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Evaluation of the Efficiency of Wastewater Treatment Plants in the Removal of Common Antibiotics from Municipal Wastewater in Hamadan, Iran

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Abstract

The presence of antibiotics in the environment, especially aquatic environments, is a major health and environmental concern. Wastewater treatment plants play an important role in the treatment of municipal and industrial wastewater and removal of contaminants. The aim of this study was to determine the concentration of prevalent antibiotics in municipal wastewater of Hamadan, Iran and to evaluate the removal efficiency of wastewater treatment plants. During 3 months (April, May, and June 2016), a total of 12 composite influent and effluent samples were collected from the wastewater treatment plants. Solid-phase extraction (SPE) was used for preparing the samples, which were then analyzed using high-performance liquid chromatography (HPLC) with UV detection. Based on the analysis of 6 antibiotics, three antibiotics, including amoxicillin, imipenem, and cefixime, were detected, and their concentrations were measured at 1.6, 10.7, and 5.8 ug/L, respectively. The removal efficiency of these antibiotics in wastewater treatment plants was 55.66%, 34.01%, and 24.33%, respectively. Due to the presence of examined antibiotics in the effluent and influent wastewater treatment plants, they might cause direct and indirect effects on human health and environment if proper measures are not taken by the authorities. Since the removal of these antibiotics from wastewater treatment plants is relatively poor, it is suggested to use advanced wastewater treatment plants to reduce antibiotics in effluent wastewater and decrease the adverse effects of these micropollutants.

Keywords: Antibiotics, Wastewater Treatment, Municipal Wastewater

1. Introduction

Presence of antibiotics in the environment, especially aqueous media, is a major concern. Antibiotics are generally used to improve human and animal health and promote growth in poultry and aquaculture farms (1). They are poorly absorbed in the body, and large quantities of these agents are disposed (without any changes or with partial changes) through urine and body excretions. They are largely introduced into wastewater channels and can reach the surface and groundwater.

Use of antibiotics in veterinary medicine for the treatment of bacterial infections in animals, besides their application as prophylactic agents, is another source of contamination (2). Low concentrations of antibiotics cause resistance in bacteria and genes (3). In addition, low concentrations of drugs and antibiotics for livestock result in the disruption of reproductive and endocrine glands. The food and drug administration (FDA) is the primary federal agency responsible for the regulation of pharmaceutical and personal-care products in the United States (4). According to previous studies, antibiotic concentrations in hospital and urban wastewater range from 0.3 to 200 μg/L (or above) (5).

Wastewater treatment plants (WWTP) have great contributions to the removal of pollutants through physical, biological, and chemical processes. However, these systems have not been designed to remove micropollutants, such as pharmaceutical pollutants (eg, antibiotics). In previous studies, different rates of antibiotic removal have been reported in WWTP systems, while in some studies, the antibiotic removal percentage has been estimated at 0% (6). In addition, in several studies, removal rates up to 80% and rarely 100% have been reported (7). Overall, antibiotic removal in WWTP systems depends on the refining system and antibiotic type (8).

There are various studies concerning the quantity of pharmaceutical materials and their metabolites in urban wastewater and potable water, while in Iran, few studies have been performed on antibiotics in environmental samples. Since no research has been conducted in Hamadan, Iran to identify antibiotics in urban wastewater, the aim of the present study was to determine common antibiotic concentrations in Hamadan urban wastewater and to evaluate the removal efficiency in WWTP systems.

2. Methods

2.1. Chemicals and Reagents

In the present study, to identify most commonly used antibiotics, hospitals, major pharmacies, stores, and deputies of food and drug, affiliated to Hamadan University of Medical Sciences, were visited. According to the surveys, the most common antibiotics in Hamadan included amoxicillin (a penicillin), cefixime (a third-generation cephalosporin), ciprofloxacin (a fluoroquinolone), erythromycin (a macrolide), and sulfamethoxazole (a sulfonamide).

