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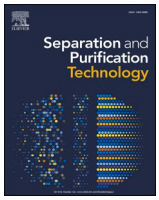

Electron beam irradiation applied for the detoxification and degradation of single ciprofloxacin aqueous solution and multiclass pharmaceutical quaternary mixture

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ABSTRACT

The application of electron beam irradiation for detoxification and degradation of single antibiotic ciprofloxacin (CPF) and in a mixture with multiclass pharmaceuticals in aqueous solutions was carried out. Ecotoxicity assays indicated that the green algae were most sensitive to antibiotic and also that the presence of several pharmaceutical increased the toxicity. After the irradiation treatment, degradation results of single antibiotic indicated reduction of 95.86 % at 1.0 kGy. Total organic carbon decreased up to 38 % at 5.0 kGy. At lower doses (1.0 kGy), no effect in toxicity was evidenced, however, increase in toxicity for *Vibrio fischeri* was observed after 2.5 kGy. For *Daphnia similis* exposure, an increase in toxicity was noted for all applied doses. In contrast, for the green algae *R. subcapitata* toxicity reduction varied from 62.3 to 81.9 % at the evaluated doses. Toxicity assays to microbes *E. coli* and *S. aureus* reduced antibacterial activity of CPF after irradiation treatment. Regarding the irradiated quaternary mixture at 2.5 kGy, reduction up to 96 % was achieved for the ciprofloxacin, metformin and acetylsalicylic acid, and 81 % removal was achieved for fluoxetine. Acute assays with *V. fischeri* indicated no increase in toxicity, while some increase was noted for *D. similis* (acute effects). Nevertheless, chronic assays data indicated low toxicity reduction (14 %) with *D. similis*, and complete detoxification was shown for the green algae after the irradiation. In addition, decrease in antimicrobial activity was noted after the treatment. Furthermore, the *in-silico* model was not enough accurate for the prediction of CIP toxicity. These findings showed that electron beam irradiation can be applied for reducing the impacts of antibiotics in aquatic ecosystem. Measuring toxicity on living-organism from different trophic levels are useful tools to evaluate the interaction of mixtures and also to assess toxicity of the generated byproducts.

1. Introduction

Antibiotics contamination on aquatic ecosystems have been regarded as a growing environmental issue of global concern due to massive use in humans and veterinary medicines. The persistence in the environment and the increasing occurrence of antibiotic-resistant pathogens, even superbugs, present a potential risk to the ecological environment and human health [1,2]. Antibiotic contamination may aid the development and spread of antibiotic resistance [3], meanwhile it possess the potential of affecting organisms on aquatic ecosystems, especially for microbial ecosystem services [4–6].

Ciprofloxacin is a second-generation fluoroquinolone with activity toward gram-negative and gram-positive bacteria [7], which has been frequently detected in several aquatic environments [2,8–13]. In addition, these contaminants do not occur individually but as complex mixtures. Some evidences indicated that mixtures are more toxic than the individual compounds and can impact the exposed biota [14]. Therefore, due to the increase in the contaminant's concentration and the complexity of urban and industrial effluents, the demand for more efficient technologies for water treatment becomes increasingly

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necessary.

Pharmaceuticals occur as complex mixtures, with evidences that mixtures are more toxic than the individual compounds and can impact the exposed biota [14]. Previous works have shown both antagonistic and synergistic features depending on the applied amounts and concentration of pharmaceuticals on mixture acute assays assessed with *Daphnia similis* [15].The authors also identified hormetic effects at low concentrations of a quaternary mixture assessed by chronic assays with daphnids, indicating that the effects of individual pharmaceuticals can underestimate the risk level of these contaminants in the environment. Fluoxetine is a selective serotonin uptake inhibitor, which is used as antidepressant and widely prescribed drugs used to treat depression and anxiety [16]. Acetylsalicylic acid has been used as analgesic, antipyretic and anti-inflammatory and is mostly consumed worldwide [17]. Metformin is extensively consumed due its medical prescription for the prevention of chronic diseases, such as type 2 diabetes mellitus [18].

Advanced oxidative processes (AOP) have been considered as an important technique for the improvement and removal of pharmaceuticals from wastewater. These processes have been commonly applied as an interesting alternative for the removal of hazardous pollutants, showing results in the mineralization of non-toxic aliphatic inorganic acids, carbon dioxide (CO_2) and water [19]. Several AOP have been evidenced great removal of CIP antibiotic (ciprofloxacin) such as photocatalyst [20], fenton [21], photo-fenton [20,22], electro-fenton [23].

Also considered an advanced oxidation process, electron beam irradiation (EBI) has been proved as green and effective technology for water and wastewater treatment, presenting the neutralization of biohazardous pollution almost instantly [24], and including the ability to convert non-biodegradable substances to more readily degradable ones [25] and proving to be efficient for removal of several contaminants [26–30]. Radiation based processing is more economical and effective on a large scale compared to other techniques used to remove persistent micropollutants [31]. This technology has been widely investigated in laboratory scale; however, recent studies have shown practical applications for long-term operation [32–34]. For instance, Wang et al., [32] reported the first full application of electron beam technology to treat dyeing wastewater with capacity of 30,000 m^3/d and operational cost of total wastewater treatment processes of about 2.0–2.5 ¥ m^{-3}. Wang and Wang [33] developed a novel combination of processes (electron beam irradiation and Fenton process) for the advanced treatment of dyeing wastewater with a cost of 1.5 ¥ per ton wastewater and the maximum treatment capacity of 5000 m^3 per day per electron accelerator. Hossain et al., [35] estimated a cost for the treatment by electron beam of 0.29 US$ m^{-3} for 10,000 m^3, and according to the authors the cost value decrease with increasing volume treated (0.041 US$ m^{-3} for the treatment of 200,000 m^3 day^{-1}).

In this study, electron beam irradiation was applied to remove toxicity and decompose the antibiotic ciprofloxacin in a single aqueous solution and also in quaternary mixture with other pharmaceuticals classess (antibiotic, antidepressant, anti-inflammatory and antidiabetic) diluted in natural water. The removal efficiency of the pharmaceuticals was quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS). The ecotoxicity was assessed by *Vibrio fischeri* bioluminescent bacteria, *Daphnia similis* microcrustacean and *Raphidocelis subcapitata* green algae. The antibacterial activity was characterized using gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*.

2. Experimental

2.1. Chemicals

Ciprofloxacin [$C_{17}H_{18}FN_3O_3$, MM = 331.34 g mol^{-1}; 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid; CAS 85721–33-1] was purchased from Sigma aldrich (>98 %). Fluoxetine hydrochloride [$C_{17}H_{18}F_3NO.HCl$; MM = 309.33 g mol^{-1}; methyl[(3S)-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl] amine]; CAS 54910–89-3] was obtained from Divis Pharmaceuticals Pvt. ltd. (98.8 %). Ciprofloxacin aqueous solutions were diluted using ultra-pure water (Millipore Milli-Q). The quaternary mixture was prepared by diluting chemicals in natural water, collected from Salto (Brazil) in order to simulate real conditions. Formic acid and acetonitrile, chromatographic grade, was purchased from Supelco/Millipore. Culture media such as Mueller Hinton was purchased from Sigma Aldrich.

2.2. Irradiation process

The liquid samples containing aqueous solutions of pharmaceuticals were irradiated in batches systems in a Dynamitron® Electron Accelerator: 37.5 kW power. The irradiator energy was fixed at 1.4 MeV, varying only the electric current. Low doses (1.0, 2.5 and 5.0 kGy) were applied to enable the operating cost and minimize energy consumption. The doses were confirmed with the Perspex dosimeter, Harwell Red, Batch KZ 4034. During irradiation, the samples were kept at room temperature, in a rectangular container (Pyrex®), with a volume of 246 mL, to ensure 4 mm of thickness, and get the maximum penetrability.

