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Occurrence, Fate, and Removal of Selected Antibiotics in Advanced Biological Urban Wastewater Treatment Plant

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İleri Biyolojik Kentsel Atıksu Arıtma Tesisinde Seçilmiş Antibiyotiklerin Oluşumu, Akıbeti ve Giderimi

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Abstract
Wastewater treatment plants (WWTPs) are considered one of the most important point sources for releasing antibiotics into the natural environment. In this study, the occurrence, fate, and removal of three antibiotics, ciprofloxacin (CIP), sulfamethoxazole (SMX), and azithromycin (AZI), belong to fluoroquinolone, sulfonamide, and macrolide groups, respectively were investigated seasonally in a large-scale urban WWTP. While the occurrence concentrations of the investigated antibiotics in raw wastewater were found between about 0.26 and 0.73 µg/L in the summer season, it was determined in the range of approximately 0.41 and 4.6 µg/L in the winter season. Although the removal efficiencies obtained for antibiotics in the pre-treatment stage of the studied advanced biological WWTP were not very high, the most treated compound in the pre-treatment stage was determined as CIP (up to 23.8%). Total removal efficiencies of the investigated antibiotics in the WWTP varied mainly based on the biological treatment stage of the WWTP. Therefore, the removal efficiencies of the antibiotics obtained in biological treatment also changed considerably depending on the season. Among all the investigated antibiotic compounds, it was determined that AZI was the compound that was least affected by the seasons in terms of the total removal efficiency obtained in WWTP. According to the annual average total removal efficiencies, it was determined that SMX (25.7%), CIP (71.3%), and AZI (89.2%) were treated poorly, moderately, and highly in WWTP, respectively. However, advanced biological WWTP was determined to be insufficient for the complete removal of total antibiotics.

Keywords: *Antibiotics; Pharmaceuticals; Removal; Seasonal distribution; Wastewater; Wastewater treatment plant.*

Öz
Atıksu arıtma tesisleri (AAT'ler), antibiyotiklerin doğal ortama geçişi için en önemli noktasal kaynaklardan biri olarak kabul edilmektedir. Bu çalışmada, sırasıyla florokinolon, sülfonamid ve makrolid gruplarına ait üç antibiyotik bileşik siprofloksasin (CIP), sülfametoksazol (SMX) ve azitromisinin (AZI) büyük ölçekli kentsel bir AAT'deki oluşumu, akıbeti ve uzaklaştırılması mevsimsel olarak araştırılmıştır. İncelenen antibiyotiklerin ham atıksulardaki oluşum konsantrasyonları yaz mevsiminde yaklaşık 0,26 ile 0,73 µg/L arasında bulunurken kış mevsiminde ise yaklaşık 0,41 ile 4,6 µg/L aralığında belirlenmiştir. Çalışılan ileri biyolojik AAT'nin ön arıtma kademesinde antibiyotikler için elde edilen giderim verimleri çok yüksek olmamak ile birlikte ön arıtma kademesinde en fazla arıtılan bileşik CIP (%23,8'e kadar) olarak belirlenmiştir. AAT'de araştırılan antibiyotiklerin toplam giderim verimleri esas olarak AAT'nin biyolojik arıtma kademesine bağlı olarak değişiklik göstermiştir. Aynı zamanda biyolojik arıtmada antibiyotikler için elde edilen giderim verimleri de mevsimlere bağlı olarak önemli ölçüde değişmiştir. İncelenen tüm antibiyotik bileşikler arasında AAT'de elde edilen toplam giderim verimi açısından mevsimlerden en az etkilenen bileşiğin AZI olduğu belirlenmiştir. Yıllık ortalama toplam giderim verimlerine göre AAT'de; SMX (%25,7), CIP (%71,3) ve AZI'nin (%89,2) sırasıyla düşük, orta ve yüksek oranda arıtıldığı belirlenmiştir. Bununla birlikte, ileri biyolojik AAT'nin toplam antibiyotiklerin tamamen giderilmesi için yetersiz olduğu belirlenmiştir.

Anahtar Kelimeler: *Antibiyotikler; Farmasötikler; Giderim; Mevsimsel dağılım; Atıksu; Atıksu arıtma tesisi.*

1. Introduction

Especially during the last century, the global consumption of drugs has increased dramatically, mainly because of the striking increase in the human population and the development of societies (Dolu and Nas 2023a, Dolu and Nas 2023b). Antibiotics, natural, synthetic, or semi-synthetic origin, are one of the most extensively consumed sub-groups of pharmaceuticals, especially for preventing and treating many infectious diseases in people and animals. Approximately 250 antibiotic compounds are used in human and veterinary medicine (Kümmerer 2009). Besides that, antibiotics are commonly used in many areas, including livestock farming, agriculture, food preservation, aquaculture, horticulture, etc. (Gao et al. 2012a, Meek et al. 2015, Tran et al. 2016). It is estimated that the total amount of antibiotic consumption in the world is between 100,000-200,000 tons (Kumar and Pal 2018, Song et al. 2019). After the antibiotics are used in various fields, they are eventually introduced into the natural environment through medical

