Monitoring of Amoxicillin and Cephalexin Antibiotics in Municipal WWTPs During Covid-19 Outbreak: A Case Study in Isfahan, Iran

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ABSTRACT: Antibiotics are non-biodegradable drugs that inhibit the expansion and growth of microorganisms. Especially with the prevalence of Covid-19, the consumption of antibiotics has increased. Therefore, the presence of most prescribed antibiotics from ß-lactams including amoxicillin and cephalexin were studied at two municipal WWTPs in Isfahan. The analytical method was to extract antibiotics from the aqueous phase and then detected them via HPLC/UV. Samples were collected from 2 WWTPs for 13 sampling periods over 2 months between February and March 2020 during the outbreak of Covid-19. In WWTP A, the average concentration of amoxicillin in influent, effluent, and its removal efficiency was 509.64 ± 161.97 µg/L, 352.96 ± 203.88 µg/L, 34.35 ± 31.38%, and the average concentration of cephalexin in influent, effluent, and its removal efficiency was 189.42 ± 176.06 µg/L, 32.6 ± 49.59 µg/L, 78.75 ± 23.81%, respectively. In WWTP B, the average concentration of amoxicillin in influent, effluent, and its removal efficiency was 2134.82 ± 3031.53µg/L, 401.09 ± 205.86µg/L, and 54.82 ± 33.29%, respectively. Also, the average concentration of cephalexin in influent, effluent, and its removal efficiency was 183.69 ± 123.48 µg/L, 23.01 ± 40.71 µg/L, and 87.65 ± 21.76%, respectively. According to Mann–Whitney test results, the concentration of antibiotics in both WWTPs had significant differences (p-value < .05), and according to results from the Spearman test no correlation between removal efficiency of antibiotics with other principles wastewater parameters was observed.

KEYWORDS: Antibiotics, amoxicillin, cephalexin, Covid-19, removal efficiency, wastewater treatment plants

Introduction

In the past decade, the presence of emerging contaminants such as pharmaceutical compounds (PhCs) in the environment, especially aquatic environments, has increasingly received great attention (Tran et al., 2018). Effluents from wastewater treatment plants (WWTPs) are the primary and constant sources of PhCs in aquatic environments (Shraim et al., 2017). The reason is that conventional treatment plants are usually designed to eliminate nitrogen, phosphorous, and biodegradable carbon (Mirzaei, Yunesian, Mesdaghinia, et al., 2018; Shokoohi et al., 2017) and they cannot remove PhCs efficiently (Shokoohi et al., 2017).

One of the most important groups of PhCs, with high usage and worldwide consumption, is antibiotics (Rodriguez-Mozaz et al., 2020). Antibiotics are used to deal with infections in humans or animals (Ngigi et al., 2020), and based on their characteristics, they are of different types (Bilal et al., 2020). On a report by the World Health Organization (WHO), antibiotics induce further antibiotic resistance genes, causing serious environmental pollution, food safety challenges, and ecological toxicity (Rodriguez-Mozaz et al., 2020; Shokoohi et al., 2020). Late the year 2019 saw an outbreak of a respiratory disease called Covid-19, which has since been rapidly progressing worldwide and affecting humans of all ages with a high number of deaths (Miranda et al., 2020). With the prevalence of Covid-19, the consumption of antimicrobial drugs to treat or prevent secondary infections has increased (Chen et al.,

2021). Current overuse of antibiotics could accelerate the emergence of the next global public health crisis caused by the resistance of different types of microorganisms against antibiotics (Chen et al., 2021; Miranda et al., 2020) and scientists are concerned about their side effects (Usman et al., 2020).

Results of the investigation of national and regional monitoring organizations from 71 countries along with analyzed data from scientific writings during the past decade have shown that the use of antibiotics is constantly increasing globally (30%) (Rodriguez-Mozaz et al., 2020). Especially in Asia, which makes up almost 60% of the world's population, antibiotics and other drugs are easily available on the markets (Tran et al., 2018). Iran, in terms of using the drug, is among the top 20 countries in the world (Aali et al., 2020) and about 13% of the pharmaceutical market in this country is made up of antibiotics (Rezazadeh, 2016).

The widespread use of antibiotics has resulted in an increased concern worldwide owing to their recurrent detection in different environmental media such as coastal (Dougherty et al., 2010), surface (Hanna et al., 2018; Ngigi et al., 2020; Shi et al., 2020), and ground (Barnes et al., 2008; Focazio et al., 2008) waters, soil (Hanna et al., 2018), edible part of vegetables (Patel et al., 2019), etc. that have potential hazards for humans and environment (Ngigi et al., 2020).

About 10% to 90% of antibiotics depending on their chemical properties will be excreted out of the body unchanged or as metabolites through urine and body excretions (Shokoohi

et al., 2017). The excreted antibiotics will be delivered into the wastewater (Honda et al., 2018; Shokoohi et al., 2017). Due to water scarcity, climate change, urbanization, and regional drought, the use of WWTPs effluent to irrigate agriculture in arid and semi-arid regions is usual (Pan & Chu, 2017) and especially in Iran, Isfahan, which is in the semi-arid region, there is no exception. Therefore, knowing the fate of antibiotics in WWTP influent and effluent and the removal efficiency of them in WWTPs is important and can estimate their potential impacts on ecology and human health (Kim & Aga, 2007; Lacey et al., 2012).

The concentration of antibiotics in WWTPs effluents greatly depends upon several factors such as the design and operational conditions of WWTP (Kim & Aga, 2007), treatment procedure, hydraulic retention time (HRT), and solids retention time (SRT) (Gao et al., 2012). Also since the usage patterns of antibiotics are different, the types and concentrations of them detected in WWTPs differ from country to country or even from plant to plant (Gao et al., 2012).

