

Article

Electrochemical Processes Used to Degrade Thiamethoxam in Water and Toxicity Analyses in Non-Target Organisms

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Abstract: Pesticides ensure greater productivity in less time; however, they spread beyond the perimeters to which they are applied to reach non-target organisms, thereby affecting plant, animal, and human health. Thiamethoxam (TMX) is considered to be one of the main agents responsible for poisoning bees and potentially contaminating surface and groundwater. Conventional water-treatment protocols are unable to degrade thiamethoxam; therefore, electrochemically advanced oxidative processes (EAOPs) have become promising alternatives owing to their ease of operation and cost-effectiveness. Herein, we examined the use of EAOPs to oxidize thiamethoxam in commercial Actara[®] and analyzed treatment efficiencies through phytotoxicity studies using cucumber and maize seeds as bioindicators. In addition, the cost of each process was analyzed based on the resulting current efficiency. The treated solutions were used to germinate seeds that were analyzed for total protein, hydrogen peroxide, lipid peroxidation (MDA), superoxide dismutase (SOD), and catalase (CAT) activities. EAOPs were found to effectively oxidize TMX, with more than 50% degraded and 80% COD removed under all treatment conditions, even when the commercial product was used. The photoelectro-Fenton process using 10 mg L⁻¹ FeSO₄ and 100 mg L⁻¹ H₂O₂ exhibited the best results, with 79% of the TMX degraded and 83% of the COD removed, additionally exhibiting the lowest estimated operating cost (USD 1.01 dm⁻³). Higher enzymatic SOD and CAT activities, total protein content, and H₂O₂ concentration were observed; however, no significant changes in MDA were recorded. This treatment protocol effectively oxidizes TMX and reduces its phytotoxicity in maize and cucumber seedlings.

Keywords: wastewater treatment; electro-oxidation process; advanced oxidative process; pesticides; phytotoxicity



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1. Introduction

Thiamethoxam (TMX) is a systemic neonicotinoid insecticide (the first second-generation neonicotinoid pesticide) that is used on several crops in Brazil and worldwide. In a similar manner to other neonicotinoids, its toxicity toward pollinating agents, such as bees, has been widely discussed in the literature; it is associated with disorders observed in the entire population, with harmful consequences for agriculture and species survival [1–5]. TMX has the potential to induce a variety of behavioral difficulties in foraging honeybees, including memory and learning dysfunction and altered navigational skills, even at sublethal doses (i.e., doses that do not lead to direct mortality) [2]. Accordingly, the individual and joint toxicities of TMX and seven other pesticides toward bees have been analyzed to elucidate how the combined actions of pesticides affect crop pollinators. Seven-day food toxicity testing revealed that TMX exhibited the greatest toxicity, with an LC₅₀ of 0.25 µg mL⁻¹ [3].

Growing evidence from non-target organisms has resulted in the European Commission's gradual restriction of the use of some neonicotinoids (thiamethoxam, imidacloprid,

and clothianidin) on bee-attracting seed crops and cereal seeds (including maize and sunflower), with the exception of winter cereals and seeds used in greenhouses [6]. In Brazil, the Standard Table of Potability for Pesticides and Metabolites indicates that a health risk is associated with TMX, with a tolerance of $36 \mu\text{g L}^{-1}$ [7].

In addition to harming bees, TMX is also a potential surface and groundwater contaminant owing to its unique properties, including low soil sorbability, high leaching capacity, and high water solubility [8–10]. Studies have shown that TMX has a high potential for leaching in heavy rain and is highly persistent (half-life of between 46 and 301 days) [11]. In fact, data acquired by monitoring the occurrence of neonicotinoids in water bodies have revealed that TMX and its main by-product (i.e., clothianidin) are frequently detected in water samples [12,13]. The problem intensifies as neonicotinoids also interact synergistically with other pesticides or formulation ingredients commonly present in aquatic environments in agricultural areas [9].

TMX is a toxic bioaccumulant that is difficult to mineralize and highly resistant to biological treatment; consequently, it presents a significant risk to aquatic ecosystems and human health [14].

In this context, the research problem addressed in this study is the search for technologies aimed at the degradation of refractory organic molecules, such as TMX in water, since conventional methods do not show satisfactory efficiency. Among the different processes available (physical, chemical, or biological), advanced oxidation processes (AOPs) have been successfully studied for the degradation of toxic and persistent organic pollutants. These processes are based on the generation of the hydroxyl radical (HO^\bullet), a strong and non-selective oxidant, which reacts with most organic compounds very quickly, guaranteeing the effectiveness of POAs both in terms of oxidation capacity and from a kinetic point of view. In general, the reaction of HO^\bullet in the presence of organic compounds occurs from the abstraction of a hydrogen atom (aliphatic) or the electrophilic addition to a double bond (aromatic), and it can react with the molecular oxygen atom, forming the radical peroxy (RO_2^\bullet) and thus initiating a sequence of oxidative degradation reactions that can lead to the complete mineralization of the contaminant [15,16].

In POAs, the generation of HO^\bullet can be carried out via chemical, electrochemical, or photochemical methods. POAs that are based on electrochemical technology are called electrochemical advanced oxidative processes (EAOPs), and they are developed for the prevention and remediation of environmental pollution, especially in water. EAOPs have several advantages over other POAs, including the absence or low addition of non-toxic catalysts, their relatively low cost, increased safety due to their use in mild environmental conditions, and the ability to be operated with simple equipment that can be easily expanded and automated at industrial scales. Furthermore, EAOPs use the energy necessary to carry out electrolysis and the energy used in UVA lights as inputs, with the only reactants being electrons and photons.

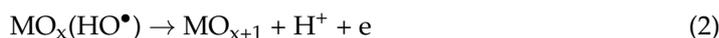
In this research, EAOPs combined with the Fenton reagent are presented, and they are called electro-Fenton and photoelectro-Fenton. Electro-Fenton is the combination of anodic oxidation and the Fenton reaction, which is already well known [17], and the photoelectro-Fenton process consists of irradiating UVA light in the electro-Fenton system and allows increasing the mineralization efficiency of the electro-Fenton process in order to increase the generation of HO^\bullet . Therefore, the combination of processes has shown that degradation rates are higher when compared to the degradation rates of individual processes [15,18,19].

The anodic oxidation of organic compounds can occur via direct electron transfer to the anode surface and the HO^\bullet formed on the anode surface during water oxidation. The production efficiency of these radicals and, consequently, the oxidation of organic compounds depend on the anode material used in the process. In this work, ruthenium and titanium oxide electrodes ($\text{Ti/RuO}_2\text{-TiO}_2$) were prepared and applied, as they have a large surface area and excellent mechanical stability and electrocatalytic activity. Furthermore, they have already been previously characterized and studied by the research group [20–24].

