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Degradation and toxicity attenuation of persistent triazole contaminants by sequence-controlled ferrate(VI)-ozone oxidation

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Abstract

Advanced oxidation processes (AOPs) offer a promising route for degrading persistent organic pollutants, yet single-oxidant systems are limited by selective reactivity and incomplete mineralization. To overcome these limitations, this study developed a sequence-controlled ferrate(VI)-ozone (Fe(VI)-O₃) process and evaluated its performance on fluconazole (FLZ) and tebuconazole (TEB), two representative triazoles. The key innovation lies in oxidant sequencing: pre-oxidation with Fe(VI), followed by ozonation, enabling dual benefits of substrate pre-modification and enhanced reactive-species formation, substantially outperforming simultaneous addition or reverse ordering. Under the selected conditions, FLZ and TEB removals reached 92.5% and 90.4% within 60 min, with corresponding TOC removals of 70.2% and 71.5%—values that far exceeded the additive responses of the single-oxidant controls (synergy indices: 52.7% for FLZ, 52.4% for TEB). Mechanistic investigations suggested that Fe(VI) pre-oxidation altered reactive sites in the target molecules, while ferrate-derived iron species were associated with enhanced ozone decomposition and stronger EPR-detectable reactive-species signals. EPR and scavenger tests identified $\cdot\text{OH}$, $\cdot\text{O}_2^-$, and $^1\text{O}_2$ as contributors, with $\cdot\text{OH}$ playing the dominant role (apparent contribution: 61.5% for FLZ, 59.9% for TEB). DFT and UHPLC-MS results further

suggested preferential attack at triazole rings and halogenated aromatic moieties, initiating hydroxylation, dehalogenation, ring-opening, and carbon-chain cleavage. Although some products retained predicted toxicity, bioassays showed attenuated biological effects after treatment, consistent with a reduction in overall toxicity. This work demonstrates that proper oxidant sequencing is an effective strategy for achieving enhanced mineralization and detoxification in the synergistic application of ferrate and ozone.

Keywords: Ferrate(VI); Ozonation; Triazole pollutants; Coupling mechanism; Ecotoxicity

1. Introduction

Triazole compounds are widely used as antifungal pharmaceuticals and agricultural fungicides, which are continuously released into aquatic environments through municipal wastewater, hospital effluents, agricultural runoff, and drainage from fungicide-treated fields¹⁻³. Their characteristic triazole rings and certain halogenated aromatic moieties confer high chemical stability and resistance to microbial degradation. Unlike many other emerging contaminants that succumb to hydroxylation or hydrolysis, the triazole ring exhibits high chemical stability, and the electron-withdrawing halogen substituents on aromatic rings further deactivate these structures toward oxidative attack. This resultant stabilization makes triazoles particularly recalcitrant to conventional oxidation⁴. Moreover, oxidative degradation of the nitrogen-rich heterocycle can generate nitrogen-containing transformation products that may maintain similar toxicity to parent compounds or even acquire higher toxicity; consequently, parent-compound disappearance does not guarantee risk reduction⁵. These inherent challenges—high structural stability, resistance to oxidative ring opening, and the potential formation of toxic intermediates—necessitate treatment strategies that achieve not only parent-compound removal but also enhanced mineralization and demonstrable toxicity attenuation.

Fluconazole (FLZ) and tebuconazole (TEB) are representative, environmentally relevant triazoles from pharmaceutical and agricultural use, respectively. Both compounds are poorly removed by conventional wastewater treatment and are frequently detected in aquatic environments⁶. Structurally, FLZ bears two triazole rings and a difluorophenyl group, whereas TEB contains one triazole ring, a chlorinated phenyl ring, and a tert-butyl side chain. These structural differences are expected to influence molecular electron distribution, oxidant accessibility, and the preferential sites of electrophilic and radical attack, potentially leading to distinct transformation pathways for FLZ and TEB⁷. These contrasting structural features provide a useful basis for examining how molecular structure governs reactivity and transformation under sequence-controlled Fe(VI)–O₃ oxidation.

Ozone (O₃) is widely applied in advanced water treatment because it can oxidize organic contaminants directly and decompose to generate highly reactive hydroxyl radicals (•OH)⁸. However, molecular ozone is inherently selective, preferentially reacting with electron-rich moieties such as C=C bonds and activated aromatic systems; the halogenated aromatic moieties in FLZ and TEB may therefore exhibit comparatively lower reactivity toward electrophilic O₃ attack. In addition, O₃ application is limited by gas–liquid mass transfer constraints, which reduce utilization efficiency, and incomplete oxidation can produce transformation products that retain biological activity. By contrast, ferrate(VI) is a high-valent iron oxidant capable of electron-transfer oxidation, providing reaction pathways that differ from the predominantly electrophilic reactions of molecular ozone⁹. Its reduction product, Fe(III), also contributes to coagulation and adsorption functions. Nonetheless, the reactivity and stability of Fe(VI) are strongly pH-dependent, reflecting changes in ferrate speciation, intrinsic reactivity, and self-decay behavior¹⁰.

Recent studies have demonstrated that coupling Fe(VI) with O₃ can enhance the oxidation of micropollutants through interactions among ferrate-derived iron species, ozone, and reactive oxygen species^{11–13}. Stepwise ferrate pre-oxidation followed by ozonation has also been reported for selected contaminants, suggesting that ferrate pretreatment may alter both pollutant reactivity and the subsequent ozonation

environment¹⁴. However, the role of oxidant addition order in governing synergistic degradation, mineralization, reactive-species evolution, transformation pathways, and detoxification of structurally distinct triazole contaminants remains insufficiently resolved. In particular, it remains unclear whether Fe(VI) pre-oxidation can strategically modify recalcitrant triazole structures and thereby enhance their susceptibility to subsequent ozonation, rather than merely producing an additive effect of the two oxidants. We therefore hypothesized that Fe(VI) pre-oxidation could transform initially less reactive molecular moieties while generating iron-containing intermediates that favor subsequent ozone-driven oxidation and reactive-species formation.

Accordingly, we systematically investigated a sequence-controlled Fe(VI)–O₃ process for FLZ and TEB with three objectives: (i) to determine the magnitude of sequence-dependent synergistic effects through systematic comparison of different oxidant-dosing configurations; (ii) to elucidate the mechanistic basis of the observed synergy by identifying reactive species and pollutant transformation pathways; and (iii) to evaluate whether enhanced oxidation leads to reduced ecological toxicity using integrated *in silico* prediction and multispecies bioassays. The broader substrate scope of the process was further evaluated using twelve antibiotics from four structural classes.

2. Materials and methods

2.1. Chemicals and reagents

Fluconazole, tebuconazole, potassium ferrate (K₂FeO₄), reactive-species scavengers, spin-trapping reagents, and analytical-grade chemicals were used as received. FLZ and TEB stock solutions were prepared in ultrapure water. Fe(VI) solutions were freshly prepared immediately before use and protected from strong light. Ozone was generated from oxygen using a laboratory ozone generator. Detailed information on reagent purity and suppliers is provided in Text S1 of the Supplementary Information.

Batch experiments were performed in a 500 mL stirred reactor equipped with an ozone gas-transfer unit, a gas-flow controller, and an off-gas destructor. Unless otherwise stated, the initial concentration of FLZ or TEB was 10 mg L⁻¹, the temperature was maintained at 25 ± 1 °C, and the initial pH was adjusted to 7.5 using 0.1 mol L⁻¹ HCl or NaOH. Fe(VI) was added at a molar ratio of n(Fe(VI)):n(contaminant) = 10:1. Ozone was continuously supplied at a nominal feed concentration of 10 mg L⁻¹ and a gas flow rate of 0.5 L min⁻¹. Detailed information on reagent purity and suppliers is provided in Text S1 of the Supplementary Information.