2.3. Analytical methods

UV–vis spectra was obtained using a Shimadzu UV Spectrophotometer (UV-1800). Total Organic Carbon (TOC) was analyzed on a Shimadzu equipment, TOC-L model, to determine mineralization after irradiation. Chromatography analysis was carried out using Agilent HPLC model 1290 coupled to Sciex QTrap model 3200. Separation conditions were: Restek Ultra Aqueous (150 × 2.1 mm × 3.0 μm) column, mobile phase (A) H_2O + 0.1 % formic acid, (B) ACN acetonitrile + 0.1 % formic acid, sample injection volume of 5.0 μL. MRM (multiple reaction monitoring) scan type was employed.

2.4. Toxicity assays

Toxicity assays were performed according to the protocols described in the literature. Acute toxicity assays with *Vibrio fischeri* bacteria followed the ABNT NBR 15411/2021 [36] , exposing the bacteria for 15 min. The results were expressed as toxicity factor (TF), which it is expressed by the value of the dilution factor that correspond to the highest concentration of the sample in which no inhibition>20 % was observed.

Acute toxicity assays with *Daphnia similis* were performed according to ABNT NBR 12713/2016 [37]. The young dafnids (6–24 h) were exposed to five concentrations. After 48 h, organisms' immobility was recorded. The toxicity results of irradiated samples were expressed in Toxicity Factor (which corresponds to the highest concentration of the sample in which no immobility >10 % was observed). Chronic toxicity assays with *Daphnia similis* were performed following the OECD guideline 211 (OECD, 2012) [38] with modification of exposure time to 14 days as described in previous works [39]. The young dafnids (6–24 h) were exposed to five concentrations. The organisms were daily fed with *Raphidocelis subcapitata* and the test medium was renewed every-two/three days. At the end of the experiment, the number of living offspring produced by each parent animal was recorded. The median effective concentration (EC50) values were determined using regression analysis, by applying a three-parameter-logistic-fit (sigmoidal logistic model).

Chronic toxicity assays with green algae *Raphidocelis subcapitata* were performed as described in previous works [40] adapted to 24-well microplates. Each well contained 2 mL of test solution, 25 μL of algal inoculum. The growth inhibition was quantified by absorbance at 450 nm after 96 h. The median inhibitory concentration (IC50) was determined by Hill model. All toxicity experiments were performed in duplicate.

After irradiation treatment, the toxicity results of chronic assays were expressed in toxicity units (TU = 100/E(I)C50). The significance of

any differences between average values for the control (non-irradiated samples) and the experimental treatments (irradiated samples) were evaluated by variance analysis (ANOVA) at a 5 % significance threshold level. When the ANOVA revealed significant differences among treatments, a post hoc Tukey test was carried out (at $p = 0.05$) to prove the existence of significant differences.

Toxicity was also determined by *in silico* methods for comparison. The toxicity parameters of ciprofloxacin and their intermediates were predicted by using the ECOSAR (version 2.0) models developed by the U.S. EPA for daphnids, fish and green algae ($LC50_{48h}$, $LC50_{96h}$ and $EC50_{96h}$, respectively).

2.5. Microbial susceptibility

Antimicrobial assays were performed to determine the sensitivity of gram-positive (*Staphylococcus aureus,* ATCC 6538) and gram-negative (*Escherichia coli,* ATCC 8739) bacteria to irradiated samples of ciprofloxacin, CIP.

The antimicrobial susceptibility was evaluated according to disk diffusion (M2) and Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically (M7) of the Clinical and Laboratory Standards Institute (CLSI) [41,42]. The bacteria were cultured aerobically in Mueller Hinton (MH) and incubated overnight at 37 °C in a shaking incubator. The microbial suspension in saline was adjusted to 10^8 UFC/mL and it was spread on the surface Mueller Hinton agar. The inoculated agar was allowed to dry for 15–30 min before the disks were applied. Then discs impregnated with 10 μL with test sample dilutions were later placed on the surface of the MH agar inoculated with the target microorganism. After 24 h incubation at 37 °C in an incubator, the diameters of the inhibition zones on the plate were determined.

Microdilution assays were also performed for the quaternary mixture to assess the antibacterial activity, in which the inhibited bacterial growth refers to antibacterial activity of the solutions tested. The bacteria were cultured aerobically in Mueller Hinton and incubated at 37 °C overnight in a shaking incubator. The cell density was set to 10^8 UFC/mL in Mueller Hinton. Then the inoculum was adjusted for a concentration of 5×10^5 CFU/mL per well. The bacteria suspensions were incubated at 37 °C at 180 rpm with different concentrations of the samples on a 96-well plate for 24 h. After incubation, resazurin was added to all wells, and further incubated for 1 h for the observation of color change. On completion of the incubation, columns with no color change (blue resazurin color remained unchanged) were scored as above the minimum inhibitory concentration (MIC) value, as described in [43].

3. Results and discussion

3.1. Effect of EBI on degradation and mineralization of single ciprofloxacin aqueous solution

The UV–vis spectra of non-irradiated and irradiated CIP solutions, under different radiation doses, are displayed in Fig. 1. Characteristic absorption band of the CIP were noted at 270, 322 and 334 nm in the non-irradiated samples [44]. After the electron beam irradiation, absorbance decrease was observed at all characteristic wavelengths of the antibiotic, indicating that EBI was effective for the removal of the antibiotic at low doses (1.0 kGy).

In addition, chromatograph analysis was performed to follow degradation of the antibiotic and reduction of 95.86 % was achieved at 1.0 kGy. By increasing the dose up to 2.5 kGy, concentration below the detection limit were noted for CIP (LOQ = 0.004 mg/L). In fact, several works have demonstrated that ionizing radiation has effective for the degradation of CIP antibiotics (doses up to 10 kGy) [45–49].

Electron beam irradiation is based on the water radiolysis, which involves the production of highly reactive radical species, such as the aqueous electron (e^-_{aq}), hydroxyl radicals (•OH) and hydrogen atoms (H•), which are able to interact and promote the oxidation, reduction,

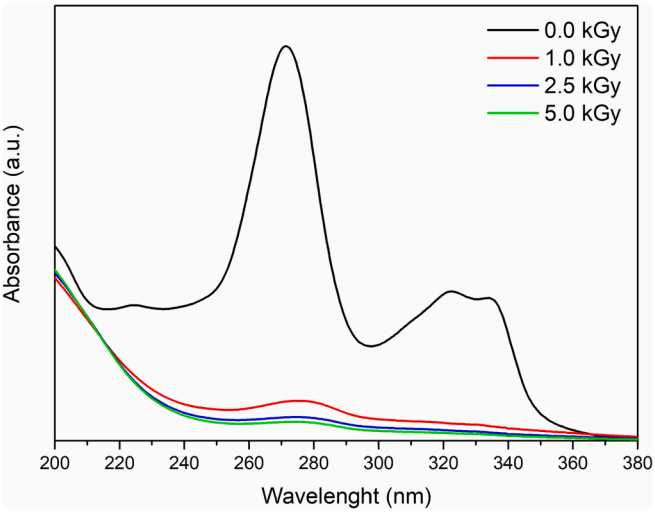


Fig. 1. UV–vis spectrum of the antibiotic ciprofloxacin irradiated at different doses. Initial concentration – 11.4 mg/L.

dissociation or degradation of various organic pollutants [50,51]. Through dose control, the degradation of organic contaminants can be controlled in order to obtain a partial decomposition (generating more biodegradable and smaller molecules) or complete decomposition (mineralization) [50,52].

