ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

PROFILE OF PRIORITY SUBSTANCES AND TOXICITY ASSESSMENTS OF WASTEWATER TREATMENT PLANTS IN ISTANBUL

Ph.D. THESIS

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Department of Environmental Engineering

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

İSTANBUL İLİ ATIKSU ARITMA TESİSLERİNDE ÖNCELİKLİ MADDELERİN PROFİLİ VE TOKSİSİTE DEĞERLENDİRİLMESİ

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Rahime İclal BİRTEK, a Ph.D. student of ITU Graduate School student ID 501122704, successfully defended the thesis/dissertation entitled "PROFILE OF PRIORITY SUBSTANCES AND TOXICITY ASSESSMENTS OF WASTEWATER TREATMENT PLANTS IN ISTANBUL", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. İzzet ÖZTÜRK** İstanbul Technical University	.....
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Date of Submission : 27 April 2023
Date of Defense : 31 May 2023

Dedication,

I dedicate my work to my dear family.

I also dedicate this work to virtuous people whose efforts enable scientific developments.

FOREWORD

This thesis is about a study of the occurrence of priority substances and their toxicity to living organisms in the wastewaters of the megacity of Istanbul. Supplying potable water to the city, Istanbul Water and Sewage Administration (ISKI) is also the responsible agency for the treatment and disposal of wastewaters. The experiments in this study were carried out during my association with ISKI from 2013–2021. I am foremost grateful to Prof. İzzet Öztürk for encouraging me to present this community-based research study as a Ph.D. thesis which included setting up of the most advanced instrumental analyses laboratory as well as Türkiye's first ecotoxicology laboratory to assess the toxicity and analyses of emerging contaminants in different matrices, including surface waters and wastewaters in Istanbul.

I would like to extend my hearth felt gratitude to many scientists and professionals for their support especially to Prof. Melek Türker Saçan who trained me on ecotoxicity and kindly provided the use of her computer programs, to Prof. Abdulbari Bener for his generous assistance on my data evaluation, to Dr. Kartal Çetinturk for his technical support on the instrumental analyses methodology section; to Prof. Süleyman Övez for his kind support, Mr.Michael Ives, Dr. Gisela Cluster, Ms. Lindsay O'Donahue, Prof. Daniel Schlenk, Mr. Steve Bay, MS, Prof. Derick G. Brown, Dr. Nur Orak, and Prof. Heidi Gough for their technical assistance in toxicity data evaluations; to Mr. Ensar Başakın, M.S. and Ms. Elif Kartal, M.S. for their help in the visualization of my data, to esteemed Prof. Glen Lawrence for inspiring and encouraging me to further my academic work as well as for proofreading my thesis. I am indebted to (late) Prof. Yaşar Bağdatlı for being a beacon in conducting scientific work.

The physicochemical and biological wet analyses through this work was supported by ISK. I would like to thank the Department of Wastewater and Central Laboratory of ISKI for their technical support and their active participation in sampling campaigns and analyses (2015–2019). I am also indebted to ISTAC for their kind collaboration in sampling of the leachate (2015–2019) and to the hospital team and Prof. Gülsüm Yılmaz for availing hospital samples. My appreciation and thanks go to my friends in R&D team in ISKI (2015–2019) for their collaboration.

Being community-based research, this work was only possible through contributions of many national and international people and organizations. Unfortunately, due to space restriction it has not been possible to mention each and everyone here and I would like to express my gratitude to them all.

Considering the community-based research part of this thesis, I also need to acknowledge the key successors at ISKI for making this project possible at the public level. Many thanks to the passionate, innovative managers of ISKI (2015–2019), namely Dr. Dursun Atilla Altay, Messr. Osman Yıldız, Alişan Koyuncu, Fatih Yıldız, Mehmet Sert, Orhan Baykan, Mses. Emine Oğuz M.S., Yasemin Özgül M.S., Elif Başoğlu for their dedication and support for the establishment of the Ecotoxicity Laboratory and most advanced instrumental laboratory within ISKI Central Laboratory. Many thanks also go to their amazing team that, without whose effort, PSs and specific pollutants in surface waters of Istanbul could not be monitored.

Lastly, I would like to thank my family for being a constant source of support during my research.

May 2023

Rahime İclal BİRTEK
(Research associate)

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ABBREVIATIONS

ACN : Acetonitrile
BOD : Biochemical Oxygen Demand
COD : Chemical Oxygen Demand
DLCs : Dioxins and dioxin-like compounds
DCB : 1,2-Dichloroethane
DCM : Dichloromethane
DEHP : Di(2-ethylhexyl) phthalate
DO : Dissolved Oxygene
EC_{50} : Median Effective Concentration (50%)
EPS : Expanded polystyrene
EU : European Union
GC-HRMS : Gas chromatography-high-resolution mass spectrometry
GC-MS : Gas Chromatography-Mass Spectrometry
GC-MS/MS : Gas Chromatography-Tandem Mass Spectrometry
HBCDD : Hexabromocyclododecanes
HC : Heptachlor
HCE : Heptachlor epoxide
HCB : Hexachlorobenzene
HCBD : Hexachlorobutadiene
HCH : Hexachlorocyclohexane
HRT : Hydraulic retention time
IC_{50} : Inhibition Concentration (50%)
IC_{25} : Inhibition Concentration (25%)
ICP-MS : Inductively Coupled Plasma Mass Spectrometry
ICP-OES : Inductively Coupled Plasma Optical Emission Spectrometry
ISKI : Istanbul Water and Sewage Administration
ISTAC : Istanbul Environment Management Industry and Trade Company
LC_{50} : Median Lethal Concentration (LC_{50})
LC-MS/MS : Liquid Chromatography-Tandem Mass Spectrometry
LOEC : Lowest Observed Effect Concentration
LLE : Liquid-Liquid Extraction
MEC : Measured Environmental Concentrations
MeOH : Methanol

MLSS : Mixed liquor suspended solids

NOEC : No Observed Effect Concentration

NP : Nonylphenols

NPDES : National Pollutant Discharge Elimination System

OP : Octylphenols

PAHs : Polyaromatic Hydrocarbons

PCP : Personal Care Product

pH : Quantitative measure of the acidity or basicity of aquaus or other liquid solutions.

PBDEs : Polybrominated Diphenyl Ethers

PeCB : Pentachlorobenzene

PFOS : Perfluorooctane Sulfonic Acid and its derivatives

PNEC : Predicted No Effect Concentration

PSs : Priority Substances

P&T GC-MS : Purge and trap gas chromatography-mass spectrometry

RQ : Risk Quotient

SBSE : Stir Bar Sorptive Extraction

SPE : Solid phase extraction

SRT : Solids retention time

TCE : Trichloroethylene

TBT : Tributyltin compounds

TCB : Trichlorobenzenes

TIE : Toxicity Identification Evaluation

TKN : Total Kjeldahl Nitrogen

TP : Total Phosphorus

TRE : Toxicity Reduction Evaluation

TSS : Total suspended solids

TUBITAK : The Scientific and Technological Research Council of Türkiye

US EPA : United States Environmental Protection Agency

XPS : Extruded polystyrene

WET : Whole Effluent Toxicity

WFD : Water Framework Directive

UF : Ultrafiltration

SYMBOLS

Cd	**:** Cadmium and its compounds
Hg	**:** Mercury and its compounds
t	**:** Time
t0	**:** The time of the test start
n0	**:** Initial cell density
tL	**:** The time of test termination
nL	**:** The measured cell density at time tL.
μ	**:** Growth rate (day^{-1})
lμi	**:** Normalized inhibition
μi	**:** Growth rate for the test batch
μc	**:** Growth rate for the control batches.
Ni	**:** Nickel and its compounds
Pb	**:** Lead and its compounds
pg	**:** Picogram
μg	**:** Microgram

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PROFILE OF PRIORITY SUBSTANCES AND TOXICITY ASSESSMENTS OF WASTEWATER TREATMENT PLANTS IN ISTANBUL

SUMMARY

Anthropogenic activities increase the wastewater formation around urban areas and cause a threat to aquatic environments. Substances found at trace concentrations (ng/L - µg/L) are called micropollutants or emerging contaminants (ECs) and they occur in urban wastewaters. Treatment of micropollutans in conventional wastewater treatment plants are found to be incomplete. Micropollutants present in urban wastewaters may have adverse effects on human and environmental health. Understanding the effects of the micropollutants and ECs on a water environment provides the opportunity for their assessment and management. Wastewater treatment plant effluents are known to be the main point sources of micropollutants in the environment. Known to have toxic, persistent, bioaccumulative and ubiquitous properties for decades, the knowledge of the presence of priority substances (PSs) in wastewaters is crucial. The Turkish Surface Water Quality Regulation adopted from the European Water Framework Directive (WFD) requires states to have a better understanding of priority substances (PSs) entering their surface, coastal, and transitional waters. Complying with the regulations, understanding of the behavior of PSs entering the receiving water bodies, especially from the wastewaters in the urban areas are important. The daily flow of effluents entering the wastewater treatment plants (WWTPs) of Istanbul shows the magnitude of the problem.

The goal of this research is to study the occurrences of PSs in the seven largest WWTPs in the provincial borders of the City of Istanbul. The Risk Quotient (RQ) approach was used to predict the potential environmental risk posed by each detected organic PS in WWTP effluents. Surveillance monitoring of the organic PSs having RQ > 1 in the WWTP effluents of Istanbul is recommended. This thesis also includes estimation of the acute toxicity of effluents from the aforementioned WWTPs. Outcomes in this thesis could be utilized by the regulators undertaking environmental risk assessments in the initiation of monitoring programs for protection of the Sea of Marmara, the Bosphorus, the Black Sea as well as other receiving aquatic environments. This also could bring the idea of improving receiving water quality, as well as introducing improvements in wastewater treatment technologies.

İSTANBUL İLİ ATIKSU ARITMA TESİSLERİNDE ÖNCELİKLİ MADDELERİN PROFİLİ VE TOKSİSİTE DEĞERLENDİRİLMESİ

ÖZET

Kentsel alanlarda insan faaliyetleri nedeniyle oluşan antropojenik (evsel/kentsel) atıksular sucul ortamlar için tehdit oluşturmaktadır. Dünya çapında nüfusun kentsel alanlara doğru kayması, buna paralel olarak endüstriyel faaliyetlerdeki ilerleme ve bunun yanında iklim değişikliğinin yol açtığı tehdit, her geçen gün kirliliğin boyutlarını artırmaktadır. Arıtılmış veya arıtılmamış atıksu deşarjının, alıcı su ortamları için bir tehdit oluşturduğu bilinmektedir. Geleneksel atıksu arıtma tesisleri (AAT), makro kirleticiler, besin yüklerinin (C, N ve P) su ortama girişlerini en aza indirmek için inşa edilir. Yapılan araştırmalar mikrokirletici ya da nevzuhur (yeni ortaya çıkan) kirleticilerin (EC (Emerging Contaminants)) de atıksularda bulunduğu ve bazılarının gideriminde geleneksel AAT'lerın yetersiz kaldığını ortaya koymuştur. Dahası, biyolojik olarak parçalanamayan mikrokirleticilerin alıcı ortamlarda varlığı endişe uyandırmıştır. Birtakım mikrokirletici ve EC'lerin su ortamlarında çok düşük konsantrasyonlarda (pg/L–ng/L) varlığının, insanlar ve ekosistem üzerinde olumsuz tesirleri gözlenmiştir. Endüstriyel ve evsel atıksular ile yüzeysel akış atıksulardaki mikrokirletici ve EC'lerin ana kaynaklarıdır.

Atıksu arıtma prosesi ile çıkış sularından uzaklaştırılamayan mikrokirleticiler dolayısıyla AAT'lerin mikrokirleticilerin alıcı su ortamlarına girişinde ana noktasal kirlilik kaynağı olduğu bildirilmektedir. Farmasötikler, kişisel bakım ürünleri, steroid hormonlar, pestisitler (herbisitler, biyositler, insektisitler ve fungisitler), plastikleştiriciler, yüzey aktif maddeler (deterjanlar gibi) ve endüstriyel kimyasallar (metaller, alev geciktiriciler vb.) kentsel atıksulardaki mikrokirleticilerin ana kaynakları olarak bilinmektedir. Birleşik sistem kanalizasyon şebekesine sahip kentlerın atıksularda bulunan mikrokirleticiler, evsel atık sularından, hastane atık sularından, endüstriyel atık sulardan, katı atık sızıntı sularından, bahçe, tarım, hayvancılık alanları ve yollardan yüzeysel akışla gelen mikrokirleticilerden kaynaklanmaktadır.

Yıllardır pek çok yerde analiz edilen sularda varlık gösteren, kalıcı, zehirli, ve biyobirikir özellik taşıyan mikrokirleticiler, öncelikli maddeler (PSs) olarak adlandırılmışlardır. Bu bileşiklerin su ortamları için yüksek risk oluşturduğu ifade edilmektedir. Avrupa Birliği Su Çerçeve Direktifi (SÇD) (2000/60/EC), yüzey suları ve yeraltı sularının "iyi durumunu" sağlamak için çevresel hedefleri tanımlar; burada "iyi durum", "iyi kimyasal ve ekolojik durum" anlamına gelmektedir. Su ortamının "iyi kimyasal durumu", her bir PS için (2013/39/EU Direktifi Ek II) Çevresel Kalite Standartları (ÇKS) tarafından belirlenen sınırların aşılmadığı su ortamlarını ifade eder. 2013'te değiştirildiği şekliyle SÇD (2000/60/EC), ÇKS ile belirlenen eşik seviyelerini aşmamak amacıyla, üye devletlerin yüzey sularında PS'lerin varlığını belirlemesini şart koşmaktadır. Türkiye Cumhuriyeti Orman ve Su İşleri Bakanlığı, yüzey sularındaki PS'ler için yukarıda belirtilen kalite standartlarını 2012 yılında benimsemiş

ve 2016 yılında güncellemiştir. ÇKS'ler, yüzey sularının kimyasal kalitesinin değerlendirilmesinde hayati bir role sahiptir. AAT çıkış sularının alıcı su ortamları için mikrokirleticilerin ana nokta kaynağı olduğu bilinmesine rağmen, şu anda AAT çıkış sularının PS veya mikrokirletici içeriği ve kısıtlaması hakkında herhangi bir düzenleme bulunmamaktadır. Oysa, SÇD'nin 16. Maddesi, PS'lerin noktasal kaynaklardan kontrolünün önemini vurgulamaktadır.

PS'ler pestisitler, polisiklik aromatik hidrokarbonlar, uçucu organik bileşikler, dioksin ve benzeri bileşikler, alkilfenoller, pilibromlu difenil eterler, endüstriyel kimyasalllar ve metaller şeklinde alt gruplara ayrılabilir. SÇD'den uyarlanan Yerüstü Su Kalitesi Yönetmeliği (YSKYY, 2012) yüzey, kıyı ve geçiş sularına giren PS'lerin etkilerinin daha iyi anlaşılması gerekliliğini ön görmektedir. Bu yönetmeliğe uyum sağlamak için özellikle kentsel alanlardaki atık sulardan alıcı su kütlelerine giren PS'lerin izlenmesi önem arz etmektedir.

AAT çıkış sularında tespit edilen PS'ler, son noktadaki deşarjlarından dolayı çevresel risklerin değerlendirilmesi konusundaki ilgiyi artırmıştır. PS'ler kalıcı, toksik, biyobirikir ve her yerde bulunma özellikleri gösterdikleri için, akıbetleri ve ekosistem için herhangi bir toksikolojik risk oluşturup oluşturmadıkları araştırılmalıdır. Avrupa Komisyonu, çevrede bulunan zararlı maddelerin insan ve çevre sağlığına oluşturabilecekleri riskleri değerlendirmek amacıyla direktif oluşturmuşlardır (EC, 1993). Ölçülen çevresel konsantrasyonun (MEC) öngörülen etkisiz konsantrasyona (PNEC) oranı risk katsayısı olarak tanımlanmaktadır (RQ). RQ mikrokirleticilerin potansiyel risklerinin değerlendirilmesinde yararlı bir araçtır ve RQ > 1.0 oranına sahip bir kimyasalın izlenmesi için yeterli risk potansiyeli taşadığı kabul edilir. RQ'nun uygulanması, yetkilileri risk yönetimi stratejilerini geliştirmeye ve belirli maddelerden kaynaklanan riskleri azaltmak için yeni düzenlemeler hazırlamaya yönlendirebilir.

Mikrokirleticilerin veya EC'lerin atıksularda izlenmesi faydalı niceliksel veriler sağlar. Bununla birlikte, Amerikan CAS kayıt servisi günümüzde 204 milyon organik ve inorganik kimyasal olduğunu belirtmektedir ki doğal olarak bu kadar kimyasalın atıksularda izlenebilmesi teknik ve ekonomik olarak mümkün değildir. Bu nedenle, EC'lerin analizleri tek başına atıksu toksisitesinin izlenmesinde yeterli olamaz. Tespit edilebilen EC'ler buz dağının sadece görünen kısmı olarak ifade edilebilir. Atıksu kalitesi izlemede etkiye dayalı yöntemler, kimyasal maruziyete dayalı yöntemleri tamamlayıcı yararlı bir yöntem olarak tanıtılmıştır. Balıklar, omurgasızlar, bitkiler ve bakteriler üzerlerinde yapılan testler, ölüm, üreme, beslenme, büyüme vb. tepkileri ölçmekte ve değerlendirmektedir. Bu biyoanalitik araçlar atıksuda bilinmeyen kimyasalların neden olduğu toksisiteyi tahmin etmeye yardımcı olmaktadır. Bu kapsamda, AAT arıtma süreci etkinliği ve arıtılmış AAT çıkış suyu kalitesi değerlendirmesinin kullanılması için biyoanalizlerden oluşan toksisite testleri dizisi önerilir. *Daphnia magna'nın* immobilizasyon testi, yeşil alg *Selenastrum carpricornutum'un* büyüme önleme testi ve *Vibrio fischeri* ile biyolüminesans inhibisyon testleri, AAT'nin giriş ve çıkışları için yaygın olarak kullanılan test bataryasıdır.

Bu tez kapsamında çalışılan AAT giriş ve çıkış sularında akut toksisite testleri yapılmıştır. İleri biyolojik arıtma tesis prosesi giriş sularındaki akut toksisitenin çıkış sularında giderildiği gösterilmiştir. Böylece biyolojik arıtımın akut toksisite gidermedeki etkinliği gözlenmiştir.

PS ve EC'ler başta olmak üzere atıksulardaki toksisitenin potansiyel varlığı ve oluşturabileceği riskler, araştırmaya değer bir konudur. İstanbul, nüfusu 16 milyonun

üzerinde olan tarihi bir metropoldür. İstanbul ilinde arazi kullanımı kabaca tarım (%25), hayvancılık (mera ve çayır) (%2), ormancılık (%48) ve diğer (%25) olarak ifade edilebilmektedir. Ancak incelenen AAT drenaj alanında tarım alanlarının kirliliğe katkısı önemli düzeyde değildir. İstanbul'da evsel atık sularla birlikte AAT'lere deşarj edilen orman ürünleri, kağıt ve kağıt hamuru, gıda, kimya, metal, inşaat, petrol ve gaz, tekstil, turizm (oteller ve yemek hizmetleri) endüstrileri bulunmaktadır. Sanayi kuruluşları genellikle organize sanayi bölgelerinde yer almaktadır. Ayrıca, kanalizasyon sistemlerine deşarj öncesi, endüstriyel deşarj limitlerine uymak üzere ön arıtma yönetmeliğine tabî sanayiler de olmakla birlikte 1990'lardan sonra çevreyi kirleten büyük tekil sanayilerin tamamına yakını İstanbul dışına taşınmıştır.

Bu tez kapsamında İstanbul il sınırları içerisinde yer alan Türkiye'nin en büyük yedi atıksu arıtma tesisi giriş ve çıkış sularında PS'ler ölçülmüş ve tespit edilen organik PS'lerin atık sularda oluşturduğu risk tahmin edilmiştir. Aynı zamanda aynı atıksularda üç taksada akut toksisite biyoanalizleri yapılmıştır. AAT giriş ve çıkışlarında tespit edilen PS'lerin konsantrasyon seviyeleri ng/L ila µg/L aralığında bulunmuştur. Sonuçlar, İstanbul ili atıksularında 73 PS arasından 48 PS'nin bulunduğunu, 25 PS'ye ise hiç rastlanmadığını göstermektedir. PS'lerin atıksulardaki en yüksek konsantrasyonları şöyledir: Pestisitler (bifenox, quinoxyfen ve endrin) 110 ng/L – 482 ng/L; PAH'lar (naftalin, benzo(k)floranten, antrasen), 14 ng/L – 1,2 µg/L; VOC'ler (triklorometan, triklorobenzen ve tetrakloroetilen), 3,7 µg/L – 12,4 µg/L; alkilfenoller (nonifenol ve oktifenol) 9,1 ng/L – 151 ng/L; metaller (267 µg/L - 18.9 µg/L). AAT deşarj yapılan sularda yaşayan organizmalar üzerinde PS'lerin oluşturduğu riski değerlendirmek için üç trofik organizma seviyesi (balık, su piresi ve algler) seçilmiştir.

Yapılan risk tahminleri sonrası; ileri biyolojik arıtım çıkış sularında endrin, endosulfan, diuron, alfa-cypermethrin, beta-cypermethrin, theta-cypermethrin, zeta-cypermethrin, quinoxyfen, aclonifen, bifenox, benzo-ghi-perilen, benzo(a)piren, floranten, indeno (1,2,3-cd)-piren, tetrakloroetilen, DEHP ve kloroalkanlar, C10-13'ın potansiyel risk (RQ > 1) oluşturdukları ve çıkış sularında gözetim amacıyla düzenli izlenmeleri önerilmiştir. Ön arıtma çıkış sularında ise endrin, endolsulfan, diuron, alpha-cypermethrin, theta-cypermethrin, zeta-cypermethrin, aclonifen, bifenox, anthracene, benzo-ghi-perylene, benzo(k)fluoranthene, fluoranthene, indeno(1,2,3-cd)-piren, tetrakloroetilen, oktilfenoller, DEHP ve kloroalkanlar, C10-13'ın potansiyel risk (RQ > 1) oluşturmaları dolayısıyla gözetim amacıyla izlenmeleri tavsiye edilmiştir.

İzleme neticesinde süreklilik arz eden her bir PS'in kaynağının araştırılması tavsiye edilmektedir. PS'lerin kaynaklarını ve döngülerini daha iyi anlamak için, yağış esnasında, kıyı sularında, çökelebilir partiküllerde, yüzey sularında, aktif çamurlarda ve atık su taşkınlarında PS'lerin varlığının araştırılması önerilmektedir.

PS ve mikrokirletici arıtım oranlarının düşük olduğu AAT'lerdeki arıtma proseslerinin iyileştirilmesi önerilmektedir. Ayrıca Avrupa Birliği İzleme Listesi'nde (2015/495/EU) listelenen 17 organik bileşiğin İstanbul atıksularında varlığının araştırılması tavsiye edilmektedir. Endüstriyel deşarjların önemli olduğu tesislerde, bilhassa AAT 1 ve AAT 3 için son arıtma olarak ozonla oksidasyon prosesinin eklenebileceği önerilmektedir.

Yapılan toksisite testleri, biyolojik arıtımın akut toksisteyi azaltıcı etki gösterdiğini ve ön arıtma tesislerinin biyolojik arıtıma dönüştürülmesinin önemini ortaya koymuştur.

IBAAT ve AAT çıkış sularında kronik toksisite testlerinin yapılması tavsiye edilmiştir. Bu çalışmadaki bulguların, Marmara Denizi, Boğazlar, ve Karadeniz başta olmak üzere alıcı su ortamlarının korunması için izleme programlarının oluşturulmasında yararlı ve çevresel risk değerlendirmeleri yapan düzenleyicilere faydalı olması umulmaktadır.

1. INTRODUCTION

Wastewaters formed due to anthropogenic activities around urban areas pose a threat to aquatic environments. The growth in industrial activity along with the worldwide urban migration, as well as the threat posed by climate change, increase the extent of pollution. The discharge of the treated or untreated wastewaters is reported to cause a threat to their receiving water environments. Conventional wastewater treatment plants (WWTPs) are constructed to minimize the nutrient loads of macropollutants (C, N, and P) entering the receiving water bodies. As, removal of some of the emerging contaminants (ECs) or micropollutants present in wastewaters is found to be incomplete in conventional wastewater treatment processes, traces of those non-biodegradable ECs were reported to be found in the receiving environments. The presence of ECs, even in very low concentrations (pg/L–ng/L) in the water environments could cause adverse effects on humans and the ecosystem. In addition to industrial emissions, domestic discharges along with urban runoffs are main contributors of ECs in WWTPs.

Understanding the presence, sources and transport of the micropollutants and ECs in wastewaters is important for assessing their impacts, and hence can help their reduction and management in the receiving environment. Micropollutants that have shown toxic, persistent, bioaccumulative, and ubiquitous properties and have been identified in aquatic environments, are designated as priority substances (PSs) by the EU Water Framework Directive (WFD). EU Member States are required to identify the presence of PSs in surface waters, in order not to exceed threshold levels specified by the Environmental Quality Standards (EQS) dictated by WFD. The Turkish Ministry of Forestry and Water Affairs adopted the aforementioned quality standards for the PSs in surface waters in 2012, and updated them in 2016. Since WWTPs are known to be main point sources of ECs entering the receiving water bodies, investigating the occurrence of PSs in wastewaters of Istanbul has generated valuable information.

This thesis aims at understanding the occurrences of PSs in the wastewaters of the megacity of Istanbul as well as assessing WWTP effluents as sources of PSs in receiving environments. The thesis also includes the acute toxicity assessment of the same wastewaters. The scope of the study includes the wastewaters of the seven largest WWTPs, a hospital wastewater and leachate of a landfill treatment plant in Istanbul.

The results of the PSs analyses allowed estimation of risks posed by the PSs in the WWTP effluents. Lists were formed to PSs showing sufficient risk (RQ > 1), and their inclusions are recommended in the surveillance monitoring programs for the effluents of advanced treatment, as well as mechanical treatment. The regulators undertaking environmental risk assessments in the initiation of monitoring programs for the protection of the Sea of Marmara, Bosphorus and the Black Sea may utilize the findings of this study.

Chapter 2 provides general information on the background of the study that is related to the aim and objective of the study. Chapter 3 comprises of information regarding the study area as well as methods on the description of all the experiments conducted through this study, namely analyses of PSs, toxicity analyses and physiochemical analyses.

Chapter 4 Results and Discussion, provides information on the results of the experiments conducted through this study (PSs analyses, toxicity analyses and physiochemical analyses), as well as discussion of those results.

Chapter 5 provides a summary of the whole thesis.

The References section includes the complete bibliography.

The Appendix includes tables, figures and pictures.

2. LITERATURE REVIEW

Wastewaters being discharged from urban areas pose significant risk to aquatic environments. Considering worldwide urban migration, increased industrial activities and climate change, the risks have upsurged. In addition to nutrient loads of macro pollutants C, N, and P, present in wastewaters, the presence of some chemicals is known to pose adverse effects on the environment and humans (Cravo et al., 2022; Heberer, 2002; McCallum et al., 2019; Mir-Tutusaus et al., 2018). Indeed, as the famous philosopher of the French Revolution stated in his 16th century book, "Persian Letters", he came across wise people warning on the damage that may arise due to the work of chemists that they could create substances that would "harm and destroy piecemeal" (Montesquieu, 1973). His warning was worthy of attention. Indeed, today modern life has introduced the use of natural and millions of synthetic chemicals that are found in the aquatic environment which are being released through run-off, atmospheric deposition, municipal and industrial waste discharges. Since the chemical industry is one of the largest industries in the world, introduction of abundant new chemicals is inevitable, although a number of others are being phased out each year. The toxicity of copper and lead was known to be an issue during Roman and Medieval times. However, the relation between water pollution and human health was not clear until the 1850s. Forbes was one of the first researchers recognizing the absence or presence of certain species and communities in an aquatic ecosystem which allowed him to classify rivers in terms of pollution according to content and state of species. The first aquatic toxicity tests are known to have been performed by Weigelt, Saare, and Schwab (1885) and Penny and Adams (1863) where the concern was raised due to toxic chemicals found in industrial discharges (Anderson et al., 2010a; Hoffman, 2003).

2.1 Micropollutants

Micropollutants and ECs are defined as synthetic or natural chemicals found in wastewaters in trace concentrations (pg/L–μg/L). Environmental pathways of entry of

ECs through possible sources are shown in Figure 2.1. There are limited number of regulations regarding their known presence in the environment. It's not technically possible to monitor all ECs present, including those that have recently been discovered or others that are unknown. Studies indicate the occurrence of micropollutants in wastewaters; hence, conventional wastewater treatments are considered to be incomplete in removing micropollutants (Virkutyte et al., 2010).

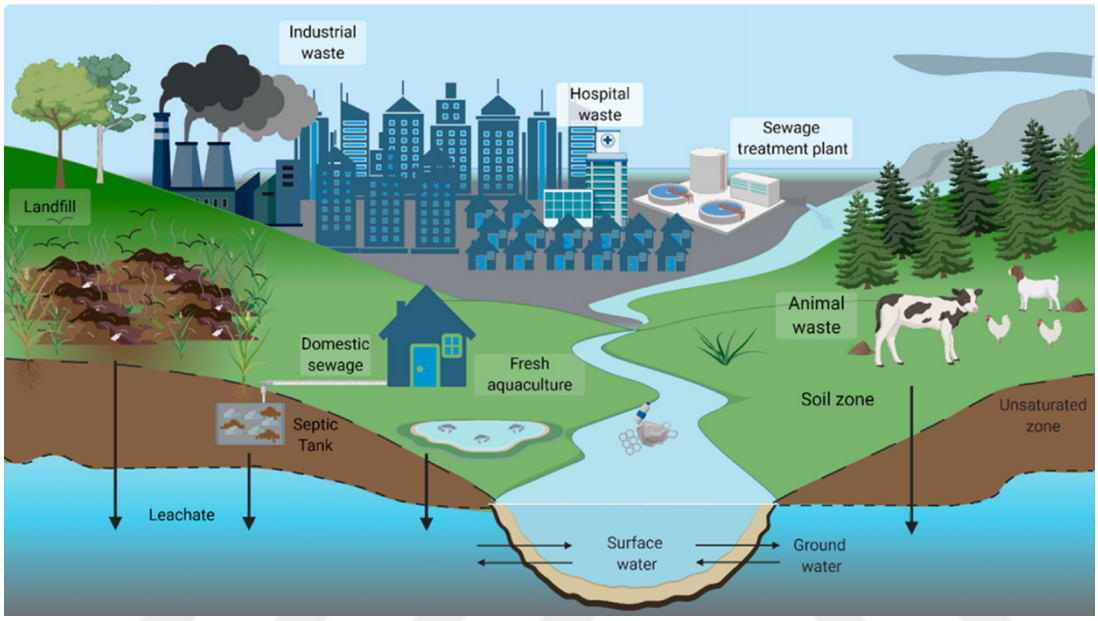


Figure 2.1 : Environmental pathways of entry of ECs through possible sources (Chacón et al., 2022).

2.1.1 Urban wastewaters as sources of ECs and their types

Municipal wastewaters are reported to be the major point source for micropollutants and ECs, and carry them to the receiving environment. Although they occur in trace concentrations in the wastewaters, considering the discharge of a megacity (4.1 million m^3/day, 1.5 x10^9 m^3/yr) through the year, may cause mass loadings in the receiving environment (Virkutyte et al., 2010). The ECs found in urban wastewaters are mainly sub grouped (Antunes et al., 2021) as:

a- Organic compounds consisting of:

- Industrial chemicals (food additives, plasticizers, gasoline flame retardants, surfactants, lubricants etc.)
- Pesticides (insecticides, herbicides, biocides, and fungicides)
- Pharmaceuticals (antibiotics, steroid hormones, prescription drugs, drugs of abuse)

- Personal care products (PCPs) (cosmetics such as sunscreens, fragrances antimicrobials etc.)

b- Inorganic compounds, consisting of:

- Trace metals

c- Particulates

- Nanoplastics
- Microplastics

Those ECs that find their ways into WWTPs originate from discharges from domestic uses, landfill leachates, hospitals, industry, as well as run off from roads (in combined systems) and gardens, from livestock and agricultural areas (Grobelak & Kowalska, 2022; Luo et al., 2014; Y. Wang et al., 2019; Wolff et al., 2018). Presence of some of the organic and inorganic ECs were within the scope of this study.

2.1.2 Chemical classification of ECs of interest

The occurrence of PSs in the wastewaters is the main target in this study. In addition, the occurrence of a number of ECs were also studied. In this section, the following classes of PSs and ECs of concern are briefly explained.

2.1.2.1 Pesticides

Pesticides are chemicals aimed at elimination of the pests with their toxic properties, in order to protect crops, where the term of pests could vary from insects to birds, from fungi to microorganisms (Aloizou et al., 2020). The pesticide use in urban settings may take place in agricultural areas, which were reported to constitute 35% of Istanbul's land use (Corine, 2018). The use of pesticides in agriculture is crop specific, while on the contrary, urban use of pesticides are reported to be more diverse (Stehle et al., 2019). Areas such as buildings, homes, gardens, yards, landscaping/turf, parks, recreational areas, urban woodlands are urban areas where pesticides are presently used (Meftaul et al., 2020). Pesticides enter urban wastewaters through urban runoff from all aforementioned sites (García-galán et al., 2020). The presence of pesticides among PSs in wastewaters is one of the points that has been of interest.

Organochlorine pesticides are stable and persistant compounds to be considered among hazardous environmental pollutants, that may lead to endocrine disruption or

cancer and found to cause problems in liver, kidney, and the central nervous system (Moawed & Radwan, 2017). Organophosphorus pesticides, which were used extensively as a substitute for organochlorine compounds, were also found to pose high acute toxicity to humans and animals (Wu et al., 2019; Zhigang et al., 2020). Used as a pesticide and plasticizer, and classified as a carcinogen, hexachlorobenzene (HCB) was widely found in water, sediment, air, and human blood (Pan et al., 2020). Triazine and urea herbicides, pyrethroid insecticides, organotin biocide, quinoline, diphenyl, and aforementioned class of pesticides that are listed among PSs and their monitoring in urban effluents are important.

2.1.2.2 Polycyclic aromatic hydrocarbons

One other group that is listed in PSs are polycyclic aromatic hydrocarbons (PAHs) due to their high persistency, hydrophobicity, low solubility properties, and wide presence in water environments (Drwal et al., 2019). PAHs draw attention due to their side effects, which include mutagenicity, carcinogenicity, and teratogenicity (Drwal et al., 2019). The release of PAHs to the atmosphere is mainly due to burning of fossil fuels from anthropogenic or natural sources (Urana et al., 2020). PAHs' presence in water bodies is primarily due to atmospheric deposition, surface run off, shipping, and both domestic and industrial effluents (Bortone et al., 2020). Therefore, sources of PAHs from the urban effluents are a concern (Ozaki et al., 2015).

2.1.2.3 Volatile organic compounds

Volatile organic compounds (VOCs) are defined by the U.S. Environmental Protection Agency as organic compounds which could vaporize under atmospheric conditions (temperature and pressure). As a common property, VOCs show high vapor pressure, low boiling point, and strong reactivity (X. Zhang et al., 2017). Among the VOCs, some show carcinogenic, mutagenic, and toxic character where they could pose risk to the environment and human health (X. Zhang et al., 2017). VOCs primarily originate from anthropogenic sources which are mainly transmitted to the air through the fossil fuel processes, whereas liquid fossil fuel sources of VOCs are due to the asphalt processes (X. Zhang et al., 2017). Wastewaters, notably industrial wastewaters, are sources of VOCs, and their presence in wastewaters is a big concern (Y. Zhang et al., 2020).

2.1.2.4 Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are synthetic chemicals named as dioxins, and they are released to the environment through human activities, where 17 of them have drawn attention internationally due to their toxicity (European Commission & Regions, 1999). Polychlorinated biphenyls (PCBs), globally manufactured chemicals whose production was prohibited by the Stockholm Convention, were found to exist in the environment. Twelve of their congeners show toxic properties similar to the dioxins and were thus named as dioxin-like PCB (PCB-DL) compounds and are also a cause for concern (Addeck et al., 2014; Commission, 2011). Dioxins and dioxin-like substances are among the Persistent Organic Pollutants (POPs), having the ability to travel long distances, and their lipophilic nature allows them to accumulate on aquatic sediments and biota, leading to their bioaccumulation and bioconcentration thorough food chains (WHO, 2010). The presence of dioxins in the environment is due to their release into air from incomplete incineration, agricultural run-off, and WWTPs (Maier et al., 2016). The aforementioned listed toxic dioxins and dioxin-like compounds are among the PSs.

2.1.2.5 Alkylphenols

Alkylphenols are anthropogenically produced chemicals having a worldwide use as surfactants in industrial and domestic applications (Kovarova et al., 2013). Nonylphenols (NP) are mainly used for the production of nonylphenol ethoxylates, as nonionic surfactants that are toxic to aquatic environments (EPA, 2010). Being used as an intermediate in the production of octylphenols ethoxylates and phenolic resins, octylphenols (OP) are reported to show estrogenic action on fish (Miyagawa et al., 2016). Being used extensively, alkylphenols end up in WWTPs, hence their effluents become the major source to the receiving water bodies (Soares et al., 2008).

2.1.2.6 Phthalates

Phthalates (phthalic acid esters) are used in plasticizers as an additive to increase the flexibility of polyvinylchloride (PVC). Di(2-ethylhexyl)-phthalate (DEHP) is widely used in the production of PVC, which is used in floorings, electric wiring, insulation, medical instruments, synthetic leathers, toys, etc. (Økland et al., 2005a). It is also used in solvents, fixatives, lubricating oils, detergents and in personal care products (Lyche,

2017). Phthalates are prone to leach out easily during use or after disposal (Lyche, 2017; Marttinen et al., 2003). DEHP are found to pose endocrine, reproductive, immuno, and embryonic toxicity (Xiu et al., 2020). DEHP are reported to be generally detected in high concentrations among phthalates in WWTP influents and effluents, and DEHP content of wastewaters of Istanbul are a concern (Marttinen et al., 2003).

2.1.2.7 Perfluorinated compounds

Perfluorinated compounds (PFCs) are hydrophobic, persistent, bioaccumulative substances that make them extensively preferred for the production of surfactants, food packages, fire-fighting foams, carpets, stain-resistant coatings, and water resistant clothing fabrics (Viberg & Eriksson, 2017). The extensive use of perfluorooctane sulfonic acid and its derivatives (PFOS) in industry result in their worldwide presence. Detection of PFOS even in human serum, blood and tap water has drawn serious attention (Hu et al., 2018a, 2019; Viberg & Eriksson, 2017) and their monitoring in wastewaters and water environments are critical. PFCs are among the POPs, and PFOS are longer-chain PFCs listed among PSs (Arvaniti & Stasinakis, 2015). Studies have shown that WWTP effluents are main sources of PFOS entering waterbodies (Y. Zhou et al., 2019).

2.1.2.8 C10-13-Chloroalkanes

C10-13-chloroalkanes are a class of chemicals used as additives in rubber, paints, textiles, and sealing compounds, that are later found to be highly bioaccumulative and persistent in biota and sediments, and a carcinogen to humans (Bettina et al., 2011).

2.1.2.9 Polybrominated diphenyl ethers and hexabromocyclododecane

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDs) were widely used brominated flame retardants which were diminished and eventually completely phased out due to their bioaccumulative and long term toxic properties on humans and the environment (Bogdal et al., 2008). Polybrominated diphenyl ethers (PBDEs), one of the POPs, were used in products such as furniture, insulation materials, electrical and electronic devices, plastics, textiles, and automobiles (US EPA, 2014). The use, of PBDEs were prohibited in 2009 in compliance with Türkiye's ratification of the Stockholm Convention (CSB, 2014). HBCDs were commonly used in polystyrene foams, as well as in insulation materials in buildings, construction, and

some household supplies such as electronic applications and cables (EPA, 2014). Due to economic reasons, HBCDs still have a use for the production of expanded polystyrene (EPS) and extruded polystyrene (XPS) foams and the like (CSB&NIRAS, 2015).

2.1.2.10 Metals

The deleterious effects of lead on biological systems are known since the Roman times. Also, methylmercury pollution in aquatic environments is known to adversely affect organisms and has raised a lot of attention. Being an essential trace element, excessive concentrations of selenium cause bioaccumulation in fish, which was related to their reproductive impairment (Hoffman, 2003). Inorganic contaminants such as heavy metals threaten the health of the environment and humans by causing bioaccumulation (Rasheed et al., 2020, Saçan et al., 2007). There are four heavy metals (Pb^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}) listed among PSs (EC, 2013).

2.1.3 Fate and behaviour of ECs in WWTP process

Understanding the fate and behaviour of ECs in the WWTP processes is one critical aspect in estimating their persistent behavior and their potential environmental impact on the receiving waterbodies, which can be a challenging task at times (Rogers, 1996). Conventional WWTP treatment consists of a primary, secondary and occasionally a tertiary treatment while none of the treatments succeed in fully eliminating the ECs. However, it is known that sorption, biodegradation, volatilization, and abiotic processes (such as hydrolysis, photolysis, oxidation etc.) are the main processes that could govern the fate as well as the behaviour of the ECs in the WWTP. Knowledge of the physical and chemical properties of ECs, as well as the operational characteristics of WWTPs are critical in estimating their fate in the WWTP (Byrns, 2001; Neczaj, 2020; Petrie et al., 2015; Rogers, 1996). The main fate of the ECs in wastewaters can be investigated in aqueous (dissolved) and particulate (bound) phases (Anderson et al., 2010a).

The physicochemical properties of ECs help to estimate their behaviour during the WWTP process, such as the sorption potential (log Kow) gives an idea of the adsorption capacity of ECs. By design, the primary treatment aims at lessening the suspended solids entering the secondary treatment. However, the vast majority of ECs, such as water soluble (hydrophilic) ones, are found not to be removed by this

treatment. Exceptions are the insoluble (hydrophobic) compounds. The mechanism of sorption on sludge could be effected by various parameters such as pH, redox potentials, and chemical characteristics (Verlicchi et al., 2012). The partition tendency of hydrophobic compounds can be described by the octanol-water partition coefficient (Kow). Sorption tendency of ECs on sewage sludge could be guided by the following: Log Kow < 2.5, low sorption potential; 2.5 < Log Kow <4.0, medium sorption potential; Log Kow > 4.0, high sorption potential (Rogers, 1996). It is a well-known fact that the higher the hydrophobicity, the better is the biosorption tendency to organic compounds, which allow ECs to be primarily removed through sedimentation or filtration of wastewater in treatment (Neczaj, 2020; Yamamoto et al., 2003).

Biodegradation is the chemical degradation process where microbes are able to extract energy from organic pollutants. Biodegradation/biotransformation and sorption are the two main mechanisms driving the removal of ECs in biological treatment, where the extent of volatilization is comparatively limited (Verlicchi et al., 2012). In WWTP processes, hydrophobic ECs are known to be partially adsorbed onto primary sedimentation. The remaining ECs in secondary treatment go through various processes such as dilution, dispersion, partition, biodegradation, and abiotic degradation. In the aerobic and anaerobic processes, there are chances that ECs could get biodegraded through mineralization or transformation products. Biodegradation is known to be influenced by factors such as the chemical nature of the ECs, half-life, characteristics of the biomass, treatment process configuration, as well as operational conditions. Therefore, the parent compound of an EC in secondary treatment is being transformed through various chemical and physical processes, biodegradation as well as sorption onto solids. If the ECs do not degrade after the secondary treatment, it is likely that they will remain stable after the discharge for a longer period of time than the hydraulic retention time of WWTP (Neczaj, 2020; Seriki, K. et al., n.d.; Verlicchi et al., 2012).

Volatilization is one other physical interaction that results in organic ECs being removed from wastewater due to their transfer to the atmosphere. Henry's Law constant (K_H) helps estimate the volatilization of ECs and along with Kow, the following can be used for the evaluation of the volatilization of ECs in WWTP processes. $K_H > 1 \times 10^{-4}$ and $K_H/Kow > 1 \times 10^{-9}$ indicate high volatilization potential while $K_H < 1 \times 10^{-4}$ and $K_H/Kow < 1 \times 10^{-9}$ indicate low volatilization potential

(Rogers, 1996). Conditions, such as temperature, aeration, and atmospheric pressure in the WWTP process also affect the volatilization process (Neczaj, 2020).