2.2.1. Single-oxidant controls

For ozonation alone, ozone was introduced at the beginning of the experiment and continuously supplied throughout the reaction. For Fe(VI) oxidation alone, freshly prepared K₂FeO₄ was rapidly added at t = 0 and the solution was continuously stirred. Samples were collected at predetermined times and immediately quenched before analysis.

2.2.2. Sequential and simultaneous Fe(VI)–O₃ treatment

Three coupled configurations were compared. In the staged Fe(VI)-O₃ process, Fe(VI) was added at t = 0 and the solution was allowed to react for 10 min before ozone introduction. In the simultaneous Fe(VI)+O₃ process, Fe(VI) addition and ozone supply were initiated at the same time. In the O₃-Fe(VI) process, In the O₃-Fe(VI) process, ozone was applied before Fe(VI) addition. Samples (2 – 5 mL) were withdrawn at 0, 5, 10, 20, 30, 45, and 60 min. Sodium thiosulfate was immediately added to terminate residual oxidants and reactive intermediates, after which samples were filtered through 0.22 µm membranes before chemical analysis.

2.3. Synergy analysis

Removal efficiency was calculated based on the ratio of the contaminant

concentration at time (t) to its initial concentration. When the removal efficiencies were expressed as fractions, the probabilistic additive response of the independent Fe(VI) and O₃ treatments was calculated as

$$R_{\text{add}} = R_{\text{Fe(VI)}} + R_{\text{O}_3} - R_{\text{Fe(VI)}}R_{\text{O}_3}$$

$$S = \frac{R_{\text{Fe(VI)-O}_3} - R_{\text{add}}}{R_{\text{Fe(VI)-O}_3}}$$

Total organic carbon (TOC) removal was used as a complementary indicator to evaluate the overall oxidation performance.

2.4. Operational parameters and water constituents

The effects of the Fe(VI)-to-contaminant molar ratio, ozone-feed concentration, and initial pH were evaluated using the staged Fe(VI)-O₃ process. The influences of Mg²⁺、Ca²⁺、SO₄²⁻、Cl⁻、NO₃⁻、CO₃²⁻ and HCO₃⁻ were also examined.

2.5. Chemical analysis

Residual Fe(VI) was determined by the ABTS method, and ozone was measured using the indigo method. TOC was measured after membrane filtration. Transformation products were analyzed by UHPLC-MS. Detailed analytical procedures for FLZ, TEB, Fe(VI), dissolved O₃, TOC, and transformation-product analysis are provided in Text S2.

2.6. Reactive-species identification and contribution assessment

Reactive species were identified by EPR using appropriate spin-trapping reagents for ·OH, ·O₂⁻, and ¹O₂. EPR spectra were collected at selected reaction times under identical instrumental conditions. Scavenger experiments were conducted using tert-butanol for ·OH, p-benzoquinone for ·O₂⁻, and L-histidine for ¹O₂. The apparent contribution of each reactive species was estimated from the decrease in contaminant removal caused by the corresponding scavenger. Because scavengers are not perfectly

selective and may affect oxidant chemistry, these values were interpreted as semi-quantitative apparent contributions rather than absolute reaction fractions. Detailed EPR settings and scavenger concentrations are provided in Text S3.

2.7. Transformation-product analysis and DFT calculations

Samples collected during oxidation were immediately quenched, filtered, extracted, and analyzed by UHPLC-MS. Transformation products were tentatively assigned primarily on the basis of the observed mass-to-charge ratios (m/z) and plausible molecular transformations, with available fragmentation information used as supporting evidence. Molecular structures were optimized at the B3LYP/6-31G(d,p) level. Electrostatic potential, frontier molecular orbitals, condensed Fukui functions, and the dual descriptor were used to identify positions susceptible to electrophilic, nucleophilic, and radical attack. Transformation pathways were proposed only when predicted reactive sites were consistent with detected mass-spectrometric features. Detailed computational procedures and Fukui-function calculations are provided in Text S4.

2.8. Toxicity assessment

Potential acute and chronic toxicity of the proposed transformation products to fish, *Daphnia*, and green algae was screened using ECOSAR. Biological toxicity was evaluated using wheat-seed germination and shoot growth, *Escherichia coli* colony formation, and zebrafish-embryo development. Untreated contaminant solutions and effluents from single-oxidant and coupled systems were compared with blank controls. Residual oxidants were completely quenched before bioassays to avoid confounding disinfection effects. Detailed ECOSAR procedures and biological toxicity assays are provided in Text S5.

2.9. Substrate-scope experiments

The substrate scope of the staged Fe(VI)-O₃ process was further evaluated using twelve representative antibiotics belonging to the tetracycline, macrolide, sulfonamide, and

fluoroquinolone classes. These compounds were selected to provide a preliminary assessment of the reactivity of the process toward structurally diverse contaminants. Unless otherwise specified, each antibiotic was tested individually under the same operating conditions used for FLZ and TEB. Fe(VI) was added at the beginning of the experiment, followed by ozone introduction after a 10 min pre-oxidation period, and the total reaction time was 60 min. Samples collected at the end of the reaction were immediately quenched and analyzed to determine the residual antibiotic concentrations. These experiments were conducted as a screening-level evaluation of substrate scope rather than as evidence of full-scale treatment applicability. The identities and initial concentrations of the selected antibiotics are provided in Table S1.

2.10. Statistical analysis

All experiments were performed using independent replicates. Results are reported as mean $\pm$ standard deviation. Differences among treatments were evaluated using an appropriate analysis of variance followed by a multiple-comparison test, with $p < 0.05$ considered statistically significant.

3. Results and discussion

3.1. Synergistic degradation and organic carbon removal under different Fe(VI)-O₃ dosing configurations

The removal of FLZ and TEB under different oxidation configurations is shown in Fig. 1. After 60 min, Fe(VI) alone and O₃ alone removed 19.3% and 30.3% of FLZ, respectively, while the corresponding removals of TEB were 21.6% and 27.3%. Coupling Fe(VI) with O₃ substantially improved contaminant removal. The O₃-Fe(VI), simultaneous Fe(VI)+O₃, and staged Fe(VI)-O₃ configurations achieved FLZ removals of 67.5%, 75.6%, and 92.5%, respectively, and TEB removals of 74.5%, 76.4%, and 90.4%, respectively. Among the tested configurations, Fe(VI) pre-oxidation followed by ozonation provided the highest removal of both contaminants.

The differences among the configurations were more pronounced for TOC removal. Fe(VI) alone and O₃ alone removed 20.3% and 21.3% of FLZ-derived TOC, respectively, whereas the staged Fe(VI)-O₃ process achieved 70.2% TOC removal. For TEB, TOC removal increased from 21.6% with Fe(VI) alone and 27.3% with O₃ alone to 71.5% in the staged process. Simultaneous Fe(VI)+O₃ treatment removed 75.6% of FLZ and 76.4% of TEB but achieved TOC removals of only 46.5% and 41.3%, respectively. The relatively high parent-compound removal but lower TOC abatement under simultaneous dosing indicates that parent transformation and carbon removal were not equally enhanced. In contrast, the staged configuration was associated with both extensive parent removal and greater oxidation of the remaining organic carbon.

