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Enhanced Removal of Common Wastewater-Derived Trace Organic Contaminants in Vertical-Flow Constructed Wetlands Amended with Fe(III)-EDTA

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ABSTRACT: Constructed wetlands (CWs) have gained scholarly attention in the last two decades as promising technologies for the attenuation of trace organic contaminants (TrOCs) from municipal wastewater effluent and combined sewer overflow discharge. Using lab-scale vertical flow constructed wetlands, we investigated amending these systems with Fe-EDTA to improve CW degradation of five representative trace organic contaminants. The study combined a 7-month monitoring campaign, 3 different hydraulic regimes, and soil extraction data to elucidate the effects of the amendment on the fate of the TrOCs within the systems. Our results indicate that Fe-EDTA contributed to the degradation of carbamazepine and sulfamethoxazole under the studied flow

TrOCs   TrOCs +Fe-EDTA

Dissolved iron amendment
↓
Lower effluent dissolved oxygen
↓
Greater removal of recalcitrant TrOCs

regimes. Iron-amended soil columns ($n = 5/9$ columns fed for 7 months with synthetic domestic wastewater) removed $12 \pm 19\%$ of influent carbamazepine (the most recalcitrant TrOC included in the study), 18% higher than the control columns. Operating the columns with periods of retention and discharge further improved carbamazepine and sulfamethoxazole removal efficiency (removal increased to $49 \pm 7.6\%$ and $81 \pm 9.2\%$ of influent concentrations, respectively). The more readily degradable compounds atenolol and trimethoprim were removed with >97% efficiency in both control and amended columns, regardless of flow. This column study positively correlates Fe-EDTA with improved removal efficiencies of environmentally recalcitrant TrOCs without affecting readily degradable TrOCs.
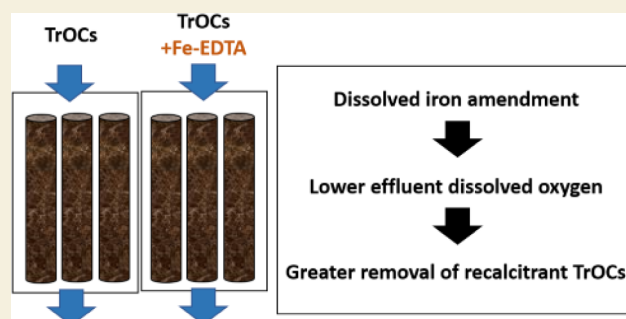
KEYWORDS: constructed wetlands, iron amendment, trace organic contaminants, EDTA, redox, biodegradation

INTRODUCTION

Trace organic contaminants (TrOCs), such as pharmaceuticals and pesticides, are increasingly associated with a myriad of adverse effects on environmental and human health.[1−3] Municipal wastewater treatment plant effluent is a primary pathway for TrOCs to enter local water bodies because conventional wastewater treatment infrastructure is not designed to remove these contaminants from wastewater streams.[4] Further, combined sewer overflows (CSOs) introduce wastewater-derived TrOCs to aquatic environments via discharge of untreated wastewater and stormwater near urban areas during large rain events.[5,6] Advanced treatment processes capable of removing TrOCs from wastewater, such as advanced oxidation,[7] high-pressure membranes,[8] and engineered biological treatment,[9] are often prohibitively expensive.

Constructed wetlands (CWs) can present a low-cost alternative to advanced treatment processes for wastewater treatment[10,11] and have the potential to both remove and degrade TrOCs in wastewater treatment plant effluent and CSOs.[12−14] In particular, the unique environments in subsurface CWs generate multiple pathways for TrOC removal

(filtration, sorption, biotransformation, and plant uptake)[10] with varying degrees of treatment efficiency.[12] Numerous case studies on full-scale, pilot, and lab-based CWs for TrOC removal over the last two decades indicate that performance is dependent on contaminant physicochemical properties and wetland design.[1,12,15,16] In a review of CW performance for TrOC removal, vertical flow CWs (VFCWs) were found to perform especially well for the removal of readily biodegradable hydrophobic compounds such as naproxen, ibuprofen, and caffeine (average removal efficiency across reviewed case studies was >75% for each compound).[12] However, poorly biodegradable compounds, such as carbamazepine, showed poor removal (average removal efficiency across the case studies reviewed was <40%) regardless of the wetland
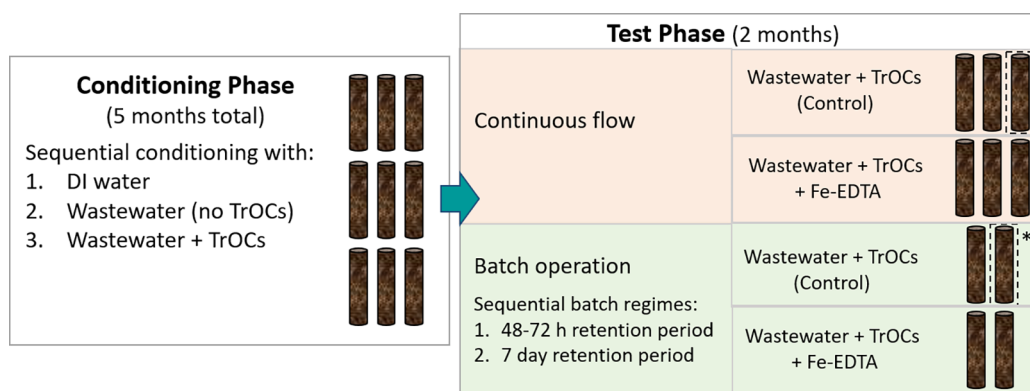
Figure 1. Experimental timeline. During the conditioning phase, all nine columns received influent from the same reservoir. During the test phase, separate influent reservoirs were used for control and Fe-EDTA amended columns. *Note that one control column was switched from continuous flow to batch operation during batch regime #2 (denoted with dashed lines).

configuration (i.e., horizontal flow, vertical flow, free water, or hybrid).[12]

To improve TrOC removal in CWs, amendments have been proposed to enhance sorption in CW soil.[17−19] While sorption is an important removal pathway, sorbed contaminants may accumulate in the subsurface if there is not adequate microbial transformation,[20] posing a potential risk of soil contamination, contaminant remobilization, and breakthrough. Therefore, amendments that promote contaminant degradation along with sorption would be advantageous.

