INVESTIGATION OF MICROPLASTICS IN ORGANIZED INDUSTRUAL ZONES AND SPECIFIC INDUSTRIAL WASTEWATERS

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ABSTRACT

INVESTIGATION OF MICROPLASTICS IN ORGANIZED INDUSTRUAL ZONES AND SPECIFIC INDUSTRIAL WASTEWATERS

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Microplastics (MPs) are a growing concern of governments as well as academic and environmental organizations due to their ease of transportation between environmental systems, increasing numbers and ability to transport other contaminants. In the last decades, plenty of studies focused on the wastewater treatment plants (WWTPs) as a contributor of MPs in the environment arising from the domestic activities. However industrial wastewater treatment plants (IWWTPs) have been studied very rarely as compared to their domestic counterparts. Few studies focusing on IWWTPs and organized industrial zones (OIZs) have demonstrated that industrial wastewaters have the capacity to surpass domestic wastewaters in terms of MP pollution. To address this gap, this study aimed to analyze MPs in two different OIZs in Ankara and two selected industries from each OIZ for their plastic use and wastewater generation. It has been found that MP concentrations varied between 3 to 149 MP/L in wastewaters and reaches a value of 183 MP/g TS in sludge and have a large variety of common polymers such as LDPE, HDPE, PET and PP. Among the industries sampled paint industry had the highest

MP concentration, however, having a pretreatment system reduced its concentration from 103 MP/L to 31 MP/L. Presence of an IWWTP in OIZs made a difference in discharges, demonstrating 90.9% removal efficiency on average. With this study, it is concluded that OIZs can be important contributors of MPs pollution in the environment.

Keywords: Industry, Microplastics, Organized Industrial Zone, Wastewater

ÖZ

ORGANİZE SANAYİ BÖLGELERİNDE VE SPESİFİK ENDÜSTRİYEL ATIKSULARDA MİKROPLASTİKLERİN ARAŞTIRILMASI

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Mikroplastikler (MP'ler), çevre sistemleri arasında kolayca taşınabilmeleri, sayıları ve diğer kirleticileri taşıyabilme kabiliyetleri nedeniyle hükümetlerin yanı sıra akademik ve çevre organizasyonlarının da giderek artan bir endişesidir. Son yıllarda yayınlanmış çok sayıda çalışmada atıksu arıtma tesislerinin (AAT'lerin), evsel faaliyetlerden kaynaklanan çevredeki MP'lere kaynak olması üzerine odaklanmıştır. Ancak endüstriyel atıksu arıtma tesisleri (EAAT'ler) evsel AAT'ler ile karşılaştırıldığında çok az incelenmiştir. EAAT'lere ve organize sanayi bölgelerine (OSB'ler) odaklanan birkaç çalışma, endüstriyel atıksuların MP kirliliği açısından evsel atıksuları geçebilecek kapasiteye sahip olduğunu göstermiştir. Bu boşluğu gidermek için, bu çalışma Ankara'daki iki farklı OSB'deki MP'leri ve her OSB'den seçilen iki endüstriyi plastik kullanımı ve atıksu üretimi açısından analiz etmeyi amaçlamıştır. Atıksularda MP konsantrasyonunun 3 ila 149 MP/L arasında değiştiği ve çamurda 183 MP/g TS değerine ulaştığı ve örneklerin LDPE, HDPE, PET ve PP gibi çok çeşitli yaygın polimerlere sahip olduğu bulunmuştur. Örneklenen endüstriler arasında boya endüstrisi en yüksek MP konsantrasyonuna sahip olduğu,

fakat bir ön arıtma sistemine sahip olduğu için konsantrasyonunu 103 MP/L'den 31 MP/L'ye düşürdüğü gözlemlenmiştir. OSB'lerde EAAT'nin varlığının deşarjlarda farklılığa sebep olduğu ve %90,9'luk ortalama giderim verimliliği gösterdiği ortaya konmuştur. Bu çalışma ile OSB'lerin çevredeki MP kirliliğine önemli katkıda bulunabileceğini gösterilmiştir.

Anahtar Kelimeler: Endüstri, Mikroplastik, Organize Sanayi Bölgeleri, Atıksu

To my family

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This thesis is dedicated to the memory of my late father, Hüseyin Karakurt, who left this earth too early and my mother, Gülseren Karakurt. Finally, I want to express my gratitude to my brother, Onur Karakurt, my uncle and cousin, Fevzi and Bahadır Karakurt. Without their support, I would not have been the person I am today.

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ABBREVIATIONS

ABS	Acrylonitrile butadiene styrene
BR	Polybutadiene
CA	Cellulose acetate
EEA	Ethylene ethyl acrylate copolymer
EPM	Ethylene propylene copolymer
EVA	Ethylene-vinyl acetate
HDPE	High-density polyethylene
LDPE	Low-density polyethylene
MP	Microplastic
OIZ	Organized industrial zone
PA	Polyamide
PC	Polycarbonate
PAN	Polyacrylonitrile
PBT	Polybutylene terephthalate
PES	Polyester
PET	Polyethylene terephthalate
PEU	Polyester urea
PMMA	Polymethyl methacrylate
POM	Polyoxymethylene
PP	Polypropylene
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PVS	Polyvinyl stearate
RB	Rubber
WWTP	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

Plastics are the defining feature of modern everyday life. They are durable and inexpensive materials with a wide variety of polymers that offer versatility in several technologies in medical, energy sectors and many other uses for society. Daily life is structured around plastics such that they are used in communication, transport, shoes and other clothing articles and packaging materials for many goods (Andrady & Neal 2009). Compared to conventional materials such as wood and metals, their unmatched chemical and physical characteristics as well as their cheaper price is a reason for this (Lee et al., 2018). With such usage in daily life, the accumulation of plastics in the environments and landfills have reached to substantial quantities such that waste plastics constitute 10 percent of municipal wastes by weight (Barnes et al. 2009). The growing concerns over this issue do not only deal with the aesthetic or land area issues but also pose an environmental pollution issue due to the negative impact of plastics on human health and the environment. Consequently, plastic pollution is expected to cause significant problems in the future including economic losses in the hundreds of millions of dollars.

Monitoring the abundance of plastic debris is necessary for the establishment of a waste inventory and to measure the efficiency of any remediation efforts. Many studies exist on the abundance of plastics on the shorelines compared to oceans or the seabed. However, there needs to be more data on the other kinds of discharges such as rivers and sewers (Ryan et al. 2009). Added to the fact is the variety of methods that are used by different countries and organizations to quantify plastic debris proves to be difficult for the comparison of such data (Cheshire et al. 2009). With such difficulties and ever-increasing production of plastics equating to almost 410 million tons in 2022 (PlasticsEurope, 2023), a new issue has risen such as those

of climate change or persistent organic pollutants (POPs) which are denoted as microplastics (MPs) (Hale et al. 2020).

MPs are small (1-5000 μm) sized polymeric substances that are affecting the environment on a global scale. MPs generally arise due to the deterioration of larger plastics (macro plastics) or can be generated as in the case of microbeads (Hale et al. 2020). These MPs are categorized into two groups that are primary microplastics which are produced in the size range of 1 to 5000 μm for use in products such as personal care products or small fibers in clothing articles. Secondary MPs are the other category that arise from the degradation of macro plastics via some mechanism in the environment such as exposure to solar radiation, mechanical breakage and weathering (Kataoka et al., 2019). As mentioned above for the general estimation of plastic debris in the environment, there also exists a gap in the standard extraction and analysis methods of MPs even with the globally increased attention on the issue. MPs can originate from various sources such as landfills, tire wear, paints, washing of clothes, wastewater treatment, industrial activities and many more (Hale et al. 2020). In order to unify these results several studies have been undertaken dealing with aspects of the procedure such as organic matter removal techniques (Hurley et al. 2018), chemical oxygen demand (COD) based degradation approach (Hatinoğlu et al. 2022) or density separation with high-density salt solutions (Lusher et al. 2020) that aims to standardize MP analysis methods.

Despite the limitations, more than a thousand scientific articles have been published annually since 2019 and rose to more than three thousand since 2022 (Yarahmadi et al., 2024). Many studies have analyzed different mediums for MPs such as municipal wastewaters (Mason et al., 2016), wastewater treatment sludge (Li et al., 2018), sediments (Lusher et al., 2020) and selected industrial wastewaters (Franco et al., 2020). With an emphasis on these mediums and considering especially the amount of work done on domestic wastewaters are relatively high, studies regarding the MP load of organized industrial zones (OIZs) to the overall environmental MP inventory are lacking in the literature. This represents the need for research in the case of

industrial wastewaters and specifically large-scale OIZs as an unaccounted factor in the global MP pollution.

In consideration of these reasons, this study aims to investigate MP pollution in organized industrial zones and selected industries contained within, while applying previously validated methods to quantify MPs and have a large number of samples with representative sample volumes.

The specific objectives of this study are to:

- Quantify and characterize the MPs in OIZs and four selected industries to show daily and seasonal variations in the MPs' amount, size, shape and polymer types.
- Include numerous samples and large volumes to be representative of the real conditions,
- Demonstrate the effectiveness of industrial wastewater treatment plant (IWWTP) and pre-treatment units of selected industries in terms of MPs removal.

To achieve these goals, two different OIZs from Ankara Türkiye have been chosen since they are among the biggest OIZs in Ankara by size, population, production and waste generation. Two different industrial sectors from each OIZ have also been included to study detailed MP generation numbers, where food and paint industries from OIZ-A and metal alongside textile industry from OIZ-B were studied. The industrial partners that have been worked with asked to be left anonymous, which is the reason they are not specified further and are classified by only their industries' general names. The novel part of this study is that MP analysis was conducted by an in-house developed and validated method (Hatinoğlu, et al., 2024) and analyzed a large number of industrial wastewaters from various sources to find the concentrations and characteristics of MPs in large-scale OIZs including seasonal and daily variations. This study is dependent on the organization of these two OIZs where OIZ-A discharges its combined effluent to the nearby wastewater collection system leading to the central urban wastewater treatment plant (WWTP), while OIZ-B treats

its combined effluent in its own industrial wastewater treatment plant (IWWTP) and discharging it into a nearby river. This factor is important for the realization of the effect of IWWTPs to reduce MPs pollution arising from industrial zones as well as demonstrating the differences between municipal wastewater and industrial wastewater treatments in terms of MPs removal.

CHAPTER 2

LITERATURE REVIEW

2.1 Plastics and Environmental Concerns

Plastic pollution is seen as a major threat on a global scale due to inadequate waste management activities despite the increasing plastic production. According to a report published on plastic production in 2022, plastic production worldwide reached about 400 million tons (PlasticsEurope, 2023). Based on 2016 data, the World Economic Forum predicts that global plastic production will double in 2025 and quadruple by 2050 (UNEP, 2020; WEF, 2016). On the other hand, only 6% to 26% of the plastics produced are recycled, thus a substantial portion is released into the environment (Kazour et al., 2019). According to the United Nations Environment Programme Report, if humanity continues its production and waste management activities with its current attitude, 12 billion tons of plastic waste will accumulate in nature by 2050 (UNEP, 2018).

2.2 Industrial Wastewaters, Generation and Characteristics

Industrial wastewater contains a complex mix of contaminants, including heavy metals such as cadmium (Cd), nickel (Ni), lead (Pb), mercury (Hg), arsenic (As), copper (Cu), and chromium (Cr). It is also rich in organic matter, synthetic dyes, chemical residues, suspended particles, and pathogenic microorganisms. Globally, around 359.4 billion cubic meters of wastewater are generated annually, with approximately 80% discharged untreated into the environment (Jones et al., 2021; Amin et al., 2022). Nitrogen (N) and phosphorus (P) are among the primary nutrients found in wastewater, with municipal and agricultural sources—especially swine

effluents—showing concentrations as high as 1180 mg/L. By contrast, industrial effluents generally contain lower levels, up to 480 mg/L (Cheng et al., 2020a). Agricultural, livestock and municipal wastewater typically exhibit higher nitrogen-to-phosphorus (N/P) ratios compared to other sources (Díaz et al., 2022).

Industrial effluents are commonly characterized by elevated levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), and fluctuating pH. The composition of these effluents varies across industries. For example, wastewater from the agricultural and food processing sectors contains prominent levels of BOD, suspended solids, and ammonia nitrogen. Effluents from confectionery production are rich in simple sugars, whereas those from meat processing plants include body fluids, antibiotics, hormones, and other organic matter. Paper and pulp industry wastewater contains significant quantities of suspended solids along with salts of inorganic acids and alkalis. Meanwhile, steel industry effluents are contaminated with gasification by-products, such as benzene, naphthalene, anthracene, cyanide, ammonia, phenols, cresols, and polycyclic aromatic hydrocarbons (PAHs) (Doble and Kumar, 2005).

Moreover, wastewater from agrochemical and pharmaceutical industries often contains complex organic pollutants, such as pesticides, organochlorides, dye residues, plasticizers (e.g., phthalates, bisphenol), and dioxins. These compounds, collectively referred to as xenobiotics, are typically resistant to standard wastewater treatment processes and are classified as persistent organic pollutants (POPs) due to their stability and environmental persistence (Rieger et al., 2002). The discharge of untreated or nutrient-rich effluents into aquatic ecosystems can result in eutrophication, the bioaccumulation of heavy metals, and the buildup of toxic organic substances, posing significant threats to aquatic life and human health (Nagarajan et al., 2022).

The treatment of industrial wastewater varies widely across sectors due to the distinct nature of pollutants generated, requiring industry-specific approaches for efficient management. In the cement, concrete, and ceramics industries, wastewater primarily

originates from cooling and cleaning operations, resulting in significant particulate matter and other contaminants. Commonly applied treatment techniques, such as sedimentation and filtration, are effective in removing suspended solids and reducing pollutant levels (Kumar et al., 2020).

Industrial wastewater treatment plants (WWTPs) differ significantly from domestic WWTPs due to the distinct and complex composition of industrial effluents. Unlike domestic wastewater, which primarily consists of organic matter and nutrients from residential activities, industrial effluents are often characterized by elevated levels of toxic pollutants, heavy metals, and industry-specific organic compounds. As a result, advanced treatment technologies, such as chemical precipitation, advanced oxidation, and specialized biological processes, are required to effectively address these contaminants (Crini & Lichtfouse, 2019; Jones et al., 2021).

Industrial WWTPs also face more stringent regulatory requirements concerning the discharge of hazardous substances, with standards varying widely depending on the industrial sector (Ahmed et al., 2021). To meet these challenges, the design and infrastructure of industrial treatment facilities are typically more sophisticated. They are built to manage fluctuations in flow rates and pollutant concentrations and increasingly incorporate resource recovery systems, enabling the extraction of valuable materials and energy generation from wastewater (Rawat et al., 2023).In summary, the treatment of industrial wastewater necessitates customized and advanced strategies to effectively manage its diverse and hazardous composition while ensuring compliance with environmental regulations.

The food processing sector produces wastewater rich in organic substances and nutrients due to its diverse processing activities. Biological treatments, including anaerobic digestion, are widely used in this industry, as they not only address organic pollution effectively but also enable the recovery of biogas as a renewable energy source (Zhang et al., 2021). In the iron and steel industry, wastewater is heavily contaminated with toxic pollutants and heavy metals due to extensive water use during production processes. Chemical precipitation combined with advanced

filtration systems is frequently employed to eliminate these contaminants, ensuring adherence to environmental standards (Singh et al., 2019).

The pharmaceutical industry generates wastewater containing complex, non-biodegradable organic substances, including hormones and antibiotics. To treat these challenging effluents, advanced oxidation processes and membrane filtration technologies are commonly implemented, enabling the breakdown of hazardous compounds and mitigating their environmental impact (García et al., 2022). Meanwhile, the textile industry discharges wastewater that is heavily laden with dyes and chemicals from wet processing. A combination of methods, such as adsorption, biological treatments, and advanced oxidation, is utilized to remove colors and detoxify the effluent, significantly improving its quality before discharge (Khan et al., 2020).

Ultimately, the adoption of customized treatment technologies is vital for ensuring compliance with environmental regulations and advancing sustainable water management practices across these diverse industrial sectors.

2.3 Microplastics as Emerging Pollutants

2.3.1 Sources of Microplastics

Plastics smaller than 5 mm are defined as microplastics (MP) and are divided into two main groups primary and secondary MPs (Gatidou et al., 2019). While primary MPs are produced in micro sizes and used in areas such as cosmetics and personal care products, secondary MPs are formed by the disintegration, degradation and abrasion of plastics due to environmental factors (Ngo et al., 2019). Based on their shape, while there is no standard form, the most used shapes of MPs are fibers, fragments, beads, foams and films (Hamidian et al., 2021).

Shapes of MPs can be an important factor in how they are transported among environmental systems, their effects on these systems and the fate of said MPs (Xiao

et al., 2024; Zhao et al., 2021; Taghizadeh et al., 2024). Xiao and colleagues (2024) found in their studies that even amongst different fiber shapes (flat or circular) there are differences in their atmospheric long-range transport capabilities. Another study by Bello and colleagues (2022) proposed MPs settling characteristics will change in the marine environments due to their shapes and changing drag characteristics. The effect of biofilms causing changes in the sinking behavior of marine MPs are observed (Gaylarde et al., 2023). As for the effects of of MPs on the environment, Zhao and colleagues demonstrated the shape of MPs can affect the soil pH and cause varied changes in the enzyme activities such as higher acid phosphatase activity dye to fibers, films and foams. In a study by Tahzizadeh and colleagues (2024), the effect of MPs shape on the surface erosion of MPs particles were observed and found that irregularly shaped fragments were more prone to surface degradation than the spherical microbeads. Semensatto and colleagues (2024) also communicated that the reporting of MPs shapes is an important factor in the higher understanding of MPs' effect on the environment and encourages other researchers to provide these data for better comparability between the MPs studies.