The monitoring of antibiotics in WWTPs has been the subject of numerous studies. Some studies have manifested that antibiotics are not eliminated thoroughly in common WWTPs (Chang et al., 2010; Karthikeyan & Meyer, 2006; Mirzaei, Yunesian, Mesdaghinia, et al., 2018; Shokoohi et al., 2017; Shraim et al., 2017; Verlicchi et al., 2013). Sara Rodriguez-Mozaz et al. analyzed the effluent of 13 WWTPs in 7 European countries and detected 17 antibiotics in effluents (Rodriguez-Mozaz et al., 2020). Yungang Shi et al, in China, analyzed 43 antibiotics in WWTP, and among them, 23 antibiotics were found both in influent and effluent (Shi et al., 2020). In Pakistan, Rabeea Zafar et al. detected five antibiotics in the effluents of two WWTPs entered the river. The concentration of detected antibiotics was high and in the range of μg/L (Zafar et al., 2021), much more than found in other counties' effluents (ng/L or less). Reza Shokoohi et al. indicated the presence of amoxicillin, cefixime, and imipenem in the effluent of WWTPs at high concentrations around 1.6 μg/L to 10.7 μg/L (Shokoohi et al., 2017). Researchers in Beijing, China investigated antibiotics in eight WWTPs, and out of 22 antibiotics, 14 were detected. The maximum concentration of antibiotics was 3.1 μg/L in influents and 1.2 μg/L in effluents (Gao et al., 2012). In some studies, the reported concentration of antibiotics in the effluent is higher than influent (Gao et al., 2012; Jelic et al., 2012; Mirzaei, Yunesian, Mesdaghinia, et al., 2018; Yan et al., 2014). Roya Mirzaei et al. found seven antibiotics among of nine antibiotics in both the influent and effluent of two WWTPs in Tehran, Iran, and some of them were detected in effluent but not detected in influent (Mirzaei, Yunesian, Mesdaghinia, et al., 2018).

It should be noted that all studies that have measured the concentration of antibiotics in WWTPs have been conducted before the outbreak of Covid-19 in the world. Studies showed that the consumption of antibiotics has been increased after the outbreak of Covid-19 (Chen et al., 2021; Usman et al.,

2020) and therefore, their concentration in WWTPs is expected to be increased. Nikaeen et al. in a study have shown an increase in the concentration of antibiotics in the WWTPs of Isfahan during the outbreak of Covid-19 (Gholipour et al., 2021). Also, more than 65% of the antibiotics that are being used in the world are ß-lactam antibiotics. In Iran, 32.6% of used antibiotics belong to ß-lactams, and penicillin, amoxicillin, and ampicillin are the most prescribed of them (Rezazadeh, 2016; Yazdanbakhsh et al., 2016). In Isfahan, Amoxicillin and cephalexin are among the most commonly prescribed antimicrobial drugs (Benito-Peña et al., 2009; Verdier et al., 2011). In addition to the high prescription, they are also cheap and most people in Iran buy and use them arbitrarily. Especially the outbreak of Covid-19 along with flu during the winter can lead to overuse of these antibiotics.

Since few studies have determined the concentration of amoxicillin and cephalexin in WWTPs, and there was no study about the concentration of antibiotics in WWTPs influents and their removal efficiency after the outbreak of Covid-19, therefore, this study aimed to monitor amoxicillin and cephalexin antibiotics concentrations in the influent and effluent of municipal WWTPs and evaluate the removal efficiency of them in winter 2019 during the outbreak of Covid-19.

Materials and Methods
Chemicals and reagents

The analytical standards of target antibiotics including amoxicillin and cephalexin were of high purity grade (N99%) and were purchased from Sigma-Aldrich (Steinham, Germany). Sodium phosphate salt also was purchased from Sigma-Aldrich (Steinham, Germany). The HPLC grade acetonitrile (ACN) and acetic acid (85%) were purchased from Merck (Darmstadt, Germany). CA Syringe Filters were purchased from Membrane Solutions Company (U.S.A). Milli-Q water was purchased from SKY Company (Iran).

Sampling sites

This study was performed in February and March 2020, during the peak of the prevalence of Covid-19 in the world and also in Iran, at two municipal WWTPs in Isfahan, Iran. WWTP A has been designated in two modules, and each of them discharges a population of approximately 250,000 inhabitants (500,000 residents in total) and treats 100,000 m³/day of municipal wastewater per day. The treatment procedure is including anaerobic ponds, aeration lagoons, and facultative lagoons. The effluent of facultative lagoons enters the transmission channel from the outlet and is reused. Figure 1 shows the flow diagram of the treatment process in WWTP A.

