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Ecotoxicological Evaluation of Dye Degradation and Photodegradation by Peracetic Acid with Sodium Carbonate

Leydiane Barbosa Bezerra [1], Thayrine Dias Carlos [2], Aline Silvestre Pereira Dornelas [3], Warlyton Silva Martins [3], Miréia Aparecida Bezerra Pereira [3], Aline Karla de Souza Nolberto [4], Gil Rodrigues dos Santos [3], Ildon Rodrigues do Nascimento [3], Rodrigo Ribeiro Fidelis [3], Nelson Luis Gonçalves Dias de Souza [4], Douglas Henrique Pereira [4], Renato Almeida Sarmento [3] and Grasiele Soares Cavallini [4,*]

1 Curso de Química Ambiental, Universidade Federal do Tocantins, Gurupi 77.402-970, Tocantins, Brazil
2 Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Amazônia Legal, Universidade Federal do Tocantins, Gurupi 77.402-970, Tocantins, Brazil
3 Programa de Pós-Graduação em Produção Vegetal, Universidade Federal do Tocantins, Gurupi 77.402-970, Tocantins, Brazil
4 Programa de Pós-Graduação em Química, Universidade Federal do Tocantins, Gurupi 77.402-970, Tocantins, Brazil
* Correspondence: grasiele@uft.edu.br

Abstract: Advanced oxidative processes (AOPs) are procedures used for the treatment of wastewater based on the generation of free radicals, such as hydroxyl (•OH) and carbonate anion ($CO_3^{\bullet -}$) radicals. However, although contaminants are degraded in these processes, the by-products generated in this transformation can be a greater source of toxicity than the original compound, making ecotoxicological tests essential for monitoring the efficacy of these treatment processes. In this study, we examined the ecotoxicity of AOP by-products generated using peracetic acid (PAA) and sodium carbonate, with and without solar radiation, for the degradation of methylene blue dye, using the planarian *Girardia tigrina* as a test organism. Ecotoxicological tests evaluated the acute toxicity of the generated by-product in terms of lethal concentrations (LC_{50}). Although in both assays the degradation of the dye was greater than 99%, higher toxicity was observed in the assay using PAA and carbonate in the absence of radiation. From the results obtained, we conclude that the by-product generated from the degradation of methylene blue dye by peracetic acid and sodium carbonate, with and without solar radiation, can pose risks to aquatic ecosystems if released directly into water bodies.

Keywords: photochemical; carbonate anion radical; planaria; by-product toxicity

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1. Introduction

Textile industries are among the main polluters of water bodies and the largest consumers of water. Raw materials and inputs, such as dyes and other chemical products, are used in several production stages. The sector's washing stage generates large volumes of wastewater, which, when inappropriately treated, causes damage to the environment [1]. The presence of these dyes in aquatic ecosystems, in addition to causing visual pollution, is also detrimental to aquatic biota and human health, as some classes of dyes can be carcinogenic and mutagenic, and concentrations of 1 mg/L can cause such damage [2].

Efficient alternatives for the treatment of industrial effluents are the so-called advanced oxidative processes (AOPs), which are primarily characterized by the changes they produce in the chemical structure of contaminants present in water and effluents [3]. The basic principle of AOP is the formation of free radicals, such as the hydroxyl (•OH) and hydroperoxyl (•OOH) radicals, based on chemical, electrochemical, or photochemical methods [4]. In the latter, •OH radicals are generated by a combination of strong oxidants, such as ozone (O_3)

and hydrogen peroxide (H_2O_2), in the presence of ultraviolet (UV) radiation or by catalysts, such as metal ions or semiconductors [5].

Recent studies of AOPs have also highlighted the formation of carbonate anion radicals ($CO_3^{\bullet -}$) in photochemical processes in the presence of carbonate anions. These radicals can be generated by the reaction between the carbonate (CO_3^{2-}) or bicarbonate (HCO_3^-) ion and the hydroxyl radical (•OH) and, similarly to •OH, $CO_3^{\bullet -}$ has a high organic contaminant degradation potential. The carbonate anion radical is nevertheless a more selective oxidant than •OH, as it reacts with electron-rich compounds such as phenols, amines, and sulfur-containing species, many of which are present in dyes such as methylene blue [6,7].

