

Article - Environmental Sciences

# Evaluating of BTX Removal of Water Contaminated with Gasoline by Fenton Process: Chemical and Ecotoxicity Analysis

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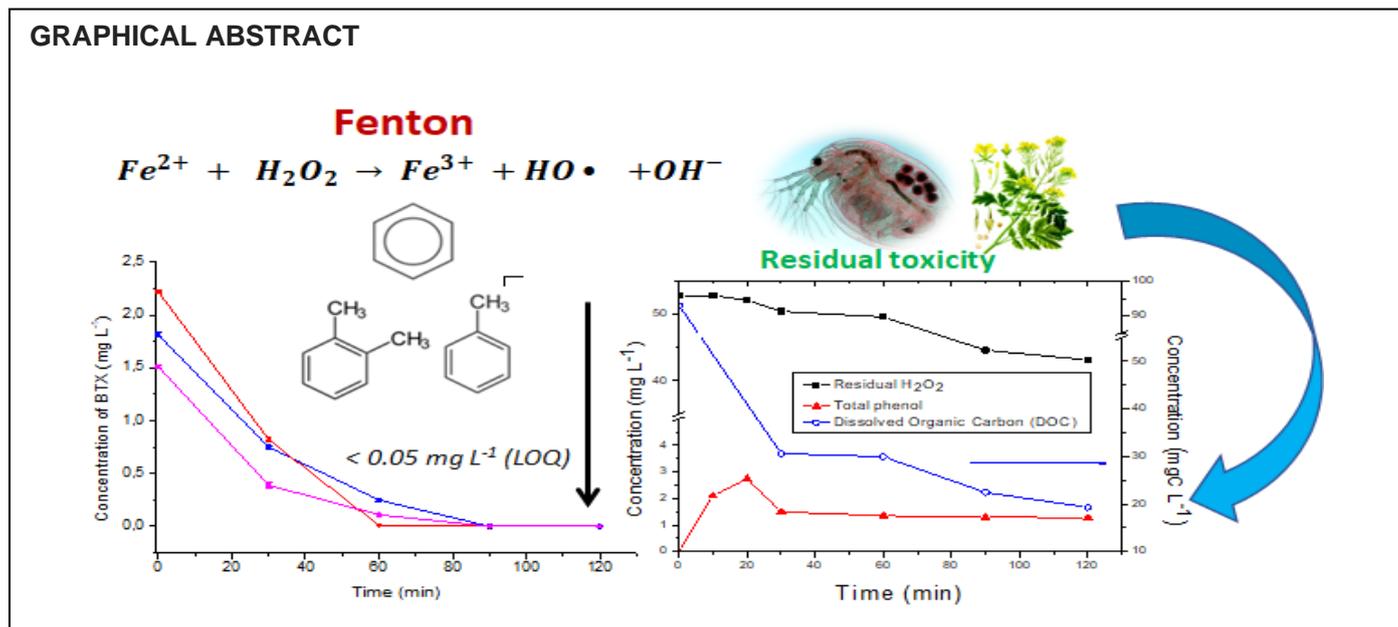
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## HIGHLIGHTS

- The Fenton process was efficient on gasoline degradation (>99% at 90 min).
- Significant removal of DOC ( $80 \pm 2\%$ ) at 120 min.
- *Daphnia magna* showed the toxicity of by products at 120 min of reaction.

**Abstract:** This study shows the results obtained of evaluation and comparison of chemical analysis and ecotoxicity for evaluated the quality of water contaminated with gasoline after Fenton process. The process Fenton was efficient to remove the aromatics organic compounds such as benzene, toluene and xylenes from water contaminated with gasoline. At 90 minutes of reaction time, the concentration measured of aromatics compounds by gas chromatography were less than limit of quantification ( $0.05\text{mg L}^{-1}$ ). This value is in accordance with the limits established by Brazilian legislation for the effluents discharge. On the other hand, analysis of total phenols and dissolved organic carbon demonstrated at 120 minutes that all dissolved organic compound and total phenols are not totally removed even though benzene, toluene and xylenes were almost completely removed. Phytotoxicity tests using *Sinapis alba* as biological response showed that water contaminated with gasoline are not toxic, however these results was probably influenced by volatilization of aromatics compounds during the assay. Differently, the ecotoxicity using *Daphnia magna* showed that water contaminated with aromatic compounds is toxic, but after 120 minutes of treatment the toxicity was reduced more than 50%. Then, *Daphnia magna* was the better bioindicator to evaluate the efficiency of Fenton process since these results were according with response of dissolved organic carbon and total phenol showing the toxicity of intermediates produced during the Fenton process.

**Keywords:** aromatic hydrocarbons; Fenton; treatment; phytotoxicity; ecotoxicity.

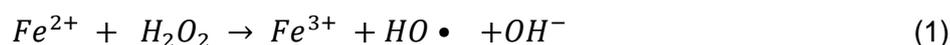


## INTRODUCTION

The quality of drinking water is important since it has a major impact on health population and must be constantly monitored and evaluated. In addition, only 0.6% of the water is available fresh water [1]. Contamination by hydrocarbons, mainly in soils and groundwater are concern due to the frequency with which they occur in the environment [2]. Some contaminants such as benzene, toluene and xylene (BTX) are considered to be one of the main contaminants, since they have high water solubility and high toxicity [3]. In accordance with Brazilian law, which establishes the conditions and standards for the discharge of effluents, and the maximum concentration permitted of benzene, toluene and xylene are  $50 \mu g L^{-1}$ ,  $2 \mu g L^{-1}$  and  $300 \mu g L^{-1}$ , respectively [4].