As the formation of HO• is a heterogeneous process, in the case of using metal oxide electrodes or oxide anodes (MO_x), the conversion/combustion process begins by discharging water, producing adsorbed hydroxyl radicals (MO_x(HO•)) according to the following reactions proposed by Comminellis [25]:



Subsequently, the adsorbed hydroxyl radicals can interact with the oxygen present, passing from the adsorbed form to the oxide anode structure and forming higher oxides (MO_{x+1}):

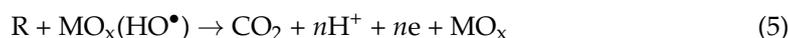


According to reactions (1) and (2), two active oxygen states can be found on the surface of the oxide's electrodes: the physical adsorption of oxygen in the form of a hydroxyl radical and the chemical adsorption of oxygen in the form of higher oxides.

In the absence of oxidizable organic compounds, the adsorbed oxygen will produce O₂ according to reactions (3) and (4), regenerating the metal oxide.



However, when there are oxidizable organic compounds, the physically adsorbed oxygen, MO_x(HO•), should preferably promote the complete oxidation of the organic compound (reaction (5)). In contrast, the chemically adsorbed oxygen in the form of higher oxides, MO_{x+1}, must promote the selective oxidation of organic compounds with the formation of oxidized organic species (RO) according to reaction (6).



Depending on the type of reaction desired, priority must be given to the way in which oxygen is adsorbed on the surface of the electrode because if the concentration of adsorbed hydroxyl radicals is higher than the concentration of higher oxides, the reaction that will probably prevail will be oxidation. A complete list of organic compounds is present. However, if the concentration of higher oxides is higher, the tendency is for the predominant reaction to be selective oxidation [25].

No studies applying the same oxide electrodes as this work to degrade TMX were found in the literature, with only some using other types of electrode materials, which will be briefly described below.

Stupar et al. [26] studied the direct electrochemical oxidation of TMX using a commercial IrO_x plate (5 cm²) as an anode, and a 10 cm² plate made of austenite stainless steel 18Cr/8Ni series 304 was used as a cathode with the aim of verifying parameters such as applied current, electrolyte pH, and TMX concentration.

Lebik-Elhadi et al. [27] studied the electrochemical oxidation of thiamethoxam using a boron-doped diamond anode (BDD) and varied operational parameters, such as the supporting electrolyte (Na₂SO₄ and NaCl), current density (4–40 mA cm⁻²), TMX concentration (1–10 mg L⁻¹ of the active ingredient in a commercial formulation), and acidity of the initial solution (pH 3–11). These authors observed that 2 mg L⁻¹ TMX degraded in 20 min by applying a current density of 16 mA cm⁻² in 0.1 mol L⁻¹ Na₂SO₄ as the medium, accompanied by slower mineralization (91% TOC removal in 120 min), which indicates that more-resistant by-products are formed.

The oxidation of TMX using Fenton's reagent with varying doses of hydrogen peroxide resulted in 40% TOC removal and 80% chemical oxygen demand (COD) removal in studies using pure TMX; TOC and COD were reduced by 40% and 60%, respectively, using commercial TMX [28].

Meijide et al. [29] performed anodic and electro-Fenton oxidation experiments with a two-electrode system: a carbon-felt cathode and a boron-doped diamond anode, which completely degraded TMX within 10 min and removed 92% of the TOC within 8h under optimal experimental conditions (300 mA and 0.2 mM Fe²⁺).

TMX oxidation mechanisms have been proposed for identifying by-products [29,30], which revealed that TMX oxidation is a complex catalytic process with several possible degradation routes, resulting in complex interconnected pathways. Studies have also shown that the catalytic degradation of thiamethoxam leads to a reduction in toxicity and the production of less toxic oxidation intermediates than the original compound [30].

In addition to studying TMX degradation, the various effects of TMX on non-target organisms also need to be studied. While TMX is known to be toxic toward non-target insects (bees), the effects of agricultural effluents contaminated with this insecticide remain poorly studied. Therefore, determining whether the presence of TMX in wastewater and/or its degradation by-products can cause phytotoxicity is important. The phytotoxic effects of insecticides range from aesthetic degradation to the significant impairment of germination, growth, development, reproduction, and yield in a variety of crop species, including soybeans, maize, tomatoes, potatoes, and other vegetables [31].

Many reactive oxygen species (ROS) accumulate in the cells of plants subjected to various biological and abiotic stresses [32], which destroy cellular molecular structures and interfere with plant physiological processes [33]. Hydrogen peroxide (H₂O₂), hydroxyl radicals (HO•), superoxide radical anions (O₂^{•-}), and perhydroxyl radicals (HO₂•) are the main ROS that accumulate [34]. While the production and elimination of ROS are dynamically balanced by regulating antioxidant systems under normal conditions, pesticides can damage biological or antioxidant defense systems [32]. Plants rely on a variety of complex enzymatic and non-enzymatic antioxidant systems that are capable of resisting oxidative stress for their defense; these systems include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathione S-transferase (GST), with other enzymatic and non-enzymatic substances that are also able to remove different types of ROS [32,35,36]. SOD is an enzyme that plays an important role in a plant's response to oxidative stress by transforming O₂^{•-} into H₂O₂ and molecular oxygen [37], while CAT dismutates H₂O₂ into H₂O and O₂. In addition to these enzymes, lipid peroxidation is a significant event associated with oxidative stress because it decreases fluidity and changes ionic permeability and other membrane-associated functions [38].

In the context presented above, we aimed to degrade commercial Actara[®] (which contains TMX as a main component) using electrochemical advanced oxidative processes and to phytotoxicologically analyze different bioindicators (cucumber and maize) in order to determine the effectiveness of treatment methods in relation to oxidative stress in plants that are non-target organisms.

2. Materials and Methods

2.1. Chemicals

The following reagents were used: thiamethoxam (3-(2-chloro-thiazol-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine, C₈H₁₀ClN₅O₃S); the Pestanal[®] analytical standard (Sigma–Aldrich, St. Louis, MO, USA) as a chromatographic standard; and the commercial Actara[®] 250WG insecticide, a commercial product based on thiamethoxam that is marketed by Syngenta, which was purchased at a local store that specializes in pesticides (São Paulo, Brazil). Actara[®] 250WG contains 250 g kg⁻¹ thiamethoxam and 750 g kg⁻¹ of other ingredients. An aqueous stock solution of TMX (20 g L⁻¹) was prepared from the commercial product and used to ensure that 100 mg L⁻¹ TMX during the experiments.

