Heliyon 10 (2024) e27266

Contents lists available at ScienceDirect

Heliyon

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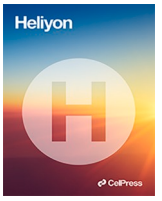

Research article

Effect of the anode material, applied current and reactor configuration on the atenolol toxicity during an electrooxidation process

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ARTICLE INFO

Keywords:
Atenolol
Electrochemical oxidation
Lactuca sativa
Sodium sulfate
Toxicity
Vibrio fischeri

ABSTRACT

Atenolol (ATL) is a beta-blocker pharmaceutical product which is excreted mainly unchanged and may represent a long-term risk for organisms present in the sea and in fresh water. Due to its low biodegradation rate, electrochemical advanced oxidation processes (EAOPs) can be used to remove this compound. In this work, ATL ecotoxicity was analyzed in the presence of sodium sulfate (Na_2SO_4), which is widely used as supporting electrolyte in EAOPs. Ecotoxicity values were expressed as the pollutant concentration that leads to a 50% inhibition of the root elongation of *Lactuca sativa* seeds in relation to the control (EC_{50}(5 days)). The obtained values for ATL showed an EC_{50}(5 days) of 1377 mg L^{-1} towards *Lactuca sativa*. When Na_2SO_4 was added, the toxicity of the sample increased but no synergy was detected between both compounds. With 2 g L^{-1} Na_2SO_4, ATL showed an EC_{50}(5 days) of 972 mg L^{-1}; and with 4 g L^{-1} Na_2SO_4 and higher concentrations, EC_{50} value for ATL was 0 mg L^{-1}. Statistical tools were used to obtain the zones of the [ATL]-[Na_2SO_4] plane which are toxic towards *Lactuca sativa*.

Solutions containing ATL and Na_2SO_4 were treated by electrooxidation. Two anode materials (a boron-doped diamond electrode and a microporous Sb-doped SnO_2 ceramic one); three operation currents (0.4, 0.6 and 1 A); and two reactor configurations (one-compartment reactor and two-compartment reactor separated by a cation exchange membrane) were used. *Lactuca sativa* seeds and *Vibrio fischeri* bacterium tests were employed to evaluate the toxicity of the solutions before and after applying the electrooxidation process. In all the tests, the ecotoxicity of the treated sample increased. This fact is owing to the persulfate presence in the solution due to the sulfate electrochemical oxidation. Nevertheless, none of the final samples were toxic towards *Vibrio fischeri* because ecotoxicity values were lower than 10 TU; and, in the case of the one-compartment reactor, practically all of them were also non-toxic towards *Lactuca sativa*. The toxicity of the treated samples increased when using the two-compartment reactor in the presence of the BDD anode, and when the operation current was increased. This is attributed to the highest formation of persulfates. Amongst all the tests performed in this work, the lowest toxicity value (i.e., 3 TU) together with the complete mineralization and degradation degrees was achieved with the two-compartment reactor using the BDD anode and operating at 0.6 A.

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https://doi.org/10.1016/j.heliyon.2024.e27266
Received 4 October 2023; Received in revised form 19 February 2024; Accepted 27 February 2024
Available online 28 February 2024

1. Introduction

Pharmaceutical products have been detected in the aquatic ecosystems as micropollutants, with concentrations values ranging from $ng{\cdot}L^{-1}$ to $\mu g{\cdot}L^{-1}$. These compounds can lead to potentially damaging environmental consequences, even in the $ng{\cdot}L^{-1}$ scale [1–4]. Among the pharmaceuticals more frequently detected in water are beta-blockers, which are usually used to treat human hypertension [5]. Atenolol, metoprolol, and propanolol are the most abundantly found antihypertensives in surface waters [6]. The presence of these therapeutic compounds in the aquatic environment can be attributed to the rise of the pharmaceutical industry; moreover, antihypertensives are among the most consumed pharmaceuticals worldwide [7].

β-Blockers may represent a long-term risk for organisms present in the sea and in fresh water. Concretely, atenolol is excreted mainly unchanged (>96%) [6] and its degradation is usually incomplete in conventional wastewater treatment plants, probably due to its low biodegradation rate [8]. Therefore, more advanced removal techniques are necessary to remove atenolol [9]. Amongst current tertiary and quaternary treatment technologies, advanced oxidation processes (AOPs) seem attractive methods to remove emerging contaminants from the aquatic ecosystems; specifically, electrochemical advanced oxidation processes (EAOPs), owing to their high effectiveness, easy handling due to the simpleness of the equipment and automation susceptibility, and low environmental impact since no chemical compounds are added and no secondary waste stream is produced [10,11]. During an EAOP, organic pollutants can be oxidized *via* two mechanisms, (i) direct oxidation, where the contaminant is oxidized directly by electron transfer from the anode surface, or (ii) indirect oxidation, where an oxidant species produced at the anode surface, such as hydroxyl radicals, mediates the electron transfer. The efficacy of both types of oxidation processes depends on the nature and concentration of the pollutant under study, the applied current, the temperature, the reaction time, the supporting electrolyte used and the pH of the solution [12,13]. A complete oxidation requires the transformation of the organic pollutant into simple oxidation by-products, which are further fragmented to harmless inorganic compounds such as CO_2 and H_2O (i.e., mineralization). However, sometimes the process can result in partial oxidation of the organic pollutant into by-products which are not mineralizable and may have harmful consequences for health [11]. For this reason, it is advisable to test water for toxicity after applying an EAOP. In fact, future investigation on EAOP should pay attention to the evaluation and minimization of toxicity, among other aspects such as technological combination, advance in new materials, lowering of energy consumption and consideration of renewable energy sources for applications on a bigger scale [14].

