EVALUATING THE EFFECTS OF ANTIBIOTICS ON THE BIOLOGICAL TRANSFORMATION OF NITROGEN AND PPCP REMOVAL FROM ONSITE WASTEWATER IN NITRIFYING SAND COLUMNS

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Abstract

Antibiotics in the environment are of concern not only with regard to the development of antibiotic resistance genes but also for their toxic effects on microbial communities. Healthy microbial communities are essential for effective biological treatment of wastewater as employed in conventional and onsite wastewater treatment systems (OWTSs). However, the effects of high concentrations of antibiotics periodically occurring in sewage on the microbial communities responsible for sewage treatment are not well understood. In order to further our knowledge on this topic, this study evaluates the effects of two commonly prescribed antibiotics – sulfamethoxazole and ciprofloxacin – on the removal of nitrogen and 23 pharmaceuticals and personal care products (PPCPs) by column-scale nitrifying sand filters. The objectives of this study are (i) to observe the effect of a realistic dose of sulfamethoxazole and of ciprofloxacin on the nitrification process and (ii) on PPCP removal in the nitrifying filter, and (iii) to evaluate the recovery of the nitrification and PPCP removal processes if an effect due to antibiotic dosing is measured. Antibiotics were continuously added to the column influent for a ten-day period at concentrations of 150 and 750 µg/L sulfamethoxazole and 300 µg/L ciprofloxacin. Nitrogen data indicated that all three antibiotic treatments temporarily inhibited nitrification compared to control columns, with NO_X concentrations during the period of antibiotic dosing averaging 37%, 47%, and 47% lower in the 150 µg/L sulfamethoxazole, 750 µg/L sulfamethoxazole, and 300 µg/L ciprofloxacin treatments respectively than in the control. However, recovery of the nitrification process occurred immediately after column exposure to the antibiotics was ended, and adsorption prevented ammonium breakthrough during the inhibition period. No effect of either antibiotic on PPCP removal was observed. These results show that at concentrations relevant to OWTSs, sulfamethoxazole and ciprofloxacin can affect nitrogen removal in unsaturated nitrifying sand filters, but the results also demonstrate the resilience of the treatment system, indicated by the recovery of the nitrification process and the lack of effect on PPCP removal. This is the first study to use environmentally relevant doses to assess antibiotic effects on microbial function in column-scale treatment systems.

Introduction

Among pharmaceuticals and personal care products (PPCPs), there has been particular interest in the environmental occurrence and impacts of antibiotics (Kelly and Brooks, 2018; Melvin, 2017). Development and horizontal transfer of antibiotic resistance genes is considered a high priority impact, but there is also focus on the effects of antibiotics on the function and structure of microbial communities (Roose-Amsaleg and Laverman, 2016; DeVries and Zhang, 2016). The impacts of antibiotics are particularly important to consider in unsewered homes relying on

onsite wastewater treatment systems (OWTSs) for sewage treatment. Most OWTSs are at least partially reliant on a microbial community for removal of nutrients and contaminants from wastewater, and effects of antibiotics on those organisms may directly affect the performance of the system. Additionally, in an OWTS serving a small residence, there is the potential for exposure to extremely high (µg/L) and variable antibiotic concentrations compared to in a conventional wastewater treatment plant (WWTP) that is integrating, and thus diluting waste from, a much larger population. Also at concentrations in the µg/L range, studies in the literature demonstrate that nitrogen transformation processes including nitrification (Gosh et al., 2009; DeVries and Zhang, 2016) and denitrification (Ahmad et al., 2014; Underwood et al., 2001; Roose-Amsaleg and Laverman, 2016; DeVries and Zhang, 2016) are likely to be affected by antibiotic addition. It is important to understand the performance and recovery of an OWTS under such an exposure to improve and ensure consistency of wastewater treatment.