WWTP B has been designed in two modules, discharges a population of nearly 900,000 inhabitants, and treats 130,000 m³/day of municipal wastewater. The treatment procedure is conventional activated sludge. The effluent of the
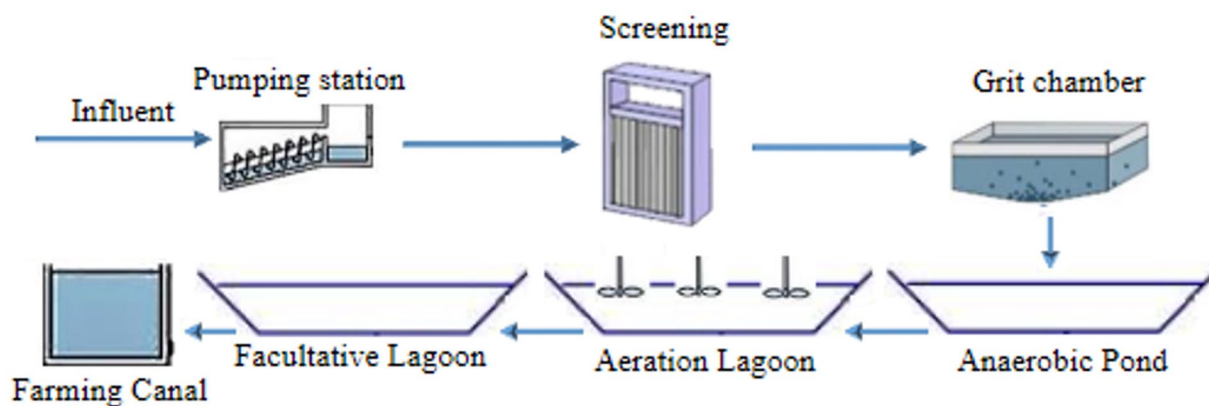
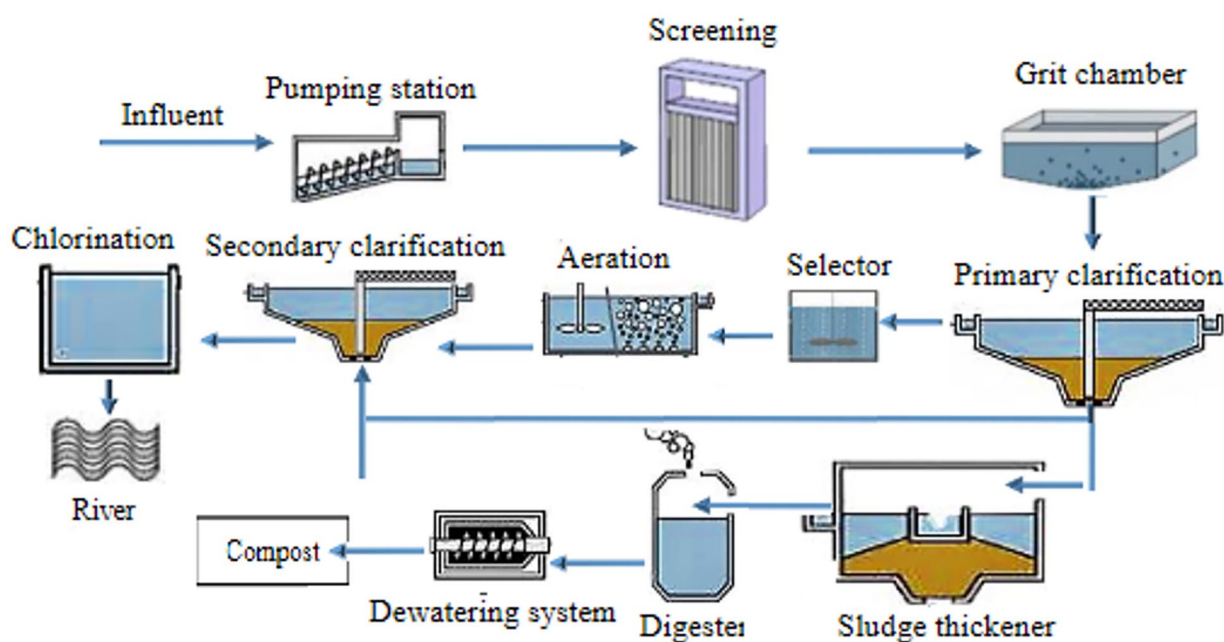**Figure 1.** Flow diagram of the treatment process in WWTP A.

Figure 2. Flow diagram of the treatment process in WWTP B.

secondary clarifier, after chlorination, is discharged into the river. Figure 2 shows the flow diagram of the treatment process in WWTP B. It should be noted that the chlorination basin has not been in operation in WWTP B for several years.

Sample collection procedure

In this study, for 2 months 26 samples were taken from each WWTP according to the standard method, 13 samples from the influent and 13 samples from the effluent, and because two treatment plants were studied, the total number of samples were 52 samples. All samples were collected in the morning at a specific time.

For sample collection, the total used glass was soaked in 10% nitric acid, washed with water, and finally with distilled water before use (Shraim et al., 2017) and left to dry.

Influent samples were collected after screening at both studied WWTPs, and effluent samples were obtained from the transmission channel of facultative lagoons in WWTP A, and the effluent of secondary clarifier in WWTP B. Samples was collected in 1 L salinized dark glass bottles and rinsed twice with the influent or effluent before sampling. The collected samples were stored at 4°C in a cool box on the way to the laboratory (Zafar et al., 2021) and were investigated as soon as entering the lab.

Since the pH of the samples was close to neutral values (pH 7.2–7.6), so there was no need for pH adjustment.

Preparation of standard solutions and samples

Each standard solution of the target antibiotic was prepared freshly in a concentration of 1 mg/mL and dissolved in Milli-Q water. To reduce the degradation of antibiotics, the test tubes containing the stock solutions were covered with aluminum foil and stored at 4°C in the refrigerator (Zafar et al., 2021).

Table 1. Optimal HPLC Conditions for the Analysis of Target Antibiotics, Their Related Calibration Curves, and Detection Limits.

ANTIBIOTICS	WAVELENGTH (nm)	RETENTION TIME (min)	CALIBRATION CURVES		LOD (µg/L)	LOQ (µg/L)	SY	RECOVERY (%)
			EQUATION	R^2				
Amoxicillin	237	4/475	$y = 1419.7x - 79037$	.9905	31.3	94.85	13465.64	91.3
Cephalexin	274	6/908	$y = 1667.2x + 5140.6$	.9996	6	18.18	3031.27	93.5
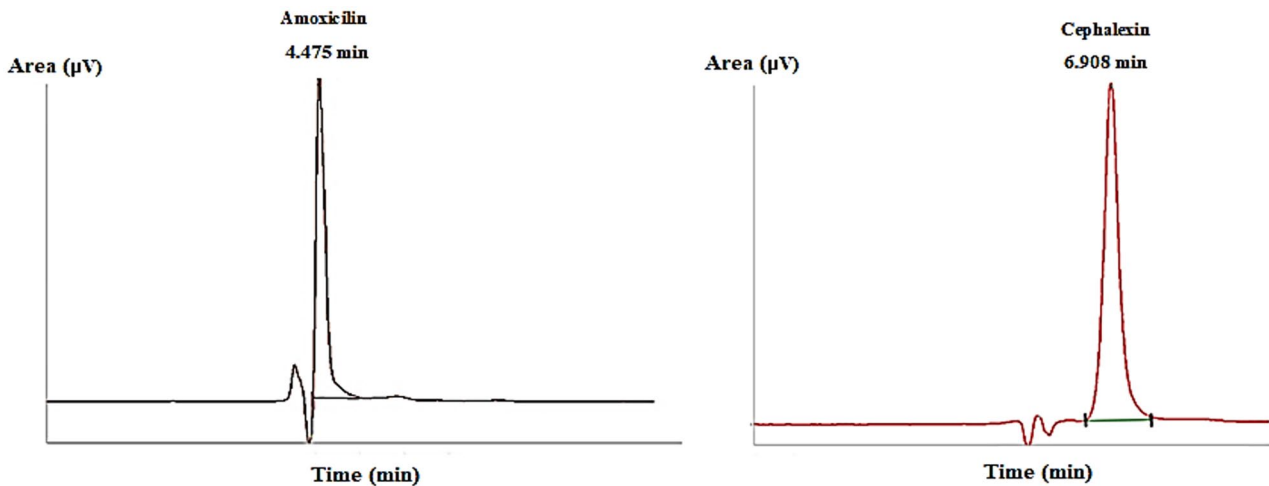
Figure 3. The chromatogram diagrams of target antibiotics.