The probabilistic additive removals derived from the single-oxidant controls were 43.8% for FLZ and 43.0% for TEB, both substantially lower than the measured removals in the staged Fe(VI)-O₃ process. The corresponding synergy indices were 52.7% and 52.4%, demonstrating that the observed enhancement could not be explained by the independent removal probabilities of Fe(VI) and O₃ alone. The enhanced performance was likely related to interactions between ferrate pre-oxidation and subsequent ozone-derived oxidation. The underlying reactive-species network and transformation mechanisms are examined in Sections 3.3 – 3.5.

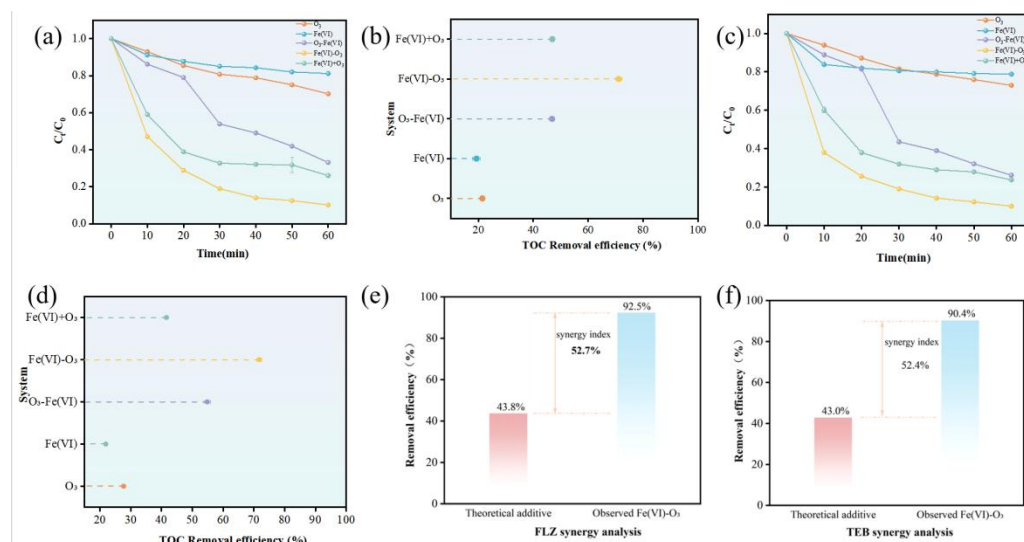


Fig. 1 Degradation of FLZ and TEB in water by different systems: (a) FLZ degradation rate, (b) TOC removal rate of FLZ in different systems (t = 60 min), (c) TEB degradation rate, (d) TOC removal rate of TEB in different systems., (e)FLZ synergy analysis ,(f)TEB synergy

analysis. Experimental conditions: Dosing ratio = 10:1, $C(O_3) = 10 \text{ mg/L}$, flow rate = 0.5 L/min, pH = 7.5, temperature = 25 °C

3.2. Effects of operational parameters and water-matrix constituents

Fe(VI) dosage strongly affected the performance of the coupled process (Fig. 2c and d). Increasing the Fe(VI)-to-contaminant molar ratio from 5:1 to 10:1 increased FLZ removal from 51.3% to 90.9% and TEB removal from 56.4% to 91.7%, respectively. The corresponding TOC removals increased from 25.2% and 29.3% to 69.3% and 70.5%, respectively. A further increase to 15:1 resulted in only marginal improvements, with FLZ and TEB removals reaching 93.7% and 92.4%. At 20:1, however, the removals decreased to 77.5% and 75.4%, respectively. This non-monotonic response indicates that increasing the Fe(VI) dose was beneficial only within an oxidant-limited range. At excessive doses, Fe(VI) self-decay, non-target reactions, and competition for reactive species may reduce effective oxidant utilization^{9,15}. Increased formation of Fe(III) precipitates may also influence contaminant mass transfer and the apparent TOC removal measured after filtration. A molar ratio of 10:1 was therefore selected as a practical compromise between treatment performance and chemical demand¹⁶.

Increasing the O_3 supply also enhanced treatment performance, although the incremental benefit diminished at higher concentrations (Fig. 2a and b). When the ozone-feed concentration was increased from 1 to 10 mg L^{-1} , FLZ removal increased from 37.8% to 88.4%, while TEB removal increased from 29.8% to 86.5%. Their corresponding TOC removals increased from 17.8% and 19.8% to 68.4% and 66.5%, respectively. Increasing the O_3 concentration further to 20 mg L^{-1} raised FLZ and TEB removal to only 95.3% and 91.6%, with TOC removals of 72.3% and 71.6%. The observed plateau suggests a transition from ozone-limited conditions to a regime increasingly controlled by competition among the parent contaminants, transformation products, and background constituents for reactive species¹⁷. Ozone loss through off-gassing and non-target decomposition may also become more important at elevated

input. Accordingly, 10 mg L⁻¹ was used in subsequent experiments to avoid the limited benefit associated with the higher ozone supply¹⁸.

The Fe(VI)-O₃ process performed most effectively under neutral to mildly alkaline conditions, with relatively high contaminant and TOC removals at pH 7.0-8.0 and lower performance at pH 5.0 and 9.0 (Fig. 2e and f). This trend likely reflects the combined effects of Fe(VI) stability, protonation state, and ozone decomposition. Although protonated Fe(VI) species have comparatively high intrinsic oxidation reactivity, rapid Fe(VI) decay under acidic conditions may shorten the effective reaction period. Near neutral pH, improved Fe(VI) persistence coincides with the presence of the relatively reactive HFeO₄⁻ species. At higher pH, the increasing predominance of the less reactive FeO₄²⁻ species may weaken direct oxidation. On the basis of treatment performance and the pH range commonly encountered in environmental waters, pH 7.5 was selected for subsequent experiments. Because different buffers were used in the original pH tests, however, the observed trend represents the combined effects of pH and buffer composition; their individual contributions require verification under buffer-free conditions¹⁹.

The tested inorganic ions produced distinct matrix effects (Fig. 2g and h). Mg²⁺, Ca²⁺, SO₄²⁻, Cl⁻ and NO₃⁻ caused only limited changes in FLZ and TEB removal, indicating a degree of tolerance to these common ions. In contrast, CO₃²⁻ and HCO₃⁻ markedly inhibited the removal of both contaminants. This inhibition is consistent with competitive scavenging of •OH by carbonate species, which converts highly reactive •OH into the more selective and less reactive carbonate radical^{20,21}. Carbonate alkalinity may also modify solution pH, Fe(VI) speciation, and ozone-decomposition pathways. These results identify alkalinity as a critical water-quality parameter for applying the Fe(VI)-O₃ process to natural waters and wastewater effluents. Nevertheless, the inorganic-ion tests alone do not represent the additional oxidant demand and radical scavenging expected in waters containing natural organic matter.