The total organic carbon measurements, TOC, indicated that the EBI process did not reach complete pharmaceutical mineralization up to 5.0 kGy, leading to the formation of several intermediate byproducts. TOC reduction of 19.04 ± 1.91 %, 31.60 ± 0.74 % and 37.62 ± 0.29 % was achieved at 1.0; 2.5 and 5.0 kGy, respectively, demonstrating dependence among mineralization and absorbed dose (Fig. 2). Moreover, a decrease in pH of CIP solutions was verified with increasing dose. The CIP pH decreased from 7.00 ± 1.23 to 4.22 ± 0.03 at 5.0 kGy, corroborating with formation of organic acids and dissolved ions resulting from EB treatment. In fact, previous works have reported formation of several byproducts after ionizing radiation treatment [45,46,48]. It is worth noting that the disappearance of toxic organic pollutants is not correlated to their complete mineralization, as well as the toxicity reduction. Based on the physiochemical properties, degradation intermediate products might present lower, equal or even higher toxicity than original pollutants [53]. Therefore, toxicity assessment during the AOP is critical

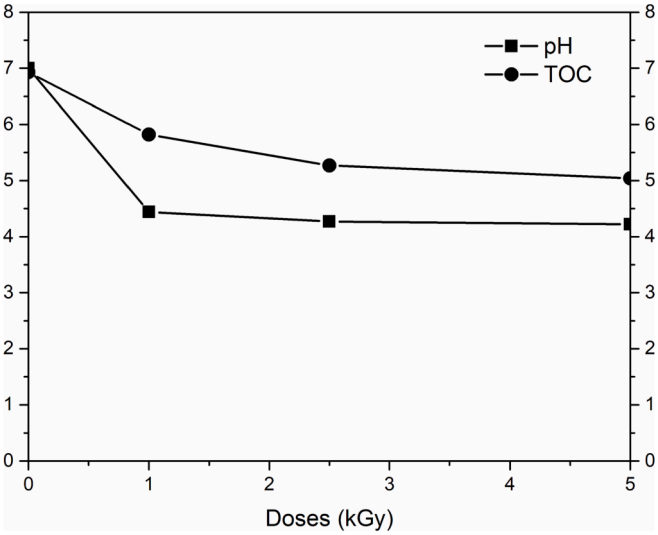


Fig. 2. TOC concentrations (circle) and pH (square) of CIP vs absorbed dose. Initial concentration of 11.4 mg/L, pH = 7.00 ± 1.23.

for accurately evaluating the performance of the studied process.

3.2. Effect of EBI on detoxification of single ciprofloxacin aqueous solution

Advanced oxidative process may be a viable technology for detoxifying a wide range of contaminants, which is one of the critical aspects of the research due to their potential risk to ecological environment and human being [54]. Toxicity changes after the treatment are still unclear since the intermediate products could exhibit equivalent or even higher toxicity compared with the original pollutants [53]. Therefore, it is necessary to evaluate the toxicity evolution during the process. After treatment of CIP antibiotic by ionizing radiation, the formation of several intermediate products has been reported [45,46,48], as shown in Table 1.

Initially, the intermediated products toxicity was predicted by ECOSAR, since it is impossible to investigate the toxicity of all the chemicals and it is hard to simulate the complex conditions in laboratory in some cases. The LC50 and EC50 were reported at Table 1 for fish, green algae and daphnid. Both formation of less toxic (P1, P2, P3, P5, P7 and P10) and more toxic (P4, P6, P8, P9 and P11) intermediates than the parent compound was identified depending on the dose range. Higher toxic intermediates (P4, P6, P8, P9 and P11) were identified at low doses (0.010. 2 kGy), suggesting more attention and suitable radiation conditions for pharmaceuticals detoxification.

Wang and Wang [53] reported that in general the toxicity change of wastewater can be affected by many factors such as: types of chemical reactive species, structure of organic pollutants, the concentration of reactive species, used toxicity assessment method, experimental parameters, residual oxidants, and heterogeneous catalysts. According to the authors, the differences on the wastewater toxicity could be caused by different organic pollutants, which present different physiochemical properties. Therefore, the toxicity of degradation intermediates could vary. Besides, the concentration of the intermediates products for causing toxicity can change along the process, so some organic pollutants or degradation products with low concentration may show higher toxicity than other organic pollutants with high concentration [53].

The toxicity results for ciprofloxacin obtained *in silico* models were also compared to experimental data. The obtained results indicated an IC50% of 27.4 ± 3.62 %, which correspond to $IC50_{96h}$ of 2.74 mg/L for green algae. Concerning for *D. similis,* Tominaga et al. [15] reported an $EC50_{48h}$ = 23.2 mg/L, while, Parente et al. [55] estimated $EC50_{15min}$ of 41 mg/L for *V. fischeri*, Therefore, Table 1 demonstrated that the obtained results were not accurate for predicting the toxicity of CIP, since lower values were reported ($EC50_{96h}$ 1.62 × 10^3 mg/L for algae, $LC50_{48h}$ 1.24 × 10^3 mg/L for daphnids and $LC50_{96h}$ 1.31 × 10^4 mg/L for fish).

In silico toxicity models are important for the assessment of toxicity data gaps and for prioritizing chemicals for further assessment. Although previous works have demonstrated accurate predictable (>92 %) for generic (Q)SAR (Quantitative) Structure Activity Relationship) [56], the obtained results indicated that there is still a need for improvement of these models. According to literature, there is still a challenge to produce accurate predictions across a wide variety of functional chemical classes [57], since not all endpoints are available for the chemical of interest and the predictive ability of QSAR for combined toxicity of mixed organic pollutants needs to be further improved [53]. Therefore, the experimental toxicity data were also assessed to investigate the toxicity of the generated intermediates.

The toxicity results of the non-treated and treated samples for *D. similis*, *V. fischeri* and *R. subcapitata* were determined in Fig. 3. Before irradiation, the low acute toxicity was noted for *D. similis* and *V. fischeri*, thus, the results were expressed in Toxic factor (TF). After EBI treatment, toxicity assays with *D. similis* indicated that the microcrustacean was the most sensitive for the generated byproducts after irradiation. Toxicity increase was noted at all doses, demonstrating an important increase in toxicity at 1.0 kGy (TF = 8), followed by a decrease at 2.5 and 5.0 kGy (TF = 4). Additionally, the obtained results corroborate with the *in-silico* data for daphnid that identified more toxic intermediates (*P*4, *P*6, P8, P9 and P11) at low doses (0.010. 2 kGy) (Table 1).

Toxicity assays with *Vibrio fischeri* bioluminescence also indicated an increase in toxicity after 2.5 kGy (TF = 2). Nevertheless, at low doses (1.0 kGy) no significant enhancement was noted (TF = 1). Lastly, toxicity assays with the green algae showed a great toxicity reduction at all evaluated doses. About 62.3, 72.8 and 81.9 % of toxicity reduction was achieved at 1.0, 2.5 and 5.0 kGy, respectively. Reduction improvement was noted with increasing dose. Statistical analysis indicated no significant differences between the irradiated samples, demonstrating that low doses are suitable for CIP treatment. In addition, although formation of more toxic intermediate products has been shown *in-silico* data for algae (P4, P6, P8, P9 and P11), decrease in toxicity of the experimental data have been noted.

