INVESTIGATION OF OCCURENCE AND FATE OF BIOCIDES IN WASTEWATER TREATMENT PLANTS AND SURFACE WATERS

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ABSTRACT

INVESTIGATION OF OCCURRENCE AND FATE OF BIOCIDES IN WASTEWATER TREATMENT PLANTS AND SURFACE WATERS

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Biocides are widely used as a preservative or as an antiseptic agent in consumer care products such as toothpaste, mouthwash, and soaps, as well as in household cleaners and even in textiles due to their high antimicrobial effectiveness. The usage of this compounds results in discharge to wastewater treatment plants and so into surface waters.

Their existence in the environment is of importance due to their negative effects on aquatic environment microorganisms and human health in terms of occurrence in surface waters and their fate in wastewater treatment plants.

In this scope, this study focuses on occurrence and fate of selected biocides, namely triclosan (TCS) and chlorhexidine (CHD), in wastewater treatment plants and in surface waters. It was aimed to determine the biocides levels in surface water and wastewater in Turkey. For the wastewater treatment plant (WWTP) studies, several WWTPs with different process configurations, namely, Tatlar WWTP, METU WWTP, Kayseri WWTP and Antalya WWTPs were selected. Composite wastewater samples were taken from various points along the WWTPs on a seasonally basis for one year period. For the surface water part, samples were taken monthly from three different sources with different pollution levels, namely, Kesikköprü Reservoir, Çamlıdere Reservoir and Eymir Lake for one year period. All water samples were analyzed for their biocide level using liquid chromatography, following solid phase cartridge extraction.

As a result of analyses, TCS concentration in surface water samples was detected as in the range of 0.65-11.15 ng/L, 0.86-48.96 ng/L and 0.86-757.7 ng/L for clean, moderately polluted and polluted water sources respectively. The recovery of solid phase extraction analyses for TCS was achieved as %92. CHD concentration was determined as in the range of < 1.33-5.31 ng/L for surface water samples and the recovery of extraction were calculated as %96 for CHD. The concentration of TCS in wastewater samples was measured as in the range of 1.77-94.47 ng/L and 1.40-15.09 ng/L for influent and effluent samples respectively. These ranges became 1.39-10.45 ng/L and <1.32-2.44 ng/L for CHD. The highest concentrations of biocides were observed in sludge samples with concentrations of 1117-3687 µg/kg and 510-2742 µg/kg for TCS and CHD. Biocide removal efficiency of primary and biological treatment together was reported as % 67.5±8.2 in January 2012 Tatlar WWTP analyses.

Key words: Biocides, wastewater, surface water, solid phase extraction method, Liquid chromatography

ÖZ

ATIK SU ARITMA TESİSLERİNDE VE YÜZEY SULARINDA BİYOSİTLERİN SEVİYELERİNİN VE AKİBETLERİNİN ARAŞTIRILMASI

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Biyositler, koruyucu ya da antiseptik madde olarak diş macunu, sabun, gargara gibi birçok kişisel bakım ürünlerinin ve temizlik malzemelerinin içerisinde, antimikrobiyal etkilerinden dolayı yaygın olarak kullanılmaktadır. Bu maddelerin kullanımı atık su arıtma tesislerine ve dolayısı ile yüzey sularına deşarj ile sonuçlanmaktadır. Yapılan birçok çalışma göstermiştir ki, biyositler doğada çok az konsantrasyonlarda bulunmalarına rağmen bakterilerde mutasyona yol açıp antibiyotiklere karşı dirençlerinde değişime sebep oldukları için, insan sağlığı açısından olumsuz etkilere sahiptirler. Bunun yanı sıra, insan ve balık dokusunda kolaylıkla birikebildikleri için vücuttaki konsantrasyonları çok yüksek seviyelere ulaşabilip insan sağlığı açısından tehdit oluşturabilmektedir. Bütün bunlar göstermektedir ki, biyositlerin atık su arıtma tesislerindeki ve yüzey sularındaki seviyeleri önem teşkil etmektedir.

Bu kapsamda, bu çalışma triclosan (TCS) ve chlorhexidine (CHD) adlı biyositlerin yüzey suyundaki konsantrasyonları ve atık sudaki akıbetlerine odaklanmıştır. Atık su numuneleri ile yapılacak çalışmalar için farklı proses konfigürasyonuna sahip Tatlar Atık Su Arıtma Tesisi, ODTÜ Atık Su Arıtma Tesisi, Kayseri Atık Su Arıtma Tesisi ve Antalya da bulunan çeşitli atık su arıtma tesislerinden numune alınmıştır. Arıtma tesislerinde, kompozit numuneler proses içinde farklı noktalardan bir yıllık süre içinde sezonsal olarak alınmıştır. Yüzey suları analizleri için ise farklı kirlilik seviyesinde bulunan Kesikköprü Barajından, Çamlıdere Barajından ve Eymir Gölünden bir yıllık dönem içerisinde her ay numune alınmıştır. Bu kapsamda, alınan tüm numunelerde biyosit seviyesi katı faz kartuş ile zenginleştirme metodu ve onu takiben sıvı kromatografi kullanılarak analiz edilmiştir.

Yapılan analizlerin sonucunda, yüzey sularındaki TCS konsantrasyonu temiz, orta dereceli kirli ve kirli su kaynakları için sırasıyla 0.65-11.15 ng/L, 0.86-48.96 ng/L ve 0.86-757.7 ng/L olarak belirlenmiştir. Katı faz ekstraksiyonu için % 92 geri kazanım sağlanmıştır. Aynı yüzey sularında CHD konsantrasyonyu ise < 1.33-5.31 ng/L aralığında saptanmış ve katı faz ekstraksiyonunda %96 geri kazanım sağlanmıştır. TCS konsantrasyonu atık sularda giriş sularında 1.77-94.47 ng/L, çıkış sularında 1.40-15.09 ng/L olarak ölçülmüştür. Bu konsantrasyon aralıkları CHD için ise 1.39-10.45 ng/L ve <1.32-2.44 ng/L olarak belirlenmiştir. Biyositlerin en yüksek konsantransyonu arıtma çamurlarında 1117-3687 µg/kg olarak TCS için ve 510-2742 µg/kg olarak da CHD için gözlenmiştir. Tatlar Atıksu Arıtma Tesisi'nde Ocak 2012'de gerçekleştirilen analizlere göre, birincil ve biyolojik arıtmanın biyosit giderim oranı % 67.5±8.2 olarak tespit edilmiştir.

Anahtar kelimeler: Biyosit, atık su, yüzey suyu, katı faz kartuş ile zenginleştirme metodu, LC/MS-MS ölçüm

To my family

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CHAPTER 1

INTRODUCTION

1.1. General

Nowadays, biocides which are chemical substances or microorganisms are widely used in medicine, agriculture and industry in order to eliminate any harmful organisms. Biocidal products are necessary to control the organisms which are harmful to human and animal health and organisms that lead to damage of natural or manufactured process. On the other hand, biocidal products can create a problem for humans, animals and environment in a different ways due to their properties. (The Biocidal Product Directive, 98/8/EC).

Biocides are classified into two groups as pesticides and antimicrobials. Pesticides include fungicides, herbicides, algaecides whereas antimicrobials include antibiotics, antibacterial, antiviral agents.

Among these antimicrobial agents, TCS and CHD are widely used ones in various application areas. These biocides are used as an ingredient for non-therapeutic cosmetic, personal care products, therapeutic products, veterinary products, pesticides, households and industrial cleaning products. Besides these, TCS can also be used for some plastics and textile products due to its antimicrobial activity against bacteria, moulds and yeast. TCS satisfies the protection of plastics from deterioration, odors and discoloration. For textile applications, the aim of TCS usage is to gain odour-protection properties to wool, synthetics, blends, and non-wovens by inhibiting the growth of bacteria and fungi on these surfaces.

Moreover, TCS is preferred to be used in body sprays such as underarm deodorants, feminine deodorants, foot and shoe deodorant sprays, in soaps including liquid hand wash, bath gels, face cleanser, eye make-up, anti acne formulation, in oral products such as toothpastes, mouthwash and in some creams such as sunscreens, insect repellents, in dishwashing detergents such as wool wash laundry detergent, bathroom surface cleaning products, kitchen surface cleanser, hospital grade disinfectant (Onodera et al. 1987;Lach et al. 2003; Arizona and Takao 2006; Inaba et al. 2006).

According to the Australian statistics, yearly 15 tons of TCS are used for households and industrial cleaning products, textile additives and plastic additives (Athanasios S.Stasinakis et al. 2008). Moreover, Sabaliunas et al. (2003) reported that TCS concentration in personal care products is in the range of 0.1-0.3 % (w/w) (Ying et al., 2006) and approximately 350 tonnes of TCS were produced annually in Europe (Singer et al.,2002). Thus, this widely usage of antimicrobial agents leads to their discharge into surface water either directly or via waste water effluents. According to Halden and Paull, 2005, more than 300 t/yr of TCS were disposed into water in USA. Moreover, biocides are known to accumulate in wastewater sludge due to their hydrophobic characteristics (Mc. Avoy et al., 2002).

The level of TCS in surface waters is so variable depending on the location of water sources. For example, Junclaus et al. (1978) reported that TCS concentration in United States fresh water was in the range of 12000-300000 ng/L on the other hand Wezel and Jagar (2002) demonstrated that TCS concentration in Netherlands fresh water was in the range of 2.1-7.7 ng/L.

Similar with surface water concentrations, biocide concentration in wastewater and sludge samples are so variable due to different input load, different configurations of WWTPs. The study of Mezcua et al. (2004) demonstrated the influent and effluent concentrations of TCS as 2300-562000 ng/L and 100-269000 ng/L, respectively for Spain WWTPs. In other respects, influent concentration of TCS was reported in the range of 0.87-0.83 ng/L and effluent concentration was in the range of 0.05-0.36 ng/L by Hing-Biu et al. (2005) for Canada WWTPs. Removal efficiency of the WWTP is also varied with respect to treatment technology. Mc Avoy et al. (2002) stated that the removal efficiency of TCS with

activated sludge treatment is approximately 96%;whereas other quoted literature figures are lying within the range of 70-90% (Singer et al., 2002; Bester, 2003; Ying and Kookana, 2007; Stasinakis et al., 2007). The concentration of TCS in sewage sludge samples is also important due to its application as biosolids to the land. The study of Bester (2003) in German WWTP demonstrated that nearly 30% of TCS was adsorbed onto the sludge. The TCS concentration of primary sludge was reported as 7500-14700 ng/g and secondary sludge concentration was reported as 900-4200 ng/g by Mc. Avoy et al. (2002) for USA WWTPs. The study conducted in 19 WWTPs of Sweeden by Svensson (2002) demonstrated that TCS concentration in primary sludge was 2100 ng/g and secondary sludge concentration was measured within the range of 470-530 ng/g.

The occurrence of biocides in environment at different level has drawn an increasing attention in environmental pollution and toxicity studies due to their microbial and algal toxicity possessed on the environment and humans (Ying et al., 2006). Some negative effects such as photochemical conversion of TCS to 2,8-dichlorodibenzo-p-dioxin, fish toxicity, formation of chlorinated and brominated derivatives of TCS were reported in literature studies (Onodera et al. 1987;Lach et al. 2003; Inaba et al. 2006). Moreover, the conversion of TCS with the help of sunlight into more toxic forms, dioxins, was reported by Latch et al. (2003). Danish Environmental Protection Agency stated that TCS can bioaccumulate in fish with 3700 to 8400 bioaccumulation factors. Similarly, the study of Winkler et al. (2007) demonstrated that TCS and its methyl derivative form can be accumulated, and so these compounds can be detected in water organisms such as fish, water plants and even human milk. Besides its negative effects on environment, TCS has also negative effects on human health. Mild itching and allergic redness on skins were reported as negative effects of TCS by Lin-Wu et al. (2007). Moreover, Jacobs et al., (2005), have reported that TCS can be accepted as endocrine disruptors since it affects the activation of the human pregnane X receptor.

The investigation of these biocides in different environmental media gained importance, while regarding the widely usage of biocides, their variable concentration in water sources and in WWTPs, their negative effects on environment and human health. In the light of this information, the objective and scope of this study was identified and given at following section.

1.2. Objective and Scope

The main objectives of this study are:

1. to determine the occurrences of biocides, namely, TCS and CHD in selected surface waters of Ankara case.
2. to determine TCS and CHD levels in municipal wastewaters in some selected Metropolitan city
3. to determine the removal efficiencies of TCS and CHD in municipal wastewater treatment plants (WWTPs) in Turkey and also to determine the TCS and CHD content of sludge originating from these WWTPs
4. to investigate the fate of TCS in a selected wastewater treatment plant in Turkey

Toward the first objective, three different surface water sources, namely, Çamlıdere Reservoir, Kesikköprü Reservoir and Eymir Lake were selected with respect to their pollution level as to represent “clean”, “moderately polluted” and “polluted”, respectively. One year monthly monitoring study was conducted between the Jan 2010 and May 2011.To the second and third purpose, six different wastewater treatment plants (WWTPs), namely, Tatlar (Ankara), Kayseri, Lara, Hurma, Kemer and METU VRM WWTPs were selected based on the treatment methodology applied. These WWTP monitoring studies were conducted between Jan 2010 and May 2011. Composite wastewater samples were taken from various points along the WWTPs on a seasonally basis (where appropriate) for one year period. Fate of biocides was investigated in the Tatlar WWTP to fulfill the fourth purpose.

1.3. Thesis Overview

This thesis includes six chapters. First chapter covers the introduction and objectives of this study. The second chapter satisfies a literature review on biocide properties, their negative effects on environment and human health, their occurrence in aquatic environment and their fate in wastewater treatment plant. Chapter 3 provides the materials and methods used. Chapter 4 illustrates the results of biocide monitoring study in surface water and waste water samples. Chapter 5 includes the conclusion and Chapter 6 includes recommendations for future studies.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

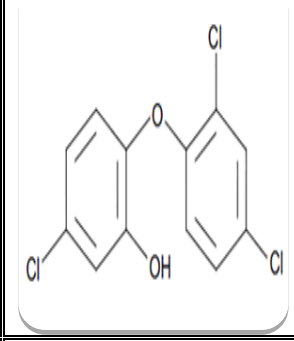
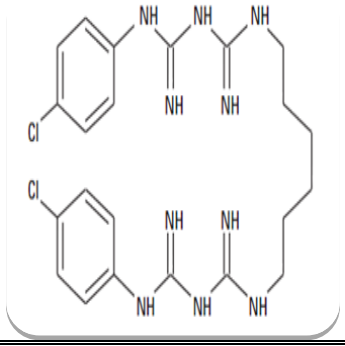
This chapter outlines the properties of biocides considered in this study, their negative effects, and their occurrence in aquatic environment and their fate in wastewater treatment plant respectively.

2.1. Background

2.1.1. Properties of Target Biocides

The wide application areas of biocides make the characteristics of them important in order to determine their fate and occurrence in different environment. Therefore, the chemical and physical properties of TCS and CHD are given in Table 1.

Table 1: Characteristics of Biocides

Properties	**TCS**	**CHD**
CAS Number	3380-34-5	55-56-1
CAS Name	5-Chloro-2-(2,4-dichlorophenoxy)phenol	N,N¢¢-Bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide
Additional Name	2,4,4-trichloro-2-hydroxydiphenyl ether	1,1¢-hexamethylenebis[5-(p-chlorophenyl)biguanide]; 1,6-bis[N¢-(p-chlorophenyl)-N5-biguanido]hexane; 1,6-bis(N5-p-chlorophenyl-N¢-diguanido)hexane; 1,6-di(4¢-chlorophenyldiguanido)hexane
Molecular formula	$C_{12}H_7Cl_3O_2$	$C_{22}H_{30}Cl_2N_{10}$
Structural formula		
Molecular weight	289.54	505.45
Melting point	54 to 57.3°C	134-136°C
Decomposition temperature	280°C to 290°C	-
Density	1.55 g/cm^3 at 22°C	1.07 g/ml
pKa	7.9	10.78 (at 25° C)
Log K_{ow}	5.4	0.08
Solubility (at water)	0.001/100g ($1*10^{-5}$ g/ml) at 20°C	0.8 g/L at 20°C

These properties of target biocides are critical for determination of fate of biocides in environment and determination of phases which biocides stay on. In this scope, the low water solubility and high octanol/water partitioning coefficient of TCS at neutral pH is the indication of significant potential for particle sorption (Halden et al., 2005).

2.1.2. Hazard of Biocides on Environment and Human Health

Besides their benefits as antimicrobial agents for commercial products, TCS and CHD have also some adverse effects on aquatic environment, biological removal process and human. Previous studies about the adverse effects of biocides are mainly focused on TCS instead of CHD due to much more widely usage of TCS.

TCS can undergo photo degradation or biodegradation when it is released into environment and this situation creates an environmental concern. The photochemical conversion of TCS to 2,8-dichlorodibenzo-p-dioxin , its fish toxicity , weak estrogen activity, and the formation of various chlorinated and brominated derivatives of TCS have been reported as a negative effects of it in previous studies (Onodera et al. 1987;Lach et al. 2003; Arizona and Takao 2006; Inaba et al. 2006). Moreover, the photodegradation can be occurred more easily when TCS is in its phenolate form (Chau et al., 2008).

Numerous studies have shown that TCS can be transformed into other potentially toxic compounds including methyl TCS, dioxins, chloroform and other chlorinated compounds. For example, when chlorinated TCS from WWTPs receive to environment, sunlight converts it into more toxic form, dioxins as reported by Latch et al. (2003). In their study, it was shown that photoconversion of TCS to 2,8-dibenzodichloro-p-dioxin with a yield of up to 12% is possible under different irradiation wavelengths. Similarly, the study of Halden et al. (2006), demonstrated that 2, 7/2, 8-dichlorodibenzo-p-dioxin in wastewater influent derived from TCS conversion. Moreover, 2,4-dichlorophenol and 2,4,6-trichlorophenol have been detected as the degradation products of TCS when low concentrations of free chlorine presents in water (Lin-Wu et al., 2007).

Regarding the adverse effects of TCS on aquatic organisms, TCS is known to be highly toxic to fish, plants and invertebrates (Department of Health and Ageing NICNAS, 2009). Wilson et al. (2003) have reported that TCS may lead an increase in Synedra bacteria while in reduction of rare genus Chlamydomonas at 15 ng/L and 150 ng/L respectively. Studies revealed that TCS is very toxic to aquatic organisms with LC_{50} or EC_{50} values < 1mg/L (Mensink et al., 1995). According to the literature review by Danish Environmental Protection Agency, TCS bioaccumulates in fish with 3700 to 8400 bioaccumulation factors. Indicating that concentration of TCS found in fish is several thousand times higher than concentration in water column.

Furthermore, TCS has a bacteriostatic activity against to gram negative and gram positive bacteria, molds and yeast when the level of TCS is even at 0.1-0.3% (w/w) (McAvoy et al.,2002). For certain type of algae, 500 ng/L has been determined as a no observed effect concentration (NOEC) and a predicted no effect concentration (PNEC) was determined as 50 ng/L by considering the commonly used safety factor of 10 (Ciba Speciality Chemical Holding, 1998 & Orvos et al. 2002). On the other hand, Hanstveit and Hamwijk (2003) stated that the most reliable NOEC for algae was considered as 0.69 µg/L. This value was also referred by Environmental Agency (2004) and by Thompson et al. (2005). According to another research, a LC_{50} value for fish was found in the range of 0.26-0.54 mg/l (Ciba 2002, Orvos et al. 2002). This study also indicated that 1.4 – 19 µg/l TCS in aquatic environment can result in inhibition of growth of algae. Moreover, acute and chronic tests indicated that TCS became much more toxic to freshwater invertebrates in neutral or acidic waters with respect to alkaline waters. However, the ecotoxicity of TCS on freshwater algae decreased in the presence of dissolved organic matter due to potential adsorption of TCS on this matter (Department of Health and Ageing NICNAS, 2009).

Beside these, TCS inhibits the enyl-acyl carrier protein reductase which causes the possible development of bacterial resistance to TCS, thereby lipid biosynthesis is blocked (Heath et al., 1999; Levy et al., 1999; Tixier et al., 2002). In addition, environmental media which is polluted by these compounds, contribute to the induction and coselection of antibiotic resistance of bacteria, molds and

yeast (Gaze et al. 2005). Due to their specific action, they can foster resistant bacteria and they may lead to increase of allergies.

High level of TCS concentration in wastewater can also be inhibitor for biological wastewater treatment process. The limited studies indicated that the inhibitory effects of TCS on activated sewage sludge micro-organisms can vary in compliance with the level of adaptation. It was found that 10 mg/L TCS concentration inhibited anaerobic sludge digestion process (Department of Health and Ageing NICNAS, 2009). Neumegen et al. (2005) studied the effect of TCS on BOD degradation rate constant and EC_{50} value was found as 1.82 mg/l. Moreover, results of the study conducted by Federle et al. (2002) revealed no adverse effects of TCS on activated sludge process when TCS concentration was increased from 0.04 to 2 mg/l. In contrast, Offhaus et al. (1978) stated that 2 mg/l TCS restrained peptone biodegradation by sewage sludge (Stasinakis et al., 2007).

The application of biosolids on the soil can also create some adverse effects on the plant. These effects may show some variation with respect to the soil type on which biosolids are applied. Recent studies demonstrated that soil TCS can be degraded more rapidly in aerobic soils with respect to anaerobic soil. Degradation process of TCS under aerobic condition occurs via the formation of methyl TCS and bound residues. In this situation, TCS disperse from water phase with the help of degradation and they pass to the sediment. On the other hand, in anaerobic condition TCS degrade very slowly and so they tend to persist in the environment. However, recent studies indicated that TCS did not affect the respiration and nitrification of soil up to 2 mg/kg concentration (Department of Health and Ageing NICNAS, 2009).

The adverse effects of TCS on human have also been investigated by several researchers. Effects including mild itching and allergic redness on sensitive skins were retained (Lin-Wu et al., 2007). Moreover, TCS can be accepted as endocrine disruptors due to its effects on activation of the human pregnane X receptor (Jacobs et al., 2005). Furthermore, TCS and its methyl derivative form can be accumulated, so these compounds were detected in water organisms such as fish, water plants and even human milk (Winkler et al., 2007).

2.2. Occurrence of Biocides in Environment

The incorporation of these compounds in wide array of products in washing and cleaning technologies results in their discharge in to the environment via effluent of wastewater treatment plants, reuse of treated effluent and disposal of sludge on soil which provides a transfer pathway for biocides from sewerage to the soil. Therefore, it is important to determine the fate of biocides in the aquatic environment in order to estimate the environmental and human exposure of biocides when their stability in the environment and hydrophobic characteristics are taken into account (Sabaliunas et al., 2003).

2.2.1. Occurrence of TCS in Environment

The recent studies have been especially focused on the occurrence of TCS in water samples due to its widespread usage, and its effects on human and natural environments, and its bioaccumulative and persistent nature due to its stable and lipophilic characteristics (Onodera et al. 1987;Lach et al. 2003; Arizona and Takao 2006; Inaba et al. 2006).

In this respect, the exposure pathways of TCS in the aquatic environment are represented in Figure 1.

