

Electrochemical Post-Treatment of Constructed Wetland Effluent for Enhanced Ketoprofen Removal and Toxicity Reduction

Isabel Maximo Corrêa Alcantara

Federal University of Mato Grosso do Sul

Thalita Ferreira Silva

University of São Paulo

Priscila Sabioni Cavalheri

Federal University of Mato Grosso do Sul

Emmanuel da Silva Côgo Miguel

Federal University of Mato Grosso do Sul

Antonio Kaique Canatto

Federal University of Mato Grosso do Sul

Lucas Melo da Silva

Unigran Capital University Center

Fernando Jorge Correa Magalhães Filho

Federal University of Rio Grande do Sul

Osvaldo Chiavone Filho

Federal University of Rio Grande do Norte

Marcos Roberto Vasconcelos Lanza

University of São Paulo

Anderson Rodrigues Lima Caires

Federal University of Mato Grosso do Sul

Amilcar Machulek Junior

`amilcar.machulek@ufrn.br`

Federal University of Rio Grande do Norte

Research Article

Keywords: Emerging contaminants, electro-Fenton, nature-based solutions, experimental design, ecotoxicity, ketoprofen

Posted Date: October 6th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11089134/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Electrochemical Post-Treatment of Constructed Wetland Effluent for Enhanced Ketoprofen Removal and Toxicity Reduction

Isabel Maximo Corrêa de Alcantara^a, Thalita Ferreira da Silva^b, Priscila Sabioni Cavalheri^c, Emmanuel Da Silva Cogo Miguel^c, Antonio Kaique Canatto^a, Lucas de Melo da Silva^d, Fernando Jorge Correa Magalhães Filho^c, Osvaldo Chiavone Filho^f, Marcos Roberto de Vasconcelos Lanza^b, Anderson Rodrigues Lima Caires^c, Amilcar Machulek Junior^{f*}

^a Faculty of Engineering, Architecture and Urbanism, and Geography, Federal University of Mato Grosso do Sul, Costa e Silva Avenue, s/n, Campo Grande, MS, Brazil.

^b São Carlos Institute of Chemistry, University of São Paulo, Trab. São Carlense Avenue, 400, São Carlos, SP, Brazil.

^c Institute of Physics, Federal University of Mato Grosso do Sul, UFMS St, s/n, Campo Grande, MS, Brazil.

^d Unigran Capital University Center, Abrão Júlio Rahe St, 325, Campo Grande, MS, Brazil.

^e Institute of Hydraulic Research, Federal University of Rio Grande do Sul, Bento Gonçalves Avenue, 9500, Porto Alegre, RS, Brazil.

^f Institute of Chemistry, Federal University of Rio Grande do Norte, Senador Salgado Filho Avenue, s/n, Natal, RN, Brazil.

*Corresponding author:

E-mail addresses: amilcar.machulek@ufrn.br, machulekjr@gmail.com

ABSTRACT

Emerging contaminants such as pharmaceuticals are poorly removed by conventional sewage treatment plants, posing risks to ecosystems and human health. Ketoprofen (KET), a widely used non-steroidal anti-inflammatory drug frequently detected in wastewater, was selected as a target contaminant. This study evaluated its removal from real sewage treatment plant effluent using an integrated system that combines a vertical flow constructed wetland (CW), a nature-based solution relying on biological and physical processes, with electrochemical advanced oxidation processes, namely electro-Fenton (EF) and photoelectro-Fenton (PEF). Both combinations, CW+EF and CW+PEF, achieved KET removal above 99.0%, with CW+PEF reaching 99.3% degradation and 45.0% mineralization, while also improving effluent quality (COD, BOD, and turbidity reductions of 86.6%, 93.5%, and 95.2%, respectively). Acute toxicity to *Danio rerio* embryos was reduced by 87.6% under CW+PEF, reclassifying the effluent from acutely to mildly toxic, while toxicity to *Artemia* sp. was eliminated and no phytotoxic effects were observed on *Lactuca sativa* (germination index > 80.0%) across all treatments. A comparative operational cost analysis further showed that CW+PEF offers the best balance between treatment performance and operating costs (77.49 USD m⁻³, versus 81.24 USD m⁻³ for CW+EF), with markedly lower energy consumption per gram of TOC removed (10.7 vs. 33.6 kWh g⁻¹ TOC). Overall, the results indicate that coupling nature-based solutions with advanced oxidation technologies is an effective and sustainable strategy for wastewater treatment, enhancing contaminant removal without pH adjustment and with reduced environmental impact.

Keywords: Emerging contaminants, electro-Fenton, nature-based solutions, experimental design, ecotoxicity, ketoprofen.

1. INTRODUCTION

Pharmaceutical products comprise a wide variety of compounds designed to produce specific biological responses and are extensively used in human and veterinary medicine for disease prevention and treatment (Khasawneh and Palaniandy 2021; Chafi et al. 2022). However, their frequent detection in effluents and aquatic environments has become a growing environmental concern (Schwartz et al. 2021; Shahid et al. 2021; Parida et al. 2021; Gupta et al. 2023). The complex chemical structure of many of these compounds hinders the effectiveness of conventional wastewater treatment processes in their removal (Majumder et al. 2019; Sánchez et al. 2022; Attig et al. 2023). As a result, these pollutants can persist in the environment and enter receiving waters through wastewater treatment plant effluents (Pacheco et al. 2021; Lopez-Herguedas et al. 2023; Andrade et al. 2024).

Ketoprofen (KET), chemically known as 2-(3-benzoylphenyl) propionic acid, is a non-steroidal anti-inflammatory drug frequently detected in aquatic environments (Guelfi et al. 2019; Casas and Matamoros 2020; Alkimi et al. 2020; Bayati et al. 2021; Ávila et al. 2021; Schwartz et al. 2021; Tyumina et al. 2023). Its

1 persistence in different water matrices raises environmental concern because it may affect aquatic organisms
2 (Alkimin et al. 2020; Tyumina et al. 2023). In addition, its potential to bioaccumulate can impact multiple
3 trophic levels, compromise aquatic biodiversity, and potentially pose risks to human health through the
4 consumption of contaminated water and food.

5 Given the limitations of conventional treatments in removing emerging contaminants such as KET, sustainable
6 alternatives have been widely investigated. Constructed wetlands (CWs), as nature-based solutions (NbS), have
7 shown good performance in the removal of emerging contaminants, including pharmaceuticals (Bayati et al.
8 2021; Ávila et al. 2021; Wu et al. 2026). These systems depend on natural processes and the interaction among
9 vegetation, soil, and microbial communities to promote effluent treatment (Cavalheri et al. 2023a; Montenegro-
10 Apraez and Machuca-Martínez 2023). However, complete removal of pharmaceuticals by CWs alone remains
11 challenging, as these systems may be insufficient for degrading highly persistent compounds. For this reason,
12 combining CWs with other treatment technologies has emerged as a promising approach (Casas and Matamoros
13 2020). In this context, a pilot-scale vertical flow constructed wetland integrated with solar photo-Fenton at
14 natural pH achieved 39.0% organic matter removal by the CW stage and up to 95.0% ketoprofen degradation
15 upon coupling with the advanced oxidation processes (AOPs) step (Cavalheri et al. 2023a), illustrating the
16 potential of hybrid CW-AOP systems for pharmaceutical removal.

17 Among the complementary technologies, AOPs stand out due to their ability to generate highly reactive
18 hydroxyl radicals ($\text{HO}^\bullet$), which promote the degradation of emerging contaminants (Friedrich et al. 2012;
19 Teodoro et al. 2014; Guelfi et al. 2019; Cavalcante et al. 2021; Cavalheri et al. 2023a; Montenegro-Apraez and
20 Machuca-Martínez 2023; López-Vinent et al. 2024). Photo-Fenton-based systems are particularly promising
21 within this group. However, electrochemical advanced oxidation processes (EAOPs) have also shown
22 significant potential for wastewater treatment, offering operational simplicity, versatility, and high efficiency
23 (Poyatos et al. 2010; Sirés et al. 2014; Gozzi et al. 2017; Sánchez et al. 2022). The conventional Fenton process,
24 based on the reaction between hydrogen peroxide (H_2O_2) and ferrous ions (Fe^{2+}) to generate hydroxyl radicals,
25 is widely used but generates large volumes of $\text{Fe}(\text{OH})_3$ sludge, which increases operational costs and limits its
26 practical application.

27 To mitigate this limitation, the electro-Fenton (EF) process was developed, allowing the *in situ* generation of
28 H_2O_2 through the electrochemical reduction of oxygen, thereby reducing the need for external peroxide addition
29 and minimizing sludge formation (Sirés et al. 2014). The incorporation of ultraviolet (UV) radiation into the
30 EF system leads to the photoelectro-Fenton (PEF) process, which further enhances contaminant degradation
31 via Fe^{2+} regeneration and additional $\text{HO}^\bullet$ production (Sirés et al. 2014).

32 This study aimed to evaluate the combination of a vertical flow CW (VFCW) with EAOPs, operating at near-
33 neutral pH, for the removal of ketoprofen from real wastewater treatment plant effluents. This is the first report
34 in the literature on the integration of these two technologies applied to the treatment of real wastewater.
35 Physicochemical parameters and acute toxicity were assessed in the treated effluents using *Artemia sp.*, *Lactuca*

sativa, and zebrafish embryos to verify the system's efficiency in reducing pollutant levels and environmental risk.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Ketoprofen (KET, CAS 22071-15-4, 99.8% purity) was purchased from Sigma-Aldrich. Anhydrous sodium sulfate (99.0% purity) and sodium hydroxide (97.0% purity) were obtained from Vetec, hydrated ferric sulfate (77.4% purity) from Neon, and trisodium citrate (99.9% purity) from Alphatec. LC-MS-grade acetonitrile (J.T. Baker) and methanol (Merck) were used for chromatographic analyses. Deionized water (resistivity > 18.0 MΩ cm) was produced using a reverse-osmosis system (Gehaka, model OS50 LX).