Iron amendments have the potential to enhance the degradation of contaminants, including TrOCs, via biological and abiotic mechanisms. For instance, iron-amended biochar was shown to remove benzofluoranthrene and ammonia in a model wetland by enhancing sorption and subsequent microbial degradation.[21] Other forms of iron (iron scraps, pyrite (FeS_2), and zerovalent iron (ZVI)) have been shown to enhance nitrogen removal by promoting the growth of autotrophic denitrifying bacteria that use Fe^{2+} as an electron donor.[22−25] In an abiotic pathway, iron may catalyze the oxidation of TrOCs in soil via the Fenton reaction under circumneutral pH conditions. For instance, in a comparison of laterite-amended and biochar-amended CW systems, 30% of the removed acetaminophen was oxidized by hydroxyl radicals produced from the Fenton reaction in the laterite system, whereas the biochar system simply immobilized the TrOC.[26] However, interpretation of the impacts of iron in amended soils is complicated by the concurrence of iron precipitation and homogeneous reactions, which can contribute to changes in redox states, formation of reactive species, and contaminant sorption.

To better understand the role of iron in enhancing TrOCs degradation in iron-amended CWs, we utilized lab-based column experiments designed to mimic VFCWs amended with Fe-ethylenediaminetetraacetic acid (Fe-EDTA). Fe-EDTA was chosen as the amendment because: (1) it is relatively cost-effective and easily accessible (i.e., it is used in commercially available fertilizers,[27] (2) it remains soluble in aqueous solution, allowing us to isolate the effects of transformation from changes in sorption that might occur if iron precipitated within the columns,[28−30] and (3) while well studied for its efficacy in metals removal in contaminated soils,[31,32] its application to TrOC treatment has not been well studied despite the known benefits of other forms of iron amendments.[22,26] Five TrOCs (trimethoprim, atenolol, fipronil,

sulfamethoxazole, and carbamazepine) that span a range of physicochemical properties representative of wastewater-derived TrOCs were dosed into the CW columns (Table S1). Contaminant concentrations in influent, effluent, and porewater samples were combined with soil extract data and transformation product measurements to distinguish between contaminant immobilization and degradation within the soil columns.

MATERIALS AND METHODS

Synthetic Wastewater

Synthetic wastewater was prepared approximately weekly with nutrient and bulk organic matter concentrations representative of un-nitrified secondary wastewater treatment plant effluent[33,34] as well as 10 μg L^{-1} of each TrOC. The solution was continually dosed into the columns from continuously mixed influent reservoirs. Concentrations, suppliers, and composition of the synthetic wastewater are listed in Section S2.1.

VFCW Column Experiments

Nine gravity-driven soil columns were constructed using 50 cm lengths of clear PVC pipe and filled in sequence with 12.5 cm of coarse gravel, 2.5 cm of quartz sand, and 30 cm of soil (EcoMedia, Veratec Engineered Products, Inc., British Columbia). A full overview of the column construction and the physical characteristics of the soil is described in Section S2.2 and Table S3.

Influent water was pumped into the columns using peristaltic pumps (Shenchen Precision Pumps, Baoding City, China); a constant hydraulic head of 40 cm was maintained above the soil layer by overflow valves. Tubing attached to the open overflow valves recycled overflow water to the influent reservoir. The columns were first conditioned with deionized (DI) water for 10 days at a flow rate of 0.25 mL min^{-1} (Figure 1). This relatively slow flow rate was selected to avoid clogging and the development of preferential flow paths within the columns. During this period, tracer tests were conducted using sodium bromide to determine that all columns had hydraulic retention times (HRT) of 40−60 h (further details in Section S2.4). Following the DI conditioning period, the columns were further conditioned with synthetic wastewater without TrOCs at a flow rate of 0.25 mL min^{-1} for 2 months, followed by 3 months of conditioning with synthetic wastewater containing TrOCs. Shortly after adding TrOCs to the influent, the flow rate increased from 0.25 mL min^{-1} to 1 mL min^{-1} to accelerate

the saturation of sorption sites and breakthrough of the TrOCs. Another tracer test was conducted to assess whether ideal flow conditions were maintained in the columns with a higher flow rate. The results indicated that the columns had HRTs of 5−15 h at the higher flow rate and were not indicative of clogging or short-circuiting (Section S2.4). This phase after DI conditioning is termed the wastewater conditioning phase.

Following the 5-month conditioning phase, iron was introduced to 5 designated "Fe-amended" columns via a second influent reservoir containing synthetic wastewater and 1 g L^{-1} of dissolved Fe-EDTA in the test phase. During the test phase, two different hydraulic regimes were tested: continuous flow and batch flow. Six of the columns were operated under continuous flow, with triplicate columns fed synthetic wastewater without Fe-EDTA ("control", designated C1, C2, and C3) or with Fe-EDTA ("Fe-amended", designated F1, F2, and F3). Three of the columns were operated under batch conditions ("batch columns") to achieve anoxic−oxic redox cycling in the soil. One batch column was fed synthetic water without Fe-EDTA ("batch control", BC1), and two were fed with Fe-EDTA ("batch Fe-amended", BF1 and BF2). The batch columns were operated under two subsequent flow regimes. In the first, effluent flow was paused for 48−72 h, holding the water fully saturated within the column. Flow was then resumed for 24 h at 1 mL min^{-1}, mimicking continuous flow operation. This regime continued for 3.5 weeks, for a total of 8 cycles. Batch regime #2 began immediately after regime #1 and consisted of 2 cycles in which the effluent flow was paused for 7 days, followed by 6 days of continuous flow. Column C3 from the continuous flow regime was switched to the batch flow regime during batch regime #2 to serve as a second batch control column along with BC1.

Flow was discontinued, and the columns were disassembled after 7 months of operation. The soil and sand in each of the columns were separated into three sections: sand, bottom soil (bottom 15 cm of soil), and topsoil (top 15 cm of soil), and samples from each section were saved for extractions and moisture analysis.