Volatile organic compounds can cause serious effects on the human body, even at low concentrations, and they are considered one of the biggest contributors to the deterioration of water quality [5]. In addition, according to Freitas and Becker [6], ethanol can impact the behavior of hydrocarbon compounds in water due its performance as a co-solvent that decrease in interfacial tension [6]. Brazil is the country that most adds alcohol to gasoline (20-26%); the reason behind this high percentage is that the government aims to produce economic benefits and reduce environmental problems due to carbon dioxide emissions. However, this addition can modify the physicochemical characteristics of gasoline, increasing the solubility and mobility of aromatic hydrocarbons [7]. The solubility of aromatic hydrocarbons in water is  $1770 mg L^{-1}$ ,  $534.8 mg L^{-1}$ ,  $160 mg L^{-1}$  for benzene, toluene and xylene, respectively [8,9].

Although natural attenuation and bioremediation have been used to treat contaminated water [10,11], advanced oxidative processes, such as Fenton has been used as alternative due its high efficiency. The Fenton oxidation was discovered by H.J. H. Fenton (Fenton, 1984) in 1894. This process has been studied and highly oxidative hydroxyl radicals ( $HO\cdot$ ) are formatted as well as include more than 20 chemical reactions. The reaction of hydrogen peroxide ( $H_2O_2$ ) with  $Fe^{2+}$  produces  $HO\cdot$  at a pH around 2.5–3.0 (Equation 1) and can degrade organic pollutants to dioxide de carbon and water [12].



Studies by Radwan and co-authors [13], Lou and Lee [14] demonstrated that Fenton process can be good alternative for water treatment after spill of gasoline. However, partial oxidation of organic compounds can result in the formation of by-products that are more toxic than the original compounds [15]. Bioassays can be used as a tool for evaluate water quality after treatment since include synergic effect of intermediates producing during the treatment.

According to Ramirez-Morales and co-authors [16], the main objective of assessing the ecotoxicological characteristics of chemicals is to prevent damage to ecosystems, which guarantees the absence of negative effects of these compounds on living organisms. For example, phytotoxicity tests can detect any substance capable of generating temporary or long-term stress on seed germination capacity and root growth [17]. Pan and Chu [18] highlight that seed germination and root elongation tests are simple, sensitive and inexpensive environmental bioassays. In addition, advantages of phytotoxicity tests are involving seeds, is a simple and

very reproductive method. Also, no requirement for major equipment minimal maintenance costs seeds is self-sufficient, only small sample size required and no seasonality seeds can be easily purchased in bulk seeds remain viable a long time (rapid germination) [19]. There is a lack of analytical procedure to evaluate the joint effect of toxic substances consequently, bioassay seed germination test has attracted a lot of attention to overcome these concerns [20].

In addition, *Daphnia magna* is widely used in toxicity tests because they are well distributed in freshwater bodies, are important in many sources of food chains, have a relatively short life cycle, are easily grown in the laboratory and are sensitive to various contaminants in the aquatic environment. There is a great deal of information about *Daphnia magna* regarding cultivation techniques, temperature, light and nutrient requirements, and its response to many toxic substances [21]. Aquatic ecotoxicology has become much more than the simple application of laboratory assays in evaluating the effects of a certain specific compound on only one selected bioindicator, an organism or group of organisms that reflects environmental quality information, and on its biochemical, physiology, or histological indicators, or biomarkers [22].

Studies reported in the literature show the efficiency of the Fenton process in the degradation of aromatic hydrocarbons present in the aqueous phase using chemical analysis such as chromatography that allow quantifying the percentage of BTX removal. These studies have been evaluated the efficiency of system of degradation considering just the limits of concentration recommend for government laws. But there are few studies that evaluated the quality of water after treatment using bioassays and also studies that compare efficiency of treatment process using both chemical analysis and bioassays. In this way, chemical analysis and toxicity tests can be complementary [23, 24].

The main objective of the present study was to evaluate the efficiency of the Fenton process in the degradation of hydrocarbons present in an aqueous sample obtained after a gasoline spill simulation using chemical analysis and bioassays (*Sinapis alba* and *D. magna* as test organisms) as well as comparison the response from both tool analysis.

## MATERIAL AND METHODS

### Chemical and Reagents

Benzene (Cinética Química, Brazil), Toluene (Nuclear, Brazil), Xylene (Nuclear, Brazil), methanol (Biotec, Brazil), Hydrogen peroxide 10% (Synth, Brazil), ferrous sulfate (Vetec, Brazil), sodium bisulfite (Biotec, Brazil), reactive Folin and Ciocalteu (Merck, Brazil), sulfuric acid (Synth, Brazil), sodium carbonate (Dinâmica, Brazil), sodium tartrate (Dinâmica, Brazil), phenol (Synth, Brazil), ammonium metavanadate (Dinâmica, Brazil), catalase (Sigma-Aldrich, German) and others reagents of the analytical grade.

### Degradation Study

The contaminated water used in this study was obtained from the simulation of a gasoline spill under a laboratory setting. The spill simulation was carried out using 3 L of tap water and 50 mL of commercial gasoline (with 27% of ethanol) in a closed container for 48 hours. In this condition one film of gasoline was formatted above the water. At intervals of 0 to 48 hours at  $22 \pm 2^\circ\text{C}$  and in the absence of light, aliquots were collected to verify the concentration of organic compounds (benzene, toluene and xylene) using the tap fixed at the bottom of system.

The degradation studies were carried out using aqueous phase after 48 hours of contamination. The Fenton process was conducted in the dark at  $\text{pH } 2.8 \pm 2$  using conventional reactor with a 200 mL capacity and a stirring magnet at temperature of  $25 \pm 2^\circ\text{C}$ . In order to control of volatilization of BTX during the experimental design water samples was maintain during 30 min without Fenton's reagents and the concentration of BTX was measured by chromatography.