2.3.2 Negative Effects of Microplastics

MPs, which enter environmental systems such as water, soil, air and sediment and accumulate due to their resistant structures to degradation, block the digestive system of animals in aquatic ecosystems and can create the potential for biofilm formation by pathogenic and antibiotic-resistant microorganisms (Galafassi et al., 2019). In addition, due to their high surface areas and hydrophobic structures, MPs can carry hazardous chemicals and cause these chemicals to be taken into the body of organisms (Zhang and Chen, 2020). Many plastics do come with additives up to 4% of their weight. These additives can include plasticizers, antioxidants, adhesives, stabilizers (Bayo et al., 2018) and WWTPs may be a source of MPs and their additives as due to accumulation and eventual release. These additives are also known to be overall toxic to fauna, flora or humans (Wang et al., 2013).

2.4 Occurrence of Microplastics in Wastewater Treatment Plants

There are studies in the literature on the amount and characterization of MPs in WWTPs, mainly domestic WWTPs. Table 2.1 demonstrates the concentration of MPs in WWTPs from several studies including domestic, industrial and mixed wastewater sources.

Yuan et al. (2020) analyzed MPs in two different WWTPs. Since industrial and domestic wastewater were mixed in both WWTPs, it was not possible to make separate analyses of the wastewaters. However, while industrial wastewater from industries such as textile, pharmaceutical, beer, machinery manufacturing, and electronics was predominant in one of the WWTPs, the proportion of domestic wastewater was high in the other WWTP, as seen in Table 2.1. Their results show that at the influent of both WWTPs, MP concentrations ranged from 10.3 MP/L to 22.05 MP/L. On the other hand, the effluents of both WWTPs showed significantly lower MP concentrations, at 0.24 MP/L and 0.34 MP/L, respectively. The WWTPs these plants used were biological treatment-based systems combined with advanced filtration techniques.

It can be concluded that while both WWTPs were effective in significantly reducing MP concentrations in treated effluents, the variations in influent MP levels suggest that industrial wastewater contributes higher levels of MPs compared to domestic sources. This underscores the importance of tailoring WWTP technologies to account for the higher pollutant load from industrial sources to achieve optimal removal efficiency.

In their study, Franco et al. (2021) examined two WWTPs with distinct wastewater sources in terms of MP content. One of these WWTPs treated industrial wastewater, primarily originating from activities such as cleaning, dismantling, maintenance, and repair of ships, which involve the use of significant amounts of paint, coatings, anti-slip materials, and abrasives. The other WWTP processed domestic wastewater. As shown in Table 2.1, the industrial wastewater influent had more than twice the MP

concentration compared to the domestic influent, with values of 1567.49 ± 413.18 MP/L and 645.03 ± 182.24 MP/L, respectively.

The MP removal efficiency of the industrial WWTP was calculated at 91.62%, while the domestic WWTP achieved a slightly higher removal rate of 97.20%. This difference can be attributed to variations in the treatment technologies employed. Overall, these results highlight the significantly higher potential for MP abundance in industrial wastewater compared to domestic sources, emphasizing the need for robust and specialized treatment processes in industrial WWTPs to manage these elevated levels effectively.

In another study, X. Xu et al. (2019) examined the MPs in 11 different WWTPs receiving industrial and domestic wastewater at different rates. It was stated that two of the 11 facilities studied (W3 and W5) received completely industrial wastewater, on the other hand, the other two (W4 and W9) received mixed industrial and domestic wastewater, with a major share of industrial wastewater. Of these, W4 received 95%, and the other received 80% (W9) industrial wastewater, mainly from textile printing and dyeing factories. Therefore, two of the 11 facilities studied can be considered as industrial WWTPs, and three can be considered as WWTPs receiving high-rate industrial wastewater.

Long et al. (2019) observed that MP concentrations in industrial wastewater entering WWTPs were 1.8 times higher than those in domestic wastewater, highlighting the substantial contribution of industrial sources to MP pollution. Limited studies corroborate these findings, indicating that industrial wastewater significantly increases MP loads compared to domestic effluents (Franco et al., 2020; Long et al., 2019; Hamidian et al., 2021). This underscores the necessity for more extensive research on industrial wastewater's role in MP contamination.

Table 2.1 MPs concentrations reported in previous studies on WWTPs

Study	Location	Wastewater Source	Results
(Yuan et al., 2020)	WWTP 1	Mostly Industrial + Domestic	Inlet: 22.05 MP/L Outlet: 0.34 MP/L
	WWTP 2	Mostly Domestic + Industrial	Inlet: 10.3 MP/L Outlet: 0.24 MP/L
(Franco et al., 2021)	WWTP 1	Domestic	Inlet: 645.03 ± 182.24 MP/L Outlet: 16.40 ± 7.85 MP/L
	WWTP 2	Industrial	Inlet: 1567.49 ± 413.18 MP/L Outlet: 131.35 ± 95.36 MP/L
(X. Xu et al., 2019)	W1	Domestic (90%)	Inlet: 144.30 ± 13.30 MP/L Outlet: 9.41 ± 1.29
	W1	Domestic	Inlet: 142.67 ± 19.48 MP/L Outlet: 11.7 ± 1.51
	W3	Industrial	Inlet: 116.00 ± 11.43 MP/L Outlet: 9.48 ± 1.09
	W4	Industrial (90%)	Inlet: 330.00 ± 9.93 MP/L Outler: 9.41 ± 1.48 MP/L
	W5	Industrial	Inlet: 198.67 ± 18.06 MP/L Outlet: 7.7 ± 0.86 MP/L
	W6	Domestic (99%)	Inlet: 110.00 ± 8.16 MP/L Outlet: 3.63 ± 0.46 MP/L
	W7	Domestic (95%)	Inlet: 250.00 ± 17.66 MP/L Outlet: 10.59 ± 0.64 MP/L
	W8	Industrial (90%)	Inlet: 79.33 ± 0.94 MP/L Outlet: 8.59 ± 1.09 MP/L
	W9	Industrial (70%)	Inlet: 260.67 ± 7.36 MP/L Outlet: 8.96 ± 0.64 MP/L
	W10	Domestic (90%)	Inlet: 342.67 ± 7.37 MP/L Outlet: 13.63 ± 2.63 MP/L
	W11	Domestic (90%)	Inlet: 182.67 ± 15.52 MP/L Outlet: 6.37 ± 0.64 MP/L
(Liu et al., 2019)		Domestic	Inlet: 79.9 ± 9.3 MP/L Outlet: 28.4 ± 7.0 MP/L
(Franco et al., 2021)	WWTP1	Domestic	Inlet: 378 MP/L Outlet: 7 MP/L
	WWTP2	Domestic	Inlet: 586 MP/L Outlet: 18 MP/L
	WWTP3	Domestic	Inlet: 275 MP/L Outlet: 8 MP/L
(Edo et al., 2019)		Domestic	Inlet: 171 ± 43 MP/L Outlet: 10.7 ± 5.2 MP/L

Phuprasert et al. (2021a) investigated MPs in an industrial WWTP that primarily processed wastewater from the automotive, electronics, and transportation industries. Their findings revealed an influent MP concentration of 101.9 ± 0.5 MPs/L and an effluent concentration of 11.04 ± 0.1 MPs/L, equating to a 90% removal efficiency. Similarly, Deng et al. (2023) analyzed a petrochemical WWTP and found MP concentrations of 10,310 items/L in the influent and 1,280 items/L in the effluent, achieving an 87.6% removal rate. Additionally, MPs accumulated in the sludge, with 4,328 items/g detected in activated sludge and 10,767 items/g in waste sludge.

Saiwaree and Kanokkantapong (2021) examined an industrial WWTP handling wastewater from food and beverage, electronics, metal, manufacturing, and chemical industries. They reported an influent MP concentration of 103.1 ± 59.5 MPs/L and a removal efficiency of 93.9%. Haque et al. (2022) focused on MP pollution in wastewater and sludge from dyeing, washing, pharmaceutical, battery, and printing industries, identifying 2,713 ± 566 MPs/L in influent water, 293 ± 47 MPs/L in effluent, and 115,878 ± 20,453 MPs/kg in sludge.

Xu et al. (2018) studied a WWTP in a textile industrial park, documenting MP concentrations of 334.1 MPs/L in the influent, 80.7 MPs/L after pre-sedimentation, 54.5 MPs/L post-sedimentation, and 16.3 MPs/L in the final effluent, with an overall removal efficiency of 95.1%. Similarly, Magalhães et al. (2022) investigated Portuguese industrial effluents from paint, resin, textile, pharmaceutical, and PVC industries, concluding that these activities, particularly paint and pharmaceutical production, significantly contribute to environmental MP pollution. However, the results of industrial wastewater show significant differences and vary by industry. This situation reveals the importance of examining the wastewater of individual industries in terms of MP.

When the results of the study were examined, it was observed that the MP concentration of wastewater with an increasing industrial rate often increased. These

results show that the MP release from industrial wastewater is higher than that from domestic wastewater, as indicated in the previous parts.

The findings from various studies consistently demonstrate that IWW contains significantly higher concentrations of microplastics (MPs) compared to domestic wastewater (Long et al., 2019; Franco et al., 2020; Saiwaree and Kanokkantapong, 2021). Industrial processes, such as those in textiles, automotive, and chemical manufacturing, contribute to higher MP loads due to the large quantities of synthetic materials, including plastics, used in these industries. For instance, studies have shown that industrial WWTPs receive influent MP concentrations that are much higher than those of domestic wastewater, often due to the variety of contaminants introduced by industrial activities (Long et al., 2019; Franco et al., 2020). As the proportion of industrial wastewater increases in a treatment plant, the MP concentrations in the influent and effluent tend to rise as well. In contrast, domestic wastewater primarily originates from household activities and typically contains fewer MPs, although they still contribute to overall MP pollution. This stark difference highlights the need for more targeted treatment strategies for industrial wastewater, as it represents a significant source of MP contamination in the environment. Consequently, industrial wastewater is a major contributor to MP pollution, and addressing it requires specialized treatment methods to effectively mitigate its environmental impact.

2.4.1 Effect of Treatment on MPs Concentration

Studies conducted in WWTPs have demonstrated that these facilities can achieve high MP removal efficiencies, with rates reaching up to 99% under optimal conditions (Castelvetro et al., 2020). It has been observed that over 90% of the removed MPs are transferred to and accumulate in wastewater treatment sludge (L. Zhang et al., 2020). However, despite the substantial removal of MPs from treated effluent, the large volumes of influent wastewater and high MP concentrations result in an average discharge of 10^5–10^8 MP into the environment daily from WWTP

changing with the capacity and type (Lares et al., 2018; Magni et al., 2019; Sun et al., 2019)

Removal efficiencies vary across various stages of treatment. During primary treatment, MPs are removed at rates ranging between 30% and 90%, depending on the process design and operating conditions, while secondary treatment contributes an additional 16% to 50% removal (Reddy & Nair, 2022; Iyare et al., 2020). In tertiary treatment, advanced technologies such as membrane filtration systems can achieve MP removal efficiencies of up to 99.9%, offering a highly effective solution for minimizing MP discharge into receiving water bodies (S.F. Ahmed et al., 2024).

Phuprasetet et al. (2021) examined MPs in two different industrial WWTPs in their study. One of these WWTPs (WWTP A) treats wastewater from approximately 200 factories, including automotive, electronics and industrial plastic manufacturers, while the other WWTP (WWTP B) treats wastewater from approximately 146 factories (mostly automotive, transportation and electronics sectors). In the study, samples were taken from different units of industrial WWTPs and MP amounts were determined. From 5 different points of WWTP A, respectively; samples were taken from the WWTP inlet (101.87 ± 0.47 MP/L), after the grit chamber (113.49 ± 0.71 MP/L), after the aeration tank, after the final sedimentation tank and finally WWTP outlet (11.04 ± 0.08 MP/L). In WWTP B, samples were taken from 4 different points; WWTP inlet (148.44 ± 0.91 MP/L), after grit chamber (150.56 ± 1.83 MP/L), after aeration tank and after the final sedimentation tank as the WWTP outlet (33.53 ± 0.55 MP/L), respectively. While the MP removal efficiency of WWTP A was 89.22%, this value was found to be 77.56% in WWTP B. It was stated that with the reverse osmosis unit, WWTP A had the highest overall MP removal efficiency of 99.54% and that increased the overall treatment efficiency.

The study by Saiwaree and Kanokkantapong (2021) provides valuable insights into the performance of an industrial WWTP treating wastewater from food and beverage, electronics, metal, manufacturing, and chemical industries across two distinct seasonal periods: rainy and dry seasons. The overall performance of the

WWTP demonstrated effective MP removal, with an efficiency of 93.86% during the rainy season. This high removal rate underscores the capability of the facility to manage significant MP loads effectively, even under varying operational conditions. The comparison between the rainy and dry seasons revealed notable differences in MP abundance across the treatment units. During the wet season, the highest MP concentration was recorded in the aeration unit (134.35 ± 20.79 MP/L), while in the dry season, it was significantly lower (31.38 ± 10.36 MP/L). Higher MP concentration in the aeration unit compared to the influent wastewater is attributed to the mechanical breakdown of the larger MPs to smaller particles. Similarly, the lowest MP levels were found at different points depending on the season: the WWTP outlet during the wet season (6.33 ± 1.36 MP/L) and the post-final sedimentation tank in the dry season (13.98 ± 4.50 MP/L), however difference between the two found to be statistically insignificant. The differences between the dry and wet seasons can be attributed to several factors. Higher water inflows during the rainy season may lead to increased dilution of MPs at certain stages, altering their concentration throughout the treatment process or the increase in the contact time of sludge may lead to higher MPs at the effluent wastewater. The grit chamber's effectiveness as the most efficient MP removal unit during the wet season (81.27%) further highlights the importance of physical separation processes in managing high-flow conditions.

MPs represent a heterogeneous group of polymeric materials characterized by varying dimensions and structures, each possessing distinct physicochemical and toxicological properties (Bonfanti et al., 2021). Their shape plays a crucial role in their classification and significantly influences their removal efficiency during wastewater treatment processes (Hidayaturrahman et al., 2019). Fibers, fragments, and films are the most commonly identified microplastic forms in wastewater (Van Do et al., 2022; Bayo et al. 2020). Of these, fibers—defined as filament-like microstructures—are the most prevalent type encountered in wastewater samples.

The diverse range of dimensions and shapes identified in the MPs samples collected from various WWTPs is summarized in Table 2.2.

Table 2.2 Characteristics of MPs in industrial wastewater influents

WWTPs Location	**Shape**	**Size Range of Particles**	**Reference**
Spain/Cabezo Beaza WWTP	Fragments (46.5%), fibers (34%), films (15.1%), foam (2.4%)	400 to 600 µm	(Bayo et al. 2020)
Vietnam/HoaCam WWTP	Fibers and fragments	1.6 to 5000 µm	(Van Do et al., 2022)
Vietnam/DaNang WWTP	Fibers and fragments	1.6 to 5000 µm	(Van Do et al., 2022)
Vietnam/HoaKhanh WWTP	Fibers and fragments	1.6 to 5000 µm	(Van Do et al., 2022)
France/Seine-Centre WWTP	(A) Flake, (B) sphere, (C) fiber, (D) filament, (E) fragment	100 to 5000 µm	(Hidayaturrahman et al., 2019)
Poland/Silesia Province WWTP, South Korea/Daegu WWTP	Microbeads, fragments, fibers	<355 µm	(Kittipongvises et al., 2022)& (Hidayaturrahman et al., 2019)
China/7 WWTPs in Xiamen	Fragments and films were the most abundant shapes	43–335 µm	(McCormick et al., 2014)
Germany/12 WWTPs in Lower Saxony	Fragments and films were the most abundant shapes	<500 µm	(Wang et al., 2022)

The size range of microplastics (MPs) detected in industrial wastewater influents varies considerably across studies, reflecting differences in sampling locations, industrial activities, and methodologies. Table 2.2 provides a detailed summary of the reported size ranges in different regions. These variations underscore the influence of industrial activities on MP characteristics, with facilities handling diverse effluents from textile, chemical, or agricultural sources typically exhibiting broader size ranges. Smaller MPs, such as those below 500 μm, are especially concerning due to their higher bioavailability and potential for accumulation in aquatic organisms. Moreover, the detection of such minute particles highlights the limitations of current wastewater treatment technologies in achieving complete MP removal, particularly for particles at the lower end of the size spectrum.

2.4.2 MP Polymer Types in WWTP

Numerous studies have investigated the types of MP polymers prevalent in WWTPs, revealing significant variations depending on the wastewater source and treatment processes. Polyethylene (PE), in its low-density (LDPE) and high-density (HDPE) forms, is among the most commonly detected polymers. For instance, Bayo et al. (2020) identified LDPE and HDPE as the most prevalent MP types in a classical WWTP utilizing an activated sludge process. Similarly, Wang et al. (2020) found that PE, along with polypropylene (PP) and polystyrene (PS), accounted for approximately 83% of the MPs detected in the influent and effluent of several WWTPs in Changzhou, China, with the majority being fragments and films smaller than 500 μm.

Mintenig et al. (2019), in their study of 12 WWTPs in Germany, reported that PE dominated MPs larger than 500 μm, comprising 59% of the total, followed by PP at 16%. For MPs smaller than 500 μm, PE remained the most abundant polymer, constituting 40% of the total. Interestingly, no significant correlation was observed between the number, size, or type of polymers and the treatment plant characteristics.

