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**Environment and Sustainable
Development Program**

Determination of Microplastics in Wastewater, a Factor towards Marine Contamination

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Abstract

Microplastic is widely spread in various segments of the environment, such as water and soil. The pollution caused by these particles is worldwide becoming an increasing environmental concern. Potential impacts on human health have been reported and still there are many doubts and mechanistic approaches need to be investigated. The ultimate sink of this ubiquitous and numerous other pollutants is water bodies. Therefore, its determination and quantification in water bodies and remediation therefrom is utmost important. Wastewater treatment plants (WWTPs) play a pivotal role in the release of microplastics (MPs) into the marine environment. This study investigated the occurrence of MPs in the receiving water flow at a facility of WWTP in the Kingdom of Saudi Arabia (KSA). It also assessed the fate of MPs by examining the abundance, morphology, and polymer type at the end of each treatment process. Identification and quantification of MPs were carried out by fluorescence microscopic observation upon application of Nile Red staining technique as well as Raman spectroscopic analysis. The study confirmed the presence of 610 particles/L in the influent, which was reduced by 64.4% in the final effluent. The secondary treatment was shown to have the highest removal efficiency for MPs with a reduction rate of 54.6%. Fibers were the dominant shape of MPs in wastewater samples and were found to be retained by the treatment processes. Among the identified polymers were polyethylene, polypropylene, polystyrene, and polyvinyl chloride. There is a substantial need for standardization of methodologies to validate the comparison of available datasets with regard to MP contamination. Future studies should investigate the potential impacts of the exposure of humans and other organisms to MPs via the reuse applications of wastewater.

Dedication

To my parents ***Nader Alzayer*** and ***Amal Aljarrash***, to my sisters, and to my whole family. Thank you all for your support and encouragement during the tough time of compilation of this work.

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Table of Contents

Abstract.....	a
Dedication.....	b
Acknowledgment.....	c
Table of Contents	d
List of Figures.....	f
List of Tables	g
Chapter 1 – Introduction.....	1
1.1 Background	1
1.2 Research Problem	4
1.3 Research Significance.....	4
1.4 Research Questions	4
1.5 Research Objectives.....	5
1.6 Research Setting	5
Chapter 2 – Literature Review and Theoretical Framework	6
2.1 Literature Review.....	6
2.2 Sources of MPs.....	6
2.2.1 Primary MPs.....	6
2.2.2 Secondary MPs.....	7
2.3 Characteristics of microplastics	7
2.3.1 Shape.....	7
2.3.2 Size	8
2.3.3 Polymer Type	8
2.4 Wastewater treatment process.....	9
2.4.1 Primary treatment	9
2.4.2 Secondary treatment	10
2.4.3 Tertiary treatment	10
2.5 MPs removal from wastewater	11

2.6	Overview of methodological approaches	12
2.6.1	Extraction of MPs	12
2.6.2	Physical characterization of MPs	13
2.6.3	Chemical characterization of MPs	14
2.7	Executive summary	16
2.8	Theoretical Framework.....	17
Chapter 3 – Methods and Procedure		19
3.1	Research Area	20
3.2	Sample preparation	20
3.2.1	Wastewater samples.....	20
3.2.2	Reference materials	21
3.3	Sample processing	21
3.3.1	Extraction process.....	21
3.3.2	Staining procedure.....	22
3.4	Mitigation of cross-contamination	22
3.5	Analysis of MP	22
3.5.1	Visual inspection	23
3.5.2	Spectrum acquisition	24
3.6	Statistical Analysis.....	25
Chapter 4 – Results and Discussion.....		26
4.1	Spiked samples.....	26
4.2	Wastewater samples – abundance of MP	29
4.3	Characterization of MP	32
4.4	Types of MPs in analyzed samples.....	35
4.5	Implementations.....	39
Chapter 5 – Environmental Risk of Microplastics.....		42
Chapter 6 – Conclusion and Recommendation.....		47
References.....		49

List of Figures

Figure 1	Analytical procedure used in the analysis of microplastics in wastewater	12
Figure 2	Layout of the study outlined in this thesis.	18
Figure 3	Procedure of the experimental work of the current study	19
Figure 4	FTIR results confirming the types of polymers in the spiked samples: polyvinylchloride (PVC), polypropylene (PP) polystyrene (PS), and polyethylene (PE).....	27
Figure 5	Plastic particles under fluorescence microscope using green filter (top) and blue filter (bottom). From left to right: polyvinylchloride (PVC), polypropylene (PP), polyethylene (PE) and polystyrene (PS).....	28
Figure 6	Non-plastic samples under fluorescence microscope with green (top) and blue (bottom) filter. Samples from left to right are steel, wood, cement, glass, and wax.....	28
Figure 7	Mean MP concentrations (particles/L) and reduction rate (in percent) in each treatment stage of the treatment process.	31
Figure 8	Recorded shapes of MP; (a & b) fiber, (c) line, (d) fragment, (e) film and (f), pellet	33
Figure 9	Distribution of MPs based on their shape (left) and sizes in μm (right).....	33
Figure 10	Abundance of fibers vs MPs of other shapes in influent and effluent wastewater samples .	34
Figure 11	Raman spectra for microplastic particles in wastewater samples.....	38
Figure 12	MPs transportation through WWTPs and human exposure.....	46

List of Tables

Table 1	Count of microplastics (particles/L) in each stage of the wastewater treatments.	29
Table 2	Chemical composition of MPs detected in the current study.	36

Chapter 1 – Introduction

1.1 Background

Plastic contamination in the natural environment is a critical issue that concerns researchers, environmental policymakers as well as the general public (Li et al., 2016). In the past 5 decades, global plastic production has increased 20-folds reaching 368 million metric tons in 2019 (Walker & Fequet, 2023). With the current increasing demand for plastic, this figure is projected to be double within the next 20 years (Walker & Fequet, 2023). Plastics are synthetic or semi-synthetic polymeric materials derived mainly from petroleum products. They are cheap, strong, and durable. These features make plastic a preferable material in the manufacturing field. The commonly used polymers are polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) to name just a few. They account for almost 90% of global plastic production (Li et al., 2016). Due to their long-lasting and corrosion-resistant properties, they tend to persist in the environment for up to a century. The limited recyclability of plastic has contributed to the massive plastic waste generation. Worldwide, only 9% of plastic waste has ever been recycled, 12% incinerated, and 79% has accumulated in the environment (Walker & Fequet, 2023). Statistics show an annual estimate of 53 million metric tons of global plastic waste which will enter the aquatic ecosystem by 2030 (Walker & Fequet, 2023).

As this massive plastic debris accumulates in the environment, it gets exposed to various environmental factors including physical, chemical, and biological processes that would make plastic debris brittle, thus breaking down into small pieces called microplastics (Almadhi et al., 2023). Exposure to wind, storms, sunlight, and water currents are important factors causing plastic fragmentation in the environment (Almadhi et al., 2023). Weathering and photodegradation result in the oxidation of polymer matrix leading to breakage of chemical bonds. Moreover, abrasion, wave current and turbulence are all mechanical forces that cause loss of structural integrity, hence the breakdown of plastic debris. Therefore, microplastic pollution has emerged from the mismanaged plastic waste that has been accumulating in the environment.

Microplastics (MP) are small pieces of plastics that are less than 5 mm in size. They are one of the emerging contaminants that have increasingly gained global attention in recent years (Bui et al., 2020). MPs are estimated to account for more than 92% of the global plastic count (Maricela

& Espinoza, 2019). Besides their abundance, their small size, ubiquity, widespread distribution and persistence in the environment are among the reasons for the increasing concerns around them (Lv et al., 2019). Researchers estimated more than 5 trillion pieces of plastic are currently floating on the sea surface (Araujo et al., 2018). In fact, this number is exponentially growing due to unsustainable plastic production as well as improper management of plastic waste. As a consequence, there would be an increase in global plastic pollution and subsequent degradation into micro-sized plastics (Al-Shaikh Ali et al., 2023; Uddin et al., 2022).

Microplastic pollution is enormously widespread in the aquatic ecosystem globally. MPs have been detected in water bodies, beach sediments and numerous marine animals from plankton to whales. For example, MPs were detected in surface water in Ireland, Australia and South Korea, and in bottom water in Brazil and the Portuguese coast (Li et al., 2016). MPs were also found in the open sea far from any possible waste source indicating that their distribution might be affected by winds and water currents making it a transboundary kind of pollution. Besides water bodies, MPs were detected in beaches worldwide. For instance, beach sediments from Malaysia, Hong Kong, the Belgian coast, California, and Hawaii (Fauziah et al., 2015; Ivar do Sul et al., 2009; Li et al., 2016). Moreover, MPs were found in multiple aquatic species worldwide including seabirds, turtles, fish, dolphins and whales. Occurrence of plastic ingestion was reported on the Brazilian coast, the USA, the Mediterranean, the Northern Pacific, Australia, India, and many European countries (Li et al., 2016). Therefore, microplastic pollution is widely spread in the marine environment threatening the sustainability of the whole ecosystem.

Microplastics are a particular threat to the environment because of their characteristics. They are durable, long-lasting, and have low biodegradability i.e. instead of disintegrating into organic substances, they simply break down into smaller and smaller pieces of plastic upon exposure to external factors (e.g. sunlight, wind, and wave-current) (Atugoda et al., 2022). Moreover, microplastics have high mobility i.e. they tend to transport through surface runoffs, stormwater, wind, waves, and currents. Their transport and distribution are facilitated by their small size, buoyancy, and prolonged periods of persistence (Atugoda et al., 2022). This translocation is what makes them widely distributed in different water metrics (surface water, coastal water, lakes, rivers, and marine sediments) threatening biota life.

MPs pose a potential threat to aquatic animals through direct ingestion from seawater. MPs are mistaken for plankton by marine organisms (Setälä et al., 2014). As a consequence, MPs enter the food chain leading potentially to bioaccumulation and biomagnification at higher trophic levels (Khattab et al., 2022). Unlike macroplastics, ingestion of micro-sized plastics may cause indirect and invisible harm to animals (Saling et al., 2020). When an animal swallows pieces of MPs, this may lead to blockage of the intestinal tract or cause other physical effects including inflammation, intestinal alterations, delays in ovulation, failure to reproduce, or even mortality (Ben-David et al., 2021; Li et al., 2016). MPs may carry chemical pollutants such as polychlorinated biphenyls (PCBs) that are toxic, bioaccumulative, and have the potential to be transferred to other animals across the trophic levels. Similarly, plastic additives, such as bisphenol A, may be released and accumulate inside the body upon ingestion causing adverse effects e.g. endocrine disruption, hepatic stress, and oxidative stress (Li et al., 2016). Many of these species are considered crucial food sources for humans, and hence this marine pollution is threatening the safety of the marine food supply. Consuming plastic-contaminated seafood could have serious toxicological impacts on humans (Akhbarizadeh et al., 2019; Fauziah et al., 2015; Ghosh et al., 2023).

Therefore, it is very important to prevent or at least minimize the number of plastics from entering the marine environment. Tracking the possible origins of microplastics is well investigated and researchers found wastewater treatment plants (WWTPs) to be an entrance route for MPs into the aquatic ecosystem (Liu et al., 2021; Talvitie et al., 2015). Treatment processes at these plants were shown to be capable of only partial removal of the MP load. A proportion of MPs was evidently found to pass these treatments (i.e. was detected in the effluent) hence entering the receiving water bodies. Due to the large volume of treated wastewater discharged to the environment on a daily basis, WWTPs are considered hotspots for MP pollution. It was recently reported that a single medium-sized WWTP could release up to 1.83×10^{10} pieces of MPs per day (Ben-David et al., 2021). However, there is a great variation in the emission figures among WWTPs depending on the MP content in the receiving water, the capacity of the plant and the removal efficiencies of its treatment processes (Ben-David et al., 2021). These observations grabbed the attention of numerous researchers who have been investigating the issue in different parts of the world (Bayo et al., 2021; Ben-David et al., 2021; Bilgin et al., 2020; Cristaldi et al., 2020; Long et al., 2019; Lv et al., 2019; Uddin et al., 2022).

1.2 Research Problem

Microplastics pose a potential threat to humans because of their high mobility in the aquatic ecosystem, their capacity to be contained within the food chain at all levels, and their ability to carry and accumulate hazardous substances. Looking at the literature that involves assessment of microplastic pollution in the Arabian Gulf region, a few studies have confirmed pervasiveness of MPs along the coastline of Qatar, in the surface seawater of Oman, in coastal areas of Kuwait, and along the eastern coast of Saudi Arabia (Abayomi et al., 2017; Al-Shaikh Ali et al., 2023; Aliabad et al., 2019; Saeed et al., 2020). However, knowing that WWTPs provide the main pathway for MP entry to the aquatic environment, there is a limited investigation of their role as a source of microplastic pollution in the Gulf Region. Recently, two studies have reported the discharge of MPs from WWTPs located on the northern coast of the Gulf (i.e. Iran and Kuwait) (Naji et al., 2021; Uddin et al., 2022). Another study has confirmed microplastics' contamination of artificial freshwater systems (ponds and channels) that are recharged with treated wastewater in KSA (Picó et al., 2021). However, there is a complete lack of baseline data in Saudi Arabia regarding the occurrence of MPs in wastewater systems and the efficiency of the treatment processes in removing them.

1.3 Research Significance

There is a knowledge gap in research and literature on the contribution of WWTPs to microplastic pollution in the Kingdom of Saudi Arabia. The current study was conducted in Saudi Arabia investigating the occurrence of MPs in wastewater along with quantification and characterization analysis. This study provides the first dataset of MP concentration in WWTPs in KSA and their potential release into the environment.

1.4 Research Questions

- What is the degree of microplastic contamination in the inflow of the specified WWTP – quantitative and qualitative analysis (e.g. count, shape, size, and type of MPs)?
- How does the MP concentration change throughout the treatment stages within the specified WWTP? Which treatment is more efficient in removing these microplastic particles?
- Considering the amount of MPs discharged from the specified WWTP (i.e. in the effluent flow), what could the consequent potential harm to the environment be?