Standard amoxicillin, ciprofloxacin, erythromycin, sulfamethoxazole, imipenem, and cefixime were purchased from Sigma Aldrich Co. (Germany). HPLC-grade methanol and ultrapure water were supplied by Merck Company (Germany). Moreover, SPE cartridges (absorbent, 200 mg; volume, 6 mL) were provided by Thailand Corporation.

A cellulose acetate filter with a pore size of 0.45 μm, as well as a cellulose acetate syringe filter with a pore size of 0.2 μm, was utilized. In addition, analytical-grade sulfuric acid and disodium ethylene diamine tetra acetate were purchased from Merck Co. (Germany). The main solution for each antibiotic was separately prepared in a mixture of methanol and water (volume ratio, 1:1) at 1 mg/L concentration and stored in a freezer at -10°C.

The mixed standard solutions (concentration range, 10 - 200 μg/L) were prepared using stock solutions in a mixture of methanol and high-purity water (volume ratio, 3:1) if necessary and stored at 4°C. All standard solutions (including stock and work solutions) were stored in a capped 50-μL volumetric balloon at -10°C. Under these conditions, antibiotic activity reached the minimum possible amount, and tendency to bind to divalent ions decreased. Finally, the samples were stored at 4°C until further extraction (9).

2.2. Sampling Method

The present descriptive applied study was conducted during 3 months from March to June 2016. In the middle of each month, two combined 500-cc samples from WWTP influent, as well as a combined sample from WWTP effluent, were collected in Hamadan; the temperature and pH were recorded for each sample. In order to confirm the reliability of some ambiguous findings, the procedures were performed in triplicate.

2.3. SPE

The SPE process was conducted using SPE cartridges with an absorbent (200 mg; volume, 6 mL). Air was pumped from the vacuum flask through a vacuum pump, and cartridges were attached to the inner space of the flask using a 1-mL pipette. The cartridges operated by passing over 4-mL methanol, followed by 6-mL deionized water. Then, 100 mL of wastewater sample (pH, 2.8 - 3) passed through the cartridge at a flow rate of 5 - 8 mL/min under vacuum at 7 - 9 inches of mercury, using extraction manifold. Under these conditions, with regard to the absorbent and antibiotic properties, major compounds were separated from the wastewater matrix and remained in the SPE absorbent. Then, 10 mL of ultrapure water passed away from cartridges for leaching, and cartridges were dried in air for 5 minutes.

Finally, the residual analytes were transferred to glass tubes using 10-mL methanol. The extracts were concentrated up to drying under a nitrogen flow and recovered to a 250-mL volume in a mixture of ultrapure water and methanol solvent for leaching (ratio, 9:1) (9). Following that, the extracts were filtered through a cellulose acetate syringe filter (pore size, 0.2 μm; diameter, 4 mm), transferred to brown vials, and stored at -15°C until analysis. In order to measure antibiotics in the samples, a chromatography system (Agilent 1200, USA), equipped with an autosampler, was utilized (9). Figure 1 indicates the schematic presentation of SPE for the studied antibiotics.