All samples were centrifuged at 4,000 rpm for 5 minutes. After Centrifugation, the liquids of different densities were separated and the solution that contains a contaminant is called a transparent part, which is formed on the top of centrifuged liquid sample. Then the transparent part was separated and passed through the CA Syringe Filters. Then, 1 mL of the filtered samples was injected into HPLC-UV.

Analytical method

The concentration of target antibiotics including amoxicillin and cephalexin in the samples was determined by an HPLC system (Jasco PU-2080, Tokyo, Japan) which was equipped with a quaternary mixing pump, an inline vacuum degasser, automatic injector (AS-2055 Plus), a C18 column (150 × 4.6 mm, Germany), packed with 5 µm particles for the separation, and an UVVis detector (UV-2075 plus).

The mobile phase that was used contains a gradient of Milli-Q water, acetonitrile, NaH_2PO_4 1M, and acetic acid 1N (909:80:10:1v/v).

Multiple runs were performed with different conditions including flow rate, run time, and wavelength to analyze each target antibiotic. The HPLC method for each analyte was presented in Table 1.

Method validation

Method validation was based on the linearity response of the results regression coefficient or R^2 which is calculated using

Excel software. Based on available sources, a regression coefficient above .99 is the best condition to ensure the linearity of the results and as mentioned in Table 1, for our target antibiotics R^2 was above .99 which indicates appropriate accuracy.

The next indicator to ensure the accuracy of the measurements is to calculate the LOD (limit of detection) and LOQ (limit of quantification), and all obtained results should be higher than them. LOD and LOQ would be calculated according to equations (1) and (2) as follow:

$$LOD = 3.3(\frac{S_y}{S}) \tag{1}$$

$$LOQ = 10(\frac{S_y}{S}) \tag{2}$$

In equations (1) and (2), S_y is the standard deviation of the response, and S refers to the slope of the calibration curve. In the end, according to equation (3), the total method recovery will be calculated.

$$Recovery\,(\%) = \frac{Real\ Concentration}{Artificial\ Concentration} \times 100 \tag{3}$$

The results obtained from the validity of the results are summarized in Table 1. The chromatogram diagrams of target antibiotics obtained from the HPLC method for targeted antibiotics can be seen in Figure 3.

Calculation of removal efficiency in WWTPs

In the present study, the concentration of target antibiotics in influent and effluent of WWTPs were obtained and then according to the results removal efficiency of antibiotics was calculated. The removal efficiency of the target antibiotic in the aqueous phase was calculated using equation (4) (Zhou et al., 2013):

$$\text{Removal Efficiency}(\%) = \frac{C_0 - C}{C_0} \times 100 \qquad (4)$$

In equation (4), C_0 refers to the concentration of the target antibiotic in the influent and C is the concentration of the target antibiotic in the effluent. In cases where the concentration of antibiotics was not quantified in effluent samples due to their concentrations below the corresponding LOD, the value of $\frac{LOD}{\sqrt{2}}$ was considered to perform analysis (Verburg et al., 2019).

Statistical analysis

Data analysis was performed using SPSS software version 20, and average, mean, minimum, maximum, and standard deviation were calculated.

Then, to check the normality of the data, the Kolmogorov-Smirnov test was performed. To investigate the significant dissimilarities among the concentrations of antibiotics in influent at both WWTPs Mann–Whitney test was used. Also, the correlation of data was examined by the Spearman method at a 95% confidence level.

Results

Concentrations of target antibiotics in WWTPs

Target antibiotics in real samples were quantified in the aqueous phase of the two WWTPs with a detection frequency of 100%. The concentrations of target antibiotics including amoxicillin and cephalexin in WWTPs A and B during the study period are presented in Figure 4.

In WWTP A, the average influent concentration of amoxicillin and cephalexin were 509.64 ± 161.97 and $189.42 \pm 176.06\,\mu g/L$, respectively. Also, the average effluent concentration of amoxicillin and cephalexin were 352.96 ± 203.88 and $32.6 \pm 49.59\,\mu g/L$, respectively. In WWTP B, the average influent concentrations of target antibiotics were $2{,}134.82 \pm 3{,}031.53$ (amoxicillin) and $183.69 \pm 123.48\,\mu g/L$ (cephalexin), and the average concentration of amoxicillin in the effluent was $401.09 \pm 205.86\,\mu g/L$ and cephalexin $23.01 \pm 40.71\,\mu g/L$. As can be seen in Figure 4(A1) in WWTP A, the maximum influent concentration of amoxicillin was $718.44\,\mu g/L$ and the minimum influent concentration was $138.99\,\mu g/L$

and the maximum effluent concentration of amoxicillin was $674.6\,\mu g/L$ and the minimum effluent concentration was $22.13\,\mu g/L$. For cephalexin in WWTP A, according to Figure 4(A2) the maximum influent concentration was $655.21\,\mu g/L$ and the minimum influent concentration was $29.7\,\mu g/L$ and the maximum and minimum effluent concentration was 184.48 and $4.24\,\mu g/L$, respectively. In WWTP B, as could be seen in Figure 4(B1), the maximum influent concentration of amoxicillin was $9032.41\,\mu g/L$ and the minimum influent concentration was $501.77\,\mu g/L$ and the effluent concentration of amoxicillin was 801.17(maximum) and 122.47(minimum)$\mu g/L$, respectively. Figure 4(B2) shows the maximum concentration of cephalexin $425.43\,\mu g/L$ (influent) and $116.4\,\mu g/L$ (effluent) and the minimum concentration of cephalexin $78.61\,\mu g/L$ (influent) and $4.24\,\mu g/L$ (effluent), respectively. As can be seen in Figure 4(C1) and (C2), in both WWTPs amoxicillin had the highest and cephalexin had the lowest concentration in the influent of both WWTPs. In WWTP A, the median influent concentration of amoxicillin and cephalexin was 558.66 and $118.9\,\mu g/L$, and the median influent concentration in WWTP B was $862.2\,\mu g/L$ (amoxicillin) and $136.5\,\mu g/L$ (cephalexin), respectively. The results showed that amoxicillin has a higher consumption than the rate of cephalexin in Isfahan.

