Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Phytoremediation of microcystins using *Myriophyllum aquaticum* can prevent sublethal effects in a Neotropical freshwater catfish

A fitorremediação de microcistinas usando *Myriophyllum aquaticum* pode prevenir efeitos subletais em um bagre de água doce neotropical

Augusto Lima da Silveira[1], *Sabrina Loise de Morais Calado*[1], *Rafael Shinji Akiyama Kitamura*[1], *Maiara Vicentini*[1], *Thomaz Aurélio Pagioro*[2], *Taynah Vicari*[1], *Ana Carolina Felipe da Silva*[1], *Maiara Carolina Perussolo*[1], *Mariana de Almeida Torres*[3], *Fernanda Rios Jacinavicius*[3], *Maritana Mela Prodocimo*[1], *Ernani Pinto*[3], *Marta Margarete Cestari*[1], *Helena Cristina Silva de Assis*[1]

ABSTRACT

Microcystins are cyanotoxins that can be produced by cyanobacteria species such as *Microcystis aeruginosa*. Due to the presence of microcystins in water bodies and aquatic organisms, it needs to be monitored. Furthermore, additional studies are required in the implementation of alternative and sustainable water treatment methods. The aim of this study was to investigate the potential of *Myriophyllum aquaticum* to prevent the harmful effects induced by *M. aeruginosa* aqueous extract in *Rhamdia. quelen* using biomarkers and to assess whether the plant can be suitable for phytoremediation in aquatic ecosystems. In the first experiment, *R. quelen* was exposed to 1 and 10 µg.L^{-1} of microcystins through an aqueous extract dissolved in the bioassay's water. Second, tanks containing the same test concentrations of microcystins were treated by phytoremediation using *M. aquaticum* at 10 g.L^{-1}, for 7 days. After that, treated water was used in a new bioassay with fish exposure. The results showed that phytoremediation decreased the microcystin concentration in water, and different biomarker analyses demonstrated that *M. aquaticum* treatment prevented DNA damage, hematological alterations, and tissue damage in *R. quelen*. The phytoremediation with *M. aquaticum* can be a sustainable and cost-effective alternative to water treatment, highlighting its role in enhancing water quality and supporting biodiversity conservation. These results support the importance of adopting more restrictive legal limits for cyanotoxins in water to protect native aquatic species and promote sustainable water resource management.

Keywords: biomarkers; cyanobacteria; cyanotoxins; ecotoxicology; *Rhamdia quelen*.

RESUMO

As microcistinas são cianotoxinas produzidas por cianobactérias como *Microcystis aeruginosa*. Em razão da presença dessas cianotoxinas em corpos d'água, é crucial monitorar os organismos aquáticos. Além disso, são necessários estudos adicionais para implementar métodos alternativos e sustentáveis para o tratamento da água. Este estudo teve como objetivo investigar o potencial de *Myriophyllum aquaticum* para prevenir os efeitos nocivos do extrato aquoso de *M. aeruginosa* em *Rhamdia quelen* utilizando biomarcadores e avaliar a adequação da planta para a fitorremediação em ecossistemas aquáticos. No primeiro experimento, biomarcadores foram avaliados em *R. quelen* expostos a 1 e 10 µg.L^{-1} de microcistinas, provenientes de um extrato aquoso dissolvido na água do bioensaio. No segundo experimento, tanques contendo as mesmas concentrações de microcistinas foram tratados por fitorremediação utilizando *M. aquaticum* a 10 g.L^{-1} durante sete dias. Após o tratamento, a água foi usada em um novo bioensaio de exposição, com avaliação de biomarcadores em *R. quelen*. Os resultados mostraram que a fitorremediação reduziu a concentração de microcistinas e análises de diferentes biomarcadores demonstraram que o tratamento com *M. aquaticum* preveniu danos ao DNA, alterações hematológicas e danos teciduais em *R. quelen*. A fitorremediação com *M. aquaticum* pode ser uma alternativa sustentável e de baixo custo para o tratamento de água, destacando seu papel na melhoria da qualidade da água e no apoio à conservação da biodiversidade. Além disso, os resultados reforçam a importância de adotar limites legais mais restritivos para cianotoxinas na água para proteger espécies aquáticas nativas e promover a gestão sustentável dos recursos hídricos.

Palavras-chave: biomarcadores; cianobactérias; cianotoxinas; ecotoxicologia; *Rhamdia quelen*.

[1]Federal University of Paraná – Curitiba (PR), Brazil.

[2]Federal University of Technology – Paraná – Curitiba (PR), Brazil.

[3]University of São Paulo – São Paulo (SP), Brazil.

Corresponding author: Augusto Lima da Silveira – Rua Francisco H. dos Santos, 100 – Jardim das Américas – CEP: 81530-980 – Curitiba (PR), Brazil. E-mail: augusto.ls13@gmail.com

Conflicts of interest: the authors declare no conflicts of interest.

Funding: this work was supported by CAPES (National Council for the Improvement of Higher Education) that partially supported this research (finance code 001). H.C. Silva de Assis received a research productivity grant from CNPq (Brazilian Agency for Science and Technology) grant number 308765/2019-2.

Received on: 07/01/2024. Accepted on: 09/09/2024.

https://doi.org/10.5327/Z2176-94782172

Introduction

Toxic cyanobacterial blooms are a significant threat to water quality and potability due to the risks they pose to human and environmental health (Wang et al., 2023). Eutrophication, coupled with global warming due to climate change, can contribute to the occurrence and persistence of toxic cyanobacterial blooms. In this context, the impact on water quality poses risks to the various potential intended uses of water resources (Zhan et al., 2022).

Within the group of cyanobacteria, the species *Microcystis aeruginosa* is frequently documented in freshwater environments. *M. aeruginosa* is a potential producer of microcystins, which are hepatotoxic cyanotoxins with documented effects on plants (Zhou et al., 2021; Zhang et al., 2022), fish, and mammals, including humans (Li et al., 2023). Microcystins are cyclic heptapeptides with a general structure of D-Ala^{1}–X^{2}–D-MeAsp^{3}–Z^{4}–Adda^{5}–D-Glu^{6}–Mdha^{7}, where X and Z represent amino acids that vary depending on the type of microcystin. Over 329 variants are presently known, with Microcystin-LR (MC-LR; L-leucine, R-arginine) being the most prevalent and toxic among them (Jones et al., 2021; Janssen et al., 2023).

For long-term exposure, the World Health Organization sets a maximum limit of 1.0 $\mu g.L^{-1}$ for microcystins, which can be up to 12.0 $\mu g.L^{-1}$ in cases of exposures not exceeding 1 week (WHO, 2021). In aquatic environments, there are records in Latin America, including concentrations that can reach up to 20,000 $\mu g.L^{-1}$ of total microcystins (Aguilera et al., 2023). However, adverse effects on nontarget organisms were observed in these concentrations, such as damages linked to the spleen, brain, liver, gonads, and kidneys of both fish and mammals, including humans (Hinojosa et al., 2019; AlKahtane et al., 2020; Zhang et al., 2021). Thus, minimizing exposure risks depends on establishing safe exposure limits and effective monitoring measures.

Some strategies, such as membrane filtration, advanced oxidative processes, ultraviolet radiation, the application of metallic species in water, and activated carbon, have been employed to mitigate harmful cyanobacterial bloom effects in water resources, aiming to ensure safe uses (Akyol et al., 2021). However, significant challenges remain, including incomplete removal of toxins, the generation of toxic byproducts, and the economic infeasibility of implementing these strategies for public water supply treatment (Munoz et al., 2021).

Some approaches indicate the potential of aquatic macrophytes for toxic cyanobacterial bloom control (Wang and Liu, 2023). *Myriophyllum aquaticum* is a native to South America aquatic macrophyte, and this species has gained recognition due to its remarkable capability to remove environmental contaminants, including microcystins (Kitamura et al., 2022a). Hence, it is evident that there are promising prospects for the utilization of phytoremediation in microcystin control (Dahedl and Urakawa, 2023). The phytoremediation potential arises from the mechanisms that occur in aquatic macrophytes when exposed to contamination. In organic compound exposure, these plants can immobilize the contaminant within their structure, biodegrade it through chemical compounds released by the plant or by microorganisms associated with roots, or dissipate it through volatilization processes (Kafle et al., 2022). However, studies evaluating the effectiveness of this treatment from the perspective of nontarget organisms are currently lacking.

Among fish species, *Rhamdia quelen* has been successfully used as a model in ecotoxicological studies in South America (Mazzoni et al., 2020; Assis, 2021; Kitamura et al., 2022b). *R. quelen* (commonly known as "jundiá") is distributed from Argentina to southern Mexico, with a predominant presence in the southern region of Brazil, where it holds significant economic importance in aquaculture activities (Mazzoni et al., 2020; Assis, 2021).

In this context, the study aimed to investigate the potential of *M. aquaticum* to prevent the harmful effects induced by *M. aeruginosa* aqueous extract in *R. quelen*. Furthermore, another aim of the study was to assess whether the plant can be suitable for phytoremediation.

Materials and Methods

Fish acclimation

Juvenile Neotropical catfish (*R. quelen*) were acquired from pisciculture in Toledo (western Paraná State, Brazil). Fish were acclimated in laboratory glass aquaria (15 L) for 20 days. Specimens were maintained at room temperature of 26±2°C in filtered and dechlorinated water with a 12 h photoperiod and constant aeration. Water parameters, including pH (7.3±0.2), nitrite (0.13±0.12 $mg.L^{-1}$), and ammonia (<0.1 $mg.L^{-1}$), were assessed using Labcon® test kits. Daily feeding using fish food (Laguna® containing 32% protein) was provided. The Animal Experimentation Ethics Committee of the Federal University of Paraná approved the experiments under certificate 1242/2018.

Aquatic macrophyte laboratory acclimation

The aquatic macrophyte *M. aquaticum* (Vell.) Verdc. was acquired from aquaculture in Mantena (eastern Minas Gerais State, Brazil). Before the phytoremediation process, the plants were acclimated to laboratory conditions for 10 days. The plant material was submerged in filtered and chlorine-free water. The species was maintained without aeration, under a 12 h photoperiod, and at room temperature of 26±2°C. The measured light intensity in the cultivation area was 116±22 $\mu mol.m^{-2}.s^{-1}$ (ICEL Luximeter, LD-511).