Water Sample Collection and Analysis

Column effluent samples were collected 3−5 times per week for the duration of column operation. During the 0.25 mL min^{-1} flow period, 50 mL samples were collected from the outflow valves over the course of 3.5 h. When the flow rate increased to 1 mL min^{-1}, 30 mL samples were collected over 30 min. Samples were collected in 50 mL polypropylene centrifuge tubes, with the tops covered in parafilm to reduce the introduction of oxygen into the sample during collection. Influent samples were taken at the same time as effluent samples using a 25-mL glass pipette. Periodically, porewater and pond water samples were also collected. pH, electrical conductivity, and temperature were measured in each sample immediately after collection using a calibrated Environmental Express Oakton PC 100 Portable pH and conductivity meter. Dissolved oxygen was analyzed immediately by using a Hach HQ30d portable DO probe. Samples were filtered by either 0.45-μm polyether sulfone (PES) syringe filters or 0.22-μm PES syringe filters and prepared for analysis of ions, carbon, dissolved iron, and TrOC concentrations, which are further discussed in Section S2.5.

Selected transformation products of TrOCs were analyzed alongside parent compounds to aid in the interpretation of removal processes. Specifically, metoprolol acid was analyzed as the primary biotransformation product of atenolol to indicate the role of biotransformation, in addition to sorption, in the columns. Two transformation products of fipronil, which form under different redox conditions in soil (i.e., fipronil sulfone, which forms under oxic conditions, and fipronil sulfide, which forms under anoxic conditions), were analyzed to provide evidence regarding the redox conditions under which biotransformation occurred.[35,36]

Soil Extractions

Two separate extraction methods were used on the soil samples collected during column decommissioning. A methanol extraction modified, from Stiegler et al.[37] was used to quantify TrOC concentrations in the soils. Spike-recovery tests were performed on soil from column BC1 and fresh soil that had never been used in a column. Soil extraction results are considered quantitative for sulfamethoxazole and fipronil, which had recoveries between 90% and 110%. Trimethoprim and atenolol recoveries were low (20−60%) and carbamazepine recovery was >140%; therefore, we consider the soil concentration data for trimethoprim, atenolol, and carbamazepine to be semiquantitative, and we only comment on relative concentrations among columns for these compounds. Further details on the soil extraction method, spike-recovery tests, and calculation of soil concentrations are found in Section S2.8.

Soil samples were extracted for iron following a modified version of the sequential iron extraction method used by Chen et al.[38] to determine concentrations of: (1) ion-exchangeable iron by 1 M CaCl$_2$, (2) surface-adsorbed Fe and Fe in low-crystalline minerals extractable by 0.5 M HCl, and (3) Fe in high-crystalline minerals extractable by 5 M HCl. Total iron content in each of the extracts was measured by UV−vis spectrophotometry using the 1,10-phenanthroline method. Each soil extraction was conducted in duplicate (full method in Section S2.8).

Carbamazepine Microcosms

Following the column study, a series of soil microcosm tests were conducted to better understand the impact of the iron amendment on the fate of carbamazepine (CBZ). Briefly, 20 mL scintillation vials were filled with approximately a 1:1 soil:water ratio. Three different soils were tested in the microcosms: soil from the control columns, soil from the Fe-amended columns, and fresh soil that was not used in the column experiment. Each type of soil was then mixed with one of four solutions: DI water + 100 μg L^{-1} CBZ, DI water + 100 μg L^{-1} CBZ + 1 g L^{-1} Fe-EDTA, synthetic wastewater +100 μg L^{-1} CBZ, and synthetic wastewater + 100 μg L^{-1} CBZ + 1 g L^{-1} Fe-EDTA. Samples were taken for CBZ concentration immediately after the microcosms were assembled (sample t0), after 7 days during which the microcosms were sealed to prevent oxygen perturbation and stored in the dark (sample t1), and after an additional 24 h during which the microcosms were unsealed and shaken for 24 h to promote oxygen diffusion (sample t2). Microcosms were conducted in triplicate. Full experimental details can be found in Section S2.9.

Statistical Analyses and Data Processing

All statistical analyses and data processing were performed with Python on the Jupyter Lab platform (© Copyright 2018−2023, Project Jupyter). Data cleaning and database manage-

Table 1. Mean Values (±Standard Deviation) of General Water Quality Parameters and Nutrients During the Conditioning and Test Phases

Column Category		pH	Dissolved Oxygen (ppm)	Dissolved Organic Carbon (mg/L)	Electrical Conductivity (μs/cm)	Ammonia (mg-N/L)	NO_3 and NO_2 (mg-N/L)
Influent conditioning with synthetic WW	**All columns**	7.56 ± 0.27 $n = 34$	7.37 ± 1.85 $n = 30$	10.05 ± 6.76 $n = 18$	422.79 ± 21.26 $n = 34$	9.78 ± 1.13 $n = 35$	1.31 ± 2.41 $n = 23$
Effluent conditioning with synthetic WW	**Continuous flow control**	6.60 ± 0.36 $n = 159$	5.28 ± 1.63 $n = 168$	29.53 ± 15.65 $n = 123$	473.03 ± 167.93 $n = 159$	2.23 ± 2.41 $n = 175$	5.87 ± 7.86 $n = 181$
	Continuous flow amended	6.51 ± 0.44 $n = 163$	4.96 ± 1.63 $n = 172$	36.01 ± 20.43 $n = 127$	478.38 ± 108.63 $n = 163$	2.37 ± 2.32 $n = 177$	3.61 ± 7.80 $n = 195$
	Batch control	6.60 ± 0.41 $n = 63$	5.22 ± 1.84 $n = 66$	33.76 ± 16.86 $n = 48$	465.65 ± 97.21 $n = 63$	2.82 ± 2.41 $n = 68$	5.46 ± 10.04 $n = 72$
	Batch amended	6.53 ± 0.42 $n = 126$	5.06 ± 1.53 $n = 132$	32.80 ± 17.55 $n = 98$	483.91 ± 132.01 $n = 126$	2.44 ± 2.22 $n = 135$	4.29 ± 9.54 $n = 144$
Influent Fe-Test	**Control**	6.61 ± 0.32 $n = 28$	5.57 ± 2.04 $n = 28$	9.48 ± 1.74 $n = 23$	332.56 ± 37.35 $n = 25$	2.69 ± 3.16 $n = 28$	10.74 ± 4.24 $n = 24$
Effluent Fe-Test	**Continuous flow control**	6.37 ± 0.34 $n = 72$	4.24 ± 1.22 $n = 71$	10.48 ± 6.68 $n = 57$	320.82 ± 33.15 $n = 72$	1.74 ± 1.52 $n = 71$	5.55 ± 5.83 $n = 62$
	Batch control	6.37 ± 0.27 $n = 77$	3.81 ± 0.98 $n = 81$	12.75 ± 6.01 $n = 61$	347.29 ± 48.57 $n = 77$	1.83 ± 1.56 $n = 77$	7.40 ± 8.11 $n = 67$
Influent Fe-Test	**Amended**	6.63 ± 0.29 $n = 28$	5.78 ± 1.97 $n = 28$	324.16[a] ± 7.11 $n = 23$	487.54 ± 24.83 $n = 25$	1.89 ± 2.20 $n = 28$	16.67 ± 3.91 $n = 25$
Effluent Fe-Test	**Continuous flow amended**	6.33 ± 0.32 $n = 51$	3.27 ± 1.57 $n = 52$	326.58[a] ± 8.82 $n = 43$	475.04 ± 44.78 $n = 51$	0.90 ± 0.80 $n = 48$	12.23 ± 7.61 $n = 45$
	Batch amended	6.36 ± 0.32 $n = 66$	2.11 ± 1.94 $n = 68$	319.69[a] ± 52.45 $n = 56$	492.35 ± 71.20 $n = 66$	0.90 ± 0.56 $n = 64$	11.29 ± 8.04 $n = 60$

