

TECHNICAL REPORT

Emerging Contaminants

Impacts of the COVID-19 pandemic on pharmaceuticals in wastewater treated for beneficial reuse: Two case studies in central Pennsylvania

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Abstract

During the COVID-19 pandemic, wastewater surveillance was leveraged as a powerful tool for monitoring community-scale health. Further, the well-known persistence of some pharmaceuticals through wastewater treatment plants spurred concerns that increased usage of pharmaceuticals during the pandemic would increase the concentrations in wastewater treatment plant effluent. We collected weekly influent and effluent samples from May 2020 through May 2021 from two wastewater treatment plants in central Pennsylvania, the Penn State Water Reclamation Facility and the University Area Joint Authority, that provide effluent for beneficial reuse, including for irrigation. Samples were analyzed for severe acute respiratory syndrome coronavirus 2 (influent only), two over-the-counter medicines (acetaminophen and naproxen), five antibiotics (ampicillin, doxycycline, ofloxacin, sulfamethoxazole, and trimethoprim), two therapeutic agents (remdesivir and dexamethasone),

Abbreviations: EC₅₀, effective concentration at which 50% of a population is affected; LC₅₀, lethal concentration at which 50% of a population is affected; MEC, measured environmental concentration; MLD, million liters per day; NOEC, no observable effect concentration; Penn State WRF, Pennsylvania State University Water Reclamation Facility; PNEC, predicted no-effect concentration; RQ, risk quotient; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UAJA, University Area Joint Authority; UV, ultraviolet; WWTP, wastewater treatment plant.

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and hydroxychloroquine. Although there were no correlations between pharmaceutical and virus concentration, remdesivir detection occurred when the number of hospitalized patients with COVID-19 increased, and dexamethasone detection co-occurred with the presence of patients with COVID-19 on ventilators. Additionally, Penn State decision-making regarding instruction modes explained the temporal variation of influent pharmaceutical concentrations, with detection occurring primarily when students were on campus. Risk quotients calculated for pharmaceuticals with known effective and lethal concentrations at which 50% of a population is affected for fish, daphnia, and algae were generally low in the effluent; however, some acute risks from sulfamethoxazole were high when students returned to campus. Remdesivir and dexamethasone persisted through the wastewater treatment plants, thereby introducing novel pharmaceuticals directly to soils and surface water. These results highlight connections between human health and water quality and further demonstrate the broad utility of wastewater surveillance.

1 | INTRODUCTION

Wastewater-based epidemiology is increasingly being viewed as a powerful tool for providing information about the health of a community, with implications ranging from identifying potential exposure to contaminants and pathogens to estimating the use of legal and illegal drugs in a community (Lorenzo & Picó, 2019). Because each person in a treatment plant's service area inevitably contributes to the wastewater, analyzing wastewater provides an unbiased assessment of the entire community. During the COVID-19 pandemic, wastewater epidemiology became particularly useful because infected individuals who are presymptomatic or asymptomatic shed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through their feces (Wang, Hu, et al., 2020; Wang, Xu, et al., 2020; Wu et al., 2020; Xiao et al., 2020; Xu et al., 2020; Zhang et al., 2020), thereby introducing the novel coronavirus to the wastewater stream. Therefore, wastewater surveillance can be and has been successfully used to monitor community spread (Balboa et al., 2021; Weidhaas et al., 2021).

Although it is believed that SARS-CoV-2 is effectively inactivated through wastewater treatment plants (WWTPs) using typical wastewater treatment methods for removing pathogens (CDC, 2020; Foladori et al., 2021), WWTPs are known to be relatively ineffective in treating many over-the-counter and prescription-strength pharmaceuticals, including those that are likely to increase in usage by people who are suffering from viral infection (Escher et al., 2011; Kibuye et al., 2019; Musee et al., 2021; Noguera-Oviedo & Aga, 2016; Wilkinson et al., 2017). For example, at the height of the 2009 H1N1 viral pandemic in England, antibiotics including sulfamethoxazole and ofloxacin were detected most frequently

and at high concentrations in wastewater compared to the late pandemic period (Singer et al., 2014). A possible explanation is that viral infections can lead to secondary bacterial respiratory infections, which can lead to a higher risk of death among patients hospitalized with a viral infection (Balcan et al., 2009; Shafran et al., 2021; Singer et al., 2011, 2014).

Over the course of the COVID-19 pandemic, people experiencing mild symptoms but not severely ill to the point of needing hospitalization were generally advised to treat their symptoms with pain relievers and cough suppressants, including common over-the-counter pain medications such as naproxen and acetaminophen (Shah et al., 2021; Yousefifard et al., 2020). Meanwhile, some hospitalized patients were treated with the malaria drug hydroxychloroquine (Gao et al., 2020) and the therapeutic antiviral drug remdesivir (Beigel et al., 2020). Dexamethasone, which is often used to treat inflammation, began to be used to provide relief to patients with COVID-19, particularly those on ventilators (Horby et al., 2021; Musee et al., 2021). Usage of some of these drugs to treat the novel coronavirus was experimental and, in some cases, controversial; however, their usage would likely be detected in the influent to WWTPs that have hospitals in their service areas. Common over-the-counter pain medications and antibiotics may be prevalent in wastewater regardless of a hospital in the wastewater service area. However, it is possible that the detection of these pharmaceuticals may increase with increasing detection of SARS-CoV-2, which could indicate an increased usage of these drugs when COVID-19 cases may be high.

By analyzing wastewater for SARS-CoV-2, prescription medications, and over-the-counter medications, valuable information regarding the well-being of an entire community can be gained without the need to interview, survey, or test

individuals. Here, we partnered with two WWTPs in the Commonwealth of Pennsylvania—the Pennsylvania State University Water Reclamation Facility (Penn State WRF) and the University Area Joint Authority (UAJA) Wastewater Treatment Plant—to monitor the concentrations of SARS-CoV-2, two pain medications (acetaminophen and naproxen), five antibiotics (ampicillin, doxycycline, ofloxacin, sulfamethoxazole, and trimethoprim), two therapeutic agents (remdesivir and dexamethasone), and hydroxychloroquine. Influent samples were analyzed for all constituents of interest, whereas effluent samples were analyzed only for pharmaceuticals.

The specific objectives of this research were (a) to investigate relationships between SARS-CoV-2 and the pharmaceuticals of interest; (b) to identify relationships between hospitalization data (i.e., number of hospitalized patients with COVID-19) and constituents of interest; (c) to understand potential ecological risks posed by effluent for pharmaceuticals with known effective and lethal concentrations at which 50% of a population is affected (EC_{50} or LC_{50} , respectively) and no observable effect concentration (NOEC) values for fish, algae, and daphnia; and (d) when possible, to compare effluent concentrations with influent concentrations to understand the persistence of novel pharmaceuticals through wastewater treatment. These objectives were selected to further qualify wastewater surveillance as a tool to inform community-scale decision-making and to estimate the impacts of the pandemic on the quality of treated wastewater for beneficial reuse activities (e.g., wastewater irrigation).

2 | MATERIALS AND METHODS

2.1 | Study sites

We partnered with two wastewater treatment plants using beneficial reuse of treated wastewater for weekly monitoring of SARS-CoV-2 and selected pharmaceuticals: the Penn State WRF and the UAJA facility. Beneficial reuse activities began at both facilities as an alternative to discharging directly to Spring Creek due to water quality concerns related to the stream's designated use as a high-quality cold water fishery under Chapter 25 Pa. Code, Section 93.4b(a)(2)(i) (Carline et al., 2011; Centre Regional Planning Agency, 2021).

Penn State's University Park campus has separate stormwater and sewer systems, and the Penn State WRF treats domestic wastewater for the campus, which has an undergraduate enrollment of ~46,000 students. The facility has an annual average hydraulic design capacity of ~11 million L d⁻¹ (MLD) and an organic loading capacity of ~6,350 kg d⁻¹ of biological oxygen demand. At the facility, (a) grit is removed from raw influent; (b) grit is sent to a landfill, and the rest of the wastewater undergoes screening and primary clarification where biosolids are removed; (c) the wastewater

Core Ideas

- Pharmaceuticals were monitored at two wastewater treatment plants through the COVID-19 pandemic.
- Novel pharmaceuticals persisted in reclaimed wastewater for irrigation activities.
- Detection of dexamethasone occurred with increasing numbers of patients with COVID-19 on ventilators.
- Pharmaceuticals in effluent posed potential acute risks to nontarget aquatic organisms.

undergoes biological treatment (i.e., activated sludge treatment with biological nutrient removal) where microorganisms remove/metabolize organic matter in the wastewater (food to microorganism ratio of 0.3); (d) the wastewater undergoes secondary clarification where any remaining solids are removed by physical settling; and (e) the wastewater is disinfected with chlorine (Kibuye et al., 2019). Although the hydraulic retention time varies widely throughout the day due to diurnal flows and throughout the year based on the number of students, the overall retention time in the entire treatment process is typically 16–24 h. The Penn State WRF produces Class B reclamation water that is beneficially reused for spray irrigation at a 245-ha mixed-use (agricultural and forested) area known as the “Living Filter” (Ferguson, 1983).

The UAJA treats domestic wastewater and some industrial waste from the community surrounding Penn State, including College, Harris, Patton, and Ferguson Townships; State College Borough; and Mount Nittany Medical Hospital. Mount Nittany is a 260-bed acute care facility with units dedicated to airborne isolation, adult intensive care, general medical/surgical procedures, and pediatric care (Mount Nittany Health, 2022; Pennsylvania Department of Health, 2021). The facility is also equipped with 25 ventilators and an intensive care unit dedicated to patients with COVID-19 (Pennsylvania Department of Health, 2021).

Like the Penn State WRF, the UAJA has a separate stormwater and sewer system. The UAJA treats ~23 MLD of wastewater (UAJA, 2018a), of which ~13 MLD is beneficially reused for commercial uses and golf course irrigation (Centre Regional Planning Agency, 2021). The remainder of the treated wastewater (~9 MLD) is discharged to Spring Creek. At the facility, raw influent is treated similarly to the primary and secondary steps (Steps *a–d*) at the Penn State WRF. After secondary clarification, wastewater undergoes secondary treatment where alum is added and small solids and dissolved phosphorous are removed. From there, effluent destined for beneficial reuse is further processed through microfilters and reverse osmosis. On the other hand, effluent

that is destined to be discharged to Spring Creek undergoes tertiary treatment where wastewater is passed through eight anthracite coal/mono-media filters and disinfected with ultraviolet (UV) filters before it is discharged into Spring Creek (UAJA, 2018b). In this study, effluent was collected after UV disinfection. Although the hydraulic retention time varies, the average retention time for the treatment plant is typically 18 h.

