Research Article

Monitoring occurrence and removal of selected pharmaceuticals in two different wastewater treatment plants

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Abstract
The occurrence of pharmaceuticals in the environment is a concern due to its potential adverse effect on human health and ecology. In this research study, the occurrence of three selected pharmaceuticals—erythromycin (ERY), sulfamethoxazole (SMZ), trimethoprim (TMP), and an antimicrobial/antifungal agent triclosan (TRL) was monitored at two Southern California wastewater treatment plants (WWTPs). Between April and October of 2017, 77 composite samples were analyzed. Results from the study revealed the presence of all four pharmaceuticals in the influent at both WWTPs. Of the four pharmaceuticals, SMZ had the highest concentration (WWTP1, 1860 ng L^{-1}, and WWTP2, 2146 ng L^{-1}). In WWTP1, the concentrations of ERY, TRL, and TMP ranged from 33 to 278 ng L^{-1}, 146 to 410 ng L^{-1}, and 410 to 1021 ng L^{-1}, respectively, while in WWTP2, their concentrations ranged from 16.7 to 209 ng L^{-1}, 0 to 160 ng L^{-1}, and 407 to 672 ng L^{-1}, respectively. Even though both plants employed secondary biological treatment protocols, their removal efficiencies are remarkably different. WWTP1 is relatively more effective in the removal of ERY, TRL, and TMP with a removal efficiency of 46%, 63%, and 61%, respectively, while at WWTP2 only 4% TMP was removed, and no removal of ERY and TRL was observed. Both plants were unable to remove SMZ. Pharmaceuticals are bioactive compounds, and as such, even at low concentration levels, they may adversely affect the environment and will require additional treatment to ensure that treated wastewater meets the specific water quality criteria for reuse.

Keywords Pharmaceuticals · Sulfamethoxazole · Trimethoprim · Erythromycin · Triclosan · Wastewater

1 Introduction

Antibiotics are considered as one of the profound scientific achievements of the twentieth century. Alexander Fleming discovered the first antibiotic in 1928; since then, many antibiotics (natural and synthetic) were developed to treat bacterial infections, transforming both human and veterinary medicine [1]. Antibiotics are used for both therapeutic and sub-therapeutic usages in human and animal health protection. The usage and consumption of antibiotics have consistently increased due to humans living longer than ever, as well as the availability of cheaper generic drugs [2]. Sulfamethoxazole (SMZ) and trimethoprim (TMP) are two commonly used antibiotics in human therapy for the treatment of urinary and respiratory tract infections. The widespread incidences of these infections and the overuse of SMZ and TMP have led to treatment complications and linked to increasing cases of bacterial resistance. Both these drugs are highly effective against a variety of bacterial infections when they are co-administered, another reason which may explain the overuse of these two drugs [3, 4]. Erythromycin (ERY) is an antibiotic of macrolide group used in human medicine for the treatment of various infections, including respiratory tract infections, chlamydia, and syphilis. It is also used in poultry and livestock production to prevent disease and

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enhance growth [5]. Due to their low removal rate during wastewater treatment, antibiotics such as SMZ, TMP, and ERY were frequently detected in aquatic bodies [6]. In recent decades, although the consumption of SMZ has been reduced, it is the most popular germicidal drug used in animal food production. Triclosan (TRL) was introduced approximately 47 years ago as an active ingredient in surgical sanitation procedure and has since been found in many household cleaning and pharmaceuticals and personal care products [7]. Widespread use of TRL led to its frequent detection in freshwater streams [8].

It is widely accepted that human/animal body does not completely absorb/metabolize the administered antibiotics, and after a short residence time, the unmetabolized portion is excreted (through urine and feces) into the wastewater treatment plants (WWTPs). It is common knowledge that WWTPs are the major sinks of pharmaceuticals in the environment. The principal sources of antibiotics in WWTPs include therapeutic usages in human and animal therapy (hospitals and veterinary clinics), unused discarded medicines from households, and pharmaceutical manufacturing facilities [9]. The occurrence of antibiotics in sewage treatment plants has been examined in many countries, including Austria, Canada, China, England, Germany, Switzerland, and the USA. The widespread occurrence of pharmaceuticals in water bodies has been reported throughout the USA. A nationwide reconnaissance of the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants was conducted from 1999 to 2000 [8]. This study was the first significant nationwide reconnaissance covering 30 states with samples from 139 streams located downstream from intense urbanization and livestock operations. The authors reported that 80% of the sampled streams had quantifiable detection of organic wastewater contaminants including triclosan, bisphenol A, erythromycin, lincomycin, sulfamethoxazole, diazinon, and estriol [8]. In fact, of the 47 samples analyzed, 23 percent of the samples have sulfamethoxazole concentration up to 1110 ng L^{-1}, indicating the frequency and magnitude of detection of these compounds. Similarly, the detection of SMZ in raw sewage in China was reported up to 8 µg L^{-1} [10]. Various studies have reported the occurrence of pharmaceuticals and personal care products, hormones, flame retardants, biocides, and their respective metabolites in the WWTP. For example, a study reported the presence of a variety of drugs including antiphlogistics, lipid regulators, and psychiatric drugs, in the treated effluent wastewater from German municipal sewage treatment plant [11].

The wastewater from animal agriculture facilities containing residual SMZ often directly discharged into the aquatic environment [12]. In human therapeutic practices, SMZ is often administered in combination with TMP and commonly analyzed together in the laboratories. TMP shows high persistence with little removal in WWTPs and is thus frequently detected up to 0.2 ng L^{-1} to 1 µg L^{-1} in wastewater influent and effluent. Also, ERY is reportedly found in various environmental matrices with concentrations ranging from 0.15 ng L^{-1} to less than 1 µg L^{-1} in sewage influent and effluent [13]. Recent studies also reported that wastewater effluent from drug manufacturing facilities could be a potential source of high concentration of active pharmaceutical ingredients. Studies conducted by Fick et al. [14] reported that processed wastewater effluent from homogenized wastewater from 90 bulk drug manufactures showed the presence of 12 pharmaceutical compounds (cetirizine, ciprofloxacin, citalopram, enalapril, enoxacin, enrofloxacin, lomefloxacin, metoprolol, norfloxacin, ofloxacin, terbinafine, and trimethoprim) with concentrations as high as 14 mg L^{-1} of ciprofloxacin, 2.1 mg L^{-1} of cetirizine, 0.43 mg L^{-1} of citalopram, and 0.21 mg L^{-1} of enrofloxacin.