2.2. Oxidative Processes

Two oxidative processes were applied in this paper, electro-Fenton and photoelectro-Fenton, to oxidize TMX from the commercial product Actara[®]. For this, the same methodology was already described by Da Silva et al. [35], which is summarized below:

- Anode: 30% RuO₂ + 70% TiO₂ (Ti/Ru_{0.3}Ti_{0.7}O₂): Oxide electrodes with a geometric area of 2 cm² and an oxide thickness of 2 μm were prepared using a standard method [22,23]. The oxide anode was fixed in a glass tube, which was inserted into an electrochemical cell (one compartment, 100 mL) during electrochemical analyses;
- Cathode: platinum spirals;
- Supporting electrolyte: 0.05 mol L⁻¹ Na₂SO₄, pH 3 (due to Fenton's reagent);
- TMX: 100 mg L⁻¹ (experimental concentration was higher than that present in aqueous environments to allow for an adequate evaluation of degradation);
- Final volume: 100 mL;
- Fenton's reagent solutions: 5 mg L⁻¹ FeSO₄ and 50 mg L⁻¹ H₂O₂ (T1), 5 mg L⁻¹ FeSO₄ and 100 mg L⁻¹ H₂O₂ (T2), 10 mg L⁻¹ FeSO₄ and 50 mg L⁻¹ H₂O₂ (T3), and 10 mg L⁻¹ FeSO₄ and 100 mg L⁻¹ H₂O₂ (T4);
- Photo: closed light chamber and exposed to artificial UV irradiation via 6 W fluorescent black light lamps;
- Electrolysis: 50 mA cm⁻² for 2 h.

2.3. Instruments and Analytical Procedures

Thiamethoxam degradation was followed by high-performance liquid chromatography (HPLC) using a Shimadzu chromatograph (Kyoto, Japan) equipped with a Prominence LC-20AT pump, a CTO-10AS column oven, an SIL-20 AC autosampler, and an SPD-10A UV-visible detector. Separations were performed using a CLC-ODS (M) column (250 × 4.6 mm id; 5 μm) protected by a Supelcosil C-18 pre-column (4.0 × 3.0 mm id; 5 μm) and eluted with 20:80 acetonitrile/water at 1.0 mL min⁻¹. The oven temperature was set to 35 °C, and UV detection was performed at 255 nm. Chromatograms were recorded and integrated using the Class-VP v5.97 SP1 software package (Shimadzu). Concentrations were determined by integrating peak areas and using a calibration curve.

COD analysis was performed using the colorimetric method [35,39]. The possible interference of H₂O₂ in these results was corrected according to a correlation equation [40].

2.4. Phytotoxicity

Phytotoxicological analyses were performed using cucumber (*Cucumis sativus* L., VALENT, TAKII SEED) and maize (*Zea mays*, cultivar Syngenta[®], NK555 VIP3) seeds as bioindicators. Germination testing was performed in quadruplicate using 60 seeds, and solutions were collected after each oxidative process. The effluent collected following treatment was not filtered. An 80 mL aliquot of the effluent was directly applied on seed-containing germination paper only on the first day. Germination proceeded over a 7 d period in a germination chamber at 24 °C with a photoperiod of 12 h. Extractions were carried out with different solvents according to the analyses carried out using 1.5 g fresh weight (FW) and 4 mL of reagent [34]. The total protein content, the extent of lipid peroxidation (malondialdehyde, MDA), H₂O₂ content, and CAT and SOD activities were evaluated according to the procedures described by Forti et al. [41].

3. Results and Discussion

TMX was quantitatively determined via HPLC after 2 h of electrolysis at 50 mA cm⁻² under electro-Fenton or photoelectro-Fenton conditions using various concentrations of Fenton's reagent (T1, T2, T3, and T4). Under the conditions described in the Methodology section, the standard TMX solution (100 mg L⁻¹) exhibited a retention time of 5.5 min, and quantification was performed by comparing the sample's peak areas against the calibration

curve. The percentage of TMX degraded in samples treated using different oxidative processes is shown in Figure 1.

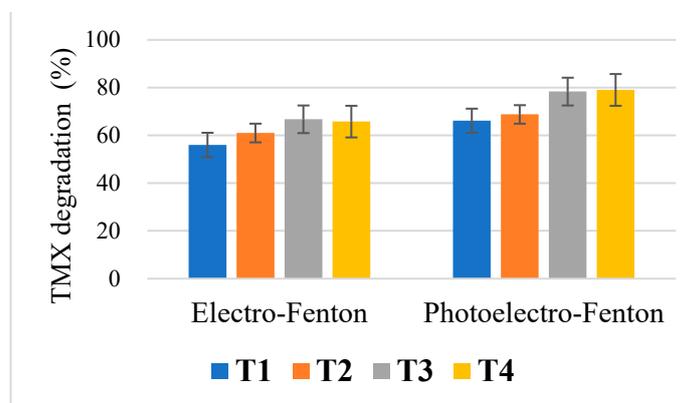


Figure 1. Degradation of thiamethoxam (TMX) (%) from the commercial product Actara[®] as a function of the different oxidative treatments applied. Conditions: $[TMX]_{initial}$: 100 mg L^{-1} ; electrode: $Ti/Ru_{0.3}Ti_{0.7}O_2$; Fenton's reagent: T1 ($FeSO_4$ 5 mg L^{-1} and H_2O_2 50 mg L^{-1}), T2 ($FeSO_4$ 5 mg L^{-1} and H_2O_2 100 mg L^{-1}), T3 ($FeSO_4$ 10 mg L^{-1} and H_2O_2 50 mg L^{-1}), and T4 ($FeSO_4$ 10 mg L^{-1} and H_2O_2 100 mg L^{-1}).

More than 50% of the TMX was removed under all process conditions. The use of UV radiation in the photoelectro-Fenton process resulted in an approximately 20% higher removal efficiency for all treatment protocols, with the best results obtained for T3 and T4, with approximately 80% TMX degraded after 2h under photoelectro-Fenton conditions.

T3 and T4 exhibited similar results in the presence and absence of UV light. The COD-removal data were analyzed to select the best operating condition (Figure 2).

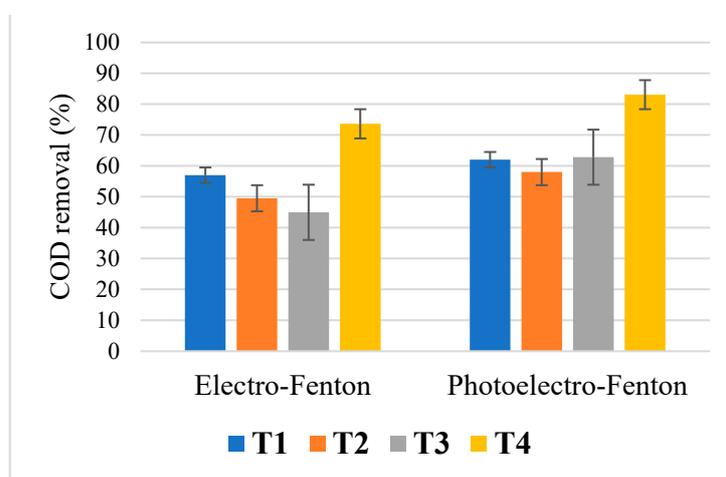


Figure 2. COD removal (%) according to the different oxidative treatments applied. Same conditions described in Figure 1.