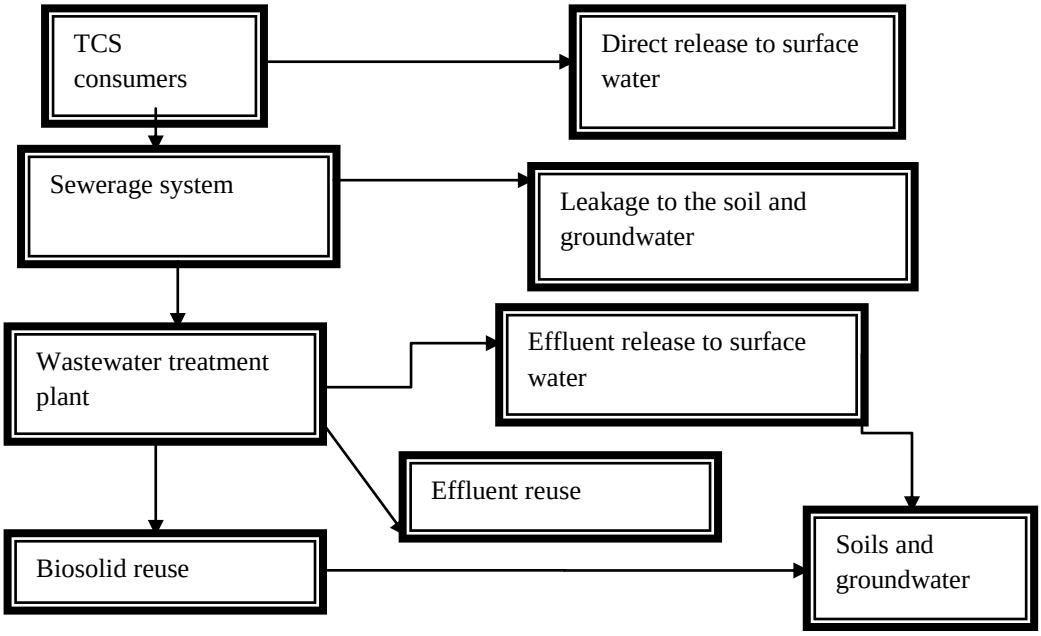


Figure 1: Exposure pathways of TCS (Department of Health and Ageing NICNAS, 2009)

The concentrations and distribution of TCS in the aquatic environment depends on many factors such as consumer usage pattern, removal rate during wastewater treatment, partitioning and degradation process in surface waters (Sabaliunas et al., 2003). Previous study conducted by Ciba (1998) indicated that TCS undergoes a complete biodegradation in a batch activated sludge test and also be removed in continuous activated sludge systems. Another removal mechanism for TCS from environment is photolysis. The photolysis of TCS may occur with the help of natural sunlight, especially at upper parts of the lakes where TCS is ready for photolysis (Tixier et al., 2002; Aranami and Readman, 2007).

The direct discharge of TCS to the surface waters can also be possible due to recreational activities including body contacts (such as swimming). During these activities, TCS containing personal care products, disinfectants, creams which have been applied to bodies may mix into the water (Department of Health and Ageing NICNAS, 2009). However, there is not any study about the interference of TCS in to surface water via recreational activities.

2.2.1.1. Occurrence of TCS in Surface Water

Several studies put forward that TCS has been found as a contaminant in rivers and lakes and open sea at ng/l levels due to its discharge into environment via wastewater effluents.

The literature values for TCS concentration measured in various surface waters of the world are presented in Table 2.

Table 2: TCS Levels in Surface Water Samples

Sampling area	Concentration	Reference
Outflow Point of Edo River	11-31 ng/l	Nishi et al., 2008
Location D	55-134 ng/l	
River water in Fo Tan industrial area (September 2005)	37.6 ng/L	Jian-Lin Wu et al., 2007
River water in Sha Tin residential area (September 2005)	26.0 ng/L	
Pearl River (September 2005)	31.6 ng/L	
Sea water Tai Po Harbour (June 2005)	16.2 ng/L	
Sea water Victoria Harbour (December 2005)	99.3 ng/L	
Sea water Victoria Harbour (March 2006)	31.9 ng/L	
Australian Surface water	14 to 75 ng/L	Ying and Kookana, 2007
Germany freshwater	30-90 ng/L	Wind et al. (2004)
Norway marine water	Not detected	Weigel et al. (2004)
Norway marine water	160 ng/L	Remberger et al. (2002)
Switzerland fresh water	≤3-74 ng/L	Poiger et al. (2002)
	5-100 ng/L	Singer et al. (2002)
Netherlands fresh water	2.1-7.7 ng/L	Wezel and Jagar (2002)
United Kingdom fresh water	19-80 ng/L	Sabaliunas et al. (2003)
United States/ Canada fresh water	4-8 ng/L	Hua et al. (2005)
	ND-2300 ng/L	Kolpin et al. (2002)
	34-785 ng/L	Morrall et al. (2004)
	110-800 ng/L	Wilkison et al. (2002)
	12000-300000 ng/L	Junclaus et al. (1978)
	600-40000 ng/L	Lopez-Avila and Hites (1980)

According to several studies conducted all over the world, the level of TCS in surface waters was so variable with respect to location of water sources and TCS concentration was reported in the range of 2.1-300000 ng/L.

2.2.1.2. Occurrence of TCS in Wastewater

In wastewater samples, several synthetic organic compounds which are originating from domestic, commercial and industrial activities were detected (Katsoyiannis and Samara, 2004). Some of these compounds are considered as inhibitor for biological wastewater treatment processes, and considered as contaminant for aquatic environment. Among these, TCS has significant importance due to its frequent detection in wastewater and also its reported physicochemical and toxicological properties. As an antimicrobial agent, the majority of TCS enters sewer systems due to its normal usage of households cleaning products and is transported to wastewater treatment plants. Therefore, TCS has been detected in sewage sludge, discharge effluent, thereby receiving surface waters and sediments (Lin-Wu et al., 2007). As a result, in order to understand the potential environmental risk posed by

antimicrobial agents, it is necessary to investigate its concentration level, behavior and fate in wastewater treatment plants and exposure in the environment (Ying et al., 2006).

The literature studies regarding the TCS existence in wastewaters are given together with the detected levels in Table 3.

Table 3: TCS Levels in Wastewater Samples

Sample name	Influent Conc. (ng/L)	Effluent Conc.(ng/L)	Reference
Sewage plants with 200000 m^3/d flow rate	1200	50	Bester,2003
Two-stage biological process	7300±1500 3200±1000 (after first aeration basin) 400±100 (after main aeration basin)	300±100	Bester,2004
One-stage biological process	4800±550 3300±950 (after first sedimentation tank) 260±1900 (after main aeration basin)	620±1500	
WWTP Sha Tin/ Hong Kong	142.0 170.2 (after primary treatment)	22.5	Jian-Lin Wu et al. (2007)
WWTP Kwun Tong/Hong Kong WWTP	213.8	177.3 (primary	Jian-Lin Wu et al. (2007)
Activated sludge WWTP	5210	240	Mc.Avoy et al.
Activated sludge WWTP	10700	410	Mc.Avoy et al.
Activated sludge WWTP	670	32	Kanda et al. (2003)
Activated sludge WWTP	1100	27	Kanda et al. (2003)
Trickling filter WWTP	2500	140	Kanda et al. (2003)
Trickling filter WWTP	3700	130	Kanda et al. (2003)
Australia 19 Activated sludge WWTPs effluents	-	23-434	Ying and Kookana, (2007)
Australia 5 WWTPs	573 -845	60.5-159	Ying and Kookana, (2007)
Australia 5 WWTPs (1995-1996)	-	<100-740	Ciba Speciality Chemicals (2003)
Canada 8 WWTPs (2004)	0.87-0.83	0.05-0.36	Hing-Biu et al. (2005)
Denmark Activated sludge WWTP (2002-2003)	750 (estimated from removal efficiency and reported effluent concentration)	90	Paxeus (2004)

Table 3 (Continued)

Denmark 2 Activated sludge WWTP (2002)	1600-3000	<1000	Pedersen and Nielson (2003)
France 2 Activated sludge WWTPs	378 (estimated from removal efficinecy and reported effluent concentration)	170-430	Paxeus (2004)
Germany WWTP (2003)	ND (<9.4)	ND(<24)	Quintana and Reemtsma (2004)
Germany WWTP (2000)	380	180	Weigel et al. (2004)
Greece 2 Activated sludge WWTPs (2002-2003)	2167(estimated from removal efficiency and reported effluent concentration)	130-190	Paxeus (2004)
Italy 3 Activated sludge WWTPs (2002-2003)	1370(estimated from removal efficiency and reported effluent concentration)	370-700	Paxeus (2004)
Norway 3 WWTPs & hospital (2002)	430-2380	160-480	Weigel et al. (2004)
Spain WWTP (2002-2003)	2300-562000	100-269000	Mezcua et al. (2004)
Spain 2 WWTPs (2002)	1300-37800	400-22100	Agüera et al.(2003)
Sweden 3 WWTPs (1993)	-	≤500	Paxeus (1996)
Sweden 6 WWTPs (1995)	100-1500	≤200	Paxeus (cited in Danish EPA,2003)
Sweden 2 Activated sludge WWTPs	381-1444	130-160	Paxeus (2004)
Switzerland 7 WWTPs (1999)	520 (primary effluent)	42-213	Singer et al. (2002)
United Kingdom Meltham WWTP **(**Trickling filter)	7500 5900 (primary effluent)	340	Sabaliunas et al. (2003)
United Kingdom Crofton WWTP **(**Activated sludge)	21900 13350 (primary effluent)	1100	Sabaliunas et al. (2003)
United Kingdom 1 Trickling filter&1 Activated sludge WWTPs (1999)	7510-11980	470 (AS) 1100 (TF)	Ciba Specialty Chemicals (1999)
United States 3 Activated sludge WWTPs (2004)	3000-3600	54-82 (AS) 28-72 (final)	Thomas and Foster (2005)
United States 1 WWTP (2002)	-	10-21	Boyd et al. (2003)
United States 1 TF WWTP	-	785	Morrall et al.(2004)

Table 3 (Continued)

United States 2 TF WWTPs (1996)	3830-16600	1610-2700 (TF)	McAvoy et al.(2002)
US mid-Atlantic Activated sludge WWTP	800-10800	10-240	Heidler et al. (2007)
Switzerland Maur WWTP (2001)	980	650	Lindström et al. (2002)
Switzerland Pfaffikon WWTP (2001)	1044	250	
Switzerland Uster WWTP (2001)	1300	110	
Switzerland Wetzikon WWTP (2001)	584	183	
Switzerland Gossau WWTP (2001)	970	136	
Switzerland Gossau WWTP (1997)	500-1000	70-100	
United Kingdom RBC WWTP	594-4945	75-322	Thompson et al.(2006)
United Kingdom Oxidation Ditch WWTP	710-5115	4-104	
United Kingdom Trickling Filter WWTP	1562-3057	35-290	
WSTP effluents (OH, USA)	-	410	W. Hua et al. (2005)

As can be seen from Table 3, the levels of TCS in influent and effluent samples for wastewater have wide range due to different location of sampling point, variable input load and different treatment technology in wastewater treatment plants. In this context, TCS concentration was stated as 0.83-562000 ng/L and 0.05-269000 ng/L for influent and effluent samples, respectively.

Final effluents concentration of activated sludge process is much lower than the effluents of trickling filter process. This situation can arise from the longer hydraulic residence times of activated sludge process with respect to trickling filter process (Thomson et al., 2005).

Ying et al. (2007) stated that the variation in TCS concentration can results from the difference in input loads, treatment technology and climate factor. Similarly, Agüera et al. (2003) have made attribution to the input load for the variation observed in TCS concentrations.

2.2.1.3. Removal of TCS in WWTPs

It is important to determine the fate and removal of biocides in wastewater treatment plants considering the afore-mentioned effects of them on an aquatic environment, human and their entrance mechanisms in to the aquatic environment. Biocides exist in effluent due to incomplete removal in WWTPs and also exist in sludge due to its hydrophobic nature (McAvoy et al.,2002; Singer et al., 2002; Bester 2003).

Many studies have been conducted in order to determine TCS occurrence in wastewater samples as well as its fate in wastewater treatment plants.

The study conducted by Samoe-Petersen et al. (2003) indicated that TCS is degradable under aerobic conditions so it can be degraded and removed in activated sludge systems. In accordance with this, Mc Avoy et al. (2002) stated that the removal efficiency of TCS with activated sludge process is

approximately 96%; whereas other quoted literature figures are lying within the range of 70-90% (Singer et al., 2002; Bester, 2003; Ying and Kookana, 2007; Stasinakis et al., 2007). On the other hand with trickling filter, TCS removal efficiency decreased to 58- 86% (Xie et al. 2008).

The remaining fraction of TCS which are not eliminated during wastewater treatment process is discharged to the aquatic environment via the effluent. Previous studies in Europe, North America, Australia and China have shown that occurrence of TCS in influent streams lies in the range of 1000-10000 ng/L whereas in effluents appears in the range of 40-2700 ng/L (Singer et al., 2002; Bester,2003; Weigel et al., 2004, Halden and Paull, 2005; Ying and Kookana, 2007). As a result of TCS discharge to the aquatic environments, TCS concentrations detected was between 50-150 ng/l, 1-35 µg/kg and 0.07-14000µg/l, in sea water, in sediments and in wastewater samples, respectively (Lopez et al., 1980; Okumura et al. ,1996; Lindström et al., 2002; Mc Avoy et al., 2002; Singer et al., 2002).

Therefore the removal efficiency of TCS in different type of treatment process becomes an important issue. Some of the previous studies on the removal efficiency of TCS in WWTPs are summarized in Table 4.

Table 4: Removal Efficiency in Different WWTPs

Sampling sites	%Removal	Reference
RBC	81	Thompson et al. (2006)
Oxidation Ditch	97	
Trickling filter	92	
Two-stage biological process	95	Bester, 2004
One-stage biological process	87	
Australia 2 Tertiary treatment plants	93-72	Guang-Guo Ying,2006
Australia WWTP Lagoon	85	
Australia Activated Sludge WWTP	89	
Australia Biological treatment plants	92	
United Kingdom Meltham WWTP **(**Trickling filter)	95.5	Sabaliunas et al. (2003)
United Kingdom Crofton WWTP **(**Activated sludge)	95	Sabaliunas et al. (2003)
Colombus WWTP (Activated Sludge)	95.4	Mc Avoy et al. (2002)
Glendale WWTP (Trickling Filter)	58	
Loveland WWTP (Activated Sludge)	96.2	
Gossau WWTP (Activated Sludge)	94	Singer et al., 2002
WWTP in Germany (vicinity of Dortmund, Activated Sludge)	>90	Bester, 2003
Australian WWTPs	72-93	Ying and Kookana, 2007
Continuous flow activated sludge systems	>90	Stasinakis et al., 2007

Based on the literature studies, it can be safely stated that TCS removal efficiency of WWTPs exhibits some difference with respect to their treatment process applied. This variation could be caused by the difference in their operational conditions and input loads.

Another important issue to consider is the main removal mechanism of TCS during the course of wastewater treatment. According to the Federle et al. (2002)'s study which was conducted in a continuous activated sludge laboratory unit; more than 80% of TCS was removed by biodegradation. On the other hand, adsorption mechanism can also play an important role during the TCS removal due to its hydrophobic characteristics. In another study, Ying et al. (2006) reported that 41% of the removal was achieved at the primary treatment level of the tertiary treatment. Similarly, Mc Avoy et al. (2002) stated that TCS removal achieved by primary treatment in WWTPs in USA was up to 48%. On the other hand, Sabaliunas et al.(2003) comparatively reported that primary removal is by 21.3% and 39% with trickling filter process and activated sludge process, respectively.

In a more study, Athanasios et al. (2008) stated that 45±27% part of TCS was accumulated in sewage sludge, while 9±6% part of TCS was removed via the effluents. Similarly, Heilder and Halden (2007) reported that 50±19 % of TCS accumulated in the sludge in WWTPs. Furthermore, the study of Bester (2003) conducted in German WWTPs revealed that 22-43% of initial TCS was sorbed to sludge, while 5% of TCS was discharged via the effluents. In contrast, in the study by Federle et al. (2002) ,more than 94% of TCS removal was reported to occur by biodegradation whereas 1.5-4.5% of TCS by accumulation onto the wasted sludge (Stasinakis et al. 2008). Moreover, according to the Singer et al. (2002), the TCS removal due to biodegradation was 79% and the remaining 15% was sorbed onto sludge. In a similar way, previous study by Athanasios et al. (2007) put forward that the main mechanism of TCS removal was biodegradation and this mechanism was enhanced by the acclimatization of the biomass (Stasinakis et al. 2007).

2.2.1.4. Occurrence of TCS in WWTPs Sludge

As stated earlier, during the removal of TCS in WWTPs, significant amounts of TCS adsorb on the sludge due to its hydrophobic characteristics. Therefore, TCS can be present at high levels (mg/kg) in sewage sludge samples. The degree of TCS partitioning to sludge depends on the wastewater treatment process (Department of Health and Ageing NICNAS, 2009).

In a study by Bester (2003) which was conducted in German WWTP, nearly 30% of TCS was adsorbed onto the sludge. Reiss et al. (2002) stated that sorption coefficient of TCS is 22000 L/kg for deactivated sludge and organic normalized sorption coefficient of TCS is 48000 L/kg.

The impact levels of TCS on activated sewage sludge micro-organisms, indicate variations with respect to the level of adaptation to TCS, however TCS can negatively affect their removal ability of ammonia (Stasinakis et al., 2008).

Literature summary for TCS levels detected on sludge samples are given in Table 5.

Table 5: TCS Concentration on Sludge Samples

Sampling Site	**Concentration**	**Reference**
Primary, secondary and anaerobic digested sludge in US	0.53-15.6 mg/kg with an average of 6.97 mg/kg	Mc. Avoy et al. (2002)
Wastewater treatment plant	400-8800 µg/kg	Bester et al. (2003)
Australia 2 WWTPs anaerobic digested sludge (1999)	50-3400 ng/g	Luke 1999
Australia 17 WWTPs anaerobic &aerobic digested sludge (2004)	90-16790 ng/g(anaerobic) 220 ng/g (aerobic)	Ying and Kookana (2007)
Canada 35 WWTPs (1999-2001)	3400-17900 ng/g (primary sludge) 5400-28200 ng/g (anaerobic sludge)	Lee and Peart (2002)
USA 4 WWTPs (1996)	7500-14700 ng/g (primary sludge) 900-4200 ng/g (secondary activated sludge) 15600 ng/g (anaerobic sludge) 530 ng/g (aerobic sludge)	McAvoy et al. (2002)
Germany 1 WWTP (April 2002)	3000 ng/g (anaerobic sludge)	Bester (2003)
Switzerland 7 WWTPs (June 1999)	580 ng/g (activated sludge)	Singer et al.(2002)
Sweeden 19 WWTPs	2100 ng/g (primary sludge) 470-530 ng/g (secondary sludge of Trickling filter) 180-3700 ng/g (activated sludge) 1200-6400 ng/g (anaerobic sludge of TF) 28-450 ng/g (aerobic sludge of activated sludge)	Svensson (2002)
Sweeden 4 WWTPs	2800-4400 ng/g (aerobic sludge)	Remberger et al. (2002)
US mid-Atlantic Activated Sludge WWTP	20000-55000 µg/kg (digested, dewatered sludge)	Heidler et al. (2007)
Spain marine sediments	0.27-130.7 µg/kg	Agüera et al. (2003)

2.2.2. Occurrence of CHD in Environment

Like TCS, CHD is another widely used antimicrobial agents especially for surgical scrubs, health care personal soaps, skin antiseptics, skin cleanser, acne creams and oral products such as toothpastes and mouth rinses (Dynes et al., 2006). CHD is a positively charged hydrophobic and lipophilic molecule and so they accumulate in the fatty tissues (e.g., lipids) of living organisms and indicate toxic effects (Castillo et al., 2004; Akaho and Fukumori, 2001).Moreover, CHD disrupts the integrity of the cell membrane and causes the leakage of intracellular components of the organisms.

In literature, there are only a few studies regarding the CHD determination in surface water and wastewater samples. These studies are illustrated in Table 6. As seen from this table, CHD in medical wastewater is expectedly much higher than that in surface water.

Table 6: CHD Concentration in Water Samples

Sample Source	Concentration	Reference
Hamamatsu School of Medicine, Medical WWTP, Japan	1.94 mg/L	Matsushima and Sakurai,1984
South Saskatchewan river, Saskatoon, Saskatcheman, Canada	100 µg/L	J.J. Dynes et al., 2006

CHAPTER 3

MATERIALS AND METHODS

3.1. Sampling Sites

3.1.1. Surface Waters

Surface waters studied in this study, are presented in Table 7. As indicated in this table, they are primarily selected based on their pollution state as such to represent a "clean", "moderately polluted" and "polluted" surface water sources. Also, their proximity to our laboratory and their functions were also considered during the selection. Çamlıdere Reservoir is one of the reservoirs supplying drinking water to Ankara, capital city of Turkey. Kesikköprü Reservoir is the one used when the main reservoirs are in lack of water during water scarcity periods. Eymir Lake serves as the recreational area in Ankara and it receives some illegal wastewater discharge(s).

Table 7: Surface Waters Studied

Surface Water	**Pollution State**	**Water Class***
Çamlıdere Reservoir	Clean	Class 1
Kesikköprü Reservoir	Moderately Clean	Class 2
Eymir lake	Polluted	Class 4

*Water Pollution Control Regulation (2004, Table 1:Criteria for inland water quality classes)

Moreover, water class identification of water sources was carried out with respect to previous studies about water quality of these water sources and Table 1 of Water Pollution Control Regulation which is given at Appendices. According to the thesis study of Tezce (2010), Total Organic Carbon (TOC) concentration of Kesikköprü Reservoir was reported as 7.705±0.32 mg/L. Therefore water class of Kesikköprü Reservoir was accepted as Class 2 with respect to water quality classes' criteria of Water Control Pollution Regulation. Similarly, TOC concentration in Çamlıdere Reservoir was reported in the range of 1.987-5.180 mg/L from July 1998 to June 1999 (Gür, 1999). Therefore water class of Çamlıdere Reservoir was accepted as Class 1. In the same way, Eymir Lake water quality analyses conducted between 1993 and 1994 in the scope of "Water Sources and Environmental Management Plan Project for Mogan-Eymir Lake" and TOC concentration was reported in the range of 2-40 mg/L and so water class of Lake was accepted as Class 4.

3.1.1.1.Çamlıdere Reservoir

Çamlıdere reservoir was constructed between 1976 and 1987 in order to supply drinking water to the Ankara. It is located on Bayındır Creek and it has 2500 hm^3 dam volume. Samples were taken from same and definite location which was near the pumping station of the reservoir, during whole sampling period.

The location of Çamlıdere Reservoir is indicated in Figure 2. This water source was selected as to represent a clean water source due to the fact that it is serving as a drinking water source for Ankara, and therefore, it is a well-protected water source.

Figure 2: The location of Çamlıdere Reservoir

3.1.1.2. Kesikköprü Reservoir

Kesikköprü reservoir is another but occasionally used drinking water source of Ankara. It was constructed between 1959 and 1966. It is located on Kızılırmak River and it has 0,9 hm^3 dam volume. Samples were taken from same and definite location which was near the pumping station of the reservoir, during whole sampling period.

The location of Kesikköprü Reservoir is illustrated in Figure 3. This water source was selected as to represent a moderately polluted water source since it is located in downstream of the Kızılırmak River which might have been receiving some wastewater discharges along the way upstream.

Figure 3: Location of Kesikköprü Reservoir

3.1.1.3. Eymir Lake

Eymir Lake is used as a recreational area in Ankara and it was accepted as polluted surface water due to some illegal possible wastewater discharges, at least in the past for years. Eymir Lake is connected to the Mogan Lake with a canal and so its main upstream source is the Mogan Lake. Eymir Lake level is 3 meter lower than that of Mogan Lake and it is fed by the waters of Mogan Lake with the help of regulator. The surface area of lake is 1.09 km^2 and its average depth is 3.80 m.

