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Intracellular microcystins degradation and acute toxicity decrease towards *Daphnia similis* by low electron-beam irradiation doses

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ABSTRACT

Ionizing radiation has attracted attention due to its ability to inhibit pathogens and microorganisms, with low doses reported as being able to degrade cyanotoxins and inhibit toxic cyanobacteria growth. In this context, the aim of the present study was to investigate how electron beam irradiation (EBI) applied at doses between 1 and 10 kGy affect intracellular and extracellular microcystin variants. Effects on *M. aeruginosa* culture recovery, as well as changes in physico-chemical cyanobacterial suspension parameters and water quality alterations were assessed through ecotoxicity assays. The findings indicate that doses over 2 kGy may are lethal to *M. aeruginosa* even at 48 h post-irradiation. Concerning physico-chemical parameters, exposure to 2–5 kGy doses decrease pH values and later stabilizing. At least 50 % of intracellular MC led to significant degradation of all variants at 3 kGy. No acute toxicity effects were observed in *Daphnia similis* exposed to sample supernatants. Statistically significant differences were observed when cladocerans were exposed to *M. aeruginosa* cells treated with EBI doses ranging from 3 to 10 kGy compared to non-treated cyanobacterial suspensions. These findings suggest that low EBI radiation doses are suitable for the control of toxic cyanobacteria in water treatment processes, providing a less toxic environment compared to non-treated solutions.

1. Introduction

New technologies are required to treat water for human consumption, as water quality declines are noted due to the proliferation of harmful cyanobacterial blooms (CHABs), caused by anthropogenic activities that directly interfere with aquatic ecosystems, altering their biological structures. Rapid phytoplankton growth is noted as a direct primary eutrophication stage and effect, with cyanobacterial species usually comprising the dominant taxonomic group [1,2].

In this regard, *Microcystis aeruginosa* is among the most studied and common strains detected in freshwater CHABs. This species is known to produce hepatotoxic cyanotoxins, such as microcystins (MC). Over 310 different MC have been described in an open database regarding secondary cyanobacteria metabolites [3]. This high number of congeners is explained by structural amino acid variations, such as the frequent substitution of L-amino acids at positions 2 and 4 or substitution of Mdha by dehydrobutyrine (Dhb) or serine at position 7, as well as a lack of amino acid methylation at positions 3 and/or 7 [4]. Toxicity levels vary according to molecular structure and organization [5–8]. In addition, the presence of D-Glu and an Adda moiety chain are associated to MC binding to and inhibition of protein phosphatases 1 (PP-1) and 2A (PP-2A) in liver cells [8–10]. At high levels, these toxins can lead to death in humans [11–13].

Despite conventional treatments being widely employed, mainly for drinking water treatment, additional technique is required for high amounts of cyanobacterial biomass [4,14]. Furthermore, the treatment itself depending on employed for the control of cyanotoxins and cyanobacterial blooms may lead to cell disruption and consequent toxin releases into the water, leading to secondary water body contamination and/or the formation of more toxic by-products [4,14]. To address such issues, advanced oxidation processes (AOP) are widely investigated as a promising technology for wastewater treatment. These processes are characterized by the production of hydroxyl radicals (HO•), highly non-selective oxidizing transient species [15–17]. The non-selective formation of HO• enables the mineralization of organic compounds, which, when complete, forms only carbon dioxide (CO2) and water. Fenton, photo-Fenton, UV/H2O2, ozonation, and ionizing radiation are the most noteworthy types of POA. The latter encompasses gamma-ray and

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electron beam irradiation (EBI).

EBI is known to inhibit pathogens and microorganisms and is widely employed in industrial services in the sterilization of medical devices and materials, as well as in food pasteurization and in the modification of polymeric materials [18–20]. This technology also displays the potential for the treatment of emerging pollutants and infectious wastes, as it generates highly energetic electrons from regular electricity. Furthermore, EBI is also capable of generating both oxidative ($HO^{\bullet}$) and reductive (H, e^{-}_{aq}) species that interact and degrade contaminants through water molecule radiolysis [15]. Although this process has led to interesting results regarding the degradation of microcystin-LR (MC-LR) and the inhibition of certain species found in CHABs [21–26], no studies concerning EBI efficiency in the degradation of other MC congeners are available.

