ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

OCCURRENCE AND FATE OF MICROPOLLUTANTS AND THEIR METABOLITES IN WWTPs

M.Sc. THESIS

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Environmental Sciences, Engineering, And Management Programme

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

MİKROKİRLETİCİ VE ARA ÜRÜNLERİNİN AAT'LERDE OLUŞUMU VE AKIBETİ

YÜKSEK LİSANS TEZİ

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Serenay Ceren Tüzün, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 501151759, successfully defended the thesis/dissertation entitled "OCCURRENCE AND FATE OF MICROPOLLUTANTS AND THEIR METABOLITES IN WWTPs", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Assoc.Prof. Dr. Elif PEHLİVANOĞLU MANTAŞ** Istanbul Technical University
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To my family and friends,

FOREWORD

The process of becoming an environmental engineer was a lot longer than one person can imagine. Since I was freshman in highschool, I wanted to be an environmental engineering. One might ask why since it was an extraordinary choice for a profession and I would answer as I really don't know. The only logical answer I can provide would be that when I first heard the name of it, it felt right to be one. The years have passed and I graduated from university with a new mindset and consideration for my profession and that is to pursue an academic career to make a difference in the world. I know that this sounds like a stretch but we should start somewhere to fulfill the purpose of our lives. This was my way to do that, to make small differences in the world. Without further ado, I must thank the people that help me write this thesis and make it a dream come true. I sincerely thank my thesis advisor Assoc. Prof. Dr. Elif PEHLIVANOĞLU MANTAŞ for sharing her profound knowledge and experiences, and supporting me throughout the year. Withouth her, this thesis would definitely not be finished. Also, I sincerely thank Assoc. Prof. Dr. Egemen AYDIN and Assoc. Prof. Dr. Serdar DOĞRUEL for their support and knowledge that guide me through process of writing this thesis. I thank the teachers and research assistants of ITU Environmental Engineering Department for their help throughout the year, The Scientific and Technological Research Council of Turkey (TUBITAK) for project 114Y487 and ITU Scientific Research Project (BAP) Department for project 39421 for their support to carry out this project which I call my thesis. I also thank my friends Zuhal ÇETİNKAYA ATEŞÇİ and Nilay BİLGİN SARITAŞ for their sincere friendships, help and understanding. To my friends that I love dearly; Eda ÇİÇEK, Elif GERDAN, Ezgi PIRLANT, Doruk DÜNDAR, Ömer Kerem KARAALİ, Gökçe ÖZTÜRK, Gül HATİNOĞLU, and Merve BULUT, from the bottom of my heart, please accept my sincere thanks for your unbelievable support, understanding, help and presence. I don't think I would make it this far without you guys. I love you all very much. To a special person whom I consider as a sister; Rumeysa AKTÜRK. I know we were very far apart but like you always say I felt the presence of you near me everyday. You were and are just by my side no matter what. Please accept my sincere thanks for being with me for this 7 years and apologies for me being a little of a bad friend during this year. I know I didn't call you enough at all. Please know that I love you to moon and back. To my family whom I can't even process the love I have, my mother and father who were and are always right there when I need them, when I need to cry, when I just need to babble. They always listen and guide me with their profound life experiences. They support me with every decision I made. They gave me the life that I have now. Is there anything more important than this? I just love you with everything I have and I will always continue to love you no matter what. Just wait a little bit more, I will be at home right beside you.

May 2017

Serenay Ceren TÜZÜN
(Environmental Engineer)

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ABBREVIATIONS

AAT : Atıksu Arıtma Tesisi
ACN : Acetonitrile
ATL : Atenolol
BPA : Bisphenol-A
CBZ : Carbamazepine
CFF : Caffein
COD : Chemical Oxygen Demand
CPX : Ciprofloxacin
DCF : Diclofenac
E1 : Estrone
E2 : Estradiol
E3 : Estriol
EC50 : Half Maximal Effective Concentration
EDC : Endocrine Disrupting Compound
EE2 : Ethinylestradiol
EU : European Union
HRT : Hydraulic Retention Time
IBF : Ibuprofen
LC50 : Half Maximal Lethal Concentration
LC-MS/MS : Liquid Chromatography Tandem Mass Spectrometry
LOD : Limit of Detection
LOQ : Limit of Quantification
MTBE : Methyl Tert-Butyl Ether
NDMA : N-Nitrosodimethylamine
NPX : Naproxen
NSAID : Nonsteroidal Anti-inflammatory Drug
NSAİİ : Non Steroidal Antienflamatuar İlaç
PhAC : Pharmaceutically Active Compound
PRP : Propranolol
PRX : Paraxanthine
SMX : Sulfamethoxazole
SPE : Solid Phase Extraction
SRT : Sludge Retention Time
TKN : Total Kjeldahl Nitrogen
TP : Total Phosphorus
TSS : Total Suspended Solids
USEPA : United States Environmental Protection Agency

SYMBOLS

logKow **:** Octanol-water Partition Coefficient
pKa **:** Acid Dissociation Constants

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OCCURRENCE AND FATE OF MICROPOLLUTANTS AND THEIR METABOLITES IN WWTPs

SUMMARY

Micropollutants can be natural or synthetic compounds such as steroid hormones and pharmaceutically active compounds, respectively. They are present in water systems at µg/L or even as low as ng/L levels. Although the presence of micropollutants were discovered in the 60's (Stumm-Zolinger & Fair, 1965), not much attention has been paid to them until late 90's due to their low concentrations and unknown effects on organisms. Although micropollutants in receiving water bodies can originate both from point and non-point sources, wastewater discharges are the most important source to investigate. Wastewater treatment plants (WWTPs) are not designed to remove micropollutants; hence, micropollutants that are insufficiently removed are discharged to receiving water bodies and they enter the water cycle. The presence of micropollutants in the water can limit its current or future beneficial uses such as irrigation or drinking water.

Being present at low levels presents a challenge to measure the micropollutants. In the past, analysis methods had high limits of detection (high µg/L or mg/L), resulting in the incorrect assumption that the reporting compounds did not exist even in cases where the micropollutants were actually present in the samples. Nowadays, micropollutants are observed more frequently in water systems in connection with the improved detection limits of the analysis methods. In addition, a higher consumption of some micropollutants might also result in more frequent detection of these micropollutants.

The detection of micropollutants in environmental waters was concerning due to fact that their effects were unknown. To evaluate their possible effects and take the necessary precautions, toxicity studies on micropollutants as acute and chronic bioassays were carried out. The results of acute toxicity studies mostly indicate that environmentally relevant concentrations of micropollutants do not present a risk since the concentrations determined to be acutely toxic are higher than the environmentally relevant concentrations. This information may incorrectly have evaluated as micropollutants do not having adverse effects on organisms at concentrations they are found in the environment, yet, the concern about micropollutants is more about the chronic effects since the organisms are being exposed to low concentrations of micropollutants for a long time. Moreover, synergistic effects of micropollutants are also unknown, but it is crucial to know their effects as they are present as mixtures in the environment rather than a single compound.

In the light of this information, considering that wastewater treatment plants are the main sources of micropollutants in the environment, the understanding of current situation in wastewater treatment plants of Istanbul has great importance. Information

obtained from this study can be used as foundation for further studies about toxicity research and advanced treatment technologies for further removal of micropollutants.

In this study, grab sampling campaigns are carried out in different treatment units of two different WWTP for each season in one year, in order to observe the possible effects that seasonal change and operational conditions may have on micropollutant concentrations.

Studied micropollutants were selected among the micropollutants most frequently detected around the world, to cover a wide range of micropollutants. The groups of studied micropollutants included NSAIDs, estrogens, industrial chemicals and, disinfection byproducts.

Due to low expected concentrations and the possible effect of wastewater matrix, solid phase extraction followed by LC-MS/MS is used for the measurement of micropollutants in samples collected from different units in the wastewater treatment plants. Both the SPE and the LC-MS/MS conditions were optimized to be able to run the method as multi-residue analysis where multiple micropollutants can be measured simultaneously. In addition to wastewater samples, sludge samples were also taken from each WWTP at 3 points in order to evaluate the fate of micropollutants by differentiation between biodegradation and adsorption to sludge.

Most of the selected micropollutants were present in the influents of the WWTPs with concentrations ranging from high µg/L (e.g., naproxen and caffeine) to ng/L (e.g., estrogens) but their fate and behavior was different. For some of the micropollutants (e.g., carbamazepine), the concentration in the effluent was not significantly different than in the influent, whereas some micropollutants (e.g., estrogens) were below detection limit (i.e., 20 ng/L) in the effluent. Moreover, although some micropollutants (e.g., caffeine) had high (>99%) removal efficiencies, their effluent concentrations are still very high due to the high influent concentrations.

The WWTPs where the samples were obtained had similar treatment processes, but received wastewaters with different characteristics. Also, the operational conditions and general efficiencies for organic and nutrient removal were different. However, these differences were not necessarily reflected on the removal efficiencies of micropollutants.

Our results suggest that seasonal changes can affect micropollutant concentrations in more than one way. Carbamazepine is an anti-epileptic drug which means similar influent concentrations in each season. However, in winter, the concentrations of carbamazepine were lower than the concentrations of summer and fall results. This can be explained by dilution of heavy rain. Due to dilution, the concentrations of NSAIDs are expected to decrease, yet, the influent concentrations of NSAIDs were higher than the results of summer and fall indicating extensive usage of NSAIDs. In addition, cold weather conditions during Winter resulted in decrease of removal efficiencies for most of the micropollutants.

For some compounds, the concentrations have increased during the treatment scheme. The increase of the concentration is believed to occur due to deconjugation of metabolites into parent compounds which were measured in the effluent as the parent compound.

The presence of micropollutants in wastewater effluent suggests that the intentional or unintentional reuse of wastewater may pose a threat to environmental and human health. Therefore, further studies on the effects of micropollutants either in surface

waters or drinking waters are needed to establish standards that need to be applied in case of wastewater reuse. Moreover, studies on removal of micropollutants in the wastewater effluents are needed and can be used to decrease the concentrations further. These studies can focus on the existing wastewater treatment systems and can establish the link between the microbial population and micropollutant removal. Also, advanced chemical or physico-chemical processes such as chlorination, ozonation, oxidation with nanoparticles and adsorption can also be applied for the removal of miropollutants before the reuse of wastewater.

MİKROKİRLETİCİ VE ARA ÜRÜNLERİNİN AAT'LERDE OLUŞUMU VE AKIBETİ

ÖZET

Mikrokirleticiler; steroid hormonlar gibi doğal madde, ilaç etken maddeler gibi sentetik madde olabilir. Su sistemlerinde, µg/L ve hatta ng/L olarak bulunurlar. Mikrokirleticilerin varlığının 60'lı yıllarda fark edilmesine ragmen (Stumm-Zolinger ve Fair, 1965), düşük konsantrasyon ve bilinmeyen etkilerinden dolayı 90'ların sonuna kadar çok ilgi gösterilmedi. Alıcı su ortamlarındaki mikrokirleticiler noktasal ve yayılı kaynaklar nedeniyle bulunsalar da atıksu deşarjı araştırılması en önemli noktasal kaynaktır. Atıksu Arıtma Tesisleri (AAT) mikrokirleticileri gidermek için tasarlanmamıştır. Bu nedenle, atıksu arıtma tesislerinde yeteri kadar giderilmeyen mikrokirleticiler alıcı su ortamlarına deşarj edilerek su döngüsüne katılmaktadır. Mikrokirleticilerin su sistemlerinde bulunması güncel ve gelecekteki sulama suyu veya içme suyu olarak kullanımını sınırlamaktadır.

Düşük konsantrasyonlarda bulunması, ölçüm konusunda sıkıntılar oluşturmaktadır. Geçmişte, analiz metotlarının yüksek ölçüm limitleri (yüksek µg/L ya da mg/L) olduğundan mikrokirleticiler numunelerde bulunduğu halde bulunmadığına dair yanlış varsayımlara neden olmaktaydı. Günümüzde, analiz metotlarının gelişmiş ölçüm limitleri dolayısıyla mikrokirleticiler su sistemlerinde daha sık rastlanmaktadır. Buna ek olarak, bazı mikrokirleticilerin daha fazla tüketimi daha sık karşılaşılmasına da neden olmaktadır.

Bilinmeyen etkilerinden dolayı, mikrokirleticilerin çevresel su sistemlerinde bulunması endişe uyandırmaktadır. Muhtemel etkilerini belirlemek ve gerekli önlemleri almak için toksisite deneyleri akut ve kronik biyoesseyler olarak yürütülmektedir.

Akut toksisite çalışmalarının sonuçları, çoğunlukla su sistemlerinde karşılaşılan mikrokirletici konsantrasyonlarının akut toksisiteye neden olan konsantrasyondan çok daha küçük olmasından dolayı herhangi bir risk oluşturmadığını belirtmektedir. Bu bilgi, yanlış bir varsayımla su sistemlerinde bulunduğu konsantrasyonlarda canlılar üzerinde olumsuz etkiye sahip olmaması olarak yorumlanabilir. Fakat; bu konudaki endişe, canlıların sürekli düşük konsantrasyonlardaki mikrokirleticilere maruz kaldığından dolayı kronik etkilerinden gelmektedir.

Dahası, mikrokirleticilerin sinerjistik etkileri de bilinmemektedir ama mikrokirleticilerin su sistemlerinde bir karışım halinde bulunmalarından ötürü sinerjistik etkilerinin bilinmesi çok önemlidir.

Bu bilgiler ışığında, atıksu arıtma tesislerinin çevredeki mikrokirletici varlığının ana sebebi olarak düşünüldüğünde İstanbul'un atıksu arıtma tesislerindeki şu anki durumun bilinmesi büyük önem arz etmektedir.

Bu çalışmadan elde edilen bilgiler, toksisite ve ileri arıtma teknolojileri gibi çalışmalar için temel olarak kullanılabilir.

Bu çalışma içerisinde, bir sene içinde her mevsim iki farklı AAT'nin farklı ünitelerinden anlık numune alımları gerçekleştirildi. Bunun nedeni ise, mevsimsel değişikliklerin ve işletme koşullarının mikrokirletici konsantrasyonları üzerindeki etkilerini gözlemlemektir.

Çalışılan mikrokirleticiler, geniş bir mikrokirletici aralığını kapsayan ve dünya genelinde sıklıkla rastlanan mikrokirleticiler arasından seçildi. Çalışılan mikrokirleticiler, NSAİİ, östrojenler, endüstriyel kimyasallar ve dezenfeksiyon yan ürünlerini kapsamaktadır.

AAT'lerin farklı ünitelerinden alınan numunelerdeki mikrokirleticileri ölçmek için, beklenen düşük konsantrasyonlardan ve atıksu matrisinin etkilerinden dolayı katı faz ekstraksiyonunu (SPE) takiben LC-MS/MS kullanıldı. SPE ve LC-MS/MS koşulları, birçok mikrokirleticiyi aynı anda ölçen ölçüm metotu olan çoklu-kalıntı analiz metotunun uygulanabilirliği için optimize edildi.

Atıksu numunelerine ek olarak, AAT'lerin 3 noktasından çamur numuneleri de alındı. Bunun amacı ise, mikrokirleticilerin akıbetini, biyolojik parçalanma ve çamura adsorplanma arasında ayırt ederek belirlemektir.

Seçilen mikrokirleticilerin çoğu, AAT'nin girişinde yüksek µg/L konsantrasyonlarından (örn., naproksen ve kafein) ng/L konsantrasyonlarına (örn., östrojenler) kadar bulunmaktadır. Fakat, akıbetleri ve davranışları farklıdır. Bazı mikrokirleticiler için (örn., karbamazepin) giriş konsantrasyonu ile çıkış konsantrasyonu arasında belirli bir fark yokken bazı mikrokirleticiler (örn., östrojenler) ise çıkışta ölçüm limiti altında bulunmaktadır (örn., 20 ng/L). Dahası, bazı mikrokileticiler (örn., kafein) yüksek (>%99) giderim verimine sahip olmasına ragmen, giriş konsantrasyonlarının çok yüksek olmasından dolayı çıkış konsantrasyonu hala yüksek olarak bulunmaktadır.

Atıksu numunelerinin alındığı AAT'ler benzer arıtma sistemlerine sahipken sistemlere giren atıksular farklı karaktere sahiptirler. Ayrıca, işletme koşulları ve genel organik ve nütrient giderimleri birbirinden farklıdır. Fakat; bu farklılıklar, mikrokirleticilerin giderimine pek yansımamıştır.

Sonuçlar, mevsimsel değişikliklerin mikrokirleticilerin konsantrasyonlarını birden fazla yolla etkilediğini göstermektedir. Karbamazepinin anti-epileptik ilaç olmasından dolayı konsantrasyonunun her mevsim sabit olması gerekirken, kış mevsiminde yaz ve sonbahara oranla daha düşük giriş konsantrasyonuna sahiptir. Bu durum, yağışlardan dolayı kosantrasyonların seyreldiğini göstermektedir. Bundan dolayı, NSAİİ'lerin de seyrelmesi beklenirken konsantrasyonları yaz ve sonbahara oranla daha yüksektir. Bu sonuç, NSAİİ'lerin kış mevsimindeki fazla tüketimiyle açıklanmaktadır. Buna ek olarak, kış mevsimindeki soğuk hava koşulları nedeniyle mikrokirleticilerin giderim verimlerinde düşüş yaşanmıştır.

Bazı mikrokirleticiler için, arıtma sırasında konsantrasyonlarda artma gözlemlenmiştir. Konsantrasyonlardaki artışın nedeni olarak, metabolitlerin ana maddeye dönüşünden kaynaklandığı düşünülmektedir.

Atıksu çıkışındaki mikrokirleticilerin varlığı, atıksuların kasıtlı ve kasıtsız tekrar kullanımının çevre ve insan hayatını tehlikeye sokabileceğini göstermektedir. Bu yüzden, yüzeysel ve içme sularındaki mikrokirleticilerin etkileri üzerine çalışmaların

yapılması, atıksuların tekrar kullanılması durumunda oluşturulması gereken standartlar için gereklidir.

Dahası, atıksu çıkışlarındaki mikrokirleticilerin giderimi üzerine çalışmalar gereklidir ve bu çalışmalar konsantrasyonları daha da azaltmak için kullanılabilir. Bu çalışmalar, var olan atıksu arıtma tesisleri üzerine odaklanabilir ve mikrobiyal nüfus ile mikrokirletici giderimi arasındaki bağlantıyı belirleyebilir. Ayrıca, klorlama, ozonlama, nanopartikül ile oksidasyon ve adsorpsiyon gibi ileri kimyasal ve fiziko-kimyasal arıtma prosesleri, atıksuların tekrar kullanımı öncesi mikrokirleticilerin gideriminde kullanılabilir.

1. INTRODUCTION

1.1 Meaning and Importance of This Study

Micropollutants have become a hot topic in the last two decades. One of the questions being studied thoroughly is the sources of micropollutants in water systems. The sources of micropollutants can be both point and non-point sources. In particular, wastewater as a point source in water systems is the most important source for micropollutants. Therefore, their occurrence and fate in wastewater treatment systems have been investigated frequently in the environmental science community. Wastewaters being the major source of micropollutants leads to the concerns about the reuse of wastewater as a water resource. Currently, in Turkey, wastewaters can only be directly reused as irrigation water. However, in some cases when there is not enough control over discharging wastewaters into receiving water bodies wastewaters can be present in surface waters which are used as drinking water sources. This situation describes unintentional and indirect reuse of wastewaters. As a result, the reuse of wastewater is threatened by the fact that wastewater treatment systems cannot remove the micropollutants since wastewater treatment systems are not designed to remove micropollutants.

As the name suggests, micropollutants are present in concentrations of µg/L or even ng/L. Although they are present in low concentrations, they have shown or are expected to have severe adverse effects on the environment.

Although the exact adverse effects are still unknown, there are many reports about acute and chronic toxicity on species exposed to micropollutants. Both for humans and other organisms, the chronic toxicity on species is more of a concern due to constant exposure to micropollutants. Moreover, while the micropollutants may have some toxicity when they are the only chemical for the organisms to be exposed to, synergistic effects of micropollutants are more important to investigate since both in drinking water and environmental water, the micropollutants are rarely present as

single chemicals and there are studies that indicate the toxicities is not additive but rather synergistic, when micropollutants are present as a mixture.

In the light of this information, first, we need to understand the current situation of micropollutant removal in wastewater treatment plants in Istanbul. The occurence data can then be used as the foundation for further studies on technologies with higher micropollutant removals.

1.2 Aim and Scope of This Study

The aim of this study is to study the occurence and fate of micropollutants and to determine the concentrations and removal efficiencies of selected micropollutants in every unit of wastewater treatment system. 14 micropollutants and their 10 metabolites are selected to monitor throughout one domestic and one municipal wastewater treatment plant. The micropollutants to be monitored are 17β-estradiol, 17α-ethinylestradiol, Atenolol, BPA, Caffeine, Carbamazepine, Ciprofloxacin, Diclofenac, Estrone, Ibuprofen, Naproxen, NDMA, Propranolol, Sulfamethoxazole. The metabolites to be monitored are Paraxanthine, 10,11-dihydro.hydroxycarbamazepine, Epoxycarbamazepine, 4OHdiclofenac, Estrone3sulfate, 1OHibuprofen, 2OHibuprofen, o-desmethylnaproxen, 4hydroxypropranolol, and Acetysulfamethoxazole.

For the studies, 4 sampling campains for each WWTP were conducted in a year. Taking samples from two different WWTPs enabled the comparison of removal efficiency differences due to different operational conditions. On the other hand, taking seasonal samples provided some information about effects of seasonal changes on concentrations and removal efficiencies of micropollutants. Moreover, to distinguish between removal through biological treatment and adsorption, sludge samples were taken from 3 points from each WWTP.

Wastewater samples are taken with 10 L teflon jerrican that prevents any absorption and interferences from the sampling equipment. Similarly, sludge samples are taken with glass jars. After sample collection, wastewater is filtered through 0.22 μm filter and stored at 4°C to minimize biological reactions. For this purpose, samples are subjected to characterization experiments to understand the possible relationship between parameters and micropollutants, then solid phase extraction procedure is

applied to overcome detection limit of LC-MS/MS. After SPE, the samples are injected to LC-MS/MS.

2. LITERATURE REVIEW

2.1 Definition of Micropollutants

Micropollutants are defined as synthetic and natural substances that arise from the human activities and are present in low concentrations such as ng/L or µg/L. To give an example for synthetic and natural compounds, pharmaceutically active compounds are considered as synthetic compounds while steroid hormones are considered as natural compounds. Even though they are present in low concentrations, they have varying adverse impacts on both human and environment health that should be considered as dangerous.

In the early years of studies on micropollutants, some of the micropollutants could not be seen, yet, nowadays their presence is acknowledged to be present even back then. The reason why they were not detected is that the analysis methods were not suitable to detect low concentrations of micropollutants. On the other hand, some of the micropollutants are present in water systems due to industrialisation and invention of micropollutants.

First publication of micropollutants is about deficient removal of steroids in wastewater treatment plants (Stumm-Zollinger and Fair, 1965). In the 1970s and 1980s, some of the human hormones and pharmaceuticals were stated for being present in the wastewater treatment plants and aquatic environments. Although there was multiple research about it, micropollutants had been ignored due to its low concentrations and limited knowledge on their toxicity (Tabak and Bunch, 1970; Tabak et al., 1981).

Until the early 1990s, main concern was persistent organic pollutants and heavy metals. With the help of extensive research and regulations, the emissions of some of the persistent organic pollutants had immense decrease throughout the years. In the last two decade, the concern switched to human and veterinary pharmaceuticals, personal care products etc. (Petrovic et al., 2003).

2.2 The Groups of The Selected Micropollutants

After the 1990s, the emissions of the persistent organic pollutants are decreased greatly with the help of regulations. Therefore, the attention switched to the compounds called emerging or new micropollutants that are not regulated and researched enough (Petrovic et al., 2003). Petrovic et al. (2003) also stated in the same report that these compounds are present in daily life and consumed in large amounts.

Micropollutant as a name defines a large group of compounds. In this thesis, 14 micropollutants and their 10 metabolites have been studied based on their presence in wastewater influent and/or effluents. These selected micropollutants are in the groups of pharmaceutically active compounds, steroid hormones, industrial chemicals, and disinfection byproducts.

2.2.1 Pharmaceutically active compounds

Pharmaceuticals include human and veterinary prescribed drugs and over-the-counter medication. There are also diagnostic agents (e.g., X-ray contrast media), vaccines, and bioactive dietary supplements called "nutraceuticals" and food supplements such as vitamins. Pharmaceuticals can be both synthetic and natural substances. They can be obtained from microorganisms (e.g., antibiotics), tissues, plants, animals etc. (Daughton, 2007). They can be acidic, alkaline and neutral compounds. Among the studied compounds carbamazepine, ciprofloxacin, diclofenac, ibuprofen and carbamazepine are present in the group of acidic compounds while propranolol is in the group of alkaline compounds (Petrovic et al., 2003). Pharmaceutically active compounds (PhACs) are refered to a single compound used in a finished pharmaceutical product and called as "pseudopersistent" pollutants because of the constant introduction into the ecosystem. PhACs have been on the spotlight last decade for having an unfavorable effects on organisms and creating antibiotic-resistant bacteria gene (Yan et al., 2014). Creating antibiotic-resitant bacteria is not the only concern. The micropollutants such as pharmaceuticals make the reuse of wastewaters for a water source quite difficult since they cannot be treated completely in the wastewater (Rosal et al., 2010). The concern arised for PhACs in water systems is the effects of combined PhACs on organisms rather than the effects on organisms exposed to the environmentally relevant concentrations. Moreover, wastewaters can be reused directly or in some cases indirectly. Indirect reuse of wastewaters are not the intention

as mentioned above. However, due to deficient removal of PhACs, PhACs can enter the water systems that can be used as a drinking water sources resulting in using wastewater with partially treated micropollutants as a water resource. Since PhAC can be found in water sytems as a mixture, not as a single compound, the synergistic and chronic effects on human become a problem to solve. Therefore, this issue is detrimental to the reuse of wastewater as a water reuse.

2.2.2 Steroid hormones

Endocrine system includes glands that are located in the some parts of the body. These glands secrete one or more hormones that are natural chemicals and regulate physilogical functions of the body (Gore et al., 2014). All streoid homones are obtained from chlesterol. They are carried by bloodstream to their target organs to regulate the various physilogical fuctions. Steroid hormones are released from three steroid glands which are the adrenal cortex, testes, and ovaries. The steroid hormones include estrogens, androgens, progestagens, glucocorticoids and mineralocorticoids. Adrenal cortex secretes glucocorticoids and mineralocorticoids while testes produce androgens (Britannica, 2017).

They bind to the receptors to get a specific response. Moreover, endocrine disrupting compounds can mimic the steps of how endocrine systems work with hormones and their specific receptors or they can inhibite the whole process. In this case, the receptors that are attacked by endocrine disrupting compounds are estrogen receptors which are located in the brain, bone, vascular tissues and reproductive tissues in both female and male although estrogen receptors are not the only receptors that are disrupted by EDCs (Gore et al., 2014).

Some of the Endocrine Disrupting Compounds are bioaccumulative, meaning that constant exposure to the EDCs can result in increased concentrations in fat tissues. Moreover, there are studies that show EDCs can change germ cells which are precursors to sperm and egg cells which means the changes made to the cell can be inherited (Gore et al., 2014).

In this thesis, the hormones that are studied are estrogens which are secreted by ovaries. Estrogens that are studied are 17β-estradiol, Estriol, Estrone, Estrone-3-sulfate, and Estron-glucuronide. In addition to natural hormones, there is one synthetically produce estrogen which is 17α-ethinylestradiol.

2.2.3 Industrial chemicals

Industrial chemicals consist of components, additives or admixtures in industrial production. These compounds enter surface waters either directly or indirectly, through wastewater treatment plants. On the other hand, some of the compounds can enter the surface when they are still in use. Industrial chemicals can be detected in the wastewater treatment plants and surface water due to their high production volumes. Some chemicals are known to have endocrine disrupting effects on organisms in the surface waters (Evaluation Report on Industrial Chemicals, 2012). The example selected to represent plasticizers within the industrial chemicals group is BPA. BPA is used for producing polycarbonate plastics for bottles and the inner coating of tins and beverage cans. Moreover, BPA is used in epoxy resins, sealing and packaging materials, as antioxidant in plasticizers (Evaluation Report on Industrial Chemicals, 2012). Furthermore, BPA is in the list of EDCs. Therefore, eliminating BPA is crucial.

2.2.4 Disinfection byproducts

NDMA has received a lot of attention as an emerging micropollutant in the last decade. USEPA stated that when concentration of NDMA is present at 10 ng/L, cancer risk is one in a million (USEPA, 2000). There must be precursor which are natural organic matter and anthropogenic pollutants for NDMA to form in water systems. If wastewaters are chlorinated when precursors are present, NDMA can form as in water treatment plants. This can result in cancer risk for humans if wastewaters with NDMA enters water systems (Bilgin, 2014).

2.3 The Properties of Selected Micropollutants

The micropollutants that are selected for this report to investigate can be seen from Table 2.1.

Table 2.1 : Properties of the selected micropollutants (Pehlivanoğlu-Mantaş, 2016).

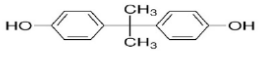
Micropollutant	Molecular Structure	Properties
Ibuprofen		Molecular Weight:206.28 g/mole Solubility:21 mg/L pKa:4.91

Table 2.1 (continued): Properties of the selected micropollutants (Pehlivanoğlu-Mantaş, 2016).

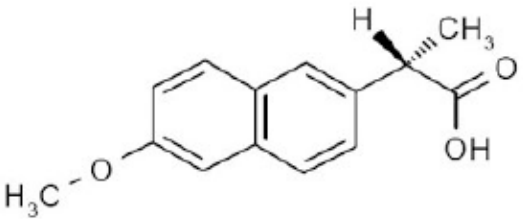
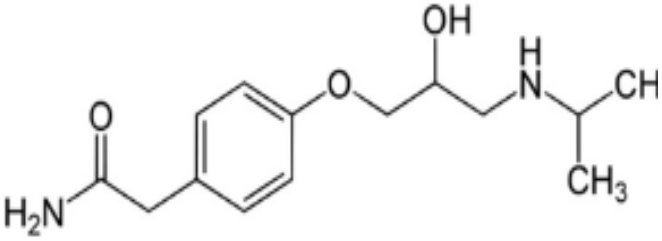
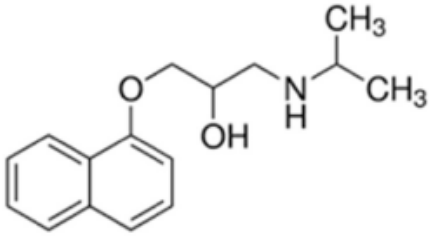
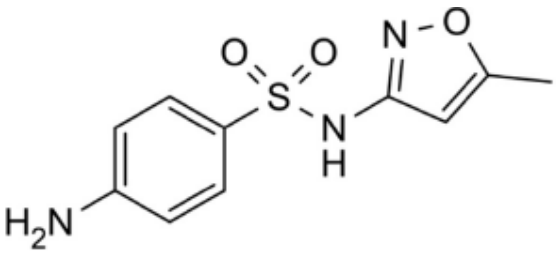
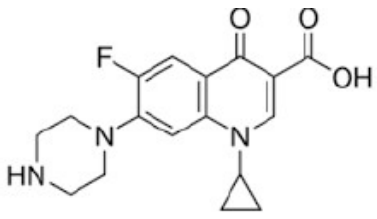
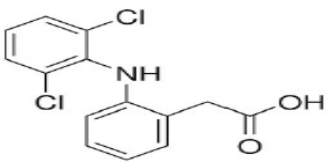
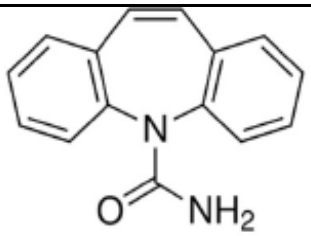
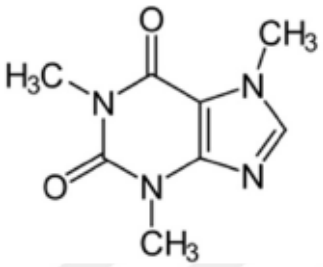
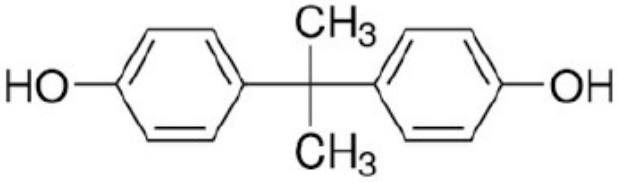
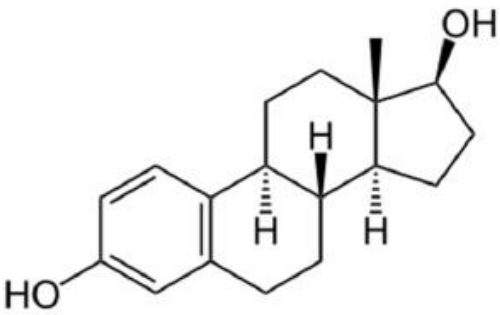
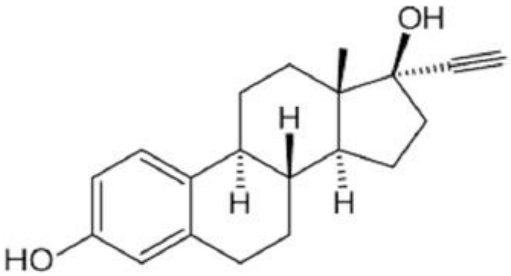
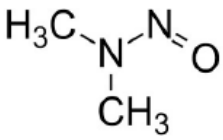
Micropollutant	Molecular Structure	Properties
Naproxen		Molecular Weight:230.25 g/mole Solubility:15.9 mg/L pKa:4.15 logKow:3.18
Atenolol		Molecular Weight:266.33 g/mole Solubility:13300 mg/L pKa:9.6 logKow:0.16
Propranolol		Molecular Weight:259.34 g/mole Solubility:61.7 mg/L pKa:9.42 logKow:3.48
Sulfamethoxazole		Molecular Weight:253.27 g/mole Solubility:500 mg/L pKa:6 logKow:0.89
Ciprofloxacin		Molecular Weight:331.34 g/mole Solubility:30000 mg/L pKa:6.09 logKow:0.28
Diclofenac		Molecular Weight:296.14 g/mole Solubility:2.37 mg/L pKa:4.15 logKow:4.51

Table 2.1 (continued): Properties of the selected micropollutants (Pehlivanoğlu-Mantaş, 2016).

Micropollutant	Molecular Structure	Properties
Carbamazepine		Molecular Weight:236.26 g/mole Solubility:18 mg/L pKa:15.96 logKow:1.51
Caffeine		Molecular Weight:194.19 g/mole Solubility:21.17 mg/L pKa:10.4 logKow:0.01
Bisphenol A		Molecular Weight:228.28 g/mole Solubility:300 mg/L pKa:10.3 logKow:3.32
17β-estradiol (E2)		Molecular Weight:272.38 g/mole Solubility:3.6 mg/L pKa:10.33 logKow:2.7
17α-ethinylestradiol (EE2)		Molecular Weight:296.40 g/mole Solubility:11.3 mg/L pKa:10.2 logKow:3.67
N-Nitrosodimetilamin (NDMA)		Molecular Weight:74.08 g/mole Solubility:100 mg/L pKa:9.3 logKow:2.15

*Solubility values is valid for water and temperature of 25-28 ºC.

In this thesis, some transformation products of the parent compounds are also studied. The reasons why there are transformation products included in this thesis are that some of the transformation products can deconjugate and form the parent compound. Transformation products can be present in the wastewater from the beginning and sometimes parent compounds conjugate in the wastewater during biological process. The metabolites that are already present in the wastewater can deconjugate and form the parent compounds resulting in increased concentrations of these micropollutants in the effluent. Some of the metabolites cannot be removed from the system while their parent compounds can be easily removed.

The metabolites of the selected PhACs and their ratio of the transformation in the body can be seen from Table 2.2.

Table 2.2 : The forms and ratios of the metabolites (Al-Omar, 2005; Kasprzyk-Hordern, 2008; Senta, 2015).

Name of PhAC	Form that is discharged via urine (%)	
	Unchanged	Metabolite
Ibuprofen	1	(+)-2-4'-(2-Hidroxy-2-methylpropyl)-phenyl propionic acid (25) (+)-2-4'-(2-carboxypropyl)- phenyl propionic acid (37) Conjugated ibuprofen (14)
Naproxen	<1	6-0-Desmethyl naproxen (<1) Conjugated naproxen (66-92)
Atenolol	50	Hydroxyl form (3)
Propranolol	<0.5	4-Hidroxypropranolol Glucuronide conjugated propranolol (20)
Sulfamethoxazole	30	N4-Acetyl metabolite
Ciprofloxacin	40-50	Desethyleneciprofloxacin Sulfo-ciprofloxacin, Oxo-ciprofloxacin N-acetylciprofloxacin (15)
Diclofenac	5-10	Glucuronide and sulfate conjugate form
Carbamazepine	3	Hydroxyl (10,11- epoxy) form Conjugated carbamazepine

The micropollutants and their metabolites selected for this thesis can be seen Table 2.3.

Table 2.3 : The micropollutants and their metabolites.

Parent Compound	Metabolite
Atenolol	
Bisphenol-A	
Diclofenac	4-OH-Diclofenac
17α-ethinylestradiol (EE2)	
17β-estradiol (E2)	
Ibuprofen	1-OH-Ibuprofen
	2-OH-Ibuprofen
Caffeine	Paraxanthine
Carbamazepine	10,11-dihydro-10-hidroxycarbamazepine
	10,11-epoxycarbamazepine
Naproxen	O-desmethylnaproxen
NDMA	
Estrone	Estrone-3-sulphate
Propranolol	4-hidroxypropranolol
Ciprofloxacin	
Sulfamethoxazole	Acetylsulfamethoxazole

The micropollutants are considered as hazard to environment and an important problem to investigate due to their existing and possible adverse effects they may exert on the environment. For this reasons, information about toxicity of micropollutants will be provided. In the past, for calculating the possible effects of toxicants, the only endpoint considered was used to be the mortality providing LC50 values. After many studies, the results show that there are many effects other than mortality that should be considered when evaluating the toxicity of a chemical. Therefore, the toxicity of micropollutants can be evaluated based on their Effective Concentrations (EC50). Some of these effects are immobilization and inhibiting the growth, and more recently other bioasssay have been develped to measure the effect of chemicals on different mechanisms in the body of the organisms such as vitellogenin or p53 or biding to estrogen receptor.

LC50 values of some micropollutants are a lot higher than environmentally relevant concentrations thus, leading to a conclusion that they are not important at environmentally relevant concentrations such as ng/L. However, the more important consideration of the toxicity of micropollutants is the chronic toxicity since the measured concentration for chronic toxicity are much less than LC50 and EC50 values.

Moreover, it is proven that synergistic toxicity of micropollutants are greater than the added toxicity of a single micropollutants.

Besides LC50 and EC50 values, there is also endocrine disrupting properties of micropollutants that are of concern. The endocrine system's working principle and how EDCs can interfere with the system was explained in Section 0. In this section, the possible effects of EDCs on organisms are going to be studied in details.

Some of the micropollutants studied are either called as suspicious EDCs or proven to have endocrine disrupting activity (Table 2.4).

Table 2.4 : EDCs among the selected micropollutants (Chang, Choo, Lee, & Choi, 2009).

Compound Class	Name of EDC
NSAID	Ibuprofen
NSAID	Diclofenac
β-Blockers	Atenolol
β -Blockers	Propranolol
Antibiotics	Sulfamethoxazole
Steroid Hormones	17β -estradiol (E2)
Steroid Hormones	17α-ethinylestradiol (EE2)
Steroid Hormones	Estrone
Personal Care Product (Stimulant)	Caffeine
Plasticizers	BPA
Disinfection by-products	NDMA

EDCs have wide range of adverse effects on organisms that are proven with studies. The most known EDCs are BPA and estrogens. BPA can increase the risk of breast cancer. It can also cause feminizing effects on men. Steroid hormones such as 17β-estradiol and estrone can have an effect of feminization (Bolong, Ismail, Salim, & Matsuura, 2009). The highest environmental risk comes from 17β-estradiol, 17α-ethinylestradiol, and ibuprofen. The concentrations of the compounds are diluted after the discharged thus, resulting in no acute risk for aquatic organisms. However, constant exposure can cause chronic effects on long-life organisms in the ecosystem (Martin, Camacho-Munoz, Santos, Aparicio, & Alonso, 2012).

The studies are conducted to determine the effects on organisms exposed to E2. The results of E2 exposure are dose-dependent and the effects of the exposure vary greatly. In the adult stage of male zebrafish, the effective concentrations are 5 and 25 ng/L for vitellogenin induction. Formation of a female-like retrogonodal cavity in male zebrafish starts to increase when exposed to 25 ng/L E2 (Brion et al., 2004).