In the last twenty years, toxicity investigations using plants have progressively replaced in most fields, tests with animals. Phytotoxicity tests, such as the seed germination rate or the inhibition of root elongation tests, present some advantages as their simpleness, low price, and that they need a relatively small quantity of sample; in addition, the seeds can be used for a long time [15]. The most common plant species used are cucumber, lettuce, radish, red clover and wheat. After comparing some of these species, Banks and Schultz [16] and Priac and coworkers [15] recommended lettuce (*Lactuca sativa* L.) as a bioindicator to analyze the toxicity of water and soil samples. Besides phytotoxicity tests, microorganisms with luminescent properties as the *Vibrio fischeri* bacterium have been employed in numerous toxicity studies too [17]. In this case, an appropriate equipment is used to measure the modification in the bacterial luminescence in contact with a toxic compound. These types of experiments stand out for their simplicity and speed to analyze the samples. For these reasons, both types of toxicity assays, i.e., *Lactuca sativa* and *Vibrio fischeri* tests, are widely used to evaluate toxicity of untreated and EAOP-treated samples [18].

The objective of the present paper is to evaluate the ecotoxicity of atenolol (ATL) in the simultaneous presence of sodium sulfate, which is a supporting electrolyte widely used in electrochemical processes, before and after its treatment by an electrochemical advanced oxidation, the electrooxidation. Moreover, the effect of three process variables, i.e., anode material, operation current and reactor configuration, on the toxicity modification during the electrooxidation was analyzed. The toxicity was developed by means of *Lactuca sativa* and *Vibrio fischeri* assays, determining the inhibition in growth and luminescence, respectively.

2. Material and methods

2.1. Solutions for Lactuca sativa tests

The effect of ATL (Fig. 1), sodium sulfate and pH on the ecotoxicity towards *Lactuca sativa* was assessed. Solutions were prepared with ATL ($C_{14}H_{22}N_2O_3$, Sigma-Aldrich) ranging from 0 to 550 ppm, sodium sulfate (Na_2SO_4, analytical grade from Panreac) varying from 0 to 6 g L^{-1} and pH ranging from 2 to 9.5. The pH was adjusted using 0.1 M sulfuric acid (H_2SO_4, J.T.Baker) or 0.1 M sodium

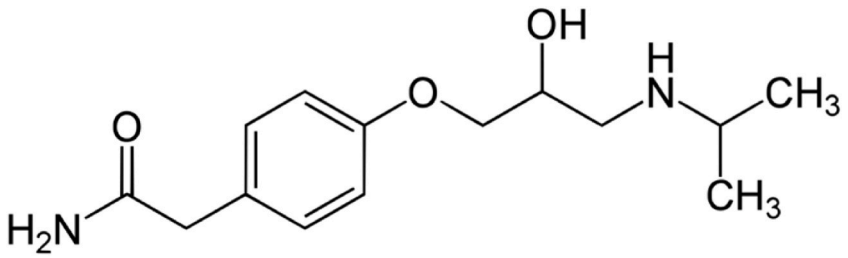

Fig. 1. Structural formula of Atenolol.

hydroxide (NaOH, analytical grade from Panreac). These ranges were chosen to match the concentrations used in the electrochemical experiments.

2.2. Electrochemical experiments

The electrooxidation process was applied for 4 h, to an initial solution containing 100 mg L^{-1} of ATL and 2 g L^{-1} or 14 g L^{-1} of Na_2SO_4 as supporting electrolyte, depending on the electrochemical reactor configuration: a one-compartment reactor and a two-compartment reactor containing a cationic exchange membrane (Nafion 117, Dupont). In order to obtain comparable results it was necessary to increase the conductivity in the solution due to the greater distance between electrodes when using the two-compartment reactor. This is why the highest concentration value of the supporting electrolyte is employed in this case. A stainless steel sheet was used as cathode and two distinct materials were employed as anodes: a microporous Sb-doped SnO_2 ceramic anode with a molar relation SnO_2/Sb_2O_3 equal to 98/2, which was characterized in a previous work [19], and a Boron-doped diamond (BDD) anode with a doping degree of 2500 mg L^{-1} (NeoCoat SA). In addition, three operation currents (0.4, 0.6 and 1 A) were applied.

The ATL final concentration was evaluated by an HPLC system provided with a Photodiode Array detector MD2018 Plus, a PU-2089 quaternary gradient pump (Jasco, Japan) and a Kinetex XB-C18 column. The total organic carbon (TOC) concentration was determined by means of a Shimadzu TNM-L ROHS TOC equipment. The persulfate concentration was quantified by iodometry titrations [20]. Finally, changes in the ecotoxicity of the solution were evaluated by means of both *Lactuca sativa* and *Vibrio fischeri* tests. Before carrying out the ecotoxicity assays, the pH of the samples was adjusted approximately to 6 using H_2SO_4 or NaOH, both described previously.

2.3. Ecotoxicity measurements towards Lactuca sativa

Commercial *Lactuca sativa* seeds (Batavia variant, Reina de Mayo) preserved at 5 °C without any pretreatment, were used to carry out ecotoxicological tests. Twenty seeds were arranged over a 90 mm diameter filter paper, soaked with 4 ml of the problem solution to be analyzed and placed inside a Petri dish. Distilled water was used as the negative control and each experiment was repeated four times. The dishes were preserved at 20 °C in the darkness during 5 days, and results were considered adequate if at least 65% of the negative control seeds germinated [21].

After the 5 days, the ecotoxicity results were evaluated in terms of the root relative growth (*RRG*) according to Equation (1):

$$RRG\ (\%) = (L/L_c) \cdot 100 \tag{1}$$

where L is the average root length developed in contact with the problem sample and L_c is the average root length for the control solution.

The normality analysis of the obtained L_i (the root length of each seed) data was assessed by the Shapiro-Wilk test [22] by means of the free software Past 3.20. For normally distributed L_i data, Tukey's pairwise test was used to compare the L means with their respective L_c mean; whereas non-normally distributed L_i data were analyzed using the Mann-Whitney test, performed with the software Past 3.20. All the statistical analysis presented in this work were performed using a 95% confidence level.