In this context, this study aimed to assess the inhibition of a cyanobacterial culture, the degradation of five MC variants and the toxicity of *M. aeruginosa* cultures following EBI treatment. To the best of our knowledge, this is the first study to employ EBI from a linear accelerator to determine the efficiency of ionizing radiation on the degradation of both intracellular and extracellular MC congeners in *Microcystis aeruginosa* and evaluate toxicity responses towards the microcrustacean *Daphnia similis*. This study provides critical information on how EBI interacts with five different MC variants, contributing to drinking water treatment and cyanobacteria control techniques through ecotoxicology assays employing aquatic organisms.

2. Material and methods

2.1. Reagents

MC-RR (MW 1038.20, purity ≥90 %), [D-Asp3, *E*-Dhb7]MC-RR (MW 1024.17), MC-LR (MW 995.17), [D-Asp3]MC-LR (MW 981.1, purity ≥90 %), MC- LA (MW 910.0, purity ≥95 %), MC-LF (MW 986.2, purity ≥95 %), MC-YR (MW 1045.19), methanol (MeOH) (CAS 67-56-1, HPLC grade), and acetonitrile (ACN) (CAS 75-05-8, purity ≥99.9 %) were purchased from Sigma-Aldrich. All the reagents used to prepare the ASM-1 culture medium were of analytical grade. Distilled water was obtained using a water distiller system (Cristófoli®).

2.2. Cyanobacterial culture

Microcystis aeruginosa (CCIBt3454) were obtained from the School of Pharmaceutical Sciences (University of São Paulo), São Paulo, Brazil, belonging to the Culture Collection of the Brazil Institute of Botany (CCIBt). The strain was cultivated in 9 L glass flasks containing 6 L of the ASM-1 medium (pH 8.0) [27] at 24 ± 1 °C under a 14:10 h (L:D) photoperiod cycle and light intensity of 4000 ± 250 lx. All culture experiments were performed in the cyanobacterial exponential phase.

2.3. Monitoring culture and cell density

Cultures were monitored for 48 h through optical density assessments at 680 nm (OD_{680}) (Shimadzu®, Model UV-1800) followed by cell density estimations for each sample through *M. aeruginosa* cells counting. The cells were counted on a compound microscope in a Neubauer counting chamber after preservation in Lugol's iodine.

Eq. (1) presents the correlation between absorbance and the cell density (cell mL^{-1}) with $R^2 = 0.9727$ used to estimate the number of cyanobacteria cells in the suspension after e-beam treatment. The assays were carried out in triplicate.

$$y = 5E+07 \times OD_{680} - 945521 \quad (1)$$

2.4. Electron beam irradiation

Electron beam irradiations were performed at the Radiation Technology Centre from the Nuclear and Energy Research Institute (IPEN/CNEN, São Paulo - Brazil). A linear electron beam Dynamitron® accelerator was employed at 1.4 MeV energy, with a 25 mA beam current and 37.5 kW beam power at 0 (control), 1.0, 2.0, 3.0, 4.0, 5.0 and 10.0 kGy.

2.5. Microcystin extraction

Extracellular and intracellular MC were extracted from 10 mL aliquots from each treated culture and from the controls. The samples were centrifuged and the supernatant and pellets were separated, stored in different tubes and freeze-dried. Intracellular MC were extracted from the pellets using MeOH 70 % (300 μL) followed by probe sonication at 30 % amplitude for 3 min employing a Soni Omni Disruptor in an ice bath. Cell debris was removed by centrifugation at 7000 ×*g* for 10 min at 4 °C using an Eppendorf 5804R centrifuge. The supernatants were filtered through 0.22 μm polyvinylidene fluoride (PVDF) syringe filters (Analítica®, Brazil) and stored in vials at −20 °C prior to the LC/MS analysis. The same extraction procedure was performed on the freeze-dried supernatants.