Regarding the mineralization of the compound as a whole (TMX + ingredients included in the commercial product + by-products formed during oxidation), the results show that T3 did not significantly remove COD, which indicates that various by-products are possibly formed during the more effective degradation of TMX (Figure 1), as reported in the literature, further suggesting that several degradation routes may be in operation [28,29,33]. While by-products were not identified in this study, previous studies have reported that the catalytic degradation of thiamethoxam leads to a reduction in toxicity by producing oxidation intermediates that are less toxic than the original compound [29].

T4 removed the most COD (>70%) in both oxidative processes. The use of UV light favors COD removal, which shows that more oxidants are generated during the oxidative process and that different oxidation mechanisms may be in operation, including the direct photolysis of generated by-products and indirect oxidation by the oxidants [42].

The COD and current efficiency (EC) data enable approximate costs associated with the various oxidative treatment methods to be calculated using Equation (7) [42], in which we considered the current cost of electricity in Brazil (approximately USD 0.15 kWh⁻¹), the results of which are shown in Figure 3.

$$\text{Cost (US\$ dm}^{-3}\text{)} = \text{EC (kWh dm}^{-3}\text{)} \times 0.15 \text{ (US\$ kWh}^{-1}\text{)} \quad (7)$$

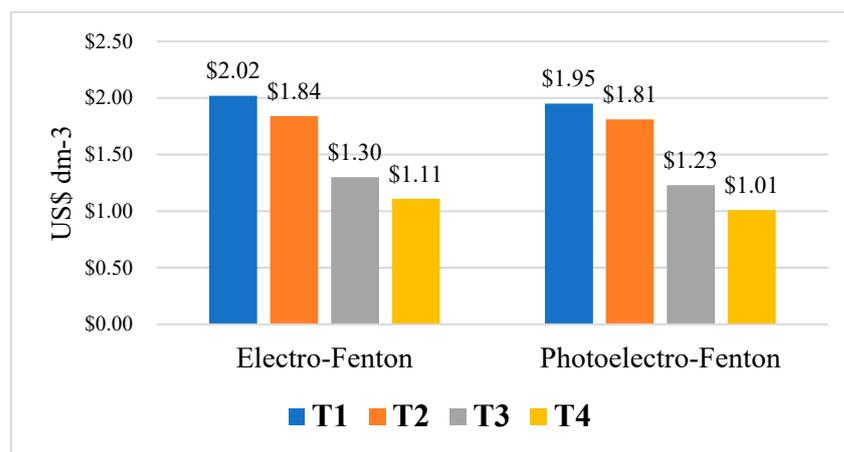


Figure 3. Cost (USD dm⁻³) of each advanced oxidative electrochemical treatment applied to degrade TMX. Same conditions described in Figure 1.

Treatment costs range between USD 1.01 and 2.02 dm⁻³, which are considered satisfactory values, again highlighting that photoelectro-Fenton processes, specifically those involving T4, are slightly more efficient (lower costs). Similar results were obtained in a previous glyphosate oxidation study [35].

To analyze the effects of the various treatment protocols, we separately germinated cucumber and maize seeds in treated solutions of effluent contaminated with Actara[®], a commercial pesticide. Seeds were also germinated using an untreated solution (i.e., 100 mg L⁻¹ of TMX; referred to as “contaminated”) and a control solution (devoid of TMX; referred to as the “reference”) for comparison purposes.

All cucumber and maize seeds germinated in the presence of solutions collected following the oxidative treatment of Actara[®], which shows that the various treatment protocols are effective in this first evaluation since the solution containing 100 mg L⁻¹ TMX (no treatment) resulted in abnormal germination. These preliminary results differ from those of a previous study on another pesticide, in which uneven germination was observed using different oxidative processes [41]. Figure 4 shows representative images of cucumber and maize seeds germinated in solutions treated under photoelectro-Fenton conditions with T4 (A and C) and in solutions contaminated with TMX (no treatment) (B and D).

Normal seedlings have the potential to continue developing into normal plants under favorable conditions. Cucumber and maize seedlings germinated in EAOP-treated TMX solutions are classified as “normal” based on the results presented above as well as their similarities to seedlings germinated in the control solution (without TMX). On the other hand, abnormal seedlings do not continue to develop into normal plants, even when grown under favorable conditions; such seedlings were observed when germinated in untreated (contaminated) solutions. In addition to these classifications, dead seeds that do not germinate at the end of the testing period, are not hard or dormant, are generally soft, and are attacked by microorganisms and those that do not show any signs of germination, while often observed [43], were not observed in this study.

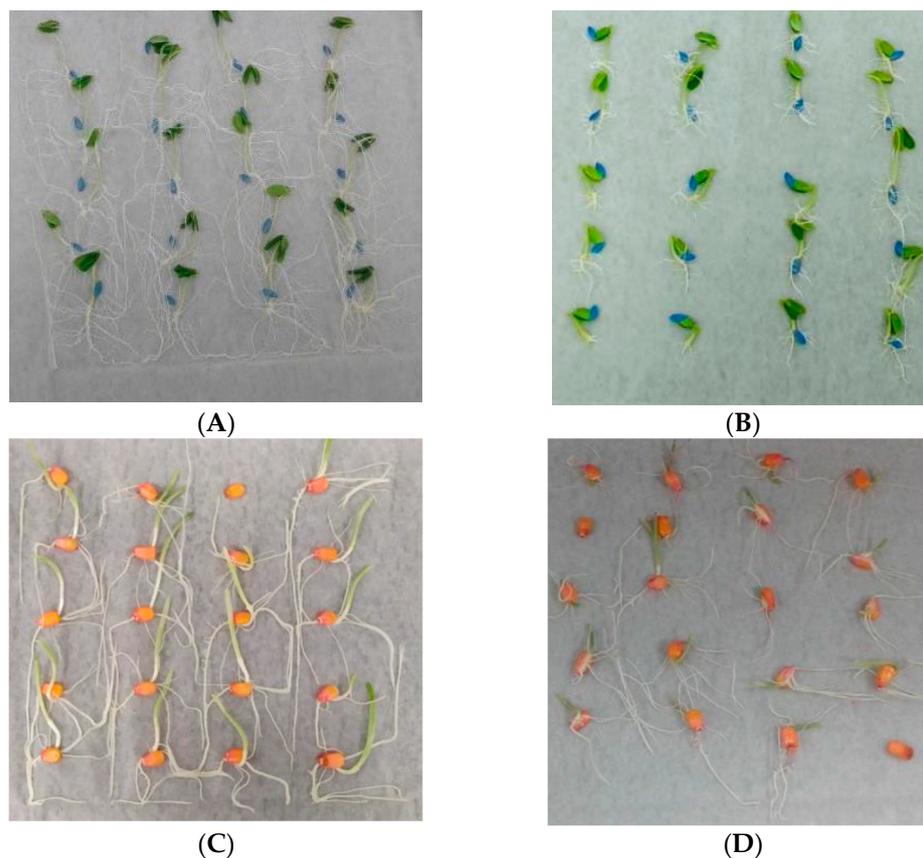


Figure 4. Seedlings of (A) cucumber germinated with TMX solution after treatment via the photoelectro-Fenton T4 process; (B) cucumber germinated with untreated (contaminated) TMX solution; (C) maize germinated with TMX solution after treatment via photoelectro-Fenton T4 process; (D) maize germinated with untreated (contaminated) TMX solution.