[a]The addition of the Fe-EDTA amendment added approximately 310 mg L^{-1} DOC due to the EDTA molecule.

ment were accomplished using the Python packages Pandas and NumPy, statistical tests were performed with the Python package SciPy, and figures were created with a combination of the Python packages Matplotlib and Seaborn. For boxplots, the range of the box and whiskers comprises the interquartile range and 1.5 times the interquartile range, respectively. Points outside these ranges are plotted as outliers.

Unless otherwise stated, we present all summary values as the sample mean ± sample standard deviation. Duplicate data values are averaged, and the error is presented as the difference between the two values. Statistical comparisons between data groups were made using the SciPy one-way ANOVA function with a p-value cutoff of 0.01. We used SciPy optimize.curve_fit to identify relationships within the data and the Python machine learning package scikit-learn r2_score function to calculate the coefficient of determination for the fitted curve.

■ RESULTS AND DISCUSSION

General Water Quality Parameters, Nitrogen, and Iron

The columns quickly began to remove nutrients and dissolved oxygen (DO) during the wastewater conditioning phase. We consistently measured effluent DO concentrations >4 ppm, which were 2.09−2.44 ppm less than the corresponding influent concentrations (Table 1). During the test phase, the dissolved oxygen concentration in the influent reservoirs was lower and more variable than the values measured in the wastewater conditioning phase. This observation was likely the result of biofilm growth over time in the influent reservoirs. Despite this variation in influent water chemistry, we continued to observe DO consumption within the columns, with the amended columns in the test phase consuming concentrations of DO that were larger than those of the corresponding control columns. Ammonia removal (7.1 ± 3.03

mg-N L^{-1} relative to influent concentrations) was also observed across all 9 columns. Total dissolved nitrate and nitrite were detected in the effluent at maximum concentrations of 66.8 mg-N L^{-1}, consistent with nitrification occurring in the soil.

During the conditioning phase, salts and organic carbon were present in higher concentrations in the effluent than in the influent, indicative of leaching from the soil. Electrical conductivity in effluent samples was higher than influent values for the first 30 days of operation, after which concentrations stabilized near influent values for the remainder of column operation (Table 1 and Figure S20), consistent with the normal operation of treatment wetlands.[10] During the conditioning period with deionized water, effluent samples contained an average of 260 ± 148 mg L^{-1} DOC, indicating significant leaching of soil organic matter (Figure S19). When synthetic wastewater was introduced, effluent DOC values remained higher than influent DOC values (35.2 ± 20.5 vs 8.8 ± 4.8 mg L^{-1}), likely due to continued gradual leaching from the soil as well as biomass produced by the microbial communities within the soil columns. During the test phase, effluent DOC concentrations resembled influent concentrations (Table 1), indicating that all readily dissociable DOC had been removed from the soil column during the conditioning period.

During the test phase, influent ammonia concentrations were lower and more variable than those observed during conditioning, likely due to partial nitrification in the influent reservoir and feed lines (further discussion of changes in influent water quality is provided in Section S3.1). Effluent ammonia concentrations were not significantly different from influent concentrations during the test phase. Influent concentrations of nitrate and nitrite during this period

averaged 10.7 mg-N L^{-1} and 16.67 mg-N L^{-1} in control and amended columns, respectively, and partial removal of the oxidized species was observed in both control (4.43 ± 8.1 mg-N L^{-1} removed) and amended columns (4.89 ± 8.7 mg-N L^{-1} removed). However, effluent concentrations of the nitrogen species were highly variable, as indicated by large relative standard deviations (Table 1 and Figure S16).

During conditioning, effluent dissolved iron concentrations were near the limits of detection of both iron analysis methods. During the test phase, the control column effluent samples continued to contain iron concentrations near the detection limits, while the effluent iron concentrations from amended columns were approximately 2 orders of magnitude greater and within a standard deviation of influent concentrations (control: 0.88 ± 1.55 mg L^{-1}; amended: 100.30 ± 37.27 mg L^{-1}; and influent: 110.01 ± 27.85 mg L^{-1}), indicating that the mass of iron introduced in the influent remained dissolved in the aqueous phase and did not substantially partition into the soil of the columns. Soil iron content measured via extractions of soil from the amended columns confirms this assumption; a very small proportion of the total mass of iron introduced in the column was retained in the soil matrix (<0.02%) (Section S3.2).

TrOcs in Continuous-Flow Columns

During the test phase of the experiment, 6 of the columns were operated under a continuous flow (CF) regime, in which effluent flow rates were maintained at 1.2 ± 0.3 mL/min^{-1} and 1.1 ± 0.3 mL min^{-1} for the control columns and amended columns, respectively. The corresponding hydraulic retention times were 0.45 ± 0.19 days and 0.40 ± 0.2 days.