1.5 Research Objectives

- To investigate the occurrence of microplastics in the influent of wastewater.
- To evaluate the removal efficiency of microplastics by the primary, secondary, and tertiary treatment processes.
- To review the risk of microplastic pollution towards marine life.

1.6 Research Setting

The analysis of this study would cover a WWTP that is located in Dhahran City in the Eastern Province of Saudi Arabia and the practical work would be conducted in the Environment and Hydrology Laboratory at King Fahd University of Petroleum and Minerals.

Chapter 2 – Literature Review and Theoretical Framework

2.1 Literature Review

Plastic waste, including macroplastics, MPs, and nanoplastics, has become of great concern globally due to the exponential growth of plastic use with no enhancement in waste management (Sipe et al., 2022). According to the United Nation's Environment Program, the global production of plastic is estimated to be 400 million tones every year and it is forecasted to reach 1,100 million tones by 2050 (Amaral-Zettler et al., 2015). In addition, almost half of the total plastic products are designed for single-use purposes, which leads to a growing amount of plastic being thrown into the environment. Unfortunately, plastics including MPs are now ubiquitous in all environmental compartments. It is estimated that by the year 2030, the annual amount of plastic waste entering the aquatic ecosystem will reach 90 million tones (Borrelle et al., 2020). MPs are pieces of plastic that are larger than 1 μm but do not exceed 5000 μm in dimensions (Ghosh et al., 2023). Such small-sized plastics are characterized by low biodegradability and high mobility. They are released over the life cycle of material products that are made of plastics including synthetic textiles and personal care products (primary MP). They can also come from a secondary source caused by the degradation of unmanaged plastic waste (secondary MP) (Nava et al., 2021). Primary and secondary microplastics may enter the environment directly (via industrial spills and transportation) or indirectly (via shoreline activities and wastewater treatment facilities) posing serious risks to terrestrial and aquatic systems (Conley et al., 2019).

2.2 Sources of MPs

Microplastics have been classified as primary and secondary based on their origin as explained hereunder:

2.2.1 Primary MPs

On an industrial scale, virgin plastic is deliberately synthesized in micro sizes for a variety of applications. For example, plastic pellets are used as a precursor for the manufacturing of a variety of plastic products (Dick Vethaak & De Kreuk, 2015). The plastic particles from there can

unintentionally get lost throughout the production processes and eventually reach the environment (Liu et al., 2021). Furthermore, plastic microbeads are specifically engineered to be used in cosmetics, cleaners, clothing, fishing nets and most importantly personal care products such as fluid soap, shower gels, scrubs and toothpastes (Conley et al., 2019). Similarly, plastic is a main constituent of synthetic fiber fabrics, and hence could be released upon washing of these fabrics. Therefore, household activities and personal hygiene activities would result in microplastics being washed-off through sinks and drains leading them to wastewater treatment plants (WWTPs) (Cristaldi et al., 2020).

2.2.2 Secondary MPs

Continuous fragmentation of plastic waste would eventually result in the formation of microplastics in the form of fibers, fragments and flakes. This process can be induced by biochemical and physical forces such as sunlight, ultra violet radiations, wind and water wave action which highly contribute to the breakdown of littered plastic items (Biyik & Baycan, 2021). Moreover, abrasive forces can result in tires, brakes and road wear which are major sources of microplastics in the environment (Sommer et al., 2018). Similarly, repetitive opening and closing of plastic-made water bottles were found to induce microplastic abrasion from the screw cap system (Giese et al., 2021).

The pathways in which secondary MP gets into the wastewater can be human-related (e.g. direct disposal of plastics through the treatment facilities of wastewater) or nature-related (e.g. stormwater runoff) (Sipe et al., 2022). Moreover, primary MPs could be broken into secondary MPs through different treatment processes (physical, chemical and biological treatments) (Liu et al., 2021). Therefore, primary and secondary MPs could end up in any environmental compartments (atmosphere, soil, and mainly surface water and freshwater), posing a potential threat of being ingested by animals, entering the food chain, and causing health hazards to human health (Cristaldi et al., 2020; Liu et al., 2021).

2.3 Characteristics of microplastics

2.3.1 Shape

Several categories of MPs are presented in the literature with regards to their visual appearance; fragments (hard particles with sharp edges), films (thin sheets of flimsy particles), lines (long

pieces of plastic), fibers (thin thread-like pieces), and pellets (rounded particles) (Ben-David et al., 2021). Studying the morphology of MPs can provide an indication of their origin e.g. fibers originate primarily from microfiber shedding due to laundry washing, while fragments originate from water runoff that carries road dust into the sewage system (Bilgin et al., 2020).

2.3.2 Size

Researchers use arbitrary classifications to divide MPs based on their size which makes it difficult to compare (Ben-David et al., 2021). Yet, it appeared that the particle size of MPs detected in wastewater samples has a great variation. A size range of 25 μm – 5 mm was reported in a systematic review published recently, (Cristaldi et al., 2020). Statistical analysis confirmed that the mean size of MPs found in the influent (500-1000 μm) was different than that in the effluent (<500 μm) (Van Do et al., 2022). A reduction in the size of MPs upon treatment may suggest that larger microplastics are easier to treat and hence smaller MPs are more likely to be released to the environment (Cristaldi et al., 2020). Additionally, size and shape could make a combined factor for the variation in the removal efficiency. For instance, a study found the influent of wastewater to consist of mainly small-sized fibers, relatively equal concentrations of small and large films, as well as low concentrations of small fragments (Bilgin et al., 2020). The small size in this study was considered as < 1 mm and the large size ranged between 1 and 5 mm. This classification helped in the analysis of MP removal based on treatments. For instance, flotation was shown to be more effective in removing large fibers and large films (46 and 59%, respectively) compared to small fibers and small films (around 40% both). Larger films (1–5 mm) may be characterized by low mass and wide surface area favoring flotation rather than sedimentation (Bilgin et al., 2020).

2.3.3 Polymer Type

Analysis of the chemical composition of suspected particles could confirm the presence, concentration, and type of MPs in the sample. According to Liu et al. (2021), twenty-nine types of polymers were confirmed to be present in wastewater samples. In fact, studying the polymer type could facilitate researchers to trace the source where these particles come from. Taking polyethylene, polypropylene, and polystyrene as examples of common types of MPs in wastewater, they usually originate from plastic products (e.g. plastic bottles, plastic silverware, containers, food packaging bags, toys, etc.) or from the mechanical crushing of plastic products

(e.g. rubber particles in road dust, tires, construction materials, etc.) (Liu et al., 2021; Lv et al., 2019). Polyamide (Nylon) and polyethylene terephthalate (PET) are among the most widely detected MPs in wastewater and are often detected as fiber-like MPs (Bayo et al., 2021). They appear to originate from textile products i.e. released from washed clothes and carpets (Cristaldi et al., 2020).

It could be perceived that making comparisons regarding the abundance of MPs in wastewater and their removal efficiency is very challenging due to the great variability in their types, size, and shape across WWTPs. Similarly, the different treatment practices in each plant could result in diverse removal rates and quantitative differences in types, shapes and sizes of MPs (Elizalde-Velázquez & Gómez-Oliván, 2021). A good understanding of these treatment techniques may help to explain the variation in MP removal efficiency. These techniques are further elaborated in the following text.

2.4 Wastewater treatment process

WWTPs concentrate large quantities of MPs coming from domestic areas (Ben-David et al., 2021). Conventional WWTPs generally have three phases of treatment: primary, secondary, and tertiary. Although these processes do not target MPs specifically, they have a considerable potential to reduce the proportion of MPs compared to that in the influent (Liu et al., 2019).

2.4.1 Primary treatment

Primary treatment intends to eliminate solid particles from the wastewater via filtration and sedimentation. This step is common at all WWTPs and it is known as the mechanical treatment since it relies on physical water treatment processes. Wastewater influent passes initially through a screen chamber and a grit chamber which blocks debris and grit from entering and damaging the plant equipment. Suspended particles that pass through these filtration chambers are removed from the sewage in a sedimentation tank (settling tank). This tank, also called a primary clarifier, allows suspended particles to settle down under the influence of gravity. Wastewater at this stage is known as the primary effluent, and it should be ready for secondary treatment.

2.4.2 Secondary treatment

Secondary treatment, also known as biological treatment, uses microorganisms to breakdown the organic matter that has bypassed through the primary step. The most common biological treatment is conventional activated sludge (CAS) which involves aeration of microorganisms in the bioreactor. These microbes utilize the provided oxygen to grow and reproduce, forming an active suspension of biological solids called activated sludge. The wastewater then flows from the aeration tank into a secondary clarifier (settling tank), where activated sludge settles down by gravity. Water that is skimmed from the surface of the clarifier is called the secondary effluent and it is considered as clear water.

The second most common biological treatment is the membrane bioreactor (MBR) where membranes are used for solid/liquid separation along with the activated sludge process. A number of microfiltration membrane modules are installed in series (usually submerged in the bioreactor) with semi-permeability that allows the transport of only specific compounds based on their relative size or electrical charge. This provides an advanced level of organic and suspended solids removal producing a higher-quality effluent.

The last step in both CAS and MBR involves recycling or return of settled biomass (called return activated sludge or RAS) back to the beginning of the aeration process for another operation cycle. This step is important to maintain an adequate concentration of activated sludge in the bioreactor which is necessary to prevent the loss of microorganisms (i.e. progress with the aeration digestion process). Moreover, the secondary effluent usually gets disinfected, by chlorination or ultraviolet irradiation, before it is discharged to surface water or tertiary treatment if needed.

2.4.3 Tertiary treatment

The final phase of wastewater treatment is the tertiary treatment which is optionally applied to remove the residual substances that are still found in the wastewater after passing through the primary and secondary phases. Examples include sand filtration, activated carbon adsorption, ozonation, and advanced oxidation.

2.5 MPs removal from wastewater

Although WWTPs are not specifically designed to remove MPs, it has been confirmed in many studies that the proportion of MPs was reduced going from the influent to effluent (Bilgin et al., 2020; Lares et al., 2018; Liu et al., 2019; Yang et al., 2019). Yet, MPs are not totally removed and their effluent concentrations fluctuate across studies (Liu et al., 2021). This variation could be attributed to the characteristics of the WWTPs (e.g. treatment process, treatment technology, flow rate, service composition and service population), sampling method, sampling frequency, as well as sample preparation and processing procedures (Cristaldi et al., 2020). In addition, the characteristics of the MPs themselves could be responsible for this inconsistency in their removal potential. For instance, a study found fibers, in particular, to have low removal efficiency due to their morphology which allows them to bypass some treatment processes (Ben-David et al., 2021). Another study stated that fragments are more likely to be removed by the sedimentation process due to their irregular 3D structure and their density which is usually greater than that of wastewater (Bilgin et al., 2020). Similarly, some studies found a specific size class of MPs to dominate at specific stages of the treatment processes e.g. those of particle size smaller than 0.5 mm were better removed during secondary treatment processes while those ranging between 0.5 and 1 mm were removed much easier by the primary treatment (Bilgin et al., 2020; Cristaldi et al., 2020). A study by Liu et al. (2021) compared the efficiency of different treatment technologies in removing MPs across global WWTPs. Similar removal potential was attributed to the primary and secondary treatment processes, while the tertiary treatment attained lower removal potential. Filter-based technologies including bio-filter and bioreactor (secondary treatment technologies) were observed to achieve the best performance in removing MPs. In fact, the total removal efficiency of an individual WWTP can vary from 54% to 99.9%, yet the consistent release of MPs from wastewater effluent toward the aquatic environment remains the main issue (Bilgin et al., 2020). Therefore, it has been proved that MPs are frequently discharged from WWTPs and accumulate in water bodies and other environmental compartments. Thus, in order to reduce MPs entering the natural aquatic system, it is urgent to study the efficiency and the mechanism of the treatment technologies of WWTPs in removing MPs, taking into consideration a specified analytical protocol to facilitate the comparability of the results (Liu et al., 2021).

2.6 Overview of methodological approaches

A considerable amount of literature has been published on the methods currently used for the analysis of MPs in real environmental samples. This section provides a brief synopsis of the various analytical procedures reported in the literature for the investigation of MPs (Figure 1).

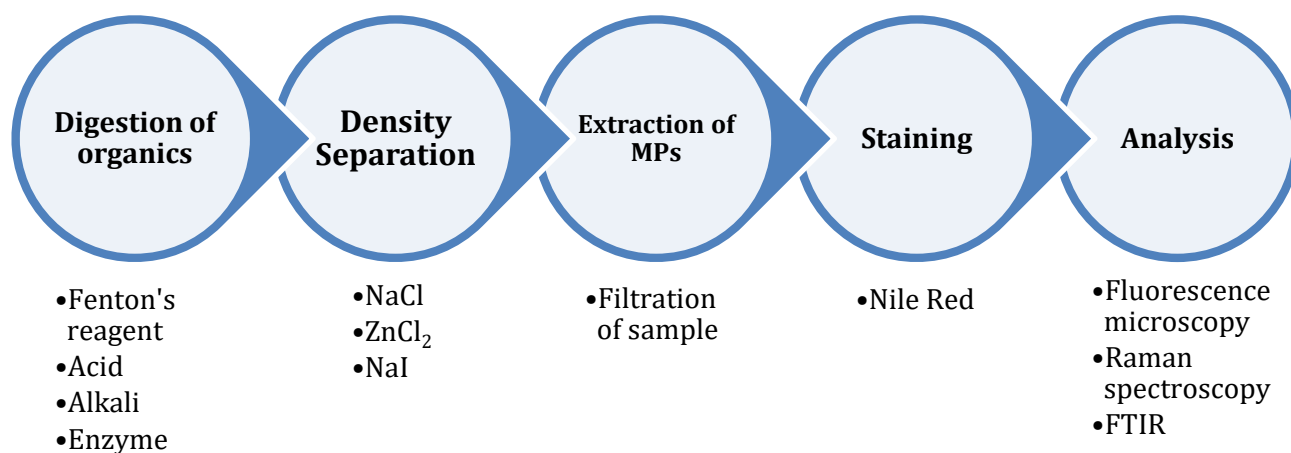


Figure 1 Analytical procedure used in the analysis of microplastics in wastewater

2.6.1 Extraction of MPs

Detection of microplastics in complex environmental samples requires a combination of sample processing techniques (Water et al., 2020). Wastewater samples, for instance, contain a high amount of organic matter that can be confused with MPs. Thus, organic matter should be initially reduced using processes that do not interfere with the structural or chemical identity of polymers/MPs. Then, MPs are supposed to be extracted from samples to facilitate quantification and characterization (Prata et al., 2019).