2.2 | Wastewater sampling

From 28 May 2020 through 26 May 2021, one 24-h composite raw influent and one 24-h composite treated effluent sample were collected every Thursday from both treatment plants (excluding the winter holiday period of 23 Dec. 2020–6 Jan. 2021), which provided a total of 200 samples (50 influent and 50 effluent at each treatment facility). Samples were collected in trace-cleaned 1-L amber glass bottles with polytetrafluoroethylene-lined caps. These samples were analyzed for selected pharmaceuticals, including pain medications and fever reducers (acetaminophen and naproxen), for which use is encouraged to treat mild COVID-19 related symptoms (Shah et al., 2021; Yousefifard et al., 2020); antibiotics (ampicillin, doxycycline, ofloxacin, sulfamethoxazole, and trimethoprim), which are used to treat secondary bacterial infections experienced by some hospitalized patients with COVID-19 (Chedid et al., 2021; Shafran et al., 2021) and known to occur in waterways and wastewater treatment plants (Ebele et al., 2017; Kibuye et al., 2019) during viral pandemics (Singer et al., 2014); and therapeutic agents (remdesivir and dexamethasone) and hydroxychloroquine, which have been used in some cases to treat hospitalized patients with COVID-19 (Beigel et al., 2020; Gao et al., 2020; Horby et al., 2021). Analysis for dexamethasone began on 1 Oct. 2020. Additionally, starting 9 July 2020, a 250-ml aliquot of the 24-h composite raw influent sample was collected into a high-density polyethylene bottle every Thursday from each WWTP for SARS-CoV-2 analysis.

2.3 | SARS-CoV-2 analysis

Sample aliquots in 250-ml high-density polyethylene bottles were pasteurized (90 min at 60 °C) to inactivate biohazardous agents. After pasteurization, each influent sample was split into three 40-ml aliquots. One aliquot was spiked with 400,000 copies of heat-inactivated SARS-CoV-2 (for a final concentration of 10,000 copies ml⁻¹). All aliquots were filtered through a 0.2-μm filter onto polyethylene glycol 8000 and NaCl at a final concentration of 10% (w/v) and 0.4 M, respectively. Samples were vortexed briefly and centrifuged at 12,000 × g for 2 h at 4 °C. The supernatant was discarded, and the concentrated pellet containing nucleic acids

and viral particles was processed using the QIAamp viral RNA mini kit (Qiagen) to isolate purified RNA, which was quantified using the 2019-nCoV Centers for Disease Control qPCR Kit (N2 gene), on an ABI 7500 Fast Real-Time PCR System. Commercial master mix was used for real-time quantitative polymerase chain reaction analysis to provide optimal reproducibility between assay measurements over time. Initially, qScript One-Step qRT-PCR, low-ROX master mix (QuantaBio) was used, later replaced by qScript 1-Step Virus ToughMix (QuantaBio) in December 2020. During a 1-mo trial period, samples were analyzed using both master mixes in parallel, yielding similar results, especially for corrected values; however, qScript 1-Step Virus ToughMix performed better for samples derived from higher-strength wastewater with more polymerase chain reaction inhibitors and was used exclusively in the later part of the study. Viral concentrations in unspiked aliquots were corrected based on the recovery of spiked virus from the same sample matrix. The limit of detection for this method was approximately one to two virus copies per 40 ml of wastewater.

2.4 | Pharmaceutical analysis

Influent and effluent samples collected in 1-L amber glass bottles underwent the same pasteurization process as noted above before they were analyzed for pharmaceuticals following USEPA Method 1694 (USEPA, 2007). First, each sample was filtered through 0.47-μm glass fiber filter paper. Filtered samples were then concentrated onto a hydrophobic-lipophilic balanced solid-phase extraction cartridge (Waters, Oasis) and eluted with 6 ml of liquid chromatography–mass spectrometry–grade methanol at a rate of three drops per second. The eluant was then blown down to 1 ml under a gentle stream of nitrogen gas in a water bath held at 45 °C and filtered through a 0.2-μm polyethersulfone membrane into a 1.8-ml amber glass autosampler vial. The concentrated sample was analyzed using a high-resolution accurate mass Q Exactive mass spectrometer (Thermo-Fisher Scientific) interfaced with an ICS-5000+ chromatography system (Thermo-Fisher Dionex) via a heated electrospray injection source. Ten microliters of sample were injected onto a 2.1 mm by 100 mm, 3-μm Hypersil Gold aQ column (Thermo-Fisher Scientific) and eluted using 0.1% formic acid and 4 mM ammonium formate in water (A) and methanol (B) under the following linear gradient: 88%A:12%B at 0 min, 100%B at 9 min with a 2-min hold, followed by a 3-min equilibration at 88%A:12%B. The flow rate during the elution was 0.3 ml min⁻¹. The mass range for the mass spectrometer was set from 80 to 1,100 m/z with a resolution of 70,000 and operated in data-dependent MS2 mode for all analytes of interest. The data-dependent MS2 mode used a resolution of 35,000 and used normalized collision energies of 20, 40, and 60 electron volts.

Each of the pharmaceuticals of interest had an instrument limit of detection of $1 \mu\text{g L}^{-1}$ ($\text{LOD} = 2 \times z_{1-\alpha} \sigma_0$, where $z_{1-\alpha}$ is the standard normal variable, and σ_0 is the standard deviation of the blank) and a method limit of quantification of $5 \mu\text{g L}^{-1}$ ($\text{LOQ} = 10 \times \sigma_0$). The recovery percentage for each analyte was determined to be $>90\%$. The instrument calibration range for each of the analytes was $5\text{--}5,000 \mu\text{g L}^{-1}$. After the samples were concentrated and adjusted for the estimated error variance, the measured concentration range of each analyte was 0.1 ng L^{-1} to $100 \mu\text{g L}^{-1}$.

2.5 | Risk quotient calculations

To assess the potential ecological risk pharmaceuticals in the treated effluent may pose to the aquatic environment, acute and chronic risk quotients (RQs) were calculated following European Commission (2003) guidelines and examples in existing literature (Kosma et al., 2014; Kumari & Kumar, 2022; Vestel et al., 2015). The RQ is defined as the measured environmental concentration (MEC) divided by the predicted no-effect concentration (PNEC) (Equation 1).

$$\text{RQ} = \frac{\text{MEC}}{\text{PNEC}} \quad (1)$$

Here, the measured environmental concentration is taken to be the measured effluent concentration, and RQs for acute and chronic toxicity (RQ_{acute} and $\text{RQ}_{\text{chronic}}$) were calculated using acute and chronic PNECs, as defined in Equations 2 and 3, where NOEC is the no-observed-effect concentration (European Commission, 2003; Kosma et al., 2014; Vestel et al., 2015):

$$\text{RQ}_{\text{acute}} = \frac{\text{MEC}}{\text{PNEC}_{\text{acute}}} = \frac{\text{MEC}}{\left(\frac{\text{EC}_{50} \text{ or } \text{LC}_{50}}{1,000} \right)} \quad (2)$$

$$\text{RQ}_{\text{chronic}} = \frac{\text{MEC}}{\text{PNEC}_{\text{chronic}}} = \frac{\text{MEC}}{\left(\frac{\text{NOEC}}{10} \right)} \quad (3)$$

Compound-specific EC_{50} , LC_{50} , and NOEC values for fish, algae, and daphnia were used to calculate PNECs, which can be found in Supplemental Table S5. In cases where these values were not available in published toxicological studies, predicted EC_{50} , LC_{50} , and NOEC values were obtained from the USEPA ECOSAR database (v2.0) (Kumari & Kumar, 2022; Nejumal et al., 2021; Sanderson et al., 2003; USEPA, 2017). Because both WWTPs direct a portion or all their effluent to beneficial reuse, RQs are used to speculate how these activities could mitigate ecological risks posed by pharmaceuticals in effluent to protect Spring Creek.

2.6 | Assessing risk to Spring Creek

Once the treated effluent from UAJA is discharged to Spring Creek, the risk of pharmaceuticals present in the effluent on the aquatic ecosystem is influenced, in part, by the streamflow. Streamflow data were downloaded for a USGS gauging station (Station no. 01546400) located 1.6 km downstream of the UAJA discharge point (USGS, 2022). Monthly minimum and maximum discharge values observed in Spring Creek were used to determine the range in the percentage contribution of UAJA effluent to the Spring Creek flow rate. This percentage was used as a dilution factor to estimate a diluted pharmaceutical concentration in Spring Creek, and these diluted pharmaceutical concentrations were used to calculate acute and chronic risk quotients that the pharmaceuticals may pose once diluted in Spring Creek. This assessment assumes homogenous mixing of effluent with influent, which is not typically realistic when effluent is discharged into waterways. Further, this assessment does not account for the influence of biogeochemical and physical factors like pH, soil/water partitioning, temperature, and sunlight, that can influence the transport/fate of contaminants and therefore the risk they pose to the aquatic environment (Kibuye et al., 2020; Li et al., 2010; McCarthy & Alvarez, 2014; Sabourin et al., 2009).

3 | RESULTS AND DISCUSSION

3.1 | Influent concentrations during the pandemic

3.1.1 | Over-the-counter pain and fever medications

Over the 1-yr study period, acetaminophen and naproxen were detected ubiquitously ($>96\%$ of weekly influent samples) at concentrations orders of magnitude higher than all other pharmaceuticals of interest, exceeding $2 \mu\text{g L}^{-1}$ on average (Table 1). Seasonal trends were observed, with higher concentrations of both acetaminophen and naproxen during the colder period (December 2020–April 2021) (Figure 1). No correlations were observed between virus concentrations and influent concentrations of either naproxen or acetaminophen. These over-the-counter medications typically exhibit seasonal trends in wastewater influent and therefore are not indicators of the prevalence of COVID-19 in the community serviced by the treatment plants.