2.2 Study area, wetland operation, and wastewater sampling

Wastewater was sampled at the outlet of a pilot-scale, partially saturated vertical flow constructed wetland (VFCW). The system was installed at a municipal wastewater treatment plant (WWTP) in Campo Grande, Mato Grosso do Sul, Brazil (latitude 20°33'28.8"S, 54°39'35.7"W), FigS1 (see Supplementary Material). Primary treatment at the WWTP is provided by upflow anaerobic sludge blanket (UASB) reactors with a capacity of 1100 L s⁻¹. UASB effluent is normally discharged directly to the Anhanduí River (Class IV, per Brazilian environmental legislation) (Mazucato et al. 2020). For this study, a slipstream of UASB effluent was diverted to feed the pilot VFCW.

Further details on the UASB reactor and the VFCW have been described in our previous studies (Cavalheri et al. 2023b; Miguel et al. 2024). To evaluate KET degradation and mineralization in a real wastewater matrix, the reservoir feeding the VFCW was spiked with KET at a concentration selected to ensure reliable quantification. The drug was added at the mg L⁻¹ level to enable degradation and mineralization studies, considering the detection limits of the techniques used, such as Total Organic Carbon analysis (TOC) and High-Performance Liquid Chromatography (HPLC).

In a preliminary trial, the VFCW outlet pulse was monitored for 10.0 h to identify the time point corresponding to the highest KET concentration. For this purpose, sequential effluent samples were collected at the system outlet, extracted by solid-phase extraction (Strata C18-E cartridges, 55 μm, 70 Å, 500 mg/6 mL, Phenomenex), following Cavalheri et al. (Cavalheri et al. 2023b), and analyzed by Total Organic Carbon (TOC) and High-Performance Liquid Chromatography (HPLC). The peak occurred approximately 8.0 h after feeding began; therefore, the effluent used in all AOP experiments was collected at this 8.0 h time point and stored at 4.0 °C until treatment.

KET concentration was determined by HPLC using a Shimadzu system (SPD-M20A) equipped with a UV-Vis detector, operating at 260.0 nm. The detailed procedures are described in the Supplementary Material. The physicochemical characteristics of the effluent from the wetland system can be found in Table S1 (see Supplementary Material).

2.3 Electrochemical and photoelectrochemical experiments

2.3.1 Experimental Setup

The electro-Fenton (EF) experiments were carried out in a jacketed electrochemical cell with a capacity of 150.0 mL, equipped with a pump for external water recirculation to maintain the temperature at 25.0 ± 2.0 °C. The electrodes consisted of a C-PTFE gas diffusion cathode (GDE, E-TEK, Somerset, NJ, USA), and a platinum plate anode (SEMPSA, Spain, $\geq 99.0\%$), both with a geometric area of 3.0 cm^2 and positioned 1.0 cm apart. The system was connected to a power supply (Instrutherm, FA-3003), and 150.0 mL of the solution was kept under constant stirring throughout the experiment (da Silva et al. 2019).

For the PEF treatment, a fluorescent tube lamp (Philips TL/4W/08, $\lambda_{\text{max}} = 360.0 \text{ nm}$, photon flux = 1.19×10^{21} photons s^{-1}) was additionally positioned above the open cell, approximately 6.0 cm from the solution surface (da Silva et al. 2019). Na_2SO_4 (0.05 mol L^{-1} , pH 6.0) was added to the wastewater as a supporting electrolyte to ensure adequate conductivity. Samples were collected at defined time intervals throughout the experiments to evaluate mineralization and the reduction in KET concentration. Anodic oxidation (AO) experiments were also performed for comparison purposes. A schematic representation of the experimental setups used in this study is shown in Fig1.

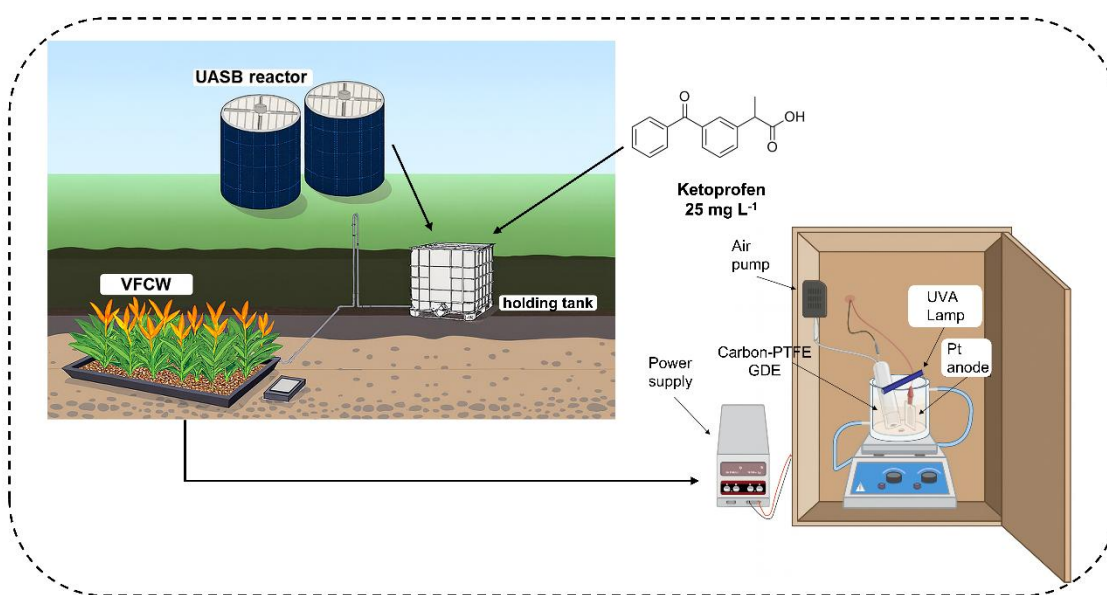


Fig1 Schematic overview of the processes applied in this work

2.3.2 Central Composite Design (CCD)

A central composite design (2^3), with the addition of triplicated central points, was employed to identify the most significant variables affecting KET degradation and the mineralization of organic matter by EF and PEF processes. Current density (j , mA cm⁻²), iron concentration ([Fe³⁺], mg L⁻¹), and the ratio between Fe³⁺ and the complexing agent sodium citrate (Na₃C₆H₅O₇) were evaluated as independent variables. The levels of these variables and the experimental range used are described in Table 1.

Seventeen experiments, including triplicate central points, were randomly generated using software Statistica Statsoft (version 12, StatSoft, Tulsa, USA) (da Silva et al. 2019; Cavalheri et al. 2023a). The resulting data were statistically analyzed by analysis of variance (ANOVA) at a 95.0% confidence level.

Table 1 Design levels and variable values, including the central point, for the EF and PEF processes

Independent Variables	Levels					
	-1.68		-1	0	+1	+1.68
j (mA cm ⁻²)	18.0	35.0	60.0	85.0		102.0
[Fe ³⁺] (mg L ⁻¹)		0.27	3.00	7.00	11.00	13.72
Fe ³⁺ :citrate ratio		0.31	1.00	2.00	3.00	3.68

2.4. Acute toxicity assays

Acute ecotoxicity was evaluated using *Artemia sp.*, *Lactuca sativa*, and *Danio rerio* as test organisms. The *Artemia sp.* assay was performed according to the Brazilian Standard NBR 16530:2016, provided by the Brazilian Association of Technical Standards (ABNT)(ABNT. NBR 16530: 2016), and the results were expressed as toxic units (TU) (Persoone et al. 2003). Phytotoxicity assays were carried out using untreated *Lactuca sativa* seeds (cultivar Aurelia; 95.0% germination rate; Topseed brand; Lot no. 065044). The detailed procedures for all toxicity assays are described in the Supplementary Material.

Adult zebrafish (*Danio rerio*) were maintained in individual tanks equipped with independent filtration systems under controlled laboratory conditions at 28.0 °C, with pH between 7.0 and 7.5 and a photoperiod of 14.0 h light/10.0 h dark. For embryo production, males and females were placed in mating tanks at a 2:1 ratio, at a density of 1.0 fish L⁻¹. After spawning and fertilization, the embryos were collected and cleaned with reverse osmosis water containing sodium hypochlorite (25.0 ppm active chlorine) to remove debris. Eggs were then incubated in E3 medium (composed of NaCl, KCl, CaCl₂, and MgCl₂) for 24.0 h at 28.0 °C. After incubation, non-viable embryos were discarded to ensure that only healthy and viable specimens were used for the toxicity assays.

Experimental exposure started at 5.0 h post fertilization (hpf), with daily medium renewal under semi-static conditions. For each treatment condition, 20 embryos were exposed in triplicate ($n = 60$ embryos per group), distributed in 24-well plates (one embryo per well). Embryonic development was staged according to Kimmel et al. (1995)(Kimmel et al. 1995), and monitoring was conducted up to 96.0 hpf following OECD Guideline No. 236 (OECD 2025). The endpoints evaluated included mortality, hatching rate, heart rate, and the incidence of morphological malformations (e.g., pericardial edema, spinal curvature, and yolk sac edema).