Aqueous extract preparation

Aqueous extract (AE) was obtained from laboratory cultures of *M. aeruginosa* (strain BB005), provided by the Federal University of São Carlos, and isolated from toxic cyanobacterial blooms in the Barra Bonita Reservoir, São Paulo, Brazil.

Cultures were maintained in an ASM-1 growth medium (Gorham et al., 1964) at a room temperature of 23±2°C and a light intensity of 45 $\mu mol.m^{-2}.s^{-1}$. Additionally, cell suspensions were continuously aerated and subjected to a 14-h light/10-h dark photoperiod. These conditions resulted in a *M. aeruginosa* suspension containing about 10^6 cells.mL^{-1} after 10 days of growth, at which point the development was interrupted to prepare the AE.

To obtain the AE, the cell suspension was centrifuged at 12300 *xg* (4°C) for 4 min. The supernatant (culture medium) was discarded, and the pellet was lyophilized for 72 h. The extraction was carried out using the solid fraction of the cultures, employing an ultrasonic bath following the H method proposed by Silva-Stenico et al. (2009). Extracts were pooled and stored at –20°C until further use.

Quantification of microcystins in the AE was conducted according to the methodology detailed in Section 2.6. Based on the MC-LR concentration, a dilution using an AE was performed with ultrapure water to achieve the desired experimental conditions regarding MC-LR equivalents (MC-LR_{eq}).

Water treatment using M. aquaticum

This bioassay was conducted at concentrations of 0.0, 1.0, and 10.0 $\mu g.MC\text{-}LR_{eq}.L^{-1}$, based on both the guidelines of the WHO (2021) and recent literature (Aguilera et al., 2023). Initially, aquaria containing 15 L of chlorine-free filtered water were prepared with a density of 10 $g.L^{-1}$ of *M. aquaticum*. The experimental temperature and light intensity conditions remained the same as the plant acclimation period. The treatment was carried out without aeration, considering n=5 in each experimental group. Subsequently, water contamination was performed by adding AE in a sufficient volume to achieve the test conditions, considering the concentration of 13,313.05 $\mu g.L^{-1}$ of MC-LR determined in the AE described below. Only filtered and chlorine-free water was added to the aquarium in control conditions. To assess the potential natural degradation of microcystins, an aquarium containing 15 L of water contaminated with the AE under the same experimental conditions, but without *M. aquaticum* was included.

The treatment lasted for 168 h and water sampling for microcystin analysis was done at the beginning (0 h) and at the end of the experiment (168 h). This period was based on previous studies (Calado et al. 2019). The phytoremediation efficiency was calculated by considering the ratio of MC-LR_{eq} removal to the initial treatment concentration.

Fish exposure experiments

The assessment of sublethal effects in *R. quelen* was conducted in two 96 h static exposure bioassays. Fish were fed daily (once a day) using the same feed during the acclimation period and were randomly distributed among the glass aquaria for the experiments.

The first bioassay involved the exposure of *R. quelen* to environmental concentrations of MC-LR_{eq} in three groups (n=12) within a 15 L aquarium (three fish/aquarium, density of 7 g of fish/L). Based on the quantification of MC-LR present in AE, dilutions were performed to achieve exposure concentrations of 1.0 and 10.0 $\mu g.MC\text{-}LR_{eq}.L^{-1}$. Additionally, a control group of 0.0 $\mu g.MC\text{-}LR_{eq}.L^{-1}$ without extract addition was included for assessment. Water quality parameters, including pH (7.3±0.2), nitrite (0.13±0.12 $mg.L^{-1}$), and ammonia (<0.1 $mg.L^{-1}$), were daily assessed using Labcon® test kits. Water parameters were monitored, and samples were collected for microcystin analysis at 0 and 96 h of the exposure period to determine the concentration of MC-LR.

In the second bioassay, *R. quelen* was exposed to posttreatment water following similar conditions to the first bioassay. Fish were exposed to water treatment for 96 h. The control condition for the 0.0 $\mu g.MC\text{-}LR_{eq}.L^{-1}$ in the treated water consisted of a group exposed to water (without adding the extract). The water quality parameters were also maintained within appropriate ranges during measurements of pH (7.1±0.3), nitrite (0.04±0.09 $mg.L^{-1}$), and ammonia (0.02±0.01 $mg.L^{-1}$). The water samples were collected at 0 and 96 h of the exposure period to determine the concentration of MC-LR.

After the 96 h exposure, fish were anesthetized with 0.1 $\mu g.L^{-1}$ of benzocaine. Blood samples were collected through caudal vein puncture using heparinized syringes for hematological and genotoxicity biomarker analyses. Subsequently, euthanasia was performed through the spinal cord section, and the weight and length of the fish were recorded. The tissues were sampled for genotoxicity (gill, liver, kidney, and brain), biochemical (gills, liver, kidney, brain, and muscle), and histopathological (gill, liver, kidney, intestine, muscle, and skin) biomarkers.

Water chemical analysis

For microcystin analysis, water samples were filtered through a glass fiber microfilter (GF-1, 47 mm). Subsequently, solid-phase extraction (SPE) was conducted using a C18 cartridge (Bond Elut 500 mg/6 mL – Agilent®) in a Manifold system with a vacuum pump.

The chemical analysis was performed on a chromatographic system (Agilent 1260 Infinity) with a triple quadrupole mass spectrometer (QqQ – 6460 Triple Quad LC/MS, Agilent Technologies, USA), featuring electrospray ionization (ESI) in positive mode at 3500 V. Nitrogen gas was employed as the nebulizer at a pressure of 45 psi (drying gas at 300°C and a flow rate of 9 $L.min^{-1}$ N_2). The chromatographic separation was carried out according to the method used by Silva et al. (2023). The calibration curves exhibited appropriate linearity for analyte quantification (R^2=0.9932; $p<0.0001$), and the quantification limit was 0.5 $\mu g.L^{-1}$. The equation of the linear model was y=1311.8x – 16352, established using the chromatographic peak areas of the standards (*m/z*) to their respective concentrations. The analyses focused on the mass-to-charge ratio (*m/z*) 995>135.

Biomarker analysis

Biomarkers of genotoxicity

Biomarkers of genotoxicity were assessed by the piscine micronucleus test (PMT) and erythrocytic nuclear alterations (ENAs), the comet assay, and the determination of the frequency of apoptotic and necrotic cells using the DNA diffusion assay.

The PMT was analyzed on blood samples, with 2000 erythrocytes assessed on each slide using an optical microscope at a 1000x magnification (Heddle, 1973; Schmid, 1976), following the frequencies of other ENAs as described by Carrasco et al. (1990). The ENAs were classified as blebbed, lobed, notched, and vacuolated. The comet assay was conducted on blood, gills, liver, kidney, and brain, according to Cestari et al. (2004) and Ferraro et al. (2004). Analysis of blood and liver of the frequency of apoptotic and necrotic cells using the DNA diffusion assay followed the methodology proposed by Ramsdorf et al. (2009). In each sample, 100 nucleoids were individually assessed and visually classified for DNA damage (from 0 to 4) as described by Azqueta et al. (2011) using an epifluorescence microscope at 400x magnification.

Hematological biomarkers

Erythrocytes (Er) were counted using a 20 µL blood sample diluted in 2 mL of citrate formaldehyde. Counting was carried out using a Neubauer chamber in an optical microscope. The total erythrocyte count was determined according to Oliveira-Júnior et al. (2009) method. The determination of hemoglobin (Hb) levels was carried out using the cyanmethemoglobin method by Collier (1944) and Drabkin (1946). The hematocrit (Ht), the blood centrifuged for 5 min at 16128 *xg*, and the percentage of the total volume occupied by erythrocytes to the total volume was followed by the method by Hine (1992). Differential counting of leukocytes and thrombocytes was performed in blood extensions using May–Grunwald–Giemsa (Tavares-Dias and Moraes, 2006).

Biochemical biomarkers

The tissues were homogenized using a potassium phosphate buffer solution in a 1:10 (w/v) ratio. The homogenates were centrifuged at 12,000 *xg* (4°C) for 30 min. For the gill, liver, and kidney samples, a 0.1 M potassium phosphate buffer, pH 7.0 was used, and the homogenate was centrifuged for 30 min at 15,000 *xg* (4°C). The acetylcholinesterase activity (AChE) was measured in the brain and muscle using the Ellman et al.'s (1961) method. For the gills, liver, and kidney samples, the antioxidant system was evaluated such as glutathione S-transferase (GST) (Keen et al., 1976), superoxide dismutase (SOD) (Gao et al., 1998), catalase (CAT) (Aebi, 1984), glutathione peroxidase (GPx) (Hafeman et al., 1974), and non-protein thiol/glutathione (GSH) levels (Sedlak and Lindsay, 1968). Additionally, lipid peroxidation (LPO) was evaluated according to Jiang et al. (1992) for potential oxidative damage.

The determination of total protein concentration followed Bradford's (1976) method.

Histopathological biomarkers

Tissue fragments were fixed for 16 h using ALFAC solution (80% ethanol, formaldehyde, and neat acetic acid). Subsequently, the materials were transferred to a 70% ethanol solution. The sample processing started with tissue dehydration, diaphanization in xylol, and embedding in Paraplast® (Sigma Aldrich). Following processing, embedded samples were cut into 5-µm-thick sections using a rotating microtome. The material fixed in slides was stained with hematoxylin and eosin (HE) and assessed on an optical microscope (Leica DMi8).

Histopathological evaluation followed Bernet et al.'s (1999) protocol with modifications done by Mela et al. (2013) for determining the occurrence and extent of lesions through a lesion index (LI).

Data analysis

Statistical analysis

The homogeneity and normality of variables were assessed using the Levene and Shapiro-Wilk tests, respectively. A two-way ANOVA followed by Tukey's test was applied for normally distributed data. Non-parametric data were analyzed using the Kruskal-Wallis test, followed by the Dunn's test. The analyses were conducted, and graphs were generated using Prism 6 (6.0.1) software (significance level $p<0.05$).

Integrated biomarkers response index

The Integrated Biomarkers Response Index (IBRv2) was used as a comprehensive tool to integrate multiple biomarkers, encompassing hematological, genotoxic, biochemical, and histological endpoints in the experiments (Beliaeff and Burgeot, 2002), which was modified by Sanchez et al. (2013).