In general, pharmaceuticals enter the aquatic environment through a variety of sources such as the discharge of treated effluent from WWTPs to surface water or groundwater, landfill leachate, leaking sewers, and manure storage tanks or lagoons, runoff, leaching from manure-fertilized farmland, and manure disposal in agricultural areas [12, 15, 16]. Treated wastewater effluent containing residual pharmaceuticals and their metabolites when discharged to the receiving water not only pollutes the receiving streams but also adversely affects the aquatic life. Since the primary pathway of these chemicals to the environment is through the WWTPs, how well the WWTPs can remove these compounds from the wastewater determines their release and occurrence in the environment [17]. However, not all WWTPs utilize the same treatment processes or technologies or have the same composition of wastewater entering the plants. The effectiveness of the WWTPs in eliminating these chemicals may depend on the form of treatment(s) being used and the makeup of the wastewater.

Of the pharmaceuticals that were routinely detected in the environment, antibiotics have gained significant attention in recent years due to their magnitude of usages, and proliferation of antibiotic resistance microorganisms [18]. Once the microbes become resistant to the antibiotic, in all likelihood, our ability to fight off opportunistic infection may be compromised, leading to the advanced/alternative treatment protocol. According to the Center for Disease Control, the fear of superbugs is aptly exemplified by the emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) that is known to be resistant to most available antibiotics in the USA. The growth of antibiotics resistance phenomenon is one of the most critical challenges to the healthcare sector in the twenty-first century [19].

Use/overuse/misuse/abuse of antibiotics has led to the occurrence of these bioactive compounds in various environmental matrices. Consequently, during the past two decades, the public, regulators, and scientists have raised concerns over the potential or unknown impacts due to the occurrence of pharmaceuticals and personal care products (PPCPs) in the environment. In the present study, we investigated the occurrence of three antibiotics: erythromycin (ERY), sulfamethoxazole (SMZ), and trimethoprim (TMP), and antibacterial agent triclosan (TRL) in the influent and treated effluent wastewater. The antibiotics were chosen as a representative group among the most frequently prescribed drugs in the USA and also due to their frequent detection in the aquatic environment [8, 20]. To develop a strategy for efficient removal of pharmaceutical compounds, before their release to the environment, it is critical that accurate quantification of the mass of pharmaceutical entering the WWTP be conducted. Monitoring information is also critical to evaluate the potential reuse of the wastewater and the potential for growth of antibiotic-resistant microorganisms. The objectives of this study are to investigate the occurrence of antibiotics and antibacterial agents in raw wastewater, and to critically evaluate its potential for direct/indirect reuse and subsequent ecological risk.

2 Materials and methods

2.1 Chemicals and standards

The certified analytical standards of the selected antibiotics and antibacterial agents with higher than 98% purity were purchased from Restek (Bellefonte, PA, USA). Figure 1 shows the molecular structures and chemical properties of the target analytes. Individual standard stock solutions (at a concentration of 200–1000 µg mL^{-1}) were purchased in pure acetonitrile and stored at − 20 °C. Through serial dilution using methanol and acetonitrile working standard solutions of varying concentrations were prepared. Solvents such as acetonitrile and methanol were purchased from Honeywell Chemicals (Morris Plains, NJ, USA). LC–MS water and HPLC-grade isopropyl alcohol were obtained from Fisher Chemical (Fair Lawn, NJ, USA). Ammonium acetate and ammonium fluoride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid and

