REMOVAL AND DISCHARGE OF MICROPLASTICS FROM THE WASTEWATER TREATMENT PLANTS IN ISTANBUL

by

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ABSTRACT

REMOVAL AND DISCHARGE OF MICROPLASTICS FROM THE WASTEWATER TREATMENT PLANTS IN ISTANBUL

Wastewater treatment plants (WWTPs) act as both receivers and sources of microplastics as they both receive microplastics from household and commercial activities and release the particles that could not be retained. This study investigated several units of a tertiary WWTP with a nutrient removal unit. Samples after physical treatment and from the effluent were also taken as 3h composite samples in order to observe fluctuation effect of influent wastewaters. Dried sludge from the WWTP was also investigated for potential hazards with application of the sludge to the soils. Particles isolated from samples were divided into fibers, fragments, nylons, pellets and glitters based on their morphological characters. Fibers were the most dominant shape, followed by fragments. 500-1000 μm and 1000-2000 μm was the most common size ranges for wastewater samples and sludge, respectively. Size distributions were different for shape classes. Particles in different size ranges demonstrated distinct behaviors for some size classes in various units of the WWTP. Fragments <500 μm were removed more efficiently where fibers with sizes 250-500 μm and 1000-2000 μm were removed better within the WWTP. 84.6 and 93.0% removal were observed with grab and composite samples. Despite the high removal rates $5{,}151 \times 10^6$ particles/d were released to Marmara Sea where, 36 days of discharge was equal to abundance value reported for Marmara Sea and $5{,}069 \times 10^6$ particles/d were present in the dried sludge. Polymer structures were not confirmed therefore, the rates calculated represent the microlitter release and microplastic release rate should be lower with a characterization step utilized.

ÖZET

İSTANBUL'DA BULUNAN ATIKSU ARITMA TESİSLERİNDE MİKROPLASTİK GİDERİMİ VE TESİSLERİN MİKROPLASTİK DEŞARJLARI

Atıksu arıtma tesisleri (AAT) evsel ve endüstriyel kaynaklardan mikroplastiklerin tesislere ulaşması ve arıtma aşamalarında tutulamayan mikroplastiklerin tesislerden deşarj edilmesi sebebiyle mikroplastik alıcıları ve aynı zamanda kaynaklarıdır. Bu çalışmada ileri nutrient giderimine sahip bir AAT'nin giriş suyu, fiziksel işlemler sonrasındaki atıksu, biyolojik işlemler sonrasındaki atıksu ve deşarj edilen su numuneleri incelenmiştir. Fiziksel işlemlerden sonraki su ve çıkış suyunun ayrıca 3 saatlik kompozit numunesi de, giriş suyundaki anlık değişimin etkilerinin anlaşılması amacıyla, alınmıştır. Toprağa uygulanması halinde oluşabilecek zararların tespitini incelemek üzere AAT'den kaynaklanan kurutulmuş çamur da ayrıca incelenmiştir. Örneklerden ayrılan partiküller fiber, parçacık, naylon, pellet ve sim olacak şekilde morfololojik karakterlerine göre ayrılmıştır. Fiberler en yaygın partikül şekli olup, parçacık biçimli partiküller fiberleri takip etmektedir. 500-1000 µm ve 1000-2000 µm boyut sınıfları atıksu ve çamur numunelerinde en sık görülen boyut sınıfları olmuştur. Farklı şekiller için boyut dağılımlarının değiştiği gözlemlenmiştir. Farklı boyut sınıflarında bulunan partiküller, AAT'nin çeşitli aşamalarında değişik biçimde davranmışlardır. Parçacık şekilli <500 µm partiküller AAT içinde daha efektif bir şekilde giderilmiş olup, fiberler ise 250-500 µm ve 1000-2000 µm boyutlarında iken daha efektif bir şekilde giderilmiştir. Partikül giderimi anlık ve kompozit numuneler için %84.6 ve %93.0 oranında gerçekleşmiştir. Yüksek giderim verimlerine rağmen, Marmara Denizi'ne günde $5{,}151 \times 10^6$ partikül deşarj edilmekte olup bu değer 36 günlük deşarj ile Marmara Denizi için literatürde verilen mikroplastik konsantrasyonuna ulaşılması demektir. Günlük olarak kuru çamurda $5{,}069 \times 10^6$ partikül bulunmaktadır. Polimer yapıları teyit edilmemiş olup, sunulan değerler tesislerden yapılan mikro-süprüntü (microlitter) salınımını yansıtmaktadır. Mikroplastiklerin deşarj değerleri polimer karakterizasyonu yapıldığı takdirde daha düşük olacaktır.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
Fe	Iron	
H_2O_2	Hydrogen Peroxide	
NaCl	Sodium Chloride	
ZnCl2	Zinc Chloride	

Abbreviation	Explanation
ABS	Acrylonitrile Butadiene Styrene
ACR	Acrylate
ACR	Acrylate
ATR	Attenuated Total Reflectance
A2O	Anaerobic-Anoxic-Oxic
BAF	Biological Active Filter
BPL	Biopolymer
DAF	Dissolved Air Floatation
DDT	Dichlorodiphenyltrichloroethane
DEHP	Di(2-ethylhexyl) Phthalate
DI	De-ionized
EPA	Environmental Protection Agency
EPR	Ethylene Propylene Rubber
EPS	Expanded Polystyrene
EU	European Union
EVA	Ethylene Vinyl Acetate
FTIR	Fourier Transform Infrared
FPA	Focal Plane Array
HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
MBR	Membrane Bioreactor
MCR	Methacrylate
MMF	Melamine
MP	Microplastic
MPF	Microplastic Fiber

MPP	Microplastic Particle
NYL	Nylon
PA	Polyamides
PAX	Polyaluminum Chloride
PAH	Polycyclic Aromatic Hydrocarbon
PC	Polycarbonate
PCB	Polychlorinated Biphenyl
PE	Polyethylene
PEP	Polyethylene Propylene
PES	Polyester
PET	Polyethylene Terephthalate
Phe	Phenanthrene
PIB	Polyisobutylene
PLA	Polylactide
PMMA	Polymethyl Methacrylate
PO	Polyolefin
POP	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PSU	Polyaryl Sulfone
PTFE	Teflon
PUR	Polyurethane
PVAL	Polyvinyl Alcohol
PVI	Polyvinyl
PVC	Polyvinyl-Chloride
RBB	Rubber
RO	Reverse Osmosis
RSF	Rapid Sand Filter
SBR	Sequencing Batch Reactor
SDS	Sodium Dodecyl Sulfate
SAN	Styrene Acrylonitrile
UV	Ultraviolet
WPO	Wet Peroxide Oxidation
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

359 million tons of plastics are produced every year (Plastics Europe, 2019). Since most of the products contain plastics and not all plastic material is readily recovered, plastics are a major component of waste stream. They were detected in rivers, lakes and oceans (Cole et al., 2011) as well as soil and air (Dris et al., 2016; Steinmetz et al., 2016; Horton et al., 2017a). Being waterproof, durable and non-biodegradable materials, plastics are used in many consumer products. However, these properties make plastics more persistent in the environment (Imhof et al, 2012; Shah et al., 2008; Barnes et al., 2009). Plastic particles that were not visible to the eye were detected in 2000's (Thompson et al., 2005). The broad classification of the plastics offered was; mega-debris (>100 mm), macro-debris (>20 mm), meso-debris (20–5 mm) and micro-debris (<5 mm) by Barnes et al. (2009). Particles <5 mm was considered as microplastics and this classification is commonly used afterwards however, no lower limit was defined (Duis and Coors, 2016). Different opinions for lowest size exist (Koelmans et al., 2015; GESAMP, 2015; Eriksen et al., 2014) in the literature however, the term is generally used for particles smaller than 5 mm and larger than 1 μm (Da Costa et al., 2016). Microplastics can further be categorized as primary and secondary microplastics. Where, plastics produced in micro-sizes on purpose are classified as primary microplastics (Fendall and Sewell, 2009; Patel et al., 2009; Sundt et al., 2014; Browne et al., 2011) and microplastics that were fragmented from larger pieces are categorized as secondary microplastics (Barnes et al., 2009; Ter Halle et al., 2016).

Primary microplastics mostly originate from personal care products, drugs and pellets used for production of plastic consumer products (Fendall and Sewell, 2009; Patel et al., 2009; Sundt et al., 2014; Browne, 2015; Napper et al., 2015). The microplastics present in personal care products entering the aquatic environments through effluent discharges of wastewater treatment plants (Browne et al., 2011; Duis and Coors, 2016; Anderson et al., 2017) and run-off or mismanagement of industries producing or storing the pellets used in various products including the personal care products (Sundt et al., 2014). Fibers are the most occurring form of secondary microplastics and they are most likely to be generated during washing activities of synthetic clothing (Browne et al., 2011; Napper and Thompson, 2016). Fibers enter the aquatic environment mostly with discharges from wastewater treatment plants. Sludge of the wastewater treatment plants also contain microplastics that were removed during the purification of the wastewater (Talvitie et al., 2017a; Li et al., 2018). Sludge generated from wastewater treatment plants, under certain conditions, can end up in soil for

agricultural and landscaping purposes as well as landfills (Zubris and Richards et al., 2015; Ng et al., 2018; Corradini et al., 2019).

Microplastics that are discharged from wastewater treatment plants can be transported to many compartments of the environment. Through rivers and water channels, they can be transported to the seas and oceans (Jambeck et al., 2015) or sedimented (Nel et al., 2018; Besseling et al., 2017). Through application on land, they can be transported to groundwaters, with rainwater they can be transported to rivers and seas and with wind they can be transported on land and in the air (Zylstra, 2013; Dris et al., 2016; Horton et al., 2017a). Microplastics can cause harm to many organisms in different levels in many different ways. Through feeding they can cause many digestive system problems, false satiation and many other symptoms in the organisms that can result even in death (Eerkes-Medrano et al., 2015). They can disturb the organisms on cellular level causing inflammation (Von Moos et al., 2012). They can accumulate persistent, toxic, bio-accumulating chemicals due to their hydrophobic properties (Eerkes-Medrano et al., 2015; Akdoğan and Güven, 2019). Apart from sorption and adsorption, they can also leach the hazardous chemicals contained in their structures (Yogui and Sericano, 2009).

Although the removal rates of microplastics are generally high in wastewater treatment plants, they still discharge considerable amount of microplastics with their effluent streams with high flow rates (Michielssen et al., 2016; Murphy et al.,2016; Edo et al., 2020). They also contain high rates of microplastics in their sludge (Leslie et al., 2017; Edo et al., 2020; Liu et al., 2019). In the literature, there was no study investigating the wastewater treatment plants in Istanbul, Turkey and there were only two studies investigating the wastewater treatment plants in Turkey, overall. Therefore, the aim of this study was to investigate wastewater treatment plants in Istanbul and specifically evaluate various units of the treatment system in order to better understand removal dynamics in different steps rather than only looking at only influent and effluent samples. Composite sampling was performed in only two sampling locations of the wastewater treatment plant. Three-hour composite samples of physical treatment effluent and final effluent were collected in order to have an idea on the effect of fluctuating streams arriving in the plant.

2. BACKGROUND AND LITERATURE REVIEW

In this chapter, microplastics and their properties are first reviewed. Subsequently, sources and fate of microplastics in the environment are given with a special emphasis on their direct and indirect health effects and potential impacts. Wastewater treatment plants as receivers of primary and secondary microplastics and removal of microplastics in these wastewater treatment plants, which are the main focus of this study, are presented along with various methods used for sampling, extraction and characterization of microplastics.

2.1. Plastic Pollution in the Environment

World plastic production figures are constantly increasing and have reached to 359 million tons in 2018 despite the production of plastics in Europe decreased, compared to 2017 (Plastics Europe, 2019). Rochman et al. (2013a) stated that if the same consumption trend continues from the year 2013 by the year 2050, the world would have contained 33 billion tons of plastics which would coincide to 891 million tons produced annually.

Prior to method of their production, plastics can be divided into two types; thermoplastics and thermoset plastics. Thermoplastics which are main types of consumer plastics are produced by melting the raw plastic material and can be re-melted and recovered (Moore, 2008). Polyethylene (PE), Polypropylene (PP), Polyethylene Teraphthalate (PET), Polystyrene (PS), Expanded Polystyrene (EPS), Polyvinyl-chloride (PVC), Polyamides (PA), Acrylonitrile butadiene styrene (ABS), Styrene Acrylonitrile (SAN), Polycarbonate (PC), Poly methyl methacrylate (PMMA), Thermoplastics Elastomers (TPE), Polyarylsulfone (PSU), Fluoropolymers are most known species of thermoplastics (Plastics Europe, 2019). Thermoset plastics, however, are set by catalysts or additives and they do scorch rather than melt when exposed to heat. Both types of plastics are present in the environment but thermoset plastics are present less in amount as they are produced less. Also, they can be recovered much less than thermoplastics due to the aforementioned factors (Moore, 2008). Polyurethane (PUR), Unsaturated PE, Epoxy resins, Melamine resins, Vinyl esters, Silicone, Phenolic and Acrylic resins are the most known species of thermoset plastics.

Due to their large area of application, plastics contain specific substances in order to improve their characteristics suitable for the industry that they will be used in. These substances include colorants, plasticizers, fillers, stabilizers for heat and UV resistance, modifiers for impact, lubricants

and flame retardants. Plastics also contain catalytic residues and monomers that are not polymerized (Enfrin et al., 2019) and there are 30,000 different polymers registered for use in the European Union (EU) and 84% of these polymers are thermoplastics (Postle et al., 2012; Horton et al., 2017a). Plastic is a good material to be used in consumer products as it is waterproof, durable and resistant to biodegradation. High molecular weight, hydrophobicity and cross-linked chemical structure are the key common characteristics of polymers (Shah et al., 2008; Gautam et al., 2007). However, this causes plastic materials to be extremely resistant to biodegradation and therefore, persistent in the environment (Imhof et al., 2012; Barnes et al., 2009).

In Europe; PP and PE are the most demanded types of plastics as they are generally used in packaging materials and they are also used by many different industries with demand rates of 19.3% and 29.7% respectively. PVC, PUR and PET and PS-EPS are also utilized with demand rates of 10%, 7.9%, 7.7% and 6.4% where other types of plastics are demanded by 19%. Most of the plastics need occur from packaging industry by 39.9% followed by construction industry (19.8%), automotive (9.9%), electrical and electronical equipment industry (6.2%), agriculture (3.4%) and household, leisure and sports industries (4.1%) where the rest constitute of only 16.7% of the plastics demand in Europe. Although the plastic consumer and packaging waste are collected across Europe, the average recycling rate of packaging waste is 42% for EU 28+2 countries and not all consumer plastics that are produced can be collected and recovered. According to Geyer et al. (2017), in 2015, 6300 million tons of plastic waste had generated since 1950's and only 9% of all of this waste was recycled, 12% was incinerated and 79% was landfilled or littered; which implies that there are still plastics in the environment which can constitute a waste problem in many different aspects along with plastics that are still being produced.

Since most of the products contain plastics and not all plastic material is readily recovered, plastics are a major component of waste stream and since oceans are downhill and downstream from anywhere where humans reside; they are most likely to be present in the rivers, lakes and oceans (Cole et al., 2011) as well as other media such as soil and air due to littering, mismanagement, losses in production, materials used in agriculture, application of fertilizers and sludge to soil and atmospheric transport processes (Dris et al., 2016; Steinmetz et al., 2016; Horton et al., 2017a; Mintenig et al., 2017).

2.2. Microplastics

Plastic litter in the oceans started to be reported in 1970's and in 1990's and many studies were conducted on their hazardous effects on living organisms (Andrady, 2011). However, in 2000's, microplastics had found to occur and defined as ''a form of man-made litter that have been accumulating in the oceans for at least over the last four decades'' (Thompson et al., 2005) and these plastics were not visible to the naked eye. Plastic debris was then classified based on their size in order to include a term for non-visible microplastics. Microplastics were classified as barely visible particles that pass through a 500 μm sieve but retained by 67 μm sieve (0.06-5 mm in diameter) by Gregory and Al (2003) and Thompson et al. (2004) referred to microscopic plastics as micro-plastics. Numerous size ranges were attributed to the term 'microplastics' such as; <10 mm (Graham and Thompson, 2009), <5 mm (Barnes et al., 2009), <2 mm (Ryan et al., 2009) and <1 mm (Browne et al., 2010; Claessens et al., 2011). The broad classification of the plastics offered was; mega-debris (>100 mm), macro-debris (>20 mm), meso-debris (20–5 mm) and micro-debris (<5 mm) by Barnes et al. (2009). Particles <5 mm was considered as microplastics and this classification is commonly used afterwards however, no lower limit was defined (Duis and Coors, 2016). The discovery of smaller particles added the term 'nanoplastics' into the literature which again, modified the definition of microplastics (Koelmans et al., 2015). Lower size limit for microplastics was suggested to be 1 μm (Da Costa et al., 2016), 20 μm and even 1 nm (GESAMP, 2015). The European Commission defines nanomaterial sizes as 1-100 nm which is also used to define nanoparticles as particles below 100 nm (Koelmans et al., 2015). Eriksen et al. (2014) defined microplastics as small MPs and large MPs if they are below or above 1 mm, respectively. To summarize, description 'microplastics' is broadly used for particles smaller than 5 mm and larger than 1 μm.

Microplastics can further be categorized as primary and secondary microplastics. Primary microplastics are microbeads manufactured for several purposes and they are used in cosmetics and other personal care products (Fendall and Sewell, 2009; Darling et al., 2015), in air-blasting media (Gregory, 1996; Sundt et al., 2014), in medicine as vector for drugs (Patel et al., 2009) or as raw materials (pellets) for producing goods (Browne et al., 2011; Wagner et al., 2014). Secondary microplastics are the fragments from breakdown of larger plastics at sea or at land (Cole et al. 2011) due to physical, chemical and biological processes or exposure to UV radiation (Browne et al., 2007; Moore, 2008; Barnes et al., 2009; Ter Halle et al., 2016).

2.3. Microplastics in the Environment

Microplastics are found in many compartments in the environment throughout the oceans, land and air. Their abundance and distribution, fate and transport and -as a material that is widely used throughout the world- their health impacts and hazards were investigated in many studies. Figure 2.1 summarize sources, fate and transport of microplastics throughout the environment, which will be explained in detail within this chapter along with their uptake and hazards.

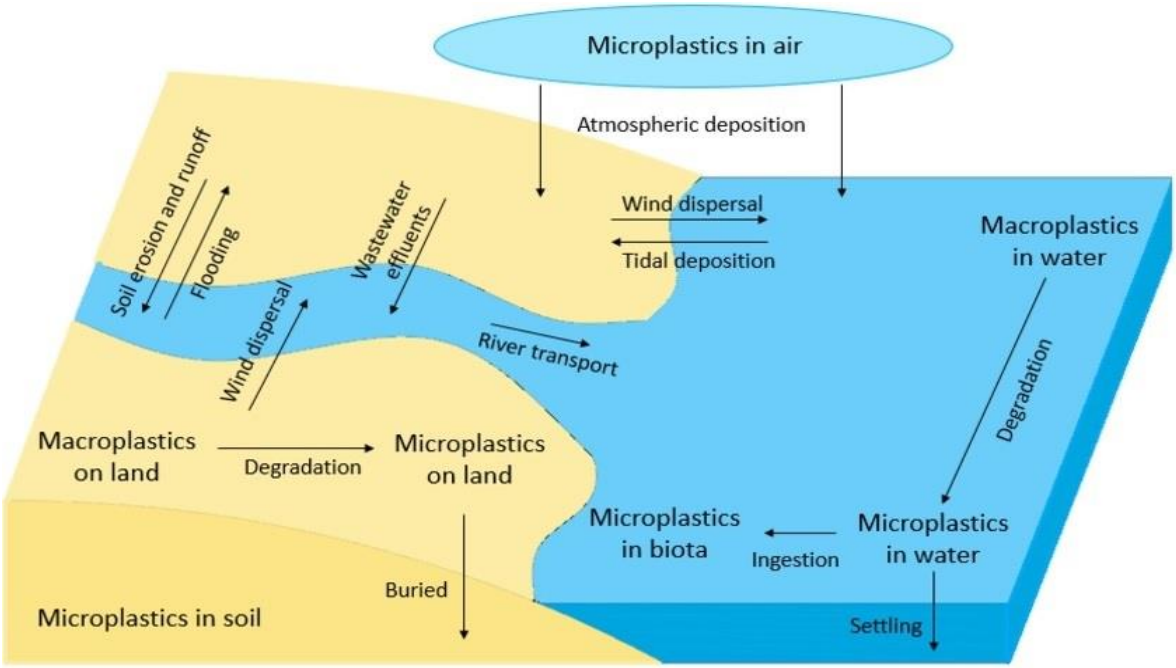

Figure 2.1. Graphical summary of sources, fate and transport of microplastics (Courtesy of Akdoğan and Güven, 2019).

2.3.1. Sources

Microplastics are quite diverse in their properties and source originations they are present in many products and have diverse application fields. Therefore, as one might expect, microplastics have various sources entering to the environment.

Primary microplastics include particles used in many domestic consumer products such as facial cleansers, tooth paste, shower gels, baby products, cosmetics such as eye shadows, scrubs, peelings, sunscreens, insect repellants and also in drugs as vectors (Fendall and Sewell, 2009; Patel et al., 2009; Sundt et al., 2014; Browne, 2015; Napper et al., 2015; Duis and Coors, 2016; Auta et al., 2017). The microplastic beads present in personal care products, cosmetics and drugs are most likely entering marine and other aquatic environments through effluent discharges of wastewater treatment plants

(Fendall and Sewell, 2009; Browne et al., 2011; Duis and Coors, 2016; Anderson et al., 2017). Primary microplastics are also found in air-blasting media, cleaning products, abrasives and drilling fluids (Sundt et al., 2014; Browne, 2015). Virgin pellets with typical sizes of 2-5 mm in diameter are also considered as primary microplastics (Andrady, 2011; Wagner et al., 2014). Microplastics present in industrial products and virgin pellets can enter the aquatic environments via industrial wastewater streams, from lack of operation in a closed system, a run-off and improper storage or disposal (Sundt et al., 2014; Duis and Coors, 2016, Horton et al., 2017a).

Fibers are the most common forms of microplastics present in the environment (Browne et al., 2011). Washing causes continual abrasion of clothes made from synthetic fabric and washing machine effluents contain considerable amount of fibers as synthetic fabrics can shed up to 1900 fibers per garment during washing (Browne et al., 2011; Napper and Thompson, 2016). Because of their fragmentation from a larger structure, fibers can be classified as secondary microplastics however, wastewater treatment plant effluents are thought to be a major and a common source for fibers in freshwaters and seas as well as primary microplastics and they result in the environment with same pathways. (Horton et al., 2017a).

Application of sewage sludge to land and use of treated wastewater for soil irrigation is also considered as a source of primary microplastics and fibers into the environment (Horton et al., 2017a; Ng et al., 2018) as treatment systems remove a major fraction of plastics from wastewater and the removed fraction is deposited in the sludge (Duis and Coors, 2016; Murphy et al., 2016; Horton et al., 2017a; Ng et al., 2018). Soils with known history of sludge application showed significantly higher concentrations of microplastics in Zubris and Richards et al. (2015). Danish EPA (Vollertsen and Hansen, 2017) study however, found out that soils received sludge showed lower concentrations of microplastics. As the effect of sludge application may depend on the rate of application, this pathway is still considered to be significant with serious expected loads to the land through these biosolids (Nizetto et al., 2016; Corradini et al., 2019).

Secondary microplastics are generated from macroplastic and mesoplastic fragmentation and with large amount of microplastics waste entering the environment, they are considered as a great contributor (Duis and Coors, 2016). Littering and release during collection and disposal of the waste cause large plastics to be released into the environment. Through various physical, chemical and biological processes, these particles can be fragmented into secondary microplastics in the soil and in the marine environment with latter being more retarded. Wind may transport the resulting particles in the soil across land and into water bodies (Zylstra, 2013; Dris et al., 2016; Horton et al., 2017a).

Soil erosion and agricultural runoff are potential sources of secondary microplastics into water bodies. Agricultural mulches used to increase crop yield and control growing conditions are also considered as another source of microplastics on land as they generally remain in the soil after germination as well as other plastics used in agriculture from bale twines to nettings (Horton et al., 2017a; Ng et al., 2018).

Tyre wear particles, plastic paint from road markings, storm drains on highways and roads and vehicle debris are other sources of secondary microplastics into the environment and, especially riverine environment (Browne et al., 2010; Lasen et al., 2015; Horton et al., 2017a; Unice et al., 2019). Atmospheric fallouts also carry particles present at land, fibers from clothes, particles from artificial turfs, construction activities, landfills and incineration (Dris et al., 2016; Magnusson et al, 2016; Cai et al., 2017). These particles can be transported across land and to water bodies and affected by environmental conditions of wind, tides, rainfall and other climatic conditions and physical forces (Akdoğan and Güven, 2019).

2.3.2. Occurrence and Distribution

Occurrence and distribution of microplastics are widely studied in the marine environment and there are many studies to date reporting microplastic pollution on sea surface, in subsurface waters and sediments (Auta et al., 2017; Rezania et al., 2018; Akdoğan and Güven, 2019). Approximately, $5.33x10^9$ mesoplastic particles were calculated floating in North Pacific Ocean by the model in Eriksen et al. (2014) study in a simplistic approach to define the scale of the pollution potential. Microplastics have been reported in a diverse geography as they were found in North and South America, Asia, Europe, Oceania, Africa, Mediterranean and even in Antarctica and Polar regions. They were reported in waters of Atlantic Ocean, Pacific Ocean, North Sea, Adriatic, Bohai and South China Seas and Arctic Pole (Goldstein et al., 2013; Dekiff et al., 2014; Lusher et al., 2014; Gajst et al., 2016; Ter Halle et al., 2016; Zhang et al., 2017; Obbard, 2018). Microplastics are also observed in deep sediments of many different seas from Baltics to Red Sea (Akdoğan and Güven, 2019). Mediterranean Sea was another location where the microplastics were observed and they were found in water and sediment samples collected from Turkey, Greece, Italy, Spain, France and Israel (Alomar et al., 2016; Gündoğdu and Çevik, 2017; Güven et al., 2017; Schmidt et al., 2018; Akdoğan and Güven, 2019). They were also found in Black Sea and Marmara Sea waters (Aytan et al., 2016; Tunçer et al., 2018). Marine plastics mostly originate from land-based sources and transported into the seas and oceans through freshwater streams, runoffs and with wind transport and fallouts (Ryan et al., 2009). Marine vessels and fishery are also sources of macroplastics or the mesoplastics (nets)

into marine environment which will fragment into microplastics (Cole et al., 2011). Major fraction of plastics assumed to be microplastics in marine environment and fibers are the most common form of microplastics in marine waters and sediments as well as biota followed by fragments (Claessens et al., 2011; Lusher et al., 2015a; Martin et al., 2017; Rezania et al., 2018; Güven and Akdoğan, 2019). PE, PP and PS are the most common types of polymers in marine environment as well as various environmental compartments (Ng and Obbard, 2006; Zhang et al., 2017).

Microplastics are abundant in beaches as they are subjected to both littering and washing ashore of fragmented particles due to the waves. Therefore, they act as reservoirs for the particles. Estuaries also accumulate microplastics due to same factors being; anthropogenic litters resulting from beaches and particle transport into estuaries due to waves. Estuaries also receive particles transported by freshwater systems (Kim et al., 2015; Herrera et al., 2017; Rezania et al., 2018; Akdoğan and Güven, 2019; Besseling et al., 2019). Coastal areas and estuaries often have the highest concentrations of microplastics due to combined effect of aforementioned factors (Antunes et al., 2018). Jambeck et al. (2015) estimated that 4.8 to 12.7 million tons of plastics are entering the oceans through coastal regions of the world. Particle loads were higher in rainy seasons (Lima et al., 2014; Cheung et al., 2016) which can be caused because of runoffs and increased transport of debris with increased flowrate of rivers. Cheung et al. (2016) also observed that seasonal differentiations occurred significantly in west coast of Hong Kong where Pearl River Estuary is located but not so significantly in the East Coast. PP, PS and PE were the most common types, with differing order of prevalence of microplastics in estuaries as expected (Ng and Obbard, 2006; Kim et al., 2015; Wessel et al., 2016).

Lakes were also investigated for microplastics and many of these studies were conducted in North America. Microplastics were reported in surface waters, shorelines and sediments. Similar studies were carried out in Europe and Asia (Eriksen et al., 2013; Ballent et al., 2016; Zhang et al., 2016; Anderson et al., 2017; Blair et al., 2017; Horton et al., 2017a). Microplastic pollution was detected even in Lake Hovsgol, Mongolia which is a remote lake with no urban interaction (Free et al., 2014).

Plastic pollution was observed in many rivers such as Great Lake tributaries, Rheine River, Ombrone River and Thames River as well as many rivers throughout the world (Mc. Cormick et al., 2014; Eerkes-Medrano et al., 2015; Mani et al., 2015; Klein et al., 2015; Baldwin et al., 2016; Blair et al., 2017; Zhang et al., 2018). Study carried out by Lechner et al. (2014) estimated 4.2 tons of large microplastics and mesoplastics discharge into Black Sea via Danube River over a two-year period using stationary drift nets in Austria. 22-36 tons of microplastic debris were found to be floating in

River Seine by Gasperi et al. (2014) and most of them were PP, PE and PET, to a lesser extent. Lebreton et al. (2017) calculated flow of 1.15 to 2.41 million tons of plastics from riverine systems to marine systems. 67% of calculated global annual input resulted from top 20 polluting rivers.

Several studies were carried out to investigate microplastic particles in rivers, urban lakes and their tributaries and fibers were the most common particles and dominant polymer compositions were PET and PP (Wang et al., 2017; Zhang et al., 2017; Zhang et al., 2018). Effect of urban activities and municipal and industrial effluents were investigated where, urban activities and mismanagement of waste was considered as an important source for microplastics pollution (Dris et al., 2015; Castaneda et al., 2014; Zhang et al., 2017; Lahens et al., 2018). Horton et al. (2017b) observed significantly higher microplastics abundance downstream of a storm drain receiving urban runoff. Morritt et al. (2014) detected higher concentrations in the sampling points where sewage outfalls are located than the other sampling points. Generally higher concentrations were detected downstream of a wastewater treatment plant in the study conducted by Kay et al. (2018). Vermaire et al. (2017) reported higher microplastic concentrations downstream of a wastewater treatment plant on Ottawa River then upstream. Wang et al. (2017) also reported higher concentrations on downstream of an urban area, which produce considerable amount of municipal waste and contains a wastewater effluent outlet. In Estahbanati and Fahrenfeld (2016) study, downstream of three wastewater treatment plants were studied. Microplastics concentrations in 125 μm category increased in three plants and for 250 μm category, concentrations increased for two plants and no significant differences were observed for 500 μm category. Higher concentration of microplastics was observed downstream of the wastewater treatment plant in Mc Cormick et al. (2014) study. Furthermore, the composition of microplastics were also different as no pellets or foams were observed upstream of the plant where they were present downstream. Klein et al. (2015) however, did not find any correlation with microplastic concentration and polymer compositions with population density or proximity to sewage or industrial discharge. Temporal differences in river flow resulted in 25 times higher amount of microplastics in winter in Nel et al. (2018) study, which is likely due to increased sedimentation on dry seasons. Sampling dates would play a significant role with observation of freshwater flow systems in order to make a comparison between the studies.