Endocrine disrupting properties of the micropollutants are mentioned above. The concerns for micropollutants are not only limited to endocrine disrupting properties. There are also toxicity definitions such as acute, chronic and synergistic toxicity. Acute toxicity is the definition of a single exposure or multiple exposure in a short period of time while chronic toxicity means constant exposure to contaminant at lower concentrations as opposed to acute toxicity. On the other hand, synergistic toxicity is still in the progress of study as well as acute and chronic toxicity. Synergistic toxicity defines the toxicity of a mixture of contaminants rather than a single contaminants. Furthermore, the synergistic toxicity is greater than the toxicity of a single compound. Therefore, the studies focus on the synergistic effects of micropollutants since they are present in the environment as a mixture. The toxicity of micropollutants will be studied in detail below.

The potential ecological risk (RQ) in STP effluents and surface waters for NSAIDs which are ibuprofen, diclofenac, and naproxen are high. Similarly, for β-blocker and antiepileptic drugs such as propranolol and carbamazepine, respectively have medium to high potential ecological risk (Hernando, Mezcua, Fernandez-Alba, & Barcelo, 2006).

The toxicity of the combined pharmaceutical is investigated. The toxicity of carbamazepine with another micropollutant called clofibrinic acid is measured. From the results, it is seen that the toxicity of the mixture of two micropollutants was greater than the individual toxicity of the micropollutants. The same results were obtained for ibuprofen and diclofenac. Moreover, the measured toxicity is greater than the predicted toxicity (Cleuvers, 2003). Although the effects are still unknown, the toxicity of combined micropollutants is reported as to be greater than the individual toxicity. Another example for the toxicity is the acute and the chronic toxicity. Ferrari claimed (2004) that one concentration value can mean two things when using predicted acute and chronic toxicity distribution. When using predicted chronic toxicity distribution

the same concentration value can affect a greater percentage of a population compared to predicted acute toxicity distribution (Ferrari, 2004).

There are studies done on diclofenac. With these studies, diclofenac made into the watch list of EU. One of the most eye opening study on diclofenac was the effects of diclofenac on vulture population in Pakistan. Oaks et al. stated (2004) that there was 95% population decline of the oriental white-backed vulture starting from 90s. In this study, the correlation between diclofenac and renal failure is made (Oaks et al., 2004).

In the selected micropollutants, there are two antibiotics and one metabolite: Ciprofloxacin, Sulfamethaxozole and Acetyl-sulfamethaxozole. Creating antibiotic-resistant bacteria in the environment is another adverse effects to be concern about since it would be difficult to regulate the water-borne diseases caused by bacteria. Jones et al. stated that the low concentration of antibiotics can contribute to creating antibiotic-resistant pathogen. Moreover, antibiotics may inhibit the microbial population in the wastewater thus, leading to decrease in the degradation of organic matter and affecting the nitrification/denitrification processes (Jones, 2007).

2.4 Removal of Micropollutants In Wastewater Treatment Plants

In other sections, the main topic was what micropollutants are and the importance of removing of them. In this section, the most common treatment technologies for micropollutants will be mentioned.

2.4.1 Biological treatment

The design purpose of biological treatment is the removal of organic carbon. Therefore, the most common system used for biological treatment is activated sludge system. In this system, organic carbon removal is achieved under aerobic conditions. However, for municipal wastewater treatment systems nutrient removal is crucial. To remove nutrients, in addition to activated sludge system, anaerobic and anoxic tanks are designed. This design is called as conventional wastewater treatment system.

Moreover, ibuprofen can be removed with efficiency more than 90%. Naproxen also can be removed with high removal efficiencies (50-80%). On the other hand, diclofenac can be removed partially (20-40%) while carbamazepine cannot be removed from the system (Joss et al., 2005). Furthermore, diclofenac can be removed

with 70% of removal efficiency in the first wastewater treatment system while in the second wastewater treatment system, there can be no removal of diclofenac. On the other hand, BPA is almost completely removed in the first wastewater treatment plant while in the second wastewater treatment plant, there is no removal observed. This might mean that degradation of BPA might depend on SRT since the second WWTP has SRT of 1 and 2 days (Clara, Strenn, et al., 2005). On the other hand, Göbel et al. (2005) stated that considering 62% of total load belonged to acetyl-sulfamethoxazole, after secondary treatment, the metabolite was removed almost completely while concentration of sulfamethoxazole stayed as 290 ng/L indicating transformation between sulfamethoxazole and acetyl-sulfamethoxazole (Göbel et al., 2005). In some cases, transformation between parent compounds and their metabolites leads to negative removals which means higher effluent concentration of parent compound than influent concentraiton. This can be because of either inaccurate sampling, inaccurate sorption coefficient for especially primary sludge or deconjugation of metabolites and transformation into original compound during biological treatment (Joss et al., 2005).

To investigate the effects of SRT on the removal of diclofenac, the removal of diclofenac is observed with different SRT values. There is data about increased concentrations of diclofenac throughout the system up to SRT of 15 days. However, significant removal efficiencies are achieved with SRT of 30+ days. On the other hand, the removal of estrone (E1) is achieved with high removal efficiency such as 80% with no dependency on SRT (Lishman et al., 2006).

The micropollutants such as diclofenac and carbamazepine have minimal adsorption to sludge because of their hydrophobic nature. Activated sludge plant with SRT of one day or less has no removal effects on ibuprofen and diclofenac (Drewes, 2007). Clara et al. stated (2005) that plant with SRT of two days without nitrification process has no removal of ibuprofen. However, the plant with SRT of 48 days with the denitrifying process has 98% removal of ibuprofen (Clara, Kreuzinger, Strenn, Gans, & Kroiss, 2005).

The biological treatment initially is not designed to remove micropollutants. Therefore, advanced treatment technologies are required. Some of the advanced treatment technologies are mentioned below.

2.4.2 UV photolysis

UV photolysis process is based on the absorption of photons. The compounds that come in contact with the photons are oxidized and the treatment of the pollutant is finalized. UV photolysis process is used in water and wastewater treatment plants for disinfection.

UV treatment can increase the removal efficiency of micropollutants. However, it is not a practical removal treatment when it comes to micropollutants. 50-80% of removal efficiency is achieved only with a dose hundred times greater than a typical disinfection dose (Bolong et al., 2009).

2.4.3 Ozonation

Ozonation is one of the most common and preferable advanced treatment technologies. Ozone is produced by exposing oxygen to high electric voltage or UV radiation. Ozonation process is a powerful oxidation process in water treatment systems. The process is carried out by ensuring that water comes in contact with ozone. Then, ozone oxidizes the organic pollutants and microorganisms. In the past, ozonation is mostly used as oxidation process in water treatment systems. Nowadays, ozonation is also used as oxidation process in wastewater treatment plants.

Oxidation by ozonation occurs in two ways: Direct contact with ozone and indirect contact with hydroxyl radicals (Altmann, Ruhl, Zietzschmann, & Jekel, 2014). Ozonation used as a pretreatment can increase the biodegradability of PhACs (Ziylan & Ince, 2011).

2.4.4 Chlorination

Poorly treated domestic wastewater creates health problems since wastewaters contain enteric bacteria, viruses, and protozoan cysts. These microorganisms can cause diseases such as cholera if wastewater is discharged to receiving water bodies withouth control. Due to this health hazard, disinfection becomes one of the most common process to inactivate the pathogenic microorganisms. Chlorine is one of the most commonly used disinfectant in domestic wastewater treatment plants. Chlorine can be applied in many ways which are chlorine gas, hypochlorite solutions, and other solid or liquid forms. Chlorination is mostly used because of its cost effectiveness, residual chlorine in the effluent for longer disinfection (EPA, 1999). Studies on chlorination

show that the reaction between chlorine and micropollutants happen quickly. For instance, 5 mg/L of chlorine concentration comes in contact with sulfamethoxazole and in 30 seconds 44% removal of sulfamethoxazole is achieved. However, after 60 minutes, there is no further decrease in concentration (B. Li, Zhang, T., 2013). With 10 mg/L chlorine concentration, more than 80% removal of BPA, diclofenac, and naproxen is achieved. On the other hand, ibuprofen has less tendency to react with chlorine than other micropollutants (Noutsopoulos, 2015).

2.5 Regulation on Selected Micropollutants

Surface Water Quality Regulation (No: 29797) entered into force in Turkey to to determine the biological, chemical, physico-chemical and hydromorphological quality of surface and coastal waters, categorise the surface waters, monitor the water quality and the amount of the water, present the purpose of use of the water considering the sustainable development objectives, protect the water, and take precautions in order to achieve good water status. In this regulation; E2, EE2, BPA, diclofenac and sulfamethoxazole are included (Table 2.5).

Table 2.5 : The regulation of selected micropollutants.

Compound Name	YA-EQS* Rivers/Lakes (µg/L)	MA-EQS** Rivers/Lakes (µg/L)	YA-EQS Coastal Waters (µg/L)	MA-EQS Coastal Waters (µg/L)
17-α-ethinylestradiol (EE2)	0.5	0.9	0.5	0.9
17-β-estradiol (E2)	0.5	0.5	0.5	0.5
Bisphenol-A	6.5	252	6.5	65
Diclofenac	100	100	100	100
Sulfamethoxazole	5	50	5	50

*Yearly Average-Environmental Quality Standard

**Maximum Allowed- Environmental Quality Standard

Even though in the regulation, the standards of micropollutants listed above are set to µg/L levels, the studies show that even at ng/L, E2 and EE2 can have adverse effects on organism. Therefore, in order to monitor the micropollutants that have possible negative effects, the watch list of EU is formed.

The aim of watch list is to monitor emerging pollutants and other substances which the monitoring data is not sufficient enough. For this purpose, micropollutants of watch list are monitored for 4 years at most. Pollutants that are proven to be a risk are determined as candidate priority substances (Carvalho et al., 2015).

In 2012 diclofenac, E2 and EE2 were suggested to be on the priority list of European Union. In addition to the micropollutants mentioned, E1 was suggested to be on the watch list of European Union. As of 2015, the watch-list includes diclofenac, E1, E2, and EE2.

2.6 Concentrations Reported In the Literature

A thorough literature survey indicated that the concentration range of micropollutants in the influents and effluents of wastewater treatment plants can be rather wide (Table 2.6). The concentrations and the removal percentages of micropollutants depend on various factors:

- Temperature
- pH
- Redox conditions
- Hydraulic retention time (HRT)
- Solids retention time (SRT)

Microorganisms that are responsible for the biodegradation process require optimum temperature conditions. If the temperature is low, there can be problems with the removal efficiencies since microorganisms that are present in the treatment plant have specific temperature conditions to live. The meaning of the redox conditions is the different removal efficiencies of anaerobic, anoxic and aerobic processes. Hidraulic retention time and Sludge retention time directly affect the removal efficiency of the pollutants.

Table 2.6 : Minimum and maximum concentration values of micropollutants.

Micropollutant	Influent	Effluent	References
Atenolol	<26.4 -11,239 ng/L	<10-7,602 ng/L	(N. A. Al-Odaini, Zakaria, Yaziz, & Surif, 2010; Najat A. Al-Odaini, Zakaria, Yaziz, Surif, & Abdulghani, 2013; Alidina et al., 2014; Anumol, Vijayanandan, Park, Philip, & Snyder, 2016; Behera, Kim, Oh, & Park, 2011; De la Cruz et al., 2013; Gros, Petrovic, & Barcelo, 2006; Klamerth, Malato, Aguera, & Fernandez-Alba, 2013; Kleywegt et al., 2016; Kostich, Batt, & Lazorchak, 2014; Kot-Wasik, Jakimska, & Sliwka-Kaszynska, 2016; Papageorgiou, Kosma, & Lambropoulou, 2016; Prieto-Rodriguez et al., 2012; B. Subedi, Balakrishna, Joshua, & Kannan, 2017; Bikram Subedi et al., 2015; B. Subedi & Kannan, 2015; Tewari, Jindal, Kho, Eo, & Choi, 2013; Vieno, Tuhkanen, & Kronberg, 2006)
Caffeine	<10.2-120,000 ng/L	<10-66,379 ng/L	(Alidina et al., 2014; Anumol et al., 2016; Arrubla Vélez et al., 2016; Behera et al., 2011; Choi et al., 2008; Gomez, Martinez Bueno, Lacorte, Fernandez-Alba, & Aguera, 2007; Klamerth et al., 2013; Kleywegt et al., 2016; Kosma, Lambropoulou, & Albanis, 2014; Kot-Wasik et al., 2016; X. Li, Zheng, & Kelly, 2013; Papageorgiou et al., 2016; Prieto-Rodriguez et al., 2012; B. Subedi et al., 2017; Bikram Subedi et al., 2015; Tewari et al., 2013; Yan et al., 2014; Yu & Chu, 2009)
Carbamazepine	<15.8-3217.1 ng/L	<4.2-5127.8 ng/L	(Alidina et al., 2014; Altmann et al., 2014; Aymerich et al., 2016; Bahlmann, Brack, Schneider, & Krauss, 2014; Behera et al., 2011; Choi et al., 2008; De la Cruz et al., 2013; Gomez et al., 2007; Gros et al., 2006; Kleywegt et al., 2016; Knopp, Prasse, Ternes, & Cornel, 2016; Kosma et al., 2014; Kostich et al., 2014; Kot-Wasik et al., 2016; Lajeunesse, Smyth, Barclay, Sauve, & Gagnon, 2012; X. Li et al., 2013; Lindholm-Lehto, Ahkola, Knuutinen, & Herve, 2016; McEneff, Barron, Kelleher, Paull, & Quinn, 2014; Öllers, 2001; Papageorgiou et al., 2016; Prieto-Rodriguez et al., 2012; B. Subedi et al., 2017; Bikram Subedi et al., 2015; B. Subedi & Kannan, 2015; Vieno et al., 2006; Yan et al., 2014)
Ciprofloxacin	66-650 ng/L	<29-1510 ng/L	(De la Cruz et al., 2013; Klamerth et al., 2013; Kleywegt et al., 2016; Kostich et al., 2014; Prieto-Rodriguez et al., 2012; Tewari et al., 2013; Vieno et al., 2006)
Diclofenac	<18.9-5164 ng/L	<16.7-28000 ng/L	(N. A. Al-Odaini et al., 2010; Alidina et al., 2014; Altmann et al., 2014; Anumol et al., 2016; Arrubla Vélez et al., 2016; Aymerich et al., 2016; Behera et al., 2011; De la Cruz et al., 2013; Gomez et al., 2007; Gros et al., 2006; Klamerth et al., 2013; Knopp et al., 2016; Kosma et al., 2014; Lindholm-Lehto et al., 2016; Madikizela & Chimuka, 2016; McEneff et al., 2014; Öllers, 2001; Papageorgiou et al., 2016; Prieto-Rodriguez et al., 2012; Tewari et al., 2013; Yan et al., 2014)
Ibuprofen	<12-221,000 ng/L	<78.3-67,900 ng/L	(Alidina et al., 2014; Arrubla Vélez et al., 2016; Aymerich et al., 2016; Behera et al., 2011; Carballa et al., 2004; Gomez et al., 2007; Gros et al., 2006; Klamerth et al., 2013; Kleywegt et al., 2016; Kosma et al., 2014; Kot-Wasik et al., 2016; X. Li et al., 2013; Lindholm-Lehto et al., 2016; Madikizela & Chimuka, 2016; Öllers, 2001; Prieto-Rodriguez et al., 2012; B. Subedi et al., 2017; Bikram Subedi et al., 2015; Tewari et al., 2013; Yan et al., 2014; Yu & Chu, 2009)
Naproxen	<26.4-38,800 ng/L	<9-5,340 ng/L	(Alidina et al., 2014; Arrubla Vélez et al., 2016; Behera et al., 2011; Carballa et al., 2004; De la Cruz et al., 2013; Gros et al., 2006; Klamerth et al., 2013; Kleywegt et al., 2016; Kosma et al., 2014; X. Li et al., 2013; Lindholm-Lehto et al., 2016; Madikizela & Chimuka, 2016; Öllers, 2001; Prieto-Rodriguez et al., 2012; Tewari et al., 2013)
Propranolol	<8.3-290 ng/L	<5.5-470 ng/L	(Gros et al., 2006; Kleywegt et al., 2016; Papageorgiou et al., 2016; Prieto-Rodriguez et al., 2012; B. Subedi et al., 2017; Bikram Subedi et al., 2015; B. Subedi & Kannan, 2015)
Sulfamethoxazole	<4.5-3,800 ng/L	<3.2-2,500 ng/L	(Alidina et al., 2014; Altmann et al., 2014; Aymerich et al., 2016; Behera et al., 2011; Carballa et al., 2004; Choi et al., 2008; De la Cruz et al., 2013; Gros et al., 2006; Klamerth et al., 2013; Knopp et al., 2016; Kosma et al., 2014; X. Li et al., 2013; Papageorgiou et al., 2016; Prieto-Rodriguez et al., 2012; B. Subedi et al., 2017; Bikram Subedi et al., 2015; Tewari et al., 2013; Yan et al., 2014)

The micropollutants listed in the table above is found to be in a very wide range of values. This might imply that the concentrations of micropollutants can vary according to country, season, and the amount of consumption of the respective pharmaceutically active compound.

The removal efficiencies of the micropollutants from the literature can be seen from Figure 2.1.

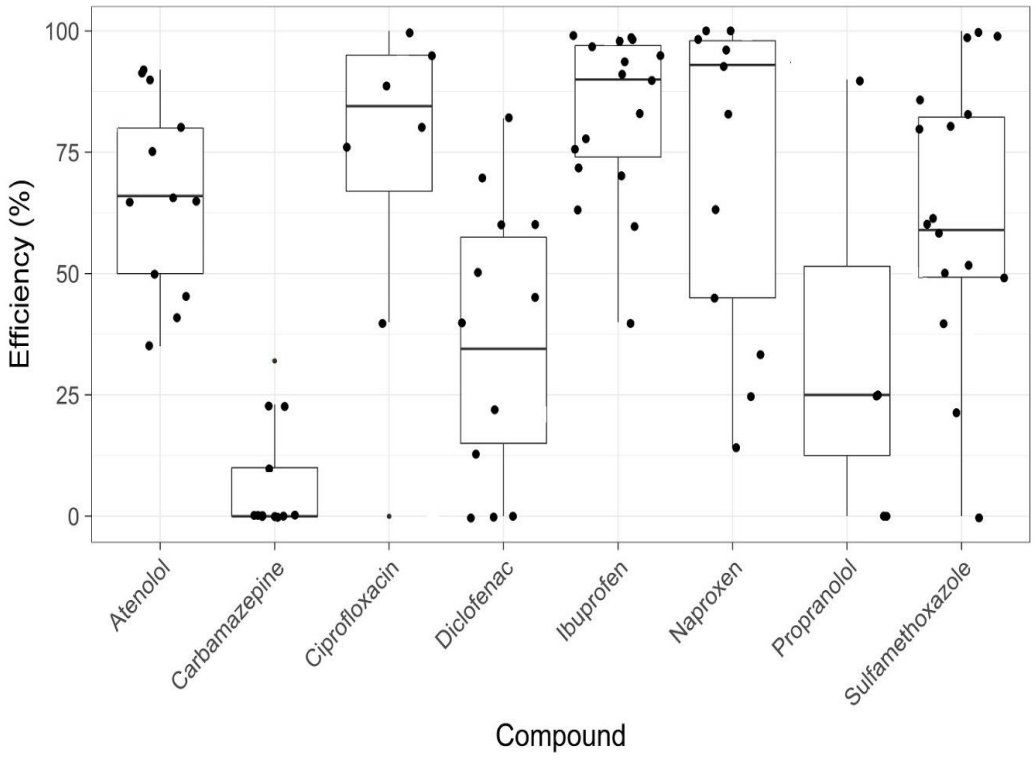


Figure 2.1 : The removal efficiencies of micropollutants reported in the literature.

It can be said that ibuprofen can be removed mostly with high removal efficiencies, yet, there are medium removal efficiencies 40%. The opposite can be said for carbamazepine with 0% removal to low removal efficiencies. The removal efficiencies for diclofenac and sulfamethoxazole differ greatly from 0% removal efficiencies to high removal efficiencies. Atenolol stays in between medium to high removal efficiencies. Similarly, ciprofloxacin differs between medium to high removal efficiencies. Moreover, the removal efficiency of naproxen is mostly high, yet, there are a few low removal efficiency. For propranolol, removal efficiency can be in a wide range. The removal efficiencies of the micropollutants can also vary greatly as concentrations. This information suggests that some micropollutants cannot be removed even though there are multiple variations among the conditions of treatment

plants. On the other hand, some micropollutants can be removed easily in most of the conditions.

3. MATERIALS AND METHODS

For the thesis, all the volumetric glasswares are washed with acetonitrile followed by distilled water. The remaining glasswares such as borosilicate glass bottles and beakers are washed with acetonitrile followed by distilled water. After the washing, glass bottles and beakers are put in to oven at 550 °C for 3.5 hours.

3.1 Sample Collection

Grab samples are taken from two different wastewater treatment plants.WWTP A and B are equipped with primary sedimentation, conventional activated sludge secondary treatment units with nitrogen and phosphate removal. WWTP A which is a domestic wastewater treatment plant has the capacity of 400,000 m^3/day and services the population of 2,500,000 people while WWTP B which is municipal wastewater treatment plant has the capacity of 300,000 m^3/day and services the population equivalent of 1,500,000 people.

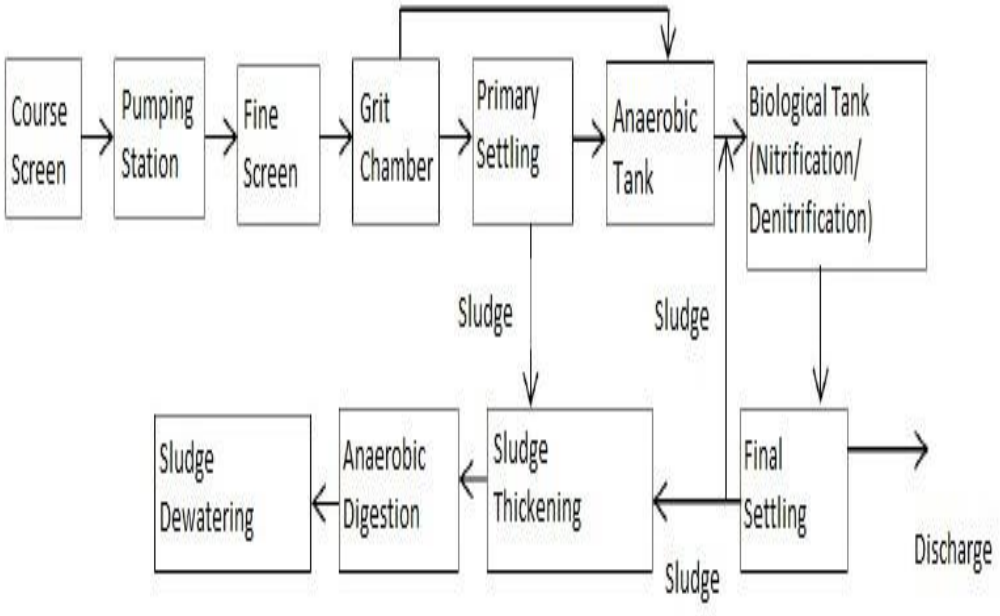


Figure 3.1 : The configuration of wwtp a

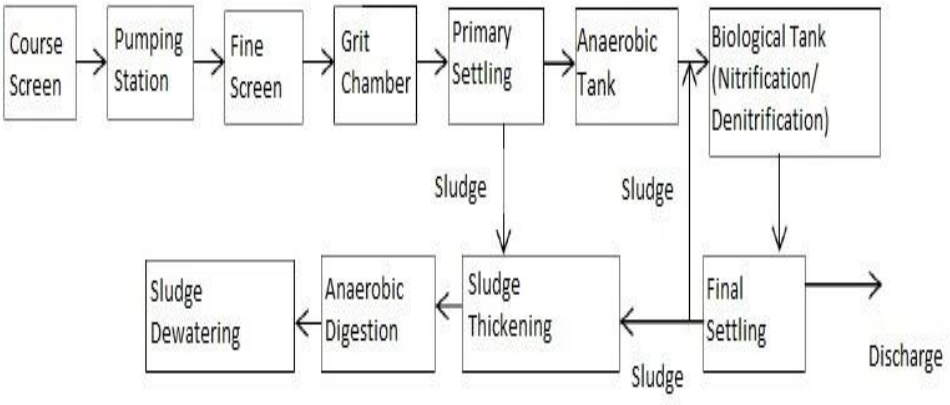


Figure 3.2 : The configuration of wwtp b.

The samples are taken with 10 L fluorinated polypropylene (FLPP) jerrican that prevents any absorption and penetration of samples to the surface. The units which samples are taken are grit chamber, primary settling, anaerobik, aerobic and anoxic tank and the discharge.

3.2 Sample Preparation and Storage of Sample

After collecting the samples, they are filtered through 0.2 µm Whatman cartridge filter, stored in 10 L FLPP jerrican at 4 °C.

3.3 The Experimental and Measurement Procedure

3.3.1 The characterization of wastewater samples

For the samples taken from the each unit, the parameters that are analyzed for the characterization and the methods used for measuring the parameters are provided in Table 3.1.

Table 3.1 : The methods used for parameters.

Parameter	Method
pH	4500-H+ B Electrochemical Method
TSS	2540 D Total Suspended Solids Dried at 103-105°C Method
COD	ISO 6060 Method
TP	4500-P B Sample Preparation ve 4500-P D Stannous Chloride Method
TKN	4500- N-org Nitrogen (Organic) B Macro-Kjeldahl Method
NH3-N	4500- NH3-N (Ammonia) C-Titrimetric Method

3.3.2 Experimental procedure of solid phase extraction (SPE)

The procedure is carried out with 250 mL of filtered wastewater samples and the experiment is performed in pairs. The procedure is applied to samples taken from each unit of WWTP.

Internal standards are injected to make sure the measurment of the micropollutants done correctly. 100 µL of 1000 µg/L internal standard solution is injected to 250 mL of sample. To aim of this is to see that 100 ng/L of the internal standard is measured after the procedure. If it is less than 100 ng/L, from this information, the accurate concentration of micropollutant can be calculated.

Since micropollutants are present in low concentrations, SPE is applied to overcome the detection limit of LC-MS/MS thus, concentrating and cleaning the samples. In order to obtain the best yield with SPE, pH conditions should be arranged. With this purpose, experiments are conducted to find the optimum pH value.

From the results, with pH of 2, the best results are achieved. Therefore, before SPE procedure, the sample's pH is lowered to 2 with using 3 N of H_2SO_4.

In order for SPE to be applied homogeneously for each sample, VacMaster Vacuum Manifold is used as shown in Figure 3.3.

Figure 3.3 : VacMaster manifold.

For the application of SPE on micropollutants except NDMA, Oasis HLB 6 cc Vac Cartridge with 200 mg Sorbent per Cartridge is used as shown in Figure 3.4.

Figure 3.4 : Oasis HLB 6 cc vac cartridge with 200 mg sorbent per cartridge.

For NDMA, the Bakerbond Activated Spherical Carbon SPE Cartridge is used since the preliminary experiments indicated that HLB cannot retain NDMA (Figure 3.5).

Figure 3.5 : Bakerbond cartridge.

However, before the procedure is applied, there should be pre-treatment of cartridges called as conditioning. For conditioning, the cartridges are washed with 5 mL of MTBE, ACN and Merck Water, respectively. After the conditioning, samples which are injected with internal standard are adjusted to pH 2 and loaded onto cartridges with the flowrate of 5 mL/min with teflon tubing. When the samples compeletly pass through the cartridges, they are washed with 5 mL of ACN:Merck Water (20:80, w/w)

to eliminate any contaminant in the cartridge. Then, the cartridges are dried under vacuum for 1 hour for a good recovery.

The concentrated micropollutants in the cartridges are eluted with 5 mL of ACN and MTBE, respectively. The elutes are evaporated until dryness under 10 bar at 40 °C by using TurboVap II (Caliper Life Sciences) shown in Figure 3.6.

Figure 3.6 : TurboVap II.

The micropollutants then are dissolved with 1 mL isopropyl alcohol:Merck Water (15:85, w/w). The solution are filtered through 0.22 µm filter then taken into 2 mL amber vial bottles for the measurement.

3.3.3 Experimental procedure of solid phase extraction for sludge samples

Sludge samples are put into centrifuge to get the solid part of the sludge. After that, solid parts of the sludge are put into Freeze-drying for 2 days to get rid of the moisture.

0.1 g sludge is weighed. 4 mL ACN, 4 mL Ace, and 4 mL MTBE are added to the sludge. This mixture of sludge and solvent is kept in ultrasonic bath for 15 minutes. Afterwards, the mixture is centrifuged at 9000 rpm for 15 minutes. Liquid phases are filtered through 0.22 µm filter. The remaining solid part of the sludge after centrifuge is subjected to the same procedure once again to increase the efficiency of recovery and liquid phases are collected and evaporated until dryness in TurboVap instrument. Then, the dried remains are reconstituted with 2 mL ACN:Ace (1:1, w/w) and 18 mL

distilled water. The obtained 20 mL of sample is subjected to the same SPE procedure as the wastewater samples.

3.3.4 Sludge detection limit experiment

Most of the micropollutants selected in this study tend to have low Kow values and hence are not expected to accumulate much in the sludge samples. To be able to report the concentration of the micropollutants in the sludge samples, mixture of micropollutants are spiked into a freeze-dried sludge sample to obtain two different concentrations (5 ng/g and 20 ng/g). are chosen. To obtain the concentrations mentioned, 25 µL and 100 µL from the stock solution with 20 µg/L concentration, has been spiked into sludge for for 5 ng/g and 20 ng/g, respectively. Then, the extraction procedure for sludge samples followed by the SPE is used to obtain to concentrations in the samples.

3.3.5 LC-MS/MS measurement method

In order to identfy the compounds, the main ion, their fragmented products and the ionization mode are determined. The highest ion intensity among the fragmented products is used for the quantitative analysis while the second highest ion is used for validation. The results can be seen from Table 3.2.

Table 3.2 : The parent ion and the fragmented product.

Compound	Parent ion	Fragmented Product 1	Fragmented Product 2	Fragmentation Energy (V)	Ionization Mode
Bisphenol-A (BPA)	227.3	212.1	133.2	20	Negative
Ibuprofen	205.3	161.3	-	12	Negative
Naproxen	229.3	169.9	185.1	20	Negative
17β-estradiol (E2)	271	145.1	182.4	35	Negative
17α-ethinylestradiol (EE2)	295.1	267.1	145	35	Negative
Atenolol	267.2	145.4	190.3	20	Positive
Propranolol	260	183.3	116.3	20	Positive
Sulfamethoxazole	254	155.7	91.9	20	Positive
Ciprofloxacin	332	314.1	230.9	20	Positive
Diclofenac	294	249.9	214.1	20	Negative
Carbamazepine	237.2	194.4	192.2	20	Positive

Table 3.2 (continued): The parent ion and the fragmented product.

Compound	Parent ion	Fragmented Product 1	Fragmented Product 2	Fragmentation Energy (V)	Ionization Mode
Caffeine	195	138	110.8	20	Positive
NDMA	75.4	43.5	58.6	17	Positive
4-OH-diclofenac	309.8	265.8	230.2	20	Negative
1-OH-ibuprofen	221.1	158.9	143.2	20	Negative
2-OH-ibuprofen	221.1	177	160	20	Negative
o-desmethylnaproxen	215.1	171.1	169.4	20	Negative
10,11 dihydro-10-hydroxycarbamazepine	255	193.8	236.8	20	Positive
10,11-epoxycarbamazepine	253	180.4	210.3	20	Positive
Acetylsulfamethoxazole	295.9	198.1	145.8	20	Positive
4-hydroxypropranolol	276	172.9	116.4	20	Positive
Estrone-3-sulphate	349	269.1	144.9	35	Negative
Paraxanthine	180.9	124	165.8	20	Positive
BPA-d16	241	223.5	-	20	Negative
Ibuprofen-d3	208.1	164.4	-	12	Negative
Naproxen-d3	231.9	170.9	173.1	20	Negative
17β-estradiol -d2	273	147.2	186.1	35	Negative
Atenolol-d9	274	226.2	179.3	20	Positive
Propranolol-d7	267	189.3	163.2	20	Positive
Sulfamethoxazole -d4	258	159.9	96.4	20	Positive
Diclofenac-d4	298	254.3	217.1	20	Negative
Carbamazepine-d10	247	204.4	201.4	20	Positive
Caffeine-d9	204	144.3	116.5	20	Positive
NDMA-d6	81.4	54.5	40.7	17	Positive
4-OH-Diclofenac-d4	313.7	269.9	-	20	Negative

Table 3.2 (continued): The parent ion and the fragmented product.

Compound	Parent ion	Fragmented Product 1	Fragmented Product 2	Fragmentation Energy (V)	Ionization Mode
o-desmethylnaproxen-d3	218	174.3	171.4	20	Negative
10,11-epoxycarbamazepine-d10	263	190.3	220.4	20	Positive
4-hydroxypropranolol-d7	283	173.1	199.4	20	Positive
Ciprofloxacin-d8	340	322.2	296.3	20	Positive

The instrument must be calibrated in order to get an accurate results. For calibration, the concentrations between 0-200 µg/L are chosen. In the calibration curve, x-axis shows the concentration while y-axis shows the ratio of the measured compound's area to area of the internal standard. "R^2" of the calibration curves for the micropollutants ranges 0.9892 to 1. This means that correlation coefficients of calibration curves are in a linear range and calibration curves that are plotted fits the obtained data.

Even though mass spectrometer does the separation by mass, when the fragmentation occurs, the similar ions can cause contamination. Therefore, there should be separation by chromatographically. The column for both negative and positive mode is the same which is Thermo Hypersil Gold with the dimensions of 100 mm x 2.1 mm, 1.9 µm. However, the mobile phase is different for the different modes shown in Table 3.3.

Table 3.3 : Mobile phases for negative and positive modes.

Negative Mode	Positive Mode
A: 10 mM NH_4OH in water	A: 0.1% Formic Acid in water
B: 10 mM NH_4OH in ACN	B: 0.1% Formic Acid in ACN

For the best separation, gradient elution is used. The gradient elution can be seen from Table 3.4.

Table 3.4 : The gradient elution program for LC-MS/MS.

Time (min)	A (%)	B (%)	µL/min (negative mode)	µL/min (positive mode)
0.00	80	20	400.0	500.0
3.00	10	90	400.0	500.0
3.50	10	90	400.0	500.0
3.60	80	20	400.0	500.0
5.00	80	20	400.0	500.0

The optimum operating conditions for LC-MS/MS are shown in Table 3.5.

Table 3.5 : The optimum operating conditions.

Parameter	Optimum Value
Injection Volume (µL)	25.0
Syringe Speed (µL/s)	8.0
Tray Temperature (°C)	10
Column Temperature (°C)	30
Collision Energy (V)	10
Skimmer Offset (V)	10
Collision Gas Presure (mTorr)	1.5

4. RESULTS AND DISCUSSION

Before taking wastewater samples, SPE method was optimized as a preliminary study (Section 4.1.1). The results of WWTP sampling campaigns are provided under Section 4.2 as seperately for WWTP A and WWTP B, from summer to spring.

4.1 Experimental Preliminary Study

4.1.1 Determination process of spe method

Before the sample collection from WWTP, two SPE methods were tried on synthetic samples. Synthetic sample is prepared by injecting the selected micropollutants into distilled water with a last concentration of 100 µg/L.

Two SPE methods which are proven to give good results in the literature are chosen and shown in Table 4.1.

Table 4.1 : Applied spe procedures.

	SPE 1	SPE 2
Conditioning	5 mL MTBE Acetone Methanol Acetonitrile Water	5 mL MTBE Acetonitrile Water
Washing	5 mL water	5 mL water
Drying	1 hour	1 hour
Elution	5 mL Acetonitrile Methanol Acetone MTBE	5 mL Acetonitrile MTBE
Evaporation	Dryness	Dryness
Dissolution	1mL 20:80 ACN:Water	1mL 20:80 ACN:Water

After comparing two methods, SPE 2 method gives the best recovery results and peak forms. Since several micropollutants are studied, to see the effects on the recovery rates, another study has been done on synthetic sample. SPE 2 procedure was studied under pH 2 and pH 10 conditions. From the information obtained SPE 2 procedure

gives the best result under pH 2 condition. Therefore, for SPE procedure, SPE 2 is chosen to be applied under pH 2.

SPE 2 procedure has applied to wastewater samples of summer. The sample volume was 250 mL. After evaporation to dryness, the micropollutants were dissolved with 0.5 mL of ACN:Water (20:80, w/w). This results in 500 times concentrated samples. After the measurement with LC-MS/MS, the matrix effect of wastewater become more obvious thus, resulting in poor results. Therefore, to minimize the matrix effect, under pH 2 conditions, different SPE procedures are examined. The procedures shown in Table 4.2 was applied on 2 L of wastewater samples.

Table 4.2 : Applied spe procedures on wastewater samples.

	SPE 3-1	SPE 3-2
Conditioning	5 mL MTBE 5 mL Acetone 5 mL Methanol 5 mL Acetonitrile 5 mL Water	5 mL MTBE 5 mL Acetonitrile 5 mL Water
Washing	5 ml water	5 ml 20:80 ACN:Water
Drying	1 hour	1 hour
Elution	5 mL Acetonitrile 5 mL MTBE	5 mL Acetonitrile 5 mL MTBE
Evaporation	Dryness	Dryness
Dissolution	1 mL 20:80 ACN:Water	1 mL 15:85 İsopropyl Alcohol:Water

From the information received, SPE 3-2 (called later on as SPE 3) has the best recovery rates. Moreover, the matrix effects are lessened thus, resulting in good peak forms. However, different SPE procedure has to be applied on wastewater samples for NDMA. The procedure is shown in Table 4.3

Table 4.3 : SPE procedure for NDMA.

SPE Procedure	SPE 4
Conditioning	5 mL acetonitrile 5 mL acetonitrile 5 mL water 5 mL water
Washing	none
Drying	1.5 hours
Elution	2 mL acetonitrile 2 mL acetonitrile 2 mL acetone 2 mL acetone
Evaporation	Between 0.5 mL-1 mL
Dissolution	Completing to 1 mL with water

The recovery rates for the selected procedures and measurement limits are shown in Table 4.4.

Table 4.4 : Recovery rates and LOQ.

Micropollutant	Recovery Rate (%)	LOQ (ng/L)
Atenolol	110±14	4
Bisphenol-A (BPA)	88±5	4
Diclofenac	109±4	4
4-OH-diclofenac	128±18	4
17α-ethinylestradiol (EE2)	82±0,2	10
Ibuprofen	111±8	4
1-OH-ibuprofen	94±23	4
2-OH-ibuprofen	87±3	4
Caffeine	94±22	4
Paraxanthine	99±23	4
Carbamazepine	107±1	4
10,11 dihydro-10-hydroxycarbamazepine	123±3	4
10,11-epoxycarbamazepine	81±1	4
Naproxen	104±1	4
o-desmethylnaproxen	129±5	10
NDMA	105±12	2
17β-estradiol (E2)	82±2	10
Estrone-3-sulphate (E1-sulphate)	69±2	20
Propranolol	105±23	4
4-hydroxypropranolol	61±24	4
Ciprofloxacin	104±15	4
Sulfamethoxazole	85±7	4
Acetylsulfamethoxazole	117±9	4

4.2 Results of Sludge Detection Limit and Concentrations of Micropollutants in Sludge Samples

Sludge detection limit experiment is carried out as it is mentioned in Section 3.3.4. The results are shown in Table 4.5.

Table 4.5 : Results of sludge detection limit.

Micropollutants	Recovery (%)	LOQ (ng/g)
BPA	72	20
Caffeine	121	20
Carbamazepine	153	20
Epoxycarbamazepine	107	20
Diclofenac	169	20
Ibuprofen	146	20
Naproxen	142	5
Sulfamethoxazole	50	5

Experimental procedure for sludge samples is carried out as it is mentioned in Section 3.3.3. Micropollutants were below detection limits show in Table 4.15. Therefore, they are not detected in the sludge. However, For WWTP B, BPA was found as 280 ng/g in primary settling sludge while it was found as 500 ng/g in return activated sludge.

4.3 The Characterization and Micropollutant Measurement Results

The obtained results are given according to WWTP and the seasons. In each title, there are characterization and micropollutant concentration results. The results of micropollutants are categorized as NSAIDs, antibiotics, steroid hormones and other micropollutants. In the group of NSAIDs, there are diclofenac, hydroxydiclofenac, ibuprofen, hydroxyibuprofen, naproxen and o-desmethylnaproxen. Antibiotics include ciprofloxacin, sulfamethoxazole and acetylsulfamethoxazole. While grouping steroid hormones, E1, E1-glucuronide, E1-sulphate, E2, E3, and EE2 are included. The rest is categorized as other micropollutants. In this group, there are atenolol, propranolol, 4-hydroxy-propranolol, BPA, caffeine, paraxantine, carbamazepine, epoxycarbamazepine, dihydro-hydroxycarbamazepine.