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wastes, animal wastes, landfill leachate, pharmaceutical industries, hospital wastewater, and effluent of wastewater treatment plants (WWTPs) (Kümmerer 2009, Luo et al. 2014). As a result of the intensive use of antibiotics and their ability to pass into the environment via many different routes cause antibiotics to be detected simultaneously in various environmental media, such as raw/treated wastewater, surface waters, groundwater, irrigation water, and even drinking water (Anjali and Shanthakumar 2019, Nas et al. 2021a, Wu et al. 2016). Moreover, the detection of antibiotics frequently in natural environments promotes the emergence and evolution of antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARB), one of the crucial global concerns of our time (Inyinbor et al. 2018, Li et al. 2019, Wang et al. 2020). In recent years, serious concerns about ARGs and ARB in the aquatic environment have been increasing due to the increasing occurrence of antibiotic-resistant pathogens, even superbugs, that pose potential risks to the ecological environment and human health (Berendonk et al. 2015, Li et al. 2022). Apart from this, it has been reported in many studies that the proportion of ARB and/or ARGs in animals like fish, chickens, pigs, and cattle has increased considerably (Van Boeckel et al. 2019, Jia et al. 2020, Ajayi et al. 2024). In addition to all these, a recently performed study has estimated that human deaths associated with ARB and ARGs are about 700,000 per year and that by 2050, approximately 10 million people may die from this cause each year (Lorenzo et al. 2018). Therefore, it can be said that antibiotic resistance is becoming a very fast-growing and crucial threat to environmental and public health.

Since a small fraction of the consumed antibiotics is metabolized or absorbed by the human body, a high percentage (up to 90%) of the used antibiotics are excreted from the body via urine and feces in an unchanged form (Cha and Carlson 2019, Kümmerer 2009). Thus, antibiotics that pass into the sewerage systems ultimately reach the WWTPs. Although WWTPs are the last barrier against the release of antibiotics into the natural environment, they cannot eliminate these pollutants effectively. Because the majority of the existing WWTPs were not designed to eliminate pharmaceuticals (Nas et al. 2021b, Nas et al. 2017). Thus, antibiotic residues are continuously being released into the aquatic environments through effluent discharges of WWTPs. For this reason, WWTPs are one of the most crucial point sources for passing antibiotic residues into aquatic environments. Many studies conducted in WWTPs have reported widely varying removal efficiencies, from substantial negative to high positive removals, for various antibiotics (Tran et al. 2016, Sabri et al. 2020, Wang et al. 2024). These differences are mainly related to the (i) physicochemical properties of the targeted antibiotic compounds and (ii) types of the studied WWTPs (activated sludge treatment processes or nature-based solutions such as wastewater stabilization ponds (WSPs) or constructed wetlands (CWs)) (Zhang et al. 2011, Khasawneh et al. 2021, Nas et al. 2021a). In a study investigating the removal of different antibiotic compounds in 12 different WWTPs located in the same city in China, total removal efficiencies ranging from -189.9% to 100% were reported (Zhang et al. 2017). In another study conducted in China, antibiotic removals ranging from 11.8% to 100% and -62.5% to 100% were reported in a WWTP with an activated sludge system and another WWTP operated as an oxidation ditch, respectively (Zhou et al. 2013).