Results and Discussion

Water chemical analyses and phytoremediation with M. aquaticum

Among the microcystin variants analyzed in the degradation control and samples (MC-RR, MC-YR, MC-LR, and MC-LA), only MC-LR was detected. Concentrations of the other variants remained below the quantification limit of the analytical method.

Natural degradation of microcystins remained insignificant throughout the phytoremediation period. No significant differences were observed between the initial and final concentrations under degradation control at two tested concentrations (Table 1). The control conditions showed no detectable concentrations of MC-LR in any of the experiments. As observed in Table 1, phytoremediation exhibited

removal efficiencies of 6.56 and 19.25% for 1 and 10 µg.MC-LR$_{eq}$.L^{-1} conditions, respectively.

Since microcystins are the most recorded cyanotoxins in the environment, research efforts have focused on effectively removing this hepatotoxin through phytoremediation. Somdee et al. (2016) evaluated six aquatic macrophyte species concerning the removal of MC-LR, obtained from toxic blooms of *M. aeruginosa*. The treatment period occurred for 7 days (168 h) in waters previously contaminated with 500 µg.L^{-1}, presenting 28.49–59.74% MC-LR removal. In the study conducted by Dong et al. (2022), employing dilutions of standard microcystin solutions, the species *Ceratophyllum demersum* demonstrated a removal rate exceeding 99% for 10 µg.L^{-1} MC-LR within 14 days; nonetheless, negative effects, such as a reduction in plant biomass, were noted.

In our study, the removal was 6.56% at 1 µg.MC-LR$_{eq}$.L^{-1} and 19.25% for the 10 µg.MC-LR$_{eq}$.L^{-1} over 7 days (168 h) of treatment. These results suggest that the species *M. aquaticum* holds the potential for microcystin phytoremediation. These removal outcomes align with those observed by Somdee et al. (2016), who also used extracts from *M. aeruginosa* blooms in their experiments. The variation in the percentage of MC-LR removal could be attributed to the presence of other metabolites produced by cyanobacteria in extracts, which may also pose toxicity to the plants (Jacinavicius et al., 2023).

Biomarkers analysis in R. quelen

Aquatic macrophytes produce substances with allelopathic potential that can be toxic not only to photoautotrophic species but also to other aquatic species (Techer et al., 2016; Li et al., 2021). However, in the present study, *M. aquaticum* possibly did not interfere with the sublethal effects, as no differences were observed between the controls of untreated and treated water bioassays.

The biomarkers used facilitated a more comprehensive assessment of phytoremediation treatment by comparing exposure to untreated and treated water with *M. aquaticum*. MC-LR presence, confirmed by water chemical analyses, led to alterations in hematological, genotoxicity, biochemical, and histopathological biomarkers. These results align with previous studies suggesting the adverse effects of microcystins on fish physiology (Rodrigues et al., 2022; Shahmohamadloo et al., 2022; Aklakur et al., 2023).

Biomarkers of genotoxicity

No significant differences were found in any treatment for the PMT, ENAs, apoptotic, and necrotic cells. However, the presence of microcystins in untreated water increased brain tissue DNA damage. Treated water caused a significant reduction in brain DNA damage when compared to untreated water at a concentration of 1 µg.MC-LR$_{eq}$.L^{-1} (p=0.0008) (Figure 1A).

The kidney of *R. quelen* also exhibited significant alterations concerning DNA damage, indicating the reduction in DNA damage, after the water treatment (p=0.0069) at 1 µg.MC-LR$_{eq}$.L^{-1} (Figure 1B). Blood, gills, and liver showed no significant DNA damage (Figure 2).

The presence of microcystins may result in the production of reactive oxygen species (ROS), which can be responsible for damaging cell membranes and DNA (Ishfaq et al., 2022). Interestingly, even after the phytoremediation, genotoxicity remained in the brain, possibly due to MC-LR concentration in treated water. Microcystins have shown effects on brain tissue due to their ability to cross the blood–brain barrier (Hinojosa et al., 2019). An apparent reduction in DNA damage was observed in the kidney by the comet assay. It can be linked to the cytotoxicity of MC-LR, which probably harms renal cells. The presence of necrosis, leukocyte infiltration, adipocytes, and melanomacrophage centers may have contributed to genotoxicity. On the other hand, after

Table 1 – Initial and final concentrations of microcystins in the phytoremediation experiments and exposure bioassays, as well as phytoremediation efficiency.

Experiment		Condition (MC-LR$_{eq}$)	MC-LR concentration (µg.L^{-1})			Phytoremediation efficiency (%)
			0 h	96 h	168 h	
Phytoremediation		Control	n.d.	-	n.d.	-
		1 µg.L^{-1}	1.22±0.00^{a}	-	1.14±0.01^{b}	6.56
		10 µg.L^{-1}	13.61±0.30^{a}	-	10.99±0.22^{b}	19.25
Bioassays	Untreated	Control	n.d.	n.d.	-	-
		1 µg.L^{-1}	1.33±0.06$^{a, A}$	1.52±0.03$^{a, A}$	-	-
		10 µg.L^{-1}	12.72±0.26$^{a, A}$	11.73±0.25$^{a, A}$	-	-
	Treated	Control	n.d.	n.d.	-	-
		1 µg.L^{-1}	1.14±0.01$^{a, A}$	1.34±0.03$^{b, A}$	-	-
		10 µg.L^{-1}	10.99±0.22$^{a, B}$	10.02±1.27$^{a, A}$	-	-

Values indicate mean±standard error. Differences within the same experiment are indicated by different lowercase letters (p<0.05). Different uppercase letters indicate differences between distinct experiments at the same MC-LR$_{eq}$. concentration condition (p<0.05). Non-detected concentrations are indicated as n.d.

treatment, DNA damage in the kidney was not significant, indicating a possible improvement in water quality.

Hematological biomarkers

The exposure to microcystins caused a decrease in erythrocyte and thrombocyte cell numbers in untreated water experiments (Figures 3A and 3B). In the erythrocyte count (Figure 3A), this difference was evident at 1 and 10 µg.MC-LR_{eq}.L^{-1} conditions when compared to control ($p=0.002$; $p<0.0001$). These effects were notably absent after the *M. aquaticum* treated water ($p<0.05$). In the bioassay with untreated water, there was also a reduction in hematocrit percentual and hemoglobin concentration at 10 µg.MC-LR_{eq}.L^{-1} (Figures 3C and 3D). The water treatment using *M. aquaticum* contributes to preventing these hematological alterations. Leukocyte counts did not reveal differences in the experimental conditions or between the bioassays.

Phytoremediation decreased the sublethal effects in *R. quelen*. While untreated water bioassay responses indicated difficulties in oxygen transport (reduction in hematological parameters), fish exposed to treated water showed no significant alterations. The hematological biomarkers suggest that microcystins may lead to a deficit in the oxygen transport capacity in *R. quelen*. Similar effects on hematological biomarkers were also observed after 12 h intraperitoneal exposure to microcystins in *Hoplias malabaricus* fish (Sakuragui et al., 2019).

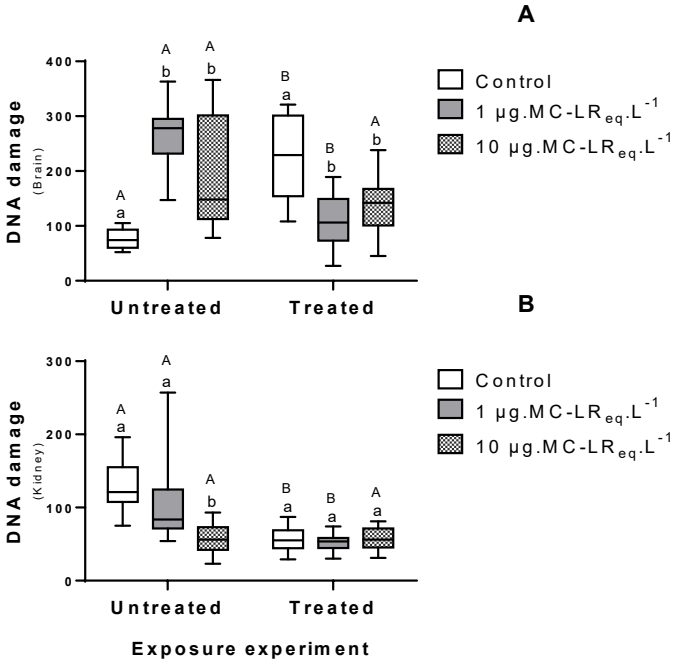

Figure 1 – DNA damage scores (comet assay) in brain (A) and kidney (B) of *Rhamdia quelen* exposed for 96 h to 1 and 10 µg.MC-LR_{eq}.L^{-1} conditions in untreated and treated water with *Myriophyllum aquaticum*.
Values presented as median±interquartile range; differences within the same bioassay are denoted by different lowercase letters ($p<0.05$); different uppercase letters indicate variations between distinct bioassays under the same MC-LR_{eq}; condition ($p<0.05$); statistical analysis was performed using Kruskal-Wallis with Dunn's test (n=12).

Biochemical biomarkers

In the liver, GPX activity (Figure 4A) decreased at 1 and 10 µg.MC-LR_{eq}.L^{-1}, while SOD activities increased and GST activities decreased (Figures 4B and 4C). These changes were observed only in the higher MC-LR_{eq} concentration in untreated water treatment. After water treatment using *M.* aquaticum, the GST activity increased at 1 µg.MC-LReq.L^{-1} (Figure 4C).

Changes in GPx, SOD activities, and GSH levels in the kidney were observed (Figures 4D, 4E and 4F). In untreated water bioassays, a decrease in GPx activity and an increase in SOD activity at 10 µg.MC-LR_{eq}.L^{-1} were observed. GSH levels were lower at the low concentration and increased at the higher MC-LR_{eq} concentration. Moreover, treated water caused a reduction in GPx activity for both tested conditions and a decrease in SOD activity for the 1 µg.MC-LReq.L^{-1}. Another response was the decrease in GSH levels when comparing the two assays (Figure 4F).