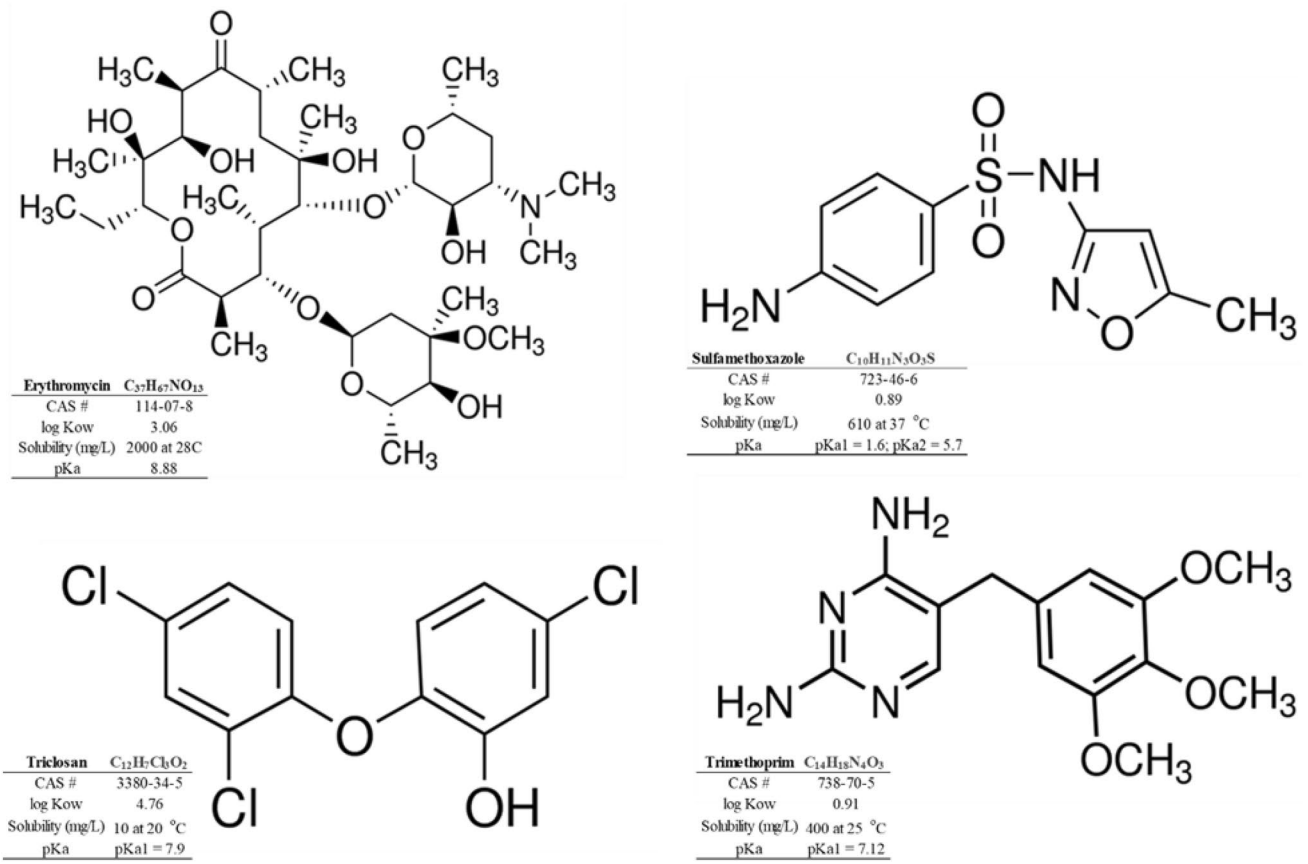

Fig. 1 Names, molecular structures, and chemical properties of the target analytes

sodium azide were obtained from Moltox Molecular Toxicology, Inc. (Boone, NC). Formic acid was purchased from Fisher Chemical (Fair Lawn, NJ, USA). Oasis HLB 6 cc cartridges (with 500 mg sorbet per cartridge) were obtained from Waters Ltd. (Milford, MA, USA). Liquid chromatography analytical column, Zorbax Eclipse Plus C18 (2.1 mm × 100 mm, 3.5 µm), was obtained from Agilent (Santa Clara, CA, USA).

2.2 Site selection and sampling

Two large wastewater treatment plants (WWTP1 and WWTP2) in Southern California operated by the Orange County Sanitation District (OCSD) were selected for the study. The two plants provide wastewater collection, treatment, recycling, and disposal from nearly 2.6 million people in central and northwest Orange County. The plants receive wastewater from residential, commercial, and industrial users. In 2017–2018, an estimated average of 114 million gallons per day (MGD) was treated at WWTP1 and 74 MGD at WWTP2 [21]. WWTP1 is located in Fountain Valley, and WWTP2 is located in Huntington Beach. The treatment processes at both plants consist of chemically enhanced primary treatment (CEPT), activated sludge (AS) (WWTP1, located in Fountain Valley), and trickling filter (TF) (WWTP2, located at Huntington Beach) treatments. CEPT involves the addition of ferric chloride followed by an anionic polymer to promote sedimentation at the primary clarifiers.

From April 2017 to October 2017 (seven sampling events and four quality control samples per event), 77 samples were collected from both plants. All samples were collected in 10-L glass bottles using automatic SIGMA samplers (Loveland, CO, USA). WWTP1 influent and WWTP2 influent were collected based on a flow-paced 24-h composite (a mixture of sample aliquots taken in a constant ratio with the flow rate of the stream being sampled). One-liter sample was transferred to pre-cleaned salinized amber glass bottles and preserved with 50 mg of ascorbic acid to quench any residual oxidant activities (e.g., chlorine). One gram of sodium azide was also added to prevent microbial degradation. Once preserved, samples were kept at 4 °C until extraction.

2.3 Sample extraction

The analytes of interest were extracted from 500 mL of samples; each was spiked with the isotope-labeled internal standard. Five hundred milliliters of samples and calibration standards was extracted with Oasis HLB cartridges using solid-phase extraction instrument (Thermo Scientific Dionex AutoTrace 280). SPE cartridges were conditioned with 10 mL of methanol followed by 6 mL of LC–MS-grade water. A volume of 100–500 mL of sample was pre-concentrated through the cartridge at 5 mL min^{-1}. Afterward, the cartridges were dried with nitrogen gas for 60 min, and finally, analytes were eluted with 6 mL of methanol. The sample extracts were evaporated to dryness and reconstituted with 1 mL of LC–MS-grade water. All extracts were then filtered into 2-mL autosampler vials with 0.2-µm disposable syringe filters and subsequently analyzed by an Agilent 1260 HPLC system coupled with an Agilent 6495A iFunnel triple quadrupole mass spectrometer (Santa Clara, CA).

2.4 Analytical methods

The chromatographic separation of the analytes was carried out using an Agilent Series 1260 high-performance liquid chromatography (HPLC) system equipped with a reversed-phase C18 Agilent Zorbax Eclipse Plus analytical column with a dimension of 100 mm × 2.1 mm and particle size of 3.5 µm (Agilent Technologies, Santa Clara, CA, USA). The column temperature was maintained at 35 °C. The HPLC consisted of vacuum degasser, autosampler, and a binary pump and was connected to an Agilent 6495A triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), which equipped with electrospray Jet Stream and iFunnel technology. Gradient separation with the combination of the mobile phase as recommended by the Environmental Protection Agency (EPA) Method 1694 was used [22]. In this research, we observed that using aqueous mobile phase with 1 mM ammonium fluoride modifier (instead of 0.3% formic acid and 0.1% ammonium formate as recommended in EPA Method 1694) improved the linearity range from 0.5–250 ng L^{-1} to 0.5–1000 ng L^{-1}. The fragmentor voltage was fixed at 380. Cell accelerator voltage and collision energies were optimized for each compound and ranged from 2 to 6 V and from 0 to 48 eV, respectively. The data acquisition was recorded with a time stamp and processed with MassHunter chromatography software (Agilent Technologies). Table 1 shows the method detection limit (MDL), minimum

Table 1 Method detection limit, method reporting limit, calibration range, and coefficient of determination for TMP, ERY, SMZ, and TRL

Compound	MDL (ng L^{-1})	MRL (ng L^{-1})	Calibration range (ng L^{-1})	(R^2)
Trimethoprim	1.3	3.8	0.1–500	0.996
Erythromycin	1.5	4.5	0.2–1000	0.997
Sulfamethoxazole	0.9	2.7	0.2–1000	0.999
Triclosan	10	10	0.2–400	0.992

reporting limit (MRL), calibration range, and coefficient of determination for the compounds that were studied.