### Analytical Methods

For the determination of the BTX concentration, gas chromatography (Allcrom GC-6000 model YL 6100) with a headspace system was used. The column used was an Elite 5MS 30m x 0.25mm x 0.25 $\mu\text{m}$  (6% cyanopropylphenyl and 94% dimethylpolysiloxane), with a flame ionization detector and nitrogen as carrier gas at a flow rate of 1 mL  $\text{min}^{-1}$ . The headspace was incubated for 30 min at  $70^\circ\text{C}$  while being agitated with a magnetic stirrer. The injection volume was 1mL with Solit of 10 with split 10 injection system. The calibration curve was in the range of 0.05 to 5 mg  $\text{L}^{-1}$ ,  $R^2=0.99$  and LOQ of 0.05 mg  $\text{L}^{-1}$ .

The total phenol was determined using a stock solution of phenol (100 mg  $\text{L}^{-1}$  of 99% Synth) that was diluted in deionized water. Different dilutions were prepared for the calibration curve using a solution buffer

(sodium carbonate and sodium tartrate) and the Folin–Ciocalteu reaction (Merck, 99%) [25]. The calibration curve was in the range of 1 to 10 mg L<sup>-1</sup> range, and the R<sup>2</sup> value was equal to 0.974 and LOQ=2.34x10<sup>-5</sup> mg L<sup>-1</sup>.

H<sub>2</sub>O<sub>2</sub> consumption was measured according to the method reported by Oliveira and co-authors [26]. The method was based on the reaction between vanadate ions and H<sub>2</sub>O<sub>2</sub> in an acid medium, which formed the peroxovanadate complex. The absorbance was measured at λ=446 nm. The calibration curve range of 20–400 mg L<sup>-1</sup> was prepared with H<sub>2</sub>O<sub>2</sub>, with R<sup>2</sup> value equal to 0.999 and LOQ=3.22 mg L<sup>-1</sup>.

The mineralization was monitored by measuring the dissolved organic carbon (DOC) content using a Shimadzu TOC-L CSH analyzer. The calibration curve between 5 and 200 mg L<sup>-1</sup> was elaborated with aqueous solutions of the potassium hydrogen phthalate. Under these conditions, a typical standard deviation of about 2% was observed, with R<sup>2</sup> = 0.999, LOQ= 5 mg L<sup>-1</sup>

## Experimental design

The conditions for studies degradation were carried using the best response obtained after planning design. The design of experiment with matrix of 2<sup>3</sup> with central point was used to evaluate the important variables on Fenton process as ions Fe<sup>2+</sup> and hydrogen peroxide concentration. The analysis of variance was evaluated using the Statistica 13.0 program.

## Ecotoxicity bioassays

For the evaluation of ecotoxicity, samples were collected at an initial time point (0 min) and at a final Fenton treatment time (120 min) in clean glass flasks. The pH was adjusted to approximately 6–7 and, when necessary, catalase was added for residual H<sub>2</sub>O<sub>2</sub> removal. Two bioassays were selected: *Sinapis alba* seeds (phytotoxicity) and *Daphnia magna* (acute ecotoxicity).

The seed germination and root elongation phytotoxicity test was conducted according to standardized protocols [27,28] using *S. alba* (mustard) seeds. Tests were carried out in Petri dishes lined with filter paper; each dish contained 15 seeds and 4 mL of the sample before and after treatment. The assay was done in triplicate. Seeds were incubated at 22 ± 2°C in the dark for 120 h. At the end of the test, the number of germinated seeds and the root elongation data were used to calculate the germination index (GI) and the relative growth index (RGI), respectively [28]. The RGI values were divided into three categories according to the observed toxicity effects: (a) inhibition of the root elongation: 0 < RGI < 0.8; (b) no significant effects: 0.8 ≤ RGI ≤ 1.2; and (c) stimulation of the root elongation: RGI > 1.2 [28]. A statistical evaluation of the data was performed with the BioEstat 5.3 software (BioEstat Software, Belém, Brazil). Data were subject to a Kolmogorov–Smirnov normality test. As data were normally distributed, they were submitted to a one-way analysis of variance (ANOVA) and Tukey test (p < 0.05). Significance values are indicated as follows: \*p < 0.05 and \*\*p < 0.01.

The acute ecotoxicity to *D. magna* was assessed according to the methodology described in the ABNT [29]. Ten neonates (6–24 hrs) were used for each of the three replicates. Samples were diluted in cultured medium at 100, 50, 25, 12.5, and 6.25% (v/v). The negative control was the standard medium and the positive control was potassium chloride (Vetec). All tests were maintained at 20±2°C. After 48 hrs of exposure, the number of immobile organisms was recorded and the results were expressed in terms of the toxicity factor (TF), which corresponds to the highest sample solution which no toxic effect is observed, represented as 1 (sample without dilution), 2, 4, 8, 16, and 32 (highest dilution) [29].

## RESULTS

### Characterization of water after contamination simulation

The water contaminated with gasoline using in this study was obtained by simulate gasoline spill. The samples of water collected after of 48 hours of contamination showed the concentration of which BTX was approximately 2 mg L<sup>-1</sup> (Table 1), the pH was 8 and COD ≅ 90 mgC L<sup>-1</sup>.

**Table 1.** Concentration of BTX obtained after simulation of contamination.

Hydrocarbon	Concentration (mg L <sup>-1</sup> )	Concentration (mg L <sup>-1</sup> )	Concentration (mg L <sup>-1</sup> )	Average	Standard deviation
<b>Benzene</b>	1.83	1.82	1.84	1.83	0.010
<b>Toluene</b>	2.27	2.25	2.24	2.25	0.015
<b>Xylenes</b>	1.58	1.60	1.57	1.58	0.015