It has been reported that the most crucial factor that directly affects the removal of pharmaceuticals, including antibiotics, in WWTPs is the biological treatment stages applied in these plants (Verlicchi et al. 2012, Tran et al. 2016, Nas et al. 2021b). Especially some operating parameters of the studied WWTPs, like solid retention time (SRT), hydraulic retention time (HRT), redox conditions, recirculation ratio, temperature, pH, and the fraction of heterotrophic and autotrophic biomass present in the activated sludge systems, affect the total removal efficiencies of the targeted antibiotics (Guerra et al. 2014, Gruchlik et al. 2018, Oberoi et al., 2019). For example, it has been stated that for various biological treatment processes, increasing the wastewater temperature up to a certain limit can increase microbial activities for the degradation of pharmaceuticals (Luo et al. 2014, Tran et al. 2018). Some studies have reported that changing wastewater temperatures depending on the seasons changes the microbial biomass composition in wastewater and accordingly affects the biodegradation and adsorption of antibiotics, which are the main removal mechanisms of antibiotics in WWTPs (Zheng et al. 2019, Zhang et al. 2023). In addition, it has been reported that the pH values of wastewater are another parameter that can cause negative removal efficiencies in WWTPs for some compounds. It has been reported that changes in pH values in wastewater change the overall dissociation and chemical nature of antibiotics in wastewater, and this affects the removal of these compounds in plants (Kumar et al. 2022). A study has reported that many antibiotic compounds, including AZI, can be eliminated more effectively from wastewater under slightly acidic environments (Urase et al. 2005). Apart from these, some studies are showing that SRT and HRT, two other critical operating parameters, should be longer than 15 days and 4-6 hours, respectively, for the effective removal of many antibiotics in WWTPs (Zhang et al. 2016, Guo et al. 2017). Similarly, Neyestani et al. (2017) reported that longer SRT provides enhanced microbial diversity, which results in the enrichment of slow-growing microbes capable of degrading the antibiotics. In another study, it has been reported that HRT should be longer than 4-6 hours for the contact time required for the efficient degradation of many sulfonamide group antibiotics, including SMX (Yang et al. 2011). One of the fluoroquinolone antibiotics, ciprofloxacin (CIP), is widely prescribed in both human and veterinary medicine across the globe due to its broad-spectrum activity against bacteria (Hom-Diaz et al. 2017). The main sources of CIP entering raw wastewater are hospitals, homes, and industries (Yi et al. 2017). CIP could prevent enzymatic activities in the DNA replication process of Gram-positive and Gram-negative bacteria, which can result in cell death (Pan et al. 2018). Sulfamethoxazole (SMX), which belongs to the sulfonamide group and is one of the most prescribed antibiotics, is widely used in various bacterial infections, especially urinary tract infections, bronchitis, and prostatitis (Shahmahdi et al. 2020). SMX is mainly present in raw wastewater produced by animal farms, pharmaceutical factories, and hospitals. It eventually ends up in surface waters via discharges from WWTPs due to its relatively persistent nature (Cui, et al. 2021). Azithromycin (AZI) in the group of macrolides, the third most widely consumed group of antibiotics, has antiviral and immunomodulatory effects (Klein et al. 2018). AZI is widely used for treating respiratory diseases and sexually transmitted infections due to its high capability to act against a wide range of bacteria (Mirzaie et al. 2022). Since CIP, SMX, and AZI antibiotics are used extensively in different areas and various diseases, these three compounds have been detected frequently in the raw wastewater of WWTPs located in different countries (Rizzo et al. 2013, Tran et al. 2016, Nas et al. 2021a).

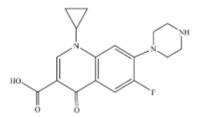
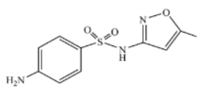
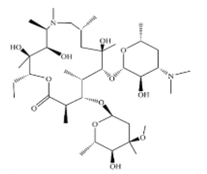
In this study, the occurrence, fate, and removal of the three most widely prescribed antibiotics were investigated seasonally (winter and summer) in a large-scale urban WWTP. With this in view, CIP, SMX, and AZI antibiotics in the group of fluoroquinolones, sulfonamides, and macrolides, respectively, were monitored. In addition, to determine the possible removal mechanisms of the targeted antibiotics in the WWTP, the pre-treatment, biological treatment, and total treatment performances of the investigated advanced biological WWTP on selected antibiotics were also determined.

2. Materials and Methods

2.1 Chemicals and reagents

The standards of the investigated antibiotics, CIP, SMX, and AZI, were provided by Toronto Research Chemicals Inc. (North York, Canada). Some physicochemical properties of the studied antibiotics are given in Table 1. Internal standards, CIP-d_8, SMX-d_4, and AZI-d_3, used in this study were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of all standards was ≥98%. HPLC grade solvent, methanol and acetonitrile, and all other chemicals utilized in this work were acquired from Sigma–Aldrich (Sigma-Aldrich Corporation, Germany). Besides, all used reagent water was provided by the Milli-Q unit (Millipore, USA) water purification system.

Table 1. Some physicochemical properties of the studied antibiotics.

Antibiotics (Chemical formula)	CAS number	Molecular structure	Molecular weight (g/mol)	Solubility (mg/L)	pK_a	Log K_{ow}	Log K_d
Ciprofloxacin (CIP)[a, b, c, d] ($C_{17}H_{18}FN_3O_3$)	85721-33-1		331.3	3×10^4 (20°C)	6.16, 8.63	0.28	2.70
Sulfamethoxazole (SMX)[a, b, e, f] ($C_{10}H_{11}N_3O_3S$)	723-46-6		253.3	610 (37°C)	1.85, 5.70	0.89	1.04
Azithromycin (AZI)[d, g, h] ($C_{38}H_{72}N_2O_{12}$)	83905-01-5		748.9	2.73 (25°C)	8.74, 9.45	4.02	2.20

Marks and abbreviations: **pK_a:** Acid dissociation constant, **K_{ow}:** Octanol-water partitioning coefficient, **K_d:** Solid-water distribution coefficient.
[a](Li and Zhang 2010), [b](Olicón-Hernández et al. 2017), [c](Wu et al. 2009), [d](Okuda et al. 2009), [e](Yu et al. 2011), [f](Martín et al. 2012), [g](McFarland et al. 1997), [h](Vermillion Maier and Tjeerdema 2018).