2.6. QqQ- LC –MS/MS parameters

Microcystins were determined employing a 1260 Infinity chromatographic system coupled to a 6460 triple quadrupole mass spectrometer (HPLC-QqQ) (Agilent Technologies, Santa Clara, USA) with an electrospray ionization source (ESI) applied 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). Compound separation was performed using a Luna 3 μm C18 column (2) (150 × 2 mm × 3 μm) (Phenomenex, USA). The mobile phases consisted of A) water containing 0.1 % formic acid and B) acetonitrile. A total of 10 μL of each sample were injected and the chromatographic separations were performed at a 0.25 $mLmin^{-1}$ flow rate at 40 °C following a gradient ratio between phases A and B, with acetonitrile gradients ranging from 25 to 90 % (Table 1). The QqQ instrument was operated in full scan mode and in the selected reaction monitoring (SRM) mode, selecting specific *m/z* transitions. Single and double-charged ions were monitored in the positive ion mode. The retention times of the eluted peaks were compared to the MC standards (Table S1). The characteristic precursor ions in the SRM were set as *m/z* 519 (RR), 512 ([D-Asp3], RR), 995 (LR), 981 ([D-Asp3] LR), 910 (LA) and 1045 (YR). Characteristic fragments considered diagnostic MC ions, namely 135 and 213 *m/z* for Adda and Glu-Mdha, respectively, were also monitored. Calibration curves were employed for MC quantitation. Linearity was assessed employing standard solutions ranging from 0.5 μgL^{-1} to 4 μgL^{-1} for MC-RR, [D-Asp3]MC- RR, MC-LR, [D-Asp3]MC- LR, MC-LA, MC-LF and MC-YR. Data were processed using the Mass Hunter Qualitative Analysis Software and Mass Hunter Quantitative Analysis Software (Agilent Technologies, USA).

2.7. Daphnia similis cultivation and ecotoxicity assays

Daphnia similis were cultivated at the Biological and Environmental Testing Laboratory belonging to the Nuclear and Energy Research Institute (São Paulo, SP - Brazil). All the procedures of culture and ecotoxicological assays were conducted following the guidelines NBR 12713/16 of the Brazilian National Standards Organization [28]. The cladocerans were cultured in filtered natural freshwater collected in a watershed area of Ribeirão Piraí (SP), water hardness adjusted between

Table 1
Acetonitrile mobile phase gradient ratio employed in the QqQ- LC –MS/MS analysis.

Time (min)	0	10	12.5	13	19
B%	25	95	95	25	25

40 and 46 mg $CaCO_3$ L^{-1} and pH 7.0–7.6. The organisms were maintained under a photoperiod of 16:8 h (L:D) at 20 ± 1 °C. A density of 1 to 5×10^5 cells mL^{-1} of *Raphidocelis subcapitata* cells was added daily to the culture as feed and water was changed once a week. The culture was analyzed monthly by sensitivity tests, using potassium chloride (KCl) as a reference substance.

Two acute assays were performed with pre- and post- EBI-treated cyanobacterial cultures, one concerning cladoceran exposure to the entire cyanobacterial culture (*M. aeruginosa* cells + supernatant) and *D. similis* exposure to only the supernatant. Tests were performed using negative controls (natural freshwater, ASM-1 medium and irradiated ASM-1 medium) and five exposure concentrations at 2-fold serial dilutions and the sample dilutions were considered the entire solution and *M. aeruginosa* density calculated at 0 h after e-beam irradiation. In each experiment, newborns (6 < age < 24 h) were isolated from the culture and twenty organisms per concentration were distributed in four tubes, filled with 10 mL of the test solutions and incubated at 20 ± 1.0 °C in the dark. After 48 h of exposure, the number of immobile organisms was recorded, and the effective concentration (EC_{50}) was calculated using the Trimmed Spearman-Karber method. Dissolved oxygen, pH and conductivity were determined for each experiment at the beginning (0 h) of the assay and after 48 h of exposure. The assays were performed in triplicate (n = 60 newborns per concentration).

2.8. Statistical analyses

Data were statistically analyzed using the GraphPad software v7. The Shapiro-Wilk test was used to verify data normality. An analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test to compare the treated and control groups. All tests were performed at a 95 % significance ($p < 0.05$).

3. Results and discussion

Cyanobacteria suspensions were irradiated and maintained under optimal conditions to assess how EBI can affect *M. aeruginosa* culture growth. The cell density estimation for each sample is presented in Fig. 1. All cultures were photographed for visual investigations (Fig. S1).