## Design of Experiments

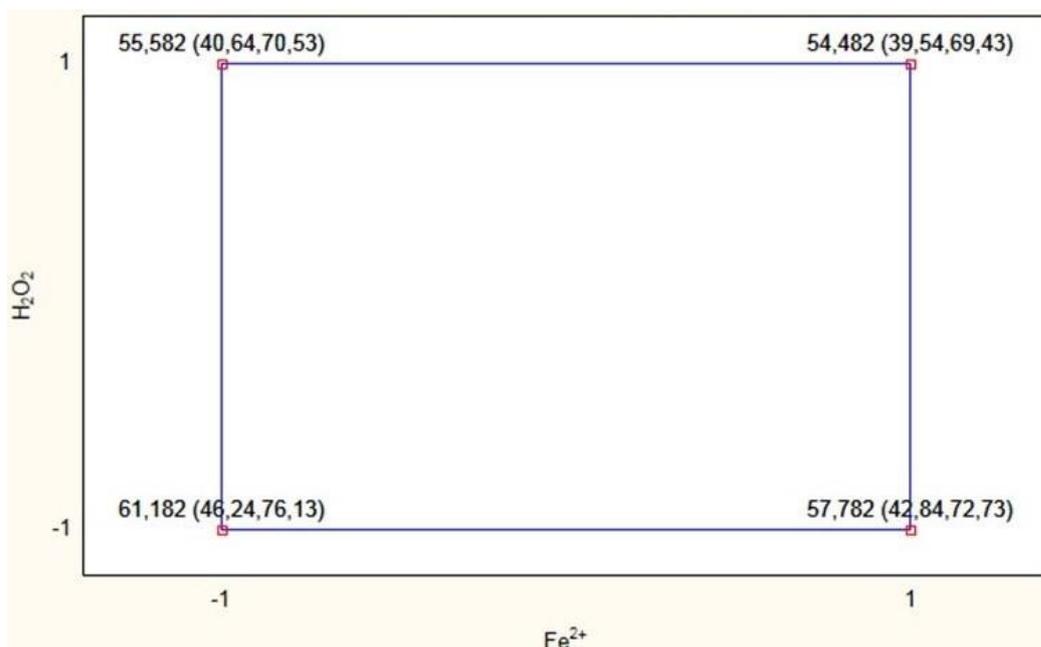
The effect of relevant experimental variables, such as  $\text{Fe}^{2+}$  and  $\text{H}_2\text{O}_2$  concentrations, on the efficiency of the Fenton process was investigated using a factorial design. The  $2^2$  experimental matrix and the average central points are presented in Table 2. The variables evaluated were the concentrations of  $\text{Fe}^{2+}$  (5–15  $\text{mg L}^{-1}$ ) and  $\text{H}_2\text{O}_2$  (50–150  $\text{mg L}^{-1}$ ) at pH 3. The BTX concentration was measured at 30 min and response expressed in removal percentage of BTX.

**Table 2.** Experimental matrix describing the factorial design and the average central point.

Experiment	Coded variables		Removal of Benzene %	Removal of Toluene %	Removal of Xylene %
	$\text{Fe}^{2+}$ ( $\text{mg L}^{-1}$ )	$\text{H}_2\text{O}_2$ ( $\text{mg L}^{-1}$ )			
1	-1	-1	64.0	64.5	74.1
2	1	-1	60.6	61.7	72.7
3	-1	1	58.4	60.8	68.9
4	1	1	57.3	62.1	70.8
5; 6; 7	0	0	53.5	58.4	67.2

**Experiment 5; 6; 7 (central point) 2.4%**

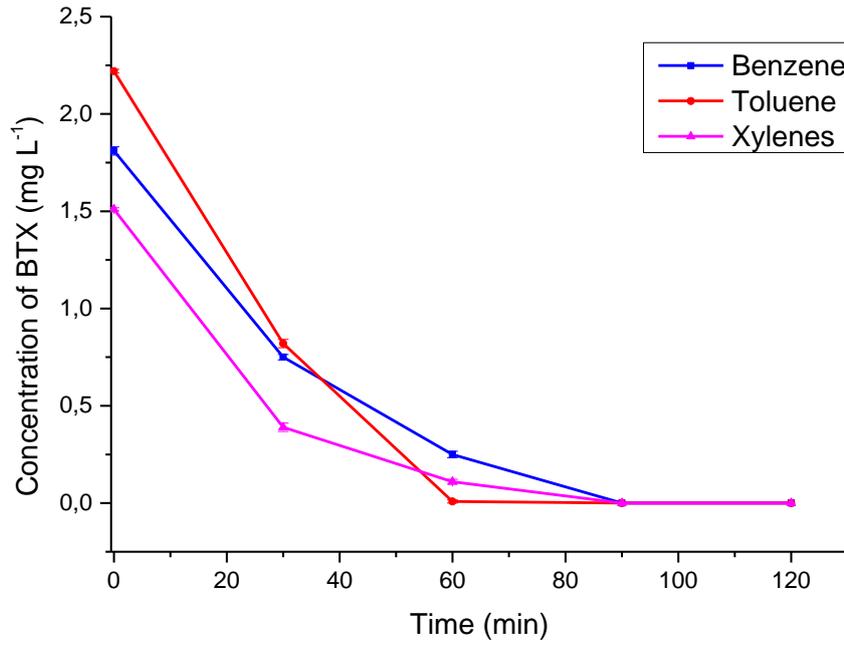
The Figure 1 shows the geometric representation of chemometric response obtained by experimental design  $2^2$  with central point. The axes represent the variables, concentration of iron ions and hydrogen peroxide, which responses were observed after 30 minutes.