2.2 Wastewater treatment plant and sampling

This study was conducted in an urban advanced biological WWTP found in Konya City, Türkiye. Studied large-scale Konya WWTP, which serves about 1.3 million people consists of three main treatment stages: (i) a primary physicochemical treatment, (ii) a secondary biological treatment, comprising nitrification and denitrification treatment zones, and finally (iii) an elimination step by disinfection through ultraviolet (UV) radiation. Konya advanced biological WWTP is being operated as 4-stage Bardenpho process (consisting of pre-anoxic, aerobic, post-anoxic, and post-aerobic treatment zones, respectively) with partial nitrogen removal and treats about 200,000 m^3 of wastewater daily. While Konya WWTP mainly receives domestic wastewater, it also receives industrial and hospital wastewater. Since there are many different sectors, especially automotive, metal, and machinery manufacturing industries in Konya City, about 15,000 m^3/day of industrial wastewater comes to Konya WWTP via a combined sewerage system. In addition, approximately 6000 m^3 of wastewater generated in hospitals per day also comes to Konya WWTP.

The schematic flow diagram and determined sampling points of the studied Konya WWTP are given in Figure 1. Sampling campaigns were conducted in the winter (February) and summer (August) seasons of the 2021 year. There are two main aims for choosing February and August, which have a six-month time difference between them, in this study. The first goal is to detect the reflection of different antibiotic usage patterns of people in the winter and summer seasons on raw wastewater. The second goal is to determine the possible changes in the treatment performance of the studied WWTP on selected antibiotics in the two seasons when the wastewater temperature difference will be highest. Wastewater samples were collected once from each of the three sampling points on non-rainy days in the second week of February and August. Wastewater samples were collected as composite samples (2-h) from the determined sampling points. Wastewater samples (0.5 L) taken every 30 min during a 2-h period were collected in amber glass bottles (2 L) to give a 2-h composite. This sampling technique provides the advantage of a more representation composite, especially during rapidly fluctuating conditions. In addition, hydraulic retention times of the wastewater treatment units of the studied WWTP were considered while sampling. All wastewater samples were kept in the ice bath until they were brought to the laboratory from the WWTP and stored in a refrigerator at 4 °C until analysis. Finally, all antibiotic analyses were carried out within 2-3 days following the day the wastewater samples were collected. In 2021, when the sampling campaigns were carried out, the average values of SRT and HRT, two important operating parameters of Konya WWTP, were 10 days and 17 hours, respectively. In addition, the average values of mixed liquor suspended solids (MLSS) and dissolved oxygen (DO), two other operational conditions of the biological treatment process of Konya WWTP, were 3,600 and 1.2 mg/L, respectively.

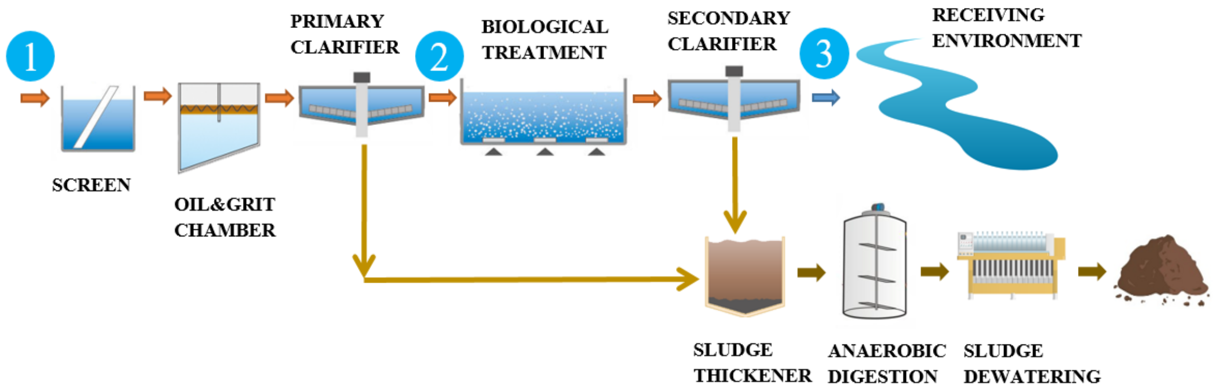


Figure 1. Schematic flow diagram and wastewater sampling points of Konya WWTP.