Industrial WWTPs exhibit different polymer profiles compared to municipal WWTPs. Van Do et al. (2022) report that polyethylene terephthalate (PET), PE, nylon, and polyvinyl chloride (PVC) were the dominant polymers in MPs from three industrial treatment facilities in Vietnam. Franco et al. (2020), in their study of five municipal WWTPs and two industrial plants in Cadiz, observed higher MP quantities in industrial plants. The influents contained diverse polymer types, including PVC, HDPE, poly (ethyl methacrylate) (PEMA), PP, PS, and PE.

Additionally, MPs originating from specific industrial sources demonstrate unique polymer compositions. For example, Wang et al. (2020) identified PE (38%) and polyamide (PAM) (32%) as the predominant polymers in wastewater from livestock farms and fishponds.

These findings underscore the predominance of PE, PP, and PET in both municipal and industrial WWTPs, with significant contributions from other polymers such as PVC and PS. The diversity in polymer types reflects the variability in wastewater sources and emphasizes the need for tailored treatment approaches to address specific MP challenges in WWTPs.

2.5 Analysis of Microplastics in Wastewaters and Sludge

2.5.1 General Approach

A striking issue in studies involving MP measurements in environmental samples is that the measured MP amounts show significant numerical differences from one study to another. For example, in a study examining 7 domestic WWTPs in the USA, the amount of MP found in the plant inlet wastewater was reported as 1 MP/L (Carr et al., 2016), while in a study examining 10 domestic WWTPs in Denmark, this value was reported as 7216 MP/L (Alst and Vollertsen, 2018). It can be expected that these values will naturally vary depending on the sewer infrastructure, wastewater character, country conditions and season. However, another important reason for the

observation of such different values is that there is no standard method accepted worldwide for MP measurements.

In recent years, the American Society for Testing and Materials (ASTM) has published two standard methods for sampling (ASTM D8332-20) (ASTM, 2020a) and preparation (ASTM D8333-20) (ASTM, 2020b) of samples to be analyzed for MPs. These methods are intended for drinking water, surface water, wastewater influent and effluent, and seawater; sewage sludge is not included in these methods. These methods, which have been published recently, have not yet been used by many of the studies reviewed, and each study has adopted a different method. Despite all these differences, MP analysis protocols typically consist of three basic steps after sampling. These steps are the pretreatment process, MP extraction, and MP counting and identification steps, respectively.

Sampling of the MPs in wastewater and sludge is critical to ensure further reliability of the results. Sampling can be carried out in two different ways: grab or composite. In grab sampling, a single sample is taken and examined at a certain time, while in composite sampling, samples are taken over a period of time. Since grab sampling does not reflect time-dependent changes, it may be insufficient compared to composite sampling in this respect (Talvitie et al., 2017; Lasee et al., 2017). On the other hand, grab sampling may be necessary and more meaningful to follow the differences during the day.

The sample volume to be taken for sampling varies in studies. In general, the organic load or the strength of the water or wastewater in the sample to be taken has been a determinant of the sample volume in most studies. In WWTP inlet waters with high organic load, the sampling volume was kept lower due to both the relatively high expected MP amounts and the fact that the sieve system could clog rapidly. However, in cases where the identification of MPs in large size ranges is critical for the study, sampling in high volumes has been recommended (Gatidou et al., 2019). It has been emphasized that not using plastic material in the sampling is critical for the accuracy of the measurement results (Vollertsen and Hansen, 2017).

2.5.2 Sample Pre-treatment

The first step of the measurement after sampling includes the pretreatment of the sample. This step is especially important for wastewater and sludge, which have matrices rich in organic matter. In the last step, MP identification, it is critical that the pretreatment step required to prevent organic matter from interfering does not affect the polymer structure of the MPs.

The main pretreatment methods used can be classified as enzymatic pretreatment and oxidative chemical pretreatment. Chemical pretreatment methods can be listed as acidic, alkaline, wet peroxide oxidation and Fenton oxidation processes (Li et al., 2020). It has been stated that a strong chemical pretreatment containing strong oxidants has the potential to reduce the mass of certain MPs by up to 60% (Hurley et al., 2018).

In addition, attention should be paid to the chemical dose used for pretreatment, to avoid wasting chemicals and to avoid unnecessarily high levels that will be ineffective in removing organic matter. It would be appropriate to determine the chemical amounts based on the relevant quantitative parameters. Among the methods, enzymatic pretreatment does not damage the polymer in the sample as much as strong oxidizing chemical treatments (Cole et al., 2015; Catarino et al., 2017; Courtene-Jones et al., 2017). However, enzymatic pretreatment is not sufficient for matrices containing high organic matter such as wastewater and sewage sludge. In addition, since the time it takes for enzymes to reach their highest activity can be in the order of days, this puts enzymes at a disadvantage in terms of the rapidity of analysis and practicality of measurement. In addition, enzymes such as proteinase-K used in the studies are generally used for microbiological purposes and have very high costs (Löder et al., 2017). Therefore, chemical pretreatment for wastewater and sludge samples is much more advantageous in terms of effective organic removal, rapid analysis and low measurement cost.

Hurley et al. (2018) emphasized that the use of acids such as hydrochloric acid (HCl) and nitric acid (HNO_3) during the pretreatment process is a highly effective pretreatment process. However, Li et al., (2020) suggested in their study that the use of low concentrations of hydrochloric acid (HCl) and nitric acid (HNO_3) can increase the extraction of MPs by 2.9-7.6% and that acids used at higher concentrations cause deterioration of MPs, especially in their structures. In the study, it was emphasized that the structural properties of the PA MP type, in particular, changed when exposed to high doses of acid. However, Li et al. (2020) stated that acids (HCl and HNO_3) used at low concentrations help to increase the efficiency of the pretreatment process while reducing the damage to MPs and that 24-hour reactions with 1M HCl or 1M HNO_3 at 60^{o}C are the most suitable procedure.

Another pretreatment process is the alkaline pretreatment process. Alkaline pretreatment is widely used for organic matter removal in sludge and wastewater samples with alkaline pretreatment. In a study, NaOH (1M and 10M) and KOH (10% (w/w)) were used while comparing different pretreatment methods, and the same procedure (at 60°C for 24 hours) was followed for both alkalis. As a result of this experiment, it was found that both NaOH and KOH could not provide sufficient organic matter removal, and mass change in MP types such as PET, polycarbonate (PC) and PS was not observed. It has been reported that they cause organic matter removal (Lusher et al., 2018; Cole et al., 2013). In literature studies, it has been stated that the alkaline pretreatment process, as in the acidic pretreatment process, damages MPs while providing low levels of organic matter removal.

One of the most popular pretreatment processes is wet peroxide (H_2O_2) oxidation (WPO). WPO is preferred especially in sludge and wastewater with complex matrix structures. Cole et al., (2015) determined in their study that H_2O_2 (35% w/w) provided 25% removal on existing organic matter with a 7-day reaction time and ambient temperature. It was also observed that the reaction time could be reduced to 12 hours when the temperature was increased to 70 °C levels (Sujathan et al., 2017). However, Lusher et al. (2018) reported that the pretreatment process carried out at 70 °C caused changes in the structures of some plastics. Lusher et al. (2017), who

compared two types of alkaline and Fenton oxidation pretreatments, namely hydrogen peroxide, KOH and NaOH, showed that Fenton oxidation is the most effective method in terms of cost and time in samples with high organic content such as sewage sludge. Similarly, Hurley et al. (2018) showed that the Fenton reagent provides effective organic matter removal in PP, LDPE, HDPE, PS, PET, PA-6.6, polymethylmethacrylate (PMMA) polymers without any damage. In addition to revealing the effects of the chemical to be used on the polymer, the importance of quantitatively reporting the organic matter removal in the sample with a parameter such as chemical oxygen demand (COD) is also critically emphasized (Nguyen et al., 2019).

2.5.3 Microplastics Extraction

The third step in MP analysis protocols is MP extraction. After the organic matter content is successfully removed without damaging the polymer structure of the MPs, the MPs should be separated from the sample.

The most common method used for this step is the density separation method, which concentrates the sample with salts such as NaCl (1.15-1.2 g/cm^3), $ZnCl_2$ (1.5-1.7 g/cm^3) and NaI (1.6-1.8 g/cm^3) and floats the low-density MPs (Li et al., 2019; X.Lv et al., 2019). In this step, it is critical to successfully extract polymers such as PET and polyacrylonitrile (PAN). Li et al. (2019) showed that the number of MPs detected increased as the salt density was increased. In a study comparing the extraction efficiencies of four different salt solutions (i.e. NaCl, NaBr, NaI, $ZnBr_2$), Quinn et al. (2017) showed that the lowest recovery efficiency (<85–95% and ~70% for MPs of 200–400 μm and 400–800 μm sizes, respectively) was for NaCl solution added to sediment samples. Therefore, three washings of the sediment were needed for the NaCl solution, while only a single washing was sufficient for NaI and $ZnBr_2$ solutions to properly remove MPs (Quinn et al., 2017). To separate and accurately quantify both low- and high-density MPs, sequential extraction with NaI and $ZnCl_2$

has been recommended in the literature despite its high cost and the relatively toxic properties of the salts (Okoffo et al., 2019; Silva et al., 2018).

2.5.4 Quantification and Identification

After MPs are separated from the environmental samples, they need to be identified and their quantities determined. In this context, there are different methods applied in literature.

The most commonly used analysis method is visual identification using optical and spectroscopic techniques together and then determining the chemical structure of the polymer (Silva et al., 2018). Depending on the size of the plastics to be examined with the visual identification method, devices such as light microscope (Lares et al., 2018), fluorescence microscope (Erni-Cassola et al., 2017), stereomicroscope (Edo et al., 2020) and scanning electron microscope (SEM) (Wang et al., 2020) are used to pre-separate the plastics whose chemical structures will be examined in the next stages from the sample. With this method, the physical properties such as size, shape, color and the number of MPs can be determined (Okoffo et al., 2019).

Nile Red (Stanton et al., 2019), Safranine-T (L.Lv et al., 2019) and various textile dyes (Karakolis et al., 2019) that do not dye organic and semi-synthetic substances but dye plastics, or Rose Bengal chemical (Campo et al., 2019) that dyes organic substances but does not dye plastics, can be used to distinguish MPs from other substances remaining in the sample under a fluorescence microscope and count them.

Although it is necessary to use visual assessment methods in MP analyses, when only this approach is used, MP numbers are overestimated. In addition, polymer types cannot be determined with this method. Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy are widely used vibrational spectroscopy methods in polymer analyses (Käppler et al., 2016). These methods are also widely used to determine the types of MPs and are non-destructive analysis methods (Frond et al., 2021; Crichton et al., 2017; J. Xu et al., 2019; Araujo et al.,

2018). Both methods have their advantages and disadvantages, for example, while Raman spectroscopy can read at lower particle sizes, the measurement quality may decrease due to the fluorescence effect. Similarly, while FTIR measurements are not affected by fluorescence, their results are negatively affected in the presence of water (Cabernard et al., 2018). However, as Cabernard et al. (2018) mentioned, particle size creates a selective effect between the two methods. Considering that 90% of the MP numbers encountered in industrial wastewater are smaller than 500 μm (Sun et al., 2019), choosing the right method is of great importance. In these cases, by using both techniques together, it is possible to scan the entire sample and reveal the overall picture.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Selection of OIZs and Industries

While selecting the OIZs to be studied within the scope of the study, three issues were considered:

1. Working with OIZs with large capacities and different sectors
2. At least one of the two OIZs selected should have a WWTP so that samples can be taken from WWTP inlet, outlet and different units
3. The OIZs should be located in Ankara and provide permission and assistance for the necessary field works.

The studies were carried out in OIZ-A and OIZ-B as well as food, paint, textile and metal industries from these OIZs. Respecting the request to be kept confidential by our industrial partners which agreed to aid this study, their identities are retained anonymous.

The food industry, located in OIZ-A, mainly produces jams, halva, honey and spices. While their processes do not directly have plastics in them, some equipment, personal protective equipment and product packaging are expected to yield some plastics in the wastewater. This industry generates about 10 m^3/day of wastewater, which is especially high inflow in the evening hours due to cleaning of the plant.

The paint industry, located in OIZ-A, produces electrostatic powder paint which has polymers as its main ingredients as well as other process-related equipment. It is expected that this industry be one of the higher MPs contributors in this study due to its main product being a plastic derivative as well as the high amount of its

production. This industry generates around 10 m^3 of wastewater daily and generally discharges it in 5 m^3 batches in the morning and afternoons.

The textile industry of OIZ-B is another plant where the raw material used in production is plastic (mainly polyesters). Their wastewater generation varies greatly, depending on the type of processes employed daily. At full production capacity, the factory generates up to 80 m^3/day of wastewater which is collected in an underground tank and treated in batches (amount unknown).

The metal industry is the biggest wastewater contributor of OIZ-B, which produces about 30% of the OIZ-B's total daily wastewater. Their process is continuous as well as their wastewater treatment and discharge (405 m^3/day). While they produce metal works from large powerline structures to small nails and bolts, none of which directly involves plastics, it is expected with such high wastewater flows, even small numbers can greatly contribute to MPs in the OIZ-B's wastewater.

3.1.2 Sampling Locations

In both sampling campaigns, samples were taken at targeted times from all selected points. Of the two sampled OIZs, OIZ-B has a full-scale IWWTP that is continuously in operation.

Both OIZs have a large capacity among OIZs in Ankara (close to 1000 hectares each with close to 100% occupancy). OIZ-A generates wastewater around 3000 m^3/day while OIZ-B generates 2500 m^3/day.

OIZ-A discharges its wastewater into the municipality sewer system from two different locations. The main discharge location compromises more than 60% of the OIZs total wastewater and it was selected as the sampling point for OIZ-A.

OIZ-B IWWTP inlet and outlet were the main sampling points and multiple samples were taken throughout the day. In addition to these, there were locations where only a single sample was taken from the secondary sludge tank, centrifuge sludge,

centrifuge reject, post-screening and aerated grit chamber. Sampling locations are given in Figure 3.1.

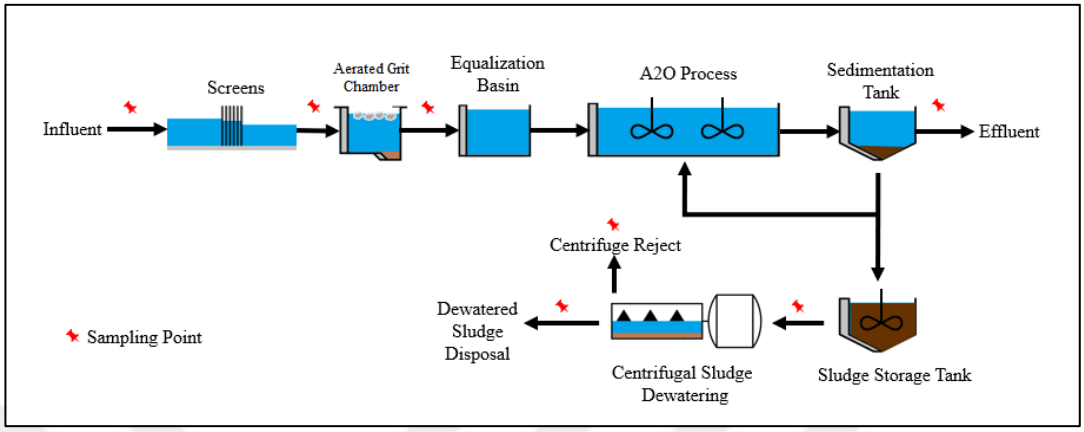

Figure 3.1 Flow diagram and sampling locations of OIZ-B IWWTP

For the specific industries, food and paint industries are located in OIZ-A while textile and metal industries are located in OIZ-B. Of the four factories, only the food industry does not have any pretreatment process and discharges directly into the OIZ-A wastewater network.

Paint, textile and metal industries all have pretreatment processes to comply with local legislation. These pretreatment units vary in size and technologies, however, all three of them are composed of some variation of chemical precipitation. Inlet and outlet designations for the wastewater samples are taken from these pretreatment units, the inlet being the raw wastewater the industry generates and the outlet being the pretreated effluent.

Pre-treatment systems in these plants operate intermittently at various intervals throughout the day depending on the generation of wastewater in some industries where wastewater due to production is intermittently formed. This occurs, for instance, once daily in a textile dyeing plant and twice daily on average in the dye industry. On the other hand, the metal production and processing plant continuously produces wastewater, and the pre-treatment system is in continuous operation.

3.1.3 Sampling Protocols and Timetable

Two sampling campaigns were concluded for OIZ-A on 06.12.2021 and 05.04.2022. For OIZ-B, these dates were 10.12.2021 and 12.04.2022. All samplings were done by adhering to a sampling protocol and simultaneous samplings were done as much as possible from multiple points by multiple people since sampling locations were not in close vicinity to each other. Several individuals were spread out throughout the OIZ area, who helped with the sampling efforts to ensure the timetable was reached. Sampling instruction used for OIZ-A and OIZ-B is provided in Appendix-A. The general sampling times, the sampling procedure, and the safety measures to be taken in case of an emergency are all covered in the sampling instructions. Accordingly, the same process was applied to each sampling location.

Sampling locations and times are given in Tables 3.1 and 3.2 for OIZ-A and OIZ-B respectively.

