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Research Article

Determination and monitoring of tetracycline and degradation products in livestock slaughterhouse wastewater treatment plant effluent

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ABSTRACT

Aim of our study was the determination and monitoring of tetracycline (TC) and degradation products (DEP) in livestock slaughterhouse wastewater treatment plant (SWWTP) effluent. For this purpose, TC and DEP values in SWWTP were investigated. The concentrations of TC and DEP were monitored for 12 months. TC, 4-epitetracycline (ETC), 4-epianhydrotetracycline (EATC), anhydrotetracycline (ATC), and physicochemical parameters of pH, suspended solids (SS), BOD5, COD, and TP were calculated. The maximum TC concentration was determined as 1.68±0.08 µg/L in March and the minimum TC was 1.08±0.05 µg/L in January. The maximum ETC was 2.93±0.14 µg/L in March and April. The minimum ETC was 1.98±0.1 µg/L in January. EATC was 10.82±0.5 µg/L in September, and minimum EATC value was determined as 9.14±0.4 µg/L in March. The maximum ATC value was 8.62±0.4 µg/L in June and the lowest ATC value was 6.61±0.3 µg/L in September. Concentrations of TC and DEP detected in SWWTP effluent were listed in descending order as EATC> ATC> ETC> TC.

1. Introduction

Antibiotics have been widely used in livestock industries as therapy or prophylaxis [1]. Tetracycline (TC) antibiotics are broad-spectrum agents that show activity against many bacteria. Favorable antimicrobial properties of TCs have extended their use in the therapy of animal infections [2]. Thus, the use of TC for purposes of veterinary is higher than for other antibiotics classes [3]. TCs are water-soluble (log Kow<1) and excreted with urine as parent compounds or metabolites [4]. Degradation products (DEP) can be as active and/or toxic as their parent [5, 6]. Excretion rates vary between 40 and 90% [7]. If intracorporal degradation takes place, it often proceeds in feces, but if an antibiotic is not metabolized, recalcitrants persist in the environment [8]. Furthermore, metabolites of antibiotics may be transformed back to the parent compound after the excretion [7, 9].

The slaughterhouse wastewaters include various pollutants. The pharmaceuticals for purposes of veterinary may be present in slaughterhouse wastewater [10, 11]. For example, the presence of TC and DEP in poultry slaughterhouse wastewater was reported [12]. Wastewaters and liquefied animal wastes loaded with veterinary drugs may flow into streams and other waterways through spills, manure runoff, and intentional releases [13]. Several slaughterhouses now have wastewater treatment plants to deal with their effluents in terms of organics, N, and P loads. Most wastewater treatment plants are not designed for removing pharmaceuticals. Pre-treated or treated slaughterhouse effluents are known to be discharged into sewer systems before being discharged to the receiving environment. The presence of drug residues in these wastewaters should be monitored due to environmental problems [14]. The contribution of slaughtering activities to pharmaceutical concentrations in the environment has not been thoroughly researched. The slaughterhouses for various animals (e.g. cows, poultry, and lambs) produce wastewater containing pharmaceuticals [15]. TC and degradation products (DEP) of it that reached to water bodies cause various environmental problems. Due to the low volatility and high hydrophilicity of TC, it tends to accumulate in surface waters [16]. TC and DEP can cause ecological risk and human health damage. Accumulation of TC in water poses a potential threat to aquatic ecosystem health [17]. Furthermore, the resistance of microorganisms is one of

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the problems. For example, TCs have been responsible for the selection of resistant organisms to that antimicrobial family [2, 18]. To date, over 100 classes of antibiotic-resistance genes have been found in bacterial isolates from different environments, with TC resistance genes occupying the greatest percentage of them [1, 19]. Resistance to TC has been a worrisome problem [20].

This study delivers a new study in the literature on monitoring of TC and degradation products (DEP) in the effluent of livestock slaughterhouse wastewater treatment plant during a period of a year. The study also provides a status that could be used as a basis for decisions on future treatment processes for antibiotics from these kinds of wastewaters.