Furthermore, a statistical analysis of the calculated *RRG* data was carried out by means of Statgraphics Plus 5.1 (Manugistics, Inc., Rockville, MA, USA) to calculate the effect of the three experimental factors (i.e., ATL concentration, Na_2SO_4 concentration, and pH value) on the response variable (*RRG*). Initially, an ANalysis Of VAriance (ANOVA) was used to assess the factors and interactions that have a statistically significant effect on the response variable (RRG). Later, the response surface methodology (RSM) was applied to develop a black box model that relates RRG with the statistically significant factors and interactions identified by the ANOVA analysis. In order to validate the statistical analysis, the four main hypotheses, i.e. statistical significance, independence, normality, and homoscedasticity, were checked. First, the statistical significance hypothesis is met since the residual number of degrees of freedom exceeds 4 in all the statistical studies presented here. Second, the independence condition is guaranteed by the selected experimental design. Third, the normality of the residues was checked using normal probability plots, and Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Finally, the homoscedasticity hypothesis was verified using predicted versus residue plots. To end, the pollutant concentration that leads to a 50% inhibition of the root elongation in relation to the control (EC_{50}) was estimated using the built black box model. The RSM is not the usual methodology for estimating EC_{50} values; however, Mao and co-workers considered it a helpful tool in ecotoxicological investigations [23] as it needs less experimental data and, therefore, fewer experiments than the standard method [24].

2.4. Ecotoxicity measurements towards Vibrio fischeri

A Microtox model M − 500 toxicity analysis equipment (Strategic Diagnostics Inc., USA) was used to evaluate the toxicity towards *Vibrio fischeri*. The bioluminescence inhibition tests were performed at 15 °C with a salinity degree of 2% NaCl, and an exposure time of 15 min. Under these conditions, the MicrotoxOmni software provides toxicity values as *n*-TU (toxicity units), being *n* the number of dilutions to be carried out with a sample to achieve a luminescence inhibition of 50% of the *Vibrio fischeri* bacteria.

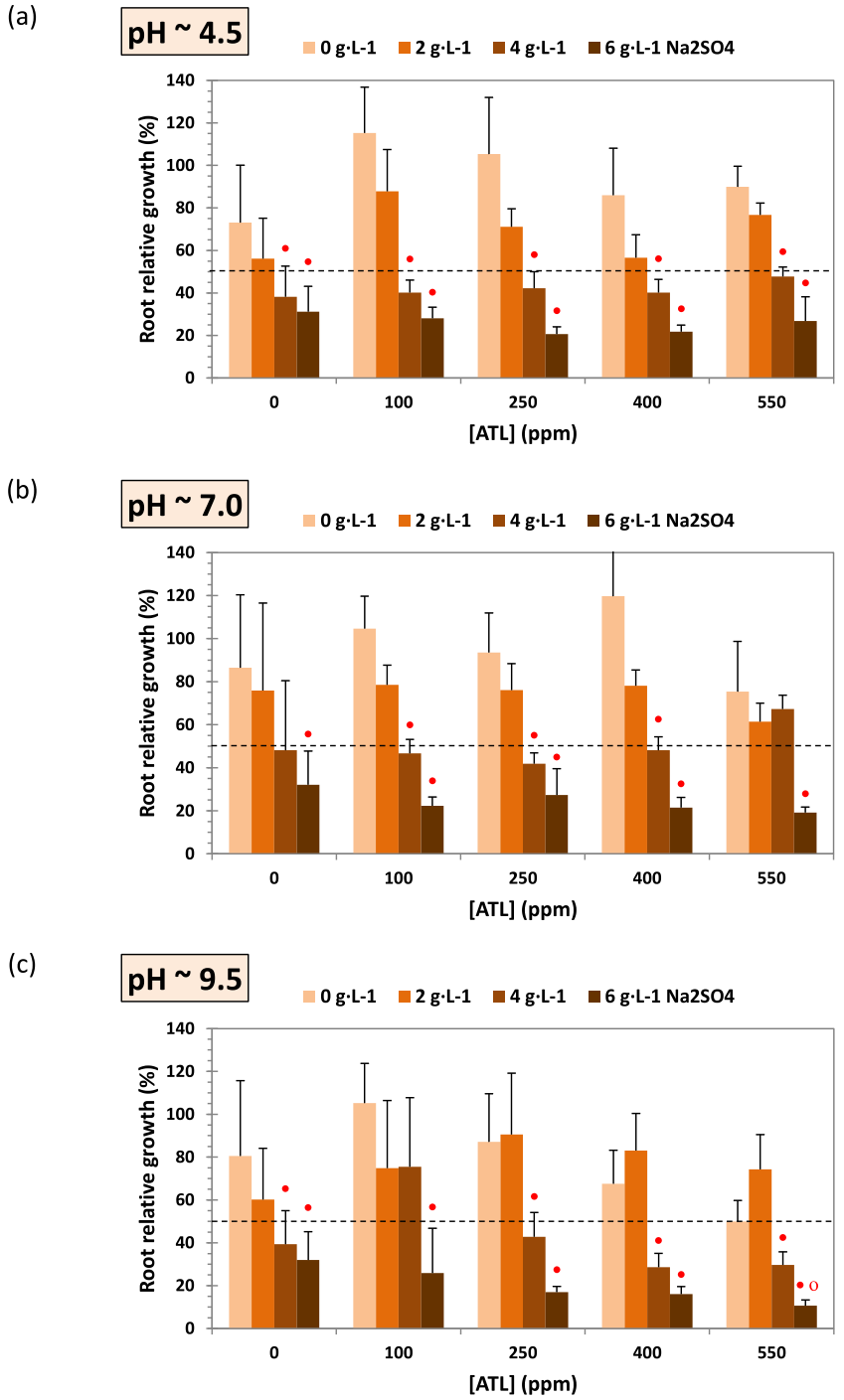

Fig. 2. Combined effect of ATL and Na_2SO_4 concentrations on root relative growth of *Lactuca sativa* at (a) pH value equal to 4.5, (b) pH value equal to 7.0, and (c) pH value equal to 9.5. Error bars indicate the standard deviation of four repetitions. Red points (•) and white points (o) indicate the samples which show a statistically significant difference from the negative control and from 0 mg L^{-1} ATL, respectively, for a confidence level of 95%. The dashed line defines the limit to consider that a sample is toxic. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results and discussion