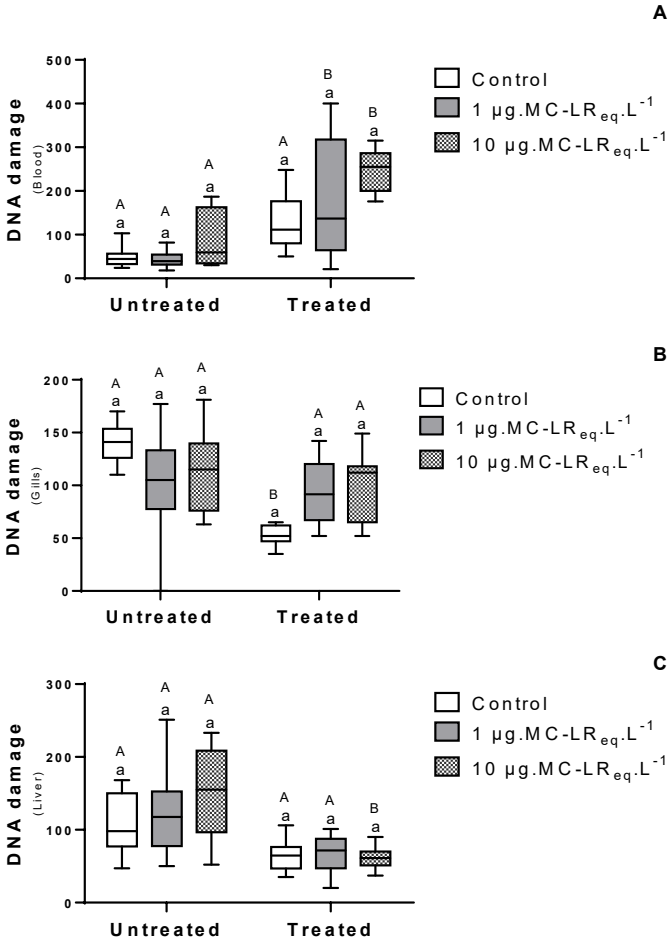

Figure 2 – DNA damage scores (comet assay) in blood (A), gills (B), and (C) liver of *Rhamdia quelen* exposed for 96 h to 1 and 10 µg.MC-LR_{eq}.L^{-1} condition ($p<0.05$).
Conditions in untreated and treated water with *Myriophyllum aquaticum*; values are presented as median±interquartile range; differences within the same bioassay are denoted by different lowercase letters ($p<0.05$); different uppercase letters indicate variations between distinct bioassays under the same MC-LR_{eq}; statistical analysis was performed using Kruskal-Wallis with Dunn's test (n=12).

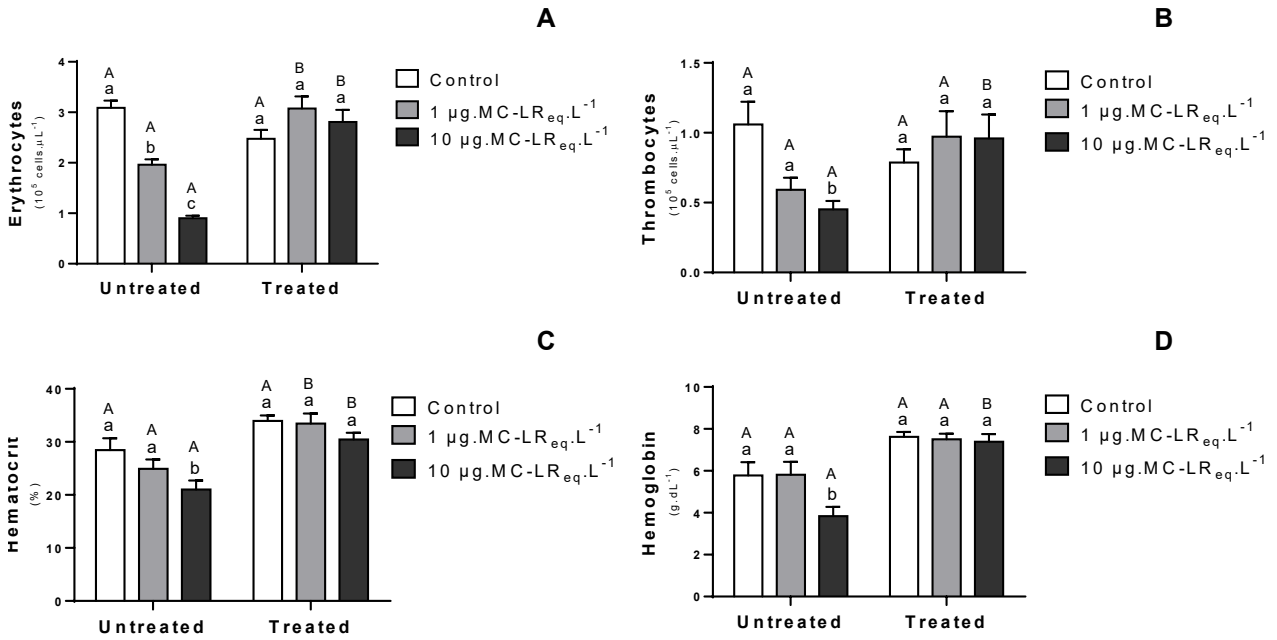

Figure 3 – Hematological biomarkers, represented as mean±standard error, in *Rhamdia quelen* exposed for 96 h to 1 and 10 µg.MC-LR$_{eq}$.L^{-1} conditions in untreated and treated water with *Myriophyllum aquaticum*. (A) Erythrocyte count, (B) thrombocyte count, (C) hematocrit, and (D) hemoglobin levels.
Differences within the same bioassay are denoted by different lowercase letters ($p<0.05$). Different uppercase letters indicate variations between distinct bioassays under the same MC-LR$_{eq}$ condition ($p<0.05$). Statistical analysis was performed using two-way ANOVA and Tukey's test (n=12).

In gill tissues there was a reduction in GSH levels and GST activity and a decrease in LPO when exposure occurred in untreated water (Figures 4G, 4H and 4I). No changes in biochemical biomarkers were observed, after the treated water bioassay.

Evaluating biochemical biomarkers allowed us to observe the activation of the antioxidant system and possibly the conjugation process for the elimination of microcystins in untreated water. In the 10 µg.MC-LR$_{eq}$.L^{-1} condition, the increase in liver and kidney SOD activity indicates antioxidant activity. The generation of ROS by microcystins requires SOD action to dismutate the superoxide radical (O^{2-}) into hydrogen peroxide (H_2O_2) to mitigate oxidative effects on tissues (Zaidi et al., 2021). Even with the reduction in GPx activity, the action of the antioxidant system, evidenced by the activation of SOD in the liver and kidney, was sufficient to prevent damage to lipids. Additionally, the decrease in GPx and GST activity may be related to the conjugation process of GSH with microcystins. In detoxification, GSH forms complexes to make the molecules more hydrophilic, allowing the transport system to eliminate these conjugates. The conjugation between microcystins and GSH forms a more soluble complex for excretion (Malbrouck and Kestemont, 2006; Martins et al., 2017). Thus, the recruitment of GSH for the excretion of microcystins is likely reflected in the liver's reduced GPx and GST activities. In fish, GPx and GST activity uses GSH as a cofactor for converting hydrogen peroxide and organic peroxides into water. Therefore, the lack of adequate levels of GSH may decrease GPx and GST activity (van der Oost et al., 2003; Zaidi et al., 2021). The highest GSH level in the kidney also occurred in the group with the lowest GPx activity. These data once again reinforce the hypothesis that GPx activity depends on the presence of GSH as a substrate.

In gills, differently from other tissues, the decrease in LPO may be associated with tissue damage. The occurrence of epithelial lifting and total hyperplasia may have damaged the tissue. The antioxidant system in the gills has less potential to neutralize ROS compared to other tissues. Therefore, the tissue becomes more vulnerable to damage from oxidative stress (Prieto et al., 2006).

Phytoremediation using *M. aquaticum* prevented the biochemical biomarker alterations. In the treated water bioassay, alterations were observed in the liver GST activity and GPx and SOD activities in the kidney due to the residual concentrations of MC-LR in the posttreatment.

Histopathological biomarkers

In untreated water, damages occurred in the skin, gills, liver, kidney, and muscle with no alteration in the gut at the higher concentration of MC-LR$_{eq}$. In muscle and skin, higher lesion indices were observed for both MC-LR$_{eq}$ conditions (Figure 5).

In the liver, changes included leukocyte infiltration (Figure 6A), necrosis (Figure 6B), and melanomacrophage centers (Figure 6C). In