Hydrolysis is the chemical breakdown of a molecule due to reaction with water. Polar molecules having high solubility are readily hydrolysed, and the rate increases with increased temperature and pH (Tran et al., 2018; J.-L. Zhou et al., 2022).

2.1.4 Effects caused by ECs

Numerous studies were conducted investigating the potential impacts of ECs in aquatic environments (Caliman & Gavrilescu, 2009; Mehdi et al., 2019; Geiger et al., 2016). The sustainability of both aquatic environments and human health was found to be potentially affected by the presence of various ECs (Alavanja et al., 2004; Birnbaum & Fenton, 2003; Chacón et al., 2022; Hu et al., 2019). The marine mussel exposed to the widely used non-steroidal anti-inflammatory drug diclofenac and lipid reducing agent gemfibrozil had lipid peroxidation, damaged DNA, and reproductive system (Schmidt et al., 2011).

Experiments done on wild fish collected downstream of the discharges of the WWTP effluents revealed biological disruptions such as male feminization, altered gene expression, decreased testosterone levels, impaired stress responsiveness, and behavioral changes. These effects were linked to the presence of ECs in the studied waters, that were believed to be discharged from the WWTP effluents (Du et al., 2019; McCallum et al., 2019). Estimation of the prevalence of human cancer due to pesticide exposures has been reported (Alavanja et al., 2013). A wide variety of studies on the presence of ECs, including antibiotics and antimicrobial agents in water environments, have been baffling inview of their therapeutic effect in animals and humans and reveal the gravity of the problem caused by ECs.

2.1.5 Environmental Risk Assessment

The process of ecological risk assessment (ERA) was developed to evaluate the possibilities of negative ecological responses due to the occurrences of stressors in the environment (U.S. EPA, 1998). This process is possible through understanding and predicting the interactions between stressors and ecological effects by evaluating the collected data, information, uncertainties, and hypothesis. The assessment may include physical, chemical or biological stressors for one or multiple stressors. Effect

assessment and exposure assessment are the main elements leading to the risk characterization and lastly the risk management steps finalize the process of ecological risk assessment. The main frame of the ecological risk assessment has been provided by the U.S. EPA (Figure 2.2).

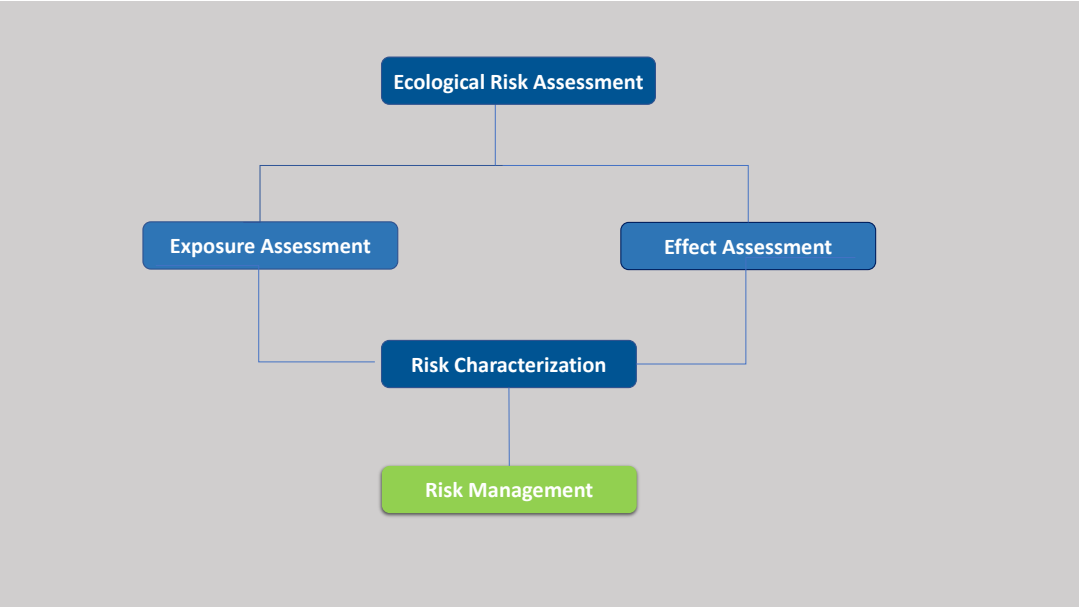


Figure 2.2 : The framework of ecological risk assessment of US EPA (U.S. EPA, 1998).

The first step in ERA includes the hazard identification. Risk can be defined as the probability of an adverse effect of the environment due to exposure to chemicals, while hazard defines whether a substance or event may cause harm. Evaluating such harm and risk due to presence of micropollutants in wastewaters are important in estimating their probable adverse effect in the aquatic organism in receiving water bodies. Labeling of chemicals and products provide such important information. Globally Harmonized System of Classification and Labelling (GHS, United Nations, 2003) provides internationally accepted labelling standards (Escher et al., 2021).

Exposure assessment and effect assessment are the key next steps implementing ERA. Exposure assessment provides the data relating to the frequency, magnitude, extent, character, and duration of the exposure to a chemical. This step provides the information of the Measured Environmental Concentration (MEC) of the PSs in this study. Effect assessment includes the PBT assessment and dose-response assessment. PBT assessment stands for the assessment of persistence (P), bioaccumulation (B) and toxicity (T) of the chemical. On the other hand, dose-response assessment provides the response of the test organisms after being exposed to a chemical in order to derive data

on Predicted No Effect Concentration (PNEC). Acute and chronic toxicity testing with fish, daphnia, algae, and bacteria are widely applied tests in conducting environmental risk assessment of wastewaters (Escher et al., 2021; U.S. EPA, 1998).

Risk characterization is conducted through estimating Risk Quotient (RQ) which is the ratio of level of exposure (MEC) over the assigned PNEC value. If RQ > 1, exposure to the acceptable levels is exceeded and a detailed risk assessment and reduction measures are required. Finally, evaluating the scientific data, risk management considers the economic, social, and political considerations for risk reduction measures to minimize the degree of exposure (Escher et al., 2021).

2.1.5.1 Environmental risk assesement of chemicals

The research conducted on water pollution in recent decades revealed the presence of many organic chemicals in wastewaters. Even though the occurrence, identification and quantification of specific ECs in the effluents of WWTPs were the main focus, the assessment of their environmental risks was essential due to their discharges to the receiving water bodies, specifically of those PSs showing ubiquitous, toxic, bioaccumulative, and persistent characteristics. Databases such as ECOTOX (US, EPA) provide information regarding the ecotoxicity of known chemicals. The European Commission Directive requires the assessment of substances in the environment (EC, 1993b). The RQ approach is helpful in conducting risk analyses of individual chemicals, the EC having RQ values above 1.0 are considered to possess sufficient risk potential and must be monitored (Aemig et al., 2020; Anderson et al., 2010; EC, 1993; Escher et al., 2021; Kobayashi et al., 2015; Regan et al, 2013).

2.2 Toxicity Evaluation

2.2.1 Environmental toxicology

Environmental toxicology is an interdisciplinary field of study that examines the basic biological and chemical mechanisms by which harmful synthetic chemicals, xenobiotics (foreign to life) interact within the biosphere (Knapp & Bromley-Challoner, 2003; Walker, 2001). Xenobiotics have drawn attention and are often classified as carcinogenic, mutagenic, or cytotoxic. Mode of the exposure, physical and chemical properties as well as the concentration of the xenobiotics are the factors affecting their absorption. Inhalation, digestive entry through gastrointestinal tract,

dermal contact as well as injections are main exposure routes of harmful chemicals (Korrapati & Mehendale, 2005; Niesink et al., 1996).

Chemical monitoring of micropollutants in wastewaters provides useful information. Advancements in chemical analysis techniques in recent decades allow the identification and quantification of a number of selected organic micropollutants in wastewaters. This has provided expansion of exposure-based assessment of organic chemicals that cause pollution in aquatic environments, allowing quantitative assessment of those chemicals to permit their regular monitoring. Chemical monitoring provides quantitative data on ECs, although identification of their transformation products, as well as other known or unknown chemicals may be limited. Since there are more than 204 million individual organic and inorganic chemicals identified by the Chemical Abstracts Services (CAS) (ACS, 2023), it is not possible to measure all chemicals present in the environment. Considering the share (five trillion dollars in 2017) of global chemical industry, a report of the United Nations Environment Programme expects the share to double by 2030 (Yang et al., 2022). Monitoring programs may include 10 to 300 chemicals. However, it is not always possible to analyze certain chemicals due to the availability of relevant chemical methods or technical infrastructure (B. Escher et al., 2021).

Monitoring micropollutants or ECs on the monitoring list provides useful quantitative data and information regarding their presence in the environment. However, since there are many ECs, it is not technically possible to analyze all ECs present in wastewaters. Therefore, analysis of ECs alone is considered the tip of the iceberg, and would not be sufficient to track the toxicity of wastewaters. Effect-based methods were introduced as a beneficial means to compliment chemical exposure-based methods. In vivo bioassays consisting of fish, invertebrates, and plants measure endpoints such as mortality, reproduction, feeding response, growth, etc. and are popular in aquatic toxicity testing (B. Escher et al., 2021). These bioanalytical tools also help estimate the mixture toxicity of wastewaters, which include the toxicity caused by unknown chemicals, as well as the interaction of all chemicals in the wastewater or environment that may end up showing synergistic or antagonistic effects (B. Escher et al., 2021; Niesink et al., 1996).

The quality of WWTP effluents is being monitored as ordained by the Turkish Regulation on Urban Wastewater Treatment, where conventional parameters such as

chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total suspended solids (TSS) are regularly monitored (MOEF, 2006). Since wastewaters are formed from various sources, including toxic substances in domestic wastewaters, industrial discharges, leachate effluents, hospital wastewaters, urban run-off, etc., they may include toxic components that pose a threat to receiving aquatic environments and the biocommunity responsible for the treatment in WWTPs (Ozturk et al., 2003). Current conventional monitoring parameters are insufficient in revealing the toxic effects.

It is necessary to monitor WWTP effluents and the receiving environment in order to protect the environment from the negative effects of wastewater discharges. Currently there are no universal regulations regarding the monitoring the toxicity testing of WWTP effluents around the globe, but exceptions are found in a number of developed countries (B. Escher et al., 2021).

Conducting aquatic quality risk assessment of ecosystems is important in protecting biodiversity. Maintaining and restoring the physical, chemical and biological integrity of water environments are important to sustain the aquatic life. Various tools have been developed for this purpose (B. Escher et al., 2021). Within this scope, a battery of toxicity tests consisting of in vitro bioassays is recommended for the use in sewage treatment efficiency and effluent quality assessment. The immobilization test of *D. magna*, the growth inhibition test of green algae *S. capricornutum*, and the bioluminescence inhibition tests of *V. fischeri* is a widely used test battery for the influents and effluents of WWTPs (B. Escher et al., 2021; Hernández Leal et al., 2012).

2.2.2 Toxicity of wastewaters in WWTP influents and effluents

Biological wastewater treatment aims at degrading pollutants that are non-toxic and soluble in wastewater. This important task is being conducted by the activity of microorganisms both in aerobic and anaerobic environments. Therefore, provision of a healthy environment to the microorganisms is crucial for a sustainable treatment. Influent toxicity tests are conducted to sustain the health of the productive biological wastewater treatment processes. Surely, in order to monitor the toxicity of influents, the bioassays shall be chosen properly to represent the biocommunity in WWTP processes (Quevauviller et al., 2006; Wilderer, 2011).

Effluent toxicity testing is conducted to estimate the effect of the sampled wastewater on the receiving aquatic environment. Bioassays chosen from the selected different bioindicator species are used. Once the wastewaters are subjected to bioassays, these indicator organisms show different endpoints such as luminescence, reproduction, immobility, etc. The bioassays may be divided by the test duration and categorized as acute or chronic toxicity tests. The chosen bioassays are expected to represent the ecosystem of the receiving body (B. Escher & Frederic, 2012; Quevauviller et al., 2006).

There is a strong similarity between toxicity of the influents and effluents. Considering the ease of applicability of the ecotoxicity tests, bioassays consisting of the acute immobilization of *Daphnia magna (D. magna)*, the growth inhibition of green algae *Selenastrum carpricornutum (S. carpricornutum)*, and the bioluminescence inhibition with *Vibrio fischeri (V. fischeri)* were applied in this study.

2.2.3 General Principles

Ecotoxicity of the wastewaters is investigated by the relation between the concentration of the mixture of wastewater vs. measure of the exposure of the organism. This is called the concentration-effect relationship (Figure 2.3) and the units provided are used for evaluation. The quantified effects are estimated in each toxicity test and refer to IC_{50} in the case of inhibition of light, LC_{50} in the case of lethal effect, etc. The concentration of the effluent refers to dilution series of 0 to 100% of the studied wastewater (Quevauviller et al., 2006; Walker, 2001).

This study was conducted to investigate the state of the toxicity of the wastewaters of the megacity of Istanbul. In this context, presence of PSs, emerging contaminants and estimation of acute toxicity in the influents and effluents of the seven largest WWTPs, a leachate treatment facility and a hospital wastewater were conducted. Currently, Turkish regulations do not regulate the monitoring of the aforementioned parameters in WWTP effluents. The data from this survey is valuable in estimating the occurences of the urban priority substances and acute toxicity of effluents of Istanbul's seven largest WWTPs. The data obtained may be useful in the endeavor to transition to the end-of-pipe approach to meeting water quality standards in receiving water bodies.

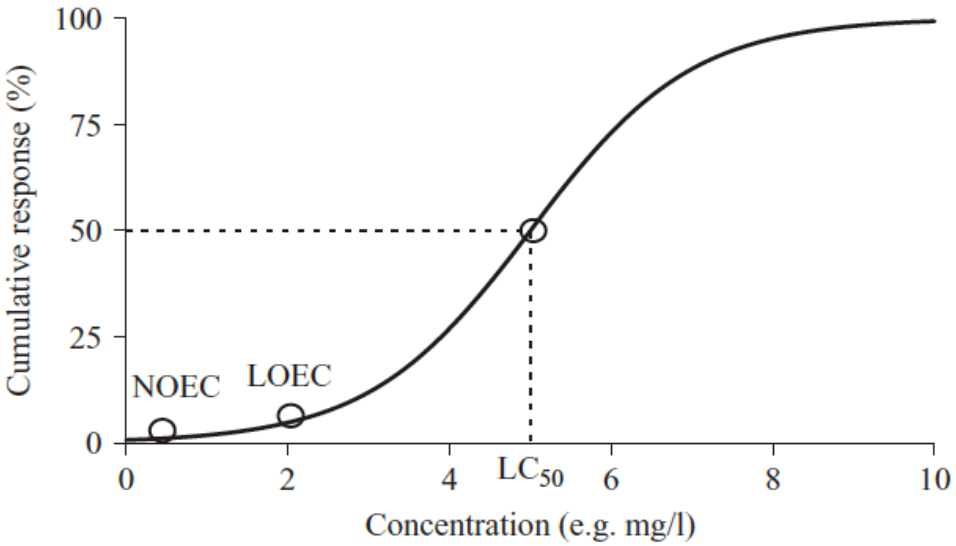


Figure 2.3 : Cumulative response (%) vs. concentration sigmoidal curve (Quevauviller et al., 2006).

LC_{50}: The median effec concentration.

LOEC: Lowest Observed Effect Concentration

NOEC: No Observed Effect Concentration

2.3 Regulation of Wastewaters in Türkiye

A number of regulations have been in force in Türkiye to ensure the protection of the environment, in line with the principles of sustainable environment and development. The directives related to water pollution in Türkiye are listed in chronological order in Table 2.1.

PSs are micropollutants having persistent, toxic, bio-accumulative, ubiquitous properties in aquatic environments. Their presence poses high risk to the water environment. According to the European Water Framework Directive (WFD) (2000/60/EC) the environmental objective is to ensure “good status” of surface waters and groundwaters. The “good status” refers to “good chemical and ecological status”. The good chemical status of the water environment means compliance with the limits set by the Environmental Quality Standards (EQS) for each PS (Annex II of Directive 2013/39/EU). WFD (2000/60/EC), as amended in 2013 (Directive 2013/39/EU), required member states to monitor the presence and quantity of PSs in surface waters, in order not to exceed threshold levels specified by the EQS. The Turkish Ministry of

Forestry and Water Affairs adopted EQS for the PSs in surface waters in 2012, and updated them in 2016. Although WWTP effluents have been found to be the main point source of micropollutants to the receiving water bodies, currently there are no regulations for PSs or micropollutants. Hence, Article 16 of WFD emphasizes the importance of control of the PSs from the point sources (EC, 2000, 2008, 2013; OSIB, 2016a; SCHEER, 2017).

The WFD also recommends monitoring of both acute and chronic toxicity testing of the water bodies, including a set of the three taxa, preferably with algae, daphnia, and fish, or other available aquatic taxa (2000/60/EC). Since Türkiye adopted the WFD, and published the Surface Water Quality Regulation, the same recommendation can apply to Türkiye. In addition, the Turkish Water Pollution Control Regulation has a fish biotest for assessment of the toxicity of effluents (MEU, 2004).

The traditional physical and chemical assessment of the WWTP effluents include monitoring of pH, temperature, COD, and specific parameters such as heavy metals or oil. This approach is known to lessen the discharge of toxic substances and help to improve the quality of the receiving aquatic environment (Concawe, 2004). WWTP effluents having a flow of 10,000 m^3/day or over are required to be monitored in cabinets that are measuring pH, conductivity, dissolved oxygen (DO), COD, and TSS, for 24 h prior to discharge in Türkiye (MEU, 2015). Some countries have additional monitoring of the biological quality parameters in evaluating the quality of the discharged wastewatwers as well as the receiving environment (Concawe, 2004). There are numerous applications to assess the mixture effluent toxicity in the developed world such as Direct Toxicity Tests in UK and Australia, and Whole Effluent Toxicity testing (WET) of National Pollutant Discharge Elimination System (NPDES) in USA. WET aimes at assessing the quality of the discharged effluents to ensure sustaining quality of the aquatic environments (B. Escher et al., 2021). WET is applied to assess the acute, short term chronic and chronic toxicity of the mixture of micro and macro pollutants in wastewater on aquatics organisms (EPA, 2002a, 2002b, 2002c). The biological endpoints are growth, survival, reproduction or fertilization and reported as NOEC, LOEC, Median Lethal Effect Concentration (LC50), Median Effect Concentration (EC50), Inhibition Concentration (25%) (IC25) (EPA, 2001, 2002a).

Table 2.1 : List of regulations in line with the prevention of water pollution

Regulation	Scope	Year established
Fisheries Regulation	To protect the fishery stocks and to economically benefit from aquaculture resources, fishing licenses, hunting for recreation, arranging the places for hunting, controlling use of explosives and harmful substances in hunting, harmful and polluting substances that are forbidden to be dumped into aquaculture production areas, quality and conditions of the means of their production and use, procedures, principles, prohibitions, limitations, obligations, precautions, regarding the conditions and use of fisheries, regulation of fisheries, trawling, fishery products, that are casually harvested, healthiness of fishery products, production of finished and semi-finished products to be made from fishery products, it covers the methods, principles, prohibitions, restrictions, responsibilities, measures, controls, and supervision regarding to all aforementioned issues.	1995
Water Pollution Control Regulation	To identify legal and technical principles in order to prevent the pollution, hence to sustain and to realize best possible use of surface and underground water potential in compliance with the sustainable development goals. This regulation replaced (now defunct: RG-17/12/2022-32046).	2004
Regulation on Control of Pollution Caused by Hazardous Substances in Water and Its Environment	To detect, prevent, and provide gradual reduction of pollution caused by dangerous substances in water and its surroundings.	2005
Regulation on Urban Wastewater Treatment	To protect the environment against the negative effects of the collection, treatment and discharge of urban wastewater and certain industrial discharges.	2006
Surface Water Quality Regulation	To determine the biological, chemical, physico-chemical and hydromorphological qualities and classification, monitoring of the quality and quantity of surface, coastal and transitional waters, establishment of consumption, purposes of these waters taking into account the balance between consumption and prevention in compliance with the sustainable development goals, and identification of methods and principles to be followed concerning the the achievement of good quality water.	2012
Regulation on Monitoring of Surface and Groundwater	To establish the current status of all surface waters and groundwaters in the country in terms of quantity, quality and hydromorphological indicators, to monitor the waters with an approach based on ecosystem integrity of waters, to determine the procedures and principles for the standardization in monitoring and for the coordination among monitoring institutions and organizations.	2014
Protection of Drinking-Domestic Water Basins Regulation	To regulate the procedures and principles regarding the protection and improvement of the quality and quantity of drinking-potable water supplied from all surface and groundwater resources.	2017
Regulation on the Water Intended for Human Consumption	To regulate the procedures and principles regarding the conformity of water intended for human consumption with the technical and hygienic conditions as well as the provision compliance with the quality standards of water, the production, packaging, labeling, sale and inspection of spring water and drinking water.	2005
Regulation on The Protection of Water Against Nitrate Pollution from Agricultural	To regulate the procedures and principles regarding the detection, reduction and prevention of pollution caused by nitrate of agricultural origin in water.	2016

Once the evaluation of WET tests conducted on effluent shows toxic effects, the procedure of effluent Toxicity Reduction Evaluations (TRE) is required. TRE aims at determining the acceptable levels of measures in effluents to prevent aquatic toxicity. Toxicity Identification Evaluation (TIE) is part of TRE and has three phases. Phase I includes characterization of the physical and chemical constituents that are the reason for the toxicity. Phase II identifies whether the effluent has ammonia, non-polar organics and metals, while Phase III include methods to confirm suspected toxicants (EPA, 1991).

Throughout this research, the analyses of the aforementioned tests became possible through establishment of an advanced instrumental laboratory as well as an ecotoxicity laboratory in ISKI. It is expected that regulations may stipulate the WWTP effluents be subjected to a total assessment in the future.

3. MATERIALS AND METHODS

3.1 Study Area and Sampling Locations

Istanbul has an estimated population of over 16 million. The estimated average daily water consumption of city is over 3 million m^3/day while the produced wastewater is 4.1 million m^3/day. Food, pulp, textile, forestry, paper, and tourism are the main industries/services in the city. The discharges generally emanate from organized industrial districts and finally reach the sewer line along with the domestic wastewater. Industrial discharges are subject to industial pre-treatment or discharge criteria (ISKI, 2013, 2020).

In this study, in order to estimate the toxicity characteristics, the PSs concentrations, acute toxicity characteristics and the physicochemical parameters of the wastewater samples from every station were investigated. In addition, monitoring of the physicochemical parameters were conducted in seven WWTPs. All of these analyses were conducted from September 2015 to December 2019. Througout this chapter, the methodology obtained and gathered through The Scientific and Technological Research Council of Türkiye-Marmara Research Center (TUBITAK-MAM) and ISKI is described for the aforementioned analyses.

The sampling points were chosen among the largest four WWTPs (WWTP 1–WWTP 4) as well as three mechanical treatment facilities (MTF = WWTP 5–WWTP 7), a landfill leachate treatment plant (LTP) and the sewer of one hospital (HWW) that serves in the City of Istanbul. While the LTP, HWW, two of the WWTPs and two of the MTFs were located on the European side, the other two of the WWTPs and one of the MTFs were located on the Asian side. Advance treatment processes consisted of activated sludge systems with biological nutrient (N, P) removal facilities. The MTFs had a bar screening and aerated grit removal. All sampling points were selected to sufficiently represent overall conditions prevalent in the city. The basic characteristics of the MTFs and WWTPs studied are given in Table 3.1. The average flow rates

(m^3/day), mixed liquor suspended solids (MLSS) (mg/L), and dissolved oxygen (mg/L) concentrations of aeration pools (for advance treatments) of each WWTPs are provided through Figure A.1 to Figure A.4. The sewer system essentially operates as a separate system and receives some industrial influents in addition to domestic influents. The flow diagrams of the advanced wastewater treatment plant and mechanical treatment process as well as their sampling points are shown in Figure 3.1. Pictures taken at sampling stations at WWTPs are provided in Figures I.1 (a)–(b).

The studied sanitary landfill leachate treatment plant (LTP) and hospital are among the largest ones in Türkiye and they both discharge into the municipal sewer system. The LTP consists of a balancing tank, denitrification-nitrification tanks, and ultra-filtration unit (Figure 3.2). The daily capacity of the LTP was 2,000 m^3/day. The daily average COD and total Kjeldahl nitrogen (TKN) values were 18,000 mg/L and 3,000 mg/L, respectively. The flow rate, COD (mg/L) and the SRT (d) rates of the LTP are given in Figure B.1. The leachate treated in LTP orginates mainly from LTP. However, in recent years it also included leachates transfered from LTP II and LTP III. The percentage of leachates treated in LTP by origin of LTPs are shown in Figure B.2. The hospital studied had a bed capacity over 1,300, while water consumption was aproximitaly 1,500 m^3/day. The hospital had a combined sewer system.

3.2 Sample Collection, Processing and Analysis

The wastewater samples were taken from the influent and effluent streams of the WWTPs, the LTP, and the sewer of the hospital. The sampling schedule was planned as one sample per season from all sampling points for quantification of the PSs and toxicity analyses, while one sample per month was arranged for the physiochemical monitoring of the WWTPs. Sampling schedule was conducted on different days through August 2015 to December 2019. All-weather refrigerated automatic samplers (4 ºC) were used to collect 24 h composite samples from each sampling point and were located after the grid chamber of each of the WWTP and MTF, as well as after the final clarifier of the WWTPs. The LTF samples were collected after the balancing tank and after the ultrafiltration process (UF). Picture taken at sampling of leachate is provided in Figure I.1 (d). The sampling arrangement from the hospital sewer network

is depicted in Figure 3.3. Picture taken at sampling of hospital wastewater is provided in Figure I.1 (c).

3.3 PSs and Emerging Pollutant Analyses

The sampling schedule for PSs and emerging contaminants was planned as one sample per season for all sampling points from December 2015 to February 2017. Also, to investigate the variations in summer and winter seasons, three consecutive weekday samplings were performed in WWTP 1, WWTP 2, WWTP 3, and WWTP 5 throughout January 2016 and August 2016. All sampling work was conducted in dry weather. Twelve one L samples were taken and stored in Teflon coated amber glass bottles, using thermostat with cooling elements to maintain them at 4 °C, and were delivered to the Cleaner Production Institute of TUBITAK-MAM and laboratories of ISKI within two to three hours. The analyses of physicochemical parameters and the volatile PSs were conducted on the same day of sample collection. Samples that could not be analyzed within a week were stored frozen at -20 °C and thawed prior to the analysis. All analyses were completed within two months. This research was only conducted on dissolved phases.

The HWW, the influent and effluent samples of WWTPs and LTP were analyzed for the presence of PSs and emerging contaminants. The analyses were conducted by the laboratories of the TUBITAK-MAM, an internationally accredited laboratory in line with ISO/IES 17025:2012 by the Turkish Accreditation Agency (TURKAK) and German Accreditation Council Deutscher Akkreditierung Rat (DAR/DAP).

3.3.1 Chemicals

Standards and internal standards used for the analyses of PSs, emerging contaminants, toxicity testing and physicochemical analyses used in this study were bought from Wellington Laboratories Inc. (Ontario, Canada), Sigma-Aldrich (Steinheim, Germany), Ehrenstorfer (Augsburg, Germany), Supelco (Bellefonte, PA, USA). Appropriate solvents (acetonitrile, acetone, methanol) were used to prepare stock solutions at the concentration of 1,000 mg/L in amber glass vials. Solvents (methanol, hexane, acetonitrile, acetone) used were purchased from Merck (Darmstat, Germany)

Table 3.1 : Characteristics of the WWTPs studied.

WWTP	Population Served	Flow Capacity (m^3/d)	Type of Wastewater Treated	Primary Treatment Units	Secondary Treatment Process	SRT (d)*	HRT (h)**	Receiving Waterbody
WWTP 1	2,000,000	400,000	Domestic + Industrial	Screening + Grit Removal + Primary Sedimentation	Activated sludge process, A2O	11–15	7	Ayamama Creek / The Sea of Marmara
WWTP 2	770,000	200,000	Domestic	Screening + Grit Removal	Activated sludge, Johannesburg Configuration	15–26	7	Riva Creek/ The Black Sea
WWTP 3	500,000	100,000	Domestic + Industrial	Screening + Grit Removal + Primary Sedimentation	Activated sludge process, A2O	9–17	12	The Sea of Marmara, Deep Sea discharge
WWTP 4	2,000,000	400,000	Domestic + Industrial	Screening + Grit Removal + Primary Sedimentation	Activated sludge process, A2O	9–22	8	Harami Creek, The Sea of Marmara
WWTP 5	4,320,000	864,000	Domestic + Industrial	Screening + Grit Removal	-	-	-	The Sea of Marmara, Deep Sea discharge
WWTP 6	3,125,000	625,000	Domestic + Industrial	Screening + Grit Removal	-	-	-	The Sea of Marmara, Deep Sea discharge
WWTP 7	2,230,000	833,000	Domestic + Industrial	Screening + Grit Removal	-	-	-	The Sea of Marmara, Deep Sea discharge

*SRT: Solids retention time, **HRT: Hydraulic retention time.

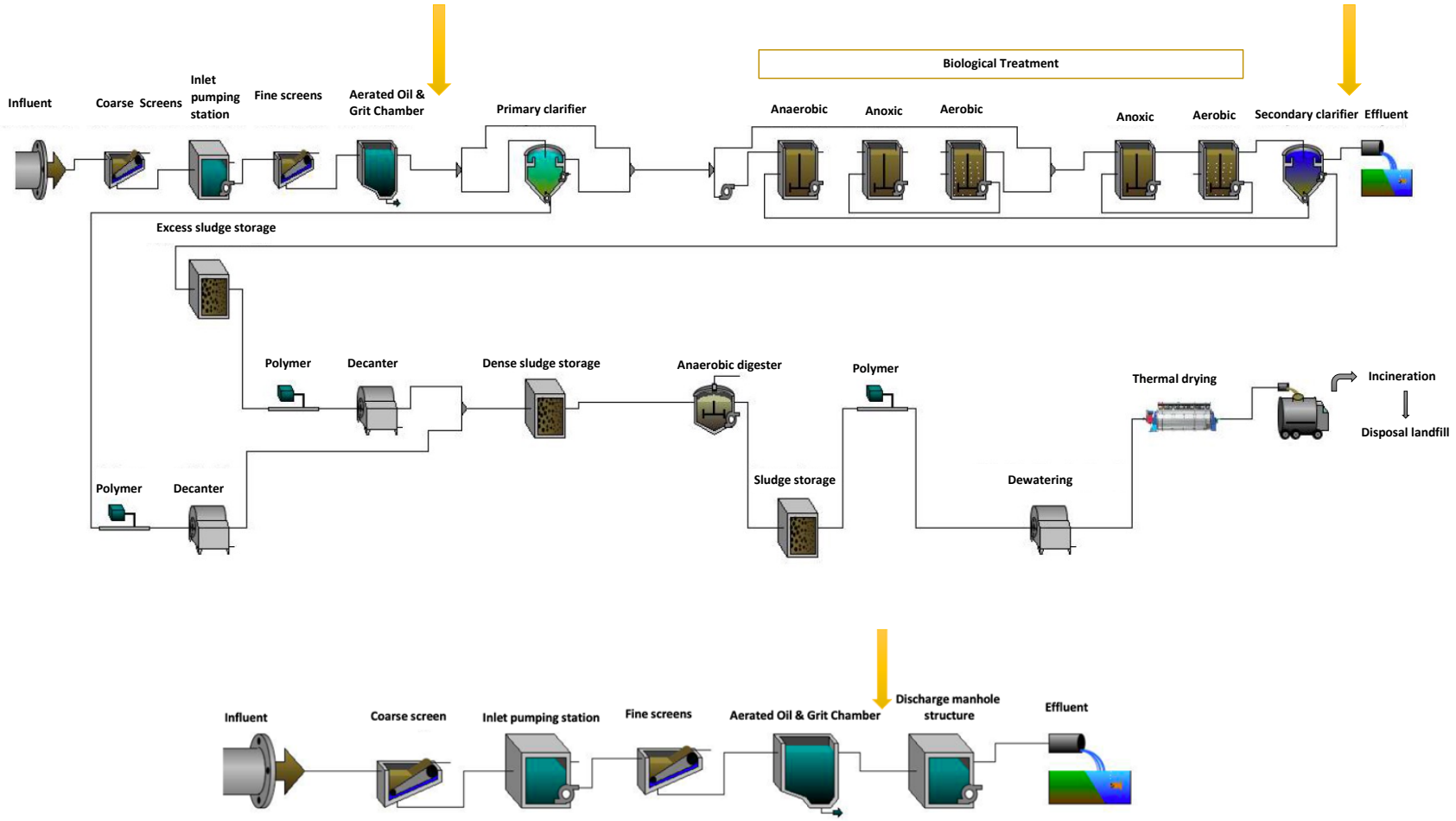


Figure 3.1 : Advanced wastewater treatment plant (above) and mechanical treatment process (below) flow diagrams showing the sampling points.

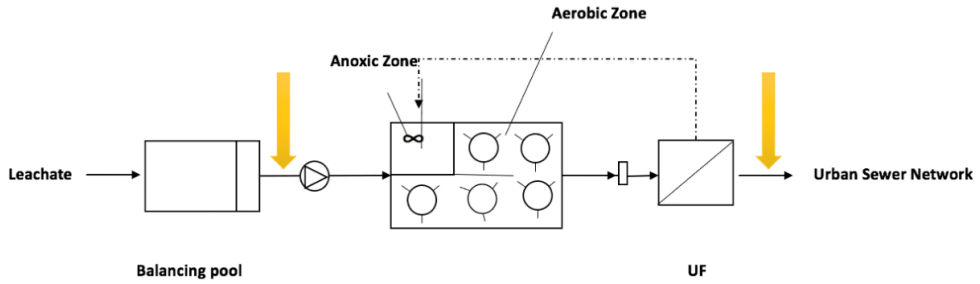


Figure 3.2 : Leachate treatment plant process flow diagram showing the sampling points (Ozturk I., 2019).

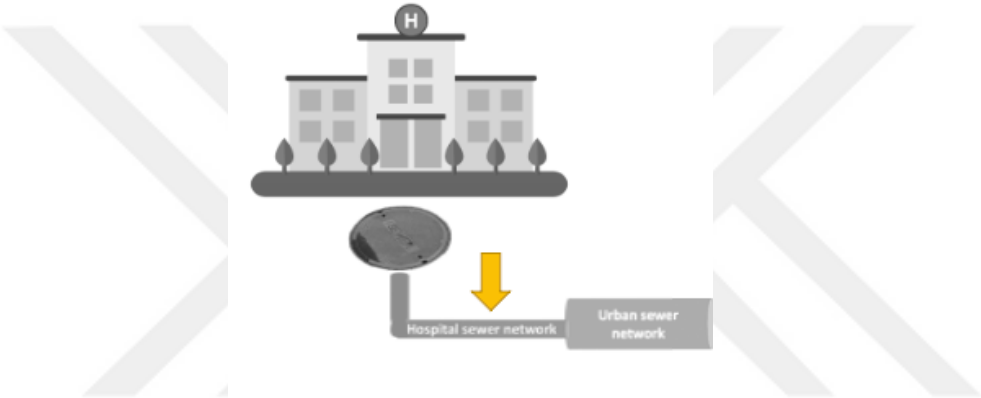


Figure 3.3 : Sampling arrangement from the hospital sewer network.

and were all analytical grade. The dilution of calibration standards (10 mg/L) were accomplished using these solvents. Water was purified using the Milli-Q Plus system (EMD Millipore, Billercia, MA).

3.3.2 Analytical methods

All organic PSs and emerging contaminants were analyzed for the dissolved phase. The analytical methods presented here were previously reported (Birtek et al., 2022). PSs that were listed in the Directive 2013/39/EU and the chosen list of emerging contaminants were analyzed by LC-MS/MS, GC-MS/MS, GC-MS, Direct Mercury Analyzer, and ICP-MS techniques. The list of PSs and emerging contaminants analyzed as classified by their analytical techniques are summarized in Table 3.2. The analytical methods used to detect PSs and emerging contaminants in the influents and effluents of WWTP and LTP, and in HWW are shown in Table 3.3.

3.3.3 Sample Pre-treatment and Analytical Methods

Pesticides, VOCs, PAHs, alkylphenols, PBDEs, DCLs, other organics, and metals among PSs were analyzed by using GC-MS, GC-MS/MS, LC-MS/MS, GC-HRMS, and ICP-MS techniques. Organic PSs were extracted according to the modified EPA 8270D method.

The LC-MS/MS analyses were conducted using a 1260 Infinity LC system 6460 triple quadrupole MS/MS (Agilent Technologies, Santa Clara, CA, USA). The column, Poroshell 120 SB-C18 3 x 100 mm, 2.7 µm, was used when flow was set at 0.6 mL/min and, the column temperature was maintained at 30 ºC. The MS/MS was used in electrospray ionization (ESI)- jet stream (JS) with dMRM mode. The capillary voltage was 3000 V. The sheath gas temperature and flow rate were 400 ºC and 12 µL/min, respectively. For the preparation of LC mobile phases, 0.3% acetic acid in water (Solvent A) and methanol (Solvent B) were used. The compounds were separated following a gradient program where 20-70 % of solvent B was run for 2 minutes, ramped to 70% of Solvent B over 6 minutes and then held at 95% for 2 minutes. The column was re-equilibrated to the starting conditions of 20% of Solvent B and 80% of Solvent A for 10 minutes. The injection volume was 50 µL. The CAS number, mass, characteristic precursor and product ions, polarity, retention time, linearity range, LOD, LOQ, correlation coefficient, recovery, and RSD of the PSs analyzed by LC-MS/MS are given in the Table 3.4.

VOC analysis extractions were conducted by using purge and trap (P&T IO Analytical Eclipse Model 4660 Sample Concentrator (College Station, TX, USA)) coupled with 6890N GC 5975C Inert MS System. A 25 mL aliquot of sample was automatically transferred to the P&T unit with the addition of internal standard (BFB, bromofluoro benzene). Samples were purged (35 ºC, 11 min, 50 mL/min) with helium and then trapped into the VOCARB 4000. A DB-5MS (60 m x 0.25 mm x 0.25 µm) column was used for the separation of VOCs. The column flow was set at 1 mL/min, the injector temperature was set to 200 ºC and split ratio was 20:1. The GC oven program was set at 40 ºC (2 min), and ramped to 200 ºC at 7 ºC/min. CAS number, retention time, characteristic quant and qual ions, correlation coefficient, LOD and mass of the PSs analyzed by P&T GC-MS are shown in Table 3.5.

Table 3.2 : List of PSs and specific pollutants analyzed classified by analytical instruments used.

LC-MS/MS	GC-MS/MS	P&T GC-MS	GC-HRMS	ICP-MS + DMA
Alachlor	PBDEs	Benzene	DLCs	Cd
Atrazine	C10-13-chloroalkanes	Carbon-tetrachloride		Pb
Chlorfenvinphos	Chlorpyrifos	1,2-dichloroethane		Ni
Diuron	Cyclodiene pesticides	Dichloromethane		Hg
Isoproturon	DDT	HCBD		
Pentachlorophenol	DEHP	Ethylbenzene		
Simazine	Endosulfan	Isopropylbenzene		
PFOS	HCB	n-propylbenzene		
Quinoxyfen	HCH	Naphthalene		
Aclonifen	NP	Tetrachloroethylene		
Bifenox	OP	Trichloroethylene		
Cybutryne	PeCB	Trichloromethane		
Dichlorvos	PAHs	Trimethylbenzene		
HBCDD	TBT	TCB		
Terbutryn	Trifluralin	Toluene		
	Dicofol	Xylene		
	Cypermethrin	Dichlorobenzene		
	HC			
	HCE			

PBDEs: Polybrominated diphenyl ethers: PBDE 28, PBDE 47, PBDE 99, PBDE 100, PBDE 153, PBDE 154

Cyclodiene pesticides: Aldrin, Dieldrin, Endrin, Isodrin

DDT: p,p'-DDE, p,p'-DDT, p,p′-DDD

DEHP: Di(2-ethylhexyl) phthalate; Dichlorobenzene: 1,3 dichlorobenzene, 1,4 dichlorobenzene, PeCB: Pentachlorobenzene

PAHs: Polyaromatic hydrocarbons: anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)-perylene, fluoranthene, indeno(1,2,3-cd)-pyrene

TBT: Tributyltin compounds; TCB: Trichlorobenzenes (1,3,5-TCB,1,2,4-TCB,1,2,3-TCB)

Trimethylbenzene: 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene;

PFOS: Perfluorooctane sulfonic acid and its derivatives

Cypermethrin: alpha-cypermethrin, beta-cypermethrin, theta-cypermethrin, and zeta-cypermethrin

HBCDD: Hexabromocyclododecanes: α-Hexabromocyclododecane, β-Hexabromocyclododecane, γ-Hexabromocyclododecane

DLCs: Dioxins and dioxin-like compounds (PCDD, PCDF, PCB-DL)

Cd: Cadmium and its compounds, Pb: Lead and its compounds, Ni: Nickel and its compounds, Hg: Mercury and its compounds

HC: Heptachlor, HCE: Heptachlor epoxide

HCB: Hexachlorobenzene, HCH: Hexachlorocyclohexane, HCBD: Hexachlorobutadiene, NP: Nonylphenols, OP: Octylphenols

Xylene: o-xylene, m-xylene, p-xylene.

Table 3.3: Analitical methods used in the analyses of subgroups of PSs and specific pollutants.

Class	Number of Chemicals	Sample Pre-treatment	Analytical Method		
			Extraction method		
			Technique	Solvent	
Pesticides	18	Filtration (0.22µm PTFE)	SPE	ACN MeOH	LC-MS/MS
	21	Filtration (0,45 µm PTFE syringe)	SBSE		GC-MS/MS
PAHs	7	Filtration (0.45µm)	SBSE		GC-MS/MS
DLCs	2	-	LLE (EPA 3510C) + SPE (EPA 1613)	Hexane DCM Toluene	GC-HRMS
PBDEs	6	EPA 3510C/EPA 3630C	LLE (EPA 3510C) + SPE	Hexane DCM Toluene	GC-MS/MS
Alkylphenols	2	Filtration (0.45µm)	SBSE	-	GC-MS/MS
VOCs	23	Filtration (0.45µm)	P&T, EPA 8260 B ISO 15680	-	GC-MS
Others*	2	Filtration (0.22µm PTFE)	SPE	ACN MeOH	LC-MS/MS
	1	Filtration (0.45µm)	P&T, EPA 8260 B ISO 15680	-	GC-MS
	3	Filtration (0,45 µm PTFE syringe)	SBSE		GC-MS/MS
Metals	3	APHA 3030 K	EPA 6020 A		ICP-MS
	1		EPA 7473		DMA-80

PBDEs: Polybrominated diphenylethers, DLCs: Dioxins and dioxin-like compounds, ACN: Acetonitrile, DCM: Dichloromethane, MeOH: Methanol.
SBSE: Stir Bar Sorptive Extraction, SPE: Solid phase extraction, LLE: liquid-liquid extraction.

DCM: Dichloromethane, TCE: Trichloroethylene, DCB: 1,2-Dichloroethane

*Others: DEHP, 1,2-dichloroethane, PFOS, C10-13-chloroalkanes, Pentachlorobenzene and Hexabromocyclododecanes.

Semi-volatile PSs were extracted using LLE (liquid, liquid extraction) and SPE (solid phase extraction) methods and their CAS numbers, and characteristic precursor and product ions, retention times, correlation coefficients are given in Table 3.6. A 2 L aliquot of sample was extracted using either SPE or LLE procedure. The final extracts were evaporated by using nitrogen flow (99.99 %). Afterwards, the analytes were injected to GC-MS/MS 7890B GC, 7000D (Agilent Technologies, CA, USA; Thermo TSQ 8000, USA), and LC-MS/MS (1260 HPLC, 6460 MS, Agilent Technologies, CA, USA). The GC oven program was initiated at 60 ºC (1 min), ramped to 170 ºC at 40 ºC/min, then ramped to 310 ºC at 10 ºC/min and held for 3 min.