3.1.2 | Antibiotics

Although COVID-19 is a viral disease for which antibiotics are not effective treatment, hospitalized patients with secondary bacterial infections have often required longer

TABLE 1 Summary statistics for each constituent of interest for the influent and effluent of the Penn State Water Reclamation Facility (WRF) and the University Area Joint Authority (UAJA)

Constituent	Penn State WRF		UAJA	
	Influent	Effluent	Influent	Effluent
Pain medications and fever reducers				
Acetaminophen	1,990 ng L ⁻¹ ; <LOD–15,800 ng L ⁻¹ ; 96	30.37 ng L ⁻¹ ; <LOD–334.6 ng L ⁻¹ ; 54	4,230 ng L ⁻¹ ; <LOD–24,600 ng L ⁻¹ ; 98	13.3 ng L ⁻¹ ; <LOD–185.1 ng L ⁻¹ ; 42
Naproxen	2,280 ng L ⁻¹ ; <LOD–11,100 ng L ⁻¹ ; 98	520 ng L ⁻¹ ; <LOD–5,150 ng L ⁻¹ ; 90	4,850 ng L ⁻¹ ; <LOD–18,200 ng L ⁻¹ ; 100	40.25 ng L ⁻¹ ; <LOD–290.5 ng L ⁻¹ ; 68
Antibiotics				
Ampicillin	9.24 ng L ⁻¹ ; <LOD–23.3 ng L ⁻¹ ; 38	10.19 ng L ⁻¹ ; <LOD–22.4 ng L ⁻¹ ; 40	8.89 ng L ⁻¹ ; <LOD–20.7 ng L ⁻¹ ; 44	10.0 ng L ⁻¹ ; <LOD–22.0 ng L ⁻¹ ; 20
Doxycycline	N/A; <LOD–22.0 ng L ⁻¹ ; 2	N/A; N/A; 0	N/A; N/A; 0	N/A; N/A; 0
Ofloxacin	3.78 ng L ⁻¹ ; <LOD–13.8 ng L ⁻¹ ; 24	4.33 ng L ⁻¹ ; <LOD–17.3 ng L ⁻¹ ; 26	10.85 ng L ⁻¹ ; <LOD–62.6 ng L ⁻¹ ; 72	8.60 ng L ⁻¹ ; <LOD–48.1 ng L ⁻¹ ; 34
Sulfamethoxazole	77.36 ng L ⁻¹ ; <LOD–301.1 ng L ⁻¹ ; 36	126.7 ng L ⁻¹ ; <LOD–486.9 ng L ⁻¹ ; 52	172 ng L ⁻¹ ; <LOD–940 ng L ⁻¹ ; 70	250 ng L ⁻¹ ; <LOD–1,010 ng L ⁻¹ ; 90
Trimethoprim	71.1 ng L ⁻¹ ; <LOD–336.8 ng L ⁻¹ ; 54	80.44 ng L ⁻¹ ; <LOD–421.7 ng L ⁻¹ ; 26	105.2 ng L ⁻¹ ; <LOD–448.3 ng L ⁻¹ ; 88	114.6 ng L ⁻¹ ; <LOD–656.7 ng L ⁻¹ ; 84
COVID-19 therapeutic agents				
Remdesivir	5.26 ng L ⁻¹ ; <LOD–6.82 ng L ⁻¹ ; 16	5.58 ng L ⁻¹ ; <LOD–7.59 ng L ⁻¹ ; 28	6.15 ng L ⁻¹ ; <LOD–11.9 ng L ⁻¹ ; 28	5.37 ng L ⁻¹ ; <LOD–7.87 ng L ⁻¹ ; 20
Dexamethasone	N/A; <LOD–11.5 ng L ⁻¹ ; 6	8.89 ng L ⁻¹ ; <LOD–10.8 ng L ⁻¹ ; 9	12.1 ng L ⁻¹ ; <LOD–17.0 ng L ⁻¹ ; 31	12.2 ng L ⁻¹ ; <LOD–15.4 ng L ⁻¹ ; 16
Hydroxychloroquine	N/A; <LOD; 0	N/A; <LOD; 0	N/A; <LOD; 0	N/A; <LOD; 0
Virus surveillance				
SARS-CoV-2	1,205 copies ml ⁻¹ ; <LOD–9,955 copies ml ⁻¹ ; 95	N/A; N/A; N/A	1,680 copies ml ⁻¹ ; 8.10–8,604 copies ml ⁻¹ ; 100	N/A; N/A; N/A

Note. Values are average; range; % >limit of detection (LOD). Average concentrations are reported for only the samples with concentrations above the LOD. N/A, not applicable.

hospitalization times and experienced higher death rates (Shafran et al., 2021). The antibiotics ampicillin, ofloxacin, sulfamethoxazole, and trimethoprim were detected more frequently in the samples from the UAJA compared with the Penn State WRF (Table 1), likely at least in part due to a hospital located within the service area of the UAJA. Doxycycline was detected in only one sample collected from the Penn State WRF and was not present at detectable levels in any samples collected from the UAJA (Supplemental Table S1). Sulfamethoxazole and trimethoprim were the most frequently detected antibiotics in the influent of both the Penn State WRF (36 and 56% of samples, respectively) and the UAJA (70 and

88% of samples, respectively). The concentrations observed at each treatment plant were generally on the same order of magnitude, with ofloxacin and ampicillin detected at average concentrations between 3 and 10 ng L⁻¹, whereas average concentrations of sulfamethoxazole and trimethoprim were each ~70 ng L⁻¹ at the Penn State WRF and >100 ng L⁻¹ at the UAJA (Table 1).

In general, the highest antibiotic concentrations were observed in the colder months (Figure 1), which aligns with other studies (Kibuye et al., 2019). At the UAJA, antibiotics were detected more frequently, and concentrations were an order of magnitude higher than the Penn State WRF influent

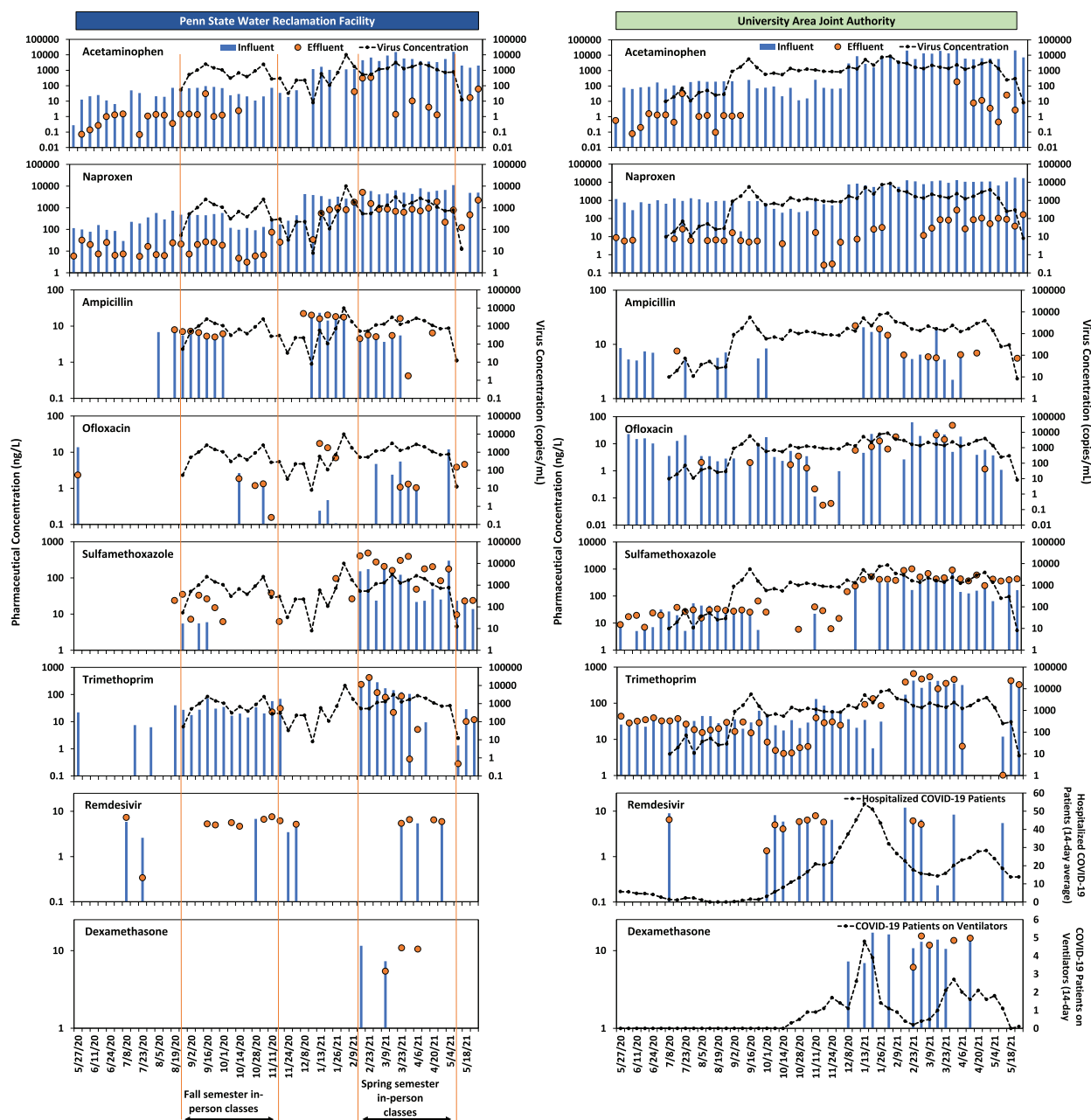


FIGURE 1 Weekly influent (bars) and effluent concentrations (dots) of select pharmaceuticals at the Penn State Water Reclamation Facility and the University Area Joint Authority (UAJA) over the 1-yr study period. Acetaminophen, naproxen, and antibiotic concentrations are plotted with severe acute respiratory syndrome coronavirus 2 concentrations detected in influent samples. At the UAJA, remdesivir concentrations are plotted with the number of hospitalized patients with COVID-19, and dexamethasone concentrations are plotted with the number of patients with COVID-19 on ventilators in the hospital within the treatment plant's service area. The 14-d average hospitalization data were obtained from the Pennsylvania Department of Health (2021)

(Table 1). At the Penn State WRF, antibiotic concentrations were further influenced by the semester schedule, typically dropping below the detection limit when students were largely away from campus for break or remote learning (Figure 1). Penn State students were a mix of remote and in-person in the fall 2020 semester and were switched to fully remote instructional mode from late November 2020 to February 2021.