Carbamazepine and fipronil removal were significantly greater in the amended columns compared to the controls (one-way ANOVA, $p \ll 0.01$) (Figure 2). Carbamazepine is notoriously recalcitrant in subsurface constructed wetland
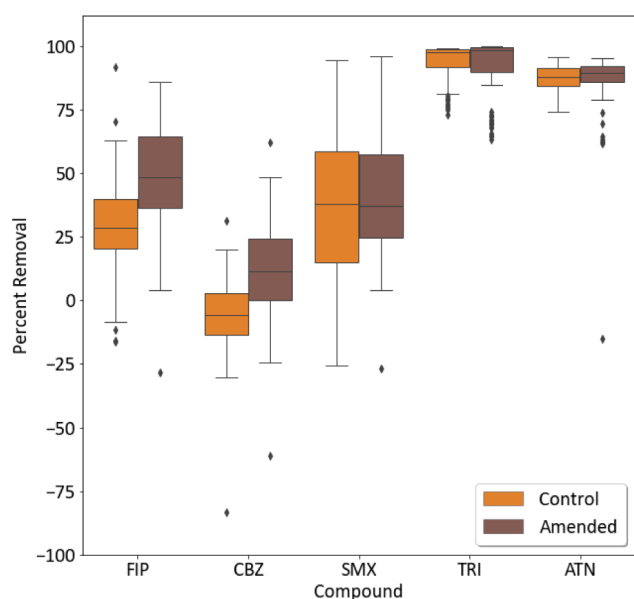

Figure 2. Box plot comparing the removal efficiencies of the 5 trace organic contaminants in the control and amended columns under continuous flow conditions during the test phase of the experiment (i.e., after day 150 when iron addition began in the Fe columns). CMX − carbamazepine; SMX − sulfamethoxazole; TRI − trimethoprim; ATN − atenolol; FIP - fipronil.

systems.[12,39] Studies that have achieved high carbamazepine removal (e.g., 94% removal in a horizontal-flow subsurface treatment wetland for secondary wastewater treatment plant effluent)[40] operated under long retention times (10 days) or with highly sorptive substrates.[12] Most vertical flow systems that resemble our setup, however, report less than 30%[12] removal of carbamazepine, with some studies indicating negative removal.[39] We observed −6 ± 15% carbamazepine removal in the control columns and 12 ± 19% removal in the amended columns.

We attribute carbamazepine removal in all soil columns primarily to sorption followed by partial degradation, as has been observed in previous studies.[12,39,41,42] Carbamazepine (log K_{OW} = 2.45) is estimated to have reached sorptive breakthrough in all the columns before we began dosing the iron amendment, after about 140 days of conditioning (Figure S22). Breakthrough is approximated as the time point at which effluent concentrations reached a steady state, implying that sorption sites throughout the columns were saturated with respect to carbamazepine, and sorption could not account for further carbamazepine removal after this point in time. However, some removal of carbamazepine was observed after breakthrough occurred, with greater removal in columns amended by Fe-EDTA. Soil extract data indicate that the dissolved Fe-EDTA amendment did not affect the sorption of carbamazepine (i.e., concentrations in the soil extracts were not significantly different between the control and amended columns). However, the addition of Fe-EDTA may have affected biodegradation by shifting the redox conditions within the amended columns. Specifically, average effluent dissolved oxygen concentrations in the control columns were approximately 1 ppm greater than in the amended columns despite similar influent DO levels (Table 1). Carbamazepine has been previously observed to preferentially degrade under anaerobic and suboxic conditions.[43,44] While effluent dissolved oxygen concentrations remained oxic in amended columns (>0.5 ppm), porewater samples indicated that dissolved oxygen was not consumed linearly with column depth, implying spatial heterogeneity in redox conditions and that effluent dissolved oxygen concentrations were not necessarily representative of conditions throughout the soil column. The presence of anaerobic microsites in otherwise oxic soils has been observed in prior studies, as has the concurrence of aerobic and anaerobic microbial activity due to heterogeneous oxygen diffusion and consumption within soil structures.[45] The evidence from the column study correlating the presence of Fe-EDTA with improved carbamazepine degradation implies that Fe-EDTA may have played a role in the propagation of anaerobic microsites within the amended columns.

Fipronil removal averaged 26 ± 31% and 50 ± 21% in the control and amended columns, respectively, during the test period (Figure 2). Unlike carbamazepine, over the course of the test campaign, the effluent profile of fipronil indicates that breakthrough was not achieved until day 180 − i.e., 30 days after the addition of the amendment to 3 of the columns (Figure S23). Prior to the test phase, the columns designated for the control exhibited a steeper breakthrough curve than the columns reserved for the iron amendment. This fact may partially explain the poorer fipronil removal in the control columns during the test phase due to the quicker exhaustion of sorption sites prior to the test (Figure S23). Fipronil is susceptible to sorption (log K_{OW} = 4.5) and biotransformation under both oxic and anoxic conditions.[46] Sorption to soil

organic matter (SOM) was likely a primary removal mechanism in our experiment, considering the high organic matter content of our soil (114.87 ± 22.9 g of SOM kg^{-1} soil). The soil extraction data are consistent with this mechanism: fipronil was found in the highest concentration in soil among the 5 TrOCs across all 9 columns (average concentration = 0.38 ± 0.01 μg g^{-1}). However, there was no significant difference between the fipronil concentrations in extracts from the soil in the control and amended columns ($p > 0.01$). Further, the mass of fipronil sorbed to the soil cannot explain the total fipronil loss (Figures S6 and S9), indicating that transformation may have occurred. Two transformation products of fipronil were also detected in the soils (Figure S5), despite being absent from column effluent samples for most of the study (Figure S12). Fipronil sulfide concentrations in the soil were approximately twice as high as fipronil concentrations and 1−2 orders of magnitude larger than concentrations of fipronil sulfone (Figure S5). As the anaerobic metabolite of fipronil, the presence of fipronil sulfide in the soil implies the occurrence of anaerobic biotransformation.[47] These findings indicate that the parent compound, fipronil, was likely first immobilized via sorption and then anaerobically transformed to fipronil sulfide in both soils. In closing the fipronil mass balance, both the control and amended columns indicated that approximately a third of the total mass of fipronil was lost to transformation (37% and 31% in control and amended soils, respectively).