3.1. Effect of ATL concentration on ecotoxicity towards Lactuca sativa at different Na_2SO_4 concentrations and pH values

Although Atenolol is the pollutant under concern, sodium sulfate and pH are important factors to be considered in an electrochemical process. The former is usually employed as supporting electrolyte because it is necessary to rise the conductivity of the solution; and the latter may change during the process due to secondary reactions. Therefore, the influence of both parameters on the toxicity of ATL must be first analyzed.

Fig. 2 shows the toxicity results achieved towards *Lactuca sativa* in terms of root relative growth for different concentrations of ATL and Na_2SO_4, and various pH values: 4.5 in Figs. 2(a), 7.0 in Figs. 2(b) and 9.5 in Fig. 2(c). The results for pH equal to 2 are not shown because no seed germination was observed for any of the ATL and Na_2SO_4 concentrations analyzed. The dashed line in Fig. 2 corresponds to a root relative growth equal to 50%. Therefore, a sample is toxic when its root relative growth is below the dashed line. Error bars indicate the standard deviation of four repetitions. Finally, the samples with a black point or a white point show a statistically significant difference from the negative control and from 0 mg L^{-1} ATL, respectively.

In general, Fig. 2 shows that the root relative growth of the *Lactuca sativa* seeds decreases as the ATL concentration increases, and therefore it leads to an increased toxicity of the samples. However, for the lowest concentrations of ATL with the smallest concentrations of supporting electrolyte, the samples appear to be less toxic than in the absence of ATL. Moreover, in the absence of Na_2SO_4, the root relative growth is greater than 100%, showing that these solutions are less toxic towards *Lactuca sativa* than the control. Furthermore, for Na_2SO_4 concentrations lower or equal to 2 g L^{-1}, the samples are not toxic towards *Lactuca sativa* in the whole range of ATL concentrations considered here; whereas for the highest Na_2SO_4 concentrations (4 and 6 g L^{-1}), practically all the samples are toxic, since they have a p-value lower than 0.05, and a root relative growth lesser than 50%. In a later section, the possible synergy between both compounds will be analyzed. So far, it seems that low concentrations of ATL benefit the root growth of *Lactuca sativa*, but the presence of Na_2SO_4 worsens it. In fact, Na_2SO_4 produces a rise in the toxicity of the solution towards *Lactuca sativa*; and, as the concentration of Na_2SO_4 rises, the value of the ATL concentration that shows a 50%-relative root growth rate decreases leading to higher toxicity values. This behavior was also detected in a previous work [25] where toxicity of Norfloxacin was analyzed using Na_2SO_4 as a supporting electrolyte.

Regardless of Na_2SO_4 concentration, the results in Fig. 2 clearly show that, as the concentration of the supporting electrolyte rises, the root relative growth of the *Lactuca sativa* seeds decreases, and consequently it leads to the increased toxicity of the samples. It is known that sulfate is an important nutrient for plants, but an excess of nutrients may be toxic to the organisms; sulfate toxicity is attributed to creating an unsustainable osmotic imbalance between the plant and its surrounding environment [26,27]. Fig. 2 shows that, in the absence of ATL, the EC_{50}(5 days) of Na_2SO_4 towards *Lactuca sativa*, i.e., the effective concentration that leads to a 50%-relative root growth, is between 4 and 6 g L^{-1} for the three pH values analyzed. This result is consistent with the value obtained in a previous work [25], where the EC_{50}(5 days) of Na_2SO_4 towards *Lactuca sativa* was calculated to be 4.8 g L^{-1} at a pH of 6.5.

Finally, no clear influence of the pH on the toxicity of the samples was observed in the 4.5–9.5 pH range. In the absence of ATL and Na_2SO_4, the neutral pH sample displays the closest to 100%-relative root growth and slightly acidic and basic solutions show slightly lower values. Only samples of pH = 2 were very toxic towards *Lactuca sativa* as any seed germinated. This behavior of toxicity values *versus* pH was also observed in a previous work [25], where the effect of pH on the Norfloxacin toxicity was studied; and it is in

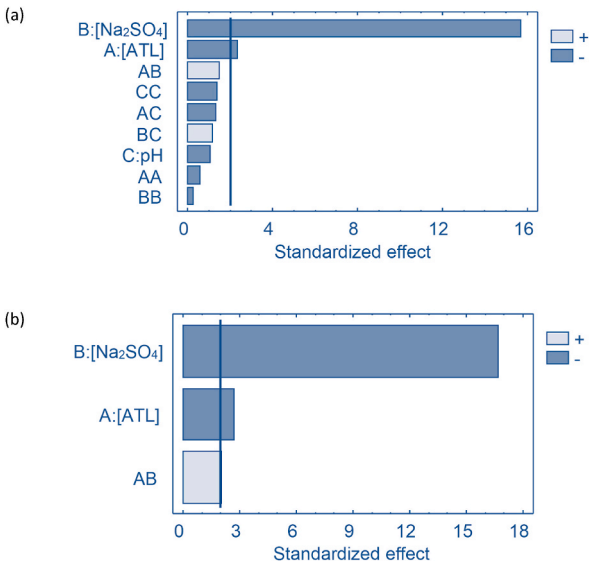

Fig. 3. Pareto charts for the root relative growth (*RRG*) of *Lactuca sativa* (a) before discarding non-statistically significant effects, and (b) after discarding them. The vertical line identifies the 95% significance threshold.

accordance with the biologically active pH interval, i.e., 5.5 to 8.0.