Concentrations of plastic particles expected to be high in soil samples with potential sources such as wastewater irrigation, sewage sludge application and plastic film/equipment debris and large surface area (Zubris and Richards, 2015; Nizzetto et al., 2016; Steinmetz et al., 2016; Horton et al., 2017a; Blasing and Amelung, 2018). Most soil samples investigated in China had low concentrations of microplastic particles (Liu et al., 2018; Zhang et al., 2018) however, with samples irrigated with

wastewater and fertilized with sewage sludge, concentrations were significantly high and even higher than most polluted marine environments (Zhang and Liu, 2018; Zhu et al., 2019). Most abundant microplastics were PP and PE and smaller microplastics (<1 mm) were the most abundant size class (Lv et al., 2019). Concentrations of microplastics can be affected by soil type as it would affect the penetration of particles in deeper soil (Horton et al., 2017a; Zhang et al., 2018). Corradini et al. (2019) investigated 30 fields with sewage application over 10-years period with different application rates differing from 1 to 5 times with a field with no sludge application as control. Study concluded that sewage application was in fact a driver of plastic pollution in farmlands and higher application rate increases this pollution rate. However, according to the study, even farmlands investigated for control contained microplastics while not receiving sludge. Particles found in atmospheric fallout could have caused these unexplained particles.

2.3.3. Fate and Transport

Size and density affect transport and fate of microplastic particles however, they are not the only determining factors of distribution. Wind, tides and water currents affect the sinking and migration processes of polymers in marine environments (Bagaev et al., 2017; Iwasaki et al., 2017). Buoyant particles (PE and PP) can be transported on the water surface where denser particles (PVC) can be transported to bottom by underlying currents (Engler, 2012; Wang et al., 2016). Microbial fouling, aggregation with organic and inorganic particles can increase particle densities and sizes causing the settling of lighter particles (Claessens et al., 2011; Vianello et al., 2013, Corcoran et al., 2015). Particulate matter and suspended sediments as well as many suspended contents of marine and estuarine environments are high and such processes most likely dominate fate and transport of microplastics in marine and estuarine environments (Besseling et al., 2017; Kooi et al., 2017; Long et al., 2017; Zhang, 2017). Microplastics abundances raised by major rainfall events in estuaries (Yonkos et al., 2014). Estuaries are accepted as major sinks for microplastic particles due to inputs resulting from rivers, beach litters and washing by surface currents (Akdoğan and Güven, 2019).

Microplastics distribution in rivers is also affected by density and particle size but not only those factors. Particles with higher densities than water can be retained in the sediment however, flooding or high flow periods can mobilize these particles retained in the sediment. Besseling et al. (2017) conducted a modeling study on retention of particles with biofilm formation, sedimentation and resuspension, aggregation kinetics, advective transportation and concluded that; for spherical particles, bigger particles are more likely to be retained in nearer distances where intermediate and small microplastics needed a lot more distance to be retained. Therefore, rivers are considered as

major pathways of microplastics into marine and estuarine environments. Schmidt et al. (2017) stated that rivers from top 10 ranked catchments have contributed 88% to 94% of total loads which was achieved by a realistically constructed model and authors concluded that reducing the loads in those rivers only, can reduce river-based loads by 45%. This contribution value was also achieved by another modeling study, reported more than 90% of plastic comes from top 122 polluting rivers which corresponds to 4% of total land mass and 36% of population. Study also verifies the temporal changes mentioned by Ballent et al. (2013), stating that more than 74.5% of total plastic inputs occurred between May and October (Lebreton et al., 2017).

Generally, degradation of plastics occurs with solar UV radiation which initiates oxidative degradation of polymers. During advanced stages of degradation, plastic particles tend to lose color and become brittle. Additives such as UV and heat stabilizers or antioxidants may retard the degradation of plastic material. Weathering of plastics are studied in various different environments but these studies focus on the early stages of degradation. There is not much information about weathering of plastics in shorelines and seawater (floating, submerged or sediments) and effects of environmental conditions and impacts such as mechanical forces, temperature, pressure, salinity, bio-fouling etc. on the rates of fragmentation. Also, there are no methods yet available to determine the age of the microplastics (Eerkes-Medrano et al., 2015; GESAMP, 2015).

Fragmentation (or weathering) would occur more rapidly in beach surfaces or exposed in air with abundant UV radiation and high temperatures (Zbyszewski et al., 2014) but relatively slow while floating in the sea (Andrady, 2011). In low oxygen or apothic zones, degradation and fragmentation are particularly slow. Ter Halle et al. (2016) showed that particles smaller than 2 mm are degraded significantly faster than larger particles which emphasize the importance of investigating smaller particles.

2.3.4. Uptake, Effects and Hazards of Microplastics

Ingestion is a widely studied path for intake of microplastics in marine environments. Zooplanktons (Cole et al., 2013; Setälä et al., 2014), adult and larval fish (Rochman et al., 2013b; Neves et al., 2015; Jabeen et al., 2017), shellfish (Van Cauwanberge et al., 2015; Tosetto et al., 2016), corals (Reichart et al., 2018), lobsters (Murray and Cowie, 2011), amphipods, lugworms and barnacles (Thompson et al., 2004; Browne et al., 2013) and marine mammals (Eriksson and Burton, 2003; Lusher et al., 2015b; Fossi et al., 2016) showed microplastics presence in their intestines and/or stomachs. Seabirds that consume fish are also affected by marine plastics pollution (Tanaka et al.,

2013). Microplastics can be disproportionately ingested for feeding by misidentification or indiscrimination (Graham and Thompson, 2009; Ory et al., 2017) or through trophic transfer (Tanaka et al., 2013; Setälä et al., 2014; Nelms et al., 2018). Estuarine organisms showed microplastics presence in various stages of the food web (Akdoğan and Güven, 2019). As microplastic concentrations are high in estuarine environments zooplanktons were investigated for occurrence (Frias et al., 2014; Lima et al., 2015). Lima et al. (2014) found out that microplastics may exceed plankton population and causing an increase in the bioavailability of microplastics. Microplastics were also found in different organs which are not related with ingestion such as gills of crabs and soft tissues of mussels and adherence was addressed as a path of uptake for certain species (Brennecke et al., 2015; Kolandhasamy et al., 2018).

Microplastics ingestion in freshwater environments were studied only for invertebrates and fish (Sanchez et al., 2014; Bratton et al., 2016; Ma et al., 2016; Campbell et al., 2017; Hurley et al., 2017; Horton et al., 2018). Digestive tracts of fishes showed microplastics presence from rivers in the USA, Canada, UK and France (Sanchez et al., 2014; Peters and Bratton, 2016; McGoran et al., 2017; Campbell et al., 2017; Horton et al., 2018). Hurley et al. (2017) also found microplastics in tissues of Tubifex worms in sediments of a River in the UK.

Microplastics can cause blocked digestive tracts, choking, sores, false satiation, debilitation, impaired feeding capacity and death on many levels of aquatic environment (Eerkes-Medrano et al., 2015). Accumulation and retention had been observed in many different organisms in different levels of the aquatic environment (Rosenkranz et al., 2009; Murray and Cowie, 2011; Wright et al., 2013). Particle's size plays a different role in retention of the particle based on particle size and density. Sea scallop *Placopecten magellanicus* retained larger and lighter particles longer than smaller and denser particles where it may cause a decrease in nutrition (Brillant and MacDonald, 2000). Ramos et al. (2012) observed lower weight of gut taxa in adult estuarine fish that ingested microplastic fragments.

Microplastics can also affect organisms at tissue and cellular levels. Ingested microplastics can cause inflammatory response in tissues. In cells of digestive system, microplastics can cause reduced membrane stability (Von Moos et al., 2012). Browne et al. (2008) observed accumulation of smaller microplastics was more than larger microplastics in tissues and translocation of smaller microplastics from gut to circulatory system occurred in 3 days and persisted for over 48 days. Bioaccumulation, liver stress response and early tumor formations were observed in Japanese medaka fish fed with virgin and marine PE fragments (Rochman et al., 2013b).

Microplastic particles may also cause growth delay, decrease in reproductivity and mortality due to their adsorptive and hydrophobic properties (De Sá et al., 2015; Eerkes-Medrano et al., 2015; Akdoğan and Güven, 2019) as they can accumulate and transport chemicals that are persistent, toxic and bio-accumulative (Teuten et al., 2007; Engler, 2012; Browne et al., 2013). Microplastics can carry both chemicals that are sorbed onto their surface and the chemicals that are in the plastics in the production phase (Yogui and Sericano, 2009; Hahladakis et al., 2018). Karami et al. (2016) observed histopathological changes in the gill and liver of African catfish *Clarias gariepinus* exposed to virgin and phenanthrene loaded microplastics such as altered blood biochemical parameters and transcription of reproductive genes of the fish. Several studies were conducted in order to compare virgin microplastics and microplastics exposed to chemicals and generally, microplastics except in nano-sizes did not affect mobility of the organisms where chemical loaded microplastics showed significant effects (Batel et al., 2016; Chen et al., 2017). Smaller particles exposed to chemicals were also found to be more hazardous to *Daphnia Magna* in Ma et al. (2016). A model study by Rochman et al. (2017) showed that polymer type is also another factor significantly affecting the concentrations of PCBs in clams.

Microplastics had been found as vectors in transport of POPs (persistent organic pollutants) such as PAHs (Polycyclic aromatic hydrocarbons) (Mendoza and Jones, 2015), DDT (Dichlorodiphenyltrichloroethane), Phe (Phenanthrene) (Bakir et al., 2014), Polychlorinated biphenyls (PCBs) (Mendoza and Jones, 2015), DEHP (di(2-ethylhexyl) phthalate) (Bakir et al., 2016; Zhang et al., 2018) from seawater to marine organisms. There are also studies showing the interaction between microplastics and heavy metals (Turner and Holmes, 2015; Brennecke et al., 2016). Adsorption potential of antibiotics on microplastics was also studied by Li et al. (2018) and adsorption capacities varied among polymer and antibiotics types and environmental conditions. Freshwater environments were more suitable for antibiotics to be absorbed. Microplastics may also carry antibiotic resistance genes and other genes due to increased permissiveness towards plasmids and therefore can cause evolutionary changes at species and population levels and their structural properties favor the biofilm growth, providing ideal conditions for collection, transport and dispersion of microorganisms over long distances (Rummel et al., 2017; Arias-Andres et al., 2018).

2.4. Wastewater Treatment Plants as Receivers and Sources of Microplastics

Wastewater treatment plants receive abundant amount of microplastics and although they remove significant amount of microplastics from the wastewater they still discharge microplastics into the environment with relation to their flow rate. The microplastics removed from the wastewater causes microplastics to be retained in the sludge resulting from the treatment units. The sludge resulting from these plants as well as discharged effluent therefore, is a source of microplastics. The effluent reaching the receiving media and sludge deposited in landfills or applied to soil makes wastewater treatment plants an important conduit for microplastics investigation.

2.4.1. Occurrence, Distribution and Fate of Microplastics in Wastewater Treatment Plants

Several studies were conducted on microplastics in wastewater treatment plants. Dris et al. (2015) investigated the microplastic contamination in Paris and investigated microplastics concentration in raw wastewater and effluents of wastewater treatment plants and surface waters. Sampling size and volumes were different throughout the sites where meshes varied between 80 µm (plankton net) to 330 µm (manta trawl). Sample identifications were carried out with stereomicroscopes. Concentrations of 260-320 particles per liter were observed in influent waters. In the final effluent, they were in 14-50 particles per liter range. Primary treatment removed around 66% of the particles and there were 50-120 particles per liter after primary treatment and secondary treatment processes removed microplastic contamination by 83-95% in total. Fibers were the most abundant shape with 99%. In surface water evaluations; plankton net samples showed 30 times higher concentrations of microplastics than manta trawl net samples, as expected. Microplastic concentrations in surface waters were 1000 times lower than treatment plant effluent. However, study did not mention the concentrations of sampling points individually to differentiate the sampling points receives or does not receive wastewater effluent.

Talvitie et al. (2015) conducted a study in Finland, Viikinmäki WWTP. Samplings of influent wastewater, wastewater after primary and secondary treatment and effluent from tertiary treatment units (biological filters) were conducted via a transparent tubular sampling device consisting of 200 µm, 100 µm and 20 µm filter segments mounted on o-rings and connected to a pump. Sample identifications were done by a stereomicroscope. Sampling method showed heavy clogging at influent wastewater sampling where, only one successful sample could be processed and the sample contained 180 fibers and 430 particles per liter. 14.2 fibers and 290.7 particles were found after primary clarification and 13.8 fibers and 68.6 particles were found after secondary clarifier. After the

treatment, average of 4.9 fibers and 8.6 particles were present per liter of water. Sediment samples from effluent discharge sites of the Viikinmäki and Suomenoja plants were collected with a corer sampler and top 30 mm was removed from each sediment. Extraction was done with NaCl solution method proposed by Browne et al. (2010). For Viikinmäki plant discharge site 1.7 fibers, 7.2 particles, 70 ring shaped particles per kg and for Suomenoja plant discharge site 4.7 fibers, 10 particles and 3.8 ring shaped particles per kg were found. In general, average fiber concentrations and particle concentrations were found to be 25 and 3 times greater than the concentrations of the receiving Baltic sea. Study observed removal of fibers on primary sedimentation and removal of particles on secondary sedimentation occurred on highest level.

Mason et al. (2016) analyzed 90 effluent samples from 17 wastewater treatment plants in the US with various capacities, sizes, treatment units and locations and of those plants, one with an advanced filtration unit. Study also included temporal differences at several treatment plants. Sampling was conducted for 2 and to 24 hours with 0.355 mm sieve stacked on 0.125 mm sieve for earlier and, only 0.335 mm sieve for the latter. Wet peroxide oxidation (WPO) method was applied to samples prior to examination and the examination of microplastic particles was done only visually with dissection microscope. Study found the particle concentrations to be 0.004-0.195 particles per liter of effluent. Most common types of microplastics were particles (59%) and fragments (33%). Smaller particles (0.125-0.355 mm) found to be slightly more than larger particles (>0.355 mm) in general of the 17 samples. Fibers identified by the study could not be confirmed since no characterization analysis was conducted. As only 75% of the fibers were verified as plastics by previous studies (Lenz et al., 2015) the microplastic counts are not exact. Study also concludes, for the plants studied, composition of particles within the effluent stream is related with the presence of combined sewers and advanced treatment systems however the overall particle concentration did not show significant positive association (15% reduction observed between influent and effluents of filter skids) with these system's presence.

Michielssen et al. (2016) conducted a study, investigating wastewater treatment plants in Detroit and Minnesota, U.S.A. and reported that tertiary treatment units were successful in removing the microplastics more than the activated sludge processes and especially MBRs are efficient in that matter in light of data collected from a pilot scale MBR plant tested in the study. Although, the removal rates were high with the WWTP, there were still 15 billion particles per day released into the Detroit River after secondary treatment. When MBR skids are operational, 1.25 billion particles still would reach Detroit River every day. Even the most efficient systems could not retain smaller particles, especially fibers as fibers are abundant in effluent of MBR pilot plant. Fiber was the most

abundant shape category (61-62%), followed by fragments (23-33%) and microbeads (11.1%) in secondary wastewater treatment plants. Tertiary treatment systems also displayed a similar character. Fibers were 54.5% of influent particles where they were raised to 84.7% in the effluent and fragments decreased from 25.9% to 13.0%. Carr et al. (2016) observed 99.9% removal from wastewater treatment plants of Southern California with different methods of treatment and investigated the particles with FT-IR spectrometry. Authors concluded that wastewater treatment plants have nearly no effect on receiving environment.

Twelve wastewater treatment plants with different capacities and wastewater compositions in Northwest Germany were investigated by Mintenig et al. (2017) by samples taken from the effluent and influent. Samples are taken with a custom device consisted of a membrane pump connected to a flow meter directing the water to a 10 μm stainless steel cartridge filter. Custom enzymatic digestion protocol (with an additional wet peroxide oxidation step for particles <500 μm) was applied for purification of water samples. Alkaline treatment according to Cole et al. (2014) was applied for sludge samples prior to micro FT-IR analysis. 10 of the 12 plant effluents contained microplastic particles >500 μm which were PE, PP, PVC, PS, PUR, Silicone and PUR based paint. PE was the most common polymer (59% avg.) followed by PP (16% avg.). 1 to 5 polymers were present in 500-7200 μm range per sample. Estimated annual releases ranged from $1x10^6$ particles/year for Lohne and $5x10^7$ particles/year for Varel treatment plants. All plant effluents contained microplastic particles <500 μm which were PE, PP, PVC, PS, PUR, PA, PET, SAN, EVA, PVAL, ABS, PLA and paint. PE was the most common polymer (40% avg.) followed by PVAL (16% avg.), PA (8% avg.) and PS (8% avg.). 3 to 12 polymers were detected per sample. Estimated annual releases ranged from $1x10^7$ particles/year for Neuharlingersiel and $5x10^9$ particles/year for Holdorf treatment plants. None of the sludges contained particles <500 μm but all of them contained particles >500 μm of PE, PP, PA and PS. Estimated annual releases ranged from $1.24x10^9$ particles/year for Schillig and $5.67x10^9$ particles/year for Scharrel treatment plants. Membrane reactors and gravity filters were found not as effective as other studies suggested however filtration through pile fabric removed effective to remove all particles >500 μm and 93% of particles <500 μm.

A wastewater treatment plant in Sweden was investigated in Magnusson and Norén (2014) study. Sampling was conducted with a Ruttner sampler and samples were filtered through 300μm meshes. h FT-IR spectrometry was utilized for characterization. Results showed more than 99% removal of all kinds of microplastics in the effluent which were categorized as fibers, flakes and fragments by shapes and dimensions. Relative particulate distributions were 70 and 49% for fibers in influent and effluent waters and in the effluent distribution fragments were increased twice. Study implies that

retained microplastics are to be found in the sewage sludge by vast majority and also investigates a facility with a disc filter final treatment system for effluent, which had considerably smaller amount of microplastics (80% less) than a similar sized facility.

Murphy et al. (2016) sampled influent wastewater after coarse screen, wastewater from grit and grease effluent, primary effluent and final effluent of a wastewater treatment plant located on Clyde River, Glasgow serving 650,000 people with an average flow rate of 260,954 m^3/d. It consists of coarse and fine screens, grit and grease removal, primary settling, aeration, and secondary clarifier. Sampling was done with steel buckets and filtered through 65µm sieve before the analysis. Characterizations were done with FT-IR spectrometry after the extraction procedures. Average of 15.7 particles/L were found in influent water where effluent had 0.25 particles/L. Despite the high removal rate, considering the flow rate average of the plant, there still is a chance of 23 billion of MPs per year released from this WWTP alone. Regarding the removal rates in liquid fraction, biggest reduction of particles was achieved at grit and grease removal unit (44.59%) and there was a further reduction in primary settling (33.7%). The most common polymers in the influent were alkyds (28%) PS-ACR (19%), PES (10%), PU (9%) and ACR (8%). Whereas the most common polymers in the effluent were PES (28%), PA (20%), PP (12%), ACR (12%), Alkyds (8%), PE (4%), PS (4%), PET (4%), respectively. Size average in liquid fractions was 0.598 mm. In solid fractions, grease sample had an average of 19.67 particles/2.5 g sample which was significantly higher than sludge cake and grit chamber sample. Size average in solid fractions was 1.342 mm. Study did not take the storm water runoff into account.

Leslie et al. (2017) investigated the influent and effluent of seven wastewater treatment plant as well as sludges in Amsterdam. Subsamples of 100 g were collected after homogenization of samples and density separation with NaCl was applied. Subsequently, extracts were filtered on a 0.7 µm filter. Particle confirmation was accomplished with an FT-IR spectrometer. Influent particle concentrations ranged from 73 to 238 particles per liter. Effluent concentrations of plants ranged from 9 to 91 particles per liter and an average of 52 particles per liter was detected for investigated seven plants where the most common type was fibers. In sludge samples, an average of 650 particles per kg wet weight was present where dry weight was 1% on average. Fixed bed carbon filters and MBR filters were tested for the removal of microplastics and similar concentrations were observed in the effluent.

Danish EPA conducted an assessment on ten wastewater treatment plants in the country by sampling raw and treated wastewater, and sludge from five wastewater treatment plants (Vollertsen and Hansen, 2017). Study also collected ten agricultural soil samples which five of them used

sludge as fertilizer and five that never received sludge. Study assessed the microplastics with sizes 20-500 μm. Pre-treatment methods of each type of samples were different and analysis were conducted with FT-IR analysis. Pre-treatment was generally conducted with applying SDS solution for all types of samples and a following wet peroxide oxidation step for wastewater samples. Concentrations of microplastics varied from 0.22-29.6 mg/L (corresponding 13,000-442,000 particles/L) with an average of 10 mg/L (130,000 particles/L) for raw wastewater and 0.037 mg/L (8,000 particles/L) for effluents which was quite high when compared to other studies. Overall plastic particle sizes and diameters were smaller in the effluent samples. The median mass value of raw wastewater was nearly two times of treated wastewater. Study discovered that particle masses less than 7 ng present more in raw wastewater, than the effluent. Digested sludge samples which had a dry mass of 25-30% contained an average of 6.5 mg/g (175,000 particle/g) of microplastics in nearly 2% of that dry mass. Most common particles in influent waters were NYL (76,8%) and PE (13,3%) where in treated wastewater the distribution was similar with NYL (78,6%) and PE (15,7%). Sludge samples showed common particles of PE (65.5 particulates %, 79.2 mass %), NYL (20.3 mass %, 31 particulates %) where a significant decrease of nylons from influent was observed. The average particle mass from agricultural land samples was 7.6 mg/kg for soils received sludge and 15 mg/kg for soils that did not receive sludge. Common particles were PP (56%) and PE (39%) for soils received the sludge and PE (89%) and NYL (10%) for soils did not receive the sludge.

In a study of Talvitie et al. (2017a) Viikinmäki WWTP in Finland was investigated which consists of screening, grit removal, pre-aeration, chemically enhanced primary sedimentation, aerobic reactor, secondary sedimentation and a biological filter. Sampling was done as grab, 24h composite and 24h sequential. Grab samples were taken with metal beakers for influent streams and pumps for other water samples and passed through meshes of 300 μm, 100 μm and 20 μm. Sludge and reject water samples were diluted before filtering. Composite samples were taken from all sites with 15 min intervals for 24 hours and sampling performed three times a week. Influent and effluent were simultaneously sampled at 1hr intervals and after sampling 3 samples were pooled together resulting in 8 sequential samples. Analysis were done with FT-IR spectrometer after separation with stereomicroscopes. From grab sample analysis it was concluded that in pre-treatment phase 97.4-98.4% of particles were removed from wastewater and further decrease but in smaller amounts of 7-20%. According to the study, tertiary biological filter did not affect particle concentrations. After biological filters, 0.7-3.5 particles per liter was detected. From composite samples, 0.4-0.8 particles per liter was found. Concentrations of 0.8 particles per liter and 1.7 particles per liter were found for night and day time. Wednesday showed the lowest effluent concentration and highest removal where

samples taken in Saturday showed a removal efficiency being significantly less. The lowest concentrations were observed at 1-4 p.m. In influent sample fibers made up around 70% of the total particles where, in effluent samples they were only present by 30%. Very small percentage of the particles were confirmed by FT-IR because of the presence of organic fibers and as there had been no pre-treatment to digest the biofilm and other organics.

Talvitie et al. (2017b) conducted another study where, several wastewater treatment facilities with different tertiary treatment methods were investigated. First one was the Viikinmäki WWTP in Helsinki which used denitrifying Biological Active Filter (BAF) system with disc filters and ferric coagulant and a cationic polymer. Second one was Kakolanmäki WWTP in Turku which used Rapid Sand Filters (RSF) with 1 m of gravel with 3-5 mm grain size and 0.5 m of quartz with 0.1-0.5 mm grain size. Third one was Paroinen WWTP in Hämeenlinna which used Dissolved Air Filtration with PAX (Polyaluminium chloride). Three aforementioned plants applied tertiary treatment processes following an activated sludge process. Fourth one was Kenkäveronniemi WWTP in Mikkeli which used an MBR system after activated sludge process and secondary clarifier. Sampling was conducted with the instrument used in Talvitie et al. (2015). Results have shown reduction from 6.9 to 0.005 particles per liter with MBR where secondary treatment effluent had 0.2 particles per liter (99.9%); reduction from 0.7 to 0.02 (97%) particles per liter with RSF; reduction from 2.0 to 0.1 particles per liter (95%) with DAF and reduction from 0.5 to 0.3 and 2.0 to 0.03 particles per liter (40-98.5%) for 10 µm and 20 µm disc filters after primary effluent respectively. Size distribution observed was similar to Talvitie et al. (2017a) and particles <100 µm are not removed by these processes. Polyesters (60% in avg.) followed by PE (14% in avg.) were the most common particles in all samples. Authors state that larger sample volumes (1000 L) after DAF and RSF showed more volumes of rare polymers than smaller sample volumes. After activated sludge process, primary and secondary plastics were found as 19% and 81% where after advanced treatment systems 9% and 91% in the effluents respectively.

Three wastewater treatment plants in Australia with different treatment processes were investigated in Ziajahromi et al. (2017) first one with post primary, second one with post primary and secondary and the third one with tertiary treatment and reverse osmosis (RO). Sampling was conducted with a custom device consisting of 500 µm, 190 µm, 100 µm and 25 µm meshes stacked on each other in a contained case and connected to a pump. Water content of samples were concentrated to 100 mL at 90^{o}C according to Masura et al. (2015) prior to digestion of organics Digestions were conducted according to Nuelle et al. (2014). 30% H_2O_2 added in differing amounts depending on sample's organic content and samples were stirred at 60^{o}C while digestion is carried

out. Density separation was done with NaI solution with a density of 1.49 mg/L and centrifuging the samples. Subsequently, buoyant particles in supernatant was filtered through 25 μm meshes. Rose-Bengal solution was then applied to all mesh surfaces in order to stain natural and non-plastic particles for 5 minutes and then the dye was washed off with DI water. FT-IR microscope was used to characterize the particles. Larger sample volumes resulted in larger amount of microplastic particles compared to the samples with lower volumes. 10% of particles suspected as microplastics were characterized as microplastics in the samples with lover volumes whereas; larger sample volumes yielded 70-78% of suspected particles confirmed as microplastics. PET fibers (80%) and PE particles (20%) were the most abundant particles in the effluent for the facility with primary treatment, PET (35%), nylon (28%), PE (23%), PP (10%) and PS (4%) were the most abundant particles after primary treatment and PET fibers and PE particles were the most abundant particles in the effluent for the facility with secondary treatment. PE (42%), PET (36%), PS (15%) and PP (8%) were the most common types of microplastics after primary treatment in the facility with tertiary and RO treatment where, effluent samples after RO contained PET fibers in abundance. 90% of microplastics were removed during advanced treatment processes.

In Lee et al. (2018) three wastewater treatment plants in South Korea with different treatment methods including A2O, SBR and Media filters were investigated. 10 L of influent samples were taken before the coarse screen and 100L of effluent samples were taken after UV sterilization and sieved on-site on a 106 μm mesh screen. Wastewater samples were treated with WPO method according to Masura et al. (2015). Sludge samples were subjected to density separation in $ZnCl_2$ solution then filtered through the sieves and stored in petri dishes. Concentrations in the influent samples were 29.9 particles/L, 16.5 particles/L and 13.9 particles/L for A2O, SBR and Media processes respectively. Whereas, concentrations of effluent waters were 0.44 particles/L, 0.14 particles/L and 0.28 particles/L for A2O, SBR and Media processes respectively. Removal rates for the sieved fraction larger than 106 μm was about 99%. There was no characterization step in order to verify the extracted particles as microplastics.

Gies et al. (2018) sampled influent, primary and secondary effluents and sludge samples from a secondary wastewater treatment plant at different dates in Vancouver, BC, Canada. Pre-treatment methods based on oil extraction differed for sample fractions and particle confirmation was accomplished with FT-IR. The most common particles obtained from the study were fibers (65.6%), fragments (28.1%) and pellets (5.4%) for influent, primary and secondary effluent samples. Study estimated 97.1 to 99.1% retention of microplastics in the treatment plant.

Water samples from influent, after primary clarification and after disinfection and, sludge samples from activated sludge, MBR sludge and digested sludge were collected from Kenkäveronniemi WWTP in Mikkeli, Finland in Lares et al. (2018). Modified version of WPO by Masura et al. (2015) was selected as pre-treatment method for water samples.Sludge samples were treated according to Murphy et al. (2016) study. Characterization of particles were accomplished with both FT-IR and Raman spectroscopy. Fibers were 91% of particles in influent samples and ranged from 50-70% in different sampling locations. Fibers accounted for 79.1% of all microplastics collected during the study and most of these fibers (96.3%) were made of PES where rest of the MPFs were PA (3.1%). PE was the most common polymer where it made up for 63.9 % of microplastic particles (MPPs), which accounted for 11.4% of the microplastics collected during the study, where the rest (36.1%) were characterized as PE, PS and PP. Study concluded that the removal efficiency for the aforementioned plant was 98.3% for overall microplastics and final effluent contained 1.0 MP/L on average.

Simon et al. (2018) investigated ten wastewater treatment plants in Denmark; 9 of which had wastewater of municipal origin and 1 had 75% of industrial origin. Pre-treatment consisted of enzymatic incubation and wet peroxide oxidation with Fenton's reagent. Chemical characterization was determined with FPA based FT-IR imaging by mapping. Removal efficiency of the plants were 99.3% on average where influent waters contained 7216 particles per liter and effluent waters contained 54 particles per liter on average. In influent wastewater the most abundant particles by particle numbers were ACR (27%) and in effluent PES (25%) and PE (27%) respectively. However, by mass (from calculations of volume and density of the observed polymers) PES and ACR contributed to 34% and 20% of the particles respectively where PE corresponded to 9%.

In a study by Li et al. (2018), 79 dewatered sludge samples from 28 WWTPs in China were sampled during 2014 and 2015. Solid contents differed from 11.8% to 51.1%. Extractions were conducted according to the method by Thompson et al. (2004) with certain modifications. 20 g of sludge was put into a flask with 300 mL of NaCl solution and stirred for 15 min and the mixture then settled for 2 hours. Supernatant was then filtered through a 37 μm sieve. The process was repeated for three times and three replicates were collected on the sieve. Subsequently, sieve was treated with 100 mL of 30% H_2O_2 overnight. Mixture then poured into 200 mL of distilled water and filtrated through a fiberglass filter and dried for 3 days in a desiccator. Authors found 1.6 to $56.4x10^3$ particles/kg dry sludge with average of $22.7x10^3$ particles/kg dry sludge. Fibers were the dominant shape (63%) followed by lines (15%), films (14%), flakes (7.3%) and spheres (1.3%). PO fibers and lines, ACR fibers, PE films, PA films, alkyd resin flakes and PS spheres were the dominant types of

each shape. Microplastic concentrations in the sludge were lower in the areas with lower population density, lower urbanization and industrialization and higher amount of forest lands. Rainfall and temperature affected the sludge's microplastics content as higher amounts of microplastics were observed with heavy rainfall and lower temperatures. Service area that the plant serves also affected microplastics concentrations where with a wider service area, microplastic concentrations decreased.