Oxidative stress negatively affects plant growth and development in addition to generating ROS that damage various macromolecules and cell structures. To determine the influence of TMX on oxidative stress and the oxidation efficiencies of the applied treatment methods, phytotoxicological testing was performed for cucumber and maize seedlings, the results of which are shown in Figures 5 and 6, respectively.

We analyzed the total protein content, which is an indicator of the nutritional status of the plant. Only cucumber showed good results (i.e., superior to those of the TMX sample) (Figure 5A), while maize exhibited good protein levels only for electro-Fenton 2 and 3 (Figure 6A).

High concentrations of H_2O_2 (Figures 5B and 6B) were observed for the two sets of seedlings relative to the reference, which is expected given that the treatment procedures involve the use of H_2O_2 . While the added H_2O_2 is mostly consumed in the oxidative process, residual amounts are expected to remain.

H_2O_2 is a ROS, and cells develop antioxidant enzymatic defense systems to eliminate these species when attacked. However, defense systems can be damaged by the overproduction of ROS, leading to cellular damage [44]. Cucumbers and maize exhibited different defense enzyme (SOD and CAT) activities. Low concentrations of SOD (Figure 5D) and CAT (Figure 5E) were observed in cucumbers relative to the reference sample. In contrast, maize exhibited higher SOD activity (Figure 6D) than cucumber, consistent with the higher concentration of H_2O_2 (Figure 6B). The presence of H_2O_2 , combined with the fact that SOD acts on $O_2^{\bullet-}$ to form H_2O_2 and O_2 , results in a greater demand for CAT to dismutate H_2O_2 into H_2O and O_2 , which was not observed for most treatment methods. In addition, HO^{\bullet} is also generated by oxidative processes and is possibly not fully removed by these treatments, thereby stimulating the production of combatant enzymes. Yang et al. [14] studied the

photocatalytic degradation of TMX by TiO_2 and concluded that HO^\bullet contributed more to photocatalytic degradation than other ROS; i.e., it acted effectively during oxidation.

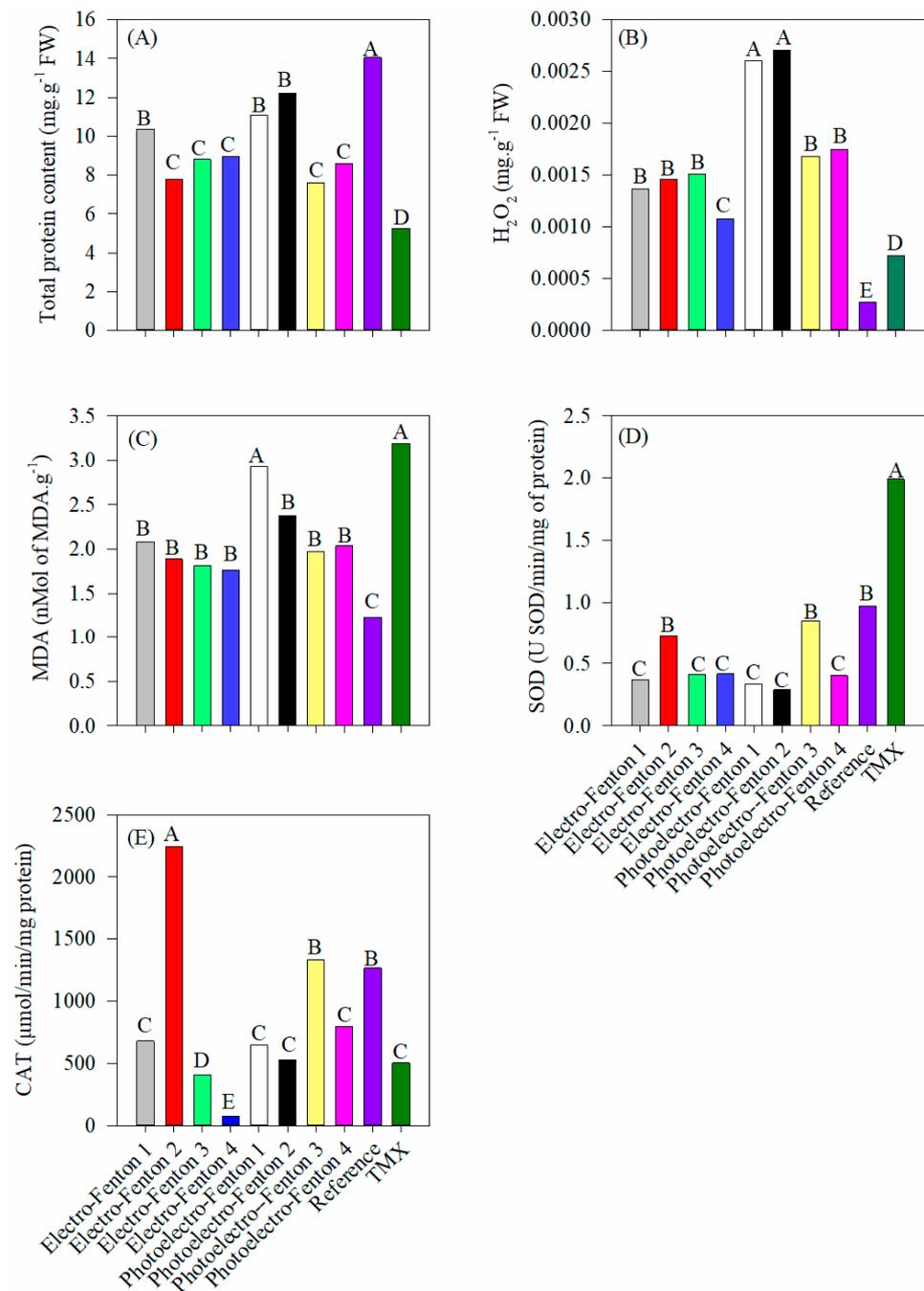


Figure 5. Phytotoxicological analyses of cucumber seeds germinated in the presence of the final solution collected after each treatment applied. (A) Total protein content, (B) hydrogen peroxide content, (C) lipid peroxidation (MDA), (D) superoxide dismutase (SOD) activity, and (E) catalase activity (CAT). Reference test: support electrolyte, without TMX. Contaminated test: 100 mg L⁻¹ of TMX from Actara[®] (no treatment). Equal letters on the bars indicate that there is no significant difference, and different letters indicate that there is a significant difference between the groups ($p < 0.05$) according to Tukey's test at 5% significance.