In the case of effluents that tend to be more resistant to degradation, a proportion of the organic matter may not undergo complete mineralization, and this partial oxidation can generate toxic by-products. In this context, evaluating the ecotoxicity of the generated by-products is of extreme importance for the safe release of effluents into water bodies. Such assessments are typically based on bioassays carried out under specific controlled experimental conditions and are used to estimate the toxicity of substances or by-products in industrial effluents discharged into water bodies [8]. Ecotoxicological assays for acute effects evaluate the impact of toxic agents on organisms over a short period using higher contaminant concentrations, with exposure times from 24 to 96 h, depending on the life span of the test organism [9].

While considering the generated effluent as a whole, ecotoxicological procedures enable valid assessments of the environmental toxicity of contaminants and contribute to determining the impacts on the ecosystem. Thus, the effects of both the residual concentrations of reagents used in the production process and the by-products generated by the chemical reactions among these reagents are evaluated. With respect to the degradation of dyes, this can readily be comprehended, given that the degradation of dyes is immediately visible in the discoloration of samples. However, as the toxic by-product generated can be colorless, this parameter is not the only condition necessary to ensure the efficiency of treatment. Consequently, the use of toxicity bioindicators has become an indispensable tool for more comprehensive assessments concerning the conservation of ecosystem diversity. Ecotoxicological tools must be considered because the observed biological responses are integrators of a complex mixture of compounds and components that exist in water bodies.

In this context, we sought in this study to evaluate the ecotoxicity of the by-products generated in the AOP degradation of methylene blue dye based on a combined treatment using peracetic acid (PAA), sodium carbonate, and solar radiation, which promotes the formation of hydroxyl and carbonate anion radicals. Evaluations were performed using bioassays, for which the planarian *G. tigrina*, a bioindicator of freshwater contaminants, served as a test organism. Planarians are aquatic organisms that can be found in puddles, streams, and springs, wherein they play important roles, serving as a food source for predatory invertebrates and vertebrates and, in turn, predating on small invertebrates, such as protozoa, rotifers, small crustaceans, snails, and insect larvae [10]. Consequently, these planarians are routinely used as bioindicators of environmental contamination in studies that evaluate the effects of the use of pesticides [9], disinfectants [10], and advanced oxidative processes for dye degradation [8,11].

2. Materials and Methods

2.1. Dye Degradation Assay

Reagents used: methylene blue (Neon), 15% peracetic acid (Thech Desinfecção, Brazil), and anhydrous sodium carbonate (Dinâmica).

The dye degradation process used in this study has been described previously by [11], and the degradation of methylene blue dye was evaluated using a solution containing 16 $\mu mol.L^{-1}$ methylene blue, 1.3 $mmol.L^{-1}$ PAA, and 3.5 $mmol.L^{-1}$ Na_2CO_3. It is worth mentioning that the commercial PAA solution used in the present study comprises 15% PAA, 23% hydrogen peroxide, and 16% of acetic acid. Thus, a solution of 1.3 $mmol.L^{-1}$ PAA also contains 4.4 $mmol.L^{-1}$ hydrogen peroxide and 1.7 $mmol.L^{-1}$ acetic acid.

Degradation of the dye was evaluated either in the presence or absence of solar radiation. In the former, samples were exposed to radiation for 2 h between 10:00 and 12:00, the daily period coinciding with the highest radiation intensity. In the assay without radiation, the dye was reacted with reagents for 24 h. As solar radiation increased the reaction rate, the test with radiation was performed after 2 h and the test without radiation was performed after 24 h, as established in a previous work [12]. Under these conditions, it is expected that both treatments will have similar residual dye concentrations, allowing the assessment of any differences in the formation of by-products in the processes.

For the assessments with solar radiation, the samples were placed in transparent PET bottles similar to the SODIS process (solar disinfection). Measurements were performed with a Mes-100 Instrutherm portable radiometer, which records radiation intensities within a wavelength range of 400 to 1000 nm (a band of solar radiation that reaches the Earth's surface in greater quantity). This monitoring is important, as the radiation received by the system varies according to weather conditions and seasons.