2.3 Instrumental analysis

A single extraction method was applied to the collected wastewater samples. Firstly, wastewater samples were filtered by syringe filters (0.45 µm). After filtration, solid phase extraction (SPE) method was applied to samples. During this process, SPE cartridges, Oasis HLB (3 mL/60 mg), were conditioned at 20 mL of methanol and 6 mL of deionized water. Followed by the conditioning process, 1 L wastewater sample was passed through the SPE cartridges.

After drying under a vacuum process (10 mL/min), the antibiotics in the cartridges were eluted with 10 mL of methanol. The eluted extracts were then concentrated using a gentle nitrogen stream. Afterward, selected internal standards were added. Finally, extracts were analyzed using the Triple Quadrupole LC-MS/MS system

(Agilent, CA, USA) according to the slightly modified method of EPA 1694. Besides, collected wastewater samples were analyzed by LC-MS/MS in two replicates. Analysis conditions of the studied antibiotics in the LC-MS/MS and achieved recoveries for antibiotics are shown in Table 2.

2.4 Calculation of removal efficiency

In this study, removal efficiencies of the three investigated antibiotics at different treatment stages of Konya WWTP were investigated seasonally. In this context, the removal performances of pre-treatment, biological treatment, and total treatment stages of Konya WWTP for antibiotic compounds were calculated separately. The formulas used to calculate the removal efficiencies of antibiotics in different treatment stages of the Konya WWTP are given in equations (1-3) below.

Removal of pre-treatment;

$$(\%) = \frac{C_{raw\ ww} - C_{pc}}{C_{raw\ ww}} \times 100 \qquad \text{Eq. (1)}$$

Removal of biological treatment;

$$(\%) = \frac{C_{pc} - C_{sc}}{C_{pc}} \times 100 \qquad \text{Eq. (2)}$$

Total removal;

$$(\%) = \frac{C_{raw\ ww} - C_{sc}}{C_{raw\ ww}} \times 100 \qquad \text{Eq. (3)}$$

"C", "ww", "pc", and "sc" used in the formulas denote concentration, wastewater, primary clarifier, and secondary clarifier, respectively.

Table 2. Analysis conditions of LC-MS/MS and obtained recoveries for studied antibiotics.

Antibiotics	Retention time (min)	Precursor ion (m/z)	Product ions (m/z)	Recovery (%)
CIP	18.65	332	314, 231	88 – 99
SMX	19.03	254	156, 92	94 – 103
AZI	20.06	749	591, 158	85 – 101

Table 3. Comparison of influent concentrations (ng/L) of investigated antibiotics in WWTPs, located in some countries in different geographical regions.

Antibiotics	Country	Occurrence concentration	References
CIP	Türkiye	725.3 (S) – 4,577.2 (W)	*This study*
	Türkiye	218.6 – 2,733.5	(Nas et al. 2021a)
	China	1,684 – 3,585	(Mostafa et al. 2023)
	Iran	552.6 – 796.2	(Mirzaei et al. 2018)
	Spain	1,270 – 149,700	(Bijlsma et al. 2021)
	Peru	2,910 – 4,700	(Nieto-Juárez et al. 2021)
SMX	Türkiye	258.9 (S) – 409.4 (W)	*This study*
	Türkiye	<50 – 179.7	(Nas et al. 2021a)
	Korea	49 – 410	(Sim et al. 2011)
	Italy	30.7 – 155.8	(Spataro et al. 2019)
	USA	1,860 – 2,146	(Phonsiri et al. 2019)
	Colombia	123 – 558	(Botero-Coy et al. 2018)
AZI	Türkiye	391.7 (S) – 788.9 (W)	*This study*
	China	1.5 – 1,687.2	(Ben et al. 2018)
	Japan	199 – 371	(Yasojima et al. 2006)
	Latvia	70 – 150	(Pugajeva et al. 2017)
	Canada	61 – 2,500	(Guerra et al. 2014)
	Egypt	<54 – 660	(Younes et al. 2019)

*S: Summer, W: Winter.

3. Results and Discussions

3.1 Occurrence of antibiotics in Konya WWTP

In Table 3, seasonal occurrence concentrations of the antibiotic compounds investigated in Konya WWTP are given together with some other studies in the literature. As given in Table 3, the compound detected in the highest concentrations in raw wastewater of Konya WWTP in both winter and summer seasons was determined as CIP among the investigated antibiotics. In addition, although there was not much difference between the two seasons, AZI was also detected in higher concentrations than SMX in raw wastewater. Annual average concentrations of CIP, AZI, and SMX in raw wastewater were determined to be about 2.6, 0.6, and 0.3 µg/L, respectively. In this context, it can be said that CIP is the most consumed antibiotic compound in Konya City among the investigated antibiotics. The variability of antibiotic concentrations detected in the raw wastewater of WWTPs of different countries shown in Table 3 can be associated with drug consumption behavior and amount in each country, daily water consumption per capita, population density, weather conditions, and type of sewer system used (Khasawneh and Palaniandy 2021, Tran et al. 2016).