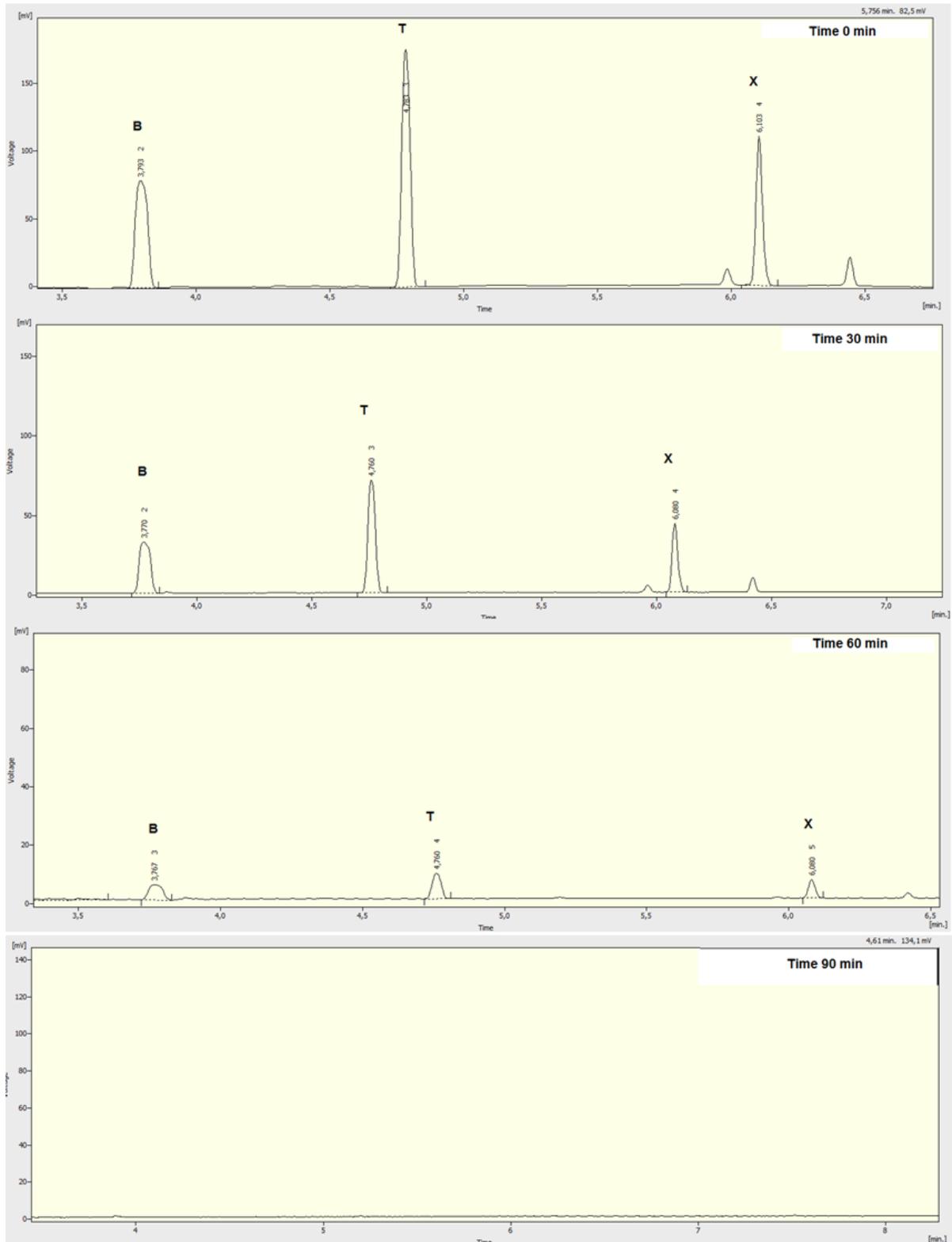
**Figure 1.** Geometric representation graph showing standardized effect estimate for BTX degradation.

## Degradation Study

After the preliminary study described above, the degradation experiments were carried out to evaluate the efficiency of the Fenton using the lower concentration of reagents. The results are presented in Figure 2 and 3. The high degradation efficiency by Fenton process was observed since at 90 min the concentration measured was lower than the quantification limit (0.05  $\text{mg L}^{-1}$ ).



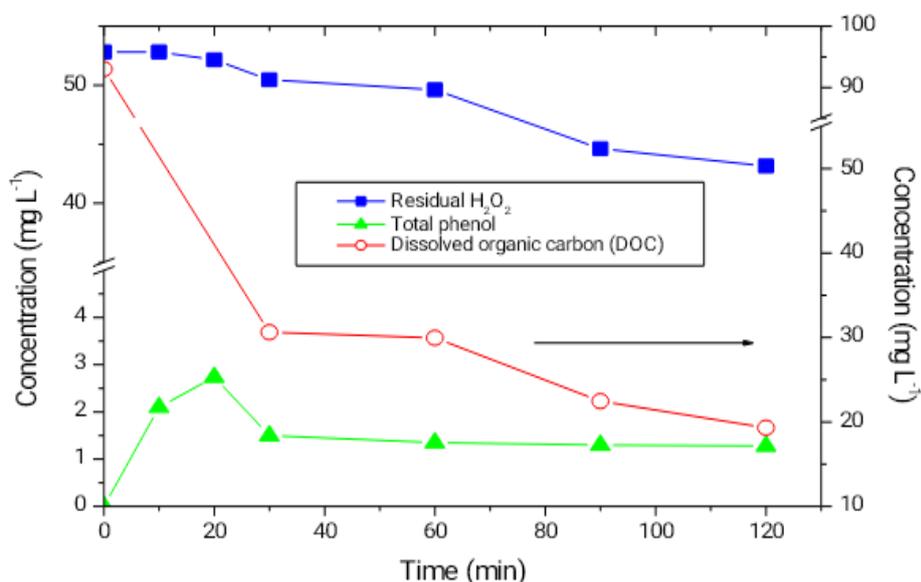
**Figure 2.** Kinetic degradation of BTX using Fenton.  $[\text{Fe}^{2+}] = 5 \text{ mg L}^{-1}$ ;  $[\text{H}_2\text{O}_2] = 50 \text{ mg L}^{-1}$ ; pH = 3.



(b)

**Figure 3.** Chromatograms of BTX degradation.  $[\text{Fe}^{2+}] = 5 \text{ mg L}^{-1}$ ;  $[\text{H}_2\text{O}_2] = 50 \text{ mg L}^{-1}$ ; pH = 3.

In the present study, additional experiments were conducted to evaluate the phenol compounds formed during the Fenton and the dissolved organic carbon (DOC) removal (Figure 4).