Beyond nutrient removal, select OWTSs are capable of removing organic contaminants including many PPCPs from wastewater via a variety of biodegradation pathways (Tilley et al. 2008; Clyde, 2021). Thus, antibiotic effects on those processes are of interest as well. The PPCPs studied here are listed in Table 1. In addition to functional effects, exposure to antibiotics has been shown to alter microbial community composition (Katipoglu-Yazan et al., 2018) – as particular organisms are affected, others thrive due to the decreased competition. Therefore, depending on the overlap of portions of the microbial community that are affected by the addition of a given antibiotic, and portions of the community that are responsible for removal of a given PPCP, removal of some PPCPs may be largely decreased by antibiotic addition while removal of others is unaffected.

Table 1. Isotope recoveries and reporting limits for the 23 PPCPs studied.

		Isotope recovery (%)		RL (ng/L)	
Compound	**Use**	**INF**	**EFF**	**INF**	**EFF**
Acetaminophen	*analgesic*	51%	62%	33	4
Atenolol	*Beta-blocker*	79%	89%	53	7
Caffeine	*stimulant*	64%	85%	45	6
Carbamazepine	*anticonvulsant*	86%	102%	59	8
Cimetidine	*H2 blocker*	77%	51%	85	11
Ciprofloxacin	*antibiotic*	80%	54%	95	13
Cotinine	*human metabolite of nicotine*	55%	76%	89	12
Deet	*insect repellent*	63%	89%	368	49
Diclofenac	*NSAID*	62%	20%	76	10
Diltiazem	*calcium-channel blocker*	16%	57%	88	12
Diphenhydramine	*antihistamine*	70%	88%	51	7
Fluoxetine	*SSRI*	22%	68%	114	15
Ketoprofen	*NSAID*	71%	66%	18	2
Metoprolol	*Beta-blocker*	66%	81%	41	6
Nicotine	*stimulant*	271%	630%	210	28
Paraxanthine	*human metabolite of caffeine*	67%	81%	66	9
Propranolol	*Beta-blocker*	62%	93%	30	4
Ranitidine	*H2 blocker*	73%	62%	167	22
Salbutamol	*bronchodilator*	92%	94%	64	9
Sulfamethoxazole	*antibiotic*	86%	88%	36	5
TCEP	*flame retardant*	79%	93%	77	10
Trimethoprim	*antibiotic*	70%	84%	49	7
Warfarin	*anticoagulant*	70%	62%	28	4

The aim of this work was to determine the occurrence and resilience of nitrogen and PPCP removal in OWTS when the system is exposed to a realistic antibiotic dose. Pre-acclimated nitrifying sand columns were dosed with either sulfamethoxazole or ciprofloxacin and monitored at the influent and effluent for 23 PPCPs and for nitrogen species during a ten-day dosing period followed by a 25-day recovery period. The results will help clarify expected performance of similar OWTSs under comparable conditions and will aid in the design of wastewater treatment

systems to withstand and recover from such shocks. This work will also be a valuable contribution to the limited literature on antibiotic effects on nitrogen cycling and will provide the first direct look at how antibiotics may influence the behavior of 23 PPCPs during wastewater treatment.

Methods

Eight columns were used in this study (Figure 1, Clyde (2021)). All columns were preconditioned with wastewater for 4.5 months, followed by further conditioning for 1.5 months with wastewater spiked with PPCPs. Immediately after the conditioning process, antibiotic dosing began in the six treatment columns, with the two remaining columns serving as controls. Determination of realistic concentrations of sulfamethoxazole and ciprofloxacin in a STE took into account the typically prescribed dose (1.6 g/day and 1 g/day respectively), the average percentage of the drug that is excreted unchanged (12.7% and 35% respectively) (RX List, 2021), and the minimum flow rate suggested for a 3-bedroom house (330 gal/day). The calculated concentrations were 163 µg/L sulfamethoxazole and 280 µg/L ciprofloxacin. Based on these calculations, the three concentrations tested in this study were 150 µg/L sulfamethoxazole, 750 µg/L sulfamethoxazole, and 300 µg/L ciprofloxacin. Therefore, two of the treatments represent realistic exposure levels in an OWTS, and the third provides a comparative higher dose of sulfamethoxazole chosen to create a condition of even higher stress and potentially increased impact to the microbial community.