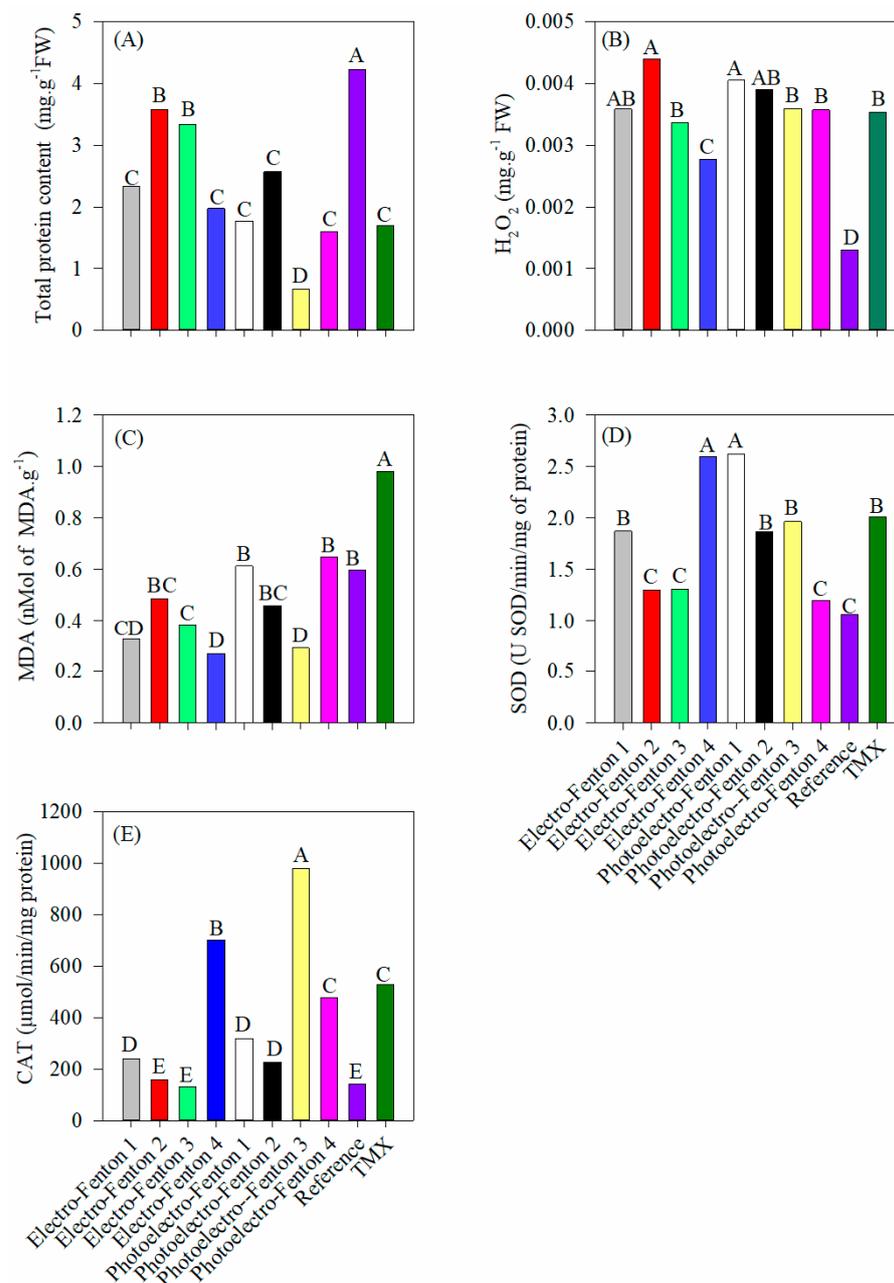


Figure 6. Phytochemical analyses of maize seeds germinated in the presence of the final solution collected after each treatment applied. (A) Total protein content, (B) hydrogen peroxide content, (C) lipid peroxidation (MDA), (D) superoxide dismutase (SOD) activity, (E) catalase activity (CAT). Reference test: Support electrolyte, without TMX. Contaminated test: 100 mg L⁻¹ of TMX from Actara[®] (no treatment). Equal letters on the bars indicate that there is no significant difference, and different letters indicate that there is a significant difference between the groups ($p < 0.05$) according to Tukey's test at 5% significance.

MDA is the main substance produced during lipid peroxidation and serves as an indicator of oxidative damage to membrane lipids [32]. Figures 5C and 6C show that the various treatment protocols were effective, as lower MDA concentrations were observed than those of the untreated samples (contaminated).

ROS are formed in response to pollutants, and antioxidant enzymes are activated to alleviate excess ROS and minimize damage to plants. Under normal conditions, ROS exist in a state of dynamic equilibrium regulated by antioxidant enzymes [36]. However, these enzymes cannot remove excess ROS in a timely manner at high pollution levels.

Furthermore, excess ROS can destroy cell membranes, which increases the MDA content (the end product of lipid peroxidation). Excess ROS and MDA can damage DNA; however, this was not observed for the treated samples; i.e., TMX was efficiently oxidized, as enzyme activities and lipid peroxidation levels that were closer to those of the reference samples were observed.

Homayoonzadeh et al. [45] exposed cucumber seedlings to two insecticides, a neonicotinoid and an organophosphate, to investigate the plant's defense system. Higher SOD and CAT activities and protein contents were observed, whereas no significant changes in H_2O_2 and MDA content were observed.

Most literature studies that report neonicotinoid-induced oxidative stress and the generation of ROS have used *in vitro* models or *in vivo* animal studies [34,46]. Quan et al. [44] studied the TMX-removal efficiency and associated biodegradation pathway using microalgae, and they showed that TMX slightly promoted microalgal growth at $5\text{--}40\text{ mg L}^{-1}$, whereas 60 mg L^{-1} severely reduced growth and was accompanied by higher MDA and SOD contents.

The treatment protocols described herein did not show behavior sufficient for highlighting one method over the others; rather all treatment methods effectively oxidized TMX and reduced phytotoxicity in maize and cucumber seedlings.

4. Conclusions

The advanced electrochemical processes used in this study effectively degraded thiamethoxam, with more than 50% of the TMX degraded under all treatment conditions and up to 80% of the COD removed, even when a commercial product that contains additional organic ingredients that increase the complexity of the oxidative process was used. The use of UV radiation (photoelectro-Fenton process) led to higher efficiencies. Among the various treatment methods, those that used T4, with a high Fenton reagent concentration ($10\text{ mg L}^{-1}\text{ FeSO}_4$ and $100\text{ mg L}^{-1}\text{ H}_2\text{O}_2$) exhibited the best removal results (79% TMX degradation and 83% COD removal), the best current efficiency, and consequently, the lowest estimated cost ($\text{USD } 1.01\text{ dm}^{-3}$).

All cucumber and maize seeds germinated in the presence of solutions collected after being subjected to various oxidative processes, which indicates that the treatment protocols are effective in this first evaluation. In addition, the obtained seedlings showed the potential to develop into normal plants. Good phytotoxicological data were obtained in terms of plant nutritional status (total proteins) and MDA levels, consistent with low lipid peroxidation. However, high H_2O_2 concentrations were observed because the applied processes use H_2O_2 , which stimulates the plant's defense enzymes (i.e., SOD and CAT). In this context, we conclude that the treatment protocols in this study effectively oxidized TMX and reduced phytotoxicity in maize and cucumber seedlings.