4.3.1 The results of wwtp a for summer season

The characterization of the summer samples and removal efficiencies are shown in Table 4.6 and Figure 4.1.

Table 4.6 : The characterization results of wwtp a for summer.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	7.51 ± 0.02	450 ± 25	790 ± 25	260 ± 15	89 ± 4	60 ± 3	7 ± 0.3
Grit Chamber	7.42 ± 0.02	360 ± 20	665 ± 20	245 ± 15	88 ± 4	57 ± 3	7 ± 0.3
Primary Settling	7.73 ± 0.02	200 ± 10	390 ± 15	180 ± 15	89 ± 4	52 ± 3	8 ± 0.4
Biological P	6.97 ± 0.02	165 ± 10 (S) 5930 ± 45	65 ± 5 (S)	40 ± 5	63 ± 3	28 ± 2	16 ± 2
Anoxic	7.11 ± 0.02	80 ± 10 1480 ± 20	45 ± 5 (S)	35 ± 5	30 ± 2	13 ± 1	6 ± 0.3
Aerobic	6.96 ± 0.02	50 ± 5 (S) 1340 ± 20	40 ± 5 (S)	30 ± 5	22 ± 2	8 ± 1	7 ± 0.3
Effluent	7.67 ± 0.02	<10	55 ± 5	40 ± 5	11 ± 1	8 ± 1	6 ± 0.3

*(S):Supernatant

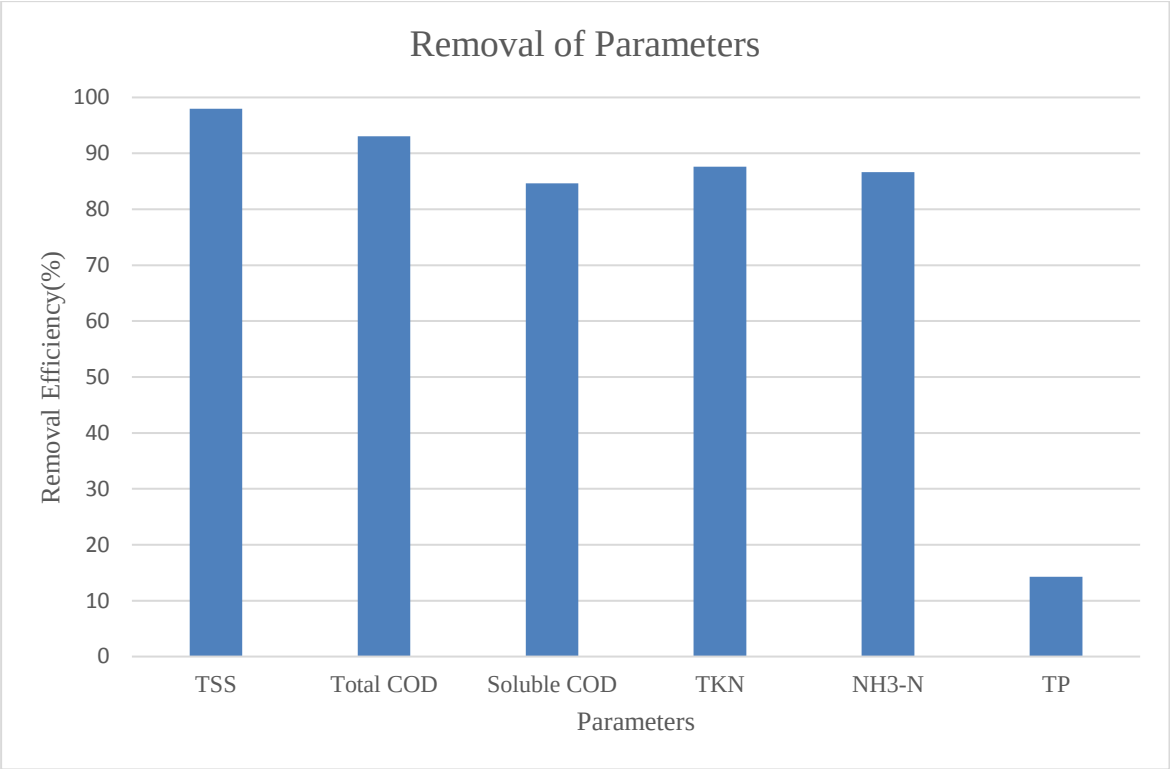


Figure 4.1 : The removal efficiency of wwtp a parameters for summer.

The concentration of TSS was presented as 450 mg/L in the influent and in the effluent, the concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 790 mg/L and 260 mg/L, respectively indicating that total COD was constituted by mostly particulate COD while in the effluent, concentration of total COD and soluble COD were found as 55 mg/L and 40 mg/L which indicated that inert COD was dominant in the effluent. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 89 mg N/L and 60 mg N/L whereas in the effluent they were found as 11 mg N/L and 8 mg N/L, respectively implying that in both influent and effluent, the dominant form of nitrogen was NH_3-N. On the other hand, concentration of TP was measured in the influent and effluent as 7 mg/L and 6 mg/L, respectively while in biological P tank, the concentration of P was increased to 16 mg/L due to the release of phosphorus in anaerobic conditions. As a result, concentrations of TKN and TP in the effluent did not meet the standards of 10 mg N/L and 1 mg/L, respectively.

The high removal efficiencies were found for TSS (98%), total COD (93%), and soluble COD (85%), TKN (88%), NH_3-N (87%). However, removal of TP was in low level (14%).

The micropollutant concentration results are shown between Figure 4.2-Figure 4.7.

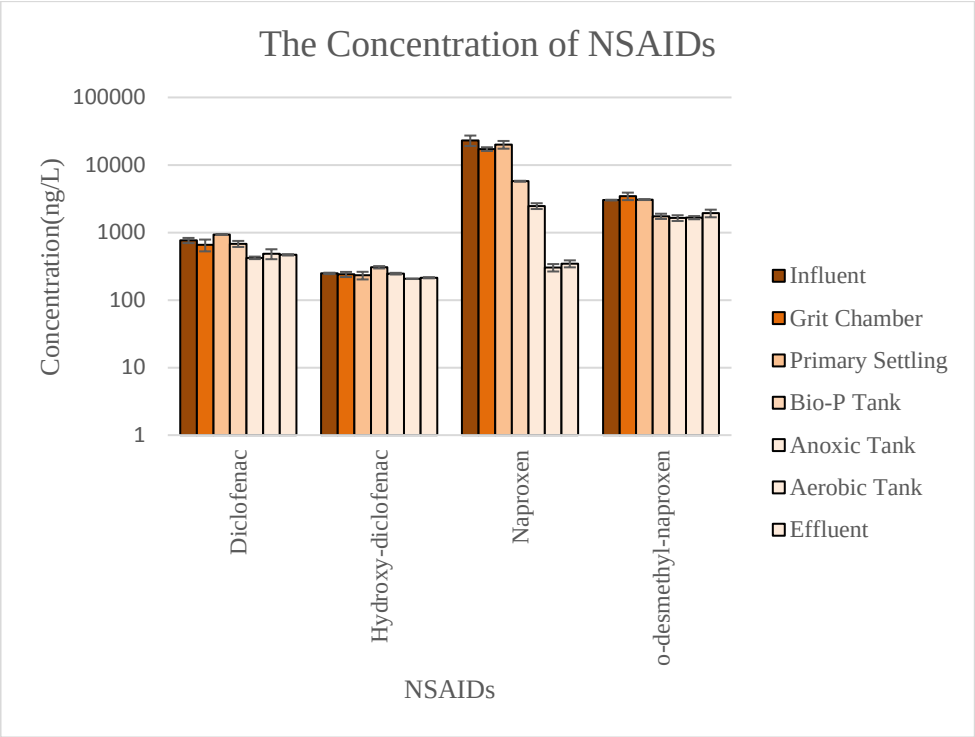


Figure 4.2 : The concentrations of NSAIDs for summer.

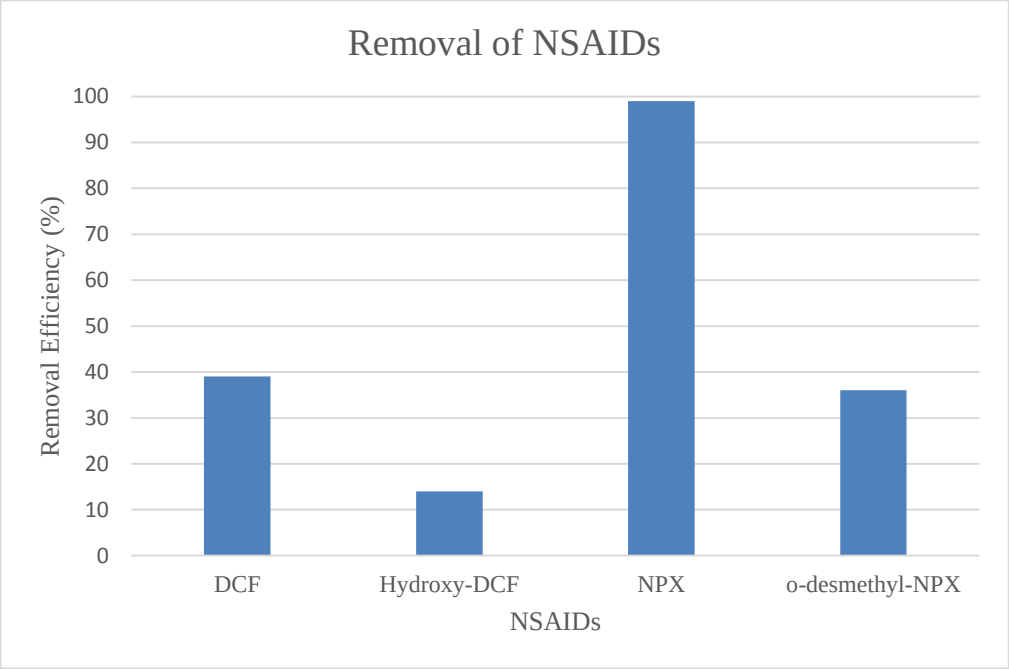


Figure 4.3 : The removal efficiency of NSAIDs for summer.

Diclofenac was present in the influent and effluent with concentrations of 765 ng/L and 467 ng/L, respectively while hydroxy-diclofenac which is the transformation product of diclofenac, was observed in the influent and effluent with concentrations of 248 ng/L and 214 ng/L, respectively. On the other hand, naproxen was found to be present in high concentration of 23,177 ng/L in the influent, yet, naproxen was present

as 346 ng/L in the effluent. o-desmethyl-naproxen which is the transformation product of naproxen was detected in the influent and effluent with concentrations of 3,019 ng/L and 1,931 ng/L, respectively.

For diclofenac (DCF), medium removal (39%) efficiency was found, yet, for hydroxy-diclofenac, low removal (14%) was measured. Furthermore, removal efficiency for naproxen (NPX) was very high (99%) whereas o-desmethyl-naproxen, was in medium levels (36%).

Concentrations of ibuprofen and its transformation products which are 1-OH-ibuprofen and 2-OH-ibuprofen were below quantification limits which are 4 ng/L as shown in Table 4.4.

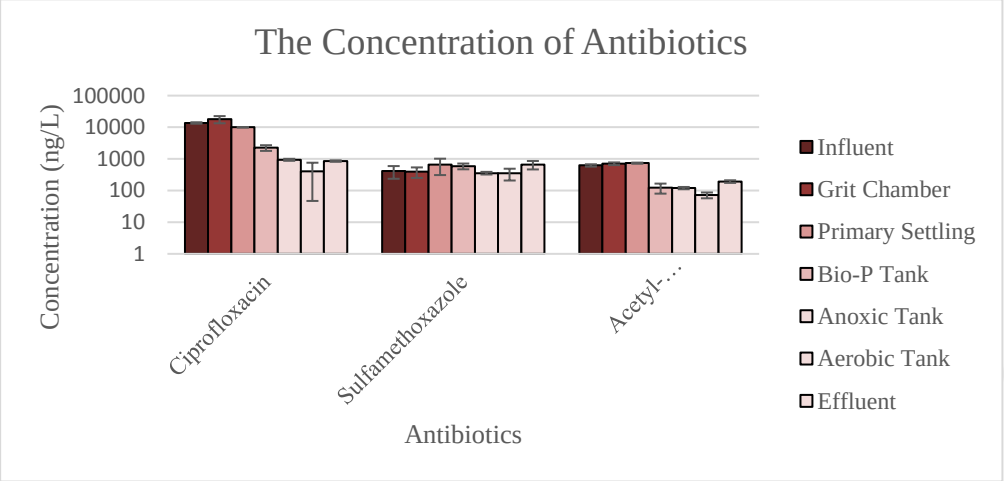


Figure 4.4 : The concentrations of antibiotics for summer.

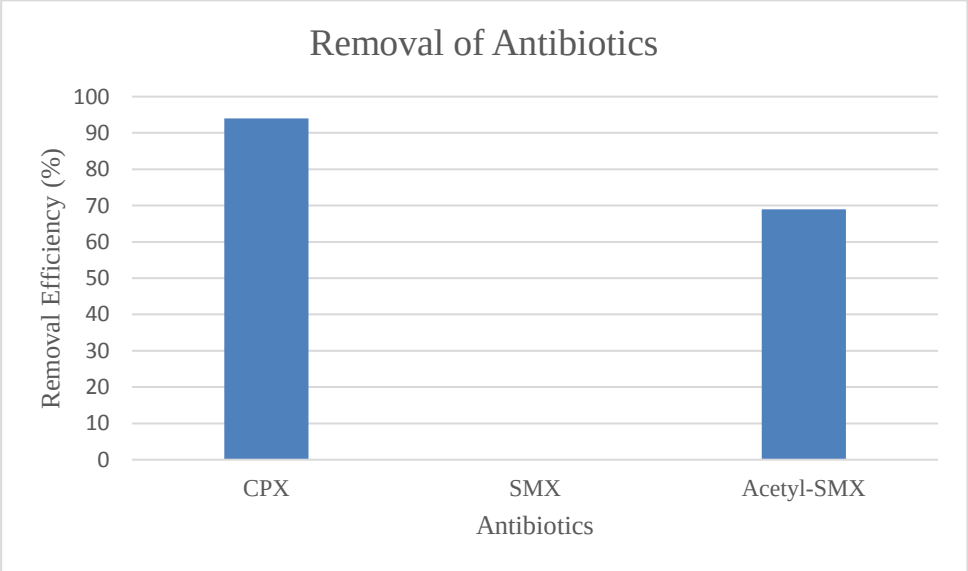


Figure 4.5 : The removal efficiency of antibiotics for summer.

Ciprofloxacin was present in the influent with high concentration of 13,673 ng/L while in the effluent, ciprofloxacin was observed as 857 ng/L. On the other hand, sulfamethoxazole was found in the influent and effluent as 415 ng/L and 663 ng/L, respectively. Higher concentration of effluent can be due to sampling inaccuracy or deconjugation of transformation product which is acetyl-sulfamethoxazole. Acetyl-sulfamethoxazole was found in the influent as 624 ng/L while in the effluent, it was 193 ng/L.

For ciprofloxacin (CPX), high removal rate (94%) was achieved yet, for sulfamethoxazole (SMX) there was negative removal. On the other hand, for acetyl-sulfamethoxazole, medium removal efficiency (69%) were achieved.

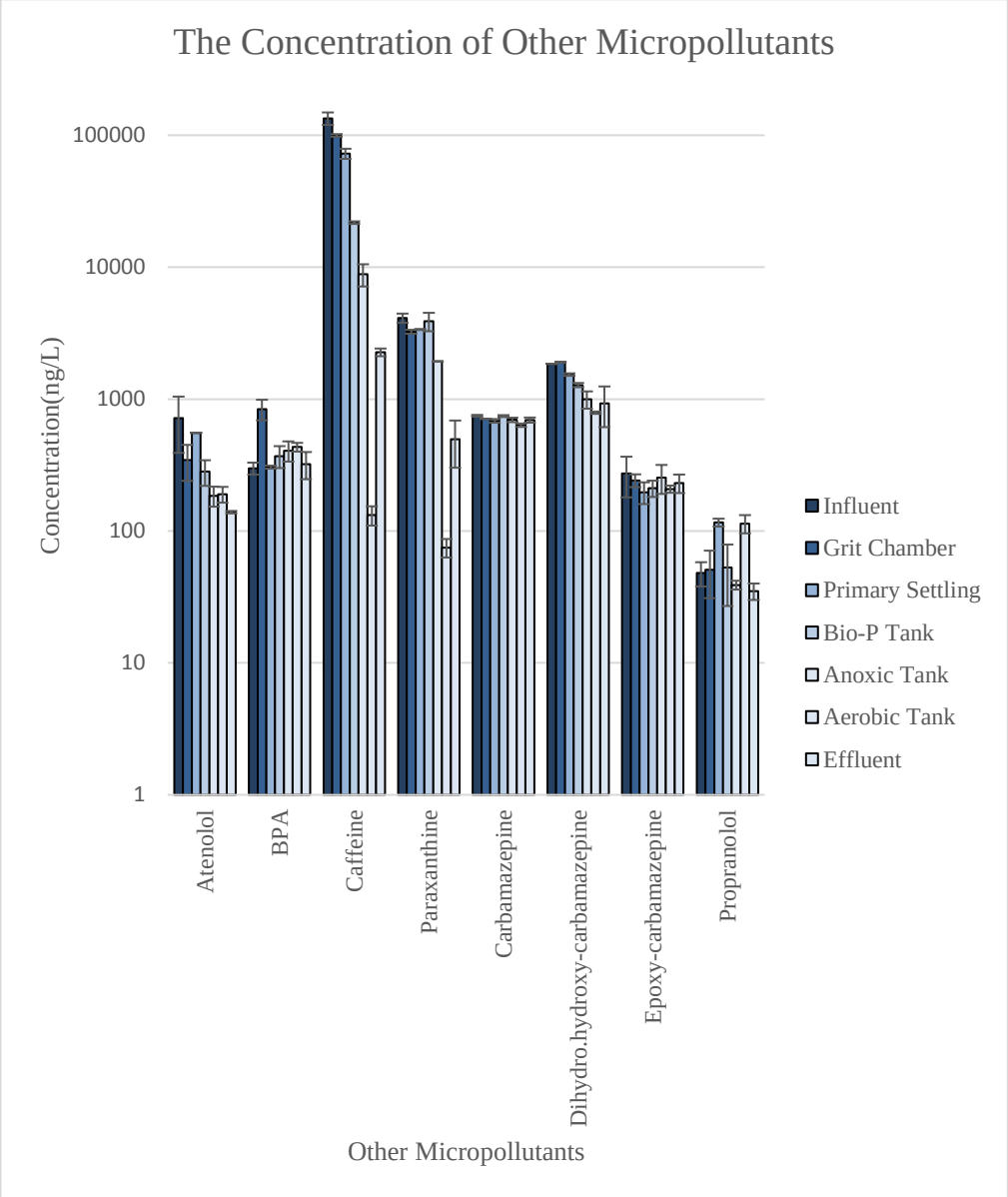


Figure 4.6 : The concentrations of other micropollutants.

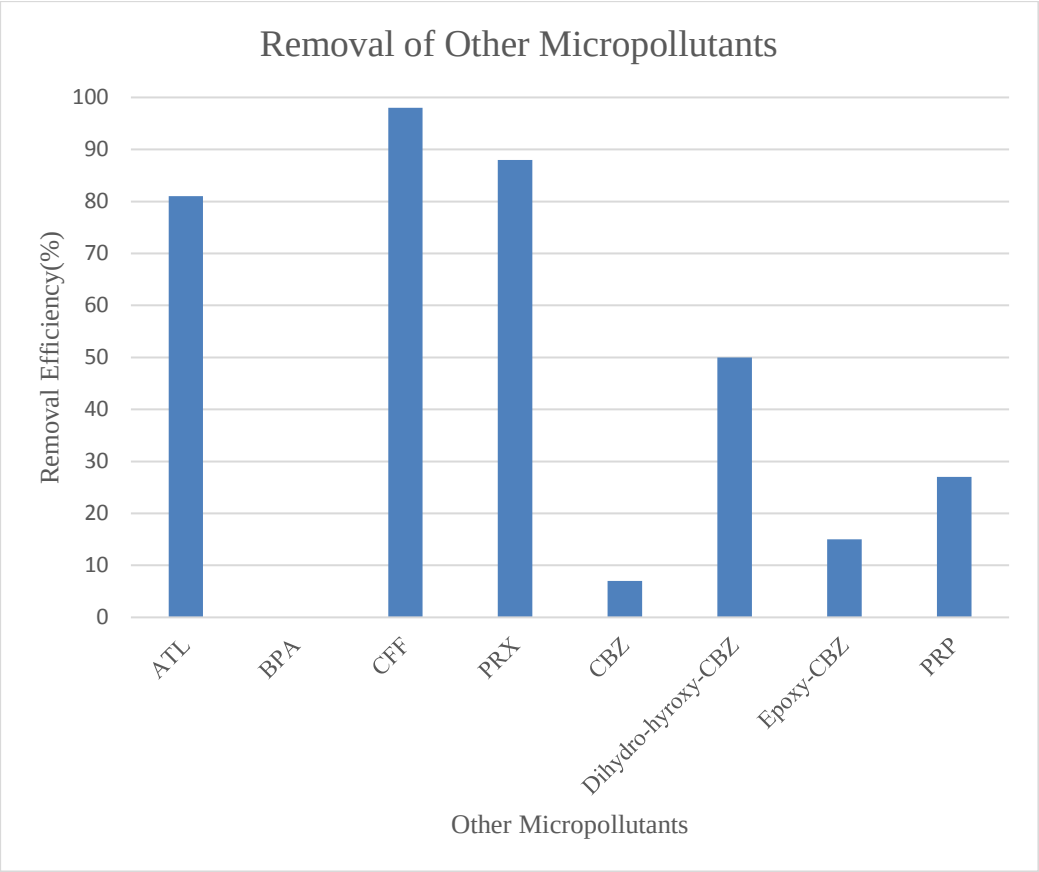


Figure 4.7 : The removal efficiency of other micropollutants for summer.

Atenolol was measured in the influent with a concentration of 718 ng/L while in the effluent, it was 139 ng/L whereas BPA was found as 299 ng/L in the influent, and in the effluent concentration of BPA was increased to 322 ng/L. Caffeine presented the highest concentration with 134,373 ng/L, yet, in the effluent concentration of caffeine was decreased to 2,266 ng/L. Paraxanthine which is the transformation product of caffeine presented high concentration as well with 4,117 ng/L while in the effluent, the concentration of paraxanthine was observed as 495 ng/L. Carbamazepine was measured as 747 ng/L in the influent, and in the effluent it was found as 694 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine which are the transformation products of carbamazepine were observed in the influent as 1,848 ng/L and 273 ng/L while in the effluent they were found as 930 ng/L and 231 ng/L, respectively. On the other hand, propranolol was measured in the influent and effluent as 48 ng/L and 35 ng/L, respectively. 4-hydroxy-propranolol which is the transformation product of propranolol was below detection limit which is 4 ng/L as shown in Table 4.4. Lastly, steroid hormones which are E1, E1-glucuronide, E1-sulphate, E2, E3, and EE2 were below detection limits. The detection limit of E1-

sulphate is 20 ng/L while both E2 and EE2 have the detection limit of 10 ng/L shown in Table 4.4.

The highest removal efficiencies were presented by atenolol (ATL) (81%), caffeine (CFF) (98%), and paraxanthine (PRX) (88%). The medium removal efficiency was found for dihydro.hydroxy-carbamazepine (50%). Low level to negative removal efficiencies were presented by BPA (negative), carbamazepine (7%), epoxy-carbamazepine (15%), and propranolol (27%). The heatmap of summer results are shown in Figure 4.8.

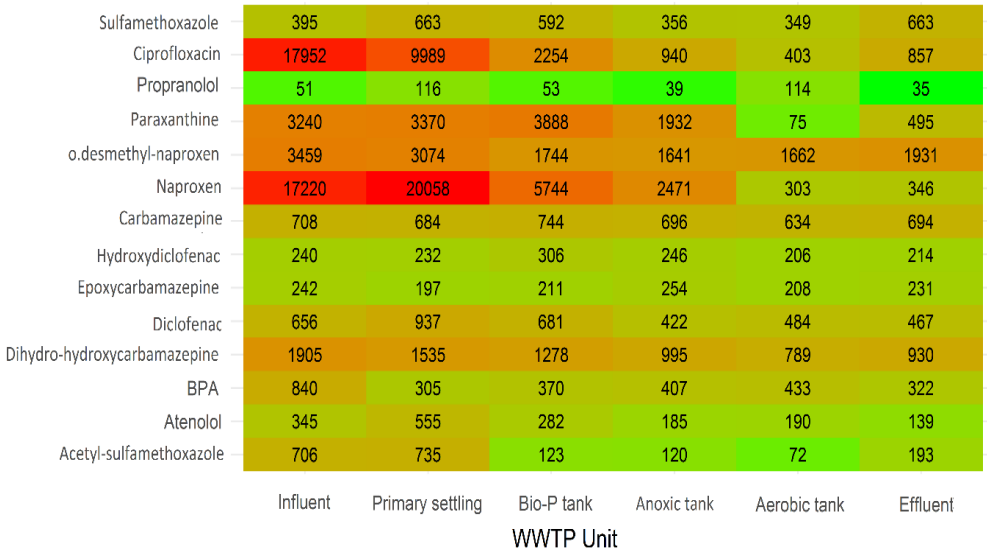


Figure 4.8 : Heatmap of wwtp a for summer.

Concentration results and removal efficiencies are converted into a heatmap to see the differences in removal efficiency in each unit. Ciprofloxacin and naproxen which are antibiotic and NSAID, respectively have high influent concentration even though the season is summer. This can be explained by decrease in flowrate due to summer weather conditions. Lajeunesse et al. (2012) stated that carbamazepine has very limited removal efficiency (0-10%). The same result is obtained for this study and carbamazepine is removed with very low removal efficiency (7%). Epoxy-carbamazepine (15%) also have very limited removal while dihydro.hydroxy-carbamazepine has 50% removal efficiency. Gomez et al. (2007) claimed that diclofenac can be removed with medium removal efficiency (40%) while in this study diclofenac can also be removed with medium removal efficiency (39%). On the other hand hydroxy-diclofenac has limited removal efficiency (14%). Sulfamethoxazole and acetyl-sulfamethoxazole have a different trend compared to others. Acetyl-

sulfamethoxazole has higher concentration than sulfamethoxazole in the influent while in the effluent, acetyl-sulfamethoxazole is removed with high removal efficiency (70%) and negative removal is observed for sulfamethoxazole. Göbel et al. (2005) stated a similar trend and concluded with a possible transformation of acetyl-sulfamethoxazole to sulfametoxazole.

4.3.2 The results of wwtp a for fall season

The characterization results and removal efficiencies for fall season are shown in Table 4.7 and Figure 4.9.

Table 4.7 : The characterization results of wwtp a for fall.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	8.15 ± 0.02	200 ± 10	475 ± 15	240 ± 15	103 ± 5	67 ± 3	9 ± 0.4
Primary Settling	7.95 ± 0.02	200 ± 10	560 ± 15	220 ± 15	83 ± 4	61 ± 3	8 ± 0.4
Biological P	6.94 ± 0.02	7,440 ± 55	80 ± 10 (S)	45 ± 5	60 ± 3	34 ± 2	17 ± 1
Anoxic	7.04 ± 0.02	7,070 ± 55	90 ± 10 (S)	40 ± 5	29 ± 2	3 ± 0,3	10 ± 1
Aerobic	7.25 ± 0.02	2,430 ± 35	85 ± 10 (S)	35 ± 5	34 ± 2	21 ± 2	15 ± 1
Effluent	7.65 ± 0.02	<10	55 ± 5	40 ± 5	36 ± 2	27 ± 2	4 ± 0.2

*(S): Supernatant

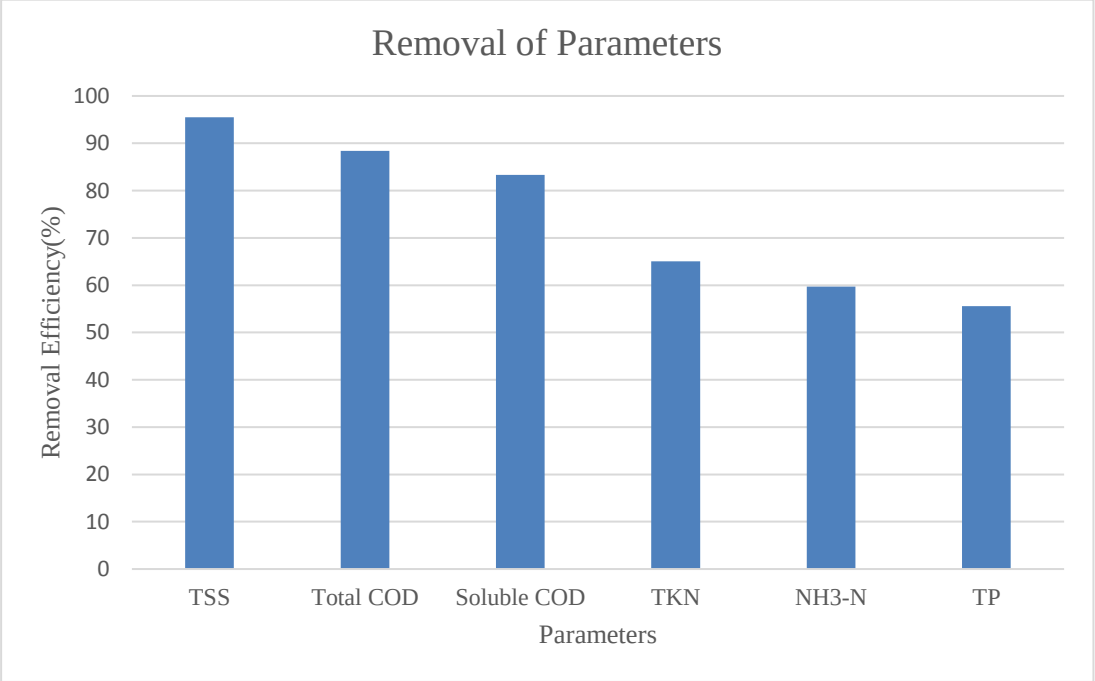


Figure 4.9 : The removal efficiency of wwtp a parameters for fall.

Concentration of TSS were presented as 200 mg/L in the influent and in the effluent, concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 475 mg/L and 240 mg/L, respectively indicating that total COD was constituted with the same quantities of particulate and inert COD while in the effluent, concentration of total COD and soluble COD were found as 55 mg/L and 40 mg/L which indicated that inert COD was dominant in the effluent. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 103 mg N/L and 67 mg N/L whereas in the effluent they were found as 36 mg N/L and 27 mg N/L, respectively implying that in both influent and effluent, the dominant form of nitrogen was NH_3-N. On the other hand, concentration of TP was measured in the influent and effluent as 9 mg/L and 4 mg/L, respectively while in biological P tank, the concentration of P was increased to 17 mg/L due to release of phosphorus in anaerobic conditions. As a result, concentrations of TKN and TP in the effluent did not meet the standards of 10 mg N/L and 1 mg/L, respectively.

The high removal efficiencies were found for TSS (96%), total COD (88%), and soluble COD (83%). The medium removal efficiencies were measured for TKN (65%), NH_3-N (60%), and TP (56%).

The micropollutant results are shown between Figure 4.10-Figure 4.17.

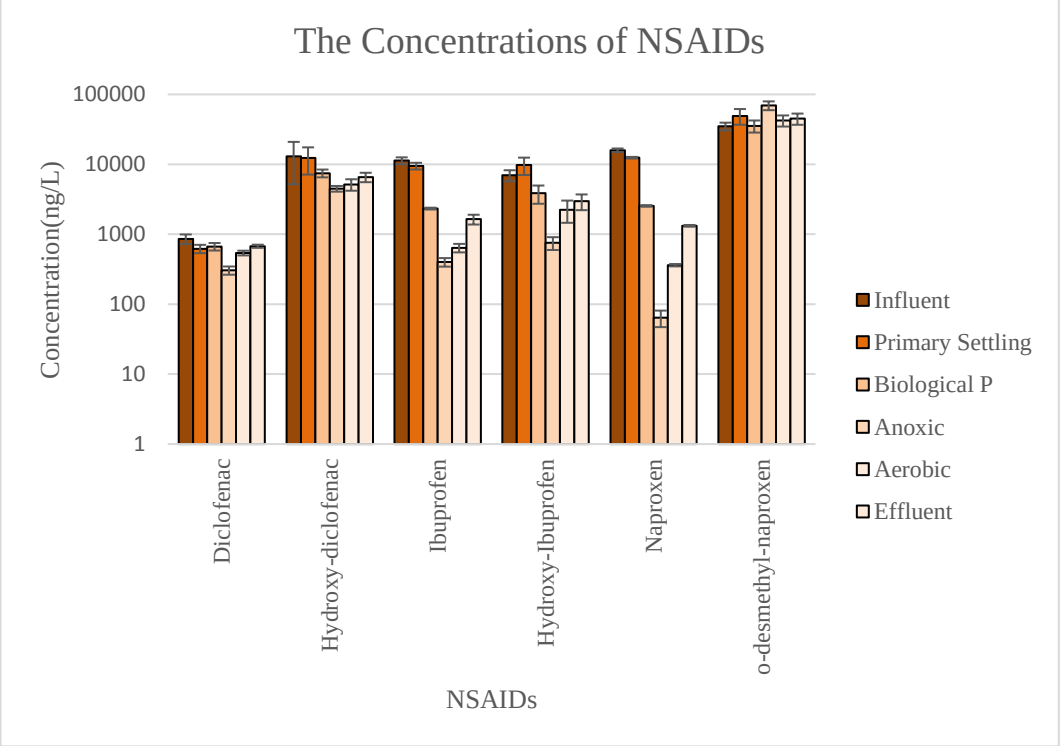


Figure 4.10 : The concentrations of NSAIDs for fall.

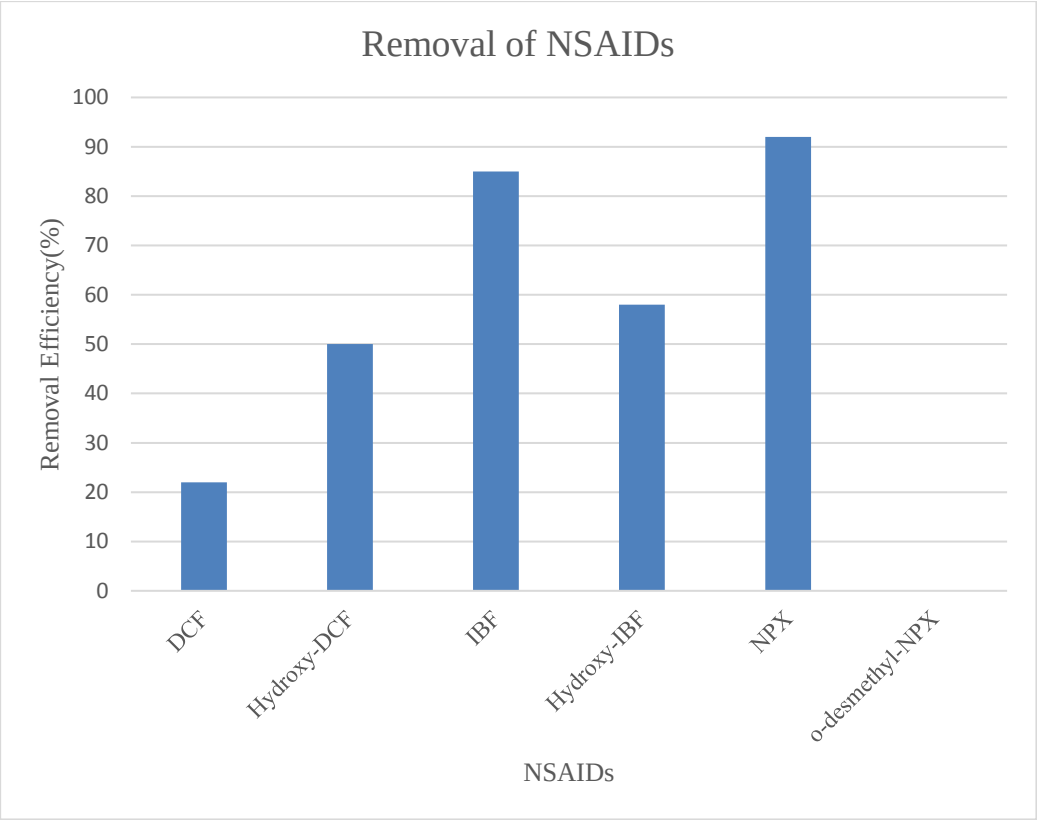


Figure 4.11 : The removal efficiency of NSAIDs for fall.

Diclofenac was present in the influent and effluent with concentrations of 859 ng/L and 674 ng/L, respectively while hydroxy-diclofenac which is the transformation product of diclofenac, was observed in the influent and effluent with high concentrations of 13,057 ng/L and 6,557 ng/L, respectively. Likewise for naproxen concentration was found to be present in high concentration of 15,899 ng/L in the influent, yet, naproxen was present as 1,320 ng/L in the effluent. o-desmethyl-naproxen was detected in the influent and effluent with the highest concentrations of 35,012 ng/L and 44,928 ng/L in NSAID group, respectively indicating higher concentration in the effluent. Ibuprofen and hiydroxy-ibuprofen were presented in the influent with high concentrations of 11,293 ng/L and 6,975 ng/L, respectively while in the effluent they were measured as 1,639 ng/L and 2,959 ng/L, respectively. Fall concentration results of NSAIDs differ from summer results of NSAIDs by their high concentration values due to extensive use of NSAIDs during cold weather conditions.

For diclofenac, low removal rate was measured (22%) , yet, for hydroxy-diclofenac, medium removal efficiency (50%) was obtained. Furthermore, removal efficiency for naproxen was very high (92%) whereas o-desmethyl-naproxen had negative removal.

On the other hand, removal of ibuprofen (IBF) was achieved with high rate (85%) whereas, removal of hydroxy-ibuprofen was in medium level (58%).

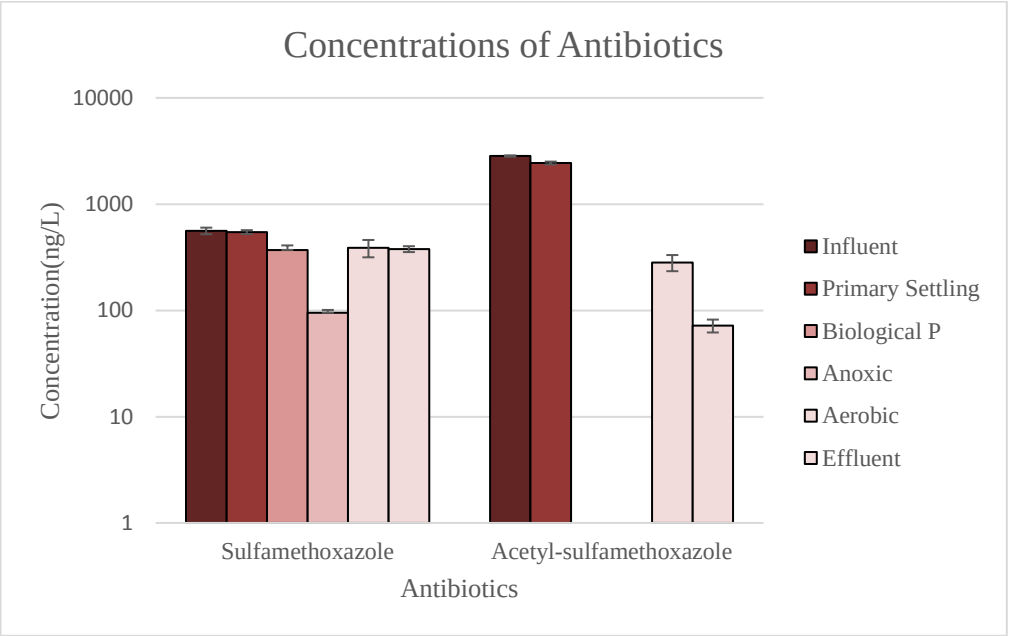


Figure 4.12 : The concentrations of antibiotics for fall.