2. Material and Method

TC, 4-epitetracycline (ETC), anhydrotetracycline (ATC) and 4-epianhydrotetracycline (EATC) were analyzed. CH_3OH, CH_2Cl_2, C_2H_3N, and CH_2O_2 were HPLC grade. HCl, NaOH, $NH_3.H_2O$, and Na_2EDTA were grade. SPE cartridges, OHLB, and OMAX (Waters Corporation, USA) were used. A SWWTP in Elazığ, Turkey was chosen as the study site in this study. Elazığ has an important place in the Eastern Anatolia Region in the field of industry. Elazığ animal products industrial zone is the first practice in terms of specialization in Turkey to remove the disorganization and incapacity of the establishments of livestock and to establish integrated facilities which are sensitive to environmental health. This industrial zone started its activities in 1997 [21]. Samples were taken from SWWTP effluent. pH was measured in SWWTP. The samples were transferred to the laboratory and immediately analyzed. pH was determined by a pH meter. SS was calculated according to the Standard Methods. COD and TP values were determined by Nova 60 Spectroquant. BOD_5 values were determined by the Hach Lange DR3800 spectrophotometer. Analyses were done according to Jia et al. [22]. In this study, SPE (solid phase extraction) and LC/MS-MS (liquid chromatography-tandem mass spectrometry) were used. All samples were filtered. 300 mL effluent was added to disodium ethylenediaminetetraacetate dihydrate sodium and brought to pH 3.0 with an acid solution. OHLB cartridge was preconditioned. The sample was passed through that OHLB. The OHLBs were passed from ultrapure water. They were dried with N2. After that, it was eluted with methanol (6 ml). Eluates were dried with N_2. The samples were reconstituted with methanol. Extracts were diluted with ultrapure water. The solutions were then applied to OMAX. The samples were concentrated to 1.5 mL. TC and DEP in SWWTP effluents were determined using UFLC-MS/MS (Shimadzu, AB). For separation of TC and DEP was performed by using C18 column (1.7µm; 2.1mm×100mm). Injection value was 10µL. Mobile phases were acetonitrile (A) and ultrapure water containing CH2O2 (v/v) (B). Data was performed using the IBM SPSS Statistics 21 programme (USA) (n=36). Data were calculated by a two-tailed Pearson correlation test for the investigation of the relationship between TC and DEP and physicochemical parameters in SWWTP effluents.

3. Results and Discussion

Parameters of pH, COD, BOD_5, TSS, and TP determined in SWWTP effluents are given in Figure 1.