The National Oceanic and Atmospheric Administration (NOAA) recommends the digestion of organic matter before the extraction of MPs from wastewater samples (Yang et al., 2019). Digestion can be done by the use of an oxidizing agent (Fenton's reagent), acid (HNO_3), alkali (NaOH), or enzymes (used for sediment samples rather than water samples). The use of Fenton's reagent (H_2O_2 with ferrous ion catalyst) has been shown to be more efficient in removing organic matter compared to acidic and alkaline digestions, with minimal degradation of polymers (Prata et al., 2019).

Another technique that is useful in the isolation of MPs from wastewater samples is density separation. Saturated salt solution including sodium chloride (NaCl), zinc chloride (ZnCl_2) and/or sodium iodide (NaI), is mixed with the samples allowing MPs to ascend and be collected in the supernatant. While ZnCl_2 was reported to be the most efficient salt in separating MPs from samples, NaCl was more commonly used as it is cheap, available and environmentally safe (Prata et al., 2019).

Moreover, the filtration of samples can be the last step in sample processing before their analysis. Filter types and pore size of the filter being applied are crucial factors that need to be considered for the filtration step. Filter papers can be made of different materials including polycarbonate, glass microfiber, aluminum oxide, cellulose nitrate, polytetrafluoroethylene, as well as gold-coated polycarbonate (Pivokonsky et al., 2018). Nava et al. (2021) have suggested factors for selecting a suitable filter for MPs analysis which include smoothness of the filter surface, strength of the fluorescence profile, possible contamination by fiber release from the filter, and possible interference from the material of the filter (Nava et al., 2021). In addition, the pore size of the filter paper can determine the lower size of MPs detected. It must be small enough to retain all relevant MPs, yet not be clogged with organic materials (Nava et al., 2021). The pore size range reported to be used in the analysis of MPs in wastewater is in the range of 0.45 to 250 μm (Prata et al., 2019).

2.6.2 Physical characterization of MPs

Several methods have been employed to detect and quantify MPs in environmental samples including wastewater. The commonly used method is visual inspection which involves direct observation of the physical characteristics of MPs (size, color and shape) using a microscope or

stereoscope. However, this method is subjective, highly time-consuming and leads to overestimation or underestimation, and therefore the method fails in many instances to distinguish biological material from MPs (Prata et al., 2019).

One of the recommended techniques to improve visual identification of the concerned particle is the use of staining dyes. Among available dyes, Nile Red (NR) is more promising and extensively used for MP detection. This is because of its intriguing characteristics such as high adsorption to plastics, short incubation time, strong affinity to various plastic polymers, and high fluorescence intensity (Shruti et al., 2022). During application, it highlights the MP particles with fluorescence making them easily distinguishable from other minerals and organics in the sample. This enhances visualization, improves the accuracy of the results, and saves time and needs less effort (Shruti et al., 2022).

Due to the lack of a well-established single technique, there is a lot of diversity in the application of NR to microplastics including the concentration of NR used, the solvent used for NR solution preparation, the incubation time and the staining method. According to a recent review of 56 published studies employing NR staining in the assessment of MP pollution, the concentration of NR dye used for staining ranged from 1 $\mu\text{g/mL}$ to $\geq 10 \text{ mg/mL}$ (Shruti et al., 2022). It was also shown that upon dissolving the NR dye in acetone, a variety of solvents were utilized to dilute the solution e.g., methanol, hexane, chloroform, ethanol, or isopropyl alcohol. Similarly, the NR-stained samples were allowed to incubate for a duration of 5 min up to overnight (approximately 12 h) at a temperature ranging from room temperature to 60°C (Shruti et al., 2022). Moreover, different methods for staining MP with NR have been outlined including adding the dye directly to the samples (before or after treatment) or adding the dye to the MP-containing filters (Shruti et al., 2022). Nevertheless, NR staining has been increasingly used for the detection of MP in environmental samples due to its easy accessibility, economic viability, and reliability. Yet, it is very labor-intensive and time-consuming, especially for MP-rich samples.

2.6.3 Chemical characterization of MPs

Chemical characterization is usually performed to confirm the results of the physical visualization of suspected MPs. Spectroscopic techniques including Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy have been shown to be effective in the identification

of MPs based on polymer type. These techniques involve producing a spectrum based on the interaction between light and the MP molecule. They both have been shown to be non-destructible and highly accurate (Prata et al., 2019).

Raman spectroscopy is rapidly gaining attraction in the analysis of MPs. It is a vibrational spectroscopic technique that relies on the vibration of functional groups and bonds in molecules leading to inelastic scattering of light producing a unique spectrum based on the chemical structure of the substance (Araujo et al., 2018). Raman spectroscopy has a high spatial resolution (up to 1 μm) that allows the detection of micro-sized plastics with minimal false positives. Compared to other spectroscopic techniques such as FTIR, Raman has wider spectral coverage, increased sensitivity to non-polar functional groups, and narrower spectral bands (Araujo et al., 2018). In addition, the NR staining method and the analysis of Raman spectroscopy were approved not to be counterproductive (Araujo et al., 2018). NR staining allows vibrational spectroscopy, and it is unlikely to cause interfering fluorescence during the Raman analysis.

Nevertheless, some limitations have been reported regarding MP analysis through Raman including (1) weak signal quality due to fluorescence interference and (2) misidentification of the material due to the presence of additive compounds (Araujo et al., 2018). Different practices intending to minimize these drawbacks have been described in the literature. Nava et al. (2021) have suggested some instrument parameters (i.e. Raman settings) that can improve the quality of the analysis which include the choice of excitation laser wavelength, acquisition times and the number of accumulations, as well as proper sample purification technique such as the use of a combination of approaches including digestion followed by density separation.

Chemical characterization of plastic particles is an intensively time-consuming process, especially for samples containing large quantities of MPs. It could be impractical to analyze the whole filter paper. Full point mapping could also be time-consuming where a whole section is analyzed with multiple spectra. Therefore, it is a common practice to analyze a selective sample of particles. This is called single point measurement where spectra are collected at the specified points only. To ensure that the selected sample gives a representative view of the extracted particles, either random selection is followed or calculation of the minimum number of particles

to be analyzed. Having a representative sample allows us to confidently generalize the findings from the sample to the entire population of detected MPs.

2.7 Executive summary

An overview of microplastics was illustrated in this section with a focus on microplastic pollution and the role of wastewater treatment plants. The section elaborated by reflecting upon the research methodologies adopted in the literature for the analysis of MPs in wastewater samples:

- MPs are pieces of plastic that are less than 5 mm in size.
- They were found in all environmental compartments but predominantly in marine environments.
- Analysis of environmental samples found MPs of different sizes, shapes, and polymer types.
- MPs may originate from primary or secondary sources and commonly enter WWTPs as a result of human activities.
- Previous studies showed that MPs were not completely removed from wastewater even after passing through the different treatment technologies of WWTPs.
- Removal efficiencies do not solely depend on the employed treatment technology, many other factors may contribute including physical and chemical characteristics of MPs, analytical procedures, and detection methods.
- Isolation of MPs from wastewater samples requires multiple steps e.g. digestion, density separation and filtration.
- Studying the morphology of MPs is often carried out by visual inspection with the aid of staining with a particular dye.
- The results of the visual inspection are usually confirmed by chemical characterization of the MPs.
- Analysis of the polymer type is performed via spectroscopic techniques.

An extensive review of the literature was very beneficial in framing the current study. The reviewed research methodologies provided justifications and logical reasons for implementing each of the methodological procedures in this research.

2.8 Theoretical Framework

The aforementioned studies shaped the present research and developed its theoretical framework. The current study aimed to confirm the occurrence of MPs in the inflow received at the selected WWTP, quantify MPs at the end of each treatment stage, evaluate the physical and chemical characteristics of the identified MPs, and finally assess the environmental toxicity of MPs with the help of literature reports.

The theoretical framework of the current study along with the employed methodological procedure is outlined as a flowsheet diagram in [Figure 2](#).

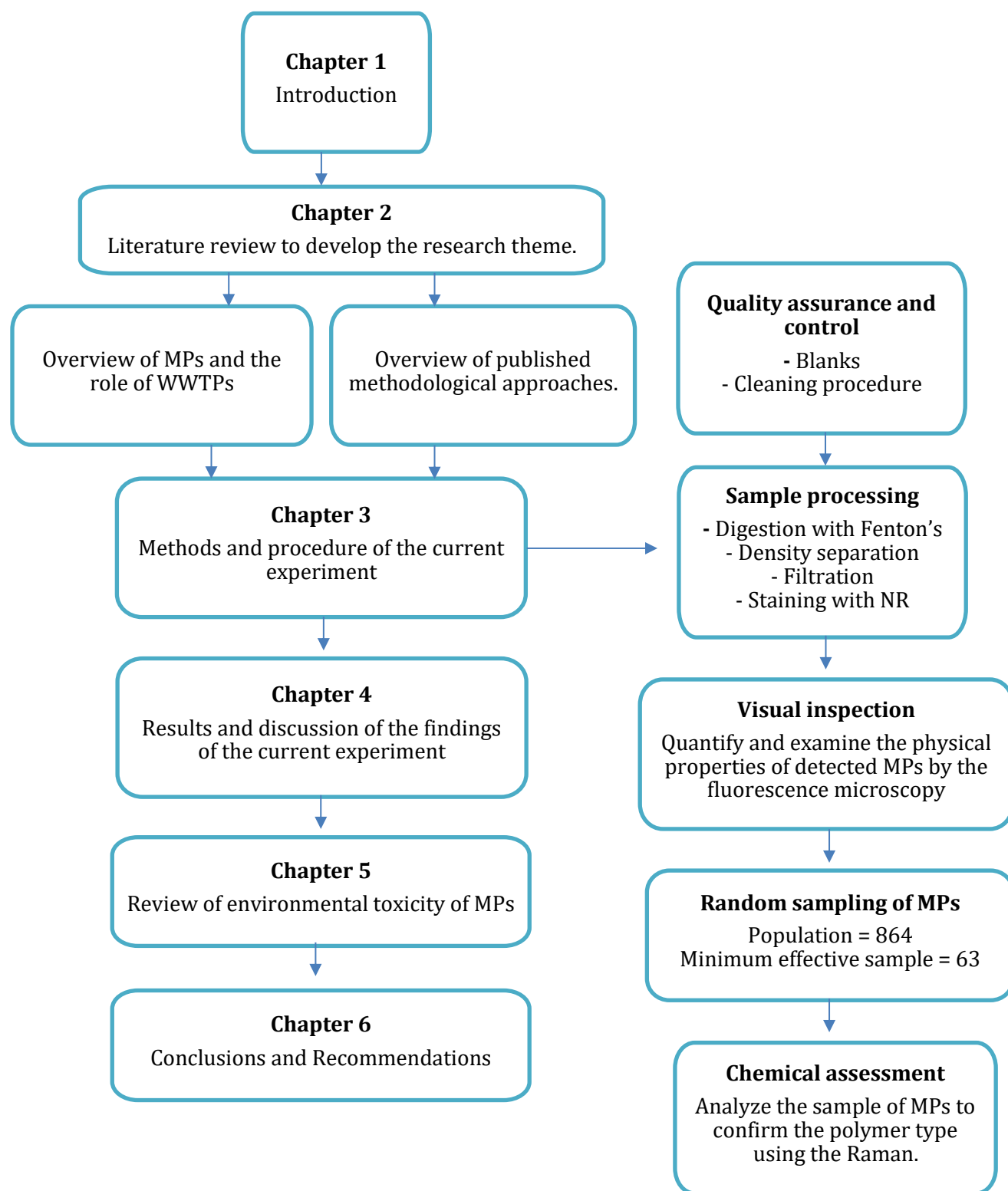


Figure 2 Layout of the study outlined in this thesis.

Chapter 3 – Methods and Procedure

In the current study, the sample-processing procedure was adopted from previous studies with some modifications and optimizations (Ben-David et al., 2021; Lv et al., 2019; Magni et al., 2019; Prata et al., 2019; Shim et al., 2016). The two main objectives of this experimental work are; the identification of MPs (count, size, and shape) and the characterization of MPs (type of polymer). The identification process depends on the staining of MPs and visualization by the microscope, while the characterization process is accomplished by Raman spectroscopy. Figure 3 provides a summarized procedure of the current experimental work.