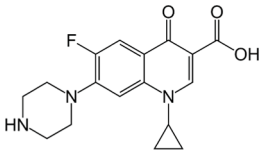
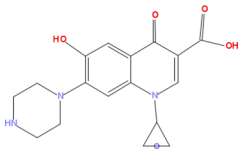
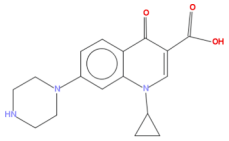
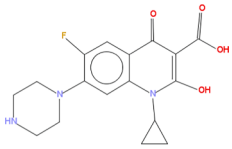
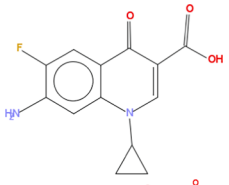
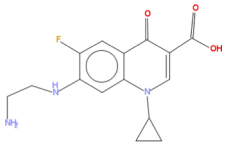
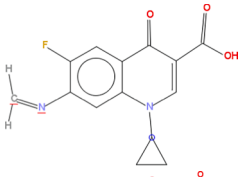
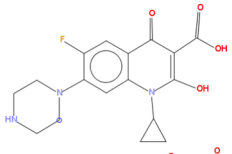
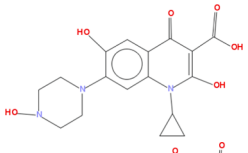
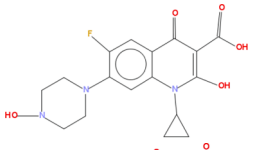
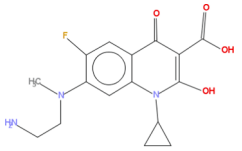
During the treatment by different AOP, three situations can be found: the decrease of toxicity; first increase and then decrease and the increase of toxicity during the AOPs [47]. The obtained results are consistent with the results of different AOPs. Tegze et al., [61] reported no toxic effects on the bioluminescence of *V. fischeri* at low doses (up 1 kGy) of CIP samples treated by gamma irradiation. However, the inhibitory effects increased by 2 kGy. Furthermore, according to Wang and Wang [53] toxicity analysis is complex, since it could be related to the evaluated toxicity method and organism-model. In fact, all the three situations were noted, and the toxicity varied according to the evaluated organism. It is worth noting that luminescent bacteria assays is one the most widely used acute toxicity assays due to its high sensibility and low cost; while water flea is an internationally recognized standard test organism, widely distributed in fresh water and a natural bait for fish, applied in both acute toxicity and chronic toxicity; and algae are the primary producers in natural water, which is the most basic link in material circulation and energy flow in aquatic ecosystem [53].

Additionally, during the irradiation, it may occur the formation of H_2O_2, especially in the presence of dissolved O_2, which may contribute to increased toxicity [58–61]. Illés et al., [61] evaluated the formation of hydrogen peroxide during the radiolysis of water from aqueous solutions containing several aromatic organic molecules, including ciprofloxacin. Hydrogen peroxide represents a negative parameter for biological assays [62], since the vast majority of aquatic organisms are highly sensitive to this compound. According to Sági et al., [63], the formation of hydrogen peroxide during ionizing radiation is often neglected, although it has a strong interference in routinely applied analytical methods. The authors evaluated the ecotoxicity of 0.1 mmol dm^{-3} solutions of sulfametazole treated by gamma radiation and verified the effects of H_2O_2 for different aquatic organisms (*V. fischeri*, *D. magna* and *R. subcapitata*). It was found that 0.5 mmol dm^{-3} H_2O_2 can cause notable effects on the mortality/inhibition of organisms, presenting ~ 96 % inhibition for algae ~ 72 % for bacteria and 100 % for mortality for *D. magna*.

Previous studies have reported toxicity removal of the treated samples after the removal of H_2O_2 [59,60,63]. Therefore, additional assays with the water flea were performed due to higher sensibility. The irradiated samples at 1.0 and 2.5 kGy were treated with catalase, as described in previous works [59,60]. About 20 % (TF = 2) mortality rates were observed for undiluted samples (100 % exposure), indicating remaining toxicity of the degradation products after the treatment process.

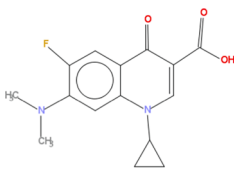
The antimicrobial activity of the untreated and treated antibiotic samples were also tested against the clinical isolates *E. coli* and *S. aureus* by using zone of inhibition assays. *E. coli* is a gram-negative bacteria, major constituent of humans and animals intestinal microbiota [64], and *S. aureus* is a gram-positive bacteria, which easily develops resistance to fluoroquinolones [65]. The results showed a strong zone of inhibition for untreated CIP for *E. coli*, while a small inhibition zone was noted for *S. aureus* (Fig. 4). After CIP E-beam treatment, for *E. coli*, the inhibition zone decreases at 1.0 kGy and disappear by 2.5 kGy, while

Table 1
Intermediate products of ciprofloxacin during ionizing radiation and ECOSAR prediction for toxicity of ciprofloxacin and transformation products.

Products				Toxicity (mg/L)		
	Chemical structure	Treatment (Doses)	Reference	Algae ($EC50_{96h}$)	Daphnid ($LC50_{48h}$)	Fish ($LC50_{96h}$)
[P0]		–	–	1.62×10^3	1.24×10^3	1.31×10^4
[P1]		Electron beam (1–10 kGy) Gamma irradiation (0.010–2 kGy)	[45,46,48]	4.88×10^3	3.19×10^3	3.65×10^4
[P2]		Electron beam (1 – 10 kGy)	[45]	2.12×10^3	1.55×10^3	1.68×10^4
[P3]		Electron beam (1 – 10 kGy) Gamma irradiation (0.010 – 2 kGy)	[45,46,48]	4.76×10^3	2.27×10^3	3.05×10^4
[P4]		Electron beam (1. 10 kGy) Gamma irradiation (0.010. 2 kGy)	[45,46,48]	128	42.8	899
[P5]		Gamma irradiation (0.145–0.870 kGy)	[48]	6.47×10^3	4.02×10^3	4.73×10^4
[P6]		Gamma irradiation (0.145–0.870 kGy)	[48]	45.1	24.6	21.0
[P7]		Gamma irradiation (0.145–0.870 kGy)	[48]	4.76×10^3	2.27×10^3	3.05×10^4
[P8]		Gamma irradiation (0.010–2 kGy)	[46]	152	119	1.25×10^3
[P9]		Gamma irradiation (0.010–2 kGy)	[46]	50.6	46.2	447
[P10]		Gamma irradiation (0.010–2 kGy)	[46]	8.16×10^3	3.59×10^3	5.03×10^4

(continued on next page)

Table 1 (*continued*)

Products				Toxicity (mg/L)		
	Chemical structure	Treatment (Doses)	Reference	Algae ($EC50_{96h}$)	Daphnid ($LC50_{48h}$)	Fish ($LC50_{96h}$)
[P11]		Gamma irradiation (0.010–2 kGy)	[46]	630	1.00×10^3	1.67×10^3

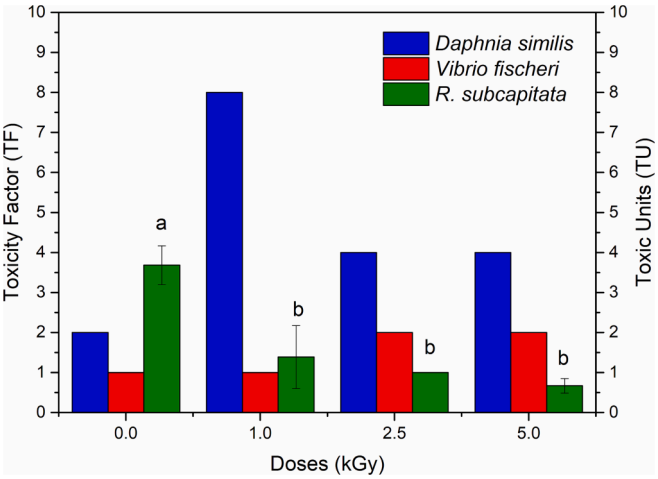


Fig. 3. Acute data (in toxic factor, TF) and chronic toxicity (in toxic units, TU = 100/EC50%) assessed for electron beam irradiated CIP at different doses using *D. similis*, *V. fischeri* and *R. subcapitata*. Initial conditions: $[CIP]_0 = 11.4$ mg/L, pH = 7.00 ± 1.23. Different letters (a–b) indicate significant differences (Tukey's test, $p < 0.05$).