Gündoğdu et al. (2018) sampled wastewater from influent and effluent of Seyhan and Yüreğir WWTPs in Adana, Turkey. Wet peroxide oxidation with Fenton's reagent was selected as the pre-treatment method for the samples. Micro Raman spectroscopy was used for characterization of the particles. Authors found out that Yüreğir WWTP had a removal rate of 79% and Seyhan WWTP had a removal rate of 73% for microplastics. Influent wastewaters contained 4825 particles/L and 2040 particles/L of microparticles in average for Seyhan and Yüreğir WWTP, respectively. Effluents contained 1249 particles/L and 351 particles/L on average for Seyhan and Yüreğir WWTP, respectively. Fibers were the most abundant group of all types of microplastics (54.8% influent and 44.4% effluent of microplastics for Seyhan and 87.7% influent and 86.5% of effluent microplastics for Yüreğir WWTP) followed by fragments (18.9% for influent and 20% for effluent samples) and films (10.9% for influent and 20% for effluent samples). Microplastic size classes were smaller in the effluent samples than influent. Average particle sizes were 1.57 mm and 1.15 mm for influent and effluent of Seyhan WWTP respectively. Where, average sizes were 1.68 mm and 1.39 mm for influent and effluent of Yüreğir WWTP respectively. PES was the most common polymer (50.8%) in Seyhan WWTP influent; followed by PE (29.2%) and PP (13.8%) while the order of the ratios was similar for the effluent samples; the percent presence in PES was reduced to 43.8% of the total amount. PE was the most common polymer (61.9%) in Yüreğir WWTP influent; followed by PE (18.8%) and PP (12.5%) while the order of the ratios was similar for the effluent samples; the percent presence in PES was increased to 68.8% of the total amount.

In Magni et al. (2019) study, a wastewater treatment plant in Northern Italy with a capacity of 400,000 m^3/day was investigated. Grab wastewater samples were taken from the influent, after settling tank and from the effluent with a volume of 30 L. Samples were subsequently filtered through 5 mm - 2 mm and 63 μm meshes on site. Density separation was applied with NaCl saturated solution and samples were then centrifuged overnight. Supernatants were filtered on 8 μm cellulose nitrate membrane filters and washed with DI water. Subsequently, organics on filters were treated with 15% H_2O_2 for 3 days in room temperature. Suspected particles were then separated and FT-IR microscope was used in order to characterize the polymers. Influent wastewaters contained 2 MPPs/L and 0.5 MPFs/L. After settling wastewaters contained 0.6 MPPs/L and 0.3 MPFs/L, meanwhile effluents

contained 0.1 MPPs/L and 0.03 MPFs/L. Indicating, 64 % removal in primary settling and 84% overall MP removal was achieved. Films were the most common MPPs in the influent (73%) followed by fragments (21%) and lines (6%). After the settling tank, films and fragments had equal presence (36%) followed by lines (28%). Lines (41%) were the most abundant shapes in the effluent followed by films (38%) and fragments (21%). In the influent stream, ABS copolymer was the most common type of polymers (40%) followed by PE (17%) and EPR (14%). After settling, PES (23%), PE (13%), PUR (13%), PA (11%) and PP (11%) were the most common types of polymers where in the effluent, PE (35%), PA (17%) and PE (10%) were the most common types. 59.5 MPPs and 53.3 MPFs were found in the sludge and 51% of them were films followed by fragments (34%) and lines (15%). Main polymers were PES (60%) and PE (35%). Sizes of MPPs were smaller in the effluent and MPP sizes were similar in sludge and primary settling.

Largest wastewater reclamation plant in Beijing, China with a capacity of 1,000,000 m^3/day was investigated by Yang et al. (2019) by taking 30 L wastewater samples from the influent, primary sedimentation, secondary sedimentation and effluent for 3 months. Samples were sieved through 5 mm and 500 µm meshes as batches of 10 L. Solids retained were transferred to a 1 L beaker and the beaker was filled with tap water. The mixture was then filtered through 10 µm nylon membranes. Subsequently, membranes were sonicated for 20 minutes and dried at 90^{o}C for 12h. 20 mL of 0.05 M $FeSO_4$ solution and 20 mL of 30% H_2O_2 were added to react for 5 minutes. The beakers were then heated to 60^{o}C while stirring for 30 minutes. $H_2O_{2\ was}$ added as 20 mL increments until the digestion was complete. Subsequently, $ZnCl_2$ was added to the mixture and HCl to facilitate dissolving of $ZnCl_2$ and solutions let to settle in a separator funnel overnight. Solids were collected from the bottom and investigated under a stereo microscope where liquid fractions were filtered on 10 µm membranes. Micro-FTIR was used in order to characterize the particles. 12 particles/L found in the influent samples and 59% removal on aerated grit chambers, 54% and 72% were removed after A2O and advanced treatment units, resulting in 95% overall removal and 0.59 particles/L in the effluent. PET (42%), PES (19%) and PP (13%) were the overall dominant polymer types while, fibers made up 86% of the microplastics.

In Conley et al. (2019) influent and effluent waters were collected from three wastewater treatment plants in the U.S. on five different dates throughout a year. 7.5–11.5 L of influent waters and 30 L of effluent waters were grab sampled. In one wastewater treatment plant 24h composite samples were also collected with volume of 3.6 L from influent and effluent waters. Approximately 0.5 L of influent waters and 1.5–15.5 L of effluent waters were filtered through a 43 µm meshes and meshes were placed into an ultrasonic bath containing DI water. Samples were then dried at 65^{o}C

until dry and digested with 30% H_2O_2 for 30 minutes on a hot plate where they were heated to 65^oC. 6 mL of 1 M HCl was then added to digest inorganics and cellulosic matter. After reactions were complete, samples were introduced into DI water again and then screened through 418, 178 and 60 μm meshes. Fractions gathered from meshes were transferred into separate petri dishes sonicated and rinsed once more. The resulting fractions then transferred into petri dishes or membrane filters and then examined under a stereo microscope. FTIR microscope with a micro-ATR accessory was used in order to characterize the particles. 80–250 particles/L were found in the influent samples and concentrations were higher in samples collected in early summer and autumn. Fibers were at least 50 % of the particles observed. 60-178 μm size range was the most abundant fraction and abundance decreased with increasing sizes throughout influent samples. 3–29 particles/L were found in the effluent samples and concentrations were again higher in early summer but no significant difference was observed in autumn. Fibers were at least 75 % of the particles observed. Tertiary treatment plants showed greater presence of fibers in the effluent. Size distributions remained similar in the effluent samples however, the ratios of 60–178 μm were lower in these samples and nearly even distribution for 60-178 μm and 178–418 μm was observed. Removal rates for the facilities were 95.9 to 98.1%, 74.8 to 97.1% and 77.6 to 88% individually.

Edo et al. (2020) investigated a secondary wastewater treatment plant near Madrid, Spain for microplastics. Primary settler effluent and secondary settler effluents were investigated for three months. Samples were filtered through 375, 104 and 25 μm meshes in the laboratory following the grab sampling. Wet peroxide oxidation and density separation methods were used in order to extract particles from the media. Particles suspected as microplastics were removed from the media and then their polymeric characters were investigated with a FTIR microscope. Sludge samples and dried soil amendment samples (which were sold commercially) (1g) were treated with 30% H_2O_2 and filtered. Primary effluent streams contained an average of 451 particles/L where, secondary effluent streams contained an average of 26 particles/L. Effluent streams contained 56% of clear fragments, 24% of colored fragments with 15 % of clear fibers 5% of colored fibers. Sludge samples in contrast, showed significantly higher amounts of fibers where 47% clear fibers, 31% clear fragments, 15% colored fibers and 7% colored fragments were observed throughout the 314 particles/g dry matter. Dried sludge, which is used as soil amendment, contained 302 particles/gram product which was similar to the sludge sample. Subsample of 172 particles were investigated for characterization with FTIR and 77 of the investigated particles were microplastics. PE, PE, cotton and PP were the most abundant structures with decreasing order in primary effluents where PE, cotton, PET fibers and PP were the most abundant structures with decreasing order where PE dominated other particles. 10.7 particles of 26 particles/L in the effluent were confirmed as MPs where, the influent sample contained 171MP/L.

Samples from grit and grease removal chamber, primary effluent, biological reactor basin and effluent after secondary clarifier in Cabezo Beaza WWTP located in Cartagena, Spain was investigated by Bayo et al. (2020). Density separation was applied to samples before filtration except effluent samples, which were directly filtered on 0.45 μm filter papers. Chemical structures of isolated particles were determined via FTIR microscope. Average of 12.43 particles/L, 9.73 particles/L, 3.21 particles/L and 1.23 particles/L observed for grit and grease unit, primary clarifier effluent, biological reactor basin and effluent stream samples, respectively. 46.9% of particles were fragments where fibers were 7.4% of the particles detected in the study. Non-plastic particles were significantly abundant than microplastic particles (73.4-74.7% non-plastics) in the plant except for biological reactor which contained significantly higher amount of plastics (66.2% MPs). An average removal of 90.3% and 90.1% was observed for microplastics and overall detected particles, respectively. LDPE (2.83 particles/L), HDPE (0.94/L), ACR (0.83/L), PP (0.64/L), PEP (0.27/L), PS (0.21/L), BPL and NYL (0.19/L), PUR (0.14 particles/L), PET (0.13/L), MCR (0.11/L), PTFE, MMF, PES, PVI, PIB and RBB (<0.1 particles/L) were observed in the samples.

Park et al. (2020) investigated 50 wastewater treatment plants in South Korea. Filtrations were conducted on a 45 μm mesh and fragments were the most dominant fraction where 68.2% and 82.3% of particles were fragments in the influent and effluent streams, respectively. Most fibers were in <45 μm range. Influent samples contained 10-470 particles/L and effluent samples contained 0.004-0.59 particles/L of microplastics. PP was the most abundant polymer where PP, PE and PET were present by 39.6%, 25.6% and 21.3% in the influent stream, respectively. PP was, again, the most dominant polymer with 63.3% followed by PE (13.8%) and PET (13.3%) in the effluent stream. The removal efficiencies for wastewater treatment plants ranged from 98.7% to 99.99%.

In Akarsu et al. (2020) influent and effluent wastewaters from Karaduvar, Tarsus and Silifke WWTPs in Mersin, Turkey were investigated for a year. Samples were sieved through 26 μm meshes and digestion was carried out on the filter papers with 35% H_2O_2 prior to microscopic examination. 2.8 particles/L, 3.1 particles/L and 1.5 particles/L were found in the influent samples of Karaduvar, Tarsus and Silifke WWTPs, respectively. Effluent samples contained 1.6 particles/L, 0.7 particles/L and 0.6 particles/L for Karaduvar, Tarsus and Silifke WWTPs, respectively. Fibers were the most dominant shape (69.7%) in all samples. Influent samples contained 79% fibers (and 21% fragments) however, in effluent waters there was an abundance of fragments up to 32.8% observed in the study. The most common polymers from the subsample selected for characterization were PE followed by PP, ACR fibers, PS and cellulose acetate. Karaduvar, Silifke and Tarsus WWTPs achieved 38%, 58% and 78% of average removal of particles, respectively.

Studies reviewed have differed in many aspects such as sampling methods, sieve pore sizes, application of digestion or lack thereof. Method of digestion when applied and post sieving/filtration pore sizes after organic materials removal were also different in each study. The characteristics of wastewater treatment plants and their removal efficiencies, along with shapes of particles and characters of polymers differed throughout the studies. Table 2.1 summarizes the studies mentioned above. Removal rates were high in most of the studies. However, with the high effluent flowrate of wastewater treatment plants, considerable amount of microplastics were released to the receiving environment.

2.4.2. Analysis of Microplastics from Wastewater and Sludge

2.4.2.1. Sampling. Sampling of wastewaters for microplastic studies can be conducted in several ways. One of which is grab sampling into containers. This can be done by collecting the wastewater with steel buckets, glass jars, metallic beakers, Ruttner samplers and automatic samplers into glass jars, glass bottles, steel buckets/containers and plastic containers. Some of these collected waters are directly transported to laboratories in the containers and stored at 4^{o}C at dark. Some studies sieved the samples on site after collecting the water by grab samplers, before transporting them to the laboratory (Magni et al., 2019; Gies et al., 2018; Lares et al., 2018; Lares et al., 2019; Leslie et al., 2017; Michielssen et al., 2016; Murphy 2016; Magnusson and Norén, 2014; Tagg et al., 2015, Dyachenko et al., 2017). Other sampling method applied was collecting grab samples by direct sieving on stack of sieves in the wastewater treatment plant. The water was passed through the sieves with a connected pump applying a steady flow rate or directly pouring the sample onto the sieves (Carr et al., 2016; Lee et al., 2018). Sieve mesh size ranges differed from 400 μm to 20 μm (Carr et al., 2016) and sieves were wrapped during and after completion of sieving. The sieves were then transported directly to the laboratory. Lee et al. (2018) used 106 μm sieves and filtered influent and effluent wastewater samples on these sieves. Particles retained were recovered from the sieves and put into zip bags and transported back to the laboratory. Magni et al. (2019) sieved samples on site with sieves of 5 mm, 2 mm and 63 μm then transported them to the laboratory. Gies et al. (2018) sieved samples on site with 5 mm and 0.25 mm and recovered particles on 0.25 mm to glass jars. Murphy et al. (2016) sieved samples on 65 μm and the debris is collected into glass bottles. Carr et al. (2016) also proposed a surface sampling device skimming the surface of the waterfall on the effluent. However, surface sampler has the drawback of collecting only the microplastics with lowest densities and, thus, miss the microplastics deeper in the water column.

Table 2.1. Summary of studies on abundance and removal of microplastics in wastewater treatment plants

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Dris et al. (2015)*	France	Secondary Wastewater	330-80	x	x	260-320	14-50	88.9
Talvitie et al. (2015)*	Finland	Tertiary WWTP (Secondary Wastewater)	200-20	x	x	610	82.4	86.5
Talvitie et al. (2015)*	Finland	(Tertiary Wastewater/Biological Filter)	200-20	x	x	N.A.	13.5	97.8
Mason et al. (2016)*	USA	17 WWTPs (Wastewater)	355-125	WPO	x	N.A.	0.004-0.195	-
Carr et al. (2016)*	USA	Several WWTPs (Wastewater)	N.A.	N.A.	N.A.	N.A.	N.A.	99.9
Leslie et al. (2017)	Netherlands	7 WWTPs (Sludge)	x	DS	x		650 / kg wet weight	N.A.

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculated.

*No characterization of samples conducted with marked studies.

Table 2.1. (continued)

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Mintenig et al. (2017)	Germany	12 WWTPs (Wastewater)	10	ED+WPO	N.A.	N.A.	4-17	-
Magnusson and Noren (2014)	Sweden	WWTP (Wastewater)	300	x	x	N.A.	N.A.	99.0
Murphy et al. (2016)	Scotland	Secondary WWTP (Wastewater)	65	X	X	15.7	0.25	98.4
Murphy et al. (2016)	Scotland	Secondary WWTP (Sludge)	x	DR	x	19.67 / 2.5 g sludge		N.A.
Leslie et al. (2017)	Netherlands	7 WWTPs (Wastewater)	X	DS	x	73-238	9-91	73.9
Conley et al. (2019)	US	Three WWTPs (Wastewater)	43	WPO	418-60	80-250	3-29	74.8-98.1

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculated.

Table 2.1. (continued)

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Vollertsen and Hansen (2017)	Denmark	10 WWTPs (Wastewater)	500-20	WPO	x	130,000	8,000	93.8
Talvitie et al. (2017a)	Finland	Tertiary WWTP (Wastewater)	200-20	x	x	380-686	3.5-0.7	99.1-99.5
Talvitie et al. (2017b)	Finland	Tertiary Wastewater (DAF)**	200-20	x	x	2	0.1	95
Talvitie et al. (2017b)	Finland	Tertiary Wastewater (Disc Filter-10 µm)**	200-20	x	X	0.5	0.3	40
Talvitie et al. (2017b)	Finland	Tertiary Wastewater (Disc Filter-20 µm)**	200-20	x	x	2	0.03	98.5
Akarsu et al. (2020)	Turkey	3 WWTPs (Wastewater)	26	WPO	X	1.5-3.1	0.6-1.6	38-78

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculate.

**Samples taken after secondary treatment. Removal efficiencies are for tertiary units.

Table 2.1. (continued)

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Ziajahromi et al. (2017)	Australia	Three WWTPs (Wastewater)	500-25	WPO+DS	25	12	0.21-1.5	87.5-98.25
Lee et al. (2018)*	South Korea	Three WWTPs (Wastewater)	106	WPO+DS	106-300	13.9-29.9	0.14-0.44	98.0-99.1
Gies et al. (2018)	Canada	Secondary WWTP (Wastewater)	x	OEP	1	24.4-37.8	0.3-0.7	97.1-99.1
Gies et al. (2018)*	Canada	Secondary WWTP (Sludge)	x	OEP	1	4.4-14.9/g sludge		N.A.
Lares et al. (2018)	Finland	WWTP (MBR)	5000-250	WPO+ED	0.8	N.A.	1	98.3
Yang et al. (2019)	China	Secondary WWTP (Wastewater)	500-5	WPO+DS	10	12	0.59	95

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculated.

*No characterization of samples conducted with marked studies.

Table 2.1. (continued)

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Simon et al. (2018)	Denmark	10 WWTPs (Wastewater)	2000-500	ED+WPO	10	7216	54	99.3
Li et al. (2018)	China	28 WWTPs (Sludge)	x	AT	37	1.6 – 56.4x10^3 / kg dry sludge		N.A.
Gündoğdu et al. (2018)	Turkey	2 WWTPs (Secondary Wastewater)	x	WPO	55	1697-5400	325-1395	73-79
Magni et al. (2019)	Italy	Secondary WWTP (Wastewater)	5000-63	DS+WPO	8	0.5-2	0.03-.0.1	84
Bayo et al. (2020)	Spain	Secondary Wastewater	x	DS	0.45	12.43	1.23	90.3
Park et al. (2020)	South Korea	50 WWTPs (Wastewater)	x	WPO+DS	45	10-470	0.004-0.59	98.7-99.9

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculated.

Table 2.1. (continued)

Author(s)	Country	Type of Sample	Pre-Sieve (µm)	Digestion Protocol	Filter Paper/ Post Sieve (µm)	Influent Concentration (Particles/L)	Effluent Concentration (Particles /L)	Removal Rate (%)
Edo et al. (2020)	Spain	Secondary Wastewater	375-25	WPO+DS	375-25	451 (Primary Effluent)	26	94.2
Edo et al. (2020)	Spain	Sludge	375-25	WPO+DS	375-25	314 / g dry sludge		N.A.
Edo et al. (2020)	Spain	Soil Amendment	375-25	WPO+DS	375-25	302/g amendment		-N.A.

AT: Alkaline Treatment, WPO: Wet Peroxide Oxidation, ED: Enzymatic Digestion , DS: Density Separation, DR: Drying, OEP: Oil Extraction Protocol, x: Not Used, N.A: Not available in the study or non-applicable for the sample type, -: data that could not be calculated.

Composite sampling was also used for sampling wastewater with auto-samplers based on 24 hours with 15-minute intervals into plastic containers. (Simon et al., 2018; Dyachenko et al., 2017; Dris et al., 2015; Talvitie et al., 2015, 2017a, 2017b). Several studies used a mobile pumping and filtration device. Mintenig et al. (2017) used a membrane pump connected to a filter housing compartment with lid containing a 10 µm steel cartridge filter and a flowmeter for sampling effluent. Ziajahromi et al. (2017) used a pump connected to a contained vessel with 500 µm, 190 µm, 100 µm and 25 µm mesh screens to sample microplastics in primary, secondary and tertiary wastewater effluents. Talvitie et al. (2015, 2017a, 2017b) used a sampling device consisting of 200, 100 and 20 µm filter segments mounted on O-rings, in transparent tubing and connected to a pump for effluent sampling. These custom devices were generally suitable for effluent waters, due to clogging of the smaller mesh sized filters with high organic loads from other units of the wastewater treatment plants. In order to sample microplastics, <500 µm sieving in the laboratory was carried on with grab samples. Dyachenko et al. (2017) used 5 mm, 1 mm, 0.355 mm, 0.125 mm sieves. 5 mm sieves were used in order to screen out larger particles and reduce clogging of the smaller sieves. To summarize, sampling of wastewaters should be done covering the sizes <100 µm. As 5 mm is the upper size limit of microplastics and 500 µm is also used as upper size limit of microplastics in many studies, 330 µm is the size of the neuston nets and this value can be found in many studies on marine and freshwater systems (Hidalgo-Ruz et al., 2012; Rocha-Santos and Duarte, 2015). 80-50 µm range is the size of the plankton nets and 2-18 µm is the wavelength of IR light and for IR microscopy, theoretical resolution is 3.5 times of this range (Imhof et al., 2012).Therefore, sampling should include both 20-50 µm and 500-5000 µm range with the ranges in between in order to capture most of microplastics, if possible.

The volume of wastewater samples collected differed in the literature. Dris et al. (2015) collected 24hr composite samples in larger volumes but processed 0.05 L aliquots for microplastics examination. 2 to 285 L of effluent samples were collected by Talvitie et al. (2015). Murphy et al. (2016) obtained 30 L samples from all units of the treatment plant investigated in the study. Michielssen et al. (2016) obtained 1 to 2 L of wastewater in the influent stream, 1 to 6 L in primary clarifier, 10 to 20 L in primary effluent and 34 to 38 L in final effluent. Carr et al. (2016) took 0.1 L of influent samples taken, where from tertiary effluent stream $1.89x10^5$ L to $2.23x10^5$ L of water was sieved. The sample volumes were dependent on the method of the sampling as well as the character of the stream.

Sludge samples were taken with 0.25 L steel cups into glass jars by Lares et al. (2018, 2019) Mintenig et al. (2017) obtained sludge samples of 500 g wet weight (sludge dry weights of ~1%) with

a shovel and refrigerated at 4°C in PVC containers. Talvitie et al. (2017a) reported that the excess sludge samples were collected into metallic beakers and the dried sludge samples were taken manually. Magnusson and Norén (2014) obtained sludge samples from slightly dewatered sludge, while Lee et al. (2018) took the sample from dehydrator prior to transportation of the samples with glass jars and the latter with a zip bag. Magni et al. (2019) reported that the amount of sludge samples taken were 7.5 g of dry weight.

2.4.2.2. Extraction and Visual Analysis. Sludge and wastewaters have considerable amounts of organics and cellulose in their matrix, especially in influent wastewaters and early stages of treatment plants. Therefore, in order to investigate the microplastics in the wastewater, the organics have to be removed to facilitate a suitable condition for the identification and characterization of these particles.

Several studies investigated microplastic particles present in wastewaters at different stages of the wastewater treatment plant, without applying a pre-treatment method under a stereo microscope on meshes of custom devices used for sampling (Talvitie et al., 2015, 2017a, 2017b), petri dishes (Michielssen et al., 2016; Carr et al., 2016) after different physical treatments, on filter papers following vacuum filtration (Murphy et al., 2016) with 7x-100x (generally 50x) zoom. Magnusson and Norén (2014) studied the 300 μm filters containing solid residues by scorching the particles under the stereo microscope. Sludge samples were also investigated by Murphy et al. (2016) under stereo microscope after mixing the sludge samples, taking a sub-sample and drying. Sludge samples were investigated without applying a pre-treatment method as well by Talvitie et al. (2015, 2017a, 2017b). Same approach was used with wastewater samples except tap water or de-ionized water was added in order to dilute the sample before filtration.

Nuelle et al. (2014) proposed exposing wastewater the samples to 35% H_2O_2 for 7 days and stated that 92% of organic matter digestion or color removal was achieved through this process. Tagg et al. (2015) applied the method from Nuelle et al. (2014) and 15 mL 30% H_2O_2 was added to wastewater from aeration after centrifuging samples and filtering the extract on polycarbonate membranes (0.2 μm pore size). Masura et al. (2015) proposed an improved method for pre-treatment of samples from organic-rich environments. Sieving (5 mm-0.3 mm), collecting particles and drying at 90°C followed by mass determination was the first step. Second part was the addition of 20 mL of 0.05 M Fe (II) aqueous solution and 20 mL of 30% H_2O_2 followed by heating and stirring at 75°C and if needed addition of 20 mL more H_2O_2 and 30 minutes of heating and stirring. The authors also suggested a density separation step using 5 M NaCl solution and heating at 75°C to dissolve the salt added followed by separatory funnel step. Floating solids were sieved on 0.3 mm sieve and sieved

particles and settled solids were tared then investigated under a stereo microscope. Tagg et al. (2017) used Fenton's reagent used by Masura et al. (2015) with ratio of 1 mL of 30% H_2O_2 to 0.5 mL catalyst in pH 5 solution but following the method by Tagg et al. (2015), where exposure time to react was 10 minutes.

Ziajahromi et al. (2017) used wet peroxide oxidation (WPO) with Fenton reagent according to Masura et al. (2015) with some modifications being; 60^{o}C for stirring and heating, NaI (99.9%) solution for density separation on dried extracts with centrifugation and filtration of buoyant particles through a 25 µm mesh. After sieving, author proposed a staining step with 5 mL of 0.2 mg/mL Rose-Bengal solution to stain non-plastic particles and washing the dye afterward.

Dyachenko et al. (2017) adapted the method from Masura et al. (2015), however, as cellulose and fatty acids were not digested by WPO process, a sequential WPO process was proposed in order to achieve a more effective digestion. After one digestion, the extract was filtered through a 0.125 mm sieve and then rinsed with hexane and washed with DI water for three times. The content on the sieve was then transferred to a beaker for another WPO digestion. 3 to 6 digestions were deemed necessary in order to remove most cellulose and other organic interferences. Extracts are concentrated onto membrane filters and particles were isolated from the filters. Study rejects centrifugation as it may cause deformation, fragmentation and compaction of the particles; rejects microwave digestion as it would lead to decomposition of the particles due to high heat. Although the method claimed success on digesting cellulose and fatty acids effectively, hexane used for rinsing the particles may cause damage on several types of particles (Herrera et al., 2018).

Lares et al. (2018) used a modified version of Masura et al. (2015). Sieved samples were dried at 75^{o}C as 90^{o}C is above the continuous operating temperatures of several polymers. Extracts were directly filtered through 0.8 µm pore size cellulose nitrate filters with 1.5 µm pore size glass fiber filters at the bottom for mechanical support. As influent samples contained cellulose fibers in large amounts, 0.25 g dry wt. were treated with Fenton's reagent and sieved on 38 µm meshes and rinsed to glass flasks. Subsequently, 250 mg of cellulase was added to 250 mg dry wt. of influent sample and sodium citrate buffer (pH 4.8) was added before placing into a controlled shaker at 4^{o}C for 24 hours. Mintenig et al. (2017) used a combination of SDS, several enzymes and for particles <500 µm an additional step of H_2O_2 application on the cartridge filters that samples were concentrated on. Particles were concentrated onto aluminum oxide filters for visual and spectroscopic examination. Simon et al. (2018) proposed another modification for WPO process with Fenton's reagent. After sieving 1 L samples on 2 mm, 1 mm and 500 µm sieves, SDS was added to 0.15 g/L, Fe (II) solution

to 2.5 g/L and H_2O_2 to 250 g/L were added to 200 mL of sieved samples for oxidation. The pH was adjusted to approximately 3 as complexation of ferric or ferrous ions occur at pH below 2 and above 4, which reduces active Fe (II) ions and therefore de-accelerating the reaction. Reactor temperature was maintained between 15 and 30^{o}C to avoid violent boiling as the reaction is exothermic and, a ferric precipitate was formed below 15^{o}C. Oxidized samples were fractionated with an 80 µm sieve mesh and filtrates of 80 µm mesh were concentrated on 10 µm meshes and suspension was incubated with cellulase and all fractions from filters and resultant extract from incubation were investigated. Gies et al. (2018) allowed solids in the samples to settle overnight and digested solids with H_2O_2 for 7 days. Suspended layer was processed with oil extraction protocol (OEP) where 5 mL of canola oil and decanted layer were poured into a 1 L separatory funnel and shaken for 30 seconds vigorously. Wastewater and canola oil layers were formed in 1-2 minutes and layers have transferred to separate flasks and the process repeated three times. The canola layer which collected microplastics were then filtered onto membrane filters. The funnel was rinsed with detergent and the rinsate was also filtered.

Influent wastewater and digested sludge samples spiked with PS, PE, PVC, PET, PA, PP and SBR were investigated for different treatment methods by Lares et al. (2019). Filtration method (device) from Talvitie et al. (2015) was tested. WPO with Fenton's reagent by Lares et al. (2018) and, modification with fractionation with a lower size mesh (20 µm) and drying and reaction at lower temperature (50^{o}C) were tested for influent samples (0.26-0.28 g dry wt.) and dried sludge samples (0.15-0.23 g dry wt.). Drying method (Murphy et al., 2016) was investigated only for sludge samples. Digestion with KOH was evaluated with the addition of 1:10 (w/v) 10 % KOH solution to samples. OEP was investigated according to Crichton et al. (2017). Density separation with KHCO2 solution (density 1.5 g/cm3) was evaluated according to Zhang et al. (2016). Total recoveries (average) were found to be between 84.8 and 92.9 % and the most efficient method was filtration for both wastewater and sludge followed by WPO at 50^{o}C and 75^{o}C, respectively. Drying at 45^{o}C did not significantly affect the recovery rates of filtration, however, it decreased processing time for wastewaters and no visible change was observed except browning of one PS bead. 20 µm mesh for WPO resulted in gain of few fibers collected. The treatment at 50^{o}C did not cause color change for PET fibers in the study but only shinier surfaces or browning on PS particles. Staining of particles with Rose Bengal solution, after pre-treatment did not prove useful in the study as it did not stain cellulose particles like it stained other organics in the sample and, it caused some color change in some PS and PVC fragments especially after WPO treatment.

Diluted solution of bleach (8.25% sodium hypochlorite) was used by Carr et al. (2016) for digesting the grab samples from skimmings, scum in aeration tanks, sewage sludge, gravity filter

backwash and biosolids before examination of particles. WPO and Fenton's reagent were also used to digest organics present in sludge samples (Sujathan et al., 2017; Li et al., 2018; Lares et al., 2018). Comparison of different treatment methods were studied on PP, LDPE, HDPE, PS, PET, PC, PMMA and PA-66 particles by Hurley et al. (2018) in order to determine the method's effects on particle structures and efficacy of the removal of organic material in organic rich samples such as sludge. Authors compared 30% H_2O_2 treatment at 60°C and 70°C, Fenton's Reagent (with ratio of 1:1), NaOH solutions in 1 M and 10 M concentrations at 60°C and 10% KOH solution at 60°C. NaOH caused surface degradation in several polymer types; even in lower concentrations such as 1 M. WPO at 60°C, Fenton's reagent and 10% KOH did cause minimal to no damage to the particles, but KOH was not appropriate for organics degradation. WPO at 70°C and Fenton's reagent were the most effective methods for organic material removal, however, WPO at 70°C caused degradation of several polymer types. Therefore, Fenton's reagent at 60°C was selected as optimum protocol in the study. Modifications of temperature adjustment between 30-50°C (~40°C optimum) and pH adjustment around 3 were recommended for better preservation of microplastics, in order to decrease decomposition of H_2O_2 and help reduce the precipitates of hydroxide species.