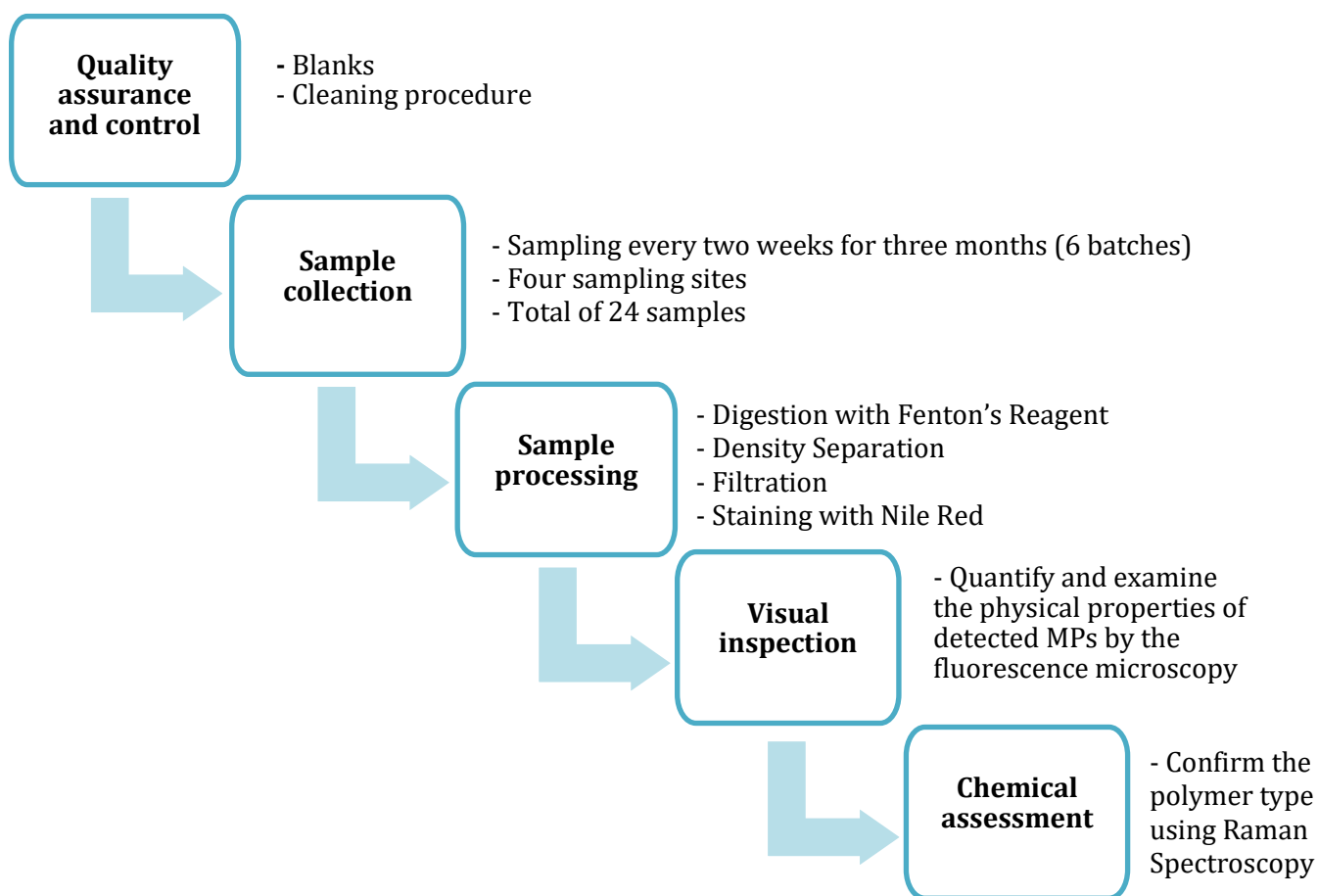


Figure 3 Procedure of the experimental work of the current study

3.1 Research Area

The selected WWTP is located in Dhahran, a densely populated city in the Eastern Province of Saudi Arabia. It receives only residential wastewater which flows into the plant via gravity sewer. According to [Mu'azu et al. \(2020\)](#), the average flow rate is approximately 52,012 m³/d. Mechanical screens and grit chambers are used to remove solid and inert materials in the primary treatment step. After that, wastewater is directed to the aeration tanks and then to a secondary settling tank to separate sludge from the treated water. Thus, the secondary treatment applied in this particular WWTP is conventional activated sludge (CAS). Then, the secondary effluent gets conveyed to the tertiary treatment facility where sand filtration is performed to remove any leftover material. The final treated water is transported to different areas in the region for the purpose of reuse in irrigation.

3.2 Sample preparation

3.2.1 Wastewater samples

Samples for this study were collected from four different sampling sites within Dhahran's WWTP. The first site is the influent (prior to any treatment) which is basically raw sewage. The other three sampling sites represent the water from the three treatment processes within the plant. Samples were collected from the primary effluent (water that has been mechanically treated in the primary step), the secondary effluent (water that has been biologically treated in the secondary treatment process), and the tertiary effluent (final water being discharged from the plant after passing through sand filtration).

Six sampling batches were carried out at each sampling site over a period of three months (November to January) making a total of 24 samples (6 samples at each one of the 4 sampling sites). Using the grab sampling method, a total of 3 L of samples were collected at each sampling site (each single sample was 0.5 L). Samples were filled in 0.5 L sterile amber glass bottles, and immediately after sample collection, they were transferred to the laboratory for preparation.

3.2.2 Reference materials

Spiked samples were prepared using four of the commonly used plastic polymers polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP) and polystyrene (PS). The polymer type of the plastic particles was confirmed using FTIR equipped with a spectra library. Similarly, four non-plastic materials were selected for analysis including steel, wood, cement, glass, and wax. All particles were sized ≤ 5 mm. To prepare the spiked samples for analysis, a portion of each material was added to 100 mL of distilled water, in a labeled glass vial, and sonicated for a few minutes to ensure particle dispersion.

3.3 Sample processing

3.3.1 Extraction process

Before processing, each sample bottle was shaken well by hand for around 10 sec. Using a graduated cylinder, 100 mL of the sample was transferred to a 500 mL beaker with a sterile magnetic stir bar. The sample was placed on the stirrer ready for processing in the fume hood.

In preparation for the separation of MP, iron(II) solution was prepared by dissolving 1.39 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 100 mL of deionized (DI) water in an acidic medium (containing 1 mL H_2SO_4). This solution was used as part of Fenton's reagent. Prior to the digestion process, samples were acidified by adding two drops of concentrated sulfuric acid to each containing beaker. Subsequently, 20 mL of freshly prepared FeSO_4 (0.05 M) was added to the sample followed by 20 mL of H_2O_2 ($\approx 30\%$). While continuously stirring, the respective beaker was covered with watch glass followed by heated at 75°C for 3 h.

All samples were allowed to cool to room temperature. Density separation was then applied to the sample of the secondary wastewater since organic contents were not completely digested. The sample was transferred to a separating funnel along with 25 mL of previously prepared density solution (50 g of ZnCl_2 + 150 g of NaCl + 300 mL of DI water). The solution was allowed to settle down for a minimum of 30 min. The supernatant was collected as expectedly the MPs should be floating in the upper portion of the solutions.

After that, all samples were filtered using a glass vacuum suction filtration apparatus and filter papers (Polycarbonate Membrane Filter, 0.45 μm pore size, gridded, 4.7 mm of diameter). Finally, the contents were washed with copious amounts of distilled water to neutralize it.

3.3.2 Staining procedure

To prepare the filtered sample (filtrate) for analysis, they were stained with NR. The dye solution was prepared by dissolving 10 mg of NR in 1 mL of acetone and then diluted with 90 mL of ethanol. Upon filtration, filters were carefully transferred onto labeled glass slides. Using a glass dropper, 2 – 4 drops of NR solution were poured into the entire filter paper. Finally, filters were stored in a covered stainless-steel box for drying (minimum of 30 min) before being examined and quantified.

Samples that had been spiked with plastic and non-plastic materials were processed in the same way as the wastewater samples i.e., treated with Fenton's reagent, filtered, and stained with NR dye. The filter papers of these samples were kept well separated from the wastewater samples.

3.4 Mitigation of cross-contamination

In the laboratory, several precautions were taken to avoid contamination with other microplastic particles/fibers. For example, wearing appropriate lab coats to cover clothing that can easily release fibers. The lab door was kept closed to minimize air currents and the sample processing was performed under a fume hood to avoid air deposition. Samples and filter papers were kept covered as much as possible. Direct contact of samples with plastic was avoided to a feasible extent. All utilized glassware, including bottles, beakers, cylinders, funnels, and pipettes, were thoroughly washed with tap water after each use, and rinsed with distilled water before each use. Laboratory blanks (2.5 L of distilled water) were prepared and analyzed in the same way as the wastewater samples to check for cross-contamination. No plastic fibers/particles were found in the blank.

3.5 Analysis of MP

Physical identification of NR-stained microplastics was performed by using a fluorescence microscope (visual inspection). Although the use of emission fluorescence has been previously

used in the literature for the detection of MP, it has not been proven to be effective for polymer-type identification (Hengstmann & Fischer, 2019; Kang et al., 2020; Meyers et al., 2022; Shim et al., 2016; Shruti et al., 2022). Therefore, in the current study, NR staining has been applied in conjugation with spectroscopic technique (spectrum acquisition). Raman spectroscopy has been increasingly utilized to accurately quantify MPs and qualitatively confirm the type of polymers as it provides a unique spectrum for each individual particle (Shruti et al., 2022).

3.5.1 Visual inspection

A fluorescence microscope (Olympus BX53) equipped with a camera and LED light was used for counting MP particles and for image acquisition. The Olympus cellSens platform, which is an image analysis software, was used to determine the diameter and other morphologies of MP. The default microscope settings used were; (gain: 2.0 x), (exposure time: 365 ms), and (two types of filters: blue and green fluorescence). The hue and saturation were set to automatic settings. Additionally, the work was all performed in the dark to avoid color distortion.

Using these settings, the spiked samples were visualized under the microscope using both the blue and green filters. Differences in the fluorescence of each reference material were noted and photos were taken to create a reference dataset. The wastewater samples were similarly visualized using identical conditions. All suspected particles were compared to the dataset and counted as MPs. Certain criteria were taken into consideration during the visualization of plastic particles, as reported by the international environmental organization KIMO (Kommunernes International Miljo Organization) (Norén, 2007):

Clarity	No organic material adhered to the particle's surface.
Thickness	No taper toward the ends of a fiber particle.
Shape	Irregular for fragments and must show a degree of bending for fibers.
Color	Clear and homogenous.

In order to prevent double-counting of particles, filter papers were scanned in a systematic way, starting from the bottom left corner, examining one square at a time, moving to the right until the end of the row, and then starting the next row above from the left side again. Counted particles

were classified based on their shape (fiber, line, fragment, or film). In addition, the size of MP was measured at their longest dimension, using the accompanied software, and categorized into five different size classes: <50, 50-150, 151-307, 308-1000, and >1000 μm . Moreover, to easily allocate particles, grids of the filter paper were numbered so that any detected particle would be linked to a number as a location on the filter paper.

3.5.2 Spectrum acquisition

To facilitate the process of chemical characterization, several particles were chosen randomly from all the extracted particles to undergo analysis by Raman spectroscopy. Random sampling ensures that each MP has an equal probability of being chosen for analysis, and thus ensures unbiased representation of the total particles. A confidence interval of 90% and a margin of error of 10% were chosen to calculate the necessary sample size. With these parameters and a population size of 864, the minimum effective sample size was calculated using the online sample size calculator (RaoSoft, Inc.,®) to be 63. All the detected particles were assigned sequential numbers in Microsoft Excel as an identity. Using the RAND function in Excel, a total of 70 pieces of MPs were randomly chosen from the samples to undergo polymer characterization by Raman spectroscopy.

Raman spectrometer (SENTERRA II, Bruker) was used to confirm the polymer nature of suspected plastic particles. The instrument was equipped with a door which was ensured to be closed throughout the analysis to prevent airborne contamination. A vibrational spectroscopy software (OPUS 8.5 SP1) was used to measure, process, and evaluate Raman spectra of the collected particle. The obtained spectra are compared with the reference spectra database. The “Polymers, Plastics and Additives Library” of the Kunststoff-Institut Lüdenscheid (KIMW) was used to confirm polymer identities (version 1.0 for additives and version 2.0 for polymers). The analysis was performed with two different laser wavelengths (532 nm and 785 nm) of 10-100 mW, a range of 400a, 50-4280 cm^{-1} and both x50 and x100 objectives (Olympus, Japan). The integration time and the number of accumulations were adjusted for each analyzed particle to improve the spectra’s quality. With regards to the mapping strategy, several points (at least three) were selected on a particle (and/or around it) to guide the laser and to overcome the impurity interference (Long

et al., 2019). Each particle spectrum is compared to the polymer library and those with a Hit Quality of more than 600 were accepted i.e. percentage match must be over 60%.

3.6 Statistical Analysis

Using the Statistical Package for the Social Sciences (SPSS 16.0 for Windows® (Chicago, IL)), a t-test was conducted to check for significant association in MPs abundance among the treatment processes. Pearson Chi-square was performed to identify significant differences in the characteristics of MPs across different treatments. For all statistical tests, $P < 0.05$ was considered statistically significant

Chapter 4 – Results and Discussion

4.1 Spiked samples

Spiked samples have been prepared and processed using the same techniques as the wastewater samples. This was intended to provide a dataset that can be used as a reference in the identification and characterization of MPs. The FTIR was used to confirm polymer types, then a fluorescence microscope was used to check for successful staining by NR dye. The FTIR results confirmed four types of spiked plastics (Figure 4). Although some particles were invisible in blue fluorescence, all of them were easily recognized in green fluorescence (Figure 5). Therefore, blue fluorescence was excluded from subsequent analyses. This observation seems to be consistent with other research which also found that the green filter was sufficiently clear to allow for the identification of several polymers such as PP, PE, PU (polyurethane), PS, PC, and EVA (polyethylene vinyl acetate) (Shim et al., 2016; Sturm et al., 2021). In addition, an interesting observation was noticed in this study in which there were differences in the fluorescence emission of each type of polymer e.g. PS showed bright green fluorescence that is prominently distinguishable from the pale blue fluorescence of PVC and the deep blue fluorescence of PP (Figure 5). This observation could potentially provide an advancement in the use of the NR staining method for the identification of polymer types. Further investigation is needed to explore and validate the correlation between the intensity of fluorescence and the polymer type.

However, none of the analyzed NR-stained non-plastic samples showed any fluorescence under the green filter of the fluorescence microscope (Figure 6). This finding supports previous research that found green fluorescence to be best suited to distinguish plastic and non-plastic particles due to very weak or absence of fluorescence for the lateral ones (Sturm et al., 2021). Consistently, organic fibers like wool, cotton, flax (linen), and hemp fabrics were found to be very weakly stained with NR dye (Meyers et al., 2022). Similarly, another study showed that sand was faintly stained with NR indicating that the dye does not bind to highly polar materials (Konde et al., 2020). However, other biological materials like cellulose tissue, sea animal shell, algae, and chitin were significantly stained which made them indistinguishable from plastic particles (Konde

et al., 2020; Uddin et al., 2022). However, in the current study, pretreating the wastewater samples with Fenton's reagent should have removed most of the biological and organic matter. Yet, digestion procedures do not guarantee the complete elimination of “false” NR-stained natural particles and thus it is advisable to apply a confirmative technique along with NR staining such as spectroscopic analysis (Shim et al., 2016).