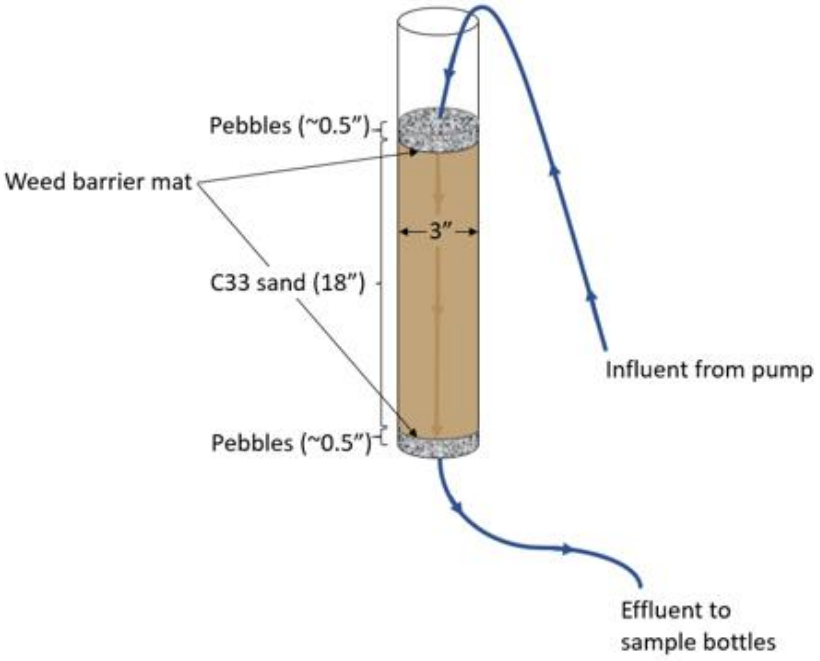

Figure 1. Schematic of column design

Sulfamethoxazole and ciprofloxacin doses were added to the influent bottles feeding the columns from day 0 to day 10 of the experiment to represent a typical antibiotic treatment period. During this time, 1 µg/mL of all PPCPs studied in this work was also added to the influent along with 0.5 mM sodium bromide. The pH of the influents was adjusted to 6.8 – 7.2 using 1 N NaOH. Beginning on day 12 of the experiment, the influent was made up with the same recipe with the exception of the high-level antibiotics which were no longer added. All influent and effluent samples were analyzed for PPCP concentrations using liquid chromatography tandem triple quadrupole mass spectrometry as described by Clyde (2021).

Results and Discussion

Effect of Antibiotics on Nitrification

Based on previously published studies, inhibition of nitrification was expected at all three of the antibiotic dose concentrations applied in this work (Ghosh et al., 2009; Parente et al., 2018). Time series data for effluent NO_X are plotted in Figure 2C. The dosing period on the columns is defined here as day 3 through day 14 of the experiment and represents the period during which the columns were exposed to high-level antibiotics. During this period, NO_X concentrations in the column effluents for the control, 150 µg/L sulfamethoxazole, 750 µg/L sulfamethoxazole, and 300 µg/L ciprofloxacin treatments were 21.9 ± 1.8 mg N- NO_X/L (standard error), 13.8 ± 1.3 mg N- NO_X/L, 12.4 ± 2.1 mg N- NO_X/L, and 12.5 ± 1.8 mg N- NO_X/L respectively, as seen in Figure 2A. A one-way ANOVA comparing these concentrations showed a significant difference between the means ($F(3,45) = 5.45$, $p = 0.00276$), and a post-hoc Tukey test indicated significant differences between each antibiotic treatment and the control ($p = 0.0252$, 0.00653, 0.00658 for 150 µg/L sulfamethoxazole, 750 µg/L sulfamethoxazole, and 300 µg/L ciprofloxacin respectively), with no significant differences between the dosed treatments. Therefore, there was a significant effect on NO_X production due to the antibiotic addition.

Although the concentrations used here are lower than those tested for effects on nitrification in previous studies, these results are similar to literature findings of inhibition of nitrification at concentrations of 1 mg/kg ciprofloxacin (Parente et al., 2018) and 1 mg/L sulfamethoxazole (Ghosh et al., 2009). The lack of inhibition seen by Ghosh et al. (2009) at 0.5 mg/L sulfamethoxazole in a nitrifying activated sludge reactor differs from the effect on nitrification during a 150 µg/L dose of sulfamethoxazole measured in this study. There are a number of possible reasons for the difference. Sulfamethoxazole may have more sorption in the sludge present in the reactor than it does in the sands used in this experiment, thereby lowering the aqueous and bioavailable concentration in the study by Ghosh et al. (2009). Additionally, differences in the microbial communities present in the two experiments could significantly change the effects of the antibiotic on the microbial function of the systems.