2.4.2.3. Polymer Characterization. Visual analysis of particles was the common method on earlier analysis, however, Dekiff et al. (2014) could only assign 49% of the particles suspected to be microplastics as plastics and Lenz et al. (2015) found that 75% of them were synthetic particles or fibers.

Pyr-GC-MS can be used to detect the polymer types. SEM-EDS/EDX or ESEM-EDS was also used in order to investigate the morphology of the particles and gather information on elemental composition in order to comment on the plastics presence. The smallest size detected by Pyr-GC-Ms reported to be was 100 μm (Dekiff et al., 2014). TED-GC-MS and LC were also utilized to characterize the particles. Although newer studies report that lower size limits are non-important for these analyses, outputs of elemental composition and mass is still not clear in order to characterize the particles (Sun et al., 2018). SWIR imaging spectroscopy was proposed for characterizing the particles by Scmidt et al. (2018), but the detection limits were 560x280 μm or 450 μm in diameter for efficient results.

Microplastic studies widely used FTIR spectrometry for chemical characterization of the particles. 4000-600 cm^{-1} wavenumber range was used with spectral resolution of 8 cm^{-1} to 4 cm^{-1}. Murphy et al. (2016) gathered 16 scans per spectrum, while Talvitie et al. (2017a, b) gathered 15 scans per spectrum. ATR module with diamond crystal was used by Carr et al. (2016) and Ziajahromi

et al. (2017), where the spectral resolution was 8 cm^{-1} and 128 scans were taken to produce spectra. Gies et al. (2018) used wavelength range of 900-3800 cm^{-1} at spectral resolution of 8 cm^{-1}, while 16 scans were accumulated for FTIR analysis. Particles >3 mm analyzed with diamond ATR accessory and particles <3 mm were analyzed with germanium micro-ATR accessory. In micro-ATR microscope slides were covered with thin layer of 2% dextrose to stabilize particles. Mintenig et al. (2017) conducted characterization analysis with FTIR spectrometer equipped with platinum ATR unit, microscope with 15x objective and 64x64 FPA detector. 25% of the filters were analyzed and two crossed 7x65 FPA arrays with assumption of overlapping area in the center of the cross only once was used. Spectral resolution was 8cm^{-1} with 6 scans with a binning factor of 4. For fibers a grid of 2 FPA fields was measured without active binning and number of scans were increased to 32. The method demanded time of approximately 10 hours for counting and characterizing the fibers. Simon et al. (2018) used 15x objective and 128x128 mercury FPA detector. 10-80 μm particles were analyzed in transmission mode and 80-500 μm particles were analyzed in reflection mode. Spectral range of 3750-950 cm^{-1} at 8cm^{-1} spectral resolution was used with 30 scans per spectra. Tagg et al. (2015, 2017) investigated filters which were split into 5 regions with FPA field of 25 μm x 25 μm, with 2 co-added scans per spectra produced where spectral resolution of 16cm^{-1} in wavenumber range 4000-650 cm^{-1} was used.

Lares et al. (2018, 2019) used wavelengths between 4000-600 cm^{-1} and spectral resolution of 4 cm^{-1} were used with 24 scans taken per spectra for micro-FTIR analysis. Baseline correction, data tune-up and normalization were done for the FTIR spectra. 4000-600 cm^{-1} wavenumber range was investigated with 15x objective and MCT single detector with 4 cm^{-1} resolution and 32 scans were taken per spectrum by Kappler et al. (2015). In the same study, spectrometer was combined with an FPA detector 4000-900 cm^{-1} wavenumber range was used with spectral resolution of 8cm-1. Fields of 6x9 were measured with FPA detector corresponding to 1000x1500 μm^2. 16 scans were taken for spectra and co-added. According to Kappler et al. (2016), microplastics >500 μm were investigated using a FTIR microscope with micro-ATR-FTIR spectrometer was used with 20x ATR objective with germanium crystal and MCT single element detector. Spectral resolution was 4cm^{-1} and wavenumber range was 4000-600cm^{-1}. 100 scans were co-added for every spectrum. Microplastics <400 μm was investigated with a FTIR spectrometer coupled with a FTIR microscope with 15x objective and area of 6x6 FPA fields (1000 μm x1000 μm) in transmission mode in a wavenumber range of 3400-900 cm^{-1}. 4x4 binning and 8 cm^{-1} spectral resolution was used where 32 scans were co-added for every spectrum. Zero filling factor 2 and 4 (Kappler et al. 2015 and 2016), Blackmann-Harris three term apodization and Mertz phase correction were used. Background was measured with same parameters.

Raman spectroscopy was another method widely used for microplastic research combined with a microscope (micro-Raman) (Lenz et al., 2015; Kappler et al., 2015, 2016; Dyachenko et al., 2017; Ossmann et al., 2017; Gündogdu et al., 2018). 532 nm laser was used by Kappler et al. (2015, 2016) with 20x objective in wavenumber range of 160-3600 cm^{-1} with laser power of 0.5-10 mW. Integration time was 500 ms and 100 scans were accumulated to acquire spectra. 532 nm laser was also used by Ossmann et al. (2017) with 600 grooves per mm, 300 µm confocal hole width, 100 µm slit width and 50x objective 1.2 and 3.2 mW laser powers were used for the lasers in wavenumber range of 150-3500 cm^{-1} with 1 second acquisition time and 2 scans were taken per spectrum. Intensity correction was performed. Gündogdu et al. (2018) used 514 nm Ar+ laser with 20x objective to investigate particles. Lenz et al. (2015) used 455 nm laser with 1200 gratings/mm with 10-50x objective. Wavenumber range was 100-3500 cm^{-1} with 0.96 cm-1 spectral resolution. Integration time was 2 seconds and 3 or more scans per spectra accumulated. Dyachenko et al. (2017) used 632.8 nm laser with 600 lines/grating to confirm cellulose particles extracted. Ossmann et al. (2017) and Gündogdu et al. (2018) used 785 nm secondary lasers to verify particles scanned with 532 or 514 nm lasers and to scan unidentified particles with aforementioned lasers.

Conducting microplastic research using filters to concentrate the particles and with transmission FTIR imaging requires a filter substrate that has to be IR transparent in a wide wavenumber range and have to be water resistant. Löder et al. (2015), recommended an aluminum oxide membrane filter (Anodisc) for FTIR imaging in transmission mode, however, the filter had self-absorption in the 1400-600 cm^{-1} range and was only usable in range of 3800-1250 cm^{-1}. Second suitable material was polycarbonate filter which had a wider range of wavenumbers, but had image outputs less clear than aluminum oxide filters which were hard to acquire a spectrum. Ossmann et al. (2017) also covered different membrane filters that are commercially available and coated white PC membranes with different metals in order to acquire a suitable filter for Raman imaging and automatic particle detection. Aluminum coated PC membrane filter was the most suitable option and showed no background with the main laser (532 nm) and showed background on 2850-3100 cm^{-1} band. Kappler et al. (2015) proposed a filter produced from Si for both FTIR and Raman imaging. Authors listed conventional IR transparent substrates as NaCl, KBr, CsI which are water soluble; KRS-5 (a solid solution from thallium bromide and thallium iodide), which is toxic and; CaF2, ZnS, etc. which are not suitable for creating holes for filtration purposes. The Si filter produced was compared to best commercially available alternative (Anodisc). Anodisc showed significant self-absorption in 1400-600 cm^{-1} range in FTIR measurement therefore, spectra were completely masked in 1250-600 cm^{-1} range. Si filter was found to represent all spectral characteristics of the polymers successfully. For direct introduction of particles, glass microscope slides, gold coated slides, ZnSe windows (in

transmission mode), microscope reflection slides (in reflection mode) and calcium fluoride (CaF_2) windows can be used to characterize isolated particles (Talvitie et al., 2017b; Simon et al., 2018; Mintenig et al., 2017).

3. PURPOSE

As discussed in the previous chapters, plastic and microplastic pollution with respect to their size, their invisibility to the naked eye and their durability in the environment is a fact that have attracted attention in the last decade. Structural properties of plastics provide manufacturers a great convenience and their production still continues in significant amounts and they are widely used in personal care products. Their direct effects on organisms in aquatic environments via feeding and indirect effect of releasing the additives which are toxic to organisms of different levels present in their structure are studied widely in the literature. Their potential to carry hydrophobic chemicals and antibiotics by sorption and desorption also, their potential to carry microorganisms over long distances were evaluated. The microorganism layer attached to the microplastic particles and transport of chemicals and antibiotics with this layer have also been mentioned recently. Therefore, microplastics is an important pollutant for investigation in the environment.

Wastewater treatment plants are major potential receivers of primary microplastics such as beads in personal care products, fibers from washing of clothes in high amounts and secondary microplastics from combined sewage systems due to stormwater runoffs. Therefore, they must be investigated to prevent any damage to the environment. Wastewater treatment plants were generally reported successful for the removal of the microplastics (70%-99.9%). However, they still discharge considerable amount of microplastics into environment and considered to be as an important point source. Regarding to the structure of microplastics and the contents of wastewaters such as organic material, various microorganisms, potential chemicals from fugitive discharge of industrial plants and from chemicals used in cleaning and antibiotics used by people; queries of potential hazards arise and therefore, the assessment of microplastics in wastewater streams is crucial both for their direct hazards and indirect hazards to the organisms that they interact with.

Sludge cakes from wastewater treatment plants also contain the retained microplastics from the water streams and they are either dried and used in furnaces or disposed into landfills at the moment in Istanbul. However, in the past they were used as fertilizers in the soils for farming and they can still be used for various purposes such as landscaping and growing fruit trees. Therefore, sludge cakes from wastewater treatment plants should also be investigated for their microplastics content in order to assess the contamination resulting from application of dried sludge and the wet sludge to the land or their storage in the landfills.

Studies considering the determination of microplastics in wastewater treatment plants differentiate in data and sample collection and processing; report the MP concentrations in different units and use different compositional segmentation due to different analyzing methods. Variability of influent loads, temporal conditions and plant operational conditions make the assessments of microplastics difficult. Reviewed studies showed that microplastics in smaller sizes are most likely to be released into environments and detection of these microplastics would lead to a more realistic approach to the microplastics problem with wastewater treatment plants being a point source. The removal of MP may vary depending upon the various units and operations used in wastewater treatment plants. In the studies reviewed from the current literature, only few studies investigated the effects of various units in WWTP on the MP removal efficiency.

This study was conducted to investigate unit-wise differentiation of wastewater treatment plants on microplastics removal. Therefore, this study aims to;

- Evaluate the overall removal performance by investigating the concentrations of microplastics in the influent and effluent of wastewater treatment plants.
- Investigate wastewater microplastic concentrations after physical treatment and biological treatment units with advanced treatment processes in order to assess the microplastics removal after each treatment stages.
- Determine the microplastics presence in WWTP sludges.
- Evaluate the possible effect of sludge when landfilled or used for landscaping purposes.
- Compare differences in MP concentrations in composite and grab samples in order to discuss the existing information in literature.

In order to reach the objectives of the study, several locations in a tertiary wastewater treatment plant with advanced nutrient removal unit was investigated for removal rate of microplastics. The samples were fractionated on stacks of sieves in order to collect solids and then these solids were digested in order to remove organics from the samples. The particles were then concentrated on filter papers by vacuum filtration. Particles suspected as microplastics due to their morphological properties were isolated from the filter papers and sorted on a clean filter paper, photographed, counted and measured.

4. MATERIALS AND METHODS

In this chapter, the experimental methodology for sampling, extraction and separation of the particles suspected to be microplastics are presented. The first section of this chapter describes the sampling of wastewaters and sludge from wastewater treatment plants and gives a general information on the facility where the sampling took place. In the following section, procedures for the digestion of organics to recover particles from the samples were explained. Following these procedures, separation of the particles under the stereo microscope followed by counting and classifying of the particles were elaborated.

4.1. Description of Investigated Facilities, Sampling Points and Sampling of Wastewater and Sludge

Samples were collected from Ambarlı WWTP, a tertiary wastewater treatment plant with a capacity of 400,000 m^3/d and an average effluent discharge of 336,820 m^3/d. Plant produces 84.1 tons of dried sludge daily. The effluent stream of the plant reaches to Marmara Sea via a water channel. Wastewater samples were collected from raw influent of the wastewater treatment plant, effluent of grit/grease removal chamber, effluent of aeration basin and, final effluent of the wastewater treatment plant. Throughout this study; these samples were referred to as influent sample (IN), physical treatment effluent (PHG for grab sample and PHC for composite sample), biological treatment effluent (BIO) and final effluent sample (EFG for grab sample and EFC for composite sample), respectively. Sludge sample was taken from dryers followed by thickeners and were referred to as dried sludge sample (SLD). Sampling was conducted on 26.12.2019. Flow chart of the advanced wastewater treatment plant investigated is illustrated in Figure 4.1, and sampling points given in Table 4.1. were marked on the illustration.

Table 4. 1. Sampling locations of Ambarlı WWTP.

No.	Name of Sampling Location	Abbreviation	Units Passed*
1	Raw influent	IN	-
2	Physical treatment effluent	PHG/PHC	Screens, Grit/Grease Removal Chamber
3	Biological treatment effluent	BIO	Phosphorus Removal Basin, Aeration Basin
4	Final effluent	EFG/EFC	Secondary Clarifier
5	Dried sludge	SLD	Thickener, Dryer

*Following the previous unit(s).

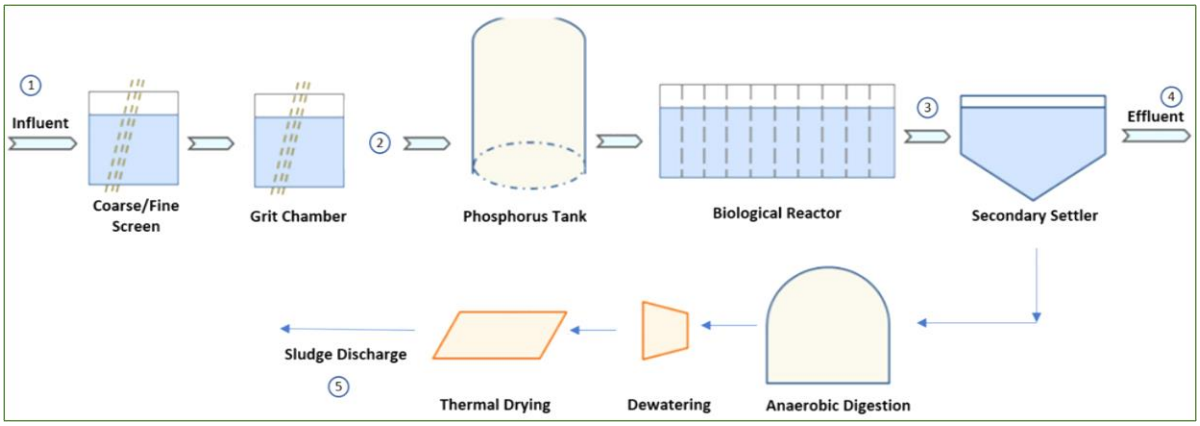

Figure 4.1. Flow diagram of WWTP and sampling points (Modified from Akarsu et al., 2020).

There is no standard method for sample collection and extraction of microplastics from organic rich media as explained in the literature review section. Grab sampling from wastewater and sludge in different volumes were conducted with respect to organic content of the waters. Wastewater sample volumes were 5 L for raw wastewater, 10 L for physical treatment effluent, 1 L for biological treatment effluent. Two identical streams were present in the wastewater treatment plant. 30 L of final effluent was sampled as a mixture of 15 L from each stream. Physical treatment effluent and final effluent were additionally sampled as 3hr composite samples with 15-minute intervals. Dried sludge sample volume was 0.25 L. Samples were held at 4^{o}C for maximum of 7 days before pre-treatment and extraction.

4.2. Pre-Treatment and Extraction

Extractions were conducted with wet peroxide oxidation method as proposed by Masura et al. (2015) with modifications according to Lares et al. (2019) and method proposed by Nuelle et al (2014) depending on the organic content of the sample. The smallest mesh for sieving was 25 µm where largest mesh size was 2 mm. Filtrations were carried out on 50 µm nylon meshes.

Collected wastewater samples were sieved through meshes with sizes of 2 mm, 1 mm, 500 µm, 125 µm, 63 µm and 25 µm. Solids retained on the sieves were collected into flasks according to size fractions of >500 µm, 500-63 µm, and 63-25 µm with minimal amount of de-ionized water. Dried sludge sample was directly subjected to wet peroxide oxidation after weighing.

Samples with low suspended solids were digested for 7 days (Nuelle et al., 2014; Tagg et al., 2015) with 10-40 mL of 35% H_2O_2. Suspended solids content was determined by visual examination. If the amount of suspended solids were high and the color of the suspended solids were yellow-brown

or brown, the sample was considered to have a high organic content. Samples with visually detectable and/or higher organic content and sludges were subjected to wet peroxide oxidation with Fenton's reagent which is 0.5 M Fe (II) SO4 solution with 30% or 35 H_2O_2. Samples let react for 5 minutes and subsequently, the solutions were stirred and heated at 50^oC for 20 minutes. The volumes of Fe (II) SO4 solution and H_2O_2 were determined by visual inspection and ranged from 0.2 mL to 20 mL for Fe (II) SO_4 solution and 20 mL to 150 mL for H_2O_2 depending on the type of the sample. pH and temperature of the mixtures were monitored throughout the study. Temperature of mixtures was around 40-50^oC while pH of mixtures was around 4 during the digestion process. If there are organics left in the samples by visual detection, H_2O_2 were added by 20 mL increments and reactions were carried out under the same conditions for 30 minutes. The peroxide addition was conducted until no reactions were observed and digestion was completed. If there were still visual organics and no reactions were not observed due to volume of the reacting flask was full, the mixture was left to settle and aqueous phase was filtered then subsequently the digestion for the settled fraction was started with same parameters as the first digestion stage. Sequential digestions were carried out until no reaction was observed. Overall reaction times were not more than 7 days for all the methods applied. Particles were then concentrated on nylon mesh filters with 50 µm mesh sizes by using vacuum filtration for the mixtures.

Two petri dishes filled with DI water were placed on the lab counters at different locations and under the hood during the studies in order to understand the potential airborne contamination. Filter papers/meshes that were cut from a larger piece were investigated under a stereo microscope after reaching the desired size and DI waters in petri dishes were filtered through these meshes in order to assess contamination during laboratory work. Lab coats were worn all times while processing the samples.

4.3. Visual Examination and Classification

Microplastics were visually investigated under a stereo microscope. The particles suspected to be MPs were separated into a clean petri dish containing a clean cellulose filter paper, previously checked for airborne contamination by using a micro-tweezer. Olympus SZX 16 stereo microscope with 30x magnification was used in order to separate the particles. 5 mega-pixel camera, which the microscope is equipped with, was utilized in order to photograph the particles detected and/or separated. ImageJ software was used for counting and measuring the particles that were separated in order to acquire the size and shape distributions.

5. RESULTS AND DISCUSSIONS

3,802 particles were detected in all samples from Ambarlı WWTP. Figures 5.1 to 5.4 display pictures of particles detected and separated from samples taken from several sampling locations in Ambarlı WWTP on 29.12.2019. Shapes, colors and sizes of the particles along with their effect on removal will be investigated in detail throughout this chapter.

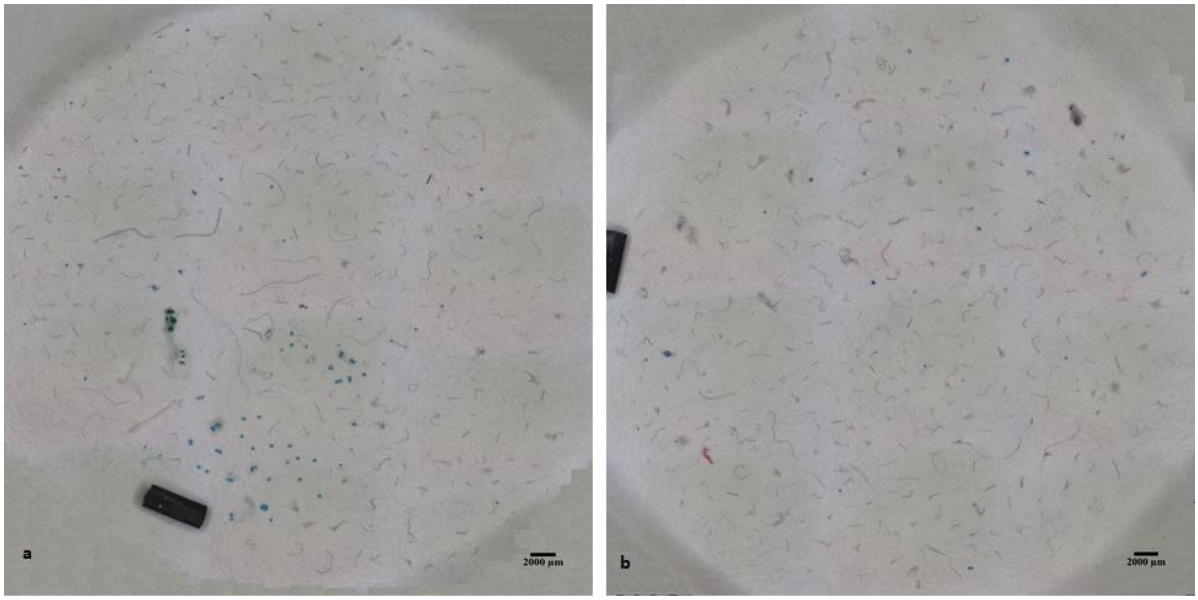

Figure 5.1 Particles separated from IN (a, b).

In the raw influent sample of 5 L, 734 particles were detected which were composed of 642 fibers, 89 fragments, 3 nylons. 643 particles were found in 10 L of grab physical treatment effluent sample composed of 608 fibers, 17 fragments, 14 nylons and 4 glitter particles. Composite physical treatment effluent sample of 10 L after physical treatment contained 718 particles where 702 of those particles were fibers and 12 and 4 particles were fragments and nylons, respectively. Biological treatment effluent sample contained 436 particles in 1 L where 396 of those particles were fibers and 23, 3 and 14 particles were fragments, nylons and glitter particles, respectively.

In the grab final effluent sample of 30 L, 704 particles were detected which were composed of 633 fibers, 27 fragments, 44 nylons. Composite effluent sample of 30 L contained 350 particles where 337 of those particles were fibers and 11 and 2 particles were fragments and nylons, respectively. 218 particles were found in dried sludge of 3 g where 202 of those particles were fibers, 15 and 1 of the particles were fragments and pellet, respectively.

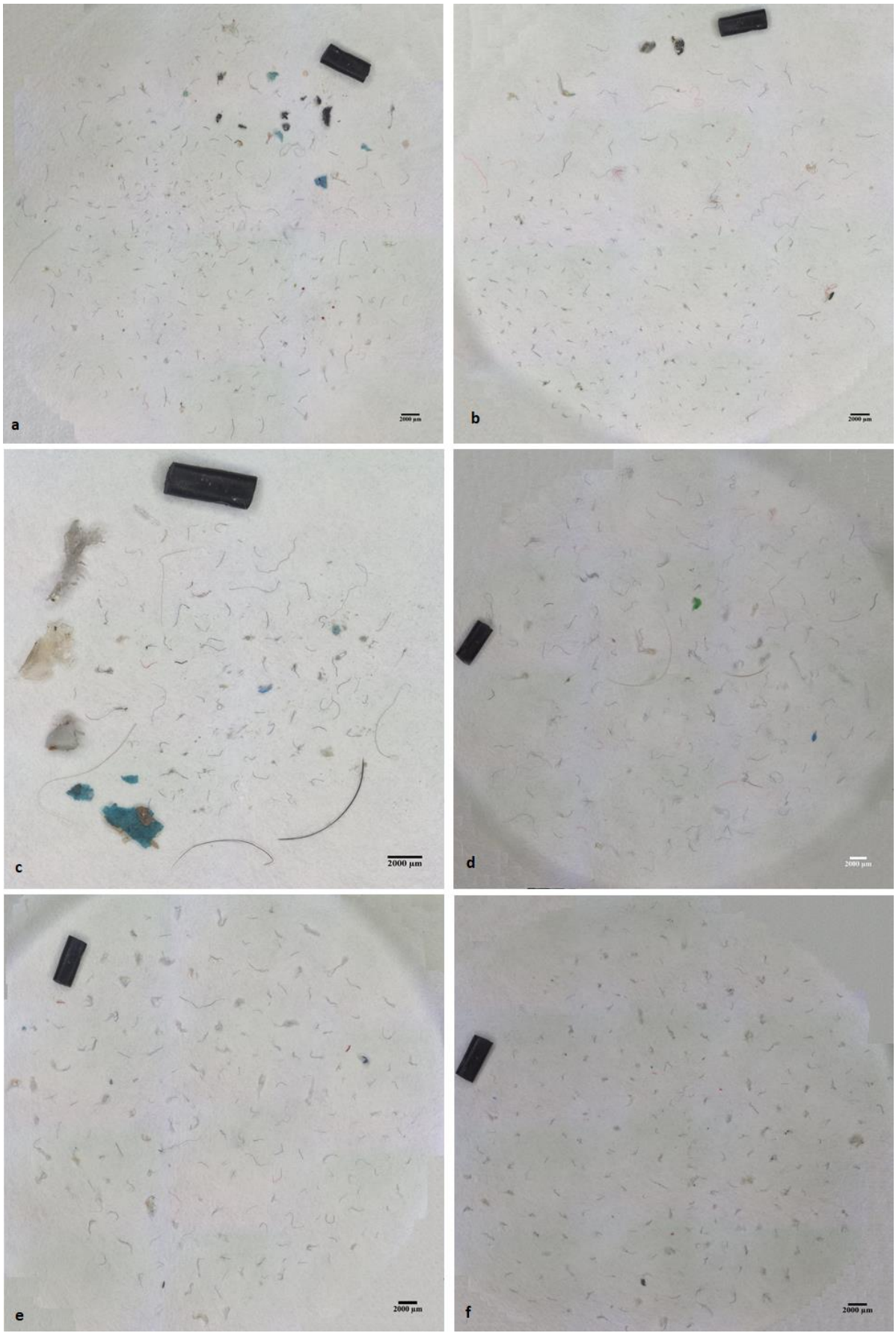

Figure 5.2. Particles separated from PHG (a, b, c) and from PHC (d, e, f).

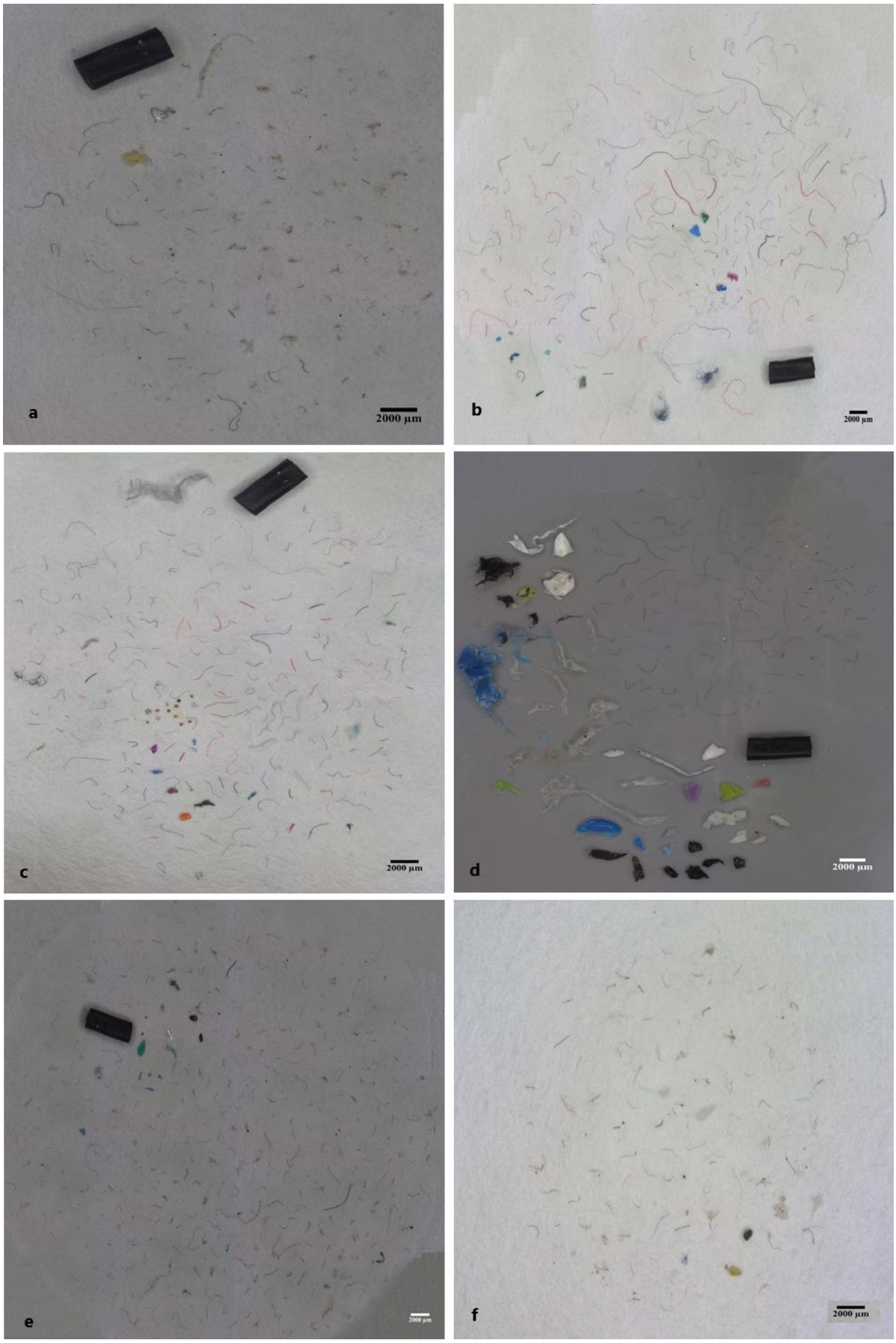

Figure 5.3. Particles separated from PHC (a), BIO (b, c) and EFG (d, e, f).