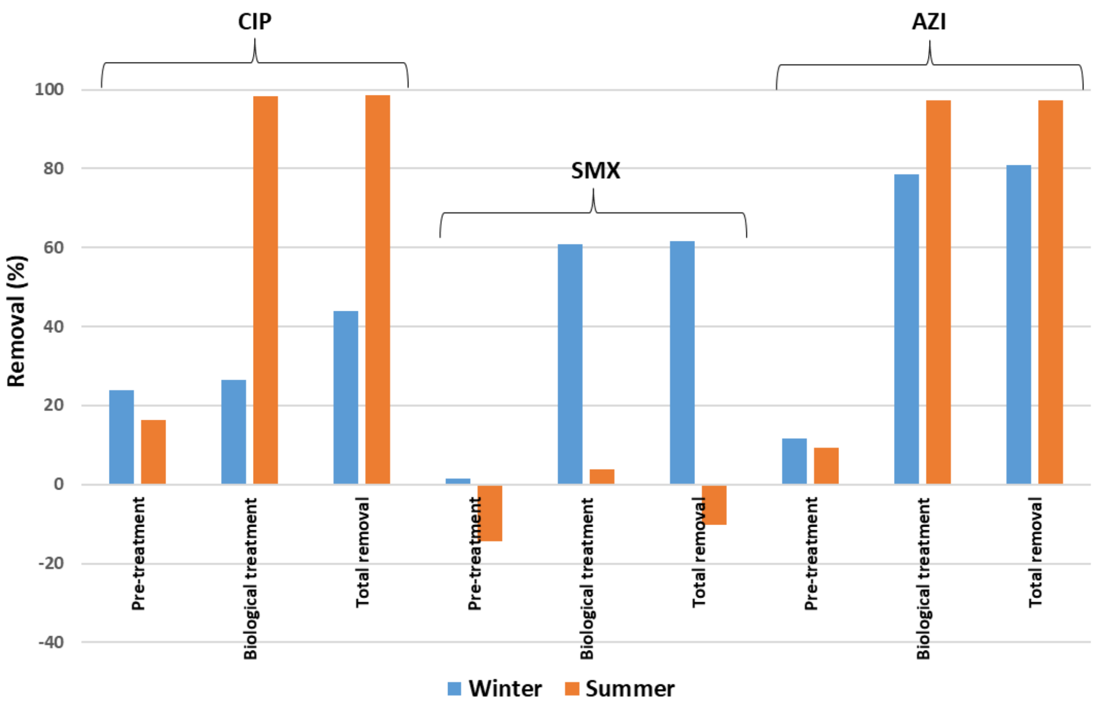


Figure 2. Removal efficiencies of studied antibiotics, CIP, SMX, and AZI, in different treatment stages of Konya advanced biological WWTP

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Another critical finding obtained within the scope of the study is that the raw wastewater concentrations detected in winter for all three investigated antibiotic compounds are higher than in summer.

This situation can be attributed to the fact that people get sick more frequently in the winter season and, accordingly, consume more drugs than in other seasons (Dolu and Nas 2023a, Nas et al. 2021b). This was observed much more prominently, especially for CIP, among the investigated antibiotics. The CIP concentration detected in raw wastewater in winter is approximately 6.3 times that determined in summer, while this ratio is about 2.0 and 1.6 for AZI and SMX, respectively. This may be related to the fact that CIP is prescribed much more often than SMX and AZI, especially for the treatment of respiratory tract infections seen in humans in the winter season.

3.2 Fate and removal of antibiotics in Konya WWTP

Figure 2 shows the removal efficiencies of the investigated antibiotics, CIP, SMX, and AZI, in different treatment stages of the Konya WWTP. As shown in Figure 2, CIP and AZI were treated in the pre-treatment of the Konya WWTP to some extent, while SMX was not. The annual average removal efficiencies achieved for CIP, AZI, and SMX in the pre-treatment stage of the plant were determined as 20.0%, 10.5%, and -6.4%, respectively. Obtained removal efficiencies are consistent with the log K_d values given for the antibiotic compounds in Table 1, showing that the sorption onto primary sludge is a valid removal mechanism for the investigated antibiotics (Dolu and Nas 2023b). Determined negative removal efficiency in the pre-treatment for SMX, which has the lowest potential for sorption onto sludge, can be associated with the tendency of the possible metabolites and/or conjugates of SMX present in wastewater to return to the main compound (Blair et al. 2015, Göbel et al. 2007).