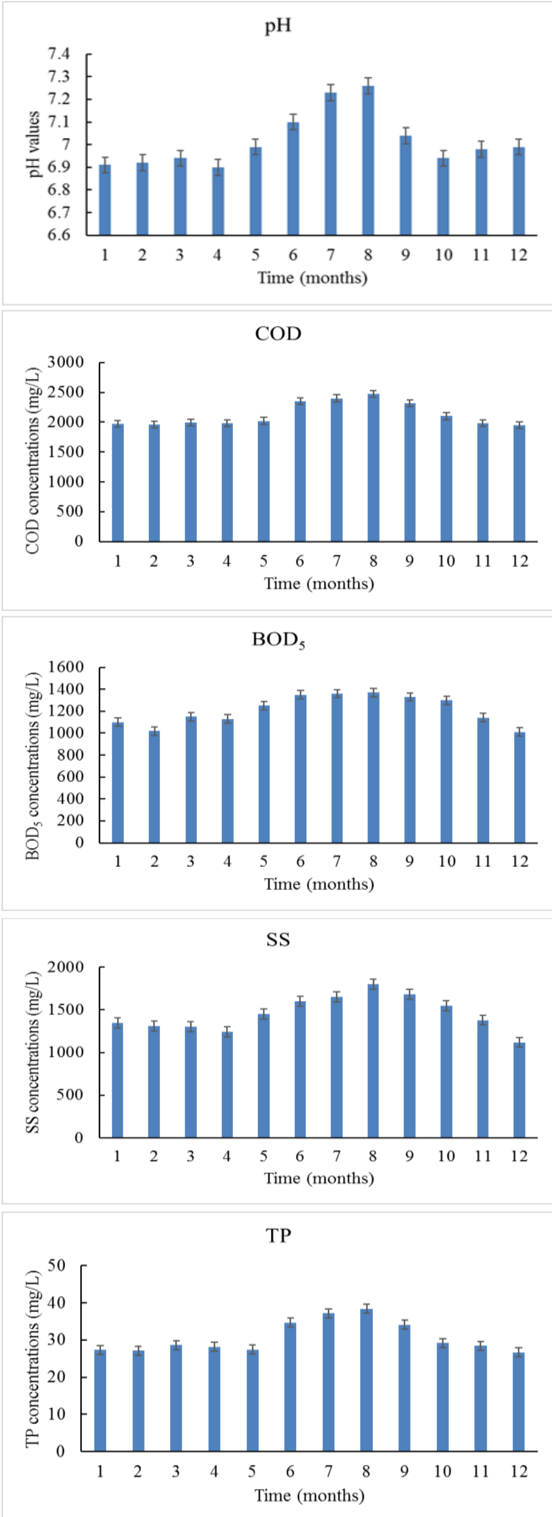

Figure 1. The pH, COD, BOD_5, TSS and TP values in SWWTP effluents

According to Figure 1, when pH values are examined, the maximum pH value was 7.26±0.4 in month 8, while the minimum pH value was 6.90±0.3 in month 4. When COD concentrations were examined, the maximum COD concentration was 2470±12 mg/L in month 8, while the minimum COD concentration was 1950±9 mg/L in month 12. The maximum BOD5 concentration was determined as 1370±17 mg/L in month 8. Also, the minimum BOD5 concentration was 1020±15 mg/L in month 12. When TSS concentrations were examined, the maximum TSS concentration was 1800±36 mg/L in month 8, while the minimum TSS concentration was 1120±15 mg/L in month 12. Similarly, the maximum TP concentration was determined as 38.4±2 mg/L in month 8. Also, the minimum TP concentration was determined as 26.7±1.3 mg/L in month 12. TC concentrations detected in SWWTP effluents are given in Figure 2.

According to Figure 2, TC concentrations determined in SWWTP effluent were determined at different concentrations by month. The maximum TC was 68±3 µg/L in March and the minimum TC was 1.08±0.05 µg/L in January. TC concentrations increased during the 1st month to the 3rd month, and not much change was observed until the 5th month. However, TC concentrations decreased from the 5th month to the 7th month and increased continuously from the 7th month. In the 12th month, the TC concentration was determined as 1.55 µg/L. Similar results were given by Ben et al. [23]. They reported TC concentrations between 1.14 and 4.62 µg/L in swine wastewater in China. Shao et al. [15] reported TC concentrations between 10-210 ng/L in effluents of slaughterhouses in China. These concentrations are lower than the ones detected by ours. In our study, as a result of a year of monitoring of SWWTP effluents, it was determined that TC concentrations received different values by the month. This is due to the change in the composition of wastewater released depending on the number of animals slaughtered in slaughterhouses. ETC concentrations detected in SWWTP effluent are given in Figure 3.

When Figure 3 is evaluated, the maximum ETC concentration in SWWTP effluent was 2.93±0.14 µg/L in March and April. The minimum ETC was 1.98±0.1 µg/L in January. There was an increase in the concentration of ETC (47.98%) from month 1 to month 4 and a decrease (44.33%) from month 4 to month 6. ETC concentrations increased again from the 6th month to the 9th month (25.09%) and decreased after the 9th month (32%). ETC concentrations in SWWTP effluent were determined to differ by month. EATC concentrations detected in SWWTP effluent are given in Figure 4. ATC concentrations detected in SWWTP effluent are given in Figure 5.