3 Results and discussion

3.1 Monitoring of antibiotics in WWTP1 and WWTP2

From April to October 2017, concentrations of antibiotics ERY, SMZ, and TMP, and an antimicrobial agent TRL were monitored in the influent of WWTP1 and WWTP2. The results of the monitoring activities indicated that the detection frequencies of all three antibiotics (ERY, SMZ, and TMP) were at 100% in all tested samples. TRL was detected at 100% frequency in both influents, primary effluent, and trickling filter effluent; however, only 38.4% frequency was observed in the activated sludge effluent. Figure 2 shows the concentrations of ERY, SMZ, TMP, and TRL in the influent of both plants. Of the compounds monitored, SMZ had the highest concentrations in both plants (1860 ng L^{-1} in WWTP1 and 2146 ng L^{-1} in WWTP2) with a mean concentration of 1372 ng L^{-1} in WWTP1 and 1354.29 ng L^{-1} in WWTP2. Similar observations describing the higher amount of SMZ (1900 ng L^{-1}) in the influent of WWTP are also reported by Gobel et al. [13]. The authors attributed the frequent abundance of SMZ to high consumption of SMZ in human medicine. On the other hand, ERY and TRL concentrations were lower compared to the other compounds in both the plants. For example, the average concentration of ERY was found to be 85.2 ng L^{-1} in WWTP1 and 60.57 ng L^{-1} in WWTP2, and the average concentration of TRL was observed to be 313.20 ng L^{-1} in WWTP1 and 59.57 ng L^{-1} in WWTP2 (Fig. 2). Although influent concentrations at both the plants exhibited the same trends and variations for the compounds analyzed, in general, WWTP1 consistently showed higher mean concentrations of all the compounds monitored. Both the plants received wastewater from similar sources, including industrial, commercial, and residential areas.

Relative differences in observed concentrations of these compounds in WWTP1 and WWTP2 could be due to various factors including sources and magnitude of discharge, seasonal variation, incidence/prevalence, duration and frequency of diseases, sample volume analyzed, and the treatment plant efficiency. In general, the variation in the concentration of antibiotics in the influent of WWTP is on the expected line, because seasonal variations affect the vulnerability of human beings to the sickness that is the incidence of sickness followed by the prescription of antibiotics and subsequent release through the fecal material in the sanitary sewer system. During the monitoring period, the concentration of ERY in the influent of both the plants consistently showed an increasing trend from April to October (Fig. 2). This increasing concentration of ERY could be attributed to the increased incidence of respiratory tract infections, which are most common in the winter season followed by treatment protocol involving ERY [13, 23, 24].

Similarly, antibiotics belonging to the same class could be prescribed individually or in combination to combat the incidence of opportunistic/seasonal infections.

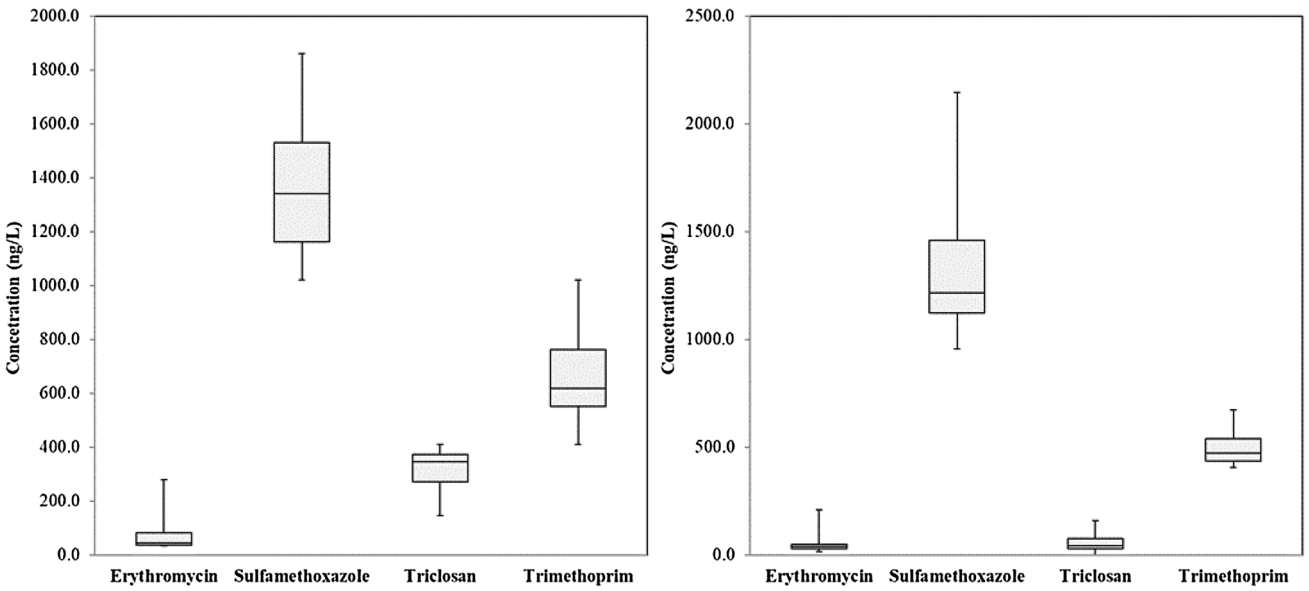

Fig. 2 Concentrations of three antibiotics—erythromycin, sulfamethoxazole, trimethoprim—and an antimicrobial agent triclosan in the WWTP1 (left) and WWTP2 (right) for sampling duration April–October 2017

Sulfamethoxazole and trimethoprim exhibit almost identical trends in their concentration range. Both these antibiotics are often prescribed individually or in combination to treat a variety of infections. In fact, according to one estimate, TMP is always used in combination with a fixed ratio of 1:5 (1 part of TMP to 5 parts of SMZ) [13]. When combined with SMZ, the bacteriostatic effect of TMP and SMZ is enhanced due to simultaneous binding of TMP and SMZ; thereby, the combination therapy produces a net bactericidal effect [13, 25]. Besides regular prescription, transient population such as tourists can also lead to increased release of pharmaceutically active compounds in the sanitary sewer either through ingestion of antibiotics as a prophylactic to combat the traveler's diarrhea.