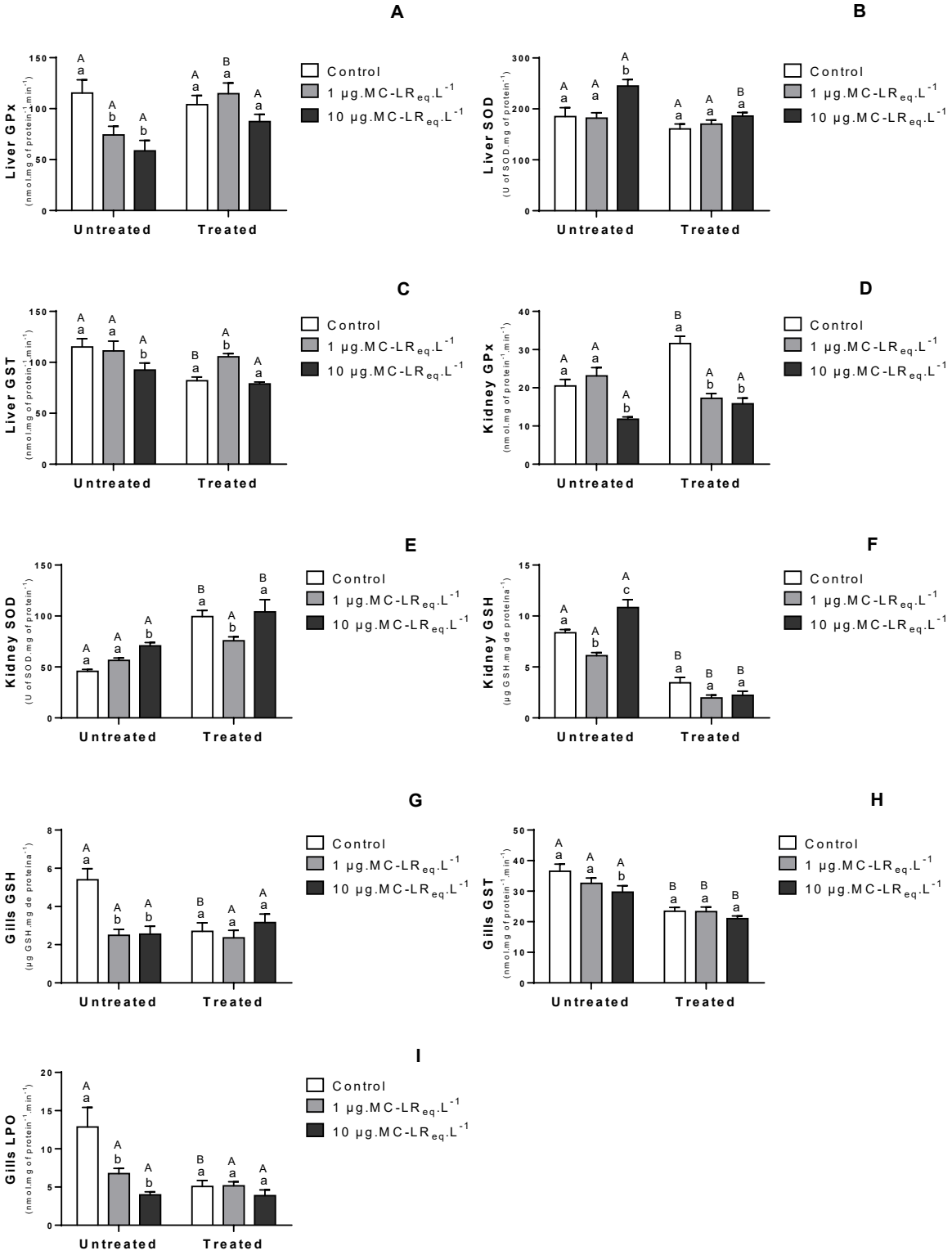

Figure 4 – Biochemical biomarkers in *Rhamdia quelen* exposed for 96 h to 1 and 10 µg.MC-LR$_{eq}$.L^{-1} conditions in untreated and treated water with *Myriophyllum aquaticum*. Effects on liver: (A) GPx, (B) SOD, (C) GST; kidney: (D) GPx, (E) SOD, (F) GSH; Gills: (G) GSH, (H) GST, (I) LPO.
GPx: glutathione peroxidase activity; SOD: superoxide dismutase activity; GSH: non-protein thiol/glutathione; GST: glutathione S-transferase activity; LPO: lipoperoxidation; values are represented as mean±standard error; differences within the same bioassay are denoted by different lowercase letters ($p<0.05$); different uppercase letters indicate variations between distinct bioassays under the same MC-LR$_{eq}$.L^{-1} condition ($p<0.05$); statistical analysis was performed using two-way ANOVA and Tukey's test (n=12).

kidney, the presence of adipocytes (Figure 6D), leukocyte infiltration, necrosis (Figure 6E), and melanomacrophage centers (Figure 6F). In muscle, leukocyte infiltration occurred (Figure 6G). Gills exhibited hyperplasia (total and partial), with lamellar fusion (Figure 6H). The skin displayed the presence of adipocytes and an increased number of club cells (Figure 6I).

In treated water bioassays using *M. aquaticum,* no significant results were observed by Bernet's index. Muscle and skin showed no damage, resulting in a null index. Liver and kidney (Figures 6J and 6K) presented necrosis and leukocyte infiltration. Gills exhibited epithelial lifting and partial hyperplasia (Figures 6L and 6M). The melanomacrophage centers, presence of adipocytes, or total hyperplasia were not observed in the tissues of this bioassay. No differences were observed between the controls in a comparison with untreated and treated water (Figure 5).

The results demonstrate that even at short-term environmental concentrations, microcystins can alter the integrity of tissues in *R. quelen*. The presence of damage, such as necrosis, leukocytic infiltrations, and melanomacrophage centers, contributed to the LI. Additionally, it was possible to observe that MC-LR presence in untreated water affected the gills, skin, and muscle (Figures 5A, 5B and 5E) in both tested conditions (1 and 10 µg.MC-LR$_{eq}$.L^{-1}), unlike other tissues that showed damage only at the higher concentration (Figures 5C and 5D). Due to the direct contact of dissolved microcystins with the gills and skin of fish, these tissues are commonly entry points for cyanotoxin, leading to structural alterations (Zamora-Barrios et al., 2019). Another evidence of phytoremediation effectiveness was the decrease in histopathological damage. The skin and muscle showed no alterations in the treated water bioassay, and the gills, liver, and kidney presented a lower LI.

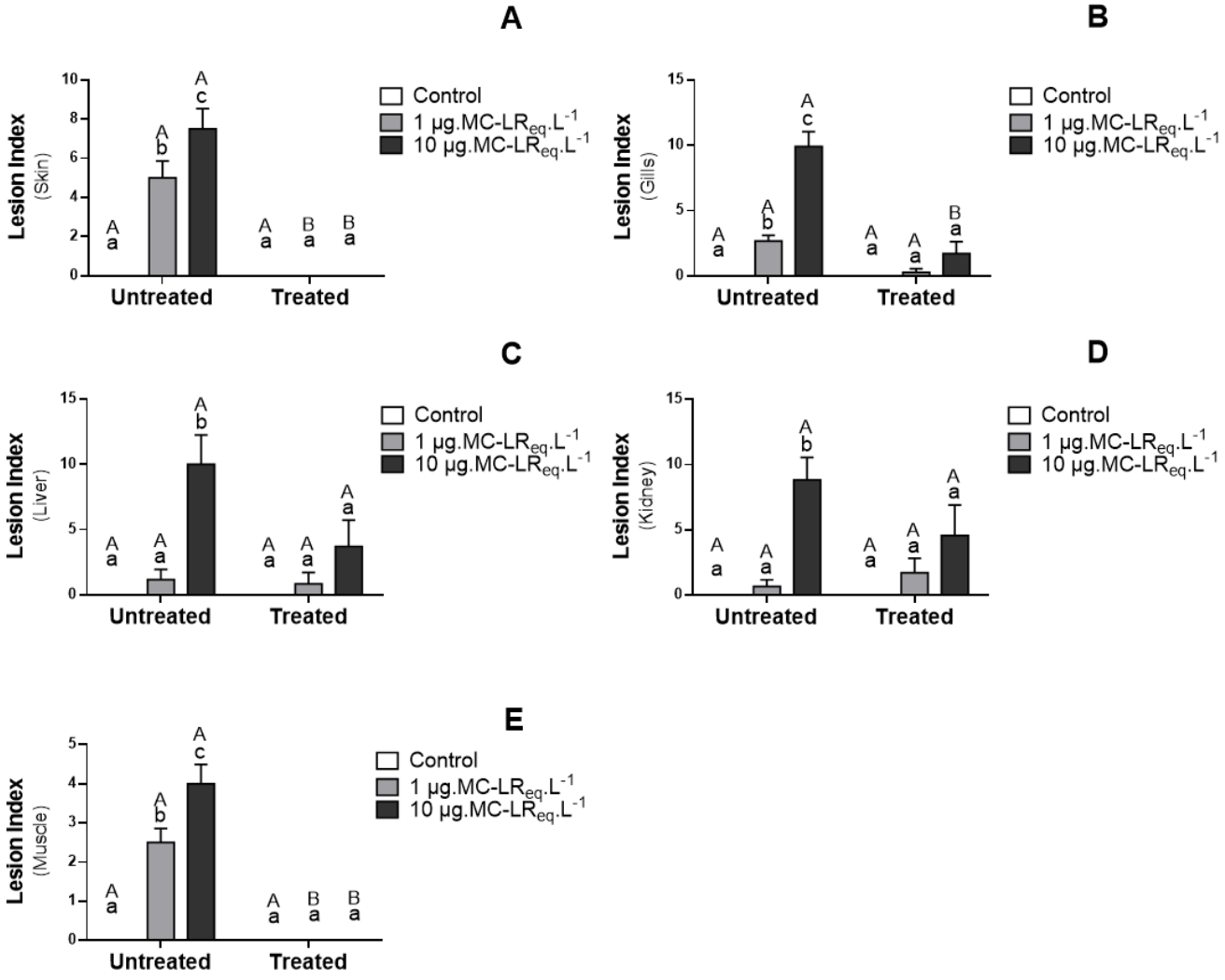

Figure 5 – Lesion index in Rhamdia quelen tissues exposed for 96 h to 1 and 10 µg.MC-LR$_{eq}$.L^{-1} conditions in untreated and treated water with *Myriophyllum aquaticum*. Values are represented as mean±standard error. (A) Skin, (B) gills, (C) liver, (D) kidney, and (E) muscle.
Differences within the same bioassay are denoted by different lowercase letters ($p<0.05$); different uppercase letters indicate variations between distinct bioassays under the same MC-LR$_{eq}$ condition ($p<0.05$); statistical analysis was performed using two-way ANOVA and Tukey's test (n=12).