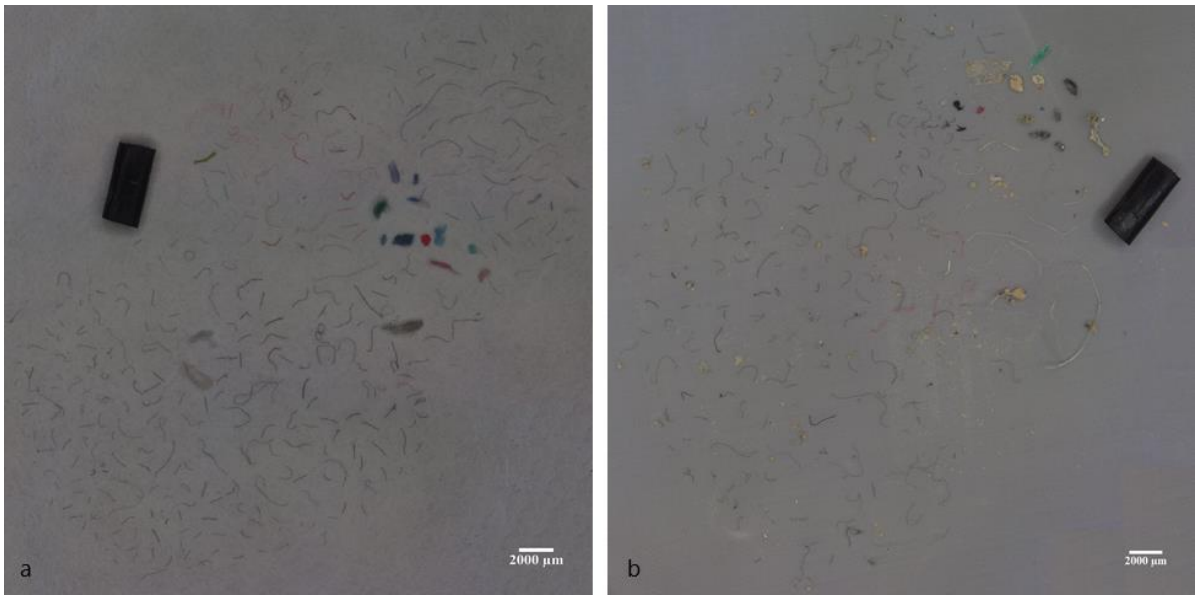

Figure 5.4. Particles separated from EFC (a) and SLD (b).

Contamination was calculated from DI water that were filled in two petri dishes during the laboratory studies and subsequently filtered from the 50 µ meshes after the laboratory work finished. Contamination was assessed as particles per L. From the control samples planted during laboratory work, the contamination was calculated as 0.73 fibers/L, daily. Average digestion and filtration times were different among the samples taken from different treatment units and contamination estimation differed from 2.92 (3) particles (for final effluent samples) to 9.69 (10) particles (for dried sludge samples) and were subtracted from amounts of fibers. A total of 122 particles isolated from the samples were excluded from the calculations, since their sizes were larger than 5 mm, which is the upper size limit of microplastics. 113 of these particles were fibers where 3 and 6 of these particles were fragments and nylons, respectively.

5.1. Distribution of Particle Shapes

Throughout the samples, fibers were the most dominant type since 92.6% of detected particles were fibers. Fragments were found as 5.2%, nylons as 1.7%, glitter as 0.5% and only one pellet was found which did not affect the percentage of the particles. Particle shapes varied in different units of WWTP, as seen from Figure 5.5 and Table 5.1. Composite samples also differed in shape distribution where, fibers were more abundant than particles in other shape categories and their grab replicates.

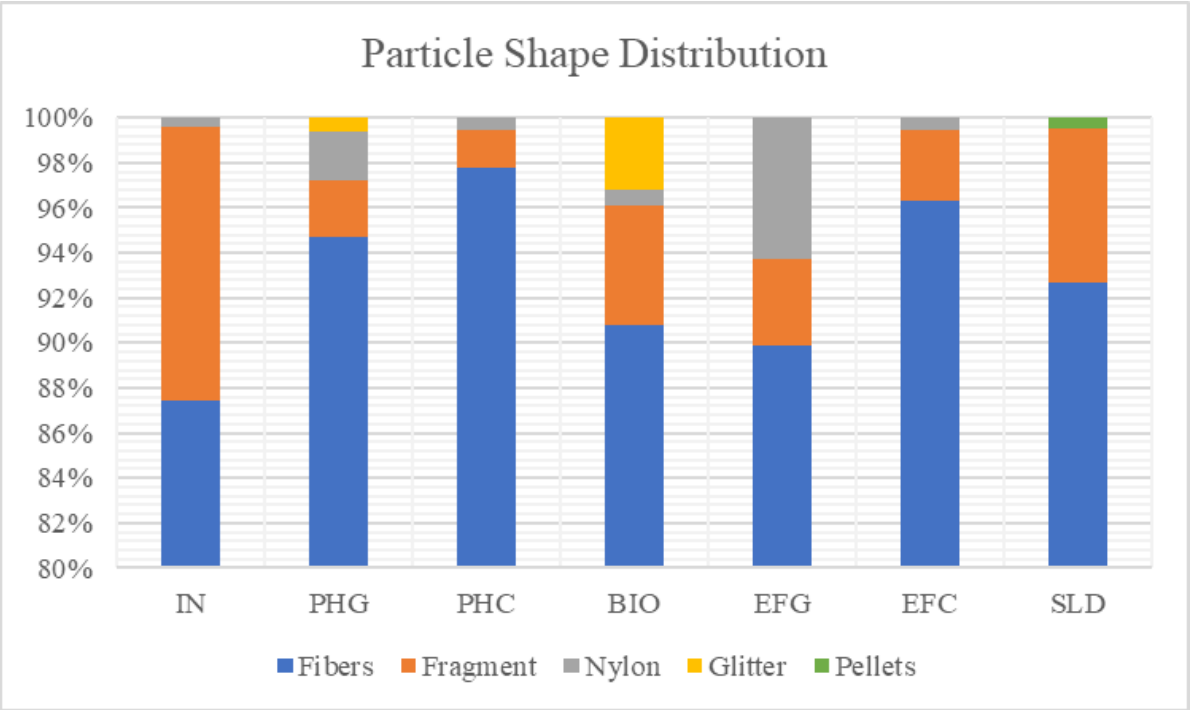

Figure 5.5. Distribution of polymer shapes throughout the WWTP.

Particle categories of nylon and fragments differed with hardness of the particles. Using the forceps, a small force is applied to the suspected particles and hard plastics classified as fragments and soft plastics are classified as nylons. Particles with hexagonal geometry that are notched in shape with straight edges, in various colors and with sizes of nearly 250 µm were classified as glitter. Glitters can contain PET films, PES films and rubber or Alkyd resins in their center (Yurtsever, 2019). A probable piece of a rubber band found in the dried sludge sample, which was sticking onto the forceps during separation, was also classified as fragment. Pellets (or microbeads) generally have spherical shapes and equal dimensions and they are produced as microparticles. Particle found in the dried sludge sample was categorized as pellet. The particle had smooth circular edges with a shape that is not spherical but slightly overcast and elliptic. Several examples of detected particles in mentioned categories can be observed in Figures 5.6 and Figure 5.7.

Fibers abundance increased from influent to effluent of the investigated wastewater treatment plant where nylons also increased and fragments decreased in abundance. Although, the trend was similar in both composite and grab samples, the rates of abundance changes were different, especially for fibers and nylons. Removal dynamics also differed between the sampling locations of the wastewater treatment plant for different shapes of particles. Removal dynamics of different shapes can be seen in Tables 5.2. and 5.3., where the changes in percent abundance throughout the units of WWTP were presented. The location pair given in tables represent two locations in the wastewater treatment plant where the change in percent abundance occurred.

Table 5.1. Particle shape distribution in sample locations of the WWTP.

Sampling Location	Fiber %	Fragment %	Nylon%	Glitter %	Pellet %
IN	87.13	12.45	0.42	0.00	0.00
PHG	94.75	2.54	2.07	0.64	0.00
PHC	97.99	1.58	0.43	0.00	0.00
BIO	90.23	5.66	0.51	3.60	0.00
EFG	90.30	3.84	6.25	0.00	0.00
EFC	96.29	3.14	0.57	0.00	0.00
WW	92.59	5.04	1.84	0.52	0.00
SLD	92.42	7.11	0.00	0.00	0.47
TOTAL	92.73	5.08	1.84	0.47	0.03

*WW represent overall wastewater samples and TOTAL represent all particles detected in different units of the WWTP.

Table 5.2. Removal dynamics based on shapes in grab samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance				
	Fiber	**Fragment**	**Nylon**	**Glitter**	**Pellet**
IN-PHG	+7.73	-10.23	+1.81	+0.69	0.00
PHG-BIO	-4.25	+3.00	-1.72	+2.97	0.00
BIO-EFG	-0.66	-1.64	+5.98	-3.66	0.00
IN-EFG	+2.83	-8.87	+6.06	0.00	0.00

*Values represent the percent change of abundances between given sampling locations.

Table 5.3. Removal dynamics based on shapes in composite samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance				
	Fiber	**Fragment**	**Nylon**	**Glitter**	**Pellet**
IN-PHC	+11.27	-11.29	+0.03	0.00	0.00
PHC-BIO	-7.78	+4.06	+0.06	+3.66	0.00
BIO-EFC	+5.43	-1.94	+0.17	-3.26	0.00
IN-EFC	+8.55	-8.85	+0.26	0.00	0.00

*Values represent the percent change of abundances between given sampling locations.

Fibers were abundant in many studies and they did account for more than 92% of particles detected in Ambarlı WWTP. Increased abundance of fibers and decreased abundance of fragments was observed from influent to effluent, along with an increase in nylons. Fibers were measured to be in abundance in one or several steps of wastewater treatment plants in the literature (Dris et al., 2015; Yang et al., (2019); Ziajahromi et al., 2017; Gies et al., 2018). Michielssen et al. (2016) reported 62.0% and 61.0% abundance of fibers followed by 22.9% and 33% of fragments for influent and effluent of a secondary wastewater treatment plant, respectively. Tertiary treatment plant showed an abundance of 54.5% and 84.7% of fibers followed by 25.9% and 13% of fragments in the influent and effluent sample respectively indicating fibers were not removed as efficiently as other particles. Similar trend of fibers increase from influent to effluent was also observed in Conley et al. (2019) in a secondary wastewater treatment plant. Blair et al. (2019) also observed increased abundance of fibers from influent to effluent of a tertiary treatment plant. Tertiary effluent sample contained more

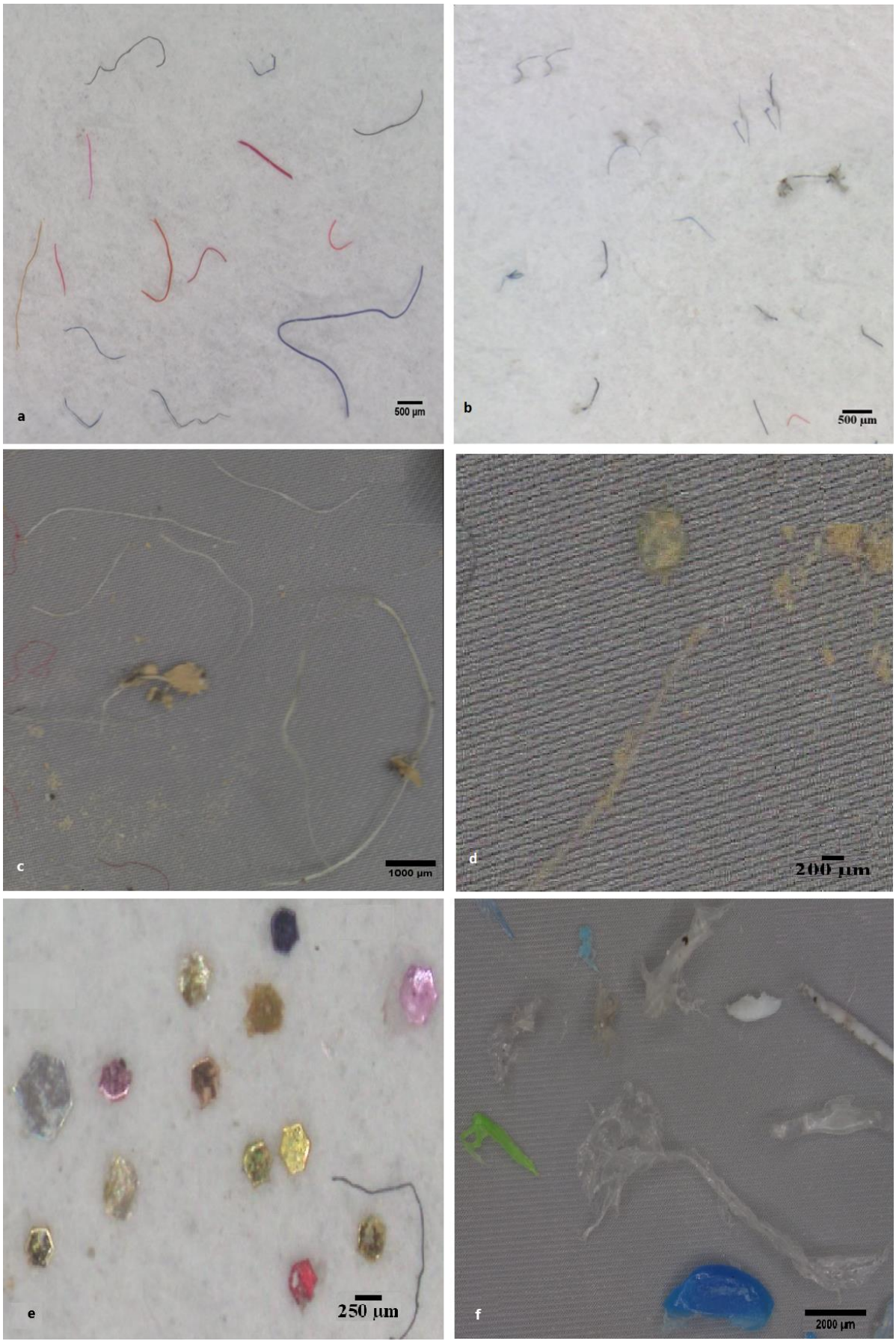

Figure 5.6. Examples of fibers (a, b, c), pellet (d-on top), glitters (e) and nylons (f-along with several fragments on the far right) isolated from samples.

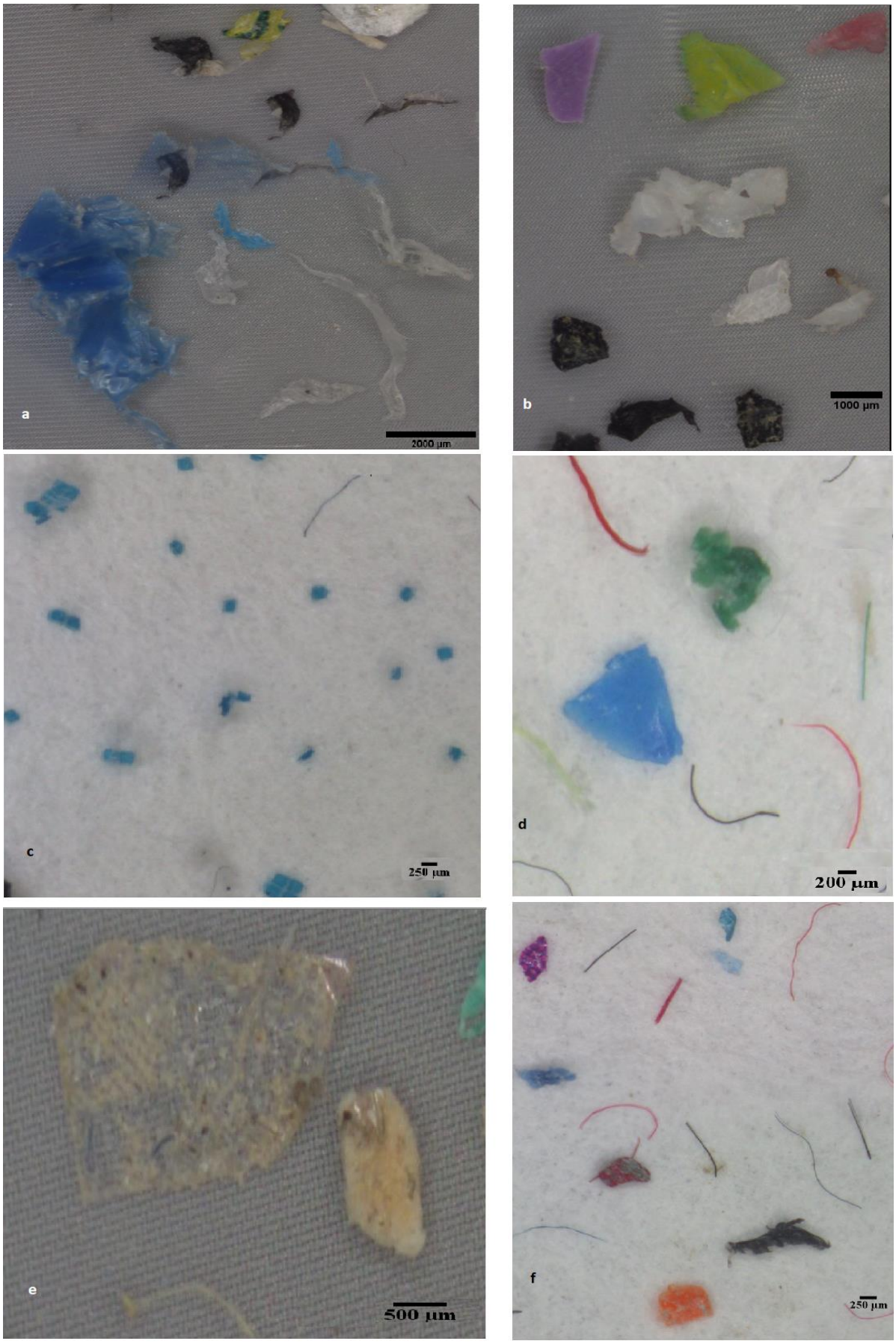

Figure 5.7. Examples of nylons (a), fragments (b, c, d, f), and a rubber band categorized as fragment (e-on the left)

fibers than secondary effluent sample, again followed by fragments. The abundance of particle shapes and their removal was similar with Ambarlı WWTP in aforementioned studies.

Lares et al. (2018) found 79.1% of fibers in samples collected from a Finnish wastewater treatment plant. Fibers were also abundant in the influent wastewaters of Talvitie et al. (2017a) study, followed by films and flakes. However, throughout the treatment steps fibers, flakes and films decreased in abundance where fragments increased. 79% fibers were found in influent samples of 3 wastewater treatment plants in Mersin, Turkey (Akarsu et al., 2020). However, fibers abundance from influent to effluent was decreased. These studies also showed high abundance of fibers however, an opposite trend of removal regarding the particle shapes.

Although fragments followed fibers in this study, with considerably lower abundances, several studies showed higher abundance of fragments than fibers (Park et al., 2020; Bayo et al., 2020). Most fibers were in <45 μm range in Park et al. (2020), which could have affected the removal dynamics. Fibers held a share of 16-38% being most abundant in sludge and influent wastewater, respectively in Edo et al. (2020) study and fragments were abundant in other units of wastewater treatment plant. Fragments abundance have increased from influent to effluent in these studies, contrary to the findings from Ambarlı WWTP.

Films which are close to our classification nylons were separated in several studies. They are generally included in the fragments category or non-fibers category in many studies (Bayo et al., 2020; Talvitie et al., 2017a). Films were nearly as abundant as fragments in a wastewater treatment plant in Adana, Turkey (Gündoğdu et al., 2018). Films dominated influent wastewater sample in Magni et al. (2019) study, they were nearly equal in primary settler and more abundant then fragments in the effluent. Other studies separated the films as a category reported lower percentages of films in their samples (Mason et al., 2016; Blair et al., 2019; Liu et al., 2019). Nylons were not abundant in our study, as reported by the aforementioned studies except in the grab effluent sample where they are more abundant than fragments. Increase of nylons observed in Ambarlı WWTP was similar with the findings of Talvitie et al. (2017a) study.

Glitters were not reported in many studies focusing on wastewater treatment plants. One of the studies is Murphy et al. (2016) study conducted in a wastewater treatment plant in Scotland. One glitter particle with dark blue color was found in sludge cake sample and reported as fragment. Second study is the Lares et al. (2018) study conducted in a wastewater treatment plant in Finland where one glitter particle in light blue color is referred to as an angular shiny fragment. The footnote states that

it might have come from wastewater sample or from the recipient lake sample. Third example is from Lusher et al. (2017) study investigating sludges of 10 wastewater treatment plant where it is defined and reported as glitter found in sludge of one of these facilities. They were also reported in studies focused on lake microplastics and microplastics in city dust (Ballent et al., 2016; Yurtsever, 2019). Pellets (referred as beads) were separated in some studies as a shape class however, they are generally reported in the fragments category such as nylons (films) and flakes (Mason et al., 2016; Michielssen et al., 2016; Li et al., 2018; Ziajahromi et al., 2017; Blair et al., 2019; Long et al., 2019; Bayo et al., 2020). Glitters were only present in grab samples of physical treatment effluent and biological treatment effluent samples in this study. One pellet was found in the dried sludge sample and no pellets were observed in wastewater samples.

Nearly equal retainment of particle shapes was observed in Lee et al. (2018) study for fragments and fibers and, shape distributions were similar in both steps. Shape distribution was similar in Liu et al. (2019) study in influent and effluent wastewater samples, respectively. Similar removal efficiencies were also observed in Gies et al. (2018) study with more than 98.0% removal for fibers and fragments, 100% removal for foam and sheets and granules and 88.6% removal for pellets and the percent distribution of shapes were similar for fibers and fragments in the effluent. However, the size distributions were not reported for the study. Two wastewater treatment plants (one secondary and one tertiary) relevant to this study were investigated in Michielssen et al. (2016). Microbeads were successfully removed from influent to effluent in both wastewater treatment plants. Fragments and fibers displayed similar removal dynamics in secondary wastewater treatment plant while, tertiary wastewater treatment plant removed most of the fragments along with the beads and others category remained similar in distribution. Fibers removal was 10% less compared to fragments in the tertiary wastewater treatment plant. One wastewater treatment plant showed better removal of fragments and fibers than films in Gündoğdu et al. (2018) study and other wastewater treatment plant removed fibers better than other shape categories. These findings contradict with the removal dynamics in this study, except the tertiary wastewater treatment plant reviewed in Michielssen et al. (2016) study.

Despite these examples, treatment systems remove fibers more efficiently were not as much efficient in removing other types of particles in other studies, especially fragments (Talvitie and Heinonen, 2014; Park et al., 2020). Fibers retained better in Talvitie et al. (2017a) study where abundances of fibers and films, along with flakes, decreased by more than half throughout the steps of the wastewater treatment plant investigated where, fragments throughout the treatment steps increased. In Edo et al. (2020) study, fibers share decreased and fragments share increased with the same rate from primary clarifier to effluent. These results contradicted with removal dynamics

observed in Ambarlı WWTP. In Lares et al. (2018) study, share of fibers decreased along with increase of fragments. However, process retained more fragments and released more fibers despite these findings. Decrease of fibers in the study showed an opposite trend with the shape distribution changes of this study, however better retainment of fragments along with escape of fibers from the system is similar to an extent with Ambarlı WWTP.

Contrary to the facilities retaining fibers more efficiently, the facilities removing other types of plastics generally removed fibers less efficiently (Conley et al., 2019; Magni et al., 2019). In Bayo et al. (2020) study, films were retained better than any other category. Fibers, beads and fragments increased where films decreased significantly. In Blair et al. (2019) study, films retained more than fragments in physical treatment units where in the effluent most films and fragments were retained and fibers share was slightly increased. These findings are in line with the removal dynamics in this study for fibers and fragments. However, nylons differ as they exhibit a decreased behavior in the study. Long et al. (2019) also observed better removal of other categories than fibers however, fibers were not as abundant as in the other studies reviewed therefore, it might have affected the dynamics of removal.

The studies reviewed are mostly conducted in secondary wastewater treatment plants and tertiary treatment plants with nutrient removal units and their removal mechanics, regarding the similarities of the processes utilized, should have been similar with our findings. Differences in removal therefore, should be influenced by other parameters such as sizes and characteristics of particles as well as parameters of influent wastewater and plant operational conditions. In Lares et al. (2018) different treatment methods reviewed yielded the same result. Where, percent abundance of fibers decreased and abundance of fragments increased. However, when size ranges are considered with particle shapes, the removal dynamics were correlated with our study where particles in different size classes had different behaviors in the treatment steps.

Overall, fibers were abundant in the samples collected from different locations in Ambarlı WWTP. High abundance of fibers is likely to occur from washing of textile. Browne et al. (2011) suggested that more than 1900 fibers can be generated per wash. Napper and Thompson (2016) reported that fibers can originate from washing of cotton and plastic blend textile and textiles made of several types of polymers, in increasing order and amount, respectively. Fibers at an average rate of 0.025 mg/g textile can be released according to Hernandez et al. (2017). Almroth et al. (2018) observed 400-2478 particles/100 cm^2 released from washing textile with detergent where washing only with water caused significantly less release of fibers to the environment. Considering that

washing generally occurs with detergents in daily activities, this finding confirms the rate of generation of Napper and Thompson (2016). From a typical wash activity of 5 kg, more than $6x10^6$ fibers were estimated to be released by De Falco et al. (2018) further support the possibility of fibers abundance resulting from textile washing activities.

84.1 and 92.3% of fibers were removed in Ambarlı WWTP, based on grab and composite samples, respectively. Physical treatment steps include coarse and fine screens followed by aerated grit and grease chamber. Fibers raised in percent abundance following physical treatment units. Particles with length to width ratio of higher than 3 were considered as fibers in Edo et al. (2020) study. Length to width ratio of 30 to 160 was reported by Li et al. (2016). Where, length to width ratio can be up to 650 for a fiber was reported by Napper and Thompson (2016). Fibers have smooth surfaces, in general (Anderson et al., 2018) and their smooth surface might have made them less resistant to water (Long et al., 2019). With the low density of fibers (Andrady, 2017) therefore, they may have escaped the grit chamber rather than settling with applied current in the wastewater stream. Fibers behavior from biological treatment effluent to final effluent differed with grab and composite samples. However, they were retained via biological treatment steps. Their characteristics of higher length to width ratio and smooth surfaces may play a role in the dynamics of retainment. As biological treatment steps include aeration via diffusors and creating a bubbled current in the aeration basin, their longer dimensions may cause them to be trapped within the foams and aggregates floating in the basin. Schmiedgruber et al. (2019) conducted a pilot scale study representing the activated sludge reactor and observed that fibers create agglomerates with the sludge flocs due to agglomeration with organic matter. This phenomenon occurred in order of minutes which was rapid compared to activated sludge retention times in wastewater treatment plants. Organic matter and fiber association can happen before entering the wastewater treatment system therefore, increase the probability of this type of a mechanism. As a result, some of the fibers should be retained in the sludge. From this perspective, they should be further retained in the final settling tank and therefore, their percent abundance should decrease at least slightly. This was the case from grab sample of effluent where, the composite sample demonstrated a contrary behavior where fibers were retained less effectively. This however, did not affect the overall removal results where, higher rate of removal was observed with composite effluent samples.

Fragments were retained better with the treatment units applied. Fragments abundance decreased following physical treatment units. Unlike fibers, fragments have a lower length to width ratio and have angular, twisted, bifurcate, curved and rough surfaces (Helm, 2017). In aerated grit chamber, fragments were removed effectively. Particles with low density (high buoyancy) or smaller size (low

resistance to currents) might have retained in the unit. Biological treatment effluent contained more fragments than physical treatment unit. As fragments have low densities and when they have larger surface, they might be more prone to floating in aeration basin and therefore, escaped the basin to final clarifier. Fragments with relatively high density and low buoyancy or smaller size then probably settled by themselves or with the flocs; which explains the decrease in percent abundance of fragments in the final effluent.

Nylons remained unchanged with composite effluent samples throughout the treatment with minor differences in composite samples. However, in grab physical treatment effluent contained more nylons compared to influent. Particles classified as nylons in the study were morphologically different in different sample types. Although this might not be applicable for all the particles present in the samples collected in this study, nylons are expected to escape the aerated grit chamber. They represented a character of lower length to width ratio compared to fibers but higher than that of the fragments. Therefore, in biological treatment units, they are likely to act closely to fibers because of their elongated shape and might be entrapped into sludge flocs. They might act as more buoyant fibers and less dense fragments in secondary effluent. They were generally retained less effectively in all of the units except biological treatment with the grab sample. Glitters were observed only in physical grab sample and biological grab sample. Therefore, a judgment based on these samples would not at all be true. However, more particles observed both in count and percent abundance in biological treatment effluent. Indicating that, they may have escaped the physical treatment units due to their lower buoyancy. Glitters were not observed in composite physical treatment sample which was collected as a 3hr composite sample which ended an hour before grab sampling began. Biological treatment effluent samples were taken from the weirs exiting the biological reactor and heading into secondary clarifier indicating that these low buoyancy particles escaped the reactor in the time period. Only one pellet was observed in the sludge. Therefore, no comments can be made on the particle apart from the fact that they were removed effectively in most of the studies reported and they are most likely to be present in the sludge samples.

Sludge characteristics was reported in several studies in the literature. Lee et al. (2018) reported that 24.5% of particles in the sludge were fibers and 75.5% were fragments, where influent samples contained 49.3% of fibers and 50.7% fragments and fibers were removed effectively while fragments increased in the effluent. Other studies with information on fractionation of particle types also showed consistent characters with the particles removed during the treatment of wastewaters (Gies et al., 2018; Lares et al., 2018; Liu et al., 2019). However, Edo et al. (2020) observed a contrary behavior with the wastewater treatment plant they have investigated. Despite the fact that fibers were removed

more efficiently than fragments, considerably high abundance of fragments was observed with wet sludge and further increase of fragments detected in the dried sludge sample. Similar distribution was observed in this study where fragments removed with a better percentage than fibers but sludge sample contained more fibers than fragments. This might be explained with abundance of particles. Both in Edo et al. (2020) and this study, the types dominated sludge samples were also considerably higher in influent and effluent samples.

One-time sampling events may not give an exact idea on how the treatment plants remove particles from wastewater. Wastewater treatment plants have many variables in their treatment steps. Aeration dynamics in physical removal units and biological reactor, solids content of wastewater, nutrients and bacterial abundances, foam and sludge flocs presence, pH levels and their changes and many other parameters that change with inflow and operation. The characteristics and loads of wastewater change in seasons, months and even days (Talvitie et al., 2017a; Conley et al., 2019). As it can be observed from Akarsu et al. (2020) study, diversity of particles observed in influent streams and the wastewater treatment plant's efficiency on retaining different types of particles can change throughout a year. Karaduvar WWTP is a good example for both cases where, Tarsus and Silifke WWTPs are good examples of how the plant responds differently to even low fluctuating influent characteristics, during different times of the year. Therefore, change of abundances may not be explained by only a morphological viewpoint. As other parameters mentioned before were not investigated in this study, a better approach might be developed by looking at the size distributions with the available findings from this study.

5.2. Size Distribution of Particles

Size distributions consider all particles detected in the sample except the particles larger than 5 mm. Although there was a fiber contamination in the laboratory experiments, the detected fibers were not uniform in length and they were not reduced from the calculations and tables in this section. Most of the particles in wastewater samples were in the range of 500-1000 µm in this study, followed by 1000-2000 µm. >2000 µm range followed these two size ranges except for influent and grab sample after physical treatment where particles in 250-500 µm range were more abundant. Composite sample after physical treatment contained slightly more particles in the 1000-2000 µm range than 500-1000 µm range. In sludge sample, most of the particles were in 1000-2000 µm range, followed by 500-1000 µm and particles >2000 µm. Overall, most particles observed were in 500-1000 µm range, followed by 1000-2000 µm range and particle sizes raised from influent to effluent.