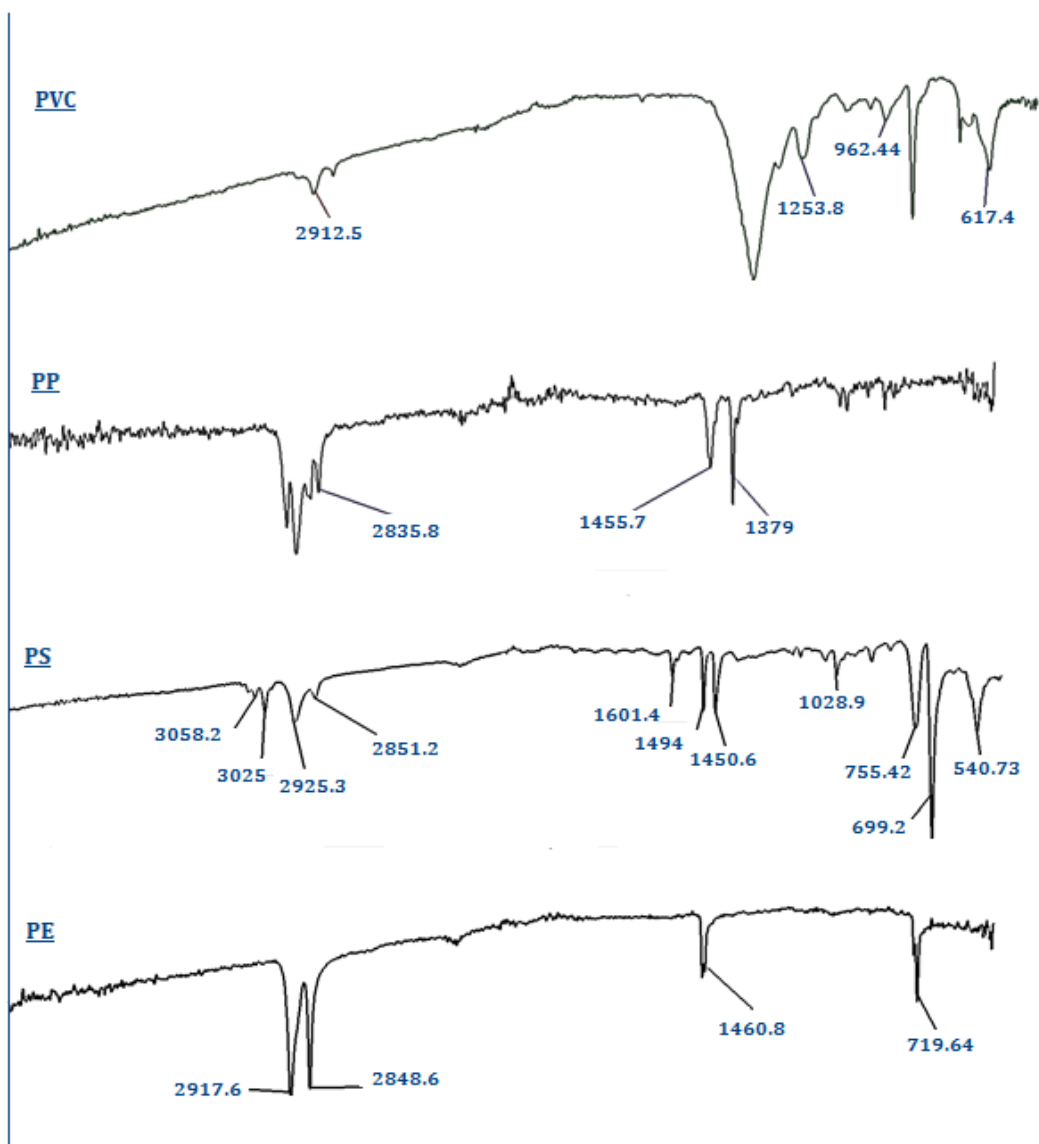


Figure 4 FTIR results confirming the types of polymers in the spiked samples: polyvinylchloride (PVC), polypropylene (PP) polystyrene (PS), and polyethylene (PE).

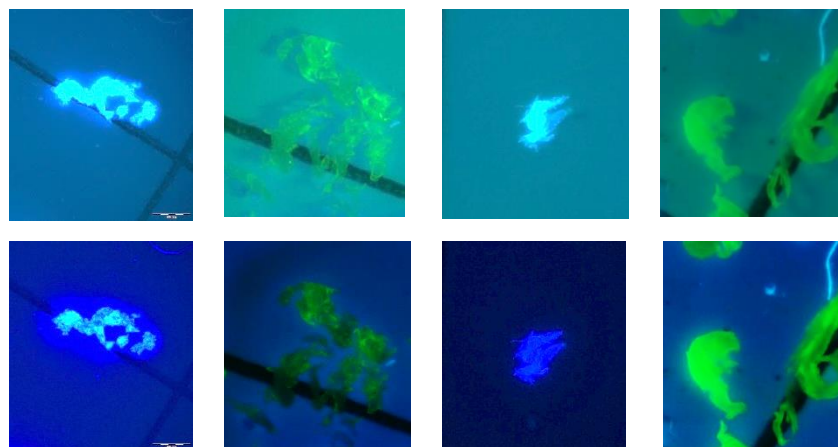


Figure 5 Plastic particles under fluorescence microscope using green filter (top) and blue filter (bottom). From left to right: polyvinylchloride (PVC), polypropylene (PP), polyethylene (PE) and polystyrene (PS)

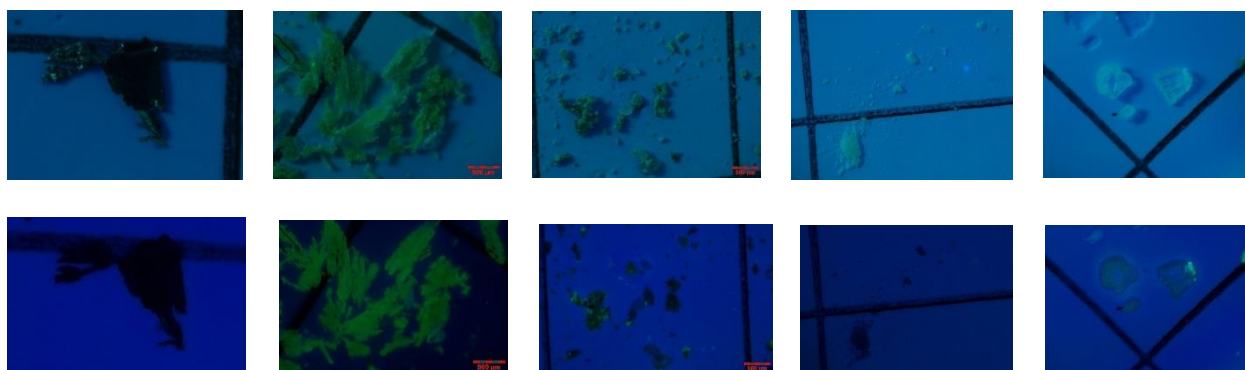


Figure 6 Non-plastic samples under fluorescence microscope with green (top) and blue (bottom) filter. Samples from left to right are steel, wood, cement, glass, and wax.

4.2 Wastewater samples – abundance of MP

In this study, the MP concentrations were counted based on the number of particles per liter (particle/L). One of the objectives was to investigate the occurrence of microplastics in the untreated water flowing into the specified WWTP. As can be seen from the table below ([Table 1](#)), MPs were found in the influent samples of all batches. Between 490 and 710 particles were detected in samples of 1 L of the raw sewage samples. This confirms that this treatment plant receives a considerable amount of MP particles and hence may greatly contribute to the MP pollution in the area.

A total of $n = 864$ pieces of MPs were detected across the three stages of the wastewater treatment process. Relatively similar numbers of MP particles were observed in all the batches collected ([Table 1](#)). The highest concentration of MPs was found in the primary effluent ranging between 330 and 630 particle/L, followed by the secondary effluent with a range of 140 and 300 particle/L. The effluent of the tertiary stage had the lowest concentration of MPs (100 – 210 particle/L). The treatment processes reveal a continuous removal of particulate/solid particles including MPs.

Table 1 Count of microplastics (particles/L) in each stage of the wastewater treatments.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Influent (raw sewage)	510	660	490	710	680	610
Primary wastewater	430	600	460	630	350	330
Secondary wastewater	300	160	260	140	150	260
Tertiary wastewater	160	210	130	100	180	130

Reported MP concentrations vary greatly among published studies around the world. For instance, MP concentrations in raw wastewater samples vary from 2 to 293,000 particle/L, and the range in effluent samples is 0.0007 to 35,000 particle/L ([Uddin et al., 2022](#)). This pattern of widely variable results is consistent even with studies of comparable parameters (social, geographical, climatic conditions, etc.). Taking a recent study carried out in Kuwait as an example (numerous

similarities to the parameters of the current study), the results were dissimilar, with the influent and the effluent MPs concentrations ranging between 119 – 230 particle/L and 1 – 12 particle/L, respectively (Uddin et al., 2022). Nevertheless, the most comparable values to the current study were 610 particle/L for influent samples in Finland and 127 particle/L for effluent samples in Denmark (Uddin et al., 2022).

The variability in MP distributions among different studies can be related to several factors. Different sampling techniques, sample preparation and processing strategies, as well as different analytical procedures with diverse materials and equipment used throughout the analysis. For example, Ben-David et al. (2021) made a comparison between two studies where they had used different sizes of mesh sieves (250 μm versus 20 μm) and found different results for the major range of MPs dimension (0.5–1 mm versus less than 300 μm , respectively). Moreover, the small size of MPs and the dependency on visual inspection and users' expertise might lead to underestimation or overestimation of the results, attributing to the observed variability between studies (Shruti et al., 2022). Seasonal variation was also reported to have a correlation with the concentration of MPs in wastewater samples (Ben-David et al., 2021). This highlights a need for a standardized procedure for the quantification of MPs to allow for direct comparison of datasets.

An overview of the trend for MP quantities throughout the treatment stages of the specified WWTP is illustrated in Figure 7. An overall decline is shown during the course of the entire process of treatment. Where the steepest decline is associated with the secondary treatment (CAS). Starting from around 600 particle/L as the mean MP concentration of the influent samples, there was an initial slight decrease (23.5%) passing through the primary treatment followed by an additional 54.6% decrease (total of 65.3% reduction) by the secondary effluent. Finally, a further gradual decline (28.3%) was observed in the tertiary samples, reaching a mean MP concentration of about 150 particles/L. Differences in MP abundance across the different treatment processes were found to be statistically significant ($P < 0.05$). The data collected during this study reveal that the overall MP removal efficiency of the present wastewater treatment plant is 75.1% (from the influent to the effluent).

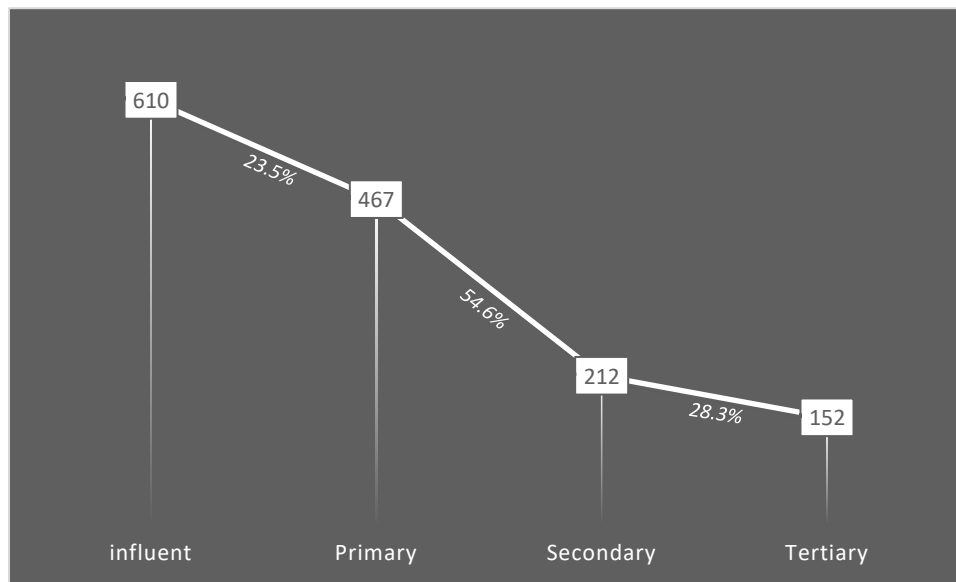


Figure 7 Mean MP concentrations (particles/L) and reduction rate (in percent) in each treatment stage of the treatment process.

The present study seems to be consistent with a study carried out in Finland revealing that the majority of MPs were removed by the primary and secondary treatment processes (Lares et al., 2018). However, a systematic study by Cristaldi et al. (2020) showed that in many cases the primary treatment demonstrated considerably high removal efficiency (ranging from 78-98%) compared to the secondary phase which was responsible for a small decrease in MPs (7-20%). This also accords with the findings of Liu et al. (2019) who intended to quantify MPs in the different treatment stages of a WWTP in China. The total removal efficiency of the plant was 64.4% with the greatest reduction in the concentration of MPs attributed to the primary (40.7%) rather than the secondary treatments (16%). A possible explanation for this observation is that MPs have the potential to adhere to suspended solids in wastewater and hence be removed by skimming and sedimentation processes of the primary phase (Carr et al., 2016; Lares et al., 2018).

It is encouraging to place the tertiary treatment in comparison with the other treatment phases with regard to MP removal efficiency. The present study found the tertiary (sand filtration) process to be less efficient in reducing the number of MPs compared with the conventional activated sludge

(the secondary treatment). This finding is consistent with an observation by other studies where the most significant decrease in MPs occurred prior to the tertiary stage (Ben-David et al., 2021; Sun et al., 2019). Similarly, a study comparing MP removal in a WWTP in Spain found that the tertiary sand filtration process was specifically less efficient than other advanced technologies i.e. MBR, ozonation, and membrane disc-filter (Bayo et al., 2020). According to Talvitie et al. (2017), among evaluated technologies, MBR was found to attain the best performance in removing MPs (99.9% removal efficiency). There are several possible explanations for this substantial high efficiency of MBR. The process is efficient because of the presence of a high concentration of mixed-liquor suspended solids (6000 – 10000 mg/L). Further, a combination of membrane filtration process and activated sludge processes add value. Another possible positive characteristic is a small membrane pore size (0.1 μm), which allows only the minimal-sized particle to cross through the system (Liu et al., 2021). Nevertheless, compared to sand filtration, MBR is very expensive and requires high energy and allows low flow (Cristaldi et al., 2020). Therefore, sand filtration may provide simple and cost-effective treatment to reasonably reduce MPs in wastewater (Magni et al., 2019).