3.2. Statistical analysis of the ecotoxicity results obtained with Lactuca sativa seeds

The results in Fig. 2 were subjected to a study of variance (ANOVA) to quantify the effects of the three experimental factors, i.e., ATL concentration, Na_2SO_4 concentration, and pH value, on the response variable (*RRG*). The obtained Pareto chart (Fig. 3(a)) shows that pH hasn't any statistically significant effect on RGG of *Lactuca sativa* in 4.5–9.5 range. This result agrees with the no clear influence of pH on the toxicity observed in Fig. 2. However, both ATL and Na_2SO_4 concentrations have a statistically significant effect on the root relative growth, for a 95% confidence level. The Na_2SO_4 concentration displays a stronger effect on the RRG than that of ATL, in the considered concentrations ranges. Moreover, both concentration values have a negative effect, indicating that an increase in any of them produces a decrease of the RRG of *Lactuca sativa*, which is consistent with Fig. 2.

The response surface method was applied, after discarding the statistically non-significant effects (Fig. 3(b)), obtaining the following black box model:

$$RRG\ (\%) = 103.578 - 0.0398659 \cdot [ATL] - 13.5173 \cdot [Na_2SO_4] + 0.00679922 \cdot [ATL] \cdot [Na_2SO_4] \quad (2)$$

Equation (2) relates the root relative growth (*RRG*) expressed in % with the ATL concentration expressed in $mg \cdot L^{-1}$, and the Na_2SO_4 concentration expressed in $g \cdot L^{-1}$. The fitted model presents an R^2 value of 85.0%, showing that the model is able to capture mostly all the variability of the experimental data. Setting RRG equal to 50% in Equation (2), it is possible to obtain the Pareto toxicity boundary, i.e., the combinations of ATL and Na_2SO_4 concentrations that lead to a 50%-RRG of *Lactuca sativa*, EC_{50}(5days):

$$[ATL] = (53.578 - 13.5173 \cdot [Na_2SO_4]) / (0.0398659 - 0.00679922 \cdot [Na_2SO_4]) \quad (3)$$

Fig. 4 presents Equation (3) in the first quadrant, where concentrations are positive. The solid line represents the Pareto toxicity boundary, so that all the combinations of ATL and Na_2SO_4 concentrations above this line represent toxic solutions towards *Lactuca sativa*.

Table 1 compares the EC_{50}(5 days) values predicted with Equation (3) with the corresponding values obtained by linear interpolation from Fig. 2 experimental data. The linear interpolations were performed without considering pH differences, because pH has not a statistically significant effect on the root relative growth of *Lactuca sativa* in 4.5–9.5 pH range, as previously evidenced. By linear interpolation, the EC_{50}(5 days) value for Atenolol in absence of supporting electrolyte is 1377 mg L^{-1}. Cleavers [28] and Hernando and co-workers [29] affirmed that ATL can be considered a non-toxic substance but, nonetheless, it can contribute to the overall toxicity of the conglomerate of pharmaceutical products and their metabolites found in the aquatic ecosystems. Comparing the EC_{50}(5 days) value of ATL towards *Lactuca sativa* with that of Na_2SO_4, ATL is more toxic than the supporting electrolyte, with an EC_{50}(5 days) ratio of around 3:1 (Na_2SO_4:ATL). Other works reported an EC_{50}(48 h) value of ATL and Na_2SO_4 towards *Daphnia magna* equal to 313 mg L^{-1} [28] and 2564 mg L^{-1} [30], respectively. Although these values lead to a 8:1 EC_{50}(48 h) ratio (Na_2SO_4:ATL), which is somewhat greater than the value obtained towards *Lactuca sativa*, they are consistent with the fact that ATL is more toxic than sodium sulfate. The addition of Na_2SO_4 to the ATL solutions causes a rise in the toxicity towards *Lactuca sativa*: the EC_{50}(5 days) values of Atenolol in the presence of Na_2SO_4 were 972, 0 and 0 mg L^{-1} for 2, 4 and 6 g L^{-1} of Na_2SO_4, respectively. Finally, as it can be seen in Table 1, the EC_{50}(5 days) predicted from Equation (3) agree with those values obtained by linear interpolation of the experimental data.

3.3. Synergy between ATL and Na_2SO_4 on ecotoxicity towards Lactuca sativa

Studies of environmental toxicity are often performed considering the individual effect of chemicals and their additive behavior in mixtures. This fact may underestimate the risk associated with mixtures, as the different chemicals may interact synergistically. When

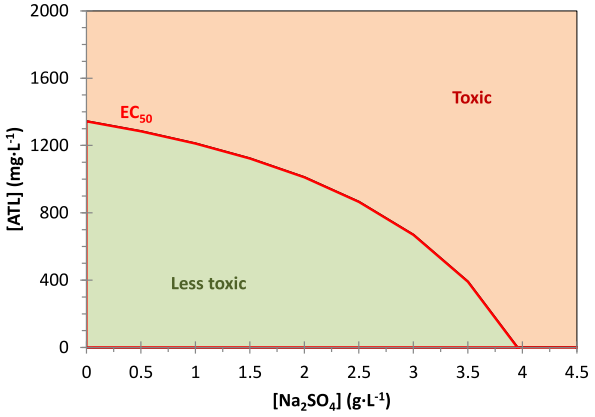

Fig. 4. Predicted EC_{50}(5 days) values towards *Lactuca sativa* for solutions containing both ATL and Na_2SO_4 in 4.5–9.5 pH range.