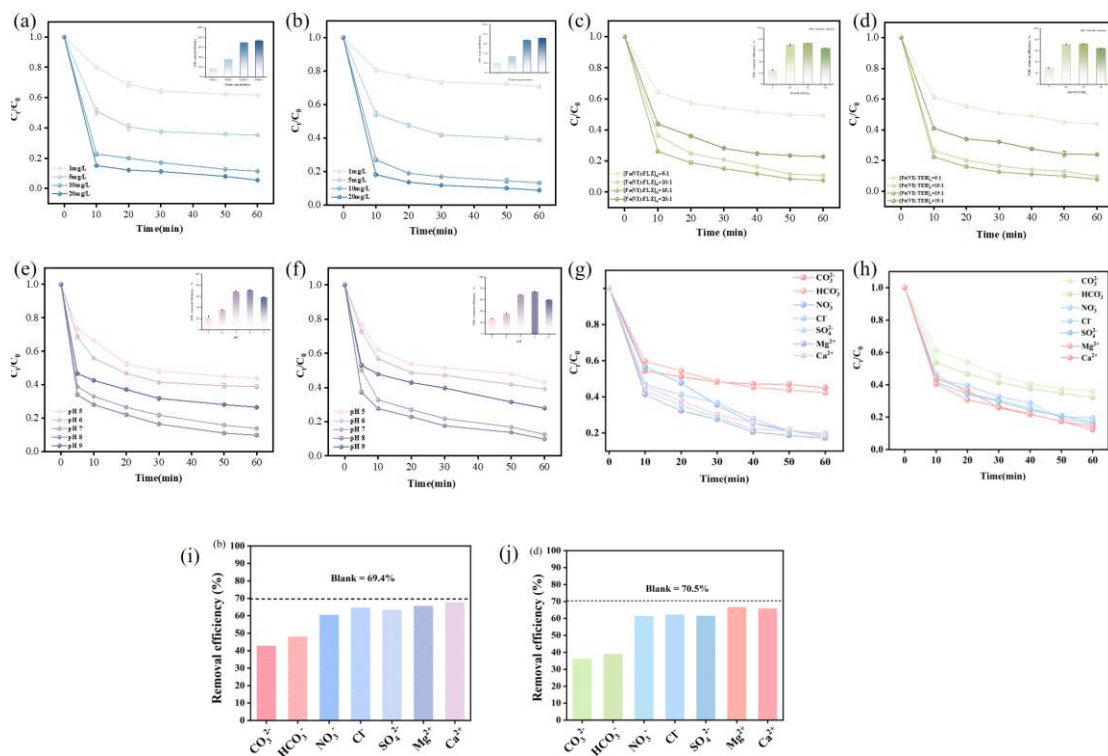


Fig. 2 Effects of key operational parameters and water-matrix constituents on FLZ and TEB degradation. a,b, Effect of O_3 concentration on the degradation kinetics of FLZ and TEB. c,d, Effect of the Fe(VI)-to-contaminant dosing ratio. e,f, Effect of initial pH. g,h, Effect of common coexisting ions. Insets show the corresponding removal efficiencies after 60 min. Experimental conditions: Dosing ratio = 10:1, $C(O_3) = 10\text{mg/L}$, flow rate = 0.5 L/min, pH = 7.5, temperature = 25 °C

3.3. Reactive species and their apparent contributions

EPR analysis indicated the formation of $\cdot OH$, $\cdot O_2^-$, and $^1O_2$ related signals in the Fe(VI)- O_3 process (Fig. 3). No evident DMPO- $\cdot OH$ or DMPO- $\cdot O_2^-$ signals were detected in the Fe(VI)-alone systems during the first 10 min, suggesting that Fe(VI) oxidation proceeded predominantly through direct electron-transfer pathways under the tested conditions. Ozonation alone generated weak signals associated with $\cdot OH$, $\cdot O_2^-$, and $^1O_2$ at 5 and 10 min, indicating limited reactive-species formation through ozone decomposition²². In contrast, Fe(VI) pre-oxidation followed by ozonation markedly enhanced all three EPR responses, with comparable trends observed for FLZ and TEB.

Simultaneous Fe(VI)+O₃ addition also promoted •OH formation, but the signal intensity was lower than that observed in the staged configuration. These results demonstrate that Fe(VI)-O₃ coupling increased both the diversity and the detectable abundance of reactive species. Nevertheless, because spin-trapping reagents may react with multiple short-lived intermediates, the EPR data were interpreted as qualitative evidence rather than absolute quantification²³.

Scavenger experiments further clarified the roles of the identified species (Fig. 4). In the absence of scavengers, 92.4% of FLZ and 93.7% of TEB were removed within 60 min. Addition of 1 g L⁻¹ tert-butanol decreased their removals to 33.6% and 34.4%, respectively, confirming •OH as the dominant oxidant. In the presence of p-benzoquinone, FLZ and TEB removals decreased to 63.7% and 68.9%, whereas L-histidine reduced the corresponding removals to 67.3% and 75.4%. These results indicate that •O₂⁻ and ¹O₂ also contributed to contaminant transformation. Based on the changes in endpoint removal, the apparent •OH contributions were estimated as 61.5% for FLZ and 59.9% for TEB. The apparent contributions of •O₂⁻ were 14.1% and 16.0%, while those of ¹O₂ were 8.7% and 12.1%, respectively. Thus, degradation of both triazoles was characterized by an •OH dominated oxidation regime supplemented by superoxide- and singlet-oxygen-associated pathways.

Although •OH accounted for the major direct oxidative contribution, •O₂⁻ and ¹O₂ likely played complementary roles. Superoxide generally has limited direct reactivity toward many persistent organic contaminants but can participate in ozone-decomposition chains and thereby sustain •OH production. Singlet oxygen may provide a more selective pathway for heterocyclic or electronically favorable reaction sites²⁴. The slightly greater apparent •O₂⁻ and ¹O₂ contributions observed for TEB may reflect differences in accessibility and reaction selectivity arising from its chlorinated aromatic group, triazole ring, and hydrophobic side chain. However, scavengers are not completely species-selective and may also perturb ozone decomposition and Fe(VI) transformation; the calculated fractions should therefore be considered semi-quantitative apparent contributions. The residual fractions of 15.7% for FLZ and 12.0% for TEB may include direct Fe(VI) and O₃ oxidation, other non-radical pathways, and

possible high-valent iron intermediates²⁵. The current evidence, however, is insufficient to assign the entire residual fraction specifically to Fe(V) or Fe(IV). The interactions among these pathways are further discussed in Section 3.4.