It is concluded that recalcitrant organic molecules, like most pesticides, require drastic treatment conditions to reduce water pollution. These results, together with the optimization of the applied parameters, will contribute to future large-scale studies using a reactor with the addition of solar radiation to reduce costs and contribute to possible industrial applications.

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References

1. De Assis, J.C.; Tadei, R.; Menezes-Oliveira, V.B.; Silva-Zacarin, E.C. Are native bees in Brazil at risk from the exposure to the neonicotinoid imidacloprid? *Environ. Res.* **2022**, *212*, 113127. [CrossRef]
2. Henry, M.; Beguin, M.; Requier, F.; Rollin, O.; Odoux, J.F.; Aupinel, P.; Aptel, J.; Tchamitchian, S.; Decourtye, A. A common pesticide decreases foraging success and survival in honey bees. *Science* **2012**, *336*, 348. [CrossRef] [PubMed]
3. Li, W.; Lv, L.; Wang, Y.; Zhu, Y.C. Mixture effects of thiamethoxam and seven pesticides with different modes of action on honey bees (*Apis mellifera*). *Sci. Rep.* **2023**, *13*, 2679. [CrossRef] [PubMed]
4. Lundin, O.; Rundlöf, M.; Smith, H.G.; Fries, I.; Bommarco, R. Neonicotinoid insecticides and their impacts on bees: A systematic review of research approaches and identification of knowledge gaps. *PLoS ONE* **2015**, *10*, e0136928. [CrossRef] [PubMed]
5. Xu, X.; Wang, X.; Yang, Y.; Ares, I.; Martínez, M.; Lopez-Torres, B.; Martínez-Larrañaga, M.R.; Wang, X.; Anadón, A.; Martínez, M.A. Neonicotinoids: Mechanisms of systemic toxicity based on oxidative stress-mitochondrial damage. *Arch. Toxicol.* **2022**, *96*, 1493. [CrossRef]
6. EU Commission. Commission Implementing Regulation (EU) No 485/2013 of 24 May 2013 Amending Implementing Regulation (EU) No 540/2011, as Regards the Conditions of Approval of the Active Substances Clothianidin, Thiamethoxam and Imidacloprid, and Prohibiting the Use and Sale of Seeds Treated with Plant Protection Products Containing Those Active Substances. *Off. J. Eur. Union L* **2013**, *139*, 12–26. Available online: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:139:0012:0026:en:PDF> (accessed on 21 February 2024).
7. BRASIL Ministério da Saúde. Portaria GM/MS n.º 888, de 04 de maio de 2021. Diário Oficial da União, n. 85. 2021; p. 127. Available online: https://bvsms.saude.gov.br/bvs/saudelegis/gm/2021/prt0888_07_05_2021.html (accessed on 7 July 2023).
8. Casillas, A.; de la Torre, A.; Navarro, I.; Sanz, P.; de los Angeles Martínez, M. Environmental risk assessment of neonicotinoids in surface water. *Sci. Total Environ.* **2022**, *809*, 151161. [CrossRef] [PubMed]
9. Morrissey, C.A.; Mineau, P.; Devries, J.H.; Sanchez-Bayo, F.; Liess, M.; Cavallaro, M.C.; Liber, K. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environ. Int.* **2015**, *74*, 291. [CrossRef]
10. Zhao, X.; Wu, C.; Wang, Y.; Cang, T.; Chen, L.; Yu, R.; Wang, Q. Assessment of toxicity risk of insecticides used in rice ecosystem on *Trichogramma japonicum*, an egg parasitoid of rice lepidopterans. *J. Econ. Entomol.* **2012**, *105*, 92. [CrossRef]
11. Gupta, S.; Gajbhiye, V.T.; Gupta, R.K. Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. *Bull. Environ. Contam. Toxicol.* **2008**, *80*, 431. [CrossRef]
12. Hladik, M.L.; Kolpin, D.W.; Kuivila, K.M. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environ. Pollut.* **2014**, *193*, 189. [CrossRef] [PubMed]
13. Struger, J.; Grabuski, J.; Cagampan, S.; Sverko, E.; McGoldrick, D.; Marvin, C.H. Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada. *Chemosphere* **2017**, *169*, 516–523. [CrossRef] [PubMed]
14. Yang, H.; Liu, H.; Hu, Z.; Liang, J.; Pang, H.; Yi, B. Consideration on degradation kinetics and mechanism of thiamethoxam by reactive oxidative species (ROs) during photocatalytic process. *Chem. Eng. J.* **2014**, *245*, 24. [CrossRef]
15. Sirés, I.; Brillas, E.; Oturan, M.A.; Rodrigo, M.A.; Panizza, M. Electrochemical advanced oxidation processes: Today and tomorrow. A review. *Environ. Sci. Pollut. Res.* **2014**, *21*, 8336. [CrossRef] [PubMed]
16. Quiroz, M.A.; Bandala, E.R.; Martínez-Huitle, C.A. Advanced Oxidation Processes (AOPs) for Removal of Pesticides from Aqueous Media. In *Pesticides-Formulations, Effects, Fate*; IntechOpen: London, UK, 2011. [CrossRef]
17. Zhang, M.H.; Dong, H.; Zhao, L.; Wang, D.X.; Meng, D. A review on Fenton process for organic wastewater treatment based on optimization perspective. *Sci. Total Environ.* **2019**, *670*, 110. [CrossRef] [PubMed]
18. Brillas, E. Fenton, photo-Fenton, electro-Fenton, and their combined treatments for the removal of insecticides from waters and soils. A review. *Sep. Purif. Technol.* **2022**, *284*, 120290. [CrossRef]
19. Brillas, E. Recent development of electrochemical advanced oxidation of herbicides. A review on its application to wastewater treatment and soil remediation. *J. Clean. Prod.* **2021**, *290*, 125841. [CrossRef]
20. Forti, J.C.; Manzo-Robledo, A.; Kokoh, K.B.; De Andrade, A.R.; Alonso-Vante, N. Electrooxidation of acetaldehyde on platinum-modified Ti/Ru_{0.3}Ti_{0.7}O₂ electrodes. *Electrochim. Acta* **2006**, *51*, 2800. [CrossRef]
21. Forti, J.C.; De Andrade, A.R. Formaldehyde oxidation on a DSA-type electrode modified by Pt or PbO₂ electrodeposition. *J. Electrochem. Soc.* **2007**, *154*, E19–E24. [CrossRef]
22. Forti, J.C.; Ribeiro, J.; Lanza, M.R.V.; De Andrade, A.R.; Bertazzoli, R. Electrochemical characterization of DSA-type electrodes using niobium substrate. *Electrocatalysis* **2010**, *1*, 129. [CrossRef]
23. Eleotério, I.C.; Forti, J.C.; De Andrade, A.R. Electrochemical treatment of wastewater of veterinary industry containing antibiotics. *Electrocatalysis* **2013**, *4*, 283. [CrossRef]
24. Forti, J.C.; Rocha, R.S.; De Andrade, A.R.; Lanza, M.R.V. Application of Oxides Electrodes (Ru, Ti, Ir and Sn) for the Electrooxidation of Levofloxacin. *Curr. Anal. Chem.* **2019**, *15*, 66. [CrossRef]