For the readily biodegradable compounds atenolol and trimethoprim, effluent concentrations were <15% of the influent concentrations (Figure 2), and no significant difference was observed between the amended and control columns ($p > 0.01$). In fact, for the duration of column operation, effluent concentrations of atenolol and trimethoprim were <25% of influent concentrations, and no breakthrough was observed despite the relative hydrophilicity of the compounds (i.e., log K_{OW} values for atenolol and trimethoprim are 0.16 and 0.91, respectively) (Figures S22 and S25). Further, soil extraction data indicated there was relatively little accumulation of either atenolol or trimethoprim in the column soil after 140 days of continuous dosing (Figures S5). The concentrations of these compounds in the soil account for <10% and <5% of the total mass of trimethoprim and atenolol introduced to the columns, respectively. Most of the mass of atenolol and trimethoprim extracted from the soil column was found in the top half of the soil column (>90% of trimethoprim and >60% of atenolol, Figure S5), indicating very slow saturation of sorption sites for these compounds. The evidence from effluent and extractable soil concentrations indicates that the CF columns primarily removed atenolol and trimethoprim via biodegradation rather than sorption.

Atenolol is known to be quickly biologically transformed under aerobic conditions[48] like those observed in the columns. Prior studies on the fate of atenolol in vertical flow constructed wetlands have also reported high removal efficiencies (e.g., 79−90%)[12,49] at a variety of hydraulic retention times and redox potentials, consistent with ready biodegradability. The detection of metoprolol acid, a metabolite of atenolol, in the effluent samples provides further evidence of the biotransformation of atenolol within the column (Figure S12). The efficient trimethoprim removal observed in this study is also consistent with reported trimethoprim removal in comparable systems (prior studies report 40−99% removal)[41,50,51] and

with prior observations of trimethoprim biotransformation via both aerobic and anaerobic processes.[51]

Sulfamethoxazole effluent concentrations were highly variable and were not significantly different in amended columns compared to controls ($p \gg 0.01$). Both sets of columns averaged an approximately 45% removal (Figure 2). The sulfamethoxazole removal efficiency in this study is consistent with reported removal efficiencies for vertical flow wetlands (54 ± 29%),[12] and sulfamethoxazole is regarded as less readily degradable than atenolol and trimethoprim.[51] Sulfamethoxazole is also relatively hydrophilic (log K_{OW} = 0.89) and negatively charged at circumneutral pH values (Table S1). We therefore expect little sorption to the substrate by either hydrophobic or electrostatic interactions, since sulfamethoxazole has similar hydrophobicity to trimethoprim and atenolol (for which little sorption was observed), and soil surface sites are predominantly negatively charged.[52] Soil extraction data confirm that very little of the total mass of added sulfamethoxazole was immobilized due to sorption to the soil (<1%) and the observed removal is largely attributed to transformations of the parent compound within the wetland columns (Figure S6).

TrOcs in Columns Operated with Batch Flow

The two batch flow regimes resulted in fluctuations in effluent dissolved oxygen concentrations, allowing us to investigate the impact of the Fe-EDTA amendment on soil redox conditions. In batch regime #1, when water was retained in the columns for 48−72 h, effluent dissolved oxygen concentrations were lower than those in the continuous flow columns during the same time period. Further, dissolved oxygen concentrations in the amended columns were consistently lower than those in the control columns (2.1 ± 1.5 and 3.45 ± 0.43 mg/L, respectively), mirroring the effect of the amendment in the continuous flow columns. However, dissolved oxygen concentrations following the retention period were not significantly different from those in samples collected after 24 h of continuous flow (Figure 3) for either the amended or control columns ($p > 0.01$).
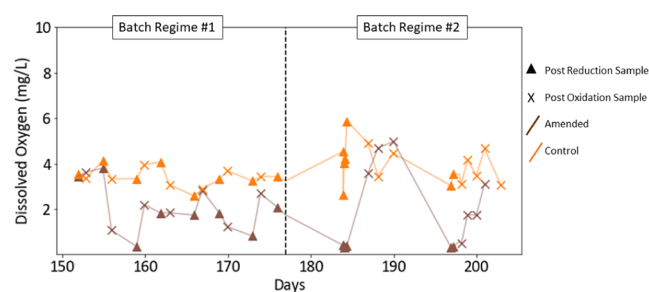

Figure 3. Dissolved oxygen influent and effluent concentrations over the course of both batch regimes. Post reduction refers to the samples collected upon opening the column effluent valves following the retention period. Post oxidation refers to the samples collected during or following the 24 h "oxidation" or discharge period.