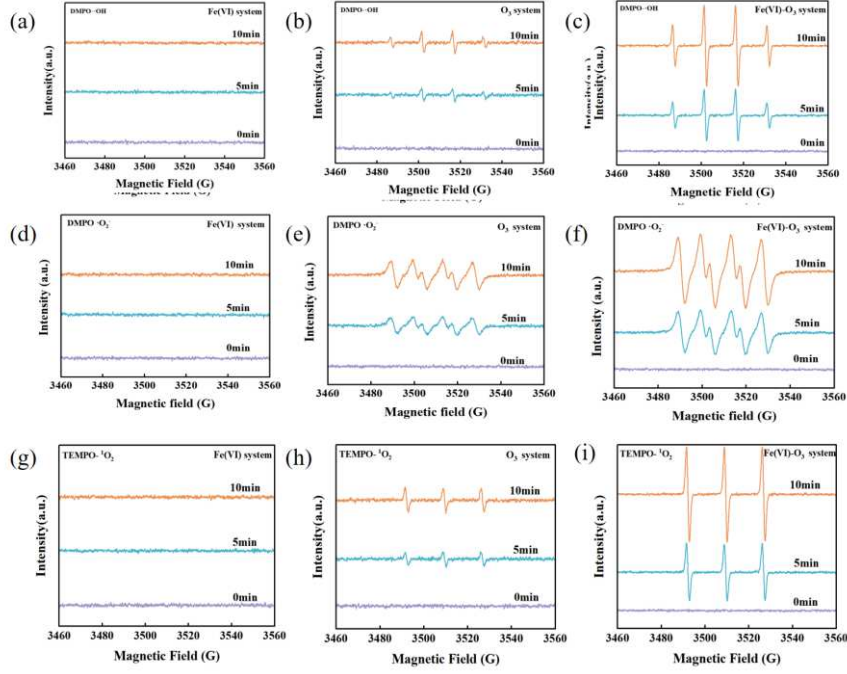
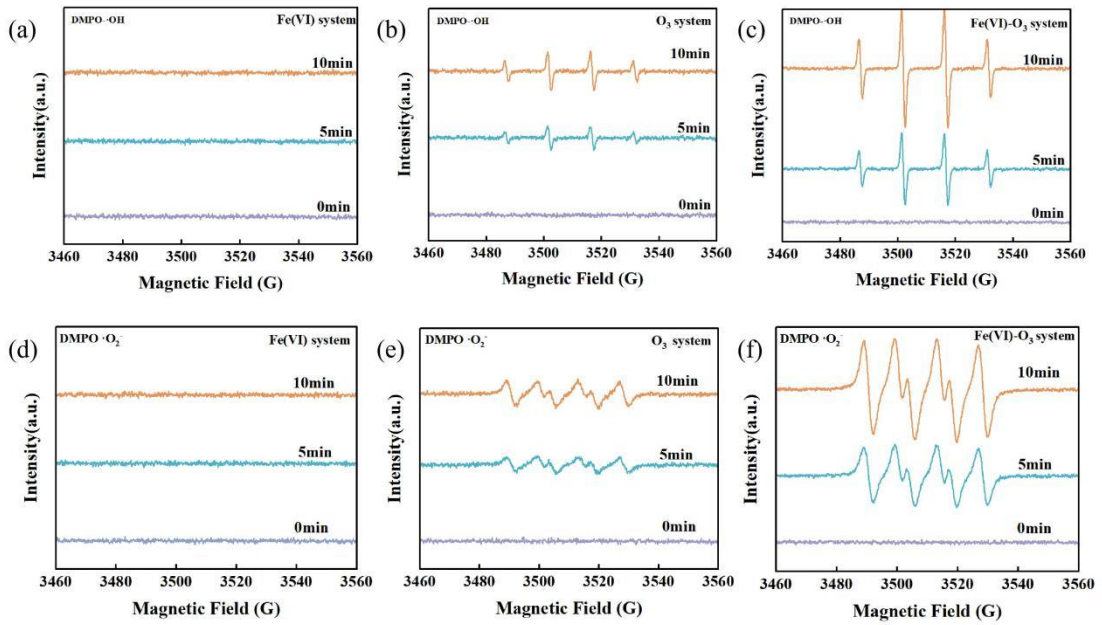


Fig. 3 EPR spectra of FLZ degradation in different systems



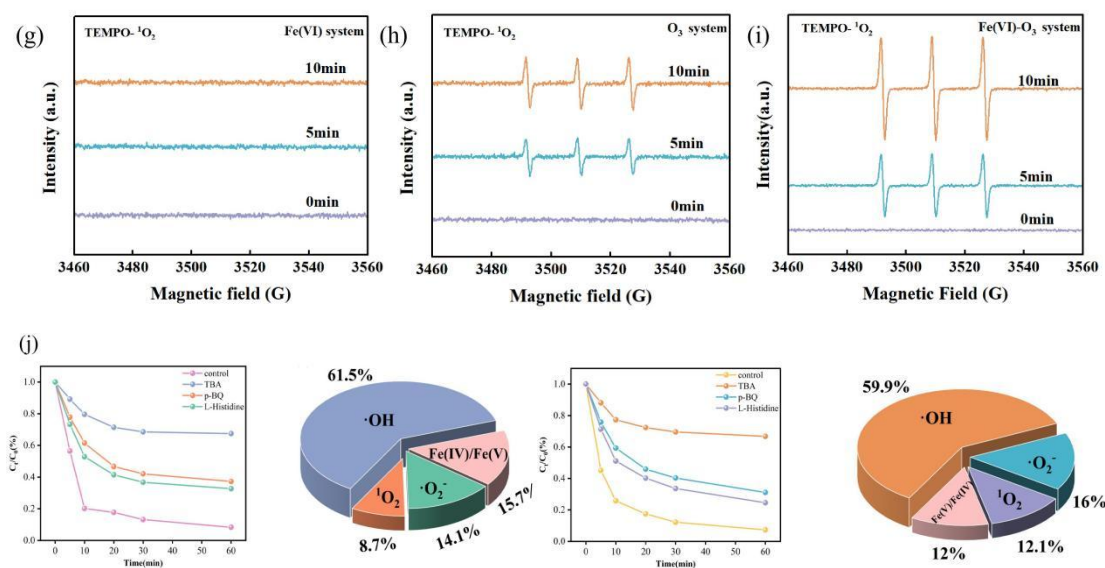


Fig. 4 Scavenger experiments and apparent contributions of reactive species during FLZ and TEB degradation.

3.4. Mechanistic interpretation of the enhanced performance in the staged Fe(VI)-O₃ process

Fe(VI)-O₃ process was not simply a consequence of combining two oxidants. As Fig. 5 shows below, the synergistic application of Fe(VI) and ozone (O₃) establishes an advanced oxidation system with multivalent iron transformations and the generation of reactive oxygen species. The advantage of the staged More importantly, Fe(VI) and O₃ acted at different stages of the reaction. Before ozonation, Fe(VI) had already reacted with FLZ and TEB, initiating oxidation at the triazole rings, halogenated aromatic groups, and side-chain positions. Ferrate reduction also produced iron intermediates and Fe(III) products²⁶. Once O₃ was introduced, stronger signals of $\cdot\text{OH}$, $\cdot\text{O}_2^-$, and $^1\text{O}_2$ were detected, indicating that the preceding Fe(VI) reaction altered the conditions governing ozone decomposition and reactive-species formation²⁷.

This staged process provided a better transition from parent-compound transformation to further oxidation of the resulting intermediates. Products formed during Fe(VI) pre-oxidation were likely more susceptible to ozone and ozone-derived reactive species than the original triazole structures. This is consistent with the

simultaneous improvement in parent removal and TOC abatement observed in the Fe(VI)-O₃ configuration. Under simultaneous dosing, direct contact between Fe(VI) and O₃ at the beginning of the reaction may have caused rapid oxidant consumption and a short-lived release of reactive species. Such a response could promote parent-compound disappearance without sustaining the oxidation of downstream products, as suggested by the lower TOC removal. When O₃ was applied first, part of the ozone may have been consumed before ferrate-derived iron species accumulated.

The synergy therefore appears to arise from the temporal coupling of selective Fe(VI) pre-oxidation with subsequent ozone-driven oxidation. Direct Fe(VI) and O₃ reactions, $\cdot\text{OH}$ dominated oxidation, and auxiliary pathways involving $\cdot\text{O}_2^-$ and $^1\text{O}_2$ likely contributed together. The present data, however, do not establish a complete Fe(V)/Fe(IV) redox cycle. In addition, adsorption or coprecipitation by Fe(III) (hydr)oxides may have contributed to the observed TOC decrease²⁸.