Particle abundances in different locations of the wastewater treatment plant, in overall wastewater samples, in sludge samples and overall particles detected in this study were given in Table 5.4. Distribution of size classes were demonstrated in Figures 5.8. and 5.9.

Table 5.4. Particle abundances in sample locations of the WWTP.

Size Class	IN	PHG	PHC	BIO	EFG	EFC	WW	SLD	TOT
<250 µm	55 (7.7%)	32 (5.1%)	13 (1.9%)	6 (1.5%)	16 (2.3%)	3 (0.9%)	125 (3.6%)	4 (1.9%)	129 (3.5%)
250-500 µm	129 (18.0%)	118 (18.8%)	95 (13.6%)	19 (4.9%)	85 (12.3%)	38 (10.9%)	484 (14.0%)	25 (11.8%)	509 (13.8%)
500-1000 µm	247 (34.5%)	257 (40.9%)	240 (34.4%)	143 (36.8%)	273 (39.5%)	157 (45.1%)	1317 (38.0%)	63 (29.9%)	1380 (37.5%)
1000-2000 µm	180 (25.2%)	159 (25.3%)	246 (35.3%)	128 (32.9%)	207 (30.0%)	93 (26.7%)	1013 (29.2%)	77 (36.5%)	1090 (29.6%)
>2000 µm	104 (14.5%)	63 (10.0%)	103 (14.8%)	93 (23.9%)	110 (15.9%)	57 (16.4%)	530 (15.3%)	42 (19.9%)	572 (15.5%)
Total	715	629	697	389	691	348	3469	211	3680

In this study, particles in 500-1000 µm range measured to be the most abundant in wastewater samples and overall samples and they were the second most abundant in dried sludge samples. They remained nearly unchanged with a slight decrease after physical treatment units regarding the composite sample where an increase was observed with the grab sample. Opposite of these trends prevailed after the biological treatment units. In the effluent samples, particles in 500-1000 µm range increased following the biological treatment and their abundance measured to be higher in the effluent samples than the influent sample. Particles in 1000-2000 µm range were measured as the most abundant particles in the dried sludge sample and overall samples and also were the second most abundant in wastewater samples. They remained nearly unchanged with a slight increase after physical treatment units regarding the grab sample where an increase was observed with the composite sample. Following the physical treatment units, their presence decreased throughout the wastewater treatment plant. Particles larger than 2000 µm were measured as the third most dominant regarding the particle sizes in all categories. Their abundance decreased after physical treatment units regarding the grab sample where, a slight increase observed with the composite sample. Their abundance measured to increase after these units to the effluent.

Overall, particles that are in <250 µm and 250-500 µm range were removed more efficiently than particles that are larger than 500 µm. However, a contrary distribution in the dried sludge was observed as particles larger than 500 µm measured as the most abundant particles where particles smaller than 500 µm only accounted for 13.7% of the particles in the sludge.

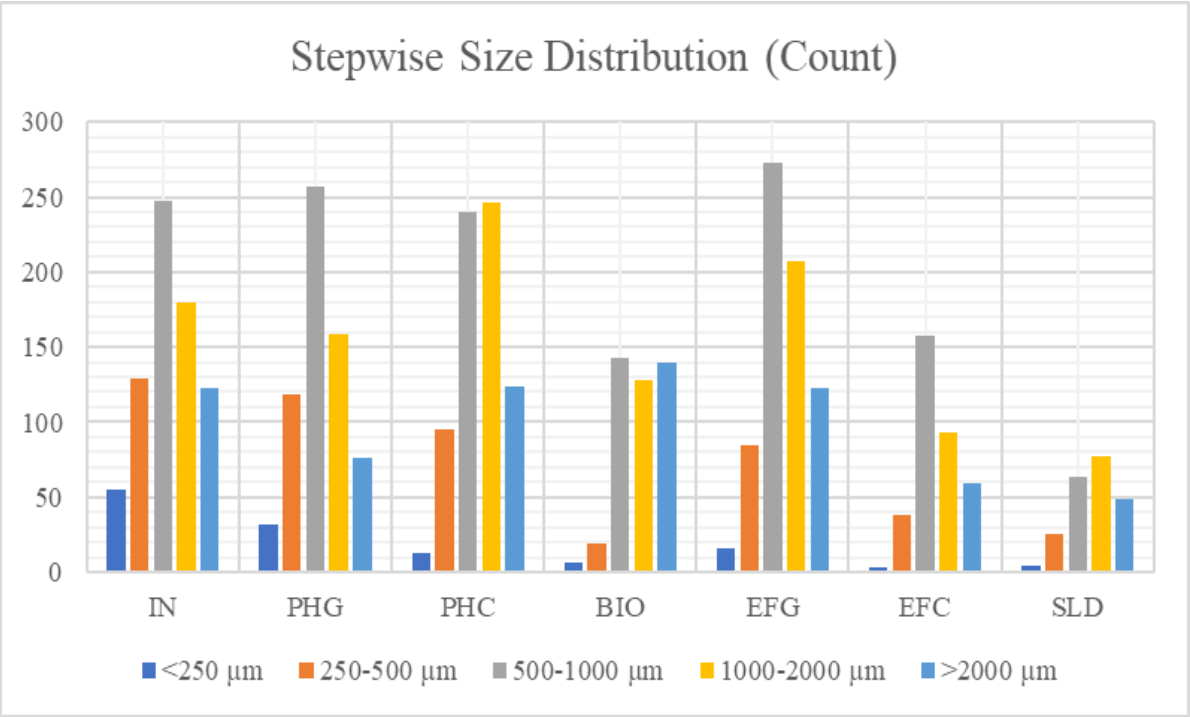

Figure 5.8 Overall size distributions of particles in different steps of WWTP (Numbers of particles).

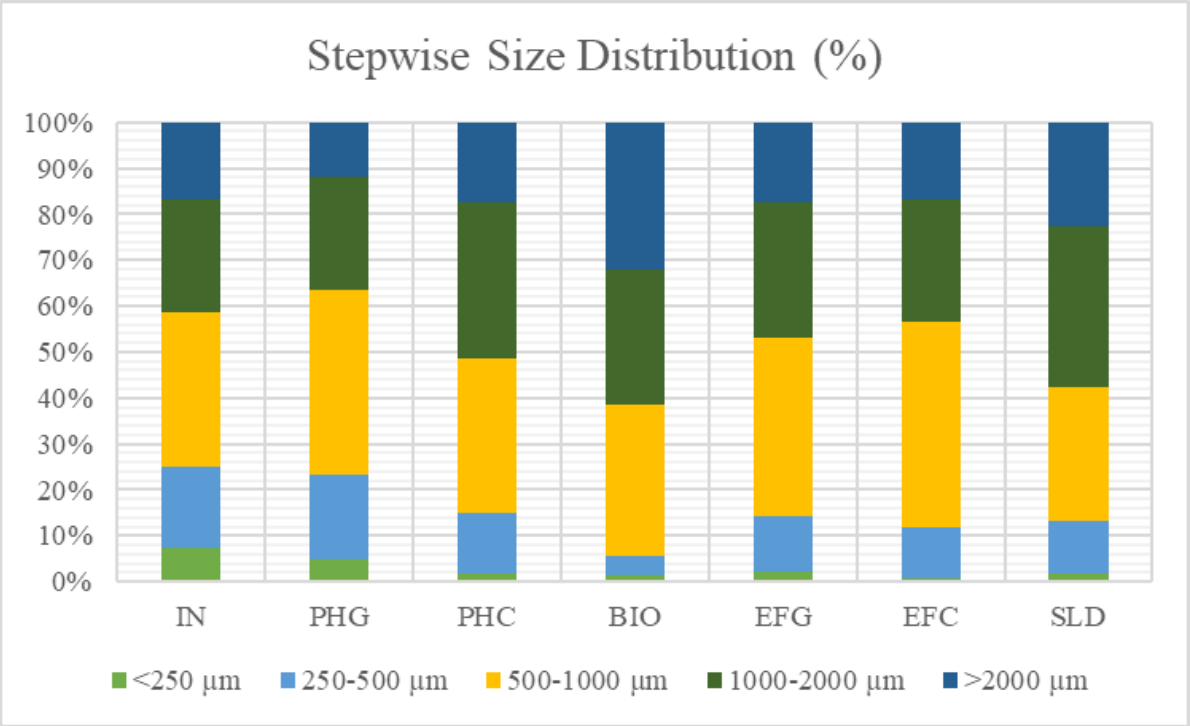

Figure 5.9. Overall size distributions of particles in different steps of WWTP (Percent particles).

Size classes of particles found in different locations of the plant also differed for shapes of the particles, as it can be seen from Figure 5.10 to Figure 5.17. The distribution of size classes of samples with respect to polymer shapes and based on particle counts and percentages, were given in Tables 5.5 and 5.6.

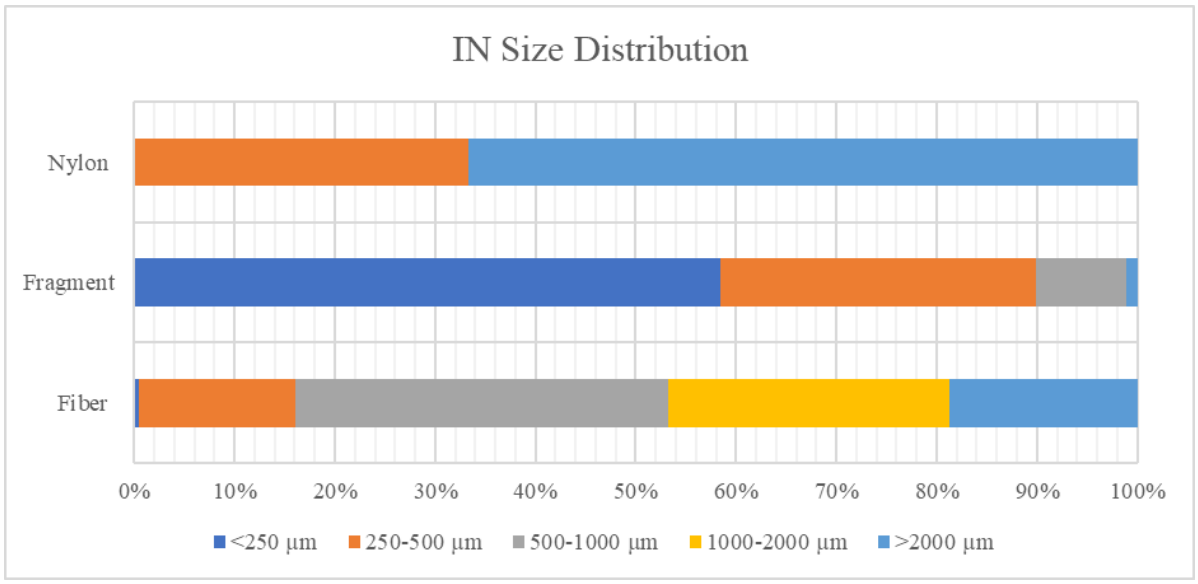

Figure 5.10. Size distribution of influent wastewater sample, with respect to polymer shapes.

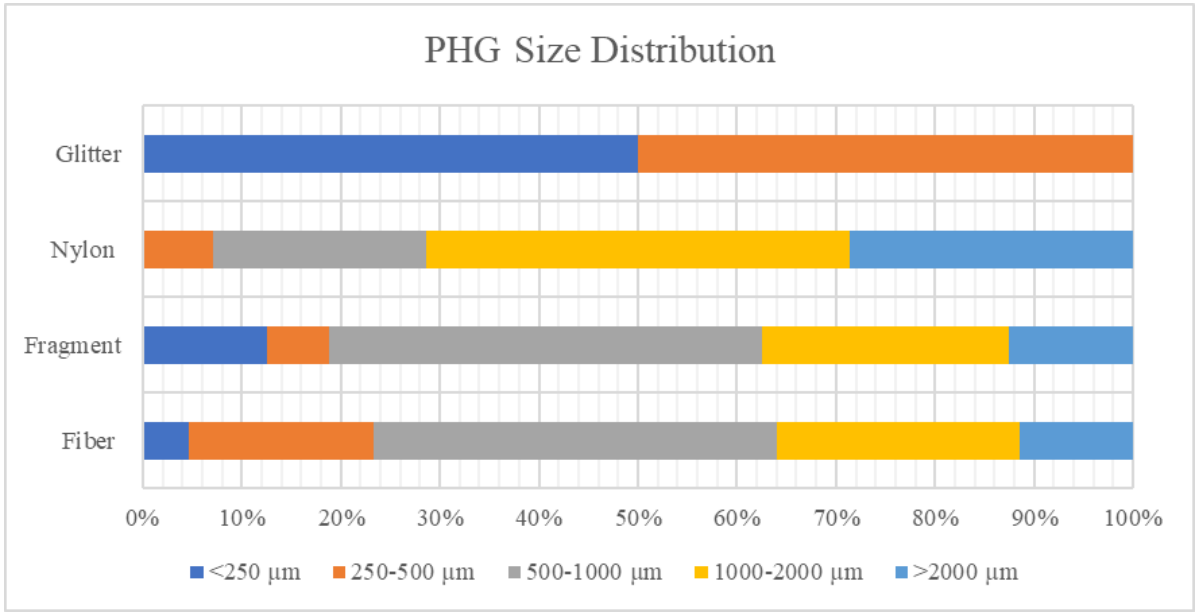

Figure 5.11. Size distribution of grab physical treatment effluent sample, with respect to polymer shapes.

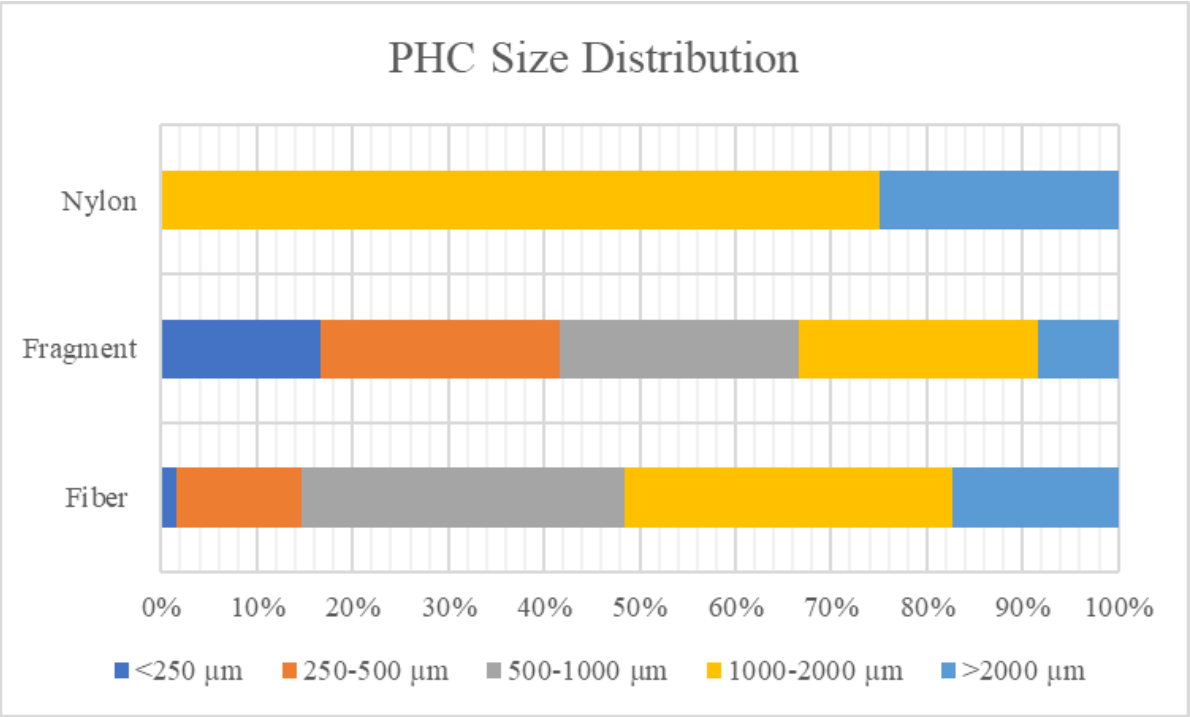

Figure 5.12. Size distribution of composite physical treatment effluent sample, with respect to polymer shapes.

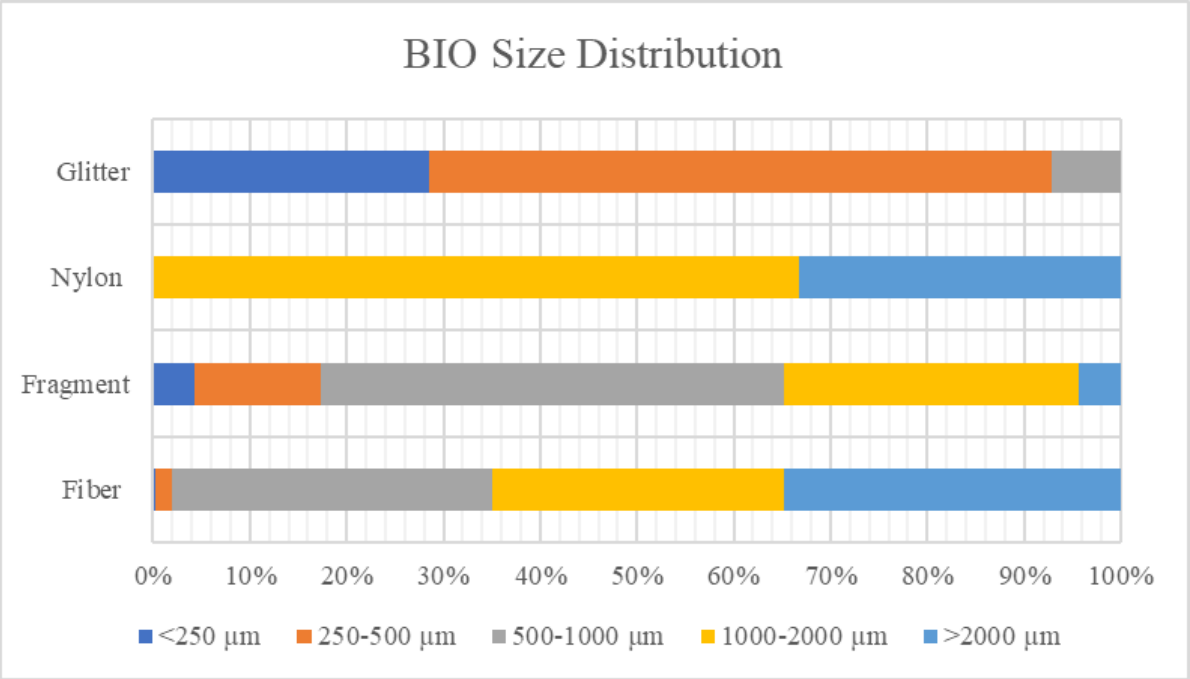

Figure 5.13. Size distribution of biological treatment effluent sample, with respect to polymer shapes.

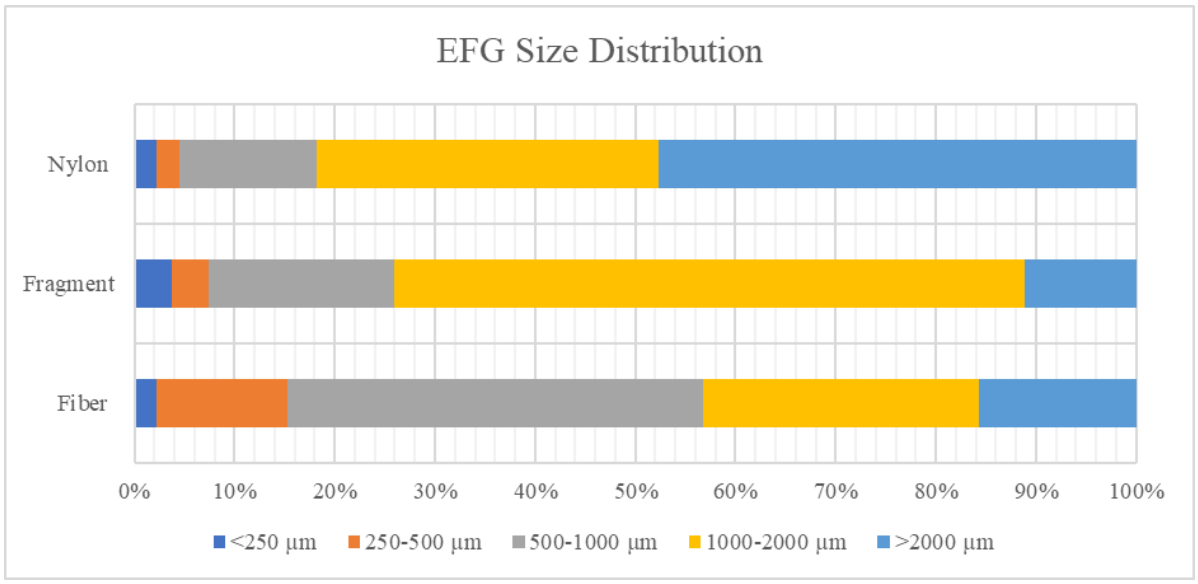

Figure 5.14 Size distribution of grab effluent wastewater sample, with respect to polymer shapes.

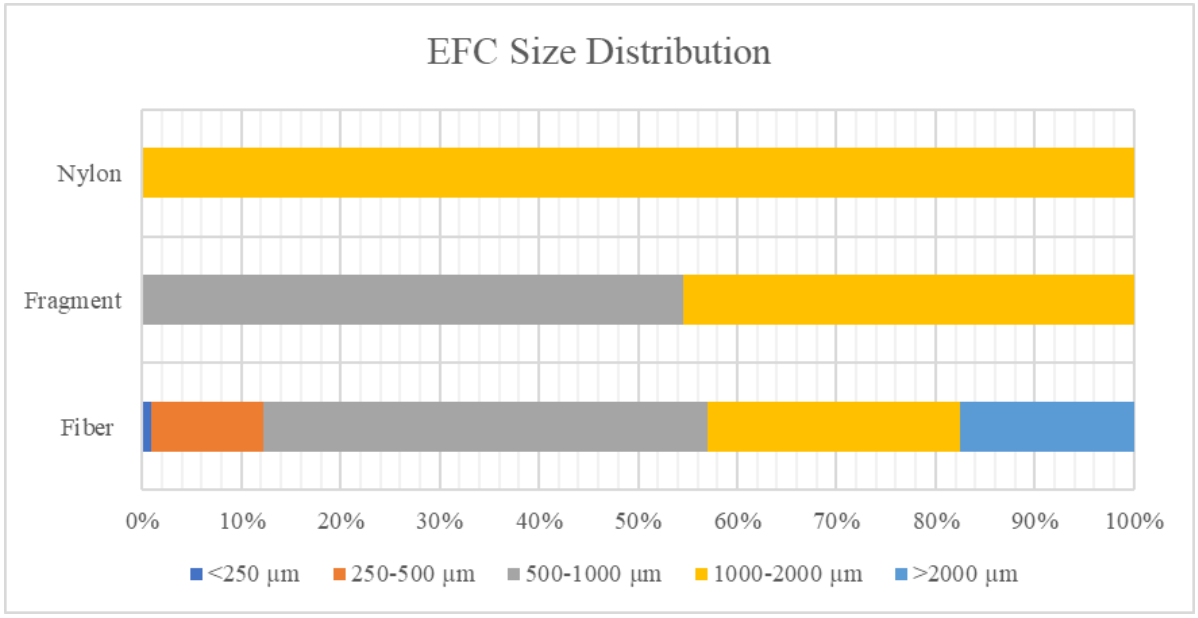

Figure 5.15. Size distribution of composite effluent wastewater sample, with respect to polymer shapes.

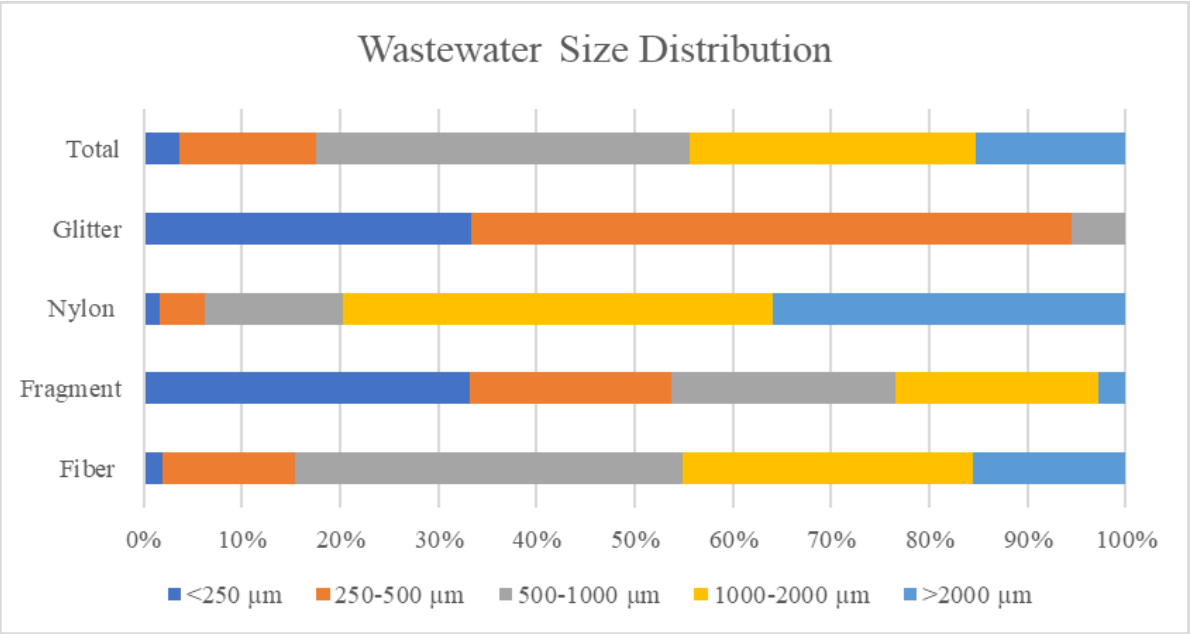

Figure 5.16. Size distribution of wastewater samples, with respect to polymer shapes.

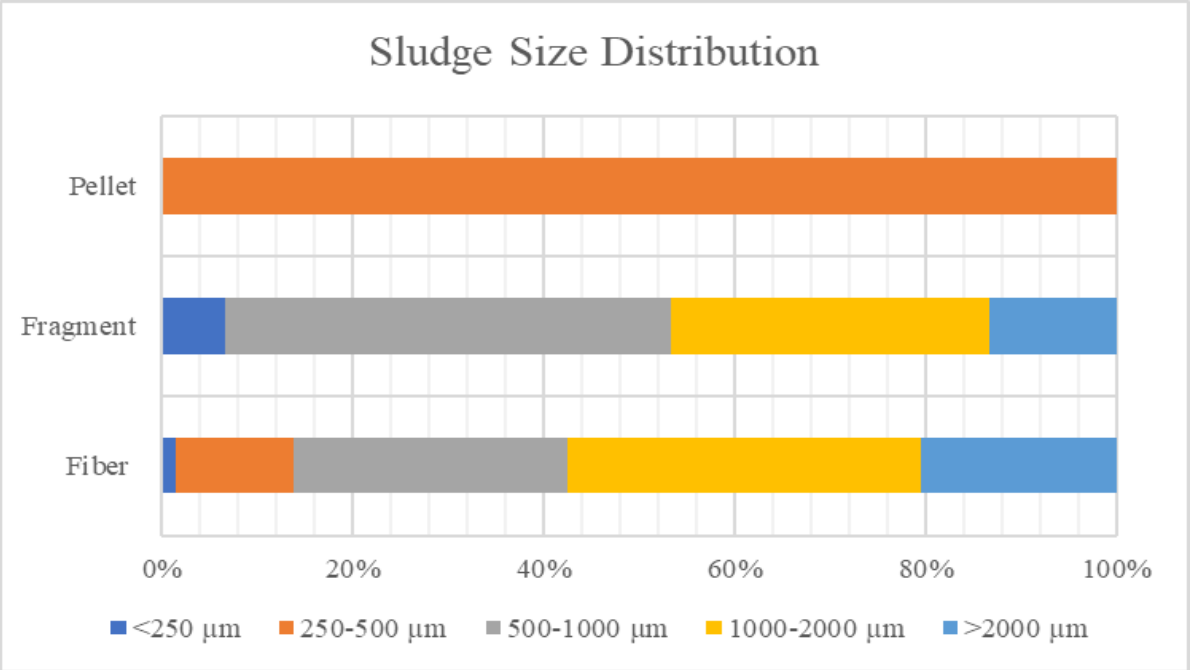

Figure 5.17. Size distribution of sludge sample, with respect to polymer shapes.

Fragments were 12.5% of the particles measured in the raw influent sample where, 58.4% of fragments were in <250 µm range followed by 250-500 µm range (31.5%). Fibers were 87.1% of the particles measured in the raw influent sample where 38.4% of them were in 500-1000 µm range followed by 1000-2000 µm range (28.9%). Fragments abundance decreased following physical treatment units as measured abundances of fragments were 2.5% and 1.6% of the particles detected in grab and composite samples respectively. In the grab sample fragments with sizes 500-1000 µm were measured to be 43.8% and fragments with sizes 1000-2000 µm were 25.0% of particles detected.

In the composite sample fragments with sizes 250-500 μm, 500-1000 μm and 1000-2000 μm were all measured to be 27.3% of particles detected. These findings indicate a better removal of fragments with lower size ranges than larger particles, except the better removal observed with >2000 μm range in the composite sample, in the physical treatment units. Fibers abundance increased following physical treatment units. In the grab sample fibers with sizes 500-1000 μm were measured to be 41.4% and fibers with sizes 1000-2000 μm were 25.0% of particles detected. In the composite sample fibers with sizes 1000-2000 were 35.1% and fibers with sizes 500-1000 μm were 34.7% of particles detected. These findings indicate a better removal of fragments with lower size ranges than larger particles, except the better removal observed with >2000 μm range in the composite sample. Larger particles decreased in abundance for both type of samples and these results indicate that fibers with lower sizes, especially <1000 μm, were removed more effectively in physical treatment units. Fibers larger than 2000 μm were also removed than particles with intermediate sizes. Increase in nylons was observed with composite physical treatment effluent sample where no change observed with the grab sample. Nylon sizes increased after physical treatment units where, glitter could not be commented on as it was only spotted in grab sample of physical treatment effluent.