In columns operated in batch regime #1, we observed enhanced removal of some TrOCs relative to continuous flow columns, but there was no significant difference in TrOC removal between the post-reduction and post-oxidation effluent concentrations ($p > 0.01$) in either the control or amended columns. As observed in the continuous flow columns, the batch columns that received the iron amendment
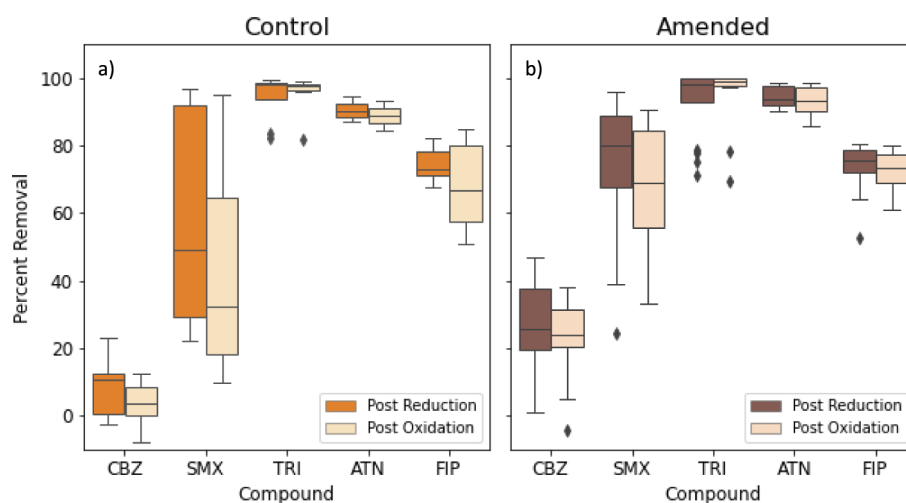
Figure 4. Box plots comparing the removal efficiencies of the 5 TrOCs in the control and amended columns during batch regime #1. Post reduction refers to the samples collected upon opening the column effluent valves following the retention period. Post oxidation refers to the samples collected following the 24 h "oxidation" period of continuous flow. CMX − Carbamazepine; SMX − Sulfamethoxazole; TRI − Trimethoprim; ATN − Atenolol; FIP - Fipronil.
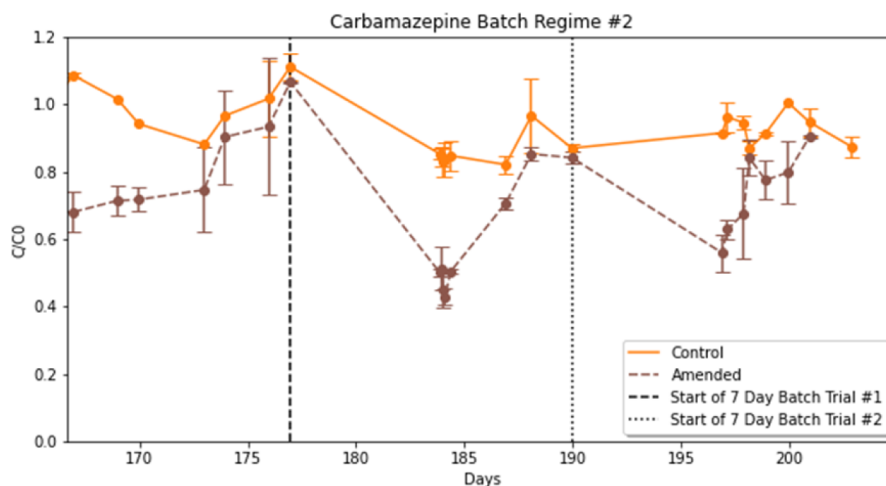

Figure 5. Effluent concentrations of carbamazepine normalized by influent concentrations during both trials of batch regime #2.

showed better removal performance for carbamazepine than the unamended batch columns during regime #1 (Figure 4). Unlike the continuous flow columns, however, fipronil removal was not enhanced by the amendment during batch regime #1. Fipronil removal efficiency in both control and amended columns was, on average, greater in the batch columns ($66 \pm 31\%$) than in the continuous flow columns ($38 \pm 29\%$) during the test phase. The longer residence time provided by the batch flow pattern may have enhanced the sorption of fipronil to organic matter and biodegradation in the soil column. Sulfamethoxazole removal efficiency was also greater in the batch columns than the continuous flow columns, averaging $37 \pm 32\%$ for both control and amended continuous flow columns and $64 \pm 27\%$ for both control and amended batch columns. We hypothesize that the batch regime may have increased the fraction of sulfamethoxazole that underwent biotransformation due to the increased residence time. Both atenolol and trimethoprim continued to exhibit high removal rates in the batch columns, as observed in the continuous flow columns.

The longer retention period in batch regime #2 resulted in a significant decrease in effluent dissolved oxygen concentrations exclusively in the Fe-amended columns ($p \ll 0.01$). The

control column effluent DO concentrations following the 7-day retention period were not significantly different from those observed post-oxidation ($p > 0.01$) (Figure 3). In the amended columns, DO levels in samples collected between 0 and 12 h after flow through the columns was resumed were consistently less than 0.5 mg L^{-1}, indicating that these columns were anoxic for a portion of the 7-day retention period. After 72 h of continuous flow, effluent dissolved oxygen concentrations increased to 3.6 ± 0.9 mg L^{-1} and 1.7 ± 0.3 mg L^{-1} for the first and second rounds of batch regime #2 operation, respectively.

Carbamazepine effluent concentrations decreased significantly in the amended columns during batch regime #2, but not in the control columns. Removal increased from $14 \pm 13\%$ under the continuous flow conditions in amended batch columns to $49 \pm 7.6\%$ in the 0−12 h following the 7-day retention period (Figure 5). As the amended columns returned to oxic conditions, carbamazepine concentrations increased to near-influent values over the course of the approximately 12-h transition period, or the time for two bed volumes of water to pass through the columns. No significant effect of the batch regime operation was observed in the control columns, and carbamazepine removal remained poor at $9 \pm 2\%$. The results

from both batch regimes and the continuous flow columns indicate that the lowest effluent carbamazepine concentrations co-occurred with DO levels less than 1 ppm (Figure S13), consistent with carbamazepine removal predominantly in oxygen-poor environments that were promoted by the presence of Fe-EDTA.

The retention periods of batch regime #2 resulted in a significant decrease in sulfamethoxazole effluent concentrations. We observed greater sulfamethoxazole removal in samples collected in the 0−12 h following the 7-day retention period (81 ± 9.2%) than in samples collected during continuous flow conditions in the same columns (50 ± 22%) (Figure S25). The observed decreases in both sulfamethoxazole and carbamazepine effluent concentrations following the 7-day retention period are consistent with recent findings by Stiegler et al. that indicated both compounds were biologically removed under sulfate- and iron-reducing conditions (i.e., oxygen-poor environments) in a pilot-scale horizontal flow constructed wetland.[43] In the pilot-scale system, carbamazepine removal was correlated to sulfate reduction. Other recent studies have also indicated that sulfamethoxazole, carbamazepine, and halogenated TrOCs may be preferentially biodegraded under oxygen-poor conditions.[53−55] Our results suggest that the addition of relatively high concentrations of Fe-EDTA may promote low-DO conditions similar to those observed in the pilot-scale horizontal flow system and similarly result in enhanced removal of some TrOCs. The retention and discharge pattern of batch regime #2 did not significantly affect effluent concentrations of fipronil, trimethoprim, or atenolol relative to continuous flow conditions.