The residual concentrations of methylene blue, PAA, and sodium carbonate remaining on completion of the degradation process were evaluated separately. The concentration of methylene blue was determined spectrophotometrically in the visible region, with readings obtained at a wavelength of 664 nm [12]. Residual PAA was determined based on the reaction between PAA and the DPD reagent in a Chemetrics® Vacu vial ampoule and then quantified spectrophotometrically in the visible region at a wavelength of 515 nm [11]. The concentrations of carbonate and bicarbonate ions were determined by titration using 0.05 mol.L^{-1} sulfuric acid as the titrant [12].

2.2. Bioassays Using G. tigrina

A culture of the planarian species *G. tigrina* is maintained at the Laboratory of Ecotoxicology (Research Group in Functional and Applied Ecology) of the Federal University of Tocantins (UFT–Campus Gurupi). These organisms were housed in culture boxes containing 1.5 L of ASTM (American Society of Tests and Materials) medium (ASTM, 1980) [13], provided with constant aeration, in an acclimatized room at 22 ± 1°C and fed weekly with beef liver, with subsequent medium renewal.

2.2.1. Acute Test with *G. tigrina*

To perform the acute toxicity test, we used organisms measuring 8 ± 0.1 mm in length, which were deprived of food for 1 week prior to experimental exposure. To determine the lethal concentration required to kill 50% of the test organisms (LC_{50}), experimental solutions of synthetic effluent treated with and without radiation were prepared by diluting a stock solution in an ASTM medium, with the ASTM medium alone being used as a control.

The concentrations evaluated were diluted while considering the percentage of the dye solution following degradation, as shown in Table 1. The concentrations were established in preliminary tests until an adequate value was obtained for the refinement of the LC_{50}.

Table 1. Nominal concentrations (%) of dye solutions used in the evaluation of acute toxicity.

Concentration (%)	C_0/ASTM	C_1	C_2	C_3	C_4	C_5	C_6
No radiation	0%	2.5%	5%	7.5%	10%	12.5%	15%
With radiation	0%	1.5%	3.0%	4.5%	6.0%	7.5%	9.0%

The physicochemical parameters of the dye solutions, namely, pH, dissolved oxygen (DO), conductivity, temperature, and total dissolved solids (TDS), were measured to monitor the conditions under which the test organisms were exposed.

The bioassays were conducted in Petri dishes containing 20 mL of the respective dye solutions and five test organisms, with five replicates being assessed for each concentration. To determine the LC_{50} values, *G. tigrina* mortality was evaluated after 24, 48, and 96 h exposures in the dark within a static system at 22 ± 1 °C, during which time the planarians

were deprived of food. Planarians that showed either immobility when exposed to light or bodily degeneration (total or partial) were considered to have died. Because they are photosensitive, living planaria move in the presence of light.

Statistical analyses were performed using GraphPad Prism software version 7.0 for Windows (GraphPad Software, La Jolla, CA, USA).

2.2.2. Chronic Tests with *G. tigrina*

Planaria organisms of 8 ± 0.1 mm in length were selected and exposed for 8 days to each treatment (nominal concentrations) at four concentrations, where the most concentrated (C4) with 10% of the LC_{50} value; C3 = C4/1.4; C2 = C3/1.4; C1 = C2/1.4; and a control with ASTM medium [10]. The planaria were inserted into glass flasks with 100 mL of experimental solution. The exposure was conducted in a controlled environment at 22 ± 1 °C in a static system without light. After exposure of 12 organisms to each treatment (totaling 60), they were decapitated with a single cut behind the atria with a previously sterilized scalpel blade. After decapitation, the organisms were individually transferred to Petri dishes with 20 mL of ASTM medium. After 48 h, the formation of photoreceptors and auricles was verified with the help of a stereoscope microscope. The results of photoreceptor regeneration and atrial appearance were reported in terms of the mean time in hours.

The sub-lethal effects of *G. tigrina* were observed separately using the same protocols and evaluated by one-way analysis of variance (ANOVA), followed by multiple comparisons according to Dunnett's post hoc test, which allows for testing for significant differences between the treatments and the control (ASTM). These calculations were performed with GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla, California, USA), with the results expressed as the mean ± SEM.