25. Comminellis, C. Electrocatalysis in the electrochemical conversion/combustion of organic pollutants for waste water treatment. *Electrochim. Acta* **1994**, *39*, 1857.
26. Stupar, S.L.; Ivanković, N.D.; Grgur, B.N.; Tomašević, A.V.; Mijin, D.Ž.; Koprivica, K. Investigation of direct electrochemical oxidation of neonicotinoid pesticide thiamethoxam in water. *Sci. Tech. Rev.* **2020**, *70*, 11. [CrossRef]
27. Lebig-Elhadi, H.; Frontistis, Z.; Ait-Amar, H.; Amrani, S.; Mantzavinos, D. Electrochemical oxidation of pesticide thiamethoxam on boron doped diamond anode: Role of operating parameters and matrix effect. *Process Saf. Environ. Prot.* **2018**, *116*, 535. [CrossRef]
28. Gomez-Herrero, E.; Lebig-Elhadi, H.; Ait-Amar, H.; Tobajas, M.; Rodriguez, J.J.; Mohedano, A.F. Thiamethoxam removal by Fenton and biological oxidation. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 913–921. [CrossRef]
29. Mejjide, J.; Gómez, J.; Pazos, M.; Sanromán, M.A. Degradation of thiamethoxam by the synergetic effect between anodic oxidation and Fenton reactions. *J. Hazard. Mater.* **2016**, *319*, 43. [CrossRef]
30. Mir, N.A.; Khan, A.; Muneer, M.; Vijayalakshmi, S. Photocatalytic degradation of a widely used insecticide Thiamethoxam in aqueous suspension of TiO₂: Adsorption, kinetics, product analysis and toxicity assessment. *Sci. Total Environ.* **2013**, *458*, 388. [CrossRef]
31. Guedes, R.N.C.; Biondi, A.; Agathokleous, E.; Nunes-Nesi, A. (Systemic) Insecticides in Plants: Phytotoxicity, Bioactivation, or Hormesis? *Agric. Commun.* **2023**, *1*, 100002. [CrossRef]
32. Liu, T.; Li, T.; Zhang, L.; Li, H.; Liu, S.; Yang, S.; An, Q.; Pan, C.; Zou, N. Exogenous salicylic acid alleviates the accumulation of pesticides and mitigates pesticide-induced oxidative stress in cucumber plants (*Cucumis sativus* L.). *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111654. [CrossRef]
33. Noctor, G.; Mhamdi, A.; Foyer, C.H. Oxidative stress and antioxidative systems: Recipes for successful data collection and interpretation. *Plant Cell Environ.* **2016**, *39*, 1140. [CrossRef] [PubMed]
34. Wang, X.; Anadón, A.; Wu, Q.; Qiao, F.; Ares, I.; Martínez-Larrañaga, M.R.; Yuan, Z.; Martínez, M.A. Mechanism of neonicotinoid toxicity: Impact on oxidative stress and metabolism. *Annu. Rev. Pharmacol. Toxicol.* **2018**, *58*, 471. [CrossRef] [PubMed]
35. Da Silva, V.E.; Tadayozzim, Y.S.; Puttim, F.F.; Santosm, F.A.; Fortim, J.C. Degradation of commercial glyphosate-based herbicide via advanced oxidative processes in aqueous media and phytotoxicity evaluation using maize seeds. *Sci. Total Environ.* **2022**, *840*, 156656. [CrossRef] [PubMed]
36. Yan, S.H.; Wang, J.H.; Zhu, L.S.; Chen, A.M.; Wang, J. Thiamethoxam induces oxidative stress and antioxidant response in zebrafish (*Danio rerio*) livers. *Environ. Toxicol.* **2016**, *31*, 2006. [CrossRef] [PubMed]
37. Racchi, M.L. Antioxidant defenses in plants with attention to *Prunus* and *Citrus* spp. *Antioxidants* **2013**, *2*, 340. [CrossRef] [PubMed]
38. Queiroz, C.G.S.; Alonso, A.; Mares-Guia, M.; Magalhaes, A.C. Chilling-induced changes in membrane fluidity and antioxidant enzyme activities in *Coffea arabica* L. roots. *Biol. Plant.* **1998**, *41*, 403. [CrossRef]
39. ASTM D1252-95; American Society for Testing and Materials, Standard Test Methods for Chemical Oxygen Demand (Dichromate Oxygen Demand) of Water. ASTM Annual Book of Standards. American Soc. Testing & Materials: Philadelphia, PA, USA, 1995. Available online: www.astm.org/Standards/D1252.htm (accessed on 12 October 2023).
40. Kang, Y.W.; Cho, M.J.; Hwang, K.Y. Correction of hydrogen peroxide interference on standard chemical oxygen demand test. *Water Res.* **1999**, *33*, 1247.
41. Forti, J.C.; Loretto, G.H.; Tadayozzi, Y.S.; de Andrade, A.R. A phytotoxicity assessment of the efficiency 2,4-D degradation by different oxidative processes. *J. Environ. Manag.* **2020**, *266*, 110588. [CrossRef] [PubMed]
42. Crispim, A.C.; Araújo, D.M.; Martínez-Huitle, C.A.; Souza, F.L.; Dos Santos, E.V. Application of electro-Fenton and photoelectro-Fenton processes for the degradation of contaminants in landfill leachate. *Environ. Res.* **2022**, *213*, 113552. [CrossRef]
43. BRASIL. Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária, Regras para análise de sementes. 2009. Available online: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise_sementes.pdf (accessed on 10 January 2024).
44. Quan, L.; Cheng, Y.; Wang, J.; Chen, Y.; Li, D.; Wang, S.; Li, B.; Zhang, Z.; Yang, L.; Wu, L. Efficient removal of thiamethoxam by freshwater microalgae *Scenedesmus* sp. TXH: Removal mechanism, metabolic degradation and application. *J. Environ. Manag.* **2023**, *332*, 117388. [CrossRef]
45. Homayonzadeh, M.; Moeini, P.; Talebi, K.; Roessner, U.; Hosseininaveh, V. Antioxidant system status of cucumber plants under pesticides treatment. *Acta Physiol. Plant.* **2020**, *42*, 161. [CrossRef]
46. Brodeur, J.C.; Fonseca Peña, S.V.D. Effects of the neonicotinoid insecticides thiamethoxam and imidacloprid on metamorphosis of the toad *Rhinella arenarum* at environmentally-relevant concentrations. *J. Toxicol. Environ. Health Part A* **2023**, *86*, 434. [CrossRef] [PubMed]

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