The GC column (HP-5MS 15m x 0.25 mm x 0.25 μm) with helium gas flow at 1 mL/min was set. Injection port was set at 300 ºC for both GC instruments, with 1 μL injection volume in splitless mode. MS/MS ion source and quadrupole temperatures were set at 300 ºC and 150 ºC, respectively. Both MS/MS systems were run in the Multiple Reaction Monitoring (MRM) mode.

The SBSE method was applied to 0.5 mm (thickness) x 20 mm (length) twister bars (Gerstel, Mulheim, Germany). A 100 mL sample was placed in an Erlenmeyer and stirred at 800 rpm for 2h. After completing the extraction, the bars were removed, dried with lint-free paper and inserted in the Thermal Desorption Unit (TDU) liners. TDU was operated in split mode. Desorption started at 40 ºC and held for 0.5 min, then ramped to 300 ºC and held for 5 min. During the desorption, Gerstel Cooled Injection System (CIS 4) was set at -20 ºC, then ramped to 310 ºC at 12 ºC/s for transferring analytes to the GC. The GC oven program was started at 50 ºC (1 min), increased to 170 ºC at 40 ºC/min and, finally to 310 ºC at 10 ºC/min, and held for 3 min. The CAS number, retention time, characteristic precursor and product ions, correlation coefficient, LOD and, mass of the PSs analyzed by GC-MS/MS are provided in Table C.1.

DLCs were extracted according to the EPA 3510C protocol and, analyzed by EPA 1613 method. DLCs were determined by using GC-HRMS (Autospec, Waters, UK). The resolution was in excess of 10,000 resolving power (10% height) coupled with DB 5ms (60m x 0.25 mm x 0.25 μm) (Agilent, Palo Alto, USA).

Table 3.4: CAS number, mass, characteristic precursor and product ions, polarity, retention time, linearity range, LOD, LOQ, correlation coefficient, recovery, and RSD of the PSs analyzed by LC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	Polarity	t_R (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2	Recovery (%)	RSD (%)
Alachlor	15972-60-8	269.77	270.1	238.1	Positive	4.79	0,001–1	7	25	0.990	103	6
Alachlor		269.77	270.1	162.2	Positive	4.79						
Pentachlorophenol	87-86-5	266.34	264.6	35	Negative	5.00	0,001–1	10	50	0.990	101	5
Atrazine	1912-24-9	215.69	216.2	174.1	Positive	3.98	0,001–1	10	50	0.990	100	8
Atrazine		215.69	216.2	96.2	Positive	3.98						
Simazine	122-34-9	201.66	202.1	124.2	Positive	3.64	0,001–1	5	25	0.996	99	6
Simazine		201.66	202.1	104	Positive	3.64						
Cybutryne	28159-98-0	253.37	254.1	83	Positive	4.22	0,001–1	5	25	0.992	99	8
Cybutryne		253.37	254.1	198	Positive	4.22						
Terbutryn	886-50-0	241.36	242.2	186.1	Positive	4.60	0,001–1	2	10	0.996	101	10
Terbutryn		241.36	242.2	91.1	Positive	4.60						
Diuron	330-54-1	233.09	232.9	72	Positive	4.04	0,001–1	3	10	0.997	107	5
Diuron		233.09	232.9	46	Positive	4.04						
Isoproturon	34123-59-6	206.32	221	79.2	Positive	4.00	0,001–1	8	25	0.980	97	6
Isoproturon		206.32	207.1	165	Positive	4.00						
Chlorfenvinphos	470-90-6	359.564	359.1	155.1	Positive	5.26	0,001–1	4	10	0.991	103	6
Chlorfenvinphos		359.564	359.1	99.1	Positive	5.26						
Diclorvos	62-73-7	220.98	221	109	Positive	5.86	0,001–1	4	20	0.990	94	8
Diclorvos		220.98	223	109	Positive	5.86						
Quinoxyfen	124495-18-7	308.13	308	214	Positive	6.43	0,001–1	15	50	0.990	102	12
Quinoxyfen		308.13	308	197	Positive	6.43						
Aclonifen	74070-46-5	264.66	265	248.1	Positive	4.95	0,001–1	4	20	0.990	103	15
Aclonifen		264.66	265	193	Positive	4.95						
Bifenox	42576-02-3	342.1	359	310	Positive	6.14	0,001–1	20	50	0.998	108	4
Bifenox		342.1	359	189	Positive	6.14						
PFOS	1763-23-1	500.13	499.2	80	Negative	6.33	0,001–1	10	30	0.990	107	8
PFOS		500.13	499.2	99	Negative	6.33						
HBCDD	*	641.70	640	79	Negative	10.20	0,001–1	10	50	0.980	98	7

*This refers to 1,3,5,7,9,11-Hexabromocyclododecane (CAS 25637-99-4), 1,2,5,6,9,10- Hexabromocyclododecane (CAS 3194-55-6), α-Hexabromocyclododecane (CAS 134237-50-6), β-Hexabromocyclododecane (CAS 134237-51-7) and γ- Hexabromocyclododecane (CAS 134237-52-8).

Table 3.5: CAS number, mass, characteristic quant and qual ions, retention time, linearity range, LOD, LOQ, correlation coefficient, and RSD values of the PSs analyzed by P&T GC-MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Quant Ion	Qual Ion	Qual Ion	t_R (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2	RSD (%)
Benzene	71-43-2	78.11	78	77	79	7.492	0,25–20,00	10	30	0.9961	0.3
Carbon-tetrachloride	56-23-5	153.82	116	118	120	13.79	5,00–20,00	40	120	0.9968	1.4
1,2-Dichloroethane	107-06-2	98.95	63	65	83	7.278	0,25–20,00	100	300	0.9975	1.2
Dichloromethane	75-09-2	84.93	84	56	51	5.339	5,00–20,00	20	60	0.9975	0.8
Hexachlorobutadiene	87-68-3	260.90	225	223	127	24.26	5,00–20,00	30	90	0.9996	0.8
1,2,3-Trichlorbenzenes	120-82-1	181.44	180	182	184	24.34	0,25–20,00	20	60	0.9995	0.7
1,2,4-Trichlorbenzenes	108-70-3	181.44	180	182	184	23.49	0,25–20,00	10	30	0.9995	0.5
1,3,5-Trichlorbenzenes	95-50-1	181.44	180	182	184	22.31	0,25–20,00	10	30	0.9985	0.3
Tetrachloroethylene	127-18-4	165.82	166	164	168	12.15	0,25–20,00	20	60	0.9963	0.8
Trichloroethylene	79-01-6	131.38	95	130	97	8.564	0,25–20,00	30	90	0.9948	0.9
Trichloromethane	67-66-3	119.37	83	85	87	6.604	0,25–20,00	20	60	0.9970	0.6
Naphthalene	91-20-3	128.17	128	127		23.8	0,25–20,00	10	30	0.9965	0.4

Table 3.6 : CAS number, mass, characteristic precursor and product ions, retention time, linearity range, LOD, LOQ and, correlation coefficient of the PSs analyzed by Thermo GC-MS/MS (Birtek et al., 2022).

Compound	CAS Number	Mass (g/mol)	Precursor Mass	Product Mass	t_R (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
PBDE 154	207122-15-4	643.6	643.5 485.85	483.7 643.5	31.74	0,004–1	4	15	0.998
PBDE153	68631-49-2	643.6	643.5 485.85	483.7 643.5	30.49	0,004–1	4	15	0.998
PBDE 100	189084-64-8	559.6	404 403.8	297 563.6	28.21	0,004–1	4	15	0.997
PBDE 99	60348-60-9	559.6	404 403.8	297 563.6	27.26	0,004–1	4	15	0.997
PBDE 47	5436-43-1	481.7	485.7 325.9	219.08 485.7	24.31	0,004–1	4	15	0.999
PBDE 28	41318-75-6	403.8	405.9	246.1	20.24	0,004–1	4	15	0.999

The oven temperature program was initiated at 140 ºC (3 min) ramped to 200 ºC at 15 ºC/min, then 3 ºC/min to 235 ºC and held for 15 min, then 4 ºC/min to 300 ºC and held for 10 min.

Column flow was held at 1.2 mL min. CAS number, retention time, correlation coefficient, LOD and, mass of the PSs analyzed by GC-HR/MS are shown in Table 3.7.

All the reported methods on PSs and specific pollutant analyses were provided by TUBITAK-MAM. The missing QA/QC information on the organic PSs analyses refer to the studies conducted at TUBITAK-MAM (Canlı et al., 2020; Güzel & Canli, 2020).

Ni, Cd, and Pb analyses were conducted according to the Standard Methods 3030K (APHA, 2012) and EPA 6020A methods (EPA, 2012). Acid digestion of samples by using microwave with temperature control (Milestone Ultrawave-Single Reaction Chamber Microwave Digestion System) was applied prior to the metal analyses. Perkin Elmer Nexion 300 XX Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) was used for Cd, Pb, and Ni analyses. ICP multi element stock solutions for each analyte were used for forming the calibration curves. ICP-MS sample flush, read delay and wash time were 45 s, 15 s, 45 s, respectively. ICP-MS operating conditions were 1500 W of RF power, 3925 V RF voltage, and 1.212 L/min of carrier gas (0.95 L/min Ar, 3.474 mL/min He, 0.05 mL/min O_2 gas flow, 20 rpm of nebulizer pump). Hg was analyzed according to EPA 7473 (US EPA, 2007) method. DMA-80 (Milestone) was used for Hg analysis. The analytes were first heated at 200º C for 70 seconds, thereafter kept at 650 ºC for 180 s to evaporate mercury. The analysis of standards for ICP-MS and DMA are listed in Table 3.8.

3.3.4 Statistical methods and data analyses

The data were coded and processed using IBM SPSS (Version 28.0). Data in general are expressed as mean and standard deviation (SD) while median, minimum, maximum, and frequency of detection (DF%) was provided for the results of the analyses of PS and specific pollutants. Student-t test was used to ascertain the significance of differences between mean values of two continuous variables and Mann-Whitney test was used for non-parametric distribution. Chi-square analysis was

Table 3.7: CAS number, mass, retention time, linearity range, LOD, LOQ and correlation coefficient of the PSs analyzed by GC- HR/MS (Birtek et al., 2022).

Compounds	CAS number	Mass (g/mol)	t_R (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
2,3,7,8-T4CDD	1746-01-6	321.8936	29.96	0,000005–0,025	0.005	0.015	0.998
1,2,3,7,8-P5CDD	40321-76-4	355.8546	38.01	0,000005–0,025	0.005	0.015	0.998
1,2,3,4,7,8- H6CDD	39227-28-6	389.8157	45.24	0,000005–0,025	0.005	0.015	0.999
1,2,3,6,7,8-H6CDD	57653-85-7	389.8157	45.40	0,000005–0,025	0.005	0.015	0.998
1,2,3,7,8,9-H6CDD	19408-74-3	389.8157	45.83	0,000005–0,025	0.005	0.015	0.998
1,2,3,4,6,7,8-H7CDD	35822-46-9	423.7766	50.32	0,00001–0,05	0.005	0.015	0.999
1,2,3,4,6,7,8,9-O8CDD	3268-87-9	459.7348	55.20	0,00001–0,05	0.005	0.015	0.999
2,3,7,8-T4CDF	51207-31-9	305.8987	28.16	0,000005–0,025	0.005	0.015	0.998
1,2,3,7,8-P5CDF	57117-41-6	339.8597	35.38	0,000005–0,025	0.005	0.015	0.998
2,3,4,7,8-P5CDF	57117-31-4	339.8597	37.37	0,000005–0,025	0.005	0.015	0.998
1,2,3,4,7,8-H6CDF	70648-26-9	373.8208	43.78	0,000005–0,025	0.005	0.015	0.999
1,2,3,6,7,8-H6CDF	57117-44-9	374.8620	43.99	0,000005–0,025	0.005	0.015	0.998
1,2,3,7,8,9-H6CDF	72918- 21-9	374.8600	46.31	0,000005–0,025	0.005	0.015	0.998
2,3,4,6,7,8-H6CDF	60851-34-5	374.8600	44.96	0,000005–0,025	0.005	0.015	0.999
1,2,3,4,6,7,8-H7CDF	67562-39-4	407.7818	48.70	0,00001–0,05	0.005	0.015	0.998
1,2,3,4,7,8,9-H7CDF	55673-89-7	407.7818	51.06	0,00001–0,05	0.005	0.015	0.998
1,2,3,4,6,7,8,9-O8CDF	39001-02-0	443.7398	55.48	0,00001–0,05	0.005	0.015	0.999
3,3',4,4'-T4CB, PCB 77	32598-13-3	289.9224	19.38	0,000005–0,025	0.01	0.030	0.998
3,4,4',5-T4CB, PCB 81	70362- 50-4	289.9224	18.65	0,000005–0,025	0.01	0.030	0.997
2,3,3',4,4'-P5CB, PCB 105	32598-14-4	325.8804	23.53	0,000005–0,025	0.01	0.030	0.996
2,3,4,4',5-P5CB, PCB 114	74472-37-0	325.8804	22.13	0,000005–0,025	0.01	0.030	0.998
2,3',4,4',5-P5CB, PCB 118	31508-00-6	325.8804	21.22	0,000005–0,025	0.01	0.030	0.999
2',3,4,4',5-P5CB, PCB 123	65510-44-3	325.8804	20.93	0,000005–0,025	0.01	0.030	0.996
3,3',4,4',5-P5CB, PCB 126	57465-28-8	325.8804	26.49	0,000005–0,025	0.01	0.030	0.997
2,3,3',4,4',5-H6CB, PCB 156	38380-08-4	359.8415	29.08	0,000005–0,025	0.01	0.030	0.998
2,3,3',4,4',5'-H6CB, PCB 157	69782-90-7	359.8415	29.36	0,000005–0,025	0.01	0.030	0.998
2,3',4,4',5,5'-H6CB, PCB 167	52663-72-6	359.8415	27.71	0,000005–0,025	0.01	0.030	0.997
3,3',4,4',5,5'-H6CB, PCB 169	32774-16-6	359.8415	31.27	0,000005–0,025	0.01	0.030	0.996
2,3,3',4,4',5,5'-H7CB, PCB 189	39635-31-9	393.8025	33.14	0,000005–0,025	0.01	0.030	0.997

Table 3.8: CAS number, mass, linearity range, LOD, LOQ and correlation coefficient, recovery, and RSD of the PSs analyzed by ICP-MS (Cd, Pb, Hg) and DMA (Hg) (Birtek et al., 2022).

Metals	CAS number	Mass (g/mol)	Linearity µg/L	LOD (µg/L)	LOQ (µg/L)	r^2	Recovery (%)	RSD (%)
Cd	7440-43-9	112.41	0–50	0.01	0.04	10000	99,7	4,10
Pb	7439-92-1	207.2	0–200	0.04	0.20	0,9997	99,5	4,36
Hg	7439-97-6	200.61	0–200	0.07	0.30	0,9995	98,5	3,51
Ni	7440-02-0	58.69	0–200	0.10	0.13	0,9998	96,8	2,27

performed to test for differences in proportions of categorical variables between two or more groups. The validity of the instruments was tested by performing the diagnostic measure of sensitivity and specificity. One-way analysis of variance (ANOVA) was employed for comparison of several group means and to determine the presence of significant differences between group means of continuous variables. Non-parametric statistical method the Kruskal -Wallis one way analysis of variance was performed to confirm the results of ANOVA and t-test. The Pearson's correlation for continuous and the Spearman rank correlation coefficient for non-parametric data was used to evaluate the strength association between the two variables. The level $p < 0.05$ was considered as the cut-off value for significance.

3.3.5 Environmental Risk Assessment

The potential risk posed by individual organic PSs present in wastewater effluents was evaluated by the Risk Quotient (RQ) method. RQs were estimated for each detected organic PS by the equation 3.1 (Aemig et al., 2020a; Anderson et al., 2010a):

$$RQ = \frac{MEC}{PNEC} \tag{3.1}$$

MEC = Measured Environmental Concentration,

PNEC = Predicted No Effect Concentration

MEC concentrations were chosen from the maximum measured concentration. PNEC provides estimates of the risk assessment of individual chemicals. PNEC values were estimated from the least tolerable ecotoxicological concentration through the most sensitive experimented species by applying an assessment factor (EC, 1993b).

From the literature, the experimental acute LC_{50}, EC_{50} and IC_{50} values were collected for each organic chemical for fish, daphnia, and algae and the most sensitive specie

was selected. The PNEC value was calculated using assessment factor of 1000 (Eq. 3.2):

$$PNEC = \frac{EC_{50}\ or\ IC_{50}\ or\ LC_{50}}{1000} \quad (3.2)$$

Chemicals with estimated RQ > 1, are added to the effluent surveillance monitoring list as they pose sufficient risk (Anderson et al., 2010; OECD, 2002).

3.4 Toxicity Test Procedures

The toxicity of wastewaters was conducted to assess the acute toxicity of the influents and effluents of the WWTPs, LTPs as well as of the hospital wastewater by using widely applied screening bioassays consisting of crustacean (*D. magna*), plant (*S. capricornutum*), and bacteria (*V. fischeri*). Each wastewater sample arriving at the laboratory, its physical and chemical parameters were analyzed, and its conformity with relevant ISO protocol standard for wastewaters was ensured. EC_{50} and IC_{50} values were calculated according to the most convenient statistical methods described in each ISO protocol using CETIS program as well as computer softwater programs provided by MicroBioTest Inc. and BIOTOX. Pictures taken before, and during toxicity experiments can be found in Figures I.1 (e)–(y). In addition, pictures taken at the established ecotoxicity laboratory and advanced instrumental analyses laboratory are provided in Figures I.2 (a)–(f).

3.4.1 *Daphnia magna* immobilization acute toxicity testing

The immobilization of *D. magna* test, a crustacean, is widely applied in acute toxicity testing of wastewaters. *D. magna* was obtained from the ephippia of DaphToxKit F (Microbiotest, Belgium) which was in accordance with protocols such as ISO and OECD. The tests started with the preparation of standard freshwater solution by filling a 2,000 mL volumetric flask with pure water. After four different nutrients (firstly vial 1 = $NaHCO_3$, followed by vial 2 = $CaCl_2$, vial 3 = $MgSO_4$, vial 4 = KCl) were added to the flask, the flask was aerated for 15 minutes. This standard freshwater solution was used as hatching medium for the ephippia as well as a dilution agent in the dilution of wastewater samples.

Hatching of the ephippia had to be started three days ahead of the experiment. The vial containing ephippia was completely passed through the microsieve and was rinsed

with water to eradicate impurities due to the storage medium. The ephippia transferred into the hatching petri dish that contained 15 mL of pre-aerated standard freshwater solution. After the hatching, petri dish was covered and incubated under steady illumination of 6,000 lux for 72 h at 20–22 °C (Figure I.1 (h)).

In order to prepare the toxicant dilutions, 100 mL calibrated flasks were labelled for the dilution series of 100%, 50%, 25%, 12.5%, 6.25% down to 0.012 % in highly contaminated wastewater samples (Figures I.1 (f) and (g)). Dilutions were prepared with standard freshwater solution on a basis of 1:1 dilution.

The vial containing spirulina powder was filled with standard freshwater solution and was shaken to be homogenized. Two hours ahead of the starting of the test, neonates were uniformly pre-fed with the algal suspension.

The test plate provided for the test had 4 replicates for each concentration of the wastewater tested as well as for the control, named as A, B, C, and D. Rows were also labeled according to the wastewater dilutions and control. Ten mL of dilution water and 10 mL of the set concentrations of wastewater samples were poured in to each well. After the plate and the petri dish containing pre-fed neonates were placed on the light table and the transfer of 5 active neonates were provided using a micropipette to each test well (counting for 20 neonates for each replicate set) (Figure I.1 (j)). In the next step, the plates were covered tightly with parafilm prior to placing under dark incubation at 20 °C. At the end of 24 h and 48 h, the plate was put on the light table or microscope and the immobilized or dead neonates were reported (Figures I.1 (k)–(o). The estimation of the EC_{50} was performed by calculating percentage immobilization for all concentrations in relation to the number immobilized at 48 h of *D. magna*. A validity test of each experiment was conducted (ISO 6341, 2012; MicroBioTests Inc., 2017).

3.4.2 Algal growth acute toxicity testing

Unicellular green microalgae *S. capricornutum* algal growth inhibition bioassay is widely used in the toxicity testing of wastewaters and it was carried out using AlgalToxKit F (Microbiotest, Belgium). AlgalToxKit F test was in compliance with OECD Guideline 201, and ISO Standard 8692. The validity of the test was checked in each single test. The test was performed using long cell test vials.

The preparation of algal culturing medium was the first step in the algal toxicity testing. The test kit included four nutrient stock solutions (A = NH_4Cl, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, B = $FeCl_3 \cdot 6H_2O$, $Na_2EDTA \cdot 2H_2O$, C = H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnCl_2$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $Na_2MoO_4 \cdot 2H_2O$, D = $NaHCO_3$). Initially, 800 mL of pure water was transferred into a 1,000 mL volumetric flask. Then 10 mL of pure water was added into the nutrient stock solution A, after being mixed well, it was transferred into the 1,000 mL flask. Later, 1 mL of each nutrient stock solutions B, C, and D were also added to the 1,000 mL flask, the volume brought to 1,000 mL with pure water and was aerated for 30 min.

Secondly, to revive the algal beads, the liquid in the tube containing algal beads was poured out and 5 mL of matrix dissolving medium was transferred into it. After the algae was totally dissolved, the tube was centrifuged at 3,000 rpm for 10 minutes. Supernatant was disposed, and replaced by 10 mL of pure water. After shaking this mixture vigorously, the tube was again centrifuged at 3,000 rpm for 10 minutes, the supernatant was discarded. Finally, algae were re-suspended in 10 mL of algal culturing medium. The optical density of the algal suspension was measured at 670 nm with a spectrophotometer. Using the regression formula provided by each AlgalToxkit, the read values of optical density were converted to algal numbers. The algal suspension was transferred into a 25 mL flask and filled to the mark to prepare the algal inoculum. Then the algal culturing medium was transferred into the calibration cell and placed in the spectrophotometer for a zero calibration. When the optical density corresponded to an algal density of $1x10^6$ cell/mL, the standard was ensured, and the preparation of the wastewater dilution series was started. Wastewater samples were vacuum-filtered using 0.45 μm membrane filter. Dilution series of 100%, 50%, 25%, 12.5%, 6.25% down to 0.012 % in highly contaminated wastewaters were labeled and prepared in each flask. One mL of the algal suspension was added to each flask and these dilutions were transferred to test vials (Figure I.1 (r)). Three replicates were prepared for each concentration and control. The set of test tubes were incubated for 72 h in an incubator under 10,000 lux for 72 h at 23 °C (Figures I.1 (s)–(x)). The optical density at 670 nm was measured at 0 h, 24 h, 48 h, and 72 h for each tube and all values were recorded. The optical density values were plotted on a growth curve for all individual test concentrations and control. After the results, the percent inhibition was calculated with equation 3.3.

$$\mu = \frac{\ln(nL) - \ln(n0)}{tL - t0} \quad (3.3)$$

μ = Growth rate (day^{-1})

t0 = the time of the test start

n0 = initial cell density

tL = the time of test termination

nL = the measured cell density at time tL

Later, the percentage inhibition was calculated for each test replicate using the following equation (3.4).

$$I\mu i = \frac{\mu c - \mu i}{\mu c} \; x\, 100 \quad (3.4)$$

$I\mu i$ = Normalized inhibition

μi = Growth rate for the test batch

μc = Growth rate for the control batches.

Next, the normalized inhibition ($I\mu i$) was plotted for each individual test concentration on a logarithmic scale. The most suitable non-linear model was chosen by regression analysis and finally the EC_{50} values were calculated.

3.4.3 Bacteria inhibition of the luminescence toxicity testing

Inhibition of the bioluminescence of the *V. fischeri* toxicity testing is getting more attention in wastewater analyses due to its ease and quick response. The bacteria test was conducted using *V. fischeri* (strain NRRL B-11177, ABOATOX, Finland) test kit. After being filtered through 0.45 μm membrane filter, wastewater samples were transferred into a 250 mL beaker Figure (I.1 (i)). The pH, of 6.5–8, salinity of 3.5%, temperature of 15–24 °C, dissolved oxygen higher than 3 mg/L of the wastewater was ensured. The reviving solution was taken out of the deep freezer and was dissolved using the thermostatically controlled thermo-block that was set to 4 °C. Twelve mL of this solution was transferred onto *V. fischeri* reagent. After mixing the bacteria, this mixture was placed in a bath system and set at 4 °C for 30 min. After the bacteria was activated, the thermostatically controlled thermo-block was set to 15 °C for 30 min. Prior to testing, the conductivity of the samples was adjusted with NaCl to 50 mS/cm. In order to prepare the toxicant dilutions, thermostatically controlled thermo-block was set for 9 serial duplicate readings. Each tube was labeled for the dilution series of 50%,

25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78% down to 0.012 % in highly contaminated wastewater samples (Figure I.1 (y)). Samples were kept in a bath system at 15 °C for 30 min. After each test, the tube was placed in luminometer and the luminescence of the *V. fischeri* was measured at 0 and 30 min. For the evaluation of the data, the correction factor was calculated using equation 3.5.

$$fkt = \frac{Ikt}{Ip} \tag{3.5}$$

fkt = Correction factor

Ikt = Luminescence intensity in the control sample after contact times in luminescence units

Ip = The maximum luminescence intensity of the control test suspension

$$Ict = Ip.\, fkct \tag{3.6}$$

Ict = The corrected peak intensity value

Ip = The maximum luminescence intensity of the control test suspension

fkct = The mean of *fkt*

The corrected peak intensity value (equation 3.6) was used to calculate the inhibitory effect (*Ht*) (equation 3.7) of the wastewater sample after the contact time.

$$Ht = \frac{(Ict - It)}{Ict}\ x\ 100 \tag{3.7}$$

Ict = The corrected peak intensity value,

It = The luminescence intensity of the test sample after contact time.

The gamma value was calculated to evaluate the interaction between concentration and effect (equation 3. 8 and 3.9).

$$\Gamma t = \frac{Htc}{(100 - Htc)} \tag{3.8}$$

Γt = Gamma value

Htc = Mean values of the *Ht*

$$lgct = b\ lg\Gamma t + lga \tag{3.9}$$

ct = % Portion of the water sample

b = Value of the slope of the line

lga = Value of the intercept of the line

Finally *ct* = EC_{50} values were calculated by calculating concentration-effect relationship for each exposure time using linear or non-linear regression analyses (ISO 21338, 2010).

In DaphToxKit, AlgalToxKit, and *V. fischeri* toxicity test, quality control was performed regularly using potassium dichromate.

3.5 Physicochemical Analyses

Physicochemical parameters and metals analyses of all influent and effluent samples of WWTP, LTP and HWW were conducted at the Central Wastewater Laboratories of ISKI (internationally accredited laboratories in line with ISO/IES 17025). The analytical methods used for trace elements and physicochemical analyses are presented in Table 3.9 and Table 3.10, respectively.

Table 3.9: Methods used in the analysis of trace elements.

Trace elements	Analytical Methods	
Cd, Cr, Cu, Pb, Ni, Zn	Standard Methods for the Examination of Water and Wastewater 22nd Edition, 3120 B, 3030 E-K:2005	ICP-OES ICP-MS

Table 3.10 : Methods used for each physicochemical analysis conducted.

Parameters	Methods	
BOD		5210 B
Chemical oxygen demand		5220 B
Total Kjeldahl Nitrogen		4500-N_{org} B
Biochemical oxygen demand		5210 B
Total Phosphorus		4500 P-I
Suspended Solids		2540 D
Oil and Grease		5520 D
Ammonia Nitrogen		4500-NH_3 D
Sulphate		4500-SO_4^{2-} D
Total Cyanide	Standard Methods for the Examination of Water and Wastewater, 22nd Edition	4500 - CN
Total Sulfur		4500-S2-E
Nitrogen (ammonia)		4500-NH3 – G
Total organic carbon		5310-B
Color measurement		2120 C
Chloride		4500 Cl^- B
Electrical conductivity		2510 B
Salinity		2520 B
Dissolved oxygen		4500 –O B
pH value		4500-H+B

4. RESULTS AND DISCUSSION

This chapter presents the findings and their discussion. Initially the results for the occurrence of PSs and specific pollutants are presented for WWTP 1–WWTP 7, LTP, and hospital wastewater. Additional graphic was provided for the detection frequencies (n =16) in equal numbers of the concurrent WWTP influents and effluents. They are followed, by the EC_{50} values defining the acute toxicity of the wastewaters for *D. magna*, *S. capricornutum*, and *V. fischeri*. In presenting the results of the occurrence of priority pollutants, specific pollutants, and acute toxicity analyses, in order to avoid any omission of data, the effluents of the three mechanical treatment facilities (WWTP 5–WWTP 7) were considered as influents as they were taken from the same spots within the studied WWTP influents (WWTP 1–WWTP 4). In addition, in order to analyze the data properly, the consecutive summer (n = 9) and winter (n = 9) days are presented in equal numbers of the concurrent WWTP influents and effluents. Finally, the results for the physicochemical analyses are presented.

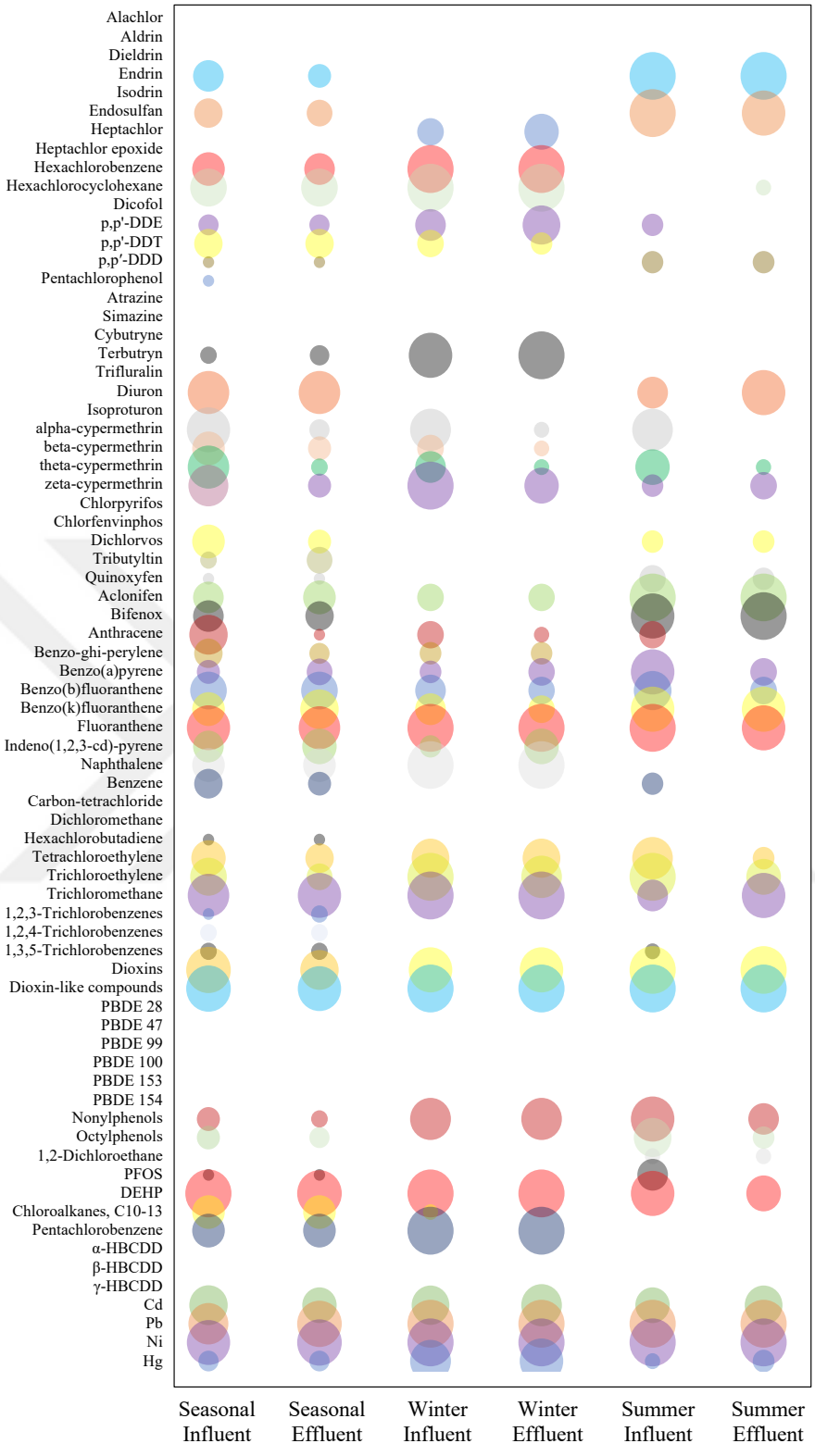
4.1 The Occurrence of Priority Substances and Specific Pollutants

Results indicated the occurrence of 48 PSs out of 73 PSs, i.e. 25 PSs were not detected in wastewaters. The detection frequency of the PSs in the influents and effluents of advanced treatment (n =16) is shown in Figure 4.1. The analyses results of PSs in influents and effluents of WWTPs are presented in Tables D.1 to D.4 In addition, the figures are provided for consecutive summer (n = 3) and winter (n = 3) days for WWTP influents and effluents through Figures 4.2.–4.8. The specific micropollutants detected in influents and effluents of WWTPs are shown in Tables E.1 to E.3 Considering the number of PSs that have been discontinued for a long time, their absence was no surprise. The presence of the detected pesticides in the urban areas was attributed to nonagricultural sources such as homes, parks, yards, recreational areas, gardens, woodlands, etc. as well as to runoff from agricultural areas (M. Meftaul et al., 2020). The results for each group are given separately.

4.1.1 Pesticides

Twenty pesticides were present in at least one sampling season in all the wastewaters analyzed in WWTPs. Thirteen of the pesticides were never detected in any sampling season. The concentrations and frequencies of the pesticides among PSs are presented through Tables D.1 to D.3. The presence of pesticides in the influents and effluents of the summer and winter consecutive days are presented in Figure 4.2. Aclonifen and cypermethrin were the most prominent pesticides detected in all sampling seasons. p,p'-DDT, HC and HCH were mostly present in winter and fall seasons. The detection of diuron, dichlorvos, endrin, and bifenox were significant during summer periods. The year-round use of pyrethroids were apparent. Their use was observed in fall, winter, summer seasons in addition to spring season for alpha-cypermethrin and zeta-cypermethrin. The wide use of cypermethrins is attributed to its high efficacy and lower levels of toxicity of cypermethrin as well as its replacement for the organophosphate and carbamate insecticides. The total maximum concentrations of pyrethroid insecticides were observed in winter season and were 89 ng/L and 61 ng/L for influents and effluents, respectively. The decreased effluent concentrations of pyrethroids were explained by their removal during the biological treatment process or their partitioning onto the sludge. Concentrations of cypermethrin and their removal rates in advance treatment were within similar ranges reported in the literature. Cypermethrins were also detected in leachate influent, effluent and hospital wastewaters and their concentrations were 6.7 ng/L, 3.12 ng/L and 35.5 ng/L, respectively. (Firouzsalari et al., 2019; Tang et al., 2018).

Diuron, an urea herbicide, was detected frequently (80%) in all seasons excluding winter. The highest influent and effluent concentrations were observed in summer and were 644 ng/L and 724 ng/L, respectively. The influent and effluent concentrations were found to be in line with previous studies. Being persistent and a carcinogen, the removal of diuron was reported to be incomplete in WWTP process in various studies (Guillossou et al., 2019; Økland et al., 2005b; Rodriguez-Mozaz et al., 2015). Some of the higher effluent concentrations of pesticides could be attributed to emerging metabolites, transformation of the products during the process in WWTP, desorption and hydrolysis from particulate matter, as well as problems related to sampling and sample preservation (Köck-Schulmeyer et al., 2013a). Leachate influent and effluent



Seasonal = The analyses conducted in four seasons, n = 16 for each WWTP influent and effluent.

Winter = Analyses conducted in consecutive days in winter, n = 9 for each WWTP influent and effluent.

Summer = Analyses conducted in consecutive days in summer, n = 9 for each WWTP influent and effluent.

Figure 4.1 : Detection frequencies of each PS in seasonal, winter and summer influents and effluents of WWTP 1–WWTP 4. (The diameters of the bubbles indicate the magnitude of frequency of detection) (Birtek et al., 2022).

concentrations of diuron were comparatively low at 51 ng/L and < LOQ, respectively (Guillossou et al., 2019; Köck-Schulmeyer et al., 2013b; Margot et al., 2013; Rodriguez-Mozaz et al., 2015).

Aclonifen was detected in all seasons, the highest detection frequencies were found in summer (95%) while the influent concentration was highest (109 ng/L) in winter and effluent was < LOQ. Aclonifen is a registered (GTHB, 2017) diphenyl ether pesticide therefore its presence was no surprise. Looking at the octanol/water partition coefficient (log Kow = 4.3), aclonifen would be expected to be adsorbed onto the active sludge, however it was not always the case (ECHA, 2018; PubChem-Aclonifen, 2020). The effluent concentrations of aclonifen in this study were lower than those reported in the literature (Aemig et al., 2020a). Aclonifen was also detected in LTP influent, effluent and hospital effluent and the concentrations were 53 ng/L, 57, and < LOQ, respectively.

HCH was one of the most detected (100%) pesticides during spring and winter seasons, where the detected maximum concentrations for influents and effluents were 21.2 ng/L and 4.9 ng/L, respectively. The agricultural use of HCH has been banned extensively since the 1980s (Y. Zhang et al., 2018). The use of HCH was prohibited in agriculture in 1985 in Türkiye. However being one of the HCH producing countries, its production was discontinued in 2017 (CSB&NIRAS, 2015b; Vijgen et al., 2011). Being an organochlorine pesticide, the detection of HCH was related to both its persistent character as well as to its transportation through run off from sediments after heavy rainfall events, especially during rainy seasons (Mishra et al., 2013; Y. Zhang et al., 2018). HCB was observed in spring and winter samples, where the detection frequency was 100% in winter and the maximum influent and effluent concentrations observed were 0.5 ng/L and 0.6 ng/L, respectively. There is a probability that the presence of HCB in winter and spring seasons may be attributed to its use in those seasons. HCH and HCB would be expected to be removed to some extent in biological treatment due to their log Kow values (3.72–4.14 and 5.73) as well as Henry's law constants ($5.14x10^{-6}$ and, $5.8x10^{-4}$ (atm-m^3)/mole)) and, log Kow values (3.72–4.14 and 5.73), respectively. The higher effluent concentrations of HCH and HCB were related to the matrix interferences that may take place during pre-sample extractions. The rates of the concentrations of HCH and HCB in effluents of WWTPs were in line with those reported in the literature (Hennebel et al., 2011; Katsoyiannis & Samara, 2005; Martí et al., 2011a). In line with its persistent character, HCH and HCB

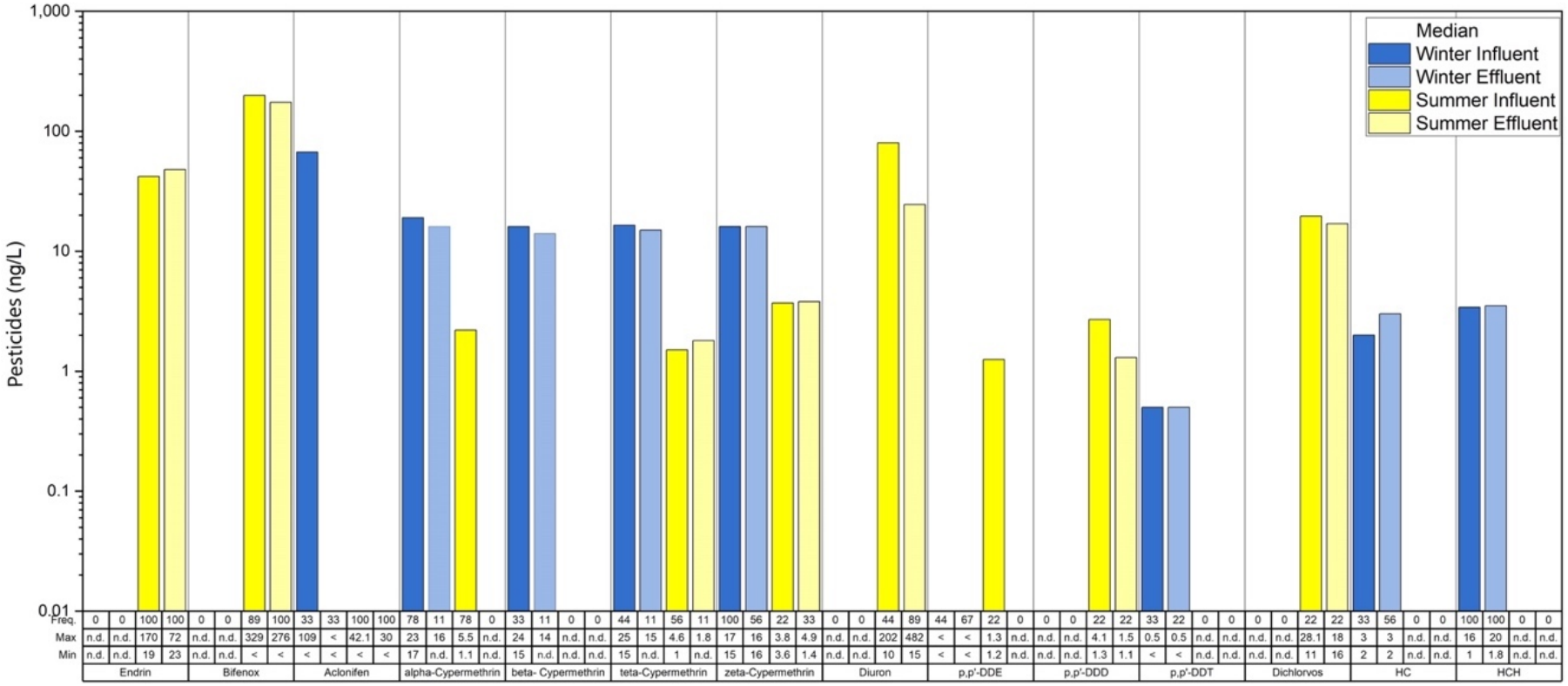


Figure 4.2 : Occurrences of pesticides among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ for each PSs. The vertical scale shows the median pesticide concentrations (ng/L, log scale). The detection frequencies, maximum and minimum concentrations of each pesticide are shown below each bar (Birtek et al., 2022).

(< LOQ) were detected in the LTP influent, effluent and hospital effluent 1.8 ng/L, 1.8 ng/L and 6.5 ng/L, respectively (PubChem-Hexachlorobenzene, 2020; PubChem-Lindane, 2020).

Dichlorvos was detected in fall and summer seasons with a detection frequency of 36%, where the highest influent and effluent concentrations were 84 ng/L and 40 ng/L in fall season, respectively. Dichlorvos was banned together with other organophosphate pesticides in Türkiye in 2011 (TOB, 2020), therefore its detection in wastewaters was unforeseen. Indeed, chlorfenvinphos and chlorpyrifos were not present in any of the samples. The use of diuron was reported to be widespread due to its reduced levels of toxicity in pest control at homes, gardens, yards, turfs (Gaonkar et al., 2019). In the presence of dissolved molecular oxygen, dichlorvos was reported to go through photodegradation; however this process is known to be suppressed by the rich organic matter, which is the case in wastewater (Bustos et al., 2019; Dimou et al., 2004). Considering its short half-life, the existence of dichlorvos in wastewaters is attributed to its unlawful use (Sahin and Karpuzcu 2020). The levels of dichlorvos in influents were found to be in line with the literature, while the effluent levels were somewhat higher (Stamatis et al., 2010). Dichlorvos was also detected in the LTP influent 47.5 ng/L, effluent (< LOQ) and hospital effluent (< LOQ).