All toxicity assays involving zebrafish were performed in accordance with national ethical guidelines and approved by the Institutional Animal Care and Use Committee of the Federal University of Mato Grosso do Sul under protocol number 1.407/2025.

3. RESULTS AND DISCUSSION

Samples of raw wastewater and effluents treated by the UASB reactor and the vertical-flow constructed wetland (VFCW) were collected to assess the presence of KET prior to pharmaceutical spiking. Three sampling campaigns were conducted ($n = 3$), and KET concentrations were determined by high-performance liquid chromatography (HPLC) after sample preconcentration by solid-phase extraction (SPE). This step enabled analyte enrichment while minimizing potential matrix interferences.

Fig2a presents the mean KET concentrations in the influent and treated effluents, together with the corresponding removal efficiencies achieved by the UASB reactor and VFCW. Removal efficiencies were calculated based on the mean KET concentrations in the influent and effluent of each treatment system. No precipitation events were recorded during the sampling periods or in the week preceding sample collection.

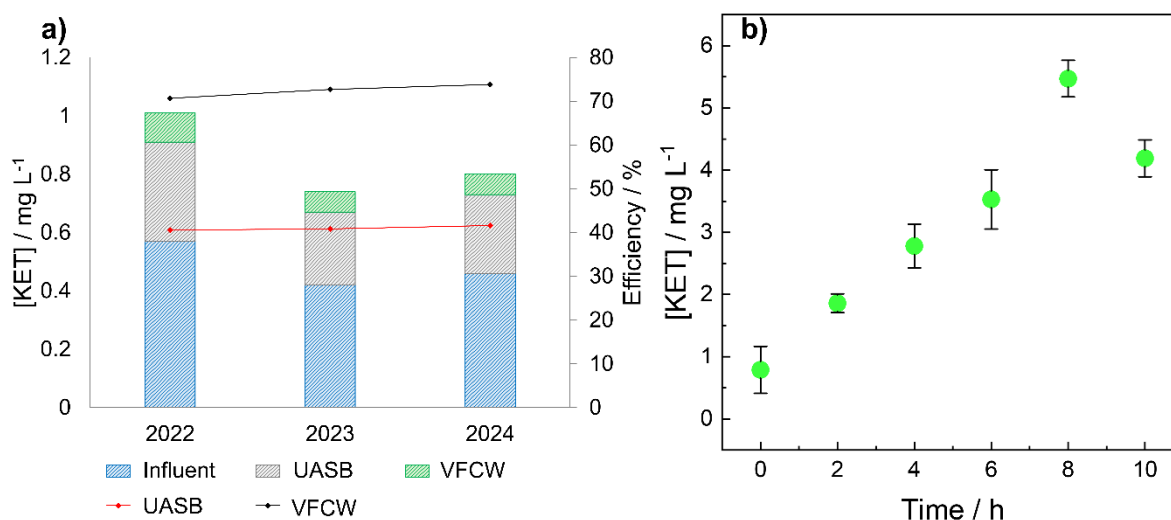


Fig2 KET removal performance and concentration behavior during wastewater treatment (a) KET concentration in raw and treated wastewater (UASB and VFCW) and removal efficiency for each treatment. (b) KET concentration profile as a function of time during the VFCW pulse mode

Fig2a demonstrates that the UASB and VFCW systems promote only partial removal of KET, with higher efficiency observed for the VFCW. However, the persistence of the pharmaceutical in the treated effluent indicates that these processes, either individually or combined, do not ensure complete removal. The outlet concentration of KET as a function of time is shown in Fig2b. The peak time was defined as the outlet time corresponding to the highest KET concentration. This peak was reached at approximately 8.0 h, with an outlet concentration of $5.47 \pm 0.29 \text{ mg L}^{-1}$. For comparison, the inlet concentration after the UASB stage was $23.71 \pm 2.64 \text{ mg L}^{-1}$, following spiking with 25.0 mg L^{-1} KET, Table S2 (see Supplementary Material). This corresponds to a KET removal efficiency of 77.0% at peak time, consistent with the 75.0% removal reported by (Cavalheri et al. 2023b).

using a similar CW system. KET was detected in all batches; in the sixth batch, however, the outlet concentration after approximately 10.0 h was lower than that observed at peak time, suggesting a decline in outlet concentration beyond this point (Fig2b). These results indicate good CW performance in contaminant removal, although post-wetland polishing treatment would be needed to achieve higher removal efficiency.

3.1 Central Composite Design for the Electro-Fenton Process

An experimental design based on Response Surface Methodology (RSM) was employed to evaluate how independent variables (factors), individually and in combination, affect the dependent variables (responses) (Lee et al. 2022). Accordingly, a 2^3 Central Composite Design (CCD), with triplicates at the central point, was applied to identify the most influential variables and the optimal experimental conditions for KET degradation in the combined CW + EF process. Table S3 (see Supplementary Material) reports the degradation percentage (%DEG) and mineralization percentage (%MIN) values obtained for the different experiments.

Among the experiments performed, the one carried out under the following operational conditions: 7.0 mg L^{-1} of $[\text{Fe}^{3+}]$, a 1:2 Fe^{3+} :citrate ratio, and a current density (j) of 102.0 mA cm^{-2} , yielded the best performance, achieving 92.7% KET degradation and 43.2% mineralization within 90.0 minutes.

Regression coefficients significant at the 95.0% confidence level, FigS2 (see Supplementary Material), were used to construct mathematical models describing %DEG and %MIN efficiencies, as shown in Equations 1 and 2, respectively. These models express the empirical relationship between the independent variables (X_i) and the response variables (Y_i) in the quadratic model.

$$\% \text{DEG} = 67.3 + 20.8j + 6.27 [\text{Fe}^{3+} : \text{citrate}] - 4.19j^2 - 6.7 [\text{Fe}^{3+}]^2 - 7.8 [\text{Fe}^{3+} : \text{citrate}]^2 + 4.39j.[\text{Fe}^{3+} : \text{citrate}] \quad (1)$$

$$\% \text{MIN} = 12.45 + 10.8j + 3.33 [\text{Fe}^{3+}] + 5.44j^2 \quad (2)$$

1 Current density was the most significant variable for both responses. Increasing j enhanced degradation and
2 mineralization, with a dominant positive linear effect on both responses, although a smaller negative quadratic
3 effect was also observed (discussed further below), indicating diminishing returns at higher current densities.
4 Pareto charts for KET degradation (Fig3a) and mineralization (Fig3b), constructed from Table S3, illustrate the
5 effects of operational parameters, their interactions, and the relationships between independent variables for
6 each response. The plots of experimentally observed versus predicted values (Figs S2a and S2b, Supplementary
7 Material) show that the statistical models are well fitted, with R^2 values of 0.9925 for %DEG and 0.9766 for
8 %MIN, indicating a good distribution of residuals around the regression line.

9 Fig3a shows that the independent variables j and $[\text{Fe}^{3+}:\text{citrate}]$, as well as the interaction $j \times [\text{Fe}^{3+}:\text{citrate}]$, were
10 significantly relevant for electro-Fenton effluent treatment, as they reached the reference line corresponding to
11 $p = 0.05$. The negative quadratic effects of j , $[\text{Fe}^{3+}]$, and $[\text{Fe}^{3+}:\text{citrate}]$ suggest a nonlinear relationship, in which
12 excessive increases in these variables may hinder the degradation process. Feng *et al.* (2014) showed that high
13 $\text{Fe}^{3+}:\text{citrate}$ ratios reduce $\text{HO}^\bullet$ production at near-neutral pH, thereby suppressing hydroxyl radical generation
14 (Feng et al. 2014). Accordingly, the signs and magnitudes of the coefficients suggest that $[\text{Fe}^{3+}:\text{citrate}]$ promotes
15 KET degradation up to a threshold, after which the effect is mitigated by the quadratic terms. The positive
16 interaction between j and $[\text{Fe}^{3+}:\text{citrate}]$ further suggests a synergistic effect, which allows simultaneous
17 adjustment of these variables to maximize degradation.

18 Fig3b further shows that j and Fe^{3+} concentration were key factors for achieving 43.2% mineralization of the
19 organic matter in 90.0 min of reaction. The variable j stood out in both its linear (L) and quadratic (Q) effects,
20 ranking first and second in importance, respectively, confirming that process optimization strongly depends on
21 this variable.