The map location of the Eymir Lake is indicated in Figure 4. Samples were taken from same and definite location which was near the restaurants area, during whole sampling period.

Figure 4: Location of Eymir Lake

3.1.2. Wastewater Treatment Plants (WWTPs)

Wastewater and sludge samples were obtained from six WWTPs located in different regions of Turkey (Table 8). Selection of these WWTPs was based on the treatment processes applied in order to see the effect of different process applications (if any) on the removal of target biocides. The treatment processes applied in these plants are also indicated in Table 8.

Table 8: Treatment Process applied in WWTPs

WWTP	**Treatment Process Applied**	**Design Capacity** (m³/day)
Ankara Wastewater Treatment Plant (Tatlar WWTP)	Activated Sludge Process	765 000
Kayseri WWTP	Activated Sludge Process with selector/bio-P basin	145 000
METU WWTP	Vacuum Rotating Membrane	150
Lara WWTP	Bardenpho Process	125 000
Hurma WWTP	Bardenpho Process	210 000
Kemer WWTP	Activated Sludge Process with Oxidation	21 415

3.1.2.1. Ankara Wastewater Treatment Plant (TATLAR WWTPs)

Tatlar Wastewater Treatment Plant is the biggest plant in Turkey and also is the fourth one in Europe. It was put into operation at August 1997. Treatment capacity of first stage of plant with activated sludge process was designed as 765.000 m³ wastewater and it was designed in order to serve nearly 4 million population equivalent. The design of the plant is compatible with 6 million population equivalent and nitrogen, phosphorus removal for later period with the construction of second and third stage. Facility area will continue to increase and comprise 182 ha at 2025 with the construction of third stage (URL 1).

The facility is located at 45 km west of city center and near the Tatlar Village. Wastewater from whole city can reach the facility location without any pumping station and this situation was very important for the selection of wastewater treatment plant location.

The wastewater treatment mechanism of Tatlar WWTP mainly consists of activated sludge with anaerobic sludge stabilization and belt filter press for dewatering of sludge. In the current situation, whole processes are taken into operation except phosphor and nitrogen removal process.

In facility, wastewater firstly passes through coarse and fine screens and then aerated grit chamber, primary sedimentation process respectively in order to elimination of suspended particles in the scope of physical treatment. After physical treatment, activated sludge process with aeration chamber start to biological treatment of wastewater via degradation of organic materials in wastewater with the help of microorganisms. Suspended particles in wastewater after aeration chamber are eliminated by secondary sedimentation process and also identified amount of activated sludge is recycled to the beginning part of the aeration chamber. Remaining part of the sludge is sent to the gravity thickener and then primary and secondary sludge are taken into operation at anaerobic sludge digester together. In the anaerobic digestion part, biogas which is formed during the digestion process is used in order to obtain electricity. This process satisfies the electricity requirement of facility at %80 amount. Digested sludge is sent to thickener in order to increase solid content at sludge. Finally digested and thickened sludge enters the belt filter press process in order to dewatered the sludge and make it ready for disposal (URL 2).

The yearly average removal efficiency of Tatlar WWTP for BOD_5 and Suspended Solid Materials are reported as greater than %85 and %90 respectively.

During sampling period, samples were taken from the primary and secondary sludge for sludge samples and from primary sedimentation tank influent, aeration basin effluent and secondary sedimentation tank effluent for wastewater samples. Analysis of wastewater and sludge samples for biocide detection was conducted with respect to Section 3.4.2.

The main design parameters and dimensions of WWTP units are given at Appendices.

3.1.2.2. METU Wastewater Treatment Plant

METU Wastewater treatment plant serves whole Middle East Technical University (METU) Campus, METU Techno city and lodging buildings. Vacuum Rotation Membrane (VRM) was preferred for wastewater treatment in this plant. VRM system is composed of ultra filtration membranes submerged with the aeration tank. This system is combination of biological wastewater treatment and high efficient solids/liquid separation. In the treatment facility, pre-screened wastewater is aerated and clarified biologically. The last step of treatment is the removal of solid particles and also bacteria/viruses from water with the help of ultra filtration membrane with respect to low pressure principle which is based on the separation of suspended solids and water with the help of pressure difference. According to this, water can pass through the membrane on the other hand solids, bacteria and viruses are retained on the membrane surface and are removed by relative movement. Therefore, wastewater is purified and the effluent of this system meets the regulation standards. During sampling period, samples were taken from the influent and effluent of the WWTP.

The system parameters of METU treatment plant are given at Appendices. The representation of VRM system is given at Figure 5.

Figure 5: Vacuum Rotating Membrane System

3.1.2.3. Kemer, Lara and Hurma Wastewater Treatment Plants

Kemer Wastewater Treatment Plant is composed of three stages. In the first stage, two oxidation ditches and three secondary clarifiers are in operation. Second stage covers two oxidation ditch and two secondary clarifiers and third stage comprises of two oxidation ditch and four secondary clarifiers. Secondary clarifiers of first and second stage are circular and surface aerators are preferred in oxidation ditches on the other hand, clarifiers of third stage are rectangular and diffusers are located at bottom of the oxidation ditch. Each stage recycles water in itself and also is connected to other stage. The first stage has 4.714 m3/day, second stage has 8.061 m3/day and third stage has 8.640 m3/day capacities. Total flow in project was designed as 21.415 m3/day with 7.923,55 kg/day BOD_5 load and 73.591 population equivalents. The flow of deep sea discharge is 313,88 lt/second and it can serve 90.431 population equivalent. Moreover, the belt filter presses with 7.5 and 18 m^3/hour capacity are preferred in order to dewatered the sludge (URL 3).

Lara Wastewater Treatment Plant is 17 km far away from the city center. This plant serves to touristic facilities in the region and a great majority of city population. Wastewaters in this region is collected with sewage system and disposed with deep sea discharge. Lara WWTP is composed of pre-treatment, biological activated sludge and sludge dewatering units and designed as four stages which each stage has 31.250 m^3/day capacities. Pre-treatment facility has treatment capacity of 500.000 population equivalent and biological treatment part can serve to 250.000 population equivalent. Wastewater coming to the facility passes through the pre-treatment units which are composed of coarse and fine screens, aerated grit chambers. After pre-treatment units wastewater enters the biological treatment units which are selector, anaerobic reactor, aeration basin, sedimentation tank. Bardenpho system is used as biological treatment in order to remove nitrogen in accordance with the nitrification and denitrification process, and to remove phosphorus with anaerobic and aerobic basins. Moreover, for sludge dewatering process, sludge storage tanks and decanter are in operation (URL 4).

Facility is operated in accordance with German ATV-131 Standards. The characteristics of wastewater treatment units are given at Appendices.

Sewage system in western part of Antalya reached to the Hurma WWTP. The construction of pre-treatment facility of Hurma WWTP started at 30 September 1996 and completed at 17 February 1999.

Since the pre-treatment unit is not sufficient in order to remove organic materials, first stage of biological treatment units with the capacity 250.000 population equivalent was taken into operation at 29 December 2001. After the construction of second stage at January 2005, facility capacity reached to 500.000 population equivalent. Finally construction of third stage at April 2011, facility capacity became 210.000 m^3/day with 1.400.000 population equivalent.

Facility mainly comprise of pre-treatment, biological treatment and sludge treatment units. Fine and coarse screens, aerated grit chamber and primary sedimentation tank was preferred for pre-treatment units. Moreover, the removal of carbon, nitrogen and phosphor is achieved by Bardenpho system with anaerobic tank, aeration basin, and secondary sedimentation tank. For the sludge treatment part of facility, mechanic thickener, anaerobic digester, decanter were preferred (URL 5). Operation parameters, design parameters and dimensions of WWTP units are given at Appendices.

Samples were taken from influent and effluent of WWTPs and from sludge units in order to conduct analysis.

3.1.2.4. Kayseri Wastewater Treatment Plant

Kayseri WWTP has been served Kayseri and its surrounding since 2003. Facility was taken into operation completely at 20 February 2004. Facility satisfies the removal of carbon, nitrogen and phosphorus from wastewater.
Treatment of wastewater is mainly satisfied by pre-treatment units which are fine and coarse screens, aerated grit chamber and primary sedimentation tank, biological treatment units which are selector/bio-P basin, aeration basin and secondary sedimentation tank. As a sludge treatment process, pre-thickener, anaerobic digester, secondary thickener and dewatering unit were preferred (URL 6).
Samples were taken from influent and effluent of WWTP and from sludge digestion unit in order to conduct thesis analysis.

3.2. Monitoring Studies

3.2.1. In Surface Waters

Biocide monitoring study was performed for each surface water source for 12 months, from May 2010 to May 2011. Not only the target biocides, but some general water quality parameters, such as TOC, TDS, pH and temperature were also monitored, so that they could be helpful during the integration of seasonal changes of biocide levels (if any). Also, relation between biocide levels and the pollution state of watercourses would be possible to see.

3.2.1.1. Sampling in surface waters

Samples were taken from the definite locations near the water pumping stations of reservoirs (near restaurant areas for Eymir Lake) and were put into amber glass bottles (2.5 L) which were transported in closed, dark and cooled conditions to the laboratory.

3.2.1.2. Parameters Monitored in Surface Waters

Parameters monitored and the basis for selecting the water quality parameters is given in Table 9.

Table 9: Parameters Monitored

Parameters	**Intention with selection**
TCS	Selected biocides
CHD	Selected biocides
TOC	Organic pollution indicator
TDS	Organic Pollution indicator
Temperature	Indicator for weather/precipitation conditions
pH	For TCS speciation/fate understanding

For surface water samples, temperature, conductivity and pH of samples were determined by on-site measurements with using the calibrated Hach-equipment. TOC of samples were determined with Schmadzu TOC device.

3.2.2. In WWTPs

Biocide monitoring study for wastewater samples was performed, from January 2010 to May 2011 in six wastewater treatment plants having different treatment technology.

3.2.2.1. Sampling in wastewaters

Composite samples were taken from the definite locations along the treatment plants with the help of peristaltic valve and were put into amber glass bottles (2.5 L) which were transported in closed, dark and cooled conditions to the laboratory.

3.2.2.2. Sampling in WWTP

Biocide concentration in WWTPs was monitored by taking sample along the treatment plants. The sampling locations along treatment plants for each WWTP are given in Table 10.

Table 10: Treatment Process applied in WWTPs

WWTP	**Sampling points**
Tatlar WWTP	Primary clarifier influent, aeration tank effluent, secondary clarifier effluent, primary sludge and secondary sludge
Lara WWTP	Primary clarifier influent, secondary clarifier effluent, sludge samples
Hurma WWTP	Primary clarifier influent, secondary clarifier effluent, sludge samples
Kemer WWTP	Primary clarifier influent, secondary clarifier effluent, sludge samples
Kayseri WWTP	Primary clarifier effluent, aerobic tank, anoxic tank, secondary clarifier effluent, secondary and digested sludge
METU VRM	Influent, UV effluent, sludge

3.3. Fate of Biocides in Selected WWTP

Fate of biocides in WWTP was monitored with January 2012 sampling in Tatlar WWTP during one month sampling period via weekly sampling.

3.4. Biocide Analysis

3.4.1. Biocide Analysis in surface water samples

Biocide analyses in water samples are composed of standard solution preparation, sample preparation, and HPLC/MSMS measurements steps, respectively. These are described in following sub-sections.

3.4.1.1. Sample preparation

An effective sample preparation and measurement method is required in order to monitor the trace pharmaceuticals in surface and waste water (Chin Kai Meng, 2007). Sample preparation is the first important step for the determination of the target biocides. Biocides, TCS and CHD, have hydrophobic characteristic and they tend to stay in solid phase and stick on the surface of sampling bottle. Therefore, the equipments which are used during the analyses must be selected properly in order to eliminate adsorption of biocides. Moreover, proper cleaning of the glassware is extremely important for biocides analyses since the analytes tend to adsorb on the glass surface and samples contaminate the glassware. Glassware which is used in the analyses must be cleaned with the

detergents for hydrophobic compounds and hot water. After detergent washing, glassware cleans with firstly tap water and then ultra-pure water. After that, they put into 150° C oven for at least 1 hour. Baking process should be minimized as far as possible since repeated baking cause permanent adsorption of analytes on the glass surface. After drying and cooling, glassware which is sealed with aluminum foil should be stored in clean and closed environment in order to prevent from any contamination. Before the reuse of glassware, they must be rinsed with acetone and methanol respectively. Moreover, identifying glassware associated with highly contaminated samples is very important since this situation may require extra cleaning. This means that, glassware which was used for highly contaminated samples should not be used for surface water samples without proper cleaning. Moreover, it is important to take necessary precautions such as wearing protective gloves and clothing to avoid contamination of the samples from the environment.

In the light of this information, the sample collection bottles and all experiment glassware which were used in this study, were washed with Alchonox detergent which is used for hydrophobic compounds washing, before the experiment.

Samples can be stored at 4° C up to 7 days, however 48 hours is recommended for extraction process in order to obtain more accurate results. If the extraction is not possible within 48 hours and we have to store samples up to 7 days, the pH of the samples must be decreased by adding sodium hydroxide or sulfuric acid solution and after this period they must have been extracted since degradation process begin. Extracted samples can be stored at -10° C and they should be analyzed within 40 days of extraction. Freezing the samples is another option in order to minimize the degradation but samples should be extracted within 48 hours after removal from the freezer. Therefore, in this study, all samples were taken from the surface water sources were immediately transferred to the laboratory in closed and cooled container and extracted within 48 hours without any pH decrease.

Sample preparation process can have some problems such as adsorption of compounds on test tube walls or on the matrix solids. However, these problems can be solved by using silanized glass ware or polypropylene materials during sample preparation and making more homogenize the samples and adding a little bit amount of solvent to the samples. Therefore, in order to solve these problems, glass wares were silanized and polypropylene materials were preferred and 5 ml of methanol as a solvent was added each 1 L samples.

Before the solid phase extraction process, surface water samples were filtered through the 0.7 micron glass fiber filter in order to eliminate the fouling of the cartridge due to large particles in samples.

3.4.1.2. Extraction of Samples

As stated in Section 2.2.1.1, the concentrations of target biocides are very low in water samples, and so the enrichment of samples seems to be essential. Besides this, samples also can contain some interference at different level according to the diversity of the sampling site especially for the wastewater samples. The concentration of these interferences can be higher than the analytes of interest. In this scope, the elimination of interferences and the enrichment of target biocides in water samples can be achieved by applying extraction.

Solid phase extraction (SPE) which is the extraction of pesticides, pharmaceuticals from water matrix, is usually preferred for sample preparation before chromatographic analyses since compounds can be adsorbed on the sorbent surface in contrast the interferences are eliminated.

Solid phase extraction become an alternative technique to the liquid-liquid extraction due to its simplicity, low cost and automation (A.Zwir,et. al, 2006). The main mechanism of SPE is based on the sorption of analytes on a solid sorbent and purification of extract. The more detail information about solid phase extraction is given at Appendices.

However, the recovery calculation and pH optimization for solid phase extraction cartridge is necessary. The recovery calculation of the cartridge can be carried out by spiking the target biocides from the stock solution to the pure samples. The equation below is used for this calculation;

Recovery (%) = (conc. Found/conc. Spiked)*100……..(1)

Incomplete elution, adsorption of analyte to the matrix, poor adsorption of analyte to the cartridge can result in poor recovery from SPE process. Therefore, the selection of cartridge volume and capacity, type of elute and pH is very important and optimization studies are necessary for each parameter.

In this scope, solid phase extraction was applied to water samples and different types of cartridge with different capacity were tried in order to find optimum one. Oasis 3cc HLB SPE cartridge was preferred for surface waters as a solid phase extraction cartridge since it was very responsive with TCS and CHD. Firstly, SPE cartridge was conditioned with 10 ml methanol by passing its own gravity. After this step, cartridge was equilibrated with 10 ml of pure water in a same way with conditioning step. Then sample was loaded to the cartridge and was passed through the cartridge by vacuum. During sample loading step, the flow rate was adjusted to 10 ml/min. In conditioning, equilibrating and sample loading step, it was not let to go cartridge being dried. At the end of sample loading through the cartridge, cartridge was dried under vacuum completely for 15-20 minutes. After this step, if there was no enough time for the elution and evaporation step, the cartridge could have been stored at - 10°C in refrigerator up to 40 days however, elution of cartridge was carried out within two days in this study. For the elution step, the cartridge was eluted with 25 ml of methanol by letting to pass through the cartridge with the help of gravity; vacuum was not used during elution process. Target analytes which were TCS and CHD, were collected in 25 ml methanol at the end of the elution step. After this step, nitrogen gas was used in order to dry the sample and separate the analyte from methanol or 50°C oven was used in order to evaporate the methanol and obtain target analyte. The final step of the extraction was collection of the dried sample to the vial with 1 ml of methanol/water mixture (% 25 methanols, %75 pure water). This means 1 liter of sample was concentrated to 1 ml this satisfy 1000 times concentrated sample in the case of %100 recovery. After collection of sample into 1 ml vial, the samples were ready for the HPLC/MSMS.

The pH adjusting and type of elute optimization was carried out for each biocide in order to get maximum recovery. Average recoveries for surface water, wastewater and sludge are given in Table 11.

Table 11: Recoveries of surface water, wastewater and sludge extraction

Sample name	% Recovery for TCS	%Recovery for CHD
Surface water samples	%92±1.67	%96± 1.94
Wastewater influent samples	%79±2.5	%86±1.3
Wastewater effluent samples	%72±2.07	%83±2.14
Sludge samples	%85±2.04	%90±0.98

3.4.1.3. Standard Solution Preparation

Target biocides standard solution could not be purchased directly and so standard solution preparation also were necessary in order to carry out measurements. TCS, with ≥ 97 purity, was purchased from Sigma-Aldrich and CHD, with ≥ 99,5 purity, was purchased from Sigma-Aldrich. TCS was solved in methanol and was prepared at least 500 ppm standard solution, on the other hand CHD has been some difficulties about solving in methanol and it was solved with methanol in warm water bath. These solutions has been kept in dark and at 4°C for 1-2 months, after this time period new solutions were prepared since concentration of solution, initially 500 ppm, reduced during this time period. Calibration samples were prepared by using these standard solutions.

3.4.1.4. HPLC –MS/MS measurements

The detection of target biocides in surface water which were taken from Çamlıdere Reservoir, Kesikköprü Reservoir and Eymir Lake, was carried out by HPLC/MSM devices. For this purpose, Agilent 6410B Triple Quadruple MSMS was used and operated in negative ESI mode in conjunction with Agilent C-18 Capillary column. The reliable measurement of target biocides on this device

depends on the reliable method development for HPLC/MSMS before the analysis of samples. The detailed information about Liquid chromatography, especially HPLC/MSMS is given at Appendices.

The method development of LC/MSMS consists of two parts which are the optimization of fragmentor voltage and collision energy for each biocides. These optimization studies were carried out in order to obtain largest signal for the precursor ion. The protonated molecule was used for the precursor ion. At each voltage, fragmentor was checked by analyzing each compound separately. According to data, optimal fragmentor signal was selected and then each compound was optimized again to select optimal collision energy for quantifying and qualifying compounds. According to the Ferrer et al. (2009) study, the optimal fragmentor voltage and collision energy for TCS was stated as 75 and 5 eV respectively. In this scope, optimization studies for both collision energy and fragmentor voltage was carried out as described in following sub-sections.

3.4.1.4.1. Method Development of HPLC/MSMS

The first step of the method development studies is the preparation of stock solution and determination of concentration of stock solution which is used for optimization analysis. Therefore, stock solution was prepared at higher concentration (100 ppm) in glass bottle which was well cleaned with Alchonox.

The analyses with the HPLC-MS/MS were carried out with the 100 ppb mixed standard solution which included TCS and CHD, in order to identify the precursor and product ions of each compounds and to determine the optimum fragmentor voltage and collision energy. Standard solution was prepared as a mixture of TCS and CHD since these biocides also exist in the environmental samples together. The aim of this application was to satisfy the elimination of the effects of biocides in chromatographic measurement to each other. This means that, if they have any effects to each other for liquid chromatography measurement, these effects can also be seen for mixed standard solution and since the method development was carried out according to mixed standard solution, the effects of biocides to each other was eliminated.

By using the 100 ppb mixed standard solution, precursor ions, product ions, fragmentor voltage and collision energy for each biocide were determined. Finding source parameters are given in Table 12.

Table 12: Precursor ion, Product ions, fragmentor voltage and collision energy of each compound

Compound name	Polarity	Precursor ion	Fragmentor voltage	Product ion	Collision energy
TCS	negative	287	70	35	4
CHD	positive	253	100	170.1(Quantifier)	12
				177.1(Qualifier)	8
	positive	505	130	336(Quantifier)	19
				184(Qualifier)	30

The polarity of the compound is important in order to determine the mode of the method: positive mode or negative mode. Two product ions which are qualifier and quantifier were determined for CHD, differ from TCS, in order to be sure that this product is exactly belongs to the desired compounds. However, TCS has only one product ion since they cannot be fragmentized into another ion. The product ion which is named as Quantifier has higher signal and more dominant than the qualifier. CHD can be charged as +1 or +2 and so it has two precursor ions. The analysis of CHD can be carried out through the one of them which has higher signal to noise ratio.

The precursor ion was found for each compound by MS2 scan mode. Then the optimum fragmentor voltage was determined for each precursor ion by using MS2sim with trying different fragmentor voltage. For TCS analysis, different fragmentor voltages from 10 to 100 were applied and the optimum one was determined as 70. On the other hand, for CHD analysis, fragmentor voltages from

50 to 150 were tried and the optimum one was determined as 100 and 130 for 253 and 505 precursor ions respectively. After that, in order to find product ion of each compound, product ion scan was carried out. The product ion for TCS was 35 m/z. On the other hand, two product ions which were qualifier and quantifier one, for each precursors of CHD were selected. For the precursor ion which has 253 m/z value, product ions were found as 170.1 and 177.1, as described afore-mentioned the product ion which had higher was selected as quantifier one. Moreover, for the precursor ion which has 505 m/z value, product ions were found as 336 and 184 for quantifier and qualifier respectively. After the determination of precursor ions, their corresponding optimum fragmentor voltages and product ions, the optimum collision energy was found by applying MRM. For collision energy determination, different collision energy levels from 2 to 20 were tried for TCS and the optimum one was determined as 4 when fragmentor voltage at 70, at the end of the analysis. For CHD, four different collision energies were selected for four product ions belongs to two precursor ions. Therefore, optimization of the HPLC/MSMS method for CHD and TCS was completed.

After completing the comprising of the method for compounds, some optimization studies related with the HPLC-MS/MS were carried out in order to obtain more accurate results.

3.4.1.4.2. Mobile Phase Optimization

The selection and preparation of mobile phase is very important for the achievement in liquid chromatography mass spectrometry analysis. The literature survey was carried out in order to determine the appropriate mobile phase for the measurement of TCS and CHD at the same time. There are different applications of the mobile phase for these compounds and in order to determine the optimum one, the mobile phase selection analysis was carried out. According to this, tested mobile phase and their results are given table below.

Table 13: Mobile Phases and Corresponding Signal Results

Content of mobile phase	Signal for TCS	Signal for CHD
5 mM Ammonium format+%0.1 Formic acid+methanol	Good	Excellent
5 mM NH_4OH+%0.5 acetic acid+methanol%94.5+%5 H_2O	Excellent	Good
10 mM ammonium format+%0.1 formic acid+methanol	Poor	Excellent

The mobile phase optimization studies firstly were carried out without any column in order to eliminate the column effect to the mobile phase performance. At the end of this study, the optimum mobile phase for both compounds was determined as 5 mM Ammonium format+%0.1 Formic acid+ methanol. Since the signal for TCS could be obtained without any background, the optimization of CHD gained much more importance and determination of optimum mobile phase was conducted according to CHD.