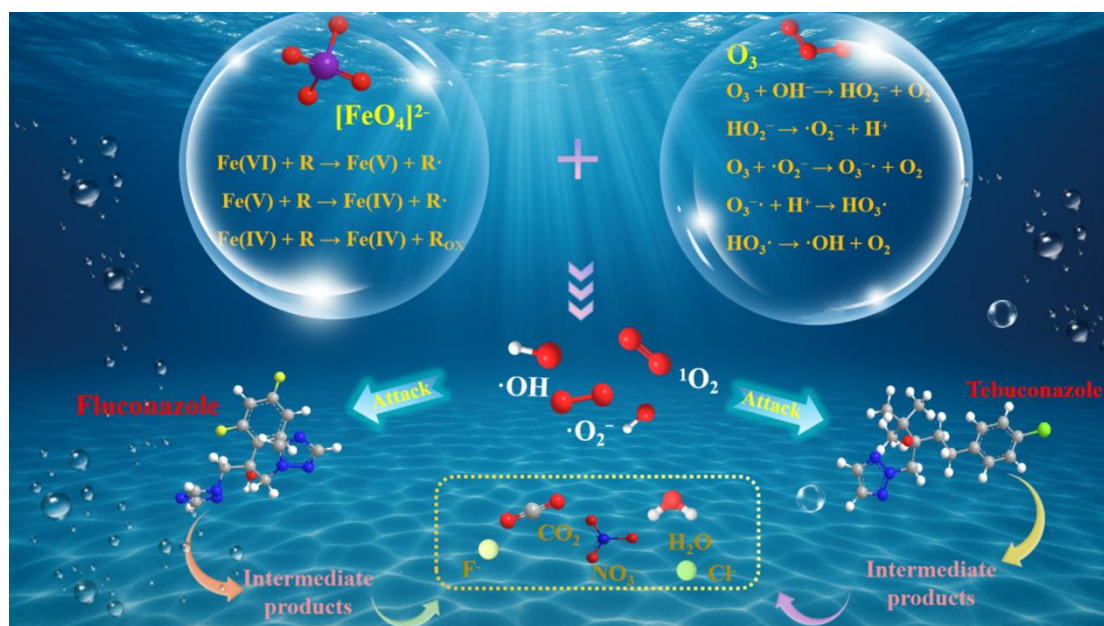


Fig. 5 Proposed degradation mechanism of FLZ and TEB in the Fe(VI)-O₃ system

3.5. Molecular transformation pathways of FLZ and TEB

DFT calculations located the most reactive regions of FLZ around the triazole rings, the difluorophenyl group, and the ether-linked moiety (Fig. 6). Nitrogen atoms in the triazole rings, selected aromatic carbon atoms, and the ether oxygen showed

relatively high electrophilic or radical susceptibility, consistent with the HOMO and Fukui-function distributions. Nine tentative FLZ transformation products were detected by UHPLC-MS (Fig. 6). One pathway involved initial hydroxylation or defluorination at the difluorophenyl group, followed by oxidation of the aromatic and triazole structures, producing intermediates at m/z 304.11, 292.09, 192.07, and 153.05. A second pathway involved cleavage near the ether linkage and opening of the triazole ring, yielding smaller products at m/z 223.05, 141.05, 69.03, and 133.04²⁹. FLZ transformation therefore involved several concurrent reactions, including hydroxyl substitution, C-N/C-O bond cleavage, and heterocyclic-ring opening³⁰.

For TEB, the predicted reactive regions were concentrated on the chlorinated aromatic ring, the triazole moiety, and the carbon side chain (Fig. 7). Two main transformation routes were inferred (Fig. 7). The first started with oxidation of the side-chain or chlorinated-aromatic region, followed by triazole-ring cleavage and formation of products at m/z 271.19, 69.03, 133.04, and 60.02. The second involved aromatic hydroxylation, side-chain oxidation, and carbonyl formation, producing intermediates at m/z 323.14, 321.12, 263.05, 182.06, and 138.03 before further carbon-chain and aromatic-framework cleavage³¹. Compared with FLZ, the hydrophobic side chain and chlorinated aromatic group of TEB provided additional sites for side-chain oxidation and aromatic transformation, whereas opening of the triazole ring was common to both compounds.

The detected mass features were broadly consistent with the DFT-predicted reactive sites and with the $\cdot\text{OH}$ -dominated, multi-species oxidation regime identified in Section 3.3. Nevertheless, the products were tentatively assigned mainly from molecular-ion signals and plausible elemental compositions. Their structures and sequence of formation should therefore be confirmed using high-resolution MS/MS fragments, isotope patterns, and time-resolved product profiles. Moreover, the approximately 70% TOC removal indicates extensive oxidation but not complete organic carbon removal. The observed pathways are thus more appropriately described as progressive fragmentation and partial organic carbon removal rather than complete conversion to CO_2 and inorganic ions.

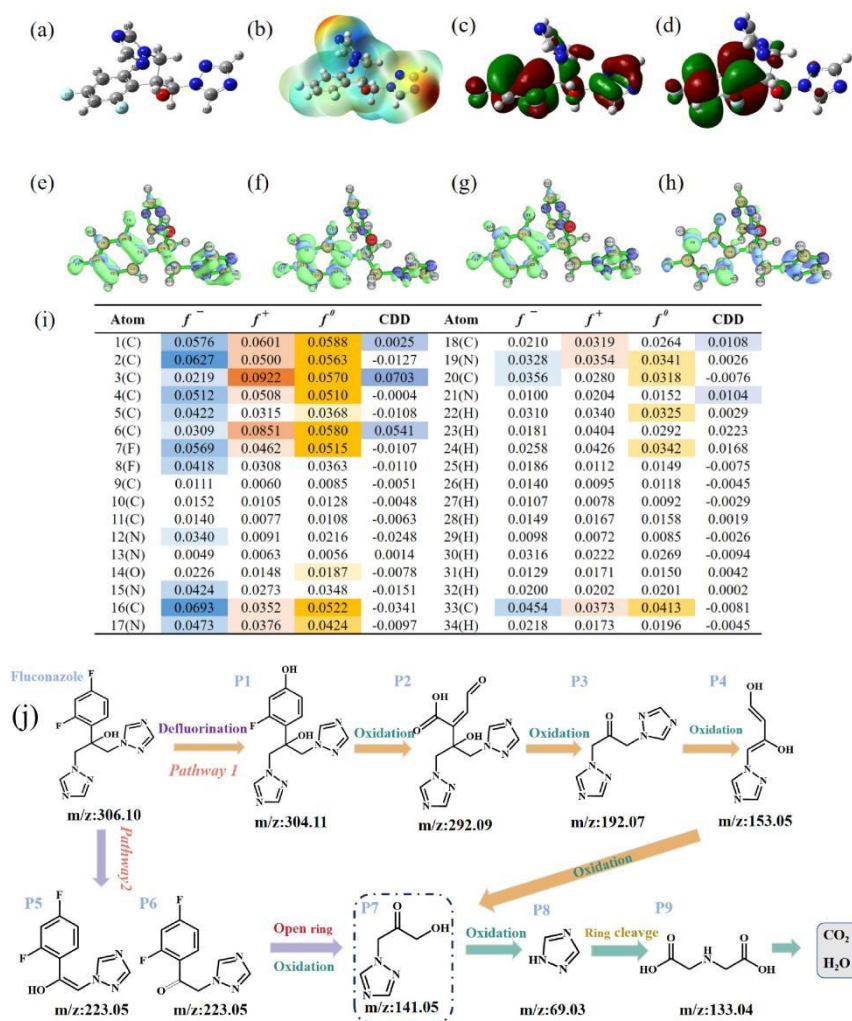


Fig. 6 DFT calculations and proposed degradation pathways of FLZ in the Fe(VI)–O₃ system.

a, Optimized molecular structure of FLZ. b, Molecular electrostatic potential (MEP) map. c,d, Frontier molecular orbital distributions of FLZ. e–h, Spatial distributions of the Fukui functions and charge-density difference (CDD), respectively. i, Condensed Fukui indices and CDD values of individual atoms, indicating the preferential reactive sites of FLZ. j, Proposed degradation pathways of FLZ based on the tentatively assigned transformation products and DFT calculations, involving defluorination, oxidation, ring opening, and ring cleavage, followed by progressive fragmentation and partial mineralization.