Table 3.1 Sampling locations and times of OIZ-A, Food and Paint Industries

Main Location	Sampling Location	1st Campaign Sampling Times	2nd Campaign Sampling Times
OIZ-A	OIZ-A main sewer discharge	-* 12:30 16:30	10:05 12:35 16:30
Food industry	Facility main sewer discharge	9:30 13:30 17:30	10:00 13:35 17:30
Paint industry	Pretreatment unit inlet/outlet	10:15/11:15 -*/15:34	10:37/11:37 -*/16:34

*Planned sample could not be taken

Table 3.2 Sampling locations and times of OIZ-B, Textile and Metal Industries

Main Location	Sampling Location	1st Campaign Sampling Times	2nd Campaign Sampling Times
OIZ-B IWWTP	Inlet/Outlet	09:00/09:30 12:30/13:10 16:00/16:30	09:00/09:30 12:30/13:00 16:00/16:30
OIZ-B IWWTP	Post-screening	10:10	10:00
	Aerated grit chamber	10:40	10:30
	Secondary sludge	15:20	14:30
	Dewatered sludge	13:30	15:00
	Centrifuge reject	12:20	14:00
Metal industry	Pretreatment unit inlet/outlet	10:00/10:02 13:02/13:06 16:00/16:01	10:00/10:00 13:00/13:00 16:00/16:00
Paint industry	Pretreatment unit inlet/outlet	16:20/16:50	15:30/16:00

Grab sampling was used to collect samples to be able to observe the diurnal variation. Wastewater samples were taken into glass jars with a volume of 5 L for measuring MPs, and the lids were then wrapped in aluminum to prevent plastic contamination.

For centrifuge reject water and WWTP effluent sampling, for each a total volume of 20 L sample was filtered through a stainless-steel sieve with a 38 μm pore opening at the sample location and the sieves were backwashed with a final volume of approximately 900 mL. This methodology is applied because these two samples were predicted to have low quantities of MPs. By increasing the sampling volume, this ensured a more representative sample for further analyses while helping with the transportation of the samples by decreasing the volume.

Grab sampling was used to collect secondary sludge and centrifugal sludge samples into a glass jar with a volume of 2 L and the caps were covered with aluminum to prevent plastic contamination.

Centrifuge sludge samples were collected from the collection bin connected to the centrifuge's conveyor belt and scooped into glass jars with metal spoons.

Following the collection of all samples, they were quickly transported via cold chain to the lab, where they were immediately placed in the refrigerator and kept there at 4 °C until use. Samples were examined as rapidly as possible.

3.2 Methods

The following methodology between parts 3.2.2-3.2.3 has been adapted with slight modifications from a previous study (Hatinoğlu et al., 2024).

3.2.1 Sample Characterization

A second sample (in 1.5 L bottles) was taken from each sampling point, and chemical oxygen demand (COD), total solids (TS), suspended solids (SS), and pH analyses were performed in order to get a general idea of the pollution load of the sampled wastewater. A single sample was collected from each sampling location during the first sampling campaign, whereas multiple samples were collected from an individual location in the second sampling campaign, in parallel with the MP samples, depending on the time of day.

The relevant methods followed for the measurements are given in Table 3.3.

Table 3.3 Measurement methods in wastewater and sludge characterization

Parameter	Method
TS, SS	Standard Method 2540 G APHA, AWWA, and WEF (2017)
pH	Standard Method 4500 H APHA, AWWA, and WEF (2017)
COD	EPA approved digestion method Jirka and Carter (1975)

3.2.2 Microplastics Analysis

The process used for MPs analysis was developed in-house by our Research Group (Hatinoglu et al., 2024) and only slight modifications were applied to the original method. The flowchart is given in Figure 3.2.

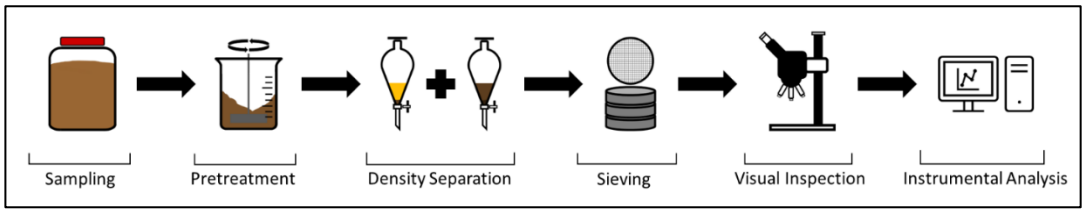

Figure 3.2 Analysis protocol developed for MPs in sludge

The process starts with sampling which is followed by volume reduction. Volume reduction is applied to help with the handling of samples and allows the use of conventionally available laboratory equipment such as 1L beakers and 600 mL separatory funnels. Additional filtration step also reduces the COD load in the sample and decreases the use of reagents.

Volume-reduced samples were then exposed to a pretreatment procedure to remove residual organic matter and microplastics were separated from the inorganics and other settleable matter by a series of density separations. Top parts of the separation funnels were passed through a stack of sieves to generate a size distribution and each sieve was backwashed onto membrane filters with a vacuum apparatus. Filters were visually inspected under a fluorescence microscope (ZEISS Axio Scope.A1) by the use of Nile Red and possible MPs were recorded. A subset of filters were analyzed by ATR-FTIR (Bruker Alpha-P) and Raman (Witec Alpha300) microscopy in order to find polymer types of MPs.

3.2.2.1 Sample Preparation

After the wastewater samples were transferred to the laboratory in a 5 L glass jar, they were filtered through a stainless steel sieve with a 38 µm pore opening, and the

sieves were backwashed and the final volume was reduced to approximately 900 mL. COD measurements were done and samples were taken for further processing.

Secondary sludges were mixed until completely homogeneous, then diluted to a COD value of 3000 mg/L using DI water. The centrifuge sludge subsamples were picked from various points of the jars and mixed with small amounts of DI water until they were able to be mixed using a magnetic stirrer to ensure sample homogeneity. Then more DI water was added until the total COD of the sample became 3000 mg/L.

300 ml of sludge mixes were used for both secondary and centrifuge sludge samples.

3.2.2.2 Organics Removal

The Fenton oxidation method, which is mentioned in the literature review to effectively remove organic matter while causing the least damage to the polymer structure, was used in the study for the pre-treatment of wastewater and sludge samples. Organic matter is oxidized by free radicals, which are formed as a result of the Fenton reaction shown in (Equation 3.1.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-} \quad \text{..... (Equation 3.1)}$$

To effectively remove organic matter in wastewater and sludge samples, the COD values of the samples were measured as explained in the sample preparation section. The required amount of hydrogen peroxide (H_2O_2) and iron (Fe^{2+}) to oxidize the determined amount of organics during Fenton Oxidation was calculated according to the theoretical amount of H_2O_2 to oxidize all the COD and Fe^{2+} is dosed with the molar ratio of 10 ($[Fe^{2+}]/[H_2O_2]$). To begin the treatment, iron solution was added to the sample beaker and the pH of the sample was reduced to 3. The sample was stirred at room temperature for 30 minutes beginning with the addition of hydrogen peroxide. Following the 30 minutes reaction time, the pH was increased to 7 and the reaction was terminated. Figure 3.3 depicts images of wastewater samples before and after Fenton oxidation.

Figure 3.3 Effect of Fenton oxidation process on the sludge samples before (A) and after (B) reaction

3.2.2.3 Density Separation

Following the Fenton reaction, the treated samples went through a two-step density-based separation process, and the MPs were extracted using flotation, as shown in Figure 3.4.

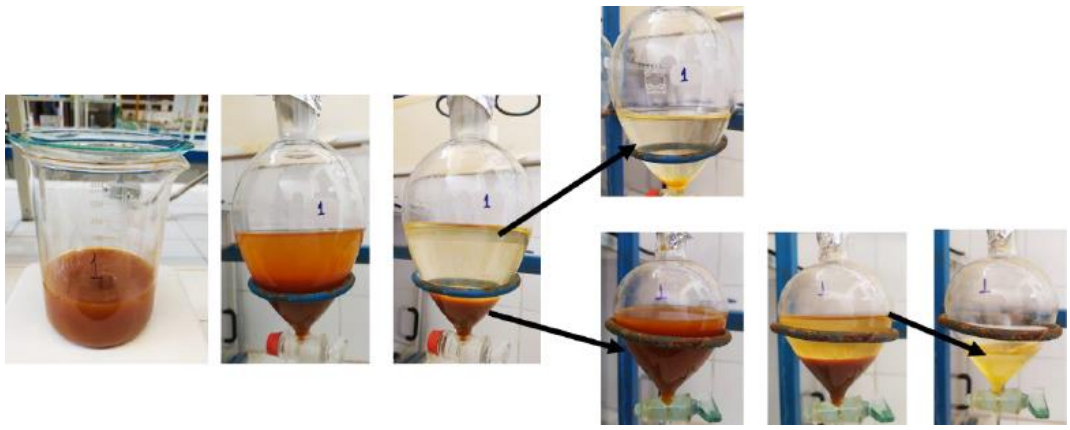

Figure 3.4 Density separation process, NaCl mixture (left) and ZnCl2 (right)

For the flotation of the MPs, the density of the sample was first increased to 1.15 g/cm^3 by adding NaCl for a final concentration of 5M. The salt was mixed

thoroughly before being transferred to a separatory funnel, where the $Fe(OH)_3$ flocs were allowed to settle to the bottom of the funnel overnight, while the clear portion containing low-density MPs (e.g. PE, PP) remained at the top. After that, the sediment was subjected to a second density separation process to extract high-density MPs (e.g., PET, PU). To accomplish this, the density of the sample was increased to 1.5 g/cm^3 using $ZnCl_2$ salt, resulting in a sample with a concentration of 5 M, which was then transferred to another separation funnel and subjected to the same process as the previous salting. As a result, higher recovery efficiency and less bias toward low density MPs were achieved. The supernatant from both NaCl and $ZnCl_2$ mixtures were combined.

3.2.2.4 Filtering

After density separation, the samples were passed through a sieve system with mesh openings of 5 mm, 1 mm, 0.425 mm, and 0.038 mm. Particles larger than 5 mm were not evaluated because they did not meet the definition of MPs. Particles on the other sieves were backwashed separately and filtered through a glass vacuum filter set, through mixed cellulose ester (MCE) filters. Membrane filters used in filtration have a diameter of 47 mm, a pore opening of 0.45 mm, and checkered surfaces. Checkered surfaces were chosen for ease of use in later steps.

3.2.2.5 Visual Inspection

Fluorescence microscopy was used to determine the number and shape of MPs. The principle of counting MPs with fluorescence microscopy is based on staining MPs with a fluorescent dye. The hydrophobic Nile Red used in this study is a fluorescent dye that attaches to MPs and some other organic substances but not to inorganic substances. Nile Red stock solution was prepared in acetone at a concentration of 250 mg/L and diluted in n-hexane to a concentration of 10 mg/L. The filters

containing the extracted MPs were stained with 200 µL of the prepared 10 mg/L Nile Red fluorescent dye and incubated in the dark for 30 minutes.

At the end of the incubation period, stained filters were analyzed using the ZEISS Axio Scope.A1 Fluorescent Microscope with GFP filter cube, 5x and 10x objectives of the Axiocam MRm camera. With the AxioVision v4.8 program running connected to the camera, the images were followed from the computer environment and the glowing particles were accepted as Nile Red positive MPs, as seen in Figure 3.5, and their numbers and shapes were recorded in an analog. The shapes of MPs were evaluated based on their similarities, and they were divided into fiber, fragment and film groups under three groups given in Figure 3.5**Hata! Başvuru kaynağı bulunamadı.**.

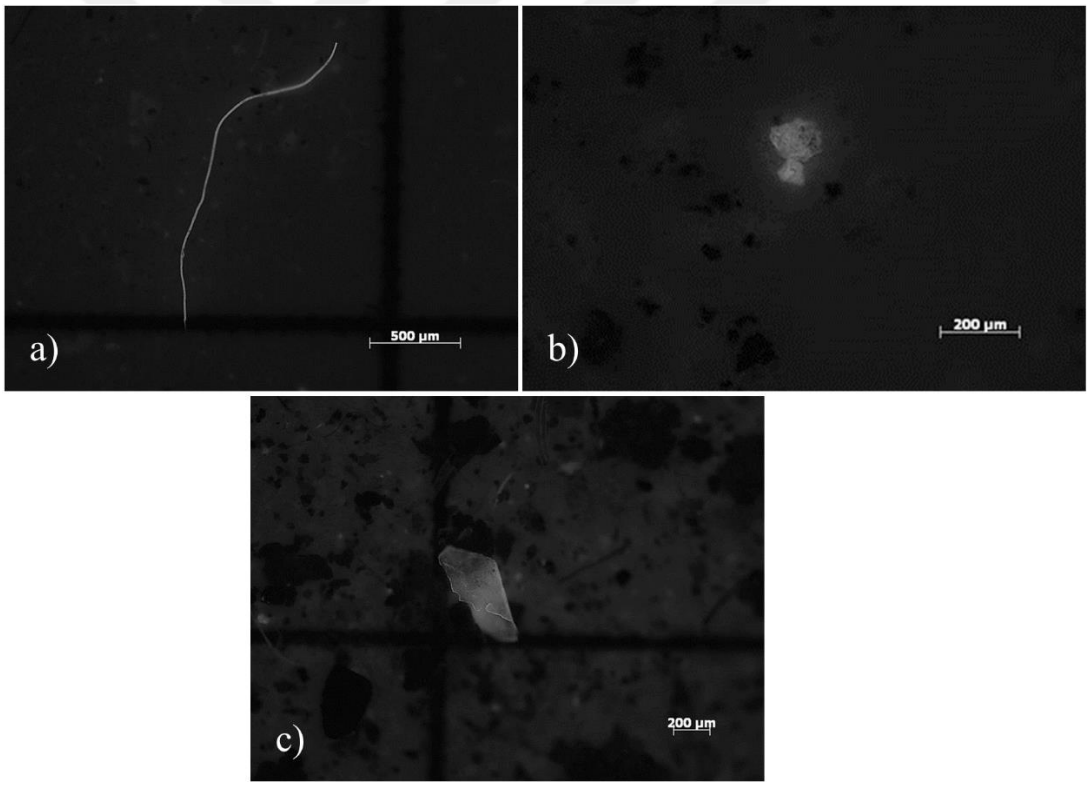

Figure 3.5 Particles obtained during MP counting. a) fiber type, b) fragment type, c) film type MPs

Also, the locations of some Nile Red positive MPs on the filter were noted using an analog to assist during FTIR and Raman spectroscopy analyses. An example of the

analog is given in Appendix-B. In addition, the sizes of the MPs were checked using the measurement module in the AxioVision program, the particles that were not in the required range were ignored if they were larger than the sieve gap used, and if they were small, they were added to the numbers of the lower filter.

Since it is known that MP pollution is generally collected in smaller sizes, the entire surface of the filters containing MPs in the 1,000-5,000 μm and 425-1,000 μm ranges were scanned, but the filters containing MPs in the 38-425 μm range only half of the filters were dyed and counted in order to lighten the workload and the other half to be used during following steps.

3.2.3 Analytical Methods

Following the visual inspection, two different methods were used in order to assess the polymer types of the MPs that were identified with the fluorescent microscope. For 5000-1000 μm and 1000-425 μm filters, FTIR measurements were taken using Bruker Alpha-P FTIR spectrometer with diamond ATR crystal and Raman microscopy measurements were taken using the SNOM-confocal Raman microscope by Witec Alpha300 series device with a laser with a wavelength of 532 nm.

Following the completion of the processing of the obtained Raman and FTIR spectra, the percentage of plastic presence for each filter was calculated, and the microscope measurements were recalculated with these ratios and reported as corrected MP numbers.

3.2.3.1 FTIR

For FTIR spectroscopy, a Bruker Alpha-P FTIR spectrometer with diamond ATR crystal and its software OPUS v6.5 were used. The ATR module was used to obtain the spectra, which had a wavenumber range of 4.000-400 cm^{-1} and a spectral resolution of 4 cm^{-1}. MPs whose locations had been determined during the

microscope counting stage were selected with tweezers, transferred to the device, and measured. Background measurements were taken using 16 replicates, and sample measurements were taken using 24 replicates. Every day before the measurement, the device's accuracy and reproducibility were tested by measuring different, untreated and pristine plastic samples. The device was cleaned with pure ethyl alcohol after each measurement.

Spectra were obtained by using the OPUS program to perform background correction and spectrum softening. The spectra were then transferred to the Thermo Scientific OMNIC program and screened through library scanning. In addition to the commercial libraries included in the program, the FLOPP and FLOPP-e libraries developed by Frond et al. (2021), which include environmental samples and increase the matching percentage, were used. During the method development phase, two different data analyses with and without the FLOPP and FLOPP-e libraries were performed, and it was discovered that using the libraries together increased mappings and decreased missing evaluations in MPs.

To account for the pollution of environmental samples, the screening process was used as a fast data processing method, and the lower limit of matching was kept at 50%. MP samples that exceeded the lower limit were first compared to other library matches, and if the matches were close, manual separation was performed by comparing them to the NIST database and spectrum books. The type of MP polymer was determined and reported along with the final comparisons. When the matching percentages of the accepted MPs were evaluated as a result of this process, it was discovered that the lower limit was around 70%.