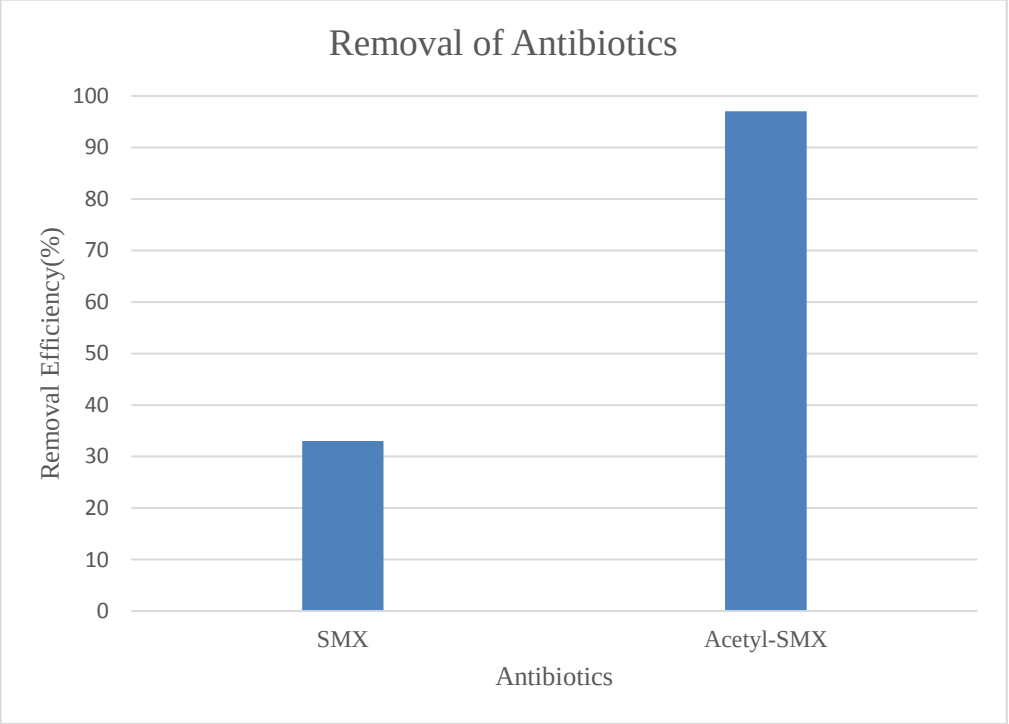


Figure 4.13 : The removal efficiency of antibiotics for fall.

Sulfamethoxazole was found in the influent and effluent as 561 ng/L and 378 ng/L, respectively. Acetyl-sulfamethoxazole was found in the influent as 2,836 ng/L while in the effluent, it was 72 ng/L. Concentration of ciprofloxacin was found to be below detection limit which is 4 ng/L shown in Table 4.4.

For sulfamethoxazole, medium removal efficiency (33%) was obtained. On the other hand, for acetyl-sulfamethoxazole, high removal efficiency was achieved (97%).

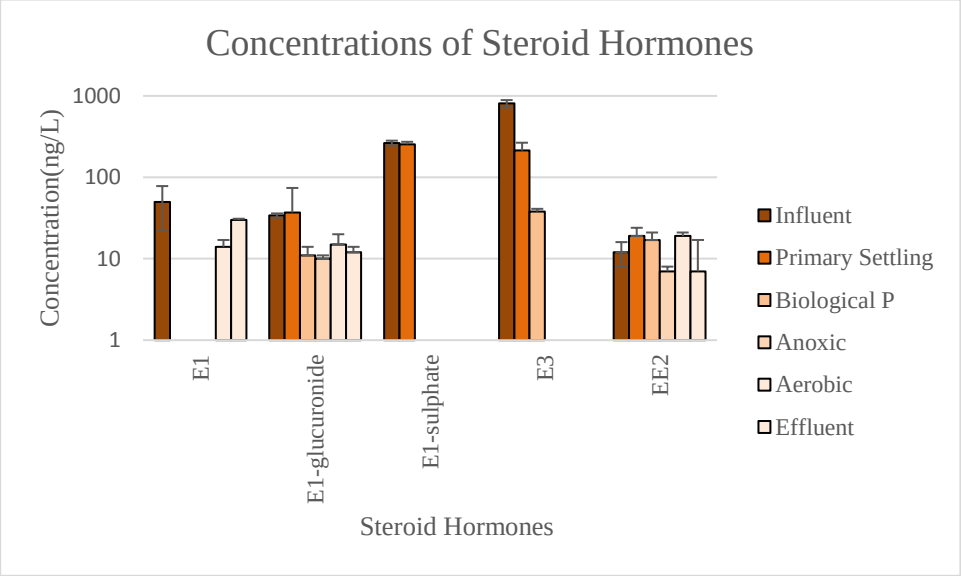


Figure 4.14 : The concentrations of steroid hormones for fall.

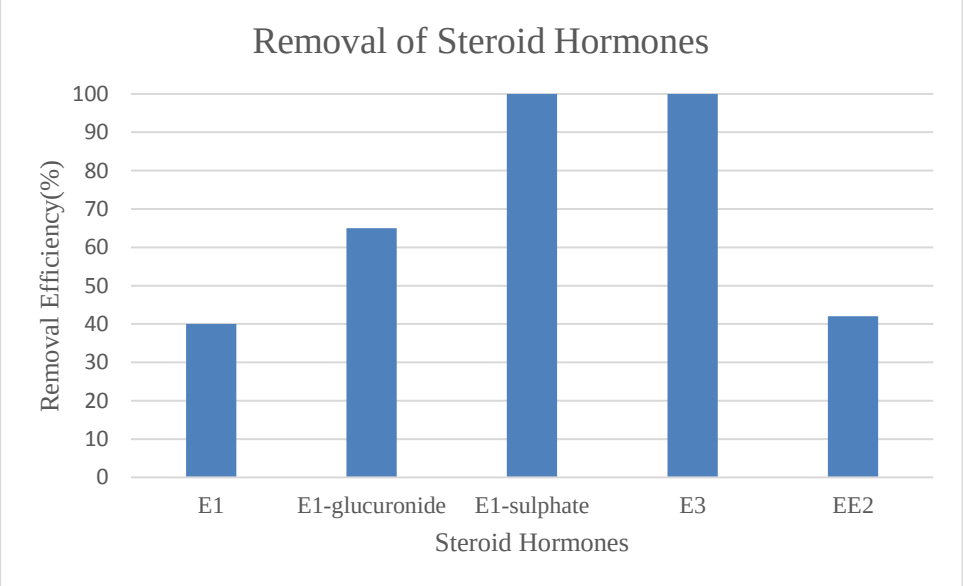


Figure 4.15 : The removal efficiency of steroid hormones for fall.

Steroid hormones were below detection limits in summer results while in fall results, they are detected above detection limits. E1 was measured as 50 ng/L in the influent while in anoxic tank, the concentration of E1 was below detection limit, the increase in concentration was observed in aerobic tank and in the effluent, the concentration of E1 was observed as 30 ng/L. E1-glucuronide and E1-sulphate which are transformation products of E1 were found in the influent as 34 ng/L and 264 ng/L, respectively while in the effluent, they were measured as 12 ng/L and <LOQ shown in

Table 4.4, respectively. Concentration of E3 was presented with a concentration of 806 ng/L in the influent and in the effluent, it was below detection limit. EE2 was measured in the influent and effluent as 12 ng/L and 7 ng/L, respectively. On the other hand, concentration of E2 was found to be below detection limit.

The highest removal efficiencies were presented for E1-sulphate (100%) and E3 (100%) while medium level efficiencies were measured for E1 (40%), E1-glucuronide (65%), and EE2 (42%). The reason for medium level removal rates might be due to low concentration in the influent.

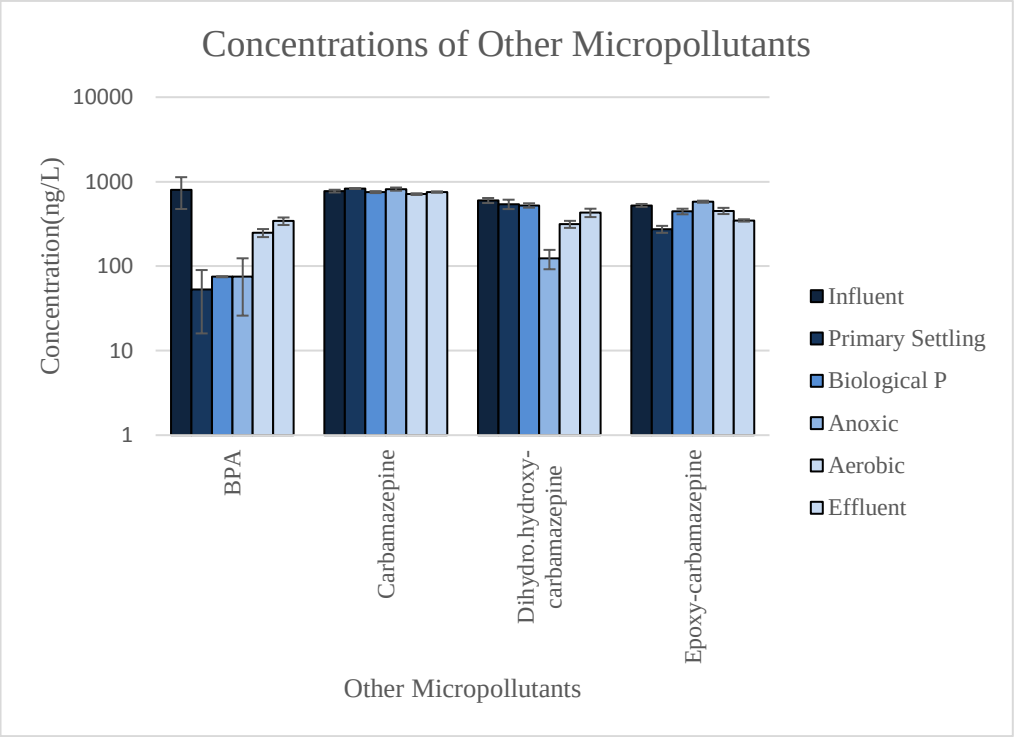


Figure 4.16 : The concentrations of other micropollutants for fall.

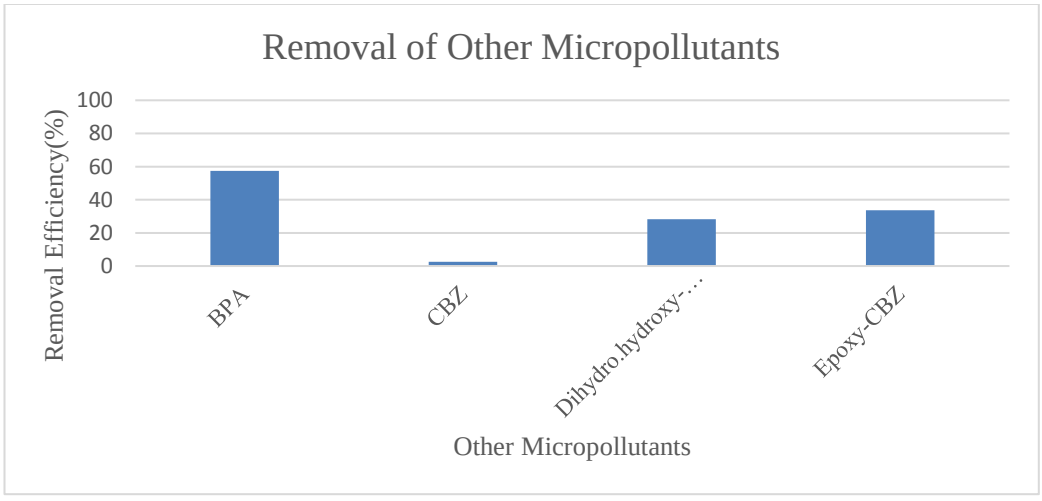


Figure 4.17 : The removal efficiency of other micropollutants for fall.

BPA was found as 803 ng/L in the influent, and in the effluent concentration of BPA was decreased to 342 ng/L. Carbamazepine was measured as 774 ng/L in the influent, and in the effluent it was found as 754 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 600 ng/L and 524 ng/L, respectively while in the effluent they were found as 430 ng/L and 348 ng/L, respectively. On the other hand, concenrations of atenolol, propranolol and 4-hydroxy-propranolol were below detection limit which is 4 ng/L for three of them. The concentrations of caffeine and paraxanthine could not detected due to possible measurement inaccuracy.

Medium removal efficiency was presented by BPA (57%). Low level removal efficiencies were presented by carbamazepine (3%), dihydro.hydroxy-carbamazepine (28%), and epoxy-carbamazepine (34%). The heatmap of fall results are shown in Figure 4.18.

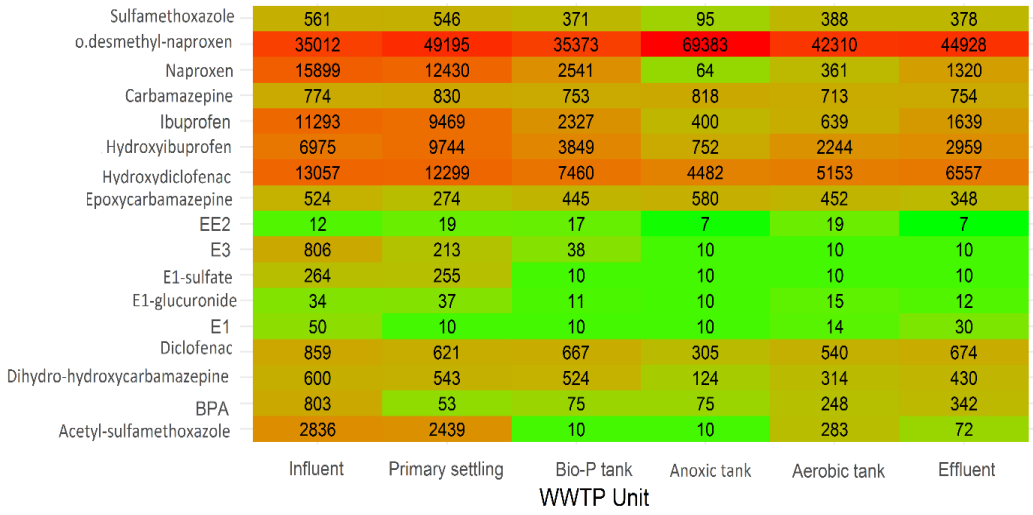


Figure 4.18 : Heatmap of wwtp a for fall.

Naproxen, ibuprofen which are NSAIDs have high influent concentration due to extensive consumption in fall season. Lajeunesse et al. (2012) stated that carbamazepine has very limited removal efficiency (0-10%). The same result is obtained for this study and carbamazepine is removed with very low removal efficiency (3%). Epoxy-carbamazepine (34%) has low removal efficiency while dihydro.hydroxy-carbamazepine has 28% removal efficiency. Knopp et al. (2016) claimed that diclofenac can be removed with medium removal efficiency (22%) while in this study diclofenac can also be removed with medium removal efficiency (22%). On the other hand hydroxy-diclofenac has medium removal efficiency (50%).

Decrease in removal efficiency of diclofenac in fall season can be explained with cold weather conditions. Sulfamethoxazole and acetyl-sulfamethoxazole have a different trend compared to others. Acetyl-sulfamethoxazole has higher concentration than sulfamethoxazole in the influent while in the effluent, acetyl-sulfamethoxazole is removed with high removal efficiency (97%), medium removal is observed for sulfamethoxazole (33%). It can be seen that most of the total load of sulfamethoxazole belongs to acetyl-sulfamethoxazole. Moreover, acetyl-sulfamethoxazole is removed with very high removal efficiencies while effluent concentration of sulfamethoxazole remains relatively the same. Göbel et al. (2005) stated a similar trend and concluded with a possible transformation of acetyl-sulfamethoxazole to sulfametoxazole. In the group of steroid hormones, influent concentration of estrone (E1) is 50 ng/L while effluent concentration of E1 is 30 ng/L. However, it can be seen from Figure 4.18 that E1 is mostly removed in primary settling tank and is started to increase in aerobic tank while E1-sulfate and E1-glucuronide have been removed from the system starting from Bio-P tank. This might be due to deconjugation of E1-sulfate and E1-glucuronide to E1. Servos et al. (2005) stated a similar conclusion and claimed that there might be deconjugation of metabolites of E1.

4.3.3 The results of wwtp a for winter season

The characterization results and removal efficiency for winter season are shown in Table 4.8 and Figure 4.19.

Table 4.8 : The characterization results of wwtp a for winter.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	7.80 ± 0.02	70 ± 10	190 ± 7	115 ± 2	48 ± 1	35 ± 1	8 ± 1
Primary Settling	7.86 ± 0.02	70 ± 6	180 ± 20	120 ± 2	48 ± 1	34 ± 1	9 ± 1
Biological P	6.97 ± 0.02	3,910 ± 10	55 ± 2 (S)	45 ± 1	43 ± 1	27 ± 1	6 ± 1
Anoxic	6.95 ± 0.02	6,825 ± 150	40 ± 1 (S)	35 ± 1	35 ± 1	18 ± 1	5 ± 2
Aerobic	7.25 ± 0.02	4,670 ± 50	55 ± 2 (S)	30 ± 1	36 ± 1	25 ± 1	6 ± 3
Effluent	7.70 ± 0.02	10 ± 2	55 ± 5	45 ± 3	37 ± 1	29 ± 1	4 ± 1

*(S):Supernatant

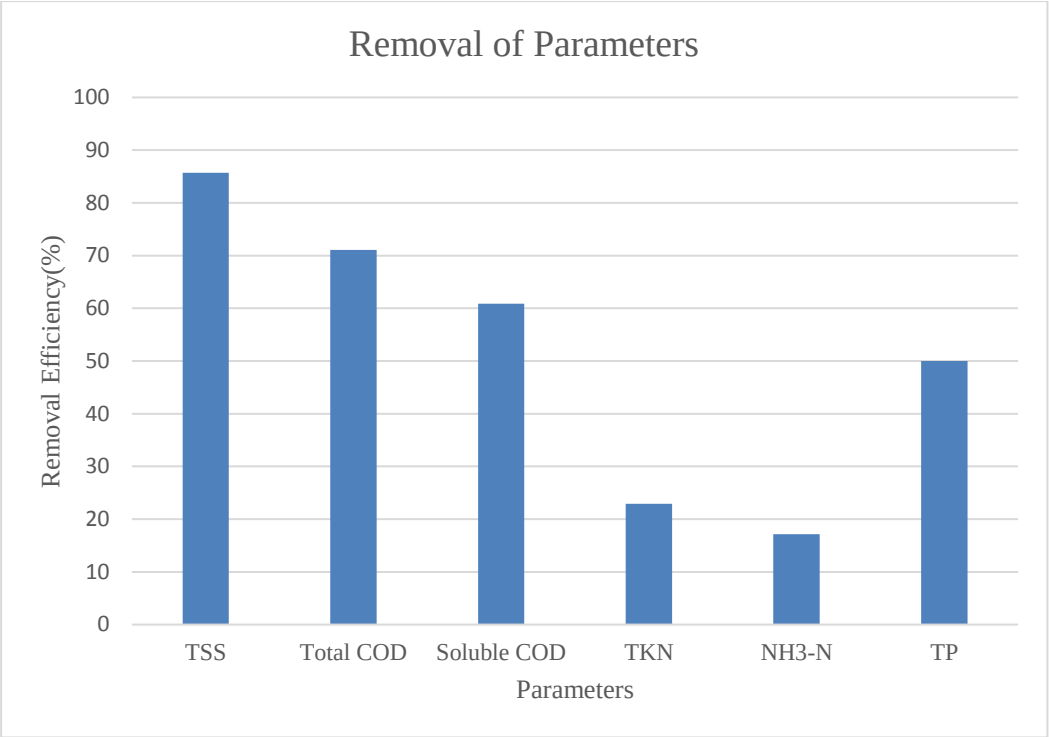


Figure 4.19 : The removal efficiency of wwtp a parameters for winter.

Concentration of TSS were presented as 70 mg/L in the influent and in the effluent, concentration of TSS was 10 mg/L. Concentrations of total COD and soluble COD in the influent were measured as 190 mg/L and 115 mg/L, respectively indicating that heavy precipitation received before sampling diluted the influent concentration and total COD was constituted with mostly inert COD while in the effluent, concentration of total COD and soluble COD were found as 55 mg/L and 45 mg/L which indicated that inert COD was dominant in the effluent. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 48 mg N/L and 35 mg N/L whereas in the effluent they were found as 37 mg N/L and 29 mg N/L, respectively implying that in both influent and effluent, the dominant form of nitrogen was NH_3-N. On the other hand, concentration of TP was measured in the influent and effluent as 8 mg/L and 4 mg/L, respectively. As a result, concentrations of TKN and TP in the effluent did not meet the standards of 10 mg N/L and 1 mg/L, respectively.

The high removal efficiencies were found for TSS (86%), total COD (71%). The medium removal efficiencies were measured for soluble COD (61%), TKN (23%), NH_3-N (17%), and TP (50%). The reason for decreased removal efficiencies for

parameters is that during cold weather conditions, efficiency of treatment plant receives a decline due to unfavourable temperature values for microorganisms.

The micropollutant results are shown between Figure 4.20-Figure 4.27.

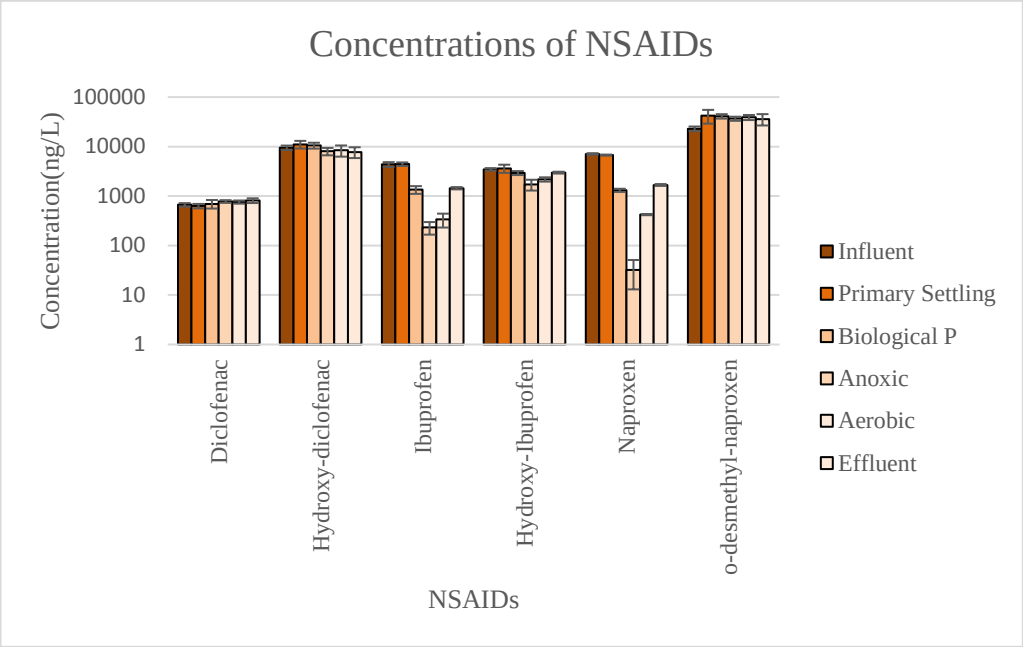


Figure 4.20 : The concentrations of NSAIDs for winter.

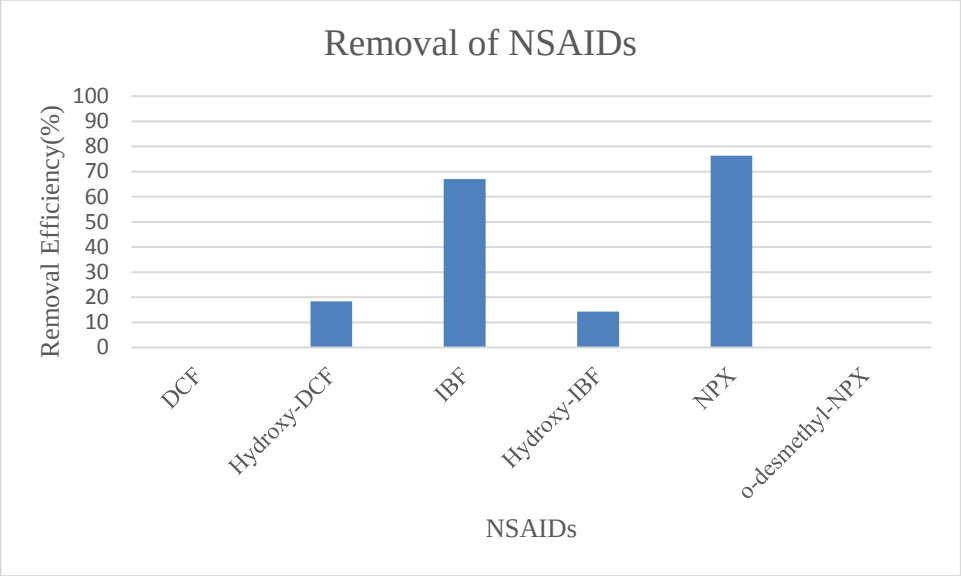


Figure 4.21 : The removal efficiency of NSAIDs for winter.

Diclofenac was present in the influent and effluent with a concentrations of 667 ng/L and 811 ng/L implying higher concentration in the effluent, respectively while hydroxy-diclofenac which is the transformation product of diclofenac, was observed in the influent and effluent with high concentrations of 9,514 ng/L and 7,771 ng/L, respectively. Likewise for naproxen concentration was found to be present in high

concentration of 7,089 ng/L in the influent, yet, naproxen was present as 1,677 ng/L in the effluent. o-desmethyl-naproxen was detected in the influent and effluent with the highest concentrations of 22,964 ng/L and 35,978 ng/L in NSAID group, respectively indicating higher concentration in the effluent. Ibuprofen and hiydroxy-ibuprofen was presented in the influent with concentrations of 4,372 ng/L and 3,474 ng/L, respectively while in the effluent they are measured as 1,441 ng/L and 2,977 ng/L, respectively.

Negative removals are presented by diclofenac and o-desmethyl-naproxen due to higher concentration in the effluent. High removal efficiency was achieved for naproxen (76%) while medium removal efficiency was observed for ibuprofen (67%). On the other hand, low removal efficiencies were found for hydroxy-diclofenac (18%) and hydroxy-ibuprofen (14%).

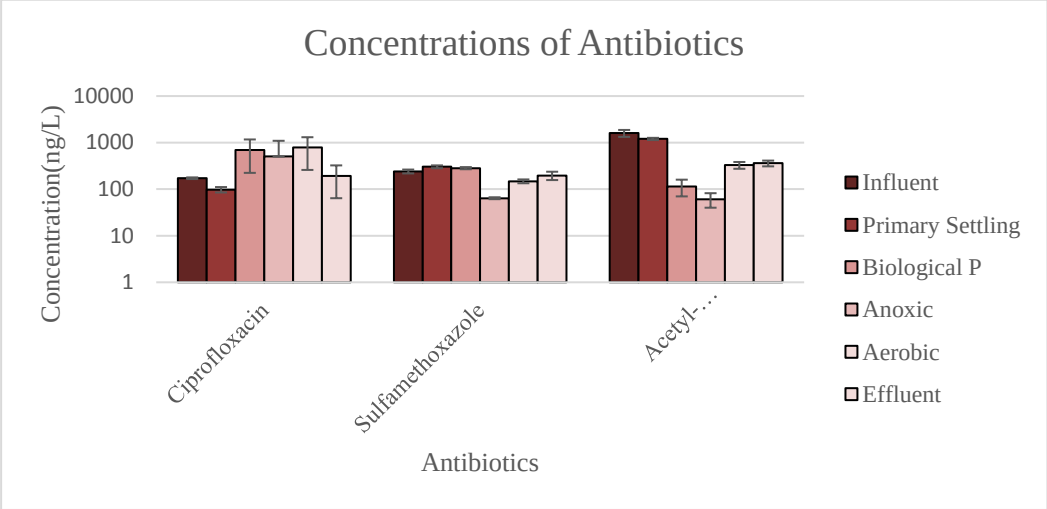


Figure 4.22 : The concentrations of antibiotics for winter.

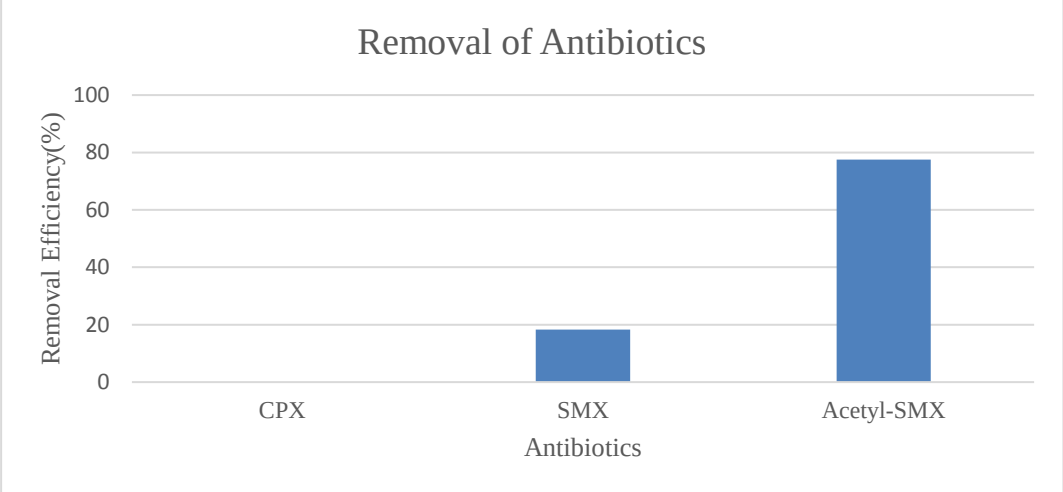


Figure 4.23 : The removal efficiency of antibiotics for winter.

Ciprofloxacin was found in the influent and effluent as 172 ng/L and 194 ng/L, respectively signifying a slightly higher concentration in the effluent. Sulfamethoxazole was observed in the influent and effluent as 241 ng/L and 197 ng/L, respectively. On the other hand, acetyl-sulfamethoxazole was found in the influent as 1,600 ng/L while in the effluent, it was 360 ng/L.

For sulfamethoxazole, low removal efficiency (18%) was found. On the other hand, for acetyl-sulfamethoxazole, high removal efficiency was achieved (78%). However, for ciprofloxacin, it was negative removal due to elevated concentration.

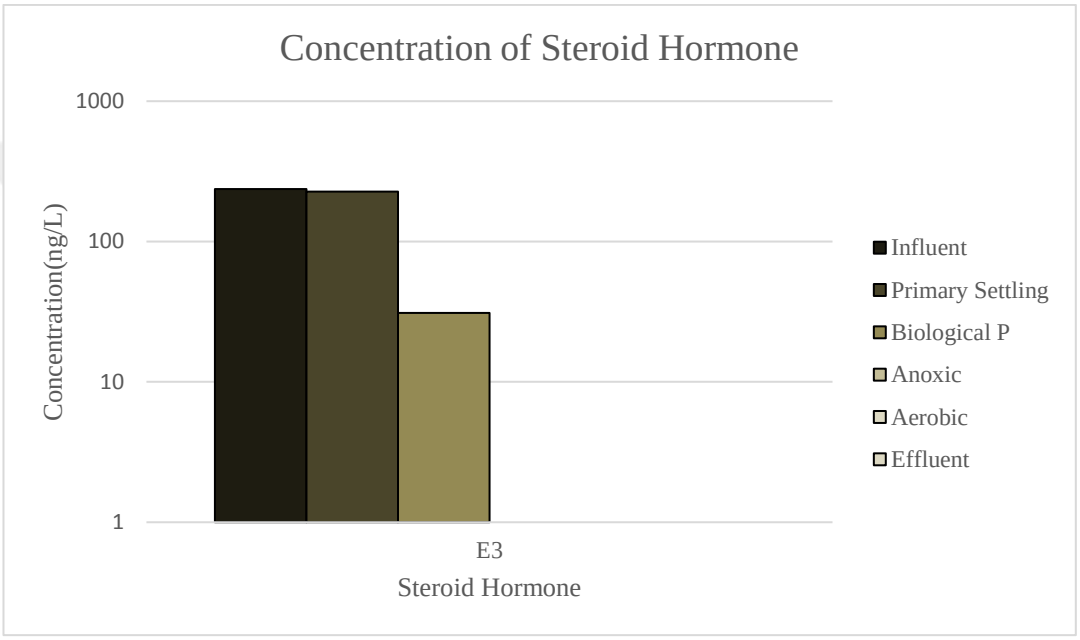


Figure 4.24 : The concentrations of steroid hormone for winter.

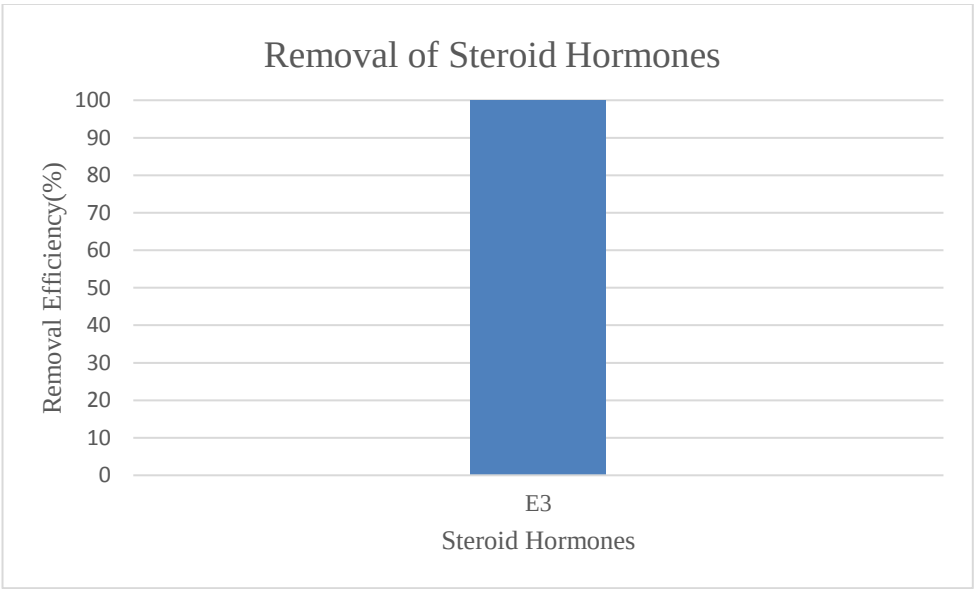


Figure 4.25 : The removal efficiency of steroid hormones for winter.

Concentrartion of E3 was observed in the influent and effluent as 237 ng/L and <LOQ, respectively. E1, E1-glucuronide, E1-sulphate, E2, and EE2 were below detection limit. The complete removal for E3 (100%) was achieved.

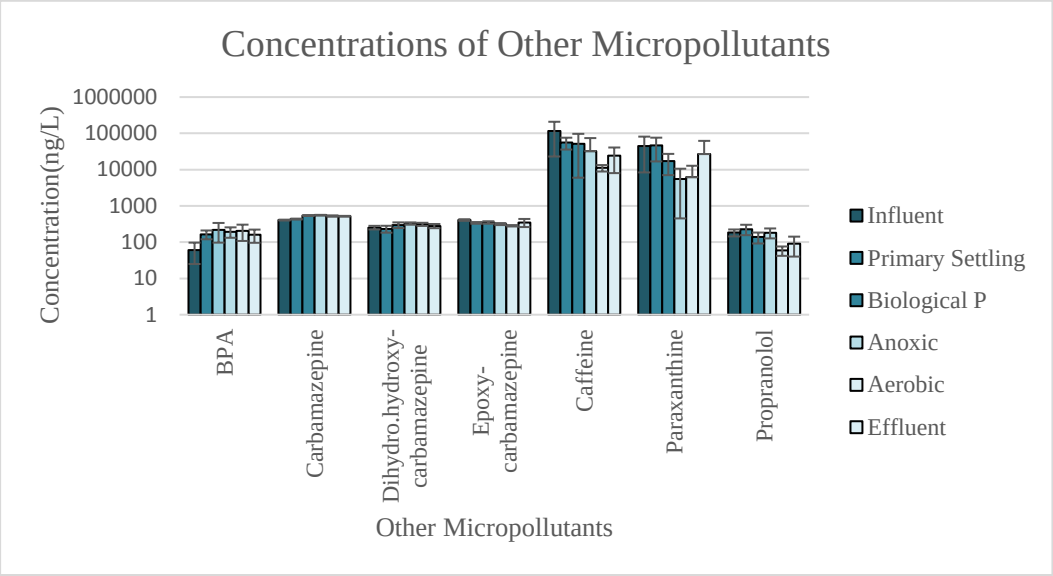


Figure 4.26 : The concentrations of other micropollutants for winter.

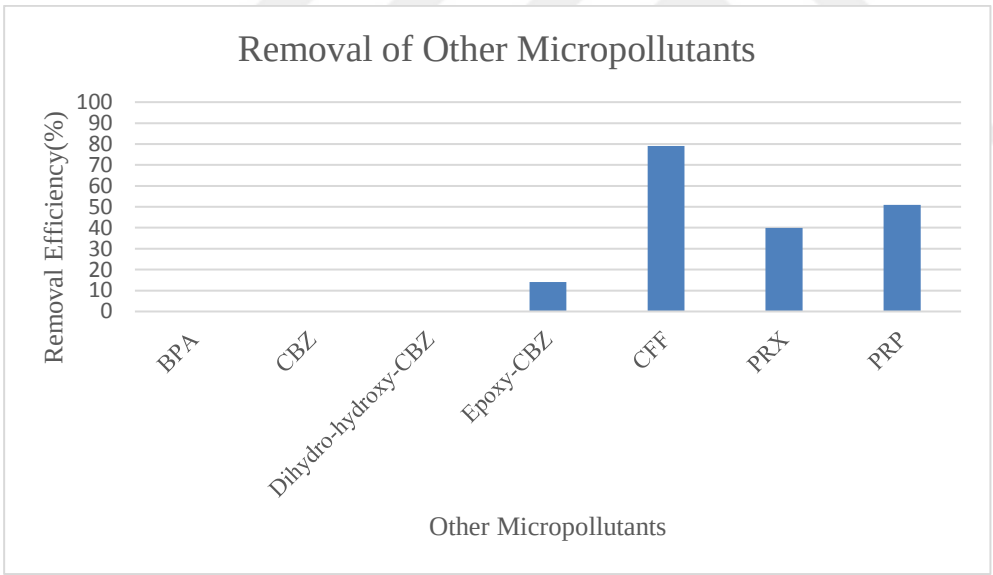


Figure 4.27 : The removal efficiency of other micropollutants for winter.

BPA was found as 61 ng/L in the influent, and in the effluent concentration of BPA was increased to 159 ng/L. Carbamazepine was measured as 406 ng/L in the influent, and in the effluent it was found as 519 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 253 ng/L and 408 ng/L, respectively while in the effluent they were found as 280 ng/L and 349 ng/L, respectively indicating a slightly higher concentration in the effluent for

dihydro.hydroxy-carbamazepine. Caffeine presented the highest concentration with 116,027 ng/L, and in the effluent concentration of caffeine was decreased to 24,368 ng/L. Paraxanthine presented high concentration as well with 44,852 ng/L while in the effluent, the concentration of paraxanthine was observed as 26,961 ng/L. On the other hand, propranolol was measured in the influent and effluent as 184 ng/L and 91 ng/L, respectively. 4-hydroxy-propranolol and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The highest removal efficiency was presented by caffeine (79%). The medium removal efficiencies were found for paraxanthine (40%) and propranolol (51%). Negative removal efficiencies were presented by BPA, carbamazepine, and dihydro.hydroxy-carbamazepine due to higher concentrations in the effluent. The heatmap of winter results are shown in Figure 4.28.

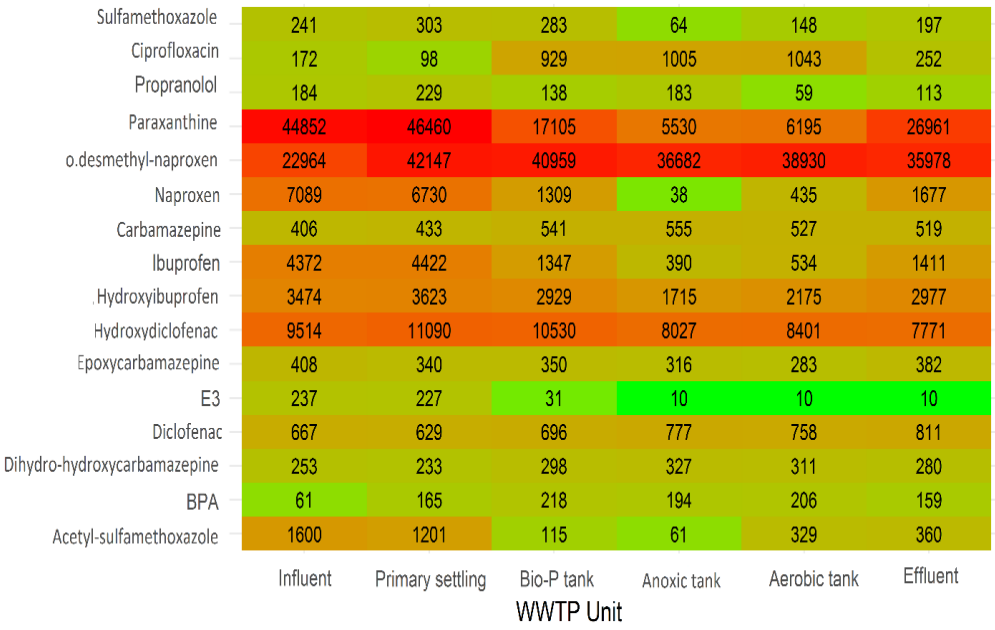


Figure 4.28 : Heatmap of wwtp a for winter.