Removal percentage of target antibiotics in two WWTPs

The removal percentage of target antibiotics in WWTPs A and B are shown in Figure 5. In WWTP A, the average removal efficiency of cephalexin is $78.75\% \pm 23.18\%$ and amoxicillin is $34.35\% \pm 31.38\%$. In WWTP B the average removal efficiency of cephalexin is $87.65\% \pm 21.76\%$ and amoxicillin $54.82\% \pm 33.29\%$.

Statistical analyses of antibiotic concentrations in WWTPs

To check the normality of the data Kolmogorov–Smirnov Test was performed. It should be noted that according to the *p*-Value (*p*-Value < .05) in both treatment plants most of the data do not follow the normality distribution. Therefore, the nonparametric tests were applied for statistical analysis. Therefore, a Mann–Whitney test was performed and the concentration of two target antibiotics was compared at both WWTPs. The results of the Mann–Whitney test are given in Table 2.

To Investigate the correlation between target antibiotics and other main parameters of wastewater including wastewater flow rate (Q), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and total suspended solids (TSS) which may affect the removal efficiency of antibiotics as well as their concentration in two studied WWTPs, Spearman's correlation was performed and results are presented in Table 3. It
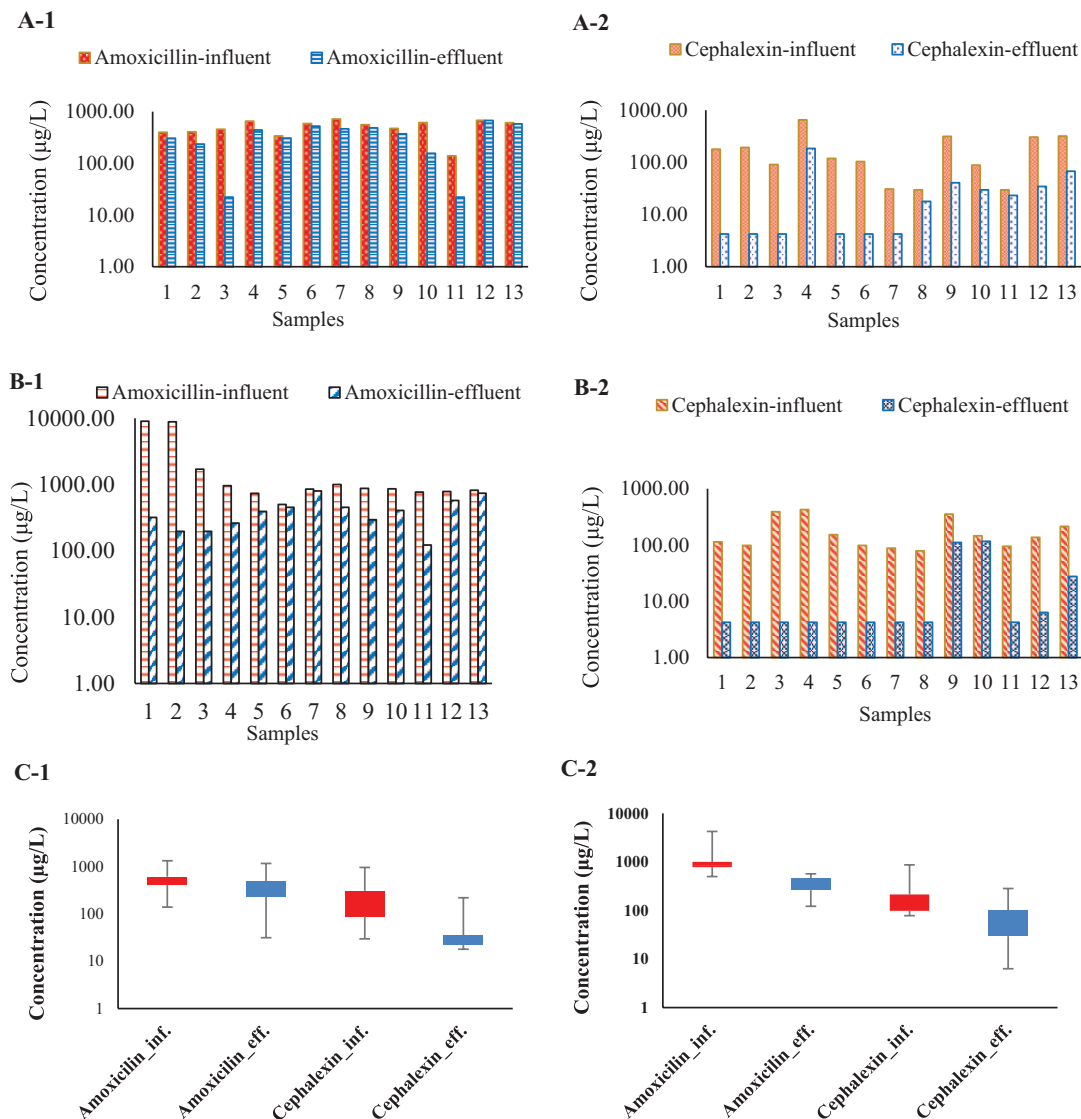
Figure 4. (A-1) Concentration of amoxicillin in WWTP A, (A-2) Concentration of cephalexin in WWTP A, (B-1) Concentration of amoxicillin in WWTP B, (B-2) Concentration of cephalexin in WWTP B, (C-1) Box plot diagram of concentration of target antibiotics in WWTP A, (C-2) Box plot diagram of concentration of target antibiotics in WWTP B.

should be noted that wastewater main parameters (Q, BOD$_5$, COD, TSS) were obtained from the laboratory of WWTPs and they are presented in Figure 6.

Spearman correlation test was also performed between the removal efficiency of target antibiotics and the removal efficiency of other main parameters (BOD$_5$, COD, TSS) in WWTPs A and B, and the results are presented in Table 4.