Moreover, column optimization was carried out with optimum mobile phase and two types of columns which are C-18 and C-8 were tried for this purpose. As a result of the analysis, C-18 column was preferred for HPLC-MS/MS system for determination of biocides. After all of these optimization studies, the optimization of physical parameters of HPLC-MS/MS method for each compound was carried out in order to increase the signal of each compound and so to decrease detection limits.

3.4.1.4.3. Optimization of the Source Parameter

Method developments and optimization of source parameters were required in order to get more accurate result. For these analyses, fragmentor voltage, collision energy, precursor and product ion of the method remained constant.

Samples were injected for TCS and CHD optimization methods separately. Moreover, more than two injections were carried out for each method in order to work parallel. All of these studies were conducted with optimum mobile phase and selected column C-18.

3.4.1.4.3.1. Source Parameter Optimization for TCS Method

In order to find optimum parameters for TCS, several runs (injections) were applied with different gas temperature. Gas temperatures were varied with keeping dwell time constant and their corresponding signal values are given in Table 14.

Table 14: Gas Temperature Optimization

Run Number	Dwell time	Capillary Voltage	Delta EMV	Injection volume (µL)	Gas Temperature	Signal Value
1	150	4000	400	10	350	$4.5*10^3$
2	150	4000	400	10	300	$4.7*10^3$
3	150	4000	400	10	250	$3.4*10^3$

As shown in the Table 14, optimum gas temperature was found as 300° C for TCS compound. Therefore, gas temperature was set to 300° C for the rest of the injections.

Another important source parameter in order to optimize the peak intensities is the capillary voltage. Therefore, the optimization studies for this parameter were also conducted.

Table 15: Capillary Voltage Optimization

Run Number	Dwell Time	Capillary Voltage	Delta EMV	Injection volume (µl)	Gas Temperature	Signal Value
1	150	3000	400	10	300	$5.0*10^3$
2	150	2000	400	10	300	$4.4*10^3$

The used capillary voltage during the method development studies for HPLC/MSMS was 4000 V. In addition to this, 3000 V and 2000 V also were tried respectively. According to the results of these studies, maximum signal was obtained when the capillary voltage was 3000 V. Therefore, the optimum capillary voltage for TCS measurement by HPLC/MSMS was determined as 3000 V and in the rest of the injections, capillary voltage was applied as 3000 V.

Delta EMV optimization is also necessary in order to increase the signal intensity for each compound. In this scope, different Delta EMV values were tested when gas temperature was 300ºC, capillary voltage was 3000 V and other parameter was kept constant. The results of Delta EMV optimization study are given in Table 16.

Table 16: Delta EMV Optimization

Run Number	Delta EMV	Signal Value
1.	300	$3.6*10^3$
2.	0	$1.1*10^3$
3.	200	$0.45*10^3$

The used Delta EMV during the method development studies for HPLC/MSMS was 400. Moreover, different Delta EMV values which were 200,300 and 0, were also tested and as a result, optimum value of Delta EMV was found out to be 400. Therefore, the rest of the injections were carried out according to this value.

The volume of injection also affects the signal intensity and chromatographic results. The volume of injection must be high enough in order to obtain high intensity but not to cause peak disturbance. Injection volume for TCS method was tried as 20 and 30 micro liters respectively with using determined optimized value for gas temperature, delta EMV and capillary voltage. The results are represented in Table 17.

Table 17: Injection Volume Optimization

Run Number	Injection Volume	Signal Value
1	20 microliter	$7.8*10^3$
2	30 microliter	$9.25*10^3$

The signal value for 20 micro liter injections had lower signal value than that of 30 micro liter injection. Moreover, first one caused the peak expansion and the retardation of retention time of TCS. Therefore, the injection volume for TCS was selected as 30 micro liters during TCS analysis in water samples.

After all of these trials for TCS method development, optimum parameters were found and whole analyses were conducted with respect to these parameters.

Table 18: Optimum Values For TCS Method

Dwell Time	Delta EMV	Capillary	Gas Temperature	Injection Volume
150	400	3000 V	300ºC	30 micro liter

3.4.1.4.3.2. Source Parameter Optimization for CHD Method

Similar analyses to the TCS method optimization were carried out in order to find optimum source parameter for CHD method in HPLC/MSMS. The tested gas temperature values with keeping other parameter constant and their corresponding signal values are given in Table 19.

Table 19: Gas Temperature Optimization

Run Number	Dwell time	Capillary	Delta EMV	Injection Volume (Microlitre)	Gas Temperature	Signal Value
1.	150	4000	400	10	350	$6.8*10^4$
2.	150	4000	400	10	300	$6.2*10^4$
3.	150	4000	400	10	325	$6.6*10^4$

After these trials, optimum value for the Gas Temperature parameter of CHD method was found as 350º C. Therefore, gas temperature was kept at 350ºC during the rest of measurement and optimization analyses.

Capillary voltage is the important source parameter which affects the signal intensity. Therefore, capillary voltage optimization studies for CHD method were also carried out.

Table 20: Capillary Voltage Optimization

Run Number	Dwell time	Capillary	Delta EMV	Injection Volume (Microlitre)	Gas Temperature	Signal Value
1	150	3000	400	10	350	$8*10^4$
2	150	2000	400	10	350	$4.8*10^4$ (distorted peak)

Optimum Capillary Voltage was taken as 3000 and this value was used in the rest of the optimization.

Delta EMV optimization studies were carried out similar to TCS method source parameter optimization.

Table 21: Delta EMV optimization

Run Number	Dwell time	Capillary	Delta EMV	Injection Volume (µL)	Gas Temperature	Signal Value
1	150	3000	300	10	350	worse
2	150	3000	400	10	350	$5.2*10^4$

Delta EMV value was optimized as 400 for all the product ions. Therefore, the rest of analyses were carried out with this value.

Moreover, the volume of injection affects the signal intensity and chromatographic results. Injection volume for CHD method was tried as 20 and 30 micro liters respectively with using determined optimized value for gas temperature, delta EMV and capillary voltage.

Table 22: Injection Volume Optimization

Run Number	Dwell time	Capillary	Delta EMV	Injection Volume (µL))	Gas Temperature	Signal Value
1	150	3000	300	20	350	$1.4*10^5$
2	150	3000	400	10	350	Worse
3	150	3000	400	30	350	$1.9*10^5$

Signal of 30 micro liter injection volumes was better than 10 and 20 micro liter and so 30 micro liter injection was determined as optimum value.

After all the injection for the CHD method, optimum parameters were found and method was applied according to this optimum values tabulated below.

Table 23: Optimum Values For CHD Method

Dwell Time	Delta EMV	Capillary	Gas Temperature	Injection Volume
150	400	3000	350	30 micro liter

3.4.1.4.4. Calibration Analysis for HPLC/MSMS

The effective biocides measurements require very sensitive calibration analysis for each target biocides. Therefore, after completing the optimization studies, calibrations analyses were carried out for each compound separately.

Calibration studies were conducted with 14 points for TCS from 0.001 ppb to 100 ppb. Dilutions from original stock solution which had 100 ppm concentration were carried out with methanol on the other hand; dilutions from ppm to ppb level were carried out with pure water/ methanol mixture (75:25). Moreover, low concentration which had low and incorrect response were omitted and calibration curve was formed with respect to this. This means that, there must be a correlation between the prepared calibration sample concentration and respected response which was obtained from chromatograph. The points (calibration sample) which did not have this correlation, was accepted as incorrect and they were omitted from the calibration curve. The R^2 value for calibration curve of each compound was above 0.99.

The calibration curve for TCS is given at Figure 6.

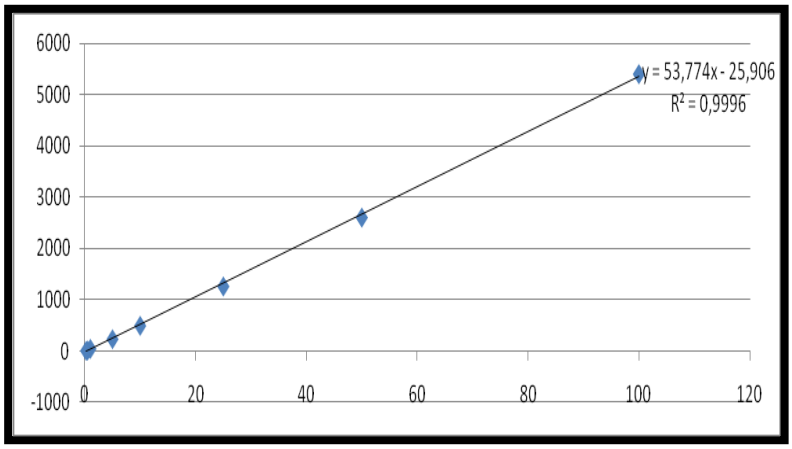


Figure 6: Calibration curve of TCS (100, 50, 25, 10, 5, 1, 0.5, 0.25 ppb)

The concentration and corresponding response value of this calibration is given in Table 24.

Table 24: Concentration and their responses of TCS

Concentration (ppb)	Responses
100	5394
50	2605
25	1266
10	503
5	243
1	54
0,5	22
0,25	17

The similar analyses for calibration of CHD were also conducted. The graphical representations of the calibration of CHD and the table of corresponding responses for CHD are given below. The accepted lowest concentration for CHD calibration was 0.1 ppb since the lower concentrations, having insufficient and incorrect response values, was omitted. It is important to conduct calibration curve with at least 5 acceptable points. The calibration curve example of CHD is illustrated at Figure 7 and corresponding responses are given in Table 25.

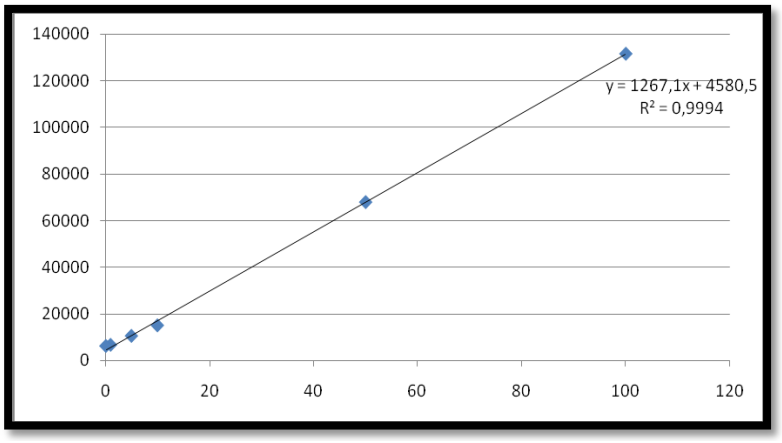


Figure 7: Calibration curve of CHD (100, 50, 10, 5, 1, 0.1 ppb)

Table 25: Concentrations and their responses for CHD calibration

Concentrations(ppb)	Responses
100	131529
50	67909
10	15033
5	10549
1	6708
0,1	6214

Another important point for chromatographic measurement is the determination of Limit of Quantification (LOQ) and Limit of Detection (LOD) value for each method.

3.4.1.4.5. LOQ and LOD Calculation

The last step before the sample measurement is the LOQ and LOD calculations. After carrying out calibration studies, the lowest concentration that can be detected by the HPLC-MS/MS was determined and this concentration was injected 10 times in order to LOQ and LOD calculations. LOQ can be expressed as the lowest concentration that can be detected exactly on the other hand; LOD is stated as the observable but not quantified limits. If the measured concentration for water samples by HPLC/MSMS is below LOQ value of the corresponding method, this concentration is stated as below LOQ and cannot be determined exactly. The equality of LOQ/LOD parameters is given below.

- LOQ=(10*S)/M ……(2)

- LOD= (3*S)/M ……(3)

where S : standard deviation of result of 10 injection

M: slope of the calibration curve

The LOQ and LOD calculations were carried out for TCS and CHD methods. In this scope, when the example calibration curve for TCS was taken into consideration, 10 injections of 0.25 ppb sample were carried out and corresponding responses are represented in Table 26.

Table 26: Results of 10 injections of 0.25 ppb TCS

Number of Injections	Responses
1	30
2	19
3	21
4	22
5	22
6	20
7	25
8	29
9	30
10	31

According to these results, the standard deviation and LOQ, LOD value for TCS was calculated and illustrated in Table 27.

Table 27: LOQ and LOD of TCS method

S	M	LOD(ppb)	LOQ(ppb)
4,677368681	53,774	0,260945923	0,869819742

Same procedure was applied for the CHD LOQ and LOD calculations by taking example CHD calibration analyses afore-mentioned. In this expect, LOQ/LOD analyses were conducted for 0.1 ppb CHD sample. The obtained results are given in Table 28.

Table 28: Results of 9 injection of 0.1 ppb CHD

Number injections	Responses
1	9805
2	8909
3	8085
4	8333
5	8278
6	8324
7	8363
8	7945
9	7756

According to these results, LOQ and LOD values for CHD method were determined as below.

Table 29: LOQ and LOD value of CHD

S	M	LOD(ppb)	LOQ(ppb)
168,0698268	1267,1	0,3979	1,32641328

The calibration correction analyses were conducted with each run for sample measurement since calibration could indicate some deviation with time. Therefore, calibration samples which had 20 to 100 ppb were also measured with water samples run. If there was any significant deviation for the retention time of the peak or signal intensity for calibration samples, new calibration set was prepared, new LOQ/LOD values were calculated and these were used for quantification of sample concentration. If there was no significant change for these parameters, the recent calibration curve was used in order to quantify the samples.

Besides the LOQ/LOD determination for the method, signal to noise ratio is also important parameter for the chromatographic measurement.

3.4.1.4.6. Signal to Noise Ratio

When conducting analysis near the lower limits of an LC method, S/N can be the restrictive factor in method performance. The term of signal to noise ratio can be defined as the ratio of signal size to that of the noise. Peaks become unclear when the magnitude of peak height is similar to the noise of the system. This noise can be occurred when there is any perturbation in the detector output of the system. Moreover, it can arise from the sensor and the associated electronics of the chromatographic system. If this is the case, the problem must be solved by increasing the signal, reducing the noise, or both.

Therefore, the signal of the peak has to be greater than that of noise in order to satisfy the identification of the peak clearly.

The noise of the system can be measured between two lines bracketing, the baseline and the signal is measured from the middle of the baseline to the top of the peak. It is important to satisfy high S/N ratio in order to improve LOQ/LOD of the method. This situation can be satisfied by either increasing the signal, decreasing the noise, or both.

In this thesis, the acceptable S/N ratio was accepted as 10 and the runs which had smaller S/N ratio were not taken into account. The optimization studies were carried out in this aspects and it was satisfied that S/N ratio always be greater than 10. Especially for TCS, S/N ratio is relatively smaller than that of CHD since their signal intensity was low in HPLC analysis. However, the background noise value for TCS was so low and this did not affect the S/N ratio in a negative way.

3.4.2. Biocide Analysis in wastewater and sludge samples

Biocide analyses in wastewater and sludge samples were conducted in a same with water analyses as mentioned in Section 3.4.1.

3.4.2.1. Sample Preparation

Sample preparation process for wastewater samples was same with surface water samples with one exception. As distinct from surface water sample analyses, wastewater samples firstly were filtered through the roughing filter and then the filtrates again were filtered through the 0.7 micron glass fiber filter before the solid phase extraction process.

Differ from water sample analyses, drying of samples at 80°C were carried out for sludge samples before extraction process.

3.4.2.2. Extraction of Samples

The extraction procedure of wastewater was nearly same with the extraction of surface water samples. The extraction procedure was same but the volume of sample to be extracted was 500 ml, 750 ml and 1000 ml for influent sample, effluent of aeration tank sample and secondary effluent sample respectively. The volumes of extracted sample were determined according to the pollution level of the samples, since wastewaters have higher concentrations of biocides than that of surface waters; the extraction amount was smaller than that of surface water. As distinct from surface water samples, 6cc Oasis cartridge was preferred for wastewater influent samples which had high impurities, due to poor analyses results of 3 cc cartridge for influent samples. The extraction process is same with 3cc cartridge except the volume of addition materials.

In order to extract the sludge samples, ultrasound-aided sequential extraction method was preferred and applied sludge samples (Sönmez et al., 2012). In this scope, 0.5 g of dried sludge sample was weighted. 100 ml methanol and 0.5 g solid sample put into 100 ml appropriate glassware which was cleaned according to afore mentioned process in order to eliminate any contamination and adsorption of biocides on the glass surface. This mixture was sonicated for 30 minutes and then was centrifuged for 10 minutes at approximately 3000 rpm. After centrifuge process, the extracts (supernatant) of the sample were taken in to 500 ml conical flask. The solid particles in the samples were stick on the bottom surface of the centrifuge container and these particles were washed with methanol, were transferred in to 100 ml glassware which was used for first extraction of the sludge samples. The extraction of sludge samples repeated 3 times and 100 ml of methanol again were added on the solid samples which were transferred from the centrifuged container, 30 minutes sonication and 10 minutes centrifuged steps were carried out each time. In this way, at the end of the extraction of the sludge samples, 300 ml methanol and analyte mixture was obtained. If the solid particles are visible in the extract, it has to be filtered through glass fiber filter (110 mm) again before drying step. The extracts did not have visible particles for sludge samples and so the filtration step was skipped out.

The extracts were directly put in to the 80°C oven and waited until all methanol portion were separated from the analyte. The final step of the extraction was collection of the dried sample to the vial with 3 ml of methanol/water mixture (% 25 methanols, %75 pure water). After collection of sample into 1 ml vial, the samples were ready for the HPLC/MSMS and HPLC/UV measurements. This extraction procedure was also applied for samples taken from Tatlar WWTP at January 2012 by evaporating water portion of samples at 100°C and obtaining solids which include biocides.

3.4.2.3. Standard Solution Preparation

Same standard solutions with water samples were used for wastewater and sludge sample analyses.

3.4.2.4. HPLC –MS/MS measurements

Similar with water sample analyses, the detection of target biocides in wastewater and sludge samples which were taken from Tatlar WWTP, Kayseri, Lara, Hurma, Kemer WWTP and METU VRM WWTP, were carried out by HPLC/MSM devices. For this purpose, Agilent 6410B Triple Quadruple MSMS was used and operated in negative ESI mode in conjunction with Agilent C-18 Capillary column.

Method development, calibration part was same with surface water samples as described under the Section 3.4.1.4.

3.4.2.5 HPLC/UV Measurements

Another preferred measurement device for biocide analyses in wastewater and sludge samples was HPLC/UV. The measurements of Tatlar WWTP wastewater and sludge samples taken at January 2012 were carried out with HPLC-UV. For this purpose, HPLC-UV was used in conjunction with HPLC-Saule Kromosil 100 C18 column. As a mobile phase Acetonitrile (%75) and Ultra Pure Water (%25) were preferred.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Biocide Monitoring Results

This chapter covers the biocide monitoring results of surface water samples and waste water samples as well as the removal and fate of biocides in WWTPs.

4.1.1. Surface Water Monitoring

As mentioned in "Section 3.1.1." surface water samples were collected from Çamlıdere Reservoir, Kesikköprü Reservoir and Eymir Lake as to represent a clean moderately polluted and polluted water sources, respectively, during one year sampling period. Samples were then subjected to target biocide analysis. Besides, water quality parameters, such as Total Organic Carbon (TOC), Total dissolved solids (TDS), pH, temperature were also monitored as to follow pollution state of the waters sources and also as to see if there exists any correlation with the biocide levels. The results obtained are presented in the following sub-sections.

4.1.1.1. Biocides in Çamlıdere Reservoir

Monitoring results for the general water quality parameters for Çamlıdere Reservoir can be depicted from Table 30.

Table 30: General Water Quality Parameters of Çamlıdere Reservoir

Sampling Time	pH	Temperature (°C)	TDS (mg/L)	TOC (ppm)
January 2010	7,4	3,3	86	1,2
February 2010	7,7	5,7	78	6,6
March 2010	7,8	8,9	83	7,3
May 2010	8,3	19,7	82	6,2
July 2010	9,0	25,7	89,2	6,5
September 2010	8,8	21,8	100	6,3
October 2010	7,24	15,7	89	5,9
November 2010	8,3	10,2	99	8,8
January 2011	6,7	5,8	91	10,8
February 2011	7,6	7,6	88	14,5
March 2011	7,5	9,3	85,7	6,3
April 2011	7,8	10,3	88,1	5,21
May 2011	7,9	16,2	88,9	5,28

As can be seen from Table 30, the temperature values indicated normal variation during the seasons of year while, TDS did not indicate any significant changes during the sampling period. Moreover, pH of water samples slightly changed and it did not indicate either sharply increase or decrease during sampling period. Actually the pH of water samples is very important due to its effect on the form of TCS in water samples. Winkler et al. (2007) demonstrated that TCS found predominantly in its phenolic form at pH<< 8.1 (pKa value of TCS) and found in its ionized form at pH>>8.1 that's why TCS became more hydrophobic at pH<<pKa.

For Çamlıdere measurements, lower TCS values observed in Oct 2010 and Jan 2011 could be attributed to lower pH conditions, as TCS would be in molecular form and hence could have been eliminated from water through its adsorption onto the solid matrix due to high hydrophobicity. However, pH of water samples did not indicate significant variation during rest of sampling period.

TOC measurement results of Çamlıdere Reservoir revealed that there was significant change from November 2010 to February 2011 since concentration of TOC reached to 14,5 ppm which is so high for water sources accepted as clean. On the other hand, the rest of samples indicated normal TOC concentrations. This means that from November 2010 to February 2011, unexpected situation occurred in Çamlıdere Reservoir. This could be arisen from the transportation of organic material from environment due to heavy rain during this time period.

The measurement of the target parameters, TCS and CHD, were conducted at laboratory conditions as described in **"Section 3.4.1"**. The concentrations of these target compounds during sampling period are tabulated in Table 31.

Table 31: Biocides concentrations in Çamlıdere Reservoir

Sampling Time	TCS Conc. (ppt)	CHD concentration (ppt)
May 2010	2,15	<1,38
July 2010	5,4	2,61
September 2010	4,44	<1,38
October 2010	0,65	<1,38
November 2010	3,61	<1,38
January 2011	<0,86	<1,72
February 2011	8,77	<1,72
March 2011	5,45	<1,72
April 2011	10,42	<1,72
May 2011	11,15	<1,72

As seen from Table 31, TCS concentrations were always higher than the CHD concentrations during the sampling period, as probably the usage of TCS is more widespread with respect to CHD. Indeed, for the samples after September 2010, concentration of CHD could not be measured exactly since the level of sample concentration was lower than the limit of quantification (LOQ) value of LC/MSMS for CHD. This situation could be arisen from decrease in method sensibility for low concentration. Therefore, the concentration of CHD for the mentioned sampling period was expressed as "below 1.38 ng/L" and "below 1.72 ng/L" which are calculated with regarding LOQ of method and % recovery of extraction. Relatively high CHD concentrations in May 2010 and July 2010 as compared to other sampling months could be attributed to the hot weather conditions leading to higher degree of evaporation and hence, higher CHD levels. On the other hand, CHD concentration did not increase back in May 2011, probably due to relatively low temperature conditions and heavy rain experienced, different than May 2010. Nevertheless, the same trend was not observed for TCS. As can be seen from Table 31, highest TCS concentration was observed in May 2011, unlike CHD. The reason for this remained unexplained. Nevertheless, when TCS variation alone was examined, it was seen that during winter season TCS concentrations were generally lower than that of summer and spring seasons, except Feb 2011.