Endrin was detected in the fall and summer seasons, where the highest influent and effluent concentrations were 170 ng/L and 123 ng/L, respectively. The detection frequency was highest during summer (95%). The removal of endrin (log Kow = 5.2) (ECHA, 2018; PubChem-Endrin, 2020) would be expected to be limited to the adsorption on the active sludge in advance treatment. However, the removal rates were inconsistent, which was in line with the findings in the literature. Being the only detected cyclodiene pesticide, its presence was attributed to its seasonal consumption. The endrin concentrations were higher than the values stated in other studies. The concentrations of endrin in LTP influent, effluent, and hospital effluent were 1.0 µg/L, 212 ng/L and 44 ng/L, respectively (Aemig et al., 2020b; Katsoyiannis & Samara, 2005; Martí et al., 2011a).

Endosulfan and bifenox were detected in fall and summer seasons with detection frequencies of 95% and 90%, respectively. The highest concentrations of endosulfan in the influents and effluents were 52.0 ng/L and 18.0 ng/L, respectively. The highest concentrations of bifenox observed in the influents and effluents were 979 ng/L and 381 ng/L, respectively. Bifenox is a diphenyl ether class herbicide and its production

is restricted (Devault & Karolak, 2020). The presence of endosulfan in wastewater samples was surprising due to its use being phased out in 2010 (MAF, 2015). Being an organochlorine biocide, its presence was related to its illegal agricultural use or to its persistent behaviour (Bacci & Campo, 2022; Økland et al., 2005a). The influent and effluent endosulfan concentrations were in line with those previously reported literature. Endosulfan and bifenox were also detected in LTP and hospital effluent (< LOQ). The concentrations of influent and effluent of LTP were 172 ng/L–43 ng/L, and 51 ng/L–1.4 µg/L , respectively (Aemig et al., 2020a; Bueno et al., 2012; Katsoyiannis & Samara, 2005; Martí et al., 2011a).

Terbutryn was the only triazine herbicide observed in winter, spring and summer seasons with the highest detection frequency of 83% in winter. Most of the reported concentrations of terbutryn were lower than their detection limits. Since the use of the terbutryn was terminated by the Ministry in 2011 (MAF, 2020), its presence could be explained by its desorption from sediments due to high flow rates in rainy seasons (Chefetz et al., 2004). Indeed, the use of atrazine and simazine were terminated in 2011 and 2010, respectively and both were never detected. Terbutryn was also detected in influent and effluent of LTP, however found values were under quantification limits.

The detection frequency of the very effective organotin biocide tributyltin was only 11%, and all of its reported values were under the quantification limit. With its lipophilic nature, the removal of tributyltin is attributed mainly to adsorption onto primary sludge and active sludge. Also, the small amount of tributyltin reported to be in a soluble phase is due to the high solid concentration in sewage and may be the reason for its presence under quantification limit (Scrimshaw et al., 2013). Being widely applied as an anti-fouling agent on ships and boats between 1960s and 1990s, its cessation provided significant reduction in its presence in coastal waters (Dipper, 2022). Being an endocrine disrupting compound, tributyltin was reported to be present in coastal waters of Türkiye (Belzunce et al., 2004). It is also known to be present in WWTP effluents due to its use as a plasticizer, in textile processing, food packaging, household products, wood preservative, and agrochemicals (Scrimshaw et al., 2013). A recently published regulation prohibits the export and import of tributyltin to be used in paint protection and limits its use as an industrial chemical, which seems to be effective in reducing tributyltin concentrations in wastewaters (MEUC, 2023).

Quinoxyfen was only present in the summer sampling campaign with a detection frequency of 32%. The only measured concentrations (> LOQ) in the influents and effluents were 280 ng/L and 380 ng/L, respectively. Quinoxyfen is currently an active ingredient in registered fungicides (MAF, 2020). Having a log Kow value of 4.66, quinoxyfen would be expected to be removed to some extent. However, due to the limited data were above the quantification limits , their evaluation could not be completed (CIRCABC, 2020).

DDTs were observed in all seasons where the highest detection frequency of 58% was observed in winter season. p,p'-DDT, p,p'-DDE, or p,p′-DDD were present at least once in a sampling period. p,p′-DDD was present in all samples of each season except winter. p,p'-DDE was present in all seasons, while p,p'-DDT was present only in fall and winter seasons. The total maximum influent and effluent concentrations were 9.0 ng/L and 6.1 ng/L, respectively. Studies conducted on the Black Sea and Sea of Marmara confirmed the occurrence of DDTs in sediments and in fish for decades. Discontinued in 1985, the prevailing detection of DDT in wastewaters was attributed to its persistent character (Cakirogullari & Secer, 2011; Coelhan et al., 2006; Fillmann et al., 2002). The extensive persistence of DDTs, albeit in low concentrations, raises concern. The persistent character of DDT causes its presence in LTP influents, effluents, and hospital effluents, where the total DDT concentrations were 11 ng/L, 6.5 ng/L, and 6 ng/L, respectively.

4.1.2 Polycyclic aromatic hydrocarbons (PAHs)

The concentrations and frequencies of the PAHs among PSs in WWTP 1–WWTP 7 are presented in Tables D.1 to D.3 The occurrence of PAHs in influents and effluents of winter and summer consecutive samples are given in Figure 4.3. PAHs were observed in all seasons, where the highest concentrations were in spring, while lowest one was observed in summer season. The total maximum highest influent and effluent PAHs concentrations were 1.22 μg/L and 0.048 μg/L, respectively. The incomplete combustion of fossil fuels is known to be the sources of PAHs in the environment. WWTP effluents are reported to be one of the sources of PAHs entering receiving water bodies.

The highest detection frequency was observed in fluoranthene per season. On the other hand, naphthalene constituted more than 60 to 97% of the total PAH concentrations in

winter and spring seasons. This was explained with the high aqueous solubility of naphthalene as compared to the rest of the PAHs (Urana et al., 2020). Being the most volatile of PAHs, the environment is exposed to naphthalene after an incomplete combustion originating from motor vehicles, heaters with fossil fuels, gasoline in both residential and industrial zones, forest fires, etc., (Gauthier et al., 2014; Preuss et al., 2003). Anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, fluoranthene, indeno (1,2,3-cd)-pyrene, and benzo-ghi-perylene were observed in all seasons while benzo(a)pyrene was present in spring, summer and winter seasons. The total concentration ranges of PAHs and their removal rates in WWTPs were in line with the stated values in the literature (Nas et al., 2020; Ozaki et al., 2015). Almost all PAHs were detected in LTP influents, effluents, and hospital effluents and their total concentrations were 0.34 µg/L, 0.02 µg/L, and 0.04 µg/L, respectively.

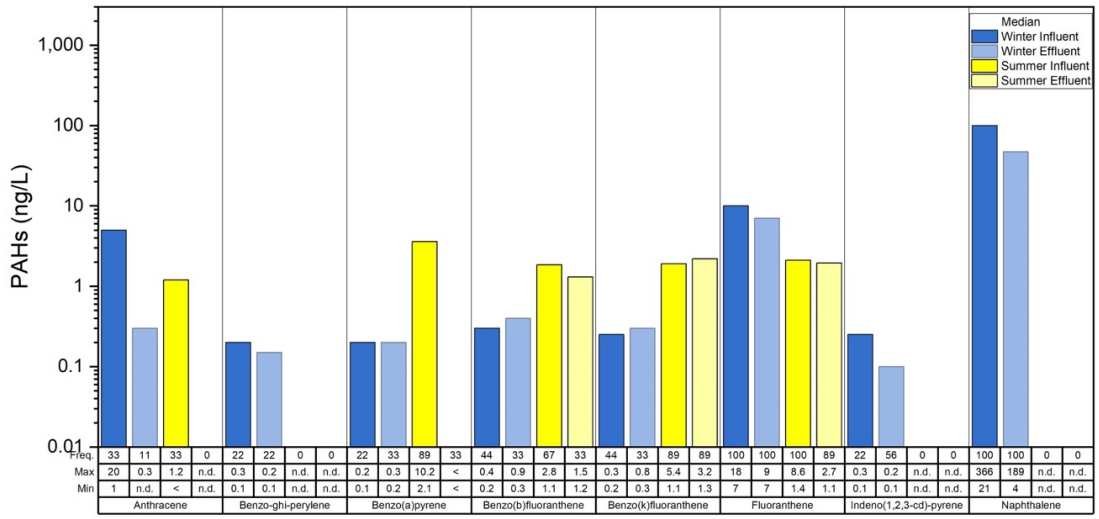


Figure 4.3: Occurrences of PAHs among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median PAH concentrations (ng/L, log scale). The detection frequencies, maximum and minimum concentrations of each PAH are shown below each bar (Birtek et al., 2022).

PAHs tend to accumulate both in tissues of the living organisms and sediments due to their high Kow values and hydrophobic character (Karacik et al., 2009). Previously the occurrence of PAHs were reported in the Bosphorus Strait of Istanbul in both mussels and sediments (Hanedar et al., 2011; Karacik et al., 2009; Taşkin et al., 2011a). Thus, PAHs contaminated sediments can undergo desorption and return to the water phase (W. Wang et al., 2021). Since WWTP effluents are known to be one of

the point sources, monitoring of PAHs content of effluents is essential. Indeed, this study revealed the occurrence of eight PAHs in wastewaters that were being discharged to the receiving seas of Istanbul. In this study, the active sludge treatment process was found to remove PAHs to some extent. This raises the question regarding the PAHs content of WWTP sludges and their fate in treatment; further studies are needed. Since today only 41% of wastewaters of Istanbul are subjected to biological treatment, it is important to increase this percentage to reduce the accumulation of PAHs in the seas of Istanbul.

4.1.3 Volatile organic compounds (VOCs)

The concentrations and frequencies of the VOCs among PSs in WWTP 1–WWTP 7 are presented in Table D.1 to Table D.3. The concentrations of VOCs in influents and effluents of winter and summer consecutive samples are given in Figure 4.4. Of all the studied PSs, VOCs were present in at least one sampling period, with the exception of carbon-tetrachloride and dichloromethane. In general, the detected VOCs were in µg/L

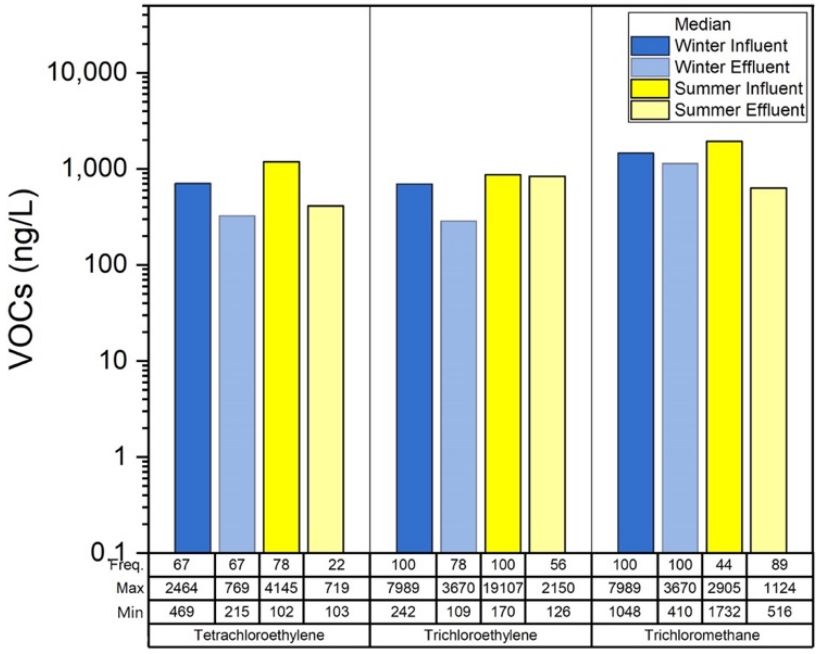


Figure 4.4: Occurrences of VOCs among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median VOC concentrations (ng/L, log scale). The detection frequencies, maximum and minimum concentrations of each VOC are shown below each bar (Birtek et al., 2022).

levels. Tetrachloroethylene, trichloroethylene, and trichloromethane were the most frequently observed VOCs in all seasons where the highest detection frequencies varied from 56% to 100%. Among those, trichloroethylene showed the highest concentration in summer and the influent and effluent concentrations were 19.1 µg/L and 2.1 µg/L, respectively. The influent and effluent concentrations of tetrachloroethylene were also observed to be the highest in summer season and were 4.1 µg/L and 2.3 µg/L, respectively. The highest concentration of trichloromethane was observed in winter at 7.9 µg/L and 3.6 µg/L for influent and effluent, respectively.

The occurrence rates of trichloroethylene are related to its wide application in industries such as textile, metal, electroplating, and automobile etc. (Kocamemi & Çeçen, 2010). Occurrence of tetrachloroethylene was associated with its utilization as a degreasing solvent in automobile cleaners, dry cleaning, textile processing, and metal degreasing (Bari & Kindzierski, 2018; Huang et al., 2014). The occurrence of trichloromethane (chloroform) is related to its use in production of some chemicals in industry, as well as emerging from the chlorination process of water treatment (Gasperi et al., 2008; Rodríguez et al., 2005). The occurrence of tetrachloroethylene, trichloroethylene, and trichloromethane in wastewaters in this study clearly reveal their use in industry. Trichloroethylene was observed in LTP influent at 0.3 µg/L while trichloromethane occurred in LTP influent, effluent and hospital effluent at 0.5 µg/L, 0.3 µg/L, and 5 µg/L, respectively.

Benzene was observed in all seasons with the highest detection frequency of 36% and the highest influent and effluent concentrations of 0.63 µg/L and 0.54 µg/L, respectively. Benzene was also observed in LTP influent and effluent, and the concentrations were 0.78 µg/L and 0.43 µg/L, respectively. The detection of trichlorobenzenes was rare in the studied samples. 1,2,3-Trichlorobenzene was only observed once in spring season and the influent and effluent concentrations were 7.1 µg/L and 2.84 µg/L, respectively. 1,2,4-Trichlorobenzene was observed in spring and fall seasons with the highest influent and effluent concentrations of 3.6 µg/L and 1.4 µg/L, respectively. 1,3,5-Trichlorobenzene was detected in the spring and summer seasons with the highest influent and effluent concentrations of 4.2 µg/L and 2.3 µg/L, respectively. The removal of trichlorobenzenes was related to the adsorption on solids considering their low biodegradation potentials and hydrophobic tendency (log Kow 3.9) (Cai et al., 2007). Being among the persistent organic pollutants (POPs) listed in

the Stockholm Convention Annex C (unintentional production), hexachlorobutadiene was observed only once in spring season with influent and effluent concentrations of 2.0 µg/L and 0.58 µg/L, respectively. The rare detection of hexachlorobutadiene was related to the success in the reduction of its use (Kajiwara et al., 2019). The removal of VOCs may be due to their emission into the atmosphere or degradation. Having high Henry's Law constants, VOCs are likely to be stripped from the wastewater (Atasoy et al., 2004; Bari & Kindzierski, 2018). The influent and effluent concentrations of VOCs in this study were in line with previous values published in the literature (Atasoy et al., 2004; Martí et al., 2011b). Their occurrence in the atmosphere have been reported (Bari & Kindzierski, 2018). Emanation of VOCs from WWTPs is a concern.

4.1.3.1 Specific micropollutants

The presence of VOCs may pose risk to human health. Since, VOCs are widely present in municipal and industrial wastewaters, the specific micropollutants were chosen from among the most detected VOCs in screened wastewaters. In addition to the previously presented VOCs included in the PSs, the results for the 11 extra specific VOCs in influents and effluents of WWTPs are shown in Tables E.1 to E.3 Indeed, all the analyzed specific VOCs were detected at least in one season. Dichlorobenzene, toluene, trimethylbenzene, and xylene had the highest detection frequency. Toluene showed the highest concentration where the maximum influent and effluent concentrations were 80 µg/L and 2.5 µg/L, respectively. Being a minor component (5%) of gasoline, its presence was related to its wide use as a solvent for glues and paints, as well as being used in the production of benzene and some other compounds. Toluene was also present in landfill leachate influent and effluent and hospital wastewater with the highest concentrations of 70 µg/L, 5 µg/L and 4 µg/L, respectively. The wide use of toluene as well as its production (PETKIM, 2010) in Türkiye explains its wide occurrence in wastewaters (Clough, 2014; Jankovic et al., 2022). The dichlorobenzenes, 1,3-dichlorobenzene and 1,4-dichlorobenzene were detected in all seasons, with the highest amounts observed in winter and the highest concentrations of 2.8 µg/L – 0.21 µg/L for influents and 2.6 µg/L – 0.20 µg/L for effllluents. 1,3-Dichlorobenzene is used in the production of dyes, pesticides and medicines, whereas, 1,4-dichlorobenzene has extensive use in the production of mothballs, deodorant blocks, and in odor control in facilities, restrooms and garbage

cans. The 1,4-dichlorobenzene contamination in surface waters is related to its presence in wastewaters, in landfill leachate, industrial discharges, as well as atmospheric deposition. Indeed, it was also present in the landfill leachate and hospital wastewater with the concentrations of 0.26 µg/L and 2.9 µg/L, respectively.

The occurrence of 1,3-dichlorobenzene in wastewaters is associated with its application in solvents in industry as well as in pesticide manufacturing (Albarrán & Mendoza, 2020). The majority of the dichlorobenzenes are released to the atmosphere through the processes of vaporization, sorption, bioaccumulation and biodegradation, processes that are also known to eventually take place in the environment (ATSDR, 2006). 1,3-Dichlorobenzene is also known to be produced during the anaerobic degradation of gamma-hexachlorocyclohexane (Vasanthy et al., 2022). Xylenes were present in each season with the highest influent and effluent concentrations of 127 µg/L and 1 µg/L and the values for ethylbenzene were 29 µg/L and n.d., respectively. Being a widely used solvent, xylene is commonly present in insecticides, paint thinners, ink, varnish and degreasers (Mirkin, 2007). It was also present in landfill leachate influent and effluent and hospital wastewater with comparatively low concentrations of 0.5 µg/L, 3 µg/L and 0.3 µg/L, respectively. Ethylbenzene was also present in leachate influent and effluent and hospital wastewaters at 1.6 µg/L, 0.2 µg/L and 0.1 µg/L, respectively. 1,2,4-Trimethylbenzene is known to emanate from automobiles along with toluene and ethylene (Liu et al., 2004). 1,2,4-Trimethylbenzene was present in all seasons with maximum influent and effluent concentrations of 13 µg/L and 0.2 µg/L, respectively and 1,3,5-trimethylbenzene was present only in influent at 4.1 µg/L. 1,2,4-Trimethylbenzene was present in leachate influent and effluent at 0.6 µg/L and 0.3 µg/L, while 1,3,5-trimethylbenzene was only present in influent at 6 µg/L. Both have a use in paints and lacquer thinner, solvent etc. Therefore, their presence in wastewaters was no surprise. They are easily released to the air, therefore exposure through breathing is possible (D.H.S.S., 2013, 2015). Measured VOCs in wastewaters were in line or higher than the values reported in the literature (Liu et al., 2004). Majority of aforementioned VOCs were measured in various studies conducted in air samples taken from WWTPs, therefore studies conducting monitoring of such VOCs are recommended to estimate their emission in WWTPs (Liu et al., 2004; Pitiriciu & Tansel, 2021).

4.1.4 Alkylphenols

The concentrations and detection frequencies of the nonylphenols (NP) and octylphenols (OP) among PSs in WWTP 1–WWTP 7 are presented in Tables D.1 to D.3. The occurrence of NPs and OPs among PSs in influents and effluents of winter and summer consecutive samples are given in Figure. 4.5. NP was observed in 79% of the samples in winter season where the maximum influent and effluent concentrations were 26 ng/L and 25 ng/L; while the observance of OP was highest in summer season with a detection frequency of 63%. The maximum influent and effluent concentrations were recorded in winter season at 205 ng/L 22 ng/L, respectively. NP and OP are highly used in the production of textiles and leather, plastics, pesticides, washing agents, personal care products, inks, and paints etc., therefore their presence in wastewaters were expected in each season. However, OP was not present in winter and spring seasons which was related to its dilution due to the rainy season. NPs were also reported to be formed during the treatment process due to a precursor compound (Loos et al., 2013). Both of metabolites of NP and OP are also reported to show more endocrine disrupting activity as well as toxicity (Berardi et al., 2019; Priac et al., 2017).

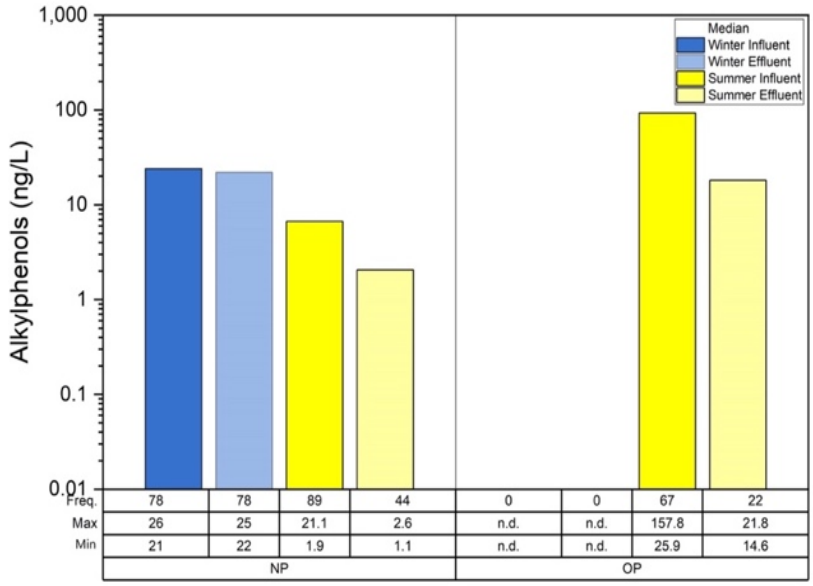


Figure 4.5: Occurrences of of alkylphenols among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median alkylphenols concentrations (ng/L, log scale). The detection frequencies, maximum and minimum concentrations of each alkylphenol are shown below each bar (Birtek et al., 2022).

Urban, industrial wastewater as well as agricultural run-off are alkylphenol sources to the receiving water environments. NP was also present in the influents and effluents of LTP with concentrations of 7 ng/L and 7 ng/L, respectively. OP was detected in the influents and effluents of LTP and hospital effluent with concentrations of 17 ng/L, 8 ng/L, and < LOQ, respectively. Having endocrine disruptive properties, presence of alkylphenols in water environments carries risks for the human and environmental health and their monitoring and treatment in WWTPs are important. (Kumari et al., 2023). The influent and effluent NP and OP concentrations and removal rates in WWTP were in line with those reported in the literature (Luo et al., 2014; Mailler et al., 2015).

4.1.5 Dioxins and dioxin-like compounds

The concentrations and frequencies of the DLCs among PSs in WWTP 1–WWTP 7 are presented in Tables D.1 to D.3. The occurrence of DLCs among PSs in influents and effluents of WWTPs in winter and summer consecutive samples are given in Figure 4.6. Dioxins and dioxin-like compounds were observed in all seasons and their maximum influent and effluent concentrations were observed in summer season at 0.42 ng/L and 0.097 ng/L for dioxins and at 2.49 ng/L and 1.39 ng/L for dioxin-like compounds, respectively. DLCs were present in all seasons with 100% detection frequency in summer season, however the majority of them were detected under LOQ. Being among the POPs whose production has been prohibited, the presence of DLCs were attributed to its persistent character (Mulvaney et al., 2017). Having a log Kow value of 6.8 and higher, DLCs are likely to accumulate on active sludge. The presence of DLCs on LTP influent, effluent, and hospital effluent were also related to its persistent character. The DLCs were above LOQ in LTP influent and effluent with concentration of 0.1 ng/L and 0.07 ng/L. The concentrations of DLCs measured in this study were lower than the values reported in the literature that covered the years (Bolzonella et al., 2010). DLCs were also detected in various industrial wastewaters in last the decade in Türkiye (Gursoy-Haksevenler et al., 2022). Since WWTP effluents are one of the routes that DLCs enter water environments, their discharge is critical (McLachlan et al., 1996).

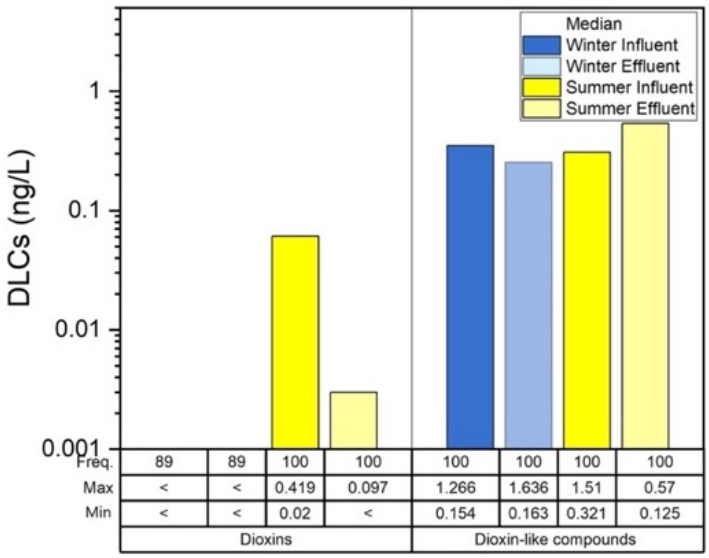


Figure 4.6 : Occurrences of DLCs among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median DLCs concentrations (ng/L, log scale). The detection frequencies, maximum and minimum concentrations of DLCs are shown below each bar (Birtek et al., 2022).

4.1.6 Other organic chemicals

The concentrations and frequencies of the other organic chemicals among PSs in WWTP 1–WWTP 7 are presented in Tables D.1 to D.3. The occurrence of other organic chemicals among PSs in influents and effluents of WWTPs in winter and summer consecutive samples are given in Figure 4.7. DEHP was one of the most detected PSs (100%) with the maximum WWTP influent and effluent concentrations of 2.3 µg/L and 0.31 µg/L, respectively.

The widespread occurrence of DEHP in the studied wastewaters is related to its leaching from PVC plumbing, drain pipes, wall coverings, packaging, flooring, perfumes, cosmetics, glues, paper coatings, paints, lacquers, inks, synthetic leather, textiles, car coatings, cables, toys, and plastic food containers. Leaching of DEHP is also expected from newly constructed sites in Istanbul and through plastic bottled water where consumption is excessive (Donner et al., 2010; Marttinen et al., 2003; Qin et al., 2021; Rule et al., 2006). Having a high log Kow value of 7.73, the DEHP in the wastewaters is expected to be adsorbed on sediments and sludges (Lee et al., 2019). Indeed, it was the case except in winter season. A study conducted on the sewage sludges in WWTPs, DEHP concentrations ranged from 18 to 490 mg/kg, revealing the presence of highest concentrations of DEHP in fall season in Istanbul, which was in

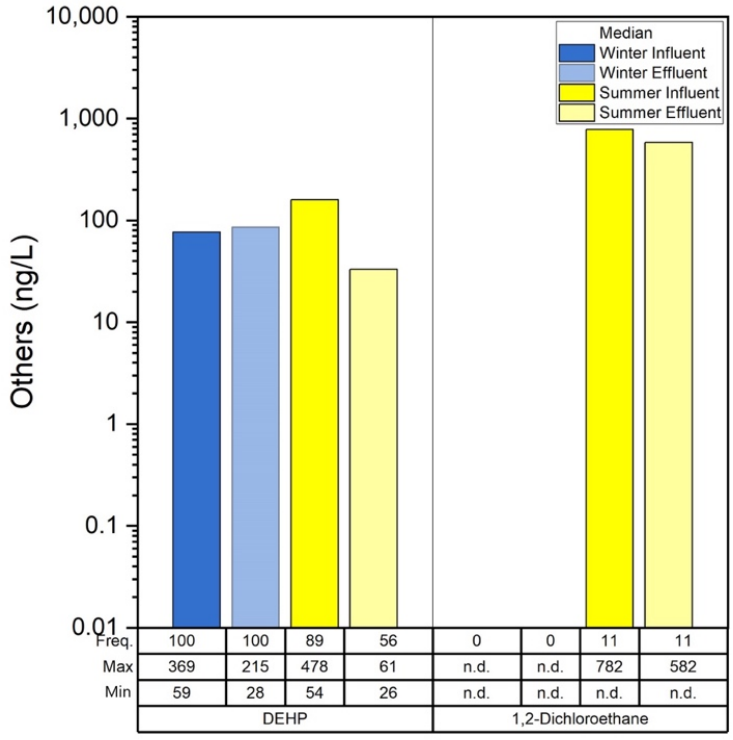


Figure 4.7: Occurrences of other chemicals among PSs in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median concentrations of other organic chemicals (ng/L, log scale). The detection frequencies and maximum and minimum concentration are shown below each bar of the measured organic chemicals (Birtek et al., 2022).

line with the findings in this study (Çifci et al., 2013). DEHP was also present in LTP influent, effluent and hospital effluent and their concentrations were 0.23 µg/L, 0.09 µg/L, and 0.12 µg/L, respectively. Being present in every wastewater studied, the persistent character of DEHP is a concern. The presence of DEHP in the influents and effluents in this study were in line with the values reported in the previous studies (Luo et al., 2014; Mailler et al., 2015).

Pentachlorobenzene was observed in 100% of winter and spring season samples, and the maximum influent and effluent concentrations of WWTPs were 1 ng/L and 3.8 ng/L, respectively. Being applied as a fungicide and flame retardant, the use of pentachlorobenzene has been limited (CSB, 2014b; Schwegler & Rasenberg, 2020). The higher effluent concentrations of pentachlorobenzene could be the result of its formation due to degradation of HCH and HCB (Rowsell, 2009). The persistence of pentachlorobenzene in the environment was related to its hydrophobic and bioaccumulative nature (log Kow 5.2) (PubChem, 2020; Rowsell, 2009).

Pentachlorobenzene was also present in LTP influent, effluents and hospital effluent and the concentrations were 0.45 ng/L, 0.45 ng/L, and 0.8 ng/L, respectively. The WWTP effluent concentrations of pentachlorobenzene were lower than those previously reported (Barco-Bonilla et al., 2013; Martí et al., 2011a).

PFOS was detected in 47% of the samples in summer and the maximum WWTP influent concentration was 41 ng/L, whereas effluent was under LOQ. PFOS is applied widely in industry, as a result its availability worldwide. It is also known to form during treatment in WWTPs due to precursor compounds. PFOS is known to pose risks on human health by developing metabolic, developmental, and immune disorders, and various cancers (EPA, 2016; Hu et al., 2018b, 2019). PFOS was also present in LTP influent at 49 ng/L, but effluent concentration was < LOQ. WWTP effluents are known to be one of the main sources of PFOS entering receiving water environments. The studies conducted on PFOS presence in WWTP effluents worldwide reported concentration range between 1.8 ng/L and 68 ng/L (US). Since the WWTP effluent PFOS values were < LOQ in this stuy, conducting further investigation of PFOS in WWTP effluents are recommended (Arvaniti & Stasinakis, 2015; Loos et al., 2013).

C10-13-chloroalkanes were observed in winter and spring seasons in WWTPs with a detection frequency of 43% and the maximum influent and effluent WWTP concentrations of 1.2 µg/L and 0.19 µg/L, respectively. C10-13 chloroalkanes are among the flame retardants that were detected < LOQ in the previously analyzed surface and wastewaters as well as in recovered products taken from resource recovery facilities (Martí et al., 2011b; P. Singh et al., 2022; Rey-Martínez et al., 2022). Having log Kow values in the ranges of 4.39 – 8.69, as well as low solubility in water 0.15 mg/L – 0.47 mg/L, C10-13-chloroalkanes were expected to be adsorbed onto the sediment and sludge during the processing in WWTPs. In this study, some removal was observed but it was not always the case due to the cold season's effect on active sludge (ECHA, 2008). 1,2-Dichloroethane was only observed in winter season with a detection frequency of 14 % of the WWTP samples where the maximum influent WWTP concentration was 4.1 µg/L and < LOQ for effluent. It was also detected in LTP influent and the concentration was 1.5 µg/L. Being used mainly in the production of polyvinyl chloride and as a solvent, the majority of the 1,2-dichloroethane is known to evaporate into the atmosphere (vapor pressure of 78.9 mm Hg at 25 °C) while some is released to water bodies (Syed & Ray, 2014). This could explain why it was only

detected in winter season. The studies conducted to track 1,2-dichloroethane in WWTPs showed their concentrations to be < LOQ. Considering its carcinogenic nature, the monitoring of 1,2-dichloroethane in air samples in WWTPs is recommended (Arjoon et al., 2013; Martí et al., 2011b; Syed & Ray, 2014).

HBCDDs and PBDEs were not detected in any of the WWTP influent and effluent samples. None of them were produced in Türkiye and their consumption was phased out in 2009 in compliance with the Stockholm Convention, which may explain their absence in the studied wastewaters (CSB, 2014b).

The list of PSs was compared with the dirty dozen of POPs and nine of them were found to be alike: DDT, aldrin, dieldrin, endrin, heptachlor, HCB, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF). The analyses conducted on the wastewaters of Istanbul indicated the presence of seven of those POPs among PSs; DDT, HCB, PCB, endrin, heptachlor, PCDD, and PCDF (Mulvaney et al., 2017). The annexes of Stockholm Convention include POP list for elimination, restriction, and unintentional production. In line with Türkiye's compliance of the Stockholm Convention (CSB, 2018), currently p,p'-DDT, HCH, endrin, endosulfan, HCB, and PeCB are included in the elimination list; PFOS, PCBs, hexachlorobutadiene, and C10-13-chloroalkanes are on the restriction list; and DLCs, HCB, PCBs, PAHs, and PeCB are on the unintentional production list (CSB, 2014c). The aforementioned PSs were all present in the studied wastewaters. Since those priority hazardous substances had been banned for a long time, their detection in wastewaters could be related to their illicit use, along with their wide range of transportability, as well as to their ubiquitous properties (EC, 2013).

4.1.7 Metals

The seasonal concentrations of metals in PSs in the influents and effluents of WWTP 1–WWTP 7 were presented in Table D.4 The occurrence of metals among PSs in influents and effluents of WWTPs in winter and summer consecutive samples are given in Figure 4.8. Nickel and lead were present in all samples in winter and summer seasons. Nickel was present in all samples and its highest WWTP influent and effluent concentrations were 267 µg/L and 123 µg/L, respectively. Lead was also present in all seasons and its highest WWTP influent and effluent concentrations were 18.9 µg/L and 1.94 µg/L, respectively. The highest cadmium detection frequency was observed

in winter season (67%) while the highest WWTP influent and effluent concentrations were observed in seasonal samples and were 2.73 µg/L and 0.11 µg/L, respectively. Mercury was mainly observed in winter season (83%) and the highest WWTP influent and effluent concentrations were 2.16 µg/L and 1.25 µg/L, respectively. All metals were present in all wastewater samples except hospital effluents.

This study included seven WWTPs from seven different sewer service areas. Looking at the pesticide profile, the concentrations of pesticides were highest during fall season in each WWTP except for WWTP 6, where the summer concentrations were the highest. Their presence could be explained with their persistent character, or seasonal applications. The concentrations of VOCs in all studied WWTPs were highest during fall season, followed by spring season. This could be due to the increased volatilization rates during summer season and dilution effect in winter season. The VOCs content of each WWTP indicates the industrial activity within each sewer service area. The content of WWTP 2 was lower than the other WWTPs, which could be related to its mainly domestic origin. The variation for concentration of PAHs were similar in general but was higher for WWTP 3. Concentration ranges of the rest of the organic PSs in WWTPs were mostly similar, with the exception of WWTP 3 where it was higher in some seasons. Metals among PSs were present in all samples. Hospital wastewaters and LTP effluents were not significant sources of PSs.

A number of studies conducted previously on the wastewater and sludges of Istanbul revealed the occurrences of emerging contaminants and micropollutants including various medicines, endocrine disrupters, antibiotics, antimicrobial agents, and x-ray contrasts etc. In addition, a number of studies reported the presence of PCDD/Fs, PCBs, and PAHs in the Sea of Marmara and sediments of the Bosphorus (Karacik et al., 2009; Okay et al., 2009; Taşkin et al., 2011b). Accordingly, the occurrence and the estimation of the concentrations of the PSs as well as emerging contaminants entering coastal waters of Istanbul through the WWTPs are critical inputs to evaluate the state of the receiving water environments. This study showed the possibility of intrusion of PSs and some specific pollutants from wastewaters in Istanbul. (Bilgin Oncu et al., 2015; Bilgin Oncu & Akmehmet Balcioglu, 2013; Dogruel et al., 2020; Karacik et al., 2009, 2009; Sari et al., 2014a, 2014a; Taşkin et al., 2011c; Yilmaz et al., 2017a).

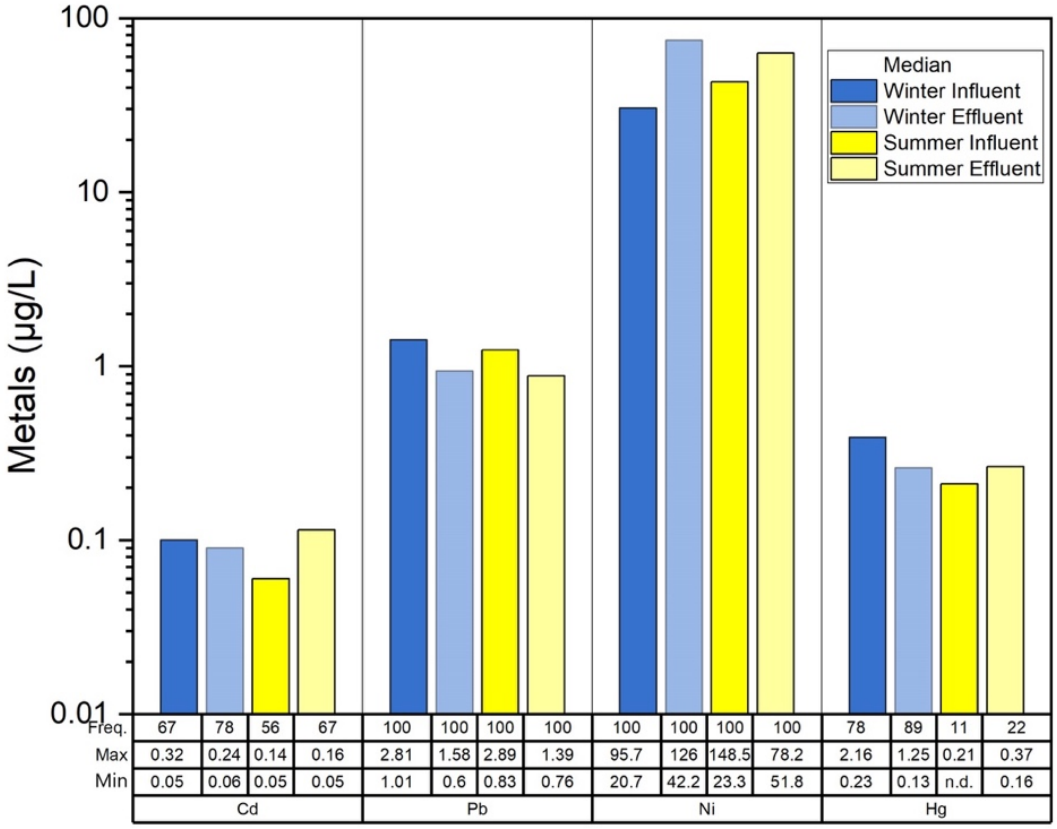


Figure 4.8: Occurrences of metal concentrations in influents and effluents of WWTP 1–WWTP 4 in consecutive days in winter (n = 9 influent, n = 9 effluent) and summer (n = 9 influent, n = 9 effluent) for samples ≥ LOQ. The vertical scale shows the median metal concentrations (µg/L, log scale). The detection frequencies and maximum and minimum concentrations of each metal are shown below each bar (Birtek et al., 2022).

4.2 Environmental Risk Estimation

The Turkish Surface Water Regulation (OSIB, 2016b) requires the monitoring of PSs in the surface, ground, transition and coastal waters. Being a source, the intrusion of PSs through WWTP effluents to the receiving Bosphorus, Sea of Marmara and Black Sea is a concern. In order to make an estimation of the presence of PSs, the environmental risk estimation of PSs present in the effluents were conducted on WWTP effluents. For the estimation of occurrences of PSs in WWTP effluents, the worst-case safety factor of 1,000 was applied in PNEC calculations. Also, PNEC was calculated by using the detected maximum concentrations. The risk estimation was conducted with PSs showed detection frequencies of more than 11%. The RQs were calculated for each of the 37 organic PSs of all the seasonal, summer and winter sampling periods in WWTP effluents that were subjected to advanced treatment (WWTP 1–WWTP 4). In addition, RQs were calculated for each of 31 organic PSs of

WWTP effluents (MTF-Eff) subjected to mechanical treatment (WWTP 5–WWTP 6). The RQ estimations for WWTP effluents for seasonal, winter and summer are given in Figure 4.9. The risk estimation conducted on more than half of the aforementioned effluents had RQ ranges less than 0.1, whilst RQ's of 11 PSs in the effluents of WWTP 1–WWTP 4, and 6 PSs in MTF effluents of WWTP 5–WWTP 6 were between 0.1 < RQ < 1, which could be related to each PS's lowered risk to the species in an aquatic environment or to inadequate data (Barco-Bonilla et al., 2013; B. Escher & Frederic, 2012).

Endrin, endosulfan, diuron, alpha-cypermethrin, beta-cypermethrin, theta-cypermethrin, zeta-cypermethrin, quinoxyfen, aclonifen, bifenox, benzo-ghi-perylene, benzo(a)pyrene, fluoranthene, indeno (1,2,3-cd)-pyrene, tetrachloroethylene, DEHP, and C10-13-chloroalkanes were the 18 PSs showing sufficient potential risk (RQ > 1) (Anderson et al., 2010b; B. Escher & Frederic, 2012) and their surveillance monitoring is recommended for the WWTP effluents prior to discharge (WWTP 1–WWTP 4). On the other hand, endrin, endolsufan, diuron, alpha-cypermethrin, theta- cypermethrin, zeta-cypermethrin, aclonifen, bifenox, anthracene, benzo-ghi-perylene, benzo(k)fluoranthene, fluoranthene, indeno(1,2,3-cd)-pyrene, tetrachloroethylene,

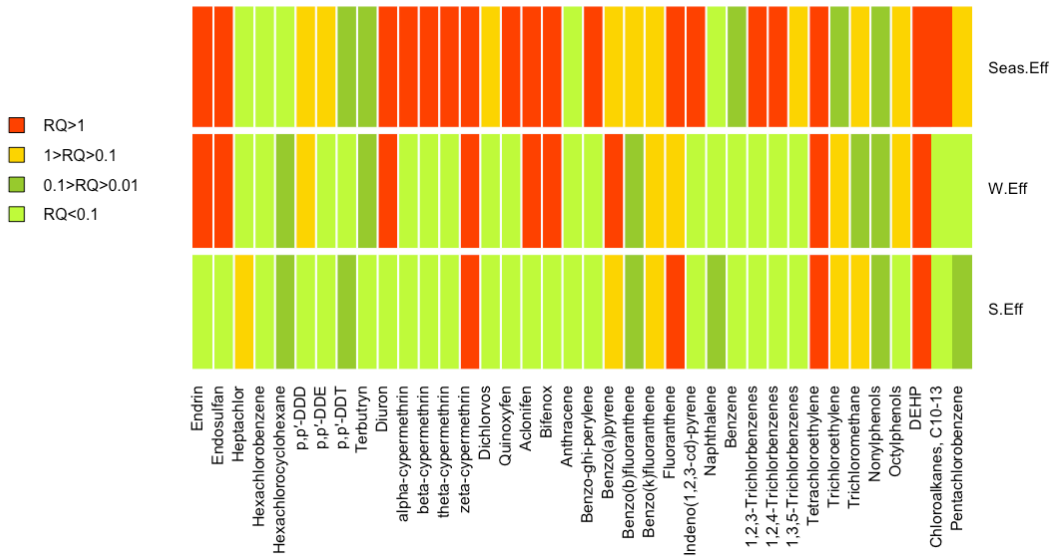


Figure 4.9 : Estimated RQs for PSs in seasonal, winter and summer WWTP effluents.

octylphenols, DEHP, and C10-13-chloroalkanes were the 17 PSs showing sufficient potential risk (RQ > 1) in MTF effluents and their surveillance monitoring is recommended for the WWTP effluents prior to their discharge (WWTP 5–WWTP 7). However, one of the MTF (WWTP 6) was converted to a biological treatment facility and new studies were recommended to trace the occurrence of PSs in the effluents of the newly established biological treatment facility. The toxicological information regarding the selected PSs (CAS numbers, corresponding most sensitive species, endpoint, PNECs, and RQs) are given in Table F.1.