Discussion

Amoxicillin is the most widely used antibiotic and over 80% of oral administration of it would be excreted out of the body after 2 hours of consumption and studies show its presence in surface water, domestic and industrial wastewater, and hospital wastewater in concentration ranges of ng/L to mg/L (Chaba & Nomngongo, 2019) and our findings were at the concentration of µg/L. In other studies, Ngoc Han Tran et al. reported the average concentrations of amoxicillin in influent 6.516 µg/L
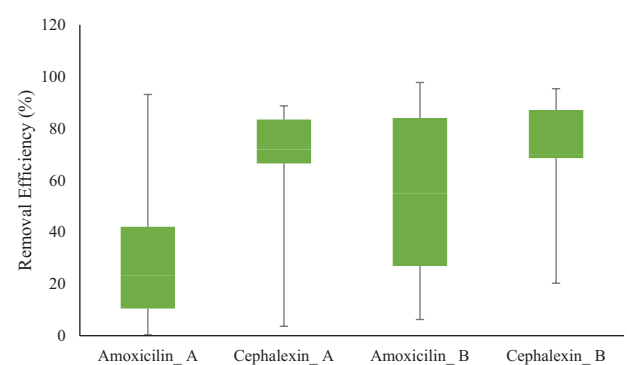
Figure 5. The removal efficiency of target antibiotics in WWTPs A and B.

and effluent 1.670 µg/L in Asian countries, and 0.190 µg/L in the effluent of European countries (Tran et al., 2018). In comparison with the concentration of antibiotics reported in this study, the concentration of them in influent and effluent

Table 2. Independent-Samples Mann–Whitney *U* Test Results for Antibiotics in WWTPs A and B.

PARAMETERS	AMOXICILLIN	CEPHALEXIN
Total *N*	26	26
Mann–Whitney U	138.000	93.000
p-Value	.000	.687

Isfahan WWTPs is too high. In the other study, Sara Rodriguez-Mozaz et al. analyzed the concentrations of antibiotics in the effluents of 13 municipal WWTPs in European countries and cephalexin was detected at concentrations ranging from $0.037\,\mu g/L$ to $0.308\,\mu g/L$ in 10 sample sites (Rodriguez-Mozaz et al., 2020). Amjad Shraim et al. reported that the average concentration of cephalexin in influent and effluent was $1.88\,\mu g/L$ and $1.55\,\mu g/L$, respectively (Shraim et al., 2017). Based on published data, the rate of antibiotic prescription in developing countries is much higher than in developed countries (Alighardashi et al., 2014). In total, the consumption of antibiotics in Iran reaches 16 times the world standard (Aali et al., 2020; Neisi et al., 2017). According to the results, it is observed that the average concentration of target antibiotics in influent and effluent municipal WWTPs in Isfahan is much higher than those from other studies. Ngoc Han Tran et al. in a study have shown that the concentration of PhACs, especially antibiotics, in wastewater and effluent of Asian countries is more than in European and North American countries (Tran et al., 2018). Considering that in Isfahan city, hospitals wastewaters are dumped into municipal wastewater after a pre-treatment, it can be concluded that this high concentration may have been due to the use of more antibiotics, especially during the Covid-19 period. It should be noted that other studies were conducted before the prevalence of Covid-19 viruses and the reason for the high concentration of antibiotics in aimed WWTPs may be the prevalence of Covid-19 and the increased consumption of amoxicillin and cephalexin antibiotics. An increase in using of antibiotics during the outbreak of Covid-19 has been reported in scientific articles (Chen

et al., 2021; Miranda et al., 2020). Especially in Isfahan, where our studied plants are located, Nikaeen et al. reported an increase in the concentration of antibiotics in the wastewater of Isfahan city during the outbreak of Covid-19. It was mentioned that based on the distribution of patients and hospitals in Isfahan, the highest inflow of wastewater from Covid-19 patients had been to WWTP B, followed by WWTP A (Gholipour et al., 2021). Therefore, it is expected that the concentration of antibiotics in WWTP B influent should be higher, which is completely true and the concentration of target antibiotics in WWTP B is higher than WWTP A, and the results obtained are consistent with this research. Table 5. was prepared to compare the concentration of antibiotics studied in the different aqueous environments. Also, the measurement time based on the prevalence of Covid-19 is given in this table.

In terms of removal efficiency, municipal wastewater treatment plants are not able to completely remove pharmaceutical compounds, especially antibiotics, and the removal efficiency in each municipal wastewater treatment system is different. In most previous studies, the removal efficiency of antibiotics in municipal WWTPs is less than 80%, and rarely removal efficiency of 100% has been observed (Shokoohi et al., 2017). In both studied WWTPs, the removal efficiency of cephalexin is higher than amoxicillin. In general, it seems that WWTP B was better in removing the target antibiotics. Unfortunately, at the time we collected samples, the chlorination unit was not in operation and we could not estimate the effect of chlorination on removing antibiotics. Studies showed that in the case of using chlorination, antibiotics can transform into more hazardous compounds and disinfection by-products (DBPs) which may have potentially hazardous effects (Guo et al., 2021; Jaén-Gil et al., 2020). Ian Zammit et al. in a study have shown that irrigating soils with treated wastewater that was disinfected using chlorination resulted in higher values of intI1(Escherichia coli) and higher levels for bla_{OXA-10} (a resistance gene) compared to before irrigation (Zammit et al., 2020).

In some previous studies, activated sludge is mentioned as a useful and available technology to eradicate pharmaceuticals from wastewater systems (Park & Oh, 2020; Pilli et al., 2020;

Table 3. Spearman's Correlation Among Wastewater Parameters in Influent.