4.3 Characterization of MP

Identified MPs were categorized based on their shape (fibers, lines, fragments, films, and pellets – refer to Figure 8) and size (<50, 50-150, 151-307, 308-1000, >1000 μm). As shown in Figure 9, fiber was by far the most abundant shape in all samples (63.0% of all detected MPs), followed by fragment (22.0%), whereas the least abundant shape was pellet (2.4%). The minimum-recorded size of MP particle was 3.76 μm while the largest piece detected was more than 3300 μm long. The average length of all detected MPs was 306.75 μm . Looking at the MP distribution among the size classes, similar quantities were observed in the three middle classes (28.7, 23.6, and 26.0%, respectively), whereas the upper and lower extremity sizes had the lowest percentage of measured MP particles (13.9% and 5.2%, respectively).

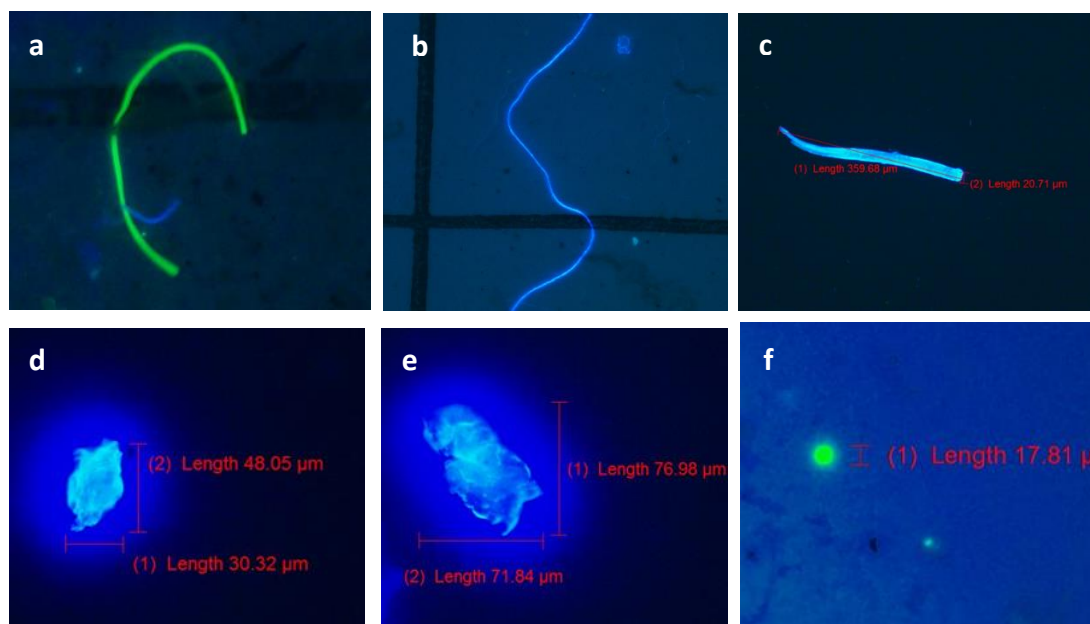


Figure 8 Recorded shapes of MP; (a & b) fiber, (c) line, (d) fragment, (e) film and (f), pellet

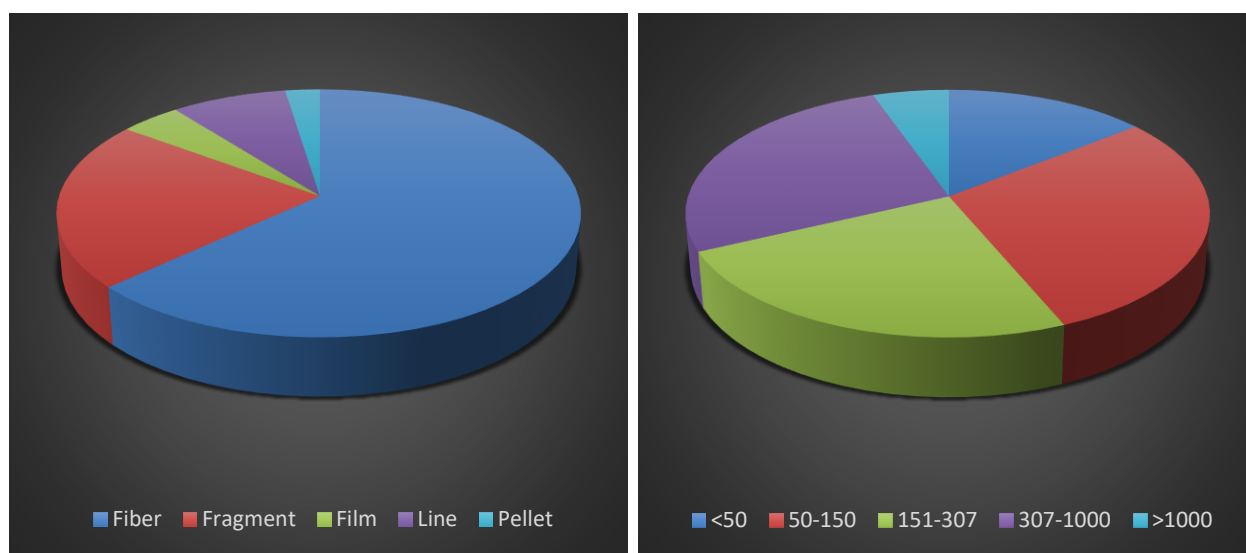


Figure 9 Distribution of MPs based on their shape (left) and sizes in μm (right).

Microplastic fibers are pieces of filament with a length-to-width ratio of almost 5:1, respectively (Cristaldi et al., 2020). Since fiber was the predominant shape of MPs in the current study, it was placed in one category versus MPs of all other recorded shapes (referred to as particles). Below, Figure 10 illustrates the proportion of fibers and particles in the influent and

effluent of the current WWTP to compare their removal efficiencies. It can be seen from the given data that fibers account for the majority of MPs detected in both the influent and effluent (62 and 70%, respectively). Interestingly, the proportion of fibers in the effluent (70%) was significantly higher than that in the influent (62%) with a P-value less than 0.05. This higher effluent abundance indicates that fibers have considerably low removal efficiency upon wastewater treatments. These results match well the reported data in earlier studies where an increase in the abundance of fibers was observed in the final effluent (90 – 93%) compared to the influent (73% – 74%) (Bayo et al., 2021; Ben-David et al., 2021). A more surprising finding was that fibers seem to bypass the most efficient treatment process in removing MPs from wastewater (the MBR). According to Bayo et al. (2020), only 57.65% of fibers were removed by MBR compared with 98.8% of particles. This might be due to the morphology of fibers, their small size as well as the high pressure in the MBR system (Cristaldi et al., 2020).

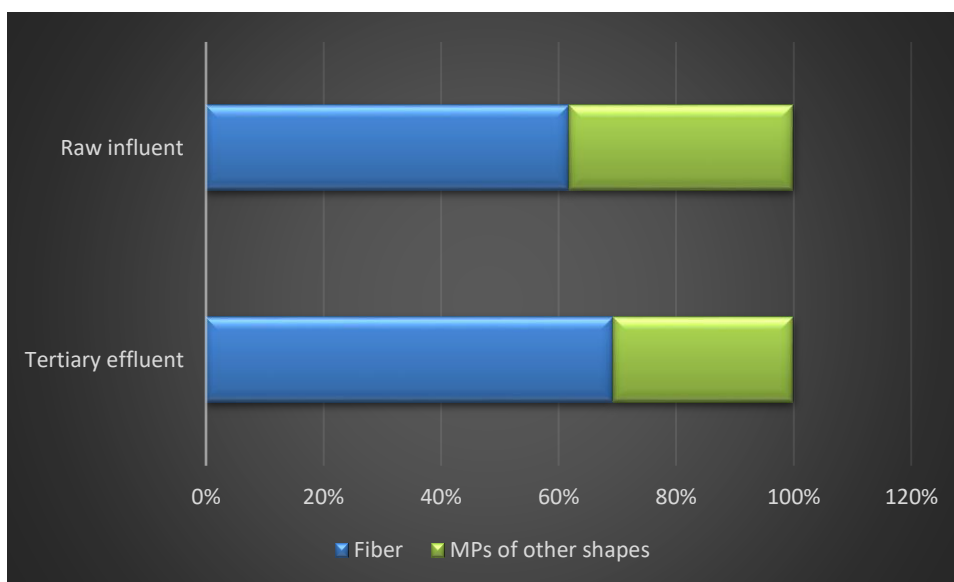


Figure 10 Abundance of fibers vs MPs of other shapes in influent and effluent wastewater samples

Many studies have reported fiber to be the dominant shape of MPs found in wastewater samples regardless of the treatment technology used – MBR, CAS, sand filtration, reverse osmosis (RO), ultrafiltration membrane, UV and chlorination (Bilgin et al., 2020; Lares et al., 2018; Picó et al., 2021; Uddin et al., 2022; Yang et al., 2019). An implication of this observation is the

possibility that wastewater could be a potential source for the release of microfiber into the environment. In fact, several studies have related the high prevalence of fibers in marine samples, including streams, lakes and rivers, to the discharge of wastewater effluent into these water bodies (Picó et al., 2021; Prata et al., 2019).

4.4 Types of MPs in analyzed samples

Using Raman spectroscopy, 70 pieces of MPs were analyzed with the criteria of a 60% match between the obtained spectrum and its comparison with the data of known polymers in the reference library. A total of 21 positive results were obtained. It reveals that 70% of the particles correspond to unidentified MPs, they did not match the specified percent match. Even after adjusting the laser power and acquisition time, poor Raman signal quality was a serious challenge to confirm the particles. This could be explained by the presence of simultaneous fluorescence interference caused by the impurity of the plastic particle due to (1) pigments and dyes, (2) degradation products, (3) additive compounds and (4) biological material or other pollutants adhered to their surface (Bayo et al., 2021). Another issue that could have altered the polymer spectra was photo-degradation leading to partial chemical changes at the MP surface (Ben-David et al., 2021). One more factor that could have hindered obtaining interpretable spectra might be related to the physical properties of the MPs including their small sizes and thickness as well as their dark color which could absorb laser light emitting intense background fluorescence and covering the Raman signal (Nava et al., 2021). In addition, the harsh environment of wastewater, the severe conditions surrounding MPs, and the arid climate of the Arabian Gulf region might have also contributed to the oxidation of some polymers making it difficult to identify the chemical properties of MPs (Bayo et al., 2021).

Due to the aforementioned challenges, a weak signal might have caused the misidentification of the polymer. Therefore, it was necessary to gain some knowledge of the main peaks and associated chemical bonds of the most common plastic polymers. The study of Nava et al. (2021) was used as a reference for the main Raman vibrations for common plastic polymers. Distinguishing the fingerprint region that characterizes a certain polymer has facilitated the

identification of the polymer spectrum. Overall, 13 types of polymers were confirmed by Raman to be present in the wastewater samples as depicted in [Table 2](#).

Table 2 Chemical composition of MPs detected in the current study.

Polymer	Common Name
PVC	Polyvinylchloride
PA66	Polyamide 66
PC	Polycarbonate
PC/PBT	Polycarbonate/ polybutylene terephthalate blend
PC/PTFE	Polycarbonate/ polytetrafluoroethylene
PC/ABS	Polycarbonate/acrylonitrile butadiene styrene blend
PE	Polyethylene
PET	Polyethylene terephthalate
PS	Polystyrene
PP	Polypropylene
UF	Urea formaldehyde resin
PUR	Polyurethane
ETFE	Ethylene tetrafluoroethylene

Polyvinyl chloride (PVC) was one of the identified types of MPs found in the samples. Its spectrum showed two high-intensity Raman bands at 638 and 694 cm^{-1} , which correspond to C–Cl bond. A peak was observed at 2914 cm^{-1} corresponding to CH_2 stretching vibration, as well as bands at 1430 and 1335 cm^{-1} are characteristic stretching vibrations of PVC structure ([Figure 11](#)).

Polyamide 66, also known as Nylon 66 (NY66), has a characteristic band in the functional group region at 2919 cm^{-1} and multiple peaks at 1441, 1298, 1128 and 1063 cm^{-1} . Similarly, polycarbonate (PC) has a distinctive band at 3074 cm^{-1} and a few adjacent ones at 2974, 2940, and 2912 cm^{-1} , as well as several other peaks at 1602, 1290, 887, 733 and 635 cm^{-1} give enough information about its existence.

Another polymer that was found in the samples is suggested on the basis of the collected data set to be polyethylene (PE) which has been identified as the predominant polymer in several wastewater studies (Bayo et al., 2021). Absorption bands for PE include C–C bond stretching vibration at 1130 and 1062 cm^{-1} , CH_2 bending region at 1470 and 1398 cm^{-1} and CH_2 stretching vibration region at 2850 and 2883 cm^{-1} .

Polyethylene terephthalate (PET) is another most commonly found polyester in wastewater samples due to its widespread applications in packaging materials and in textiles (Bayo et al., 2021; Yang et al., 2019). Two absorption bands at 1295 cm^{-1} and at 1730 cm^{-1} correspond to symmetric and asymmetric stretching of carbonyl ($\text{C}=\text{O}$) bond, a peak at 1615 cm^{-1} is predominantly assigned to characteristic vibration of the aromatic ring, and 1096 cm^{-1} corresponds to C–H stretch.

Polystyrene (PS) was easily identified by the intense band at 1001 cm^{-1} associated with the breathing mode of the ring carbons and the overlapped bands at 3054 cm^{-1} are assigned to the aromatic C–H bond stretching of the phenyl ring. Other vibrations for PS include 1602, 1583, and 1155 cm^{-1} .