This situation can arise from evaporation of water from reservoir basin during hot weather condition and also heavy rain during winter period. Temperature variation during sampling period can be depicted from Figure 8 in order to see if it affects TCS levels.

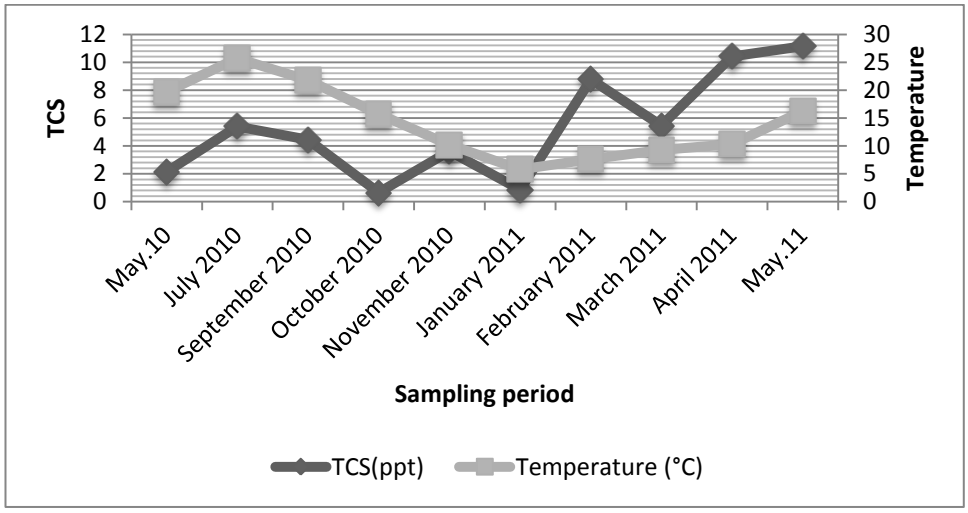


Figure 8: TCS concentration and temperature variation in Çamlıdere Reservoir

As seen from Figure 8, generally, when temperature of water samples increased TCS concentration increased. However, TCS concentration exhibited a small increase in November 2010 although temperature of water samples was decreasing. This could be reasoned by heavy rain which transports the organic material to the Çamlıdere Reservoir via its tributaries. This could also be attributed, though indirectly, to the possible excess public usage of antimicrobial agent owing to the great public concern raised on the inflectional flue disease (H1N1) during those days. Similarly, a small decrease in TCS concentration was observed in March 2011 when temperature of water samples continued to increase. This decrease in TCS concentration could derive from the dilution with the help of melting of remaining winter snow near the reservoir and on the dam layer with increasing temperature.

Therefore other parameter must be investigated in order to determine the reason of this variation in TCS concentration. In this scope, comparison of TCS level and TOC of water samples is given below.

The concentrations of Total Organic Carbon (TOC) in water samples can be accepted as a good indicator for water quality. Therefore, it was expected that TCS concentrations were high when TOC levels were also high in surface waters. In this scope, Figure 9 illustrates the variation of TOC and TCS concentrations in Çamlıdere Reservoir during the sampling period from May 2010 to May 2011.

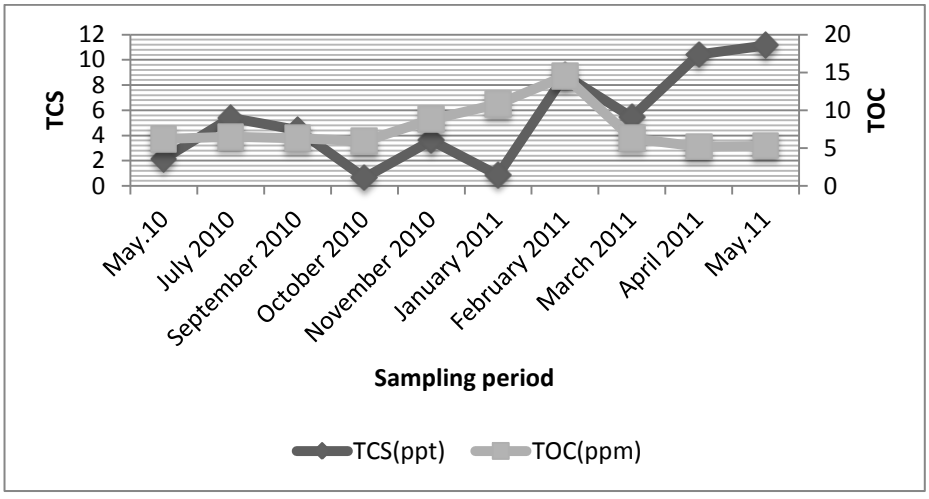


Figure 9: TCS and TOC concentration variation in Çamlıdere Reservoir

According to analyses results, there was a direct relationship between TCS and TOC concentration of samples except a few samples. It was expected that TCS concentration was high during winter time period due to highly consumption of antibacterial agents during this time period. However, for January 2011, TCS concentration indicated a decrease abruptly although TOC concentrations continued to increase. The rainy days in winter might have been the reason of this decrease in TCS concentration since this rain satisfied the dilution effects. On the other hand, rain could not create dilution effect on TOC concentration due to transportation of high amount of organic material from environment. However, in this situation it is necessary to be sure about the amount of transported biocides to the reservoir due to heavy rain.

Moreover, for April and May 2011 samples, TCS and TOC concentrations indicated different trends with respect to each other and TOC concentrations decreased while TCS concentrations was rising. This increase in TCS concentration during April and May 2011 could be the results of evaporation due to hot weather conditions or overturn in surface water during spring. Adsorbed TCS on the sediment layer could be mixed with upper part of the water body and so TCS concentration could arise. On the other hand, the decrease of TOC could arise from the uptake of dissolved organic carbon in water samples by microorganisms/bacteria with increase in temperature, however at this situation TCS concentrations was not affected and not decreased like TOC. This might have been the results of low biodegradation of TCS in aquatic environments. In order to be sure the reason of TCS decrease and increase in aquatic environment exactly, it is necessary to determine the main elimination mechanisms of TCS in environment.

4.1.1.2. Biocides in Kesikköprü Reservoir

Samples taken from Kesikköprü Reservoir were analyzed for same water quality parameters with other surface water samples, Çamlıdere Reservoir and Eymir Lake. The results are tabulated in Table 32.

Table 32: General Water Quality Parameters of Kesikköprü Reservoir

Sampling Time	pH	Temperature (°C)	TDS (mg/L)	TOC (ppm)
January 2010	8,5	5,9	885	1,1
February 2010	8,1	7,1	877	5,5
March 2010	8,7	11,4	855	6,2
May 2010	8,4	21,6	831	3,7
July 2010	8,3	23,5	826	4,7
September 2010	8,5	20,8	834	4,7
October 2010	7,6	15,2	951	4,3
November 2010	7,7	12,4	1035	5,9
January 2011	NM*	-1,0	1100	4,3
February 2011	7,5	8,0	824	5,2
March 2011	7,8	10,7	847	5,7
April 2011	7,9	11,5	862	4,2
May 2011	8,1	15,8	874	4,9

*NM: Not measured

Similar with Çamlıdere Reservoir analyses, temperature, pH and Total Dissolved Solids measurement for Kesikköprü Reservoir were carried out as mentioned at "**Section 3.2.1.2"**. Since Kesikköprü Reservoir was known as moderately polluted water sources with respect to Çamlıdere Reservoir, it was expected that pollutant concentration was higher than that of Çamlıdere. In this scope, TDS concentration of Kesikköporü Reservoir was so high with respect to Çamlıdere. However, TOC concentration for both water sources did not indicate significant difference. The temperature values indicated normal variation during the seasons of year with respect to weather conditions. Moreover,

TOC concentration in Kesikköprü Reservoir was at regular level during sampling period except some months. This means that there was no significant change in pollution level of the Kesikköprü Reservoir during sampling period. As mentioned for Çamlıdere Reservoir, pH is an important parameter for biocides, especially for TCS, due to its effects on hydrophobic characteristics of biocide. However, pH variation in Kesikköprü Reservoir remained low and did not lead to any significant change in biocide concentration.

Beside these parameters, target compounds CHD and TCS measurements for Kesikköprü Reservoir were carried out as other surface water samples. Concentrations of these target compounds are given in Table 33.

Table 33: Biocides Concentration of Kesikköprü Reservoir

Sampling Time	TCS Conc. (ppt)	CHD concentration (ppt)
May 2010	2,37	5,31
July 2010	8,21	3,97
September 2010	16,47	<1,38
October 2010	2,03	<1,38
November 2010	5,00	<1,38
January 2011	2,92	<1,72
February 2011	<0,86	<1,72
March 2011	11,3	<1,72
April 2011	15,52	<1,72
May 2011	48,96	<1,72

Similar with Çamlıdere Reservoir analyses results, TCS concentration was higher than CHD concentrations during the sampling period except May 2010. It was also expected according to the usage amount of biocides in daily life. Moreover, since the concentration of CHD was generally so low in water samples and the sensibility of method became worse day to day, its concentration remained under the detection and quantification value of method and so it could not be measured accurately. Therefore, the concentration of CHD was almost expressed as lower than the value calculated with respect to LOQ value of the method and % recovery of extraction.

As mentioned before, CHD was so low during the sampling period and it was generally under limit of quantification (LOQ) of the measurement method. However, TCS concentration could be monitored during whole sampling period and it was reported that TCS concentrations remained low during winter season with respect to spring and summer time. Although highly usage of antibacterial agents was expected for winter time, this widely consumption could not reflect to TCS concentration as a raise. The relatively high concentration of TCS in spring and summer time might also arise from evaporation of water from reservoir during sunny days.

As already mentioned, the reason of variation in TCS concentrations could be temperature changes and so evaporation of water from reservoir during sampling period. In this scope, Figure 10 illustrates variation of TCS concentrations with respect to temperature of Kesikköprü Reservoir samples.

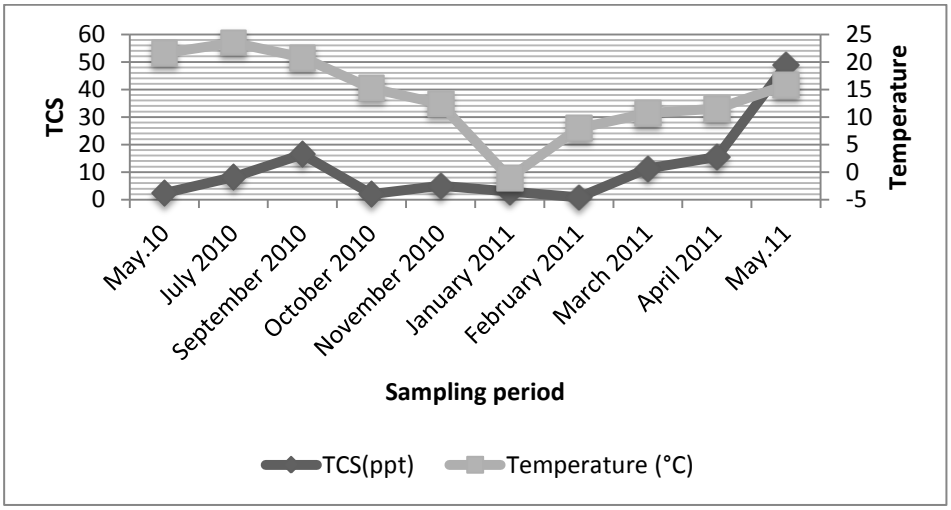


Figure 10: TCS concentration and temperature variation in Kesikköprü Reservoir

According to the analyses results, TCS concentration was generally compatible with temperature. However, there was a significant difference between May 2010 and May 2011 TCS concentration although temperature values did not indicate significant change. The reason of this difference might be excess rain and so precipitation amount during 2010 spring season. However, other parameters which affect the TCS occurrence in water samples must be investigated in order to determine the exact reason.

An indicator for pollution level of water samples is Total Organic Carbon (TOC) and it was also monitored during sampling period. It was expected that there was linear relationship between TCS and TOC concentration. This means that TCS concentration was expected to be high when TOC levels were also high in surface waters. In this scope, Figure 11 illustrates the variation of TOC and TCS concentrations in Kesikköprü Reservoir.

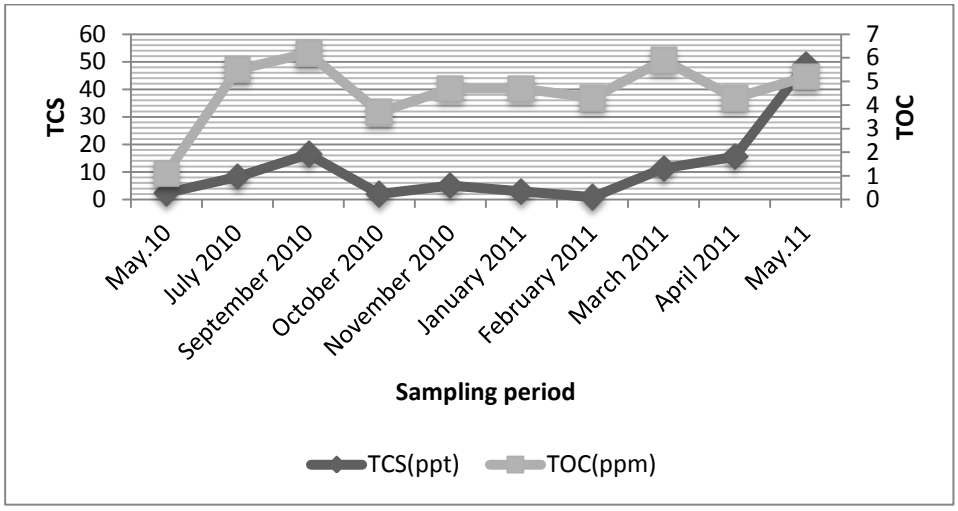


Figure 11: TOC and TCS concentration variation in Kesikköprü Reservoir

Kesikköprü Reservoir was selected as moderately polluted water sources however; TOC concentrations of water samples were generally lower than that of Çamlıdere Reservoir. Correlation between TCS and TOC was not so strong. However, reverse relationship between TCS and TOC concentration was observed during March 2011 to April 2011 sampling period.

During this sampling period, TOC concentration in aquatic environment started to decrease however, TCS concentration reached to their maximum concentrations. This situation could have arisen from the overturn in aquatic environment during spring season, resulting in the TCS previously bound tightly to the bottom sediments to release to the overlying water.

4.1.1.3. Biocides in Eymir Lake

Samples taken from Eymir Lake were analyzed for same water quality parameters with other surface water samples. The results are tabulated in Table 34.

Table 34: General Water Quality Parameters of Eymir Lake

Sampling Time	pH	Temperature (°C)	TDS (mg/L)	TOC (ppm)
January 2010	9,5	3,0	1534	19,9
February 2010	8,6	7,1	1360	22,3
March 2010	8,8	12,5	1205	14,5
May 2010	8,6	23,2	1344	6,1
July 2010	8,5	26,0	1423	10,6
September 2010	8,1	22,0	1600	6,7
October 2010	7,2	16,1	1583	6,1
November 2010	8,3	12,0	1983	15,6
January 2011	6,8	4,6	1490	3,3
February 2011	7,0	5,9	1416	3,6
March 2011	7,63	10,2	1379	6,3
April 2011	8,02	10,9	1409	4,4
May 2011	8,25	17,1	1447	6,8

Since Eymir Lake was known as the most polluted water sources among whole sampling area in this study, it was expected that pollutant concentration was higher than that of Çamlıdere and Kesikköprü Reservoir. This expectation came into view for TDS concentration of Eymir Lake since it was so high with respect to Çamlıdere and Kesikköprü. Furthermore, TOC concentrations were generally detected at high level in Eymir except January and February 2011. During sampling period, it was known that the lids between Eymir and Mogan Lake were destroyed due to heavy rain and so water transfer occurred from Mogan to Eymir Lake. This situation brought on augmentation of water in Eymir Lake and this was reflected as an enhancement in water quality of Eymir Lake. The temperature values indicated normal variation during the seasons of year with respect to weather conditions. Moreover, pH of samples was also monitored during sampling period in order to determine the possible effects of pH on antibacterial agent occurrence in aquatic environment. In this scope, pH of samples were detected as low when compared to other sampling time at October 2010, January 2011, February 2011. At October 2010 and February 2011, TCS concentration at water samples were also detected as low however, TCS concentration reached its maximum value at January 2011 when its pH had lowest value.

Beside these parameters, target compounds CHD and TCS measurements for Eymir Lake were carried out like other surface water samples. Concentrations of these target compounds are given in Table 35.

Table 35: Biocides Concentration of Eymir Lake

Sampling Time	TCS concentration (ppt)	CHD concentration (ppt)
May 2010	9,88	1,95
July 2010	2,54	2,34
September 2010	2,02	<1,38
October 2010	<0,95	<1,38
November 2010	17,59	<1,38
January 2011	757,7	<1,72
February 2011	<0,95	<1,72
March 2011	23,67	<1,72
April 2011	35,92	<1,72
May 2011	17,41	<1,72

According to the analyses results, TCS concentration was almost higher than the CHD concentrations during the sampling period. TCS concentration for Eymir Lake had generally highest level among surface water samples especially at January 2011. Analyses were carried out with duplicate samples and three injections for same samples were made and the average values were given as a result. For January 2011 sample, whole injection results were at that level. On the other hand, CHD concentration remained under Limit of Quantification (LOQ) value of method during sampling period and at January 2011 also.

As the analyses results of Kesikköprü and Çamlıdere Reservoir, CHD was so low during the sampling period in Eymir Lake and it could not be detected generally since it was under limit of quantification (LOQ) of the measurement method. However, TCS concentration reached its highest concentration among surface water samples. Especially, at January 2011, TCS concentration was measured as 757.7 ppt which was very high also for surface water with respect to literature study findings which are mentioned at Section 2.2.1.1. Beside this, pH of water sample at January 2011 was detected as 6.8 and so it was expected that TCS existed at molecular form at this pH and adsorbed on solid particles in water body. Therefore, this high TCS concentration remained unexplained. At February 2011, a sharply decrease of TCS concentration was observed at Eymir Lake and the reason of this decrease could be related with some physical parameters of lake. Since CHD concentration could not be measured exactly, only TCS variation in lake was taken into consideration.

As mentioned before, the reason of TCS concentration variation could be temperature changes and so evaporation of water from reservoir during sampling period. In this scope, Figure 12 depicts variation of TCS concentrations with respect to temperature of Eymir Lake samples.

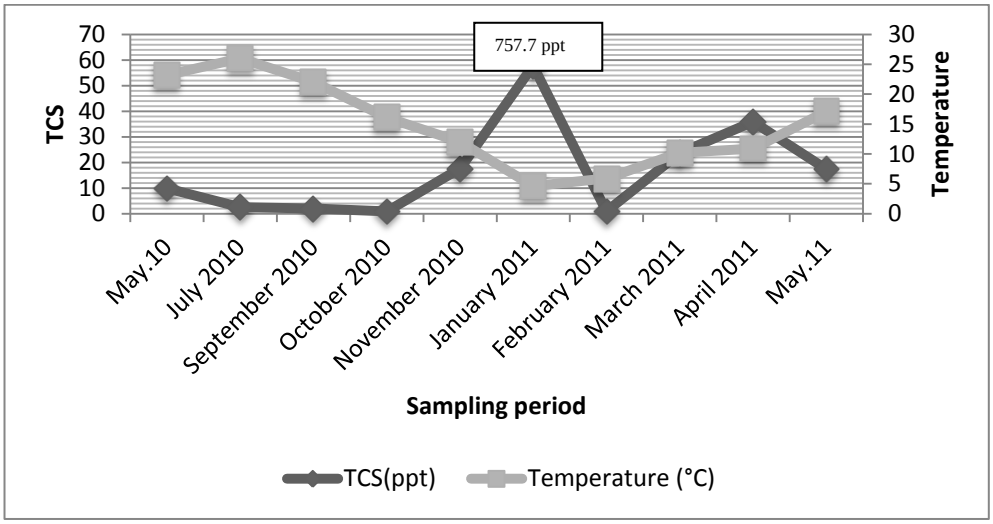


Figure 12: Variation in TCS concentration and temperature of Eymir Lake

According to the analyses results, differently from other surface water samples, Kesikköprü and Çamlıdere, TCS concentration was not generally correlated with temperature. The highest TCS concentration was measured at January 2011 while temperature of water sample was measured as the lowest value of the sampling period for Eymir Lake. The reason of this increase in TCS concentration could be the illegal discharge of wastewater including antibacterial agent. In this scope other pollution related parameters must be investigated for January 2011. Furthermore, the reverse relationship between temperature and TCS concentration was also observed at May 2011. At this time period, temperature increased and so it was expected that TCS concentration increased also due to evaporation of water however, TCS concentration indicated a decrease. This decrease could be originated from heavy rain during this sampling period.

As mentioned before, in order to determine the reason of high TCS level especially at January 2011, other pollution related parameter must be investigated. In this scope, Total Organic Carbon (TOC) concentrations which were also monitored during sampling period, can be evaluated in water samples. The variations of TOC and TCS concentrations in Eymir Lake are given at Figure 13.

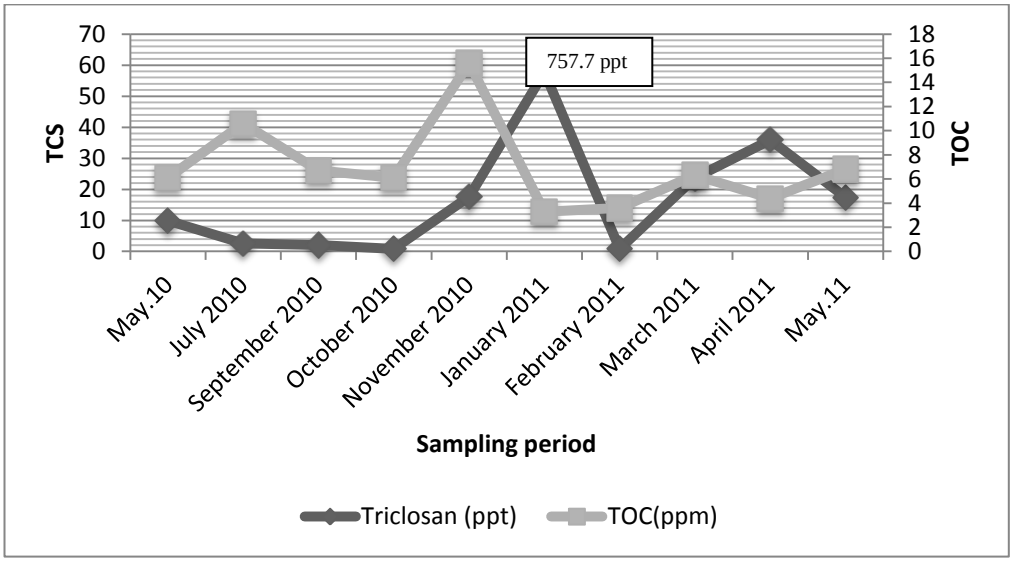


Figure 13: TCS and TOC concentration variation in Eymir Lake

TOC concentration of Eymir Lake was generally higher than that of other surface water samples. However, it was measured at lowest level in January 2011 when TCS concentration reached to highest level. According to TOC level of the lake, the discharge of wastewater could not be possible for January 2011. Therefore, the reason of this sharply increase in TCS concentration must have been different.