PARAMETERS	WWTP A		WWTP B	
	AMOXICILLIN	CEPHALEXIN	AMOXICILLIN	CEPHALEXIN
Amoxicillin$_{in}$	1.000	0.209	1.000	0.058
Cephalexin$_{in}$	0.209	1.000	0.058	1.000
Q$_{in}$	−0.304	−0.119	0.728**	0.265
BOD$_{5in}$	0.275	0.007	0.270	0.645*
COD$_{in}$	0.275	0.007	0.621*	0.275
TSS$_{in}$	0.617*	−0.087	−0.198	−0.179

**Correlation is significant at the .01 level. *Correlation is significant at the .05 level.
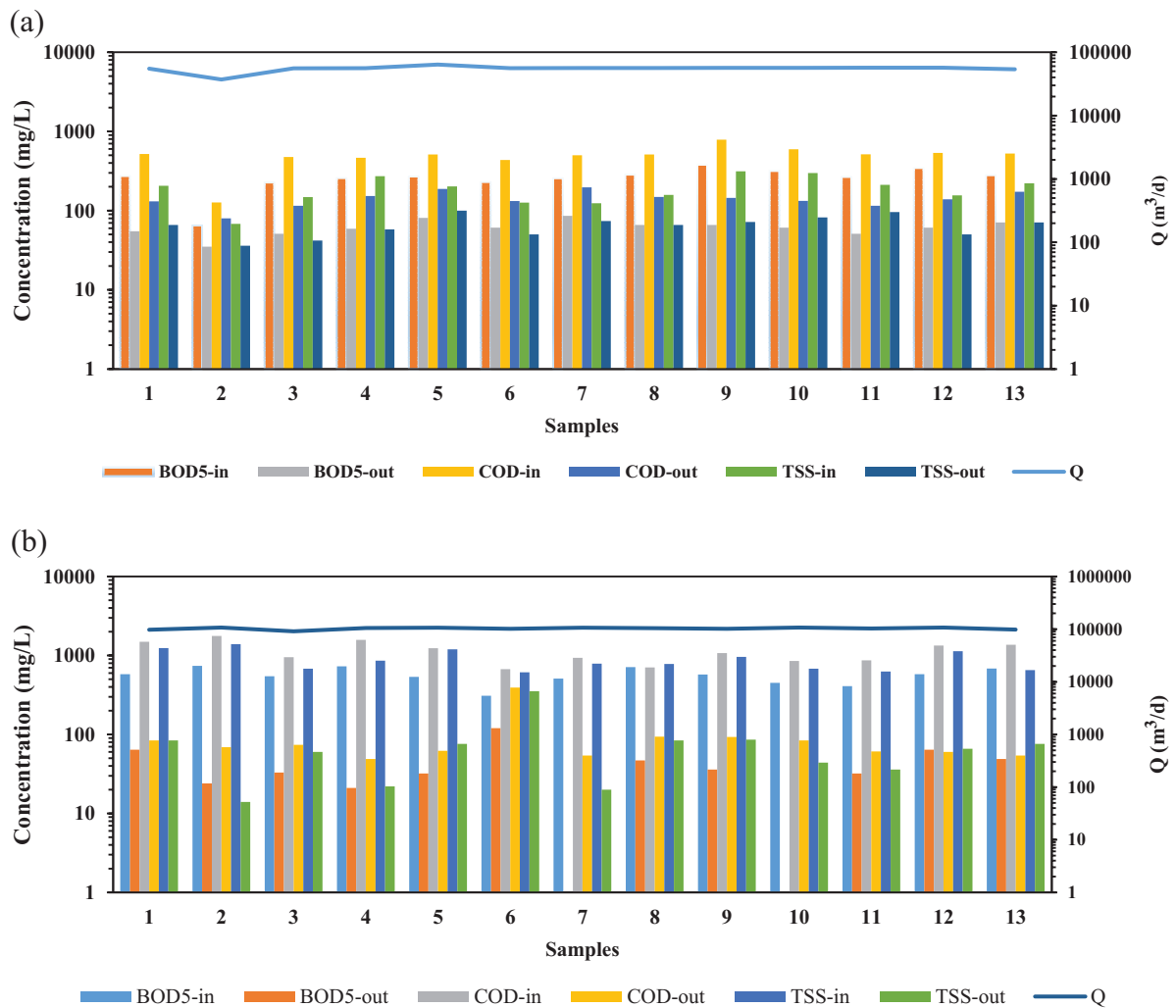
Figure 6. Wastewater treatment plants' main parameters: (a) WWTP A and (b) WWTP B.

Table 4. Spearman's Correlation Among Removal Efficiencies in WWTPs.

PARAMETERS	WWTP A		WWTP B	
	REMOVAL OF AMOXICILLIN	REMOVAL OF CEPHALEXIN	REMOVAL OF AMOXICILLIN	REMOVAL OF CEPHALEXIN
Removal of Amoxicillin	1.000	−0.511	1.000	0.041
Removal of Cephalexin	−0.511	1.000	0.041	1.000
Removal of BOD_5	0.215	−0.141	0.047	−0.006
Removal of COD	0.255	−0.174	−0.028	0.713**
Removal of TSS	−0.174	−0.315	0.423	0.283

**Correlation is significant at the 0.01 level.

Quintelas et al., 2020; Wang et al., 2020) and our findings showed that WWTP B by activated sludge system has removed antibiotics better than WWTP A. In other studies, It was estimated that the average removal efficiency of antibiotics in municipal WWTPs was 57% to 95% (Shi et al., 2020). Despite the high concentration of antibiotics in the effluent in this study, the removal efficiencies of target antibiotics are in this range, and it seems that the two studied WWTPs have

performed well at removing antibiotics. Reza Shokoohi et al. estimated the removal efficiency of amoxicillin in the municipal wastewater treatment plant of Hamedan at 55.66% (Shokoohi et al., 2017), but in general, it seems that both our studied WWTPs have performed poorly in removing amoxicillin. Roya Mirzaei et al. reported the average removal efficiency of amoxicillin in the Ekbatan WWTP at 75.21%, and 65.46% in other WWTP in Tehran (Mirzaei et al., 2018). In

Table 5. The Concentration of Target Antibiotics in the Different Aqueous Environments Before and After the Outbreak of COVID-19.