Cell densities decreased from 8.72 % to 52.49 % immediately after irradiation at 3–10 kGy, comprising the range of doses presenting the best cyanobacterial suspension inhibition results for all investigated time frames. An exception was observed for the cell density of *M. aeruginosa* after exposure to 1 kGy, in which the density exceeded the initial concentration by 49 % after 48 h of irradiation (Fig. 1). These findings corroborate Folcik et al. [29], in which *M. aeruginosa* exposed to doses over 2 kGy presented no culture biomass recovery and cell death. The same authors noted that growth rates of cultures irradiated with 1 kGy were lower than those of untreated cultures, with no increase in chlorophyll absorbance up to 5 days of incubation following irradiation. In another study, Badri et al. [30] evaluated the resistance of *Arthrospira* sp. (PCC 8005), a free-floating filamentous cyanobacterium, to ionizing radiation, reporting that 3.2 or 5 kGy doses led to photosystem II quantum yield declines and decreased pigment, lipid, and secondary metabolite syntheses. Furthermore, gamma radiation induced the transcription of photo-sensing and signaling pathways, as well as thiol-based antioxidant systems. Because of this, the number of *M. aeruginosa* CCIBt3454 cells following radiation doses of over 2 kGy may be associated to the transcription of several genes coding for "chromophore" proteins, affecting both cyanobacterial and intracellular MC survival, as indicated below.

3.1. Physico-chemical property irradiation effects

Altered cyanobacteria suspension electrical conductivity, dissolved oxygen and pH values following EBI treatments were observed (Fig. 2). After 24 h, all suspensions irradiated with 3-10 kGy presented stable electrical conductivity values. Electrical conductivity increases were evidenced by the formation of radicals due to water radiolysis, MC degradation and cell release promoted by the applied electron beam [31]. The formation of oxidative and reductive radicals through water radiolysis under different quantum yields, as indicated in Eq.(2), is due to interactions with MC cells and toxins. Furthermore, the noted pH decrease is probably due to the generation of H_2O_2 and H_3O^+ ions [32].

$$H_2O \rightarrow [2.7]OH\cdot + [2.6]e^-_{aq} + [0.6]H\cdot + [2.6]H_3O^+ + [0.45]H_2 + [0.7]H_2O_2 \quad (2)$$

It is important to note that pH values were stable following irradiation from 3 to 10 kGy, ranging from 7.5 to 8.5, similar to the electrical conductivity behavior noted for the same doses. The stability of both pH and electrical conductivity may indicate that EBI induces a maximum

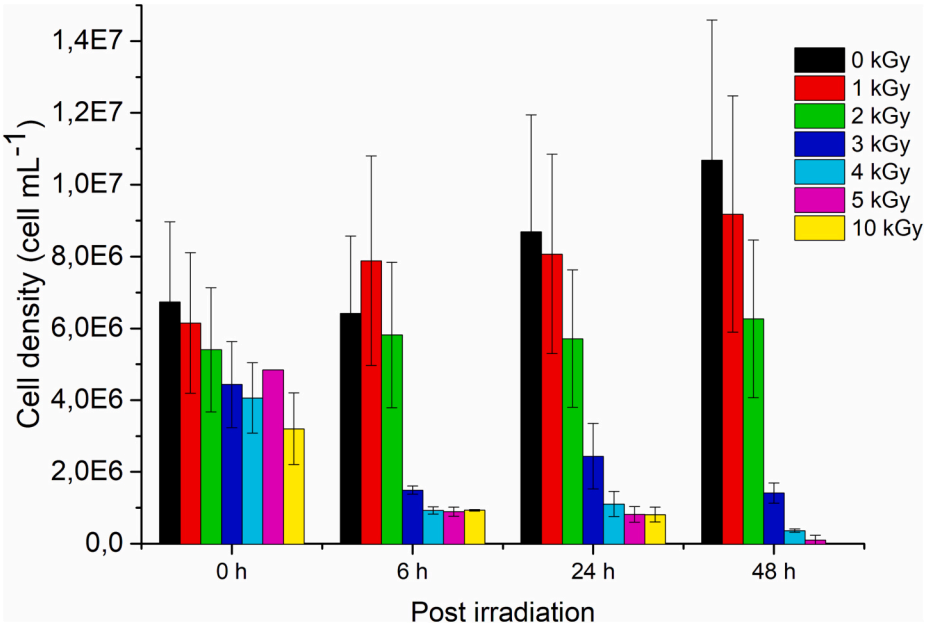

Fig. 1. *M. aeruginosa* culture cell densities (cell mL^{-1}) at 48 h post EBI irradiation. Results are expressed as means ± standard deviations.