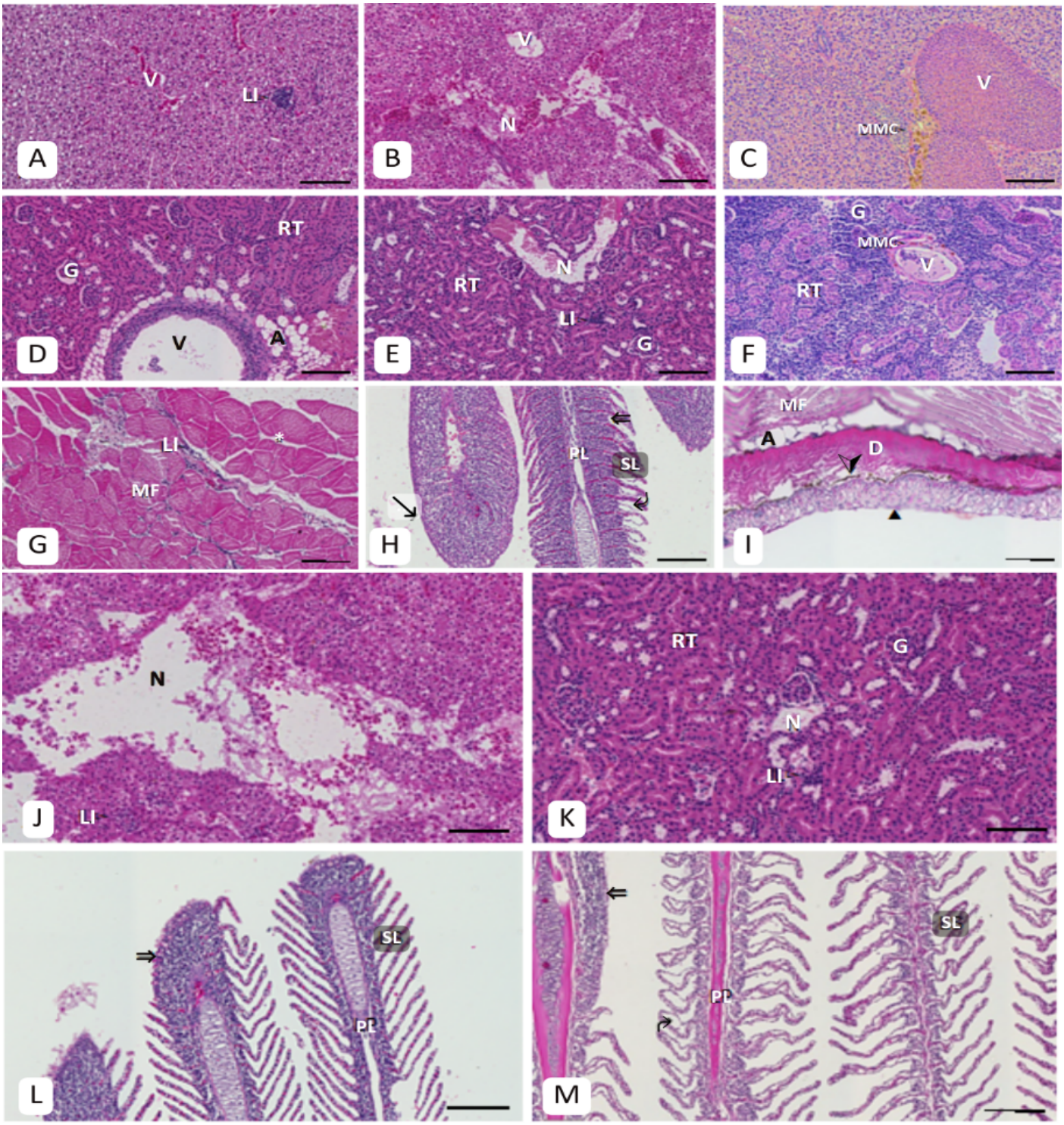

Figure 6 – Histological sections in *Rhamdia quelen* tissues exposed for 96 h to 1 and 10 µg.MC-LR$_{eq}$.L^{-1}. Untreated water (A-I) and treated water (J-M). (A) Liver in 1 µg.MC-LR$_{eq}$.L^{-1}, (B) liver in 10 µg.MC-LR$_{eq}$.L^{-1}, (C) liver in 10 µg.MC-LR$_{eq}$.L^{-1}, (D) kidney in 1 µg.MC-LR$_{eq}$.L^{-1}, (E) kidney in 10 µg.MC-LR$_{eq}$.L^{-1}, (F) kidney in 10 µg.MC-LR$_{eq}$.L^{-1}, (G) muscle in 10 µg.MC-LR$_{eq}$.L^{-1}, (H) gills in 10 µg.MC-LR$_{eq}$.L^{-1}, (I) skin in 10 µg.MC-LR$_{eq}$.L^{-1}, (J) liver in 10 µg.MC-LR$_{eq}$.L^{-1}, (K) kidney in 10 µg.MC-LR$_{eq}$.L^{-1}, (L) gills in 1 µg.MC-LR$_{eq}$.L^{-1}, and (M) gills in 10 µg.MC-LR$_{eq}$.L^{-1}.
Codes indicate V: blood vessel surrounded by hepatocytes; LI: leukocyte infiltration; N: necrotic area; MMC: melanomacrophage centers around a blood vessel; G: glomerulus; RT: renal tubules; MF: cross-sectional muscle fibers; * perimysium; PL: primary lamella; SL: secondary lamella; (⇒) partial hyperplasia of the secondary lamella; (æ) total hyperplasia causing fusion of the secondary lamellae; (↵) epithelial lifting; D: dermis; A: adipocytes presence, (▲) increased club cells in the epidermis, (Ú) presence of pigments—melanophores between the dermis and epidermis. Hematoxylin/eosin staining. Scale bar=100 µm.

Integrated Biomarkers Response Index

Respective control conditions were used as the baseline in the biomarkers' integrated assessment for both untreated and treated water bioassays. The increase in MC-LR$_{eq}$ concentration led to a more significant deviation from the baseline condition. In the untreated water bioassay, the condition with 1 µg.MC-LR$_{eq}$.L^{-1} exhibited an IBRv2 of 45.24, while the higher concentration exposure had an IBRv2 of 66.61. Similarly, in the treated water bioassay, there was an increase in deviation from the baseline level at higher MC-LR$_{eq}$ concentrations. For the 1 µg.MC-LR$_{eq}$.L^{-1}, IBRv2 was 53.49, for the 10 µg.MC-LR$_{eq}$.L^{-1}, it was 63.00. Figure 7 provides a graphical representation of IBRv2 for both bioassays. In comparison with untreated and treated water bioassays, a similar trend was observed, with an increase in IBRv2 at 10 µg.MC-LR$_{eq}$.L^{-1} concentrations. From the graphical representation of IBRv2, it is evident that histopathological biomarkers in both bioassays played a crucial role in evaluating the effects of exposure to untreated and treated water. This characteristic provides valuable insights not only into the treatment itself but also into MC-LR mechanisms of action.

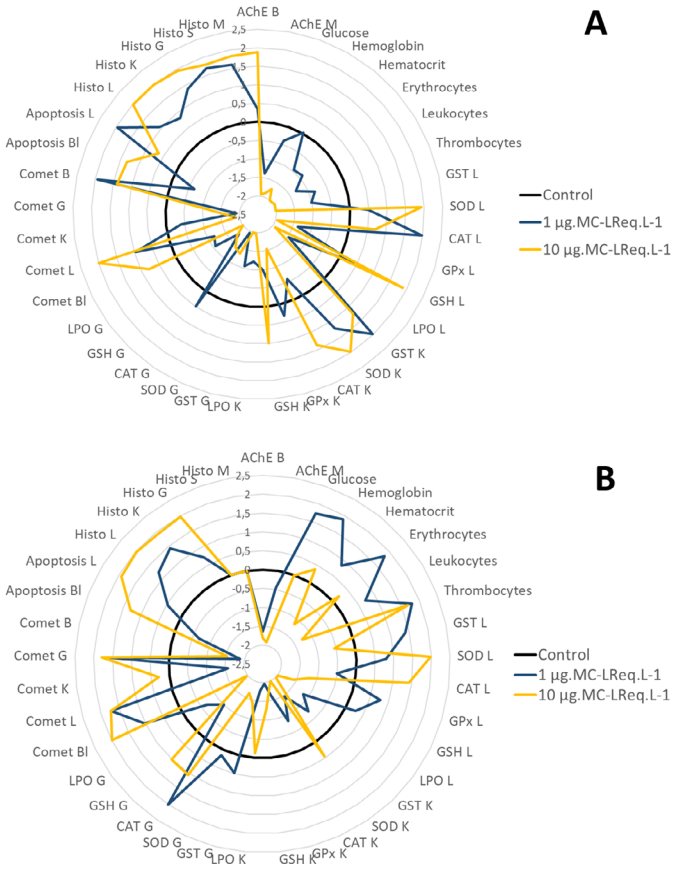

Figure 7 – Integrated Biomarker Response Index (IBRv2) in *Rhamdia quelen* exposed for 96 h to 1 and 10 µg.MC-LR$_{eq}$.L^{-1} conditions in untreated and treated water with *Myriophyllum aquaticum*. (A) IBRv2 in untreated water bioassay; (B) IBRv2 in treated water bioassay.
AChE: acetylcholinesterase activity, GPx: Glutathione peroxidase activity, SOD: Superoxide dismutase activity, CAT: Catalase activity, GSH: Non-protein thiol/glutathione, GST: Glutathione S-transferase activity, LPO: Lipoperoxidation. B: Brain, Bl: Blood, G: Gills, K: Kidney, L: Liver, M: Muscle, S: Skin.

Conclusion

Our results demonstrated that phytoremediation using *M. aquaticum* can be a sustainable and cost-effective technology that contributes to water quality improvement, leading to a consequent reduction in sublethal effects and histopathological damage. This approach can contribute to achieving the sustainable development goals, particularly target 6.3, which aims to improve water quality by reducing pollution. Although microcystin removal did not reach the expected levels within the 7-day treatment period, the results demonstrate the efficacy of the plant in reducing the concentration of this cyanotoxin. This reduction, achieved without additional energy input for aeration or plant supplementation, highlights the potential of this method, particularly in resource-limited scenarios. The present study is among the first to assess the effect of microcystins on juvenile *R. quelen*. The occurrence of damage such as necrosis and leukocyte infiltrations observed in histopathological evaluations highlights the need to discuss legislation regarding water quality monitoring. These findings support the importance of adopting more restrictive legal limits for cyanotoxins in water to protect native aquatic species and promote sustainable water resource management. Furthermore, the phytoremediation with *M. aquaticum* can contribute to water treatment by mitigating deleterious effects, reducing damage to native species, and enhancing the resilience of freshwater aquatic ecosystems.

Authors' contributions

Silveira, A.L.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. **Calado,** S.L.M.: conceptualization, methodology, supervision, writing – review & editing. **Kitamura**, R.S.A.: conceptualization, methodology, writing – review & editing. **Vicentini**, M.: conceptualization, methodology, writing – review & editing. **Pagioro,** T.A.: methodology, resources, supervision, writing – review & editing. **Vicari**, T.: methodology, writing – review & editing. **Da Silva**, A.C.F.: methodology, writing – review & editing. **Perussolo**, M.C.: methodology, writing – review & editing. **Torres**, M.A.: methodology, writing – review & editing. Jacinavicius, F.R.: methodology, writing – review & editing. **Prodocimo**, M.M.: methodology, writing – review & editing. **Cestari,** M.M.: methodology, supervision, writing – review & editing. **Assis**, H.C.S.: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing.

References

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymology, v. 105, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3

Aguilera, A.; Almanza, V.; Haakonsson, S.; Palacio, H.; Benitez Rodas, G.A.; Barros, M. U. G.; Capelo-Neto, J.; Urrutia, R.; Aubriot, L.; Bonilla, S., 2023. Cyanobacterial bloom monitoring and assessment in Latin America. Harmful Algae, v. 125, 1568-9883. https://doi.org/10.1016/j.hal.2023.102429.