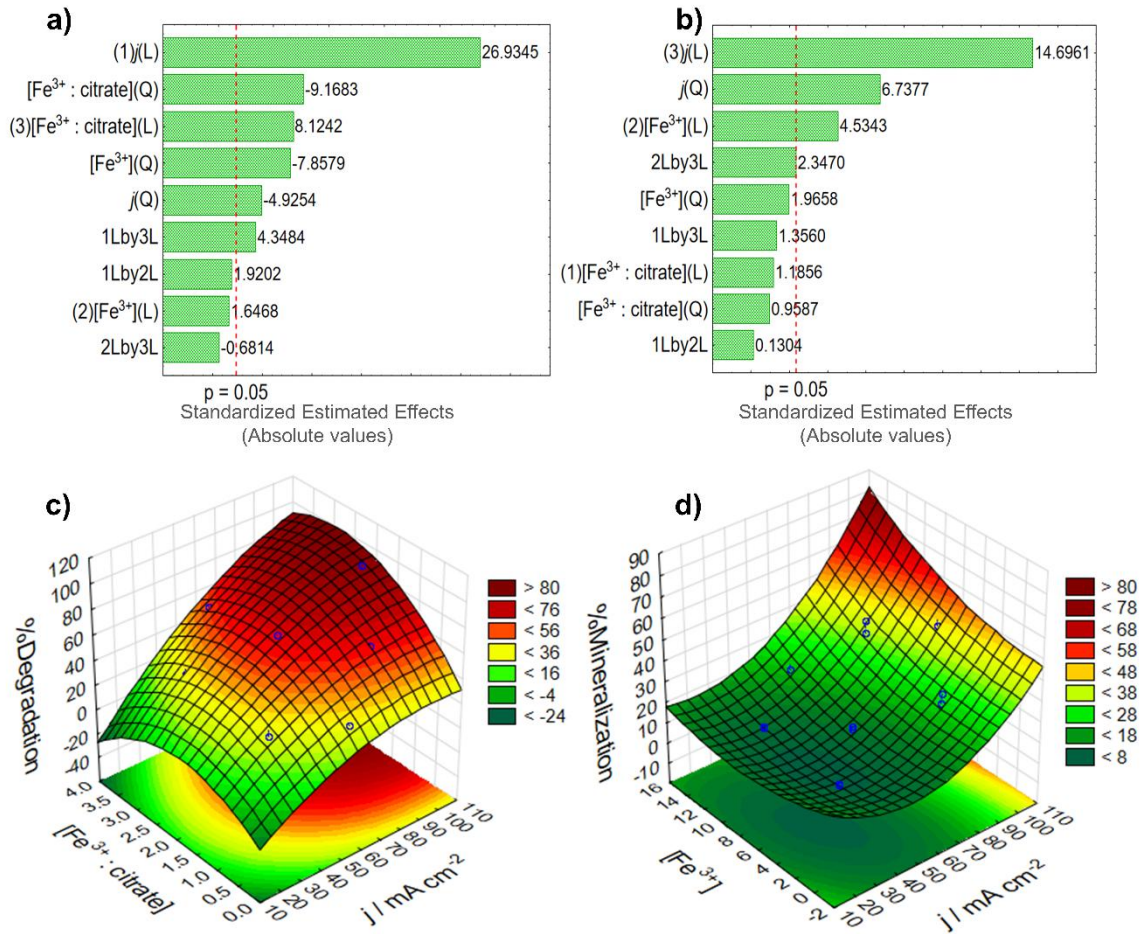


Fig3 KET removal experiments using CW and results of the 2³ Central Composite Design for the EF process
 (a) Pareto chart of the central composite design results for %DEG. (b) Pareto chart for %MIN (c) Response surface plot of %DEG (d) Response surface plot of %MIN showing the significant interaction between current density (j) and the [Fe³⁺:citrate] ratio

Surface plots were generated based on the experimental data. Fig3c shows the response surface for %DEG, illustrating the significant interaction between j and [Fe³⁺:citrate]. Higher degradation levels (%DEG > 80) are achieved at moderate to high j values and intermediate [Fe³⁺:citrate] ratios. The effect of j on the response is influenced by [Fe³⁺:citrate], indicating that these variables do not act independently. When [Fe³⁺:citrate] is at either low or high levels, the influence of j on degradation becomes less pronounced. The parabolic shape of the surface suggests that both j and [Fe³⁺:citrate] exhibit significant quadratic effects.

Regarding the mineralization of organic matter by the EF process, Fig3d suggests a synergistic effect between [Fe³⁺] and j . The effect of j is more pronounced at intermediate [Fe³⁺] concentrations, whereas very high or very low concentrations of [Fe³⁺] reduce the impact of j . The optimal region features moderate-to-high j values and controlled concentrations of [Fe³⁺].

3.2 Central Composite Design for the photoelectro-Fenton Process

To compare the results obtained through the EF process, the 2^3 CCD design was employed to investigate the variables and experimental conditions involved in the removal of KET by PEF process, using effluent collected from the constructed wetland (CW + PEF process).

The experimental results from the PEF process design corresponding to the response variables (%DEG and %MIN), are shown in Table S4 (see Supplementary Material). A 2^3 CCD was adopted, applying the independent variables and experimental ranges described in Table 1.

A maximum degradation of 99.3% for KET and 45.0% mineralization of organic matter was achieved after 90 min of reaction under the following operating conditions: 3 mg L⁻¹ of [Fe³⁺], a Fe³⁺:citrate molar ratio of 1:1, and a j of 85 mA cm⁻². Conversely, the experiment using 7 mg L⁻¹ of [Fe³⁺], a Fe³⁺:citrate molar ratio of 1:2, and a j of 17.95 mA cm⁻² showed the worst efficiency, with only 15.6% degradation and 5.7% mineralization in 90 min. These results demonstrate that the PEF process is more effective at lower concentrations of Fe³⁺ and its complex.

The statistical model shows high reliability in the estimation of both response variables, as shown in FigS3 (see Supplementary Material), with $R^2 = 0.9975$ for %DEG and $R^2 = 0.9915$ for %MIN.

ANOVA identified six statistically significant factors (p -value < 0.05) for %DEG and five for %MIN. Based on these results, regression equations were obtained for %DEG (Equation 3) and %MIN (Equation 4).

$$\% \text{DEG} = 70.34 + 24.87 j - 7.94 [\text{Fe}^{3+}] - 4.71 [\text{Fe}^{3+}:\text{citrate}] - 4.67 j^2 - 2.12 [\text{Fe}^{3+}]^2 - 2.27 [\text{Fe}^{3+}] \cdot [\text{Fe}^{3+}:\text{citrate}] \quad (3)$$

$$\% \text{MIN} = 34.39 + 12.42 j - 3.05 j^2 - 2.76 [\text{Fe}^{3+}]^2 - 2.48 [\text{Fe}^{3+}:\text{citrate}]^2 - 2.36 j \cdot [\text{Fe}^{3+}] \quad (4)$$

Pareto charts (Figs 4a and 4b), based on standardized effects from ANOVA, show the linear effect of current density j as the most significant and positive for both responses, favoring degradation and mineralization. However, its quadratic term limits the benefit beyond an optimal point.

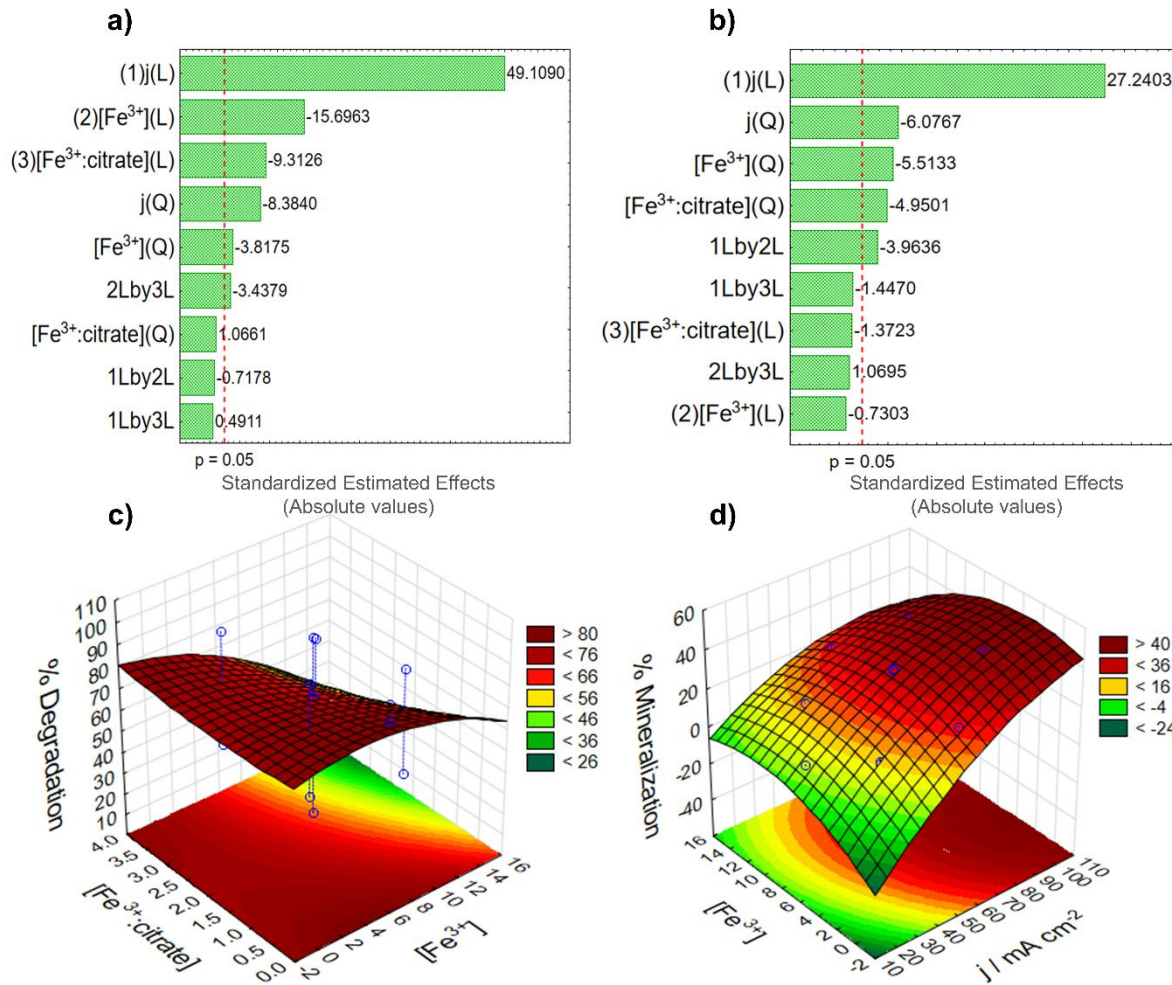


Fig4 Results of the 2³ CCD with degradation and mineralization responses for the PEF process. Pareto chart of the central composite design results for (a) %DEG and (b) %MIN. (c) Response surface plot for %DEG showing the significant interaction between [Fe³⁺] and [Fe³⁺:citrate] ratio. (d) Response surface plot for %MIN showing the significant interaction between *j* and [Fe³⁺]