Sixteen PSs detected in WWTP and MTF effluents were identical, showing either those PSs are in use or are still present due to their persistent character. A number of PSs subjected to advanced treatment showed lower concentrations of PSs in effluents compared to MTF effluents. This was related to the treatment potential of active sludge, as well as to the sedimentation process. However, there were cases that the effluent concentrations of PSs were higher in the effluents of advanced treatment plants, mainly in winter season, due to the negative effect of the low temperature on active sludge behavior.

Parallel with the practices around the globe, compliance with the environmental quality standards were investigated in samples withdrawn from the confluences of discharged WWTP effluents with the river and seas. Due to this practice, the information on dilution at the WWTP discharges into the receiving seas or river may be useful knowledge in evaluating the load of pollutants. In line with the Surface Water Regulation (OSIB, 2016b), minimum dilution in the confluence zone for the discharges into the rivers was assumed to be 3 fold (1 part wastewater + 2 parts river water) for WWTP 1, WWTP 2, and WWTP 4 during the June to October period, and 10 fold during other months. Therefore, a dilution of the conventional pollutants of 1/3 to 1/10 could be assumed at the mixture zones of the WWTP discharges. In the case of WWTP 3, WWTP 5, WWTP 6, and WWTP 7 effluents are discharged to the bottom layer of the Sea of Marmara at ~45 m to ~70 m depth by means of a diffuser installed at the end of the deep-sea discharge pipe. The deep-sea discharge system is expected to increase the initial dilution by 40 times at the end of the confluence zone of deep-sea discharge diffusers (COB, 2010). As a result, the conventional pollutants to be discharged to the bottom layer of the Sea of Marmara would be expected to be diluted by 1/40 at the mixing zone.

It would be necessary to point out that since the environmental risk assessment conducted in this study was based on the toxicity of individual trackable compounds, possible synergistic effects have not been taken into account.

This thesis has tracked the occurrence and magnitude of PSs present in the influents and effluents of WWTPs in the City of Istanbul as well as estimated the toxicity they may pose to the Sea of Marmara, the Black Sea and the Bosphorus. The data produced in this research may be beneficial to the governmental authorities and city regulators that conduct risk management strategies.

4.3 Acute Toxicity Tests

This section presents the results of the toxicity tests of the wastewaters, that were conducted after the establishment of the first ecotoxicity laboratory of Türkiye in a water utility. All tests had been carried out precisely according to their ISO standards. The standards of the ecotoxicity laboratory were very well attained and none of the tests conducted failed due to contamination.

The ecotoxicity testing of the wastewaters were conducted using a bioassay including *D. magna, S. capricornutum, and V. fischeri* and the results were represented through EC_{50} or IC_{50}. Due to the nature of the data, this was chosen as the best way to estimate toxicity of the studied wastewaters. A hazard classification system proposed previously (Persoone et al., 2003) was used which allowed the application of the conversion of EC_{50} or IC_{50} values to toxicity unit (TU), which made the demonstration of the toxicity data easier to follow. All the EC_{50} or IC_{50} values obtained from *D. magna, S. capricornutum,* and *V. fischeri* were converted to TU using the following equation (4.1).

$$TU = \frac{1}{EC_{50} or\ IC_{50}} \times 100 \qquad (4.1)$$

The classification of the TU was concluded accordingly (Figure 4.10). Acute toxicity test results conducted using *D. magna, S. capricornutum,* and *V. fischeri* are estimated for WWTP 1–WWTP 7, hospital wastewaters as well as LTP influents and effluents and the TU are given in Figures 4.11–4.17.

All acute toxicity tests conducted with *D. magna, S. capricornutum,* and *V. fischeri* for WWTP 1 (Figure 4.11), showed acute toxic character (TU > 1) of the wastewater

excluding the response of *S. capricornutum* in summer and fall seasons. In addition, the toxicity response of *S. capricornutum, and V. fischeri* in spring was higher, showing high acute toxic (TU > 10) character in the WWTP effluents.

TU	CLASSES	TOXICITY	SYMBOL
< 0.4	I	No acute toxicity	
0.4 < TU <1	II	Slight acute toxicity	
1 < TU < 10	III	Acute toxicity	
10 < TU < 100	IV	High acute toxicity	
TU > 100	V	Very high acute toxicity	

Figure 4.10: Hazard classification system proposed by Persoone (Persoone et al., 2003).

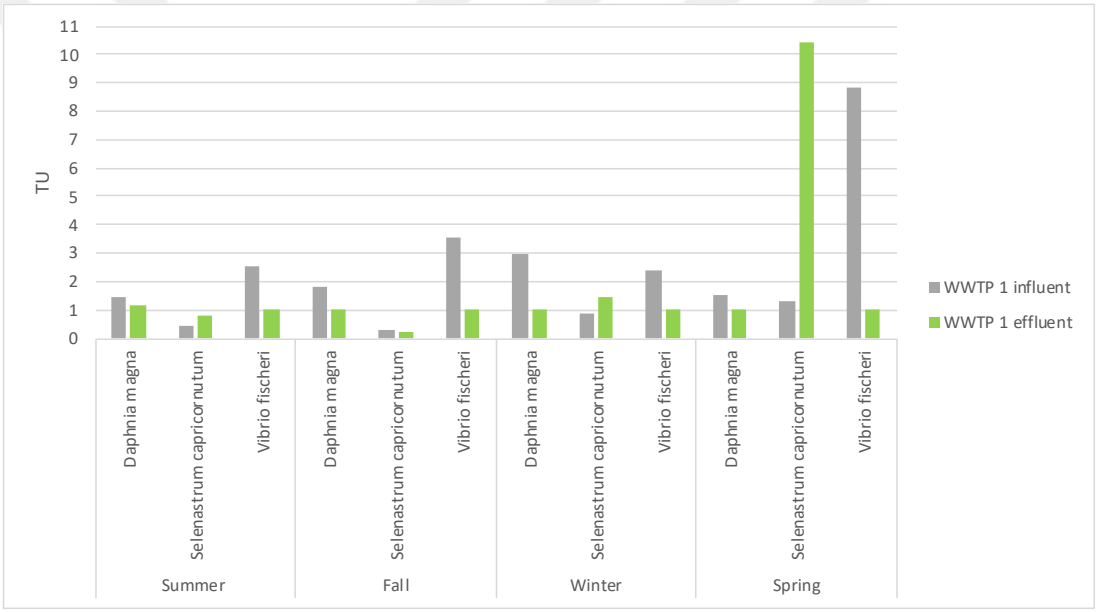


Figure 4.11: Acute toxicity test results of WWTP 1 with *Daphnia magna, Selenastrum capricornutum,* and *Vibrio fischeri* represented by TU. The vertical line shows the TU, first bars indicate the TU for WWTP influents, while the adjacent second bars represent the TU for WWTP effluents.

Having domestic character, the wastewaters of WWTP 2 showed acute toxicity (TU > 1) to *D. magna* and *V. fischeri* while toxicity to *S. capricornutum* showed TU > 1 only in spring season (Figure 4.12).

The acute toxicity of *S. capricornutum* was higher in WWTP 3 effluents in all seasons as shown in Figure 4.13. Specifically, the toxicity of the WWTP 3 effluents of the biological and advance treatment was higher in all seasons except for the winter effluents of biological treatment. Those effluent toxicities can be explained by release of toxic substances from the active sludge that is affecting *S. capricornutum* through

the warmer parts of the year. In addition, the effluent toxicity on *D. magna* was higher in fall season which may be related to the release of toxic substances from active sludge causing acute toxicity due to seasonal disturbances. *V. fischeri* and *D. magna* exhibited acute toxicity (TU > 1) to the wastewaters in each season. The biological and advance wastewater treatment in WWTP 3 seems to reduce the acute toxicity of the effluents toward *V. fischeri.*

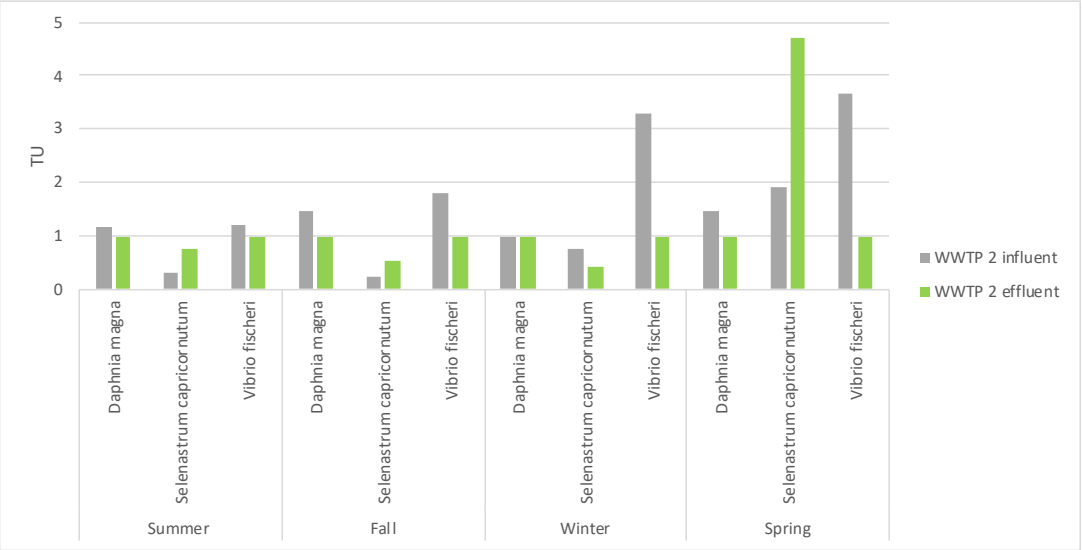


Figure 4.12 : Acute toxicity test results of WWTP 2, conducted testing with *Daphnia magna, Selenastrum capricornutum, and Vibrio fischeri* represented by TU. The vertical line shows the TU, first bars indicate the TU for WWTP influents, while the adjacent second bars represent the TU for WWTP effluents.

Wastewaters were always acutely toxic (TU > 1) to *V. fischeri* and *D. magna.* in WWTP 4 (Figure 4.14) in all seasons, whereas *S. capricornutum* showed high acute toxicity to WWTP 4 effluent only in the spring season. The fact that effluents were more toxic to *S. capricornutum* in summer, fall and winter seasons can be explained by release of a toxic substance to *S. capricornutum* from the active sludge.

MTF effluents WWTP 5–WWTP 7 were more acutely toxic to *V. fischeri* and *D. magna* in all seasons except for fall season for WWTP 6 (Figure 4.15). On the other hand, effluent toxicity toward *S. capricornutum* was greatest in spring at WWTP 7.

In addition to the findings presented in Figures 4.11–4.15, further seasonal toxicity analyses were conducted with influents and effluents of WWTP 1–7 on *D. magna*, *S. capricornutum*, and *V. fischeri.* Their EC_{50} values were estimated by the computer program provided by toxkits and represented by TU with single boxplot for each *D. magna*, *S. capricornutum*, and *V. fischeri* can be found in Figure A.5. Results show

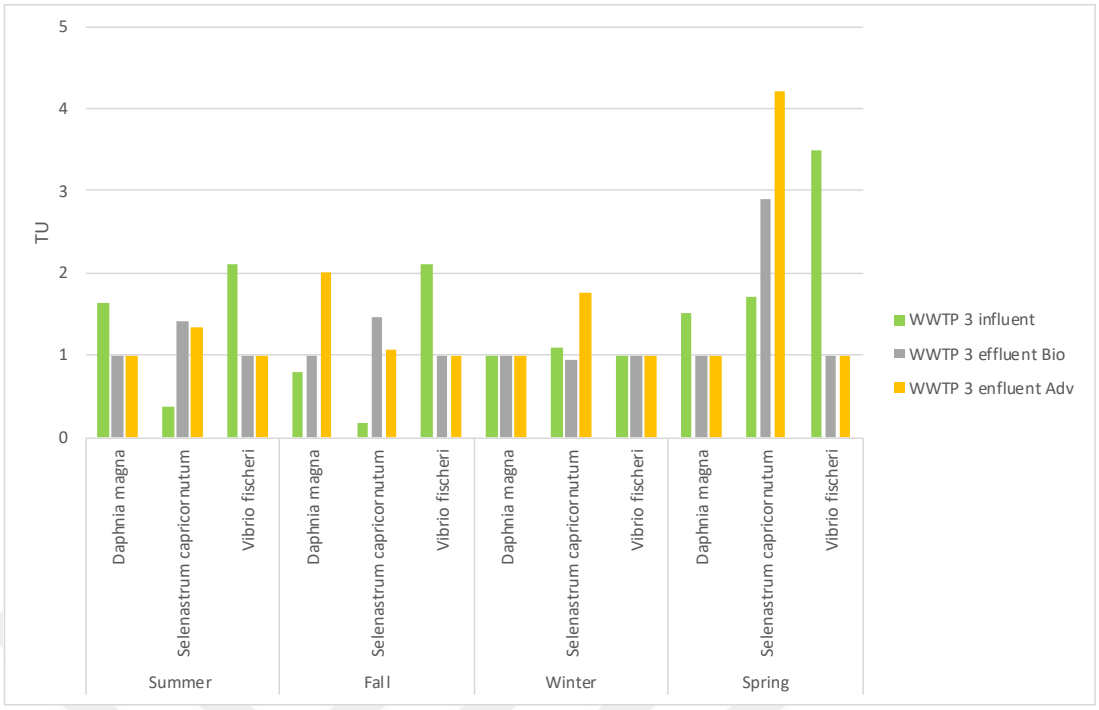


Figure 4.13 : Acute toxicity test results of WWTP 3 with *Daphnia magna, Selenastrum capricornutum,* and *Vibrio fischeri* represented by TU. The vertical line shows the TU, first bars indicate the TU for WWTP influents, while the adjacent second and third bars represents the TU for WWTP effluents of biological treatment and advance treatment, respectively.

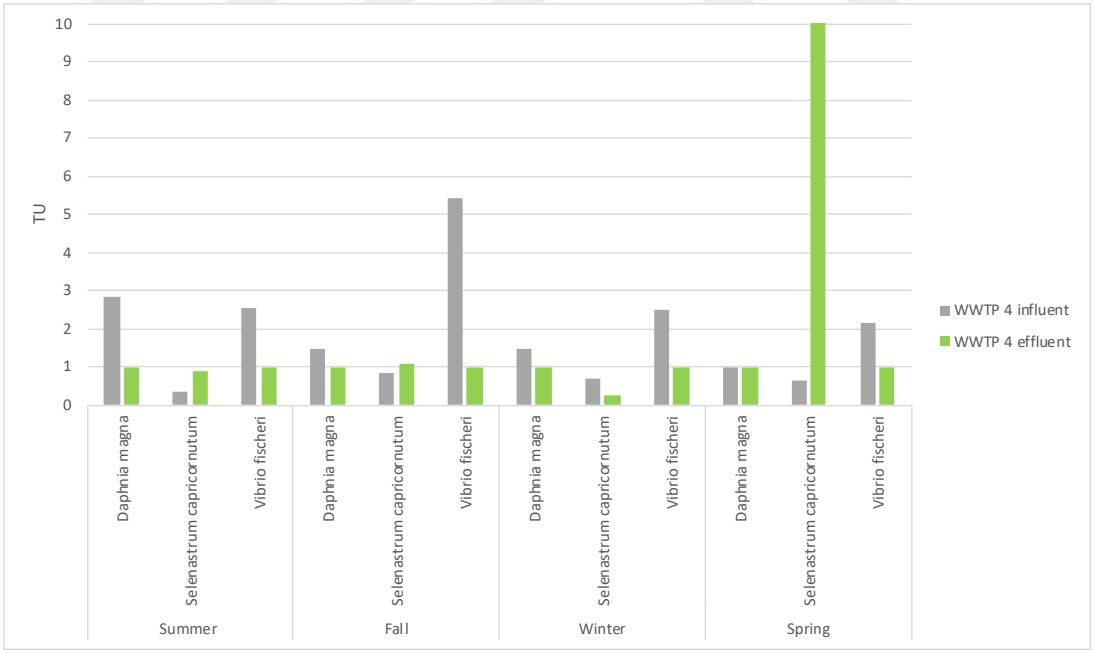


Figure 4.14 : Acute toxicity test results of WWTP 4 with *Daphnia magna, Selenastrum capricornutum,* and *Vibrio fischeri* represented by TU. The vertical line shows the TU, first bars indicate the TU for WWTP influents, while the adjacent second bars represent the TU for WWTP effluents.

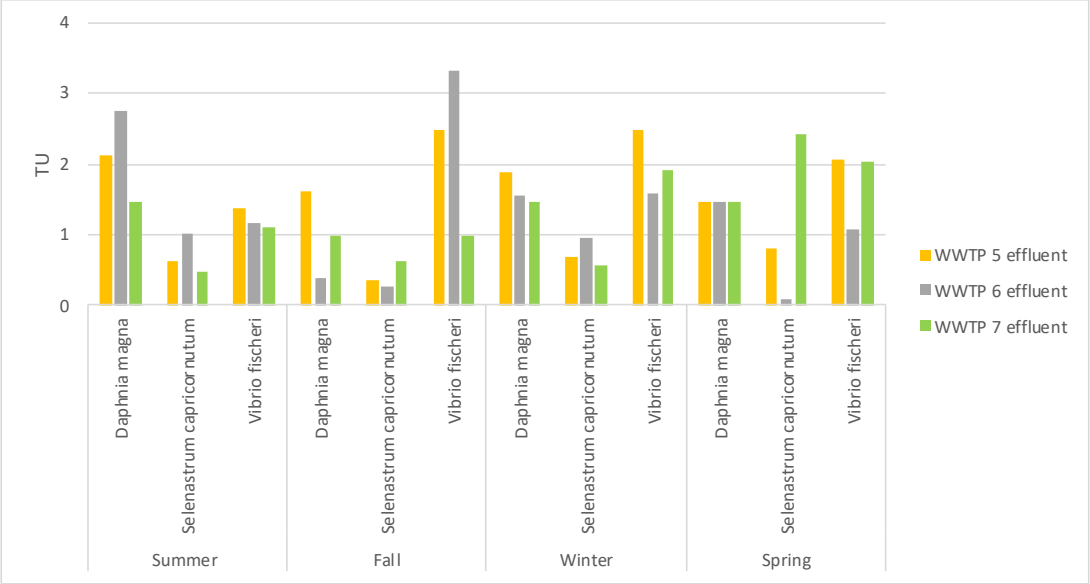


Figure 4.15: Acute toxicity test results of the effluents of WWTP 5–7 with *Daphnia magna, Selenastrum capricornutum, and Vibrio fischeri* represented by TU. The vertical line shows the TU, the consecutive three bars indicate TU for the effluents of WWTP 5–WWTP 7 of the MTF, respectively.

that the acute toxicity response variation of *S. capricornutum* was the highest in WWTP effluents, indicating its importance on evaluating effluent toxcity. In addition, CETIS program was found to be more precise in estimating toxicity calculations.

Hospital wastewaters were acutely toxic (TU > 1) to *D. magna* and *V. fischeri* in each season (Figure 4.16), whereas they showed acute toxicity to *S. capricornutum* only in winter and spring seasons, with the toxicity for spring by far the highest. The number of patients and amount of medicine used is known to increase in Istanbul in fall, winter and spring due to flu and chronic diseases. Hospital wastewaters are known to include traces of pharmaceutically active compounds.; consequently it acted like a domestic effluent (Quevauviller et al., 2006; Yilmaz et al., 2017b).

LTP influents showed high (TU > 10) or very high (TU > 100) acute toxicity to *D. magna* and *V. fischeri*. *D. magna* had the highest toxicity in LTP effluents during the fall season, whereas *S. capricornutum* showed the highest toxicity in spring season (Figure 4.17). In addition to the findings presented in Figure 4.17, further seasonal toxicity analyses were conducted with influents and effluents of LTP on *D. magna*, *S. capricornutum*, and *V. fischeri.* Their EC_{50} values were estimated by the computer program provided by toxkits and represented by TU with single boxplot for each *D. magna*, *S. capricornutum*, and *V. fischeri* are presented in Figure B.3. In general, results showed similar trend with Figure 4.17. The acute toxicity ranges of TU of LTP

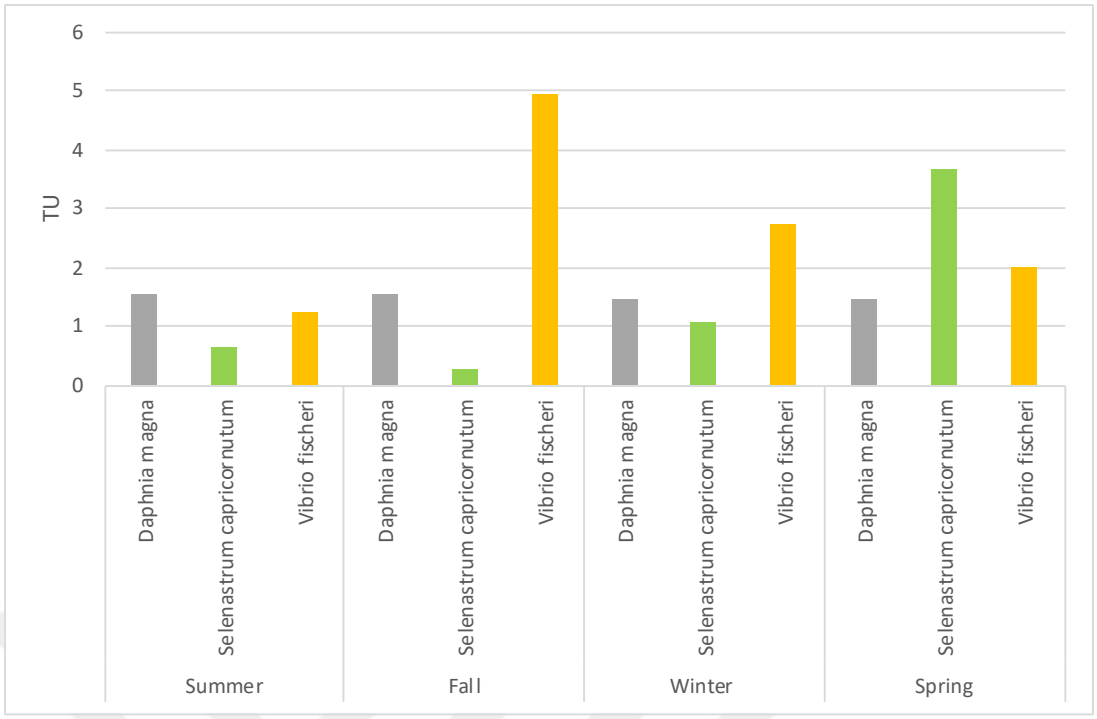


Figure 4.16 : Acute toxicity test results of hospital wastewaterwith *Daphnia magna*, *Selenastrum capricornutum*, and *Vibrio fischeri* represented by TU. Vertical line shows the TU.

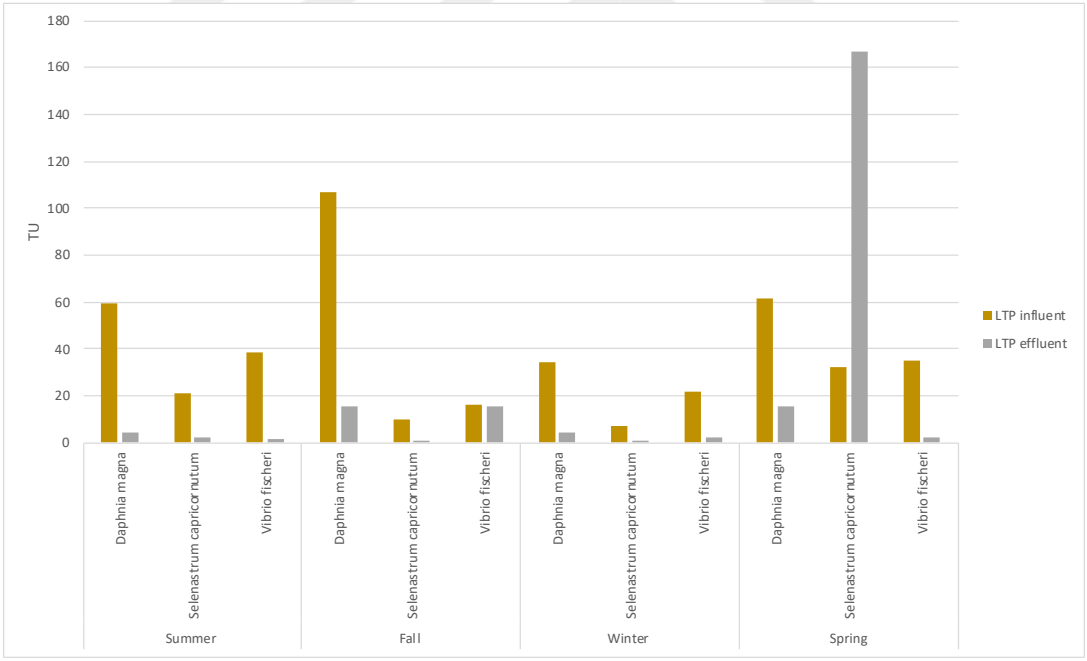


Figure 4.17 : Acute toxicity test results of LTP influents and effluents using *Daphnia magna, Selenastrum capricornutum,* and *Vibrio fischeri* represented by TU. The vertical line shows the TU, first bars indicate the TU for LTP influents, while the adjacent second bars represent the TU for LTP effluents.

Influent was much higher than LTP influents. The LTP process, which includes the UF process, was successful in reducing acute toxicity in *D. magna.* and *V. fischeri*, as well as in *S. capricornutum* except in the spring season where there was a large

increase in acute toxicity for the effluent vs influent. Considering the cities do not have LTP process, acute tests showed the importance of the acute toxicity reduction by the LTP and the extent of the acute toxicity of the leachate is likely to pose risk to its receiving environment.

Seasonal changes in the toxicity units of wastewaters where the tests had been conducted using a bioassay including *D. magna, S. capricornutum,* and *V. fischeri* is shown above. *S. capricornutum* gave the most toxic responses in the spring season. This may be due to the dense application of algicides for the water treatment process during that period in Istanbul. Also, even control of the beginning of the algal growth test was performed prior to each individual experiment and the suitability of the test has been ensured. The robustness of the algae was found to be very weak in the spring season due to its natural cycle. Indeed, within the frame of the time span provided by each test kit providers, as compared to *D. magna* and *V. fischeri* test kits, the shelve life of *S. capricornutum* found to be the least durable. Compared to *D. magna* and *V. fischeri*, the toxicity tendencies of *S. capricornutum* toward wastewaters were different and provided valuable data for interpretation. This shows the importance of doing tests in at least three taxa (de Zwart, 2005). On the other hand, the toxicity ranges for *D. magna*, which increased in summer and winter samples, may be explained by the availability of the soluble pollutants to *D. magna* during those seasons. The wastewater toxicity response of *V. f*ischeri was comparatively high in fall and spring season.

The BOD_5/COD ratio (> 0.5) of the wastewaters studied (Figure 4.18) shows the biodegradability of the studied wastewaters (Tchobanoglous et al., 2014). Ammonia is known to be toxic to the organisms under water where the main toxicity is due to the un-ionized form of ammonia (NH_3) passing through epithelial membranes of the organisms. However, ammonia concentration in water is known to be dependend on the pH where the ammonia concentration contributes 11% of the total ammonia concentration at pH 8.5 at 20 ºC and decreases as the pH drops. Ammonia concentrations in WWTP influents ranging from 30–54 mg/L, where the pH ranges 7.42–7.91, may lessen the ammonia toxicity responses for *D. magna*, *S. capricornutum*, and *V. fischeri.* Since ammonia concentrations over 23 mg/L is reported to be toxic on invertebrates and *D. magna,* the immobilization rate in 100% WWTP influent can be due to ammonia toxicity in some seasons. In addition, the conductivity of wastewaters exceeding 10,000 µS/cm is known to be toxic. The

conductivity range of WWTP influents are 720–11,410 µS/cm, and this also can explain the *D. magna* toxicity at in higher concentration. Indeed, ammonia concentrations and conductivity ranging <1–20 mg/L and 368–9386 µS/cm, respectively in WWTP effluents show that there was no acute toxicity due to ammonia and conductivity in effluents to *D. magna*. Phytoplankton and aquatic vascular plants are known to be more tolerant to ammonia (AGI, 2000; Eremektar et al., 2007; Selçuk et al., 2006; Tchobanoglous et al., 2003).

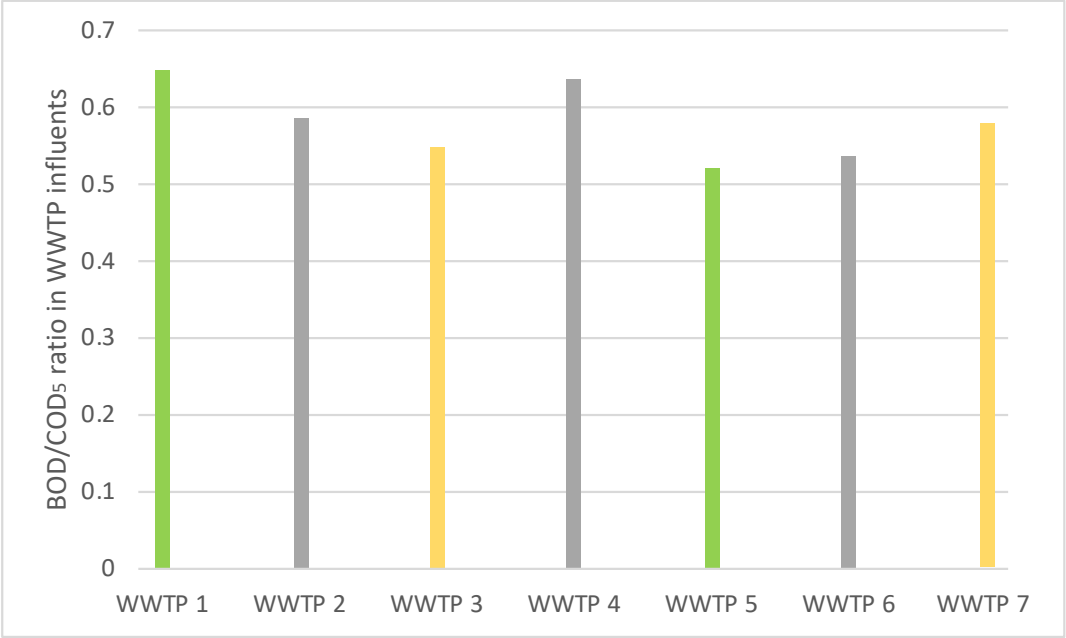


Figure 4.18 : The average BOD_5/COD ratios in the influents of WWTPs

4.3.1 Comparison of the responses of the toxicity tests kits to wastewaters

The ease of application, availability of high sensitivity, low costs as well as being a substitute for fish tests to avoid ethical problems are important factors for choosing biotests (Aydin et al., 2015). With the recommended WWTP wastewater dilution series, *D. magna* showed strong dose-response curves in the influent wastewaters, while almost no response was observed in the advanced treatment effluents. Immobility (%) vs. wastewater concentrations are shown in Figure 4.19 and Figure 4.20. In Figure 4.19, 100%, 75% and 50% concentrations of wastewaters (WWTP 2) showed toxic effect on *D magna*, indicating the toxicity character of the studied wastewaters. Acute toxicity was reduced with increasing dilutions, which can be explained by the ionic composition of the wastewater that is getting more balanced after decreasing dilutions of wastewater so *D. magna* can survive. Comparing the

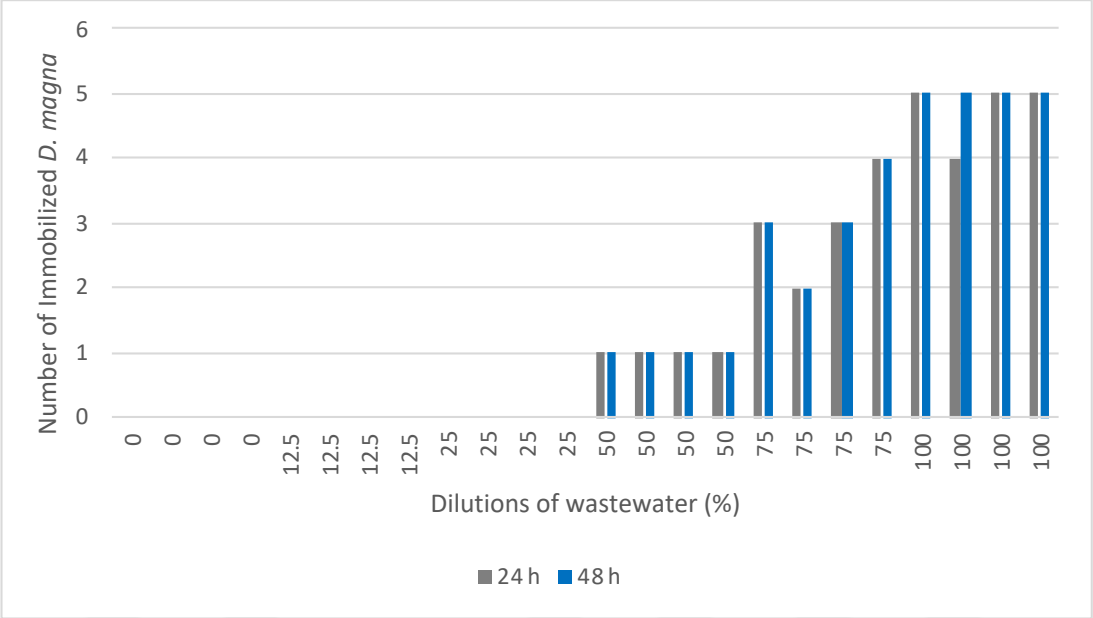


Figure 4.19 : The immobility rates of *Daphnia magna* in dilutions of influent wastewaters (100%, 75%, 50%, 25%, 12.5% and control 0%).

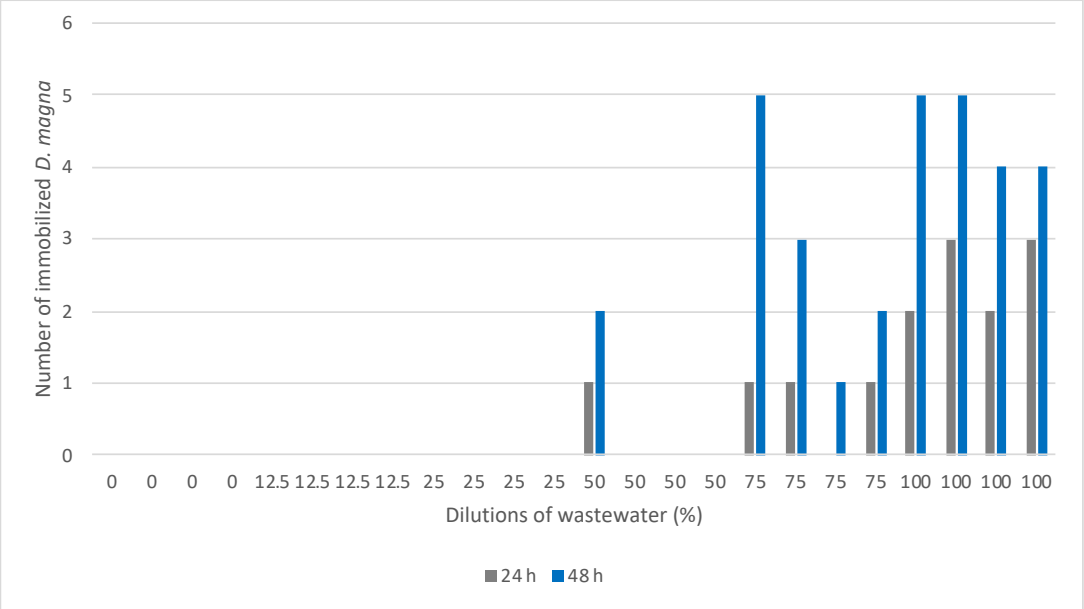


Figure 4.20 : The immobility rates of *Daphnia magna* in dilutions of influent wastewaters (100%, 75 %, 50%, 25%, 12.5% and control 0%).

immobility rate of *D. magna* between 24 h and 48 h in Figure 4.19, the rates would be expected to rise in the presence of chronic toxins in wastewater with time, however it was almost not the case. Hence, WWTP 2 mainly receives domestic wastewater. Since the acute *D magna.* test lasts for 48 h, it's important to notice the change in immobility rates between 24 h and 48 h in Figure 4.20. This may indicate the persistence of toxic ingredients of the studied wastewaters. Conducting a chronic test is recommended.

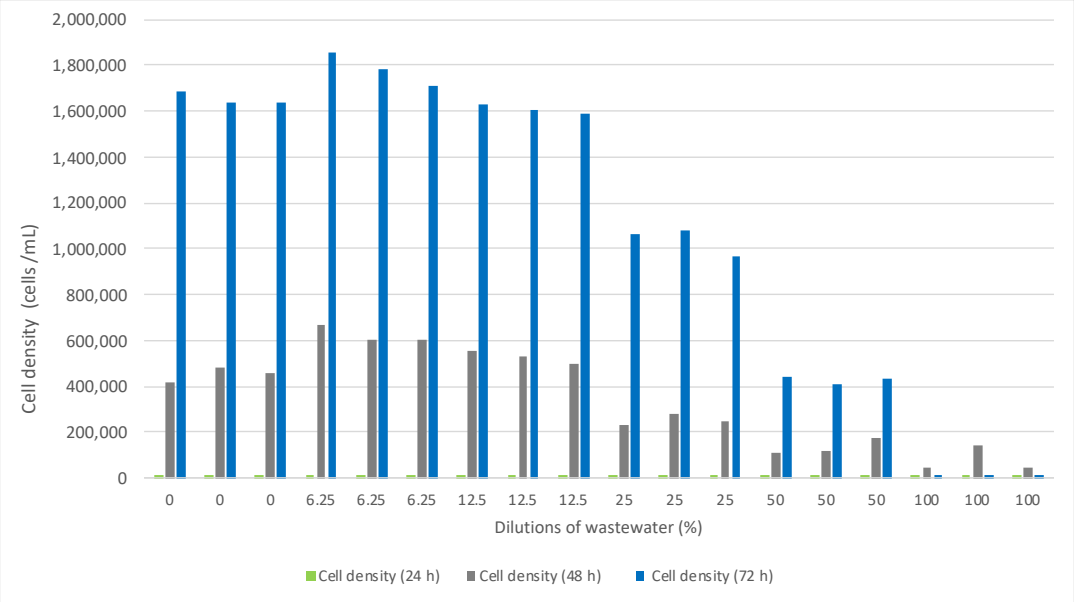


Figure 4.21 : The cell density rates of *Selenastrum capricornutum* in differing dilutions of influent wastewater (100%, 50%, 25%, 12.5%, 6.25%, and control 0%).

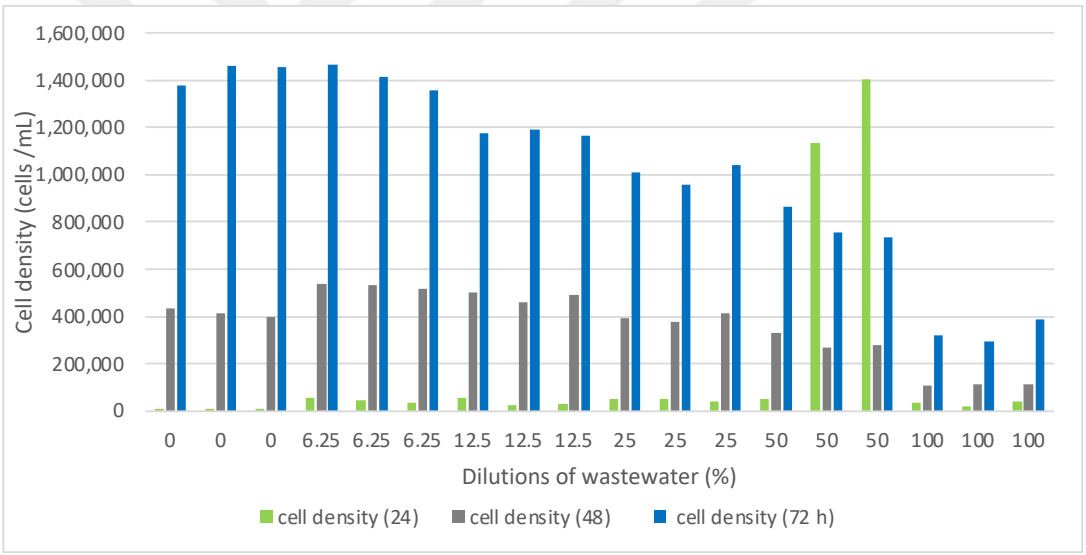


Figure 4.22 : The cell density rates of *Selenastrum capricornutum* in differing dilutions of effluent wastewaters (100%, 50%, 25%, 12.5%, 6.25%, and control 0%).

Second tests conducted with *S. capricornutum*, towards which wastewaters showed both toxic as well as stimulatory effects. The optical density in different dilutions of wastewaters (%) vs. time graphs show the different responses in 24 h, 48 h and 72 h periods (Figures 4.21–4.22) There was a mixture of stimulatory and inhibitory factors at 50 % concentrations in WWTP effluents in Figure 4.22. There were additional peaks of growth (Figure 4.22), whereas, reproduction was diminished after 24 h. The additional peak of growth was not related to the turbidity of the studied sample.

Therefore, it may indicate the presence of substances in effluents that show toxic effect to *S. capricornutum.*

The third test was conducted by exposing wastewater samples to *V. fischeri.* Compared to *D. magna* and *S. capricornutum* tests, *V. fischeri* tests required shorter time (30 min) which made it the most practical test. Luminescence vs. concentration graphics are shown in Figure 4.23 and Figure 4.24. The luminescence response of *V. fischeri* was higher at the beginning of the test (0 minute) and was reduced in 30 minutes. The effluent showed no effect, within experimental error whereas the influent showed a definite inhibition of luminescence going from 0.78% to 50%.

Figures 4.19–4.24 show the responses of three species to the wastewater influent and effluents. Indeed, each responded differently. The acute toxicity test results showed the importance of using at least three different taxonomic groups in toxicity testing to estimate significant differences that may occur in the receiving water community. Along with the WWTP effluent toxicity testing, it is also very important to conduct toxicity testing of the receiving water environments. Currently, testing of the receiving water bodies are not controlled by wastewater utilities in Türkiye, however the change in regulation may help to better evaluate the effects of WWTP effluents on receiving waters. In the evaluation of the toxicity of wastewaters response of *S. capricornutum* varied while *D. magna.* was more stable. Indeed, a study conducted in Türkiye showed that *D. magna* was one of the recommended toxicity test kits in evaluating industrial wastewaters (Aydin et al., 2015). Although short in duration, the response of *V. fischeri* was not found as sensitive as *S. capricornutum.* and *D. magna*, the luminescence inhibition was pretty steady and the replacement with fish toxicity testing is recommended. Considering the hardship of conducting fish testing and ethical concerns, since WWTP effluents are finally discharged into the seas of Istanbul, fish toxicity testing is recommended. Indeed, fish toxicity test is the only recognized toxicity test by the regulation in Türkiye (MEU, 2004).

The effluents of WWTPs had less toxicity than the influents, showing the effect of advance treatment in removing toxic substances. Since 41% of the wastewaters of Istanbul are subjected to advance treatment, conversion of the biological treatment is important for the remaining 59%. The occurrences of the PSs in effluents also points at the need for an upgrade of treatment technologies at the current advanced treatment plants, for better elimination of PSs and ECs to protect the health of receiving seas.