Additionally, transient non-community water usages also contribute to seasonal variations in pharmaceutical compounds in the wastewater stream. Given the sampling duration (April to October), which is a tourist season when a large number of people usually visit Southern California, increased concentration of these antibiotics in the WWTP is expected. Temperature is one of the critical factors that determine the removal efficiency at the WWTP. Warmer sewage temperature during summer enhances microbial degradation, while during the winter season, lower temperature inhibits microbial degradation, thereby affecting overall removal rates between summer and winter [26].

Although samples were not collected during the winter months for this study, other studies reported seasonal variation and increased concentrations of various pharmaceutically active compounds including TMP in the influent wastewater, treated effluent, and processed drinking water with a significantly higher concentration in winter compared to summer [23, 27, 28]. During winter, increased concentrations of pharmaceuticals in the influent wastewater could be attributed to two primary factors: (1) increased consumption of antibiotics and (2) relatively lower rate of removal of pharmaceuticals through slower biotic and abiotic degradation mechanisms [23].

3.2 Removal of antimicrobials at the wastewater treatment plants

Removal of ERY, SMZ, TRL, and TMP was investigated in two wastewater treatment plants. Of the antimicrobials investigated, negative (increase in concentration after final treatment) or no removal of SMZ was observed in both the plants; instead, a higher concentration of SMZ was observed in the final treated effluent (+ 36% in WWTP1 and + 71% in WWTP2). Straub [29] reported that rapid absorption and metabolism of SMZ leads to the formation N4-acetyl-SMZ (NAcSMZ) and glucuronide conjugates (GluSMZ) which subsequently converts back to the parent form leading to net negative removal in the wastewater treatment plant.

Furthermore, biodegradation studies conducted by Richardson and Bowron [30] indicated that both ERY and SMZ are non-biodegradable by the wastewater treatment processes. Biodegradation studies under laboratory condition using Zahn–Wellens test and CO_2 evolution test reported that ERY, SMZ, and TMP are non-biodegradable [31]. Given these and other findings, the results obtained through this study are consistent with the findings reported in the literature that traditional wastewater treatment plants are unable to remove certain antimicrobials during conventional wastewater treatment.

Overall, the mean percent removal of antimicrobial was higher in WWTP1 compared to WWTP2. Furthermore, results indicate that WWTP2 is inefficient in the removal of all four antimicrobials except TMP, which had 4% percent removal. Compared to WWTP2, WWTP1 had higher removal of ERY (46%), TRL (63%), and TMP (61%). Figure 3 shows the comparison between influent and effluent concentrations at both plants. Removal of TRL appears to be consistent in both plants except for the unusually higher concentration of TRL observed in the final treated effluent of both plants. A single abnormally higher concentration (1670 ng L^{-1}) of TRL in the treated effluent of both the plants reported in July has skewed the results. The effluent concentrations in July could be an outlier since effluent concentration in other months throughout the monitoring duration were less than 25 ng L^{-1}.

Differences in removal efficiency of both the plants could be attributed to the robustness of the treatment regime employed at WWTP1. Secondary treatment protocol at WWTP1 significantly differs from the one at WWTP2. In WWTP1, the effluent from primary treatment is processed through three secondary treatment processes—activated sludge treatment (optimized for BOD removal through the addition of anoxic cells), activated sludge at WWTP2 (newer enhanced nitrogen removal), and trickling filter. Given the robust secondary treatment protocol at WWTP1, it produces the very high quality of secondary effluent and exhibits better removal efficiency for the antimicrobials investigated. On the other hand, secondary treatment at WWTP2 comprised of oxygen-activated sludge that relies on pure oxygen for BOD removal followed by trickling solid filter contact. Furthermore, not all the effluent from the primary settling basin goes through both the treatment processes. Nearly 60% of the effluent from primary is treated at the trickling filter solid contact, while the remaining 40% is treated at the oxygen-activated sludge. Since these treatment regimens are not identical in efficiency, it produces the relatively poor quality of secondary treated wastewater with no removal of antimicrobial compounds investigated.

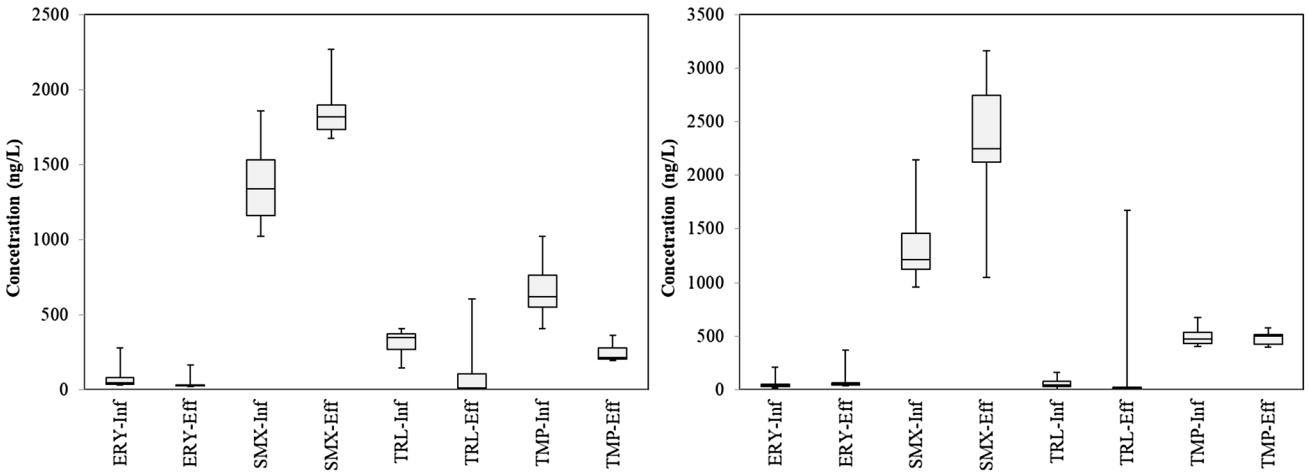

Fig. 3 Removal of ERY, SMZ, TRL, and TMP in WWTP1 (left) and WWTP2 (right). A higher concentration of SMZ in Plant No. 2 effluent shows inadequate removal in Plant No. 2