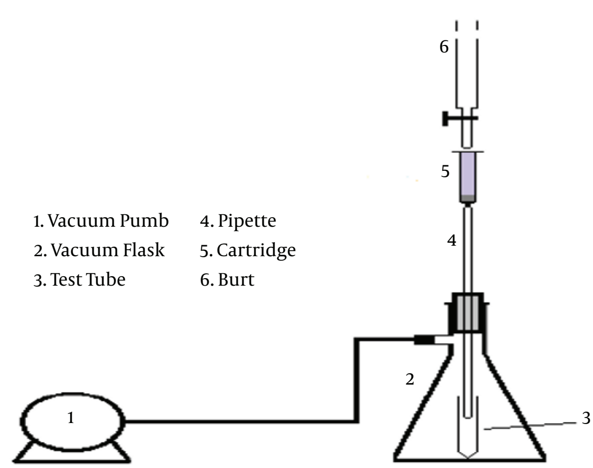

Figure 1. The Schematic Presentation of SPE for the Studied Antibiotics

2.4. Measurement via HPLC

Considering the low accuracy of simultaneous analyses, specific and separate methods were utilized for measuring antibiotics in the present study. A chromatography system (Agilent 1200, USA), equipped with an autosampler, was used. The extracted liquid simultaneously passed through a C18 column (ODS-3; 250 × 4.6 mm; 5 μm), using the mobile phase of acetate (pH, 4) and acetonitrile (ratio, 89:11), along with 0.1% formic acid at 30°C and a flow rate of 0.8 ml/min. The chromatographic conditions, required for each antibiotic, are presented in Table 1. It should be noted that the sample, prior to injection into the column, was filtered using 0.2-μm syringe filters.

2.5. Statistical Analysis

Data were analyzed using SPSS version 22. One-sample Kolmogorov-Smirnov test was used to determine the distribution of the results related to antibiotics in the samples. Then, Kruskal-Wallis test was used to compare the mean of different antibiotics in the sewage samples. Finally, Excel software was used to draw the diagrams.

3. Results and Discussion

3.1. Assessment of Chromatography Validity

In order to calculate the linearity index, different concentrations of each antibiotic were prepared. After analysis and data collection, a calibration curve was plotted, and the regression coefficient index (R^2) was calculated to achieve linearity. For imipenem, amoxicillin, and cefixime, 7 measurement points at 1 - 80, 1 - 70, and 1.5 - 50 μg/L were determined, respectively, to plot the calibration curve and determine the linearity index in the present study.

Calibration curves with an acceptable linearity index were measured at nearly 0.9997 for amoxicillin, 0.9992 for cefixime, and 0.9996 for imipenem, which represent the accuracy of calibration curves and concentrations. For estimating the method recovery (R%), three samples of each water matrix were fortified at 500 ng/L and subjected to an analytical procedure, as mentioned earlier (1).

3.2. Antibiotic Concentration in Urban Wastewater

According to the results, the mean concentrations of amoxicillin, imipenem, and cefixime were 1.6, 10.7, and 5.8 μg/L in WWTP influent and 0.75, 7.54, and 4.42 μg/L in the effluent, respectively; other antibiotics were not identified in the influent or effluent wastewater (Table 2). The efficiencies of the studied WWTP system for the removal of identified antibiotics were measured at 55.66%, 34.01%, and 24.33%, respectively (Figure 2). The chromatogram of antibiotic concentrations is presented in Figures 3 - 5.

As discussed earlier, among the studied antibiotics, only amoxicillin, imipenem, and cefixime were identified in the present study, among which imipenem (a carbapenem) showed the highest concentration. Based

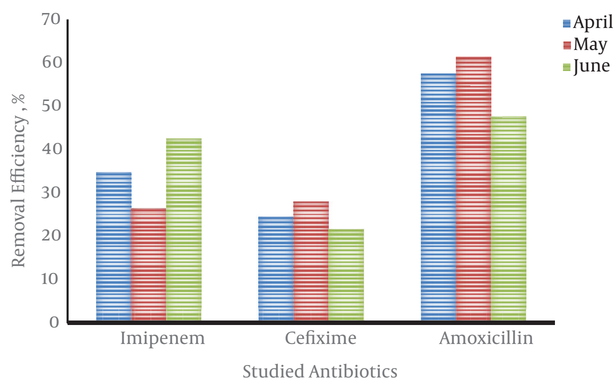

Figure 2. The Efficiency of the Studied WWTP System in the Removal of Identified Antibiotics