As shown in Figure 2, the antibiotic compounds were mainly eliminated in the biological treatment stage of the plant. The annual average removal efficiencies for CIP, SMX, and AZI in the biological treatment of Konya WWTP were determined as >62.5%, 32.4%, and >87.8%, respectively. The relatively high removals achieved in the biological treatment stage of the plant for the investigated antibiotics reveal that the removal mechanisms that are mainly effective in the elimination of these compounds are biodegradation and/or biotransformation (Nas et al. 2021a, Verlicchi and Zambello 2015). On the other hand, it was determined that the removal efficiencies achieved in the biological treatment stage of Konya WWTP were affected by the seasons for all three investigated antibiotics. This situation is much more evident for both CIP and SMX than for AZI. In the biological treatment of the plant, the removal efficiency achieved for CIP in the summer season increased significantly compared to the winter season, while the opposite situation was observed for SMX. Apart from the specific physicochemical properties of the investigated compounds, it can be explained by the fact that the compounds can behave quite differently in the biological treatment stages of WWTPs based on the wastewater temperature changing depending on the seasons (Golovko et al. 2014, Nas et al. 2021b, Sun et al. 2014). Besides, it is a remarkable finding that unlike the other investigated two antibiotics, CIP and SMX, AZI was highly removed in the biological treatment stage of the plant regardless of the seasons. This can be explained by the rapid biodegradation and/or transformation of AZI during the biological treatments of the WWTPs (Golovko et al. 2021, Verlicchi and Zambello 2015). In addition, Senta et al. (2019) reported that many metabolites of AZI were detected in wastewater along with AZI and that the removal efficiencies of AZI obtained in WWTPs largely depend on the biological transformation reactions between AZI and the existing metabolites.

Different total removal efficiencies were achieved during the two seasons in Konya WWTP for the investigated antibiotics, mainly based on the removals obtained in the plant's biological treatment. While the total removals for CIP, SMX, and AZI in Konya WWTP were 44.0%, 61.6%, and 81.0% for the winter season, respectively, it was determined as >98.6%, -10.1%, and >97.4% in the summer season. While the total removal efficiencies of CIP and AZI achieved in the studied WWTP were determined to be higher in the summer season, the opposite situation was experienced for SMX. As well as the seasonal variations seen for the occurrence concentrations of antibiotics in WWTPs, total removals obtained for antibiotic compounds in WWTPs are also closely related to many seasonal environmental factors (solar irradiance, rainfall, temperature, microbial biodegradation, etc.) (Beltrán de Heredia et al. 2023, Golovko et al. 2014). For example, in addition to providing the energy source for photosynthesis, especially in WSPs, light also provides a major pathway for the degradation of micropollutants, including antibiotics, through direct or indirect UV photodegradation (Gruchlik et al. 2018). It has been reported that the photodegradation rate of pharmaceuticals varies depending on the intensity of solar radiation according to the seasons, and higher removals may occur for photodegradable compounds in summer due to higher solar radiation compared to winter (Matamoros et al., 2015).

Some studies have reported that environmental antibiotic concentrations decrease during the summer due to photodegradation and/or microbial biodegradation promoted by stronger solar radiation intensities and higher temperatures (Osińska et al. 2020, Jiang et al. 2021). It has been stated that heavy rainfall, another factor affecting the removal of antibiotics in WWTPs, deteriorates the removal of antibiotics in plants, especially in the winter (Vieno et al. 2007). In addition, it has been reported that rainfall reduces antibiotic concentrations via dilution in WWTPs, which causes suppression of microbial activities and negatively affects the removal of antibiotics together with ARB and ARGs in plants (Yang et al. 2022, Gao et al. 2022).

Based on the average of the two seasons, CIP (>71.3%) and AZI (>89.2%) were removed from moderate to high levels in the Konya advanced biological WWTP, while SMX (25.7%) was poorly removed. In another study conducted in the same WWTP in the summer season, the determination of total removal higher than 94% for CIP was compatible with our study results. In the same study, the reported total removal efficiency of -133.4% for SMX is consistent with our study result in terms of determining negative removal for SMX in the summer season but differs in terms of the achieved total negative removal (Nas et al. 2021a). Supporting our results, in a study conducted in China, positive removal efficiencies (4.6% and 26.3%) were determined for SMX in the winter season in two different WWTPs with a similar configuration to the Konya WWTP, while negative removal efficiencies (-19.7% and -40.4%) were reported in the summer season (Zhang et al. 2015).