3.2.3.2 Raman Spectroscopy

Raman spectroscopy analyses were performed using the SNOM-confocal Raman microscope located at Bilkent University National Nanotechnology Research Center (UNAM). A laser with a wavelength of 532 nm was used in the Witec Alpha300

series device, measurements were made with 1.300 rel 1/cm spectral center and DV401_BV CCD detector, integration time 1.03 seconds and 30 repetitions. During the measurement, the status of the sample was checked from the monitor and the laser power was adjusted manually. In cases where fluorescent effects were observed, fluorescence degradation was ensured by keeping the MPs under low laser power for a few minutes and the measurement was taken again. The repeatability of the device was tested by measuring different, untreated and clean plastic samples every day before the measurement. The obtained spectra were then screened by using the CRAN package created by Renner et al. (2019) to be used in MPs measurements with Raman spectroscopy, by searching the library with the RamanMP module and PublicSpectra database in the R program. The pre-screening process was used as a fast data processing method and the lower limit of matching was kept at 50 percent, as in FTIR, taking into account the pollution of environmental samples. MP samples exceeding the lower limit were then separated by manual comparisons. Manual separation was performed by comparing the spectra in the literature and the Infrared and Raman Users Group Spectrum Database (Prize et al., 2014). The MP polymer type was determined and reported with the final comparisons. As a result of this process, when the matching percentages of the accepted MPs were evaluated, it was seen that the lower limit was around 70%.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Wastewater Characterization

The wastewater samples collected at separate times from various points were analyzed in triplicates for their conventional pollution load characteristics, i.e., COD, TS, TSS, and pH. During the first sampling campaign, only a single sample for each location was taken, however, this has been changed in the second sampling campaign to include taking samples for all sampling times. Table 4.1 shows the results of these analyses. Other analyses, resulting from the first sampling campaign can be seen in Appendix-C. For OIZ-A, the wastewater characteristics vary from morning to noon and afternoon, and COD value is getting close to 1000 mg/L which constitutes the discharge limit of the local municipalities. On the other hand, COD value of OIZ-B wastewater is much lower, both at the inlet and the outlet of the WWTP, indicating that the COD load of the two OIZs are different from each other. Similar observation can also be made for TSS value as well, where OIZ-A is higher in concentration compared to OIZ-B wastewater. This indicates that the nature of the industries generating wastewater are different. The pH values on the other hand, are mostly similar values close to neutral following the treatment process except for the effluent of the metal industry which shows pH values close to 10. Considering the acid wash step in the manufacturing process of the metal industry, followed by neutralization, these pH values are expected.

Table 4.1 Characterization results of sample locations in the second sampling campaign

			pH	TSS (mg/L)	COD (mg/L)
OIZ-A	Discharge Point	Morning	7.3	180.0±5.3	671.7±19.9
		Noon	6.8	570.7±5.8	1.180.7±27.2
		Afternoon	7.0	392.0±29.5	1.150.0±83.0
OIZ-B IWWTP	Inlet	Morning	7.9	62.0±2	366.7±8.7
		Noon	8.5	278.7±10.1	507.7±28.8
		Afternoon	8.4	163.3±4.2	575.7±7.6
	Outlet	Morning	7.6	30.0±2	331.7±16.2
		Noon	7.4	4.7±1.2	361.0±33.3
		Afternoon	7.3	8.7±1.2	376.0±12.1
Food Industry	Discharge Point	Morning	9.0	144.0±8.0	566.7±5.8
		Noon	6.8	79.3±5.8	502.0±23.1
		Afternoon	6.9	91.3±5.8	840.3±2.5
Paint Industry	Inlet	Morning	6.8	153.3±6.4	635.0±22
		Afternoon	6.9	106.7±8.3	570.7±13.6
	Outlet	Morning	6.9	30.7±1.2	498.7±40.2
		Afternoon	6.9	30.7±1.2	499.7±28.4
Textile Industry	Inlet	Morning	8.6	67.3±4.2	1.477.0±11.8
	Outlet	Morning	5.6	32.7±4.2	1.461.0±16.7
Metal Industry	Inlet	Morning	2.3	26.7±6.1	502.3±5.1
		Noon	2.4	38.7±1.2	498.7±12.7
		Afternoon	2.5	138.0±8.7	504.7±11.7
	Outlet	Morning	9.6	24.0±2	552.3±13.3
		Noon	9.7	18.7±4.6	604.0±14.7
		Afternoon	9.6	14.7±1.2	603.0±11.1

4.2 Microplastics Concentrations

MPs concentrations are categorized as direct microscope counts, which are all the particles that are Nile Red positive under fluorescense microscope and FTIR/Raman corrected results which are derived by the subsampling of Nile Red positive particles using instrumental analyses. Plastic occurrence percentage is also included in the results as given in Table 4.2, which indicates the percentage of particles confirmed to be plastics with FTIR or Raman.

Although Nile Red dye stains plastics and enables them to be distinguished with its fluorescent effect using fluorescence microscopy, it can also stain other organic substances and give false positive images. Therefore, as explained in the methodology section, after analysis with fluorescence microscopy, samples were subjected to FTIR (or Raman) analysis. By applying analytical instruments, it was clearly determined whether the particles were plastic or not and the ratio between the numbers detected with the microscope and the numbers detected with FTIR/Raman measurements are presented as "Plastic Occurrence (%)" in Table 4.2. The expression of corrected MP numbers shows the MP numbers clarified after FTIR. The rate of correct detection of plastics by counts with the microscope varied between 11% and 82%. On average, microscope analyses correctly detected plastics by 38% in the first sampling campaign and 48% in the second sampling campaign. This finding is similar to a study that found that particles previously analyzed with a light microscope and thought to be MP were 32.4% real MP after FTIR analysis (Gies et al., 2018). The numbers in Table 4.2 are given after dividing the results obtained in wastewater sampled as 5 L or 20 L by the sample volume and converting to MP/L.

The MP numbers measured in all wastewater samples analyzed in the two sampling campaigns were between 3-149MP/L, and this number was measured as 119-183 MP/g TS for the sludge samples which were reported on the basis of dry sludge mass. In this context, the highest amount was measured with 183 MP/g TS in the secondary

sludge of the OIZ-B central WWTP (second sampling). These values are consistent with those reported in the literature (Hatinoğlu and Sanin, 2021).

For the wastewater MPs concentrations, the highest observed being 149 MPs/L in OIZ-A, which are consistent with some studies (X. Xu et al., 2019; Edo et al., 2019) but is lower than the other examples provided in Table 2.1. Although changes were observed in MP numbers depending on the sampling hour, these changes were largely irregular rather than following a trend. When the two sampling campaigns are evaluated, one can see the differences in some samples in terms of MP numbers.

Table 4.2 Concentration of MPs in all sampling locations categorized by the seasonal and daily sampling times

Sampling Location	Sampling Time	1. Sampling			2. Sampling		
		Microscope Count	FTIR/Raman Correction		Microscope Count	FTIR/Raman Correction	
		MP/L	MP/L	Plastic Occurrence (%)**	MP/L	MP/L	Plastic Occurrence (%)**
OIZ-A Discharge Point	Morning	Could not be sampled*			211	149	68
	Noon	223	129	58	174	96	52
	Afternoon	194	115	59	211	139	62
OIZ-B IWWTP Influent	Morning	175	91	52	116	89	60
	Noon	153	87	57	178	113	50
	Afternoon	147	88	60	199	136	46
OIZ-B IWWTP Effluent	Morning	31	13	42	22	6	27
	Noon	42	11	26	21	7	33
	Afternoon	36	12	33	22	6	27

Table 4.2 Continued

Sampling Location	Sampling Time	1. Sampling			2. Sampling		
		Microscope Count	FTIR/Raman Correction		Microscope Count	FTIR/Raman Correction	
		MP/L	MP/L	Plastic Occurrence (%)**	MP/L	MP/L	Plastic Occurrence (%)**
OIZ-B IWWTP Post Screening	-	84	69	82	164	126	58
OIZ-B IWWTP Aerated Grit Chamber	-	138	83	60	146	56	36
OIZ-B IWWTP Secondary Sludge	-	272	130	48	280	183	65
OIZ-B IWWTP Centrifuge Sludge	-	258	119	46	189	132	70
OIZ-B IWWTP Centrifuge Reject	-	36	16	44	49	29	59

Table 4.2 Continued

Sampling Location	Sampling Time	1. Sampling			2. Sampling		
		Microscope Count	FTIR/Raman Correction		Microscope Count	FTIR/Raman Correction	
		MP/L	MP/L	Plastic Occurrence (%)**	MP/L	MP/L	Plastic Occurrence (%)**
Paint Industry Pretreatment Influent	Morning	181	103	57	149	112	84
Paint Industry Pretreatment Effluent	Morning	138	47	34	61	21	39
Paint Industry Pretreatment Influent	Afternoon	Coult not be sampled*			136	94	44
Paint Industry Pretreatment Effluent	Afternoon	Coult not be sampled*			86	26	57
Food Industry Effluent	Morning	202	69	34	178	39	36
	Noon	128	32	25	102	39	64
	Afternoon	135	31	23	156	82	46

Table 4.2 Continued

Sampling Location	Sampling Time	1. Sampling			2. Sampling		
		Microscope	FTIR/Raman Correction		Microscope	FTIR/Raman Correction	
		MP/L	MP/L	Plastic Occurrence (%)**	MP/L	MP/L	Plastic Occurrence (%)**
Textile Industry Pretreatment Influent Sample	-	73	36	49	108	48	27
Textile Industry Pretreatment Effluent Sample	-	95	16	17	69	25	26
Metal Industry Pretreatment Influent	Morning	67	15	23	77	33	66
	Noon	94	10	11	86	29	38
	Afternoon	99	14	14	115	24	27
Metal Industry Pretreatment Effluent	Morning	22	4	17	88	30	31
	Noon	21	3	15	108	29	22
	Afternoon	60	8	14	107	16	7

* Samples could not be taken due to equipment failure or lack of wastewater generation

** Ratio of confirmed plastics by analytical methods, to the entire subsample

4.2.1 Organized Industrial Zones

For the OIZ-A, while showing higher concentrations, the second sampling campaign also demonstrates high variability in the results. In addition, due to the unavailable third sample in the first sampling, it further disincentivizes making general assumptions on the effect of seasonal variations on OIZ-A. For the OIZ-B however, it is seen that higher concentrations are achieved in the second sampling campaign for the influent and a decrease in effluent concentrations. This increase in removal efficiency does require further research to understand the mechanism of MPs removal's dependence on seasonal variations. In fact, there are only about 4 months between the two samplings. Due to the limited period of the project (total of 1 year), it was only possible to separate the two samplings by this much time. So, the variations may not necessarily be too different from each other.

4.2.2 OIZ-B WWTP

In OIZ-B IWTTP units, all units except the aerated grit chamber show increasing MPs concentrations in the second sampling campaign. Since OIZ-B influent also demonstrated higher concentrations in the second sampling campaign and higher removal efficiencies, this result was expected. In Figure 4.1, MP concentrations throughout the OIZ-B WWTP from inlet to outlet are shown. It can be observed that MPs concentrations decrease as the treatment process goes on, with the exception in the 1st sampling campaign's grit chamber sample and 2nd sampling campaign's post screening sample, inlet is the highest concentration while effluent is the lowest concentration for wastewater samples.

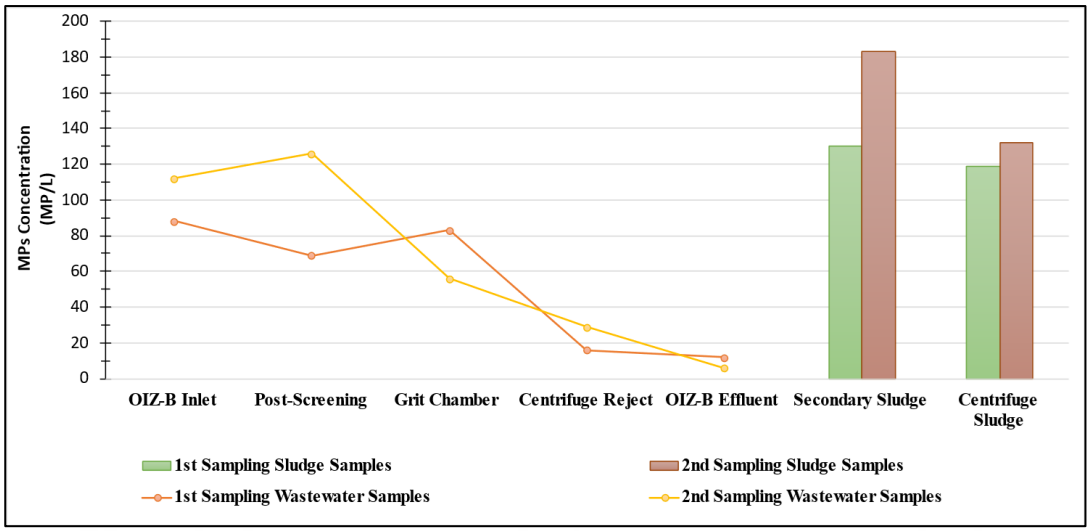

Figure 4.1 MPs concentration from inlet to outlet of OIZ-B IWWTP

Sludge samples given in Figure 4.1 are given in MP/g TSS units.

4.2.3 Specific Industries

When evaluated on a sectoral basis, the number of MPs measured in paint industry influent wastewater (103 MP/L in the first sampling, 103 MP/L on average in the second) stands out compared to other sectors. Removal efficiency of MPs changes between 37-81% in both sampling campaigns which comes to 57% removal in average, showing high variance between the sampling campaigns. However, considering the unavailable data in the first sampling campaign, it is difficult to ascertain a trend on seasonal variations. Since no similar studies could be found in the literature, these high numbers observed in the paint industry could only be compared with the number observed in a shipyard where ship repair, dismantling and painting operations were carried out. It was stated that the MPs measured in the shipyard example were much higher than in the other sectors examined (food, leather processing, slaughterhouse, furniture, etc.). Due to the high number of activities, it was understood that it contained much higher MPs (1567 MP/L) compared to those measured in this study (Franco, et al., 2020). Having the highest TSS concentration

amongst the selected industries, the high MPs concentration seems appropriate since MPs themselves are a source of TSS.

The average MP count in the food industry, which was taken as a sample and did not have a pretreatment system, was determined as 44 MP/L in the first sampling and 53 MP/L in the second sampling. While literature is scarce in the MPs analysis in food industry wastewaters directly, several food packaging studies are available (Sezer et al., 2024; Du et al., 2020; Hernandez et al., 2019). In Sezer and colleagues (2024) study, a food packaging plant's wastewater was analyzed which came into contact with the process and found 43 MPs in 500 mL samples, which is two times higher than in this study. Considering the highest TSS concentration in the second sampling occurring at the afternoon sample but highest TSS concentration is observed in morning, the trend between the TSS and MPs concentrations do not exactly match up as much as the paint industry.

For textile industry, 36 MP/L was measured in the pretreatment inlet wastewater in the first campaign and 48 MP/L in the second campaign. At the pretreatment outlet, these numbers were determined as 16 and 25 MP/L, resulting in 52% removal on average. An unexpected finding is that the MP numbers of the textile industry selected for the study were determined to be quite low. On the other hand, textile washing is seen as one of the biggest MP pollution sources and a comparable situation is expected in the textile sector wastewater. The reason for this can be said to be that the textile products used in the facility are still raw materials and do not leave high MPs since they are not repeatedly exposed to stress like clothing and other textile products. This finding is also similar to the findings of Magalhães et al. (2022). Alipour et al. (2021) determined that particles of 81 MP/L and 38 MP/L were released in two different carpet washing plants in Iran. Considering the substantial number of carpet washing plants throughout the country, they emphasized that this process could be a significant source of particles smaller than 300 μm being released into the environment. Xu et al. (2018) stated that they found 334 microfibers/L of plastic etc. structure at the inlet of a textile dyeing industry wastewater treatment plant; they stated that this number decreased to 16 microfibers/L after treatment

(95% removal efficiency) which shows higher removal than the results obtained in this study. This shows that while pretreatment systems do remove MPs from textile industry wastewaters, they are not as efficient as full scale IWWTPs. Comparing the TSS concentrations to the MPs concentrations, it is seen that inlet to outlet concentrations correlate between MPs and TSS, inlet being higher in both cases.

The sector with the lowest MP numbers among the examined sectors was the metal production and processing plant. In the plant inlet wastewater, 13 MP/L was measured in the first sampling and 29 MP/L in the second sampling, and following the pretreatment process, 5 MP/L was determined in the first campaign and 25 MP/L in the second campaign. Wang et al. (2020) stated that they found 8 MP/L as a result of steel production processes and 36 MP/L as a result of electroplating processes. These values measured in similar sectors are close to the numbers measured in this study. There were cases in the metal industry where more MP was found in the pretreatment outlet compared to the pretreatment inlet. While the exact reason could not be determined, the relatively lower MP counts can be one explanation for this. Alternatively, it is thought that the reason for this situation is that the treatment chemicals are polymeric (polyelectrolyte use is common in chemical treatment) and this may have led to false positive results. Having the lowest MPs concentrations, as with the paint industry, TSS concentrations correlate with the MPs concentration.

In general, between the industries, MPs concentrations and TSS concentrations show some similarities, but further research is needed to establish concrete evidence for this behavior.

4.3 Microplastics Shape and Size Distribution

4.3.1 Specific Industries

Size and shape distributions of MPs in specific industries are given in Figure 4.2 and Figure 4.3, respectively.