In winter, concentration of carbamzepine is lower than the concentration of fall and summer. However, carbamazepine is an antiepileptic which means patients must use it constantly. Decrease in influent concentration of carbamazepine compared to summer and fall can be explained by high influent flowrate due to heavy precipitation. Due to cold weather conditions of winter, removal efficiencies of carbamazepine and its metabolites are decreased. Naproxen is removed with high removal efficiencies while negative removal is observed for o-desmethyl-naproxen. The metabolites of naproxen and diclofenac enter the system with higher concentrations than their parent

compounds. This can be explained by their excretion ratio of metabolites. In the body, naproxen is excreted as unchanged with a ratio of <1 while diclofenac is excreted as unchanged with a ratio of 5-10%. It means that metabolites can be found in wastewater with higher concentrations due to higher transformation ratios. Estriol (E3) can be removed completely in winter. This result can be observed in summer and fall. It can be said that steroid hormones can be removed easily due to being natural compounds under most conditions.

4.3.4 The results of wwtp a for spring season

The characterization results and removal efficiency for winter season are shown in Table 4.9 and Figure 4.29. In WWTP A, the sample was not taken from primary settling due to technical problems with primary settling.

Table 4.9 : The characterization results of wwtp a for spring.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	8.22 ± 0.02	302 ± 6	380±5	130±2	66 ± 4	64 ± 1	10 ± 1
Biological P	7.10 ± 0.02	13840 ± 560	270±5	100±6	55 ± 2	46 ± 1	23 ± 3
Anoxic	7.06 ± 0.02	9380 ± 420	150±2	60±2	7 ± 1	3 ± 1	18 ± 4
Aerobic	7.35 ± 0.02	2300 ± 100	110±4	60±2	18 ± 1	14 ± 1	12 ± 2
Effluent	7.65 ± 0.02	18 ± 2	85±3	40±4	32 ± 1	31 ± 1	6 ± 2

*(S):Supernatant

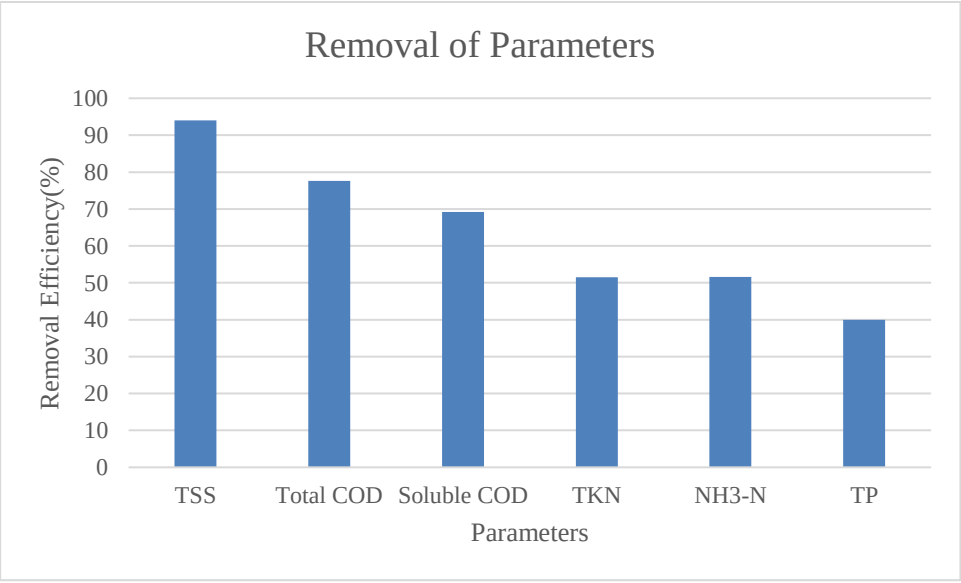


Figure 4.29 : The removal efficiency wwtp a parameters for spring.

Concentration of TSS were presented as 302 mg/L in the influent and in the effluent, concentration of TSS was 18 mg/L. Concentrations of total COD and soluble COD in the influent were measured as 380 mg/L and 130 mg/L, respectively indicating total COD was constituted with mostly particulate COD while in the effluent, concentration of total COD and soluble COD were found as 85 mg/L and 40 mg/L which indicated that inert COD was slightly dominant in the effluent. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 66 mg N/L and 64 mg N/L whereas in the effluent they were found as 32 mg N/L and 31 mg N/L, respectively implying that in both influent and effluent, the dominant form of nitrogen was NH_3-N. On the other hand, concentration of TP was measured in the influent and effluent as 10 mg/L and 6 mg/L, respectively. As a result, concentrations of TKN and TP in the effluent did not meet the standards of 10 mg N/L and 1 mg/L, respectively.

The high removal efficiencies were found for TSS (94%), total COD (78%). The medium removal efficiencies were measured for soluble COD (69%), TKN (52%), NH_3-N (52%), and TP (40%).

The micropollutant results are shown between Figure 4.30-Figure 4.37.

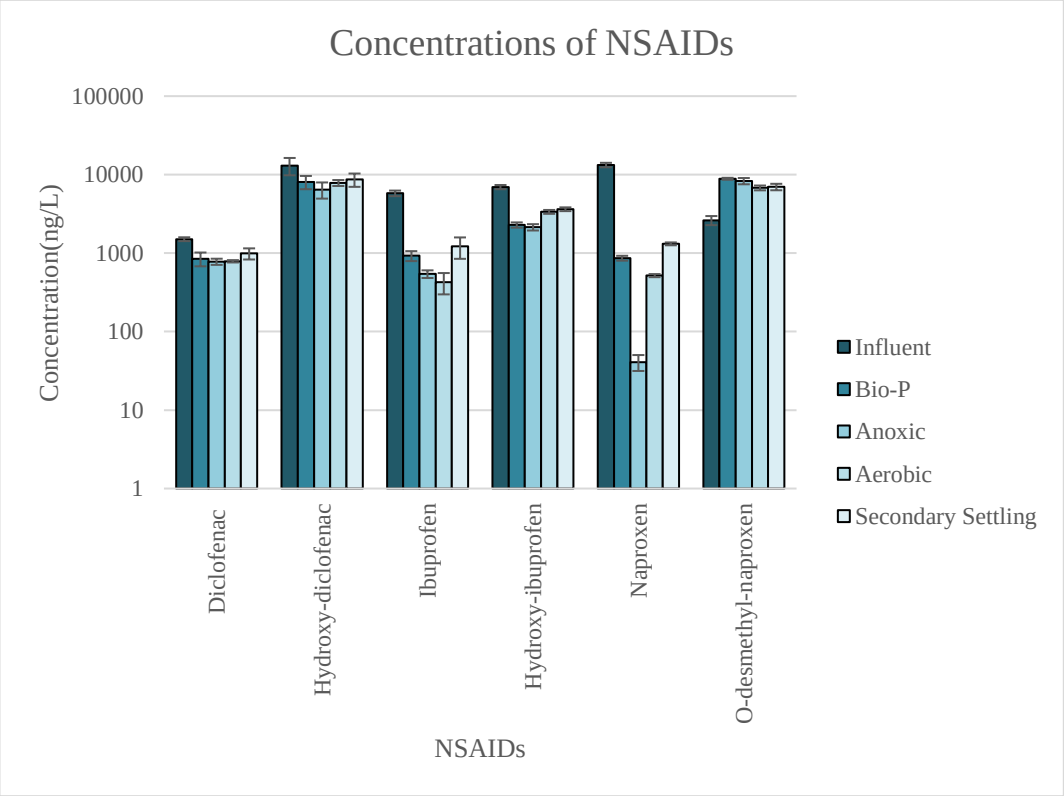


Figure 4.30 : The concentrations of NSAIDs for spring.

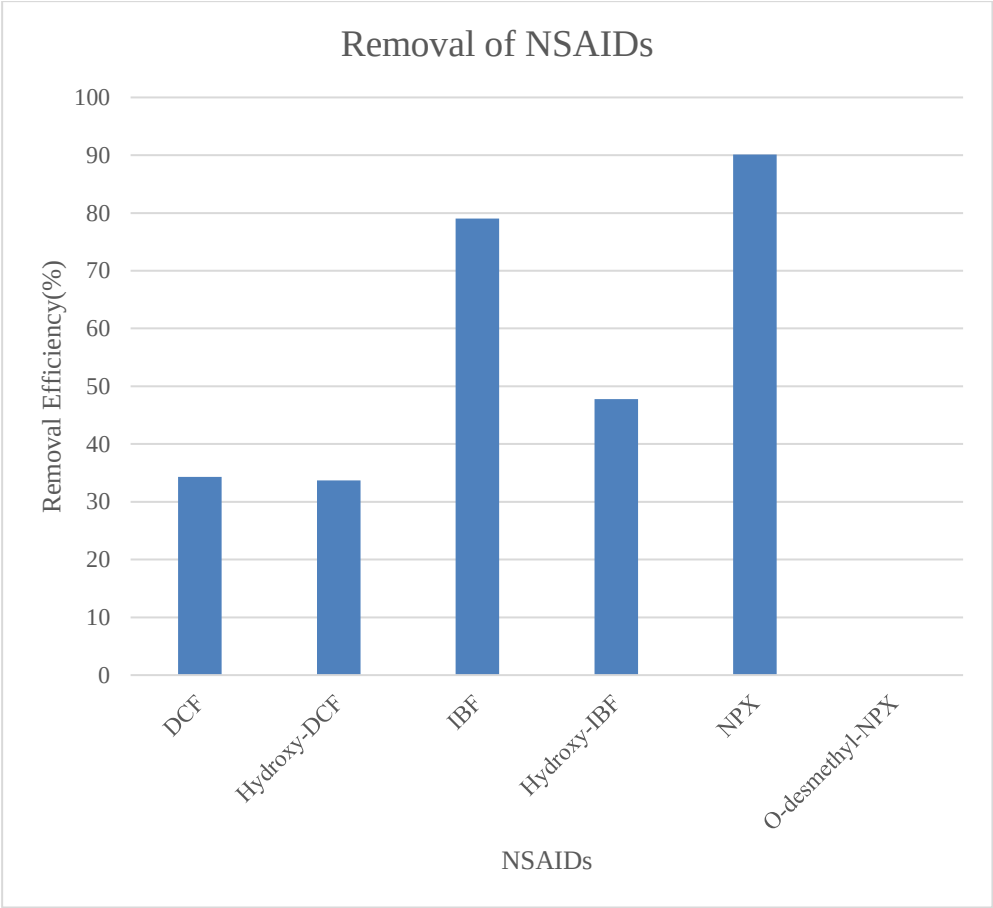


Figure 4.31 : The removal efficiency of NSAIDs for spring.

Diclofenac was present in the influent and effluent with a concentrations of 1506 ng/L and 989 ng/L, respectively while hydroxy-diclofenac which is the transformation product of diclofenac, was observed in the influent and effluent with high concentrations of 13,054 ng/L and 8,658 ng/L, respectively. Likewise for naproxen concentration was found to be present in high concentration of 13,272 ng/L in the influent, yet, naproxen was present as 1,313 ng/L in the effluent. o-desmethyl-naproxen was detected in the influent and effluent with the concentrations of 2,619 ng/L and 6,981 ng/L in NSAID group, respectively indicating higher concentration in the effluent. Ibuprofen and hiydroxy-ibuprofen was presented in the influent with concentrations of 5,789 ng/L and 6,945 ng/L, respectively while in the effluent they are measured as 1,215 ng/L and 3,626 ng/L, respectively.

Negative removals are presented by o-desmethyl-naproxen due to higher concentration in the effluent. High removal efficiency was achieved for naproxen (90%) and ibuprofen (79%). On the other hand, low removal efficiencies were found for diclofenac (34%), hydroxy-diclofenac (34%) and hydroxy-ibuprofen (48%).

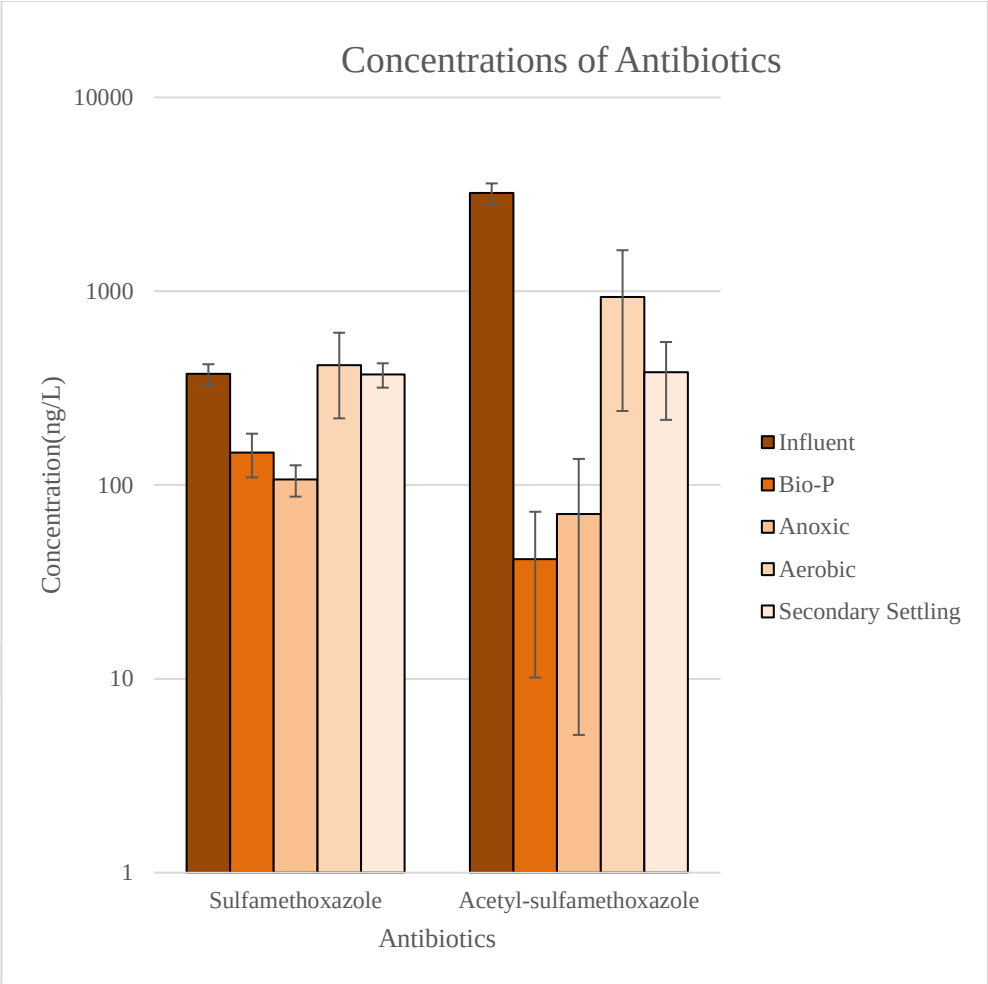


Figure 4.32 : The concentrations of antibiotics for spring.

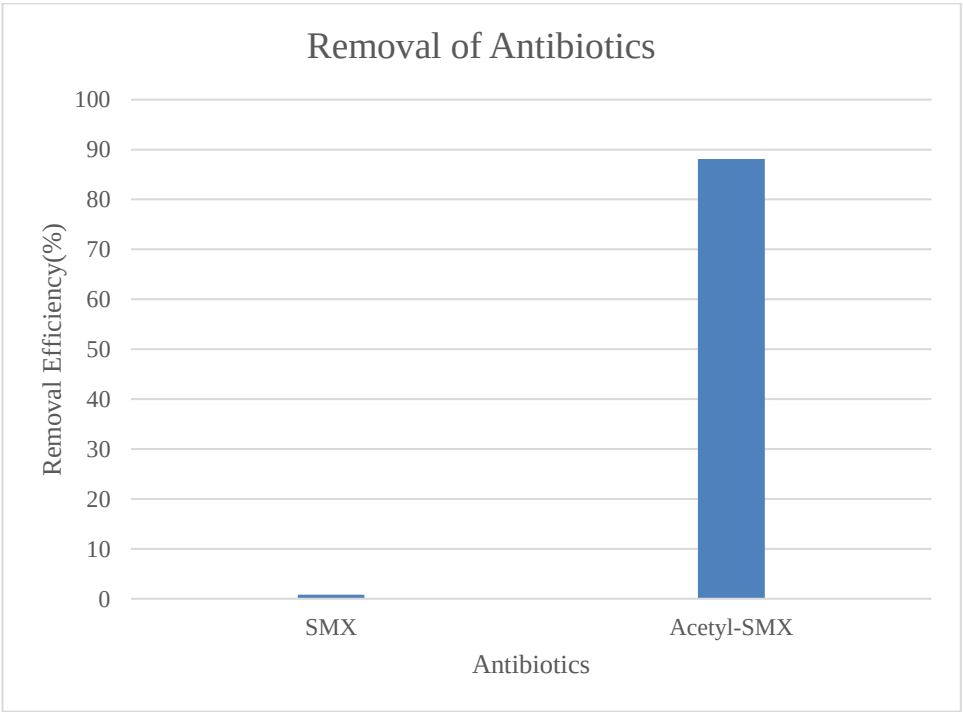


Figure 4.33 : The removal efficiencies of antibiotics for spring.

Ciprofloxacin was not detected in spring sample. Sulfamethoxazole was observed in the influent and effluent as 374 ng/L and 371 ng/L, respectively. On the other hand, acetyl-sulfamethoxazole was found in the influent as 3,210 ng/L while in the effluent, it was 382 ng/L.

For acetyl-sulfamethoxazole, high removal efficiency (88%) was found. On the other hand, for sulfamethoxazole, very low (1%) removal efficiency was achieved.

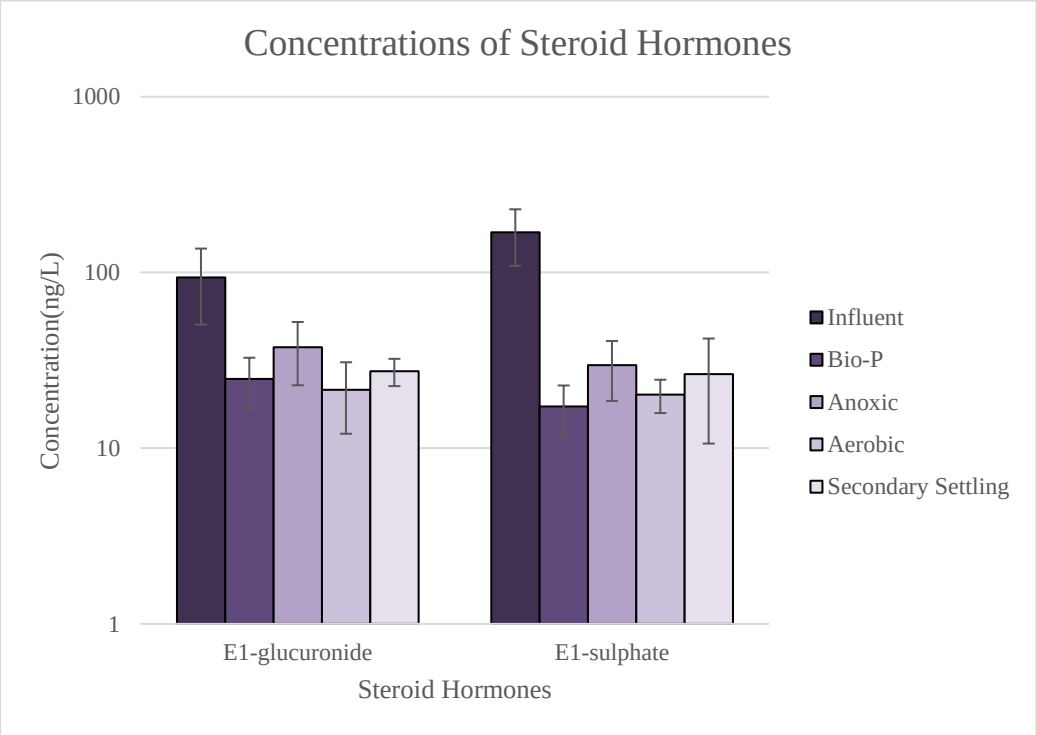


Figure 4.34 : The concentrations of steroid hormones for spring.

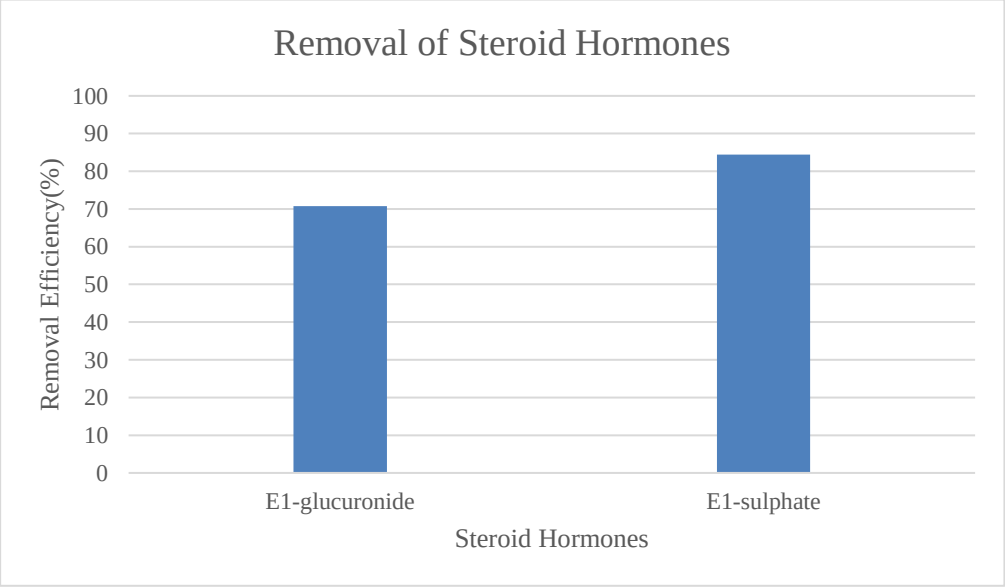


Figure 4.35 : The removal efficiencies of steroid hormones for spring.

E1-sulphate was measured as 169 ng/L in the influent and in the effluent, the concentration of E1-sulphate was observed as 26 ng/L. Concentration of E1-glucuronide was presented with a concentration of 94 ng/L in the influent and in the effluent, it was 27 ng/L. On the other hand, concentrations of E1, E2, E3, and EE2 was found to be below detection limit.

The high removal efficiencies were presented for E1-sulphate (84%) and E1-glucuronide (71%).

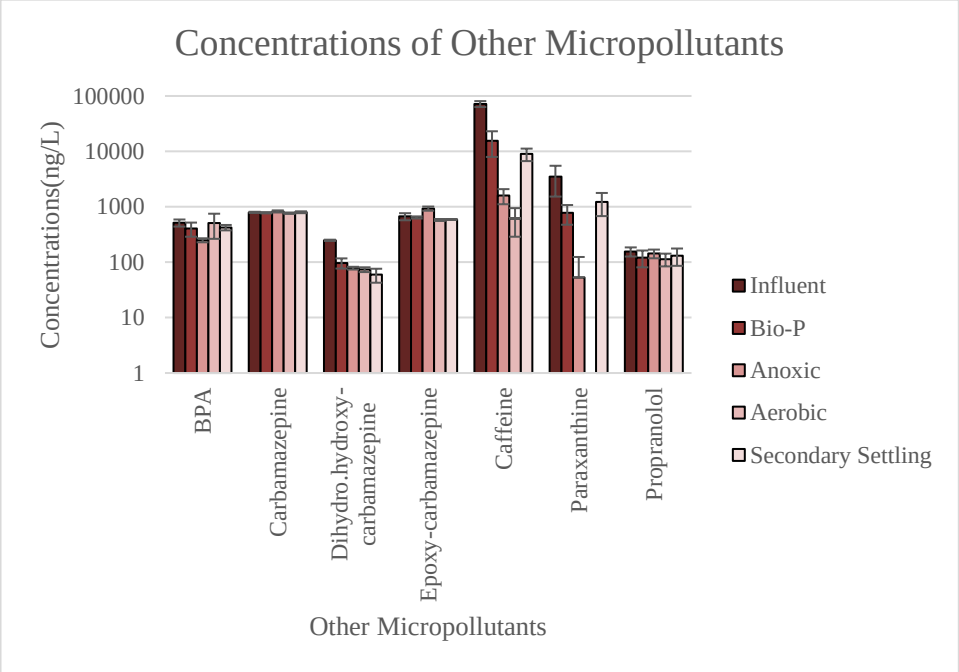


Figure 4.36 : The concentrations of other micropollutants for spring.

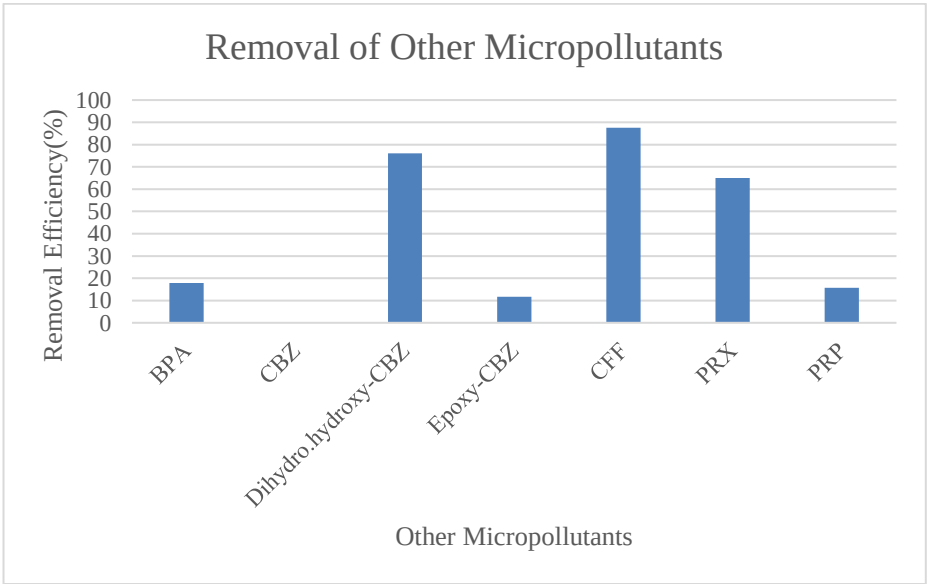


Figure 4.37 : The removal efficiencies of other micropollutants for spring.

BPA was found as 515 ng/L in the influent, and in the effluent concentration of BPA was decreased to 422 ng/L. Carbamazepine was measured as 793 ng/L in the influent, and in the effluent it was found as 797 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 248 ng/L and 669 ng/L, respectively while in the effluent they were found as 59 ng/L and 590 ng/L, respectively. Caffeine presented the highest concentration with 71,958 ng/L, and in the effluent concentration of caffeine was decreased to 8,950 ng/L. Paraxanthine presented high concentration as well with 3,515 ng/L while in the effluent, the concentration of paraxanthine was observed as 1,228 ng/L. On the other hand, propranolol was measured in the influent and effluent as 156 ng/L and 131 ng/L, respectively. 4-hydroxy-propranolol and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The highest removal efficiency was presented by caffeine (88%) and dihydro.hydroxy-carbamazepine (76%). The medium removal efficiencies were found for paraxanthine (65%). Low removal efficiencies are found for BPA (18%), epoxy-carbamazepine (12%), and propranolol (16%). Negative removal efficiencies were presented by carbamazepine due to higher concentrations in the effluent. The heatmap of spring results are shown in Figure 4.38.

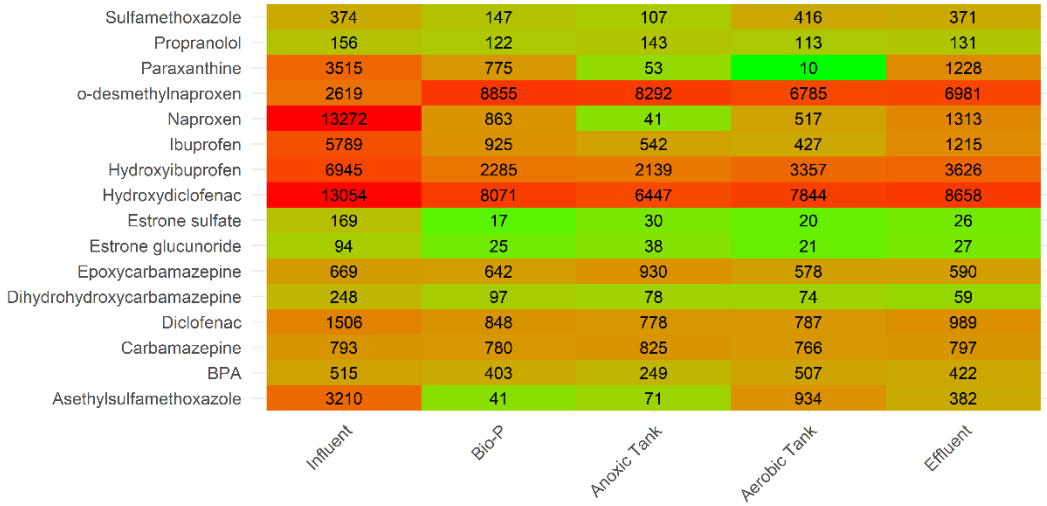


Figure 4.38 : Heatmap of wwtp a for spring.

Naproxen, ibuprofen which are NSAIDs have high influent concentration due to extensive consumption in spring season. For carbamazepine, negative removal is observed while epoxy-carbamazepinehas low (12%) removal efficiency while dihydro.hydroxy-carbamazepine has high (76%) removal efficiency. Highest removal

effciency for dihydro.hydroxy-carbamazepine is achieved during spring while carbamazepine had negative removal. This might be due to transformation of dihydro.hydroxy-carbamazepine to carbamazepine. Gomez et al. (2007) claimed that diclofenac can be removed with medium (40%) removal efficiency while in this study diclofenac can also be removed with medium (34%) removal efficiency. Hydroxy-diclofenac has also medium removal efficiency (34%). Sulfamethoxazole and acetyl-sulfamethoxazole have a different trend compared to others. Acetyl-sulfamethoxazole has higher concentration than sulfamethoxazole in the influent while in the effluent, acetyl-sulfamethoxazole is removed with high removal efficiency (88%), low removal is observed for sulfamethoxazole (1%). It can be seen that most of the total load of sulfamethoxazole belongs to acetyl-sulfamethoxazole. Moreover, acetyl-sulfamethoxazole is removed with very high removal efficiencies while effluent concentration of sulfamethoxazole remains relatively the same. Göbel et al. (2005) stated a similar trend and concluded with a possible transformation of acetyl-sulfamethoxazole to sulfametoxazole. In the group of steroid hormones, E1-glucuronide and E1-sulphate can be removed with high removal efficiencies (71% and 84%, respectively). How removal efficiency changes according to seasons is shown in Figure 4.39.

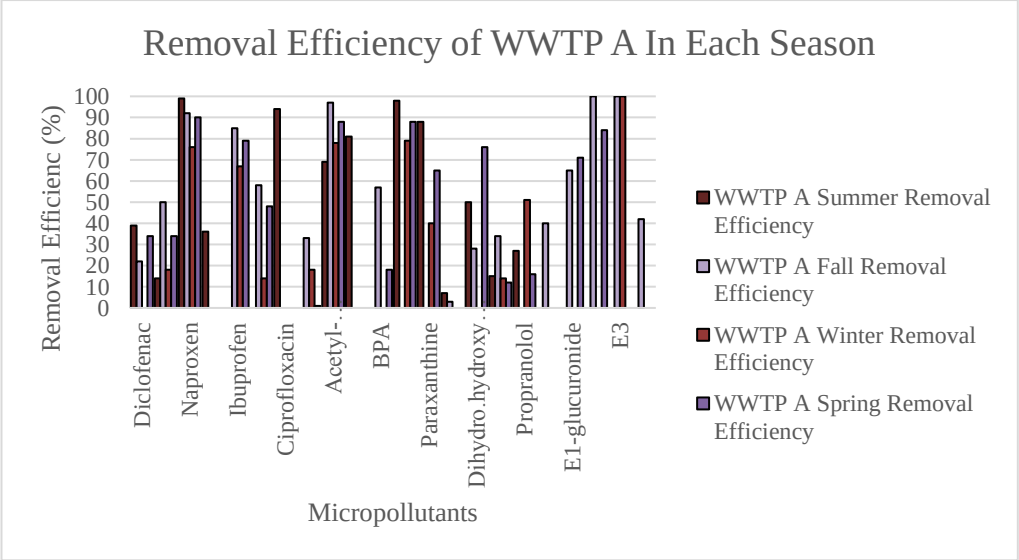


Figure 4.39 : Removal efficiency in each season.

Some of the micropollutants were not detected in each season which are atenolol, E1, E1-sulphate, E1-glucuronide, and EE2. Therefore, comparison of these micropollutants cannot be done due to missing data. Naproxen showed decline in efficiency in fall after summer and even more decline in winter while increasing in

spring. Similarly, dihydro.hydroxy-carbamazepine and diclofenac had similar trends while for carbamazepine and o-desmethyl-naproxen, similar trend was observed until spring. In spring, their removal efficiencies did not increase. Ciprofloxacin declined in winter to 0% from 94% in summer. There was no detection in fall and spring. The removal efficiencies of caffeine and paraxanthine showed similar trends. Removal efficiencies of ibuprofen and hydroxy-ibuprofen declined in winter after fall while increasing in spring. There was no detection in summer for both of them. On the other hand, some of the micropollutants had higher removal efficiencies in fall than summer such as sulfamethoxazole, acetyl-sulfamethoxazole, BPA, and epoxy-carbamazepine. Even though they had higher efficiencies in fall, the efficiencies in winter still dropped significantly. On the other hand, removal efficiency of E3 stayed as 100% in fall and summer while not detected in spring. Removal efficiencies of literature review and WWTP A are compared in Figure 4.40.

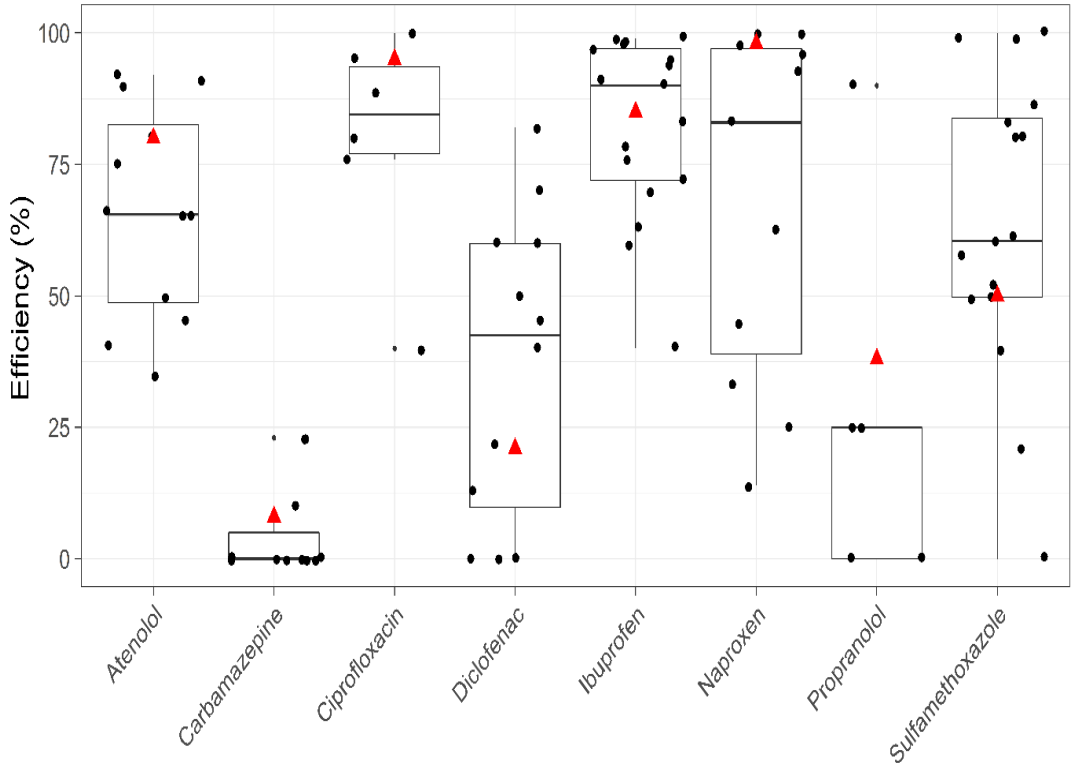


Figure 4.40 : Comparison between wwtp a and literature values.

Horizontal line in boxes defines median values of literature. Removal efficiencies of atenolol, carbamazepine, ciprofloxacin, naproxen, and propranolol in WWTP A can be seen as higher than the median values. On the other hand, removal efficiencies of diclofenac, ibuprofen, and sulfamethoxazole in WWTP A are lower than the median

values. Even though they are below median values, there are still similar values in the literature. Moreover, operational conditions and configurations of WWTPs in the literature review vary greatly. Therefore, removal efficiencies for each micropollutants are in a wide range as it can be seen for diclofenac, naproxen, and sulfamethoxazole. The highest removal efficiency achieved in units of WWTP A for micropollutants is shown in Table 4.10. Some calculations are done in order to evaluate which micropollutants have the highest removal efficiency in which unit of WWTP. From Table 4.10, it can be seen that there are some evaluations to make about some of the micropollutants. Naproxen, ibuprofen, and acetylsulfamethoxazole can be removed with highest removal efficiencies in Biological-P Tank in each season. Similar conclusion can be made for carbamazepine in Aerobic Tank. On the other hand, epoxy-carbamazepine can be removed in Primary Settling for summer, fall, winter and spring seasons, in spring it can be removed with highest efficiency in Aerobic Tank while sulfamethoxazole can be removed in Anoxic Tank for summer, fall, winter and spring seasons and for spring it can be removed in Biological-P Tank. On the other hand, diclofenac can be removed with highest removal efficiency in Anoxic Tank for both summer and fall, yet, in winter it is primary settling and in spring it is Biological-P Tank. Hydroxy-diclofenac can be also removed the highest in Anoxic Tank for both summer and winter but for fall and spring, it is Biological-P Tank. Moreover, dihydro.hydroxycarbamazepine can be removed with highest removal efficiency in Primary Settling for summer, in Anoxic Tank for fall, in Secondary Settling for winter, and in Biological-P Tank in spring. Similar conclusion can be said for o-desmethyl-naproxen in Biological-P Tank for summer, in Aerobic Tank for fall and spring, and in Anoxic Tank for winter. These results may indicate that the micropollutants that change their units with the highest removal efficiencies according to seasonal change may behave differently in different seasons. However, there some micropollutants that they stayed in the same unit with highest removal efficiency in summer, fall, winter and spring seasons. These micropollutants are naproxen, ibuprofen, acetyl-sulfamethoxazole, and carbamazepine. This may indicate that the behavior of these micropollutants does not change and they do not prefer any other units other than the units they are removed with the highest removal efficiencies according to seasonal change.

Table 4.10 : Highest removal efficiency achieved for micropollutants in wwtp a.

Micropollutants	*Highest Removal Efficiency Achieved for Micropollutants*			
	Summer	Fall	Winter	Spring
Diclofenac	Anoxic Tank	Anoxic Tank	Primary Settling	Bio-P Tank
Hydroxy-diclofenac	Anoxic Tank	Bio-P Tank	Anoxic Tank	Bio-P Tank
Naproxen	Bio-P Tank	Bio-P Tank	Bio-P Tank	Bio-P Tank
o-desmethyl-naproxen	Bio-P Tank	Aerobic Tank	Anoxic Tank	Aerobic Tank
Ibuprofen		Bio-P Tank	Bio-P Tank	Bio-P Tank
Hydroxy-ibuprofen		Bio-P Tank	Anoxic Tank	Bio-P Tank
Ciprofloxacin	Primary Settling		Secondary Settling	
Sulfamethoxazole	Anoxic Tank	Anoxic Tank	Anoxic Tank	Bio-P Tank
Acetyl-sulfamethoxazole	Bio-P Tank	Bio-P Tank	Bio-P Tank	Bio-P Tank
Atenolol	Grit Chamber			
BPA	Primary Settling	Primary Settling	Secondary Settling	Anoxic Tank
Caffeine	Bio-P Tank		Primary Settling	Bio-P Tank
Paraxanthine	Anoxic Tank		Bio-P Tank	Bio-P Tank
Carbamazepine	Aerobic Tank	Aerobic Tank	Aerobic Tank	Aerobic Tank
Dihydro.hydroxy-carbamazepine	Primary Settling	Anoxic Tank	Secondary Settling	Bio-P Tank
Epoxy-carbamazepine	Primary Settling	Primary Settling	Primary Settling	Aerobic Tank
Propranolol	Secondary Settling		Aerobic Tank	Bio-P Tank
E1		Primary Settling		
E1-glucuronide		Bio-P Tank		Bio-P Tank
E1-sulphate		Bio-P Tank		Bio-P Tank
E3		Primary Settling	Bio-P Tank	
EE2		Secondary Settling		

4.3.5 The results of wwtp b for summer season

The Characterization results of WWTP B and removal efficiencies for summer season are shown in Table 4.11 and Figure 4.41.