S. aureus inhibition zone disappeared even at 1.0 kGy. These finding corroborates with previous works, which demonstrated the antibacterial activity of CIP solutions treated by gamma irradiation was eliminated for *S. aureus* for doses higher than 2 kGy [47].

3.3. Effect of EBI on a quaternary mixture (degradation, toxicity and antimicrobial activity)

The toxicity results of quaternary mixture containing ciprofloxacin, fluoxetine, metformin, and acetylsalicylic acid indicated an increase in toxicity for the green algae. Single CIP solutions presented a TU of 3.68 ($[CIP]_0 = 11.4$ mg/L), while the mixture showed a TU of 127.37 ($[CIP]_0 = 0.789$ mg/L). Low toxicity has been reported for metformin and acetylsalicylic acid, while high toxicity have been noted for fluoxetine for the green algae *R. subcapitata*. Kusk et al., [66] determined an $EC50_{48h}$ of 241.0 mg/L for acetylsalicylic acid, while Moermond and Smit [67] verified an $EC50_{72h} > 77.24$ mg/L for metformin and Minguez et al., [68] calculated and $IC50_{72h}$ de 202.9 μg/L for fluoxetine. The obtained results indicated an increase of toxicity of the mixture; however, further studies should be conducted to assess the synergetic effects of these contaminants.

Regarding the treatment, the acute toxicity results indicate that the bacterium and the water flea were less sensitive for the pharmaceutical (results expressed in TF), while the chronic assays indicated greater sensibility for the mixture exposed to green algae (data expressed in TU) (Fig. 5). It was possible to conclude that there was no increase in toxicity after treatment (*V. fischeri* data). Furthermore, additional chronic assays were performed for *D. similis*. Although acute data for *D. similis* showed an increase in toxicity, chronic assays indicated a low toxicity reduction (14 %) for the quaternary mixture, reinforcing the importance of chronic exposure for toxicity assessment. Besides, a complete toxicity removal was achieved for the *R. subcapitata* green algae, since no inhibition effect was noted for the treated sample.

For antibacterial activity of the untreated and treated mixture, both zone of inhibition assays and microdilution experiments were performed. The results of inhibition test showed no formation of zone of inhibition for both bacteria and samples. Moreover, microdilution experiments were assessed to determine the viability of untreated and irradiated samples at 2.5 kGy (Table 2). The results indicated that *E. coli* was most sensitive than *S. aureus* for the mixture, and also, the irradiation treatment was effective for reducing cytotoxicity, since reduction of the antibacterial activity was noted for the treated samples.

The obtained results proved that electron beam irradiation may be an effective technology for removing pharmaceuticals from an aquatic

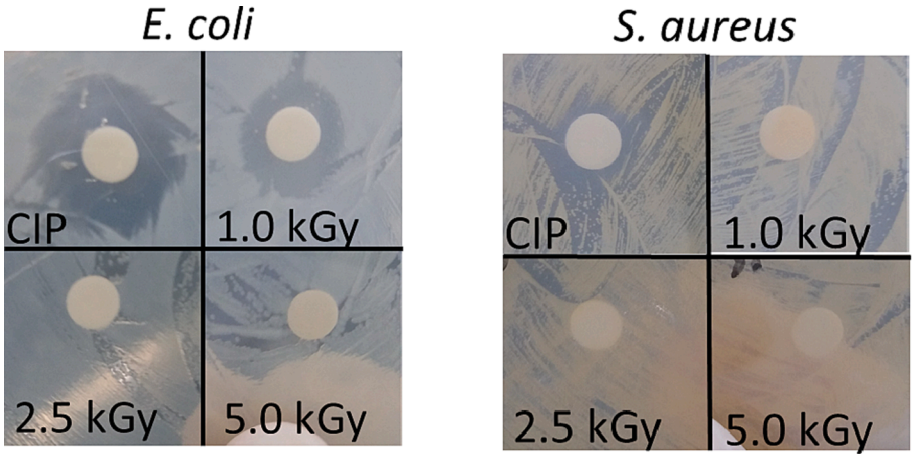


Fig. 4. Inhibition of microbial activity of untreated and 1.0 kGy, 2.5 kGy and 5.0 kGy irradiated samples of ciprofloxacin (CIP) for *E. coli* and *S. aureus*.

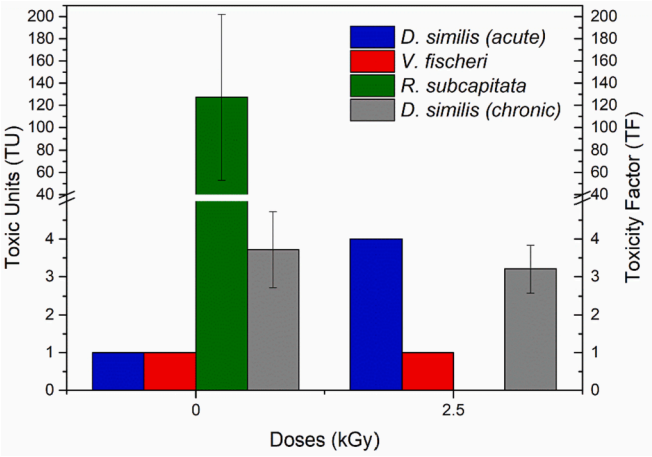


Fig. 5. Acute and chronic toxicity assessed for electron beam irradiated mixture of CIP, FXT, MET and ASA at 2.5 kGy assessed by different organisms. *V. fischeri* and *D. similis* acute data were reported in toxic factor (TF), while chronic results of *D. similis* and *R. subcapitata* were expressed in toxic units (TU = 100/EC50%). Initial conditions: $[CIP]_0 = 0.79$ mg/L, $[FXT]_0 = 1.12$ mg/L, $[MET]_0 = 1.50$, $[ASA]_0 = 0.77$ pH = 7.85 ± 0.99.

Table 2
Determination of the minimum inhibitory concentration (MIC, %) of the samples treated by Electron Beam Irradiation for *E. coli* and *S. aureus*. Initial conditions: $[CIP]_0 = 0.79$ mg/L, $[FXT]_0 = 1.12$ mg/L, $[MET]_0 = 1.50$, $[ASA]_0 = 0.77$ pH = 7.85 ± 0.99.

Bacteria	MIC (%) − 0.0 kGy	MIC (%) − 2.5 kGy
Escherichia coli	12.5	50
Staphylococcus aureus	25	> 50

ecosystem. EBI was effective in the reduction of the pharmaceutical in the quaternary mixture (reduction > 80 %). Degradation reduction of 96.32 %, 81.25, 97.93 % and 99.48 % was achieved for ciprofloxacin, fluoxetine, metformin, and acetylsalicylic acid, respectively.