3.1.3 | Therapeutic agents for COVID-19

Of the three experimental pharmaceuticals used for treatment of patients with COVID-19, hydroxychloroquine was not detected in any of the influent samples collected at either treatment plant (Table 1). Given its controversial usage and ineffectiveness for treating patients with COVID-19, its lack of detection in the influent samples is in line

with current understanding of hydroxychloroquine's utility for treating patients with malaria, rather than patients with COVID-19, and malaria's rare occurrence in the United States (Gao et al., 2020). However, remdesivir and dexamethasone, which have been shown to effectively treat patients with COVID-19 (National Institutes of Health, 2021), were detected in both wastewater treatment plants (Table 1; Figure 1). The frequency of detection of both pharmaceuticals in the Penn State influent was low, with detections occurring in only 16% of samples for remdesivir and 6% of samples for dexamethasone.

The source of remdesivir in the Penn State WRF wastewater is unclear because the WRF does not receive wastewater from hospitals, and remdesivir is primarily used to treat hospitalized patients with COVID-19 (Beigel et al., 2020). However, remdesivir is used in some experimental research settings on Penn State's campus, and it is not treated as hazardous waste. Therefore, it is possible that remdesivir was present in the treatment plant due to laboratory experiments conducted on campus and not from sick patients. In the case of dexamethasone, it was detected in Penn State influent only when campus activities resumed for the spring semester. At that time, dexamethasone concentrations in UAJA influent samples were also increasing along with the number of patients with COVID-19 on ventilators (Figure 1). Considering dexamethasone's half-life in the body is 36–72 h (Cronin et al., 2012), excretion likely continues for up to 1 wk after treatment with dexamethasone stops. Therefore, it is possible that the presence of dexamethasone in the Penn State WRF could be from students or staff returning to campus after being treated. It is also possible that the presence of dexamethasone could be from treatments unrelated to COVID because dexamethasone is also used as an anti-inflammatory/immunosuppressive agent in some human and veterinary medicines (Lalone et al., 2012). According to the Mayo Clinic (2022), aside from reducing inflammation, dexamethasone can be used to relieve redness/itching and to treat severe allergic reactions, skin conditions, adrenal problems, arthritis, asthma, blood/bone marrow problems, and kidney problems. Specifically, dexamethasone has been used in treatments of meningitis, myeloma, and bronchiolitis (Musee et al., 2021).

Influent from the UAJA, which does contain a hospital in its service area, had detectable concentrations of remdesivir and dexamethasone in 28 and 31%, respectively, of samples collected during the study period. Detection of remdesivir appeared to follow the number of patients hospitalized with COVID-19 (14-d averages obtained from the Pennsylvania Department of Health [2021]) (Figure 1). Detections generally coincided with increases in patients with COVID-19 at the local hospital during the late fall and winter surges in COVID-19 hospitalizations (Figure 1). Remdesivir was also

detected following the Fourth of July holiday in July 2020 (Figure 1). On the other hand, detections of dexamethasone appeared to increase as the number of hospitalized patients with COVID-19 on ventilators increased in winter and spring 2021 (Figure 1). This could be due to the primary use of dexamethasone to reduce severe upper respiratory inflammation for patients requiring ventilators (Horby et al., 2021; Musee et al., 2021). Detection of dexamethasone in the wastewater influent occurred five times on days when the number of hospitalized patients on ventilators was as low as two, and three times when the number of hospitalized patients on ventilators was one (Pennsylvania Department of Health, 2021). Overall, detection of dexamethasone and remdesivir at the wastewater treatment plant despite the small number of patients likely receiving these drugs relative to the entire population in the UAJA service area demonstrates the sensitivity of wastewater surveillance.

3.2 | Comparison of influent and effluent concentrations

3.2.1 | Over-the-counter pain and fever medications

Whereas acetaminophen was detected in nearly all (>95%) of the influent samples, it was present at detectable levels in only 40–50% of the effluent samples (Table 1; Figure 2). Further, the concentrations were reduced by several orders of magnitude, with average removal rates >90% for both treatment plants (Figure 3) and average effluent concentrations of 30 and 13 ng L⁻¹ for the Penn State WRF and the UAJA, respectively (Table 1). Although naproxen was similarly well removed (>80%), it persisted in a higher percentage of the treated effluent samples for both treatment plants, with a frequency of detection of 90% for the Penn State WRF and 68% for the UAJA (Table 1). The ability of the treatment plants to remove acetaminophen and naproxen nearly fully from influent is consistent with other studies (Kibuye et al., 2019; Lin et al., 2009; Luo et al., 2014; Shreve & Brennan, 2019).

Seasonal trends were also observed in the effluent at both treatment plants, particularly for naproxen, with concentrations generally highest in the winter (Figure 2). Similar trends for pain/fever reducers have been observed in other studies (Franklin et al., 2018; Kibuye et al., 2019; Zhang et al., 2015). This is likely due to increased consumption of pain/fever reducers in the colder months (Zhang et al., 2015). Reduced metabolic activity of activated sludge microorganisms at lower temperatures can also contribute to lower removal efficiencies and increased effluent concentrations in the colder months (Onesios et al., 2009; Vieno et al., 2007; Zhang et al., 2015).

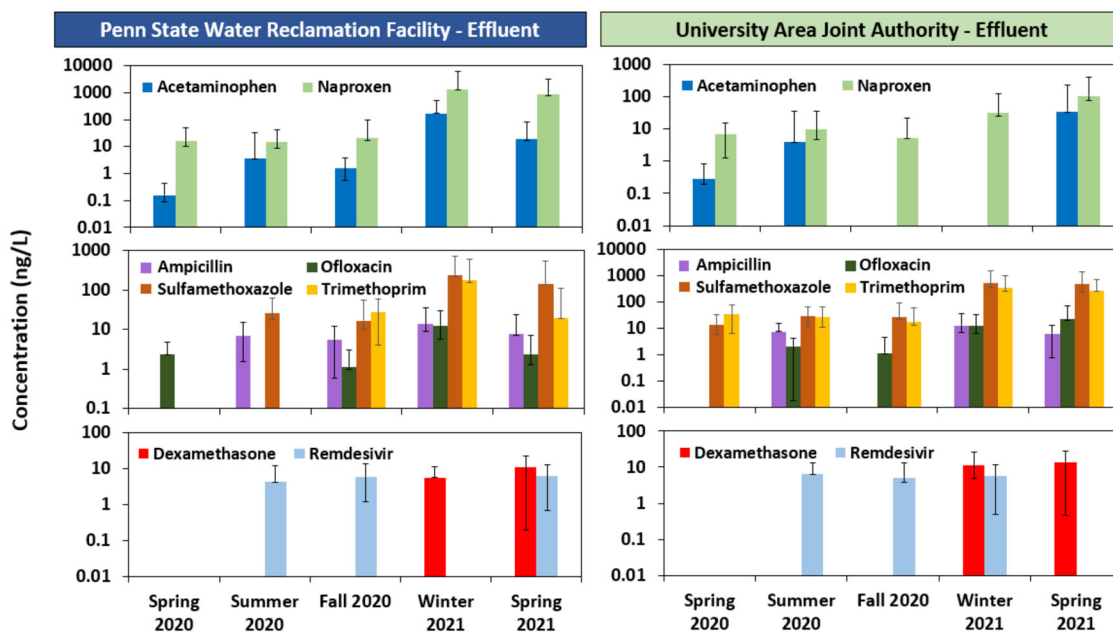


FIGURE 2 Seasonal mean effluent concentrations of each pharmaceutical of interest collected from the Penn State Water Reclamation Facility and the University Area Joint Authority wastewater treatment plant during spring 2020 (27 May–17 June), summer 2020 (24 June–16 September), fall 2020 (23 September–1 December), winter 2021 (8 December–16 March), and spring 2021 (23 March–25 May). Error bars represent the highest and lowest concentrations detected each season for each pharmaceutical at each facility. Pharmaceutical concentrations present below the limit of detection are not shown on the figure

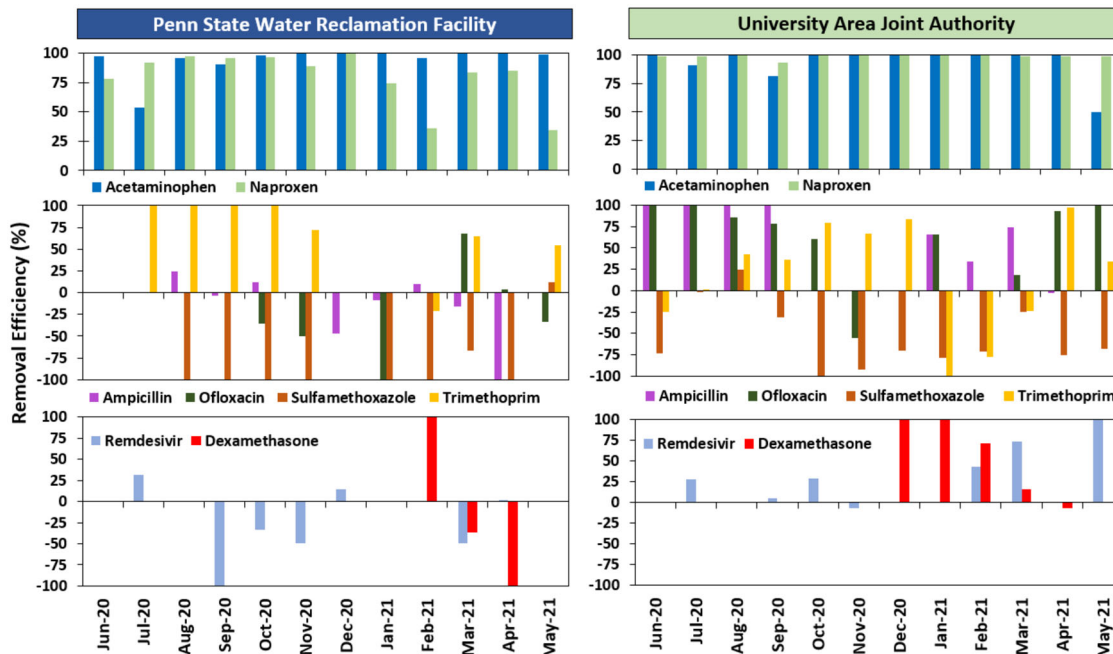


FIGURE 3 Mean removal efficiencies for each pharmaceutical of interest collected from the Penn State Water Reclamation Facility and the University Area Joint Authority wastewater treatment plant throughout the 1-yr study period. Negative values indicate that the effluent concentration was higher than the influent concentration. In cases where an effluent concentration was recorded in the absence of an influent concentration, the removal efficiency was -100%. In cases where the removal efficiency was below -100%, the removal efficiency was regarded as -100%