Table 4.11 : The characterization results of wwtp b for summer.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	7.88 ± 0.02	910 ± 25	795 ± 25	220 ± 15	66 ± 3	46 ± 2	12 ± 1
Primary Settling	7.82 ± 0.02	170 ± 10	450 ± 20	220 ± 15	52 ± 3	47 ± 2	5 ± 0.2
Biological P	7.18 ± 0.02	70 ± 5 (S) 4840 ± 35	100 ± 10 (S)	85 ± 10	36 ± 2 (S)	23 ± 2	2 ± 0.1
Aeration Tank	6.89 ± 0.02	55 ± 5 (S) 8810 ± 65	75 ± 10 (S)	65 ± 5	24 ± 2(S)	2 ± 0.2	1 ± 0.1
Effluent	7.00 ± 0.02	<10	85 ± 10	65 ± 5	14 ± 1	2 ± 0.2	1 ± 0.1

*(S): Supernatant

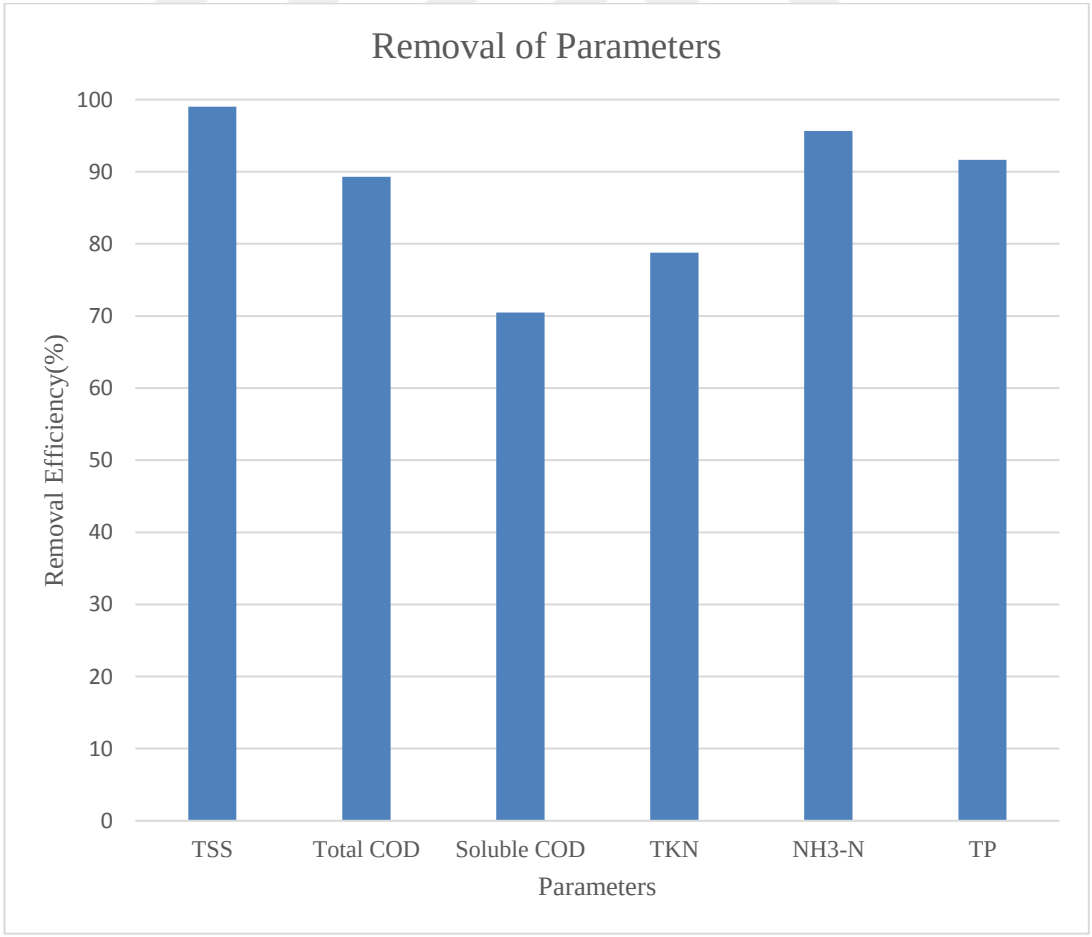


Figure 4.41 : The removal efficiency of wwtp b parameters for summer.

Concentration of TSS were presented as 910 mg/L in the influent and in the effluent, concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 795 mg/L and 220 mg/L, respectively indicating that total COD was constituted with mostly particulate COD while in the effluent, concentration of total COD and soluble COD were found as 85 mg/L and 65 mg/L which indicated that inert COD was dominant in the effluent. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 66 mg N/L and 46 mg N/L indicating that in the influent the dominant form of nitrogen was NH_3-N whereas in the effluent they were found as 14 mg N/L and 2 mg N/L, respectively implying that the dominant form of nitrogen was organic nitrogen. On the other hand, concentration of TP was measured in the influent and effluent as 12 mg/L and 1 mg/L, respectively. As a result, concentrations of TKN did not meet the standards of 10 mg N/L.

High removal efficiencies were achieved for TSS (99%), total COD (89%), soluble COD (70%), TKN (79%), NH_3-N (96%), and TP (92%).

The micropollutant results are shown between Figure 4.42-Figure 4.49.

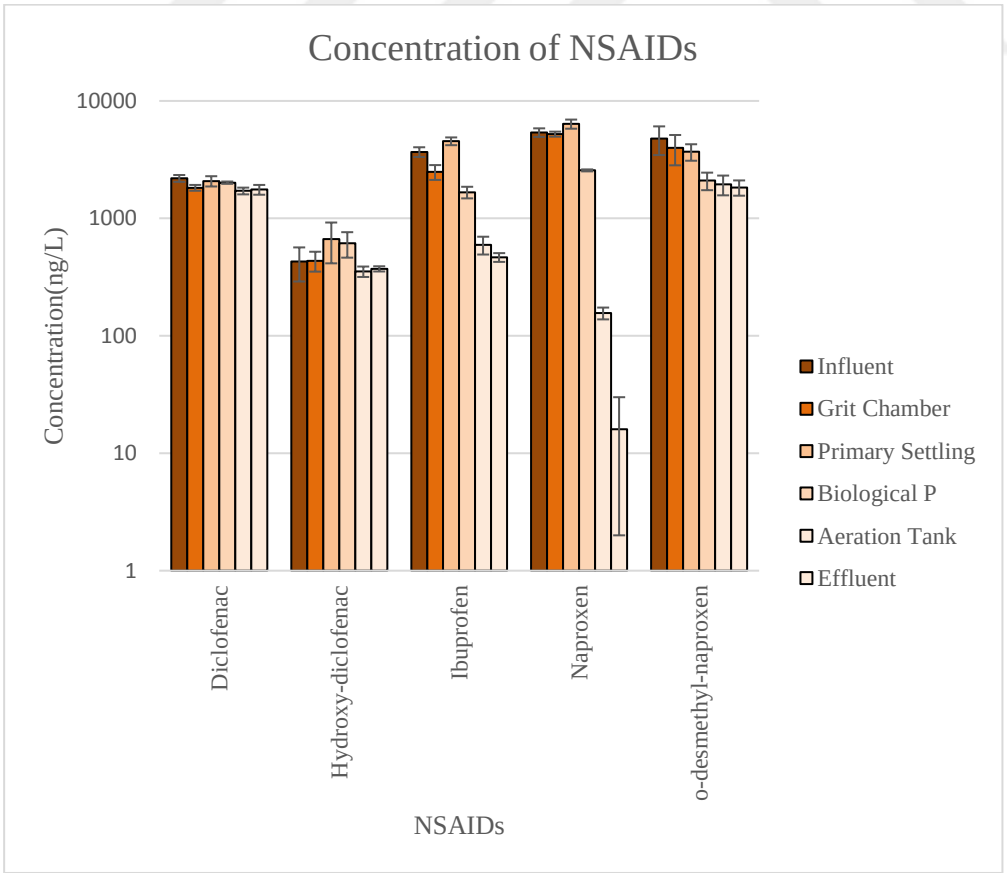


Figure 4.42 : The concentrations of NSAIDs for summer.

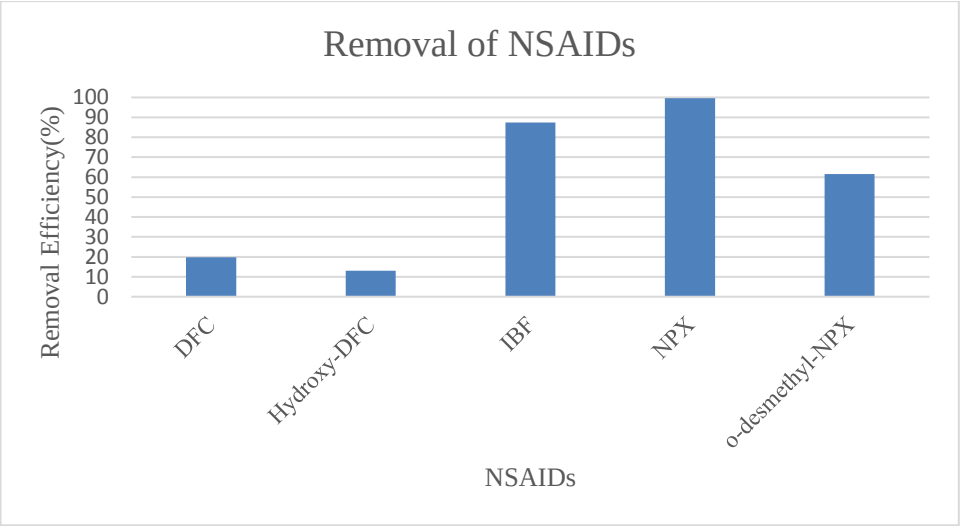


Figure 4.43 : The removal efficiency of NSAIDs for summer.

Diclofenac was present in the influent and effluent with concentrations of 2,193 ng/L and 1,758 ng/L, respectively while hydroxy-diclofenac was observed in the influent and effluent with a concentrations of 428 ng/L and 372 ng/L, respectively. On the other hand, ibuprofen was found to be present in high concentration of 3,683 ng/L in the influent, yet, ibuprofen was present as 466 ng/L in the effluent while naproxen was measured in the influent and effluent as 5,390 ng/L and 16 ng/L, respectively. o-desmethyl-naproxen was detected in the influent and effluent with concentrations of 4,767 ng/L and 1,832 ng/L, respectively. Hydroxy-ibuprofen was present as below detection limit which is 4 ng/L.

For diclofenac, low removal rate was found (20%). Likewise, for hydroxy-diclofenac the removal efficiency was at low level (13%). Ibuprofen had high removal efficiency (87%). Furthermore, removal efficiency for naproxen was very high (100%) whereas o-desmethyl-naproxen, was in medium levels (62%).

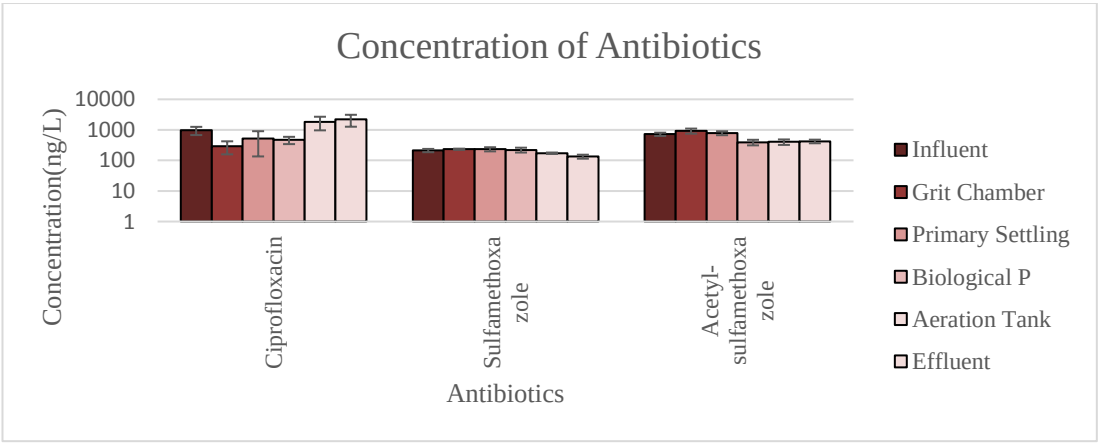


Figure 4.44 : The concentrations of antibiotics for summer.

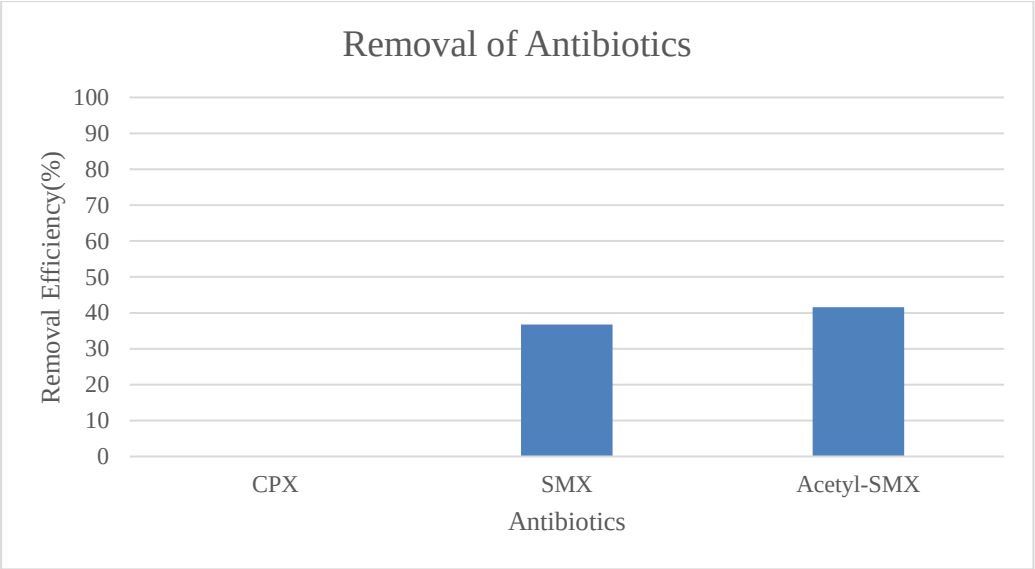


Figure 4.45 : The removal efficiency of antibiotics for summer.

Ciprofloxacin was present in the influent and effluent with concentrations of 966 ng/L and 2,189 ng/L, respectively implying higher concentration in effluent. On the other hand, sulfamethoxazole was found in the influent and effluent as 212 ng/L and 134 ng/L, respectively. Acetyl-sulfamethoxazole was found in the influent as 721 ng/L while in the effluent, it was 421 ng/L.

Negative removal was found for ciprofloxacin due to higher concentration in the effluent. Medium removal efficiencies were measured for sulfamethoxazole (37%) and acetyl-sulfamethoxazole (42%).

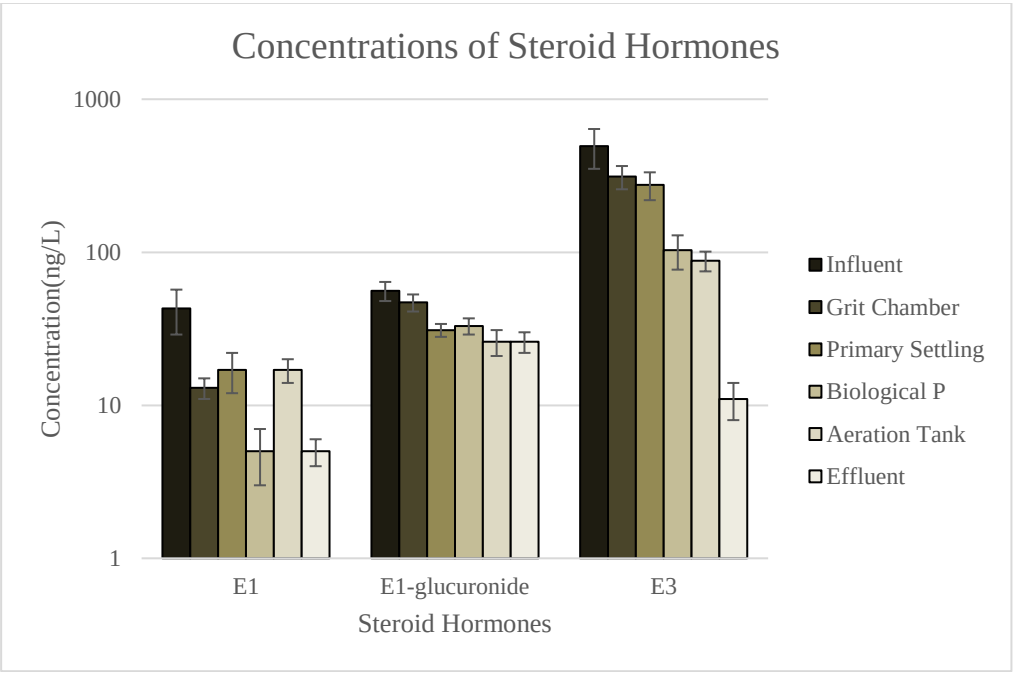


Figure 4.46 : The concentrations of steroid hormones for summer.

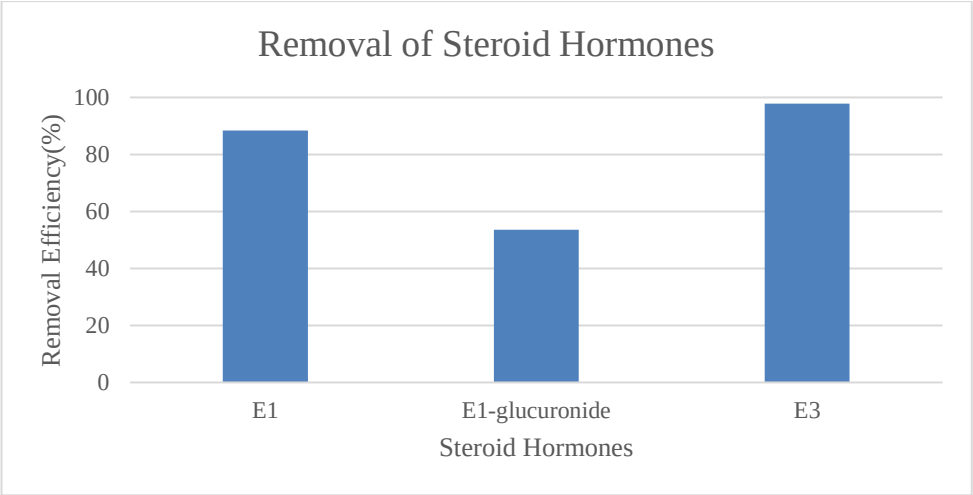


Figure 4.47 : The removal efficiency of steroid hormones for summer.

E1 was measured as 43 ng/L in the influent and in the effluent, the concentration of E1 was observed as 5 ng/L. E1-glucuronide was found in the influent and effluent as 56 ng/L and 26 ng/L, respectively. Concentration of E3 was presented with a concentration of 495 ng/L in the influent and in the effluent, it was 11 ng/L. On the other hand, concentrations of E1-sulphate, E2, and EE2 was found to be below detection limit.

The highest removal efficiencies were presented for E1 (88%) and E3 (98%) while medium level efficiencies was measured for E1-glucuronide (54%). The reason for medium level removal rates might be due to low concentration in the influent.

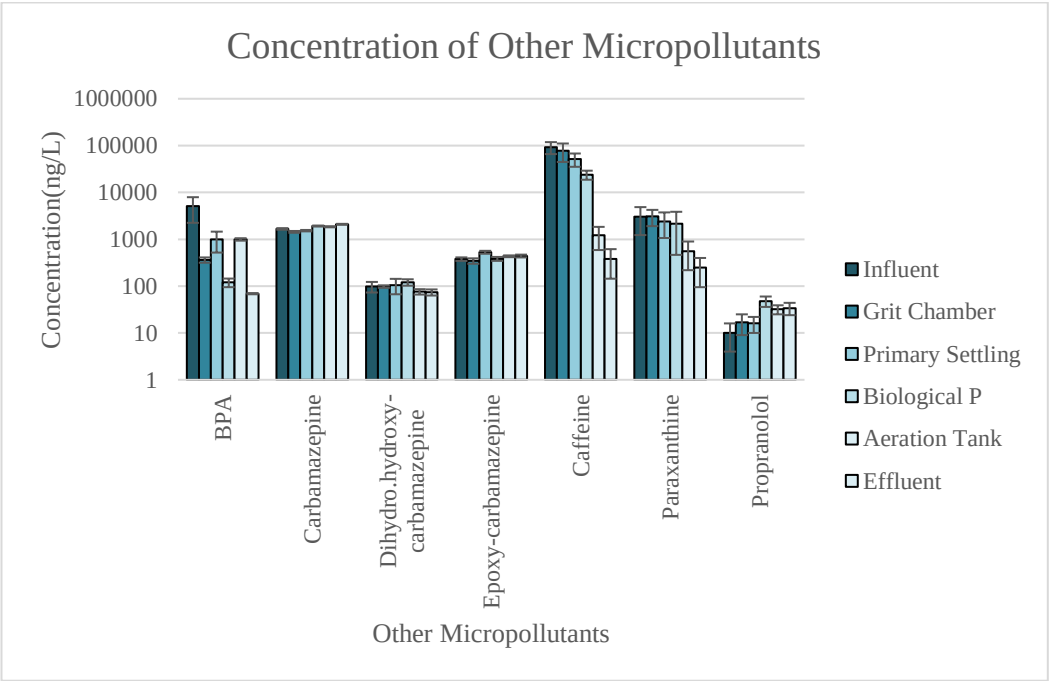


Figure 4.48 : The concentrations of other micropollutants for summer.

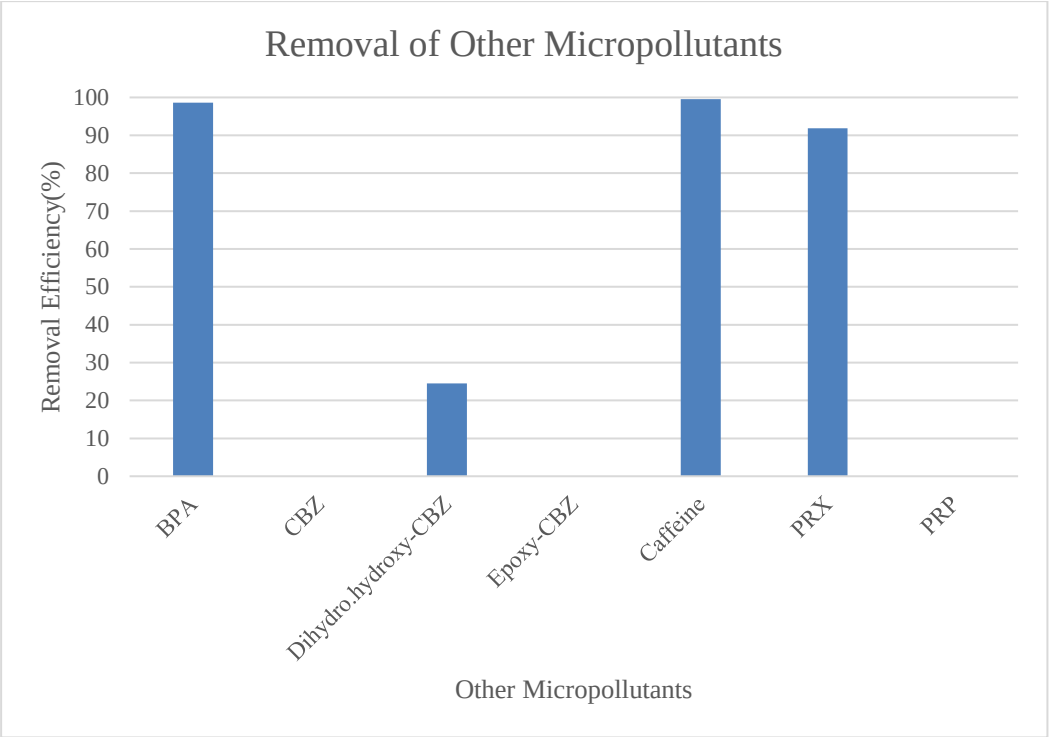


Figure 4.49 : The removal efficiency of other micropollutants for summer.

BPA was found as 5,079 ng/L in the influent, and in the effluent concentration of BPA was decreased to 69 ng/L. Carbamazepine was measured as 1,662 ng/L in the influent, and in the effluent it was found as 2,098 ng/L implying higher concentration in the effluent. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 98 ng/L and 378 ng/L, respectively while in the effluent they were found as 74 ng/L and 440 ng/L, respectively indicating slightly higher concentration for epoxy-carbamazepine. Caffeine presented the highest concentration with 92,257 ng/L, and in the effluent concentration of caffeine was decreased to 380 ng/L. Paraxanthine presented high concentration as well with 3,046 ng/L while in the effluent, the concentration of paraxanthine was observed as 247 ng/L. On the other hand, propranolol was measured in the influent and effluent as 10 ng/L and 34 ng/L, respectively. This might due to inaccurate measurement due to low concentrations. 4-hydroxy-propranolol and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The highest removal efficiency was presented by BPA (99%), caffeine (100%), and paraxanthine (92%). The low removal efficiency was found for dihydro.hydroxy-carbamazepine (24%). Negative removal efficiencies were presented by carbamazepine, epoxy-carbamazepine, and propranolol due to increased

concentrations in the effluent. The heatmap of summer results are shown in Figure 4.50.

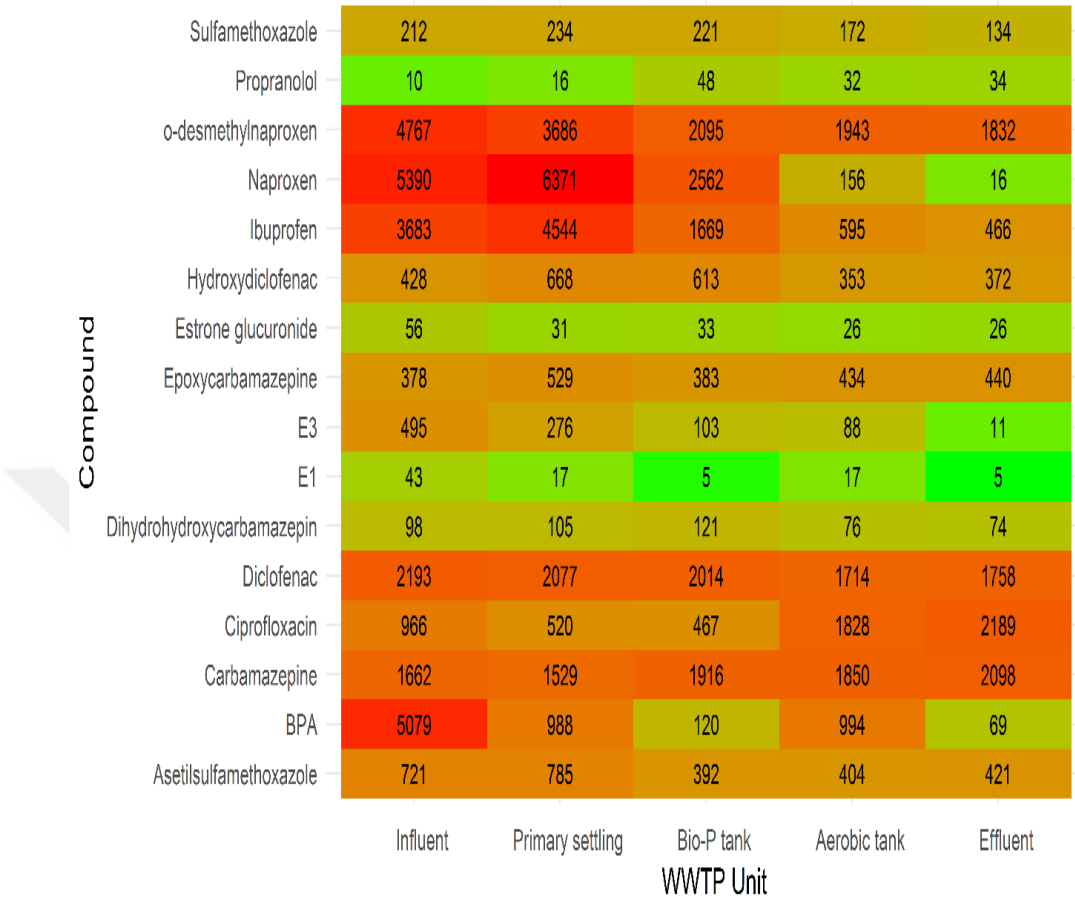


Figure 4.50 : Heatmap of wwtp b for summer.

Naproxen, ibuprofen, diclofenac, and ciprofloxacin which are NSAIDs and antibiotic, respectively have high influent concentration even though the season is summer. This can be explained by decrease in flowrate due to summer weather conditions. For carbamazepine and epoxy-carbamazepine, negative removal is observed while dihydro.hydroxy-carbamazepine has 24% removal efficiency. Knopp et al. (2016) claimed that diclofenac can be removed with low removal efficiency (22%) while in this study diclofenac can be also removed with low removal efficiency (20%). On the other hand, hydroxy-diclofenac has limited removal efficiency (13%). In WWTP B, different trend is observed for sulfamethoxazole and acetyl-sulfamethoxazole compared to WWTP A. Sulfamethoxazole and acetyl-sulfamethoxazole have similar removal efficiencies (37% and 42%, respectively). Subedi et al. (2016) stated a similar removal efficiency for sulfamethoxazole (40%). On the other hand, BPA is removed almost completely even though influent concentration is very high.

4.3.6 The results of wwtp b for fall

The characterization results and removal efficiencies for fall season are shown in Table 4.12 and Figure 4.51.

Table 4.12 : The characterization results of wwtp b for fall.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	8.06 ± 0.02	320 ± 10	630 ± 15	285 ± 10	86 ± 2	59 ± 5	0.7 ± 0.3
Primary Settling	7.90 ± 0.02	465 ± 20	760 ± 15	300 ± 5	86 ± 2	59 ± 5	0.7 ± 0.3
Biologic P	7.34 ± 0.02	4190 ± 100	55 ± 5	35 ± 5 (S)	45 ± 3 (S)	27 ± 3	4 ± 1
Aeration Tank	7.05 ± 0.02	4225 ± 125	<30	<30 (S)	21 ± 1 (S)	3 ± 0.2	4 ± 1
Effluent	7.01 ± 0.02	<10	<30	<30	9 ± 1	3 ± 0.2	0.2 ± 0.1

*(S):Supernatant

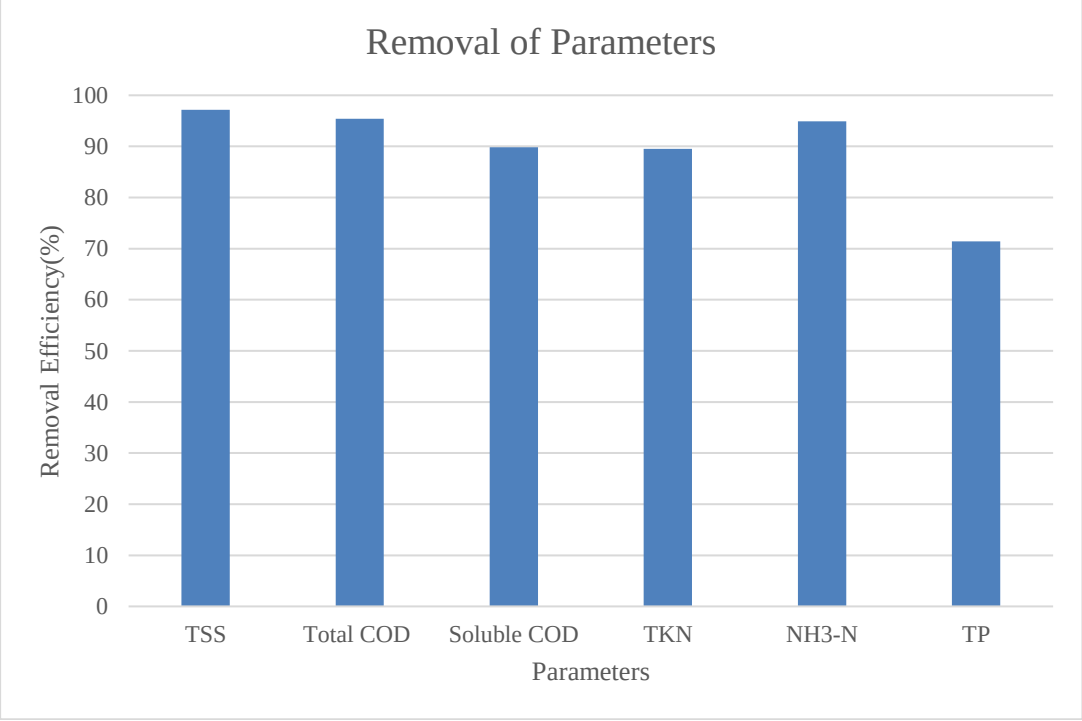


Figure 4.51 : The removal efficiency of wwtp b parameters for fall.

Concentration of TSS were presented as 320 mg/L in the influent and in the effluent, concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 630 mg/L and 285 mg/L, respectively indicating that total COD was constituted with mostly particulate COD while in the effluent, concentration of total COD and soluble COD were both found as below 30 mg/L. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 86 mg N/L and 59 mg N/L whereas in the effluent they were found as 9 mg N/L and 3 mg N/L, respectively implying that the dominant form of nitrogen was organic nitrogen. On the other hand, concentration of TP was measured in the influent and effluent as 0.7 mg/L and 0.2 mg/L, respectively. As a result, all the parameters met the standards.

High removal efficiencies were achieved for TSS (97%), total COD (95%), soluble COD (90%), TKN (90%), NH_3-N (95%), and TP (71%).

The micropollutant concentrations results are shown between Figure 4.52-Figure 4.59.

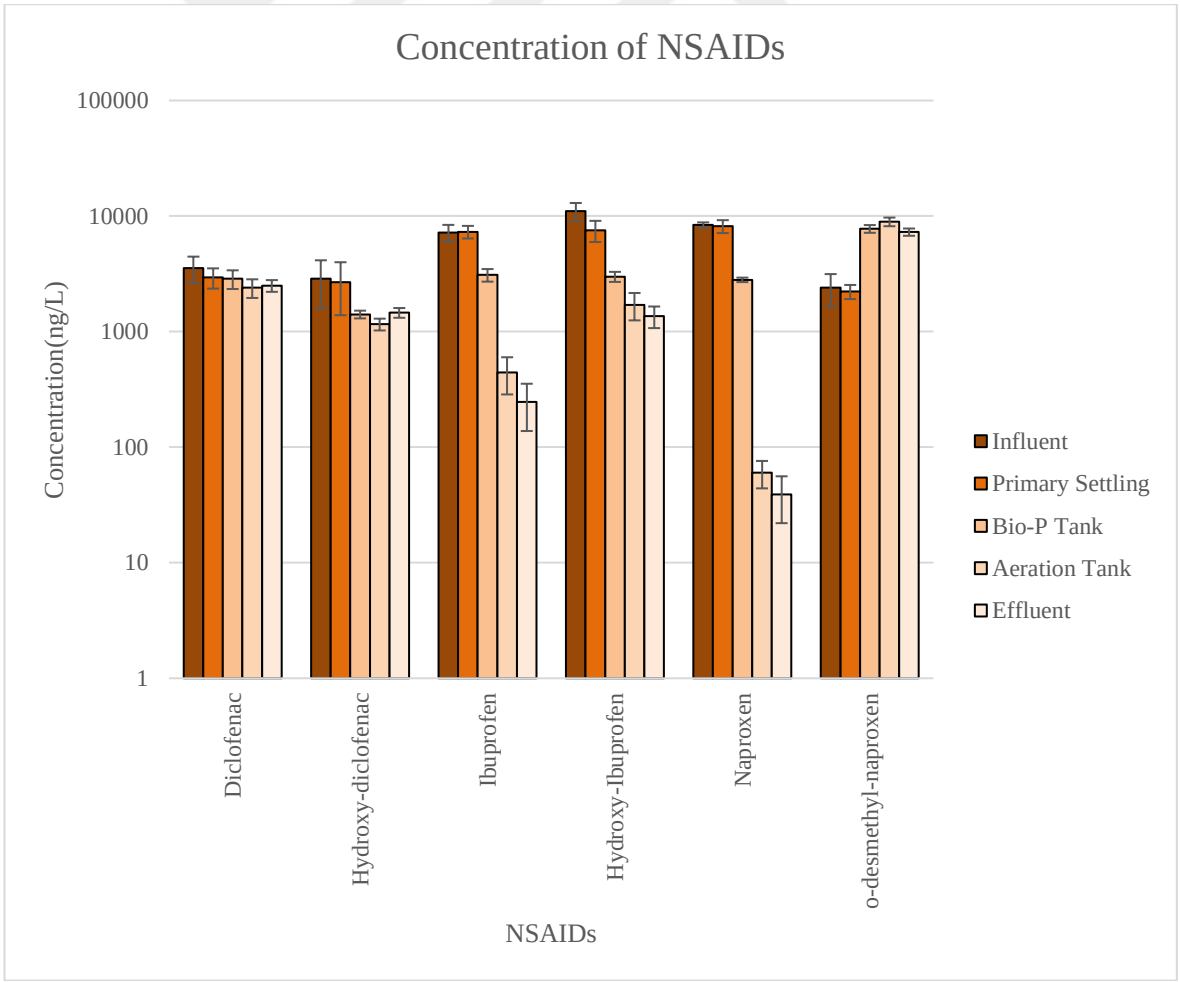


Figure 4.52 : The concentrations of NSAIDs for fall.

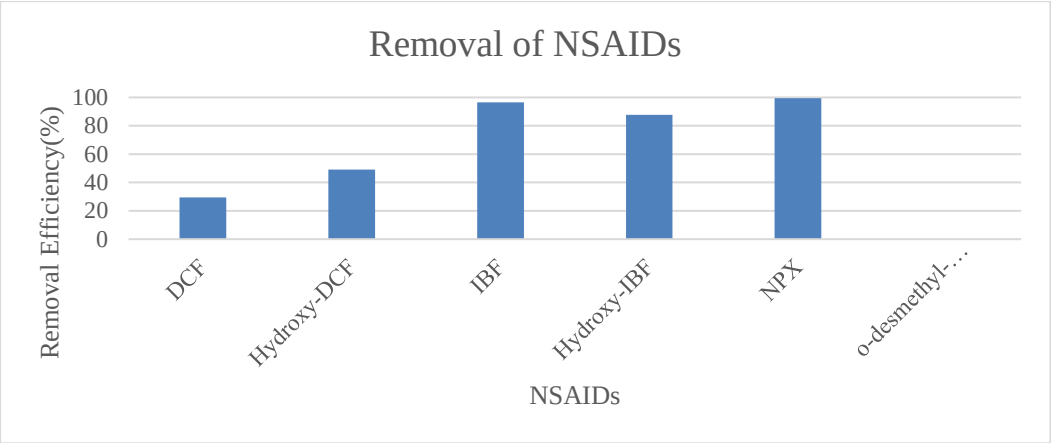


Figure 4.53 : The removal efficicency of NSAIDs for fall.

Diclofenac was present in the influent and effluent with a concentrations of 3,543 ng/L and 2,500 ng/L, respectively while hydroxy-diclofenac was observed in the influent and effluent with concentrations of 2,867 ng/L and 1,459 ng/L, respectively. Naproxen concentration was found to be present in the influent and effluent with concentrations of 8,373 ng/L and 39 ng/L, respectively. o-desmethyl-naproxen was detected in the influent and effluent with concentrations of 2,395 ng/L and 7,276 ng/L, respectively indicating higher concentration in the effluent. Ibuprofen and hiydroxy-ibuprofen were presented in the influent with high concentrations of 7,204 ng/L and 11,049 ng/L, respectively while in the effluent they are measured as 246 ng/L and 1,361 ng/L, respectively.