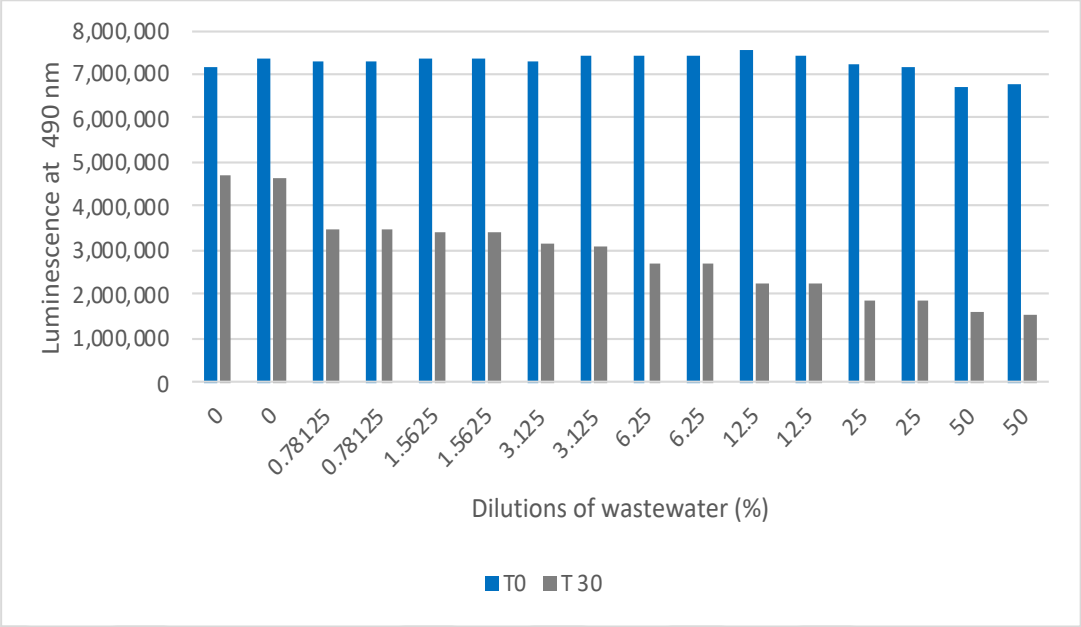


Figure 4.23 : Luminescence of *Vibrio fischeri* in differing dilutions of influent wastewaters (50%, 25%,12.5%, 6.25%, 1.56%, 0.78%, and control 0%).

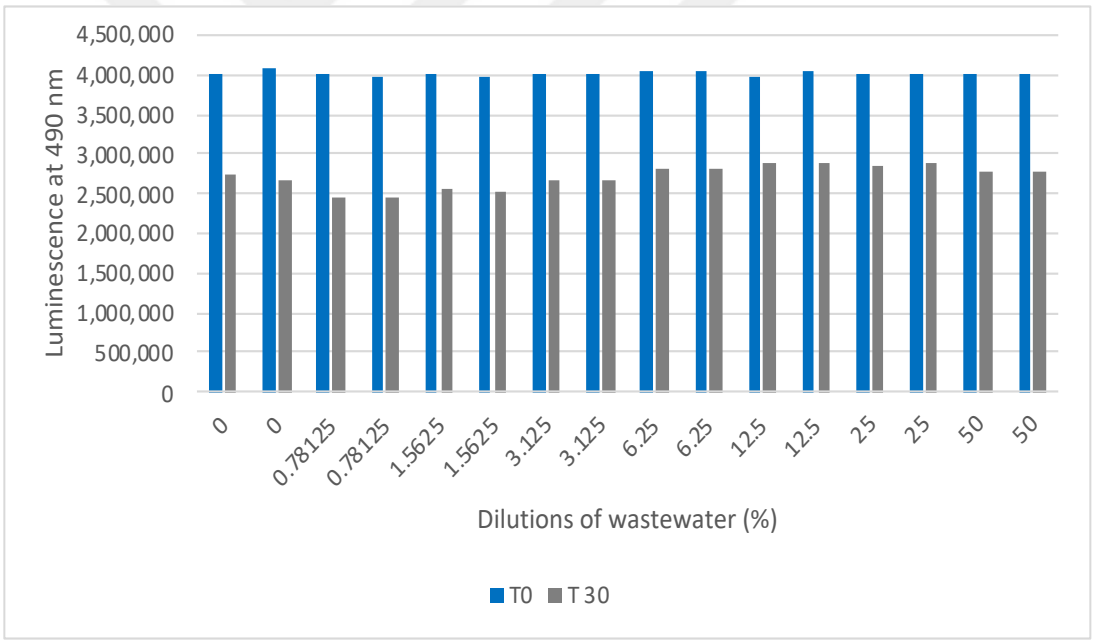


Figure 4.24 : Luminescence of *Vibrio fischer*i in differing dilutions of effluent wastewaters (50%, 25%, 12.5%, 6.25%, 1.56%, 0.78%, and control 0%).

Reducing the discharges of ECs and micropollutants from WWTP effluents through upgrading treatment by adding post treatments units such as ozonation and active carbon treatment processes can help protect environment. Ozonation was found to be efficient in eliminating (> 79 %) wide range of micropollutants. However, after ozonation, post treatment is also needed to eliminate ecotoxic substances formed during ozonation process such as oxidation by-products and biodegradable ozonation

transformation products. A study conducted on the efficiency of sand filtration, fixed bed, and moving bed as the post treatment units after ozonation, sand filtration gave the best results. For the abatement of emerging contaminants upgrading treatment at WWTPs are important (Bourgin et al., 2018; Hollender et al., 2009; Salhi et al., 2018).

In conclusion, the toxicity test conducted in this study revealed the complementary biological, effect-based testing in the wastewaters of Istanbul. PSs and ECs analyses conducted in this study as well as of those reported in the literature revealed the presence of active and endocrine disrupter compounds in the wastewaters of Istanbul (Sari et al., 2014b, 2014a; Yilmaz et al., 2017a). The difference in the toxicity of leachate influent and effluents were very significant, showing the importance of LTP treatment process protecting the environment by removing the acute toxicity of leachate to an extent, while it was not always the the case in removing PSs. The toxicity of hospital wastewater is expected to have high toxicity due to its contents such as pharmaceutically active compounds, antiseptics etc. However, it showed similar characteristics with urban wastewaters which was in line with the previous findings. This was explained with the trace concentrations of pharmaceuticals not showing acute toxic effects in the short term. The presence of active ingredients of pharmaceuticals, personal care products, pesticides, industrial chemicals as well as all endocrine disrupters in the environment have been a cause for concern in recent decades. Also considering the number (>1600) of health care facilities present in Istanbul, management of hospital wastewaters should be taken more seriously. The pharmaceutically active compounds content of hospital wastewaters can negatively affect the receiving water bodies as well as the WWTP process. Considering the studied seven sewer areas in this study (WWTP 1–7), which included 81% of the total number of health facilities in Istanbul (Figure B.4), a further investigation should be taken on the hospital wastewaters. The application of highly sensitive bioassays, such as cyrotoxicity (anti-) androgenicity, mutagenicity, and (anti-) estrogenicity as well as chronic toxicity tests are recommended in the investigation of effluents. (B. Escher & Frederic, 2012; B. I. Escher et al., 2005; Quevauviller et al., 2006).

4.4 Physicochemical Parameters

This section includes the results for regular monitoring of conventional parameters in the studied wastewaters. The mean values of five wastewater quality parameters taken in experimental dry days and their standard deviations at the studied WWTPs were presented in Table 4.1. The results of the COD (mg/L), BOD_5 (mg/L), TSS (mg/L), TP (mg/L) and pH for WWTP 1–WWTP 7 are shown in box plots (Figures G.1–G.5). The results include influents and effluents of WWTP 1–WWTP 4 and effluents of WWTP 5–WWTP 7 in dry and rainy days throughout the monitoring years, respectively. The boxplot of each parameter follows the order through WWTP 1–WWTP 7.

The chemical characteristics of the WWTPs effluents sampled prior to discharge is subjected to Regulation on Urban Wastewater Treatment of Türkiye which was previously adapted from EU Directive 91/271/EEC. The BOD_5/COD ratio of each WWTP varied between 0.52–0.65. This ratio indicates the biodegrading character (> 0.5) of the wastewaters of Istanbul (Tchobanoglous et al, 2004). The average concentrations of conventional wastewater parameters and their standard deviations in influent and effluent of each WWTPs are given in Table 4.1. Regarding the chemical characteristics of the WWTP effluents, the discharge limits of the WWTP effluents are subject to Regulation on Urban Wastewater Treatment of Türkiye (adopted from Directive 91/271/EEC) (MOEF, 2006).

Table 4.2 provides the analyses results of conventional parameters in LTP influents and effluents. The LTP effluents being discharged to the receiving water bodies directly, are subjected to Turkish “Regulation on Water Pollution Control” Table 20.6 (Wastewater Discharge Standards into Receiving Body for Landfill Leachate) by Ministry of Environment and Urbanization (SKKY, 2008). The wastewaters being discharged to the sewer system are subject to the discharge standards for water pollution control established by Turkish “Regulation on Water Pollution Control” Table 22 by Ministry of Environment and Urbanization (SKKY, 2008). In addition, wastewaters discharging into sewage in Istanbul are subject to “Regulation on Discharge of Wastewater to Sewage” of ISKI.

Table 4.1: Average concentrations of conventional wastewater parameters and their standard deviations in influent and effluent of each WWTP.

		COD (mg/L)	BOD (mg/L)	TKN (mg/L)	TP (mg/L)	SS (mg/L)	pH
WWTP 1	Influent	558 ± 106	324 ± 119	66 ± 8.7	5.7 ± 2.4	383 ± 132	7.4
	Effluent	68 ± 18	16 ± 7	25 ± 14	1.6 ± 0.9	41 ± 21.8	7.0
WWTP 2	Influent	483 ± 107	308 ± 60	66 ± 13	4.7 ± 2.1	327 ± 144	7.75
	Effluent	27 ± 9	6.7 ± 2.8	4 ± 2.4	0.9 ± 0.6	16 ± 7.2	8.00
WWTP 3	Influent	626 ± 197	± 29	72 ± 19	5.6 ± 3.0	598 ± 255	7.78
	Effluent	87 ± 16	15 ± 5.7	2.4 ± 3.9	1.1 ± 1	37 ± 23	7.42
WWTP 4	Influent	441 ± 108	242 ± 88	54 ± 16	6.3 ± 2	340 ± 108	7.22
	Effluent	38 ± 9	6.7 ± 2.8	2.5 ± 3.2	0.8 ± 1	15 ± 4.6	7.25
WWTP 5							
	Effluent	471 ± 112	245 ± 45	46.6 ± 19	4.1 ± 1.1	277 ± 123	7.73
WWTP 6							
	Effluent	530 ± 78	285 ± 33	55.7 ± 21	6 ± 2.4	362 ± 197	7.54
WWTP 7							
	Effluent	546 ± 207	317 ± 57	52.1 ± 18	4.1 ± 0.9	299 ± 169	7.63

Table 4.2: Average concentrations of conventional wastewater parameters and their standard deviations in LTP influent and effluent.

		COD (mg/L)	BOD (mg/L)	TKN (mg/L)	TP (mg/L)	SS (mg/L)	pH
LTP	Influent	18,716 ± 10,933	9,470 ± 10,471	12,080 ± 27,290	26.6 ± 36	894 ± 727	8.03
	Effluent	1,427 ± 1,131	470 ± 433	622 ± 395	5.7 ± 6.6	18.7 ± 11.4	8.03

The conventional wastewater parameters and their standard deviations in hospital wastewaters are shown in Table 4.3. Turkish "Regulation on Water pollution Control" defines hospital wastewaters as domestic wastewaters. However, hospital wastewaters being discharged to the receiving water bodies directly, are recently subjected to Turkish "Regulation on Water Pollution Control" Table 24 (SKKY, 2008).

Table 4.3: Average concentrations of conventional wastewater parameters and their standard deviations in hospital wastewater.

		COD (mg/L)	BOD (mg/L)	TKN (mg/L)	TP (mg/L)	SS (mg/L)	pH
Hospital	Effluent	482 ±215	135 ±50	58 ±19	5.5 ± 1.7	203 ± 114	8.0

Figure G.1 shows the median pH ranges of WWTP 1–WWTP 7 in dry and rainy days. The pH of WWTP 3 was slightly lower than the rest of the WWTPs. Figure G.2 shows the boxplots for COD (mg/L) content of WWTP influents and effluents in dry and rainy days. The figure also indicates the COD removal rates by advance treatment and the effectiveness of advance treatment on COD removal.

Figure G.3 shows the boxplots for TKN concentrations in each WWTP influent and effluent in dry and rainy days. The boxplot for TKN shows higher concentrations of the TKN levels in effluent of WWTP 3 as compared to WWTP 1, WWTP 2, and WWTP 4. However, it's still lower than the input coming from MTFs. Comparing the industrial load that the WWTP 3 receives, the high concentrations of TKN in effluent from WWTP 3 reflects the toxicity findings (Figure 4.13). Hence the concentrations of PSs and specific pollutants originating from industrial discharges were significant in WWTP 3. In addition, the acute toxicity to *S capricornutum* was higher in effluents of WWTP 3 in all seasons. In addition, the toxicity of the effluents of the advance treatment was higher than the biological treatment in winter and spring seasons. This may be an example of the inhibition of a treatment process due to the entrance of highly toxic substances to the WWTP 3. Where needed, the application of WET tests in the WWTP influents and effluents are highly recommended.

Figure G.4 shows the boxplots for TSS concentrations in each WWTP influent and effluent in dry and rainy days. The comparably higher concentrations of TSS in WWTP 1–WWTP 3 may be related to the open channel effect prior to the wastewaters' arrival in those WWTPs. Advance treatment is effective in reducing the TSS concentrations before being discharged into receiving water bodies.

Figure G.5 shows the boxplots for TP concentrations in each WWTP influent and effluent in dry and rainy days. According to Regulation on Urban Wastewater Treatment, TP concentrations shall be less than 1 mg/L, however there were times that WWTPs were unable to attain the abatement of TP. Since Sea of Marmara had mucilage in recent years, measures should be taken to reduce phosphorus levels in effluents. Hence, being an essential resource, recycling phosphorus from wastewaters of Istanbul can help alleviate the situation.

In addition to the previous findings, number of physicochemical as well as heavy metal analyses conducted in labrotories of ISKI. The salinity range of the studied

wastewaters were 288–10840 (‰). The color of the WWTP influents and effluents were in a range of 38–467 pt/co and 8–200 pt/co respectively. The LTP influents were highly colored and the reported values for LTP influent and effluent were > 500 pt/co. The ranges of sulfate in WWTP influents and effluents were (< 50–700 mg/L) and (< 50–485 mg/L), respectively and were 241–308 mg/L in leachate. The oil and grease ranges were 20–255 mg/L in WWTP influents while mostly reported as < 10 mg/L for effluents. The oil and grease ranges were narrower for leachate < 100 mg/L. Alkalinity ranges of WWTP influents and effluents were 121–593 mg $CaCO_3$ /L and 128390 mg $CaCO_3$ /L, respectively and were 8000–12000 mg $CaCO_3$ /L in leachate. Aliminum and iron levels of WWTP influents were in the range (1.23–6.95 mg/L) and (1.36–7.82 mg/L), while copper and zinc were in the range (0.23–0.6 mg/L) and (0.4–1.82 mg/L), respectively. Being a public utility, some measurements are mainly reported as censored data and the results were as follows for the WWTP influents and effluents: Cyanide < 0.05 mg/L, nitrate < 0.05 mg/L, nitrite < 0.50 mg/L, free chlorine < 0.1 mg/L, copper < 0.1 mg/L, zinc < 0.1, floride < 0.300 mg/L, nickel < 0.02, and selenium < 0.05.

Daily samples were taken from WWTPs between the periods of 06.00 am–10.00 am, 10.00 am–14.00 pm, 14.00 pm–18.00 pm within a week in summer season to observe the daily load routine of the wastewaters (Figure A.6). The loads were lowest during the 06.00 am–10.00 am period and were highest in 14.00 pm–18.00 pm period, indicating the effects of daily activity on the wastewaters in Istanbul.

The holiday seasons in Istanbul were also monitored. The reduced water consumption in the city (10 %) (ISKI, 2019) during holiday was an indication of the lessened population as well as the industrial activity. The mean, standart deviation, minimum and maximum concentration values of physicochemical parameters taken in holiday seasons, summer seasons, and all seasons through 2015–2019 are shown in Figures H.1, H.2 and H.3, respectively. The results showed that in holiday season the toxicity of the wastewaters of Istanbul was reduced due to paused industrial activity within city. The industrial activity in the city is found to exert stress on the quality of wastewaters. It is also important to mention the illegal discharges to sewage within in the city and the importance of tracking each party and ensure registration of each industrial activity. This may help to increase control over wastewater management

within the city, which would also help affirm the holistic approach in managing the wastewaters of Istanbul.

The relevant agencies are responsible to control and protect the health of the operation of the advanced treatment plant. Currently all industrial discharges are subjected to Regulation on Urban Wastewater Treatment as well as Regulation on Discharge of Wastewater to Sewage of ISKI. Regular monitoring of physicochemical analyses is very important. Monitoring the mentioned (RQ > 1) PSs in wastewaters can provide important information regarding their entrance into the receiving water environment. In addition, complementary assessment of the biological effects of the wastewaters would provide greater opportunity to integrate assessment of the wastewaters to the receiving water body.

5. CONCLUSIONS AND RECOMMENDATIONS

Istanbul is one of the mega cities that were formed as a result of global urban migration, with an estimated population over 16 million. It discharges 4,100,000 m^3/day of wastewater to the Sea of Marmara, the Black Sea and the Bosphorus. This research aimed to study deleterious compounds, the PSs, that are finally discharged to its seas. The aim also included assessment of the acute toxicity of those wastewaters. This research identified its scope to include the seven largest WWTPs in Istanbul, an LTP and a hospital wastewater.

This study established that wastewaters of Istanbul contained 48 PSs out of a total of 73 PSs. As a result of the risk estimation for PSs in the WWTP effluents, for the effluents of advance treatment, surveillance monitoring of endrin, endosulfan, diuron, alpha-cypermethrin, beta-cypermethrin, theta-cypermethrin, zeta-cypermethrin, quinoxyfen, aclonifen, bifenox, benzo-ghi-perylene, benzo(a)pyrene, fluoranthene, indeno (1,2,3-cd)-pyrene, tetrachloroethylene, DEHP, and C10-13-chloroalkanes is recommended.

For the effluents of MTF, surveillance monitoring of endrin, endolsulfan, diuron, alpha-cypermethrin, theta- cypermethrin, zeta-cypermethrin, aclonifen, bifenox, anthracene, benzo-ghi-perylene, benzo(k)fluoranthene, fluoranthene, indeno(1,2,3-cd)-pyrene, tetrachloroethylene, octylphenols, DEHP, and C10-13-chloroalkanes is recommended and special attention should be paid to investigate and control sources of PSs.

This thesis also included the estimation of the acute toxicity of WWTP influents and effluents in the seven largest WWTPs. Results revealed that advance treatment was effective in reducing the acute toxicity of wastewaters. Therefore, considering the health of the environment, the conversion of the mechanical treatment facilities to an advanced treatment is necessary to lessen the acute toxicity arriving at the receiving seas.

In addition to above mentioned aims, being community-based research, this study had important practical outcomes. The first ecotoxicity laboratory among water utilities of

Türkiye was established in 2017, after five years of hard work at ISKI. Also, after another five years of effort, the most advanced analytical water analysis laboratory was established in 2020. As a result, ISKI has since become equipped to monitor the selected priority substances, emerging contaminants and conduct ecotoxicity analyses of its wastewater influents and effluents, as well as in water sources. This may be considered the biggest practical contribution of this study. Consequently, ISKI can help implement the Turkish Regulation on Surface Water Quality by monitoring a list of the PSs present during surveillance monitoring in WWTP effluents in Istanbul and Türkiye. Having the said technical background, ISKI is also able to track the toxicity reduction evaluation tests in their WWTPs whenever needed.

Investigations in the occurences of PSs in active sludge, settleable particles, receiving coastal waters, wastewater overflows during rain events and surface waters are recommended to further understand the sources and cycles of the PSs in Istanbul. Once the main sources of PSs are defined special attention should be paid to those sources to control PSs. The quality of the PSs measurement in the aforemention spots shall be ensured by accredited laboratories of ISKI.

In the instances of low PS removal rates, upgrading of the treatment processes at the WWTPs should be considered. Ozone post treatment is recommended for WWTPs where the industrial discharges are involved, specifically at WWTP 1 and WWTP 3. The occurences of 17 organic compounds listed in the Watchlist List for European Union Monitoring (2015/495/EU) should also be investigated in wastewaters. It is highly recommended to conduct acute and chronic toxicity testing in WWTP effluents as well as upstream and downstream of the discharge points for efficient monitoring purposes. Findings in this study could be utilized by the regulators that are undertaking environmental risk assessments in the initiation of monitoring programs for the protection of the marine environment.

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APPENDICES

APPENDIX A : Additional information on the studied WWTPs

APPENDIX B : Additional information of the LTP and hospital wastewater

APPENDIX C : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS

APPENDIX D : Assay results of the PSs analyzed in seasonal, consecutive summer and winter influents and effluents

APPENDIX E : Assay results of the specific micropollutants analyzed in seasonal, consecutive summer and winter influents and effluents

APPENDIX F : Toxicological data for the selected PSs available in literature

APPENDIX G : Assay results of the COD (mg/L), BOD_5 (mg/L), TSS (mg/L), TP (mg/L) and pH analyses for WWTP 1–WWTP 7

APPENDIX H : Physicochemical assay results of holiday and regular seasons

APPENDIX I : Pictures related to sampling, experiments and laboratories

APPENDIX A : Additional information on the studied WWTPs

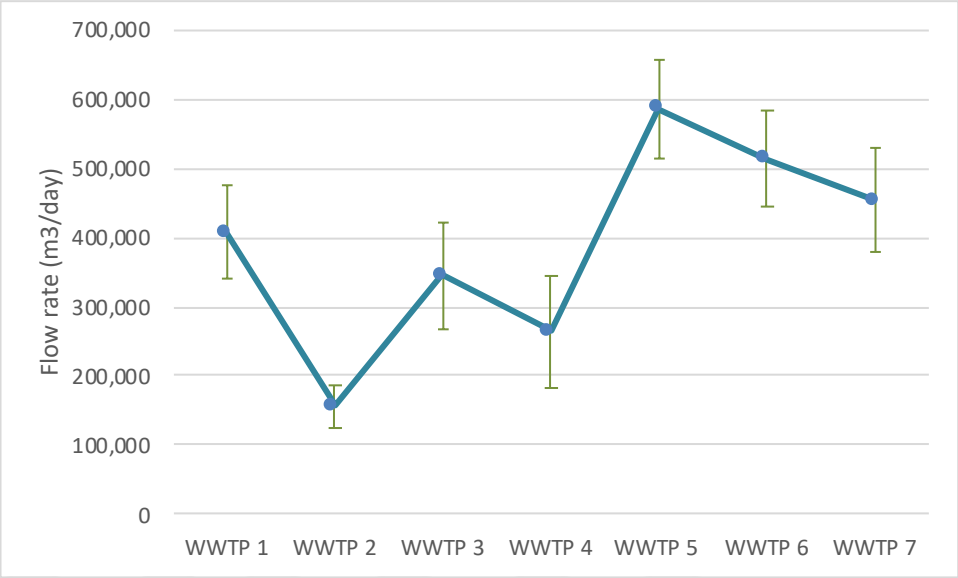


Figure A.1 : Flow rates (m^3/day) of the studied wastewaters of WWTP 1–WWTP7 (n = 27), bars indicate standard deviation.

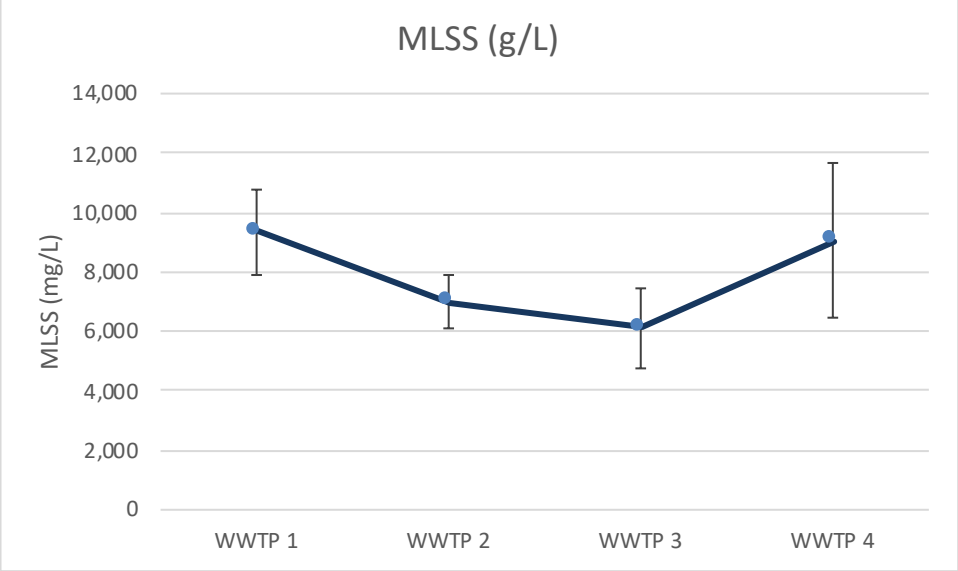


Figure A.2 : MLSS concentrations (mg/L) of the studied wastewaters of WWTP 1–WWTP 4 (n = 27), bars indicate standard deviation.

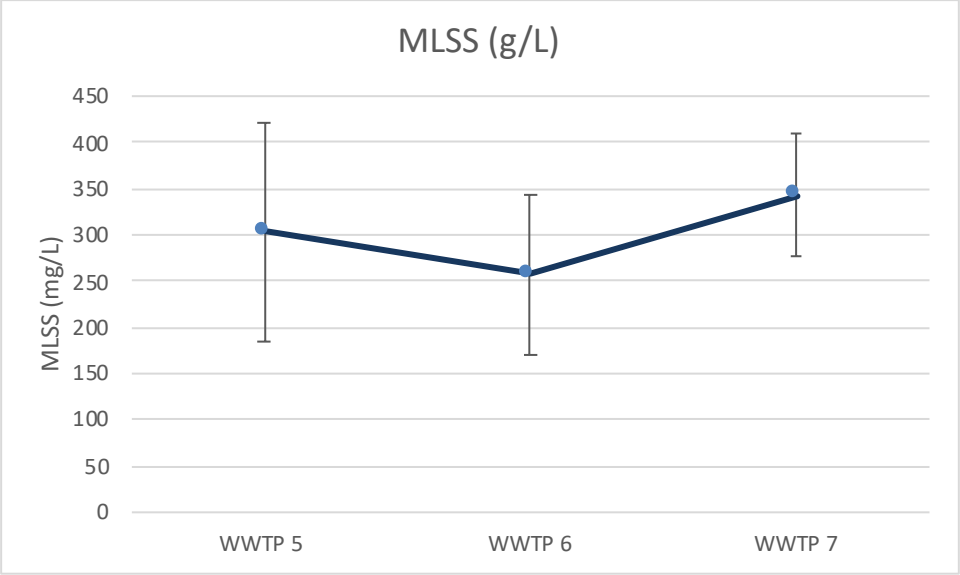


Figure A.3 : MLSS concentrations (mg/L) of the studied wastewaters of WWTP 5–WWTP 7 (n = 27), bars indicate standard deviation.

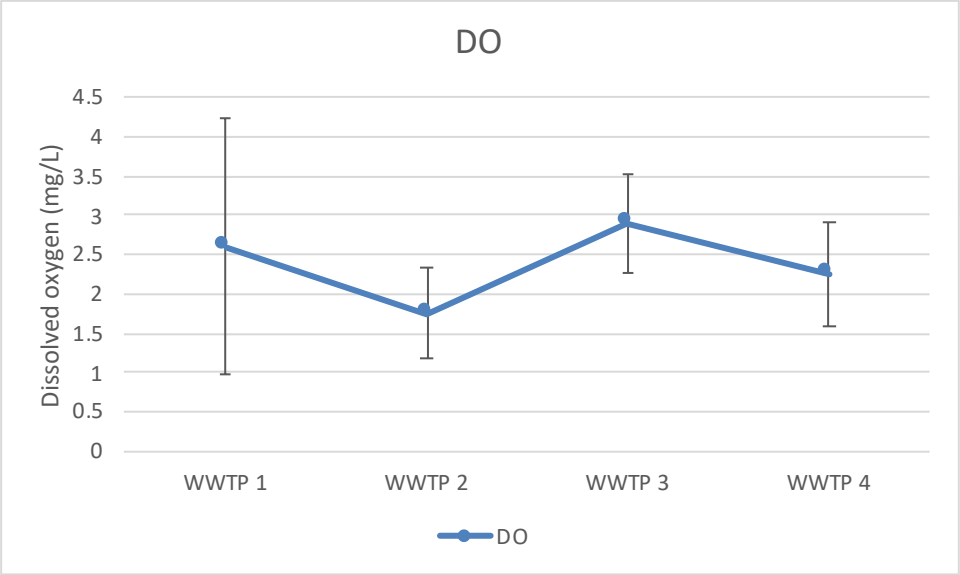


Figure A.4 : Dissolved oxygen (mg/L) concentrations of the aeration pools of WWTP 1–WWTP 4 (n = 27), bars indicate standard deviation.

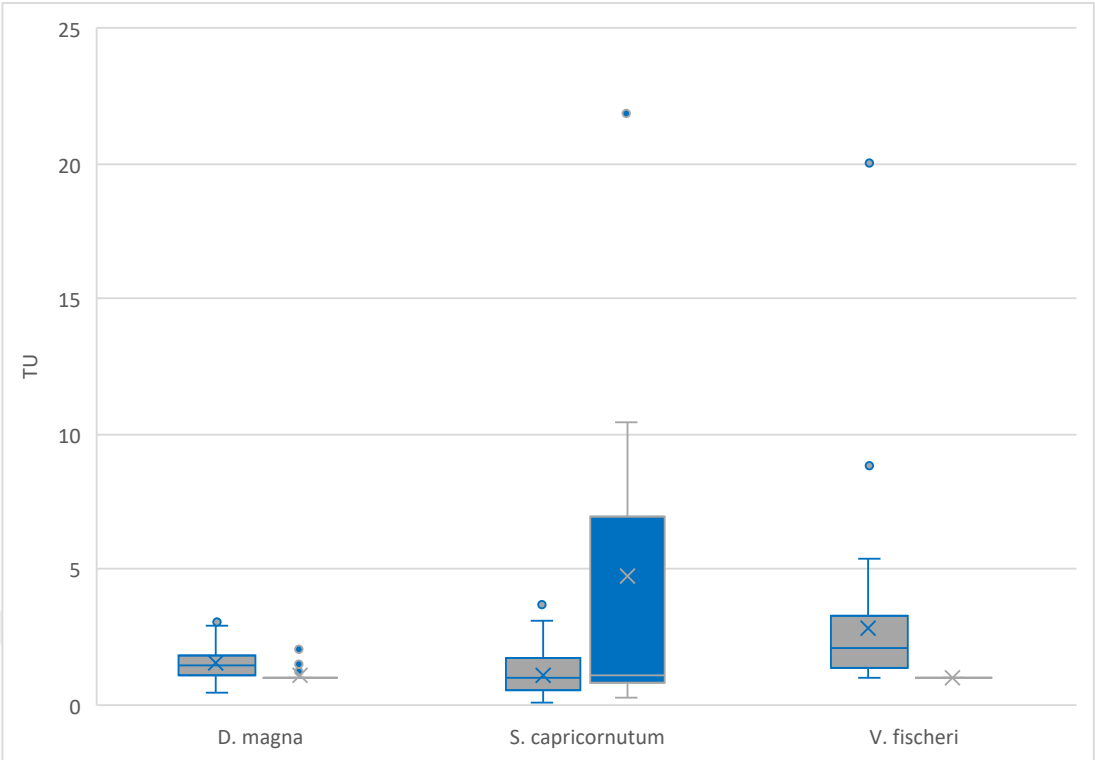


Figure A.5 : Boxplot for a range of estimated toxicity units (TU) of *D. magna, S. capricornutum*, and *V. fischeri* subjected to WWTP influents and effluents for n = 9 seasonal sampling.

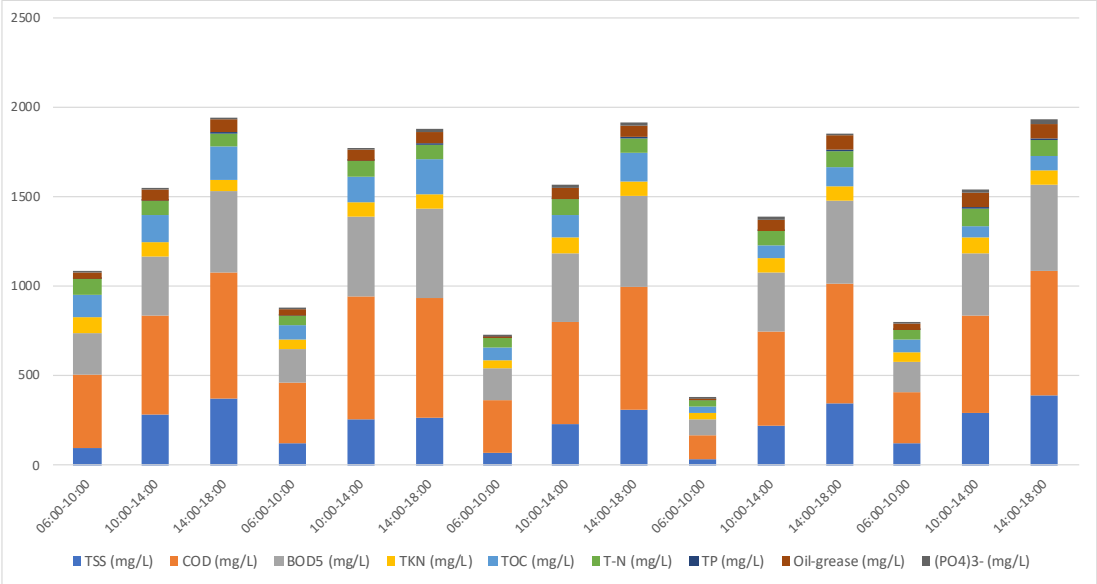


Figure A.6 : Stacked columns representing monitoring of daily total load of TSS (mg/L), COD (mg/L), BOD_5 (mg/L), TKN (mg/L), TN (mg/L), TOC (mg/L), TP (mg/L), $(PO_4)^{3-}$ (mg/L) and oil-grease (mg/L) in WWTP influent, n =105 for time periods of 06:00–10:00 am, 10:00 am–14:00 pm, and 14:00–18:00 pm, respectively.

APPENDIX B : Additional information on the LTP and hospital wastewater

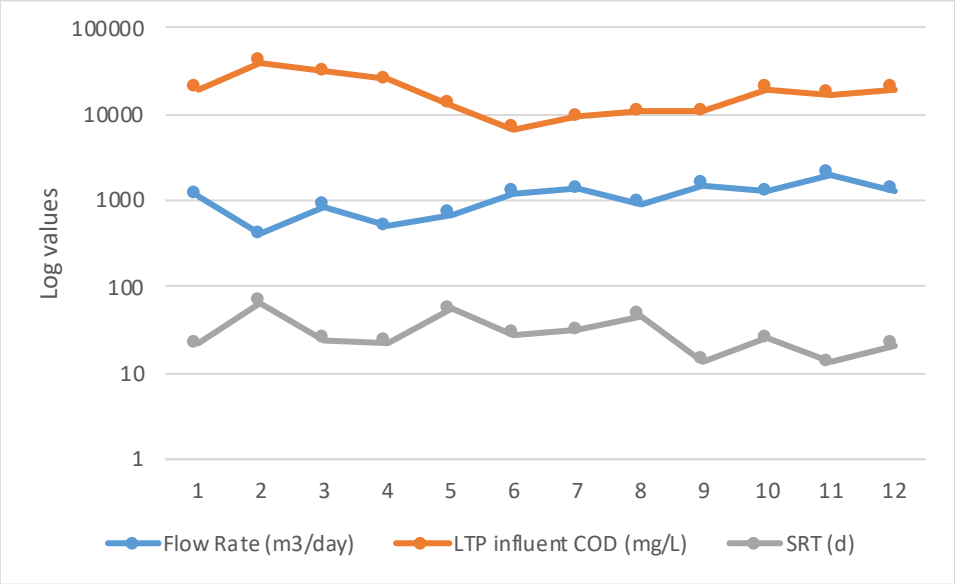


Figure B1 : Flow rate (m^3/day), COD(mg/L) and SRT (d) values of the LTP, X axis indicates sampling sequence.

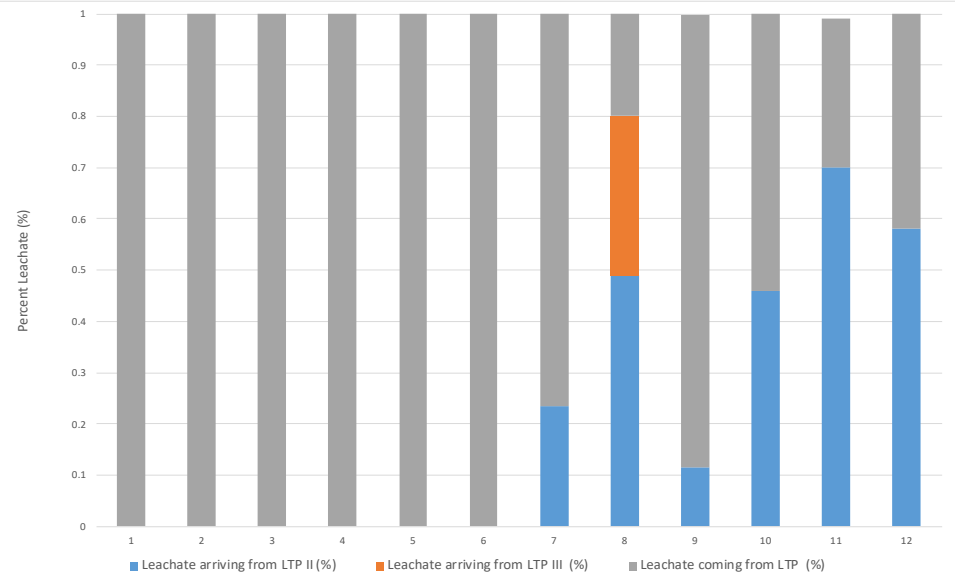


Figure B.2 : Percentage of leachate treated in LTP by origin of LTPs, X axis indicates sampling sequence; LTP II and LTP III indicates leachate arriving from other leachate treatment plants.

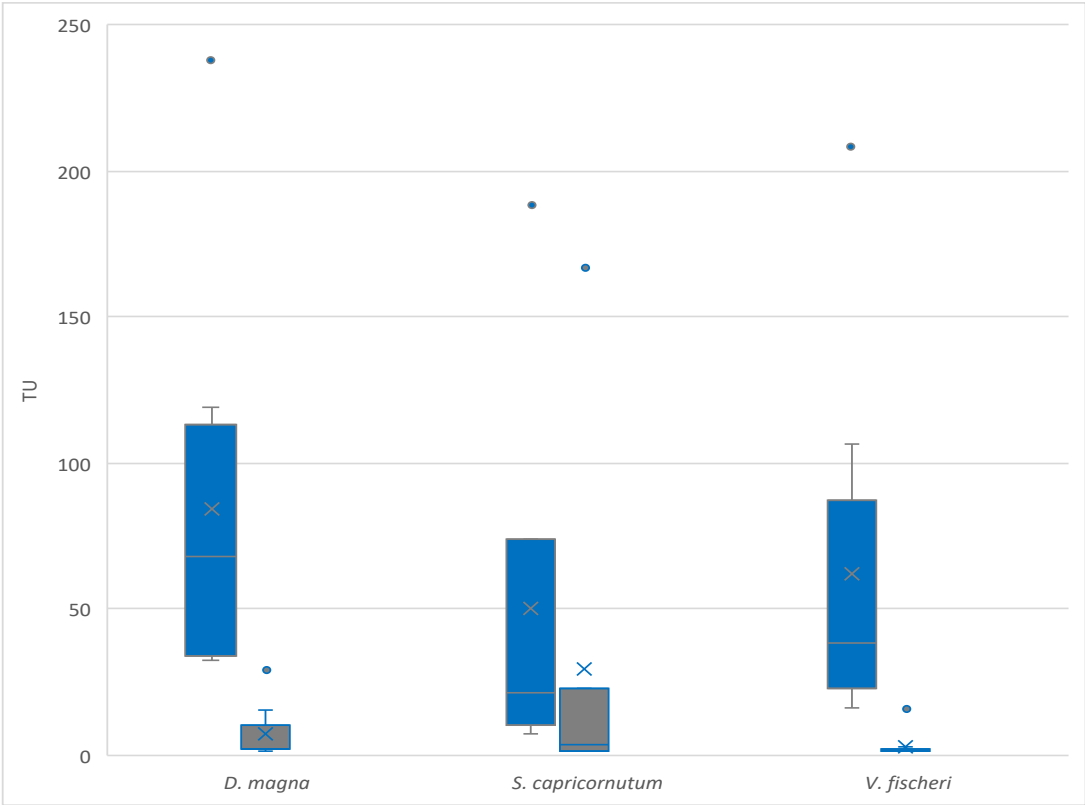


Figure B.3 : Boxplot for a range of estimated toxicity units (TU) of *D. magna*, *S. capricornutum* and *V. fischeri* subjected to LTP influents and effluents for n = 9 seasonal sampling

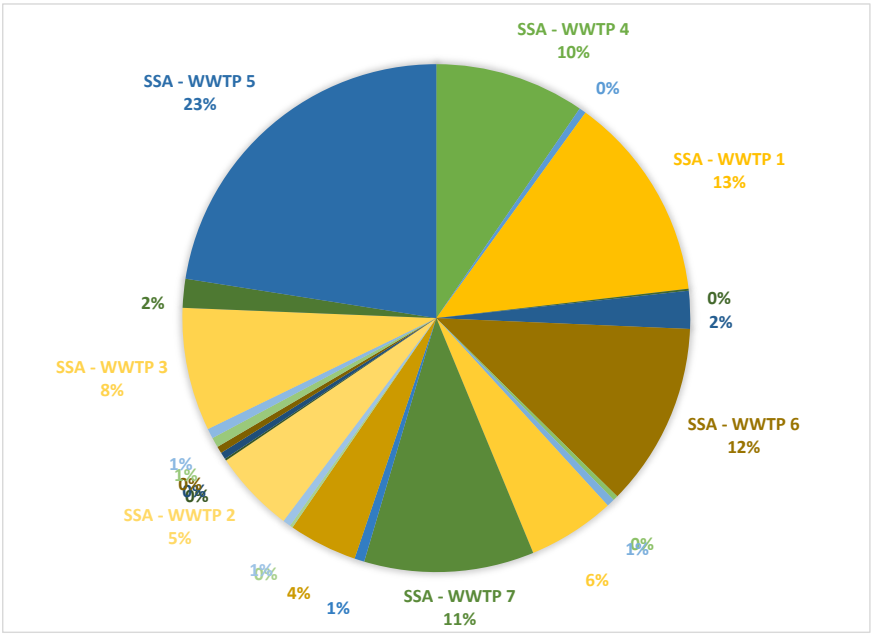


Figure B.4 : Percentage of healthcare facilities located in each sewage service area (SSA) of Istanbul including SSA WWTP 1–7.

APPENDIX C : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS.