3.2.2 | Antibiotics

Antibiotics generally persisted through both wastewater treatment plants, with average concentrations mostly remaining on the same order of magnitude in the effluent compared with the influent (Table 1). The ability of each treatment plant to remove antibiotics present in the influent was mixed. The Penn State WRF was best able to reduce the influent concentrations of trimethoprim, with an average removal efficiency of 63%, whereas its ability to remove ofloxacin, sulfamethoxazole, and ampicillin was generally poor (Figure 3). The UAJA treatment plant also experienced difficulties reducing sulfamethoxazole and trimethoprim from the influent; however, it was better than the Penn State WRF at reducing ampicillin and ofloxacin, with average removal efficiencies of these antibiotics of 71 and 54%, respectively. The UAJA facility produces class A+ reclaimed water through advanced treatment processes, likely increasing its ability to remove ampicillin and ofloxacin relative to the Penn State WRF, which produces Class B reclaimed water (Kibuye et al., 2019; UAJA, 2018b). As part of its advanced treatment process, UAJA uses UV disinfection. Because De la Cruz et al. (2012) found that ofloxacin was 65–100% removed with UV disinfection, this advanced treatment step likely allowed for better removal of this antibiotic.

Sulfamethoxazole showed the poorest removal efficiency in both WWTPs (average, less than –50%) (Figure 3). Negative removal efficiencies of sulfamethoxazole through treatment plants have been documented in other studies (Kibuye et al., 2019; Shreve & Brennan, 2019; Zhang et al., 2017). For example, the median removal efficiency of sulfamethoxazole during integrated fixed-film activated sludge treatment across six treatment plants was –44% (Shreve & Brennan, 2019). Microorganisms involved in the activated sludge process drive the removal of nutrients and the breakdown of organic parent compounds into their metabolites (Ebele et al., 2017; Onesios et al., 2009). However, for some compounds, like sulfamethoxazole, it is likely that microorganisms convert primary metabolites back to their parent form, increasing effluent concentrations (Göbel et al., 2007; Luo et al., 2014; Shreve & Brennan, 2019).

Like naproxen, seasonal trends were observed for antibiotics at both treatment plants, with mean effluent antibiotic concentrations higher in winter compared with other seasons (Figure 2). This trend aligns with previous studies where ofloxacin, sulfamethoxazole, and trimethoprim concentrations were higher in wastewater during the winter (Franklin et al., 2018; Kibuye et al., 2019; Zhang et al., 2015). In addition to seasonal trends, influent and effluent concentrations of antibiotics were driven by changes in instructional mode (e.g., switch from in-person to remote learning) (Figure 1). In contrast, antibiotic concentrations in the UAJA effluent did

not follow the Penn State semester schedule but had more consistent detections throughout the entire study (Figure 1).

3.2.3 | Therapeutic agents for COVID-19

When remdesivir and dexamethasone were present in wastewater influent, the Penn State WRF was generally poor at removing these compounds, whereas the UAJA was more capable (Figure 3). Average removal efficiencies for remdesivir and dexamethasone at the Penn State WRF were less than –12%. At the UAJA, the average removal efficiency for remdesivir and dexamethasone was 39 and 56%, respectively. Interestingly, remdesivir was detected more frequently in the effluent than in the influent throughout the study period (Figure 1). Although there was not a hospital source of remdesivir to the Penn State WRF, some experiments that were conducted on campus used a metabolized form of remdesivir known as remdesivir triphosphate. It is possible that the detection of remdesivir in the Penn State WRF effluent was due to the conversion of this metabolite back to the parent form, a phenomenon that has been documented for some emerging contaminants, like estrogens (Wilkinson et al., 2017) and antibiotics (Göbel et al., 2007; Luo et al., 2014; Shreve & Brennan, 2019). However, this was not observed at UAJA, where remdesivir was only detected in the effluent at the same times that it was detected in the influent (Figure 1). Regarding dexamethasone, transformation of the metabolites back to the parent compound has been observed in wastewater treatment that utilizes chlorination disinfection (Musee et al., 2021). Because the Penn State WRF utilizes this treatment, this could explain why removal of dexamethasone was lower than in the UAJA (Figure 3). These results suggest that surveillance efforts should include metabolized forms of pharmaceuticals to better understand their potential to enter, transform, and/or persist through wastewater treatment plants before entering the terrestrial or aquatic environments through irrigation activities or direct discharge to receiving surface water bodies.

3.2.4 | Ecological risks

To understand potential ecological risks that the treated effluent could pose once released into the environment, risk quotients (RQs) were calculated for six pharmaceuticals (acetaminophen, naproxen, ampicillin, ofloxacin, sulfamethoxazole, trimethoprim, and dexamethasone) on three representative trophic levels (fish, algae, and daphnia). Remdesivir could not be calculated because EC₅₀ and NOEC values could not be found in the literature or through the ECOSAR database. Risk quotient values ≥ 1 suggest a high ecological

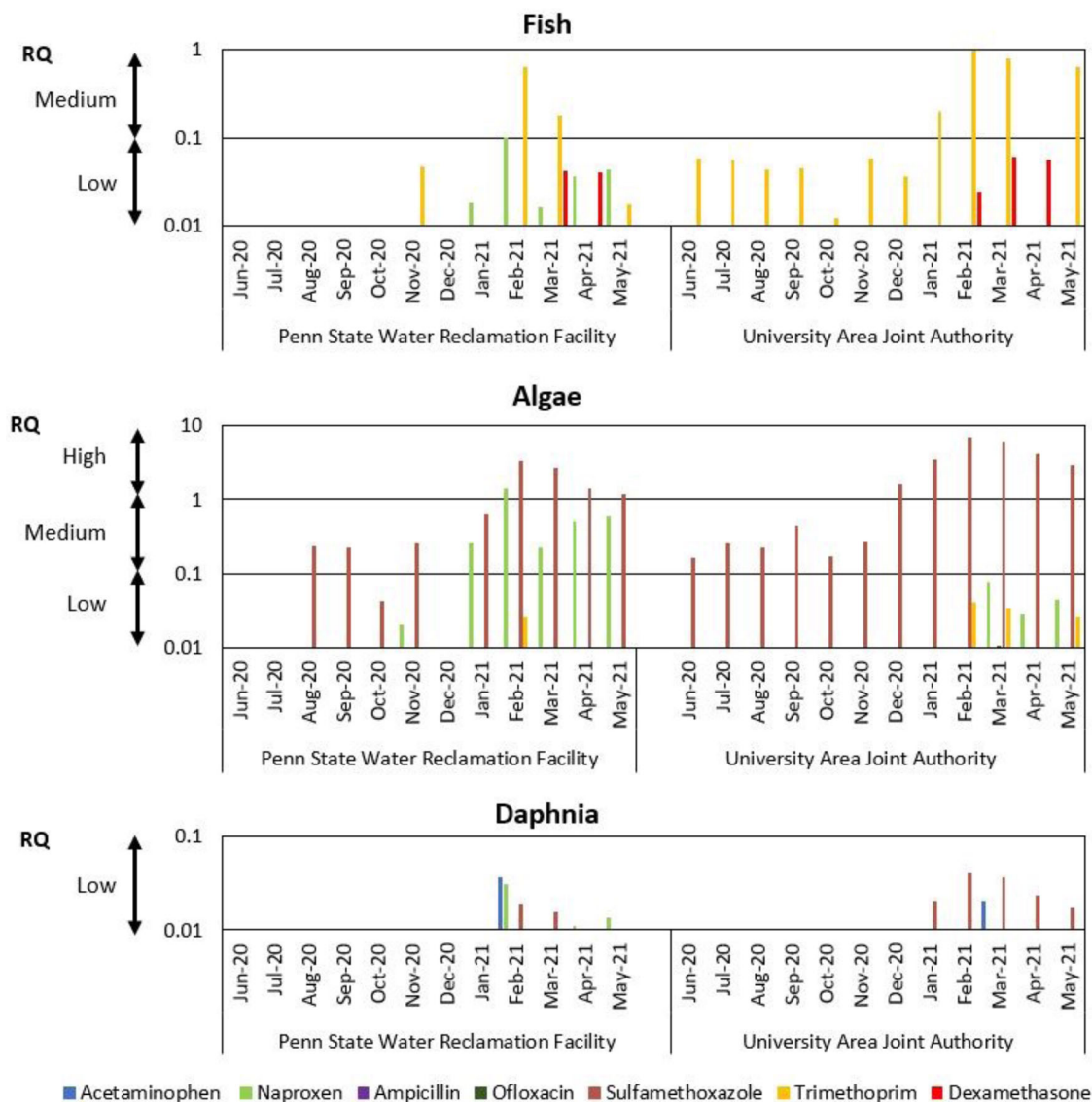


FIGURE 4 Risk quotients (RQs) to evaluate acute toxicity for fish, algae, and daphnia using the maximum effluent concentration measured each month (from June 2020 through May 2021) at the Penn State Water Reclamation Facility and the University Area Joint Authority

risk, values between 0.1 and 1 suggest a medium risk, and values between 0.01 and 0.1 suggest a low risk. These thresholds were set by Hernando et al. (2006) and have been used in other publications (Ginebreda et al., 2010; Kosma et al., 2014; Sanderson et al., 2003).

Acute and chronic RQs with values at or above the lowest possible risk (0.01) for fish, algae, and daphnia are shown in Figures 4 and 5. These values and all other values can be found in Supplemental Tables S7–S10. For estimating acute toxicity, RQs were based on the highest measured effluent concentration each month; for chronic toxicity, RQs were based on the average concentration for each month. Concentrations of pharmaceuticals measured in effluent samples are provided in Supplemental Tables S2 and S4.