4.1.1.4. Comparison of Biocide and Pollution level of Three Surface Water Sources

As mentioned before, pollution related water quality parameters are TOC and TDS. In this scope, the measurements of these parameters were carried out during one year sampling period in order to verify the pollution level of water samples. At the beginning of the study, Çamlıdere Reservoir was selected as clean water source, Kesikköprü was selected as moderately polluted one and Eymir Lake was selected in order to represent polluted surface water sample.

First polluted related parameter in this study is Total Organic Carbon and the variations in TOC concentration for three water sources are given at Figure 14.

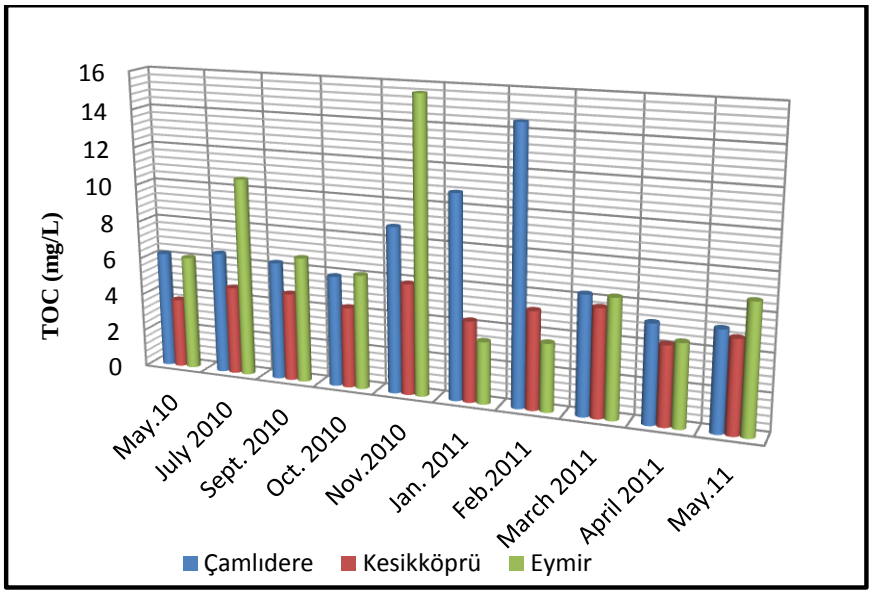


Figure 14: TOC concentrations of three water sources

Although Kesikköprü Reservoir was selected as moderately polluted sample for surface water, it was seen that it had lowest TOC concentration among three surface water sources and TOC concentration of Kesikköprü Reservoir stayed almost steady during the sampling period. On the other hand, in January 2011 and February 2011, Eymir Lake had lowest TOC concentration unexpectedly. As mentioned before, this situation probably originated from the dilution effect due to water transfer from Mogan Lake to Eymir Lake through opening of lids between two lakes as a result of heavy rain. Furthermore, TOC concentrations of Çamlıdere exhibited a remarkable increase from November 2010 to February 2011 and even exceeded that of Eymir Lake.

Total Dissolved Solids (TDS) concentration being another indicator of pollution level of surface waters was also monitored. In this scope, the analyses results for three surface water samples are compared in Figure 15.

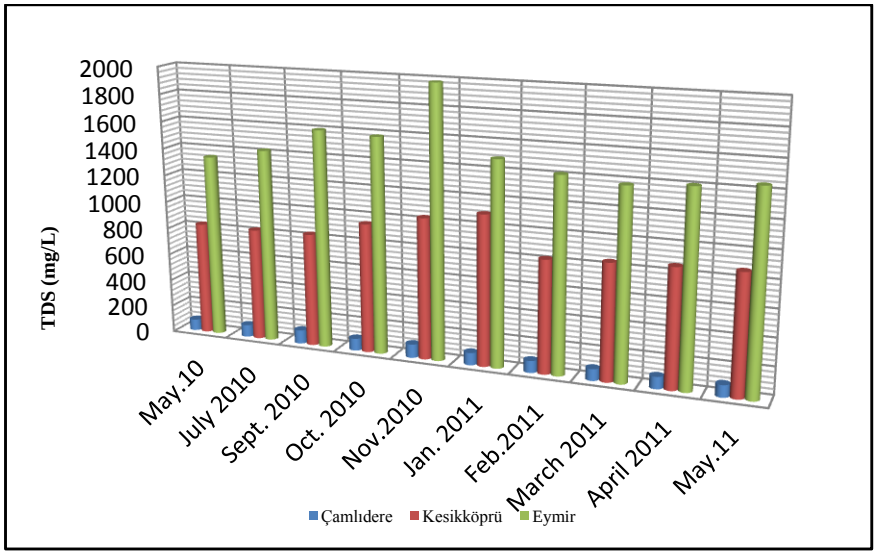


Figure 15: TDS concentrations of three water sources

As distinct from TOC concentrations of three surface water samples, TDS concentrations were compatible with expectation about pollution level of surface waters. Eymir Lake had the highest concentrations among three surface water samples as a polluted one and Çamlıdere Reservoir had the lowest concentrations.

According to the literature study, antibacterial agent concentrations were detected at high level for polluted surface water where treated wastewater effluent or direct wastewater discharge occurred. Therefore, it was expected that highest concentration of target antibacterial agent would be observed at Eymir Lake. As mentioned before, CHD concentration could not be measured generally at surface water samples and it was stated as below the value calculated with regarding LOQ of method and %recovery of extraction process. In this scope, variations of TCS concentration in three surface water sources are taken into account and given at Figure 16.

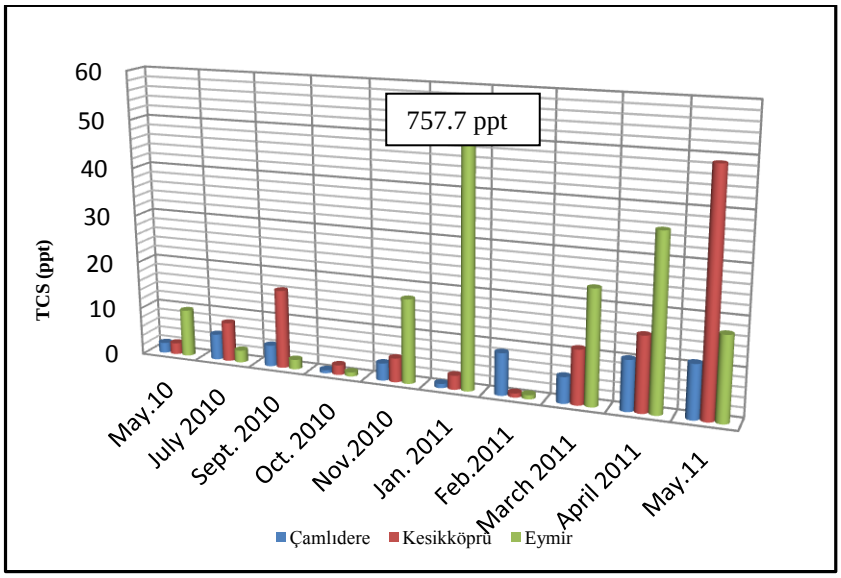


Figure 16: TCS concentration of three water sources

TCS concentration in surface water samples indicated a variation especially in Eymir Lake and it was not compatible with other pollutant parameters of these surface water samples. Although, Kesikköprü Reservoir was stated as moderately polluted, TCS concentration was much higher than that of Eymir Lake at July 2010, September 2010, October 2010 and May 2011. Similarly Çamlıdere Reservoir which was the least polluted one, had highest TCS concentration among three surface water samples at February 2011.

As a result of analysis of three surface water sources during one year sampling period, TCS and CHD concentration range in surface water of Turkey were determined and tabulated in Table 36.

Table 36: Summary of Biocides Level in Surface Water Sources

Name of water sources	TCS concentration (ppt)	CHD concentration (ppt)
Çamlıdere Reservoir (clean water sources)	0.65-11.15 (5.78±3.63)	< 1.38-2.61
Kesikköprü Reservoir (moderately polluted)	<0.95-48.96 (12.53±14.72)	< 1.38-5.31
Eymir Lake (polluted)	<0.95-757.7 (108.34±262.62)	< 1.38-2.34

As mentioned at Section 2.2.1.1., TCS concentration in surface water samples was detected in the range of 2.1-300000 ng/L with respect to the literature studies. For surface water sources in Ankara, Turkey, this range became 0.65-757.7 ppt (ng/L) and it is compatible with literature findings. Moreover, probabilistic description of Çamlıdere and Kesikköprü Reservoir data was carried out with SPSS software. According to statistical analyses, distribution of reservoirs' data indicated log-normal characteristics. The mean and standard deviation of data for log-normal distribution were reported as 1.75 and 0.988, respectively. The representation of distribution is given in Figure 17.

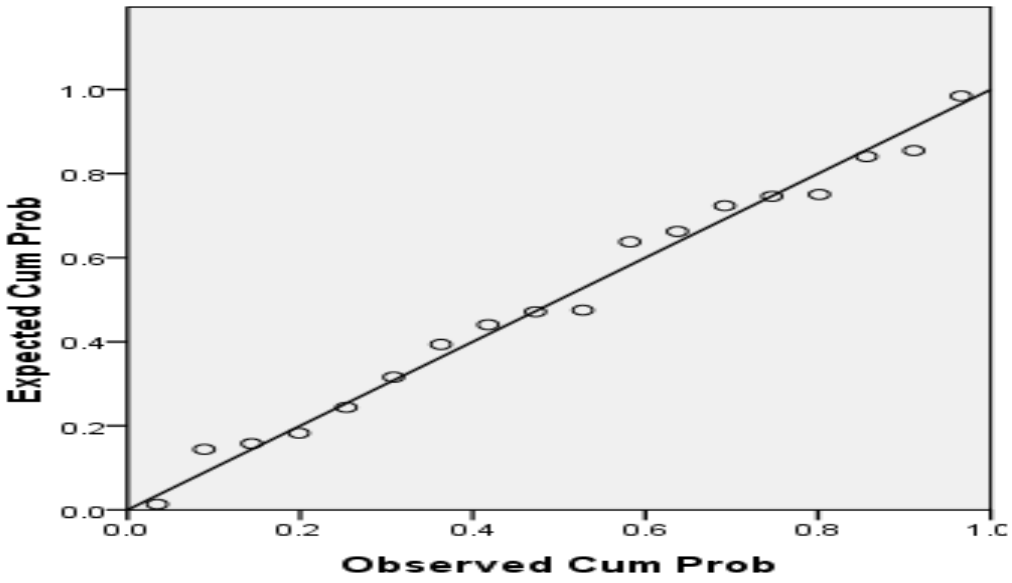


Figure 17: Log-normal distribution of Reservoirs' data

By the help of Eqn. (4), it was found that TCS data in reservoirs were below 20.58 ppt with 90% confidence level. 20.58 ppt was so low with respect to EC_{50} and NOEC concentration of TCS given in literature.

$Z = (X-\mu)/\sigma$……Eqn. (4)

Where ; μ= Mean

σ = Standard deviation

Z= Standard score (obtained from Standard Normal Probability Table (Z table))

Beside these, water distribution network studies were conducted in May 2010 and May 2011 for biocide concentration. In the former sampling, samples were taken from 10 different residential areas in Ankara and in the latter ones samples were taken from 16 different residential areas. For both sampling period, samples, were taken from Ankara Drinking Water Treatment Plant, were also analyzed in terms of TOC and biocide content. Results are represented in Table 37.

Table 37: Fate of Biocides in the Water Treatment Plant and in the Distribution Network

Source	**TCS, ppt**		**CHD, ppt**		**TOC, mg/L**	
	May 2010	May 2011	May 2010	May 2011	May 2010	May 2011
Inlet of the treatment plant	2.51	10.51	<1.33	<1.33	6.5	5.5
Outlet of the treatment plant	<0.87	<0.87	<1.33	<1.33	3.9	3.1
Distribution network	<0.87	<0.87	<1.33	<1.33	-	-

As seen from this table, biocide levels in outlet of treatment plant and in distribution network were below the detection limits and so could not be detected. Comparison of inlet and outlet values put also forward that TCS is being removed in the treatment plant, possibly by adsorption onto the sludge formed during coagulation/flocculation process.

4.1.2. Wastewater and Sludge Monitoring

As mentioned in "Section 3.1.2." wastewater and sludge samples were collected from Tatlar WWTP, METU WWTP, Kayseri WWTP, Lara WWTP, Hurma WWTP and Kemer WWTP. Influent and effluent samples as well as samples at different points (where appropriate) along the treatment plant were taken. Sludge samples were also taken. All these samples were subjected to biocide analysis. The results are presented in the following sub-sections.

4.1.2.1. WWTP Monitoring

Samples were taken from Tatlar, Kemer, Hurma, Lara, Kayseri and METU VRM WWTPs between May and November 2010. Biocide concentrations measurements are presented in Table 38.

Table 38: Biocides in WWTPs

WWTP	Treatment Process	Sampling date	Sample name	TCS (ppt)	CHD (ppt)
Tatlar WWTP	Activated Sludge Process	July 2010	Primary clarifier influent	3.34	*ND
			Primary clarifier effluent	4.24	ND
			Secondary clarifier effluent	8.92 9.94 (aeration tank effluent)	ND
		November 2010	Primary clarifier influent	94.47	ND
			Primary clarifier effluent	39.22	ND
			Secondary clarifier effluent	13.2	ND
			Secondary clarifier influent	38.3	ND
Kemer WWTP	Activated Sludge Process with Oxidation Ditch	May 2010	Kemer influent	ND	3.65
			Kemer effluent	ND	<1.60
		July 2010	Kemer influent	ND	3.52
			Kemer effluent	1.90	<1.60
		September 2010	Kemer influent	4.11	ND
			Kemer effluent	2.39	ND
Lara WWTP	Bardenpho Process	May 2010	Lara influent	ND	<3.07
			Lara effluent	ND	1.97
		July 2010	Lara influent	ND	ND
			Lara anoxic basin effluent	1.85	6.72
			Lara effluent	2.42	2.44
		September 2010	Lara influent	3.23	ND
			Lara effluent	1.40	ND
Hurma WWTP	Bardenpho Process	May 2010	Hurma influent	ND	<3.07
			Hurma effluent	ND	<1.60
		July 2010	Hurma influent	ND	1.39
			Hurma anoxic basin	3.04	<1.76
			Hurma effluent	2.28	<1.60
		September 2010	Hurma influent	1.77	ND
			Hurma effluent	3.01	ND
Kayseri WWTP	Activated Sludge Process with selectopr/bio-P basin	April 2010	Primary effluent	2.31	10.45
			Aerobic tank	22.59	1.582
			Anoxic tank	22.57	<1.76
			Secondary effluent	15.09	<1.60
METU WWTP	VRM	July 2010	Influent	11.44	<3.07
			UV effluent	15.05	<1.60

*ND: Not detected

For Tatlar WWTP, the concentration of CHD could not be measured since its peak in chromatographic view was dispersed. This situation could be expressed as peak tailing for chromatographic measurements. In order to eliminate this, measurement method and also extraction parameters were reviewed and some optimization studies were carried out for November 2010

measurements. Moreover, other substances in wastewater samples could bind or sorb the biocides during extraction procedure and so biocides could not have been measured correctly. In July 2010, although influent concentration of TCS was detected as 3.34 ppt, secondary clarifier effluent concentration was detected higher than this (8.92 ppt). This was odd as TCS accumulation in supernatant part was not expected. This observation could have arisen from matrix effects during the influent TCS measurement, so lower TCS concentration than actual was measured. Beside this possibility, grab sampling employed cause such inconsistency between influent and effluent TCS concentrations. Because, in wastewater treatment plant, wastewater portion which entered the system, could reach the secondary clarifier at the end of the hydraulic retention time, so the effluent sample was not the one really belonging to the influent of the system. One would argue here that it would not be the cause under steady state operation of the system. However, it is of great possibility that influent TCS concentration is not steady, changing even hourly. For example, Nishi et al. (2008) reported the daily even hourly variation of TCS input load. According to this study, highest input load for TCS was observed at a time between 10.0 and 12.00. Moreover, the study of Heidler et al. (2007) conducted with hourly samples demonstrated that TCS concentration was in the range of 0.8-10.8 μg/L and the average concentration of TCS was 4.8±3 μg/L. In order to solve this problem, in November 2010, it was decided to conduct composite sampling and 24 hours composite samples were taken with the help of peristaltic pumps equipped with timer. And, this attempt was proved successful as the highest concentration was detected in influent sample whereas the lowest one belonged to secondary effluent, as expected. Therefore, composite sampling seems to be more convenient than grab sampling for biocide analyses in wastewater samples. Moreover, TCS concentrations measured were higher in samples of November 2010 than that of July 2010. The reason of this difference can be the widely usage of antimicrobial agents in autumn season with respect to summer times. People tend to use more antimicrobial agents in autumn and winter time period especially during flu epidemic. Furthermore, level of TCS measured along the WWTP was also compatible with literature findings (0.83-562000 ng/L for influent samples and 0.05-269000 ng/L for effluent samples). The TCS removal efficiency of Tatlar WWTPs was calculated as 86% with regarding TCS influent (94.47 ng/L) and TCS effluent (13.2 ng/L) concentrations in November 2010 measurements. For CHD measurements in November 2010, same problem with July 2010 samples were observed although optimization of some method parameters were carried out. Therefore, CHD concentration in wastewater samples could be low to be detected or other substances in wastewater samples could bind biocides during extraction and so biocides could not have been measured correctly. One point that needs to be mentioned regarding the influent biocide concentrations (i.e in the primary clarifier influent), is that the measured primary clarifier influent values might be lower than the real level in the raw wastewater. Because, as stated earlier (Sec 1.1), TCS and CHD have hydrophobic characteristics, so, they tend to stick on solid particles in wastewater. Hence, one would expect that huge portion of biocides could be eliminated at screening parts of WWTP. Therefore, only dissolved or not sorbed fraction of TCS in wastewater could be measured via solid phase extraction process.

For Hurma, Lara and Kemer WWTPs, TCS and CHD measurements could not be carried out accurately for May and September 2010 samples, respectively, due to peak tailing in chromatographic results. Same situations also occurred for influent samples of July 2010. The reason of this could be same with Tatlar WWTP's. TCS concentrations in influent samples of Kemer, Lara and Hurma WWTPs were in the range of 1.77-4.11 ppt. For effluent samples this range became 1.40-3.01 ppt. Moreover, CHD concentrations in these WWTPs were detected as 1.39-3.65 ppt and <1.32-2.44 ppt for influent and effluent samples respectively.

For Kayseri WWTP, the problem of erroneous biocide detection in influent samples (i.e. influent value being smaller than effluent value), faced in Tatlar case, was also experienced for TCS, but not for CHD. Effluent CHD concentration could not be detected since it was below the limit of quantification (LOQ) value of HPLC/MSMS method (i.e. 1.32 ppt). Therefore, actual CHD removal percentage of treatment facility could not be calculated. However, it would be safely stated that the CHD removal efficiency is > %87. Regarding the TCS removal efficiency of Kayseri WWTP, its calculation was not possible due to the matrix effect problem experienced during the measurement of influent TCS concentration.

For METU WWTP, CHD could not be detected as both influent and UV effluent concentrations remained under LOQ value of the method. Moreover, TCS concentration in influent sample was found to be less than in the effluent. This situation was also observed in other WWTPs and the reason of these could be the matrix effects.

4.1.2.2 Sludge Monitoring

Besides, influent and effluent sample analyses along the treatment plant, biocides content of the sludge, were also monitored for six WWTPs. The results of these analyses are given in Table 39.

Table 39: Biocide Content of Sludges Originating from WWTPs

WWTP Name	Sampling date	Sample name	TCS conc. (µg/kg)	CHD conc. (µg/kg)
Tatlar WWTP	October 2010	Primary Sludge	2718	-
		Secondary Sludge	1854	-
	May 2011	Primary Sludge	2107	2240
		Secondary Sludge	1117	1582
Lara	July 2010	Lara sludge	1727	510
Hurma	July 2010	Hurma sludge	3687	1055
Kemer	July 2010	Kemer sludge	2928	2255
Kayseri WWTP	April 2010	Secondary sludge	3318	2216
		Digester sludge	3508	2742
METU	July 2010	Sludge	2844	1248

As seen from Table 38, biocide concentrations in sludge samples were higher than that of wastewater samples. This could be due to the tendency of biocides to be adsorbed onto solid particles and hence their participation in sludge nature. It was observed that both TCS and CHD contents of primary sludge samples were higher than that of secondary sludge samples. This could be explained as those biocides are exposed to primary sludge first and therefore majority is adsorbed. The levels of biocide in sludge samples were also compatible with literature findings which were in the range of 28-55000 µg/kg. Moreover, TCS concentration in sludge samples was also higher than CHD concentrations. The widely usage of TCS is the reason of its high concentration in environment.

4.1.2.3. Range of Biocide levels detected in WWTPs

As summary, target biocide concentration ranges obtained, for wastewater and sludge samples are given in Table 40.

Table 40: Concentration range of biocides in WWTPs

Sample name	TCS conc.	CHD conc.
Wastewater influent samples (ppt)	1.77-94.47	1.39-10.45
Wastewater effluent samples (ppt)	1.40-15.09	<1.60-2.44
Sludge samples (µg/kg)	1117-3687	510-2742

The range of TCS concentration in influent, effluent and sludge samples were compatible with literature values (0.83-562000 ng/L, 0.05-269000 ng/L and 28-55000 µg/kg, for influent, effluent and sludge samples, respectively).

4.1.2.4. Fate of Biocide in Selected WWTP

Sampling was carried out between 7 and 22 January 2012, on a weekly basis. Tatlar WWTP has two lines and each line has four aerated grit chambers and four primary sedimentation tanks. Effluents of grit chambers are combined and then divided again in-to four primary sedimentation tanks. Effluent of primary sedimentation tanks and recycled activated sludge are merged and portioned out to four aeration basins. After aeration basin, eight secondary clarifiers exist in each line. Samples were taken from the same line and the same tanks during each sampling time.

Biocide analyses of Tatlar WWTP during January 2012 were conducted. Analysis results of these samples are given in Table 41.

Table 41: TCS concentration in wastewater and sludge samples and corresponding % removal

Date of sampling	Primary clarifier Influent (ppb)	Secondary clarifier Influent (ppb)	Primary Sludge (µg/kg)	Secondary Sludge (µg/kg)	% Removal
07.01.2012	0,131	0,043	169,8	24,3	67,18
08.01.2012	0,218	0,058	110,7	78,6	73,39
14.01.2012	0,159	0,047	126,3	58,7	70,44
21.01.2012	0,203	0,095	223,9	136,4	53,20
22.01.2012	0,192	0,053	415,3	297	72,40
MEAN	**0,181**	**0,059**	**209,2**	**119**	**67,32**
STD	**0,035**	**0,021**	**123,3**	**107,5**	**8,24**

According to the analyses results, TCS concentrations were detected in the range of 0.131-0.218 ppb and 0.043-0.95 ppb for influent and secondary clarifier influent samples respectively. Moreover, concentration of TCS was reported as 0.181±0.035 ppb, for influent concentration. On the other hand, TCS concentration for effluent samples became 0.059±0.021 ppb. TCS concentrations, especially in influent samples, could be demonstrated accurately at January 2012 differ from WWTP Monitoring studies at Section 4.1.2.1. This situation could arise from extraction process applied. For January 2012 samples, ultrasound-aided sequential extraction method was applied to whole samples. In solid phase extraction, only dissolved TCS could be measured however, with the application of ultrasound-aided extraction, total TCS concentration could be obtained.