Table C.1 : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	tR (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
Aldrin			262.9	192.9					
Aldrin	309-00-2	364.91	254.9	220	9.77	0,005–1	0.5	1.5	0.997
Aldrin			262.9	190.9					
Dieldrin			277	241					
Dieldrin	60-57-1	380.91	262.9	193	11.529	0,005–1	0.5	1.5	0.998
Dieldrin			262.9	191					
Endrin			262.8	193					
Endrin	72-20-8	380.91	244.8	173	11.862	0,005–1	0.5	1.5	0.998
Endrin			316.7	280.8					
Isodrin			193	123					
Isodrin	465-73-6	364.91	193	157	10.27	0,001–1	0.5	1.5	0.996
Isodrin			195	123					
Endosulfan			194.9	159					
Endosulfan	115-29-7	406.93	194.9	160	11.125	0,005–1	5	15	0.989
Endosulfan			194.9	125					
Heptachlor			271.7	236.9					
Heptachlor	76-44-8	373.32	273.7	238.9	9.183	0,001–1	0.1	0.3	0.99
Heptachlor			273.7	236.9					

Table C.1 (continued) : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	tR (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
Heptachlor epoxide			352.8	262.9					
Heptachlor epoxide	1024-57-3	389.32	354.8	264.9	10.513	0,001–1	0.1	0.3	0.995
Heptachlor epoxide			262.9	193					
Hexachlorobenzene	118-74-1	284.78	283.8	248.8	7.656	0,001–1	0.05	0.2	0.981
Hexachlorobenzene			281.8	211.9					
Hexachlorocyclohexane			216.9	181					
Hexachlorocyclohexane	608-73-1	290.85	218.9	183	7,52-8,36	0,001–1	0.3	0.9	0.986
Hexachlorocyclohexane			180.9	145					
Dicofol			139	111					
Dicofol	115-32-2	370.49	139	75.1	9.847	0,005–1	1	3	0.997
Dicofol			141	113					
DDT-p,p'			235	165.2					
DDT-p,p'	50-29-3	354.48	237	165.2	12.83		0.04	0.1	0.977
DDT-p,p'			235	199.2					
DDE-p,p'			246.1	176.2					
DDE-p,p'	72-55-9	318.02	315.8	246	11.437	0,001–1	0.04	0.1	0.977
DDE-p,p'			317.8	246					
DDD-p,p'			234.9	165.1					
DDD-p,p'	72-54-8	320.04	236.9	165.2	12.169		0.04	0.1	0.996
DDD-p,p'			234.9	199.1					

Table C.1 (continued) : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	tR (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
Trifluralin	1582-09-8	335.28	305	264	7.04	0,001–1	0.5	1.5	0.996
Trifluralin			264	160					
Cypermethrin alpha	52315-07-8	416.30	163.1	127.1	16.181-16.373	0,005–1	0.2	0.6	0.973
Cypermethrin beta			163.1	91					
Cypermethrin teta			165.1	91.1					
Cypermethrin zeta			181.2	152.1					
Chlorpyrifos	2921-88-2	350.58	196.9	169	9.775	0,005–1	1	3	0.976
Chlorpyrifos			198.9	171					
Chlorpyrifos			313.8	257.8					
Chlorpyrifos			313.8	285.8					
Tributyltin	36643-28-4	209	291	179	6.04	0,005–1	0.05	0.2	0.976
Tributyltin			291	235					
Tributyltin			289	177					
Tributyltin			207	123					
Anthracene	120-12-7	178.23	178.1	152.1	8.266	0,001–1	1	3	0.996
Anthracene			178.1	151.1					
Anthracene			176.1	150.1					
Benzo-ghi-perylene	191-24-2	276,34	138	137	19.339	0,001–1	0.1	0.3	0.994
Benzo-ghi-perylene			137	136					
Benzo-ghi-perylene			276.1	274.1					

Table C.1 (continued) : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	tR (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
Benzo-a-pyrene			252.1	250.1					
Benzo-a-pyrene	50-32-8	252.31	125	124.1	16.676	0,0001–1	0.1	0.3	0.996
Benzo-a-pyrene			250.1	248					
Benzo-b-fluoranthene			252.1	250.1					
Benzo-b-fluoranthene	205-99-2	252.31	126	113.1	16.041	0,001–1	0.1	0.3	0.996
Benzo-b-fluoranthene			250.1	248.1					
Benzo-k-fluoranthene			252.1	250.1					
Benzo-k-fluoranthene	207-08-9	252.31	126.1	113.1	16.089	0,001–1	0.1	0.3	0.998
Benzo-k-fluoranthene			250.1	248.1					
Fluoranthene			201.1	200.1					
Fluoranthene	206-44-0	202.25	202.1	152.1	10.51	0,001–1	0.3	0.9	0.996
Fluoranthene			200.1	174					
Indeno-123-cd-pyrene			137	136					
Indeno-123-cd-pyrene	193-39-5	276.33	276.1	274.1	18.892	0,001–1	0.2	0.6	0.996
Indeno-123-cd-pyrene			138.1	125.1					
4-Nonyl phenol			220	107					
4-Nonyl phenol	84852-15-3	220.39	107	77	8.492	0,001–1	0.1	0.3	0.964
4-Nonyl phenol			107	51					
t-octyl phenol			135	107					
t-octyl phenol	140-66-9	206.32	135	77	6.594	0,001–1	1	3	0.979
t-octyl phenol			107	77					

Table C.1 (continued) : CAS number, mass, characteristic precursor and product ions, retention time, LOD, LOQ, and correlation coefficient of the PSs analyzed by GC-MS/MS (Birtek et al., 2022).

Compound	CAS number	Mass (g/mol)	Precursor Ion	Product Ion	tR (min)	Linearity (µg/L)	LOD (ng/L)	LOQ (ng/L)	r^2
4-octyl phenol	1806-26-4	206.32	107	77	7.734	0,001–1	1	3	0.976
4-octyl phenol			107	51					
4-octyl phenol			206	107					
DEHP	117-81-7	390.56	149	65	14.133	0,001–1	1	3	0.956
DEHP			167	149					
DEHP			149	93					
DEHP			149	121					
Pentachlorobenzene	608-93-5	250.34	249.9	215	6.114	0,005–1	0.08	0.2	0.98
Pentachlorobenzene			248	213					
Pentachlorobenzene			251.9	217					
Chloroalkanes	85535-84-8	142	103	65	9.00-14.00	0,005–1	50	0.2	0.998
Chloroalkanes			102	67					
Chloroalkanes			91	53					
Chloroalkanes			89	53					

APPENDIX D : Assay results of the PSs analyzed in seasonal, consecutive summer and winter influents and effluents

Table D.1 : Assay results of the PSs analyzed in seasonal influents (n =28) and effluents (n =16): Median concentration (ng/L), range (min-max), and detection frequency (%).

		Seasonal					
		WWTP Influent			WWTP Effluent		
Pesticides	LOQ	Median	Range	D.F.	Median	Range	D.F.
Alachlor	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Aldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Dieldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endrin	1.5	68.5	5–110	43	68.5	6.5–157	25
Isodrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endosulfan	15.0	<	<–52	36	<	<–18	31
Heptachlor	0.3	n.d.	n.d.	0	n.d.	n.d.	0
Heptachlor epoxide	0.3	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobenzene	0.2	<	<–0.5	43	0.30	<–0.6	44
Hexachlorocyclohexane	0.9	1.15	<–21.2	71	2.00	<–4.9	63
Dicofol	3.0	n.d.	n.d.	0	n.d.	n.d.	0
p,p′-DDD	0.1	3.50	<–6	21	5.00	3–6	19
p,p'-DDE	0.1	<	<–2.5	36	<	<–0.1	38
p,p'-DDT	0.1	<	<–0.5	21	<	<	6
Pentachlorophenol	50.0	<	n.d.–<	4	n.d.	n.d.	0
Atrazine	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Simazine	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Cybutryne	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Terbutryn	10.0	<	<–66	14	<	<–34	19
Trifluralin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Diuron	10.0	<	<–79	64	18.00	<–60	81

Table D.1 (continued) : Assay results of the PSs analyzed in seasonal influents (n = 28) and effluents (n = 16): Median concentration (ng/L), range (min-max), and detection frequency (%).

		Seasonal					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Pesticides							
Isoproturon	25.0	n.d.	n.d.	0	n.d.	n.d.	0
alpha-cypermethrin	0.6	3.00	<–24	82	<	<2.0	19
beta-cypermethrin	0.6	1.80	<–16	46	<	<–18	25
theta-cypermethrin	0.6	2.15	<–11	79	3.15	1.6–4.7	13
zeta-cypermethrin	0.6	2.60	<–23	68	0.55	0.1–1.0	25
Chlorpyrifos	3.0	n.d.	n.d.	0	n.d.	n.d.	0
Chlorfenvinphos	10.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichlorvos	20.0	25.5	<–84	36	32.0	10–40	25
Tributyltin	0.2	<	<–<	11	<	<–<	31
Quinoxyfen	50.0	279.6	279.6–279.6	4	379.5	n.d.–379.5	6
Aclonifen	20.0	20.0	<–42	50	16.5	<–48	50
Bifenox	50.0	221.0	69–979	43	167.5	73–381	38
PAHs							
Anthracene	3.0	<	<–14	64	<	n.d.–<	6
Benzo-ghi-perylene	0.3	2.80	1.0–9	32	5.00	1–5	19
Benzo(a)pyrene	0.3	0.30	<–1	25	0.30	0.2–0.4	31
Benzo(b)fluoranthene	0.3	1.30	<–4.7	61	1.10	0.1–2.7	63
Benzo(k)fluoranthene	0.3	2.90	<–17.2	54	2.00	0.05–4.1	69
Fluoranthene	0.9	5.00	<–10.8	89	1.50	0.09–7.5	81
Indeno(1,2,3-cd)-pyrene	0.6	5.00	<–13	46	1.00	0.2–7	56
Naphthalene	30.0	35.00	<–1154	46	9.00	2–21	50

Table D.1 (continued) : Assay results of the PSs analyzed in seasonal influents (n = 28) and effluents (n = 16): Median concentration (ng/L), range (min-max), and detection frequency (%).

		Seasonal					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
VOCs							
Benzene	30.0	192.00	109–627	36	261.50	199–536	25
Carbon-tetrachloride	120.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichloromethane	60.0	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobutadiene	90.0	2,000	n.d.–2,000	4	571.0	n.d.–571	6
1,2,3-Trichlorobenzenes	60.0	7,100	n.d.–7,100	4	1,424	5 - 2,842	13
1,2,4-Trichlorobenzenes	30.0	2,113	626–3,600	7	1,230	1,016–1,444	13
1,3,5-Trichlorobenzenes	30.0	796.8	3.5–1,590	7	160.8	5.6–316	13
Tetrachloroethylene	60.0	250.5	118–3,695	50	312.5	120–1,929	38
Trichloroethylene	90.0	560.0	160–2,650	43	138.0	2.4–406	31
Trichloromethane	60.0	1,742	134–12,420	82	832.5	112–1,873	88
DLCs							
Dioxins	0.015	<	<–0.055	93	<	<	69
Dioxin-like compounds	0.030	<	<–1.713	93	<	<–0.71	88
PBDEs							
PBDE 28	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 47	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 99	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 100	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 153	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 154	15.0	n.d.	n.d.	0	n.d.	n.d.	0

Table D.1 (continued) : Assay results of the PSs analyzed in seasonal influents (n = 28) and effluents (n = 16): Median concentration (ng/L), range (min-max), and detection frequency (%).

		Seasonal					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Alkylphenols							
Nonylphenols	0.3	3.10	1.2–9.1	29	2.40	1.2–3.6	13
Octylphenols	3.0	22.8	1.1–151	29	10.0	<–17.4	19
Others							
DEHP	3.0	112	16–2,301	100	64.0	8.0–311.0	94
1,2-Dichloroethane	300.0	1,628	<–4,111	14	<	<	0
PFOS	30.0	41.0	n.d.–41	4	<	<	6
C10-13-chloroalkanes	150.0	179	<–1262	43	132.5	<–196	50
Pentachlorobenzene	0.2	0.50	<–1	50	0.75	<–3.8	50
α-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
β-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
γ-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0

LOQ: Limit of quantification (ng/L)
DF: Detection frequency (%)
<: Under the limit of quantification
PSs: Priority substances.
n.d.: Not detected

Table D.2 : Assay results of the PSs in consecutive summer WWTP influents (n = 19) and effluents (n = 15): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Summer					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Pesticides							
Alachlor	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Aldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Dieldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endrin	1.5	46.00	17–170	95	54.00	6–123	100
Isodrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endosulfan	15.0	<	<–30.3	95	<	<–37.3	80
Heptachlor	0.3	n.d.	n.d.	0	n.d.	n.d.	0
Heptachlor epoxide	0.3	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobenzene	0.2	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorocyclohexane	0.9	1.20	1.2–11.1	16	1.70	1–32	27
Dicofol	3.0	n.d.	n.d.	0	n.d.	n.d.	0
p,p′-DDD	0.12	1.30	1–4.1	16	1.10	1–1.5	20
p,p'-DDE	0.1	1.30	1.2–1.9	16	n.d.	n.d.	0
p,p'-DDT	0.1	n.d.	n.d.	0	n.d.	n.d.	0
Pentachlorophenol	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Atrazine	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Simazine	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Cybutryne	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Terbutryn	10.0	n.d.	n.d.	0	<	<–11	20
Trifluralin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Diuron	10.0	67.00	10–644	37	21.00	15–724	87

Table D.2 (continued) : Assay results of the PSs in consecutive summer WWTP influents (n =19) and effluents (n =15): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Summer					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Pesticides							
Isoproturon	25.0	n.d.	n.d.	0	n.d.	n.d.	0
alpha-cypermethrin	0.6	2.25	1.1–6.4	53	4.60	4.6–4.6	7
beta-cypermethrin	0.6	n.d.	n.d.	0	n.d.	n.d.	0
theta-cypermethrin	0.6	1.50	1–4.6	47	1.10	1.1–1.8	20
zeta-cypermethrin	0.6	3.60	2.7–3.8	16	2.75	1.4–4.9	27
Chlorpyrifos	3.0	n.d.	n.d.	0	n.d.	n.d.	0
Chlorfenvinphos	10.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichlorvos	20.0	<	<–28.1	16	<	<–<	27
Tributyltin	0.2	n.d.	n.d.	0	n.d.	n.d.	0
Quinoxyfen	50.0	<	<	32	<	<–<	20
Aclonifen	20.0	<	<–42.1	95	<	<–57.2	100
Bifenox	50.0	127	<–357	90	174	<–276	100
PAHs							
Anthracene	3	<	<–7.1	42	n.d.	n.d.	0
Benzo-ghi-perylene	0.3	9.6	1–18.1	11	1.10	n.d.–1.1	7
Benzo(a)pyrene	0.3	3.60	2–152.1	63	2.80	<–8.8	33
Benzo(b)fluoranthene	0.3	1.75	1–33.7	63	1.40	1.2–2	27
Benzo(k)fluoranthene	0.3	2.00	1.1–11.6	90	2.10	1.3–3.4	87
Fluoranthene	0.9	3.60	1.3–37.5	90	2.05	1.1–2.8	80
Indeno(1,2,3-cd)-pyrene	0.6	22.9	22.9–22.9	5	n.d.	n.d.	0
Naphthalene	30	n.d.	n.d.	0	n.d.	n.d.	0

Table D.2 (continued) : Assay results of the PSs in consecutive summer WWTP influents (n = 19) and effluents (n = 15): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Summer					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
VOCs							
Benzene	30.0	349	250–448	11	153	n.d.–153	7
Carbon-tetrachloride	120.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichloromethane	60.0	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobutadiene	90.0	n.d.	n.d.	0	n.d.	n.d.	0
1,2,3-Trichlorobenzenes	60.0	n.d.	n.d.	0	n.d.	n.d.	0
1,2,4-Trichlorobenzenes	30.0	n.d.	n.d.	0	n.d.	n.d.	0
1,3,5-Trichlorobenzenes	30.0	2272	2,083–2,460	11	n.d.	n.d.	0
Tetrachloroethylene	60.0	703	102.0–4,145	79	374	103–2,312	33
Trichloroethylene	90.0	635	170–19,107	84	696	126–2,150	53
Trichloromethane	60.0	1859	641–3,891	37	608	464–1,124	87
DLCs							
Dioxins	0.015	0.05	<5–0.420	100	<	<–0.097	100
Dioxin-like compounds	0.030	0.62	0.21–2.49	100	0.35	0.13–1.39	100
PBDEs							
PBDE 28	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 47	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 99	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 100	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 153	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 154	15.0	n.d.	n.d.	0	n.d.	n.d.	0

Table D.2 (continued) : Assay results of the PSs in consecutive summer WWTP influents (n = 19) and effluents (n = 15): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Summer					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Alkylphenols							
Nonylphenols	0.3	9.50	1.4–21.1	79	2.40	1.1–8.9	33
Octylphenols	3.0	110.25	25.9–204.7	63	18.20	14.6–21.8	13
Others							
DEHP	3.0	127	54–1991	90	43.0	26.0–222	60
1,2-Dichloroethane	300.0	782	n.d.–782	5	582	n.d.–582	7
PFOS	30.0	<	<–<	47	<	n.d.–<	7
C10-13-chloroalkanes	150.0	n.d.	n.d.	0	n.d.	n.d.	0
Pentachlorobenzene	0.2	n.d.	n.d.	0	n.d.	n.d.	0
α-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
β-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
γ-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0

LOQ: Limit of detection (ng/L)

<: Under the limit of quantification

PSs: Priority substances.

n.d.: Not detected

Table D.3 : Assay results of the PSs analyzed in consecutive winter WWTP influents (n = 12) and effluents (n = 9): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Winter					
		WWTP Influent			WWTP Effluent		
Pesticides	LOQ	Median	Range	D.F.	Median	Range	D.F.
Alachlor	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Aldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Dieldrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Isodrin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Endosulfan	15.0	n.d.	n.d.	0	n.d.	n.d.	0
Heptachlor	0.3	2	2–3	42	3.00	2–3	56
Heptachlor epoxide	0.3	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobenzene	0.2	<	<–0.2	100	<	<–0.2	100
Hexachlorocyclohexane	0.9	3.90	1.0–16.0	100	3.50	1.8–20	100
Dicofol	3.0	n.d.	n.d.	0	n.d.	n.d.	0
p,p′-DDD	0.1	n.d.	n.d.	0	n.d.	n.d.	0
p,p'-DDE	0.1	<	<	58	<	<	67
p,p'-DDT	0.1	0.50	<–0.5	50	0.50	<–0.5	22
Pentachlorophenol	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Atrazine	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Simazine	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Cybutryne	25.0	n.d.	n.d.	0	n.d.	n.d.	0
Terbutryn	10.0	<	<–<	83	<	<–<	100
Trifluralin	1.5	n.d.	n.d.	0	n.d.	n.d.	0
Diuron	10.0	n.d.	n.d.	0	n.d.	n.d.	0

Table D.3 (continued) : Assay results of the PSs analyzed in consecutive winter WWTP influents (n = 12) and effluents (n = 9): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Winter					
		WWTP Influent			WWTP Effluent		
Pesticides	LOQ	Median	Range	D.F.	Median	Range	D.F.
Isoproturon	25.0	n.d.	n.d.	0	n.d.	n.d.	0
alpha-cypermethrin	0.6	19.0	17–23	75	16.0	n.d.–16	11
beta-cypermethrin	0.6	16.0	15–24	42	14.0	n.d.–14	11
theta-cypermethrin	0.6	17.0	15–25	58	15.0	n.d.–15	11
zeta-cypermethrin	0.6	16.0	15–17	100	16.0	16–16	56
Chlorpyrifos	3.0	n.d.	n.d.	0	n.d.	n.d.	0
Chlorfenvinphos	10.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichlorvos	20.0	n.d.	n.d.	0	n.d.	n.d.	0
Tributyltin	0.2	n.d.	n.d.	0	n.d.	n.d.	0
Quinoxyfen	50.0	n.d.	n.d.	0	n.d.	n.d.	0
Aclonifen	20.0	67.0	<–109	25	<	<	33
Bifenox	50.0	n.d.	n.d.	0	n.d.	n.d.	0
PAHs							
Anthracene	3.0	5.00	<–20	42	0.30	n.d.- 0.3	11
Benzo-ghi-perylene	0.3	<	<–0.3	25	<	<–<	22
Benzo(a)pyrene	0.3	<	<–<	25	<	<–0.3	33
Benzo(b)fluoranthene	0.3	0.30	<–0.4	42	0.40	0.3–0.9	33
Benzo(k)fluoranthene	0.3	<	<–0.3	42	0.30	0.3–0.8	33
Fluoranthene	0.9	12.0	7–18	100	7.00	7–9	100
Indeno(1,2,3-cd)-pyrene	0.6	<	<–<	25	<	<–<	56
Naphthalene	30.0	109	<–366.0	100	47.00	<–189	100

Table D.3 (continued) : Assay results of the PSs analyzed in consecutive winter WWTP influents (n = 12) and effluents (n = 9): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Winter					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
VOCs							
Benzene	30.0	n.d.	n.d.	0	n.d.	n.d.	0
Carbon-tetrachloride	120.0	n.d.	n.d.	0	n.d.	n.d.	0
Dichloromethane	60.0	n.d.	n.d.	0	n.d.	n.d.	0
Hexachlorobutadiene	90.0	n.d.	n.d.	0	n.d.	n.d.	0
1,2,3-Trichlorobenzenes	60.0	n.d.	n.d.	0	n.d.	n.d.	0
1,2,4-Trichlorobenzenes	30.0	n.d.	n.d.	0	n.d.	n.d.	0
1,3,5-Trichlorobenzenes	30.0	n.d.	n.d.	0	n.d.	n.d.	0
Tetrachloroethylene	60.0	948	469–2,464	75	326	215–769	67
Trichloroethylene	90.0	724	242–7,989	100	287	109–3,670	78
Trichloromethane	60.0	2076	1,048–7,989	100	1140	410–3,670	100
DLCs							
Dioxins	0.015	<	<	92	<	<	89
Dioxin-like compounds	0.030	0.31	0.154–1.27	100	0.31	0.163–1.636	100
PBDEs							
PBDE 28	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 47	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 99	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 100	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 153	15.0	n.d.	n.d.	0	n.d.	n.d.	0
PBDE 154	15.0	n.d.	n.d.	0	n.d.	n.d.	0

Table D.3 (continued) : Assay results of the PSs analyzed in consecutive winter WWTP influents (n = 12) and effluents (n = 9): LOQ, median concentration (ng/L), range (min-max), and detection frequency (%).

		Winter					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Alkylphenols							
Nonylphenols	0.3	23.5	21–26	67	22.0	22–25	78
Octylphenols	3.0	n.d.	n.d.	0	n.d.	n.d.	0
Others							
DEHP	3.0	76.5	59.0–369	100	86.0	28–215	100
1,2-Dichloroethane	300.0	n.d.	n.d.	0	n.d.	n.d.	0
PFOS	30.0	n.d.	n.d.	0.	n.d.	n.d.	0
C10-13-chloroalkanes	150.0	<	<	8	n.d.	n.d.	0
Pentachlorobenzene	0.2	<	<–0.2	100	0.20	<–0.7	100
α-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
β-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0
γ-HBCDD	50.0	n.d.	n.d.	0	n.d.	n.d.	0

LOQ: Limit of quantification (ng/L)
DF: Detection frequency (%)
<: Under the limit of quantification
PSs: Priority substances.
n.d.: Not detected

Table D.4 : Assay results of the metals analyzed in seasonal WWTP influents (n = 28) and effluents (n = 16), consecutive summer WWTP influents (n = 20) and effluents (n = 15), consecutive winter influents (n = 12) and effluents (n = 9): Median Concentration (µg/L), range (min–max), and detection frequency (%).

		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Seasonal							
Cd	0.01	0.20	0.05–2.73	61	0.07	0.028–0.11	56
Pb	0.04	1.88	0.77–18.9	82	1.60	0.71–1.97	94
Ni	0.07	44.6	9.62–267	89	90.3	40.6–123	94
Hg	0.10	0.24	0.15–0.27	21	0.20	0.19–0.33	19
Summer							
Cd	0.01	0.06	0.05–0.29	37	0.12	0.05–0.27	60
Pb	0.04	1.11	0.83–2.89	100	0.90	0.7–1.45	7
Ni	0.07	43.9	23.3–148.5	100	63.10	43.8–94	100
Hg	0.10	0.21	0.16–0.25	16	0.16	0.15–0.37	20
Winter							
Cd	0.01	0.10	0.05–0.32	67	0.09	0.06–0.24	78
Pb	0.04	1.42	1.01–2.81	100	0.94	0.6–1.58	100
Ni	0.07	33.8	20.7–152	100	74.7	42.2–126	100
Hg	0.10	0.41	0.19–2.16	83	0.26	0.13–1.25	89

Seasonal: Samples taken in four seasons.

Summer: Samples taken in five consecutive summer days.

Winter: Samples taken in three consecutive winter days.

D.F.: Detection frequency.

LOQ: Limit of quantification (µg/L).

APPENDIX E : Assay results of the specific micropollutants analyzed in seasonal, consecutive summer and winter influents and effluents

Table E.1 : Assay results of the specific micropollutants analyzed in seasonal influents (n = 28) and effluents (n = 16): Median concentration (ng/L), range (min-max), and detection frequency (%).

		Seasonal					
		WWTP Influent			WWTP Effluent		
	LOQ	Median	Range	D.F.	Median	Range	D.F.
Specific VOCs							
Toluene	30	5,864	422.0–80,369	64	3084.0	1,284–2,553	43.8
o-Xylene	30	398	115.0–3,841	43	n.d.	n.d.	0
m-Xylene	30	359	115.0–9,902	71	203.0	143.0–172.0	31.3
p-Xylene	30	350	100.0–7,676	68	204.0	124.0–162.5	31.3
1,2,4-Trimethylbenzene	30	299	107.0–13,276	46	327.5	n.d.–209	12.5
1,3,5-Trimethylbenzene	30	327	122.0–4,101	29	114.0	n.d.	6.3
1,3-Dichlorobenzene	30	175	103.0–249.0	25	112.5	n.d.–103	12.5
1,4-Dichlorobenzene	30	186	104.0–627.0	57	165.0	109.0–119.0	31.3
Ethylbenzene	30	202	22.0–1,379	29	466.0	n.d.–160	12.5
Isopropylbenzene	30	116	n.d.	4	n.d.	n.d.	0
n-Propylbenzene	60	701	215.0–1,187	7	n.d.	n.d.	0

LOQ: Limit of quantification (ng/L).
<: Under the limit of quantification.
n.d.: Not detected.

Table E.2 : Assay results of the specific micropollutants analyzed in consecutive summer WWTP influents (n = 19) and effluents (n = 9): LOQ, median concentration (ng/L), range (min–max), and detection frequency (%).

		Summer					
		WWTP Influent			WWTP Effluent		
Specific VOCs	LOQ	Median	Range	D.F.	Median	Range	D.F.
Toluene	30	n.d.	n.d.	0	n.d.	n.d.	0
o-Xylene	30	1,131	97–16,060	60	161	n.d.–161	7
m-Xylene	30	3,255	107.0–62,713	70	224	n.d.–224	7
p-Xylene	30	2,525	188.0–48,671	70	355	289–421	13
1,2,4-Trimethylbenzene	30	546.0	118.0–3,763	85	553	n.d.–553	7
1,3,5-Trimethylbenzene	30	284.0	115.0–1,007	40	110	n.d.–110	7
1,3-Dichlorobenzene	30	n.d.	n.d.	0	n.d.	n.d.	0
1,4-Dichlorobenzene	30	247.0	118.0–573.0	90	144	132–321	40
Ethylbenzene	30	5,801	119.0–29,382	30	n.d.	n.d.	0
Isopropylbenzene	30	118.5	115.0–122.0	10	n.d.	n.d.	0
n-Propylbenzene	60	116.0	107.0–279.0	25	n.d.	n.d.	0

LOQ: Limit of quantification (ng/L).

<: Under the limit of quantification.

n.d.: Not detected.

Table E.3 : Assay results of the specific micropollutants analyzed in consecutive winter WWTP influents (n = 12) and effluents (n = 9): LOQ, median concentration (ng/L), range (minmax), and detection frequency (%).

		Winter					
		WWTP Influent			WWTP Effluent		
Specific VOCs	LOQ	Median	Range	D.F.	Median	Range	D.F.
Toluene	30	3739	1,054–12,964	100	432	158–1001	100
o-Xylene	30	378	110.0–4,427	83	n.d.	n.d.	0
m-Xylene	30	454	127,0–4,380	83	n.d.	n.d.	0
p-Xylene	30	530.5	177,0–9,027	83	n.d.	n.d.	0
1,2,4-Trimethylbenzene	30	867.5	262.0–8,254	67	n.d.	n.d.	0
1,3,5-Trimethylbenzene	30	225.0	114.0–1,358	75	n.d.	n.d.	0
1,3-Dichlorobenzene	30	357.0	126.0–2,599	92	147	105–202	56
1,4-Dichlorobenzene	30	326.0	134.0–2,752	100	156	105–214	78
Ethylbenzene	30	465.0	107.0–2,982	50	n.d.	n.d.	0
Isopropylbenzene	30	171.0	n.d.–171.0	8	n.d.	n.d.	0
n-Propylbenzene	60	217.0	101.0–633.0	25	n.d.	n.d.	0

LOQ: Limit of quantification (ng/L).

<: Under the limit of quantification.

n.d.: Not detected.

APPENDIX F

Table F.1 : Toxicological data for the selected PSs available in literature: CAS number, corresponding most sensitive species, endpoint, PNECs (PNECs were gathered through environmental toxicity data available in literature and ECOSAR), calculated RQs for seasonal (RQseas), winter (RQw) and summer (RQs) analyses results, and references. The lowest toxicological value was divided per 1,000*.

Chemicals	CAS number	Most sensitive species	Endpoint	PNEC (ng/L)	RQseas	RQw	RQs	Reference
Endrin	72-20-8	Fish	EC50-mortality	4.20	37.38	29.29	0.00	(Zeng et al., 2018)
Endosulfan	115-29-7	Fish	EC50-mortality	0.69	26.09	54.06	0.00	(Lemke, A. E., 1981)
Heptachlor	76-44-8	Fish	EC50-mortality	27.00	0.00	0.00	0.11	(Zeng et al., 2018)
Hexachlorobenzene	118-74-1	Daphnia	EC50-immobilization	65.00	0.01	0.00	0.00	ECOSAR
Hexachlorocyclohexane	608-73-1	Fish	EC50-mortality	1300.00	0.00	0.02	0.02	(Canton et al., 1978)
p,p′-DDD	72-55-9	Daphnia	EC50-immobilization	9.00	0.67	0.17	0.00	(Zeng et al., 2018)
p,p'-DDE	72-55-9	Daphnia	EC50-immobilization	0.10	1.00	0.00	0.00	(Zeng et al., 2018)
p,p'-DDT	50-29-3	Daphnia	EC50-immobilization	9.00	0.01	0.00	0.06	(Zeng et al., 2018)
Terbutryn	212-950-5	Fish	EC50-mortality	820.00	0.04	0.01	0.00	(Mayer & Ellersieck, 1986)
Diuron	330-54-1	Algae	EC50-growth inhibition	2.70	22.22	268.1	0.00	ECOSAR
alpha-cypermethrin	67375-30-8	Fish	EC50-mortality	0.16	12.20	0.00	0.00	ECOSAR
beta-cypermethrin	65731-84-2	Fish	EC50-mortality	8.50	2.12	0.00	0.00	ECOSAR
theta-cypermethrin	71697-59-1	Fish	EC50-mortality	0.36	13.20	0.00	0.00	ECOSAR
zeta-cypermethrin	52315-07-8	Fish	EC50-mortality	0.03	33.33	163.3	533.3	ECOSAR

Table F.1 (continued): Toxicological data for the selected PSs available in literature: CAS number, corresponding most sensitive species, endpoint, PNECs (PNECs were gathered through environmental toxicity data available in literature and ECOSAR), calculated RQs for seasonal (RQseas), winter (RQw) and summer (RQs) analyses results, and references. The lowest toxicological value was divided per 1,000*.

Chemicals	CAS number	Most sensitive species	Endpoint	PNEC (ng/L)	RQseas	RQw	RQs	Reference
Dichlorvos	62-73-7	Daphnia	EC50-immobilization	93.92	0.43	0.00	0.00	(EC, 2011c)
Quinoxyfen	124495-18-7	Daphnia	EC50-immobilization	27.00	14.06	0.00	0.00	(EC, 2011b, ECOSAR)
Aclonifen	74070-46-5	Fish	EC50-mortality	7.86	6.11	7.28	0.00	ECOSAR
Bifenox	42576-02-3	Algae	EC50-growth inhibition	1.50	254.0	184.0	0.00	(EC, 2011a)
Anthracene	120-12-7	Daphnia	EC50-immobilization	3.90	0.00	0.00	0.00	(ECHA, 2011)
Benzo-ghi-perylene	191-24-2	Daphnia	EC50-immobilization	0.13	37.70	0.00	0.00	ECOSAR
Benzo(a)pyrene	50-32-8	Algae	EC50-growth inhibition	0.98	0.41	8.97	0.31	(EC, 2011d)
Benzo(b)fluoranthene	205-99-2	Daphnia	EC50-immobilization	22.30	0.12	0.09	0.04	ECOSAR
Benzo(k)fluoranthene	207-08-9	Daphnia	EC50-immobilization	4.57	0.90	0.74	0.18	ECOSAR
Fluoranthene	206-44-0	Daphnia	EC50-immobilization	3.98	1.88	0.70	2.26	ECOSAR
Indeno(1,2,3-cd)-pyrene	193-39-5	Daphnia	EC50-immobilization	1.16	6.03	0.00	0.00	ECOSAR
Naphthalene	91-20-3	Daphnia	EC50-immobilization	1960	0.00	0.00	0.10	(Verbruggen, 2012)
Benzene	71-43-2	Fish	EC50-mortality	11639	0.05	0.00	0.00	ECOSAR

Table F.1 (continued): Toxicological data for the selected PSs available in literature: CAS number, corresponding most sensitive species, endpoint, PNECs (PNECs were gathered through environmental toxicity data available in literature and ECOSAR), calculated RQs for seasonal (RQseas), winter (RQw) and summer (RQs) analyses results, and references. The lowest toxicological value was divided per 1,000.

Chemicals	CAS number	Most sensitive species	Endpoint	PNEC (ng/L)	RQseas	RQw	RQs	Reference
1,2,3-Trichlorbenzenes	12002-48-1	Daphnia	EC50-immobilization	350.0	8.12	0.00	0.00	(Calamari et al., 1983; Friberg et al., 2005)
1,2,4-Trichlorbenzenes	12002-48-1	Algae	EC50-growth inhibition	1170.0	1.23	0.00	0.00	(Calamari et al., 1983)
1,3,5-Trichlorbenzenes	12002-48-1	Algae	EC50-growth inhibition	900.0	0.35	0.00	0.00	(Calamari et al., 1983)
Tetrachloroethylene	127-18-4	Daphnia	EC50-immobilization	663.3	2.91	3.49	1.16	(EC, 2005)
Trichloroethylene	79-01-6	Daphnia	EC50-immobilization	10774	0.04	0.20	0.34	(Niederlehner et al., 1998)
Trichloromethane	67-66-3	Fish	EC50-mortality	18300	0.10	0.06	0.20	ECOSAR
Nonylphenols	84852-15-3	Algae	EC50-growth inhibition	330.0	0.01	0.03	0.08	ECOSAR
Octylphenols	140-66-9	Algae	EC50-growth inhibition	48.20	0.36	0.45	0.00	ECOSAR
DEHP	117-81-7	Algae	EC50-growth inhibition	2.50	124.4	88.80	86.00	ECOSAR
C10-13-chloroalkanes	85535-84-8	Daphnia	EC50-immobilization	43.00	4.56	0.00	0.00	ECOSAR
Pentachlorobenzene	608-93-5	Daphnia	EC50-immobilization	10.01	0.38	0.00	0.07	ECOSAR

* The lowest toxicological value was divided by an uncertainty factor of 1,000 due to differences in species sensitivity, acute to chronic and lab to field ratio.

APPENDIX G : Assay results of the COD (mg/L), BOD5 (mg/L), TSS (mg/L), TP (mg/L) and pH analyses for WWTP 1–WWTP 7

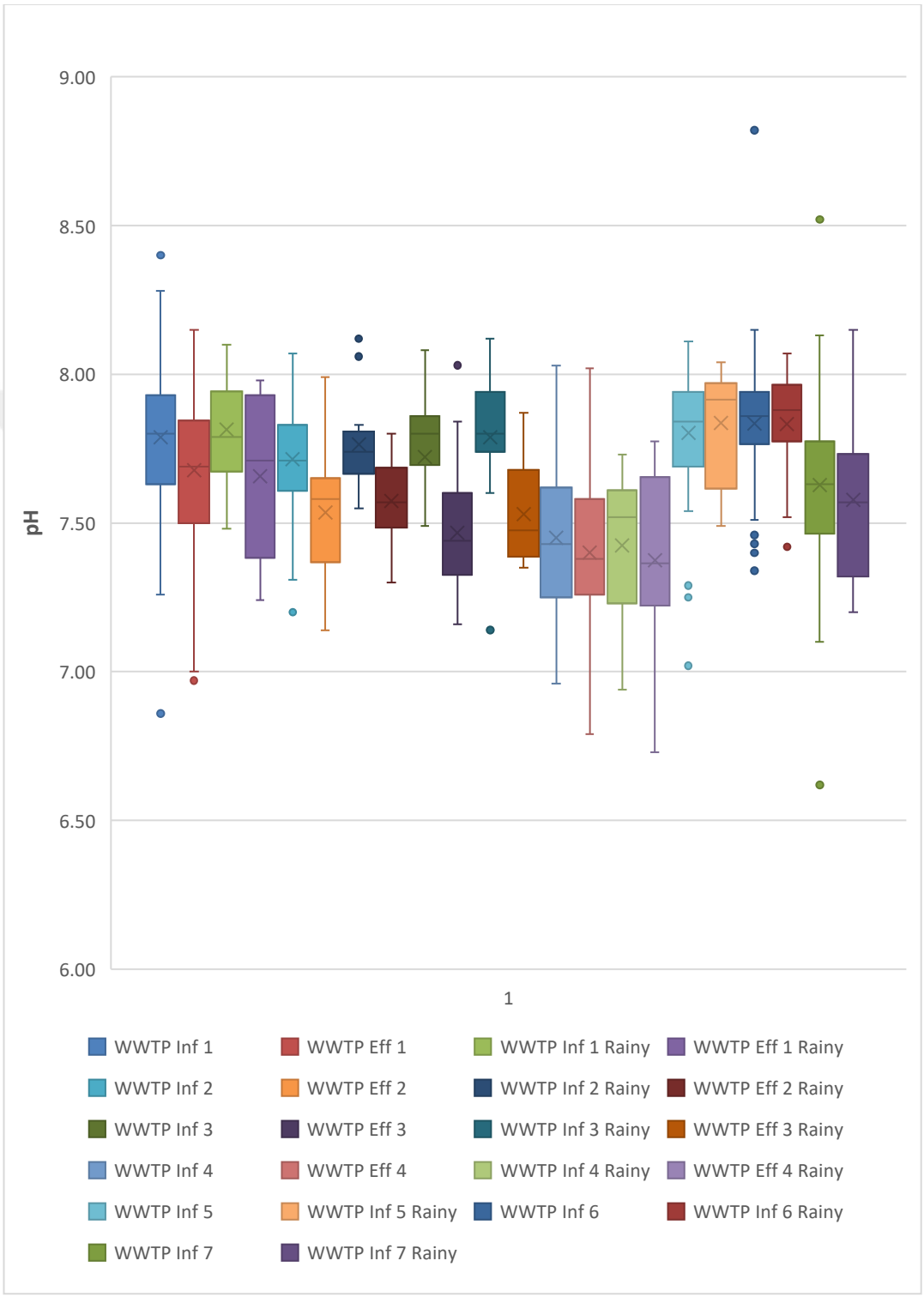


Figure G.1 : Boxplots for pH levels in each WWTP influent and effluent in dry and rainy days, outliers are excluded.

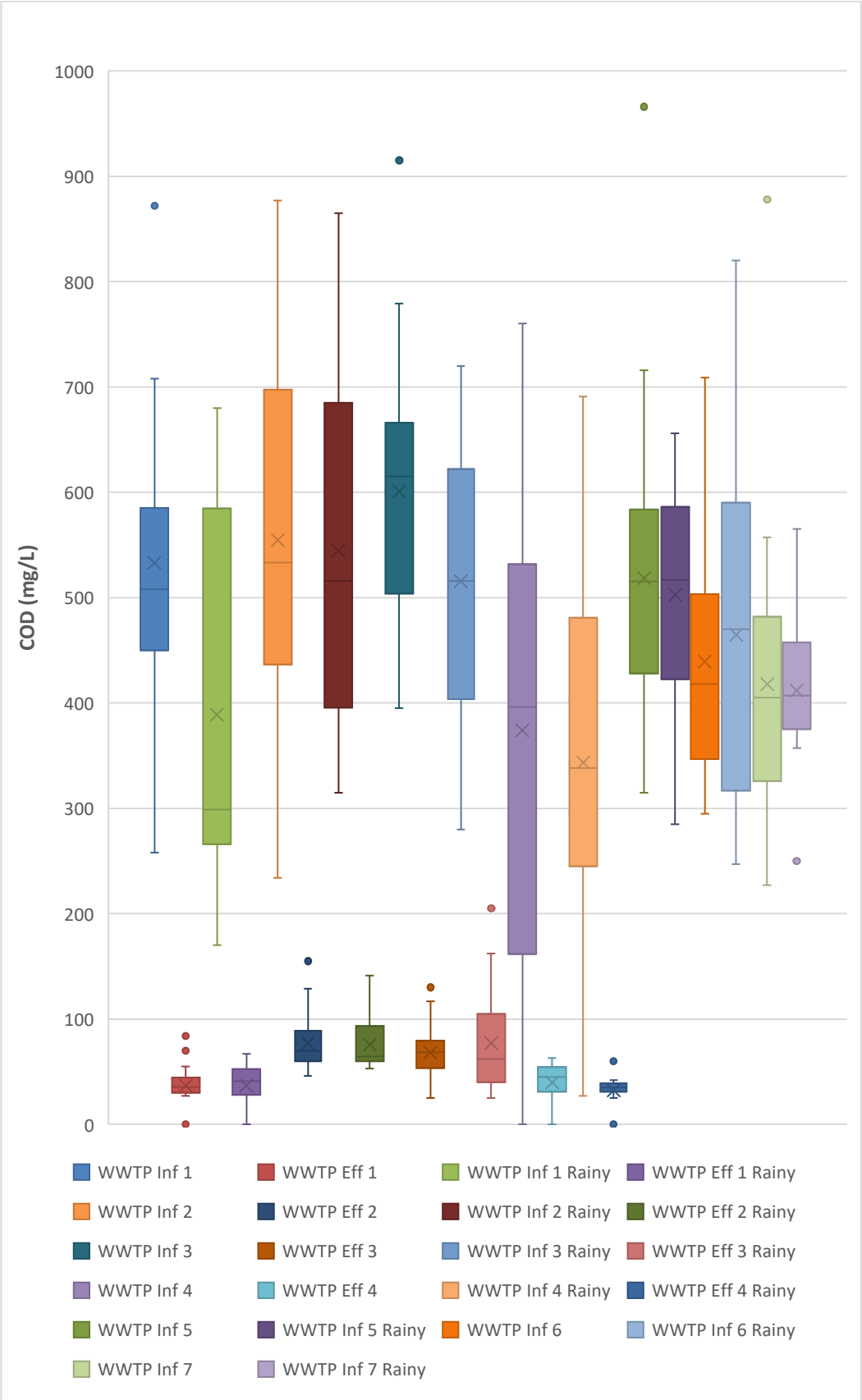


Figure G.2 : Boxplots for COD concentrations (mg/L) in each WWTP influent and effluent in dry and rainy days, outliers are excluded.