Most pharmaceuticals posed low to medium risk to sensitive aquatic organisms. Of the three species, algae were the most sensitive, which aligns with previous studies that consider the toxicity of pharmaceuticals on algae (Kosma et al., 2014; Kumari & Kumar, 2022). Because algae can accumulate a variety of contaminants, bioaccumulation and biomagnification are also of concern (Kumari & Kumar, 2022). Of all the pharmaceuticals, sulfamethoxazole posed the highest risk, with an acute risk to algae >0.1 nearly every month, peaking in February 2021 when students returned to campus after being remote for the beginning of the Spring 2021 semester. At the same time, sulfamethoxazole posed a low chronic risk to algae at the UAJA. At the Penn State WRF, naproxen primarily posed a low to medium acute risk

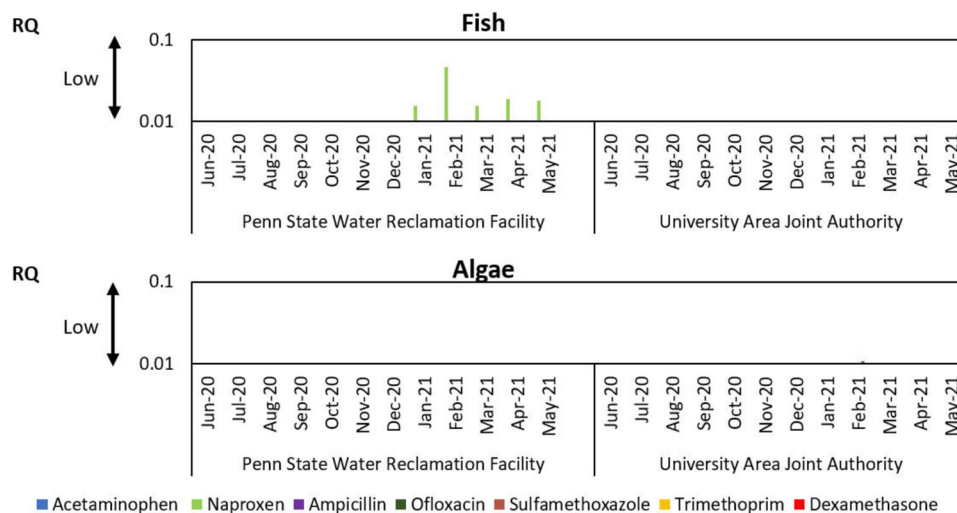


FIGURE 5 Risk quotients (RQs) characterizing chronic toxicity for fish and algae using the mean effluent concentration measured each month (from June 2020 through May 2021) at the Penn State Water Reclamation Facility and the University Area Joint Authority. Risk quotients were also estimated for daphnia, but concentrations were all below the lowest possible risk, so they are not depicted in this figure

to fish, algae, and daphnia and a low chronic risk to fish, mainly in the winter and spring. At the UAJA, trimethoprim posed a low to medium acute risk to fish nearly every month and a low acute risk to algae in the spring. At both treatment plants, dexamethasone posed a low acute risk to fish during or after February 2021. Despite the high effluent concentrations of sulfamethoxazole, naproxen, and trimethoprim at the Penn State WRF (and corresponding elevated RQs), the beneficial reuse for irrigation of agricultural and forested areas at the Living Filter has the potential to reduce the concentrations (and associated RQs) by the time the effluent infiltrates through the soil profile and enters the groundwater. For example, Kibuye et al. (2019) found that concentrations of naproxen and acetaminophen were several orders of magnitude lower in receiving groundwater than in Penn State WRF effluent that was spray irrigated onto the Living Filter. At the same site, Franklin et al. (2018) found that trimethoprim in effluent degraded quickly through the Living Filter and did not reach groundwater. However, the same study found that ofloxacin and sulfamethoxazole did not degrade as readily through the soil profile and that sulfamethoxazole was the most mobile and had the highest concentrations in groundwater compared with the other antibiotics. In the same way, the beneficial reuse of UAJA effluent to irrigate golf courses could reduce the RQs of compounds that posed a medium and/or high acute risk (trimethoprim and sulfamethoxazole) before they reached groundwater.

For the UAJA, ~9 MLD of treated effluent that is not beneficially reused is discharged into Spring Creek, and therefore the risk posed to the ecosystem in the receiving water body is likely mitigated to some extent due to dilution of the treated effluent by the streamflow in the creek. During the 1-yr study period, the UAJA effluent made up 1–17%

of total stream discharge in Spring Creek (Supplemental Table S11). During the drier months (fall and early winter), effluent made up 6–17% of stream discharge. Based on these dilution factors, the level of risk that the treated effluent poses on its own could be reduced once it is discharged into Spring Creek, with trimethoprim posing a medium acute risk to fish and sulfamethoxazole posing a low to medium acute risk to algae. Further, these dilution factors suggest that no compounds posed a significant acute risk to daphnia and that no compounds posed a chronic risk to fish, daphnia, or algae (Figure 6; Supplemental Tables S12 and S13).

Although dilution of the effluent in Spring Creek could theoretically reduce the risk pharmaceuticals pose to the aquatic ecosystem, this assumes that the effluent is uniformly mixed into Spring Creek, which is not necessarily true. Furthermore, this assessment does not account for physical or biogeochemical factors, such as temperature and pH, that can influence the ability of compounds to partition in or out of streambed sediment and be taken up by aquatic organisms (Kibuye et al., 2020; Li et al., 2010; McCarthy & Alvarez, 2014; Sabourin et al., 2009). Therefore, although dilution can help to reduce overall risk, there are other confounding factors that influence the fate and risk of pharmaceuticals in the aquatic environment.

Finally, although the RQs calculated in this paper considered the individual risks that each compound could pose on aquatic life, this calculation does not account for the potential risks that could come from the synergistic effects of these compounds in a mixture. For example, a mixture of several pharmaceuticals, including sulfamethoxazole and naproxen, was shown to have an additive acute and chronic effect on bioluminescent bacteria (Neale et al., 2017). Therefore, it is possible that the individual risks of these compounds could

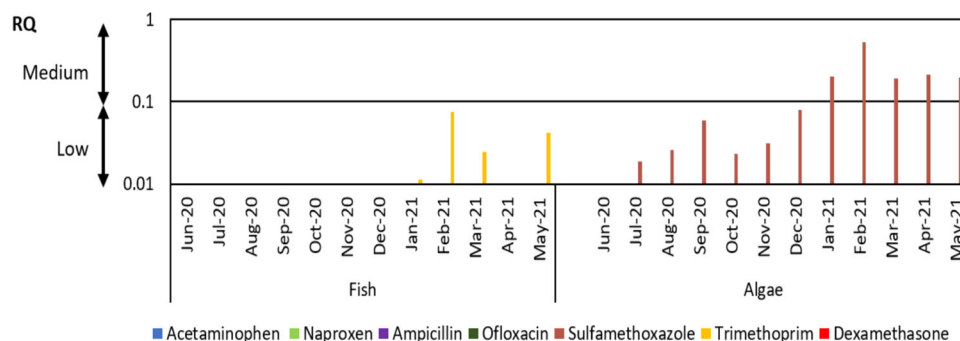


FIGURE 6 Risk quotients (RQs) characterizing acute toxicity for fish and algae based on diluted maximum effluent concentrations calculated each monthly (from June 2020 through May 2021) at the University Area Joint Authority. Risk quotients were also estimated for daphnia, but concentrations were below 0.01, so they are not depicted in this figure

underestimate the collective risk that these compounds pose as a mixture (Godoy et al., 2019; Kosma et al., 2014; Kumari & Kumar, 2022).

4 | CONCLUSION

To explore the potential effects of the COVID-19 pandemic on water quality, the presence of SARS-CoV-2, over-the-counter pain medications, antibiotics, and therapeutic agents was analyzed in the influent and effluent for two WWTPs in central Pennsylvania. Pharmaceutical concentrations did not correlate to SARS-CoV-2 concentrations in influent samples. Over-the-counter pain medications were detected most frequently and had the highest concentrations in influent samples but also exhibited the greatest removal efficiencies. Pain medications and antibiotic concentrations were typically highest in the winter and spring. At the Penn State WRF, antibiotic concentrations were further influenced by decisions regarding mode of learning.

The two WWTPs included in this study enabled a comparison between the removal efficiency of pharmaceuticals through a facility that produces Class B wastewater reuse (Penn State WRF) and a facility that produces Class A+ wastewater reuse (UAJA). Although both treatment plants consistently removed naproxen and acetaminophen with removal efficiencies typically >75%, they were unable to remove sulfamethoxazole. The UAJA consistently removed ampicillin but experienced mixed performance results in removing ofloxacin and trimethoprim. In contrast, the Penn State WRF was consistent in reducing trimethoprim but typically was unable to reduce ofloxacin from the influent. Finally, our data suggest that the Penn State WRF was often unable to remove remdesivir and dexamethasone from the influent, but these two compounds were removed at least to some extent in the UAJA facility. Overall, producing Class A+ wastewater appears to offer some benefits for improved removal of remde-

sivir, dexamethasone, ampicillin, and ofloxacin compared with producing Class B wastewater.

For the pharmaceuticals that persisted in the treated effluent, sulfamethoxazole posed the highest acute risk to aquatic life, especially when normal campus activities resumed in February 2021. Therefore, the ability of the Living Filter site to mitigate these risks highlights its important ecosystem services to protect Spring Creek. In UAJA influent, the detection of remdesivir co-occurred with increasing hospitalization of patients with COVID-19, whereas detection of dexamethasone co-occurred with increasing cases of patients with COVID-19 on ventilators. The ecological risks posed by beneficial reuse of UAJA effluent containing remdesivir are unclear due to a lack of toxicological data. Overall, this study highlights the opportunity that wastewater surveillance provides to understand the effects of human health on the quality of wastewater influent and the potential risks the quality of wastewater effluent could pose to ecological health.