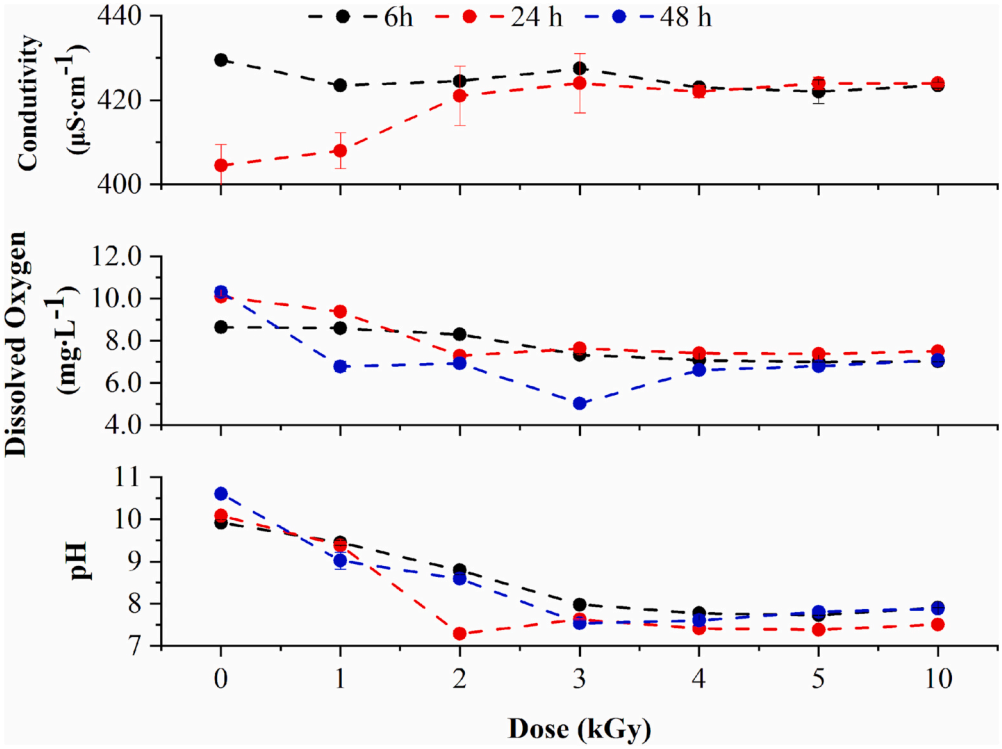

Fig. 2. Conductivity, dissolved oxygen and pH cyanobacterial culture alterations following exposure to different radiation doses (EBI). Results are expressed as means ± standard deviations.

effect on physico-chemical parameters in the tested solutions up to 2 kGy. These findings corroborate Liu et al. [31], who observed decreased pH values in an electron-beam-irradiated *M. aeruginosa* suspension and noted that cultures irradiated with doses between 2 and 5 kGy maintained their pH between 8.0 and 8.25 after one day, close to the values reported herein. The authors also emphasized that cultures irradiated with 1 kGy presented increased photosynthesis up to 7 days after the radiation process. These reports also corroborate our cyanobacterial suspension growth results.

The initial pH values observed herein and in other radiation studies indicate that this parameter is extremely important for water radiolysis efficiency and the generation of radicals that act in toxin degradation. The radicals formed through water radiolysis react with the dissolved oxygen present in biological samples, decreasing CO_2 concentrations and, consequently, pH values [31].

3.2. Effect on MC concentrations

M. aeruginosa (CCIBt 3454) exposure to 1 kGy significantly decreased total intracellular MC concentrations in 35.22 % compared to the controls. All doses of radiation used showed a significant difference compared to the control ($p < 0.0001$). When irradiated with doses between 2 and 10 kGy, total MC removal percentages increased from 47.73 to 93.95 %, respectively (Table 2).