3. Results

3.1. Dye Degradation

The results obtained for the dye degradation assays using PAA + Na_2CO_3, with or without radiation, are presented in Table 2, along with the residual concentrations of the reagents.

Table 2. Results of the dye degradation assay showing the concentrations of degraded dye and residual reagents.

	Initial Concentrations	**Concentrations after the Degradation Process**	
		No Radiation (24 h)	**With Radiation * (2 h)**
Methylene blue	16.0 μmol.L $^{-1}$	0.13 μmol.L^{-1}	0.06 μmol.L^{-1}
PAA	1.3 mmol.L^{-1}	0.017 mmol.L^{-1}	0.019 mmol.L^{-1}
Carbonate	3.5 mmol.L^{-1}	2.511 mmol.L^{-1}	2.229 mmol.L^{-1}
Bicarbonate	0	0.222 mmol.L^{-1}	0.220 mmol.L^{-1}
% dye removal		99.18%	99.62%

* Radiation dose 1870 Whm^{-2}.

3.2. Ecotoxicological Tests

3.2.1. Acute Test with *G. tigrina*

The LC_{50} values for *G. tigrina* exposed to PAA + Na_2CO_3 in the presence or absence of solar radiation for 96 h are presented in Table 3. It should be noted here that for planarians subjected to the control conditions, we detected no mortality or any morphological or behavioral changes at the end of the exposure period. However, at concentrations greater than the LC_{50} value, we detected bodily deformations and subsequent complete disintegration.

Table 3. Results of the acute toxicity assay using *G. tigrina*.

Test Type	Exposure (h)	LC_{50} (%) *	Minimum and Maximum (%)
No radiation	96	4.163	3.750–5.000
With radiation	96	6.434	6.091–6.789

* 95% CI.

The results presented in Table 3 show that an LC_{50} value of approximately 4.163% was obtained in treatments without radiation. Taking into consideration the residual values shown in Table 2, it can be estimated that the evaluated solution contains the following residual concentrations: 7.1 $\mu mol.L^{-1}$ PAA, 0.0054 $\mu mol.L^{-1}$ methylene blue, 5.5 $\mu mol.L^{-1}$ carbonate, and 63.7 $\mu mol.L^{-1}$ bicarbonate. Similarly, in the presence of radiation, we calculated an LC_{50} value of approximately 6.434%, with corresponding estimated residual values of 12.2 $\mu mol.L^{-1}$ PAA, 0.0038 $\mu mol.L^{-1}$ methylene blue, 8.5 $\mu mol.L^{-1}$ carbonate, and 90.0 $\mu mol.L^{-1}$ bicarbonate.

3.2.2. Chronic Tests with *G. tigrina*

Concentrations for the chronic assay were established after the acute assay. Thus, for the chronic tests, the concentrations used are shown in Table 4.

Table 4. Concentrations used in the chronic assay.

		Methylene Blue	PAA	Carbonate	Bicarbonate
Control	C0	0	0	0	0
No radiation	C1	0.19 $nmol.L^{-1}$	26 $\mu mol.L^{-1}$	0.20 $\mu mol.L^{-1}$	2.32 $\mu mol.L^{-1}$
	C2	0.27 $nmol.L^{-1}$	0.36 $\mu mol.L^{-1}$	0.28 $\mu mol.L^{-1}$	3.25 $\mu mol.L^{-1}$
	C3	0.38 $nmol.L^{-1}$	0.51 $\mu mol.L^{-1}$	0.39 $\mu mol.L^{-1}$	4.55 $\mu mol.L^{-1}$
	C4	0.54 $nmol.L^{-1}$	0.71 $\mu mol.L^{-1}$	0.55 $\mu mol.L^{-1}$	6.37 $\mu mol.L^{-1}$
With radiation	C1	0.14 $nmol.L^{-1}$	0.44 $\mu mol.L^{-1}$	0.31 $\mu mol.L^{-1}$	3.28 $\mu mol.L^{-1}$
	C2	0.19 $nmol.L^{-1}$	0.61 $\mu mol.L^{-1}$	0.43 $\mu mol.L^{-1}$	4.59 $\mu mol.L^{-1}$
	C3	0.27 $nmol.L^{-1}$	0.86 $\mu mol.L^{-1}$	0.61 $\mu mol.L^{-1}$	6.43 $\mu mol.L^{-1}$
	C4	0.38 $nmol.L^{-1}$	1.2 $\mu mol.L^{-1}$	0.85 $\mu mol.L^{-1}$	9.0 $\mu mol.L^{-1}$