Aklakur, M.; Bakli, S.; Deo, A.D.; Singh, D.K.; Pailan, G.H., 2023. Cyanobacteria toxicity in aquaculture system and its impact on fish physiology. Journal of Aquaculture & Marine Biology, v. 12 (1), 28-33. https://doi.org/10.15406/jamb.2023.12.00353.

Akyol, Ç.; Ozbayram, E.G.; Accoroni, S.; Radini, S.; Eusebi, A.L.; Gorbi, S.; Vignaroli, C.; Bacchiocchi, S.; Campacci, D.; Gigli, F.; Farina, G.; Albay, M.; Fatone, F., 2021. Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. Environmental Pollution, v. 286, 117535. https://doi.org/10.1016/J.ENVPOL.2021.117535.

AlKahtane, A.A.; Abushouk, A.I.; Mohammed, E.T.; ALNasser, M.; Alarifi, S.; Ali, D.; Alessia, M.S.; Almeer, R.S.; AlBasher, G.; Alkahtani, S.; Aleya, L.; Abdel-Daim, M.M., 2020. Fucoidan alleviates microcystin-LR-induced hepatic, renal, and cardiac oxidative stress and inflammatory injuries in mice. Environmental Science and Pollution Research, v. 27 (3), 2935-2944. https://doi.org/10.1007/S11356-019-06931-Z.

Assis, H.C.S., 2021. Pharmaceutical pollutants. Aquaculture Toxicology, 107-31. https://doi.org/10.1016/B978-0-12-821337-7.00008-6.

Azqueta, A.; Meier, S.; Priestley, C.; Bjerve Gutzkow, K.; Brunborg, G.; Sallette, J.; Soussaline, F.; Collins, A., 2011. The influence of scoring method on variability in results obtained with the comet assay. Mutagenesis, v. 26 (3), 393-399. https://doi.org/10.1093/mutage/geq105.

Beliaeff, B.; Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environmental Toxicology and Chemistry, v. 21 (6), 1316-1322. https://doi.org/10.1002/etc.5620210629.

Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt-Holm, P.; Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of Fish Diseases, v. 22 (1), 25-34. https://doi.org/10.1046/j.1365-2761.1999.00134.x.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, v. 72 (1-2), 248-254. https://doi.org/10.1006/abio.1976.9999.

Calado, S.L.M.; Esterhuizen-Londt, M.; Silva de Assis, H.C.; Pflugmacher, S., 2019. Phytoremediation: green technology for the removal of mixed contaminants of a water supply reservoir. International Journal of Phytoremediation, v. 21 (4), 372-379. https://doi.org/10.1080/15226514.2018.1524843.

Carrasco, K.R.; Tilbury, K.L.; Myers, M.S., 1990. Assessment of the piscine micronuc eus test as an *in situ*. Canadian Journal of Fisheries and Aquatic Sciences, v. 47 (3), 2123-2136. https://doi.org/10.1139/f90-237.

Cestari, M.M.; Lemos, P.M.M.; Ribeiro, C.A.O.; Costa, J.R.M.A.; Pelletier, E.; Ferraro, M.V.M.; Mantovani, M.S.; Fenocchio, A.S., 2004. Genetic damage induced by trophic doses of lead in the neotropical fish *Hoplias malabaricus* (Characiformes, Erythrinidae) as revealed by the comet assay and chromosomal aberrations. Genetics and Molecular Biology, v. 27 (2), 270-274. https://doi.org/10.1590/S1415-47572004000200023.

Collier, H.B., 1944. Standardization of blood haemoglobin determinations. Canadian Medical Association Journal, v. 50 (6), 550. PMID: 20323122.

Dahedl, E.K.; Urakawa, H., 2023. Differential effects of hydrogen peroxide and l-lysine treatments on the growth of freshwater Cyanophyta and Chlorophyta. Ecologies, v. 4 (2), 355-370. https://doi.org/10.3390/ecologies4020023.

Dong, J.; Dai, D.; Yang, Y.; Wang, F.; Li, X.; Yuan, J.; Chen, Y.; Gao, Y.; Zhang, M.; Gao, X.; Li, M.; Zhang, J., 2022. Responses of submerged macrophyte *Ceratophyllum demersum* to the gradient concentrations of microcystin-LR (MC-LR). Environmental Science and Pollution Research, v. 9 (47), 71257-71269. https://doi.org/10.1007/s11356-022-20917-4.

Drabkin, D.L., 1946. Spectrophotometric studies XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. Journal of Biological Chemistry, v. 164 (2), 703-723. https://doi.org/10.1016/S0021-9258(17)41272-5.

Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, v. 7 (2), 88-95. https://doi.org/10.1016/0006-2952(61)90145-9.

Ferraro, T.N.; Golden, G.T.; Smith, G.G.; Martin, J.F.; Lohoff, F.W.; Gieringer, T.A.; Zamboni, D.; Schwebel, C.L.; Press, D.M.; Kratzer-Zhao, S.H.O.; Berrettini, W.H.; Buono, R.J., 2004. Fine mapping of a seizure susceptibility locus on mouse chromosome 1: nomination of Kcnj10 as a causative gene. Mammalian Genome, v. 15 (4), 239-251. https://doi.org/10.1007/s00335-003-2270-3.

Gao, R.; Yuan, Z.; Zhao, Z.; Gao, X., 1998. Mechanism of pyrogallol autoxidation and determination of superoxide dismutase enzyme activity. Bioelectrochemistry and Bioenergetics, v. 45 (1), 41-45. https://doi.org/10.1016/s0302-4598(98)00072-5.

Gorham, P.R.; McLachlan, J.; Hammer, U.T.; Kim, W.K., 1964. Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb.) de Bréb. Verhandlungen der Internationalen Vereinigung für Theoretischeund Angewandte Limnologie, v. 15 (2), 796-804. https://doi.org/10.1080/03680770.1962.11895606.

Hafeman, D.G.; Sunde, R.A.; Hoekstra, W.C., 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. Journal of Nutrition, v. 104, 580-587. https://doi.org/10.1093/jn/104.5.580.

Heddle, J.A., 1973. A rapid *in vivo* test for chromosomal damage. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, v. 18 (2), 187-90. https://doi.org/10.1016/0027-5107(73)90035-3.

Hine, P.M., 1992. The granulocytes of fish. Fish Shellfish Immunology, v. 2 (2), 79-98. https://doi.org/10.1016/S1050-4648(05)80038-5.

Hinojosa, M.G.; Gutiérrez-Praena, D.; Prieto, A.I.; Guzmán-Guillén, R.; Jos, A.; Cameán, A.M., 2019. Neurotoxicity induced by microcystins and cylindrospermopsin: a review. Science of the Total Environment, v. 668, 547-565. https://doi.org/10.1016/j.scitotenv.2019.02.426.

Ishfaq, P.M.; Mishra, S.; Mishra, A.; Ahmad, Z.; Gayen, S.; Jain, S.K.; Tripathi, S.; Mishra, S.K., 2022. Inonotus obliquus aqueous extract prevents histopathological alterations in liver induced by environmental toxicant microcystin. Current Research in Pharmacology and Drug Discovery, v. 3, 100118. https://doi.org/10.1016/j.crphar.2022.100118.

Jacinavicius, F.R.; Geraldes, V.; Fernandes, K.; Crnkovic, C.M.; Gama, W.A.; Pinto, E., 2023. Toxicological effects of cyanobacterial metabolites on zebrafish larval development. Harmful Algae, v. 125, 102430. https://doi.org/10.1016/J.HAL.2023.102430.

Janssen, E.M.-L.; Jones, M.R.; Pinto, E.; Dörr, F.; Torres, M.A.; Rios Jacinavicius, F.; Mazur-Marzec, H.; Szubert, K.; Konkel, R.; Tartaglione, L.; Dell'Aversano, C.; Miglione, A.; McCarron, P.; Beach, D.G.; Miles, C.O.; Fewer,

D.P.; Sivonen, K.; Jokela, J.; Wahlsten, M.; Niedermeyer, T.H.J.; Schanbacher, F.; Leão, P.; Preto, M.; D'Agostino, P.M.; Baunach, M.; Dittmann, E.; Reher, R., 2023. S75 | CyanoMetDB | Comprehensive database of secondary metabolites from cyanobacteria (NORMAN-SLE-S75.0.2.0) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.7922070

Jiang, Z.Y.; Hunt, J.V.; Wolff, S.P., 1992. ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Analytical Biochemistry, v. 202 (2), 384-389. https://doi.org/10.1016/0003-2697(92)90122-N.

Jones, M.R.; Pinto, E.; Torres, M.A.; Dörr, F.; Mazur-Marzec, H.; Szubert, K.; Tartaglione, L.; Dell'Aversano, C.; Miles, C.O.; Beach, D.G.; McCarron, P.; Sivonen, K.; Fewer, D.P.; Jokela, J.; Janssen, E.M.L, 2021. CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria. Water Research, v. 196, 117017. https://doi.org/10.1016/j.watres.2021.117017.

Kafle, A.; Timilsina, A.; Gautam, A.; Adhikari, K.; Bhattarai, A.; Aryal, N., 2022. Phytoremediation: mechanisms, plant selection and enhancement by natural and synthetic agents. Environmental Advances, v. 8, 100203. https://doi.org/10.1016/j.envadv.2022.100203.

Keen, J.H.; Habig, W.H.; Jakoby, W.B., 1976. Mechanism for the several activities of the glutathiones transferases. Journal of Biological Chemistry, v. 251 (20), 6183-6188. PMID: 977564.

Kitamura, R.S.A.; Soares da Silva, A.R.; Pagioro, T.A.; Rocha Martins, L.R., 2022a. Use of *Myriophyllum aquaticum* to inhibit microcystis aeruginosa growth and remove microcystin-LR. Revista Brasileira de Ciências Ambientais, v. 57 (3), 434-41. https://doi.org/10.5327/z2176-94781309.

Kitamura, R.S.A.; Vicentini, M.; Perussolo, M.C.; Lirola, J.R.; Santos, C.F.C.; Brito, J.C.M.; Cestari, M.M.; Prodocimo, M.M.; Gomes, M.P.; Assis, H.C.S., 2022b. Sublethal biochemical, histopathological and genotoxicological effects of short-term exposure to ciprofloxacin in catfish *Rhamdia quelen*. Environmental Pollution, v. 300, 118935. https://doi.org/10.1016/J.ENVPOL.2022.118935.