Another polymer identified in wastewater samples is polypropylene (PP) which was one of the most prevalent MPs in another study (Yang et al., 2019). The most intense vibrations in the spectrum of PP are 2952, 2885, 1458, 1330, 1152, 841, and 398 cm^{-1} . These peaks and their relative region where they appear give sufficient justification for the existence of PP in water samples.

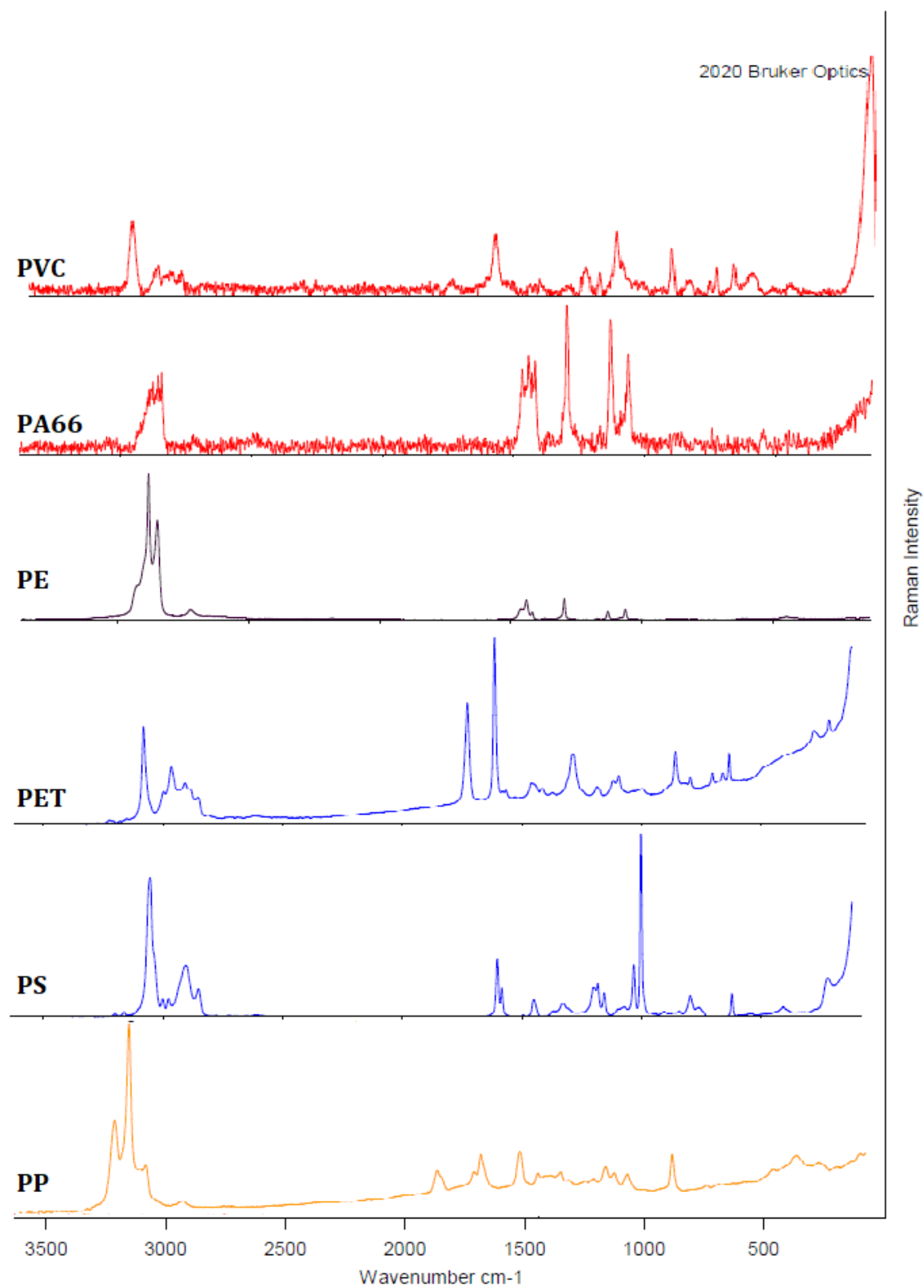


Figure 11 Raman spectra for microplastic particles in wastewater samples.

Identification of the polymeric composition of plastic particles might provide an indication of the main sources behind the occurrence of MPs in the current WWTP. The presence of NY66 and PET suggests that textiles and synthetic clothing, which are the main sources of household MPs (as microfiber detach from clothing during laundry washing), could be a potential origin. Moreover, the detection of PE, PP, and PS provides an indication that plastic products are frequently used by the community that this plant serves. Bathing and hygienic activities including the use of toothpaste, shower gel, and face cleanser could be other potential sources of these synthetic polymers. Another possible source of these types of polymers in wastewater is the usage and disposal of wipes in the toilet, as these wipes (both flushable and non-flushable) were shown to contain PET, PE, PP, PS and PUR ([Pantoja Munoz et al., 2018](#)). Polyurethane was also detected in the current study which was suggested to originate from dishwashing activities where the sponge used in the process is often made of PUR particles which could be lost over repeated use ([Jessieleena et al., 2023](#)). One researcher has attributed the occurrence of PVC in wastewater to the equipment used in WWTP processes which should be carefully evaluated as a potential source in future MP research ([Magni et al., 2019](#)).

4.5 Implementations

Although the current study was able to confirm the presence of 13 types of MPs in the investigated WWTP, there is room for improvement in the employed analysis. Several automated techniques could improve the Raman analysis and replace the time-consuming manual visualization including the use of software for particle pre-selection, filter auto-scanning, fluorescence-based particle detection, and Raman mapping. Examples were discussed by [Araujo et al. \(2018\)](#), including the use of (1) automated algorithm that removes any background disturbing fluorescence to facilitate detection of underlying polymers; (2) automated full point mapping where motorized stage is used to scan one whole section of a gridded filter; (3) particle search software that automatically detects and scans all particles in a given area; and (4) image analysis software which creates a map of all particles after enhancing their contrast with the filter and hence Raman spectra are only collected at the automatically found particles. These technologies seem to be promising in providing accurate readings while reducing the required time and effort needed for the Raman MPs analysis.

In addition, the method that was employed in the current study for investigating the count and the size measurement of NR fluorescent MPs is very time-consuming because it was done manually by observation of each individual particle. However, specialized software could be employed to facilitate the process. For instance, Galaxy Count Software could be used for the automatic quantification of particles while Image J Software could be used for automatically calculating the size of NR-fluorescent MPs based on the size of the filter paper (Shruti et al., 2022). Such technologies would enable fast detection of particles with minimal operation time and will make fast onsite analysis possible.

Another study by Meyers et al. (2022) described an innovative approach involving the use of Artificial Intelligence for MP analysis. Images of NR-stained particles were used to train two machine-learning models using differences in fluorescence intensity of different types of plastics. One model was called the “Plastic Detection Model” which allows for MPs identification and the other one was called the “Polymer Identification Model” which predicts with high accuracy the polymer type of MPs. Both models were successfully validated by using them to detect and identify MPs in spiked environmental samples. Therefore, these machine-learning models provide reliable, cost- and time-effective method for MP analysis, and seems to be straightforward i.e. without needing expertise in the field of Artificial Intelligence.

Although studying MPs through NR fluorescence has a significant potential for fast and reliable detection of particles, there is a true need for a harmonized methodology to allow for comprehensive comparison among results (Shim et al., 2016). Examples include specifications for an appropriate solvent for NR staining, dye concentrations, staining temperature, incubation time, as well as standardized excitation and emission wavelengths (Shruti et al., 2022). Having homogeneity among studies involving the use of NR for MP investigations could lead to improvements in the application of this technique.

Moreover, the present study observed variations in the results when compared with other research studies globally. This could be due to the absence of a unified methodological procedure which could consequently lead to biased conclusions. For instance, using filters of different pore sizes may influence the size of the MPs being sampled in each study (Prata et al., 2019). There is a dire need for a validated protocol to ensure uniformity of the methodological procedure.

Harmonization of the following factors among studies would allow for accurate comparison of the dataset allowing for more conclusive results;

1. Sampling methods e.g. where and how to collect samples, frequency of sampling, and amount to be sampled.
2. Sample processing e.g. defined extraction method with a specific filter's pore size, defined density separation technique with a specific salt-saturated solution, defined digestion protocol that is quick and effective and measures to reduce cross-contamination.
3. Criteria for visual identification and characterization e.g. sample-based criteria for particle detection, defined size classes, and standards to ensure representativeness ([Lv et al., 2019](#); [Prata et al., 2019](#); [Song et al., 2015](#)).

From future perspectives, it is recommended to conduct extensive studies of the current WWTP with more sampling sites, a larger sample size (100 L), and sampling frequencies that allow for checking on an hourly basis, daily and seasonal variation, etc. Moreover, continued studies are recommended to investigate any correlation to the increasing trend for plastic production, proper usage and management. Moreover, by utilizing the recommended software technologies, future researchers should aim to obtain Raman results for all detected particles to prevent overestimation or underestimation of the results.

Furthermore, future studies should investigate the potential effect of MPs existence in reclaimed wastewater. The outflow discharged from the investigated WWTP has been partially reused for different purposes including irrigation of landscape and golf courses, as well as construction of artificial recreational water bodies. The Kingdom of Saudi Arabia has set the aspiring goal of recycling 100% of treated wastewater by 2025, as part of the 2030 Saudi Vision and the National Water Strategy ([Saudi Ministry of Environment Water and Agriculture, 2018](#)). Therefore, there is a potential for using the effluent of the current WWTP for useful purposes like food crop irrigation, recharge of potable aquifers as well as surface water augmentation ([Aburizaiza & Mahar, 2016](#)). Thus, the impact of MP contamination should be further investigated and actively considered in the formulation of reuse policies.

Chapter 5 – Environmental Risk of Microplastics

In the current study, the total removal efficiency of the studied WWTP was 64.4%. However, considering that the plant treats around 52 million liters of wastewater on a daily basis, the amount of MPs potentially released to the receiving aquatic systems (based on the MPs mean concentration in the final effluent) is approximately 7.9 billion particles/day. Therefore, despite the high removal performance (could reach 99% in some studies), WWTPs could be a significant source of MPs in the environment (Lv et al., 2019). In fact, the daily discharge of MPs by WWTPs could greatly vary as shown in the literature. For example, although the WWTP studied by Talvitie et al. (2015) had a daily flow rate of almost five folds the WWTP selected in the current study, it released comparably less amount of MPs ranging between 1.7×10^6 to 1.4×10^8 particle/day. Nevertheless, due to the large volume of effluent discharged constantly by WWTPs, an extensive quantity of MP particles is daily discharged into the environment worldwide. Therefore, WWTPs are nowadays considered a potential pathway for MPs entry into marine environments (Figure 12).

Microplastics in the aquatic environment have received considerable attention over the past decade (Talvitie, Mikola, Setälä, et al., 2017). Several studies have confirmed the occurrence of MPs in different water metrics including surface water, coastal water, estuaries, reservoirs, lakes, rivers, and canals (Ivar do Sul et al., 2009; Pironti et al., 2021; Pivokonsky et al., 2018). These studies originated from different countries around the world including mainly European countries, Scandinavian countries, Southeast Asia, Australia, New Zealand, as well as North America (Gao et al., 2022; Ivar do Sul et al., 2009). Interestingly, MPs were likewise detected in remote and uncontaminated areas such as the sea around Antarctica, marine sediments, and sea ice from the area (Kelly et al., 2020). This suggests that MP particles may transport and accumulate in all marine habitats indicating potential for cross-boundary contamination. In fact, China, North America and some European countries were considered top-listed places for MP contamination (Ricciardi et al., 2021). However, many areas around the globe remain poorly investigated for MP distribution (e.g. Africa, North Asia and South America), thus further studies are needed to gather more information on the temporal and geographical distribution of MPs and build a comprehensive overview of the global MPs pollution (Prata et al., 2019; Ricciardi et al., 2021).

Although still there is a lack of a full picture of MP distribution in marine environments, the existing dataset is causing serious concerns since the associated environmental impacts are still being less studied (Ricciardi et al., 2021). Assessing the effects of MPs in the marine environment is a complex process influenced by many factors including (but not limited to) chemical and physical properties of different polymers, degree of fragmentation and degradation of MPs, complexity of water and environmental matrices, as well as variability of toxic effects which could be acute, chronic, mutagenic, etc. (Saling et al., 2020). A number of researchers have proposed to assess MPs like other emissions with the systematic and quantifiable approach in life cycle assessment (LCA) (Saling et al., 2020). Selected toxic effects of MPs were combined with the kinetics of plastic fragmentation and degradation to consider MPs under LCA perspectives. With this approach, a characterization model was developed addressing the fate of MPs, the impacts of different types of polymers and their toxicity to different organisms (Saling et al., 2020). This model should be counted as the first in a series of steps toward a systematic assessment of the impacts of MPs on marine life.

A recent Saudi-based study has assessed the ecological risks of MPs in artificial freshwater systems feeding with treated wastewater. The assessment was based on the Hazard Index (HI) which considers the chemical toxicity of polymers and the Pollution Load Index (PLI) which takes into account the MP concentrations (Picó et al., 2021). The study revealed that the identified polymers (PP, PE, PET, and PS) were categorized to have a moderate risk to the environment based on the HI index, while the MPs count (0.2 – 3.2 particle/L) revealed a very low risk based on the PLI value. Overall, there may be a potential risk for acute toxicity in these ponds and channels i.e. loss of biodiversity in these aquatic ecosystems.

MPs are of concern because they can be ingested by a variety of marine organisms, and potentially be transferred within food webs (Talvitie, Mikola, Setälä, et al., 2017). A study by Setälä et al. (2014) evaluated the potential of 12 planktonic organisms to ingest MPs and pass them to organisms at higher trophic levels. Organisms were exposed to fluorescent polystyrene microspheres. After confirmation of ingesting the provided MPs, these organisms were offered to aquatic animals feeding on the plankton e.g., mysid shrimp. Microscopic observation showed the presence of the prey as well as the microspheres in the intestine of mysid after 3 h of incubation.