When Figure 4 is evaluated. maximum EATC was determined as 10.82±0.5 µg/L in September and minimum EATC in March as 9.14±0.4 µg/L. It was determined that EATC concentrations in SWWTP effluent did not change much according to months. EATC concentrations were found to be high compared to TC and ETC concentrations. When evaluated from this perspective, it is possible to rank as CEATC> CETC> CTC.

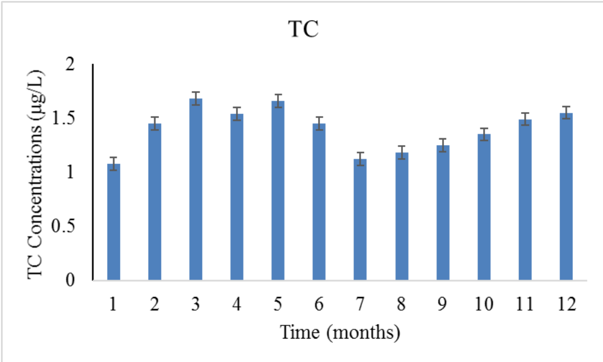

Figure 2. TC concentrations in SWWTP effluents

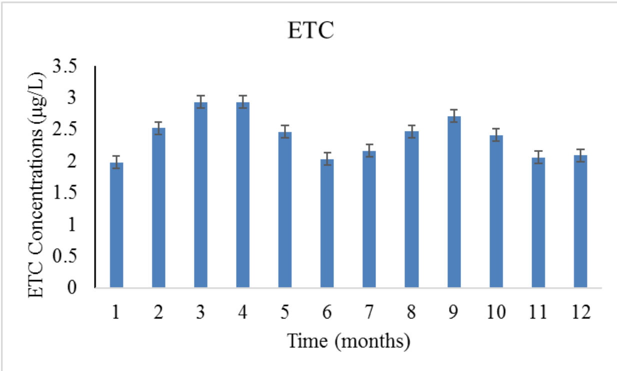

Figure 3. ETC concentrations in SWWTP effluents

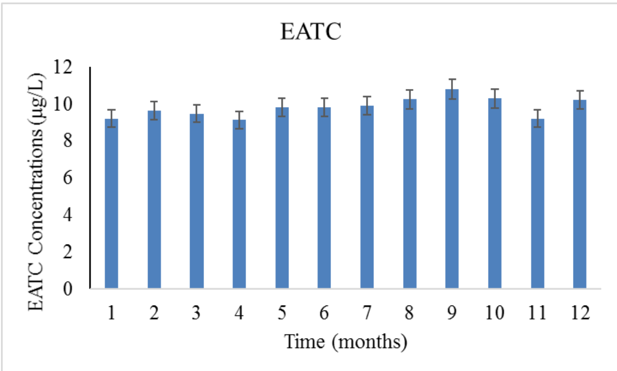

Figure 4. EATC concentrations in SWWTP effluents

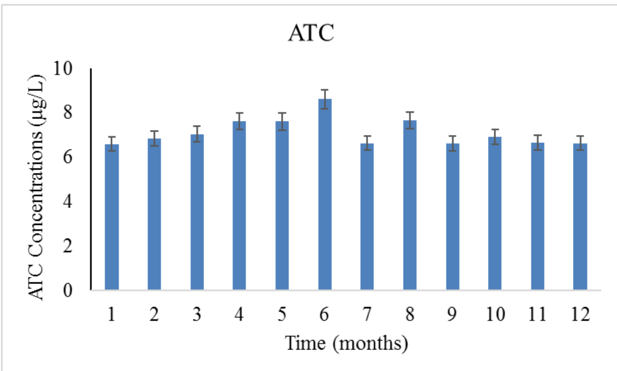

Figure 5. ATC concentrations in SWWTP effluents

When Figure 5 is evaluated, the maximum ATC in SWWTP effluent was 8.62±0.4 µg/L in June, and the lowest ATC in September was 6.61±0.3 µg/L. Similar to TC and ETC, there were increases and decreases in ATC concentrations according to months. ATC concentrations increased from 6.6±0.3 µg/L to 8.62±0.4 µg/L over the 1st month to the 6th month and decreased to 6.64±0.3 µg/L over the 6th month to the 12th month. ATC concentrations can be listed as CEATC> CATC> CETC> CTC compared to TC, ETC, and EATC. The percentages of TC and DEPs detected in SWWTP effluent are shown in Figure 6 and Figure 7.

According to Figure 6, the maximum percentage detected in SWWTP was determined for EATC (49%) in month 1. The minimum percentage was determined for TC (6%) in month 1. ETC and ATC percentages were 10 and 35%, respectively. The percentages of TC and DEPs calculated in SWWTP effluent were TC<ETC<ATC<EATC in month 1. When month 2 was examined, the maximum percentage was 47% (EATC), while the minimum percentage was 7% (TC). ETC and ATC percentages were 12 and 34%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 2. When month 3 was examined, the maximum percentage was 45% (EATC), while the minimum percentage was 8% (TC). ETC and ATC percentages were 14 and 33%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 3. When month 4 was examined, the maximum percentage was 43% (EATC), while the minimum percentage was 7% (TC). ETC and ATC percentages were 14 and 36%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 4. When month 5 was examined, the maximum value was 46% (EATC), while the minimum percentage was 8% (TC). ETC and ATC percentages were 11 and 35%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 5. When month 6 was examined, the maximum value was 45% (EATC), while the minimum value was 7% (TC). ETC and ATC values were 9 and 39%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 6. When month 7 was examined, the maximum value was 50% (EATC), while the minimum value was 6% (TC). ETC and ATC values were 11 and 33%, respectively.