3.3 Quality assurance protocol

Method detection limit studies were carried out using EPA guidelines of the 40 Code of Federal Register (CFR) 136 Appendix B, August 28, 2017. The guideline requires laboratory to process a minimum of seven method blank samples and seven spiked samples. Also the samples used for MDL must be prepared at the minimum in three batches, on three separate calendar dates and analyzed on three separate dates. Analyzing 11 blank spikes (minimum of seven is required) prepared in three batches on three separate days established the accuracy and precision. The spike concentration is 5 ng L^{-1} for all compounds. As illustrated in Table 1, the relative standard deviations (RSD) are between 0.32 and 3.17%, and recoveries are between 65 and 94%, which is well within the stipulated USEPA Method 1694 guideline (Fig. 4).

For this study, the EPA Method 1694 was optimized, validated, and used to analyze the target analytes. While only one multiple reaction monitoring (MRM) transition was used in the original method, two MRM transitions (a quantifier and a qualifier ion for each compound) were used in this study to enhance the accuracy of the confirmation. Similarly, not all labeled internal standard was available in the original method; each compound was quantified by isotopic dilution with matched labeled internal standard to compensate for ion suppression or enhancement caused by co-eluting compounds for this study. Finally, the method detection limits were established, and method performance criteria proved to be equal to or better than those listed in the original method.

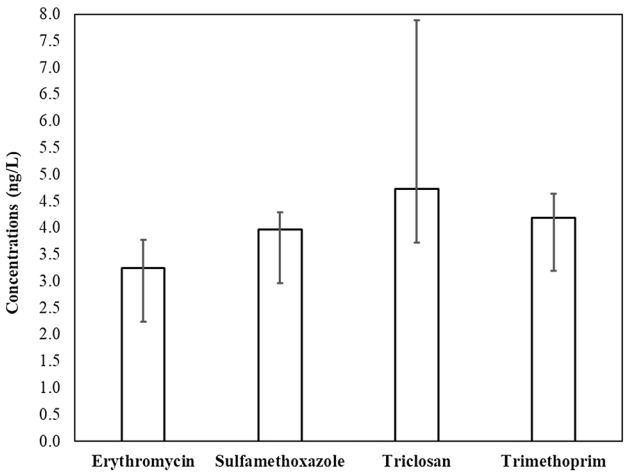

Fig. 4 Accuracy and precision of the erythromycin, sulfamethoxazole, triclosan, and trimethoprim

3.4 Reuse of wastewater and potential consequences

Increased demand for water resources has placed a renewed focus on the use of treated wastewater to mitigate the water demand. In the drought-prone areas and areas with prolonged drought conditions, treated wastewater is currently used for artificial aquifer recharge. Reusing wastewater in such a scenario appears to be a sustainable solution to conserve water resources. Various countries in Europe (England and Belgium), North America (USA), Africa (Namibia), and Asia (Singapore) have successfully developed a protocol for reusing treated wastewater effluent for indirect potable reuse [32]. California is one of the leading states that have successfully harnessed this

valuable resource through managed artificial recharge projects. Often when considering reuse of treated wastewater for disposal in water bodies such as a river, lake, or ocean, it only must meet the National Pollutant Discharge Elimination System (NPDES) effluent treatment standard. These standards include conventional pollutants such as biodegradable organic material, pH, and total suspended solids. Even when the treated wastewater effluent meets the NPDES regulation, there is no guarantee that it may not have any residual pharmaceutical compounds in it. To overcome prolonged drought conditions, recently, a large number of WWTPs are producing recycled water by providing additional treatment above and beyond the NPDES requirement, such that the produced water can be used for irrigation, groundwater recharge, or other indirect potable reuse.

The indirect potable reuse (IPR) of treated effluent appears to be a promising option, especially for recharging the groundwater in drought-prone areas. However, the cost associated with recharge needs additional consideration. For example, if the treated secondary effluent is not further polished, then groundwater recharge may not be efficient and may result in frequent clogging of the recharge basins. On the other hand, if the treated effluent is polished to such an extent that it meets and surpasses the existing primary drinking water standards, as is the case with the Groundwater Replenishment System in California, it might cost prohibitive. Given these circumstances, IPR may hold a promise to provide sustainable drinking water resources; besides cost, other factors such as socio-psychological factors need to be taken into account.

The unregulated discharge of treated wastewater effluent containing pharmaceuticals can potentially contaminate the drinking water sources [33]. Currently, there are no effluent standards for bioactive substances such as pharmaceuticals and endocrine-disrupting compounds even though various studies have documented the presence of these substances in treated wastewater. For example, studies have reported that both the Colorado River and the Sacramento–San Joaquin River Basin from where Southern California gets its potable water showed the presence of various PPCPs in the raw and finished drinking water with the concentrations in raw water in summer months approaching the concentrations found in the reclaimed wastewater [33]. Similarly, the presence of various classes of pharmaceuticals including sulfonamides, fluoroquinolones, and macrolides was detected in the influent of Croatian municipal wastewaters with total concentrations ranging from 2 to 20 μg L^{-1} [24].

The use of treated wastewater for irrigation purposes also poses several challenges. Not only long-term application of treated wastewater leads to accumulation of salt and heavy metals such as lead and cadmium, it also leads to the accumulation of pharmaceuticals and personal care products, endocrine-disrupting compounds, drugs metabolites, other illicit drugs, and their transformation products in soil and plants [34, 35]. A knowledge gap exists between applications of treated wastewater containing pharmaceutical residues and its impact on non-target species, transport of residues in the food chain through plant uptake, behavior mixture of compounds and their interaction (synergistic or otherwise), and finally the growth of antibiotic-resistant microorganisms [34].