The negative sign for [Fe³⁺] and [Fe³⁺:citrate] in degradation (Fig4a) indicates that higher levels decrease efficiency. In mineralization (Fig4b), high concentrations of these variables are detrimental due to negative quadratic terms and interactions. Moreover, the interactions [Fe³⁺]·[Fe³⁺:citrate] and *j*·[Fe³⁺] reveal an antagonistic effect on degradation and mineralization, respectively, reducing overall process efficiency. The response surface plot (Fig4c) illustrates how %DEG varies as a function of [Fe³⁺] and the [Fe³⁺:citrate] ratio. The inclined surface reveals a sharp decline at high [Fe³⁺], with maximum %DEG at low [Fe³⁺] concentrations (< 5 mg L⁻¹) and a Fe³⁺:citrate ratio between 1.5 and 2.5.

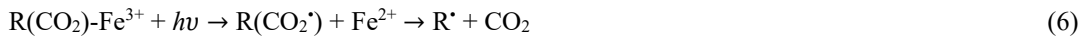
For %MIN (Fig4d), high [Fe³⁺] concentrations (> 12 mg L⁻¹) cause a steep decline, especially at low *j*. Based on the contour plot analysis, the optimal operational conditions for the interaction between these variables are achieved when *j* is between 70 and 90 mA cm⁻² and [Fe³⁺] is between 4 and 10 mg L⁻¹.

3.3 Degradation and mineralization of KET under different AOPs

After experimental planning using the 2^3 CCD, the optimal conditions for the EF and PEF processes were comparatively analyzed. Photolysis (UVA) was applied as a blank, and all experiments were conducted over 90 minutes of reaction time.

Fig5a shows that PEF achieved almost complete KET removal and substantial organic matter mineralization (Fig5c) within 90 min. Results indicate 75.7% and 92.7% KET degradation for anodic oxidation (AO) and EF, respectively, and approximately 100.0% for PEF. EF also showed significant degradation, but over a slightly longer time. Despite KET being a photoactive compound, photolysis alone was insufficient, with limited degradation even after 90 min UVA exposure (Attig et al. 2023). These findings highlight the synergy of electrical current and UV radiation in the Fenton-based oxidation mechanism, promoting photolysis of intermediates and accelerating their conversion to CO_2 , thus improving mineralization efficiency.

As shown in Fig5b, a strong correlation exists between degradation rates and the apparent kinetic constants. PEF and EF exhibited higher k_{app} values, indicating a faster degradation kinetics, while AO and UVA were the least effective treatments. PEF presented the highest rate constant ($k_{app} = 0.045 \text{ min}^{-1}$; $R^2 = 0.978$), followed by EF ($k_{app} = 0.032 \text{ min}^{-1}$; $R^2 = 0.974$) and AO ($k_{app} = 0.015 \text{ min}^{-1}$; $R^2 = 0.991$). UVA exhibited the lowest value ($k_{app} = 0.004 \text{ min}^{-1}$; $R^2 = 0.911$), confirming its limited ability to promote degradation. These results indicate that the oxidative efficiency of the treatments follows the order: PEF > EF > AO > UVA. In the PEF process, UV irradiation enhances $\text{HO}^\bullet$ generation via photolysis of $[\text{Fe}(\text{OH})]^{2+}$ and simultaneously promotes the regeneration of Fe^{2+} from Fe^{3+} (Equations 5 and 6), which explains the improved degradation observed in Fig5b.



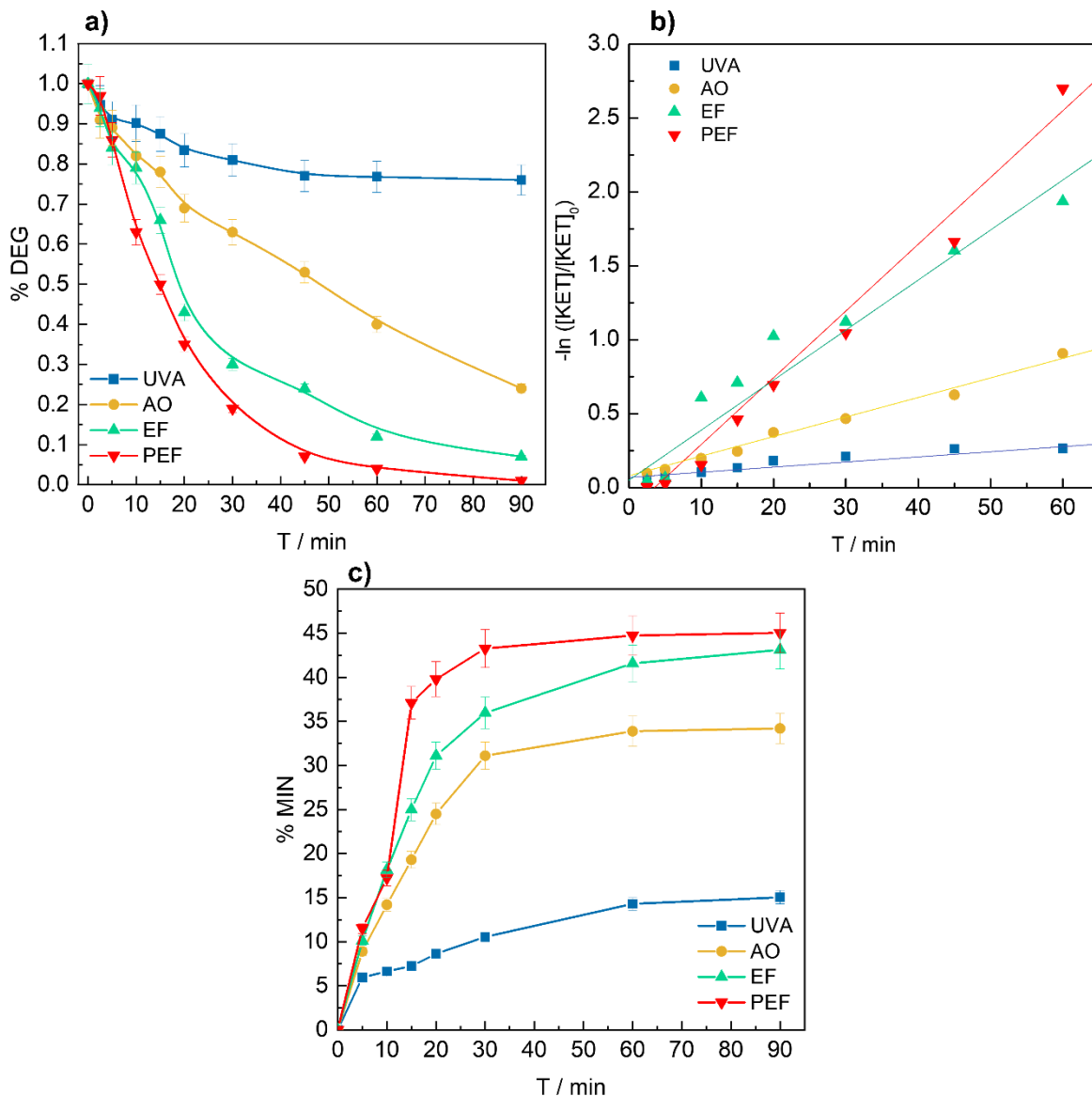


Fig5 Comparison of KET removal under optimized conditions. (a) degradation curves of KET, (b) pseudo-first-order kinetics for degradation, (c) mineralization of organic matter under optimal conditions for the electro-Fenton and photoelectro-Fenton processes

Choosing an ideal treatment method requires balancing cost, efficiency, versatility, safety, automation ease, environmental compatibility, and energy parameters for practical electrochemical feasibility (Brillas and Martínez-Huitle 2015). Table 2 shows PEF as the most efficient treatment in terms of degradation and mineralization performance, combined with the lowest energy consumption per gram of TOC removed, suggesting favorable potential for scale-up, although all experiments in this study were conducted at laboratory scale.

Table 2 Results of KET removal from real wastewater by EF and PEF under optimal conditions

Process	Parameters	%DEG	%MIN	EC _{TOC} /kWh (g TOC) ⁻¹
EF	$j = 102 \text{ mA cm}^{-2}$	92.7	43.2	33.6
	$[\text{Fe}^{3+}] = 7 \text{ mg L}^{-1}$			
	$[\text{Fe}^{3+}:\text{citrate}] = 1:2$			
PEF	$j = 85 \text{ mA cm}^{-2}$	99.3	45.0	10.7
	$[\text{Fe}^{3+}] = 3 \text{ mg L}^{-1}$			
	$[\text{Fe}^{3+}:\text{citrate}] = 1:1$			

3.4 Results of physicochemical parameters

Combined systems have been used to enhance the efficiency of conventional treatment processes and advanced oxidation processes (Brasil 2005, 2011; Granzoto et al. 2021; Antonio da Silva et al. 2021). Table 3 presents the results of the physicochemical analyses of the studied effluents.