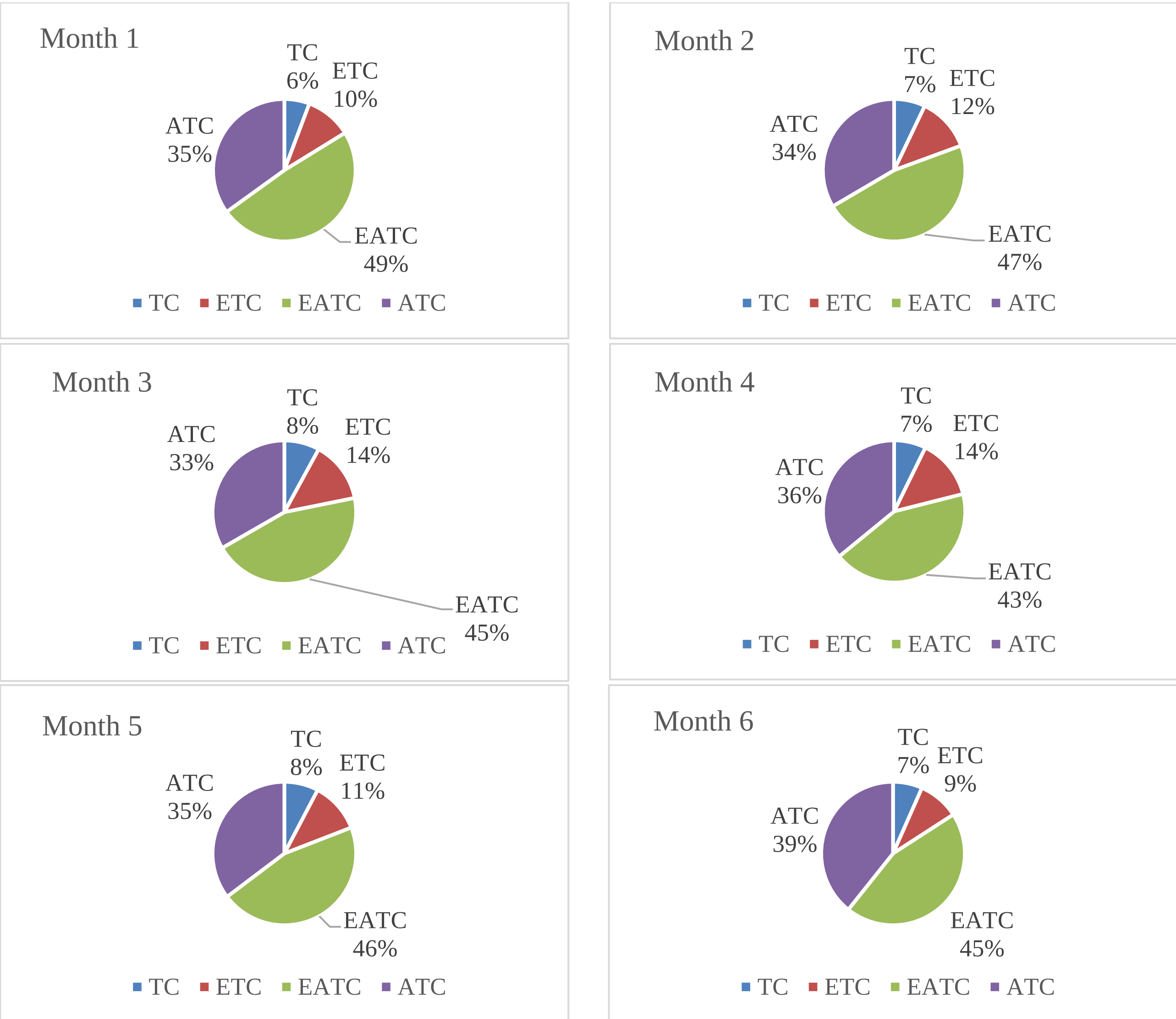

Figure 6. The percentages of TC, ETC, EATC, and ATC detected in SWWTP effluent

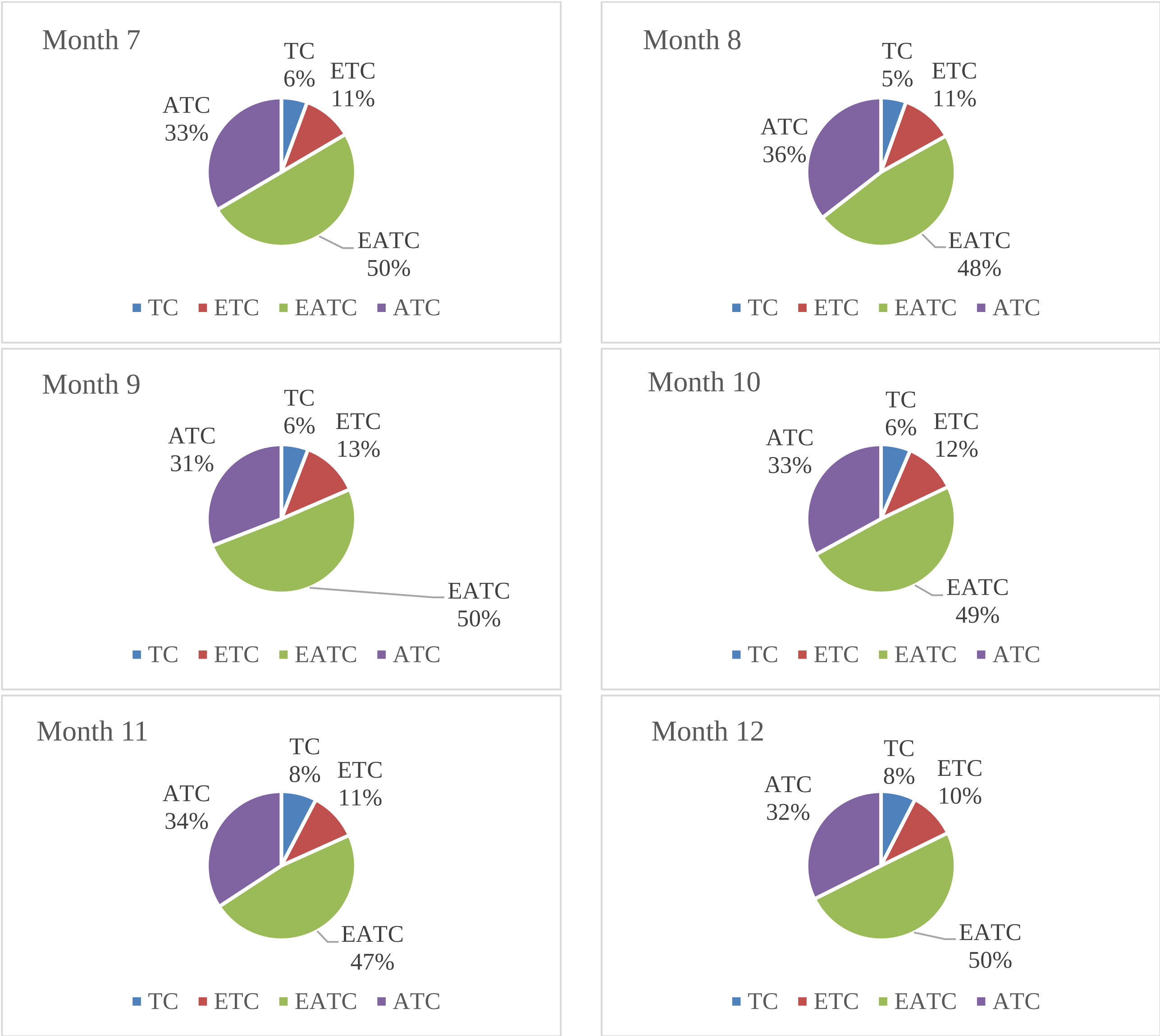

Figure 7. The percentages of TC, ETC, EATC, and ATC detected in SWWTP effluent

According to Figure 7, TC and DEP percentages were TC<ETC<ATC<EATC in month 7. When month 8 was examined, the maximum value was 48% (EATC), while the minimum value was 5% (TC). ETC and ATC values were 11 and 36%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 8. When month 9 was examined, the maximum value was 50% (EATC), while the minimum value was 6% (TC). ETC and ATC values were 13 and 31%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 9. When month 10 was examined, the maximum