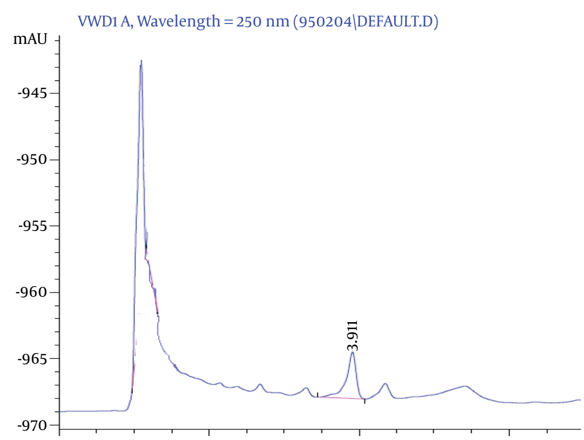

Figure 3. The Output Peak Sample for Amoxicillin

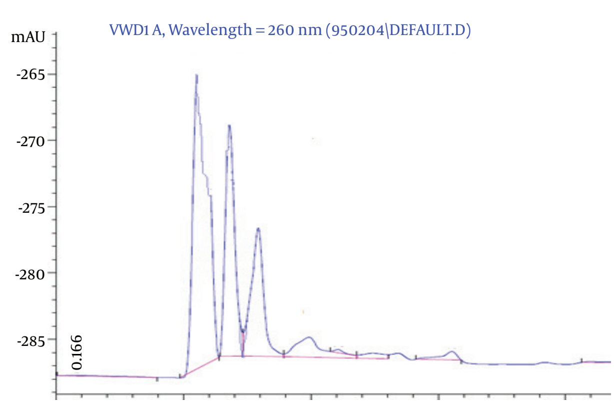

Figure 4. The Output Peak Sample for Imipenem

Table 1. The Chromatographic Conditions

Antibiotics	Injection volume, μL	Flow velocity, mL/min	Column temperature, °C	Wavelength, nm
Cefixime	25	0.9	20	290
Amoxicillin	25	0.9	20	250
Imipenem	25	0.8	20	260
Sulfamethoxazole	25	1	25	235
Ciprofloxacin	25	1	20	279
Erythromycin	25	1	25	210

Table 2. The Concentrations of Studied Antibiotics in Hamadan Urban Wastewater

Antibiotics/Mo		Amoxicillin	Cefixime	Imipenem	Ciprofloxacin	Sulfamethoxazole	Erythromycin
April	Influent	1.86 ± 0.7	6.54 ± 1.5	11.8 ± 2.3	ND*	ND	ND
	Effluent	0.79 ± 0.4	4.94 ± 2.5	7.71 ± 3.5	ND	ND	ND
May	Influent	1.5 ± 0.15	5.7 ± 1.5	10.6 ± 3.4	ND	ND	ND
	Effluent	0.58 ± 0.27	4.1 ± 1.20	7.8 ± 2.39	ND	ND	ND
June	Influent	1.7 ± 0.5	5.4 ± 2.47	9.95 ± 3.5	ND	ND	ND
	Effluent	0.89 ± 0.42	4.23 ± 1.25	5.73 ± 2.6	ND	ND	ND

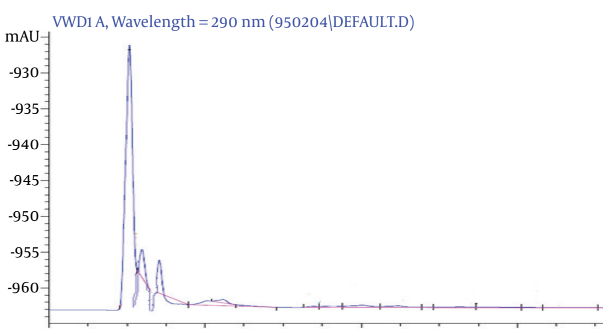

Figure 5. The Output Peak Sample for Cefixime

on the results, β-lactams, which include penicillin, cephalosporin, monobactams, and carbapenems, have the greatest application in human communities. The share of these antibiotics is almost 50% to 70% of total antibiotic consumption (10-12). Moreover, imipenem is an antibiotic from the carbapenem group, with the widest range; it is generally the last resort against antibiotic-resistant organisms.

In a study by Gulkowska et al. in Hong Kong, the highest concentration of antibiotics was associated with cephalexin, a β-lactam, ranging from 0.67 to 2.9 μg/L; on the other hand, erythromycin had the lowest concentration (0.47 μg/L) (13). Moreover, Zu et al. found that trimethorpim concentration ranged from 0.072 to 0.162 μg/L, while lincomycin content ranged from 0.044 to 0.129 μg/L. Overall, the concentrations of 15 studied antibiotics ranged from 4.58 to 942 ng/L (14).