**Figure 4.** Variation of total phenol and peroxide consumption during the degradation of BTX by Fenton and photo-Fenton processes.  $[\text{Fe}^{2+}] = 5 \text{ mg L}^{-1}$ ;  $[\text{H}_2\text{O}_2] = 50 \text{ mg L}^{-1}$ ;  $\text{pH} = 3$ .

As presented in Figure 3, the results indicate that the Fenton process produces a significant concentration of phenolic compounds in the first 20 min, and further decrease was observed at the end of the experiment. The peroxide oxygen consumed during the Fenton process also evaluated (Figure 3).

### Ecotoxicity Assays

In addition to the commonly used physical–chemical parameters, toxicity tests have been used to assess the quality of effluent and contaminated water after treatment. According to Antonopoulou and co-authors [30], the toxicity evaluation during the degradation process is essential for assessing the feasibility of water treatment. The bioassays may be important for obtaining information about safe disposal. In the present study, the ecotoxicity before and after the Fenton and photo-Fenton process was evaluated using *Sinapis alba* and *Daphnia magna*.

Phytotoxicity was evaluated using *S. alba* (mustard) seeds. The toxic effects were expressed as a function of the seed germination rate and root elongation endpoints. The results are presented in Table 3.

**Table 3.** Evaluation of the phytotoxicity of water contaminated with gasoline using *Sinapis alba* seeds before and after treatment with Fenton.

Sample	Mean root length (cm)	RGI	GI%	Effect
Control	$5.8 \pm 1.5^a$	-----	----	-----
Fenton 0	$5.4 \pm 1.4^{a*}$	0.9	100	NSE
Fenton 120	$1.8 \pm 0.4^{b**}$	0.3	100	I

Fenton 0: initial time; Fenton 120: after 120 min of treatment; GI%, germination index; I, inhibition; NSE, no significant effect; RGI, relative growth index. Mean values with different letters (a, b, c) are significantly different (Tukey's test,  $p < 0.05$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ .

The acute toxicity was also evaluated using *D. magna*, and the results are presented in Table 4. Part of the acute toxicity was removed after the Fenton process, which was based on the observation of a decrease in the immobility of organisms (from 30% to 10%) and the toxicity factor (2 to 1).

**Table 4.** Evaluation of acute ecotoxicity in relation to *Daphnia magna* before and after treatment by Fenton process.

Sample	Immobility (%)	TF
Control	100	-----
Fenton 0	30	2
Fenton 120	10	1

Fenton 0: initial time; Fenton 120: after 120 min of treatment; TF: toxicity factor

## DISCUSSION

The medium concentration of BTX measured (2 mg L<sup>-1</sup> of which one) in water after simulation of contamination of passive system was similar of the literature [31] and also with their solubility in water [8, 9]. Differently, Andrade and co-authors [32] obtained higher concentrations of BTX (>3 mg L<sup>-1</sup>) and the main difference observed in these concentrations was due the procedure during simulation of spill, the sample was obtained after agitation between gasoline and water. The BTX, C<sub>3</sub>-alkylbenzes and naphthalene are hydrocarbon compounds that are the major of environmental concern. However, the gasoline is mixture of hundreds of hydrocarbons from C<sub>3</sub> to C<sub>12</sub> range [33] and the significant concentration of DOC obtained (≈90 mgC L<sup>-1</sup>) was expected due the presence of these others hydrocarbon in aqueous solution such as n-propilbenzene, ethylbenzene, naphthalene and others. [31]

The region studied for planning design was used considering the concentration range of hydrogen peroxide quantity necessary for total mineralization of BTX (Equation 2) [34] and the relation of Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> 1:10. Concentration of Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> are important because their affect Fenton process performance. In addition, excessive concentration of the H<sub>2</sub>O<sub>2</sub> increase operational costs and enhance the scavenging effect of HO· by H<sub>2</sub>O<sub>2</sub> (Equation 3). [12]



The response obtained from the removal of the three hydrocarbons in all experiments from planning design followed the same behaviour (Table 2). The analysis of variation (ANOVA) was used to evaluate the statistical difference between of response of BTX and the results showed that it was not difference significant and the percentage removal of benzene was used because benzene its chemical structure is the most stable among the three aromatic hydrocarbons.

The response observed (Table 2), showed that when the lowest level of both Fenton reagents (assay 1 and 4) increased to highest level the lower removal rates of BTX was obtained. The experiment 3 and 1, in which the lowest level of ions Fe<sup>2+</sup> was fixed and the level of hydrogen peroxide was changed from the lowest to the maximum level higher removal was obtained. In addition, when the highest level of Fe<sup>2+</sup> was fixed and the level of hydrogen peroxide was changed from the lowest to the maximum level (assay 2 and 4) the rate of removal decrease. Also, when the lowest hydrogen peroxide was fixed and the increase o Fe<sup>2+</sup> concentration the efficiency was lower (assay 1 and 2). Besides, at the highest hydrogen peroxide concentration was fixed and the level of Fe<sup>2+</sup> was changed from the lowest to the maximum level minor efficiency was observed (assay 3 and 4). At last, when the experimental conditions were adjusted to the lowest level (50 mg L<sup>-1</sup> of H<sub>2</sub>O<sub>2</sub> and 5 mg L<sup>-1</sup> of Fe<sup>2+</sup>) higher percentages of benzene degradation were obtained more of 64% for all BTX (Figure 1). The better response observed at low levels could be due to excess H<sub>2</sub>O<sub>2</sub> and Fe<sup>2+</sup> can decrease the efficiency of the process. These species can react with hydroxyl radical, the main radical species reported as responsible for the degradation of organic compounds, producing radicals with less oxidation power such as HO<sub>2</sub>· (E<sup>0</sup>=1.5 V) when compared with HO· (E<sup>0</sup>=2.8 V) (Equation 3). Also, de excessive Fe<sup>2+</sup> enhances the scavenging effect HO· by Fe<sup>2+</sup> (Equation 4). Besides, the recombination of hydroxyl radical can take place and decrease the efficiency of Fenton process (Equation 5).