As can be seen in Figure 2B, after the dosing period, effluent NO_X concentrations in the dosed columns immediately returned to concentrations aligned with those of the control columns. No statistically significant difference was found between mean NO_X concentrations in the effluents across treatments from day 16 through day 34 using a one way ANOVA at the $p < 0.05$ level ($F(3,44) = 0.35$, $p = 0.787$), as illustrated by Figure 2B. These results show rapid recovery of the nitrifying sand columns after antibiotic doses were stopped. This implies that while the concentration of antibiotic excreted by a single resident on a typically prescribed dose is likely to inhibit nitrification in a full-scale unsaturated nitrifying sand filter, some nitrification can still occur, and the system is expected to recover immediately after the treatment is complete.

The limited literature on this subject does not provide comparisons for the rapid recovery reported here. However, an exploration of sulfamethoxazole and ciprofloxacin provide insight into the potential for such immediate response. Sulfamethoxazole is a bacteriostatic antibiotic, and so it works by inhibiting bacteria instead of killing them. This combined with the flow rate in the system and the lack of sorption of sulfamethoxazole reasonably results in a short recovery time since the microbial community does not need to regrow after exposure to a dose.

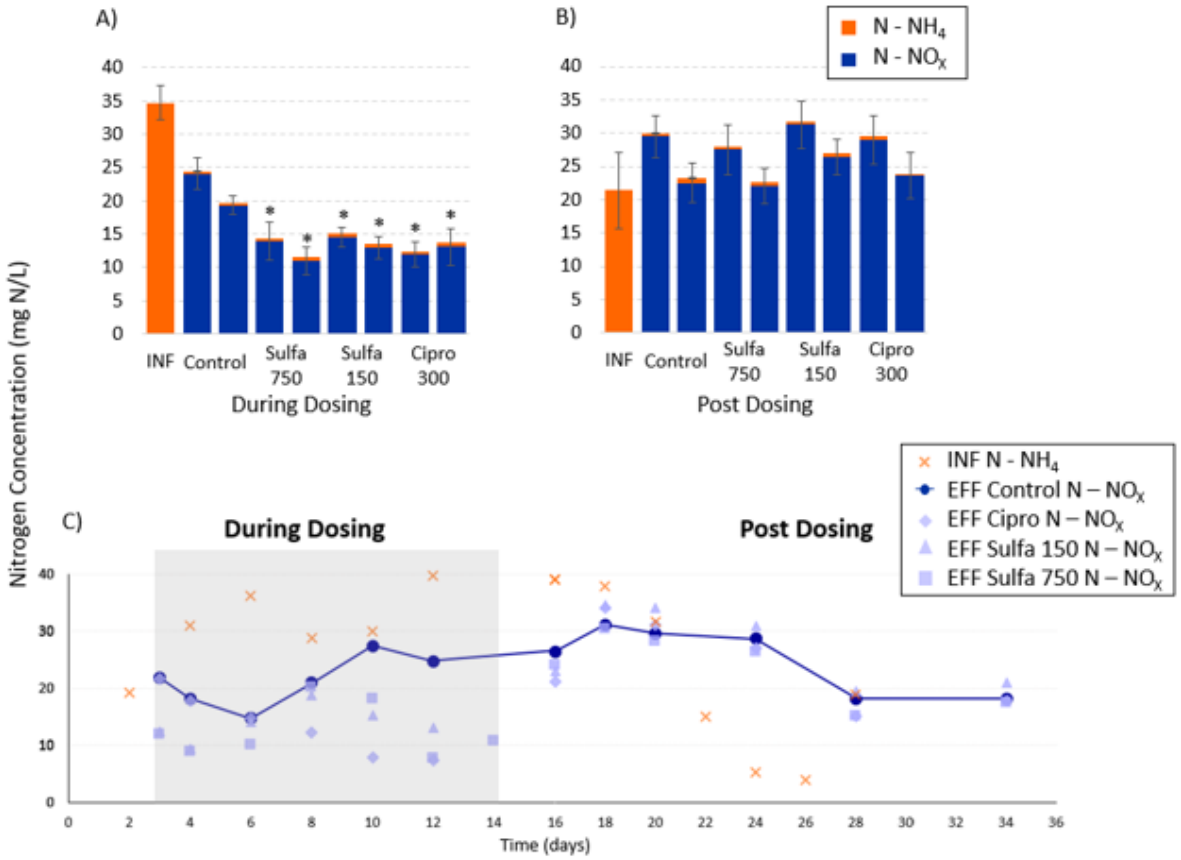

Figure 2. Effects on nitrogen transformation. A) average values during the dosing period (day 3 – day 14), B) average values during the post-dosing period (day 15 – day 34), and C) full time series with points plotted as a two-day trailing average. In C, influent NO_X concentrations and effluent NH_4^+ concentrations are not shown because they contribute minimally to the TIN as seen in A and B.

Ciprofloxacin has been shown to act as bacteriostatic or bactericidal based on the concentration of the dose. In a pure-culture study, Silva et al. (2011) found bacteriostatic effects of ciprofloxacin on *Escherichia coli* at concentrations up to 8 µg/L and bactericidal effects at 16 and 32 µg/L. Although these concentrations are lower than the 300 µg/L applied in this study, the nitrifying community in the sand columns may be protected from some portion of the applied dose by the diversity of the microbial community, the effects of the developed biofilms, or a decrease in the effective concentration due to lack of bioavailability from sorption. Thus, in both cases it is plausible that the rapid increase in NO_X effluent concentrations seen after the dosing period in each treatment is due to recovery of the nitrifying community in the sand.