Table 2
Total intracellular MC concentrations (µg L^{-1}) in cyanobacterial cells following different irradiation doses. Results are expressed as means ± standard deviations.

Dose (kGy)	Total intracellular MC (µg L^{-1})	*p*-value
0	6.442 ± 0.359	–
1	4.173 ± 0.178	<0.0001
2	3.367 ± 0.370	<0.0001
3	1.606 ± 0.244	<0.0001
4	1.297 ± 0.155	<0.0001
5	1.238 ± 0.222	<0.0001
10	0.390 ± 0.120	<0.0001

A total of five congeners are produced by *M. aeruginosa* (CCIBt3454) (Fig. S2). The descending concentration order of each identified congener was MC-RR > MC-YR > [D-Asp^3]MC-YR > [D-Asp^3]MC-RR > MC-1044 (Fig. 3). Exposure to 3 kGy led to significant degradation of all MC variants, reaching at least 50 %. MC-RR presented a mean concentration of 3.185 µg L^{-1}, corresponding to 49.44 % of the mean total MC concentration (6.442 µg L^{-1}). The second most abundant variant was MC-YR, at 17.37 %. It is possible that the *m/z* of 1031 corresponds to [D-Asp3]MC-YR [33]. The other congeners comprised about 33.19 % of total intracellular MC concentrations.

MC-RR was the predominant congener, even after irradiation, with a 35.93 % decrease in intracellular concentrations compared to the controls following exposure to 1 kGy. The [D-Asp^3]MC-RR variant was completely degraded only after exposure to the highest applied dose (10 kGy). MC-1031 ([D-Asp3]-MC-YR) was not observed from 3 kGy, indicating 100 % degradation to a final concentration below the limit of detection limit at this dose.

Normalized MC concentrations (C/C_0) with increasing irradiation doses allow for the plotting of $\ln(C/C_0)$ vs. the applied dose as a regression analysis, with A and B applied as constants (Table 3). The correlation coefficients values for two of the five variants were over 0.99, indicating that EBI induces the degradation of these MC variants following pseudo-first order kinetics under different initial concentrations.

Irrespective of the MC congener, the main hydroxyl radical attack takes place in the Adda chain, due to the presence of double bonds in this group [34–36].

Considering MC variations, previous studies have demonstrated that the amino acids in positions 2 and 4 influence the degradation process and, consequently, their ratio constant. Arginine is more reactive to •OH than leucine [37], with loss of the guanidine moiety during oxidation [38]. According to Kim & Lee [39], MC-YR degradation may indicate that the phenolic group in the tyrosine portion of MC is an important