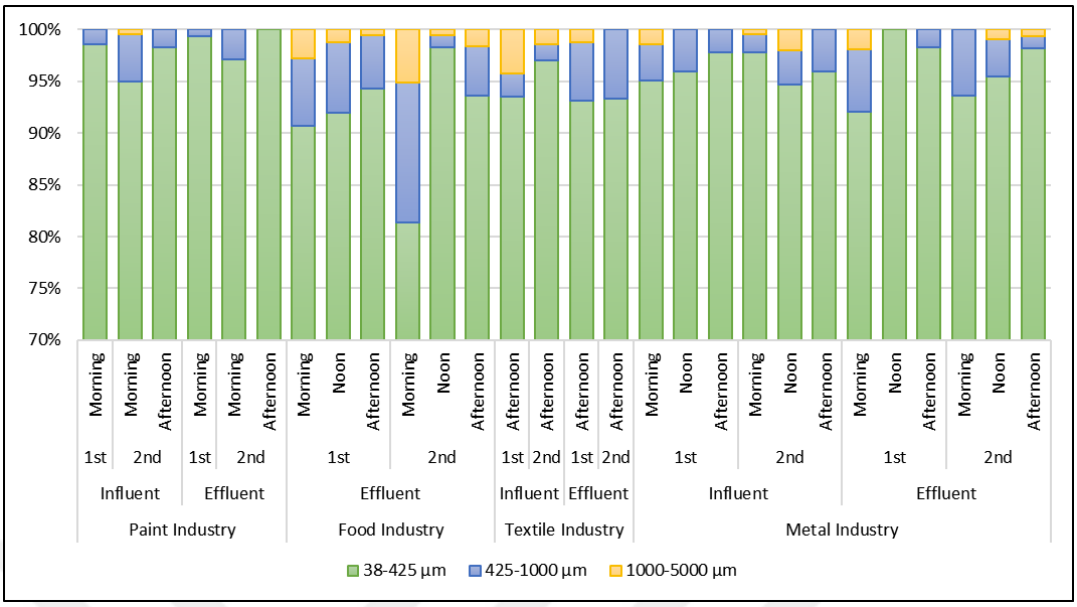

Figure 4.2 Size distribution of MPs in selected industries (1st and 2nd sampling campaign).

The size distributions show a significant trend where the smallest sized (38-425 µm) MPs are dominant in all of the samples from different industries. On the other hand, the most abundant shape is fragment, which is in agreement with Bayo et al. (2020) and Rodrigues et al. (2018). The most obvious change is observed in the metal industry MP properties, showing the highest fiber and film percentage among all samples, followed by the textile industry. However, there is no observed trend among pretreated industries as to how pretreatment affects the MPs shape or size distributions since there are both increases and decreases following the treatment such can be seen in the metal industry second sampling campaign where in morning fragment percentage decreases, in the afternoon sample the fragment percentage increases and the opposite effect is seen for the fiber particles.

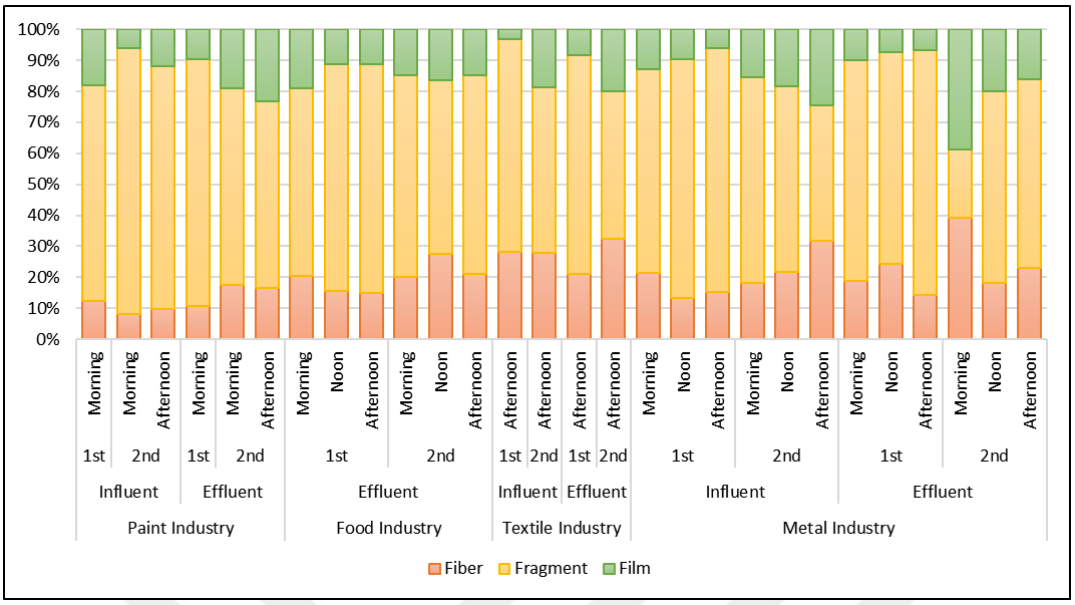

Figure 4.3 Shape distribution of MPs in selected industries (1st and 2nd sampling campaign).

4.3.2 Organized Industrial Zones

Size and shape distributions of MPs in OIZ-A effluent, OIZ-B IWWTP influent and effluent are given in Figure 4.4 and Figure 4.5, respectively.

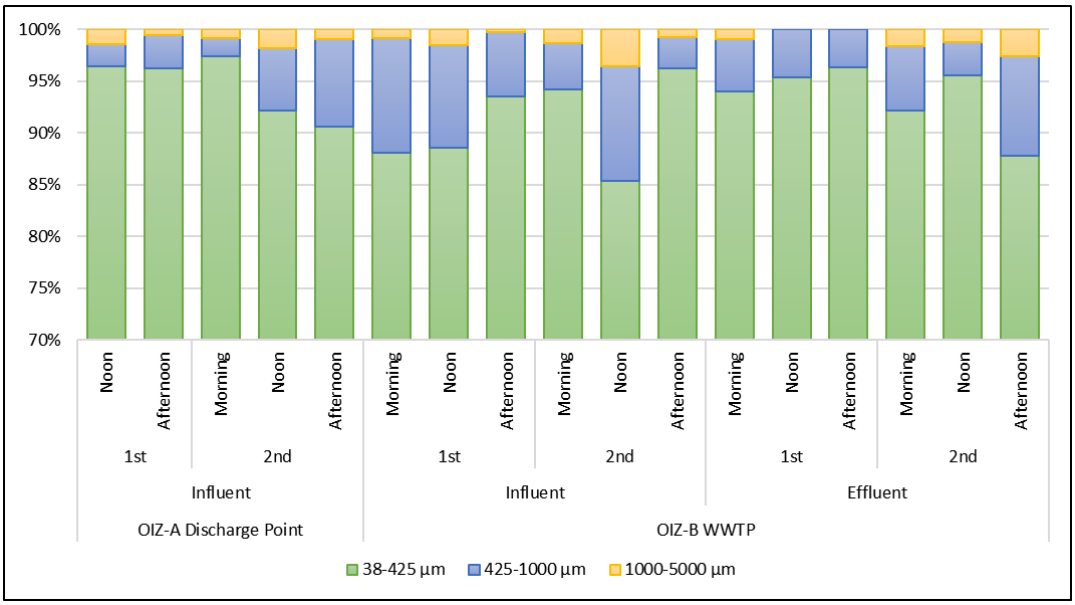

Figure 4.4 Size distribution of MPs in both OIZs (1st and 2nd sampling campaign).

As in specific industries, size and shape trends indicate that smaller sizes and fragment shapes also predominate in OIZ-A's discharge point and OIZ-B's wastewater treatment plant influent and effluent. As with the selected industries' pretreatments, WWTP does not show a concise trend in terms of particle shapes and opposing effects are demonstrated in Figure 4.5 for the OIZ-B IWWTP influent and effluent between 1st and 2nd sampling campaigns. However, smaller sizes increasing in abundance after the treatment process is in accordance with Van Do and colleague's (2022) study which also demonstrated that MPs shapes do not change significantly following the treatment process.

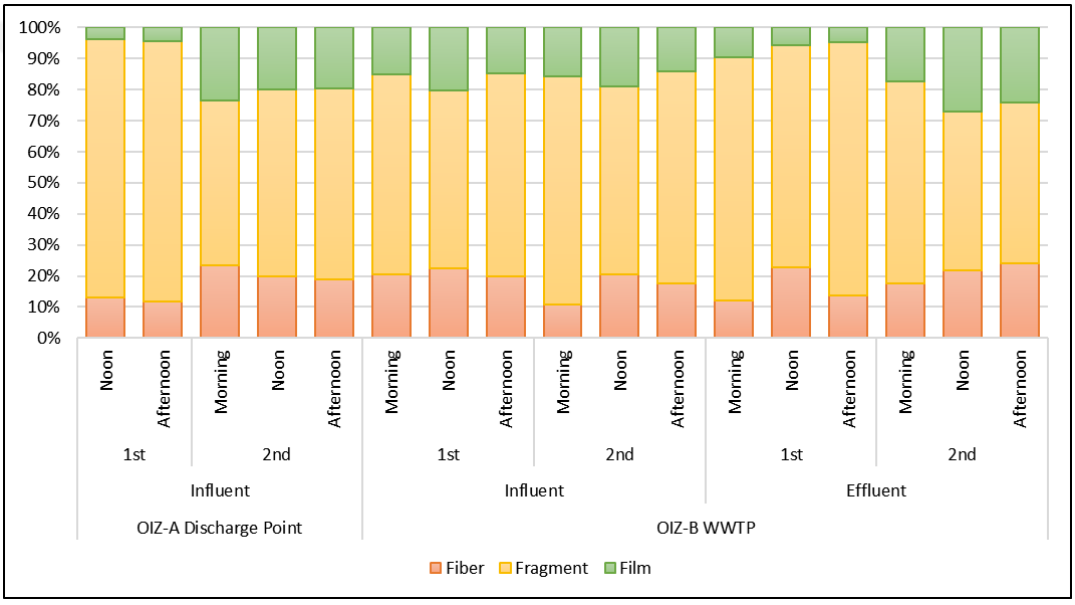

Figure 4.5 Shape distribution of MPs in OIZs (1st and 2nd sampling campaign)

4.3.3 OIZ-B IWWTP Units

The size and shape distributions of MPs in OIZ-B's WWTP units are given in Figure 4.6 and Figure 4.7, respectively.

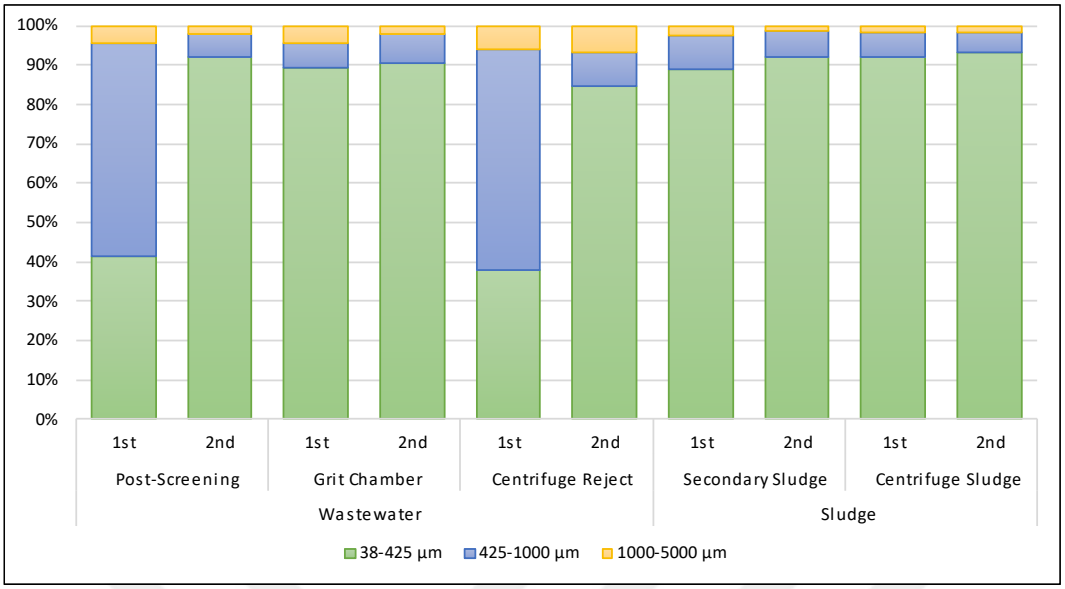

Figure 4.6 Size distribution of MPs in OIZ-B IWWTP units

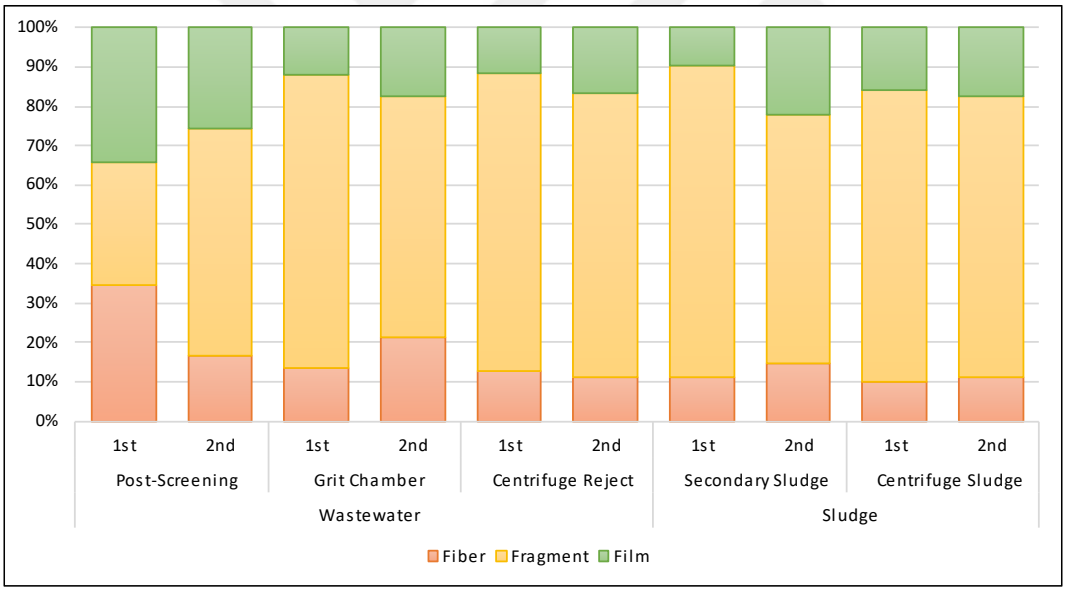

Figure 4.7 Shape distribution of MPs in OIZ-B IWWTP units

As with certain industries and total OSB wastewater, the dominance of smallest size and fragment shape is observed in all OSB-B WWTP units. However, as in the study of Murphy et al. (2016), an increase in MPs size is observed in secondary sludge compared to wastewater, which suggests further sampling is required to establish an encompassing trend.

4.4 Polymer Type Distribution of Microplastics

Polymer types of all MPs are given as the overall average for each sampling location and sampling campaign. For polymer types lower than 5% in abundance are grouped together as other for readability.

Polymer types acquired from the selected industrial wastewaters are given in Figure 4.8 and 4.9 for first and second sampling campaign below.

The polymer distribution of selected industries is given in Figure 4.8 and 4.8 are taken as the daily averages between the samples for that specific sampling location. Among the selected industries, HDPE, LDPE, PA, PP, PES and PS polymers show the highest abundances which are reported to be generously used throughout the world in all kinds of industries (Geyer et al., 2017). Moreover, while the effect is small, pre-treatment also affects the polymer diversity, causing a decrease in overall observed MPs polymer types which is supported by (Petroody et al., 2021).

In the paint industry, influent includes 9 different MPs, whereas effluent includes 5 different MP types with HDPE, LDPE, PES, PET and PS being encountered for both sampling campaigns.

The food industry includes the common polymers, LDPE, HDPE, PA, PET and PP which are shared amongst 1st and 2nd sampling campaigns.

The textile industry wastewaters only share two polymers between the two sampling campaigns. Between the influent and effluent, 1st sampling campaign share PP and PS polymers while 2nd sampling campaign shares PA, PES and PU polymers. The polymers in 2nd sampling campaign show all present in the influent are present in the effluent too, whereas in the 1st sampling campaign PVC shows up only in the

Metal industry wastewater also includes MPs that only show up in influent (EEA, PP and PTFE) and effluent (PES, ABS, PEU and RB) only. The common plastics such as HDPE, LDPE and PP are encountered in both sampling campaigns, while

EEA, PTFE and RB show up only in the 1st sampling and PEU only shows up in the 2nd sampling.