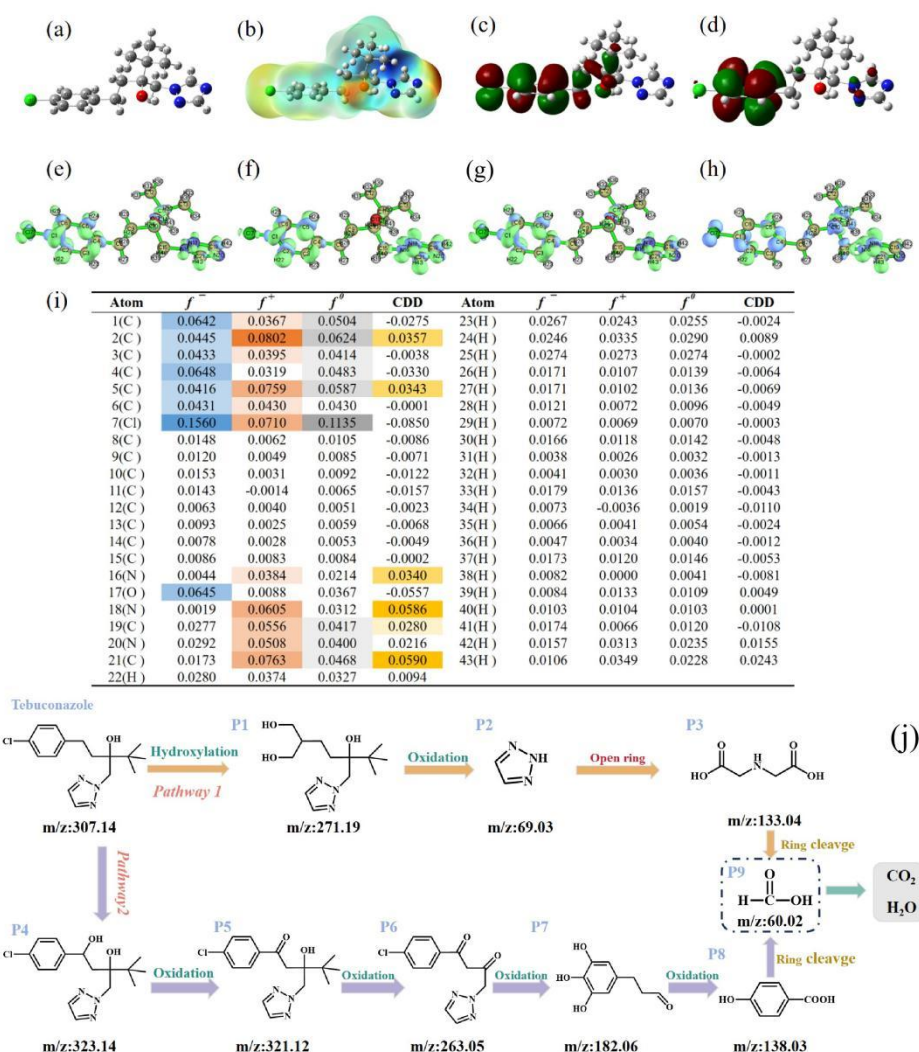


Fig. 7 DFT calculations and proposed degradation pathways of TEB in the Fe(VI)-O₃ system. a, Optimized molecular structure of TEB. b, Molecular electrostatic potential (MEP) map. c,d, Frontier molecular orbital distributions of TEB. e-h, Spatial distributions of the Fukui functions and charge-density difference (CDD), respectively. i, Condensed Fukui indices and CDD values of individual atoms, indicating the preferential reactive sites of TEB. j, Proposed degradation pathways of TEB based on the tentatively assigned transformation products and DFT calculations, involving hydroxylation, oxidation, ring opening, and ring cleavage, followed by progressive fragmentation and partial mineralization.

3.6. Evolution of ecological toxicity during treatment

ECOSAR analysis did not indicate a consistent decrease in toxicity after FLZ and TEB transformation (Fig. 8). Several FLZ products, particularly P2, P4, and P7, were identified as potential toxicity drivers for fish, *Daphnia*, or algae. Among the TEB products, P6 showed the greatest predicted concern, while P3, P5, P7, and P8 retained moderate potential effects. Parent-compound removal therefore did not necessarily reduce ecological risk immediately, because some intermediates could remain biologically relevant until further oxidation³². As ECOSAR predictions are based on molecular structure and do not account for mixture effects, they were used as a screening tool rather than direct evidence of effluent toxicity³³.

The bioassays showed clearer differences among the treatment systems (Fig. 8). Untreated FLZ and TEB solutions, as well as samples treated with Fe(VI) or O₃ alone, inhibited wheat germination and shoot growth. After Fe(VI)-O₃ treatment, germination recovered to 98.0%, close to the blank control. After 120 h, mean shoot lengths reached 6.5 cm for the treated FLZ sample and 5.8 cm for the treated TEB sample, compared with 0.5 and 0.3 cm in the untreated solutions and 8.0 cm in the control. A similar pattern was observed for *E. coli*. Both parent compounds strongly reduced colony formation, whereas single-oxidant treatment produced only partial recovery. Colony abundance after Fe(VI)-O₃ treatment was closest to that of the control. Residual oxidants were quenched before exposure to minimize interference from the direct disinfecting effects of Fe(VI) or O₃.

Zebrafish embryos provided further evidence of toxicity attenuation. At the tested concentration of 10 mg L⁻¹, untreated TEB caused pronounced developmental arrest and embryo darkening, whereas FLZ mainly induced delayed development and body curvature. These abnormalities were visibly reduced after Fe(VI)-O₃ treatment, particularly the developmental blockage associated with TEB. Considered together with parent removal and TOC abatement, the plant, bacterial, and embryo responses indicate a substantial reduction in the overall biological toxicity of the treated solutions.