value was 49% (EATC), while the minimum value was 6% (TC). ETC and ATC values were 12 and 33%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 10. When month 11 was examined, the maximum value was 47% (EATC), while the minimum value was 8% (TC). ETC and ATC values were 11 and 34%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 11. When month 12 was examined, the maximum value was 50% (EATC), while the minimum value was 8% (TC). ETC and ATC values were 10 and 32%, respectively. TC and DEP percentages were TC<ETC<ATC<EATC in month 12. As a result, when TC and DEP were examined, TC, ETC, EATC, and ATC values were between 5-8%, 9-14%, 43-50%, and 31-39%, respectively. The percentages of TC, ETC, EATC, and ATC detected in SWWTP effluent did not change. This situation can be explained by the operation of the treatment plant under optimum conditions. To date, there is no information about the statistical relationship between TC and DEP and physicochemical parameters detected in SWWTP effluents. Therefore, in this study, the statistical relationship between the parameters was determined. The statistical relationship between TC and DEP and physicochemical parameters were given in Table 1.

According to Table 1, there is a negative relationship between TC-EATC (r=-0.257) and ATC-EATC (r=-0.70). The highest relationship occurred between TC and ETC (r=0.379). It was determined that there is an important negative relationship between TC and SS (r=-0.585, p=0.05).