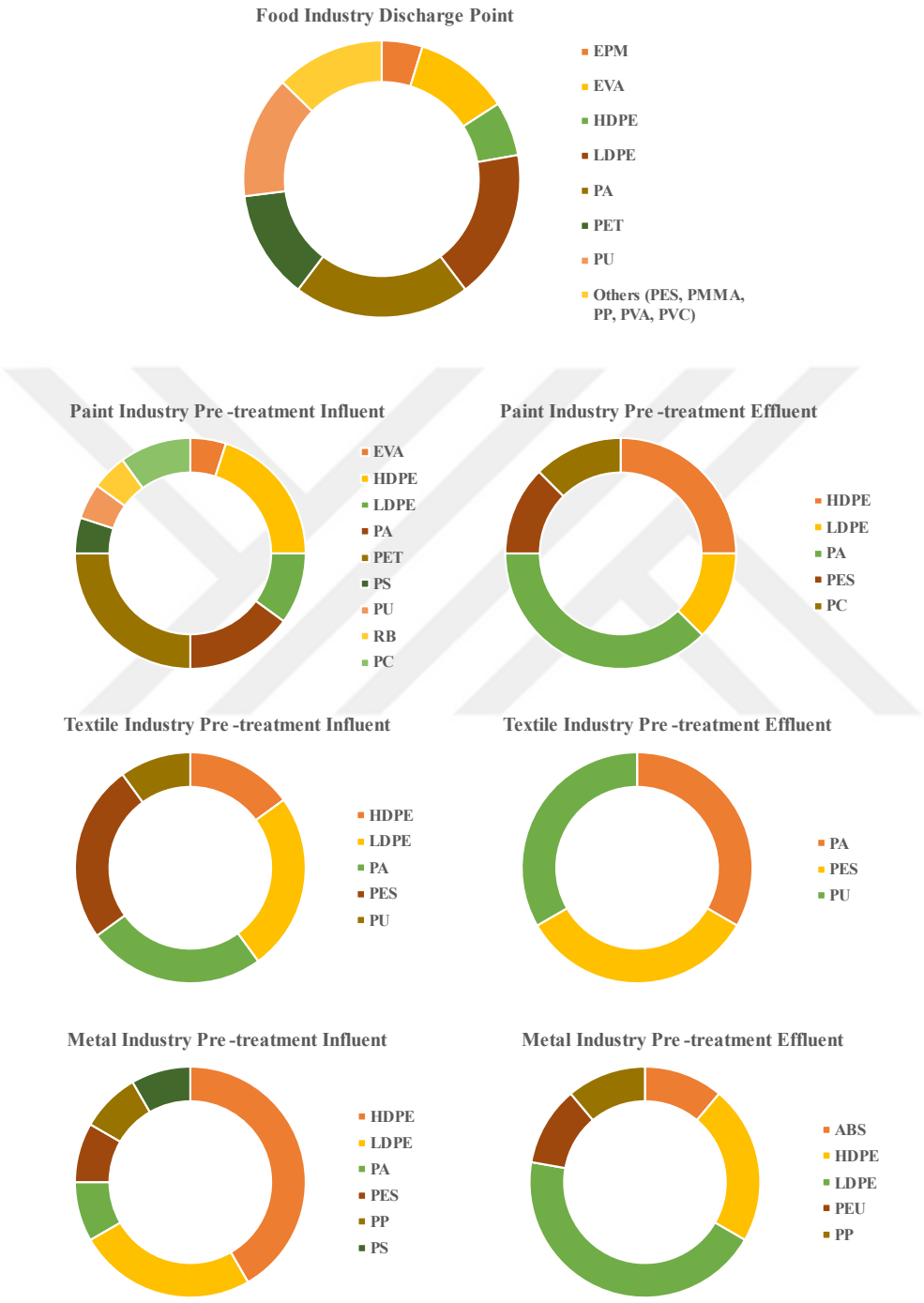

Figure 4.8 Polymer type distribution of 1st sampling campaign of industrial wastewaters

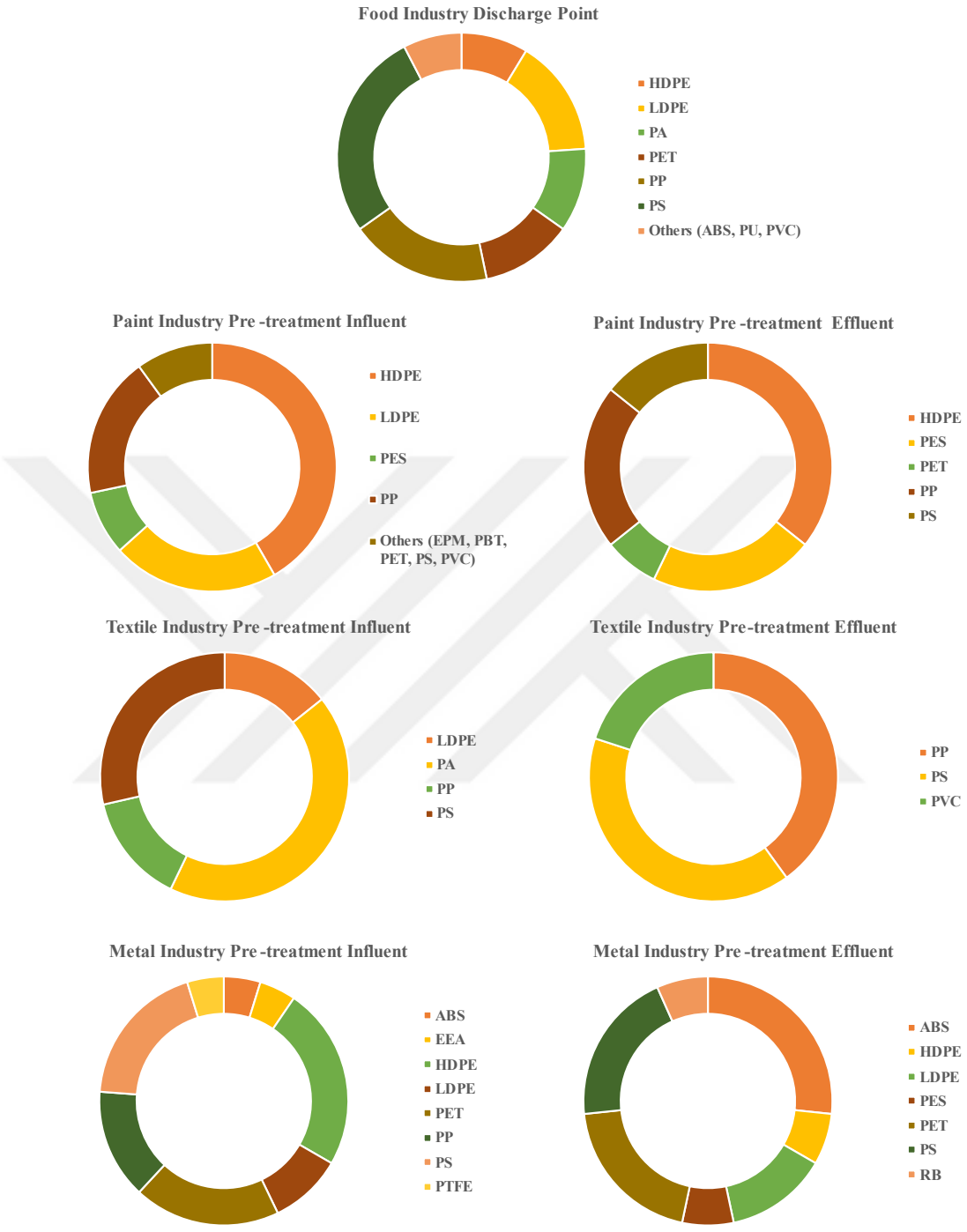

Figure 4.9 Polymer type distribution of 2nd sampling campaign of industrial wastewaters

Polymer types acquired from the selected industrial wastewaters are given in Figures 4.10 and 4.11 below.

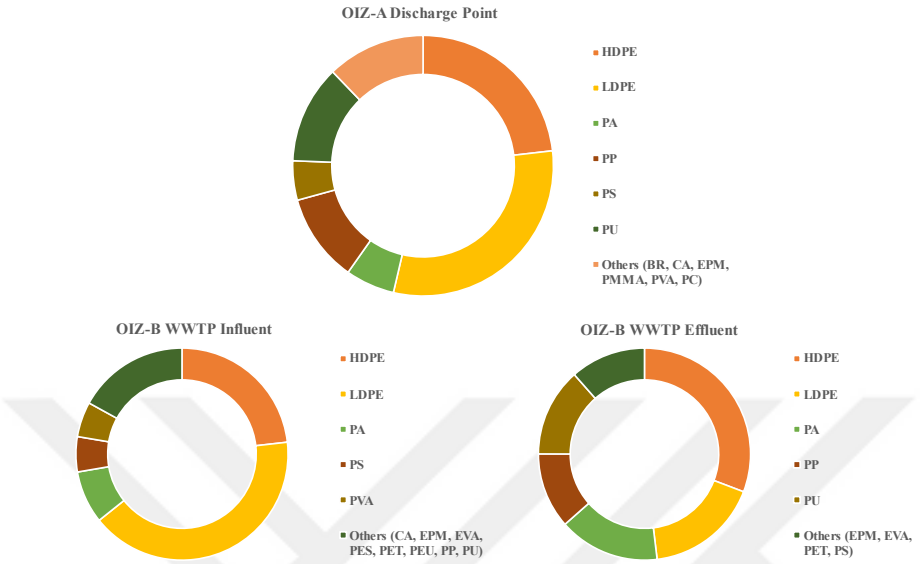

Figure 4.10 Polymer type distribution of 1st sampling campaign of OIZ wastewaters

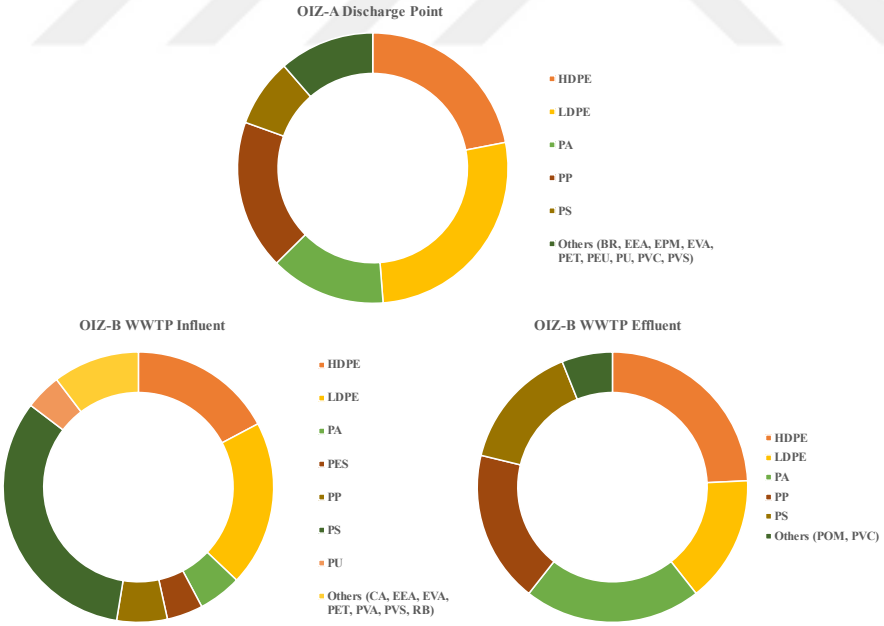

Figure 4.11 Polymer type distribution of 2nd sampling campaign of OIZ wastewaters

The polymer distribution of OIZ-A and OIZ-B wastewaters is given in Figures 4.10 and 4.11, where the inlet and outlet polymer distributions of OIZ-B are given separately. As in the pretreatments of selected industries, the OIZ-B IWWTP causes a decrease in plastic species diversity, but it is a more radical change from the pretreatment units where 13 different species were reduced to 9 polymers in the 1st sampling campaign and 14 to 7 in the 2nd sampling campaign. Although Bayo et al. (2020) did not find a significant decrease in polymer species, they did show that the relative abundance of polymers decreases further down the treatment line, which may indicate the more diverse a sample with enough low percentage polymers, there is a possibility of complete elimination of some polymer species. Both the OIZ-A outlet and OIZ-B inlet show the highest polymer diversity, which provides a robust extraction/identification methodology considering that many different industries are included in both. OIZ-A wastewater showed that HPDE, LDPE, PA, PP and PS were the most abundant polymer species, which is consistent with (Geyer et al. 2017) in terms of polymer abundance in the industries. The OIZ-B influent contains several plastics (CA, EEA, EVA, PES, PET, PU, PVA, PVS and RB) that are not present in the effluent, and vice versa, the effluent shows the absence of CA, PES, PEU, PMMA, PVA, POM and PVC in the influent.

Polymer types acquired from the IWTTP units of OIZ-B are given in Figures 4.12 and 4.13 below.

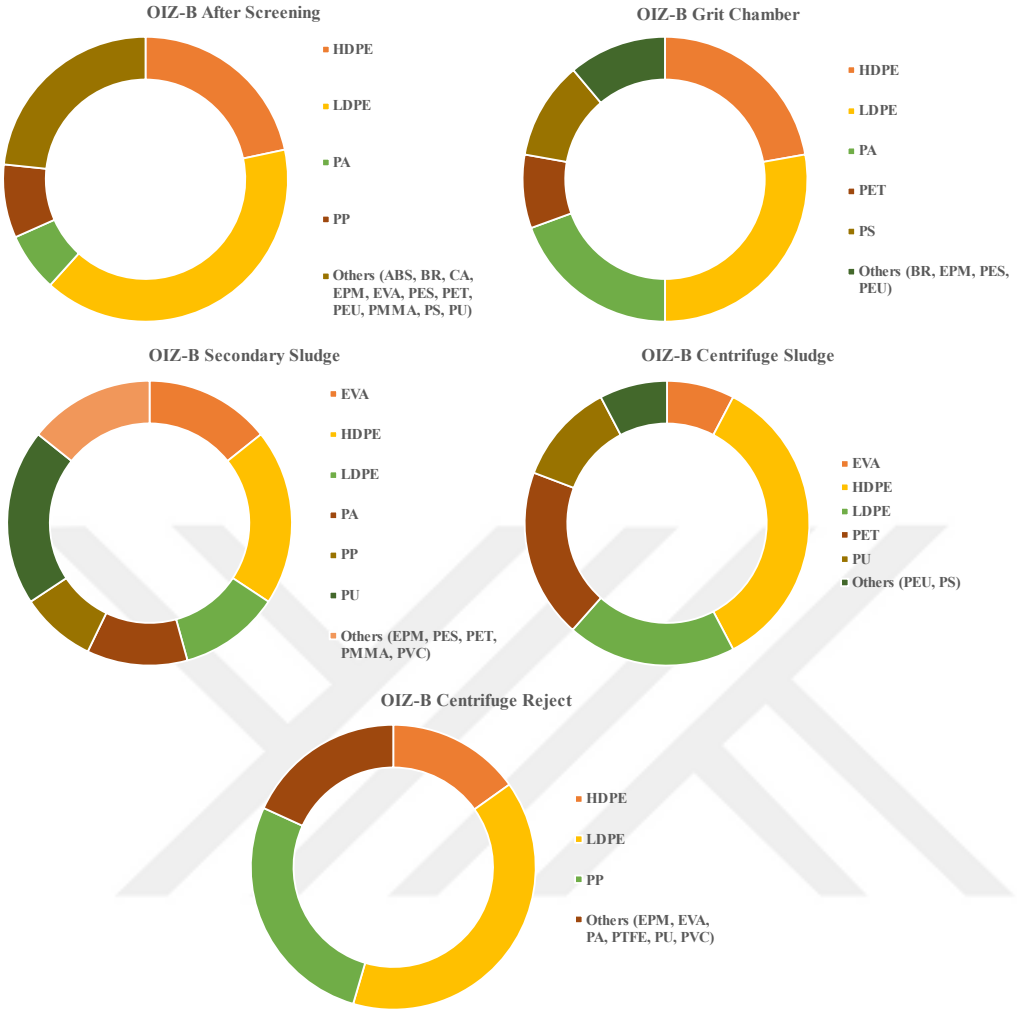

Figure 4.12 Polymer type distribution of 1st sampling campaign of OIZ-B IWWTP units

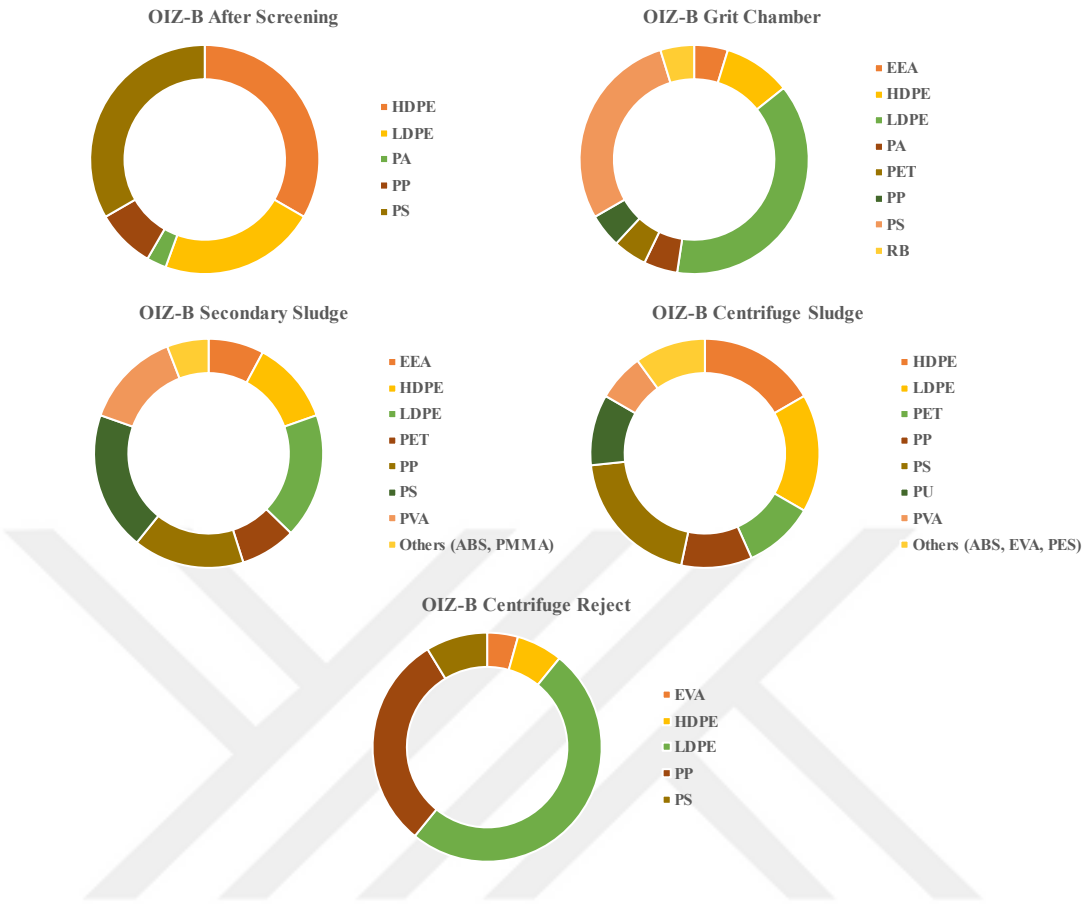

Figure 4.13 Polymer type distribution of 2nd sampling campaign of OIZ-B IWWTP units

The OIZ-B IWWTP units show a decrease in diversity, where the most diverse sample is the post-screening (15 different polymer types). After screening, the sample shows comparable properties to the OIZ-B influent wastewater but shows a few more polymer types than the influent. The aerated grit chamber with oil and grease removal, generally produce plastics that are less dense than water, as expected, and contain all polymer types seen after screening. The secondary sludge and dewatered sludge samples show comparable proportions of related plastics, with denser plastics such as PU and PES appearing after centrifugation. The centrifuge reject contains all plastics in the secondary sludge and shows an increase in the abundance of lighter plastics, as expected in a density-based separation unit. The abundance of LDPE, HDPE, PP, PS in the sludge samples is consistent with the work of L. Wang et al. (2022). The general plastic types obtained in this study show that

the most abundant polymers are LDPE, PS, HDPE, PP, PA, PS and PET, which is consistent with the findings of Geyer and colleagues (2017).