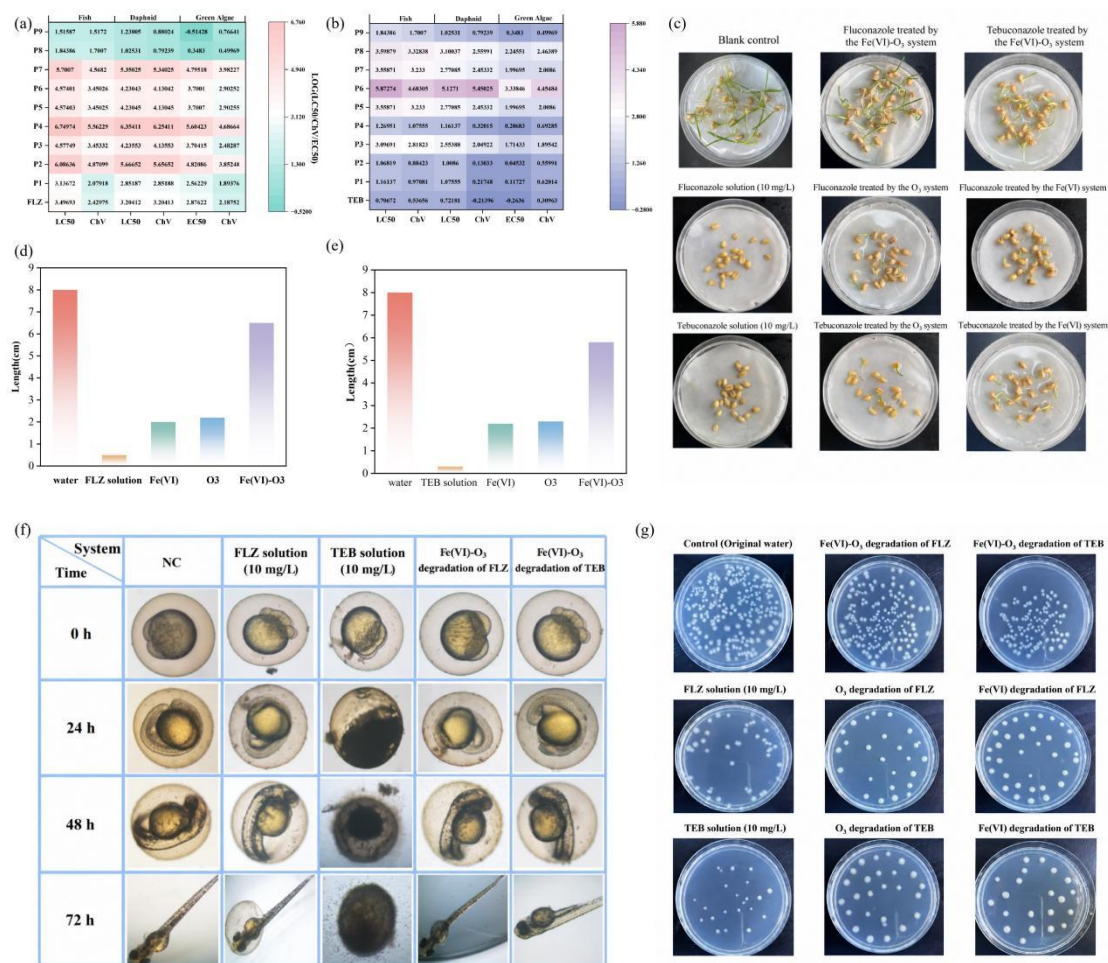


Fig. 8 Ecotoxicity assessment of FLZ and TEB before and after Fe(VI)-O₃ treatment.

3.7. Substrate scope of the Fe(VI)-O₃ process

The substrate scope of the Fe(VI)-O₃ process was examined using twelve antibiotics from four structural classes. Under the same reaction conditions, removal efficiencies ranged from 82.2% to 95.6%. Tetracyclines showed the highest removal, ranging from 93.1% to 95.6%, followed by fluoroquinolones at 87.5%-89.8%. Sulfonamides and macrolides were removed by 82.2%-90.4% and 82.5%-86.3%, respectively³⁴. These results show that the process was not restricted to FLZ and TEB and retained substantial reactivity toward structurally diverse nitrogen-containing contaminants.

Differences among antibiotic classes were likely related to molecular structure and the accessibility of susceptible sites. Tetracyclines contain several phenolic, amino, and

conjugated moieties that can react with Fe(VI), O₃, and ozone-derived oxidants, which may explain their relatively high removal³⁵. Macrolides have larger molecular frameworks and greater steric constraints, whereas the reactivity of sulfonamides and fluoroquinolones is influenced by substituent effects and heterocyclic structure³⁶. Together with the results in Section 3.3, the broad substrate response is consistent with an oxidation regime dominated by $\cdot\text{OH}$, while direct reactions with Fe(VI) and O₃ also contributed. These experiments demonstrate reaction breadth, and Section 4 below discusses the implications for treatment in complex water matrices.

4. Environmental implications

The staged Fe(VI)-O₃ treatment improved parent-compound removal and TOC abatement, and reduced the measured biological toxicity after treatment. > 90% of both FLZ and TEB were degraded with approximately 70% TOC abatement. Treated samples also caused weaker responses in the wheat, *Escherichia coli*, and zebrafish embryo assays. Parent-compound disappearance was not the only removal mechanism: transformation products formed during the initial ferrate oxidation were further oxidized during subsequent ozonation. Fe(III) solids produced from ferrate decomposition may have removed some organic matter through adsorption or coagulation; the present data do not separate this contribution from oxidative TOC loss.

The staged Fe(VI)-O₃ process could be relevant to polishing secondary effluents with persistent pharmaceuticals or pesticides in some industrial wastewaters. Its favorable performance near neutral pH is compatible with the conditions of many secondary effluents. Water composition, however, likely determines the required oxidant dose. Inhibited contaminant removal by carbonate and bicarbonate suggested that alkalinity reduces effective oxidant exposure. Natural organic matter could also compete for Fe(VI), O₃, and the reactive species generated during treatment³⁷. Consequently, operating parameters established in controlled laboratory solutions should not be applied directly to high-alkalinity or high-DOC waters without site-specific optimization.

In addition, ozone exposure was not fully normalized across the different dosing configurations, and the observed performance differences may therefore reflect dosing order, contact time, and effective oxidant input. The contaminant concentrations used were also higher than those commonly found in environmental waters, while the effects of natural organic matter, suspended particles, and contaminant mixtures were not systematically examined. In addition, TOC loss after filtration may include oxidation, adsorption, and coprecipitation. Further work should use environmentally relevant concentrations and real water matrices, with equalized ozone input, gas-contact time, and total reaction time³⁸. Ozone and iron mass balances, separate measurements of dissolved and particulate organic carbon, and continuous-flow tests of chemical demand, residual iron, and sludge production are also needed before assessing the process's technical feasibility for wastewater polishing or industrial-effluent treatment.

One feasible layout could be ferrate (Fe(VI)) pre-oxidation followed by an ozonation contactor. Ferrate performs initial transformation, and the downstream ozonation stage oxidizes remaining parent compounds and intermediates. Subsequent solids separation (sedimentation/filtration) can remove Fe(III) precipitates and adsorbed organics. Separating the stages allows independent control of ferrate contact time and ozone dosing, which can minimize mutual scavenging and optimize overall oxidant exposure. However, this is a conceptual process arrangement instead of a validated engineering design. Practical implementation will require engineering validation of ozone mass transfer and off-gas destruction, quantification and management of residual iron and sludge, and development of appropriate solids handling and disposal strategies.

5. Conclusions

Among the tested dosing configurations, ferrate pre-oxidation followed by ozonation achieved the highest removal of FLZ and TEB and the highest TOC reduction. Under the selected conditions, removal of both compounds exceeded 90%, while TOC abatement reached approximately 70%. The process performed well at near-neutral pH,

but carbonate and bicarbonate noticeably suppressed contaminant removal, suggesting that water alkalinity may constrain treatment performance in practice.

Radical-quenching test and EPR analytical results showed that $\cdot\text{OH}$ was the dominant reactive species, with additional contributions from superoxide ($\cdot\text{O}_2^-$), singlet oxygen ($^1\text{O}_2$), and direct reactions by Fe(VI) and O_3 . Transformation-product analysis indicated that FLZ and TEB underwent heterocycle cleavage, side-chain cleavage, dehalogenation, and subsequent fragmentation. Bioassays using wheat, *Escherichia coli*, and zebrafish embryos showed reduced toxic responses after treatment, consistent with reduced biological effects as parent-compound concentrations decreased.

The Fe(VI)- O_3 sequence also achieved high removal across multiple antibiotic classes, suggesting a broader substrate response beyond the two target triazoles. These findings support staged Fe(VI)- O_3 treatment as a promising approach for the advanced oxidation of persistent nitrogen-containing contaminants. Nevertheless, its efficacy in real waters and under continuous-flow, low-concentration conditions remains to be confirmed.

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