According to analyses results, removal efficiency was demonstrated as % 67.5±8.2 for primary and biological treatment together. However, the removal efficiency of secondary clarifier could not be calculated since secondary effluent samples could not be extracted with ultrasound-aided extraction due to its low solid content. According to the study of Winkler et al.(2007), the removal efficiency of secondary clarifier (4.7% removal) seemed to be relatively small with respect to overall removal in WWTP. Moreover, Thompson et al. (2005) reported that the main removal of TCS was observed between the influent and the end of activated sludge process with an approximately % 95 removal.

In addition, the levels of TCS were measured in the range of 110.7-415.3 µg/kg and 24.3-297 µg/kg for primary and secondary sludge respectively. Although these concentrations are present in the range of literature findings (28-55000 µg/kg), they are so low with respect to WWTP monitoring results which are given at Section 4.1.2.2. This situation could arise from different measurement device with different sensitivity. It is known that HPLC/MSMS device is much more sensitive to biocide analyses with respect to HPLC-UV device since it can detect samples which have concentration at ppt (ng/L) level.

CHAPTER 5

CONCLUSION

In this study, target biocides, TCS and CHD occurrence in surface waters and their fate in wastewater treatment plants are demonstrated. In this scope, three surface water sources, Çamlıdere, Kesikköprü and Eymir were selected in order to determine biocide level. Moreover, concentration of biocides was monitored at Ankara, METU, Kayseri and Kemer, Lara, Hurma WWTPs and fate of biocides in WWTP was investigated at Ankara WWTP.

Çamlıdere Reservoir was selected as clean water sources. According to the analysis results, TOC level of water source was higher than that of other surface water sources at January and February 2011. However, TDS level was observed as expected during whole sampling period. The target biocides, TCS and CHD concentrations were detected in the range of 0.65-11.15 ng/L and < 1.33-5.31 ng/L respectively. CHD concentrations could not be measured accurately since the level of CHD was generally lower than the limit of quantification (LOQ).

Kesikköprü Reservoir was selected as moderately polluted water sources. According to analyses results, TOC level of water source was always smaller than that of other surface water sources during whole sampling period. On the other hand, TDS level of reservoir was smaller than that of Eymir Lake and higher than that of Çamlıdere Reservoir during whole sampling period as expected. The main target of surface water analyses is to determine target biocide level in water samples. In this scope, TCS and CHD concentrations were measured in the range of 0.86-48.96 ng/L and < 1.33-5.31 ng/L, respectively. Similar with Çamlıdere Reservoir, CHD concentrations could not be measured accurately since the level of CHD was lower than the limit of quantification (LOQ) and also sensibility of the method had become much worse during sampling period.

Eymir Lake was selected as polluted water sources in this study. According to analyses results, TOC level of water source indicated some variations during sampling period. Especially at January and February 2011, it reached its lowest level and also TOC concentration of lake was smaller than that of other water sources during this time period. However, TDS level of reservoir was measured as expected. This means that its concentration was always larger than that of other water sources during sampling period. Moreover, target biocides concentrations were detected in the range of 0.86-757.7 ng/L and < 1.33-5.31 ng/L for TCS and CHD respectively. The range of TCS concentration is very broad and the highest concentration of TCS was also observed at Eymir Lake. This situation was expected since Eymir Lake was stated as polluted water sources in this study. However, 757.7 ng/L TCS concentration is very high for surface water samples. The reason of high TCS concentration could be the discharge of wastewater including antibacterial agent to the Lake during this time period.

Biocide concentrations in wastewater and sludge samples were carried out with different WWTPs which had different capacity and configuration. TCS concentrations were in the range of 1.77-94.47 ng/L and 1.40-15.09 ng/L for influent and effluent samples, respectively. On the other hand, CHD concentrations were detected in the range of 1.39-10.45 ng/L and 1.60-2.44 ng/L for influent and effluent samples, respectively. Moreover, fate of TCS in WWTP was investigated with weekly sampling from Tatlar WWTP in January 2012. According to analyses results, influent TCS concentration was detected as 0.181±0.035 ppb while 0.059±0.021 ppb was reported at effluent concentration. The removal efficiency of primary treatment and activated sludge system was reported as 67.5±8.2%.

Sludge samples from different WWTPs indicated that biocides mainly participated in sludge content in wastewater treatment plants. The range of biocide concentration in sludge samples can be expressed as 1117-3687 µg/kg and 510-2742 µg/kg for TCS and CHD respectively. The results of January 2012 sampling also demonstrated that high portion of biocides participate into sludge during WWTP treatment.

Therefore, one of the important mechanisms for biocide removal became adsorption onto sludge besides biological degradation. Moreover, Ying and Kookana et. al, (2007) reported that sludge application to soil affected the nitrification and respiration capacity of soil with TCS concentration higher than 2 mg/kg. Since TCS concentration in sludge samples were detected as higher than 2 mg/kg, especially in primary sludge sample, biocide concentration should be important parameter for sludge application and there would be some limitations about its applicable concentration.

In conclusion, TCS concentrations of surface water samples were observed as expected. Çamlıdere had the lowest and Eymir Lake had the highest concentrations. The level of biocide in surface water samples was also compatible with literature findings. Moreover, it was found that TCS data in Çamlıdere and Kesikköprü Reservoirs were below 20.58 ng/L with 90% confidence level and this concentration remained under the identified NOEC value for algae and LC_{50} value for fish (500 ng/L and 0.26-0.54 mg/l, respectively). Therefore, it can be concluded that the levels of biocides detected in selected surface water sources fall behind the toxic levels for the aquatic environment effects. However, considering the bioaccumulative nature of these biocides still there might be some chronic evidence effects on the biota. Moreover, possibility of antibiotic resistance development on bacteria needs to be considered when the concern is harmful effects of biocides on both public and ecosystems' health. In an ongoing project (BIOHYPO) of which this thesis is a part, antibiotic resistance development by biocides is being investigated. Based on the unpublished results of this project, biocide use throughout the food chain appears to be far from representing a risk for clinically relevant antibiotic resistance in pathogens. For wastewater samples, influent, effluent and sludge sample concentrations were detected as compatible with literature findings. However, biocide concentration in influent samples remained smaller than that of effluent sample in some of sampling. This observation could arise from matrix effects during influent TCS measurement or grab sampling. Therefore, composite sampling was applied for November 2010 samples for Tatlar WWTP and TCS concentration was detected as 94.47 ng/L and 13.2 ng/L for influent and effluent samples, respectively. The highest concentrations of biocides were reported for sludge samples as expected since biocides mainly tend to participate in sludge portion in WWTPs.

CHAPTER 6

RECOMMENDATIONS

As shown by this study, the level of biocides and their existence in aquatic environment and in wastewater treatment plant varied with respect to location, input load and removal mechanism. For surface water analyses, especially in running environment, it would be important to determine how far downstream TCS concentration could be detected and how far downstream TCS concentration remained under LOQ. Moreover, considering the sorption capacity of biocides, sediment analyses should also be carried out in order to demonstrate the fate of biocides in aquatic environment. Different removal mechanism such as photodegradation besides adsorption and biodegradation should also be investigated in order to determine the main removal mechanism of biocides from environment.

For wastewater sample analyses, monthly sampling with 24 hours composite samples during one year period would be better in order to observe seasonal variation in input load and in removal efficiency of WWTPs. Advance treatment mechanisms for biocides such as membrane filtration, ozonation should also be investigated in order to decrease the effluent concentration of biocides in WWTPs as even low concentration of biocides can affect the aquatic environment and human health negatively. However, the possibility of formation of more toxic compounds, such as dioxin, from triclosan with advance oxidation process would be investigated. Moreover, research related with sludge application would gain much more importance due to its high biocide concentration. It can be concluded that future studies about the environmental existence, fate and level of biocides will focus on sludge samples since there is a risk of contamination of biocide to soil and other environment via sludge application on soil. Therefore, biocide concentration in soil samples should also be analyzed in order to determine the level of biocide penetration due to application of sludge. Moreover, considering the possibility of applying the sludge on soil, aerobically and anaerobically digested sludge should be comparatively investigated for their biocide contents in order to represent the effects of aerobic and anaerobic conditions on biocide degradation.

In order to represent the biocides concentrations in Turkey various other fresh water and waste water sources, different sampling locations and treatment facilities from different region of Turkey would be included in future research. In a way, the biocide concentration profile of Turkey's surface waters and removal efficiency with different treatment processes could be determined. Especially surface water sources which expose to treated effluent discharge from WWTPs and are preferred as fresh water source, should be included in this research in order to determine whether the level of biocides in these water sources exceed the PNEC value or EC_{50} for aquatic organisms or not. Moreover, triclosan was known as endocrine disruptor chemicals and so some research about the effects of triclosan on fish should be investigated in order to represent its endocrine disruptor effects on living organisms. Furthermore, studies about the formation of Trihalomethane (THM) in fresh water sources should be conducted in order to investigate the contribution of triclosan on THM formation in the existence of free chlorine.

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APPENDICES

A: Solid Phase Extraction

The principle of SPE is also similar to the liquid-liquid extraction procedure in terms of partitioning of solutes between two phases. In liquid-liquid extraction partitioning occurs between two liquid phases on contrary in solid phase extraction process partitioning occurs between liquid phase and solid phase.

Solid phase extraction process has some advantages over liquid-liquid extraction process for especially pharmaceuticals applications. These advantages can be listed as below;

1. Faster sample preparation process (nearly 2/3 time reduction)
2. Less consumption of solvent and so less hazardous waste generation for the sample preparation process
3. Obtaining greater recoveries since sample transfer is optimized
4. Obtaining greater accuracy since the elimination of other undesired compounds can be satisfied accurately
5. Satisfaction of easy automation since simultaneous batch processing of multi-samples can be achieved.

Solid phase extraction procedures satisfy not only extraction of organic compound from samples but also eliminate the interfering components of the complex matrices. SPE is typically applied by passing the complex sample through the preconditioned column. If the concentration of target compounds in the sample is too low, SPE can concentrate the component to be measured. If the sample has complex matrix, SPE satisfy the elimination of undesired components in the samples and the clean, informative chromatograph can be obtain in this way. In other words, the SPE process eliminates interfering matrix components and concentrates the samples to be detected. This extraction process can be carried out through the interaction of the sorbent, the analyte and the solvent. The solvent must attract the analyte more than the matrix.

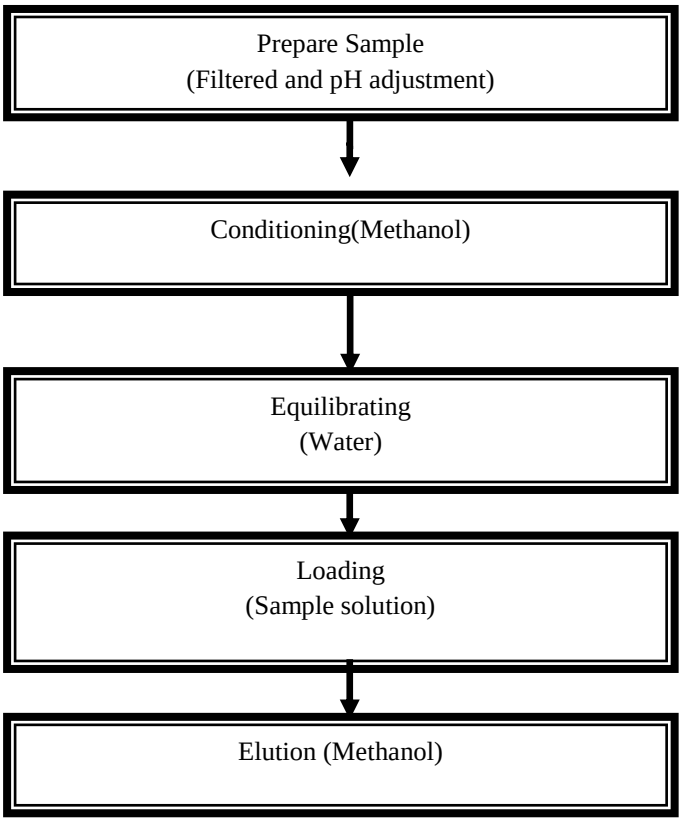
The selection of proper SPE sorbent depends on the interaction between the sorbent and the desired analyte. Hydrophobic, polar and non ionogenic properties of solute and sorbent must be known. Sorption of analytes from sample on to solid phase extraction cartridge is based on the attractive forces between the carbon-hydrogen bonds in the analyte and functional group of the sorbent surface. Polymer based sorbents are used especially to retain hydrophobic compounds. The pores on the polymers let the arrival of small hydrophobic organic compounds to the cartridge surface while other undesired compounds are eliminated from the bonded silica by the polymer. It is important to select proper sorbent type and this depends on the sample matrix and the analytical method. If the target analyte has polar properties, normal phase extraction can be selected on contrary if the analyte is less polar, reverse phase extraction can be preferred. The type of analyte and elution solvents can be classified as below.

Table A1: Type of Sorbent and Elution Solvents

Sorbent	**Analyte Type**	**Dissolving Solvents**	**Elution solvents**
Octadecyl, Octyl, Ethyl, Cyclohexyl, Phenyl	Nonpolar	Methanol/water, acetonitrile/water	For nonpolar analytes: hexane, chloroform For polar analytes:methanol
Cyano, Amino, Diol	Slightly-moderately polar-strongly polar	Hexane, chloroform	methanol
Silical gel, Florisil, alumina	Slightly-moderately polar-strongly polar	Hexane, chloroform	Methanol (dependent on type of analyte)

There are some limitations about flow rates and risks of plugging of the cartridge when the samples have suspended solids such as surface water and wastewater samples. If the sample has not been filtered before the SPE process, the typical volume of sample can be 500 ml but in the case of filtration of sample, the volume can be larger than 500 ml. There are important factors such as volume of sample, final volume of purified sample and ability to retain all analytes, when determination of optimum cartridge size is taken into account.

Solid phase extraction process is based on four steps which are conditioning, equilibrating, sample addition, and elution respectively. The elution steps can be indicated as below;



1. **Conditioning:** SPE cartridge must be conditioned with passing the methanol from the column before the sample loading step. The amount of methanol is important in order to obtain accurate conditioning and this amount can be determined as a result of optimization studies and also can be determined according to the manufacturer information. However, optimization of added methanol amount seems to be more accurate.
2. **Equilibrating:** After conditioning step, equilibration procedure is applied by addition of pure water. The amount of water added for equilibration is generally same as amount of methanol added for conditioning step. In this step column is cleaned from the impurities before the adding of the samples.
3. **Sample Loading:** After the completion of two steps, samples start to load and pass through the column with the 10 to 15 min/ml flow rate. Whole surfaces of sorbent are filled with target biocides in solid phase extraction cartridge.
4. **Elution:** Solid phase extraction cartridge is eluted with methanol in order to obtain target analytes from the adsorbed samples. The preferred solvent and pH of the sample is important in order to get maximum elution of target analytes.

The schematic representation of this extraction procedure can be illustrated as Figure A2.

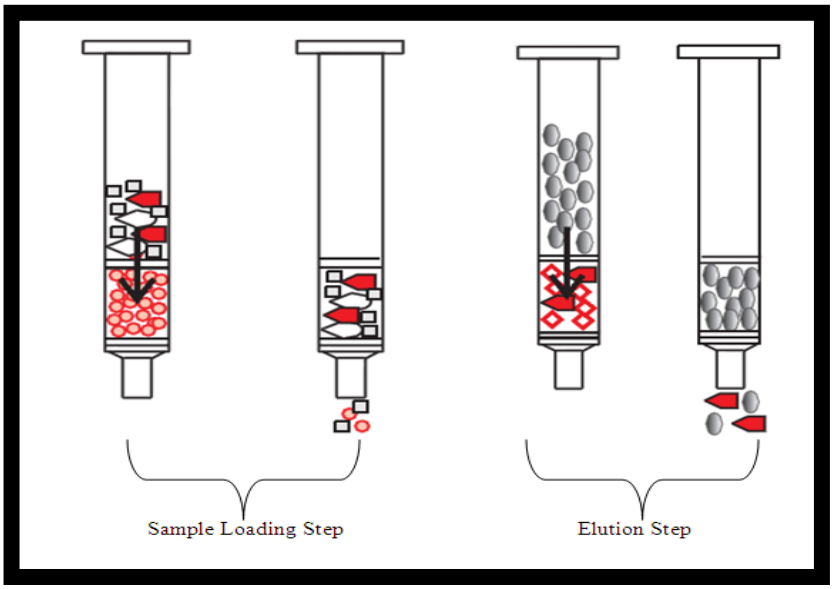


Figure A2: Schematic Representation of Extraction

Some problems can be occurred during the extraction process especially in sample loading process. These possible problems can be listed as below;

- ✓ Cartridge can be conditioned improperly. It is important to prevent the cartridge from being dried.
- ✓ Volume or mass of the sample cannot be suitable for chosen cartridge therefore the larger one can be preferred.
- ✓ Elution process also cannot be achieved due to the strength retention of the compound and so the selection of the solvent as a elution solution is very important.
- ✓ In some situation, incomplete removal of interferences due to the similar characteristics of analytes and interferences can be occurred. In this case, the pH adjusting of the wash or elute process can be necessary.

Beside these problems, the performance of the SPE cartridges which have same lot numbers can also indicate some differences. Therefore, the efficiency of SPE cartridge must be checked at least once before the usage. Identified amount of reagent is spiked into the sample in order to check the performance.

Moreover, it is very important not to let SPE cartridge go dry during conditioning process for the performance of SPE and not to exceed the range of flow rate during the loading process In this situation, the extraction of 1 L samples take long time and the usage of the vacuum manifold for the multiple samples become necessary.

However, matrix effects can be important problem for the water sample analysis especially for the sludge and the wastewater samples. There are lots of interferences in the wastewater samples since they are composed of different constitute. These interferences can eliminate the target biocide during the solid phase extraction process and so the chromatographic results are affected by this situation, the results become less accurate. The optimization of the pH of the samples and the usage of the internal standard can be solution for the elimination of the matrix effects. If the usage of the internal standard requires, it must be added to the sample at the beginning, before the sample preparation step (solid phase extraction step).

They must be included in the sample at same level for each sample and the amount of the internal standard must be enough to be quantified but not be too high to eliminate the signal of the analyte. The important point is to estimate the concentration of the target compound roughly in the sample and according to this, the amount of the internal standard which must be added in every sample, can be determined. If the concentration of the target compound is estimated as 100 fg to 25 pg, the internal standard amount might be 5 to 10 pg for each sample.

The isotopically labeled version of the target compound can be a good internal standard since they will indicate similar extraction recovery and ionization response in ESI mass spectrometry and they have similar chromatographic retention time. A chlorinated version of the parent compound is the typical internal standard since they have all necessary characteristics of internal standards.

B: Liquid Chromatography

HPLC/MS Measurement (Single Quadrupole Mass Spectrometry)

HPLC-MS associates the physical separation capability of HPLC with the mass analysis capability of MS. This technique has high sensitivity and especially is proper for determination of low concentration compounds in water samples (especially for relatively clean water samples).

In single quadrupole mass spectrometry, mass spectrometry is simply based on the analysis of ions which move with the help of vacuum. The ionization of sample takes place in ion source part of the device. The motion of ions is controlled by mass filter part during their travel to the detector in order to be turned into actual signals. The schematic representation of single quadrupole mass spectrometer can be illustrated as below.

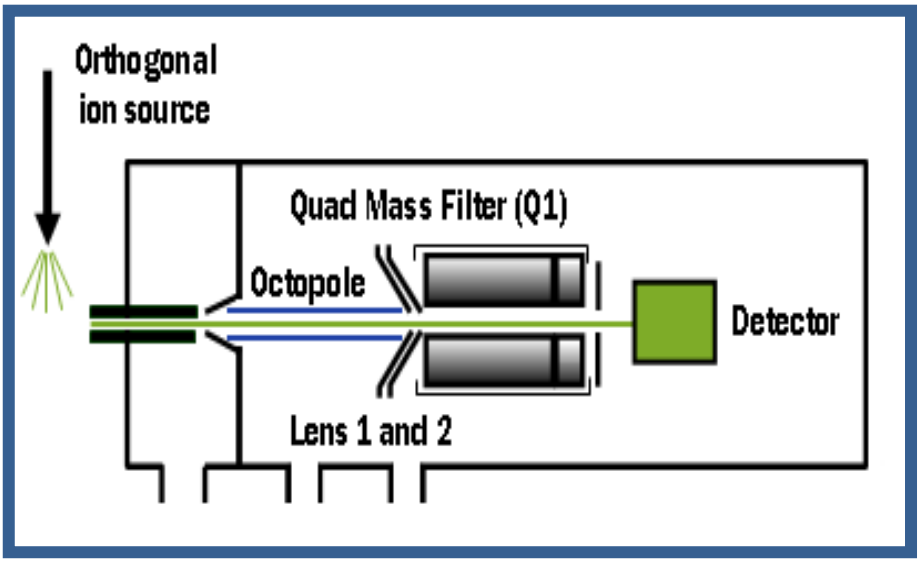


Figure B1: Schematic for single quadrupole mass spectrometer

Four parallel rods comprise the quadrupole mass analyzer parts. The rods select one or more particular m/z values according to the applied voltage. The working principle of the mass analyzer is mainly based on the selection of proper ions according to m/z values. Firstly specific voltages are set and applied, in this way only ions of the corresponding m/z value can reach the detector by passing through the quadrupole. Different ions can pass through the quadrupole by changing the voltages. Very simple presentation of working principle of single quadrupole figures in below.

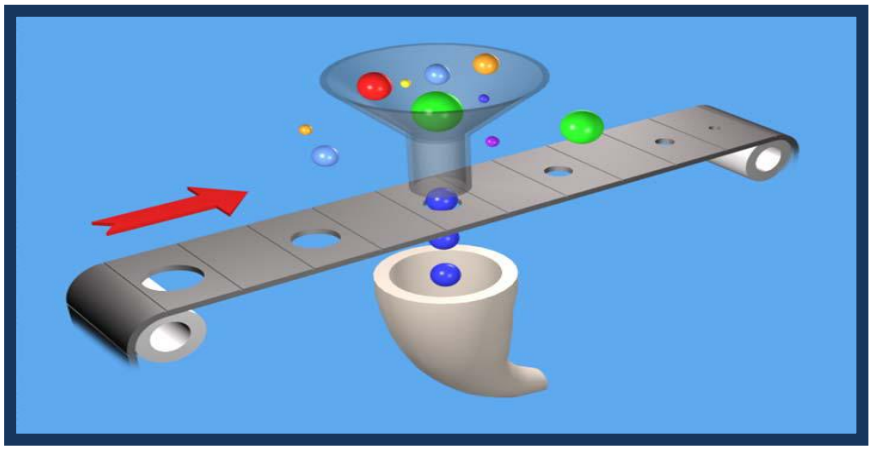


Figure B2: Conceptual model of a single quadrupole mass spectrometer

In this model, samples are ionized in external ionization source part and whole ions with different m/z values (represented as different color in the model) are collected in a funnel. The quadrupole part of the device is represented as moving belt which satisfies the selection of ions when they pass through different sizes openings. The selected ions pass from the funnel and filter here and then they reach to the detector. In this model, collecting funnel below the filtering belt in used in order to represent the detector part of the device.

In this system, it is possible that different m/z values pass through the mass spectrometer by moving the belt or changing the voltages on the rods. However, without any change in position, detector goes on to detect same m/z value during the scan period. Single quadrupole mass spectrometer cannot achieve the MS/MS process since it is not possible to identify the origin of product ions. In order to achieve this, triple quadrupole mass spectrometry which is the enhanced version of single quadrupole mass spectrometry, can be used.