Fibers decreased and fragments increased following biological treatment units in the biological treatment effluent sample. Increase of fragments with sizes 500-1000 μm and 1000-2000 μm observed compared to both grab and composite physical treatment effluent sample as well as decrease with sizes <250 μm. Increase in fragments with sizes 250-500 μm was observed with grab sample where decrease observed with the composite sample. In general, fragments with sizes <250 μm and >2000 μm removed better than other size ranges and especially fragments with sizes 500-200 μm measured to have raised in abundance following biological treatment units. Decrease of fibers with sizes <250 μm, 250-500 μm observed compared to both grab and composite sample after physical treatment units as well as increase of fibers with sizes >2000 μm. Compared to grab sample of physical treatment effluent, fibers with sizes 500-1000 μm decreased in abundance where, fibers with sizes 1000-2000 μm increased. Contrary behavior was observed with composite sample of physical treatment effluent. In general, fibers with sizes <500 μm and >2000 μm have been removed better than the fibers with intermediate sizes. Nylons with sizes 250-100 μm and >2000 were removed better where, nylons with sizes 1000-2000 μm were removed less as they increased in measured abundance in biological treatment effluent sample compared to grab physical treatment effluent sample. They were not removed compared to composite physical treatment effluent sample. Nylons overall, decreased considering the grab sample of physical treatment effluent and increased considering the composite sample. Glitter particles were also detected in an increased amount in the biological

treatment effluent sample however, they were not present in the following steps. Therefore, they could not be commented on.

Final effluent samples displayed an increase of measured abundance with fibers compared to biological treatment effluent sample and a decrease with fragments. In the final settler following biological treatment units, fragments with sizes <500 μm measured to have decreased in abundance where fragments with sizes 1000-2000 μm raised for both grab and composite samples of final effluent. Fragments with sizes 500-1000μm increased with composite sample of the final effluent where they decreased with the grab sample. Measured abundance of fragments with sizes >2000 μm remained unchanged for composite sample of the final effluent, where they raised in the grab final effluent sample. Measured abundance of fibers increased for fibers <1000 μm and decreased for fibers >1000 μm in both grab and composite final effluent samples. Nylons with sizes 1000-2000 μm did not display any change after the final settler regarding the composite final effluent sample where, nylons belonging in other size classes increased and nylons in 1000-2000 μm size class decreased in the grab final effluent sample. Grab final effluent sample showed an increase of nylons where composite final effluent sample showed a slight decrease of nylons.

Overall, despite the differences of removal mechanics between composite and grab final effluent samples and the raw influent sample, fibers with sizes 250-500 μm and 500-100 μm raised in measured abundance where 250-500 μm and 1000-2000 μm decreased. Fragments with sizes <500 μm were removed almost completely in wastewater treatment plant units where the rest generally increased in measured abundance. Several nylon size categories that have detected in the raw influent sample could not be detected in the composite final effluent sample however, size average decreased. Several other categories detected in the grab final effluent sample and size average, again, displayed a slight decrease. Glitters were only detected in grab physical treatment effluent and biological treatment effluent with increased abundance however, grab samples were taken in a short time span and it would not be correct to discuss their dynamics of removal. Sludge sample contained the most fibers in 1000-2000 μm size range which is in correspondence to our removal dynamics results however, the following range of >2000 μm remained slightly increased or decreased compared to the aforementioned size range in composite and grab samples, respectively. Fragment size ranges that displayed the most significant decreases from influent to effluent were low or absent in the dried sludge sample and the particles in size ranges that increased in the final effluent sample were the most abundant. No nylons and glitter were detected in the dried sludge sample and there was one pellet which was not observed in wastewater samples collected in this study.

Table 5.5. Numbers and percentages of particles isolated from wastewater samples from sampling locations with respect to their size classes and shapes.

Sampling Point	Size Class	Fiber	Fragment	Nylon	Glitter	Total	%	Per Liter
Raw Influent	**<250 µm**	3 (0.5%)	52 (58.4%)	0 (0.0%)	0 (0.0%)	55	**7.7**	11.0
	250-500 µm	100 (16.1%)	28 (31.5%)	1 (33.3%)	0 (0.0%)	129	**18.0**	25.8
	500-1000 µm	239 (38.4%)	8 (9.0%)	0 (0.0%)	0 (0.0%)	247	**34.5**	49.4
	1000-2000 µm	180 (28.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	180	**25.2**	36.0
	>2000 µm	101 (16.2%)	1 (1.1%))	2 (66.7%)	0 (0.0%)	104	**14.4**	24.6
	Total	**623 (87.1)**	**89 (12.5%)**	**3 (0.4%)**	**0 (0.0%)**	**734**	**100**	146.8
Physical Treatment Effluent (Grab)	**<250 µm**	28 (4.7%)	2 (12.5%)	0 (0.0%)	2 (50.0%)	32	**5.1**	3.2
	250-500 µm	114 (19.1%)	1 (6.3%)	1 (7.7%)	2 (50.0%)	118	**18.8**	11.8
	500-1000 µm	247 (41.4%)	7 (43.8%)	3 (23.1%)	0 (0.0%)	257	**40.9**	25.7
	1000-2000 µm	149 (25.0%)	4 (25.0%)	6 (46.2%)	0 (0.0%)	159	**25.3**	15.9
	>2000 µm	58 (9.7%)	2 (12.5%)	3 (23.1%)	0 (0.0%)	63	**10.0**	6.3
	Total	**596 (94.8%)**	**16 (2.5%)**	**14 (2.1%)**	**4 (0.6%)**	**629**	**100**	62.9
Physical Treatment Effluent (Composite)	**<250 µm**	11 (1.6%)	2 (18.2%)	0 (0.0%)	0 (0.0%)	13	**1.9**	1.3
	250-500 µm	92 (13.5%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	95	**13.6**	9.5
	500-1000 µm	237 (34.7%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	240	**34.4**	24.0
	1000-2000 µm	240 (35.1%)	3 (27.3%)	3 (100%)	0 (0.0%)	246	**35.2**	24.6
	>2000 µm	103 (15.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	103	**14.8**	10.3
	Total	**683 (98.0%)**	**12 (1.6%)**	**3 (0.4%)**	**0 (0.0%)**	**697**	**100**	69.7

*Percentages given in Total row represent the overall % of the shape in the column.

**Percentages in size class rows represent % abundance of shape in that category.

Table 5.5. (continued)

Sampling Point	Size Class	Fiber	Fragment	Nylon	Glitter	Total	%	Per Liter
Biological Treatment Effluent	**<250 µm**	1 (0.3%)	1 (4.5%)	0 (0.0%)	4 (28.6%)	6	**1.5**	6.0
	250-500 µm	7 (2.0%)	3 (13.5%)	0 (0.0%)	9 (64.3%)	19	**4.9**	19.0
	500-1000 µm	131 (37.3%)	11 (50.0%)	0 (0.0%)	1 (7.1%)	143	**36.8**	143.0
	1000-2000 µm	119 (33.9%)	7 (31.8%)	2 (100%)	0 (0.0%)	128	**32.9**	128.0
	>2000 µm	93 (26.5%)	0 (0.0%)	0 (0.00%)	0 (0.0%)	93	**23.9**	93.0
	Total	**351 (90.2%)**	**22 (5.7%)**	**2 (0.7%)**	**14 (3.6%)**	**389**	**100**	389.0
Final Effluent (Grab)	**<250 µm**	14 (2.2%)	1 (3.8%)	1 (2.4%)	0 (0.0%)	16	**2.3**	0.5
	250-500 µm	83 (13.3%)	1 (3.8%)	1 (2.4%)	0 (0.0%)	85	**12.3**	2.8
	500-1000 µm	262 (42.0%)	5 (19.2%)	6 (14.6%)	0 (0.0%)	273	**39.5**	9.1
	1000-2000 µm	175 (28.0%)	17 (65.4%)	15 (36.6%)	0 (0.0%)	207	**30.0**	6.9
	>2000 µm	90 (14.4%)	2 (7.7%)	18 (43.9%)	0 (0.0%)	110	**15.9**	3.7
	Total	**624 (90.3%)**	**26 (3.8%)**	**44 (6.3%)**	**0 (0.0%)**	**691**	**100**	23.0
Final Effluent (Composite)	**<250 µm**	3 (0.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3	**0.9**	0.1
	250-500 µm	38 (11.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	38	**10.9**	1.3
	500-1000 µm	151 (45.1%)	6 (54.5%)	0 (0.0%)	0 (0.0%)	157	**45.1**	5.2
	1000-2000 µm	86 (25.7%)	5 (45.5%)	2 (100%)	0 (0.0%)	93	**26.7**	3.1
	>2000 µm	57 (17.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	57	**16.4**	1.9
	Total	**335 (96.3%)**	**11 (3.2%)**	**2 (0.6%)**	**0 (0.0%)**	**348**	**100**	11.6

*Percentages given in Total row represent the overall % of the shape in the column.

**Percentages in size class rows represent % abundance of shape in that category.

Table 5.6. Numbers and percentages of particles isolated from dried sludge sample with respect to their size classes and shapes.

Sampling Point	Size Class	Fiber	Fragment	Nylon	Pellet	Total	%	Per g
Dried Sludge	**<250 µm**	3 (1.5%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	4	**1.9**	1.3
	250-500 µm	24 (12.3%)	0 (0.0%)	0 (0.0%)	1 (100%)	25	**11.8**	8.3
	500-1000 µm	56 (28.7%)	7 (46.7%)	0 (0.0%)	0 (0.0%)	63	**29.9**	21.0
	1000-2000 µm	72 (36.9%)	5 (33.3%)	0 (0.0%)	0 (0.0%)	77	**36.5**	25.7
	>2000 µm	40 (20.5%)	2 (13.3%)	0 (0.0%)	0 (0.0%)	42	**19.9**	14.0
	Total	**195 (92.4%)**	**15 (7.1%)**	**0 (0.0%)**	**1 (0.5%)**	**211**	**100**	70.3

*Percentages given in Total row represent the overall % of the shape in the column.

**Percentages in size class rows represent % abundance of shape in that category.

Average sizes were calculated for sampling locations in the WWTP, overall wastewater samples and for all particles observed in the study. Average length was 1223 µm for all particles detected when SD/AvL (Standard Deviation/Average Length) was 0.73, maximum length was 5.00 mm and minimum length was 0.05 mm for 3680 particles. Average sizes for different shapes observed throughout the samples were also calculated. Table 5.7. and Table 5.8. summarize the average sizes for different steps and different shapes of particles and they also can be seen from Figure 5.18. and Figure 5.19.

Table 5. 7 Average sizes in sampling locations of WWTP.

Type	IN	PHG	PHC	BIO	EFG	EFC	WW	SLD	TOT
Count	715	629	697	389	691	348	3469	211	3680
SD/AvL	0.82	0.77	0.71	0.63	0.69	0.67	0.73	0.68	0.73
Max. (mm)	4.99	4.90	4.89	5.00	4.96	4.80	5.00	4.57	5.00
Min. (mm)	0.10	0.05	0.11	0.23	0.07	0.18	0.05	0.14	0.05
Avg. (µm)	**1111**	**1012**	**1235**	**1684**	**1221**	**1205**	**1213**	**1376**	**1223**

*Count: Particles included in the calculation, Max: Max size encountered, Min: Minimum size encountered, Avg.: Average size calculated.

**WW represent overall wastewater samples and TOTAL represent all particles detected in different units of the WWTP.

Table 5. 8. Average sizes for different shapes observed in the samples.

Type	Fiber	Fragment	Nylon	Glitter	Pellet
Count	3407	190	64	18	1
SD/AvL	0.72	0.89	0.55	0.28	0
Max. (mm)	5.00	3.65	4.60	0.50	0.4
Min. (mm)	0.07	0.05	0.25	0.22	0.4
Avg. (µm)	**1243**	**725**	**1876**	**292**	**395**

*Count: Particles included in the calculation, Max: Max size encountered, Min: Minimum size encountered, Avg.: Average size calculated.

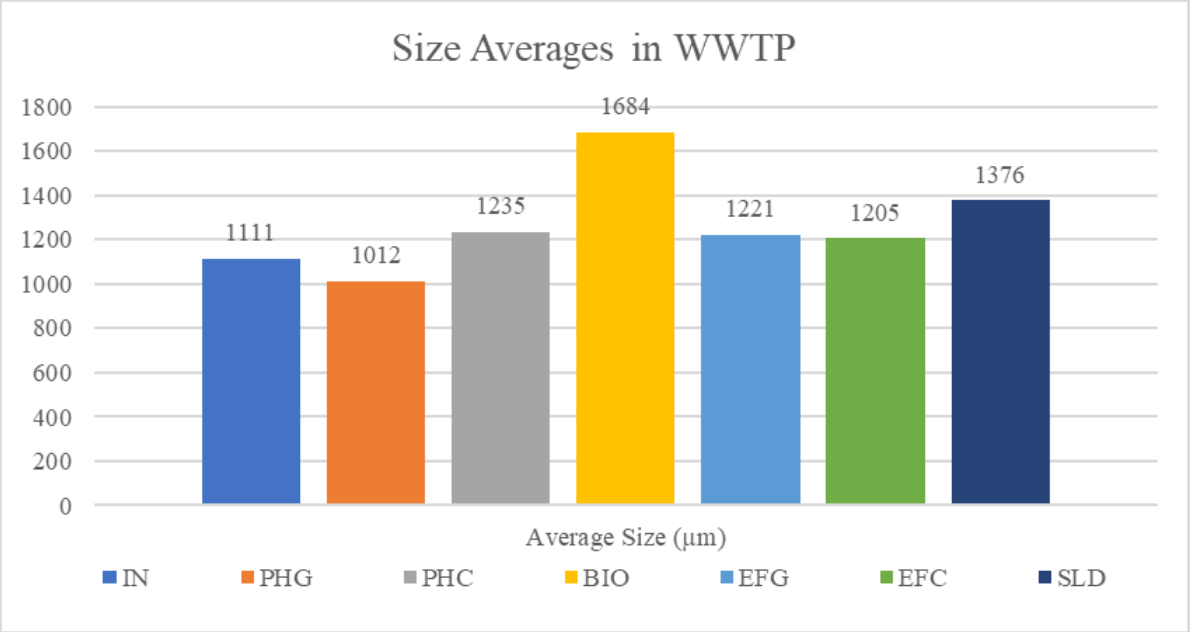

Figure 5. 18. Average particle sizes in WWTP.

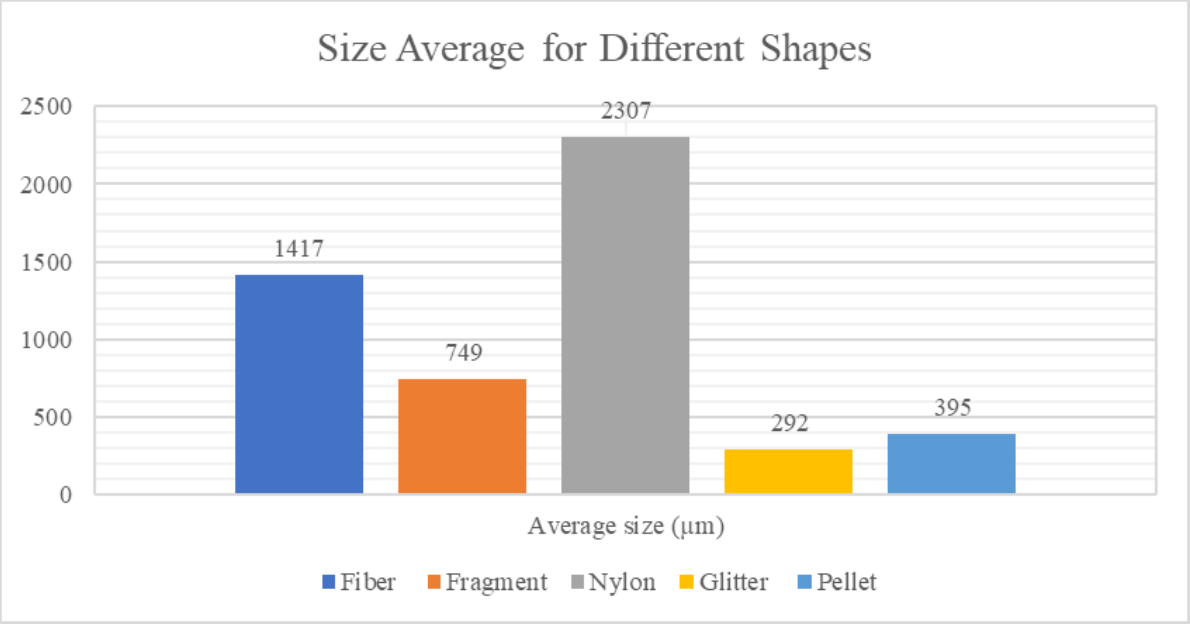

Figure 5. 19. Average particle sizes for different shapes of particles.

In the investigated wastewater treatment plant, the removal dynamics based on size classes were listed from influent to effluent in Table 5.9. and Table 5.10., where the percent abundance changes throughout the sampling locations were given. The values given in tables represent two sampling locations of the wastewater treatment plant where the change in percent abundance occurred.

Table 5.9. Removal dynamics based on sizes grab samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHG	-2.60	+0.72	+6.31	+0.10	-4.53
PHG-BIO	-3.55	-13.88	-4.10	+7.63	+13.89
BIO-EFG	+0.77	+7.42	+2.75	-2.95	-7.99
IN-EFG	-5.38	-5.74	+4.96	+4.78	+1.37

*Values represent the percent change of abundances between given sampling locations.

Table 5.10. Removal dynamics based on sizes in composite samples between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHC	-5.83	-4.41	-0.11	+10.12	+0.23
PHC-BIO	-0.32	-8.75	+2.33	-2.39	+9.13
BIO-EFC	-0.68	+6.04	+8.35	-6.18	-7.53
IN-EFC	-6.83	-7.12	+10.57	+1.55	+1.83

*Values represent the percent change of abundances between given sampling locations.

Removal dynamics for intermediate locations, with respect to particle shapes and size classes, were given in Tables 5.11. to Table 5.16., where the change of percent abundance throughout the sampling locations were given. The values given in tables represent two steps of the wastewater treatment plant where the change in percent abundance occurred.

Table 5.11. Removal dynamics of fibers, based on sizes in grab samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Fibers				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHG	+4.22	+3.08	+3.08	-3.89	-6.48
PHG-BIO	-4.41	-17.13	-4.12	+8.90	+16.76
BIO-EFG	+1.96	+11.31	+4.67	-5.86	-12.07
IN-EFG	+1.76	-2.75	+3.62	-0.85	-1.79

*Values represent the percent change of abundances between given sampling locations.

Table 5.12. Removal dynamics of fragments, based on grab samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Fragments				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHG	-45.93	-25.21	+34.76	+25.00	+11.38
PHG-BIO	-7.95	+7.39	+6.25	+6.82	-12.50
BIO-EFG	-0.70	-9.79	-30.77	+33.57	+7.69
IN-EFG	-54.8	-27.61	+10.24	+65.38	+6.57

*Values represent the percent change of abundances between given sampling locations.

Table 5.13. Removal dynamics of nylons, based on grab samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Nylons				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN- PHG	0.00	-25.64	+23.08	+46.15	-43.59
PHG-BIO	0.00	-7.69	-23.08	+53.85	-23.08
BIO-EFG	+2.44	+2.44	+14.63	-63.41	+43.90
IN-EFG	+2.44	-30.89	+14.63	+36.59	-22.76

*Values represent the percent change of abundances between given sampling locations.

Table 5.14. Removal dynamics of fibers, based on composite samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Fibers				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHC	+1.13	-2.58	-3.66	+6.25	-1.13
PHC- BIO	-1.33	-11.48	+2.62	-1.24	+11.42
BIO-EFC	+0.61	+9.35	+7.75	-8.23	-9.48
IN-EFC	+0.41	-4.71	+6.71	-3.22	+0.80

*Values represent the percent change of abundances between given sampling locations.

Table 5.15. Removal dynamics of fragments, based on composite samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Fragments				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHC	-40.25	-4.19	+18.28	+27.27	-1.12
PHC- BIO	-13.64	-13.64	+22.73	+4.55	0.00
BIO-EFC	-4.55	-13.64	+4.55	+13.64	0.00
IN-EFC	-58.43	-31.46	+45.56	+45.45	-1.12

*Values represent the percent change of abundances between given sampling locations.

Table 5.16. Removal dynamics of nylons, based on composite samples, between sampling locations of the WWTP.

Sampling Locations	Change of % in Abundance of Nylons				
	<250 µm	**250-500 µm**	**500-1000 µm**	**1000-2000 µm**	**>2000 µm**
IN-PHC	0.00	-33.33	0.00	+100.00	-66.67
PHC- BIO	0.00	0.00	0.00	0.00	0.00
BIO-EFC	0.00	0.00	0.00	0.00	0.00
IN-EFC	0.00	-33.33	0.00	+100.00	-66.67

*Values represent the percent change of abundances between given sampling locations.

Liu et al. (2019) observed an increase with smaller particles from influent to effluent. Particles with size 20-300 µm were increased by 21%, 30-1000 µm were decreased by 16%, 1000-2000 µm were decreased by 4%, 2000-5000 µm were decreased by 2%. 70% of the particles in the effluent sample belonged in 20-300 µm size class. Samples were sieved on a nearly identical mesh used in this study with 47 µm pore size and following the density separation, particles were concentrated on 8 µm filter papers. The study achieved retention of smaller sized particles than the first sieve's pore size and showed an opposite change of distribution compared to findings from Ambarlı WWTP. This might have resulted from the dominance of fragments and other types of plastics in the samples observed in the study. Another reason could be the different size ranges of particles in the influent wastewater, where particles <1000 µm dominated. Removal rates for fragments and nylons were greater for particles <500 µm in our study, except nylons with sizes >2000 µm. Therefore, the study also conflicts with this study regarding the removal dynamics, considering the particle sizes. From influent to effluent of the wastewater treatment plant investigated in Talvitie et al. (2017a) study, particles with sizes 20-100 µm reported to have increased by 30% in abundance. 14% decrease and 16% decrease were observed for particles 100-300 µm and >300 µm. Fibers were abundant in the influent sample where they were decreased throughout the treatment steps. Dominant shape was fragments in the effluent and average sizes were decreased. Particles with sizes 20-100 µm increased by 2-fold in mechanical treatment steps reaching up to 82% and started to decrease after chemical and biological treatment steps, resulting in 70% in the effluent. The removal dynamics were opposite in this study, considering overall change of abundance from influent to effluent, especially for fibers. This can mainly be caused because of the lower sizes of particles received by the treatment plant and 300-20 µm meshes were used for filtering the particles.

Findings of this study were similar to an extent with the findings of Magni et al. (2019) study, where particles in 500-1000 µm size range were the most abundant in influent, primary settling and effluent (36%, 58% and 52%, respectively). Similar abundance distribution in influent with 34.5% abundance in this size class was observed in this study. Abundances based on grab samples of physical treatment effluent and final effluent were 40.9% and 39.5%, respectively. Abundances based on composite samples of physical treatment effluent and final effluent were 34.4% and 45.1%, respectively and 1000-2000 µm range was slightly more abundant in the physical treatment effluent. The distributions of shapes in that study differed with the plant investigated in this study as higher abundances of fragments and films than fibers, in general, was reported. Fiber size ranges were not elaborated clearly, however, they were removed by more than 95% within the wastewater treatment plant. Size and shape distributions were provided only for fragments in Magni et al. (2019) study. The final distributions show a larger percentage of particles <500 µm than expected percentages from

the findings in our study. Park et al. (2020) observed removal of fibers more than fragments in a wastewater treatment plant in South Korea. Fibers were less abundant in the influent sample and decreased even further with the treatment. Fiber lengths were reported as <45 μm, in general. Findings from Ambarlı WWTP shows a better removal with fibers with sizes 250-500 μm than <250 μm and they tend to escape the system more than 250-500 μm range. The result from the Korean WWTP were not similar with our findings as, fragments should have been removed better than the fibers in both size ranges according to our findings.

The average sizes observed in Ambarlı WWTP were similar with the study conducted by Yang et al. (2019) with a 50 μm mesh used for collecting the particles. Average size reported for fibers was 1110 μm and average size for fragments was 680 μm where they were 1243 μm and 725 μm, respectively, in Ambarlı WWTP. In Yang et al. (2019) study 85.2% of all particles detected were fibers and the rest were classified as particles with different shapes. However, the study does not elaborate the distribution of particles. In Akarsu et al. (2020) study, size averages and particle shapes of influent and effluent wastewaters were reported. Overall average sizes for 3 wastewater treatment plants was 1135 μm for influent and 1309 μm for effluent samples, collected for 12 months. Average lengths of particles for Silifke and Tarsus WWTP were similar as 1057 μm and 1095 μm for influent and effluent samples, respectively. Karaduvar WWTP, which applies tertiary treatment for phosphorus removal, showed higher average particle sizes of 1242 μm in influent and 1499 μm in effluent samples. Even with use of 26 μm meshes for filtering the particles, study recovered particles with larger sizes compared to this study. Based on 17% increase in Karaduvar WWTP (which is the tertiary treatment plant in the study chosen for comparison) there is 8.0-9.2% less increase in average sizes in our study. There was no clear correlation with particle shapes and differences observed with average sizes in the study. From the presented distributions of particle shapes, it can roughly be said that with more abundance of fibers and soft plastics, there was a higher average length value in the study. Size distributions were also not provided, therefore, removal dynamics could not be compared thoroughly.

In a South Korean wastewater treatment plant (Lee et al., 2018), particles in 106-300 μm range decreased while >300 μm increased with increased abundance of fibers with the treatment steps. This result correlates with removal dynamics in this study for particles <500 μm and also correlates with raise in the abundance of fibers found in this study. Conley et al. (2019) observed increase of fibers and decrease of fragments from influent to effluent with increase of abundance with particles >418 μm where, sizes 60-178 μm were more abundant in the influent. Fibers were the most abundant shape in the effluent with increased abundance. These results are also consistent with removal dynamics

findings of this study, regarding removal of particles <500 μm, especially particles in 250-500 μm range. Bayo et al. (2020) reported slightly increased size rate from influent to effluent of a wastewater treatment plant in Spain. Most of the particles in the study were in 400-600 μm range and fragments and films were abundant in overall particles detected. There was only 1% of particles <200 μm which were in the biological reactor. Average sizes decreased from influent to primary treatment and biological treatment steps. However, the size average was again increased in the effluent. From influent to effluent, particles that are in size range 200-600 μm decreased from 51% to 38%, 600-1000 μm increased from 28% to 41%, 1000-2000 μm increased from 12% to 20% and particles >2000 μm slightly decreased from 7% to 6%. Overall retention, considering the particle shapes and size ranges was correlated with composite samples collected in our study. Stepwise retention dynamics in our study however, differentiated with the Spanish wastewater treatment plant. Grab sample after physical treatment displayed a decrease in sizes like the study where, composite sample showed an increase in sizes. In biological effluent sample, increase in particles with sizes >2000 μm and >1000 μm was observed compared to the composite sample and grab sample after physical treatment, respectively. Effluent samples had higher sizes than biological reactor in the study. However, particles in lower size classes raised at least as much as the particles >2000 μm in the treatment plant reviewed in our study, resulting in a smaller size increase. Overall, considering the particle shape and size classes in particle removal dynamics, those findings indicate removal of smaller fragments and small to mid-sized fibers throughout the treatment steps more efficiently than the other size classes. Size shift towards 500-1000 μm range can also be explained through findings from composite samples observed in our study.

From primary effluent to final effluent of another Spanish wastewater treatment plant, size classes of 25-104 μm and 104-375 μm decreased while size class of 375-5000 μm increased in by around 6%, 6% and 12%, respectively (Edo et al., 2020). Size of particles, as in the lowest and highest dimensions of length or width, ranged from 53-2100 μm for fragments in primary clarifier effluent and 41-2890 μm for fragments in final effluent. Sizes for fibers ranged from 104-4000 μm in primary clarifier effluent and 144-1824 μm for final effluent. Study reported that, 88% of the particles detected were in 25-375 μm range and the rest was in 375-5000 μm range. Most of the particles in primary clarifier effluents were fragments where fibers held 31% of the share of the particles. 72% of the particles were in the same size range in the final effluent where, fibers share decreased to 20% and overall particle sizes were increased. The study defined only fragments and fibers as shape classes. These findings were in line with our study to an extent. In this study, from physical treatment effluent to final effluent; fibers increased in sizes >500 μm and fragments increased in 1000-2000 μm size class for grab samples. Fibers increased in 500-1000 μm and >2000 size range and fragments

increased in 500-1000 µm and 1000-2000 µm range composite samples. Therefore, abundance increase >500 µm was expected when other sizes were decreasing. However, the fibers should have been removed better than fragments between physical treatment and the effluent based on findings from Ambarlı WWTP. In summary, removal dynamics were similar for grab samples.

Fibers abundance showed a slight decrease in Yüreğir WWTP, where they were still present by 86.5% in the effluent in Gündoğdu et al. (2018) study. 55 µm meshes were used for filtration of samples, which was nearly identical to the mesh size of this study. Average sizes decreased from influent to effluent, as particles<100 µm, 100-500 µm and 500-1000 µm increased in the effluents and particles in 1000-5000 µm range decreased by 18.7% for both Yüreğir and Seyhan WWTPs. In Seyhan WWTP, fibers showed a greater decrease however, particle sizes >1000 µm decreased at the same rate with Yüreğir WWTP. Films and particles with sizes 500-1000 µm and <100 µm raised in Seyhan WWTP. Films with sizes 500-2000 µm and fibers in size classes <250 and 500-1000 µm were found to have retained less in our study. Increase in abundance with 100-500 µm size range in Seyhan WWTP may be explained by abundance of films. Nylons in <250 µm, 500-1000 µm and 1000-2000 µm size classes abundance were increased from influent to effluent by the treatment process in our study, considering grab samples. Partial explanation can be derived from nylons <250 µm and partially from fibers not retained that are <250 µm. The retention values of the study are in accordance with findings in this study, regarding the removal dynamics of nylons, especially with grab samples.

Although the influent samples in this study have a similar size distribution with Lares et al. (2018) and average size to the effluent was also observed to increase, the increase of sizes observed in our study was higher for both grab and composite samples. In composite sample for physical treatment effluent, similar distribution was observed with 5.0% more abundance in particles >1000 µm which was still enough to increase the average size as share of particles <500 µm were also less in abundance in our study. Grab sample however, contained 6.8% more particles in 250-500 µm category, 5.9% more in 500-1000 µm category and 9.7% less particles >1000 µm. In the effluent, for both grab and composite samples, particles in 250-500 µm range were 11.7-13.1% less and particles >1000 µm were 13.1-15.9% more than the study compared. Compared to Lares et al. (2018) particles with sizes <250 µm and >1000 µm were retained more and particles with size 250-500 µm and 500-1000 µm were retained less in the physical treatment, according to grab samples in this study. Biological treatment and final settling characteristics were similar for particles <250 µm where particles with sizes 250-500 µm and 500-1000 µm were retained more in this study and contrary was observed in compared study. Particles with sizes >1000 µm were retained more in the compared study and therefore, the size averages for this study were significantly higher. In composite samples, the

retention of particles with sizes <250 μm and 500-1000 μm were more than the compared study and particles with size 250-500 μm and >1000 μm were retained less than the compared study following the physical treatment. After biological treatment and final settling particles with sizes <250 μm, 500-1000 μm and >1000 μm were retained more than this study where, particles with sizes 250-500 μm were retained less. However, particles with sizes 500-1000 μm were retained better with the grab effluent sample. 9.1% and 13.9% increase of particles >2000 μm in biological treatment step with composite and grab sample, respectively, may have contributed to this outcome. Fibers were also dominant in the compared study with 79.1% abundance and 64% of them were <1000 μm where half of those particles were <500 μm. In our study 55% of fibers were <1000 μm where 28% of those fibers were <500 μm. 30% of fibers were in 1000-2000 μm range and 16% of fibers were >2000 μm. Therefore, size averages were also higher in our study, considering both grab and the composite samples.