Table 3 Results of physicochemical parameters of effluents and post-treatments with AOPs

Sample	pH	Conductivity (mS cm ⁻¹)	Turbidity (NTU)	COD (mg L ⁻¹)	BOD (mg L ⁻¹)			
						Turbidity	COD	BOD _{5,20}
UASB	6.6 ± 0.20	1.0 ± 0.01	91.3 ± 7.77	452.8 ± 4.59	162.3 ± 9.09			
UASB-KET	7.3 ± 0.36	1.0 ± 0.01	90.9 ± 5.65	491.2 ± 2.47	186.9 ± 11.44			
VFCW	5.8 ± 0.19	0.6 ± 0.09	22.8 ± 3.84	133.4 ± 7.64	24.8 ± 3.18	75.0	70.5	84.8
VFCW-KET	5.8 ± 0.09	0.6 ± 0.03	24.6 ± 4.52	158.0 ± 2.83	25.3 ± 1.39	73.1	65.1	84.4
CW+UVA	5.9 ± 0.11	0.5 ± 0.10	15.0 ± 1.05	152.0 ± 2.23	22.0 ± 1.41	83.5	66.4	86.5
CW+AO	5.8 ± 0.02	8.9 ± 0.04	14.6 ± 0.64	88.0 ± 11.31	18.0 ± 2.83	84.1	80.6	88.9
CW+EF	6.0 ± 0.04	8.8 ± 0.03	7.9 ± 0.86	73.5 ± 6.36	13.5 ± 1.20	91.3	83.8	91.7
CW+PEF	6.0 ± 0.01	8.6 ± 0.08	4.4 ± 0.99	60.5 ± 4.95	10.5 ± 1.41	95.2	86.6	93.5
Release limits standard**	6.0 – 9.0	-	-	-	< 120			
Methods*	4500 B	2510A	2130 B	5220 B	5210 B			

NOTE: n = 3 samples; (±) standard deviation; UASB = UASB reactor inlet effluent; UASB-KET = UASB post-reactor effluent with KET doped; COD = Chemical Oxygen Demand; BOD = Biochemical oxygen demand; *Release limit values in class IV river (Conselho Estadual de Recursos Hídricos do de Mato Grosso do Sul (CERH/MS) no 52 2018). There are no limits for turbidity, COD, and conductivity in Brazilian legislation for class IV rivers.

Turbidity, resulting from suspended solids, is a critical parameter in AOPs employing UV or visible radiation, as it can hinder photon transmission and reduce treatment efficiency (Miguel et al. 2024). In this study, the VFCW achieved a 75.0% reduction in turbidity, supporting its role as an effective pre-treatment step for AOPs.

The integrated CW+EF and CW+PEF systems achieved COD removal efficiencies of 83.8% and 86.6%, BOD removal efficiencies of 91.7% and 93.5%, and turbidity reductions of 91.3% and 95.2%, respectively. All treated effluents met the thresholds established by Brazilian environmental standards. COD and BOD, key indicators of organic pollution, were substantially reduced, and final BOD concentrations remained below 20.0 mg L⁻¹, in compliance with Resolution No. 430/2011 of Brazilian Environment National Council (CONAMA) for water reuse (Brasil 2011)

Although electrical conductivity increased due to the addition of sodium sulfate as the supporting electrolyte, the final values remained within acceptable regulatory limits. Overall, both integrated systems performed better than conventional treatments, significantly improving effluent quality and supporting potential reuse.

3.5 Acute toxicity

Acute toxicity tests are an important first step in toxicity assessment. *Artemia sp.* larvae are widely used due to their ease of handling and sensitivity to pollutants. These results complement the physicochemical analyses and provide an indication of treatment efficacy (Cavalheri et al. 2023a).

Acute toxicity in *Artemia sp.* (Fig6a) was mild for UASB effluent but increased to toxic levels following KET (UASB-KET). CW-based treatments reduced toxicity, with CW, CW+AO, CW+EF, and CW+PEF effluents all reaching non-toxic levels. This pattern was also observed in previous work by our group (Cavalheri et al. 2023b; Miguel et al. 2024).

Based on the number of germinated seeds and radicle growth measurements, the Germination Index (GI) was calculated. Fig6b shows that all treatments yielded GI values above 80.0%, indicating the absence of phytotoxic effects on *Lactuca sativa*. Notably, even in cases where acute toxicity to *Artemia sp.* was detected (e.g., CW-KET and CW+UVA), the effluents did not impair seed germination or root elongation, suggesting differential species sensitivity to the residual pollutants present. CW+PEF and CW+EF achieved the highest GI values, indicating slightly better performance in minimizing residual phytotoxicity.

Acute toxicity and phytotoxicity responded differently to the same treatment, likely reflecting differences in species sensitivity or specific pollutants. CW+PEF was the most effective treatment overall, eliminating acute toxicity to *Artemia sp.* while maintaining high germination rates in *Lactuca sativa*, indicating the absence of harmful byproducts for either species.

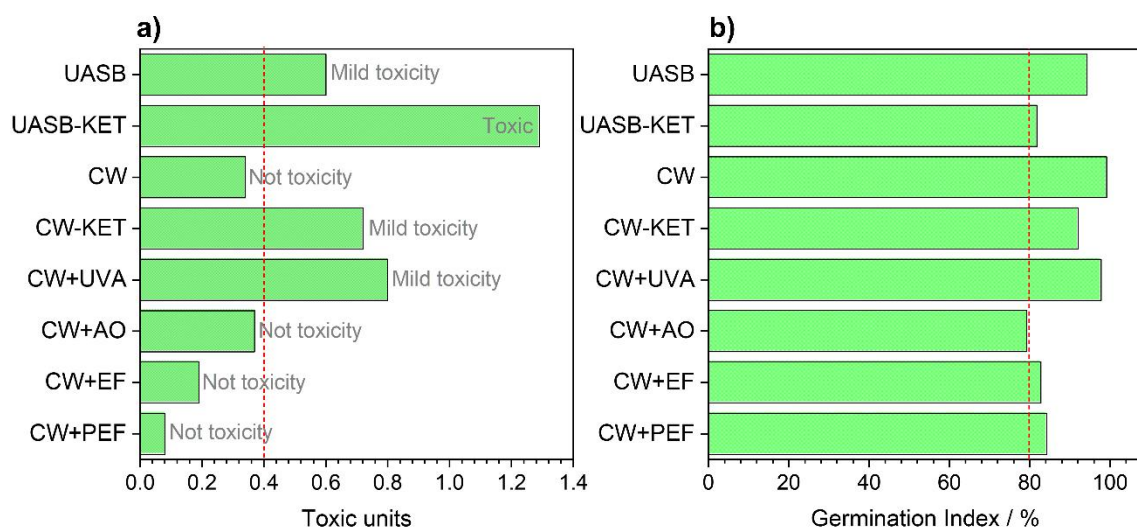


Fig6 Acute toxicity assays of the effluents before and after treatment with CW and CW+AOPs. a) Toxic Units (TU) and corresponding toxicity classification using *Artemia sp.* as a bioindicator. b) Germination Index (%) determined from *Lactuca sativa* seeds

After optimization of the operational parameters, acute toxicity assays were conducted using *Danio rerio* embryos to evaluate the ecotoxicological profile of the final effluent, specifically assessing whether the PEF process or the formation of degradation byproducts induced adverse effects.

Table 4 Toxicity parameters and effluent classification before and after treatment under optimal conditions

Samples	LC ₅₀	TU	Toxicity removal (%)	Toxicity index*
UASB-KET	12.50 ± 0.83	8.00	-	Acute toxic
CW-PEF (optimal conditions)	100.45 ± 19.51	0.99	87.60	Mild acute toxicity

Values with statistical significance ($p < 0.05$). * Toxicity index classified by Persoone (Persoone et al. 2003).

Table 4 shows that the UASB-KET effluent exhibited pronounced acute toxicity to *Danio rerio* embryos ($LC_{50} = 12.50$; 8.00 TU), indicating that conventional anaerobic treatment is ineffective in reducing the biological risks associated with ketoprofen-contaminated wastewater. In contrast, the integrated CW+PEF system achieved an 87.6% reduction in acute toxicity ($LC_{50} = 100.45$; 0.99 TU), reclassifying the treated effluent as mildly toxic (Persoone et al. 2003). This improvement is consistent with the near-complete removal of KET (>99.0%) and the marked reduction in organic load and turbidity (86.6% COD, 93.5% BOD, and 95.2% turbidity removal; Table 3), indicating effective removal of the bioactive organic fractions responsible for ecotoxicological effects.

The integration of the CW and the PEF process enhanced toxicity mitigation, as the pretreatment reduced suspended solids and optical shielding, improving photon penetration and HO[•] utilization during PEF and promoting deeper oxidation of recalcitrant pharmaceuticals and toxic intermediates (López-Vinent et al. 2023, 2024). This study provides the first demonstration of a VFCW coupled with PEF applied to real municipal wastewater at near-neutral pH, showing that this NbS-EAOP hybrid can simultaneously maximize micropollutant removal and significantly reduce residual ecotoxicity, supporting its use as a robust polishing step for advanced treatment and water reuse (Berberidou et al. 2017).