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AUTHOR CONTRIBUTIONS

Kathryn R. Hayden: Conceptualization; Data curation; Formal analysis; Methodology; Visualization; Writing-original draft. Matthew Jones: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Validation. Kyle R. Elkin: Data curation; Methodology. Michael J. Shreve: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Validation; Writing-review & editing. William Irvin Clees II: Data curation; Formal analysis; Investigation. Shirley Clark: Conceptualization; Investigation; Project administration; Supervision. Michael L. Mashtare: Conceptualization; Supervision; Writing-review & editing. Tamie L. Veith: Conceptualization; Funding acquisition; Investigation; Resources; Supervision; Writing-review & editing. Herschel A. Elliott: Conceptualization; Funding acquisition; Methodology; Supervision. John E. Watson: Conceptualization; Funding acquisition; Resources; Supervision. Justin Silverman: Conceptualization; Investigation; Methodology; Project administration; Resources; Software. Thomas L. Richard: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision. Andrew F. Read: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation. Heather E. Preisendanz: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-original draft.

CONFLICT OF INTEREST

Authors T. L. Veith and H. E. Preisendanz reside in the same household. The authors declare no other conflict of interest.

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REFERENCES

- Balboa, S., Mauricio-Iglesias, M., Rodriguez, S., Martínez-Lamas, L., Vasallo, F. J., Regueiro, B., & Lema, J. M. (2021). The fate of SARS-COV-2 in WWTPS points out the sludge line as a suitable spot for detection of COVID-19. *Science of the Total Environment*, 772. <https://doi.org/10.1016/J.SCITOTENV.2021.145268>
- Balcan, D., Colizza, V., Singer, A. C., Chouaid, C., Hu, H., Gonçalves, B., Bajardi, P., Poletto, C., Ramasco, J. J., Perra, N., Tizzoni, M., Paolotti, D., Van den Broeck, W., Valleron, A., & Vespignani, A. (2009). Modeling the critical care demand and antibiotics resources needed during the fall 2009 wave of influenza A(H1N1) pandemic. *PLOS CURRENTS*, 1, RRN1133. <https://doi.org/10.1371/CURRENTS.RRN1133>
- Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., Hohmann, E., Chu, H. Y., Luetkemeyer, A., Kline, S., Lopez de Castilla, D., Finberg, R. W., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T. F., Paredes, R., Sweeney, D. A., Short, W. R., ... Lane, H. C. (2020). Remdesivir for the treatment of COVID-19 – Final report. *New England Journal of Medicine*, 383(19), 1813–1826. <https://doi.org/10.1056/NEJMoa2007764>
- Carline, R. F., Dunlap, R. L., Detar, J. E., & Hollender, B. A. (2011). *The fishery of Spring Creek - A watershed under siege*. Pennsylvania Fish and Boat Commission. https://www.fishandboat.com/Fish/Fisheries/TroutPlan/Documents/springck_fishery.pdf
- Center for Disease Control and Prevention (CDC). (2020). *Water and COVID-19 FAQs: Information about drinking water, recreational water and wastewater*. <https://www.cdc.gov/coronavirus/2019-ncov/php/water.html>
- Centre Region Planning Agency. (2021). *Wastewater treatment and beneficial reuse in the Centre region*. <https://storymaps.arcgis.com/stories/e54e024ed5684198ad5eb6739bb75ae1>
- Chedid, M., Waked, R., Haddad, E., Chetata, N., Saliba, G., & Choucair, J. (2021). Antibiotics in treatment of COVID-19 complications: A review of frequency, indications, and efficacy. *Journal of Infection and Public Health*, 14(5), 570–576. <https://doi.org/10.1016/J.JIPH.2021.02.001>
- Cronin, J., Kennedy, U., McCoy, S., Nic an Fhailí, S., Cirspino-O'Connell, G., Hayden, J., Wakai, A., Walsh, S., & O'Sullivan, R. (2012). Single dose oral dexamethasone versus multi-dose prednisolone in the treatment of acute exacerbations of asthma in children who attend the emergency department: Study protocol for a randomized controlled trial. *Trials*, 13, 141. <https://doi.org/10.1186/1745-6215-13-141>
- De la Cruz, N., Giménez, J., Esplugas, S., Grandjean, D., De Alencastro, L. F., & Pulgarín, C. (2012). Degradation of 32 emergent contaminants by UV and neutral photo-fenton in domestic wastewater effluent previously treated by activated sludge. *Water Research*, 46(6), 1947–1957. <https://doi.org/10.1016/j.watres.2012.01.014>
- Ebele, A. J., Abdallah, M. A., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3(1), 1–16. <https://doi.org/10.1016/j.emcon.2016.12.004>
- European Commission. (2003). *Technical guidance document on risk assessment part II*. https://echa.europa.eu/documents/10162/987906/tgdpart2_2ed_en.pdf/138b7b71-a069-428e-9036-62f4300b752f
- Escher, B. I., Baumgartner, R., Koller, M., Treyer, K., Lienert, J., & McArdell, C. S. (2011). Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater. *Water Research*, 45(1), 75–92. <https://doi.org/10.1016/j.watres.2010.08.019>

- Ferguson, B. K. (1983). Environmental planning for wastewater land application: Lessons from Penn State's "Living Filter." *Landscape Planning*, 10(3), 205–218. [https://doi.org/10.1016/0304-3924\(83\)90048-5](https://doi.org/10.1016/0304-3924(83)90048-5)
- Franklin, A. M., Williams, C. F., & Watson, J. E. (2018). Assessment of soil to mitigate antibiotics in the environment due to release of wastewater treatment plant effluent. *Journal of Environmental Quality*, 47(6), 1347–1355. <https://doi.org/10.2134/jeq2018.02.0076>
- Foladori, P., Cutrupi, F., Cadonna, M., & Manara, S. (2021). Coronaviruses and SARS-CoV-2 in sewerage and their removal: Step by step in wastewater treatment plants. *Environmental Research*, 207, 112204. <https://doi.org/10.1016/j.envres.2021.112204>
- Gao, J., Tian, Z., & Yang, X. (2020). Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *BioScience Trends*, 14(1), 72–73. <https://doi.org/10.5582/bst.2020/01047>
- Ginebreda, A., Muñoz, I., de Alda, M. L., Brix, R., López-Doval, J., & Barceló, D. (2010). Environmental risk assessment of pharmaceuticals in rivers: Relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). *Environment International*, 36(2), 153–162. <https://doi.org/10.1016/j.envint.2009.10.003>
- Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., & Giger, W. (2007). Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, 372(2–3), 361–371. <https://doi.org/10.1016/j.scitotenv.2006.07.039>
- Godoy, A. A., Oliveira, Á. C. d., Silva, J. G. M., de Jazevedo, C. C., Domingues, I., Nogueira, A. J. A., & Kummrow, F. (2019). Single and mixture toxicity of four pharmaceuticals of environmental concern to aquatic organisms, including a behavioral assessment. *Chemosphere*, 235, 373–382. <https://doi.org/10.1016/j.chemosphere.2019.06.200>
- Hernando, M. D., Mezcua, M., Fernández-Alba, A. R., & Barceló, D. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, 69(2), 334–342. <https://doi.org/10.1016/j.talanta.2005.09.037>
- Horby, P., Lim, W. S., Emberson, J., Mafham, M., Bell, J. L., Linsell, L., Staplin, N., Brighling, C., Ustianowski, A., Elmahi, E., Prudon, B., Green, C., Felton, T., Chadwick, D., Rege, K., Fegan, C., Chappell, L. C., Faust, S. N., Jaki, T., ... Landray, M. J. (2021). Effect of dexamethasone in hospitalized patients with COVID-19. *New England Journal of Medicine*, 384, 693–704. <https://doi.org/10.1056/NEJMoa2021436>
- Kibuye, F. A., Gall, H. E., Veith, T. L., Elkin, K. R., Elliott, H. A., Harper, J. P., & Watson, J. E. (2020). Influence of hydrologic and anthropogenic drivers on emerging organic contaminants in drinking water sources in the Susquehanna River Basin. *Chemosphere*, 245, 125583. <https://doi.org/10.1016/j.chemosphere.2019.125583>
- Kibuye, F., Gall, H. E., Elkin, K. R., Ayers, B., Veith, T. L., Miller, M., Jacob, S., Hayden, K. R., Watson, J. E., & Elliott, H. A. (2019). Fate of pharmaceuticals in a spray-irrigation system: From wastewater to groundwater. *Science of the Total Environment*, 654, 197–208. <https://doi.org/10.1016/j.scitotenv.2018.10.442>
- Kosma, C. I., Lambropoulou, D. A., & Albanis, T. A. (2014). Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment. *Science of the Total Environment*, 466–467, 421–438. <https://doi.org/10.1016/j.scitotenv.2013.07.044>
- Kumari, M., & Kumar, A. (2022). Environmental and human health risk assessment of mixture of Covid-19 treating pharmaceutical drugs in environmental waters. *Science of the Total Environment*, 812, 152485. <https://doi.org/10.1016/j.scitotenv.2021.152485>
- Lalone, C. A., Villeneuve, D. L., Olmstead, A. W., Medlock, E. K., Kahl, M. D., Jensen, K. M., Durhan, E. J., Makynen, E. A., Blanksma, C. A., Cavallin, J. E., Thomas, L. M., Seidl, S. M., Skolness, S. Y., Wehmas, L. C., Johnson, R. D., & Ankley, G. T. (2012). Effects of a glucocorticoid receptor agonist, dexamethasone, on fathead minnow reproduction, growth, and development. *Environmental Toxicology and Chemistry*, 31(3), 611–622. <https://doi.org/10.1002/etc.1729>
- Li, H., Helm, P. A., & Metcalfe, C. D. (2010). Sampling in the Great Lakes for pharmaceuticals, personal care products, and endocrine-disrupting substances using the passive polar organic chemical integrative sampler. *Environmental Toxicology and Chemistry*, 29(4), 751–762. <https://doi.org/10.1002/ETC.104>
- Lorenzo, M., & Picó, Y. (2019). Wastewater-based epidemiology: Current status and future prospects. *Current Opinion in Environmental Science & Health*, 9, 77–84. <https://doi.org/10.1016/j.coesh.2019.05.007>
- Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, 473, 619–641. <https://doi.org/10.1016/j.scitotenv.2013.12.065>
- Lin, A. Y. C., Yu, T. H., & Lateef, S. K. (2009). Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. *Journal of Hazardous Materials*, 167(1–3), 1163–1169. <https://doi.org/10.1016/j.jhazmat.2009.01.108>
- Mayo Clinic. (2022). *Dexamethasone (oral route) description and brand names*. <https://www.mayoclinic.org/drugs-supplements/dexamethasone-oral-route/description/drg-20075207>
- McCarthy, K. A., & Alvarez, D. A. (2014). *Time-integrated passive sampling as a complement to conventional point-in-time sampling for investigating drinking-water quality, McKenzie River Basin, Oregon, 2007 and 2010–11* (Scientific Investigations Report 2013–5215). USGS. <https://doi.org/10.3133/sir20135215>
- Mount Nittany Health. (2022). *Mount Nittany Medical Center summary*. <https://www.mountnittany.org/mount-nittany-medical-center#summary>
- Musee, N., Prica Kebaabetswe, L., Tichapondwa, S., Tubatsi, G., Mahaye, N., Leareng, S. K., & Nomngongo, P. N. (2021). Occurrence, fate, effects, and risks of dexamethasone: Ecological implications post-COVID-19. *International Journal of Environmental Research and Public Health*, 18(21), 11291. <https://doi.org/10.3390/ijerph182111291>
- National Institutes of Health. (2021). *COVID-19 treatment guidelines | Therapeutic management of hospitalized adults with COVID-19*. <https://www.covid19treatmentguidelines.nih.gov/management/clinical-management/hospitalized-adults-therapeutic-management/>
- Neale, P. A., Leusch, F. D. L., & Escher, B. I. (2017). Applying mixture toxicity modelling to predict bacterial bioluminescence inhibition by non-specifically acting pharmaceuticals and specifically acting antibiotics. *Chemosphere*, 173, 387–394. <https://doi.org/10.1016/j.chemosphere.2017.01.018>
- Nejuma, K. K., Sreejith, M. V., Dineep, D., Aravind, U. K., & Aravindakumar, C. T. (2021). Identification and ecological hazard analysis of contaminants of emerging concerns (CECs) in water bodies located in a coastal metropolitan environment. *Water, Air, & Soil*