Table 1
EC_{50}(5 days) values for different mixtures of ATL and Na_2SO_4, obtained with *Lactuca sativa* seeds.

Pollutant	EC_{50}(5 days) (mg L^{-1}) obtained from Equation (3)	EC_{50}(5 days) (mg L^{-1}) obtained from linear interpolation of experimental data
Only Na_2SO_4	3960	3840
Only ATL	1344	1377
ATL with 2 g L^{-1} Na_2SO_4	1010	972
ATL with 4 g L^{-1} Na_2SO_4	0	0
ATL with 6 g L^{-1} Na_2SO_4	0	0

the real effect of a mixture is equal to the theoretic sum of the individual effects, a simple additive effect occurs. However, both values may be different: a synergy phenomenon is observed when the real effect is greater than the theoretic sum; and conversely, an antagonistic response takes place if the real effect is lower than the theoretic sum. In terms of toxicity, the synergistic case is the most damaging.

The synergy between ATL and Na_2SO_4 on toxicity towards *Lactuca sativa* was analyzed in terms of the diminution in root elongation (*DRE*) in relation to the control, which was calculated according to Equation (4):

$$\text{DRE}\ (\%) = 100 - \text{RRG}\ (\%) \tag{4}$$

Fig. 5 shows the predicted decrease in root elongation *versus* the observed decrease in root elongation for the distinct concentrations of ATL with 2, 4 and 6 g L^{-1} of Na_2SO_4. The predicted decrease is the sum of the individual effects, i.e., sum of the root elongation reduction for ATL and Na_2SO_4 individually. The dashed line in the figure corresponds to the bisector. Therefore, if the points of the graphic are located on the bisector, there is a simple additive effect between ATL and Na_2SO_4 on toxicity towards *Lactuca sativa*; if the points are above the bisector, an antagonistic effect is observed; and, finally, if the points are below the bisector, there is a synergistic effect between both compounds. The solid lines of the graph show the linear fit of the points corresponding to each concentration of supporting electrolyte.

A clear antagonistic effect between ATL and Na_2SO_4 on toxicity towards *Lactuca sativa* is observed in Fig. 5, for the lowest concentration of Na_2SO_4 analyzed (2 g L^{-1}), because the predicted decrease in root elongation is greater than the real decrease observed. However, as the Na_2SO_4 concentration rises, the antagonist effect's magnitude is reduced; and a simple additive effect is finally observed for the greatest Na_2SO_4 concentration (6 g L^{-1}). Mera and co-workers [27] observed that sodium sulfate shows a dual antagonistic/synergistic effect on cadmium toxicity towards a microalgae (*Chlamydomonas moewussi*) depending on the concentration values of the former. Sulfate is an important nutrient which can induce physiological responses to mitigate cadmium toxicity, but an excess of nutrients can be toxic to organisms, enhancing the toxic behavior of this metal. However, these authors observed that the antagonistic effect between cadmium and Na_2SO_4 is stronger than the synergistic one.

An antagonism phenomenon was also observed between Norfloxacin and Na_2SO_4 for concentrations of 2, 4 and 6 g L^{-1} of the latter compound [25]. The toxicity towards *Lactuca sativa* of Norfloxacin (336 mg L^{-1}) is greater than that determined for ATL (1377 mg L^{-1}). Therefore, it seems that the magnitude of the antagonist effect is greater when the toxicity difference between the pollutant and

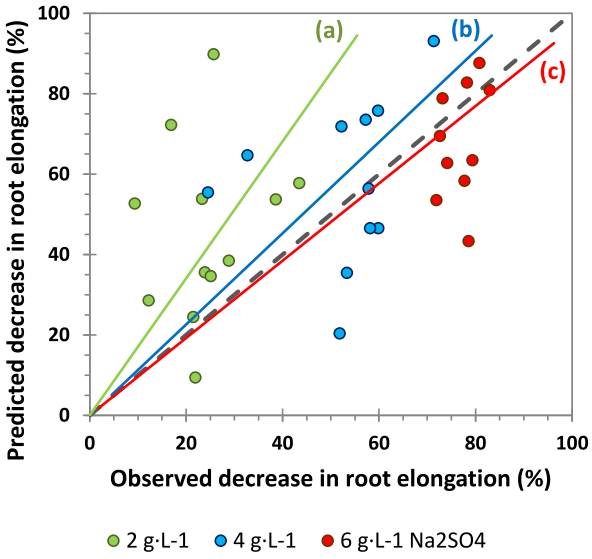

Fig. 5. Synergy analysis between ATL and Na_2SO_4 on toxicity towards *Lactuca sativa*: comparison between the sum of the individual effects (predicted decrease) and the observed decrease in root elongation. The dashed line corresponds to the bisector. The solid lines show the general trend of the points corresponding to (a) 2 g L^{-1} Na_2SO_4; (b) 4 g L^{-1} Na_2SO_4; and (c) 6 g L^{-1} Na_2SO_4.

sodium sulfate is larger.

3.4. Ecotoxicity of solutions after applying electrochemical oxidation

Table 2 contains the ecotoxicity results generated with the *Vibrio fisheri* assay after applying an electrochemical oxidation process to a solution contaminated with ATL and containing Na_2SO_4. As can be seen, both initial solutions are non-toxic towards *Vibrio fischeri*. When working with the one-compartment reactor, samples remain non-toxic regardless of the anode material and the applied current. Therefore, using the *Vibrio fischeri* test, possible small differences in toxicity cannot be detected, since all the values are equal to 0 TU. However, when working with the two-compartment reactor, almost all solutions show a certain level of toxicity, although they can be considered non-toxic because the values are less than 10 TU [31,32]. Despite these toxicity values are very similar, they seem to increase slightly when the applied current increases and when the BDD electrode is used.