In another study, Guerra et al. measured the mean concentrations of penicillin, azithromycin, and lincomycin to be 0.05, 0.29, and 0.022 μg/L, respectively (15). The mean concentrations of identified antibiotics in the present study were several times higher than other studies, especially those conducted in European countries, which could be attributed to the higher rate of antibiotic consumption in Iran. According to previous studies, each person in Iran consumes drugs 4 times more than the global mean. Moreover, according to reports by the food and drug organization, antibiotic consumption in Iran is equal to the total consumption of all European countries and seems to be growing exponentially. In 2012, 3 billion antibiotics were consumed in Iran, which indicates a 2% growth rate compared to 2011 (16).

Among the studied antibiotics, erythromycin, sulfamethoxazole, and ciprofloxacin were not identified, which could be due to their degradation during collection and/or their lower concentration in comparison with the detection limits of the instruments. In a previous study in Australia, Watkinson et al. found that the lowest concentration of antibiotics in the WWTP influnet was attributed to sulfamethoxazole and trimethoprim (0.36 and 0.34 μg/L, respectively) (17). Sulfamethoxazole, which was not identified in the present study, is a member of the sulfonamide group and a pharmaceutical agent. Moreover, erythromycin is an old drug from the macrolide group, which has been replaced by more novel drugs due to its lower tolerance by patients and similar effects to clarithromycin and azithromycin (12).

The efficiency of the studied WWTP system in the removal of amoxicillin, imipenem, and cefixime was measured at 55.66%, 24.33%, and 34.01%, respectively. As it can be observed, the highest removal rate was associated with amoxicillin. The antibiotic structure, as well as WWTP system type, has a great contribution to antibiotic removal efficiency. In this regard, Angela et al. carried out a study in USA and measured the efficiency of 4 wastewater WWTP systems (range, 33% to 97%). In their study, residence time played an important role in antibiotic removal efficiency. Therefore, a rotating biological contactor (RBC) system with 4-hour residence time showed greater efficiency, compared to a gross oxygen activated sludge system with residence time of 1 hour.

Additionally, in a study from Switzerland, the removal rates of 3 antibiotics, including sulfonamide, macrolide, and trimethoprim, were studied in some WWTP systems, activated sludge, fixed substrate, and membrane reactors (MBRs). The highest antibiotic removal was observed in the MBR WWTP system. The removal rates of macrolide and trimethoprim with residence time of 16 - 33 days exceeded 50% (8). Moreover, in a study performed in New Zealand in 2012, a fixation pond, containing a high level of algae, was utilized for tetracycline removal. The antibiotic removal rate in these pilot systems was reported at 69% (62 days of residence time) (13). Therefore, the removal rates in different studies vary depending on the type of WWTP system and antibiotics.

4. Conclusions

The results of the present study indicated the presence of amoxicillin, cefixime, and imipenem in the influent and effluent WWTP systems at high concentrations. The presence of antibiotics in urban wastewater represents the higher rates of antibiotic consumption in hospitals and other treatment facilities. Increased antibiotic use leads to an increase in the antibiotic resistance of bacterial species, promotes allergies in humans and animals, damages algae, bacteria, and other microorganisms, and disturbs biological refinement of urban WWTP systems. Therefore, serious actions should be taken to decrease and control antibiotic use. In fact, if proper measures are not taken by authorities, unfavorable and uncompensable damages to human health and environment are inevitable. The present study indicated the importance of more intensive investigations to identify antimicrobial compounds and bacterial resistance in aqueous environments, such as potable water or wastewater treatment facilities in Iran. Additionally, since antibiotic removal efficiency in the studied WWTP system was low, advanced wastewater refinement procedures should be utilized for a more effective antibiotic removal to decrease the undesirable effects of micropollutants.

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