Effect of Antibiotics on the Removal of Other PPCPs

The concentration and removal of PPCPs before, during, and after the period of antibiotic shock in treatment columns was compared to control columns to identify changes attributed to the dosing. One-way ANOVA showed no significant difference with $p = 0.05$ in the average influent concentration before, during, and after the dosing period for any of the analytes in any of the treatments, with the exception of ciprofloxacin and sulfamethoxazole concentrations during the dosing period in the treatments in which they were specifically added at higher levels. Similarly,

one-way ANOVAs indicated no significant increase at the $p = 0.05$ level in the average effluent values in the columns dosed with ciprofloxacin or sulfamethoxazole versus the control columns for any of the analytes during any of the three time periods. Figure 3 shows effluent concentration over time for representative compounds along with box and whisker plots with data separated by period. The data demonstrates the lack of difference between the control column effluents and the treatments at any point for a compound with high removal (acetaminophen, >98%), a compound with intermediate removal (warfarin, 83%), and a compound with low removal (TCEP, 43%). These results are consistent with the lack of effect of a 500 µg/L dose of ciprofloxacin on caffeine degradation found by Dorival-García et al. (2013), and as caffeine removal was used as a proxy of aerobic respiration in that work, the result here implies that these 17 compounds were removed by aerobic degradation or a similarly efficient mechanism since the same concentration which inhibited nitrification did not affect removal of these PPCPs. Although nitrification inhibition was significant in the present work, it was less complete than that achieved by Park et al. (2017) by using allythiourea as a selective inhibitor in a batch study. The difference may help explain the lack of significant effect on trimethoprim, atenolol, metoprolol, propranolol, and diltiazem removal in this work since co-metabolism by ammonium-oxidizing bacteria may have been only minorly affected by the antibiotic doses.