To explain these toxicity results, Table 2 also shows the final values of ATL degradation ($[ATL]/[ATL]_0$) and mineralization (TOC/TOC_0) achieved. As can be seen, in most of the experiments, ATL is completely degraded; but not completely mineralized. A possible hypothesis would be that the toxicity of the final solutions could be attributed to the intermediate organic products formed during the electrooxidation process. Nevertheless, this hypothesis is rejected because the samples for which complete mineralization has been achieved, present a certain degree of toxicity; while the toxicity value for some solutions without complete mineralization is equal to zero.

The total oxidation of ATL takes place according to Ref. [20]:

$$C_{14}H_{22}N_2O_3 + 25\ H_20 \rightarrow 14\ CO_2 + 2\ NH_4^+ + 64\ H^+ + 66\ e^- \quad (5)$$

As shown in Equation (5), ammonium is produced during the mineralization of ATL. The EC_{50}(48 h) value of ammonium sulfate for *Daphnia magna* is 121.7 mg L^{-1} [33], and the corresponding EC_{50}(48 h) value of ATL is 313 mg L^{-1} [28]. Consequently, the molar EC_{50}(48 h) ratio between ATL and ammonium sulfate is approximately equal to 1:1, i.e., 1 mol of ATL provides the same toxicity degree to a solution as 1 mol of ammonium sulfate. Supposing a complete oxidation of ATL in accordance with Equation (5), the molar ratio of these compounds is 1:1, so for each mol of ATL 1 mol of ammonium sulfate is generated. Therefore, the increase in toxicity of the final solutions cannot be attributed to the ammonium formation. However, ammonium can be oxidized to nitrite and nitrate with a molar ratio equal to 1:1:1 (NH_4^+:NO_2^-:NO_3^-). The EC_{50}(48 h) values of sodium nitrite and sodium nitrate for *Daphnia magna* are 15.4 mg L^{-1} [34] and 3581 mg L^{-1} [35], respectively. According to these values, if ammonium is oxidized to nitrite, the toxicity of the solution will increase; but if ammonium is completely oxidized to nitrate, the toxicity of the sample will decrease. Table 2 shows that when the two-compartment reactor is used with the BDD electrode, complete mineralization of ATL is achieved; however, for these experimental conditions with greater oxidizing power the toxicity values of the final samples are higher. This observation rules out the ammonium hypothesis.

In the electrochemical experiments, sulfates can be oxidized to persulfates according to the following reaction:

$$2\ SO_4^{=} \rightarrow S_2O_8^{=} + 2\ e^- \quad (6)$$

Sodium persulfate has an EC_{50}(48 h) value for *Daphnia magna* equal to 133 mg L^{-1} [36], being much more toxic than sodium sulfate with a corresponding value of 2564 mg L^{-1} [30]. From these values, 1 mol of sodium sulfate provides the same toxicity degree to a solution as 0.03 mol of sodium persulfate. According to Equation (6), for each mol of sodium sulfate 0.5 mol of sodium persulfate is generated. Therefore, the toxicity increase of the final samples may be attributed to persulfate formation. To verify this hypothesis, persulfates were quantified in the final samples, and Table 2 contains the results. As can be seen, the persulfate concentration in the final sample increases with the operation current, and is larger when a BDD electrode is used, and with the two-compartment reactor. This fact agrees with the mineralization degree achieved and with the final toxicity values. Other authors [37–39] also observed the

Table 2
Ecotoxicity analysis (*Vibrio fischeri* test), degradation and mineralization degrees, and persulfates concentration, of solutions containing 100 mg L^{-1} of ATL and 2 or 14 g L^{-1} of Na_2SO_4 treated by electrooxidation in both, a one-compartment and a two-compartment reactor, respectively.

Reactor type	Anode material	Applied current (A)	Degradation degree ($[ATL]/[ATL]_0$)	Mineralization degree (TOC/TOC_0)	Persulfates concentration (ppm)	Toxicity EC_{50} (TU)
Initial solution	–	–	1.000	1.000	0	0
One-compartment reactor	BDD	0.4	0.045	0.230	96	0
		0.6	0.000	0.070	115	0
		1.0	0.000	0.060	153	0
	Ceramic	0.4	0.095	0.395	0	0
		0.6	0.000	0.311	0	0
		1.0	0.000	0.180	10	0
Two-compartment reactor	BDD	0.4	0.000	0.092	672	3
		0.6	0.000	0.000	870	3
		1.0	0.000	0.000	1305	4
	Ceramic	0.4	0.050	0.363	60	0
		0.6	0.000	0.116	240	3
		1.0	0.000	0.171	1017	3

persulfate formation from supporting electrolytes containing sulfates when they applied an electrochemical oxidation process.

As previously stated, for the one-compartment reactor, no differences in the toxicity values towards *Vibrio fischeri* could be observed as a function of the current applied or the anode material; and neither between the treated samples and the initial solution. For this reason, the toxicity towards *Lactuca sativa* of these samples was also analyzed and the results are shown in Fig. 6. As can be seen, the initial solution and practically all the treated samples can be considered non-toxic towards *Lactuca* sativa. The statistical analysis supports this statement because no significant difference, with a 95% confidence level, in the root elongation means between the solutions and the control is observed. However, a rise in the toxicity of the treated samples is noticed with respect to the initial solution after the electrooxidation. Thus, the *Lactuca sativa* test shows greater sensitivity for small toxicity values than the *Vibrio fischeri* test. In addition, in general, the toxicity values seem to increase slightly when the applied current increases, the same as was observed with the *Vibrio fischeri* assay for the two-compartment reactor. This observation shows a strong correlation with the measured persulfate concentration (see Table 2). However, the toxicity of the samples does not seem to increase when the BDD electrode is used; actually, it seems somewhat higher when the ceramic electrode is used. Table 2 shows that the experiments carried out with the one-compartment reactor and with the ceramic electrode are those that reach the lowest mineralization degree for ATL. Therefore, since the oxidation is not complete, the toxicity of these samples may be due to the nitrites formation instead of the persulfates formation, in fact, the concentration of persulfates is null for these samples. It seems that the oxidation mechanism, and hence the by-products formed, depends largely on the anode material used, and consequently affects the final toxicity values.