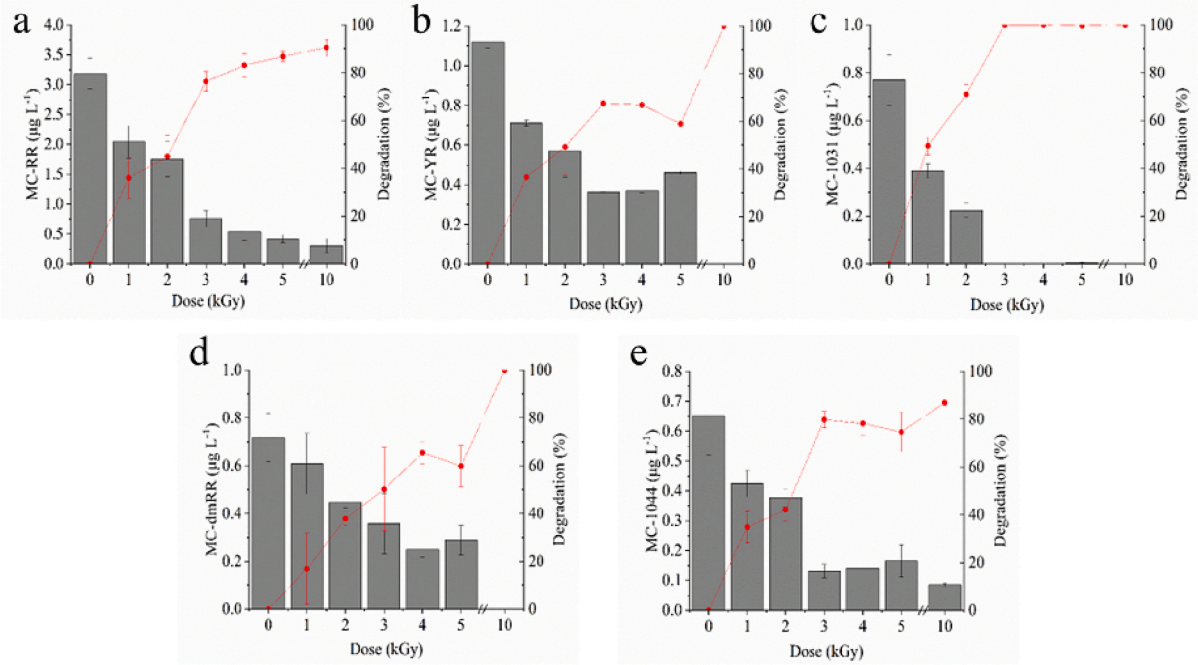

Fig. 3. Intracellular MC variant concentrations (µg L^{-1}) in cyanobacterial cells following exposure to different irradiation doses. All results are expressed as means ± standard deviation.

Table 3
Pseudo first-order equations and their correlation coefficients.

	lnC/C0 = lnA − B × Dose	R^2	Rate constant (kGy^{-1})
MC-RR	y = −0.241x − 0.3735	0.8019	0.2410
[D-Asp^3]MC-RR	y = −0.2715x + 0.0596	0.9915	0.2715
[D-Asp^3]MC-YR	y = −0.6181x − 0.0209	0.9966	0.6181
MC-YR	y = −0.1958x − 0.2191	0.7197	0.1958
MC-1044	y = −0.1951x − 0.3751	0.7497	0.1951

primary oxidation site. Our findings demonstrate the degradation rate constant order as MC-YR > [D-Asp^3]MC-RR > MC-RR > MC-1031 ([D-Asp3]-MC-YR) > MC-1044, corroborating Schneider & Bláha [37] who evaluated toxins diluted in aqueous solutions.

Unlike for intracellular MC, EBI did not alter mean total extracellular MC concentrations with increasing doses (0.654 and 0.583 µg L^{-1} for the controls and the 3 kGy dose, respectively). No significant differences were noted for any of the data when comparing the irradiated samples to the controls. Only two variants were identified and quantified, namely MC-RR and [D-Asp^3]MC-RR. MC-RR corresponded to about 50 % of the total extracellular MC for all suspensions, including the controls. It is also important to note that all the extracellular MC concentrations were below the safety limit recommended by Brazilian and World Health Organization guidelines for total MC in aquatic systems, of 1 µg L^{-1} [4,40].

3.3. Acute toxicity effect towards Daphnia similis

D. similis exposed for 48 h to cultures treated by EBI were categorized into two groups, one exposed to suspensions exposed to doses up to 2 kGy and one exposed to doses between 3 and 10 kGy. Concerning the first group, treatment with 1 and 2 kGy did not result in statistically significant differences compared to the controls, according to the previously reported culture growth results. The opposite, however, of statistically significant differences ($p < 0.001$), was observed for the second group. The best result was noted for the cyanobacterial suspension irradiated with 3 kGy, which reached an EC_{50} of 51.37 % (Fig. 4).

High cyanobacteria suspension toxicities to microcrustaceans are not

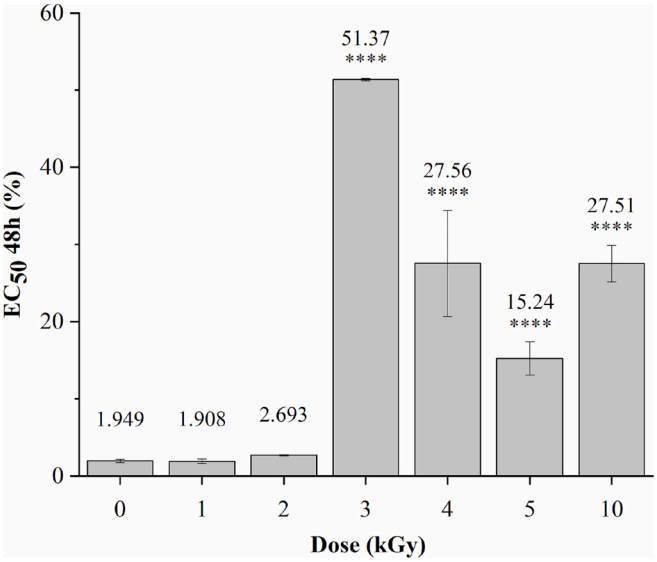

Fig. 4. *D. similis* responses to EBI-treated and control cyanobacteria suspensions (**** p value <0.001).