In the *G. tigrina* photoreceptor regeneration test (Figure 1), only the test without radiation showed a significant effect on the concentrations evaluated, that is, there was a delay in the formation of photoreceptors from the C1 concentration (0.26 $\mu mol.L^{-1}$ PAA, 0.19 $nmol.L^{-1}$ methylene blue, 0.20 $\mu mol.L^{-1}$ carbonate, and 2.32 $\mu mol.L^{-1}$ bicarbonate). Concentrations that demonstrated a significant effect are marked with an asterisk.

In the *G. tigrina* auricle regeneration test (Figure 2), the delay in regeneration was observed in both assays and was observed from the C3 concentration, which, for the test without radiation, corresponds to the following concentrations: 0.51 $\mu mol.L^{-1}$ PAA, 0.38 $nmol.L^{-1}$ methylene blue, 0.39 $\mu mol.L^{-1}$ carbonate, and 4.55 $\mu mol.L^{-1}$ bicarbonate. For the test with radiation, these concentrations were: 0.86 $\mu mol.L^{-1}$ PAA, 0.27 $nmol.L^{-1}$ methylene blue, 0.61 $\mu mol.L^{-1}$ carbonate, and 6.43 $\mu mol.L^{-1}$ bicarbonate.

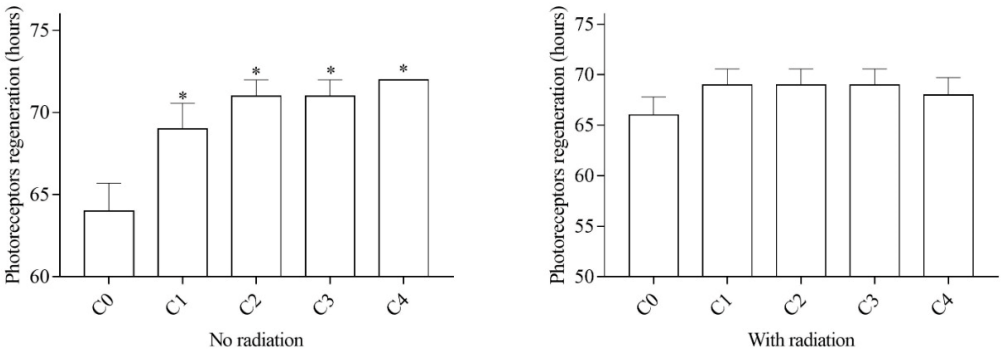

Figure 1. Chronic tests with *G. tigrina*: Photoreceptors regeneration (No radiation $F_{4,55} = 6.994$, $p < 0.05$; With radiation $F_{4,55} = 0.6275$, $p < 0.05$). * Each bar represents mean ± SD (n = 5 animals/group/replicates). Compared to the control, a significantly increased delay in the formation of photoreceptors was measured in the C1–C4 treated groups (Dunnett's test).