The analysis of Pareto with 95% of confidence level showed that the hydrogen peroxide variable had the most significant effect peroxide variable had the most significant effect. The H<sub>2</sub>O<sub>2</sub> is the most important operational parameter because affect the performance Fenton process and the cost [35]. The H<sub>2</sub>O<sub>2</sub> concentration control supply of HO· as commented before (Equation 1 and 2), but the excess can cause

decrease of efficiency (Equation 4 e 5) [35]. And none of these effects were statistically significant in terms of the efficiency of BTX degradation. For this reason, the studies of degradation were carried out using a low concentration of iron and peroxide. The main advantage of these operational conditions is low quantities of reagents that decrease of cost of treatment and the same time the concentration of  $Fe^{2+}$  is according to the Brazilian law that establish maximum of  $15\text{ mg L}^{-1}$  to effluent discharge.

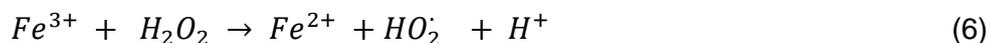


Equally important is the volatilization of BTX during the assays and under these conditions the volatilization of benzene, toluene and xylenes were  $5.67\pm 0.01\%$ ,  $6.96\pm 0.01\%$  e  $7.60\pm 0.01\%$ , respectively at 30 min. However, considering the degradation mechanism by hydroxyl radical, the intermediates formatted during the first minutes of reaction present higher polarity the parent compounds. Consequently, it is expect that these intermediates have more solubility in water than parent compounds and lower volatilization than observed in this experiment.

After setting the quantities of Fenton's reagents, the degradation experiments were carried out to evaluate the efficiency of the Fenton process. The high efficiency was observed at 90 min (Figure 3a), the BTX concentration measured was lower than the quantification limit ( $0.05\text{ mg L}^{-1}$ ) (Figure 3b) and this value observed is in compliance of ordinance with CONAMA 430 for discharge of effluent that is  $0.5\text{mg L}^{-1}$ . Lou and Lee [14] observed similar results in terms of the way in which the BTX solution ( $1\text{ mg L}^{-1}$ ) was effectively oxidized in an aqueous solution with the Fenton reagent at 10 min.

According to the literature [36], the degradation of benzene by the Fenton system is accompanied by the formation of by-products, such as biphenyl, and further oxidation compounds of phenol. Also, the photochemical degradation of aromatic compounds such as BTX involves the preliminary addition of hydroxyl radicals and the generation of phenolic compounds [37]. Recently, [38] studying the performance of ferrate (VI) in the degradation of halogenated benzene, showed that they were able to identify degradation products using the gas chromatography-mass spectrometry (GC/MS) method. Intermediate chlorobenzene products such as 3-methylbut-1-ene and 2,2-dichloroacetic acid; and bromobenzene sugar as isobutyraldehyde and ethane-1,2-diol, were identified. [39] studied the benzene, toluene, ethylbenzene, and xylene (BTEX) using  $CaO_2$  based Fenton system and based on the detected intermediates products by GC/MS the BTEX degradation pathways were proposed. According to the authors, the  $HO\cdot$  and intermediates radicals contributed to BTEX degradation and  $HO\cdot$  is the key active radical to degradation leading to benzene rings opening and complete mineralization.

This way, the variation of total phenol during the reaction may provide complementary information with respect the efficiency of system to by-products degradation. The maximum of phenol total produced during the Fenton process observed was at 20 min (Figure 2) and after this time the concentration decrease demonstrating that phenolic compounds are degraded with the reaction time. However, after 60 min phenolic compound concentration maintained constant until at 120 min. The consumption of peroxide consumed during the reaction is agreement with phenolic compounds evaluation significant decrease in  $H_2O_2$  after 20 min of reaction time but the significant concentration stable until the 120min. The reason of this performance was due the fact that the reaction rate of  $Fe^{3+}$  ions and  $H_2O_2$  is much slower than the decomposition of  $H_2O_2$  in the presence of  $Fe^{2+}$  [40] about 6000 times, which stop the efficient cycling of  $Fe^{3+}$  and  $Fe^{2+}$  and leads to accumulation of  $Fe^{3+}$  in the solution (Equation 6) [12].



The results, presented in Figure 3, showed significant removal of DOC ( $80 \pm 2\%$  at 120 min). The fraction of DOC remaining after 2 hours was probably due to the presence of phenolic compounds measured by colorimetric method and other by-products does not quantified in this study (Figure 4). The benzene has stable structure, but many intermediates are generated during the oxidation of benzene such as phenolate species, quinone, maleates, acetates, and ketones [41]. These results showed the incomplete mineralization of the organic compounds present in the water contaminated with BTX. Fenton process can be limited by accumulated  $Fe^{3+}$  and by  $H_2O_2$  concentration which is the main source of hydroxyl radical [39]. The low  $H_2O_2$  consumption observed during the process indicated that although the experiments were carried out at a low concentration of ions ferrous and  $H_2O_2$  the dose of  $H_2O_2$  used was not the optimum concentration for achieving total mineralization. For this reason, to improve the concentration of hydroxyl radicals and achieve better results of mineralization the planning design needs to be conducted using DOC as the response. Besides, unlike results can be observed in different matrixes. Natural water sources have an important content of natural organic matter and inorganic ions such as  $HCO_3^-$ ,  $NO_3^-$ ,  $NO_2^-$ ,  $Cl^-$ ,  $SO_4^{2-}$  and  $NH_4^+$  and these compounds can be scavenged by organic matter and some ionic species [42]. [39] studied the BTEX

degradation in ultrapure water and groundwater, the results showed that the components of groundwater affect the reactive species in the system. Besides, the authors demonstrated that different molar rates (CaO<sub>2</sub>/Fe(II)/BTEX) also influence the degradation of BTEX. Comparing these rates using rates of (5/5/1), (10/10/1), and (40/40/1) the decrease in efficiency was by half, one third, and only 10%, respectively.