Many studies have reported that negative removal efficiencies were obtained for some antibiotic compounds in the biological wastewater treatment process (Gobel et al. 2007, Blair et al. 2015, Kumar et al. 2022). For example, Gobel et al. (2007) reported that negative removal efficiencies were achieved for many antibiotics, including SMX (−107 ± 8%). Similarly, Blair et al. (2015) have determined negative removal efficiencies for some antibiotics, including SMX (−35.8%), during biological wastewater treatment. The authors explained that the negative removal efficiencies for antibiotic compounds were the result of the transformation of the conjugated forms into the parent compounds during biological treatment by microorganisms. N-acetyl-SMX, one of the most important metabolites of SMX, generally accounts for more than 50% of an administered dose in human excretion and can be found in influents of WWTPs at concentrations 2.5-3.5 times higher than the parent compound SMX (Zhang et al. 2015). It has been reported that in some studies, negative removal efficiencies were obtained for the parent compound SMX during secondary treatment, while removal efficiencies of up to 96% were determined for N-acetyl-SMX. The negative removal efficiencies obtained for SMX in these studies were mainly explained by the deconjugation of N-acetyl-SMX to SMX during wastewater treatment and the resulting increase in SMX concentration (Joss et al. 2006, Göbel et al. 2007, Brown et al. 2018, Zhang et al. 2023).

In an urban WWTP in Sweden with a wastewater treatment capacity of 20,000 m^3, which is being operated as the activated sludge process, the overall removal efficiency for CIP has been reported as 90% (Zorita et al. 2009). Total removal efficiencies of approximately 67.0% and 85.3% were reported for CIP and SMX in a one-week study every day in the spring season at a WWTP located in Saudi Arabia (Mostafa et al. 2023). While the total removal efficiency obtained for CIP in the mentioned study is in excellent agreement with our study results, the high removal efficiency achieved for SMX in the same study is not compatible with our study results. These differences can be attributed to the configuration and operating conditions of the studied WWTPs and the seasons in which the studies were conducted. In general, studies in the literature report a total removal efficiency of 50% and above in WWTPs for CIP (Mohapatra et al. 2016, Verlicchi et al. 2012), while a much wider range of total removals, including from negative removals to high positive elimination rates, have been determined for SMX (Phonsiri et al. 2019, Khasawneh and Palaniandy 2021).
Total removal efficiencies of 65% and 71% were reported for AZI in two advanced biological WWTPs in the USA (Gao et al. 2012) and Iran (Mirzaei et al. 2018), where 51,000 and 24,000 m^3/day of wastewater were treated, respectively. The total removal efficiencies obtained for AZI in both studies are more compatible with our removal efficiency obtained in the winter season for AZI than in the summer season. On the other hand, some studies have reported poor removals of AZI in the WWTPs. For example, the mean total removal efficiencies of AZI at two different WWTPs in India were determined as 22% and 31% (Arun et al. 2022). In another study, as a result of the sampling campaigns carried out every day for a week at two different WWTPs in Colombia, the total mean removals of AZI in both plants remained below 40% (Botero-Coy et al. 2018). As a result, although diverse removals have been determined for AZI in various types of WWTPs, it was determined that the average removal efficiency obtained for AZI in our study was higher than in the literature.

4. Conclusions

In this research, the occurrence, fate, and removal of the three typical antibiotics, CIP, SMX, and AZI, were investigated in a large-scale urban WWTP in a metropolitan city in the winter and summer seasons. For all three investigated antibiotics, the occurrence concentrations detected in raw wastewater in winter were higher than in summer. It was determined that the pre-treatment stage of the studied advanced biological WWTP was not very effective (<20%) in eliminating the antibiotic compounds. Liquid phase concentrations of the antibiotics mainly changed in the biological treatment stage along the wastewater line of the WWTP. While CIP and AZI behaved quite differently depending on the seasons in the plant's biological treatment, AZI exhibited similar behavior. The different behaviors observed for antibiotics in the biological treatment stage can be interpreted as the change in microbial activity as a result of the changing wastewater temperature during seasons. Depending on both specific physicochemical properties of the investigated antibiotics and different seasons, divergent seasonal total removal efficiencies were achieved in Konya advanced biological WWTP. CIP (44%) and SMX (61.6%) were moderately treated in the WWTP in the winter season. However, in the summer season, CIP (>98.6%) was almost completely removed in the plant, while SMX (-10.1%) was not removed at all. The other antibiotic compound investigated, AZI, was eliminated at high removal efficiencies (81.0% and >97.4%) in WWTP in both seasons and was determined as the antibiotic compound that was least affected by the seasons. As a result, it has been observed that the removal performance of advanced biological WWTP for antibiotics varies significantly based on the seasons and is insufficient for preventing the spread of antibiotics to the receiving environments.

Declaration of Ethical Standards
The authors declare that they comply with all ethical standards.

Credit Authorship Contribution Statement
Author-1: Conceptualization, investigation, methodology, sampling and analyzing, validation, visualization, writing – original draft, and writing – review and editing.
Author-2: Investigation, methodology, and writing – review and editing.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement
The authors declare that the main data supporting the findings of this work are available within the article.

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