HPLC/MSMS Measurement (Triple Quadrupole Mass Spectrometry)

A triple quadrupole mass spectrometer comprise of ion source which is followed by ion optics, and is different from single quadrupole in terms of existence of Q_3. The triple quadrupole tandem mass spectrometer is a combination of two quadrupole mass spectrometers in series. In this system, first and third quadrupoles operate as mass filters on the other hand, second quadrupole operate as a collision cell. The schematic representation of triple quadrupole system can be indicated as below.

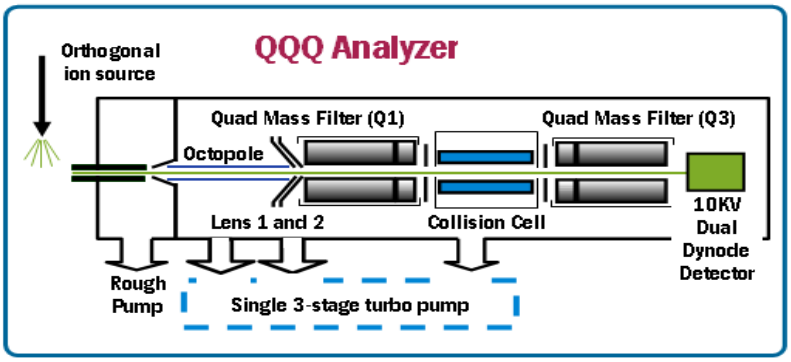


Figure B3: The Agilent Triple Quad MS

In this system, selected ions are filtered by hyperbolic rods before transferring to the collision cell part of the device. Collision cell which is named as second quadrupole, fragment the ions and it fills with nitrogen which is also used in ion source part. An inert and non-reactive collision gas is required and so nitrogen is used for this purpose. After the formation of fragment ions, third quadrupole comes for second filtering step in order to obtain one precursor and one product ion. Product ion which is an ion formed as the product of reaction including a particular precursor ion and precursor ion definitions and their scan period are very important for the mass spectrometry analysis.

In precursor ion scan period, precursor masses are scanned in the first mass analyzer and then the product ion is selected by the second mass analyzer. This type of scan cannot be done with time based MS instrument.

In product ion scan period, product ion which is scanned in the second mass analyzer is detected in the detector which is located after second mass analyzer.

Schematic representation of scanning and filtering of samples by triple quadrupole mass spectrometer can be indicated as below.

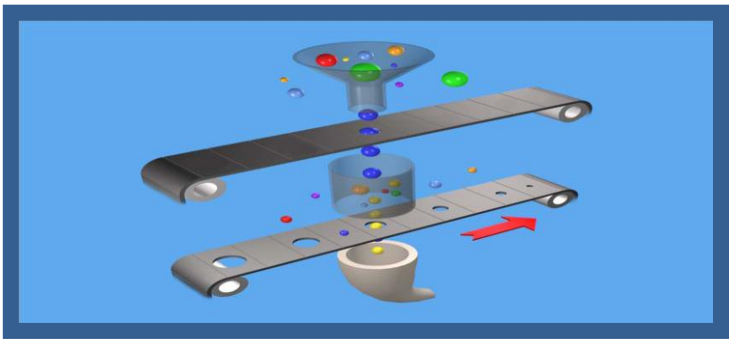


Figure B4: Conceptual model of a triple quadrupole mass spectrometer

In this conceptual model, mass analyzer is represented as moving belts and the first belt serves in order to determine that which precursor ion can reach to the collision cell. The applied voltages to the collision cell and quadrupole have to be different in order to make better the transfer of whole product ions to the third quadrupole.

In first quadrupole, a precursor ion is filtered and passes to the collision cell part in order to satisfy fragmentation. The fragments are again scanned in third quadrupole part in the scope of product ion scan. The fragment ions (product ions) actually represent whole structure of the precursor molecule and so triple quadrupole devices can determine the fingerprint of the initial compound. The determination of target compound can be obtained in a sensitive manner by fixing belt and monitoring specific precursor and in connection with product ion. In triple quadrupole mass spectrometry system, this mode is named as “selected reaction monitoring (SRM)”. Moreover, triple quadrupole mass spectrometry has multiple running SRMs options for same precursor ions, and this process is named as “multiple reaction monitoring (MRM)”.The quantification of impurities can be carried out by tandem mass spectrometry (QQQ) with using multiple reaction monitoring (MRM) for sensitivity and selectivity.

The monitoring work flow of the triple quadrupole mass spectrometer for target compound can be indicated as below.

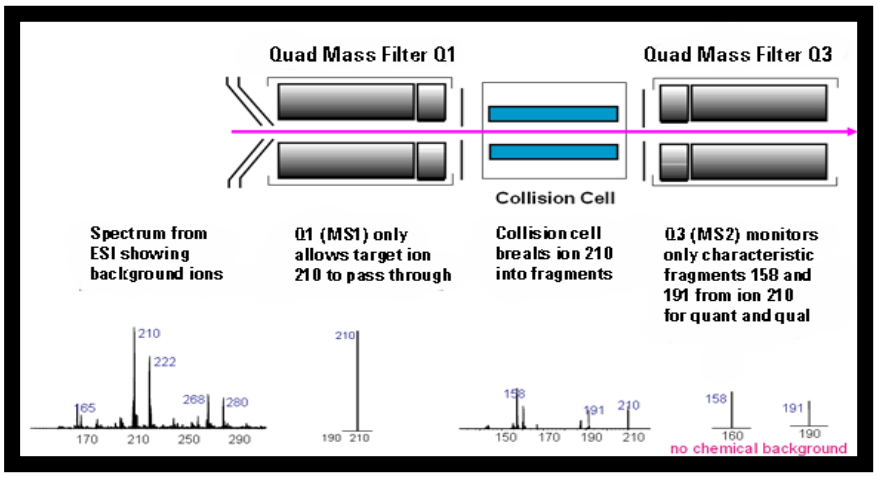


Figure B5: Triple quadrupole mass spectrometry analysis of compound which has 210 m/z value as a precursor ion

In general, first quadrupole (Q1) allows only target ion to pass through the collision cell and then this target ion breaks into fragment at collision cell. These fragmented ions are monitored at third quadrupole (Q3) in order to determine which one is used as quantifier ion and which one is used as qualifier ion. The ion which has more intense signal than other, become quantifier ion and it is used for quantification. In this situation other ion becomes qualifier ion and it is used for confirmation. For this example, this situation can be explained that a compound which has 210 m/z value pass through the quad mass filter (Q1) and Q1 let only passing of target ion and then this ion is broken apart in a collision cell. After that, Q3 detect only fragments which have 158 and 191 m/z value as a quantifier and qualifier. In this way, detection of target compound can be achieved, a compound which has 210 m/z as a precursor ion and 158, 191 m/z value as a product ions must be target compounds.

Tandem mass spectrometry can be used in both time and space. The physical separation of triple quadrupole mass spectrometer and Quadrupole time-of-flight mass spectrometer are involved in tandem MS in space on the other hand tandem MS in time includes the usage of ion trap.

Another advantage of the triple quadrupole mass spectrometer with respect to single quadrupole mass spectrometer process is to reduce the noise especially for low-level quantification in dirty matrix and this satisfy the high sensitivity.

The mechanisms of main parts of the triple quadrupole mass spectrometry, ESI ion source and collision cell, are described at following section.

ESI Ion Source

In liquid chromatography, Electrospray ionization (ESI) is preferred as the ionization technique for polar/acid/base compounds on the other hand APCI is preferred for non polar compounds.

ESI ion source eliminate the introduction of undesired compounds that can interfere to the analysis. ESI ion source can be operated at negative ion or positive ion mode according to the target compounds in order to obtain more sensitive scanning results The working principle of the ESI is based on the spreading of the liquid which includes the target analytes into a fine aerosol by electrospray. Solvents for electrospray ionization include the mixture of water and volatile organic compounds such as acetonitirile and methanol since ion formation process requires solvent evaporation. The addition of the compounds, like acetic acid and formic acid, to the solvent solutions assist to decrease the initial size of droplet and this helps the electrospray ionization.

The schematic representations of ESI Source and flow of analyte in the system can be indicated as below.

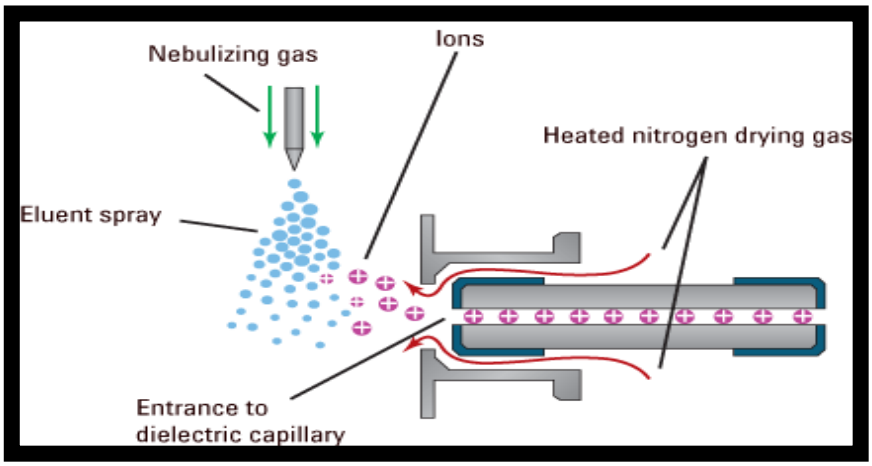


Figure B6 : The schematic representation of ESI Source

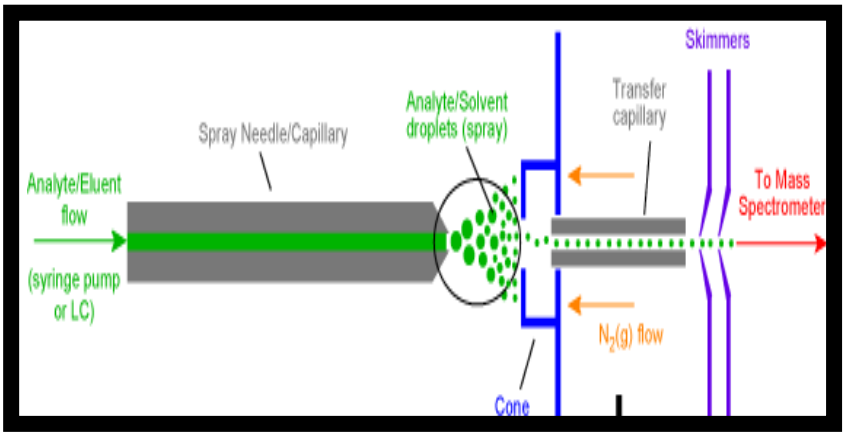


Figure B7 : The representation of analyte flow in ESI Source

The analyte solution enters to the source from a syringe pump or from liquid chromatography. Ion formation is induced by charging the droplets with high voltage electrode. In order to achieve this, the solution flows through the spray needle which has high potential difference according to the counter electrode and then droplets start to be repelled from the needle to the source sampling cone. When the droplets pass through the needle tip and cone, solvent evaporation occurs. Moreover, there are two infrared lamps in order to dry residual droplets. At the end of the process, charged ions reach to the mass spectrometer part.

Collision Cell

The collision cell in triple quadrupole mass spectrometry is defined as Q2. In collision cell, ions collide with neutral gas, generally nitrogen gas is preferred like in ion source part, and fragment in this gas phase. Nitrogen gas is used in order to satisfy collision induced dissociation of selected precursor ion which is selected by first quadrupole in the system. After that, fragments are accelerated out of the collision cell and enter the Q3. Moreover, collision cell satisfies more sensitive and specific detection since it enables the detection of a target compound in the presence of other compounds which have same molecular weight by looking for specific fragment ion. In addition to this, it can reduce the

background, increase the limit of detection and optimize MS/MS fragmentation at very low dwell times.

The location of the collision cell (Q2) in triple quadrupole mass spectrometry can be indicated below.

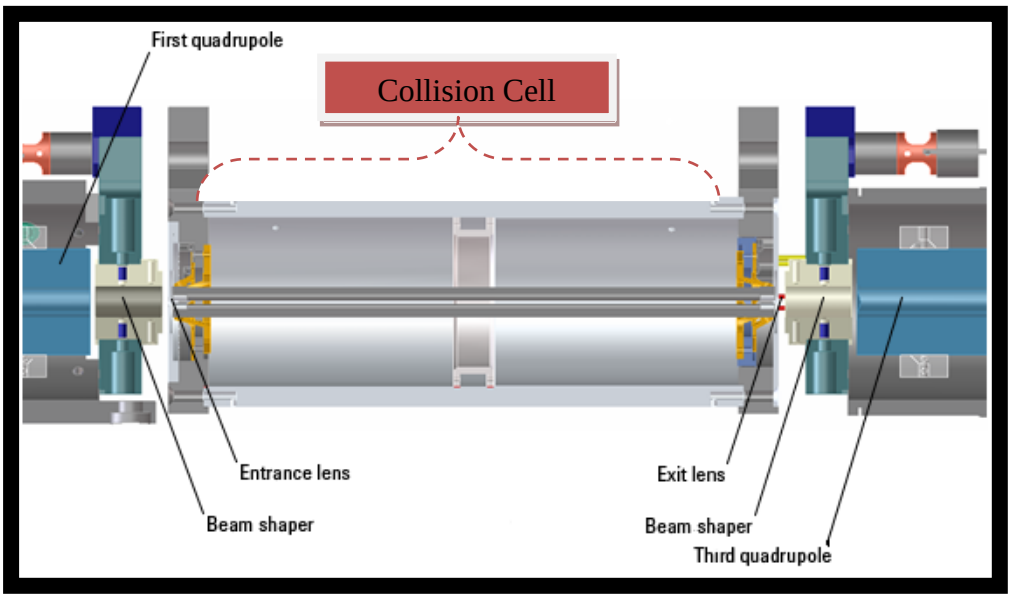


Figure B8: The representation of collision cell in triple quadrupole mass spectrometer

HPLC-UV Measurement

HPLC-UV is another chromatographic measurement method which is based on the measurement of absorption of radiation from chromophores in eluted samples over the range of 190-400 nm with the help of ultraviolet (UV) detectors.

The UV detector of the HPLC which is located after the stationary phase in order to detect compounds when they are eluted from the column, emits a response and signals a peak on the chromatograph. The main working principle of the detector is based on the measurement of the ability of sample chromophores which are part of molecule responsible for their, color to absorb UV light. This process can be achieved at one or several wavelengths in the range of 190-400 nm. There are two types of detectors which are fixed wavelength type and variable wavelength detectors. First one can measure at a single wavelength, usually 254 nm, on the other hand, second one can make sequential measurement of individual wavelengths and can detect over a wide range. There are many application of detection of organic compounds by HPLC-UV methods According to the literature study, TCS can be detected at 280,270 or 205 nm in the HPLC-UV analysis. (Kinetics of TCS oxidation by aqueous ozone and consequent loss of antibacterial activity: Relevance to municipal wastewater ozonation, Sonia Saurez et al., 2007, Determinations and residual characteristics of TCS in household food detergents of Taiwan,Shih-Wei Tsai et al., 2008).

C: Tatlar WWTP Design Parameter

Table C1: Design criteria for Tatlar WWTP

WWTP Parameters	**Target Year**		
	2002 (1st stage)	**2010 (2nd stage)**	**2025 (3rd stage)**
Number of process line	21/2	3	4
Population forecasting	3.277.000	3.970.000	4.859.000
Population equivalent	3.920.000	4.833.000	6.288.000
Average wastewater amount (m^3/day)	765.000	971.000	1.377.000
Average dry weather flow (m^3/second)	8,85	11,24	15,94
Maximum dry weather flow (m^3/second)	10,19	12,93	18,33
Maximum rainy weather flow (m^3/second)	17,71	22,48	31,88
BOD_5 load (60 g/person/day),kg/day	235.175	290.000	377.300
Raw and excess sludge (%1.5 SP, not thickened),m^3/day	20.907	25.778	33.538
Digested sludge (%3.3 SP) m^3/day	6272	7733	10.061
Sludge cake after belt filter press (%30 SP) m^3/day	704	869	1130
Treated effluent *BOD_5 conc. mg/L	<30		
*Filterable solids, mg/L	<30		

The dimensions of Tatlar Wastewater Treatment Plant units are given in Table C2.

Table C2: Dimensions of Tatlar WWTP Units

WWTP Units	**Dimensions**
Coarse Screens (Screen opening)	40 mm
Fine Screens (Screen opening)	15 mm
Aerated grit chamber * Chamber Volume m^3	584
* Surface Area (m^2)	209
* Retention time (minute)	11

Primary sedimentation tank *Volume (m^3) *Diameter (m) *Retention time (minimum)	7600
	50
	1,5 hour
Aeration chamber *Volume (m^3) * Surface area (m^2) * Retention time	13.005
	2600
	4 hour
Secondary sedimentation tank *Volume (m^3) *Diameter (m) *Retention time (minimum)	9.200
	55
	3 hour

D: METU WWTP Operation Parameters

Table D1: Operation parameters of METU WWTP

Technical parameters	**Operation Value**
Operating time	2,5 months
Surface area of membrane	540 m^2
Flow	150 m^3/day
Influent COD concentration	400-700 mg/l
Effluent COD concentration	< 10 mg/l
Influent BOD_5 concentration	230-450 mg/l
Effluent BOD_5 concentration	≈ 0 mg/l
Turbidity	0.1-0.5 NTU
Effluent fecal coliform	0 /100 ml
$MLSS_b$	7,2 g/l
$MLSS_{FD}$	14 g/l

E: Lara, Hurma, Kemer WWTPs Design Parameters

Table E1: Design Parameters of Lara WWTP

Treatment Units	Number of Units	Properties of each unit
Coarse Screens	3	Screen openings: 5 cm
Fine Screens	2	Screen openings: 3 cm
Selector	1	Volume:1500 m^3
Anaerobic Reactor	3	Volume:1500 m^3
Aeration basin	4	Volume: 17 000 m^3
Sedimentation Tank	2	Volume: 5 000 m^3
Sludge storage tank	2	Volume: 1 600 m^3
Decanter	2	100 m^3/hour

Table E2: Operation parameters for Hurma WWTP

Parameters	Influent Concentration	Effluent Concentration
COD (mg/l)	700	35-45
BOD_5 (mg/l)	400	5-15
TSS (mg/l)	500	15-25
TN (mg/l)	60	4-7
TP (mg/l)	12	1-2
pH	6-9	6-8

Table E3: Capacity and dimensions of Hurma WWTP units

WWTP Unit	Parameter
Anaerobic reactor	Width:7 m
	Length: 47 m
	Water depth: 7 m
	Volume: 4400 m^3
	Retention time: 1,4 hour
Aerated grit chamber	Length: 45 m
	Width:5 m
	Depth: 6,3 m
	Aeration rate: 540 Nm^3/hour

Primary sedimentation tank	Hydraulic Retention time: 1-1.5 hour
	Volume: 2188 m^3
Bio-P basin	Retention time:1.5-2 hour
	Volume: 8800 m^3 (2 basin) Volume :16 000 m^3 (2 basin)
Secondary clarifier (total 8 clarifier)	Diameter: 47 m (4 of them) Diameter: 52,4 m (4 of them)
	Depth: 4,5 m (4 of them) Depth: 5,2 m (4 of them)
Anaerobic digester	Height: 26,9 m
	Diameter: 23,3 m
	Volume: 9000 m^3

F: Water Pollution Control Regulation (Table 1: Criteria for inland water quality classes)

	Water Quality Class			
Water Quality Parameter	I	II	III	IV
A) Physical and inorganic-chemical parameters				
1. Temperature (^{o}C)	25	25	30	> 30
2. pH	6.5-8.5	6.5-8.5	6.0-9.0	outside 6.0-9.0
3. Dissolved oxygen (mg O_2/l)[a]	8	6	3	< 3
4. Oxygen saturation (%)[a]	90	70	40	< 40
5. Chlorine ions (mg Cl^-/l)	25	200	400[b]	> 400
6. Sulfate ions (mg $SO_4^=$/l)	200	200	400	> 400
7. Ammonia nitrogen (mg NH_4^+-N/l)	0.2[c]	1[c]	2[c]	> 2
8. Nitrite nitrogen (mg NO_2^--N/l)	0.002	0.01	0.05	> 0.05
9. Nitrate nitrogen (mg NO_3^--N/l)	5	10	20	> 20
10. Total phosphorus (mg PO_4^{-3}-P/l)	0.02	0.16	0.65	> 0.65
11. Total dissolved matter (mg/l)	500	1500	5000	> 5000
12. Color (Pt-Co units)	5	50	300	> 300
13. Sodium (mg Na^+/l)	125	125	250	> 250
B) Organic parameters				
1. COD (mg/l)	25	50	70	> 70
2. BOD (mg/l)	4	8	20	> 20
3. Organic carbon (mg/l)	5	8	12	> 12
4. Total Kjeldahl-nitrogen (mg/l)	0.5	1.5	5	> 5
5. Emülsified oil and grease (mg/l)	0.02	0.3	0.5	> 0.5
6. Methylene blue active substances (MBAS) (mg/l)	0.05	0.2	1	> 1.5
7. Phenolic substances (volatile) (mg/l)	0.002	0.01	0.1	> 0.1
8. Mineral oils and derivatives (mg/l)	0.02	0.1	0.5	> 0.5
9. Total pesticides (mg/l)	0.001	0.01	0.1	> 0.1

	Water Quality Class			
Water Quality Parameter	I	II	III	IV
C) Inorganic pollution parameters[d]				
1. Mercury (µg Hg/l)	0.1	0.5	2	> 2
2. Cadmium (µg Cd/l)	3	5	10	> 10
3. Lead (µg Pb/l)	10	20	50	> 50
4. Arsenic (µg As/l)	20	50	100	> 100
5. Copper (µg Cu/l)	20	50	200	> 200
6. Chromium (total) (µg Cr/l)	20	50	200	> 200
7. Chromium (µg Cr^{+6}/l)	indeterminable	20	50	> 50
8. Cobalt (µg Co/l)	10	20	200	> 200
9. Nickel (µg Ni/l)	20	50	200	> 200
10. Zinc (µg Zn/l)	200	500	2000	> 2000
11. Cyanide (total) (µg CN/l)	10	50	100	> 100
12. Florine (µg F^{-}/l)	1000	1500	2000	> 2000
13. Free chlorine (µg Cl_2/l)	10	10	50	> 50
14. Sulfur (µg $S^{=}$/l)	2	2	10	> 10
15. Iron (µg Fe/l)	300	1000	5000	> 5000
16. Manganese (µg Mn/l)	100	500	3000	> 3000
17. Boron (µg B/l)	1000[e]	1000[e]	1000[e]	> 1000
18. Selenium (µg Se/l)	10	10	20	> 20
19. Barium (µg Ba/l)	1000	2000	2000	> 2000
20. Aluminum (mg Al/l)	0.3	0.3	1	> 1
21. Radioactivity (pCi/l)				
alfa-activity	1	10	10	> 10
beta-activity	10	100	100	> 100
D) Bacteriological parameters				
1. Fecal coliform (MPN/100 ml)	10	200	2000	> 2000

	Water Quality Class			
Water Quality Parameter	I	II	III	IV
2. Total coliform (MPN/100 ml)	100	20000	100000	> 100000

(a) It is sufficient to ensure concentration and percentage saturation of only one of the parameters

(b) It may be necessary to lower the limit of this concentration for irrigation of chlorine-sensitive plants

(c) The concentration of free ammonia may not exceed 0.02 mg NH_3-N/l depending on pH

(d) Criteria in this group give total concentrations of chemical derivatives constituting the parameters

(e) These criteria may have to be lowered to 300 □g/l for irrigation of boron-sensitive plants