Despite contaminants occur at complex mixture, the majority obtained data focus on single degradation of antibiotics [45,46,48]. Although there is an increase of studies focusing on mixtures [26–28,49,60], further studies are needed to comprehend the degradation mechanisms and detoxification at real and complex matrices. For instance, Guo et al., [46] evaluated the effect of organic additives on CIP removal by gamma irradiation (Humic acid, 2-propanol, and *tert*-butanol) and authors noted remarkable decrease of CIP removal in the presence of organic additives at low absorbed dose, demanding higher doses for the direct decomposition of CIP molecules, which suggests that there is a competitive relationship between CIP and other organic compounds in water during the irradiation. Reinholds et al., [49] reported decomposition yields of 91–93 % for CIP at wastewater samples after EB treatment up to 5 kGy, revealing the presence of relatively high residual antibiotics concentrations. Moreover, EBI can also be combining treatment technologies for wastewater treatment. Tegze et al., [47] showed that gamma irradiation of CIP samples led to conversion of non-biodegradable CIP to accessible compounds for metabolic process of microorganism. Therefore, further investigations are required to optimize aquatic environment effluent impacts.

4. Conclusions

Electron beam irradiation was effective for removing ciprofloxacin in aqueous solution, achieving 95.86 % reduction after 1.0 kGy, which also reduced antibacterial activity (*E. coli* and *S. aureus*). 62.3 % up to 81.9 % was the detoxification obtained for irradiated ciprofloxacin exposed to *R. subcapitata* green algae. In contrast, an increase on toxicity was obtained for *Vibrio fischeri*, and *D. similis* when exposed to ciprofloxacin irradiated solution. Similar data was noted when EB radiation was applied for pharmaceutical quaternary mixture. Reduction > 80 %, for the mixture of ciprofloxacin, fluoxetine, metformin, and acetylsalicylic acid, reduction of 96.32 %, 81.25, 97.93 % and 99.48 % was achieved for ciprofloxacin, fluoxetine, metformin, and acetylsalicylic acid, respectively. Acute and chronic effects were obtained for different living organisms which were exposed to pharmaceuticals, and it is imperative for the reduction of biological effects when developing advanced oxidative process as well as for helping environmental risk analysis for target compounds and considering distinct levels of organisms.

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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References

[1] K.K. Sodhi, M. Kumar, B. Balan, A.S. Dhaulaniya, P. Shree, N. Sharma, D.K. Singh, Perspectives on the antibiotic contamination, resistance, metabolomics, and systemic remediation, SN Appl. Sci. 3 (2021) 1–25, https://doi.org/10.1007/s42452-020-04003-3.
[2] J. Wang, L. Chu, L. Wojnárovits, E. Takács, Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: an overview, Sci. Total Environ. 744 (2020), 140997, https://doi.org/10.1016/j.scitotenv.2020.140997.
[3] A. Tello, B. Austin, T.C. Telfer, Selective pressure of antibiotic pollution on bacteria of importance to public health, Environ. Health Perspect. 120 (2012) 1100–1106, https://doi.org/10.1289/ehp.1104650.
[4] D. Kim, L.N. Nguyen, S. Oh, Ecological impact of the antibiotic ciprofloxacin on microbial community of aerobic activated sludge, Environ. Geochem. Health. 42 (2020) 1531–1541, https://doi.org/10.1007/s10653-019-00392-6.
[5] D.C. Stuckey, S. Oh, Effect of ciprofloxacin on methane production and anaerobic microbial community, Bioresour. Technol. 261 (2018) 240–248, https://doi.org/10.1016/j.biortech.2018.04.009.
[6] C. Girardi, J. Greve, M. Lamshöft, I. Fetzer, A. Miltner, A. Schäffer, M. Kästner, Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities, J. Hazard. Mater. 198 (2011) 22–30, https://doi.org/10.1016/j.jhazmat.2011.10.004.
[7] M.P. Chrysouli, C.N. Banti, N. Kourkoumelis, E.E. Moushi, A.J. Tasiopoulos, A. Douvalis, C. Papachristodoulou, A.G. Hatzidimitriou, T. Bakas, S.K. Hadjikakou, Ciprofloxacin conjugated to diphenyltin (IV): a novel formulation with enhanced antimicrobial activity, Dalt. Trans. 49 (2020) 11522–11535, https://doi.org/10.1039/D0DT01665A.
[8] M.A.F. Locatelli, F.F. Sodré, W.F. Jardim, Determination of antibiotics in Brazilian surface waters using liquid chromatography–electrospray tandem mass spectrometry, Arch. Environ. Contam. Toxicol. 60 (2011) 385–393, https://doi.org/10.1007/434 s00244-010-9550-1.
[9] R.L.N. de Jesus, D.C. Silva, R.A.S. Dias, L.K. Silva, M. dos Santos Diniz, J.C.F. Goiabeira, A. das M.P. Ferreira, Determination of Antibiotics in Water Samples from Anil River, in Northeast of Brazil, Mod. Environ. Sci. Eng. (2019) 9. Doi: 10.15341/mese(2333-2581)/01.05.2019/002.
[10] A.R. Mahmood, H.H. Al-Haideri, F.M. Hassan, Detection of antibiotics in drinking water treatment plants in Baghdad City, Iraq, Adv. Public Heal. 2019 (2019), https://doi.org/10.1155/2019/7851354.
[11] M. Papageorgiou, I. Zioris, T. Danis, D. Bikiaris, D. Lambropoulou, Comprehensive investigation of a wide range of pharmaceuticals and personal care products in urban and hospital wastewaters in Greece, Sci. Total Environ. (2019). 10.1016/j.scitotenv.2019.07.371. 10.1016/j.scitotenv.2019.07.371.
[12] P. Guerra, M. Kim, A. Shah, M. Alaee, S.A. Smyth, Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five