4.5 Comparison of the Findings with Domestic Wastewaters

In order to provide a better understanding of industrial wastewaters as a source of MPs pollution, several studies providing insight onto domestic wastewaters in terms of MPs concentrations have been compiled in Table 4.3.

Table 4.3 MPs concentrations from various domestic WWTPs

Study	Influent (MP/L)	Effluent (MP/L)
(Liu et al., 2019)	79.9 ± 9.3	28.4 ± 7.0
(Edo et al., 2019)	171 ± 42	10.7 ± 5.2
(Shan et al., 2021)	71.2 ± 8.2	3.1 ± 0.5
	38.8 ± 1.8	0.5 ± 0.2
(Yang et al., 2019)	12.03 ± 1.29	0.59 ± 0.22
(Setiadewi et al, 2023)	17.1 ± 5.65	1.41 ± 0.01
OIZ-A (This study)*	125.6 ± 20.77	-
OIZ-B (This study)	100.67 ± 19.89	9.17 ± 3.19

*Since there is no WWTP, OIZ-A combined wastewaters are reported as Influent numbers

As can be seen from Table 4.3, MPs concentrations of six different domestic wastewater treatment plants average 65 MP/L in the effluent and 6 MP/L in the effluent while showing high variability. Compared to the average, MPs observed in the OIZs influents are more than doubled in some cases except for of Edo and colleagues (2019) study which showed 171 MP/L in the influent. As with the influent MPs concentrations, effluent MPs concentrations in the industrial wastewaters sampled in this study show higher occurrences compared to literature values. In this study, lowest observed MPs were observed in the metal industry effluents (3 MP/L), which is higher than three of the referenced domestic wastewaters. Unexpectedly, high effluent concentrations in the Liu and colleagues (2019) study is similar to the effluents of paint and textile industries as well as the second campaign values of the metal industry., However, compared to the influent concentrations of these

industries, only the paint industry is observed to be higher, due to the low removal efficiencies of textile and metal industries pre-treatments.

Additionally, in Edo and colleagues (2019) work, where they analyzed sludge samples alongside with wastewater, they found 133 ± 59 MP/g TS, which is close to the MPs observed in this study, between 130-183 MP/g TS, which further justifies the effect of MPs accumulating in the sludge during treatment.

Overall, most of the industrial wastewaters showed higher MPs concentrations compared to the domestic wastewaters.

CHAPTER 5

CONCLUSION

Using the prior in-house developed oxidation-based two-stage separation analysis method that continued with fluorescence microscopy having Nile Red to identify MPs, it is observed less than 50% of particles in microscopy are non-plastics. As a result of the sampling and analyses, it was seen that the highest MP count was in OIZ-A discharge (149 MP/L - second sampling) in particular. The highest MP count in the industries for wastewater appeared in the paint industry influent wastewater (112 MP/L – second sampling). The highest observed MPs in sludge samples were observed in OIZ-B secondary sludge for WWTP units (183 MP/g TSS – second sampling). The lowest MP numbers were observed in the metal industry effluent (3 MP/L) and OIZ-B IWWTP effluent (6 MP/L). It was evaluated that the obtained results were consistent with the expectations and were significant.

MP sizes were similar throughout the OIZs. The most common size range observed was 38-425 μm discarding the two results in the OIZ-B IWWTP units out of the 56 samples where the smallest size range is less than 75%. These findings are also in agreement with the literature. In terms of MP shapes, it was determined that fragment-shaped MPs were the majority in all samples. On the other hand, no difference was observed in MP sizes between the sampling campaigns discarding two samples where the fragment type is not the most dominant shape. However, it is not possible to obtain information on whether these changes are due to seasonal changes or changes in industrial activities.

Except for the metal industry, where the MPs counts were very low, it was observed that pretreatment units showed an effective change in MP numbers. In addition, it was observed that the treatment efficiency of the paint industry was low during the first sampling campaign, but an average of 57% treatment efficiency was obtained

for MP in the second sampling, which is also observed in the textile sector with an average of 52% removal.

On the other hand, considering the significant industrial wastewater contribution in OIZ-B, it is thought that values close to the MP removal efficiencies encountered in a typical secondary treatment system are observed (79% and 88%). Similarities were observed between the discharge points and the MP numbers at the treatment plant inlets and outlets between the sampling campaigns.

However, differences were detected in the OIZ-B and metal industry treatment outlets because of higher MPs concentration in the inlet compared to outlets, which may be attributed to the chemical additions during the process. In the future, it will be useful to study the structures of the materials, especially the chemicals used in treatment, and the conditions that may lead to MP contamination. During the first sampling campaign, there were some samples that showed great differences in terms of MP characteristics. In the wastewater treatment plant of OIZ-B, larger pieces were found in the size distribution of MPs after the coarse screen and in the aerated grit chamber, and almost only fiber-shaped MPs were found in the aerated grit chamber. In addition, only MPs in the range of 425-1,000 μm were found in the outlet of the pretreatment of the textile sector, and particles in the range of 38-425 μm, which are usually the highest, were not found. However, these unusual findings were not encountered again in the second sampling campaign. In the second sampling campaign, the difference was seen in the morning sample of the pretreatment outlet of the metalworking sector, the fragment shape was found in the lowest amount, while the film and fiber shapes were found in similar amounts and high.

Lastly, the textile industry showed unexpected result with low MPs concentrations. These were attributed to the raw material being processed in the plant showing higher resistance to releasing MPs compared to the studies in the literature where processes are either not using raw materials or having much harsher processes.

CHAPTER 6

RECOMMENDATION FOR FUTURE WORK

Based on the laboratory work conducted in this study as well as the results obtained, following recommentations can be made for future works:

- Although it has been demonstrated that the analysis method used in this study is effective and applicable, visual observation step of the methodolgy still includes human error. While this errors can be mitigable with experienced laboratory workers and better standard operating procedures, use of computer controlled microscope settings will be better off in the majority of cases. This approach may also increase the analysis speed since microscope step is one of the slowest steps in the methodology. Use of automated systems for the Fenton oxidation, microscope, Raman and FTIR analyses can all be performed by various automised machines such as computer controlled reaction vessels for Fenton oxidation and mechanized scanning microscopes (including FTIR and Raman measurements). While some of these technologies are still slower than some human operators, they are getting faster by the day with increased accuracy. In the future, these machines can be a part of standardized MPs analysis procedures.
- In this study, effluent MPs concentration of metal industry is observed to be higher than the influent MPs concentration. This may suggest there may be an input of MPs during the process of the metal industry's pretreatment system. This needs to be examined further with repeated samplings and understanding/analyzing the inputs of pretreatment process such as coagulants.
- Textile industry showed unexpected MP counts compared to studies in the literature. Further work in different areas of textile sector to analyze MP content may bring extra information and further confirmation on MP counts

in textile sector. This can be further expanded to the metal industry for having higher MP counts in the effluent wastewater than the influent wastewaters and how this phenomena occurred must be investigated further.

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APPENDICES

A. Sampling Procedure Example for OIZ-B IWWTP

OIZ-B WWTP SAMPLING INSTRUCTIONS Tarih: 12.04.2021

A total of 11 samples will be taken from the OIZ-B wastewater treatment plant. The hours and locations of the samples are given in the table below.

Sampling Locations	Saat										
	09:00	09:30	10:00	10:30	12:30	13:00	14:00	14:30	15:00	16:00	16:30
WWTP Inlet	X				X					X	
WWTP Outlet		X				X					X
Post Screening			X								
Grit Chamber				X							
Centrifuge Reject							X				
Secondary Sludge								X			
Centrifuge Sludge									X		

In order to collect samples, you must be provided with 5 labeled 5L glass jars, 1 5L glass jar filled with distilled water, 4 2L glass jars and 2 38µm metal sieves, 2 glass measuring cylinders, a washbasin, a metal bucket and a backwash container.

Please make sure that the jars you have are named as given below, otherwise make the corrections as indicated:

- For WWTP inlet wastewater samples:
    - | DS-OIZ-B-02-GRŞ-01 / 12/04/2022 | 09:00 sample,
    - | DS-OIZ-B-02-GRŞ-02 / 12/04/2022 | 12:30 sample,

- o | DS-OIZ-B-02-GRŞ-03 / 12/04/2022 | 16:00 sample.

• For coarse screen end wastewater and oil trap wastewater samples:

- o | DS-OIZ-B-02-KS / 12/04/2022 | coarse screen end wastewater sample,
- o | DS-OIZ-B-02-YT / 12/04/2022 | oil trap wastewater sample.

• For final sedimentation sludge and centrifuge sludge samples:

- o | DS-OIZ-B-02-SÇÇ / 12/04/2022 | final sedimentation sludge sample,
- o | DS-OIZ-B-02-DÇ / 12/04/2022 | centrifuge sludge sample.

The procedure for the samples given above is as follows:

1. It is confirmed that the equipment from which the sample will be taken is clean in advance, if not, it is cleaned.
2. The area must be ready at least 10 minutes before the sample collection time.
3. A labeled jar suitable for the sample to be taken is selected and the sample is taken as a grab and emptied into it.
4. A photograph of the point where the sample is taken is taken.
5. Before the sample jar is closed, it is checked that its lid is covered with aluminum foil.
6. A photograph of the sample is taken.
7. The sample jar is covered with a bag and stored in a dry and dry place.
8. All equipment used in sampling is washed with sufficient water.
9. The sampling schedule given at the end of the document is filled.

• For centrifuge reject and outlet wastewater samples:

- o | DS-OIZ-B-02-DÜS-01 / 12/04/2022 | centrifuge reject sample,
- o | DS-OIZ-B-02-ÇKŞ-01 / 12/04/2022 | 09:30 outlet sample,
- o | DS-OIZ-B-02-ÇKŞ-02 / 12/04/2022 | 13:00 outlet sample,
- o | DS-OIZ-B-02-ÇKŞ-03 / 12/04/2022 | 16:30 outlet sample.

The procedure for the samples given above is as follows:

1. It is confirmed that the equipment from which the sample will be taken is clean in advance, if not, it is cleaned.
2. The area must be ready at least 10 minutes before the sample collection time.
3. With the provided measuring cylinder, a total of 20 liters of wastewater is passed through a 38µm aperture metal sieve.
4. A photo of the point where the sample is taken and the sieve after the filtration process is completed are taken.
5. The metal sieve is backwashed using 900 ml of water and it is ensured that nothing is left on it. In case of doubt about the cleanliness of the sieve, the amount of water backwashed can be increased up to 1.5 liters.
6. The sample collected in the backwash container is transferred to a jar with an appropriate label.
7. Before the sample jar is closed, it is checked that its lid is covered with aluminum foil.
8. A photo of the sample is taken.

9. The sample jar is covered with a bag and stored in a dry and dry place.
10. All equipment used in sampling is washed with sufficient amount of water.
11. The sampling schedule given at the end of the document is filled.

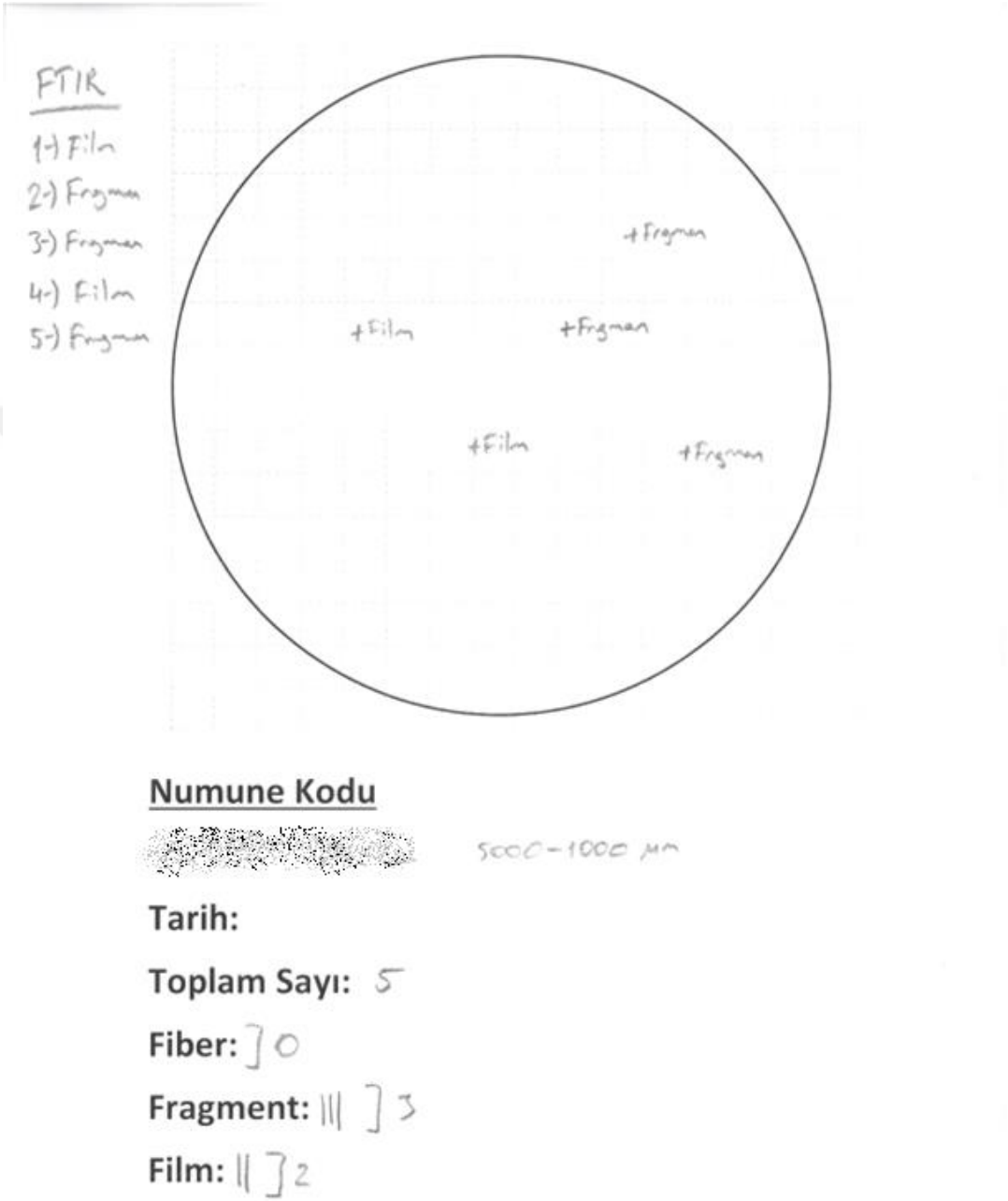
Points to consider when taking samples:

1. Samples should not interact with any plastic equipment, the equipment to be sampled should be glass or metal.
2. When filling sample jars, care should be taken to avoid spillage and contamination, and should not be rushed.
3. If necessary, Ozan Karakurt or Prof. Dr. Dilek Sanin should be contacted.

Label	Planned Sampling Time	Time Sampled	Notes
DS-OIZ-B-02-GRŞ-01 / 12/04/2022	09:00		
DS-OIZ-B-02-ÇKŞ-01 / 12/04/2022	09:30		
DS-OIZ-B-02-KS / 12/04/2022	10:00		
DS-OIZ-B-02-YT / 12/04/2022	10:30		
DS-OIZ-B-02-GRŞ-02 / 12/04/2022	12:30		
DS-OIZ-B-02-ÇKŞ-02 / 12/04/2022	13:00		
DS-OIZ-B-02-DÜS-01 / 12/04/2022	14:00		
DS-OIZ-B-02-SÇÇ / 12/04/2022	14:30		
DS-OIZ-B-02-DÇ / 12/04/2022	15:00		

DS-OIZ-B-02-GRŞ-03 / 12/04/2022	**16:00**		
DS-OIZ-B-02-ÇKŞ-03 / 12/04/2022	**16:30**		

B. Filter Counting Analogue for Fluorescence Microscope



C. Wastewater Characteristics of Second Sampling Campaign

		pH	TSS (mg/L)	COD (mg/L)
OIZ-A	Discharge Point	7.18	168.3	878.5
OIZ-B IWWTP	Inlet	6.74	213.3	553.5
	Outlet	6.35	20.0	159.5
Food Industry	Discharge Point	6.70	343.4	1149.5
Paint Industry	Inlet	7.05	48.3	237.0
	Outlet	7.15	25.0	294.5
Textile Industry	Inlet	6.74	213.3	2425.0
	Outlet	6.35	20.0	365.0
Metal Industry	Inlet	2.64	65.0	615.5
	Outlet	8.76	33.3	647.0