ANTIBIOTICS	COUNTRY AND CITY	SOURCE OF DETECTION	CONCENTRATION (μG/L)	TIME OF DETECTION	REFERENCE
Amoxicillin	Iran-Tehran	Influent WWTP	0.1145-0.5863	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al. (2018)
	Iran-Tehran	Effluent WWTP	0.02452-0.09457	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al. (2018)
	Iran-Tehran	River	0.01555	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al. (2018)
	Iran-Hamedan	Influent WWTP- Activated sludge	1.6	Before COVID 19	Shokoohi et al. (2017)
	Iran-Hamedan	Effluent WWTP- Activated sludge	0.75	Before COVID 19	Shokoohi et al. (2017)
	Iran-Karaj	River	2.305	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Iran-Karaj	Influent WWTP	7.24	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Iran-Karaj	Effluent WWTP	5.289	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Iran-Isfahan	Influent- WWTP A- Stabilization ponds	509.64	After COVID 19	This study
	Iran-Isfahan	Effluent- WWTP A- Stabilization ponds	352.96	After COVID 19	This study
	Iran-Isfahan	Influent- WWTP B- Activated sludge	2134.82	After COVID 19	This study
	Iran-Isfahan	Effluent-WWTP B- Activated sludge	41.09	After COVID 19	This study
Cephalexin	Iran-Tehran	Influent WWTP	0.122-0.46	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al (2018)
	Iran-Tehran	Effluent WWTP	0.02893	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al (2018)
	Iran-Tehran	River	0.1844	Before COVID 19	Mirzaei, Yunesian, Mesdaghinia, et al (2018)
	Iran-Karaj	River	2.325	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Iran-Karaj	Influent WWTP	6.799	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Iran-Karaj	Effluent WWTP	4.608	Before COVID 19	Mortazavi and Norozi Fard (2017)
	Saudi Arabia-Almadinah Almunawarah	Influent WWTP	0.00188	Before COVID 19	Shraim et al. (2017)
	Saudi Arabia-Almadinah Almunawarah	Effluent WWTP	0.00153	Before COVID 19	Shraim et al. (2017)
	Iran-Isfahan	Influent- WWTP A- Stabilization ponds	189.42	After COVID 19	This study
	Iran-Isfahan	Effluent- WWTP A- Stabilization ponds	32.6	After COVID 19	This study
	Iran-Isfahan	Influent- WWTP B- Activated sludge	183.69	After COVID 19	This study
	Iran-Isfahan	Effluent-WWTP B- Activated sludge	23.01	After COVID 19	This study

previous studies, the efficiency of the conventional activated sludge process at amoxicillin removal was predicted at 84%, and the removal efficiency of cephalexin was 76% (Mirzaei et al., 2018). Therefore, it seems that WWTP B has removed cephalexin relatively well by the activated sludge process, but it has performed poorly at removing amoxicillin. Recent studies in Saudi Arabia estimated the removal efficiency of cephalexin in the Almadinah Almunawarah municipal WWTP at 18.16% (Shraim et al., 2017), comparing this number with the removal efficiencies obtained in this study, cephalexin removal efficiency in both treatment plants is more effective. The reason is that cephalexin is a biodegradable antibiotic and in Tehran WWTP with an activated sludge process the removal efficiency that has been reported for this antibiotic was approximately 100% (Mirzaei et al., 2018).

According to the results of the Mann–Whitney test, which are given in Table 2, it is clear that there is no significant difference between the concentration of cephalexin in WWTP A and B. But the concentration of amoxicillin in the two WWTPs has a significant difference. The reason is that wastewater from hospitals of Isfahan is discharged to WWTP B and also amoxicillin is among the most commonly used oral antibiotics that are easily available on the markets.

According to the results of spearman's correlation presented in Table 3, it is observed that in WWTP A there is no significant correlation between the concentration of antibiotics in influent and other parameters, but in WWTP B there is more correlation. Especially, there was a significant correlation between the Q_{in} of WWTP B and the concentration of amoxicillin at a 99% confidence level in this WWTP. That is by increasing Q in WWTP B, the concentration of amoxicillin would be increased, too. Also according to the results that are presented in Table 4, in WWTP A, there was no correlation between the removal efficiency of antibiotics and the removal efficiency of other wastewater parameters. In WWTP B no correlation was observed between the removal efficiency of antibiotics and the removal efficiency of none of the parameters and there was only a significant correlation between the removal efficiency of cephalexin and the removal efficiency of COD at a 99% confidence level. The reason for the different correlations in the two studied WWTPs maybe is due to the different treatment processes of the two studied WWTPs.

Conclusion

The concentrations of antibiotics were monitored during the outbreak of Covid-19 in 2 WWTPs of Isfahan and it is observed that the average concentration of target antibiotics including amoxicillin and cephalexin in influent and effluent Isfahan WWTPs is higher than other studies, and this indicates the careless use of antibiotics in Iran and Isfahan. In most previous studies from other countries, especially developed countries, the concentration of antibiotics in WWTPs is in the range of ng/L or even less. However, in the present study, the concentration of antibiotics is too high. It should be noted that,

except for clinical usage, unfortunately, antibiotics are easily available on Iranian markets. Specifically, amoxicillin is well known among people and they used it for flu, toothache, and any other infections arbitrarily and the occurrence of Covid-19 can increase the use of antibiotics, too. The removal efficiency of antibiotics in studied wastewater treatment plants was not 100% and the removal efficiency of amoxicillin was very low, 34.35% in WWTP A and 54.82% in WWTP B. Particularly, due to water scarcity in Isfahan, farmers use effluents of WWTPs to irrigate agricultures, and as mentioned before, antibiotics can accumulate in edible parts of the plants. So the presence of this concentration of antibiotics in the effluent is problematic. For example, the consumption of agricultural products irrigated using effluent-containing antibiotics by people or even animals would lead to antibiotics resistance in the future and treatable diseases will be untreatable and cause a lot of deaths. From the statistical point of view, the removal efficiency of the target antibiotics in the two studied treatment plants was completely independent of main wastewater parameters such as influent BOD_5, COD, and TSS, and no significant correlation was observed between the removal efficiency of antibiotics and the removal efficiency of BOD_5, COD, and TSS parameters.

Authors' Contributions

Hossein Movahedian Attar: Supervision, Conceptualization, Review & Editing. Mehri Samandari: Investigation, Writing Original Draft, Formal analysis, Laboratory experiments. Karim Ebrahimpour: Methodology, Experimental advisor. Farzaneh Mohammadi: Data investigation, Statistical analysis, Review & Editing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability

All data analyzed during this study are included in this published article, and the datasets used during the current study (Q, BOD_5, COD, and TSS) are available from the corresponding author on reasonable request.

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