For diclofenac, low removal rate was found (29%), yet, for hydroxy-diclofenac, medium removal efficiency (49%) was obtained. Furthermore, removal efficiency for naproxen was very high (100%) whereas o-desmethyl-naproxen had negative removal due to higher concentration in the effluent. On the other hand, removal of ibuprofen (97%) and hydroxy-ibuprofen (88%) were achieved with high rates.

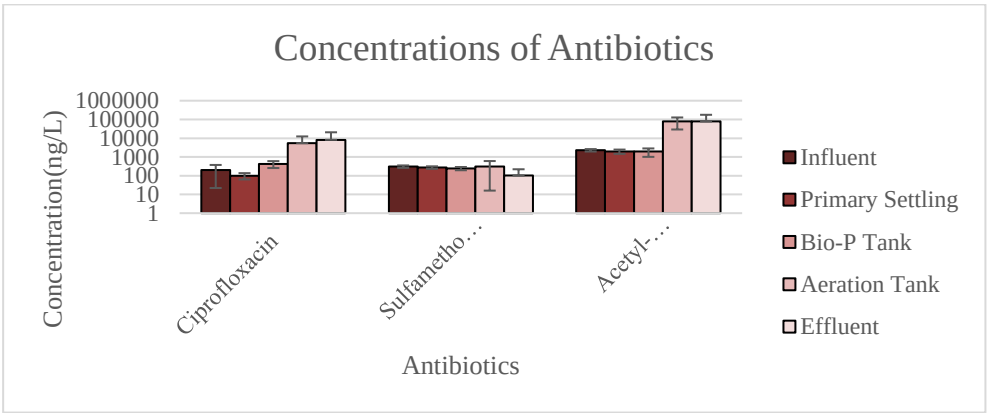


Figure 4.54 : The concentrations of antibiotics for fall.

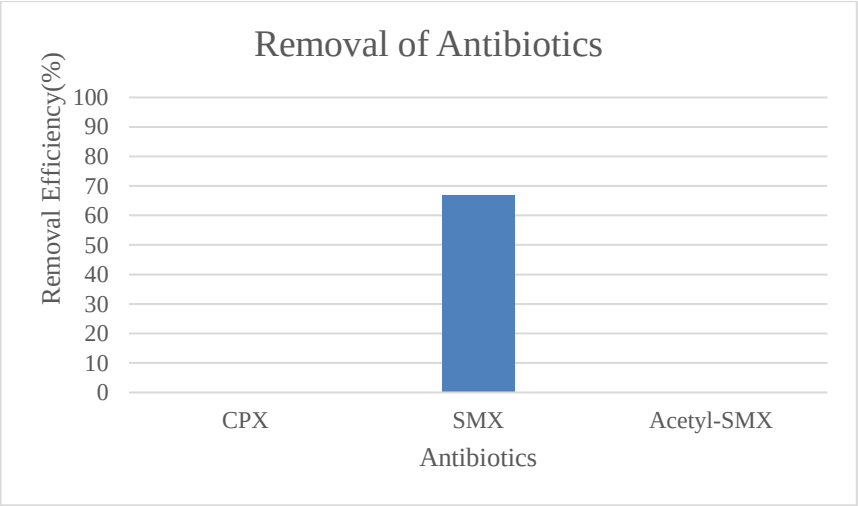


Figure 4.55 : The removal efficiency of antibiotics for fall.

Ciprofloxacin was present in the influent with low concentration of 198 ng/L and in the effluent with high concentration of 8,206 ng/L implying higher concentration in the effluent. On the other hand, sulfamethoxazole was found in the influent and effluent as 307 ng/L and 102 ng/L, respectively. Acetyl-sulfamethoxazole was found in the influent as 2,282 ng/L while in the effluent, it was 78,404 ng/L due to internal standard's inability to ionize.

Negative removal were found for ciprofloxacin and acetyl-sulfamethoxazole due to higher concentration in the effluent. Medium removal efficiencies was measured for sulfamethoxazole (67%). The negative removal may not be correct due to problems with internal standards.

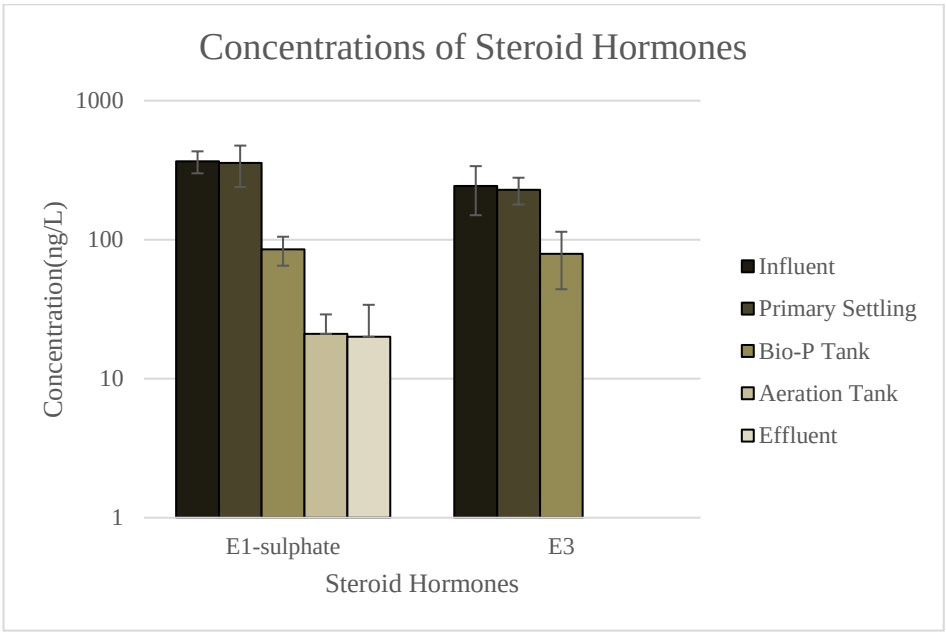


Figure 4.56 : The concentrations of steroid hormones for fall.

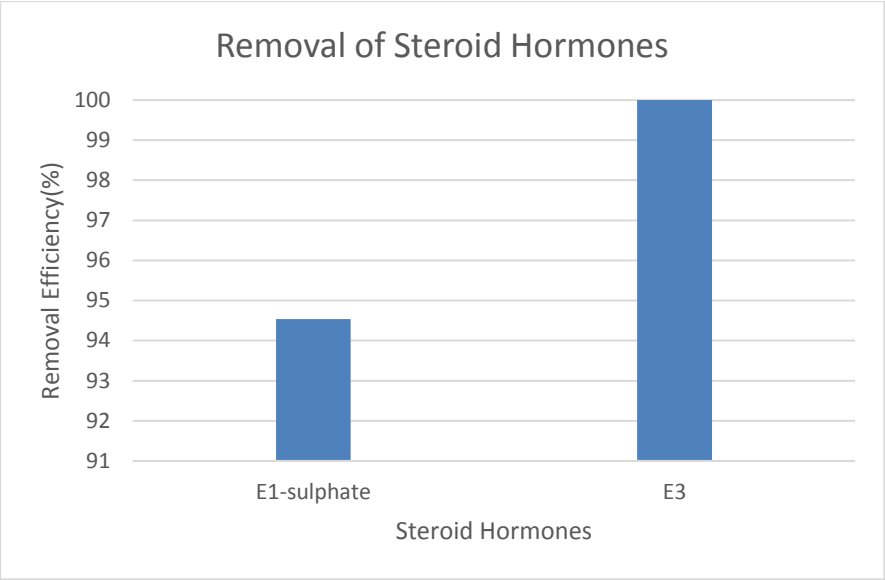


Figure 4.57 : The removal efficiency of steroid hormones for fall.

E1-sulphate was measured as 366 ng/L in the influent and in the effluent, the concentration of E1-sulphate was observed as 20 ng/L. Concentration of E3 was presented with a concentration of 244 ng/L in the influent and in the effluent, it was below detection limit. On the other hand, concentrations of E1-glucuronide, E2, and EE2 was found to be below detection limit.

The high removal efficiencies were presented for E1-sulphate (95%) and E3 (100%).

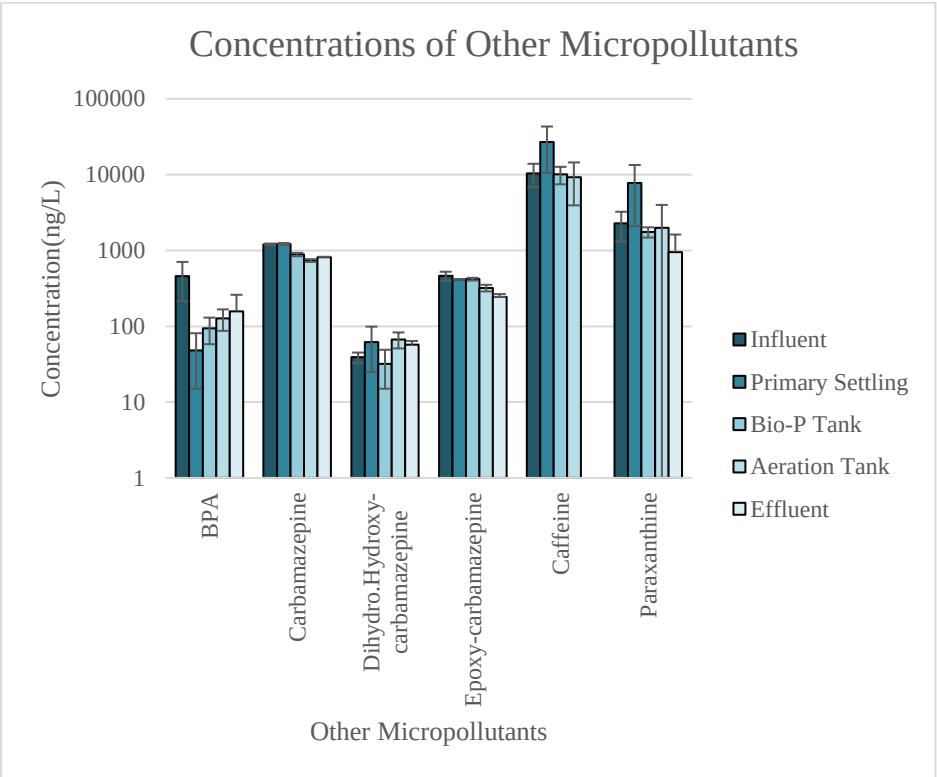


Figure 4.58 : The concentrations of other micropollutants for fall.

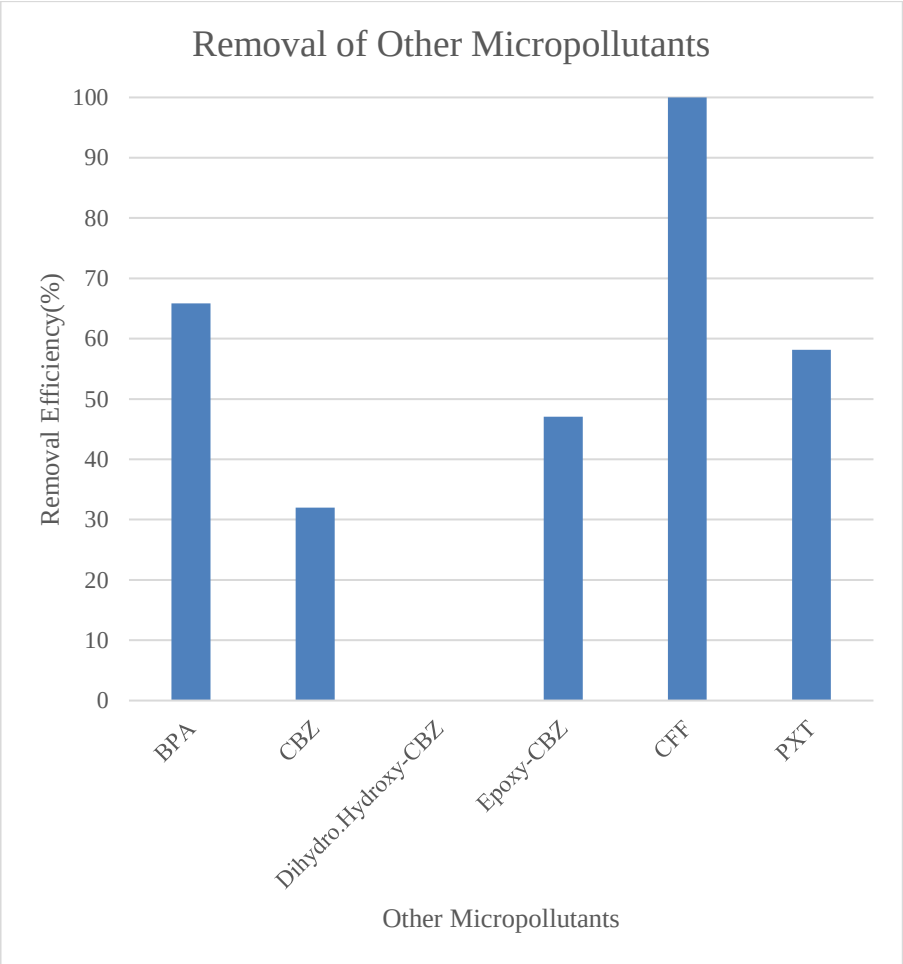


Figure 4.59 : The removal efficiency of other micropollutants for fall.

BPA was found as 460 ng/L in the influent, and in the effluent concentration of BPA was decreased to 157 ng/L. Carbamazepine was measured as 1,200 ng/L in the influent, and in the effluent it was found as 816 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 39 ng/L and 461 ng/L, respectively while in the effluent they were found as 57 ng/L and 244 ng/L, respectively indicating slightly higher concentration of dihydro.hydroxy-carbamazepine in the effluent. Increase in the concentration might be due to low concentration in the influent. Caffeine presented the highest concentration with 10,369 ng/L, and in the effluent concentration of caffeine was below detection limit which is 4 ng/L. Paraxanthine presented high concentration as well with 2,275 ng/L while in the effluent, the concentration of paraxanthine was observed as 952 ng/L. On the other hand, propranolol, 4-hydroxy-propranolol, and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The highest removal efficiency was presented by caffeine (100%). Medium removal efficiencies were found for BPA (66%), paraxanthine (58%), and epoxy-carbamazepine (47%). The low removal efficiency was found for carbamazepine (32%). Negative removal efficiencies was presented by dihydro.hydroxy-carbamazepine due to increased concentrations in the effluent. The heatmap of fall results are shown in Figure 4.60.

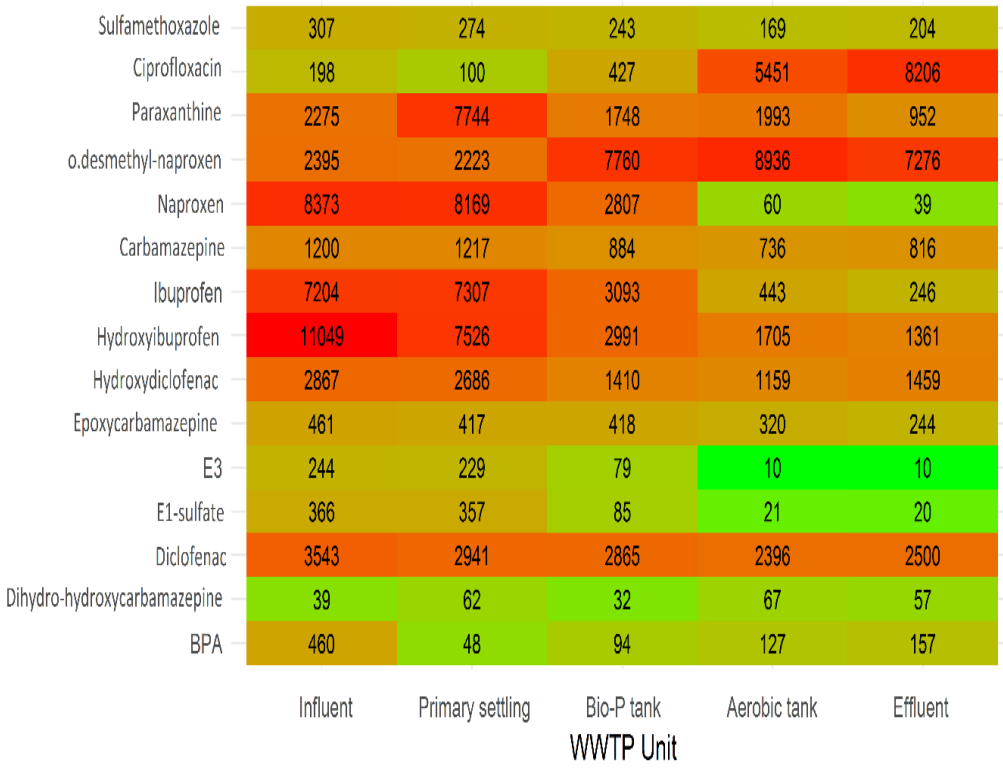


Figure 4.60 : Heatmap of wwtp b for fall.

Naproxen, ibuprofen, and diclofenac which are NSAIDs have high influent concentration due to extensive consumption in fall. Choi et al. (2008) stated that carbamazepine can be removed in the range of 0-50%. The same result is obtained for this study and carbamazepine is removed with low removal efficiency (32%). Removal efficiency of epoxy-carbamazepine is higher than carbamazepine (47%) while negative removal is observed for dihydro.hydroxy-carbamazepine. Knopp et al. (2016) claimed that diclofenac can be removed with low removal efficiency (22%) while in this study diclofenac can also be removed with low removal efficiency (29%). On the other hand, hydroxy-diclofenac has medium removal efficiency (49%). Influent concentration of ciprofloxacin is 198 ng/L while in the effluent, concentration as high as 8206 ng/L. This can be explained by inaccurate sampling.

4.3.7 The results of wwtp b for winter

The characterization results and removal efficiencies for winter are shown in Table 4.13 and Figure 4.61.

Table 4.13 : The characterization results of wwtp b for winter.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	8.07 ± 0.02	260 ± 15	855 ± 15	520 ± 8	71 ± 2	58 ± 1	6 ± 0.4
Primary Settling	8.12 ± 0.02	200 ± 4	510 ± 6	275 ± 10	71 ± 2	55 ± 1	5 ± 0.3
Biological P Tank	7.07 ± 0.02	10750 ± 100	60 ± 10	50 ± 2 (S)	32 ± 2 (S)	15 ± 0.2	6 ± 1
Aeration Tank	7.06 ± 0.02	12580 ± 100	75 ± 1	60 ± 2 (S)	34 ± 6 (S)	13 ± 0.2	6 ± 1
Effluent	7.40 ± 0.02	<10	80 ± 4	60 ± 2	25 ± 1	13± 0.2	0.8 ± 0.1

*(S):Supernatant

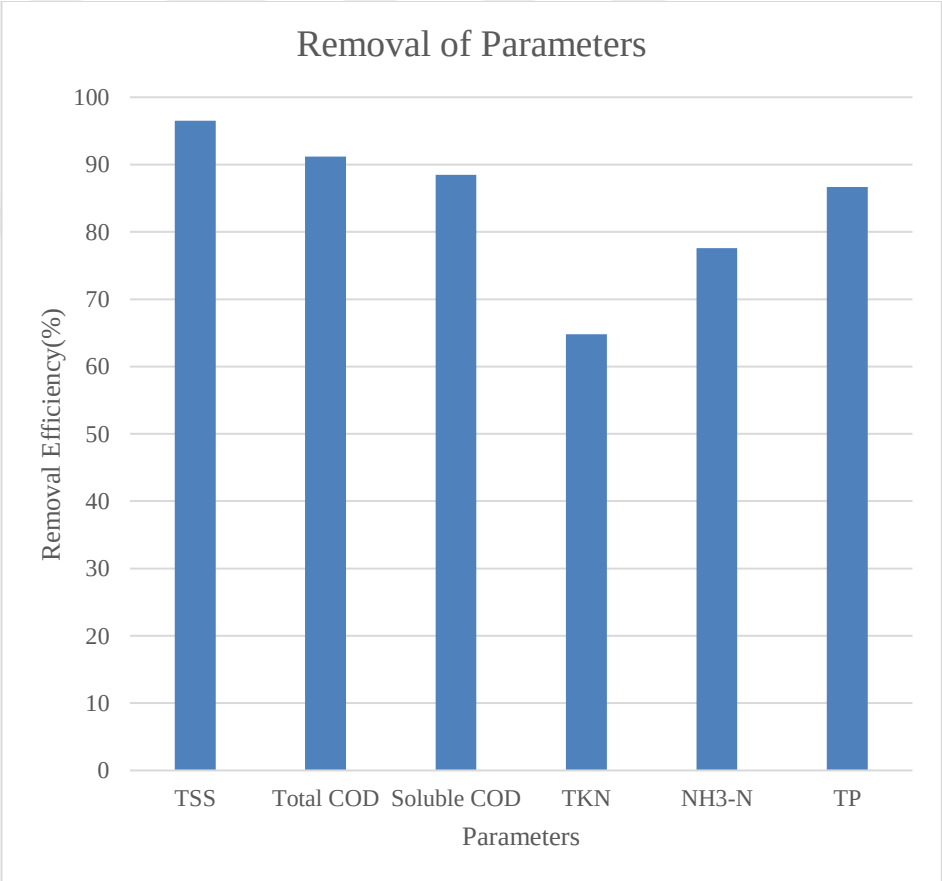


Figure 4.61 : The removal efficiency of wwtp b parameters for winter.

Concentration of TSS were presented as 260 mg/L in the influent and in the effluent, concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 855 mg/L and 520 mg/L, respectively while in the effluent,

concentration of total COD and soluble COD were found as 80 mg/L and 60 mg/L, respectively indicating that in both influent and effluent total COD was constituted by mostly inert COD. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 71 mg N/L and 58 mg N/L whereas in the effluent they were found as 25 mg N/L and 13 mg N/L, respectively implying that the dominant form of nitrogen was NH_3-N in the influent and equal quantity of organic nitrogen and NH_3-N in the effluent. On the other hand, concentration of TP was measured in the influent and effluent as 6 mg/L and 0.8 mg/L, respectively. As a result, TKN did not meet the standards.

High removal efficiencies were achieved for TSS (97%), total COD (91%), soluble COD (88%), NH_3-N (78%), and TP (87%) while medium efficiency was found for TKN (65%).

The micropollutant concentration results are shown between Figure 4.62-Figure 4.68.

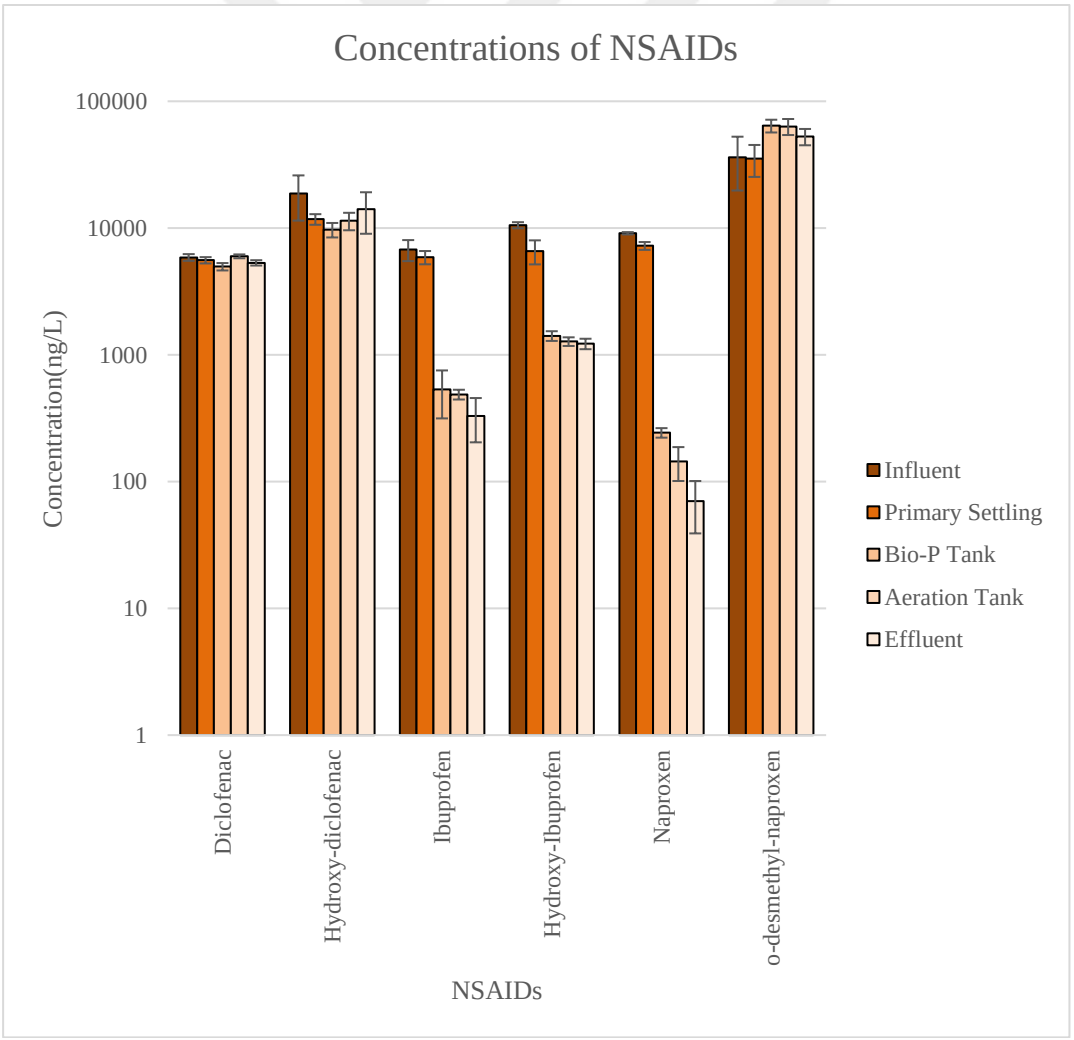


Figure 4.62 : The concentrations of NSAIDs for winter.

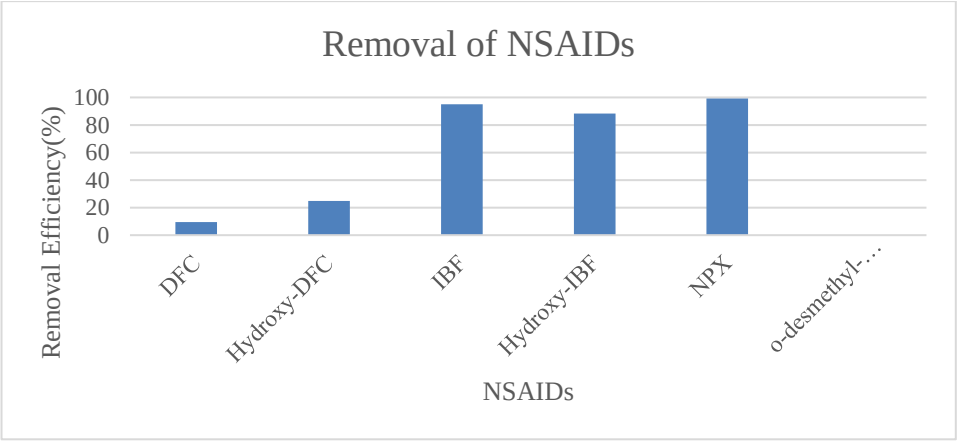


Figure 4.63 : The removal efficiency of NSAIDs for winter.

Diclofenac was present in the influent and effluent with high concentrations of 5,870 ng/L and 5,318 ng/L, respectively while hydroxy-diclofenac was observed in the influent and effluent with high concentrations of 18,733 ng/L and 14,087 ng/L, respectively. Naproxen concentration was found to be present in the influent and effluent with concentrations of 9,094 ng/L and 70 ng/L, respectively. o-desmethyl-naproxen was detected in the influent and effluent with highest concentrations of 36,170 ng/L and 52,731 ng/L in NSAID group, respectively indicating higher concentration in the effluent. Ibuprofen and hydroxy-ibuprofen were presented in the influent with high concentrations of 6,759 ng/L and 10,539 ng/L, respectively while in the effluent they are measured as 330 ng/L and 1,223 ng/L, respectively.

High removal efficiencies were achieved for ibuprofen (95%), hydroxy-ibuprofen (88%), and naproxen (99%). Low removal efficiencies were found for diclofenac (9%), hydroxy-diclofenac (25%). On the other hand, o-desmetyl-naproxen had negative removal due to increased concentration in the effluent.

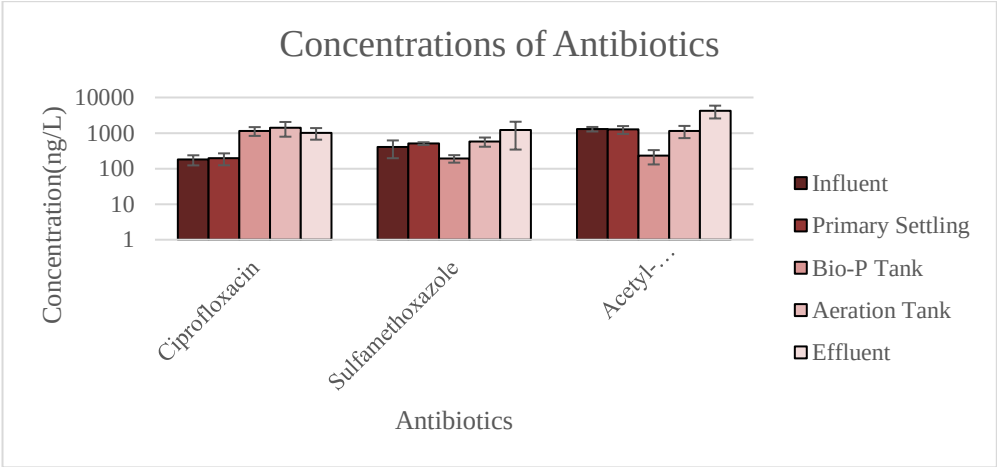


Figure 4.64 : The concentrations of antibiotics for winter.

Ciprofloxacin was present in the influent with low concentration of 180 ng/L and in the effluent with concentration of 1,015 ng/L implying higher concentration in effluent. Similarly, sulfamethoxazole was found in the influent and effluent as 408 ng/L and 1,212 ng/L, respectively. Acetyl-sulfamethoxazole was found in the influent as 1,295 ng/L while in the effluent, it was 4,236 ng/L.

Negative removals were found for ciprofloxacin, sulfamethoxazole, and acetyl-sulfamethoxazole due to higher concentration in the effluent.

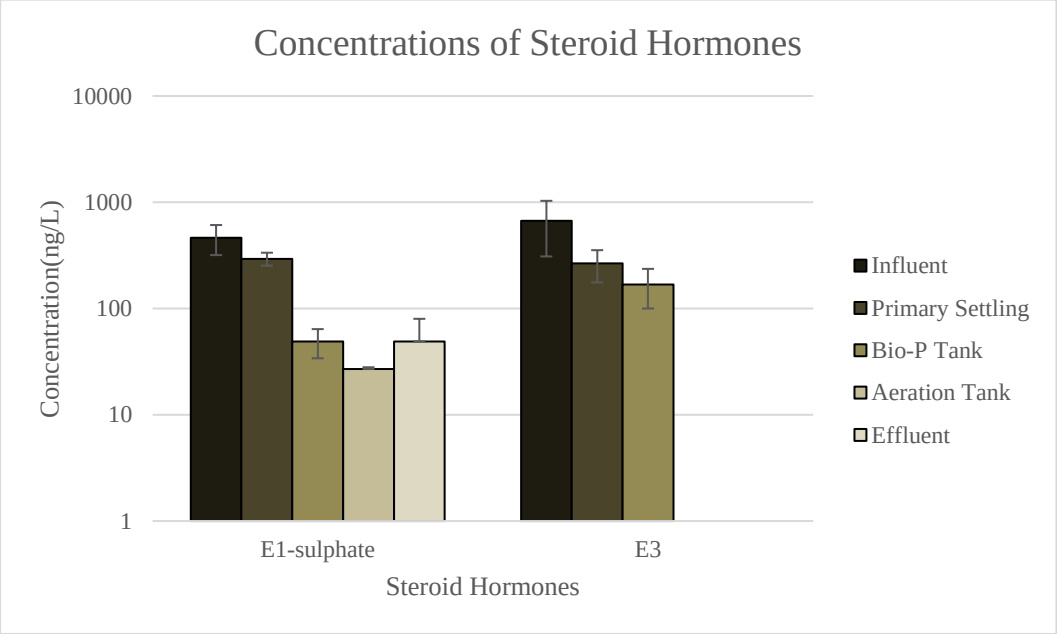


Figure 4.65 : The concentrations of steroid hormones for winter.

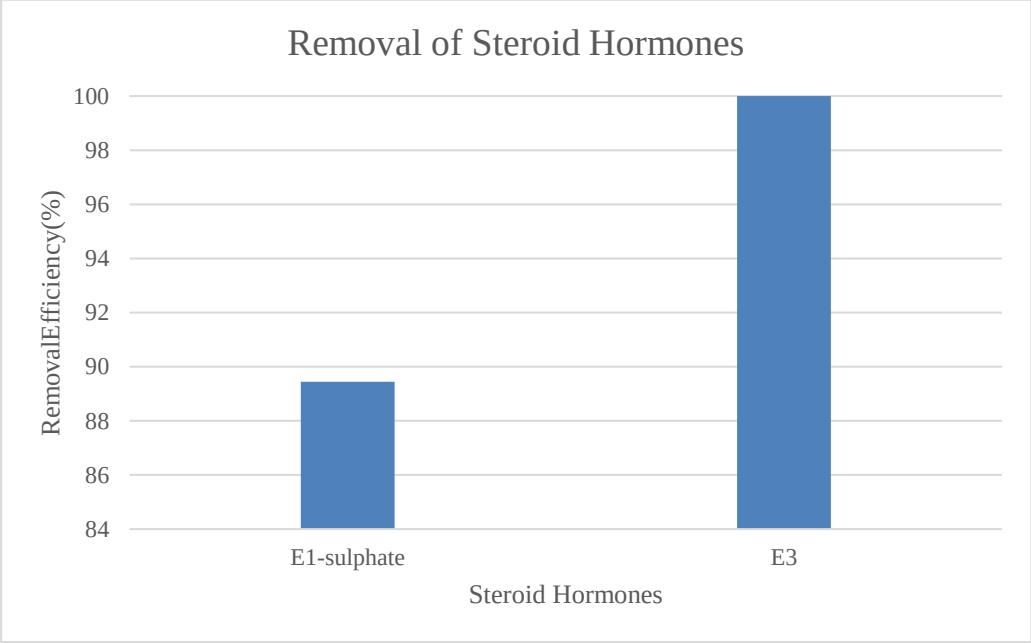


Figure 4.66 : The removal efficiency of steroid hormones for winter.

E1-sulphate was measured as 464 ng/L in the influent and in the effluent, the concentration of E1-sulphate was observed as 49 ng/L. Concentration of E3 was presented with a concentration of 670 ng/L in the influent and in the effluent, it was below detection limit. On the other hand, concentrations of E1-glucuronide, E2, and EE2 was found to be below detection limit.

The high removal efficiencies were presented for E1-sulphate (89%) and E3 (100%).

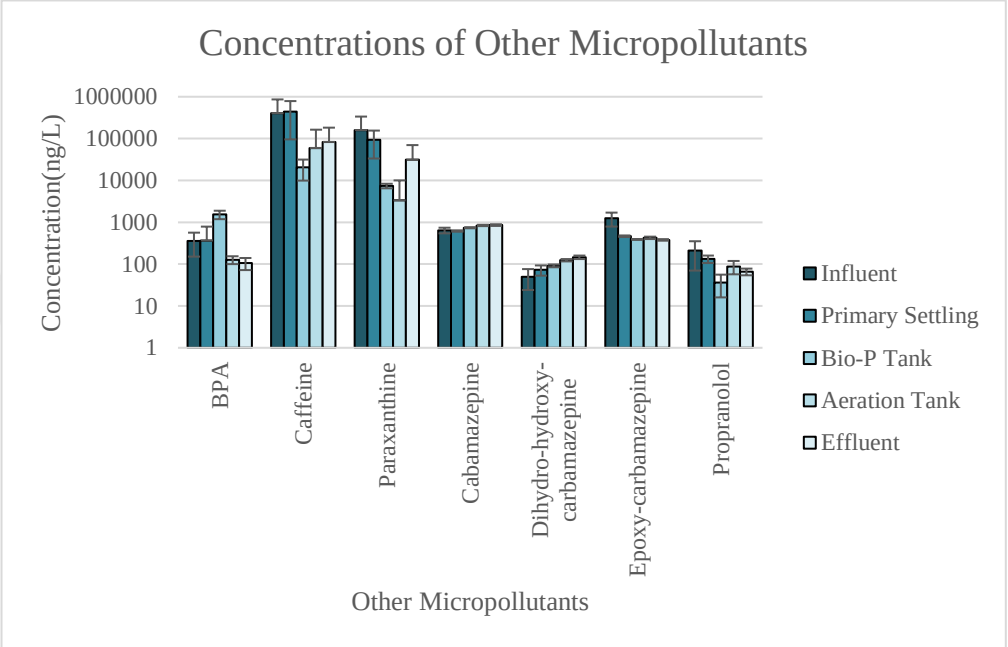


Figure 4.67 : The concentrations of other micropollutants for winter.

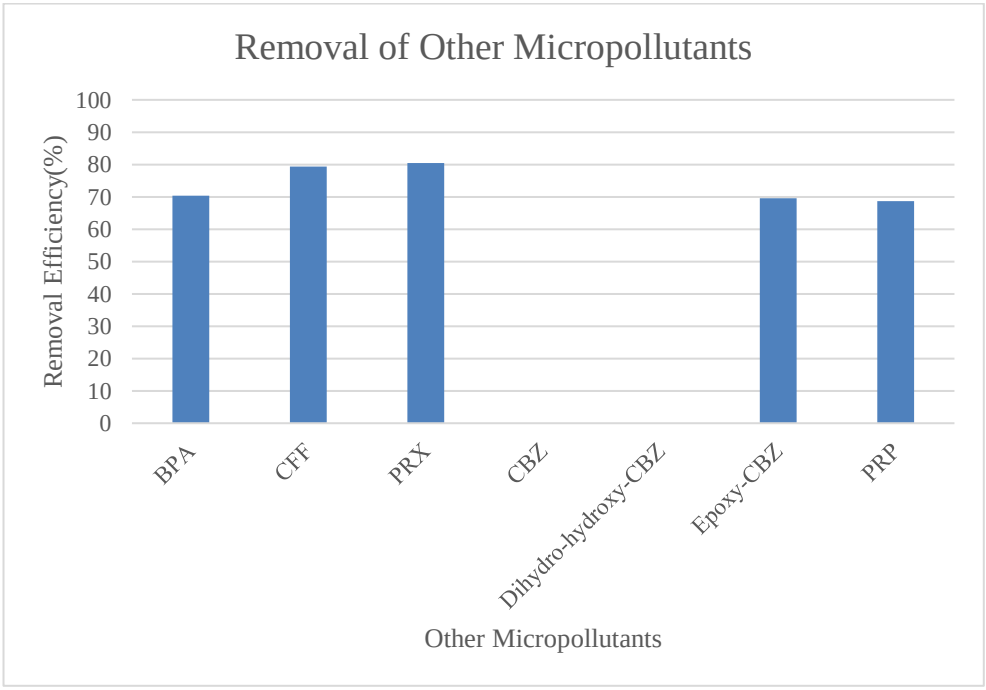


Figure 4.68 : The removal efficiency of other micropollutants for winter.

BPA was found as 358 ng/L in the influent, and in the effluent concentration of BPA was decreased to 106 ng/L. Carbamazepine was measured as 642 ng/L in the influent, and in the effluent it was found as 856 ng/L implying higher concentration in the effluent. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 50 ng/L and 1,245 ng/L, respectively while in the effluent they were found as 146 ng/L and 379 ng/L, respectively indicating slightly higher concentration of dihydro.hydroxy-carbamazepine in the effluent. Increase in the concentration might be due to low concentration in the influent. Caffeine presented the highest concentration with 404,760 ng/L, and in the effluent concentration of caffeine was found as 83,444 ng/L. The high influent concentration for caffeine is incorrect due to internal standard's inability to ionize. Paraxanthine presented high concentration as well with 159,612 ng/L while in the effluent, the concentration of paraxanthine was observed as 31,147 ng/L. On the other hand, propranolol was found in the influent and effluent as 211 ng/L and 66 ng/L,respectively.

Lastly, 4-hydroxy-propranolol, and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The high removal efficiency were presented by paraxanthine (80%), caffeine (79%), BPA (70%), epoxy-carbamazepine (70%), and propranolol (69%). Negative removal efficiencies were presented by carbamazepine and dihydro.hydroxy-carbamazepine due to higher concentrations in the effluent. The heatmap of winter results are shown in Figure 4.69.