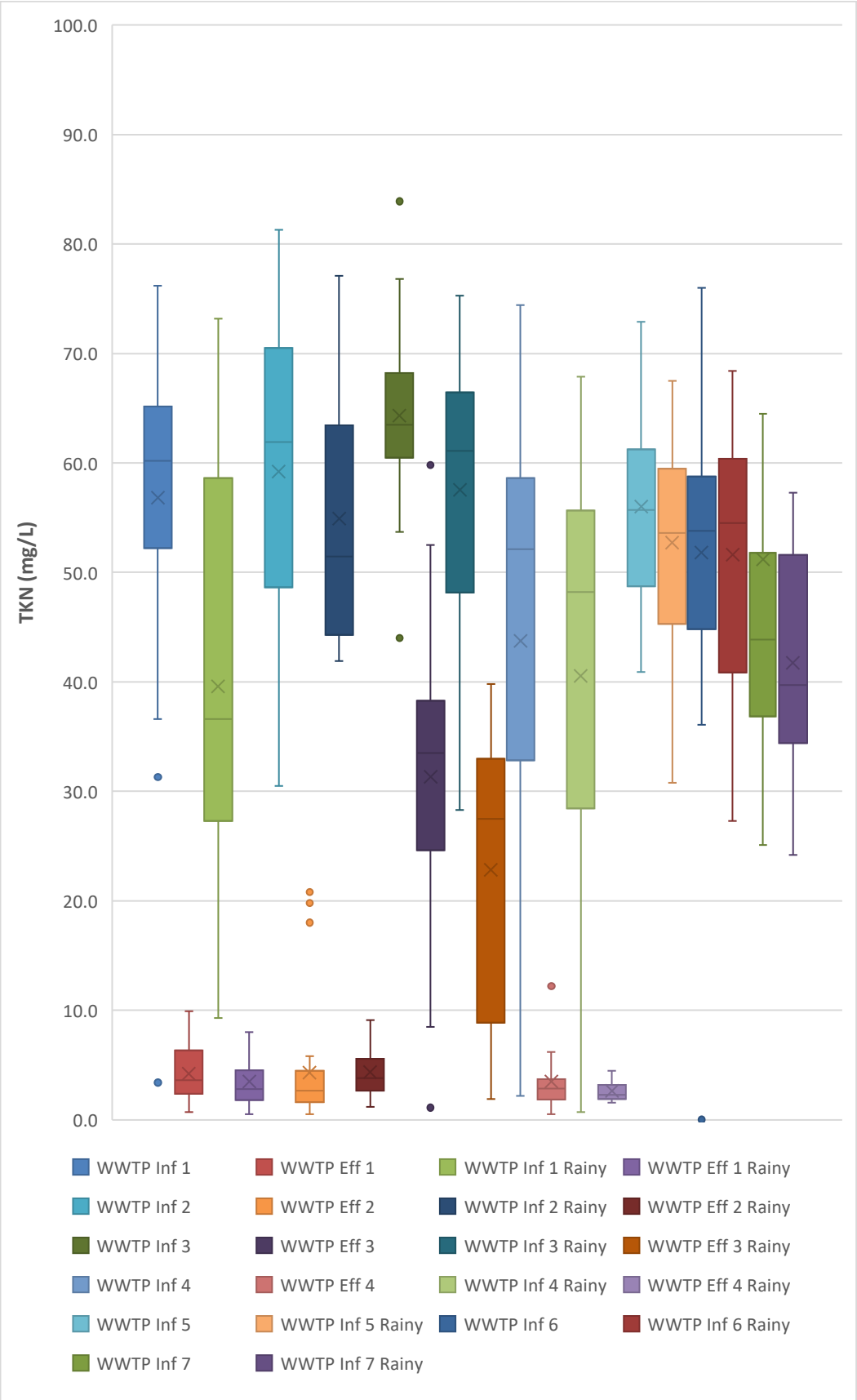


Figure G.3 : Boxplots for TKN concentrations in each WWTP influent and effluent in dry and rainy days, outliers are excluded.

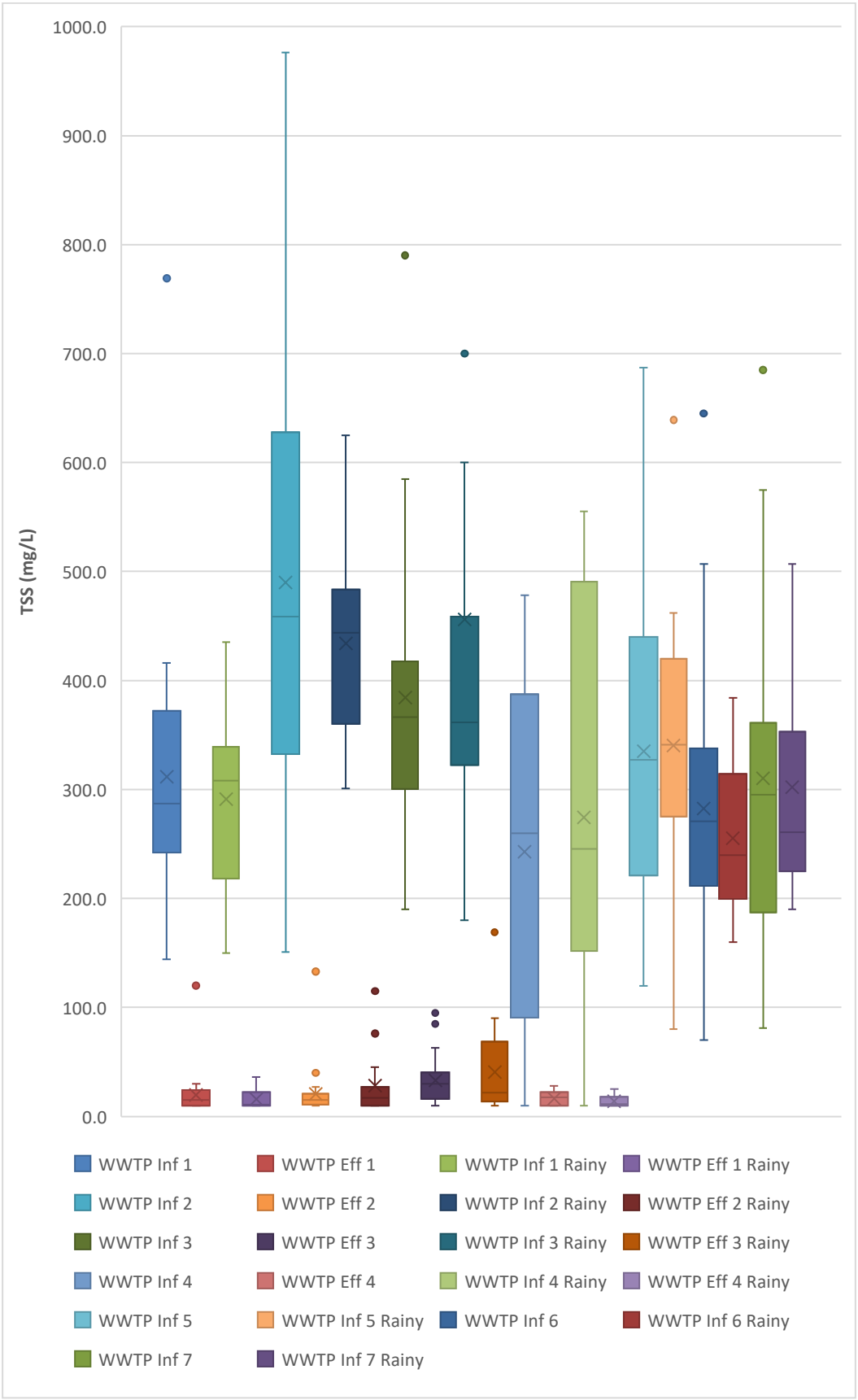


Figure G.4 : Boxplots for TSS concentrations in each WWTP influent and effluent in dry and rainy days, outliers are excluded.

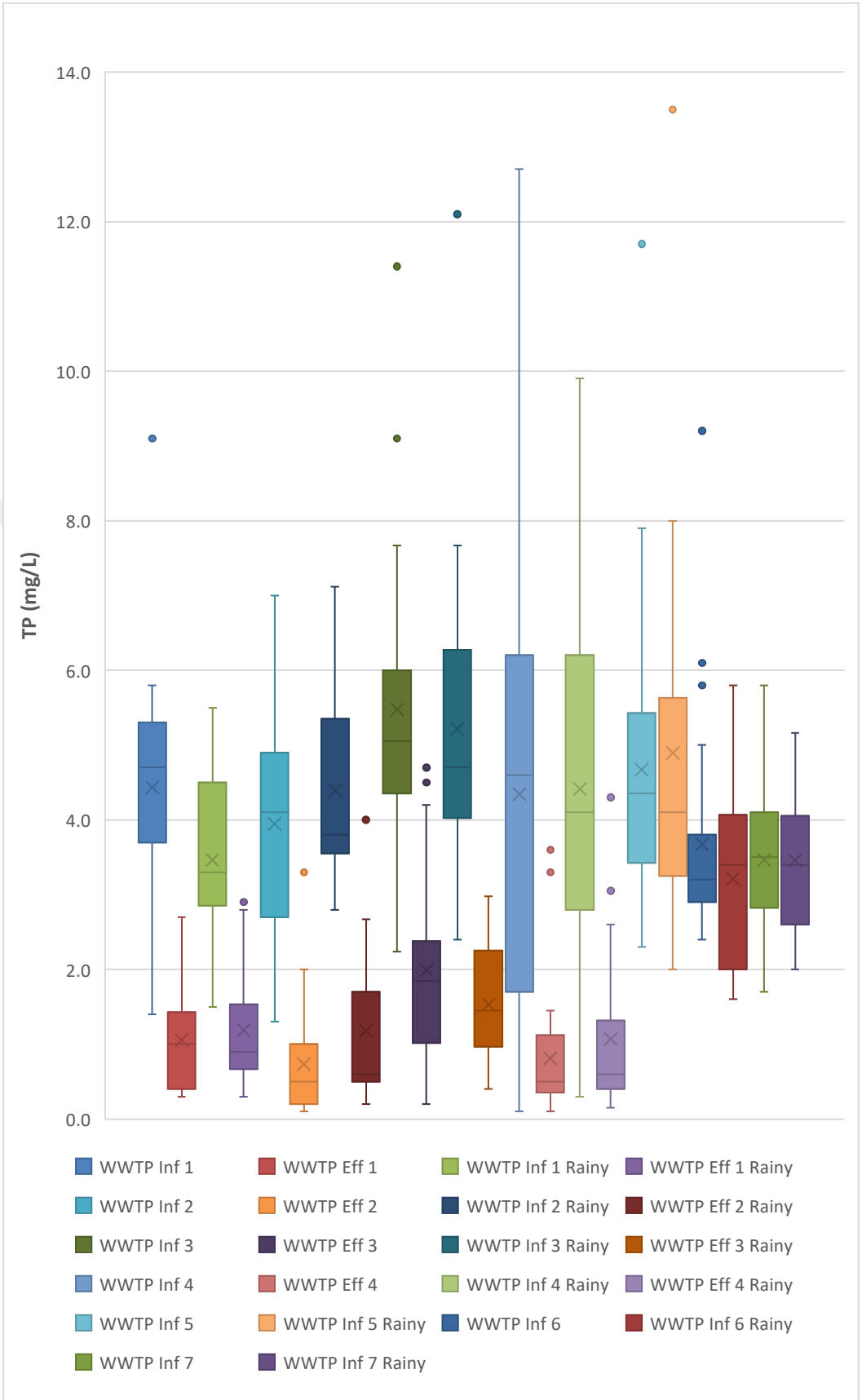


Figure G.5 : Boxplots for TP concentrations in each WWTP influent and effluent in dry and rainy days, outliers are excluded**.**

APPENDIX H : Comparisons of the physicochemical results of holiday and regular seasons

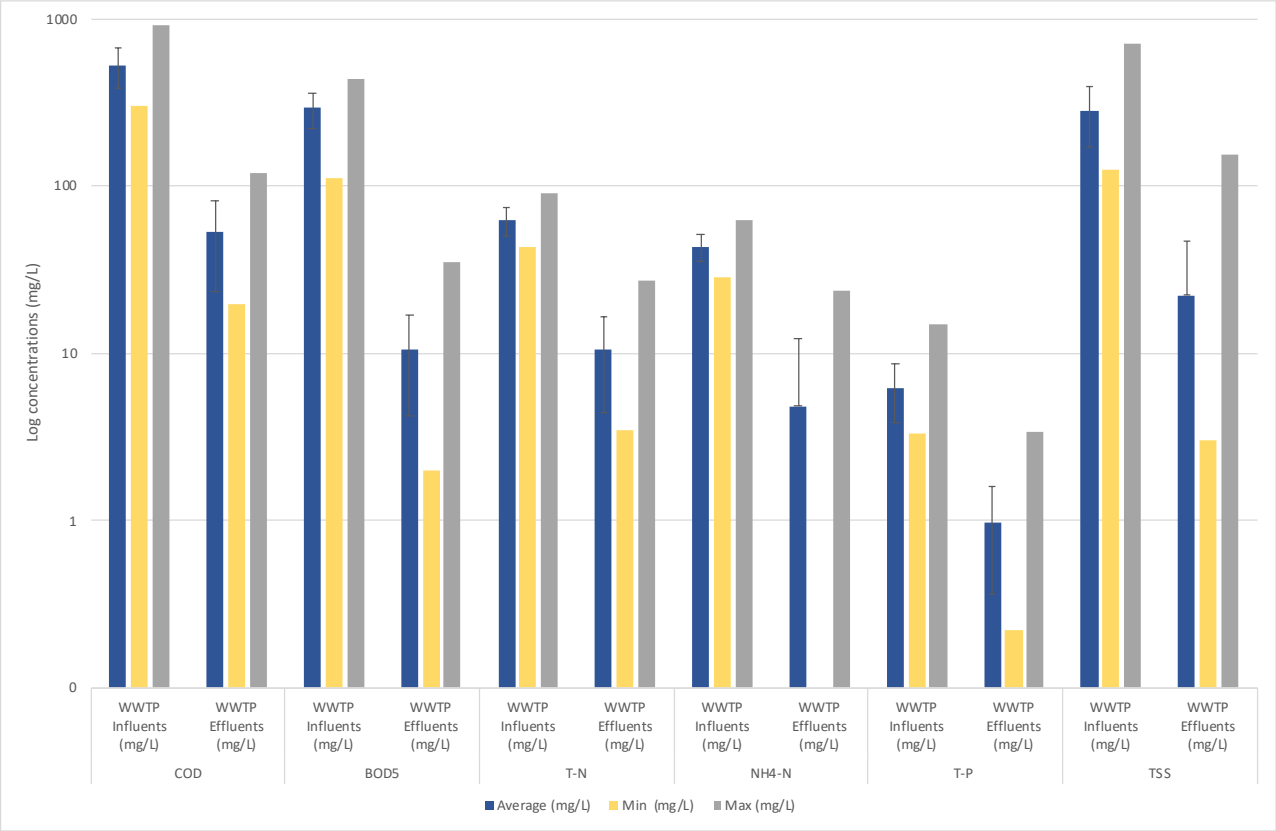


Figure H .1 : The average (bars indicating standart deviation), maximum, and minunum concentrations (mg/L, log scale) of conventinal parameters of WWTP influents (n = 175) and effluents (n =100) of holiday season in June through September 2015–2019.

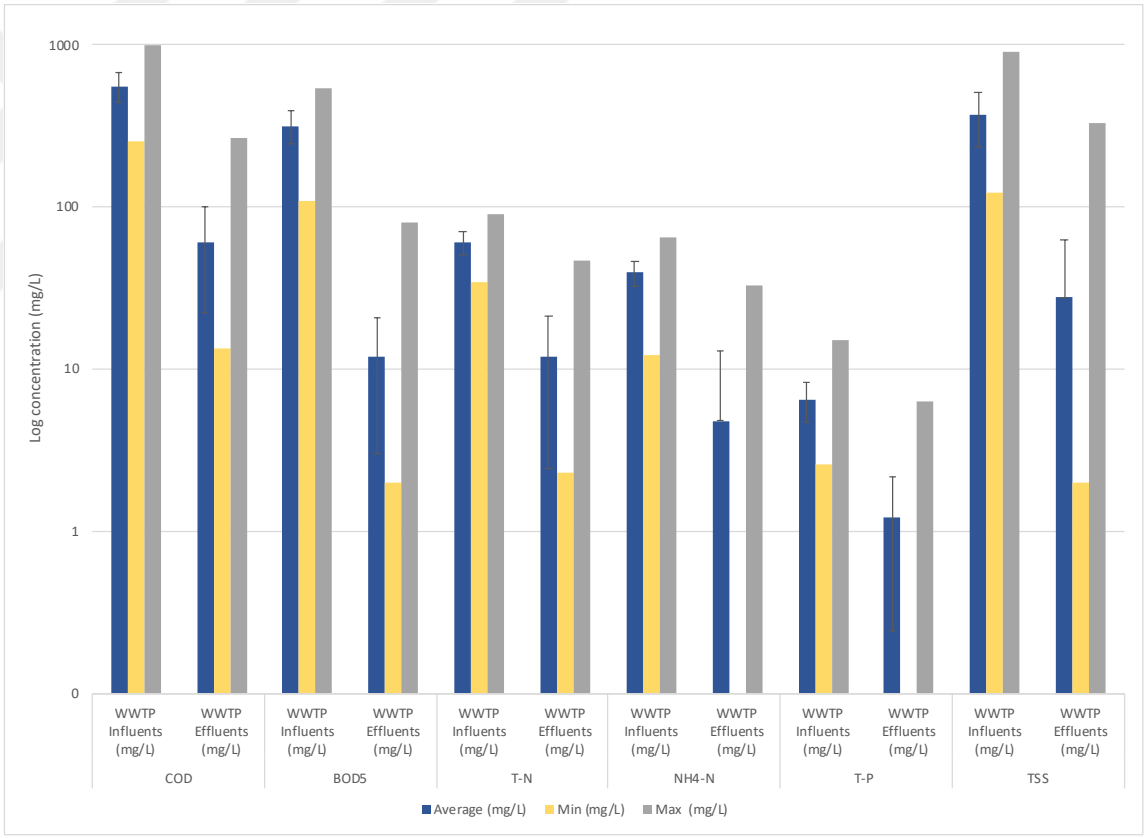


Figure H.2 : The average (bars indicating standart deviation), maximum, and minunum concentrations (mg/L, log scale) of conventinal parameters of WWTP influents (n = 1,350) and effluents (n = 1,175) between the days in June and September 2015–2019 except holidays. Outliers are excluded.

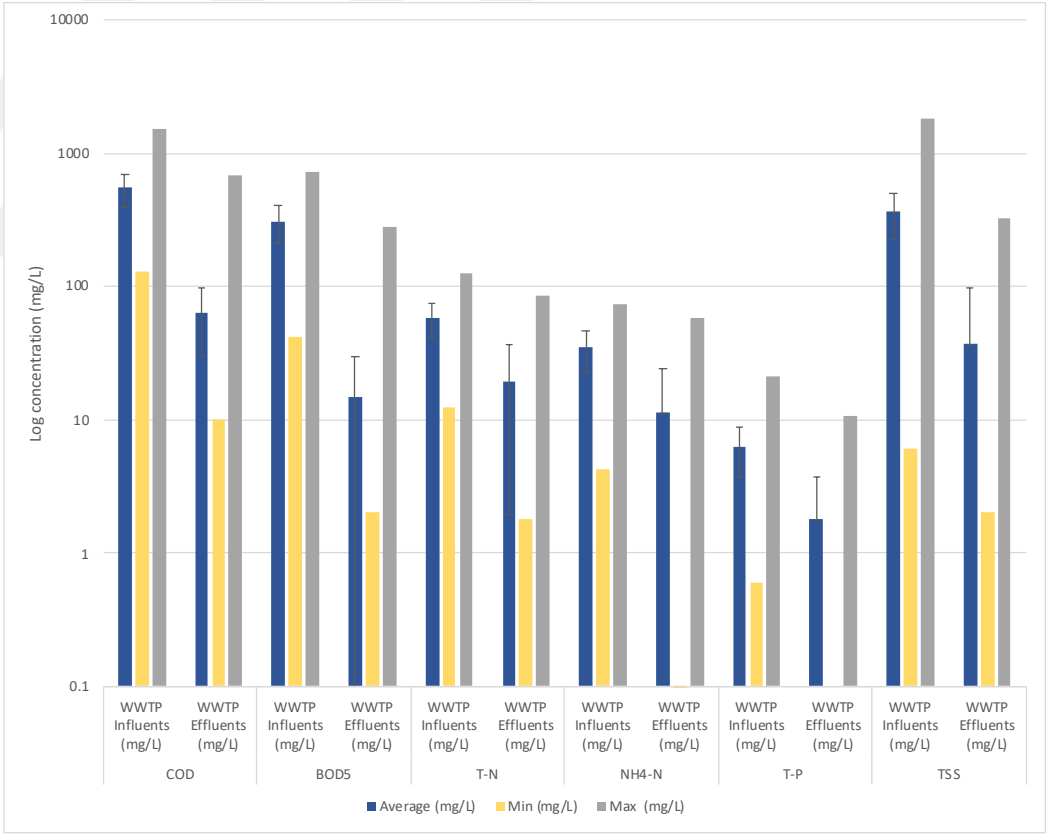


Figure H.3 : The average (bars indicating standart deviation), maximum, and minimum concentrations (mg/L, log scale) of conventinal parameters of WWTP influents (n = 5,470) and effluents (n = 4,640) (between 2015–2019) in all seasons. Outliers are excluded.

APPENDIX I : Pictures related to sampling, experiments and laboratories

(a)

(b)

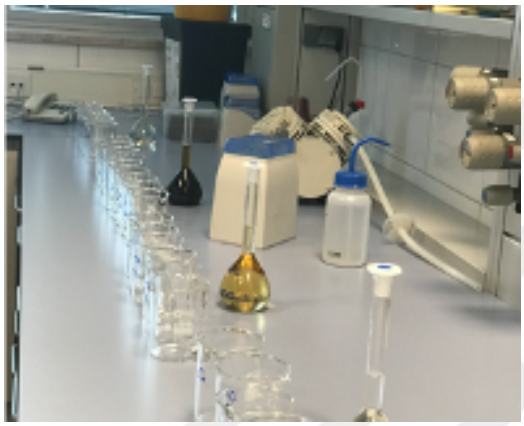
(c)

(d)

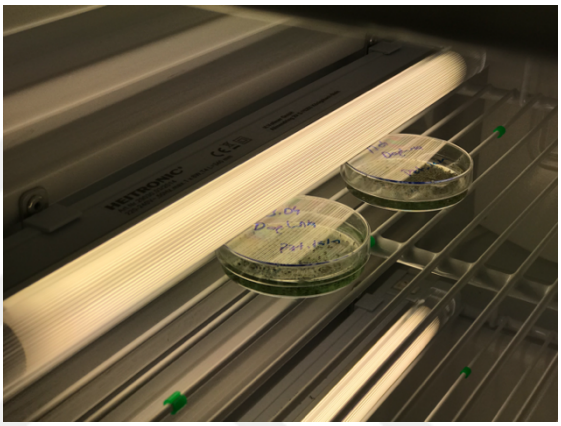
Figure I.1 Pictures taken before or during experiments: (a)–(b) 24 h composite sampling from WWTPs. (c) Composite sampling taken from hospital sewer line. (d) 24 h composite sampling from LTP.

(e)

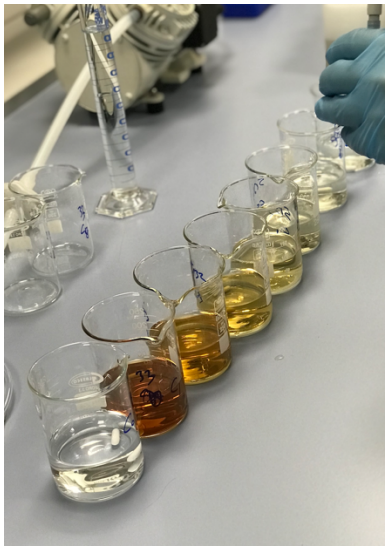
(f)



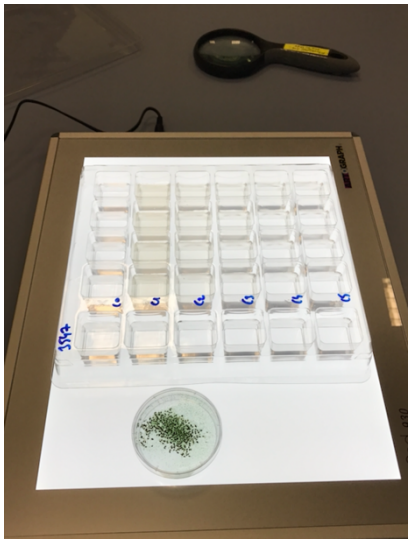
(g)



(h)

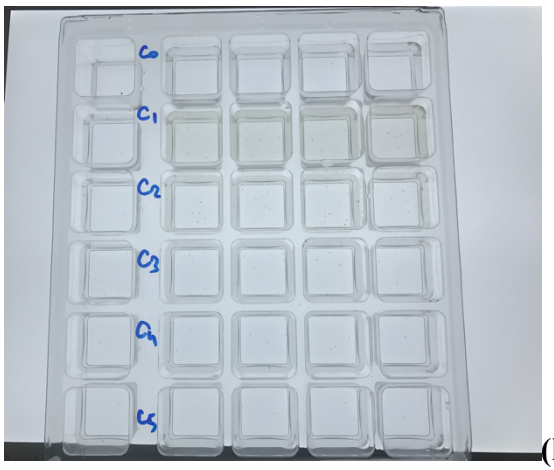


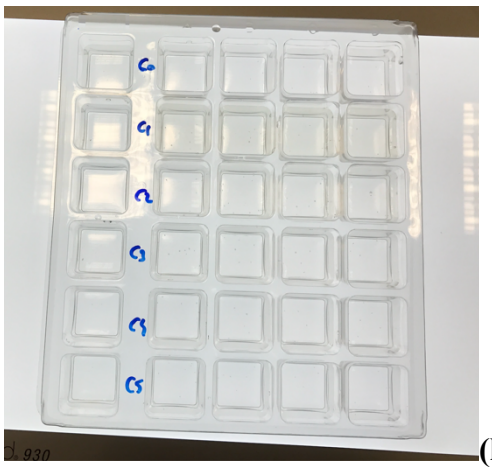
(i)

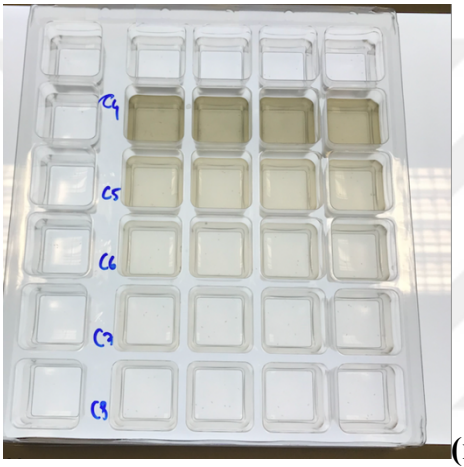


(j)

Figure I.1 (continued) : Pictures taken before or during experiments : (e) Image of initial measurements prior conducting experiments. (f) Preparation of wastewater dilution series. (g) Preparation of leachate dilution series. (h) Hatcing of ephippia under incubation. (i) Image of LTP effluent dilution series. (j) Transfer of the neonates to each test well on light table.


(k)


(l)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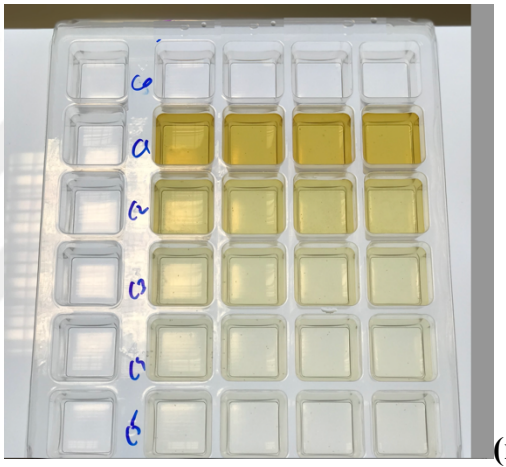
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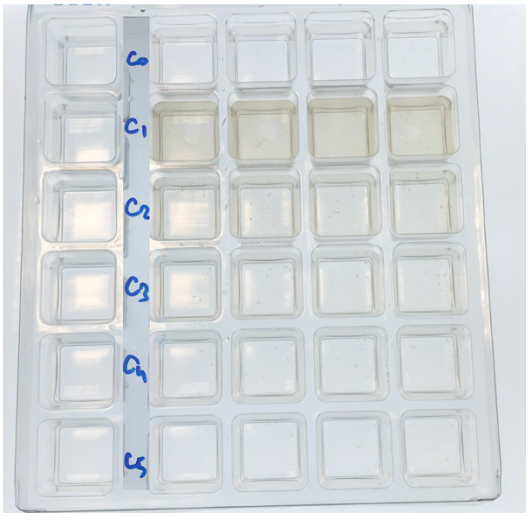
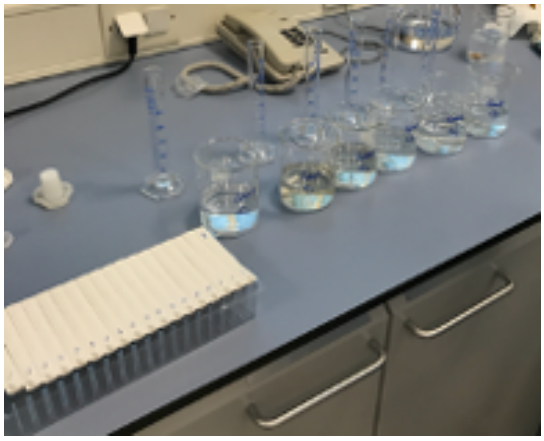
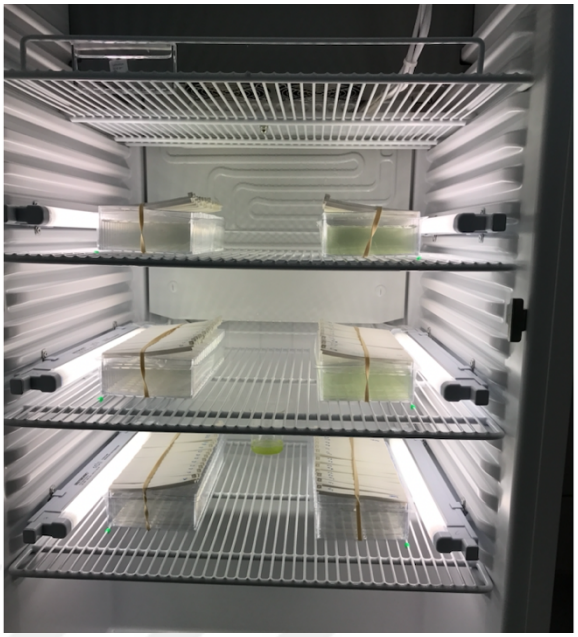

(o)


(p)

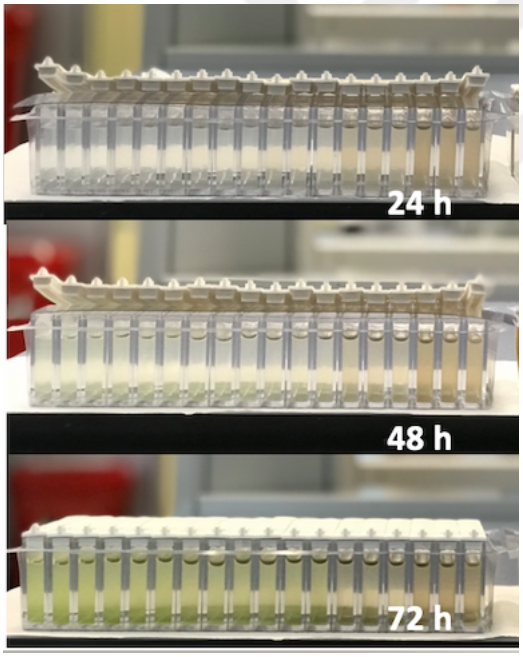
Figure I.1 (continued) : Pictures taken before or during experiments: (k) 48 h *D. magna* toxicity experiment in WWTP influent. (l) 48 h *D. magna* toxicity experiment in WWTP effluent. (m) 48 h *D. magna* toxicity experiment in LTP influent. (n) 48 h *D. magna* toxicity experiment in LTP effluent. (o) 48 h *D. magna* toxicity experiment in hospital wastewater. (p) Microscope image of *D. magna*.



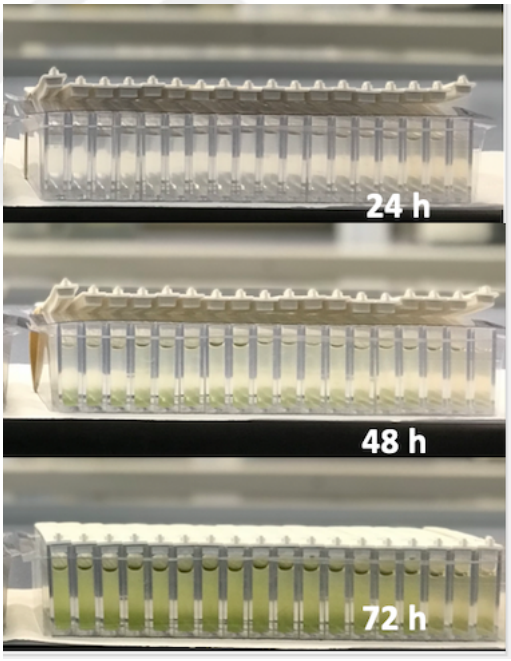
(r)



(s)

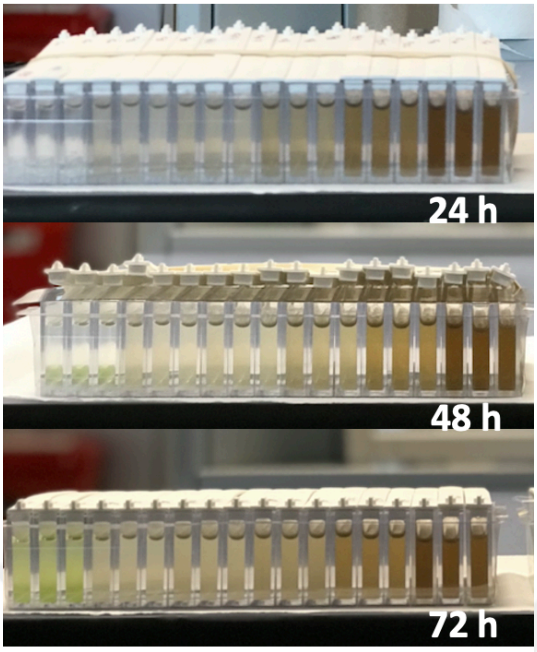


(t)

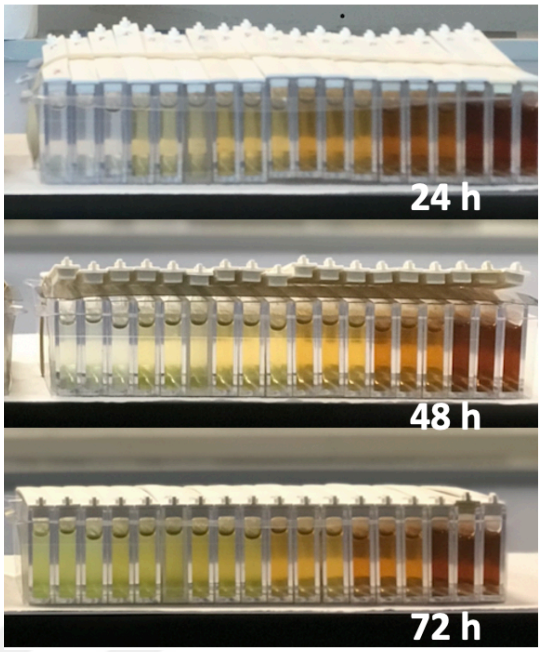


(u)

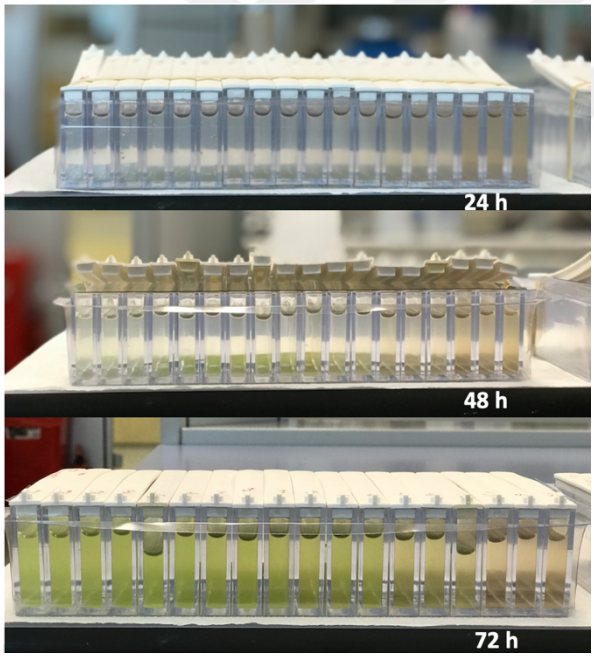
Figure I.1 (continued) : Pictures taken before or during experiments: (r) Transfer of the algae-wastewater dilutions into test vials. (s) Incubation of the test vials. (t) Image of *S. capricornutum toxicity* experiments at 24 h, 48 h and 72 h in WWTP influent. (u) Image of *S. capricornutum toxicity* experiments at 24 h, 48 h and 72 h in WWTP effluent.



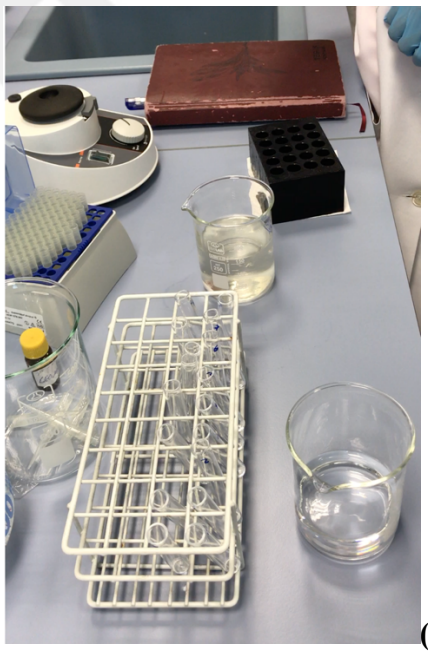
(v)



(w)



(x)



(y)

Figure I.1 (continued) : Pictures taken before or during experiments: (v) Image of *S. capricornutum toxicity* experiments at 24 h, 48 h and 72 h in LTP influent. (w) Image of *S. capricornutum* toxicity experiments at 24 h, 48 h and 72 h in LTP effluents. (x) Image of *S. capricornutum* toxicity experiments at 24 h, 48 h and 72 h in unfiltered hospital wastewater. (y) Image of *V. fischeri* toxicity experiment.

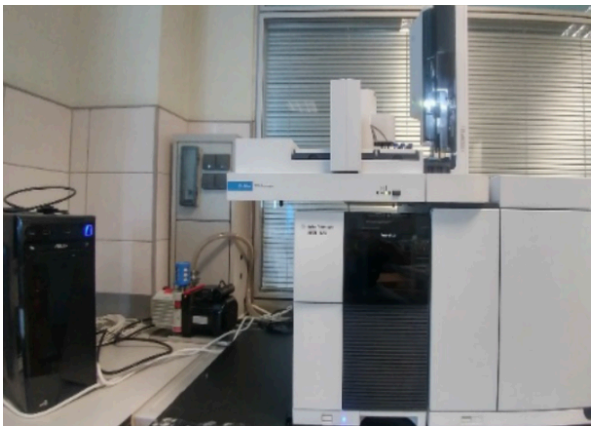
(a)

(b)

(c)

(d)

(e)



(f)

Figure I.2 : Pictures of the established laboratories: (a), (b) and (c) shots from exotoxicity laboratory (established in 2017). (d), (e) and (f) shots from the advanced instrumental analysis laboratory (established in 2020).

CURRICULUM VITAE

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EDUCATION **:**

- **B.S. Course Work** **:** 2013 Istanbul Technical University, Department of Environmental Engineering, Türkiye
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PROFESSIONAL EXPERIENCE AND REWARDS:

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- 2010–2011 Brooklyn Cultural center, Science Teacher, Brooklyn, NY, USA
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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- **Birtek, R. I.,** Karpuzcu, M. E., Ozturk, I. (2022). Occurrence of Priority Substances in Urban Wastewaters of Istanbul and the Estimation of the Associated Risks in the Effluents [Publication]. Environmental monitoring and assessment, 194(6), 426. https://doi.org/10.1007/s10661-022-09840-w

- **Birtek, R. I.,** Ozturk, I. (2022, June 5–8). Landfill Leachate as a Source of Toxicity: A Case Study in Istanbul [Presentation]. World Environmental & Water Resources Congress, Atlanta, GA, United States.
- **Birtek, R. I.**, Ozturk, I. (2022, June 5–8). Occurrence of BTEX in Wastewaters of Istanbul [Presentation]. World Environmental & Water Resources Congress, Atlanta, GA, United States.
- **Birtek, R. I.,** Ozturk, I. (2022, March 30). Potential Associated Risks Posed by the Presence of Bis(2-ethylhexyl) phthalate (DEHP) in Wastewaters of Istanbul [Virtual presentation]. ASCE EWRI Global Science Exchange Series, United States.
- **Birtek, R. I.,** Ozturk, I. (2021, October 3–6). Seasonal Characterization and Ecotoxicity of Wastewaters: A Case Study in Istanbul [Virtual presentation]. Canadian Ecotoxicity Workshop, Halifax, Nova Scotia, Canada.
- **Birtek, R. I.,** Ozturk, I. (2021, September 20–22). Hospital Wastewater as a Source of Priority Substances to the Wastewaters [Virtual presentation]. SETAC Africa 10th Biennial Conference, Africa.
- **Birtek, R. I.**, Ozturk, I. (2021, June 7–11). Landfill Leachate as a Source of Priority Substances to the Wastewaters of Istanbul [Virtual presentation]. World Environmental & Water Resources Congress, Milwaukee, WI, United States.
- **Birtek, R. I.**, Ozturk, I. (2021, June 7–11). The Seasonal Characterization and Ecotoxicity of Hospital Wastewater: A Case Study in Istanbul [Virtual presentation]. World Environmental & Water Resources Congress, Milwaukee, WI, United States.
- **Birtek, R. I.**, Ozturk, I. (2021, June 2). Occurrence of Priority Substances in Urban Wastewaters: A Case Study in Istanbul [Virtual presentation]. ASCE Women-Water Nexus (WWN) 5th short-conference session, United States.
- **Birtek, R. I.**, Ozturk, I. (2019, October 6–9). Characterization of the Festival of Sacrifice Period Wastewaters and their Effect on the Inlet-Polluting Roads in Istanbul Municipal Wastewater Treatment Plants [Presentation]. Canadian Ecotoxicity Workshop, Québec City, Quebec, Canada. Birtek, R. I., Ozturk, I. (2022, June 5–8).
- **Birtek, R. I.**, Ozturk, I. (2017, May 21–25). Adoption of European Union Water Framework Directive to Turkey: A Case Study on Micropollutants in Istanbul [Poster presentation]. World Environmental & Water Resources Congress, Sacramento, CA, United States.

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- **Birtek, R. I.** (2018). Studies on the Determination of Micropollutants in Waters in Istanbul [Publication]. ISKI Water and Innovation Scientific and Technological Research Bulletin 02, 132–135, Istanbul, Türkiye.

- **Birtek, R. I.** (2017). Environmental Toxicity Assessment Studies in the Wastewaters of Istanbul [Publication]. ISKI Water and Innovation Scientific and Technological Research Bulletin 01, 58–66, Istanbul, Türkiye.
- Lawrence, G. D., **Birtek, R. I.**, & Manne, S. (2013, August 11–16). Measurement of alpha-dicarbonyl compounds from sugar autoxidation [Presentation]. IUPAC 44th World Chemistry Congress, Istanbul, Türkiye.
- **Birtek, R.I., (2010).** The in vitro effects of vanadium and copper on fructose degradation, [Thesis, Long Island University], HERO ID: 1507381, NY, USA. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/1507381.