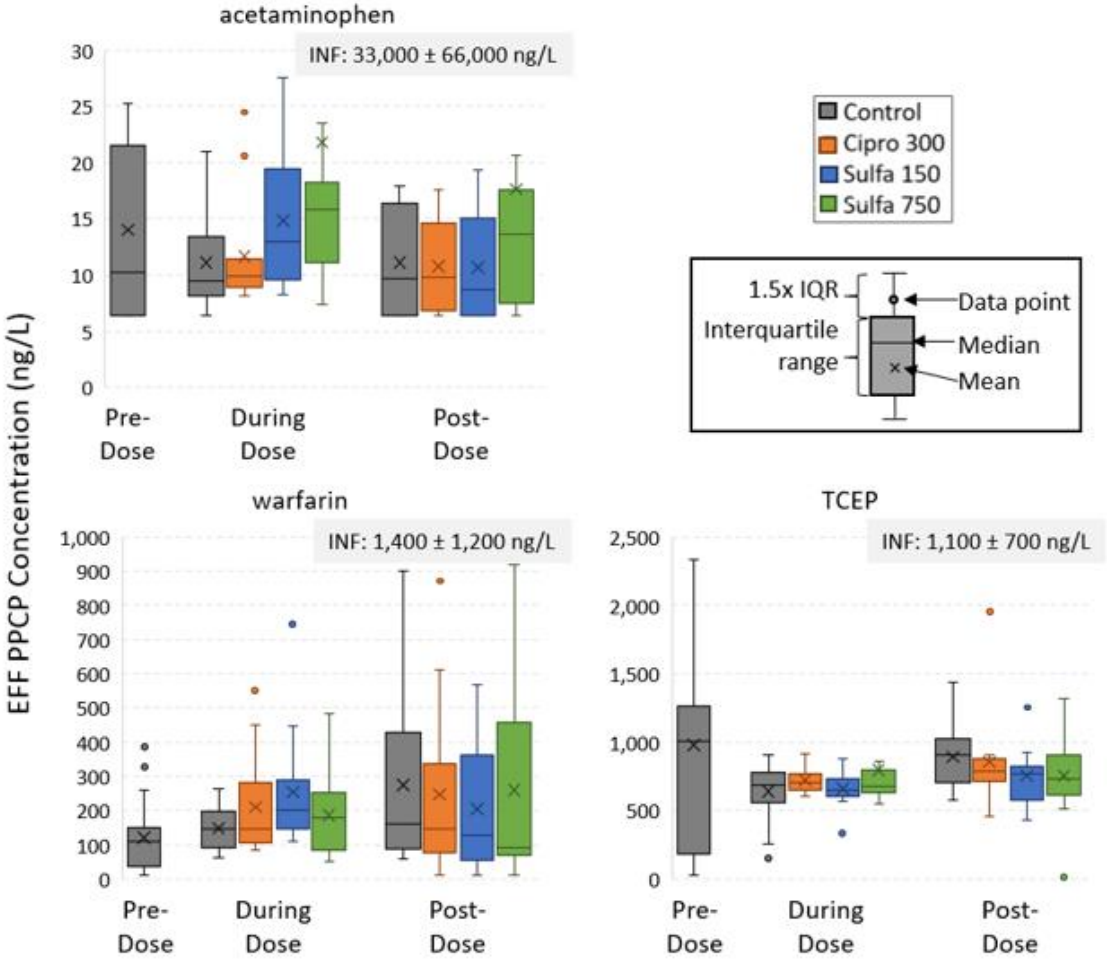

Figure 3. Effluent concentrations of three PPCPs in each treatment during three periods (pre-dose, during dose, and post-dose).

Conclusions

The results of this study demonstrate the resilience of acclimated sand filter systems to realistic shocks of highly prescribed antibiotics. To the best of our knowledge, this is the first study to examine effects of ciprofloxacin and sulfamethoxazole on nitrification and PPCP removal at these low, yet relevant concentrations. The concentrations used are consistent with a single resident of a 3-br home on a standard prescription, and therefore represent a shock potentially faced by installed OWTSs. Although nitrification was temporarily inhibited in this study, the recovery of the system was immediate, suggesting that nitrification in OWTSs will be resilient as well. Additionally, monitoring of PPCPs indicated no negative impacts on their removal due to antibiotic additions. Together these results demonstrate the ability of unsaturated nitrifying sand filters to perform consistently when faced with a realistic antibiotic dose, promoting their potential as reliable treatment systems for removing nitrogen and PPCPs. This work motivates future studies on the mechanisms of PPCP removal and the overall mechanism of resilience in these columns, potentially contributing to the literature on biofilm protection.

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