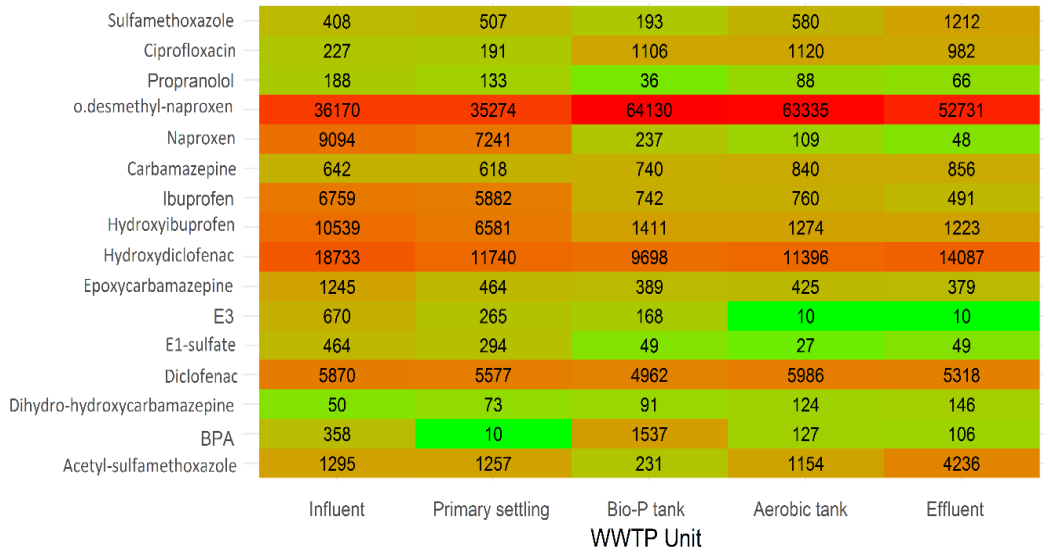


Figure 4.69 : Heatmap of wwtp b for winter.

Naproxen, ibuprofen, and diclofenac which are NSAIDs have high influent concentration due to extensive consumption in winter. Negative removals are observed for carbamazepine and dihydro.hydroxy-carbamazepine. Removal efficiency of epoxy-carbamazepine is high (70%) Diclofenac can be removed with low removal efficiency (9%). On the other hand, hydroxy-diclofenac has higher removal efficiency than diclofenac (25%). Influent concentrations of carbamazepine in summer and fall are 1,662 and 1,200 ng/L while in winter it is 642 ng/L. This is observed in WWTP A. This can be explained by dilution due to precipitation.

4.3.8 The results of wwtp b for spring

The characterization results and removal efficiencies for winter are shown in Table 4.14 and Figure 4.70.

Table 4.14 : The characterization results of wwtp b for spring.

Sample	pH	TSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TKN (mg N/L)	NH_3-N (mg N/L)	TP (mg/L)
Influent	7.71 ± 0.02	360 ± 5	730 ± 5	295 ± 10	57 ± 1	52 ± 0	5 ± 0.3
Primary Settling	7.84 ± 0.02	375 ± 30	730 ± 5	285 ± 10	64 ± 1	57 ± 1	2 ± 0.3
Biological P	6.96 ± 0.02	16375 ± 275	130 ± 20	75 ± 10 (S)	7 ± 1 (S)	3 ± 0.8	3 ± 0.2
Aeration Tank	7.05 ± 0.02	6985 ± 25	105 ± 5	60 ± 10 (S)	7 ± 0 (S)	2 ± 0.5	< 1
Effluent	7.37 ± 0.02	<10	100 ± 5	65 ± 5	4 ± 0	1± 0.1	< 1

*(S):Supernatant

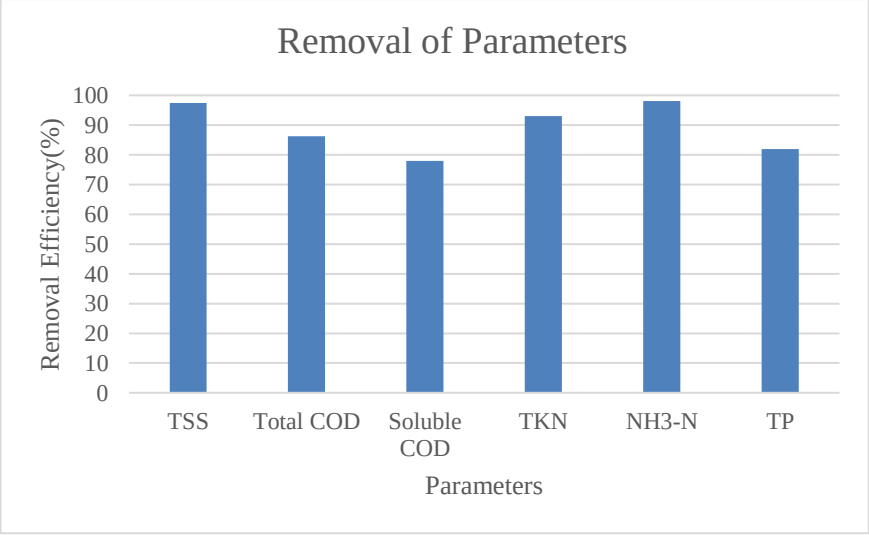


Figure 4.70 : The removal efficiency of wwtp b parameters for spring.

Concentration of TSS were presented as 360 mg/L in the influent and in the effluent, concentration of TSS was below 10 mg/L. Concentrations of total COD and soluble COD were measured as 730 mg/L and 295 mg/L, respectively indicating that in influent total COD was constituted by mostly particulate COD while in the effluent, concentration of total COD and soluble COD were found as 100 mg/L and 65 mg/L, respectively indicating that in effluent total COD was constituted by mostly inert COD. When it comes to concentrations of TKN and NH_3-N, in the influent concentrations of TKN and NH_3-N were measured as 57 mg N/L and 52 mg N/L whereas in the effluent they were found as 4 mg N/L and 1 mg N/L, respectively implying that the dominant form of nitrogen was NH_3-N in the influent and effluent. On the other hand, concentration of TP was measured in the influent and effluent as 5 mg/L and <1 mg/L, respectively. As a result, all parameters met the standards.

High removal efficiencies were achieved for TSS (98%), total COD (86%), soluble COD (78%), TKN (93%), NH_3-N (98%), and TP (82%).

The micropollutant concentration results are shown between Figure 4.71-Figure 4.76.

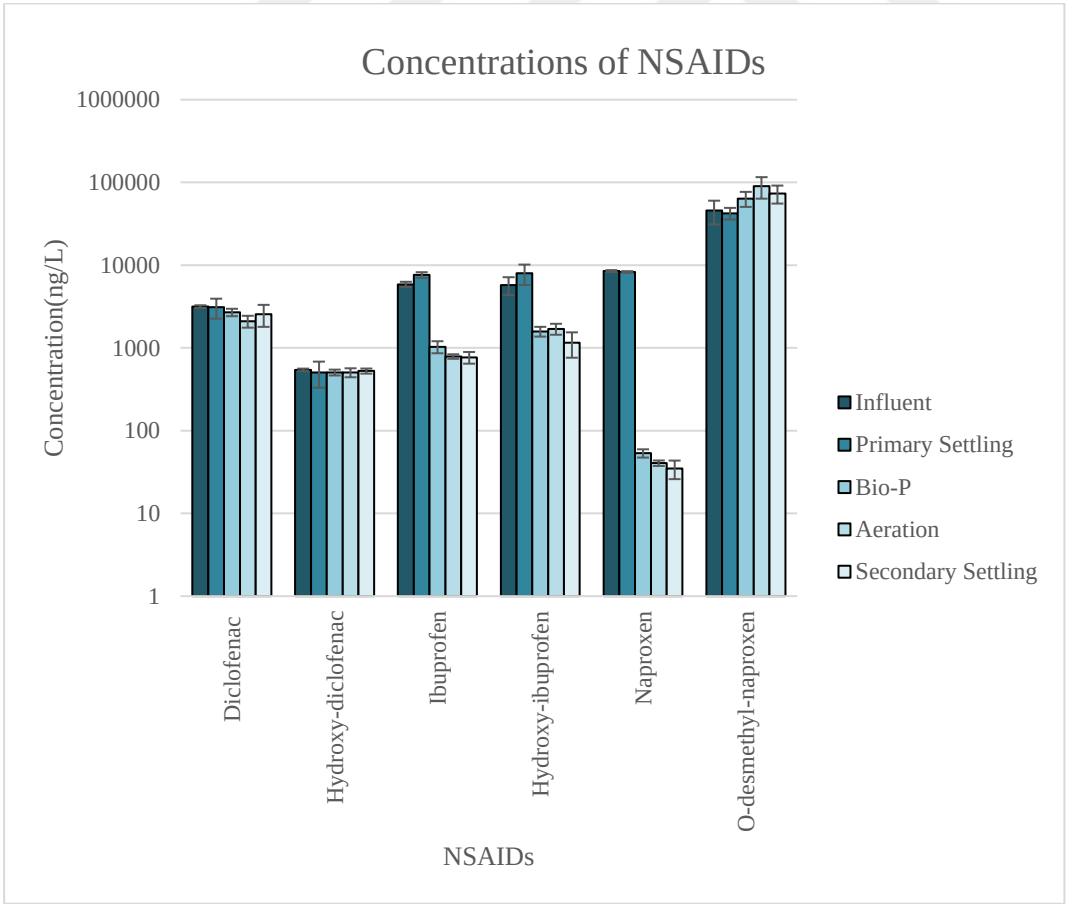


Figure 4.71 : The concentrations of NSAIDs for spring.

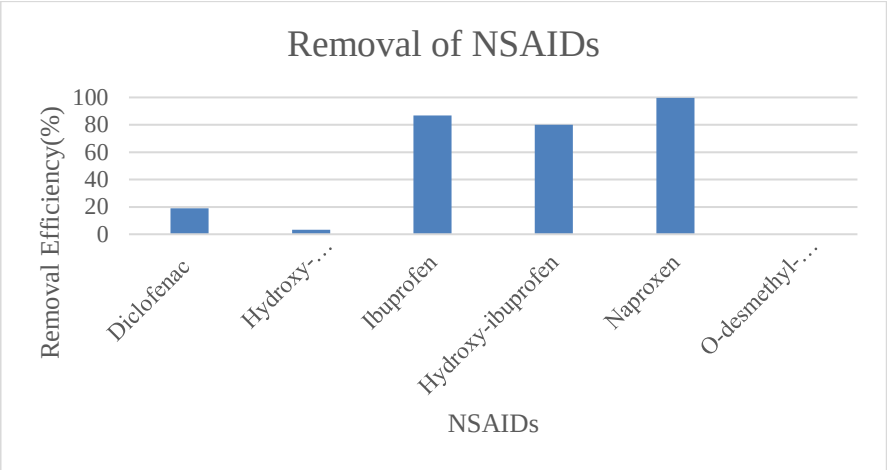


Figure 4.72 : The removal efficiencies of NSAIDs for spring.

Diclofenac was present in the influent and effluent with high concentrations of 3,158 ng/L and 2,557 ng/L, respectively while hydroxy-diclofenac was observed in the influent and effluent with high concentrations of 544 ng/L and 526 ng/L, respectively. Naproxen concentration was found to be present in the influent and effluent with concentrations of 5,758 ng/L and 35 ng/L, respectively. o-desmethyl-naproxen was detected in the influent and effluent with highest concentrations of 45,669 ng/L and 73,588 ng/L in NSAID group, respectively indicating higher concentration in the effluent. Ibuprofen and hydroxy-ibuprofen were presented in the influent with high concentrations of 5,868 ng/L and 5,758 ng/L, respectively while in the effluent they are measured as 769 ng/L and 1,152 ng/L, respectively.

High removal efficiencies were achieved for ibuprofen (87%), hydroxy-ibuprofen (80%), and naproxen (100%). Low removal efficiencies were found for diclofenac (19%), hydroxy-diclofenac (3%). On the other hand, o-desmetyl-naproxen had negative removal due to increased concentration in the effluent.

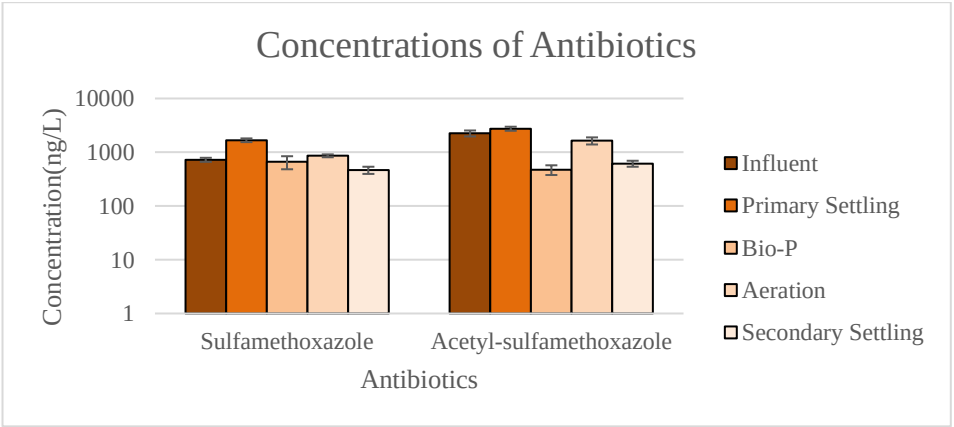


Figure 4.73 : The concentrations of antibiotics for spring.

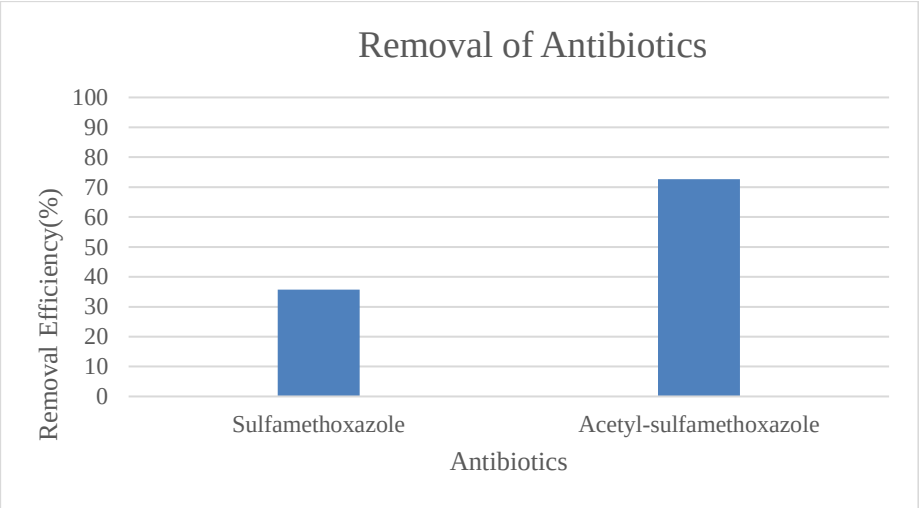


Figure 4.74 : The removal efficiencies of antibiotics for spring.

Sulfamethoxazole was found in the influent and effluent as 726 ng/L and 467 ng/L, respectively. Acetyl-sulfamethoxazole was found in the influent as 2,256 ng/L while in the effluent, it was 616 ng/L. Ciprofloxacin was not detected due to being under LOQ value.

High removal efficiency was found for sulfamethoxazole while medium removal efficiency was found for acetyl-sulfamethoxazole (36%).

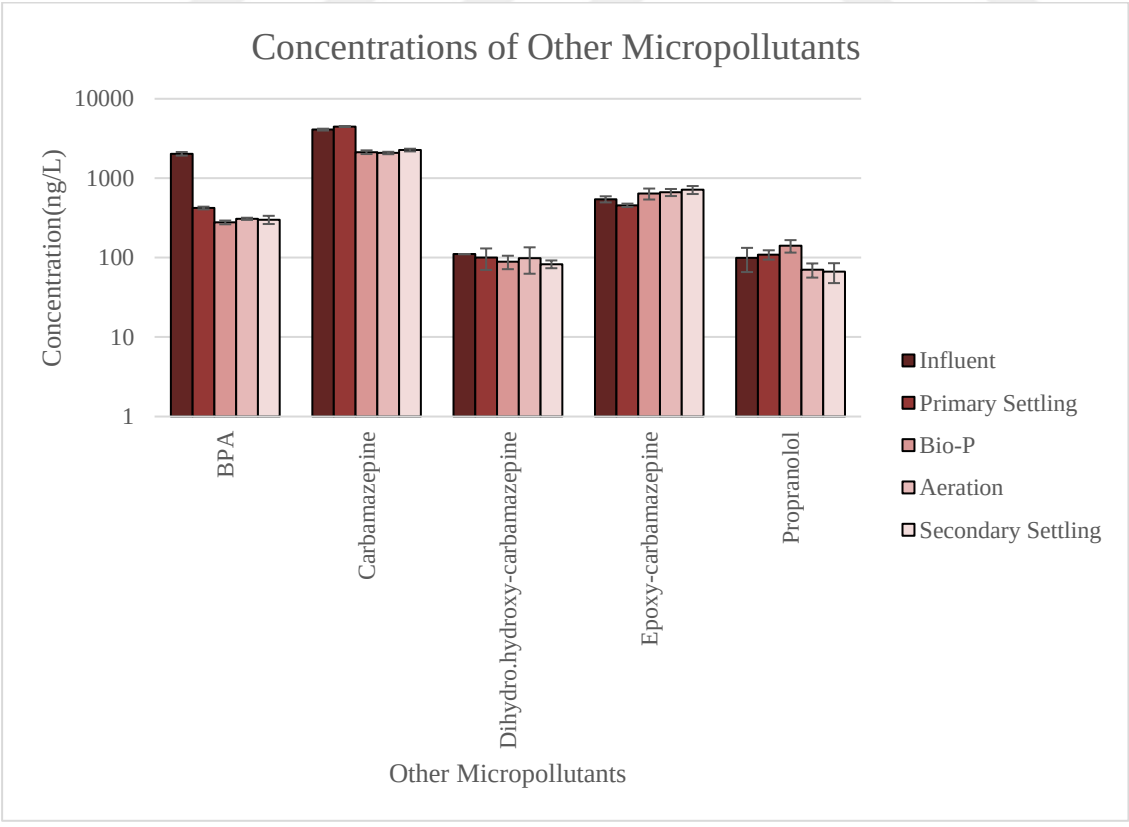


Figure 4.75 : The concentrations of other micropollutants for spring.

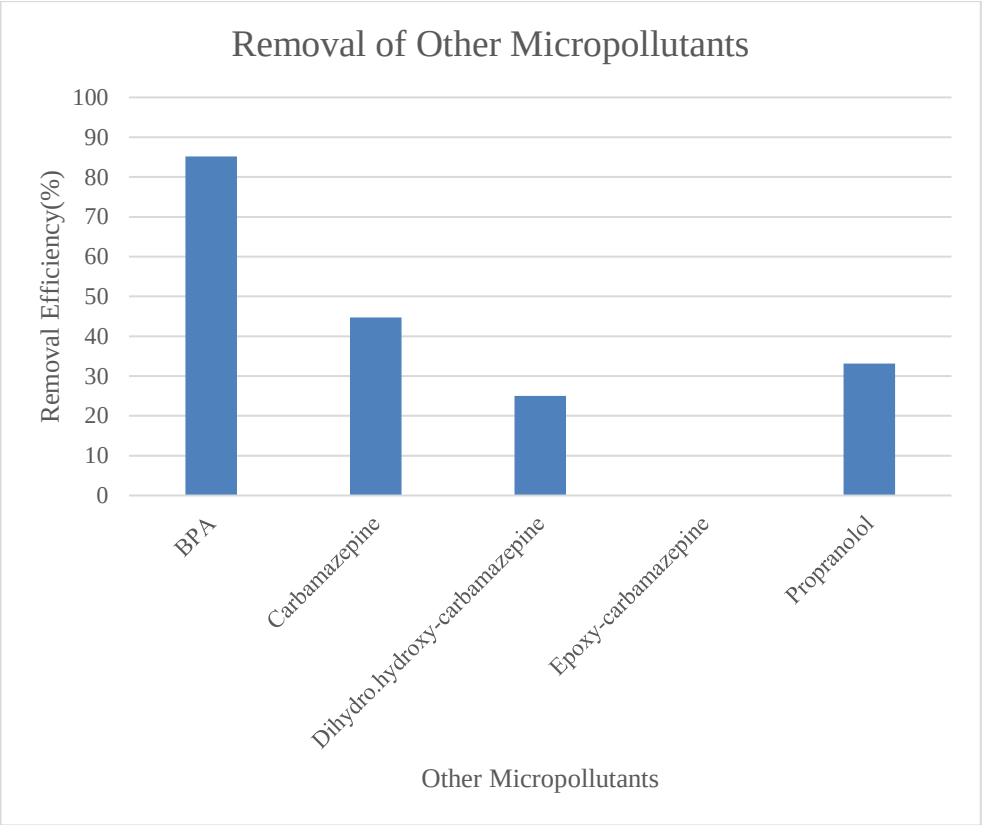


Figure 4.76 : The removal efficiencies of other micropollutants for spring.

BPA was found as 2,032 ng/L in the influent, and in the effluent concentration of BPA was decreased to 301 ng/L. Carbamazepine was measured as 4,093 ng/L in the influent, and in the effluent it was found as 2,263 ng/L. Dihydro.hydroxy-carbamazepine and epoxy-carbamazepine were observed in the influent as 111 ng/L and 544 ng/L, respectively while in the effluent they were found as 83 ng/L and 715 ng/L, respectively indicating higher concentration of epoxy-carbamazepine in the effluent. On the other hand, propranolol was found in the influent and effluent as 99 ng/L and 66 ng/L,respectively. Caffeine and paraxanthine cannot be measured due to internal standard's inability to ionize.

Lastly, 4-hydroxy-propranolol, and atenolol were below detection limit which is 4 ng/L as shown in Table 4.4.

The high removal efficiency were presented by BPA (85%). Low removal efficiency was found for dihydro.hydroxy-carbamazepine (%25) while medium removal efficiencies were found for carbamazepine (%45), and propranolol (%33). Negative removal efficiency was presented by epoxy-carbamazepine due to higher concentrations in the effluent. The heatmap of spring results are shown in Figure 4.77.

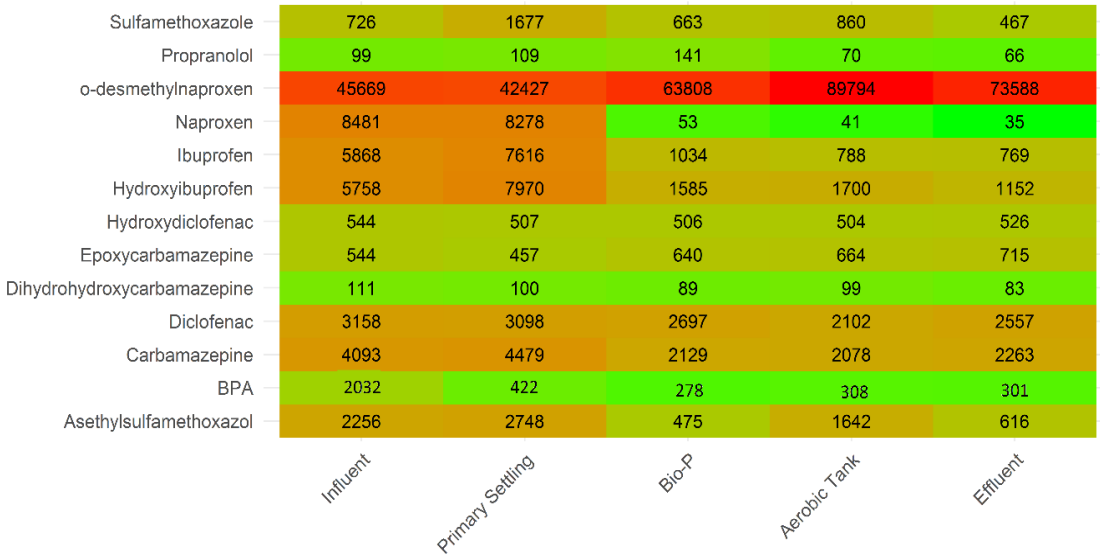


Figure 4.77 : Heatmap of wwtp b for spring.

Naproxen, ibuprofen, and diclofenac which are NSAIDs have high influent concentration due to extensive consumption in spring. Negative removals are observed for epoxy-carbamazepine. Removal efficiency of BPA is high (85%). Diclofenac can be removed with low removal efficiency (19%). On the other hand, hydroxy-diclofenac can be removed with very low removal efficiency (3%). How removal efficiency changes according to seasons is shown in Figure 4.78.

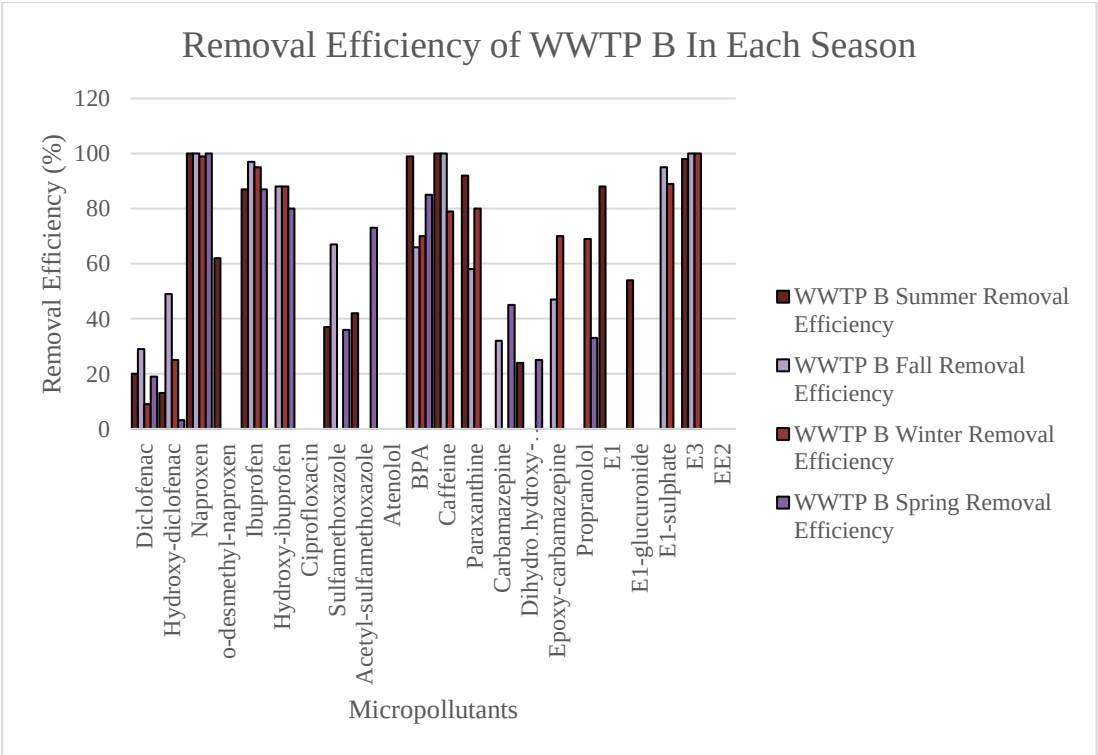


Figure 4.78 : The removal efficiency in each season.

Some of the micropollutants were not detected in each season which are hydroxy-ibuprofen, atenolol, ciprofloxacin, caffeine, paraxanthine, E1, E1-sulphate, E1-glucuronide, and EE2. Therefore, comparison of these micropollutants cannot be done due to missing data. However, o-desmethyl-naproxen showed decline in efficiency in fall after summer and negative removals are observed afterwards. Similarly, dihydro.hydroxy-carbamazepine, BPA, acetyl-sulfamethoxazole, and had similar trends, yet, in spring their removal efficiencies are increased significantly except dihydro.hydroxy-carbamazepine. On the other hand, some of the micropollutants had higher removal efficiencies in fall than summer such as diclofenac, hydroxy-diclofenac, ibuprofen, sulfamethoxazole, carbamazepine, and epoxy-carbamazepine. Even though they had higher efficiencies in fall, the efficiencies in winter still dropped significantly except for epoxy-carbamazepine, it gradually increased in each season while in spring it decreased to 0%. There were micropollutants that cannot be put in groups mentioned above such as naproxen, ciprofloxacin, caffeine, paraxanthine, propranolol, and E3. Naproxen, caffeine, and E3 were not affected by season change. Therefore, their removal efficiencies remained high in each season even though removal efficiency of caffeine declined in winter, yet, it still stayed as high as 79%. On the opposite, removal efficiency of ciprofloxacin remained as 0% in each season. On the other hand, removal efficiency of paraxanthine was high (92%) in summer, decreased to 58% in fall, increased in winter (80%). Removal efficiencies of literature review and WWTP B are compared in Figure 4.79. Horizontal line in boxes defines median values of literature.

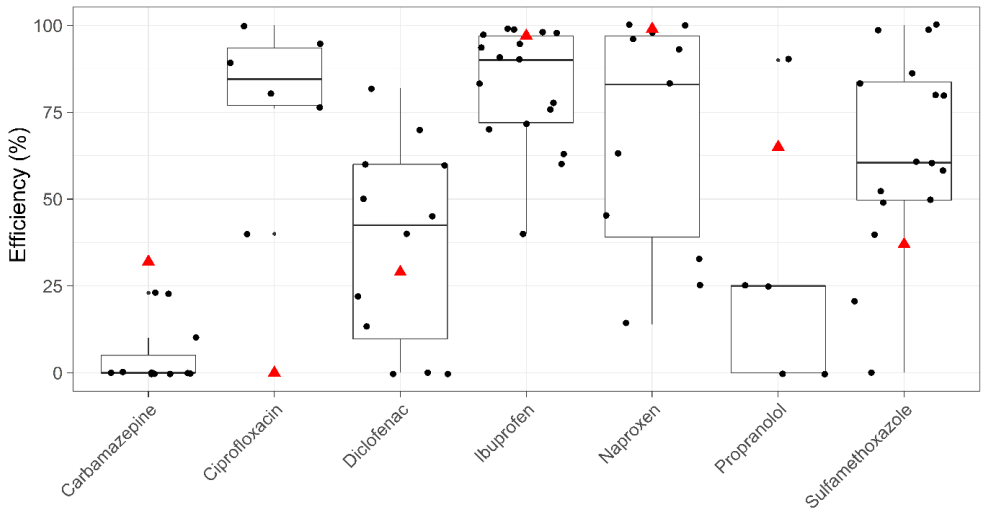


Figure 4.79 : The comparison between wwtp b and literature values.

Removal efficiencies of carbamazepine, ibuprofen, naproxen, and propranolol in WWTP B can be seen as higher than the median values. On the other hand, removal efficiencies of ciprofloxacin, diclofenac, and sulfamethoxazole in WWTP B are lower than the median values. Even though they are below median values, there are still similar values in the literature except of ciprofloxacin. Moreover, operational conditions and configurations of WWTPs in the literature review vary greatly. Therefore, removal efficiencies for each micropollutants are in a wide range as it can be seen for diclofenac, naproxen, and sulfamethoxazole. The highest removal efficiency achieved in units of WWTP B for micropollutants is shown in Table 4.15. Some calculations are done in order to evaluate which micropollutants have the highest removal efficiency in which unit of WWTP. From Table 4.15, it can be seen that there are some evaluations to make about some of the micropollutants. Naproxen, and ibuprofen can be removed with highest removal efficiencies in Bio-P Tank in each season. For naproxen and ibuprofen, the same results are obtained in WWTP A. It can be said that naproxen and ibuprofen prefer Bio-P Tank for highest removal in each season. On the other hand, the highest removal efficiency changes for diclofenac which are Anoxic+Aeration Tank in summer and spring, Primary Settling in fall, and Bio-P Tank in winter. Acetylsulfamethoxazole can be removed with the highest removal efficiency in Bio-P Tank for summer, in Primary Settling for fall, and Bio-P Tank for winter and spring again whereas epoxy-carbamazepine and sulfamethoxazol are removed with the highest removal efficiencies in Bio-P and Anoxic+Aeration Tanks for summer, in Anoxic+Aeration and Secondary Tanks for fall, in Primary Settling and Bio-P Tanks for winter, and in Primary Settling and Bio-P Tank for spring, respectively. Dihydro.hydroxy-carbamazepine can be removed with the highest removal efficiency in Anoxic+Aeration Tanks for summer, in Bio-P tank for both fall,winter, and spring. These results may imply that micropollutants such as naproxen, and ibuprofen do not prefer units other than Bio-P Tank based on seasonal change. This may be due to their unchanging behaviours. On the other hand, there are some micropollutants such as diclofenac, sulfamethoxazole, acetylsulfamethoxazole, dihydro.hydroxy-carbamazepine, and epoxy-carbamazepine. These micropollutants may change their behavior in wastewater treatment plants according to seasons and require different conditions provided by each units under different temperature conditions.

Table 4.15 : Highest removal efficiency achieved for micropollutants in wwtp b.

Micropollutants	Highest Removal Efficiency Achieved for Micropollutants			
	Summer	Fall	Winter	Spring
Diclofenac	Anoxic+Aeration Tank	Primary Settling	Biological P	Anoxic+Aeration Tank
Hydroxy-diclofenac	Anoxic+Aeration Tank	Biological P	Primary Settling	Primary Settling
Naproxen	Biological P	Biological P	Biological P	Biological P
o-desmethyl-naproxen	Biological P	Secondary Settling	Secondary Settling	Secondary Settling
Ibuprofen	Biological P	Biological P	Biological P	Biological P
Hydroxy-ibuprofen		Biological P	Biological P	Biological P
Ciprofloxacin	Grit Chamber	Primary Settling	Secondary Settling	
Sulfamethoxazole	Anoxic+Aeration Tank	Secondary Settling	Biological P	Biological P
Acetyl-sulfamethoxazole	Biological P	Primary Settling	Biological P	Biological P
Atenolol				
BPA	Grit Chamber	Primary Settling	Anoxic+Aeration Tank	Primary Settling
Caffeine	Biological P	Biological P	Biological P	
Paraxanthine	Anoxic+Aeration Tank	Biological P	Biological P	
Carbamazepine	Grit Chamber	Biological P	Primary Settling	Biological P
Dihydro.hydroxy-carbamazepine	Anoxic+Aeration Tank	Biological P	Biological P	Secondary Settling
Epoxy-carbamazepine	Biological P	Anoxic+Aeration Tank	Primary Settling	Primary Settling
Propranolol	Anoxic+Aeration Tank		Biological P	Anoxic+Aeration Tank
E1	Grit Chamber			
E1-glucuronide	Primary Settling			
E1-sulphate		Biological P	Biological P	
E3		Biological P	Primary Settling	
EE2	Grit Chamber			

5. CONCLUSIONS AND RECOMMENDATIONS

Currently in Turkey, wastewater effluents can only be intentionally reused in irrigation, although unintentional reuse happens through discharge of effluents into receiving waters. Water scarcity all over the world will require a higher intentional reuse of wastewater, thus the presence of micropollutants in wastewater effluents will be even more important when public and environmental health is considered.

In this study, 23 micropollutants were classified into 4 groups as NSAID, antibiotics, steroid hormones and other micropollutants. The concentrations and fate of these micropollutants in WWTPs depend on several factors such as the general wastewater characteristics, WWTP treatment scheme, operational parameters in the WWTP, temperature and other seasonal changes.

Some of the micropollutants such as NSAID and antibiotics are expected to have higher concentrations in winter since a higher usage of these pharmaceuticals is expected in winter. On the other hand, the total wastewater flowrate also increases due to rainfall and hence presents a possibility of dilution of micropollutant concentrations in winter. For the general evaluation of micropollutant concentrations in different seasons and the effect of dilution due to rainfall, Carbamazepine which is one of the micropollutants studied, is used. Since Carbamazepine is an anti-epileptic drug which has to be taken at the same dose throughout the year, any decrease in its concentration can be taken as an indication of heavy rainfall and dilution.

Among the NSAIDs, highest and lowest influent concentrations (23,000 ng/L and 770 ng/L) were determined for Naproxen and Diclofenac, respectively. The removal percentage for Naproxen was close to 99% in all cases, whereas for Diclofenac, the removal percentage ranged between 9 and 34%. It is also important to note that the transformation products of NSAIDs have very high (e.g., 46,000 ng/L for o-desmethylnaproxen) concentration and very low removal efficiencies.

In the antibiotics group, the ranges for Ciprofloxacin concentration (approximately 170-13,670 ng/L) and removal percentage (0-94%) were rather wide. Sulfamethoxazole had a smaller range for influent concentration (210-726 ng/L) but its removal percentages ranged from negative

(suggesting an increase in the concentration) to 67%. The fate of Sulfamethoxazole should be evaluated together with the fate of its transformation byproduct, acetyl-sulfamethoxazole since previous research suggest a transformation of acetyl-sulfamethoxazole to Sulfamethoxazole (Göbel et al., 2005). In some influent samples, the concentration of Acetyl-sulfamethoxazole was higher than Sulfamethoxazole and it had a higher removal percentage than Sulfamethoxazole as well. Considering the possible transformation, it is not possible to distinguish between the "original Sulfamethoxazole in the influent" and Sulfamethoxazole that has been transformed from the "original Acetyl-sulfamethoxazole in the influent" when calculating the removal percentages by measuring Sulfamethoxazole in the effluent. Although measuring Sulfamethoxazole alone suggest a small or even no biodegradation, it is possible that it is being removed but the removal is not observed due to the formation of "new" Sulfamethoxazole.

E3 had the highest concentration among the steroid hormones (800 ng/L) with the other hormones (E1 and EE2) were detected only in some samples at concentrations below 100 ng/L. The removal efficiencies for all E3, E1 and it metabolites were >99% with concentrations below detection limits in the effluents. EE2 had a lower removal percentage (42%) which could be due to its xenobiotic structure since it is the only synthetic hormone studied. One of the important findings of this study is that the measurement of metabolites of the micropollutants may add valuable knowledge to the understanding of removal mechanisms and fate of micropollutants. For example, the decrease in E1 concentrations in primary settling tank followed by an increase in the aerobic tank makes sense when we know that E1-sulfate and E1-glucuronide are removed from the system starting from Bio-P tank. Then, the increase in the E1 concentration can be explained by the deconjugation of E1-sulfate and E1-glucuronide to E1 as also stated in the literature (Servos et al., 2005).

The last group of micropollutants were classified as the other micropollutants and included Caffeine, BPA, Carbamezapine etc. Caffeine's concentration was always at least one order of magnitude higher than the other micropollutants ranging between approximately 10,400 to 134,400 ng/L. Caffeine is one of the other micropollutants with removal percentages almost always >80% in all samples, which can be explained by it being a natural compound and not a man-made chemical. On the other hand, Carbamezapine's concentration did not change significantly in the treatment plant with removal percentages always <30% and mostly <10%. The low removal efficiency (0-50%) for carbamezapine has also been observed by other researchers (Lajeunesse et al., 2012; Behera et al., 2011; Choi et al., 2008). The concentration

of Carbamezapine for both WWTP in Summer and Fall samples were around 1000 ng/L (750-793 in WWTP A and 1200-1600 ng/L in WWTP) but decreased almost 50% in Winter influent samples, indicating the effect of rainfall on the dilution of wastewater. Based on this information and the relatively high influent concentration of naproxen, ibuprofen and, diclofenac in Winter samples, it can be stated that the consumption of these pharmaceuticals are high enough to overcome the dilution. For carbamezapine, two different transformation byproducts were measured. Similar to Sulfamethaxazole, the negative removal for carbamezapine can be explained by the transformation of these products (epoxy-carbamazepine and dihydro.hydroxy-carbamazepine) to carbamazepine since their concentrations decrease slightly in the treatment plant.

For all the compounds studied, the removal in the WWTPs equals to biodegradation since these micropollutants were not detected in the sludge samples taken from the WWTPs. For the compounds that have been subjected to biodegradation, the highest removals were achived mostly in the Bio-P or anoxic tanks. Also, our results indicate the importance of measuring the transformation products in addition to the compounds themselves for a better understanding of the pathways of biodegradation and transformation in WWTPs.

Since the wastewater treatment plants are not specifically designed to remove micropollutants most of which are xenobiotics and are present at low concentrations, further studies on removal of micropollutants in the wastewater effluents are needed to decrease the concentrations of micropollutants further. These studies can focus on the existing wastewater treatment systems and study the effect of SRT on the removal since higher removal efficiencies are found at higher (e.g., >30 days) SRT values. However, changing the existing WWTPs to operate at such long SRT may not be easy or feasible since it will affect the sludge characteristics. It is also possible to study the microbial population directly to find a group of microorganisms that are particularly good for micropollutants removal. Also, advanced chemical or physico-chemical processes such as chlorination, ozonation, oxidation with nanoparticles and adsorption can also be applied for the removal of micropollutants. Especially the processes which can also be used for disinfection can provide additional benefits before the reuse of wastewater.

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