Although larger particles escaped from the treatment steps applied, mean sizes of fragments were still less than 50% of fibers. Nylons abundance decreased with sizes >2000 μm and 250-500 μm where, it increased in sizes 500-1000 μm and 1000-2000 μm, respectively. This may have resulted in a shift towards 500-2000 μm range and therefore, an increase in abundance for nylons. However, nylons were even rarer than fragments in samples collected from the facility, except the grab effluent sample to affect the abundance distribution. Overall fibers abundance is a factor that is likely to contribute to the higher size averages and particle abundances in larger size classes observed in this study.

In Mason et al. (2016) study, size range distribution was not clearly elaborated however, distributions of different shapes were given. In 125-355 μm size range, fibers and fragments were abundant by 46% and 44%, respectively. Particles >355 μm fibers and fragments were abundant by 80% and 14% respectively indicating an increased fibers abundance in larger sizes. More fibers presence was correlated with higher size averages where, more fragments presence resulted in lower size averages in Gündoğdu et al. (2018) study. Akarsu et al. (2020) also found a similar correlation in their study. Mason et al. (2016) also observed more fibers presence in samples from wastewater treatment plants applying advanced or tertiary steps, than secondary wastewater treatment plants.

High abundance of fibers compared to all other types in this study therefore, might explain the higher size average observed regarding the correlations demonstrated by aforementioned studies. The plant investigated in this study, being a tertiary wastewater plant with a nutrient removal step, is more likely to contain higher percentages of fibers. Nylons were also contributors to the larger dimensions

observed in the samples but not as much as fibers, as fibers were considerably abundant than any other size class. Possible reasons for high fibers abundance was discussed in Section 5.1. and textile washing was reported as an important factor. Napper and Thompson (2016) reported that fibers with average lengths of 4.99 mm to 7.79 mm can originate from washing. A more recent study showed lower sized fibers originating from textile washing can occur (Hernandez et al., 2017). The study found fibers with lengths 100-800 µm from textile washing. Fiber lengths of 100-1500 µm observed in De Falco et al. (2018). These findings may explain the larger abundances fibers present in the samples and the higher size averages in this study.

Samples collected from Ambarlı WWTP had a high organic content in all the samples, especially in phosphorus removal and activated sludge reactor. Visible organic content was present even in the effluent wastewater, when settled. Higher suspended solids were correlated with abundance of more MPs >1000 µm and lower suspended solids were correlated with MPs <1000 µm in Bayo et al. (2020). Therefore, this might also be a contributing factor for higher average sizes observed compared to other studies in the literature and especially the higher size for fragments occurred in this study as the aforementioned study displayed a dominance of fragments and films.

Daily fluctuation of concentration was confirmed as Talvitie et al. (2017) study found that daytime microplastic concentrations were higher than nighttime concentrations. Although lowest concentrations were observed around 1-4 pm., highest concentrations were observed in early evening hours. Authors state that this trend was in-line with daytime activities for the population the plant serves. This finding is supported with differences in morning size average of 660 µm and afternoon size average of 790 µm found in Bayo et al. (2020) study. These may further contribute to the increase in size, apart from the removal dynamics, as the samples in this study were collected in the afternoon.

In summary, the removal of particles depends on the sizes of particles as well as the shapes. The overall removal dynamics for all these parameters combined were similar to most of the studies conducted on tertiary wastewater treatment plants. The results mostly contradicted the studies with lower mesh sizes. Smaller mesh sizes used for screening the microplastics should be considered in order to compare the results with those studies. The parameters of influent wastewaters also affect the treatment of microplastics. In this study, fibers with sizes <250 µm and 500-1000 µm; fragments and nylons in sizes 500-2000 µm escaped the treatment more than the others remained more in the system and the results are similar to other studies compared. Differences in facilities with similar treatment units and similar influent distributions might imply that WWTP design and operation can be another factor affecting the removal of the particles. These removal dynamics can also be resulting

from suspended solids content as mentioned above, pH levels, humic acids presence (Li et al., 2018), incoming particle sizes to the WWTP and many other parameters changing in the wastewater treatment plant influent and these effects should be investigated in interdisciplinary studies.

5.3. Color Distribution of Particles

Black (57.7%) was the dominant color, followed by blue (28.3%), red (8.9%), brown (1.3%), green (1.1%), transparent (1%), orange (0.5%), pink, grey, purple, yellow and white (<0.5%, each) in all wastewater samples. Particle colors detected in sludge were black (56.0%), blue (20.6%), red (7.3%), white (6.0%), green (5.5%), brown (4.1%) and transparent (0.5%). Highest color diversity was observed in the biological reactor effluent sample. Color distributions of wastewater samples in different steps and overall color distributions of wastewater and sludge are displayed in Figure 5.20. Stepwise color distributions and overall color distributions of wastewater and sludge samples depending on the shape classes are given in Figures 5.21 and 5.22.

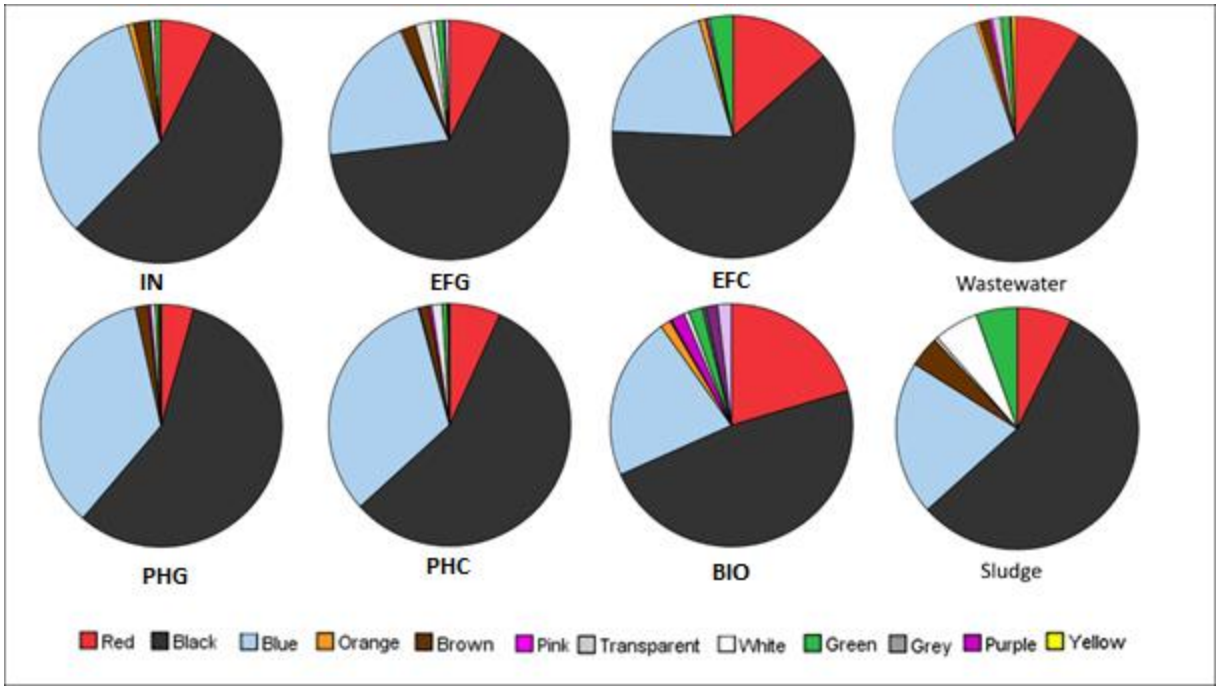

Figure 5. 20. Color distributions in samples of different steps of wastewater treatment plant.

Black (55.0%) and blue (33.4%) were the most dominant colors followed by red (7.2%) in influent sample and these colors belonged mostly to fibers. Fibers were black (63.0%), blue (25.4%), red (8.3%), brown (2.3%), orange (0.6%) and green (0.5%). Blue (92.1%) was the dominant color in fragments followed by green (3.4%), orange and transparent (2.2%, each) in decreasing abundance. Nylons were transparent (66.7%) and pink (33.3%).

Black (56.8%) and blue (35.4%) were the most dominant colors followed by red (4.4%) in grab sample after physical treatment. Fibers were black (58.4%), blue (35.5%), red (4.0%), brown (1.8%), pink and green (0.2%, each). Black (50.0%) was the most common color in fragments followed by blue (31.3%), grey, pink and transparent (6.25%, each) in decreasing abundance. Nylons were blue (42.9%), transparent (21.4%), black (14.3%), red, green and yellow (7.1%, each). Glitters were red (75%) and grey (25%). Black (56.4%) and blue (32.6%) were the most dominant colors followed by red (6.8%) in composite sample after physical treatment. Fibers were black (57.4%), blue (32.5%), red (6.8%), brown (1.3%), transparent (1.0%), green (0.6%), pink (0.3%) and orange (0.1%). Blue (50.0%) was the most common color in fragments followed by black (16.7%), red, pink, green, and grey (8.3%, each) in decreasing abundance. Nylons were transparent (75%) and yellow (25%).

Black (49.3%) and blue (22.4%) were the most dominant colors followed by red (20.8%) in sample after biological treatment. Fibers were black (52.8%), red (21.5%), blue (21.2%), pink, orange, purple (1.3%) and green (0.8%). Blue (43.3%) was the most common color in fragments followed by green (21.7%), red (13.0%), pink, transparent, orange, purple, and grey (4.3%, each) in decreasing abundance. Nylons were transparent (66.7%) and blue (33.3%). Yellow (57.1%) was the most common color in glitters followed by pink (14.3%), red, brown, grey and purple (7.1%, each) in decreasing abundance.

Black (65.6%) and blue (20.3%) were the most dominant colors followed by red (7.4%) in grab effluent sample. Fibers were black (70.1%), blue (19.1%), red (7.9%), brown (2.1%), green, orange (0.3%, each), and yellow (0.2%). Blue (33.3%) was the most common color in fragments followed by black (29.6%), green, white (11.1%, each), transparent (7.4%), red and purple (3.7%, each) in decreasing abundance. Nylons were blue (29.6%), transparent (27.3%), black (22.7%), yellow, white (6.8%, each), green (4.6%) and red (2.27%). Black (62.3%) and blue (19.7%) were the most dominant colors followed by red (13.4%) in composite effluent sample. Fibers were black (64.7%), blue (19.3%), red (13.1%), green (2.1%), orange (0.6%), and pink (0.3%). Blue (36.4%) was the most common color in fragments followed by black, red (27.3%, each), and orange (9.1%) in decreasing abundance. Nylons were blue and transparent (50%, each).

Transparent was the dominant color (Liu et al., 2019; Akarsu et al., 2020) and second dominant color (Long et al., 2019; Yang et al., 2019) in samples from wastewater treatment plants mostly located in China. White and beige was observed as dominant colors in Long et al. (2019) and Bayo et al. (2020) studies. 20 to 30% of black and yellow particles in Long et al. (2019) were confirmed as microplastics. Black was the dominant color in Yang et al. (2019) and Fortin et al. (2019) studies

where it was second dominant in Talvitie et al. (2017a) and Akarsu et al. (2020) and third dominant in Bayo et al. (2020) and Liu et al. (2019) studies. Blue was also one of the dominant colors in several studies (Talvitie et al., 2017a) and one of the most dominant (Murphy et al., 2016; Yang et al., 2019; Bayo et al., 2020). Red was also another dominant color (Murphy et al., 2016) and one of the most dominant colors (Talvitie et al., 2017a) Green was also observed in Murphy et al. (2016) with a high abundance and detected in Bayo et al. (2020) with a considerable percentage, regarding other colors. Brown was the second dominant color in samples of Akarsu et al. (2020), especially in effluent samples and Liu et al. (2019).

Overall, the dominant colors observed in samples collected from the investigated wastewater treatment plant did not change significantly. This finding correlates with Long et al. (2019) study where no significant difference was observed on color diversity of particles with the treatment steps.

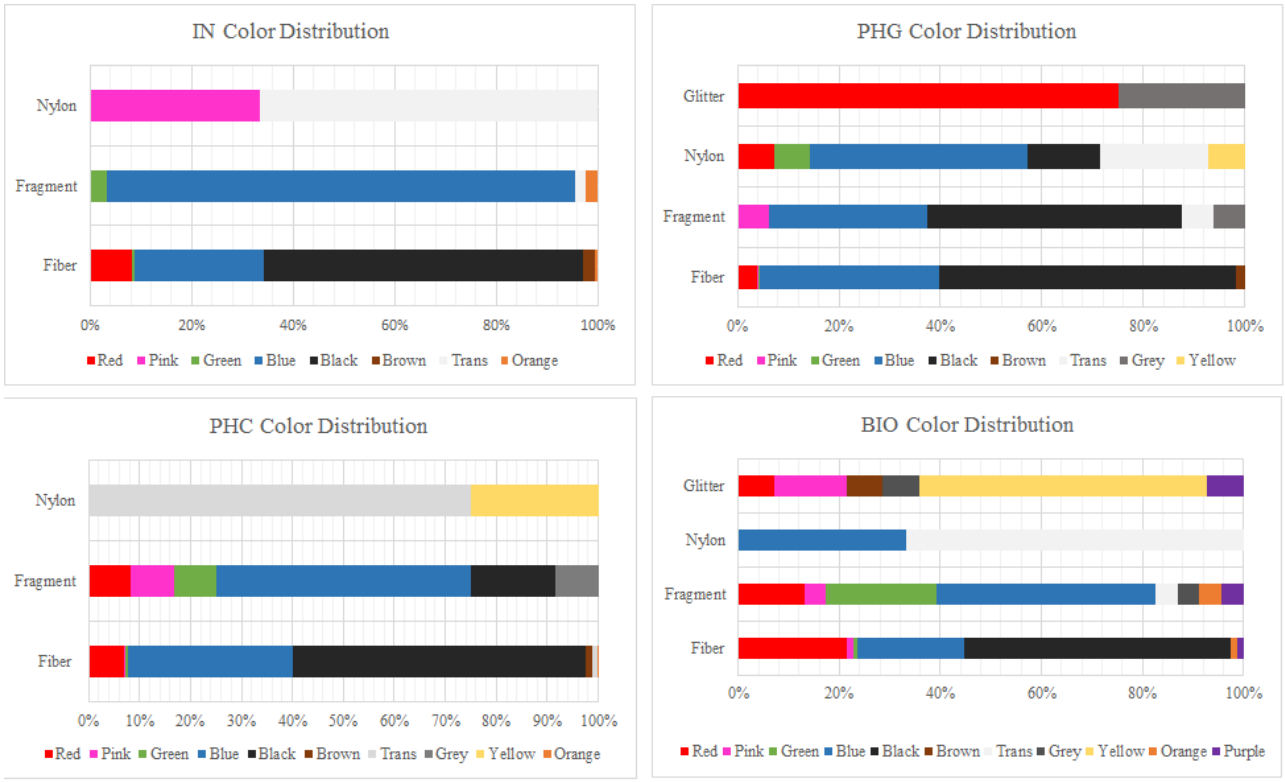

Figure 5. 21. Color distributions of different shapes of polymers present in influent sample, grab and composite physical treatment effluent samples and biological treatment effluent sample.

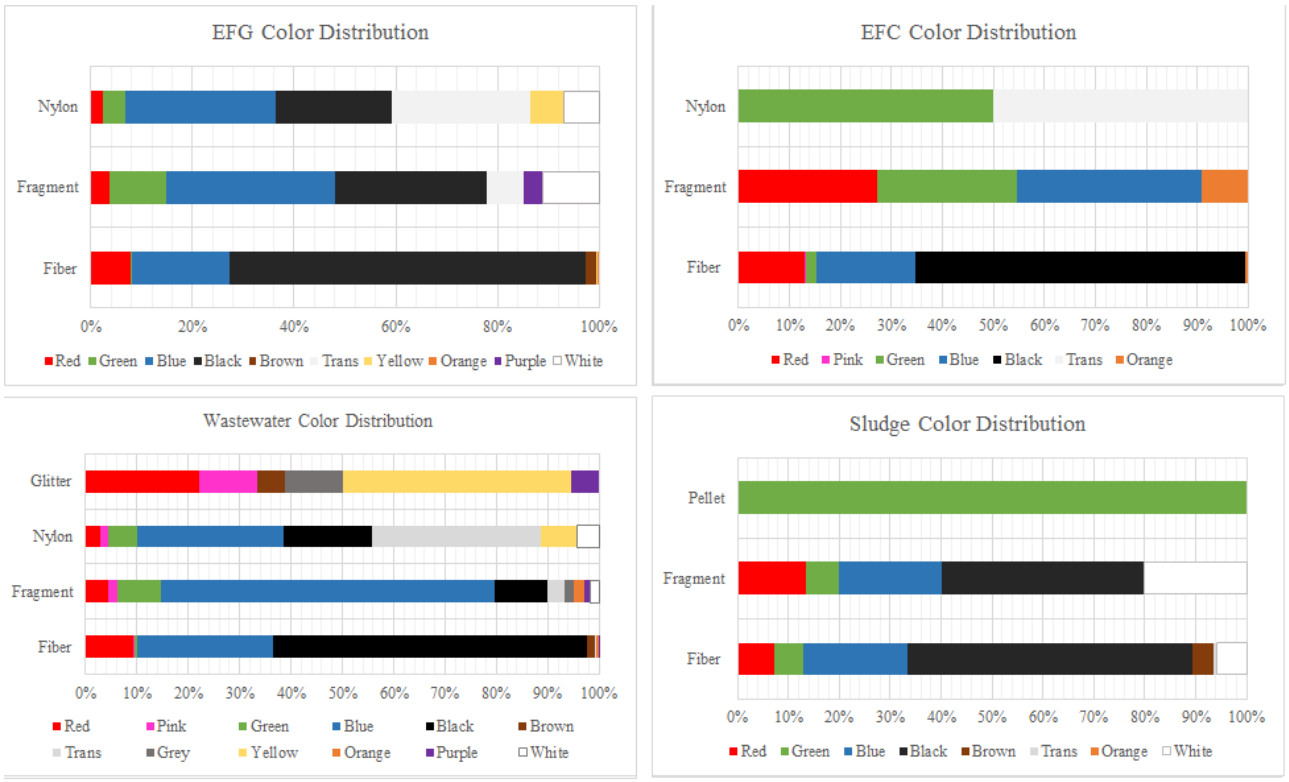

Figure 5. 22. Color distributions of different shapes of polymers present in grab and composite effluent Samples, overall wastewater samples and sludge sample.

5.4. Removal and Discharge Rates of the Wastewater Treatment Plant

Total of 137.0 particles/L entered the Ambarlı WWTP where 21.0 particles/L left the system with effluent according to grab effluent sample after contamination corrections. Composite effluent sample had a concentration of 9.6 particles/L. Table 5.17. shows concentrations of particles in different steps of the wastewater treatment plant.

Table 5. 17. Particle concentrations with applied correction for particles >5 mm.

Particles/L	IN	PHG	PHC	BIO	EFG	EFC	SLD (Particles/g)
Fiber	118.6	54.6	63.3	344.0	18.8	9.2	55.0
Fragment	17.8	1.6	1.10	22.0	0.9	0.4	5.0
Nylon	0.6	1.3	0.3	2.0	1.4	0.1	0.0
Glitter	0.0	0.4	0.0	14.0	0.0	0.0	0.0
Pellet	0.0	0.0	0.0	0.0	0.0	0.0	0.33
Total	**137.0**	**57.9**	**64.7**	**382.0**	**21.0**	**9.6**	**60.3**

Based on grab samples, physical treatment units removed 54.0% of fibers, 91.0% of fragments where nylons have increased by 116.7% in concentration. Removal efficiency for physical treatment was 57.7%. Biological treatment steps further removed 65.6% of fibers 45.8% of fragments and 100% of glitter where rise of nylons in share continued but at a lower rate (5.1%). Removal efficiency for

biological treatment was 65.6%. Fibers were removed by 84.1%, fragments removed by 92.3% and nylons increased by 127.8% throughout the wastewater treatment plant units. Overall, removal rate of particles was 84.6%. Based on composite samples, physical treatment removed 46.6% of fibers, 93.8% of fragments and 50.0% of nylons. Removal efficiency for physical treatment was 52.8%. Biological treatment further removed 85.5% of fibers, 66.7% of fragments and 77.8% of nylons. Removal efficiency for biological treatment was 85.5%. Fibers were removed by 92.3%, fragments removed by 97.9% and nylons removed by 88.9% throughout the wastewater treatment plant units. Overall, removal rate of particles was 93.0%.

Removal efficiencies ranged from 38% to 99.9% in the studies reviewed. 72 to 99.9% removal was observed with the studies conducted in Europe where 64-99.9% removal was observed in studies conducted in Asia. Wastewater treatments plant reviewed in North America varied between 74.8-99.9%. Most of the treatment plants reviewed had lower effluent concentrations (<2 particles/L) compared with this study (Murphy et al., 2016; Gies et al., 2018; Lares et al., 2018; Lee et al., 2018; Blair et al; 2019; Long et al., 2019; Magni et al., 2019; Park et al., 2020; Bayo et al., 2020) as their influent concentrations were lower, regardless of the removal rates. Several studies had effluent concentration results, ranging for different times of sampling and different treatment plants but lower (0.7-10 particles/L) than our findings because of their influent concentrations were also lower (Talvitie et al., 2017a; Mintenig et al., 2017). Particle concentrations in the effluent were similar (10-30 particles/L) in some studies with different wastewater treatment plants investigated (Conley et al., 2019, Edo et al., 2020; Leslie et al., 2017), despite the removal rate being lower in one of these plants (Liu et al., 2019). One of the studies (Leslie et al., 2017) reported higher (56-65 particles/L) concentrations in several wastewater treatment plants where seven of them were investigated where chemical character of particles was not confirmed. Edo et al. (2020) reported concentrations as both MPs (10.7 MP/L) and microlitter (overall particles detected in the sample) (26 particles/L). Concentration of microlitter after biological treatment units was also similar (451 particles/L) to this study. Removal rate was not changed significantly with microlitter and microplastics. Considering that there was no characterization conducted with samples from Ambarlı WWTP, the concentrations of microlitter were similar in both studies.

Two studies were conducted in wastewater treatment plants in Turkey. 38-78% removal of particles was observed in Akarsu et al. (2020) study and 73-79% removal was observed in Gündoğdu et al. (2018) study. Concentrations observed in effluent samples varied from 4.1-7.2 particles/L for Gündoğdu et al. (2018) and 0.6-1.6 particles/L for Akarsu et al. (2020) which were lower in concentrations compared to our study. This difference can be explained with the plants receiving

lower concentrations of particles according to the data presented in the studies. Mesh pore size for analysis used in Ambarlı WWTP was nearly identical with Gündoğdu et al. (2018) and larger than Akarsu et al. (2020) and both applied grab sampling method as this study. Overall, the wastewater treatment plant investigated in this study performed better than other plants studied to date in Turkey. Findings from Ambarlı WWTP also confirmed that considerable amounts of particles were removed during physical treatment steps as stated in previous studies (Murphy et al., 2016; Michielssen et al., 2016; Lares et al., 2018; Yang et al.; 2019; Magni et al.; 2019). Results obtained in our study were also in line with the studies implicating that wastewater treatment plants with tertiary treatment units following secondary treatment and wastewater treatment plants with phosphorus/nutrient removal units which stated to have removed MPs more efficiently (Park et al., 2020). Ambarlı WWTP was in the range with European wastewater treatment plants, regarding the overall removal rates of microlitter and microplastics.

Sludge samples differed in their particle concentrations where 1-314 particles/L found in the studies in the literature. Particle concentrations in sludge were also affected by influent concentrations as well as the effluent sample concentrations (Murphy et al., 2016; Leslie et al., 2017; Mintenig et al., 2017; Lares et al., 2018; Talvitie et al., 2017a). The particle concentrations in the sludge were higher than influent concentration except Edo et al. (2020) study and Lee et al. (2018) study. Concentration of particles in the sludge was also lower than the influent concentration in this study. Particle concentrations were in the range of an average value that can be gathered from these studies.

Ambarlı WWTP discharges a final effluent with concentrations ranging from 9.6-21.0 (avg. of 15.3) particles/L; consisting of 9.2-18.8 fibers/L (average of 14.0), 0.4-0.9 (avg. 0.65) fragments/L and 0.1-1.4 (avg 0.75) nylons/L. This corresponds to $3{,}230 \times 10^6$-$7{,}072 \times 10^6$ (average of $5{,}151 \times 10^6$) particles/day released in the receiving water channel. On average, 3957 particle/capita.day was released into the Marmara Sea from the plant through the water channel. These values were significantly high compared to 100 MP/capita.day and 133 MP/capita.day that were found in Murphy et al (2016) and Magni et al. (2019) studies, respectively. Similar release rates with our study were given in Conley et al. (2019) and Michielssen et al (2016) with rates $3{,}808 \times 10^6$ and $9{,}000 \times 10^6$ for several plants reviewed in the studies, which also did not employ a characterization step and therefore reported the release rate of microlitter than microplastics. Talvitie and Heinonen (2014) however, reported even higher values of effluent concentrations with more than 21×10^9 particles were released as particles and fibers where, again, polymer characterization was not conducted.

There was only one study in Marmara Sea investigating microplastic concentrations. In surface water with 10 cm depth, Tunçer et al. (2018) reported 12.63 particles/m^3 with a manta trawl net with 330 μm meshes. Indicating that, with increased depth and lower mesh sizes there could be a lot more microplastics in the Marmara Sea. Considering the volume of Marmara Sea (14,605x10^6 m^3) and the abundance value reported; the particle loading of 35.8 days with the effluent stream corresponds to the total particle abundance reported in the study for surface waters.

Plant also produces dried sludge with a concentration of 60.3 particles/g consisting of 55.0 fibers, 5.0 fragments and 1.0 pellet. This corresponds to 5,069x10^6 particles/d leaving the wastewater treatment plant for use in different applications with 84,070,000 kg of dried sludge leaving the plant. This value is in line with 1,600-5,640x10^6 particles/day of particles found in dry sludge samples from the wastewater treatment plant in Li et al. (2018) study.

Microlitter is a term used to describe particles that are suspected as microplastics and fibers regardless of their origin, which are smaller than 5 mm and generally used in studies where no characterization data is presented. Since there was no chemical characterization step applied in this study, the values reported are actually microlitter values than microplastic values. Characterization steps can result in 26% to 75% (Bayo et al., 2020; Lenz et al., 2015) of particles identified as microplastics. 35 to 45% of particles were identified as microplastics in the reviewed studies. Therefore, these results are likely to be in line with Edo et al. (2020). Therefore, the discharge of microplastics from the wastewater treatment plant is most likely to be lower than 9.6-21.0 particles/L. However, with lower size ranges introduced into the studies, more particles can be captured and these values might increase. Particles with lower sizes tend to fractionate at a higher rate (Ter Halle et al., 2016) and they were expected to be more abundant than larger particles.

6. CONCLUSIONS

This study showed that wastewater treatment plant under investigation was efficient in removing microlitter and essentially microplastics. Quantification of particles resulted with 9.6-21.0 particle/L of microlitter in the effluent sample and 60.3 particles/L of microlitter in the dried sludge sample. Influent sample contained 137.0 particles/L of microlitter. Removal rates of particles were 84.6% and 93.0% for grab effluent sample and composite effluent sample, respectively.

Fiber was the most dominant shape followed by fragment and nylon. Effluent wastewater sample was the only exception where, nylons were more abundant than fragments. Glitters were observed in grab sampling of physical treatment and biological treatment effluents. One pellet was observed in the sludge sample. Particles were mostly in the 500-1000 μm size range followed by 1000-2000 μm range in all wastewater samples observed in this study. Particles with sizes of 500-100 μm were 34.4-45.1% of the particles detected in different steps of the wastewater treatment plant and particles with sizes of 1000-2000 μm were 25.2-35.3% of particles detected. Sludge samples had higher average sizes where 1000-2000 μm range was more abundant (36.5%) than 500-1000 μm range (29.9%).

Fibers in 250-500 μm and 1000-2000 μm size ranges were removed more effectively in the plant investigated. Fragments were removed more efficiently when they were in <500 μm range. Nylons were removed more efficiently with sizes 250-500 μm and >2000 μm. Glitters and pellets were not present throughout the steps of the wastewater treatment plant. Therefore, removal dynamics of these particles could not be commented on in this study. These findings for removal dynamics were in line with the studies presented in the literature for studies investigated particles >50 μm. Wastewater treatment plant's removal rate within the range of European wastewater treatment plants. The average particle sizes were nearly equal or higher in the final effluent samples compared to the raw influent sample. Presence of nutrient removal step, effect of diurnal patterns on incoming particle size averages and removal behavior of particles >25-50 μm could have caused this phenomenon.

Our results showed that despite the high removal rates, particles escape the wastewater treatment plant contribute to the microplastic pollution. Daily release of $5{,}151 \times 10^6$ particles was estimated to reach Marmara Sea from the wastewater treatment plant where $5{,}069 \times 10^6$ particles were present in the wastewater sludge. Average load was estimated to be 14000 particles/m^3 to the receiving sea which was 1109 times higher than that of the reported abundance in the literature. Discharge of 36 days was equal to the reported amount of abundance of fibers detected in the surface waters (of 10

cm depth) of Marmara Sea. This represent a significant release to Marmara Sea from a single wastewater treatment plant however, the removal rates were significantly high.

Lower particle sizes should be covered in order to understand the microplastics contamination as fractionation of microplastics excels with smaller sizes. This study did not cover characterization of the particles collected from various units of the wastewater treatment plant. Considering that 35-45 % microparticles detected in wastewater and sludge samples were confirmed as plastics in the literature, calculated releases with the current experimental methodology might overestimate the potential microplastic pollution. Therefore, future work should include lower size ranges and characterization of particles in order to estimate more accurate release rates of microplastics.

The effect of sizes and polymer structures combined can also explain more about the dynamics of removal investigated in this study. Other parameters such as conventional parameters of wastewater on removal performance, effect of diurnal patterns on incoming particle sizes and concentrations, effect of presence of advanced nutrient removal steps on abundance of size ranges should also be investigated in order to achieve a better understanding on removal of microlitter and therefore, microplastics. The plant operation, design and units that were used in wastewater treatment plants should also be studied in order to evaluate the effect on microplastics removal.

Wastewater treatment plants are receivers and sources of microplastics as they receive a high load of microplastics but not being able to retain all the micro-sized particles with applied treatment technologies. Therefore, reducing the loads of microplastics in the WWTPs should be considered in order to reduce the emissions from the effluent of these facilities. Sweden and France have banned the use of microbeads in personal care products and microplastics use is restricted in several other countries. Throughout the world and in Turkey, same approach on restricted use of microplastics and higher rate of plastics recycling should be implemented through legislation in order to reduce the load of microplastics to wastewater treatment plants.

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