- Pollution*, 232(10), 434. <https://doi.org/10.1007/s11270-021-05374-y>
- Noguera-Oviedo, K., & Aga, D. S. (2016). Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials*, 316, 242–251. <https://doi.org/10.1016/j.jhazmat.2016.04.058>
- Onesios, K. M., Yu, J. T., & Bouwer, E. J. (2009). Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: A review. *Biodegradation*, 20(4), 441–466. <https://doi.org/10.1007/S10532-008-9237-8/TABLES/3>
- Pennsylvania Department of Health. (2021). *COVID-19 data for Pennsylvania*. <https://www.health.pa.gov/topics/disease/coronavirus/Pages/Cases.aspx>
- Sabourin, L., Beck, A., Duenk, P. W., Kleywegt, S., Lapen, D. R., Li, H., Metcalfe, C. D., Payne, M., & Topp, E. (2009). Runoff of pharmaceuticals and personal care products following application of dewatered municipal biosolids to an agricultural field. *Science of the Total Environment*, 407(16), 4596–4604. <https://doi.org/10.1016/j.scitotenv.2009.04.027>
- Sanderson, H., Johnson, D. J., Wilson, C. J., Brain, R. A., & Solomon, K. R. (2003). Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids, and algae by ECOSAR screening. *Toxicology Letters*, 14(3), 383–395. [https://doi.org/10.1016/S0378-4274\(03\)00257-1](https://doi.org/10.1016/S0378-4274(03)00257-1)
- Shafraan, N., Shafraan, I., Ben-Zvi, H., Sofer, S., Sheena, L., Krause, I., Shlomai, A., Goldberg, E., & Sklan, E. H. (2021). Secondary bacterial infection in COVID-19 patients is a stronger predictor for death compared to influenza patients. *Science Reports*, 11, 12703. <https://doi.org/10.1038/s41598-021-92220-0>
- Shah, F. H., Lim, K. H., & Kim, S. J. (2021). Do fever-relieving medicines have anti-COVID activity: An in silico insight. *Future Virology*, 16(4), 293–300. <https://doi.org/10.2217/fvl-2020-0398>
- Shreve, M. J., & Brennan, R. A. (2019). Trace organic contaminant removal in six full-scale integrated fixed-film activated sludge (IFAS) systems treating municipal wastewater. *Water Research*, 151, 318–331. <https://doi.org/10.1016/J.WATRES.2018.12.042>
- Singer, A. C., Colizza, V., Schmitt, H., Andrews, J., Balcan, D., Huang, W. E., Keller, V. D. J., Vespignani, A., & Williams, R. J. (2011). Assessing the ecotoxicologic hazards of a pandemic influenza medical response. *Environmental Health Perspectives*, 119(8), 1084–1090. <https://doi.org/10.1289/EHP.1002757>
- Singer, A. C., Järhult, J. D., Grabic, R., Khan, G. A., Lindberg, R. H., Fedorova, G., Fick, J., Bowes, M. J., Olsen, B., & Söderström, H. (2014). Intra- and inter-pandemic variations of antiviral, antibiotics and decongestants in wastewater treatment plants and receiving rivers. *PLOS ONE*, 9(9), e108621. <https://doi.org/10.1371/journal.pone.0108621>
- University Area Joint Authority (UAJA). (2018a). *Plant history and statistics*. <https://uaja.com/treatment/history-statistics/>
- University Area Joint Authority (UAJA). (2018b). *Plant tour*. <https://uaja.com/treatment/plant-tour/>
- USEPA. (2007). *Method 1694: Pharmaceuticals and personal care products in water, soil, sediment and biosolids by HPLC/MS/MS*. USEPA Office of Water and Office of Science and Technology. EPA-821-R-08-002. https://www.epa.gov/sites/production/files/2015-10/documents/method_1694_2007.pdf
- USEPA. (2017). *Ecological Structure-Activity Relationship Model (ECOSAR) v. 2.0*. https://www.epa.gov/sites/default/files/2017-11/documents/operationmanual_v2.0.pdf
- USGS. (2022). *USGS water data for the nation*. <http://waterdata.usgs.gov/nwis/>
- Vestel, J., Caldwell, D. J., Constantine, L., D'Aco, V. J., Davidson, T., Dolan, D. G., Millard, S. P., Murray-Smith, R., Parke, N. J., Ryan, J. J., Straub, J. O., & Wilson, P. (2015). Use of acute and chronic ecotoxicity data in environmental risk assessment of pharmaceuticals. *Environmental Toxicology and Chemistry*, 35(5), 1201–1212. <https://doi.org/10.1002/ETC.3260>
- Vieno, N., Tuhkanen, T., & Kronberg, L. (2007). Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Research*, 41(5), 1001–1012. <https://doi.org/10.1016/J.WATRES.2006.12.017>
- Wang, D., Hu, B., Zhu, F. Z., Liu, X., Zhang, J., Wang, V., Xiang, H., Chen, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., & Peng, Z. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *Journal of the American Medical Association*, 323(11), 1061–1069. <https://doi.org/10.1001/jama.2020.1585>
- Wang, W., Xu, L., Gao, R., Lu, R., Han, K., Wu, G., & Tan, W. (2020). Detection of SARS-CoV-2 in different types of clinical specimens. *Journal of the American Medical Association*, 323(18), 1843–1844. <https://doi.org/10.1001/jama.2020.3786>
- Weidhaas, J., Aanderud, Z. T., Roper, D. K., VanDerslice, J., Gaddis, E. B., Ostermiller, J., Hoffman, K., Jamal, R., Heck, P., Zhang, Y., Torgersen, K., Vander Laan, J., & LaCross, N. (2021). Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. *Science of the Total Environment*, 775. <https://doi.org/10.1016/J.SCITOTENV.2021.145790>
- Wilkinson, J., Hooda, P. S., Barker, J., Barton, S., & Swinden, J. (2017). Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field. *Environmental Pollution*, 231, 954–970. <https://doi.org/10.1016/j.envpol.2017.08.032>
- Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., Kuang, L., Fang, X., Mishra, N., Lu, J., Shan, H., Jiang, G., & Huang, X. (2020). Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *The Lancet. Gastroenterology & Hepatology*, 5(5), 434–435. [https://doi.org/10.1016/S2468-1253\(20\)30083-2](https://doi.org/10.1016/S2468-1253(20)30083-2)
- Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., & Shan, H. (2020). Evidence of gastrointestinal infection of SARS-CoV-2. *Gastroenterology*, 158(6), 1831–1833. <https://doi.org/10.1053/j.gastro.2020.02.055>
- Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., Guo, Q., Sun, X., Zhao, D., Shen, J., Zhang, H., Liu, H., Xia, H., Tang, J., Zhang, K., & Gong, S. (2020). *Nature Medicine*, 26, 502–505. <https://doi.org/10.1038/s41591-020-0817-4>
- Yousefifard, M., Sali, A., Zarghi, A., Neishaboori, A. M., Hosseini, M., & Safari, S. (2020). Non-steroidal anti-inflammatory drugs in management of COVID-19: A systematic review on current evidence. *International Journal of Clinical Practice*, 74(9), e13557. <https://doi.org/10.1111/ijcp.13557>
- Zhang, H., Du, M., Jiang, H., Zhang, D., Lin, L., Ye, H., & Zhang, X. (2015). Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China. *Environmental Science: Processes & Impacts*, 17(1), 225–234. <https://doi.org/10.1039/c4em00457d>
- Zhang, W., Du, R.-H., Li, B., Zheng, X.-S., Yang, X.-L., Hu, B., Wang, Y.-Y., Xiao, G.-F., Yan, B., Shi, Z.-L., & Zhou, P. (2020). Molecular and serological investigation of 2019-nCoV infected patients:

Implications of multiple shedding routes. *Emerging Microbes & Infections*, 9(1), 386–389. <https://doi.org/10.1080/22221751.2020.1729071>

Zhang, X., Zhao, H., Du, J., Qu, Y., Shen, C., Tan, F., Chen, J., & Quan, X. (2017). Occurrence, removal, and risk assessment of antibiotics in 12 wastewater treatment plants from Dalian, China. *Environmental Science and Pollution Research*, 24(19), 16478–16487. <https://doi.org/10.1007/s11356-017-9296-7>

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