3.6 Economic evaluation of combined processes

The operational cost of wastewater treatment systems is a pivotal factor in determining their scalable feasibility. Beyond versatility and safety, selecting an appropriate treatment method must account for operating expenses (OPEX) (Goffi et al. 2018; Ćetković et al. 2022; Alevizos et al. 2023). Although several variables influence total costs, this study provides an estimate based on treated effluent volume (USD m⁻³), focusing specifically on chemical reagents and electricity consumption (Table 5).

Due to integration with the constructed wetland, the energy required for the pump delivering effluent to the CW system (45 min m⁻³) was added to all assessments. Unit costs were established as follows: sodium citrate (USD 12.40 kg⁻¹), iron (III) sulfate (USD 16.11 kg⁻¹), and sodium sulfate (USD 9.17 kg⁻¹). Electricity was estimated at USD 0.162/kWh, based on average Brazilian retail electricity prices in 2025. While laboratory-grade reagents were used for this baseline estimate, operational costs are expected to decrease significantly in full-scale applications using industrial-grade inputs.

Table 5 Operational cost estimates (USD m⁻³) for integrated CW and AOP treatments

Treatment	Electricity Cost (USD m ⁻³)	Reagent Cost (USD m ⁻³)	Total Cost (USD m ⁻³)	Efficiency Notes
CW + AO	9.49	65.14	74.63	Lowest cost; limited degradation/mineralization
CW + EF	9.85	71.39	81.24	Highest cost; higher reagent demand for ketoprofen removal
CW + PEF	10.46	67.03	77.49	Most efficient; highest mineralization/ degradation, justifies extra cost

Note: Electricity cost estimates were calculated using the average price of USD 0.162/kWh. Reagent costs were based on supplier quotes.

The CW+AO configuration again showed the lowest total cost, making it the most economical option. However, its limited performance in terms of degradation and mineralization may restrict its use when high-quality effluent standards are required. In contrast, the CW+EF process had the highest total cost, mainly because of the higher concentrations of iron(III) sulfate and sodium citrate needed for effective KET degradation.

The CW+PEF system stood out as the most efficient method, achieving the highest mineralization and degradation rates. Although it involves the highest electricity cost due to UVA irradiation, its intermediate reagent costs make it a viable option in situations where stringent contaminant removal is required, justifying the additional investment. As a result, while CW+AO is the least expensive option, CW+PEF offers the best balance between cost and performance for pharmaceutical removal.

4. CONCLUSIONS

This study showed that the integration of nature-based solutions, such as vertical flow constructed wetlands, with electrochemical advanced oxidation processes (AO, EF, and PEF) is an effective strategy for KET removal in real municipal wastewater. Among the three configurations, CW+AO achieved 75.7% KET degradation, while CW+EF and CW+PEF achieved removal efficiencies above 99.0%, reflecting a clear performance gradient across processes. All integrated systems improved effluent quality, with the CW+PEF system reaching the highest reductions in COD (86.6%), BOD (93.5%), and turbidity (95.2%).

Ecotoxicological assessments confirmed treatment success: acute toxicity to *Artemia sp.*, was eliminated across all CW-integrated treatments. Toxicity to *Danio rerio* embryos was reduced by 87.6% under CW+PEF, reclassifying the effluent as mildly toxic. In addition, no phytotoxic effects were observed on *Lactuca sativa*. These multi-species results indicate that the hybrid systems mitigate ecotoxicological risk beyond simple pharmaceutical removal.

CW+PEF showed the best overall performance, balancing high mineralization with the lowest energy consumption (10.7 kWh g⁻¹ TOC, versus 33.6 kWh g⁻¹ TOC for CW+EF), as UV radiation accelerated Fe²⁺ regeneration and HO• production. Note that operational costs here reflect only reagent and electricity use; capital costs (Pt anode, GDE cathode, UVA lamp) were not included, so conclusions on full-scale economic feasibility should be considered preliminary.

The feasibility of operating at near-neutral pH without continuous chemical adjustment supports the practicality of this technology. Future work should address electrode durability, pilot-scale validation under variable wastewater conditions, and applicability to other emerging contaminants. In conclusion, this study provides the first evidence that coupling CW with photoelectrochemical processes in real wastewater is a robust, promising alternative for advanced treatment of emerging contaminants, supporting water reuse and reducing the environmental impact of persistent pharmaceuticals.

REFERENCES

ABNT. NBR 16530: (2016) Ecotoxicologia Aquática - Toxicidade Aguda - Método de Ensaio Com *Artemia* Sp (Crustacea, Brachiopoda). Rio de Janeiro

- Alevizos V, Georgousis I, Kapodistria A (2023) Toward Climate Neutrality: A Comprehensive Overview of Sustainable Operations Management, Optimization, and Wastewater Treatment Methods. *Pollutants* 3:521–543. <https://doi.org/10.3390/pollutants3040036>
- Alkimin GD, Soares AMVM, Barata C, Nunes B (2020) Evaluation of ketoprofen toxicity in two freshwater species: Effects on biochemical, physiological and population endpoints. *Environmental Pollution* 265:114993. <https://doi.org/10.1016/j.envpol.2020.114993>
- Andrade HN de, Oliveira JF de, Siniscalchi LAB, et al (2024) Global insight into the occurrence, treatment technologies and ecological risk of emerging contaminants in sanitary sewers: Effects of the SARS-CoV-2 coronavirus pandemic. *Science of The Total Environment* 921:171075. <https://doi.org/10.1016/j.scitotenv.2024.171075>
- Antonio da Silva D, Pereira Cavalcante R, Batista Barbosa E, et al (2021) Combined AOP/GAC/AOP systems for secondary effluent polishing: Optimization, toxicity and disinfection. *Sep Purif Technol* 263:118415. <https://doi.org/10.1016/j.seppur.2021.118415>
- Attig J Ben, Lourdes Souza F, Latrous L, et al (2023) Advanced oxidation and a metrological strategy based on CLC-MS for the removal of pharmaceuticals from pore & surface water. *Chemosphere* 333:138847. <https://doi.org/10.1016/j.chemosphere.2023.138847>
- Ávila C, García-Galán MJ, Uggetti E, et al (2021) Boosting pharmaceutical removal through aeration in constructed wetlands. *J Hazard Mater* 412:125231. <https://doi.org/10.1016/j.jhazmat.2021.125231>
- Bayati M, Ho TL, Vu DC, et al (2021) Assessing the efficiency of constructed wetlands in removing PPCPs from treated wastewater and mitigating the ecotoxicological impacts. *Int J Hyg Environ Health* 231:113664. <https://doi.org/10.1016/j.ijheh.2020.113664>
- Berberidou C, Kitsiou V, Lambropoulou DA, et al (2017) Evaluation of an alternative method for wastewater treatment containing pesticides using solar photocatalytic oxidation and constructed wetlands. *J Environ Manage* 195:133–139. <https://doi.org/10.1016/j.jenvman.2016.06.010>
- Brasil (2011) CONAMA - Conselho Nacional do Meio Ambiente. Resolução nº 430, de 13 de maio de 2011. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução no 357, de 17 de março de 2005.
- Brasil (2005) CONAMA - Conselho Nacional do Meio Ambiente. Resolução no 357, de 17 de março de 2005. Dispõe sobre as condições e padrões de lançamento de efluentes.
- Brillas E, Martínez-Huitle CA (2015) Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods. An updated review. *Appl Catal B* 166–167:603–643. <https://doi.org/10.1016/j.apcatb.2014.11.016>
- Casas ME, Matamoros V (2020) Novel Constructed Wetland Configurations for the Removal of Pharmaceuticals in Wastewater. pp 163-190
- Cavalcante RP, de Oliveira DM, da Silva L de M, et al (2021) Evaluation of the main active species involved in the TiO₂ photocatalytic degradation of ametryn herbicide and its by-products. *J Environ Chem Eng* 9:105109. <https://doi.org/10.1016/j.jece.2021.105109>
- Cavalheri PS, Machado BS, da Silva TF, et al (2023a) Ketoprofen and diclofenac removal and toxicity abatement in a real scale sewage treatment Plant by photo-Fenton Process with design of experiments. *J Environ Chem Eng* 11:110699. <https://doi.org/10.1016/j.jece.2023.110699>