Based on our findings, we can reasonably suggest potential explanations for the enhanced removal of carbamazepine and sulfamethoxazole in iron-amended columns under batch flow conditions. We observed low DO concentrations in the effluent samples corresponded with the lower carbamazepine and sulfamethoxazole concentrations, indicating the enhanced removal did not likely occur due to reactions that required DO (i.e., the Fenton reaction for hydroxyl radical generation.[56,57] Instead, we hypothesize that the degradation occurred due to reactions that are preferential to anoxic environments. Results from supplementary microcosm experiments containing carbamazepine indicated that, regardless of the amendment addition, aqueous carbamazepine was lost during the anoxic period (between time points t0 and t1) and not during the oxidizing period (S14). While we cannot discount the role of sorption in the microcosm experiments (soil extractions were not performed following the microcosm study), this finding is consistent with previous studies that indicate carbamazepine is preferentially removed under anoxic conditions.[12,43]

Sulfamethoxazole and carbamazepine transformation may have occurred either abiotically or biologically under the anoxic conditions induced by Fe-EDTA amendment. In some cases, reduced iron species are directly capable of reducing organic contaminants in anoxic environments.[58] Notably, Mohatt et al. found that sulfamethoxazole was rapidly transformed by microbially reduced Fe(II).[59] Alternatively, the unique environments created by the iron amendment and the 7-day batch period may have supported the growth of microbial communities capable of carbamazepine and sulfamethoxazole biotransformation. Considering that all amended columns consumed more dissolved oxygen than the controls, the amendment may have increased the overall

microbial metabolic activity. This hypothesis is consistent with a previous study that found increased microbial diversity and activity in constructed wetland systems fed with combinations of ferric iron and zerovalent iron.[25] In our experiments, when the water was held during the 7-day retention period, the long residence time may have enabled soil microbial communities to utilize the Fe(III)-EDTA as an electron acceptor, encouraging the growth of iron-reducing microorganisms, which have been found to directly metabolize sulfamethoxazole.[60] EDTA may also contribute to enhanced TrOC biotransformation. EDTA is poorly biodegraded in most environments[61] and contributed approximately 95% of the influent dissolved organic carbon to amended columns in our experiments (Table 1). Therefore, almost all of the carbon supplied to the microbial community in amended columns was not readily biodegradable, which may have created selective pressure for microorganisms to grow that are able to metabolize more recalcitrant carbon substrates.[62] Microbial communities adapted to using recalcitrant organic carbon sources for growth are more likely to be able to cometabolize recalcitrant TrOCs.[62,63] This phenomenon was suggested as a mechanism for the enhanced carbamazepine removal in suboxic soil columns with low concentrations of biodegradable dissolved organic carbon, and for the enhanced sulfamethoxazole removal in both oxic and suboxic environments with low concentrations of biodegradable dissolved organic carbon.[44] It is plausible that the removal of carbamazepine and sulfamethoxazole in this study could be considered a byproduct of cometabolism by microbes that produce enzymes capable of metabolizing EDTA.

■ ENVIRONMENTAL IMPLICATIONS

Overall, our experiments indicated multiple effects of an Fe-EDTA amendment on the performance of soil columns mimicking vertical-flow constructed wetlands. Under all operating conditions (continuous flow, batch regime #1, and batch regime #2), the iron amendment resulted in lower effluent dissolved oxygen concentrations, likely by increasing microbial oxygen consumption. The iron amendment, coupled with a long batch period, increased carbamazepine and sulfamethoxazole removal, resulting in comparable performance to previous observations in constructed wetland systems operating with much longer HRTs.[43] Fipronil removal was also greater in columns operated in batch mode compared with continuous flow. The removal of the other, more readily degradable TrOCs was similar under continuous flow and batch operating conditions and was not affected by the Fe-EDTA amendment. As a result, incorporating batch flows into Fe-amended vertical-flow systems may help improve the removal of contaminants that are recalcitrant under continuous flow conditions without sacrificing performance for more readily degradable compounds. Potential design configurations for operating batch flow designs include the use of multiple wetlands operating in parallel with alternating influent flow or adjustments in flow control in a single wetland with an upstream holding pond.

Our findings contribute to the fundamental understanding of interactions between iron amendments and TrOCs by demonstrating that a dissolved iron-ligand amendment may enhance the removal of recalcitrant TrOCs via degradation. Considering the more common use of iron amendments to increase sorptive capacity, the capability of iron amendments to accelerate biotransformation may enhance the removal of

hydrophilic TrOCs and extend the operational time for iron-amended systems prior to contaminant breakthrough. The addition of iron-ligand amendments may also enable systems with relatively short HRTs to reach reducing conditions, under which contaminants that are recalcitrant in aerobic environments are more rapidly transformed.

While our results provide evidence of enhanced biotransformation in the presence of iron-ligand amendments, further research is needed to advance this approach to contaminant degradation. In particular, we investigated a relatively high Fe-EDTA dose for this proof-of-concept study. However, dosing 1 g L^{-1} is likely impractical at the pilot or full scale, and the effects of amendment concentration on contaminant transformation rates require further investigation. In addition, alternative ligands besides EDTA should be investigated. EDTA is a poorly biodegradable ligand,[61] which may have increased contaminant transformation rates but also has the potential to result in adverse downstream impacts. Therefore, further research is needed to ensure that the use of iron-ligand amendments does not contribute to adverse impacts on downstream water quality. Finally, while our study suggests that the Fe-EDTA amendment improved microbial transformation of some TrOCs, further research is needed to more quantitatively assess the role of abiotic effects, such as the possibility for Fenton-like hydroxyl radical formation,[64,65] within Fe-ligand amended systems. Abiotic reactions are likely to be affected by redox and flow perturbations, and further research could elucidate methods to enhance these reactions, for instance, by integrating electrochemical and wetland treatment systems. Overall, our results indicate an important pathway by which iron amendments can enhance TrOC degradation in constructed wetlands via changes in redox conditions and biotransformation, with implications for future research regarding dissolved and solid iron amendments for the treatment of TrOC-containing waters.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.5c00017.

> The Supporting Information includes analytical methods, further information on the experimental set up, and calculations for mass balance estimates. Detailed information on soil extraction methods and validation is also provided. Effluent and influent concentrations for all analytes are plotted vs time for the duration of the experiment, as well as plots describing the fate of each TrOC in each column (PDF)

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Notes

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