Table 1. The statistical relationship between TC and DPs and physicochemical parameters

		TC	ETC	EATC	ATC	pH	COD	BOD_5	SS	TP
TC	Pear.Cor.	1								
ETC	Pear.Cor.	,379	1							
	Sig. (2-tailed)	,224								
EATC	Pear.Cor.	-,257	,043	1						
	Sig. (2-tailed)	,421	,893							
ATC	Pear.Cor.	,276	,081	-,070	1					
	Sig. (2-tailed)	,385	,802	,830						
pH	Pear.Cor.	-,486	-,241	,457	,244	1				
	Sig. (2-tailed)	,110	,451	,135	,445					
COD	Pear.Cor.	-,567	-,095	,573	,335	,906**	1			
	Sig. (2-tailed)	,055	,768	,052	,287	,000				
BOD_5	Pear.Cor.	-,406	-,002	,511	,398	,730**	,884**	1		
	Sig. (2-tailed)	,191	,994	,089	,200	,007	,000			
SS	Pear.Cor.	-,585*	-,061	,554	,261	,772**	,914**	,930**	1	
	Sig. (2-tailed)	,046	,852	,062	,412	,003	,000	,000		
TP	Pear.Cor.	-,572	-,068	,481	,295	,925**	,986**	,844**	,880**	1
	Sig. (2-tailed)	,052	,833	,113	,352	,000	,000	,001	,000	

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

The highest relationship among parameters was determined as TP-COD (r=0.986, p=0.01). There was a positive and significant relationship between parameters SS-BOD5 (r=0.930), pH-TP (r=0.925), SS-COD (r=0.914), pH-COD (r=0.906), BOD5-COD (r=0.884), TP-SS (r=0.880), TP-BOD5 (r=0.844), pH-SS (r=0.772) and pH-BOD5 (r=0.730), respectively. Also, there was a negative correlation between parameters TC-EATC (r=-0.257), ATC-EATC (r=-0.70), pH-TC (r=-0.486), pH-ETC (r=-0.241), TC-COD (r=-0.567), COD-ETC (r=-0.950), BOD5-TC (r=-0.406), BOD5-ETC (r=-0.02), SS-ETC (r=-0.61), TP-TC (r=-0.572) and TP-ETC (r=-0.68), respectively.

4. Conclusions

TC and DEP (ETC, EATC, and ATC) were detected in SWWTP effluent and monitored for one year. It was determined that TC and its DEP were at the ppb level. TC, ETC, EATC and ATC concentrations detected between 1.68±0.08 - 1.08±0.05 μg/L, 2.93±0.14 - 1.98±0.1 μg/L, 10.82±0.5 - 9.14±0.4 μg/L, and 8.62±0.4 - 6.61±0.3 μg/L, respectively. The concentrations of TC and DEP detected in SWWTP effluent are listed as CEATC> CATC> CETC> CTC. In addition, TC and ETC concentrations varied by month, while EATC and ATC concentrations did not differ much by month. As a result, TC and DEPs were detected in the SWWTP effluent. Considering the environmental impacts of TC and its DEP, it was revealed with this study that the residues in question should be constantly monitored and also determined their concentrations. Furthermore, it should also be added that there is a need for effective treatments that can treat various wastewaters as reported in the literature [24, 25].

Declaration

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. The authors also declared that this article is original, was prepared in accordance with international publication and research ethics, and ethical committee permission or any special permission is not required.

Author Contributions

M. Topal developed the methodology. M. Topal, E. I. Arslan Topal and E. Obek performed the analysis. M. Topal supervised and improved the study. M. Topal, E. I. Arslan Topal and E. Obek wrote the manuscript together. M. Topal proofread the manuscript.

Nomenclature

TC : Tetracycline
ETC : Epitetracycline
EATC : Epianhydrotetracycline
ATC : Anhydrotetracycline
COD : Chemical Oxygen Demand
BOD : Biochemical Oxygen Demand
SS : Suspended Solid

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