3.5 Occurrence of pharmaceuticals in the environment and ecological risk

The occurrence of pharmaceuticals in the influent and effluent of wastewater treatment plant has been documented by many studies [4, 8, 11, 36, 37]. The presence of bioactive compounds in treated wastewater often ends up in other water bodies. Wastewater is a heterogeneous mixture of various organic and inorganic compounds, and as such, it is difficult to quantify the risk to non-target species attributed explicitly to exposure to pharmaceuticals present in wastewater. Also, pharmaceuticals tend to act differently in the presence of other compounds compared to their impact on the same organism when acting alone. One of the measures to evaluate the potential ecotoxicological risk to non-target species is the comparison between concentrations of antimicrobials in the treated effluent and predicted no-effect concentration (PNEC) levels that are based on the chronic and sub-chronic ecotoxicity data. Since the measured mean concentrations of ERY, SMZ, and TMP in both WWTP1 and WWTP2 in treated wastewater effluents are higher than the reported deterministic PNEC values found in the literature, there is a likelihood of adverse effect due to the occurrence of ERY, SMZ, and TMP on non-target species [36–38]. Similarly, the PNEC values of TRL in both influent and effluent are well below the PNEC levels; it is unlikely to have any adverse effect on non-target species.

Hazard quotient (HQ) is another numeric indicator of potential adverse effect to non-target species and is often used as a predictor of potential ecotoxicological risk due to exposure to pharmaceuticals. The HQ can be calculated as a ratio of the predicted or measured environmental concentration to the PNEC. If the HQ ≥ 1, it indicates potential adverse ecological risk; if HQ < 0.1, it indicates low risk; and 0.1 < HQ < 1 indicates medium risk [36, 37]. Although EC50 and LC50 values are currently available to determine the PNEC for the pharmaceutical compounds investigated, for simplicity, in this study, we have used the PNEC values for each pharmaceutical derived from the most sensitive (lowest) toxicity data point [37]. Based on the PNEC values and

the measured concentration of antibiotics in the treated effluents in WWTP1 and WWTP2, all three antibiotics ERY, SMZ, and TMP pose a significant adverse risk (Table 2), while TRL does not pose a risk to various non-target aquatic species. Since ERY, SMZ, and TMP have HQ values greater than 1, aquatic species such as fish, daphnia, and algae may expect perturbation. Risk evaluation entirely based on the HQ might not reflect the real environmental concern because of the potential loss of concentration due to dilution (the minimum probable initial dilution is 181:1), and other abiotic degradation mechanisms such as hydrolysis and photolysis.

Given the findings of this research, it is imperative that long-term consequences due to the occurrence and persistence of pharmaceuticals compounds in the aquatic environment need serious consideration to prevent potential ecotoxicological impact on non-target species.

3.6 Detection of pharmaceuticals in WWTP practical challenges

Since the enactment of Clean Water Act in 1972, EPA has always been a primary agency to promulgate analytical testing procedures for the industries and municipalities to analyze the physical, biological, and chemical components of wastewater and other environmental samples that are required by regulations under the Clean Water Act. Many studies have published about occurrence and detection of pharmaceuticals and personal care products, endocrine-disrupting compounds, and natural and artificial hormones in treated and untreated wastewaters. It was not until December 2007 that the EPA finally issued a standardized testing procedure in the form of EPA Methods 1694 and 1698. Until then, the reporting of these chemicals was based on customized testing procedures specific to each laboratory. The EPA Method 1694 was published as a guideline and screening method for analyzing 74 pharmaceuticals in environmental samples [22]. Although these methods have been peer-reviewed, they have not undergone a multi-laboratory validation; consequently, they are not yet approved for NPDES compliance monitoring purposes.

In this study, we modified EPA Method 1694 by making a change in chemistry to improve the linearity range. Method modifications that improve the accuracy and precision with individual subsets of compounds or individual matrices are allowed, only when such modifications are documented, and provide the performance of equal to or better than that specified in the original method. While Method 1694 is not officially approved for NPDES monitoring purposes, it is one of the methods permitted by the regulator to be used to monitor PPCPs in wastewater discharge.

Table 2 Percent removal of pharmaceutical compounds ERY, SMZ, TRL, TMP and their respective hazard quotients using lowest toxicity data points

Parameters	ERY	SMZ	TRL	TMP
WWTP1				
Mean influent (ng L^{-1})	85.52	1372.43	313.20	668.76
Mean effluent (ng L^{-1})	46.43	1861.29	121.86	248.29
Percent removal (%)	45.71	−35.62	61.09	62.87
PNEC (ng L^{-1})	20.00	27.00	2600.00	50.00
HQ	2.32	68.94	0.05	4.97
WWTP2				
Mean influent (ng L^{-1})	60.53	1354.29	59.57	499.86
Mean effluent (ng L^{-1})	96.57	2313.71	247.57	480.14
Percent removal (%)	−59.55	−70.84	−315.59	3.94
PNEC (ng L^{-1})	20.00	27.00	2600.00	50.00
HQ	4.83	85.69	0.10	9.60

4 Conclusions

In this study, we investigated the occurrence and removal of the three most commonly used pharmaceuticals and an antimicrobial agent in two different wastewater treatment plants. Both plants employ secondary biological treatment, but the treatment protocol is significantly different. WWTP1 has a treatment regime that includes activated sludge treatment followed by enhanced and newer activated sludge treatment process and trickling filter, while WWTP2 has traditional activated sludge treatment followed by a trickling filter. Given the rigorous treatment protocol of WWTP2, it was found more efficient in the removal of ERY, TRL, and TMP. None of the plants was able to remove SMZ. Both the treatment plants are in Southern California, and reuse of treated secondary effluent of WWTP1 is considered a priority for reuse to combat extended drought condition. Occurrence and partial removal of antimicrobial compounds in the treated effluent is a concern. Additional treatment for the removal of these compounds should be considered before its reuse to prevent any potential ecological consequences. The hazard quotient calculated for ERY, SMZ, TRL, and TMP is purely based on the concentration levels observed in the treated effluent and predicted no-effect concentrations levels found in the literature. The hazard quotient does not take into consideration the dilution effect that could be significant. In this study, the observed concentration of antimicrobial compounds in the treated effluent is in the ng L^{-1} with further dilution in the post-discharge scenario; the concentration will be significantly lower. Concentration levels in the post-discharge scenario are critical to

assess the potential threat and to ensure the protection of aquatic species. Future studies are required to monitor the actual concentration levels and their persistence in the post-discharge scenario to better evaluate the risk to human health and aquatic species.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author declares that there is no conflict of interest.