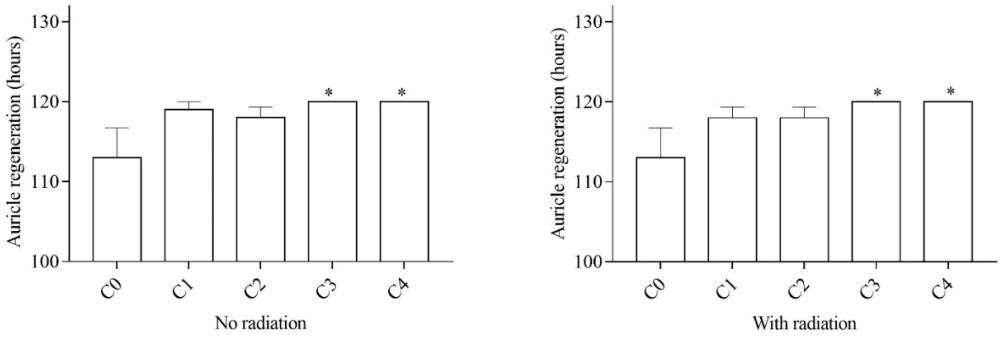

Figure 2. Chronic tests with *G. tigrina*: Auricle regeneration (No radiation $F_{4,55} = 2.513$ $p < 0.05$; With radiation $F_{4,55} = 2.313$, $p < 0.05$). * Each bar represents mean ± SD (n = 5 animals/group/replicates). Compared to the control, a significantly increased delay in the formation of photoreceptors was measured in the C1–C4 treated groups (Dunnett's test).

Figure 3 shows the regeneration of *G. tigrina* photoreceptors and auricles.

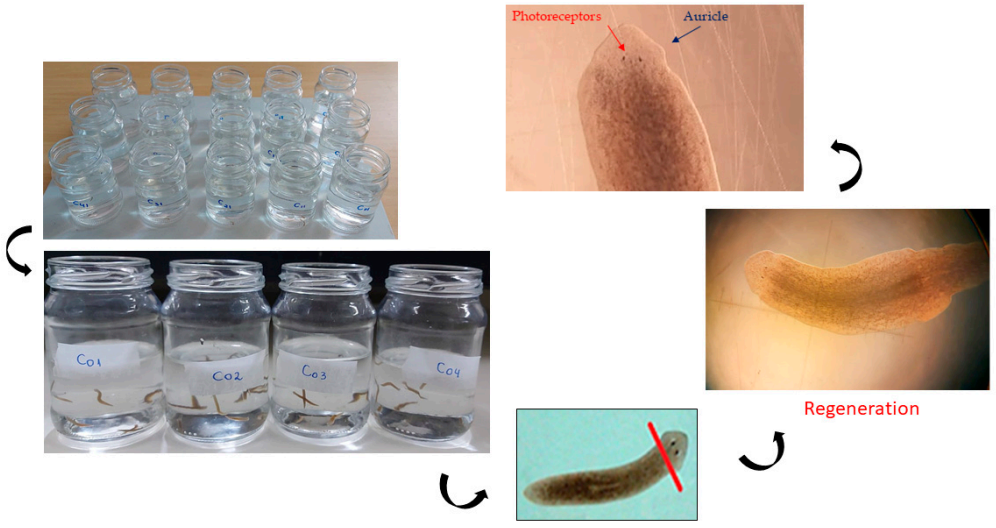

Figure 3. Photograph of the regeneration of photoreceptors and auricles of *G. tigrina*.

The physical-chemical parameters for monitoring the tests are shown in Table 5.

Table 5. Physicochemical parameters for monitoring ecotoxicological tests.

		Physicochemical Parameters				
		pH	**DO (mg.L^{-1})**	**Conductivity (µS.cm^{-2})**	**Temperature (°C)**	**TDS (mg.L^{-1})**
No radiation	0%	7.34	6.1	626	27.4	0.29
	2.5%	7.19	4.7	601	27.6	0.28
	5%	7.21	4.6	584	27.7	0.27
	7.5%	7.13	4.6	589	27.6	0.27
	10%	7.09	5.0	592	27.6	0.27
	12.5%	7.05	4.8	593	27.5	0.27
	15%	7.04	4.9	599	27.5	0.28
With radiation	0%	7.25	3.9	620	27.8	0.29
	1.5%	7.22	4.1	617	27.9	0.28
	3.0%	7.20	4.1	614	27.8	0.28
	4.5%	7.21	4.1	617	27.9	0.28
	6.0%	7.23	4.5	613	27.9	0.28
	7.5%	7.25	4.2	613	28.1	0.28
	9.0%	7.30	4.5	607	28.0	0.28

4. Discussion

The combination of PAA and sodium carbonate, with and without radiation, promoted degradations in the methylene blue dye greater than 99%, thereby corroborating the results obtained by [12], who were the first to propose this treatment. Moreover, we established that the residual concentrations of the reaction reagents were low and similar in both processes, even under different kinetic conditions.