- Cavalheri PS, Machado BS, da Silva TF, et al (2023b) Optimization of a combined system of vertical flow constructed wetland and solar photo-Fenton for ketoprofen removal in sewage and landfill leachate. *Chemical Engineering Journal* 475:146282. <https://doi.org/10.1016/j.cej.2023.146282>
- Ćetković J, Knežević M, Lakić S, et al (2022) Financial and Economic Investment Evaluation of Wastewater Treatment Plant. *Water (Basel)* 14:122. <https://doi.org/10.3390/w14010122>
- Chafi S, Azzouz A, Ballesteros E (2022) Occurrence and distribution of endocrine disrupting chemicals and pharmaceuticals in the river Bouregreg (Rabat, Morocco). *Chemosphere* 287:132202. <https://doi.org/10.1016/j.chemosphere.2021.132202>
- Conselho Estadual de Recursos Hídricos do de Mato Grosso do Sul (CERH/MS) no 52 (2018) Dispõe sobre o enquadramento dos corpos de águas superficiais em consonância com as diretrizes estabelecidas na DELIBERAÇÃO CECA/MS No 36, de 27 de junho de 2012, e dá outras providências., *Diário Oficial Do Estado Do Mato Grosso Do Sul*.
- da Silva LM, Gozzi F, Cavalcante RP, et al (2019) Assessment of 4-Aminoantipyrine Degradation and Mineralization by Photoelectro-Fenton with a Boron-Doped Diamond Anode: Optimization, Treatment in Municipal Secondary Effluent, and Toxicity. *ChemElectroChem* 6:865–875. <https://doi.org/10.1002/celec.201801651>
- Feng L, Oturan N, van Hullebusch ED, et al (2014) Degradation of anti-inflammatory drug ketoprofen by electro-oxidation: comparison of electro-Fenton and anodic oxidation processes. *Environmental Science and Pollution Research* 21:8406–8416. <https://doi.org/10.1007/s11356-014-2774-2>
- Friedrich LC, Zanta CL de P e S, Machulek Jr. A, et al (2012) Interference of inorganic ions on phenol degradation by the Fenton reaction. *Sci Agric* 69:347–351. <https://doi.org/10.1590/S0103-90162012000600001>
- Goffi AS, Trojan F, de Lima JD, et al (2018) Economic feasibility for selecting wastewater treatment systems. *Water Science and Technology* 78:2518–2531. <https://doi.org/10.2166/wst.2019.012>
- Gozzi F, Sirés I, Thiam A, et al (2017) Treatment of single and mixed pesticide formulations by solar photoelectro-Fenton using a flow plant. *Chemical Engineering Journal* 310:503–513. <https://doi.org/10.1016/j.cej.2016.02.026>
- Granzoto MR, Seabra I, Malvestiti JA, et al (2021) Integration of ozone, UV/H₂O₂ and GAC in a multi-barrier treatment for secondary effluent polishing: Reuse parameters and micropollutants removal. *Science of The Total Environment* 759:143498. <https://doi.org/10.1016/j.scitotenv.2020.143498>
- Guelfi DRV, Ye Z, Gozzi F, et al (2019) Ensuring the overall combustion of herbicide metribuzin by electrochemical advanced oxidation processes. Study of operation variables, kinetics and degradation routes. *Sep Purif Technol* 211:637–645. <https://doi.org/10.1016/j.seppur.2018.10.029>
- Gupta AK, Uddameri V, Majumder A, Nimbhorkar SK (2023) *Wastewater Engineering*. CRC Press, New York
- Khasawneh OFS, Palaniandy P (2021) Occurrence and removal of pharmaceuticals in wastewater treatment plants. *Process Safety and Environmental Protection* 150:532–556. <https://doi.org/10.1016/j.psep.2021.04.045>
- Kimmel CB, Ballard WW, Kimmel SR, et al (1995) Stages of embryonic development of the zebrafish. *Developmental Dynamics* 203:253–310. <https://doi.org/10.1002/aja.1002030302>

- Lee BCY, Mahtab MS, Neo TH, et al (2022) A comprehensive review of Design of experiment (DOE) for water and wastewater treatment application - Key concepts, methodology and contextualized application. *Journal of Water Process Engineering* 47:102673. <https://doi.org/10.1016/j.jwpe.2022.102673>
- Lopez-Herguedas N, Irazola M, Alvarez-Mora I, et al (2023) Comprehensive micropollutant characterization of wastewater during Covid-19 crisis in 2020: Suspect screening and environmental risk prioritization strategy. *Science of The Total Environment* 873:162281. <https://doi.org/10.1016/j.scitotenv.2023.162281>
- López-Vinent N, Cruz-Alcalde A, Santacruz AP, Sans C (2024) Green approach for micropollutants removal: Study of constructed wetlands as pretreatment of solar photo-Fenton catalyzed by organic fertilizers. *Catal Today* 430:114540. <https://doi.org/10.1016/j.cattod.2024.114540>
- López-Vinent N, Santacruz AP, Sales-Alba A, et al (2023) Nature-based solution as an efficient wastewater pretreatment to enhance micropollutants abatement by solar photo-Fenton at natural pH. *J Environ Chem Eng* 11:110834. <https://doi.org/10.1016/j.jece.2023.110834>
- Majumder A, Gupta B, Gupta AK (2019) Pharmaceutically active compounds in aqueous environment: A status, toxicity and insights of remediation. *Environ Res* 176:108542. <https://doi.org/10.1016/j.envres.2019.108542>
- Mazucato VSH, Santos VB dos, Cavalheri PS, Magalhães Filho FJC (2020) Comportamento De Wetland Construído De Fluxo Vertical Com Fundo Parcialmente Saturado Na Remoção De Matéria Orgânica Nitrogenada E Fósforo. *Brazilian Journal of Development* 6:56506–56520. <https://doi.org/10.34117/bjdv6n8-168>
- Miguel E da SC, Machado BS, Teles APS, et al (2024) Optimizing sewage treatment by UV/H₂O₂ process and vertical flow constructed wetland integration. *Journal of Water Process Engineering* 64:105580. <https://doi.org/10.1016/j.jwpe.2024.105580>
- Montenegro-Apraez D, Machuca-Martínez F (2023) Analysis of scientific and technological trends in the incorporation of activated carbon in advanced oxidation processes-a bibliometric study. *Environmental Science and Pollution Research* 32:10623–10639. <https://doi.org/10.1007/s11356-023-31120-4>
- OECD (2025) Test No. 236: Fish Embryo Acute Toxicity (FET) Test, Section 2. OECD Publishing, Paris
- Pacheco CR, Terán Hilaes R, Colina Andrade G, et al (2021) Emerging contaminants, SARS-COV-2 and wastewater treatment plants, new challenges to confront: A short review. *Bioresour Technol Rep* 15:100731. <https://doi.org/10.1016/j.biteb.2021.100731>
- Parida VK, Saidulu D, Majumder A, et al (2021) Emerging contaminants in wastewater: A critical review on occurrence, existing legislations, risk assessment, and sustainable treatment alternatives. *J Environ Chem Eng* 9:105966. <https://doi.org/10.1016/j.jece.2021.105966>
- Persoone G, Marsalek B, Blinova I, et al (2003) A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. *Environ Toxicol* 18:395–402. <https://doi.org/10.1002/tox.10141>
- Poyatos JM, Muño MM, Almecija MC, et al (2010) Advanced oxidation processes for wastewater treatment: State of the art. *Water Air Soil Pollut* 205:187–204. <https://doi.org/10.1007/S11270-009-0065-1>

- Sánchez M, Ruiz I, Soto M (2022) The Potential of Constructed Wetland Systems and Photodegradation Processes for the Removal of Emerging Contaminants-A Review. *Environments* 9:116. <https://doi.org/10.3390/environments9090116>
- Schwartz H, Marushka L, Chan HM, et al (2021) Pharmaceuticals in source waters of 95 First Nations in Canada. *Canadian Journal of Public Health* 112:133-153. <https://doi.org/10.17269/s41997-021-00499-3>
- Shahid MK, Kashif A, Fuwad A, Choi Y (2021) Current advances in treatment technologies for removal of emerging contaminants from water - A critical review. *Coord Chem Rev* 442:213993. <https://doi.org/10.1016/j.ccr.2021.213993>
- Sirés I, Brillas E, Oturan MA, et al (2014) Electrochemical advanced oxidation processes: today and tomorrow. A review. *Environmental Science and Pollution Research* 21:8336–8367. <https://doi.org/10.1007/s11356-014-2783-1>
- Teodoro A, Boncz MÁ, Júnior AM, Paulo PL (2014) Disinfection of greywater pre-treated by constructed wetlands using photo-Fenton: Influence of pH on the decay of *Pseudomonas aeruginosa*. *J Environ Chem Eng* 2:958-962. <https://doi.org/10.1016/j.jece.2014.03.013>
- Tyumina E, Subbotina M, Polygalov M, et al (2023) Ketoprofen as an emerging contaminant: occurrence, ecotoxicity and (bio)removal. *Front Microbiol* 14:. <https://doi.org/10.3389/fmicb.2023.1200108>
- Wu B, Xiong J, Zhou J, Zhang Q (2026) Constructed wetland-microbial fuel cells: A critical review on the decoupling of power generation and pollutant removal efficiency. *Journal of Water Process Engineering* 89:110285. <https://doi.org/10.1016/j.jwpe.2026.110285>

STATEMENTS & DECLARATIONS

Funding Information

The authors gratefully acknowledge the financial support from the Brazilian funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – grants #303943/2021-1, #401122/2025-5, #403863/2025-2, and #304490/2025-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – Finance Code 001), São Paulo Research Foundation (FAPESP – grants #2024/10952-3, #2025/13616-7, and #2022/12895-1), and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceptualization and study design were performed by Isabel Maximo Corrêa de Alcantara, Thalita Ferreira da Silva, Priscila Sabioni Cavalheri, and Amilcar Machulek Junior. Methodology and material preparation were carried out by Isabel Maximo Corrêa de Alcantara, Thalita Ferreira da Silva, Priscila Sabioni Cavalheri,

Emmanuel Da Silva Côgo Miguel, Antonio Kaique Canatto, Lucas de Melo da Silva, Fernando Jorge Correa Magalhães Filho, and Amilcar Machulek Junior. Data curation, validation, visualization, and formal analysis were performed by Isabel Maximo Corrêa de Alcantara, Thalita Ferreira da Silva, Priscila Sabioni Cavalheri, and Amilcar Machulek Junior. The first draft of the manuscript was written by Thalita Ferreira da Silva and Priscila Sabioni Cavalheri, and all authors commented on and revised previous versions of the manuscript. Funding acquisition, project administration, supervision, and resources were provided by Amilcar Machulek Junior. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)