References

1. Aminov RI (2009) The role of antibiotics and antibiotic resistance in nature. Environ Microbiol 11(12):2970–2988
2. Daughton CG (2003) Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy. Environ Health Perspect 111:757–774
3. Guneysel O, Onur O, Erdede M, Denizbasi A (2009) Trimethoprim/sulfamethoxazole resistance in urinary tract infections. J Emerg Med 36(4):338–341
4. Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N (2003) Trimethoprim–sulfamethoxazole revisited. Arch Intern Med 163:402–410
5. Louvet JN, Giammarino C, Potier O, Pons MN (2010) Adverse effects of erythromycin on the structure and chemistry of activated sludge. Environ Pollut 158:688–693
6. Jelic A, Gros M, Ginebreda A, Cespedes-Sanchez R, Ventura F, Petrovic M, Barcelo D (2011) Occurrence, partition, and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res 45:1165–1176
7. Hinther A, Bromba CM, Wulff JE, Helbing CC (2011) Effects of triclocarban, triclosan, and methyl-triclosan on thyroid hormone action and stress in frog and mammalian culture systems. Environ Sci Technol 45:5395–5402
8. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ Sci Technol 36:1202–1211
9. Behera SK, Kim HW, Oh J, Park H (2011) Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci Total Environ 409:4351–4360
10. Peng X, Wang Z, Kuang W, Tan J, Li K (2006) A preliminary study on the occurrence and behavior of sulfonamides, ofloxacin, and chloramphenicol antimicrobials in wastewaters of two sewage treatment plants in Guangzhou, China. Sci Total Environ 371:314–322
11. Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Res 32(11):3245–3260
12. Heberer T, Adam M (2004) Transport and attenuation of pharmaceutical residues during artificial groundwater replenishment. Environ Chem 1(1):22–25
13. Gobel A, Thomsen A, McArdell CS, Joss A, Giger W (2005) Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ Sci Technol 39(11):3981–3989
14. Fick J, Soderstrom H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ (2009) Pharmaceutical and personal care products in the environment: contamination of the surface, ground, and drinking water from pharmaceutical production. Environ Toxicol Chem 28(12):2522–2527
15. Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Indic 8:1–13
16. Li WC (2014) Occurrence, sources, and the fate of pharmaceuticals in the aquatic environment and soil. Environ Pollut 187:193–201
17. Hernando MD, Mezcua M, Fernandez-Alba AR, Barcelo D (2006) Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters, and sediments. Talanta 69:334–342
18. Levy BS, Marshall B (2004) Antibacterial resistance worldwide: causes, challenge, and responses. Nat Med Suppl 10(12):S122–S129
19. CDC-Center for Disease Control. https://www.cdc.gov/hai/organisms/cre/index.html. Accessed 15 Nov 2017
20. Drillia P, Dokianakis SN, Fountoulakis MS, Kornaros M, Stamatelatou K, Lyberatos G (2005) On the occasional biodegradation of pharmaceuticals in the activated sludge process: the example of the antibiotic sulfamethoxazole. J Hazard Mater 122:259–265
21. OCSD (2018) Orange County Sanitation District: facts and key statistics https://www.ocsd.com/Home/ShowDocument?id=19430. Accessed 30 May 2019
22. EPA (2007) Method 1694. pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. https://www.epa.gov/sites/production/files/2015-10/documents/method_1694_2007.pdf. Accessed 04 Jan 2018
23. Golovko O, Kumar V, Fedorova G, Randak T, Grabic R (2014) Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111:418–426
24. Senta I, Terzic S, Ahel M (2013) Occurrence and the fate of dissolved and particulate antimicrobials in municipal wastewater treatment. Water Res 47:705–714
25. Poe M (1976) Antibacterial synergism: a proposal for chemotherapeutic potentiation between trimethoprim and sulfamethoxazole. Science 194:533–535
26. Sui Q, Huang J, Deng S, Chen W, Yu G (2011) Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ Sci Technol 45:3341–3348
27. Valcarcel Y, Alonso SG, Rodriguez-Gil JL, Castano A, Montero JC, Criado-Alvarez JJ, Miron IJ, Catala M (2013) Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). Environ Sci Pollut Res 20(3):1396–1412
28. Yu Y, Wu LS, Chang AC (2013) Seasonal variation of endocrine disrupting compounds, pharmaceuticals, and personal care products in wastewater treatment plants. Sci Total Environ 442:310–316
29. Straub JO (2016) Aquatic environmental risk assessment for human use of the old antibiotic sulfamethoxazole in Europe. Environ Toxicol Chem 35(4):767–779
30. Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. J Pharm Pharmacol 37:1–12
31. Gartiser S, Urich E, Alexy R, Kummerer K (2007) Ultimate biodegradation and elimination of antibiotics in inherent tests. Chemosphere 67:604–613
32. Rodriguez C, Buynder PV, Lugg R, Blair P, Devine B, Cook A, Weinstein P (2009) Indirect potable reuse: a sustainable water supply alternative. Int J Environ Res Public Health 6:1174–1209

33. Loraine GA, Pettigrove ME (2006) Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. Environ Sci Technol 40:687–695
34. Fatta-Kassinos D, Kalavrouziotis IK, Koukoulakis PH, Vasquez MI (2011) The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci Total Environ 409:3555–3563
35. Rusan MJM, Hinnawi S, Rousan L (2007) Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination 215:143–152
36. Gros M, Petrović M, Ginebreda A, Barceló D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36:15–26
37. Verlicchi P, Aukidy MA, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load, and environmental risk after a secondary treatment—a review. Sci Total Environ 429:123–155
38. Blair BD, Crago JP, Hedman CJ, Klaper RD (2013) Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern. Chemosphere 93:2116–2123