP and the biomass proxies (eg.: biovolume, density, chlorophyll). It indicates that the availability of P may favor toxic strains inducing the production of MC and microginins without necessarily increasing the cyanobacteria biomass. Studies have shown that at higher SRP concentrations, the growth rates of toxic *Microcystis* exceeded non-toxic strains and influenced MC concentration in laboratory experiments (Vézie et al., 2002) and field studies (Davis et al., 2009; Su et al., 2015). In Funil Reservoir, the TP concentration was not statistically different between seasons; however, we observed ~2.5 times more TP when total MC exceeded 0.8 μg L^{-1} than in months with a concentration under 0.8 μg L^{-1}. The same pattern was observed in MC producer biovolume, with a difference of ~2.4 more biovolume when total MC exceeded 0.8 μg L^{-1} and increased *Microcystis* species. SRP concentrations were low, with slight variation throughout the study (9.04 ± 8 μg L^{-1}). This suggests that the primary producers rapidly uptake dissolved inorganic phosphorus when available, justifying the absence of correlation between this variable and MC.

We did not observe significant correlations in Pearson analysis between WT and MCs or biomass proxies, but a positive correlation was observed with aeruginosins. Additionally, this variable was negatively associated with MC-RR, [D-Asp3]-RR in RDA analysis, as well as positively associated with aeruginosins. Laboratory experiments and field observations indicate that toxic strains within a bloom may benefit more significantly from warming than non-toxic strains (Davis et al., 2009; Dziallas and Grossart, 2011; Lehman et al., 2010; Li et al., 2017). However, it is essential to note that temperature correlations are only sometimes consistently observed (Rinta-Kanto et al., 2009). A meta-analysis of 106 studies showed that temperature positively correlated to MC ($r = 0.30$) (Buley et al., 2022). In the meantime, Bui et al. (2018) demonstrated that when the temperature was above 27 °C, the total MC concentration decreased by 35% in tropical *Microcystis* strains with MC-LR as the dominant variant and by 94% in strains with MC-RR and, most importantly, the effect of temperature on MC is strain-dependent and seems more pronounced in strains producing less toxic MC variants (dmMC-RR, MC-RR). Another survey with tropical *Microcystis* strains demonstrated that not only MC concentration but also cell quota was affected negatively by higher temperatures (Trung et al., 2022). Conversely, a study tested warm temperatures (20–30 °C) and high levels of P and N synergically showed MC concentrations were much higher at the increased temperature and nutrient treatment than under warming alone (Lürling et al., 2017). The temperature also apparently influences the MC profile; a higher proportion of MC-LR at higher temperatures (28 °C) than at lower temperatures (20 °C), and the opposite for MC-RR was recorded for *Microcystis aeruginosa* population, concentrated from a field sample of the reservoir in Argentina (Amé et al., 2003). Here, we observed an abrupt decrease in total MC when the water temperature exceeded 27 °C (February–April 2019). The increase in total MC was only observed from July when the temperature approached 21 °C. Regarding aeruginosins, although many studies on compounds in this class have been published, there are still no studies evaluating the conditions that affect their synthesis or production in laboratory or in field.

Within the global dataset and our findings, these contradictory reports do not necessarily undermine the significance of variables with low or non-correlation with MCs. Instead, they underscore the challenges in extrapolating promoting conditions to MC and other peptides occurrences. Consequently, additional laboratory and field studies are imperative, especially covering other classes of peptides. These studies

should investigate the mechanisms mediating interactions linked to cyanobacteria promotion in tropical regions. Such research is crucial as it will aid water resource managers in gaining a deeper understanding of the conditions contributing to cyanopeptides concentration.

5. Conclusion

This study investigated the dynamics of MC and other peptides in Funil Reservoir, a large and deep tropical reservoir, unprecedented study for Brazilian aquatic systems. Our analysis reveals a notable temporal pattern in MC concentrations and microginins, where higher concentrations is recorded during months with significant rainfall. Correlation and redundancy analyses emphasized the positive link between rainfall, dissolved oxygen, nitrate and TP with MC variants and microginins, while water temperatures appear to favor aeruginosins class. During higher MC time, *Microcystis* spp. emerge as the dominant species, suggesting its role as the primary MC producer in the reservoir. In addition, a comparison with historical data indicates a decrease in total phosphorus, overall biomass, and cyanobacteria populations, hinting at improved water quality. Nonetheless, this contrast with increased cyanobacterial toxicity observed over the past eight years.

Moreover, our study underscores the pressing need to explore cyanopeptides beyond MCs in tropical aquatic systems further. Additionally, it highlights the significance of establishing reference standards to facilitate extended research on these compounds' presence, persistence, and potential toxicity.

Funding

R.S.A.'s PhD scholarship was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Brazil (CAPES)- Finance Code 001; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil (CNPq), Grant 403515/2016-5; Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Brasil (FAPERJ), Grant 303572/2017-5.

Ethical statement

No animal or human subjects were used in the study encompassed by the manuscript titled "Cyanopeptides Occurrence and Diversity in a Brazilian Tropical Reservoir: Exploring Relationships With Water Quality Variables".

CRediT authorship contribution statement

Renan Silva Arruda: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fernanda Rios Jacinavicius:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Natália Pessoa Noyma:** Writing – review & editing. **Erick Drummond:** Writing – review & editing. **Davi Almeida Barreto:** Writing – review & editing. **Lúcia Helena Sampaio da Silva:** Writing – review & editing, Project administration. **Vera Lucia Huszar:** Writing – review & editing, Resources, Project administration. **Ernani Pinto:** Writing – review & editing, Project administration. **Miquel Lürling:** Writing – review & editing. **Marcelo Manzi Marinho:** Writing – review & editing, Validation, Supervision, Resources, Project administration.

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.

Acknowledgements

We would like to thank the members of the Laboratory of Ecology and Physiology of Phytoplankton (UERJ) and Laboratory of Toxins and Natural Products from Algae and Cyanobacteria (LTPNA - USP) for technical support. We also thank the International Water Quality Management Foundation (SIWA) for the SIWA research support grant awarded to Arruda, R.S.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124051.

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