unusual. Genes related to digestive enzyme protease CT383, glutathione peroxidase, catalase, glutathione S-transferase, thioredoxin, and histone, for example, are deregulated by the presence of toxic *M. aeruginosa* cells, even at low concentrations, while the influence of other secondary metabolites, such as cyanopeptolin-A, aerucyclamides A and D, aeruginosins (602 and 684), cyanopeptolins (B and 963), and aerucyclamides (B and C), is also noted [41].

The cyanobacteria supernatants, on the other hand, exhibited no acute toxicity to *D. similis*, indicating that this cladoceran is less sensitive to toxins dissolved in aqueous solutions compared to cell. In fact, some authors have also reported the absence of acute responses for several *Daphnia* species exposed to crude extracts containing MC, depending on MC concentrations. *D. magna* survival, for example, was not affected

following exposure to a crude extract containing 5 μg L^{-1} of dissolved MC [42]. Similar findings were reported by Lürling and van der Grinten [43] for the same species following exposure to 3.5 μg L^{-1} of dissolved MC for 7 days. Both studies corroborate the findings reported herein, in which total MC concentrations did not affect *D. similis* survival.

The toxicity variation on the cyanobacteria suspension may be explained by the formation of different by-products formed during the irradiation process of each dose. Wang and Wang [44] reported that the toxicity on wastewater can change during the AOPs, with a decrease and/or increase the toxicity during the AOPs. The differences of response are related with the types of reactive species, structure of organic pollutants, the concentration of reactive species, used toxicity assessment method, experimental parameters, and residual oxidants.

For instance, different compounds may be formed during the ozonation of cyanobacterial culture [45]. The reason is that two pathways may occur, being the first pathway is the oxygen loss, which yields monohydroxylamine, oximes, and degradation products. The second pathway involves the alpha carbon (α- carbon) atom, which results in the formation of C═O compounds, such as aldehydes [46]. Besides, in addition, a slight increase in the BOD/COD ratio is observed when simulated mixed effluent is exposed to the electron beam up to a dose of 2 kGy, while the BOD/COD ratio has a slight reduction when the sample was exposed to doses of 5-80 kGy [47]. These results may explain the oscillation of toxicity in the *M. aeruginosa* suspension after EBI treatment and the lower toxicity shown when exposed to the 3 kGy dose.

4. Conclusions

The results reported in the present study demonstrate that *M. aeruginosa* in water samples is affected by EBI treatment, with low irradiation doses (2–5 kGy) being enough for the inhibition of cyanobacteria growth. Besides, the same low doses can be enough for the degradation of intracellular MC. After 1 kGy, intracellular MC-RR decreased 35.93 % compared to the controls, 15.17 % to [D- Asp 3] MC-RR, 49.37 % to MC-RR, 36.5 % to [D-Asp3]MC-YR, and 34.84 % to MC-1044. The rate constant for each MC congener was calculated considering the ionizing radiation degradation rates determined for each applied irradiation dose, indicating the specific order of [D-Asp^3] MC- YR > [D-Asp^3]MC-RR > MC-RR > MC-YR > MC-1044 (rate constants of 0.6181–0.1951 kGy^{-1} for [D-Asp^3]MC-YR and MC-1044, respectively). Concerning the ecotoxicology results, *D. similis* was more sensitive to cultures treated with doses up to 2 kGy, although no acute effects were observed following exposure to sample supernatants. These results reinforce the applicability of ionizing irradiation as a promising technology for cyanotoxin degradation and/or toxic cyanobacteria inhibition.

CRediT authorship contribution statement

Thalita Tieko Silva: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Fernanda Rios Jacinavicius:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Ernani Pinto:** Writing – review & editing, Funding acquisition. **Sueli Ivone Borrely:** Conceptualization, Validation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.103086.

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