It is important to emphasize here that in this ecotoxicological study, we evaluated the effects of different contact times between the dye and reagents, namely, 2 h for the assay with radiation and 24 h in the absence of radiation. However, as a consequence of photocatalysis, we obtained similar dye degradation efficiencies. This comparison is important in that it enabled us to evaluate the ecotoxicity of the by-products generated, with low interference from the residual concentrations of the reagents, given that high concentrations of AOP reagents indicate that the dosages used were inappropriate.

On the basis of the bioassay results obtained, we established that the process performed in the absence of radiation resulted in greater toxicity to the test organism, *G. tigrina*, than the process with radiation. With respect to the LC_{50}, we obtained a residual methylene blue concentration of 0.0054 µmol.L^{-1} in the process without radiation, which was higher than the residual concentration of 0.0038 µmol.L^{-1} obtained in the process with radiation. In this regard, [8], using the same test organism, calculated an LC_{50} value of 29.52 µmol.L^{-1} for methylene blue, indicating that a higher concentration of methylene blue was not the factor determining the greater toxicity associated with the non-radiation process. These same authors also observed that, compared with the non-degraded dye, degradation of the dye using the Fenton reagent gave rise to a higher toxicity, which may be attributed to the partial mineralization of the dye [14]. Nevertheless, despite an increase in toxicity, this does not invalidate the process but merely serves to highlight that AOP should not be the final treatment. Comparing the treatments, it was possible to perceive that the processes presented very similar toxicity, that is, the use of solar radiation did not increase the toxicity of the process.

Regarding the residual concentration of PAA, in the process with radiation, we obtained the highest LC_{50} value at a residual concentration of 7.1 µmol.L^{-1}, whereas the corresponding concentration in the non-radiation process was 12.2 µmol.L^{-1}. Previously, ref. [10] performed similar toxicity tests by exposing *G. tigrina* to PAA and obtained an

LC_{50} value of 3.16 mg·L^{-1} (41.57 µmol.L^{-1}), thus indicating that the effect of PAA alone would not be sufficient to reach the LC_{50}.

Given that the concentrations of bicarbonate and carbonate in the reaction mixtures used in the present study are lower than those detected in natural waters, we must assume that the increased toxicity of degraded dye is due to the generation of the reaction's by-products. One possibility in this regard is the formation of aromatic amines, such as phenylamines, which have been established to be highly toxic and have indeed been detected as the by-products of methylene blue degradation using AOPs [15]. Furthermore, processes without radiation are considered more conducive to the generation of toxic by-products, which can be attributed to the fact that these processes are less effective in generating radicals.

Regarding the chronic effects, the photoreceptor regeneration process of *G. tigrina* interfered in the treatment without radiation, while no delay in photoreceptor regeneration was observed for the radiation treatment. Auricle regeneration was affected by both treatments. The reactive oxygen species (ROS) can affect neuroregeneration [16], these chemical species can be formed by the decomposition of PAA or by the reaction between PAA and carbonate ions. Photoreceptors are used by planarians to detect prey and predators, so the delay in regeneration implies an elevated threat to their survival [10].

A further consideration regarding the interactions between dyes and treatment reagents is the alteration of physicochemical conditions (pH, DO, temperature, conductivity, and TDS) that enhance ecotoxicity. However, given that the reaction samples assessed in the present study were relatively highly diluted, we did not detect any significant alterations in these parameters during bioassays.

5. Conclusions

On the basis of our findings in this study, it can be concluded that by-products generated from the degradation of methylene blue dye using peracetic acid and sodium carbonate, in the presence or absence of solar radiation, can pose risks to aquatic ecosystems if the effluents thus treated are released directly into water bodies. Accordingly, it is important that effluents undergo supplementary treatments subsequent to the AOP prior to environmental release.

Although, in addition to the speed of reactions, photodegradation is conducive to generating fewer toxic effluents, in common with the processes performed in the absence of radiation, it should not be considered a final treatment process.

In general, the ecotoxicological monitoring of effluents treated using AOPs should be a mandatory step to ensure the safety of aquatic ecosystems, as they are essential for effectively evaluating the toxicity of generated by-products. Given that AOPs are recommended for degrading substances that are generally relatively resistant to degradation, the likelihood of by-product formation must be taken into consideration, even in the presence of strongly oxidizing radicals.

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