Li, B.; Yin, Y.; Kang, L.; Feng, L.; Liu, Y.; Du, Z.; Tian, Y.; Zhang, L., 2021. A review: application of allelochemicals in water ecological restoration — algal inhibition. Chemosphere, v. 267, 128869. https://doi.org/10.1016/J.CHEMOSPHERE.2020.128869.

Li, T.; Fan, X.; Cai, M.; Jiang, Y.; Wang, Y.; He, P.; Ni, J.; Mo, A.; Peng, C.; Liu, J., 2023. Advances in investigating microcystin-induced liver toxicity and underlying mechanisms. Science of The Total Environment, v. 905, 167167. https://doi.org/10.1016/J.SCITOTENV.2023.167167.

Malbrouck, C.; Kestemont, P., 2006. Effects of microcystins on fish. Environmental Toxicology and Chemistry, v. 25 (1), 72-86. https://doi.org/10.1897/05-029R.1.

Martins, N.D.; Yunes, J.S.; Monteiro, D.A.; Rantin, F.T.; Kalinin, A.L., 2017. Microcystin-LR leads to oxidative damage and alterations in antioxidant defense system in liver and gills of *Brycon Amazonicus* (SPIX & AGASSIZ, 1829). Toxicon, v. 139, 109-16. https://doi.org/10.1016/j.toxicon.2017.10.006.

Mazzoni, T.S.; Bombardelli, R.A.; Quagio-Grassiotto, I., 2020. Reproductive biology of neotropical fishes: a guide to identification to the gonadal morphology during the reproductive cycle of Catfish *Rhamdia quelen* (Siluriformes: Heptapteridae). Aquatic Science and Technology, v. 8 (2), 15. https://doi.org/10.5296/ast.v8i2.17102.

Mela, M.; Guiloski, I.C.; Doria, H.B.; Randi, M.A.F.; De Oliveira Ribeiro, C.A.; Pereira, L.; Maraschi, A.C.; Prodocimo, V.; Freire, C.A.; Silva de Assis, H.C., 2013. Effects of the herbicide atrazine in neotropical catfish (*Rhamdia quelen*). Ecotoxicology and Environmental Safety, v. 93, 13-21. https://doi.org/10.1016/j.ecoenv.2013.03.026.

Munoz, M.; Cirés, S.; de Pedro, Z.M.; Colina, J.A.; Velásquez-Figueroa, Y.; Carmona-Jiménez, J.; Caro-Borrero, A.; Salazar, A.; Fuster, M.-C.S.M.; Contreras, D.; Perona, E.; Quesada, A.; Casas, J.A., 2021. Overview of toxic cyanobacteria and cyanotoxins in Ibero-American freshwaters: challenges for risk management and opportunities for removal by advanced technologies. Science of the Total Environment, v. 761, 143197. https://doi.org/10.1016/j.scitotenv.2020.143197.

Oliveira-Júnior, A.A.; Tavares-Dias, M.; Marcon, J.L., 2009. Biochemical and hematological reference ranges for amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. Research in Veterinary Science, v. 86 (1), 146-151. https://doi.org/10.1016/j.rvsc.2008.05.015.

Prieto, A.I.; Jos, Á.; Pichardo, S.; Moreno, I.; Cameán, A.M., 2006. differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (*Oreochromis* sp.). Aquatic Toxicology, v. 77 (3), 314-321. https://doi.org/10.1016/J.AQUATOX.2005.12.012.

Ramsdorf, W.A.; Guimarães, F.S.F.; Ferraro, M.V.M.; Gabardo, J.; Trindade, E.S.; Cestari, M.M., 2009. Establishment of experimental conditions for preserving samples of fish blood for analysis with both comet assay and flow cytometry. Mutation Research, v. 673 (1), 78-81. https://doi.org/10.1016/j.mrgentox.2008.11.010.

Rodrigues, N.B.; Pitol, D.L.; Figueiredo, F.A.T.; Lima, A.C.T.C.; Henry, T.B.; Issa, J.P.M.; Umbuzeiro, G.A.; Pereira, B.F., 2022. Microcystin-LR at sublethal concentrations induce rapid morphology of liver and muscle tissues in the fish species *Astyanax altiparanae* (Lambari). Toxicon, v. 211, 70-78. https://doi.org/10.1016/J.TOXICON.2022.03.006.

Sakuragui, M.M.; Paulino, M.G.; da Silva e Souza, N.E.; Tavares, D.; Terezan, A.P.; Pesenti, E.; Giani, A.; Fernandes, J.B.; Cestari, M.M.; Fernandes, M.N., 2019. crude extract of cyanobacterium *Radiocystis fernandoi* strain R28 induces anemia and oxidative stress in fish erythrocytes. Toxicon, v. 169, 18-24. https://doi.org/10.1016/j.toxicon.2019.08.002.

Sanchez, W.; Burgeot, T.; Porcher, J.M., 2013. A novel 'integrated biomarker response' calculation based on reference deviation concept. Environmental Science and Pollution Research, v. 20 (5), 2721-2725. https://doi.org/10.1007/s11356-012-1359-1.

Schmid, W., 1976. The micronucleus test for cytogenetic analysis. Chemical Mutagens, 31-53. https://doi.org/10.1007/978-1-4684-0892-8_2.

Sedlak, J.; Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical Biochemistry, v. 25, 192-205. https://doi.org/10.1016/0003-2697(68)90092-4.

Shahmohamadloo, R.S.; Ortiz Almirall, X.; Simmons, D.B.D.; Poirier, D.G.; Bhavsar, S.P.; Sibley, P.K., 2022. Fish tissue accumulation and proteomic response to microcystins is species-dependent. Chemosphere, v. 287, 132028. https://doi.org/10.1016/J.CHEMOSPHERE.2021.132028.

Silva, T.T.; Jacinavicius, F.R.; Pinto, E.; Borrely, S.I., 2023. Intracellular microcystins degradation and acute toxicity decrease towards daphnia similis by low electron-beam irradiation doses. Algal Research, v. 72, 103086. https://doi.org/10.1016/j.algal.2023.103086.

Silva-Stenico, M.E.; Cantúsio Neto, R.; Alves, I.R.; Moraes, L.A.B.; Shishido, T.K.; Fiore, M.F., 2009. Hepatotoxin microcystin-LR extraction optimization. Journal of the Brazilian Chemical Society, v. 20 (3), 535-542. https://doi.org/10.1590/S0103-50532009000300019.

Somdee, T.; Thathong, B.; Somdee, A., 2016. The removal of cyanobacterial hepatotoxin [dha7] microcystin-lr via bioaccumulation in water lettuce (*Pistia stratiotes* L.). Bulletin of Environmental Contamination and Toxicology, v. 96 (3), 388-394. https://doi.org/10.1007/s00128-015-1715-1.

Tavares-Dias, M.; Moraes, F.R., 2006. Hematological parameters for the *Brycon orbignyanus Valenciennes*, 1850 (Osteichthyes: Characidae) intensively bred. Hidrbiológica, v. 16 (3), 271-274.

Techer, D.; Fontaine, P.; Personne, A.; Viot, S.; Thomas, M., 2016. Allelopathic potential and ecotoxicity evaluation of gallic and nonanoic acids to prevent cyanobacterial growth in lentic systems: a preliminary mesocosm study. Science of The Total Environment, v. 547, 157-165. https://doi.org/10.1016/J.SCITOTENV.2015.12.164.

van der Oost, R.; Beyer, J.; Nico, P.E. Vermeulen. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, v. 13 (2), 57-149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Wang, S.; Zhang, X.; Wang, C.; Chen, N., 2023. Multivariable integrated risk assessment for cyanobacterial blooms in eutrophic lakes and its spatiotemporal characteristics. Water Research, v. 228, 119367. https://doi.org/10.1016/J.WATRES.2022.119367.

Wang, T.; Liu, H., 2023. Aquatic plant allelochemicals inhibit the growth of microalgae and cyanobacteria in aquatic environments. Environmental Science and Pollution Research, v. 30 (48), 105084-105098. https://doi.org/10.1007/S11356-023-29994-5.

World Health Organization (WHO), 2021. Guidelines for water quality. 4. ed. WHO, Geneva.

Zaidi, H.; Amrani, A.; Sedrati, F.; Maaref, H.; Leghrib, F.; Benamara, M.; Amara, H.; Wang, Z.; Nasri, H., 2021. Histological and chemical damage induced by microcystin-lr and microcystin-RR on land snail helix aspersa tissues after acute exposure. Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology, v. 245, 109031. https://doi.org/10.1016/j.cbpc.2021.109031.

Zamora-Barrios, C.A.; Nandini, S.; Sarma, S.S.S., 2019. Bioaccumulation of microcystins in seston, zooplankton and fish: a case study in Lake Zumpango, Mexico. Environmental Pollution, v. 249, 267-276. https://doi.org/10.1016/J.ENVPOL.2019.03.029.

Zhan, Q.; Teurlincx, S.; van Herpen, F.; Vasantha Raman, N.; Lürling, M.; Waajen, G.; de Senerpont Domis, L.N., 2022. Towards climate-robust water quality management: testing the efficacy of different eutrophication control measures during a heatwave in an urban canal. Science of The Total Environment, v. 828, 154421. https://doi.org/10.1016/J.SCITOTENV.2022.154421.

Zhang, Y.; Vo Duy, S.; Munoz, G.; Sauvé, S., 2022. Phytotoxic effects of microcystins, anatoxin-a and cylindrospermopsin to aquatic plants: a meta-analysis. Science of the Total Environment, v. 810, 152104. https://doi.org/10.1016/j.scitotenv.2021.152104.

Zhang, Z.; Fan, X.; Peijnenburg, W. J. G. M.; Zhang, M.; Sun, L.; Zhai, Y.; Yu, Q.; Wu, J.; Lu, T.; Qian, H., 2021. Alteration of dominant cyanobacteria in different bloom periods caused by abiotic factors and species interactions. Journal of Environmental Sciences (China), v. 99, 1-9. https://doi.org/10.1016/j.jes.2020.06.001.

Zhou, C.; Chen, H.; Zhao, H.; Wang, Q., 2021. Microcystin biosynthesis and toxic effects. Algal Research, v. 55, 102277. https://doi.org/10.1016/J.ALGAL.2021.102277.