In summary, the aim of an electrooxidation process must not only be to reach a great degradation and mineralization degrees of the pollutant, but also to minimize the generation of intermediate compounds that can augment the toxicity of the final effluent. For this reason, both, the operation parameters and the concentration of the supporting electrolyte must be selected appropriately. All the experimental conditions applied in this work provide a final solution that is considered non-toxic towards *Vibrio fischeri*; and, with the one-compartment reactor, practically all the treated solutions are also considered non-toxic towards *Lactuca sativa*. However, only two experiments allowed total degradation and mineralization of ATL. Among them, the minimum final toxicity was achieved using a two-compartment reactor, operating at 0.6 A with a BDD anode. Similar mineralization degree and toxicity results were achieved with an operation current of 0.4 A, thereby decreasing the energy consumption; or working with the same current intensity but using the one-compartment reactor, obtaining in this case a lower final toxicity.

4. Conclusions

In this work, Atenolol (ATL) ecotoxicity towards *Lactuca sativa* was analyzed, obtaining an EC_{50}(5 days) value equal to 1377 mg L^{-1}. When Na_2SO_4 was added, the toxicity of the solution increased. Specifically, with 2 g L^{-1} Na_2SO_4, ATL showed an EC_{50}(5 days) of 972 mg L^{-1}; and with 4 g L^{-1} Na_2SO_4 and higher concentrations, EC_{50} value for ATL was 0 mg L^{-1}. Statistical tools have been applied to build a black box model, which was used to determine the Pareto toxic boundary, i.e., the zones of the [ATL]-[Na_2SO_4] plane which are toxic towards *Lactuca sativa*. Although the presence of Na_2SO_4 increases ATL ecotoxicity, no synergy was observed between both compounds; but quite the opposite, an antagonistic effect between them on toxicity has been observed for the lowest concentration of Na_2SO_4 (2 g L^{-1}). However, this effect loses importance as the concentration of Na_2SO_4 rises and a simple additive effect was finally observed for the greatest concentration of Na_2SO_4 (6 g L^{-1}).

In addition, the toxicity of a solution that contains ATL and Na_2SO_4 was analyzed after employing an electrochemical oxidation process. The ecotoxicity of the treated sample increased in every operation condition assessed in this study. This fact is principally attributed to the persulfate generation by sulfate electrooxidation. Nevertheless, none of the final samples were considered toxic towards *Vibrio fischeri* because ecotoxicity values were lower than 10 TU; and, in the case of the one-compartment reactor, practically all of them were also non-toxic towards *Lactuca sativa*. The toxicity of the treated solutions increased when using a two-compartment

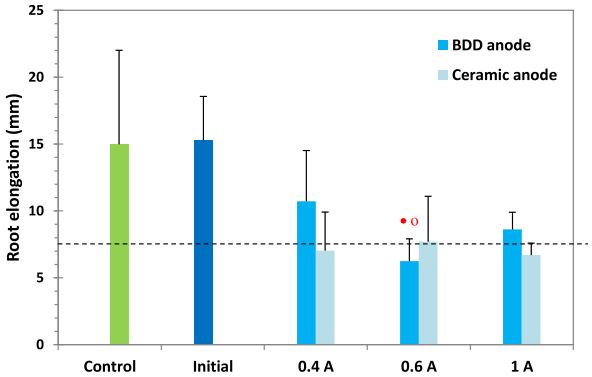

Fig. 6. Toxicity towards *Lactuca sativa* of samples which contain 100 mg L^{-1} of ATL and 2 g L^{-1} of Na_2SO_4 and treated by electrooxidation in a one-compartment reactor. Error bars indicate the standard deviation of four repetitions; red points (•) indicate statistically significant difference from the negative control (distilled water) for a confidence level of 95%, and white points (o) indicate statistically significant difference from 0 mg L^{-1} ATL for a confidence level of 95%. The dashed line defines the limit to consider that a sample is toxic. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reactor separated by a cation exchange membrane and the BDD anode, and when the operation current was increased. This is attributed to the highest formation of persulfates.

With an electrooxidation process, a great degradation and mineralization degrees of the pollutant must be reached, but the generation of intermediate compounds that can augment the toxicity of the final effluent must be minimized too. Amongst all the tests performed in this study that accomplished total degradation and mineralization of ATL, the lowest toxicity value (i.e., 3 TU) was reached with the two-compartment reactor operating at 0.6 A with the BDD anode.

Data availability statement

The data that support the results presented in this work are available from the corresponding author, upon reasonable demand.

Ethics declarations

Review and/or approval by an ethics committee, nor informed consent, was not required for this work.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

M.T. Montañés: Writing – original draft, Validation, Supervision, Formal analysis, Conceptualization. **M. García-Gabaldón:** Writing – review & editing, Supervision, Methodology. **J.J. Giner-Sanz:** Writing – review & editing, Validation, Software. **J. Mora-Gómez:** Writing – review & editing, Methodology. **V. Pérez-Herranz:** Resources, Project administration, 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.

Acknowledgements

The authors express their gratitude to the Generalitat Valenciana (GVA-THINKINAZUL/2021/013 Project) and to the Next Generation EU (European Union NextGenerationEU/PRTR) for the financial aid received. J.J.G.S. thanks the Ministerio de Ciencia e Innovación, the Next Generation EU, and the Agencia Estatal de Investigación, for their support through a Juan de la Cierva-Incorporación fellowship (IJC2020-044087-I) funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

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