wastewater treatment processes, Sci. Total Environ. 473 (2014) 235–243, https://doi.org/10.1016/j.scitotenv.2013.12.008.
[13] A.M. Botero-Coy, D. Martínez-Pachón, C. Boix, R.J. Rincón, N. Castillo, L.P. Arias-Marín, L. Manrique-Losada, R. Torres-Palma, A. Moncayo-Lasso, F. Hernandez, An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater, Sci. Total Environ. 642 (2018) 842–853, https://doi.org/10.1016/j.scitotenv.2018.06.088.
[14] T. Backhaus, Environmental risk assessment of pharmaceutical mixtures: demands, gaps, and possible bridges, AAPS J. (2016), https://doi.org/10.1208/s12248-016-9907-0.
[15] F.K. Tominaga, N.F. Boiani, T.T. Silva, V.S.G. Garcia, S.I. Borrely, Acute and chronic ecotoxicological effects of pharmaceuticals and their mixtures in Daphnia similis, Chemosphere. (2022), https://doi.org/10.1016/j.chemosphere.2022.136671.
[16] I. Fulone, S. Barberato-Filho, M.F. Dos Santos, C. De Lima Rossi, G. Guyatt, L.C. Lopes, Essential psychiatric medicines: Wrong selection, high consumption and social problems, BMC Public Health. (2016). 10.1186/s12889-015-2589-1.
[17] M.R. Montinari, S. Minelli, R. De Caterina, The first 3500 years of aspirin history from its roots. A concise summary, Vascul. Pharmacol. (2019), https://doi.org/10.1016/j.vph.2018.10.008.
[18] E.P. Ambrosio-Albuquerque, L.F. Cusioli, R. Bergamasco, A.A. Sinópolis Gigliolli, L. Lupepsa, B.R. Paupitz, P.A. Barbieri, L.A. Borin-Carvalho, A.L. de Brito Portela-Castro, Metformin environmental exposure: a systematic review, Environ. Toxicol. Pharmacol. (2021), https://doi.org/10.1016/j.etap.2021.103588.
[19] A.O. Oluwole, E.O. Omotola, O.S. Olatunji, Pharmaceuticals and personal care products in water and wastewater: a review of treatment processes and use of photocatalyst immobilized on functionalized carbon in AOP degradation, BMC Chem. 14 (2020) 1–29, https://doi.org/10.1186/s13065-020-00714-1.
[20] A.S. Giri, A.K. Golder, Ciprofloxacin degradation in photo-Fenton and photo-catalytic processes: Degradation mechanisms and iron chelation, J. Environ. Sci. 80 (2019) 82–92. 1 464 0.1016/j.jes.2018.09.016.
[21] A. Gupta, A. Garg, Degradation of ciprofloxacin using Fenton's oxidation: Effect of operating parameters, identification of oxidized by-products and toxicity assessment, Chemosphere. 193 (2018) 1181–1188, https://doi.org/10.1016/j.chemosphere.2017.11.046.
[22] M. Bobu, A. Yediler, I. Siminiceanu, F. Zhang, S. Schulte-Hostede, Comparison of different advanced oxidation processes for the degradation of two fluoroquinolone antibiotics in aqueous solutions, J. Environ. Sci. Heal. Part A. 48 (2013) 251–262, https://doi.org/10.1080/10934529.2013.726805.
[23] E. Aseman-Bashiz, A. Rezaee, G. Moussavi, Ciprofloxacin removal from aqueous solutions using modified electrochemical Fenton processes with iron green catalysts, J. Mol. Liq. 324 (2021), 114694, https://doi.org/10.1016/j.molliq.2020.114694.
[24] A.V. Ponomarev, B.G. Ershov, The green method in water management: Electron beam treatment, Environ. Sci. Technol. 54 (2020) 5331–5344, https://doi.org/10.1021/acs.est.0c00545.
[25] S.I. Borrely, A.C. Cruz, N.L. Del Mastro, M.H.O. Sampa, E.S. Somessari, Radiation processing of sewage and sludge. A review, Prog. Nucl. Energy. 33 (1998) 3–21, https://doi.org/10.1016/S0149-1970(97)87287-3.
[26] N.F. Boiani, V.H.O. Silva, V.S.G. Garcia, S.V. Del Sole, S.I. Borrely, Electron beam irradiation of pharmaceuticals aiming at toxicity reduction: a binary mixture of fluoxetine and propranolol, Ecotoxicol. Environ. Contam. (2019), https://doi.org/10.5132/eec.2019.01.06.
[27] F.K. Tominaga, A.P. Dos Santos Batista, A.C. Silva Costa Teixeira, S.I. Borrely, Degradation of diclofenac by electron beam irradiaton: Toxicitiy removal, by-products identification and effect of another pharmaceutical compound, J. Environ. Chem. Eng. (2018). 10.1016/j.jece.2018.06.065.
[28] V.H.O. Silva, A.P. dos Santos Batista, A.C. Silva Costa Teixeira, S.I. Borrely, Degradation and acute toxicity removal of the antidepressant Fluoxetine (Prozac®) in aqueous systems by electron beam irradiation, Environ. Sci. Pollut. Res. (2016). 10.1007/s11356-016-6410-1.
[29] J. Wang, L. Chu, Irradiation treatment of pharmaceutical and personal care products (PPCPs) in water and wastewater: an overview, Radiat. Phys. Chem. 125 (2016) 56–64.
[30] J. Wang, R. Zhuan, L. Chu, The occurrence, distribution and degradation of antibiotics by ionizing radiation: an overview, Sci. Total Environ. 646 (2019) 1385–1397, https://doi.org/10.1016/j.radphyschem.2016.03.012.
[31] J. Rivera-Utrilla, M. Sánchez-Polo, M.Á. Ferro-García, G. Prados-Joya, R. Ocampo-Pérez, Pharmaceuticals as emerging contaminants and their removal from water, A review, Chemosphere. 93 (2013) 1268–1287, https://doi.org/10.1016/j.chemosphere.2013.07.059.
[32] S. Wang, J. Wang, C. Chen, S. He, J. Hu, Y. Zhang, First full-scale application of electron beam technology for treating dyeing wastewater (30,000 m^3/d) in China, Radiat. Phys. Chem. 196 (2022), 110136, https://doi.org/10.1016/j.radphyschem.2022.110136.
[33] S. Wang, J. Wang, Electron Beam Technology Coupled to Fenton Oxidation for Advanced Treatment of Dyeing Wastewater: from Laboratory to Full Application, ACS ES&T Water. 2 (2022) 852–862, https://doi.org/10.1021/acsestwater.2c00040.
[34] J. Wang, S. Wang, C. Chen, J. Hu, S. He, Y. Zhou, H. Zhu, X. Wang, D. Hu, J. Lin, Treatment of hospital wastewater by electron beam technology: Removal of COD, pathogenic bacteria and viruses, Chemosphere. 308 (2022), 136265, https://doi.org/10.1016/j.chemosphere.2022.136265.
[35] K. Hossain, Y.A. Maruthi, N.L. Das, K.P. Rawat, K.S.S. Sarma, Irradiation of wastewater with electron beam is a key to sustainable smart/green cities: a review, Appl. Water Sci. (2018), https://doi.org/10.1007/s13201-018-0645-6.
[36] ABNT, 2019. Ecotoxicologia aquática - Efeito inibitório sobre a bioluminescência de *Vibrio fischeri* - Parte 3: Método utilizando bactérias liofilizadas, ABNT NBR 15411-3, Assoc. Bras. Normas Técnicas. (2019).
[37] ABNT, 2016. Ecotoxicologia aquática — Toxicidade aguda — Método de ensaio com Daphnia spp (Crustacea, Cladocera), ABNT NBR 12713, Assoc. Bras. Normas Técnicas (2016).
[38] OECD, Test guideline No. 211: Daphnia magna reproduction test, Organ. Econ. Co-Operation Dev. (2012).
[39] F.I. Vacchi, P.C. Von der Ohe, A.F. de Albuquerque, J.A. de S. Vendemiatti, C.C.J. Azevedo, J.G. Honório, B.F. da Silva, M.V.B. Zanoni, T.B. Henry, A.J. Nogueira, G. de A. Umbuzeiro, Occurrence and risk assessment of an azo dye - The case of Disperse Red 1, Chemosphere. (2016). 10.1016/j.chemosphere.2016.04.121.
[40] I. Berrebaan, L. Montassir, B. Said, E. Mustapha, H. Bessi, Evaluation of ecotoxicity of ibuprofen and paracetamol on the freshwater green microalgae "*Pseudokirchneriella subcapitata*", Eng Technol J. 2 (2017) 303–309.
[41] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disk Suspectibility Tests, Approved Standard-Eleventh Edition. Clinical and LAboratory Standards Institue.
[42] Clinical and Laboratory Standards Institute, Clinical and Laboratory Standards Institute Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically Standard,, Approval CDM-A..
[43] M. Elshikh, S. Ahmed, S. Funston, P. Dunlop, M. McGaw, R. Marchant, I.M. Banat, Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants, Biotechnol. Lett. 38 (2016) 1015–1019, https://doi.org/10.1007/s10529-016-2079-2.
[44] M. Zupančič, I. Turel, P. Bukovec, A.J.P. White, D.J. Williams, Synthesis and Characterization of Two Novel Zinc (II) Complexes with Ciprofloxacin. Crystal Structure of [C17H19N3O3F]2·[ZnCl4]·2H2O, Croat. Chem. Acta. 74 (2001) 61–74.
[45] J.Y. Cho, B.Y. Chung, K.-B. Lee, G.-H. Lee, S.A. Hwang, Decomposition reaction of the veterinary antibiotic ciprofloxacin using electron ionizing energy, Chemosphere. 117 (2014) 158–163, https://doi.org/10.1016/j.chemosphere.2014.06.039.
[46] Z. Guo, S. Zhu, Y. Zhao, H. Cao, F. Liu, Radiolytic decomposition of ciprofloxacin using γ irradiation in aqueous solution, Environ. Sci. Pollut. Res. 22 (2015) 15772–15780, https://doi.org/10.1007/s11356-015-4715-0.
[47] A. Tegze, G. Sági, K. Kovács, R. Homlok, T. Tóth, C. Mohácsi-Farkas, L. Wojnárovits, E. Takács, Degradation of fluoroquinolone antibiotics during ionizing radiation treatment and assessment of antibacterial activity, toxicity and biodegradability of the products, Radiat. Phys. Chem. 147 (2018) 101–105, https://doi.org/10.1016/j.radphyschem.2018.02.015.
[48] M. Sayed, M. Ismail, S. Khan, S. Tabassum, H.M. Khan, Degradation of ciprofloxacin in water by advanced oxidation process: kinetics study, influencing parameters and degradation pathways, Environ. Technol. 37 (2016) 590–602, https://doi.org/10.1080/09593330.2015.1075597.
[49] I. Reinholds, I. Pugajeva, I. Perkons, E. Lundanes, J. Rusko, G. Kizane, V. Nikolajeva, O. Mutere, Z. Petrina, L. Baumane, V. Bartkevics, Decomposition of multi-class pharmaceutical residues in wastewater by exposure to ionising radiation, Int. J. Environ. Sci. Technol. (2017), https://doi.org/10.1007/s13762-017-1290-6.
[50] A.G. Capodaglio, High-energy oxidation process: an efficient alternative for wastewater organic contaminants removal, Clean Technol. Environ. Policy. (2017), https://doi.org/10.1007/s10098-017-1410-5.
[51] G. V Buxton, An overview of the radiation chemistry of liquids, 2008.
[52] A.G. Capodaglio, Contaminants of emerging concern removal by high-energy oxidation-reduction processes: State of the art, Appl. Sci. 9 (2019) 4562, https://doi.org/10.3390/app9214562.
[53] J. Wang, S. Wang, Toxicity changes of wastewater during various advanced oxidation processes treatment: An overview, J. Clean. Prod. 315 (2021), 128202, https://doi.org/10.1016/j.jclepro.2021.128202.
[54] D.S. Babu, V. Srivastava, P.V. Nidheesh, M.S. Kumar, Detoxification of water and wastewater by advanced oxidation processes, Sci. Total Environ. 696 (2019), 133961, https://doi.org/10.1016/j.scitotenv.2019.133961.
[55] C.E.T. Parente, J. Sierra, E. Martí, Ecotoxicity and biodegradability of oxytetracycline and ciprofloxacin on terrestrial and aquatic media, Orbital Electron. J. Chem. 10 (2018) 262–271. https://doi.org/10.17807/orbital.v10i4.1063.
[56] H. Sanderson, M. Thomsen, Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals. Assessment of the (Q) SAR predictability of pharmaceuticals acute aquatic toxici, Toxicol. Lett. 187 (2009) 84–93, https://doi.org/10.1016/j.toxlet.2009.02.003.
[57] F. Melnikov, J. Kostal, A. Voutchkova-Kostal, J.B. Zimmerman, P.T. Anastas, Assessment of predictive models for estimating the acute aquatic toxicity of organic chemicals, Green Chem. 18 (2016) 4432–4445, https://doi.org/10.1039/C6GC00720A.
[58] B.H.J. Bielski, D.E. Cabelli, R.L. Arudi, A.B. Ross, Reactivity of HO2/O−2 Radicals in Aqueous Solution, J. Phys. Chem. Ref. Data. (1985), https://doi.org/10.1063/1.555739.
[59] L. Szabó, T. Tóth, R. Homlok, G. Rácz, E. Takács, L. Wojnárovits, Hydroxyl radical induced degradation of salicylates in aerated aqueous solution, Radiat. Phys. Chem. (2014), https://doi.org/10.1016/j.radphyschem.2013.11.039.
[60] F.K. Tominaga, T.T. Silva, N.F. Boiani, J.M.S. de Jesus, A.C.S.C. Teixeira, S. I. Borrely, Is ionizing radiation effective in removing pharmaceuticals from wastewater? Environ. Sci. Pollut. Res. 28 (2021) 23975–23983, https://doi.org/10.1007/s11356-020-11718-8.