This study showed the potential of plastic particles to transfer and possibly accumulate in the food web.

Other studies confirmed the uptake of MPs by a variety of other sea animals which can happen through two different mechanisms; passive (through gill water filtration) or active (though direct ingestion of particles or contaminated prey) (Pironti et al., 2021). MPs were detected in many marine organisms such as many fish species (pelagic, demersal, and benthic fish), whales, oysters, lugworms, shrimps, mussels, clams and scallops (Ricciardi et al., 2021). Many of these are edible seafood for humans e.g., Atlantic cod (*Gadus Morhua*), red mullet (*Mullus barbatus*), European pilchard (*Sardina Pichardus*) and European hake (*Merluccius Meluccius*) (Pironti et al., 2021). The risk associated with consuming these species depends on the contaminated part of their bodies i.e., MPs are often detected in the gastrointestinal tract of these animals which is often discarded rather than consumed. However, studies showed that MPs could be detected in the muscles, gills, and the external surface of many marine organisms that are counted as food sources for humans (Akhbarizadeh et al., 2019). Therefore, these observations give rise to concern regarding the potential transfer of MPs from the marine web into humans (Pironti et al., 2021).

Besides seafood, other possible routes for human exposure to MPs through the marine ecosystem include the consumption of sea salt and potable water. Previous studies confirmed the presence of MPs in 128 brands of table salt originating from different parts of the world ranging from 600 to 20,000 particles/kg (Pironti et al., 2021). Contaminated sea salt is probably caused by contaminated seawater since sea salt is processed by simply evaporating seawater with some sort of purification. Similarly, drinking water derives predominantly from freshwater bodies (e.g. surface and ground sources), in which any MPs contamination to the lateral one would be transferred to the drinking water (Sipe et al., 2022). According to Pivokonsky et al. (2018), the inflow received at the examined water treatment plant (raw drinking water) had a concentration of MP particles that ranged between 1383 and 4464 particles/L. Treatment processes of drinking water were shown to remove a significant portion of these MPs (70–82% removal efficiency). Nevertheless, the concentrations of MPs in the treated water were not negligible (338–628 particle/L) suggesting that drinking water could be a predominant source of MPs to humans (Pivokonsky et al., 2018).

The toxicity of MPs is related to their chemical composition as they usually include additives, heavy metals, and traces of monomers that can be very toxic (Talvitie, Mikola, Setälä, et al., 2017). For example, bisphenol-A and phthalates are two chemicals often found conjugated with polymers. These are endocrine disruptors with carcinogenic properties that can pose risks to human health (Pironti et al., 2021). Moreover, MPs can be carriers of pathogenic contaminants and organic compounds such as polycyclic aromatic hydrocarbons which may accumulate in the marine food chain when ingested by benthic animals (Liu et al., 2021). Other compounds reported to be combined with MPs include polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenyl ethers (PBDEs) (Pironti et al., 2021). A previous study showed that MPs play a key role as vectors for metal ions that were found at concentrations of mg/kg (such as aluminum, iron, zinc, nickel, chromium, molybdenum, manganese, copper, lead and cobalt) or µg/kg (such as arsenic, cadmium, vanadium and rubidium). These metals are easily absorbed on the surface of MPs exerting potential toxicity for marine biota after chronic exposure (Squadrone et al., 2022).

Overall, MP pollution caused by wastewater effluent discharge could in turn contaminate the aquatic ecosystems posing a serious risk upon exposure to humans and marine organisms. MPs act as a vector for many pollutants that can accumulate in the human body *via* the processes of bioaccumulation and biomagnification (Pironti et al., 2021). Even substantial low concentrations of these pollutants in the human body may increase the risk of contracting serious health issues such as cancer, endocrine dysregulation, and reproductive or immune system disorders (Cristaldi et al., 2020). Up till now, the toxicological effects of MPs were evaluated on cell cultures only (Ricciardi et al., 2021). The translocation of MPs in the human body and their interaction with the different body apparatus remain uninvestigated. Deeper investigations are needed in this regard to better understand the consequent effects of MPs on human health (Ricciardi et al., 2021).

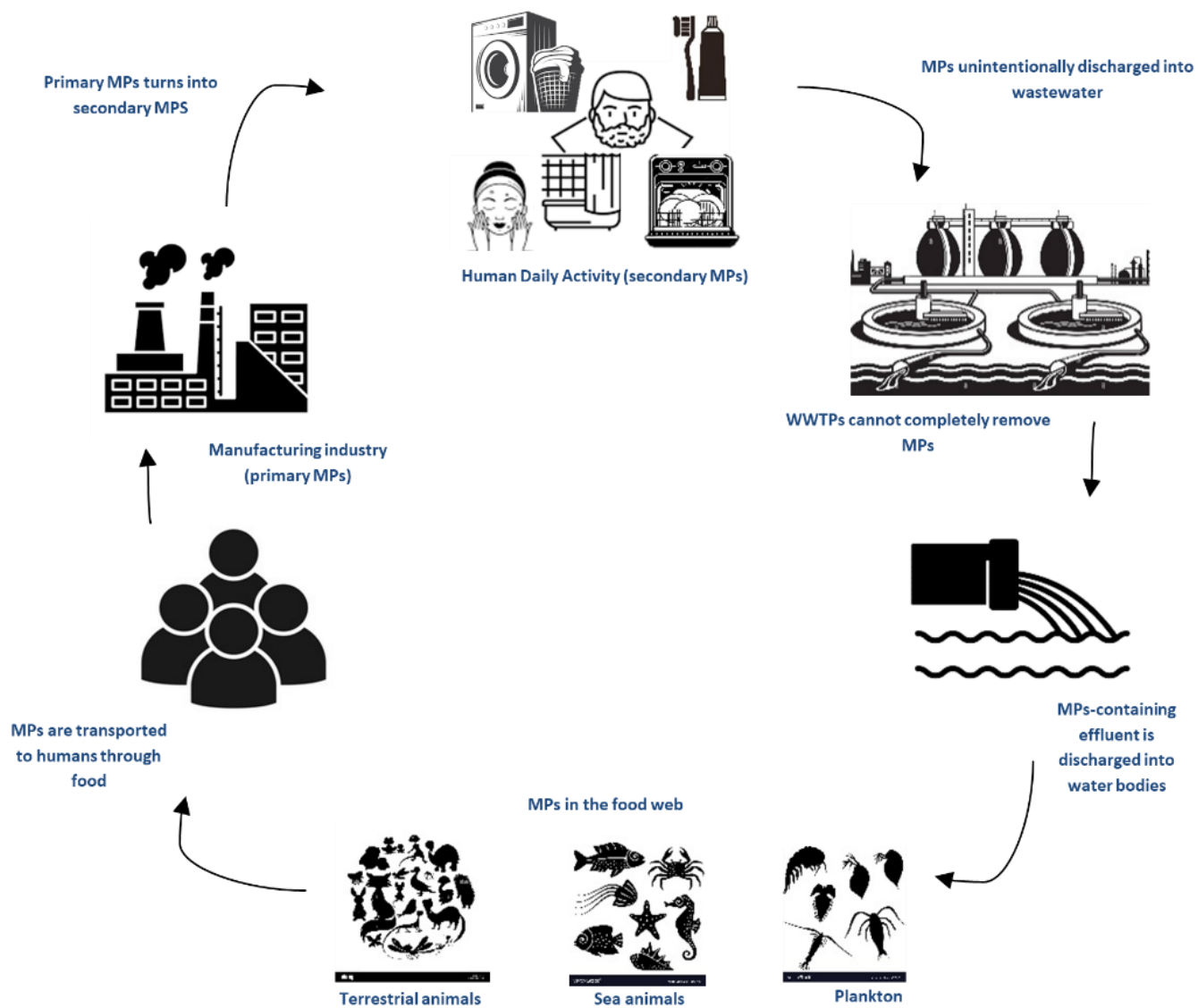


Figure 12 MPs transportation through WWTPs and human exposure.

Chapter 6 – Conclusion and Recommendation

This is the first study investigating MPs at full-scale WWTP in Saudi Arabia. The investigation involved the concentration, shape, size, polymer type and fate of MPs at the WWTP. Reliable procedures were adopted which involved removal of organics and staining of particles for improved visualization of suspected MPs. A combination of fluorescence microscopy and spectroscopic methods were employed to accurately obtain information on the abundance and polymer types of MPs. The research led to the following conclusions:

1. The occurrence of MPs in the inflow received at the WWTP was confirmed. Although the secondary treatment was shown to have higher removal efficiency compared to the primary and the tertiary treatments, all the treatment processes contributed to removing MPs from wastewater. It suggests that treatments of wastewater have a respectable capacity to eliminate MPs with some variations.
2. The concentration of MPs that was found in the final effluent of the current WWTP was not negligible (152 particle/L). Considering the high volume of wastewater being discharged daily from this plant, a corresponding emission may reach 7.9 billion microplastic particles per day. This finding proposes potential contamination of the water compartments where the effluent is discharged.
3. The dominant shapes of detected MPs were fibers followed by fragments with sizes mainly distributed between 50 and 1000 μm . Fiber was found to be highly retained in WWTP suggesting that wastewater could be a potential source for the release of microfiber into the environment.
4. Chemical characterization of MPs revealed 13 polymer types including PET and PA66 which usually account for the majority of detected fibers proposing that laundry activities are the main source of MPs received at WWTP. In addition, PE and PP were also detected in the samples suggesting that personal care and kitchen products are probably the major contributors to MPs pollution.
5. Wastewater was confirmed as a potential source of MPs in the marine environment. On one hand, MPs were themselves considered emerging pollutants as they can cause mechanical stress when ingested by organisms. On the other hand, they were shown to expose aquatic biota to various hazardous substances such as heavy metals.

Some recommendations were provided for future implementation in research studies:

1. Standardization of methodologies should be a top goal in future studies. Lack of uniformity in the analysis methods, made it a challenging task to precisely compare the findings of the current study with the available dataset. Examples include extraction procedures (e.g., protocols for digestion and density separation), staining procedures (e.g., standards for concentration of the dye, use of proper solvent and incubation time), and measurement settings (e.g., standardized excitation and emission wavelength).
2. Several automated techniques can be used to improve the Raman analysis and replace the time-consuming manual visualization including the use of software for particle pre-selection, filter auto-scanning, fluorescence-based particle detection, and for Raman mapping.
3. The potential impact of reusing treated wastewater that is contaminated with MPs should be investigated in future studies. Environmental impacts should be assessed for each one of the reuse applications (irrigation, agricultural reuse, groundwater recharge, and surface water augmentation), with a focus on human exposure.

In conclusion, resolving the issue of the entrance of MPs into the environment via wastewater is a very complex phenomenon. The WWTPs are not primarily designed to remove MPs, and advancement in MP-targeted treatment processes could not be economically viable. Preventive strategies may substantially contribute to narrowing the scale of the problem. Examples include banning or limiting the use of plastic bags, removing plastic microbeads from personal care products, modifications in the filter of washing machines to retain fibers, improving the maintenance of sewer systems, and application of membrane filtration. Moreover, strategies should be implemented to prevent secondary pollution from MPs in recycled wastewater. Effective waste management approaches should be considered to prevent the entry of MPs into water bodies and food webs. Public initiatives, local regulations, and international legislation should collaborate to explore alternative materials and reduce the extensive use of plastic and its products.

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ملخص الدراسة

ينتشر البلاستيك الدقيق (بحجم المايكرو) على نطاق واسع في قطاعات مختلفة من البيئة، مثل الماء والترربة حيث أصبح التلوث الناجم عن هذه الجسيمات في جميع أنحاء العالم مصدر قلق متزايد لما له من تأثيرات على استدامة البيئة وصحة الإنسان. إن المصرف الأخير لهذه الملوثات المنتشرة في كل مكان والعديد من الملوثات الأخرى هو المسطحات المائية. ولذلك فإن تحديد مصدر البلاستيك الدقيق ودراسة كميته وأنواعه أمر في غاية الأهمية. تلعب محطات معالجة مياه الصرف الصحي دوراً محورياً في إطلاق و نشر المواد البلاستيكية الدقيقة في البيئة البحرية. بحثت هذه الدراسة عن وجود البلاستيك الدقيق في مياه الصرف الصحي، من خلال فحص عينات مأخوذة من محطة لمعالجة مياه الصرف الصحي في المملكة العربية السعودية. تمت المراقبة المجهرية الفلورية للعينات بعد تطبيق صبغة النيل الأحمر بالإضافة إلى تحليل رامن الطيفي. كما قامت بتقييم مصير هذا الملوث من خلال فحص الوفرة والتشكل ونوع البوليمر في نهاية كل عملية معالجة. وأكدت الدراسة وجود 610 جسيمات/لتر في المياه المستقبلية، والتي انخفضت بنسبة 64.4% في التدفق النهائي. تبين أن المعالجة الثانوية تتمتع بأعلى كفاءة من حيث إزالة البلاستيك الدقيق بمعدل تخفيض قدره 54.6%. وكانت الألياف هي الشكل السائد لهذه الجسيمات في عينات مياه الصرف الصحي، ووجد أنها الأكثر مقاومة لعمليات المعالجة. ومن بين البوليمرات التي تم تحديدها البولي إيثيلين والبولي بروبيلين والبوليسترين والبولي فينيل كلورايد. اتضح من هذه الدراسة أن هناك حاجة كبيرة لتوحيد المنهجيات للتحقق من صحة المقارنة بين مجموعات البيانات المتاحة فيما يتعلق بالتلوث البلاستيكي الدقيق. يجب أن تبحث الدراسات المستقبلية في الآثار المحتملة لتعرض البشر والكائنات الحية لهذه الملوثات عبر إعادة استخدام مياه الصرف الصحي.

جامعة البحرين

برنامج البيئة و التنمية المستدامة



كلية العلوم

دراسة البلاستيك الدقيق في مياه الصرف الصحي، وتقييمه كأحد عوامل التلوث البحري

هذه الرسالة مقدمة استكمالاً لمتطلبات درجة الماجستير في برنامج البيئة والتنمية المستدامة

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