In particular, the data of toxicity of BTX are relatively abundant and including algae, daphnia, and fish [43]. However, during the degradation process BTX concentration decrease with the reaction time and at same time are producing others compounds with different characteristics physical chemical and toxicity. The ecotoxicity can be an indicator to evaluate the level of conservation of living biota and integrity of the environmental services provided by it. Some chemicals have toxic effects when released into water bodies in concentrations that affect living organisms, the function and structure of the effects of isolates substances, but also the interaction between them in each environment, disturbing the existing balance between organisms. Synergistic, additive, and antagonistic interactions that can increase or reduce the harmful effects on aquatic organisms should be considered [44].

The results presented in Table 3 show that the aromatic hydrocarbons present in water contaminated with gasoline at low concentration ( $\pm 2\text{ mg L}^{-1}$  of BTX) did not caused any adverse effects on seed germination or root elongation. As commented before, it was evaluated the volatilization of BTX until 30 minutes for estimate the percentage of BTX volatilized during the planning design and after 30 min almost 6% of BTX were volatilized. For this reason, any adverse effect was not detected probably due aromatic compounds lost during experiment time that carried out until 120 hours. Usually, the germination taken place several days and can be divided into three steps, inhibition, radicle emergence and radicle elongation. The uptake of water is the major process of seed germination during the first step which can affect by high salinity of compost. The radicle emergence the low molecular weight organic acids of compost could be the primary inhibitor and radicle elongation could be inhibited by NH<sub>4</sub><sup>+</sup>. This speculation partly supports can be used to examine the compost with high toxicity and radicle growth can be used to examine the compost with low toxicity [20]. Servedrup and co-authors [45] investigated the effect of eight polycyclic aromatic compounds (PAHs) on the seed germination and root grow of three terrestrial plants (*Sinapis alba*, *Trifolium pratense* and *Lolium perenne*) and comparing the toxicity of four PAHs for each of the three species the studies showed that the relative effects from four substances differed between species and exposure levels. Then, showing a rather large difference in sensitivity between species studied.

However, after the treatment significant decrease in the growth of radicle was observed, which may be associated with the formation of by-products, such as phenolic compounds detected after 10 min of reaction time (Figure 3) and, also DOC residual at 120 min. Although, the mechanism of degradation is not determinate in this study the main pathway according to literature degradation of benzene under advanced oxidation process follow hydroxyl radicals degrade through electrophilic substitution, addition reaction and oxidation with bond breaking. Also, oxalic acid, alcohols, acetic and other small molecule acids are producing from the ring-opening of benzoquinone by HO<sup>•</sup>; and finally mineralized to CO<sub>2</sub> and H<sub>2</sub>O [46]. For instance, carboxylic acids may be considered phytotoxic, Himanen and co-authors [47] studied the phytotoxicity of the six carboxylic acids (C<sub>1</sub>–C<sub>6</sub>): formic, acetic, propionic, butyric, valeric, and caproic. These authors measured the acute toxicity (72 or 120h) and subchronic (21 days) and the results showed a trend that toxicity increases with the length of the carbon chain. Zazo and co-authors [48] studied the evolution of intermediates and the ecotoxicity during the oxidation of the Fenton reagent in aqueous solution. The results showed that some intermediates, mainly hydroquinone and p-benzoquinone, demonstrated toxicity levels much higher than phenol itself. On order hand, by-products generated, and their concentrations may be different depending on the operating conditions, and those differences may affect the ecotoxicity response.

Differently the using *D. magna* as bioassay the results showed that water contaminate with BTX is toxic indicating that BTX present cause environmental risk due the acute toxicity, but after 120 min the toxicity reduced significantly more than 50%. Consequently, *D. magna* bioassay was more sensitivity to the aromatic hydrocarbons present after simulation of spill (initial time) (Table 4) than the tests using *S. alba*. Particularly, comparing the results of chemical analysis (DOC and total phenol) the bioassay using as bio indicator demonstrated that *D. magna* can be a good tool to indicate the efficiency of Fenton process for mineralization. Even though after 90 minutes the concentration of BTX was lower the limits by Brazilian law for effluent discharge the bioassay show that effluent can cause deleterious effect in aquatic system. This way, the bioassay can reflect the capacity of mineralization of BTX since the DOC removal and decrease of total phenol is concordance with lower toxicity after 90 min of reaction time. According Salomão and co-authors [22], continuous or frequent verification of contaminant concentrations in the water medium should be carried out, especially for intermittent and discontinuous discharges. In addition, analytical methods limitations must be observed when evaluation contaminant concentration in the assessed bioindicators, their pathways and

potential degradation products. Furthermore, the mixtures of these compounds can bring information with respect synergism and antagonism that can induces higher or lower toxicity [49].

## CONCLUSION

The high efficiency of Fenton process was observed on removal BTX present in water samples artificially contaminated with commercial gasoline. At 90 min of reaction time concentration measured was lower than limit of quantification using gas chromatography as analytical method ( $<0.05 \text{ mg L}^{-1}$ ) thus according to Brazilian legislation regarding the discharge of effluents. Although, the significant DOC removal and total phenol were observed after 120 min significant residual concentration of DOC and total phenol remained. The ecotoxicity studies demonstrated that the *Daphnia magna* was more sensible bioindicator. However, the volatile characteristics of aromatic hydrocarbon may had interfered on response observed in assays using *Sinapis alba*. Also, increase of toxicity was observed showing presence of toxic substances, for example, phenolic compounds measure by colorimetric method. Besides, the bioassay using *Daphnia magna* could be a good tool for evaluation of mineralization because the decrease of toxicity followed same behaviour with reduction of DOC and total phenol, showing residual toxicity after 120 min. The main advantages of the ecotoxicity tests are their simplicity and low cost comparing the physical-chemicals analysis and can be a good alternative to evaluate the efficiency of Fenton process considering the by-products generated during the process.

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