[61] E. Illés, A. Tegze, K. Kovács, G. Sági, Z. Papp, E. Takács, L. Wojnárovits, Hydrogen peroxide formation during radiolysis of aerated aqueous solutions of organic molecules, Radiat. Phys. Chem. (2017), https://doi.org/10.1016/j.radphyschem.2016.12.023.

[62] S.I. Borrely, A.A. Gonçalves, H. Oikawa, C.L. Duarte, F.R. Rocha, Electron beam accelerator for detoxification of effluents. When radiation processing can enhance the acute toxicity?, in: Radiat. Phys. Chem., 2004. 10.1016/j.radphyschem.2004.03.087.

[63] G. Sági, A. Bezsenyi, K. Kovács, S. Klátyik, B. Darvas, A. Székács, L. Wojnárovits, E. Takács, The impact of H_2O_2 and the role of mineralization in biodegradation or ecotoxicity assessment of advanced oxidation processes, Radiat. Phys. Chem. 144 (2018) 361–366, https://doi.org/10.1016/j.radphyschem.2017.09.023.

[64] G. Teophilo, R. dos Fernandes Vieira, D. dos Prazeres Rodrigues, F.R. Menezes, *Escherichia coli* isolated from seafood: toxicity and plasmid profiles, Int. Microbiol. 5 (2002) 11–14, https://doi.org/10.1007/s10123-002-0052-5.

[65] N.D. Gade, M.S. Qazi, Fluoroquinolone therapy in *Staphylococcus aureus* infections: where do we stand?, J. Lab. Physicians. 5 (2013) 109–112. DOI: 10.4103/0974-2727.119862.

[66] K.O. Kusk, A.M. Christensen, N. Nyholm, Algal growth inhibition test results of 425 organic chemical substances, Chemosphere. 204 (2018) 405–412, https://doi.org/10.1016/j.chemosphere.2018.04.047.

[67] C.T.A. Moermond, C.E. Smit, Derivation of water quality standards for carbamazepine, metoprolol, and metformin and comparison with monitoring data, Environ. Toxicol. Chem. (2016), https://doi.org/10.1002/etc.3178.

[68] L. Minguez, R. Bureau, M.-P. Halm-Lemeille, Joint effects of nine antidepressants on Raphidocelis subcapitata and Skeletonema marinoi: A matter of amine functional groups, Aquat. Toxicol. 196 (2018) 117–123, https://doi.org/10.1016/613 j.aquatox.2018.01.015.