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Environmental Technology & International Affairs

Occurrence of Microplastics in the Effluent of Wastewater Treatment Plants

Growing Threat to the Environment?

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"Master of Science"

supervised by
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Affidavit

I, **CHRISTINA TIRLER**, hereby declare

1. that I am the sole author of the present Master's Thesis, "OCCURRENCE OF MICROPLASTICS IN THE EFFLUENT OF WASTEWATER TREATMENT PLANTS - GROWING THREAT TO THE ENVIRONMENT?", 59 pages, bound, and that I have not used any source or tool other than those referenced or any other illicit aid or tool, and
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Abstract

Microplastics have become an emerging environmental concern. Plastic production is increasing on an international scale. Some of the plastic produced ends up in the environment as a persistent pollutant. This issue has first been raised by scientists decades ago. While consequences of macroplastics are well studied, adverse effects of microplastics are still not completely understood. Former studies focused on the occurrence and impact of microplastics in marine ecosystems. Recently, a shift towards presence and effects of microplastics on freshwater systems could be observed. In order to rate the risk, microplastics are posing to those habitats, also sources and the amount of microplastics emitted to freshwater systems have to be investigated. Wastewater treatment plants are thought to be a main contributor to microplastic pollution in freshwater systems. However little data is available on the contamination of effluent water with plastic particles in the size range below 5 mm. This is true for all countries, including Austria. Sampling and analytical methods differ widely between studies and there is still no agreed standardized analytical method. A common approach to sampling and analysing microplastics not only in wastewater is a prerequisite in order to compare data from different sources. This study intends to provide a snap shot of microplastics occurring in the effluent of an Austrian wastewater treatment plant on the one hand side. On the other hand two different methodologies to quantify microplastics were used and compared. Whilst the first methodology is based on visual inspection, the second approach is based on an elemental analysis. In 14,5 m^3 of water a total of 126 microplastic particles (>1mm, <5mm) was found. Microplastics occurred in all the samples taken. Visual inspection turned out to be the favoured methodology to detect microplastic particles in wastewater samples, as the use of threshold values for the elementary analysis are not suitable for the proof of microplastic particles in wastewater.

Keywords:
Microplastic, wastewater, visual inspection, elemental analysis, freshwater system

Table of contents

List of abbreviations

C	**Carbon**
DDEs	**Dichlorodiphenyldichloroethylene**
EC	**European Commission**
EEC	**European Economic Community**
EU	**European Union**
H	**Hydrogen**
m^3	**Cubic metre**
mm	**Millimetre**
µm	**Micrometre**
MSFD	**Marine Strategy Framework Directive**
N	**Nitrogen**
O	**Oxygen**
OECD	**Organisation for Economic Co-operation and Development**
PBTs	**Persistent bio accumulative and toxic substances**
PCBs	**Polychlorinated biphenyls**
PCDT	**Poly-1, 4-cyclohexylene-dimethylene terephthalate**
PE	**Polyethylene**
PEMRG	**Plastics Europe's Market Research and Statistics Group**
PET	**Polyethylene terephthalate**
POPs	**Persistent Organic Pollutants**
PP	**Polypropylene**
PVC	**Polyvinyl chloride**
S	**Sulphur**
t	**Tons**
TU	**Technische Universität**
USA	**United States of America**
WFD	**Water Framework Directive**
WWTP	**Wastewater Treatment Plant**

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1. Introduction

Nowadays plastic is a frequently used material. It is part of our everyday life. Plastics are used mostly for packaging and for a myriad of household items like toothbrushes, shopping bags and pens (Fendall et Sewell, 2009). Several sectors use plastic for commercial, medical, municipal as well as industrial purposes (Wright et al., 2013).

Large-scale plastic production started in the 1940s (Cole et al., 2011). The amount of plastic being produced in Europe stayed relatively constant over the past 10 years, while world plastic production has been increasing over the last few decades (Duis and Coors, 2016).

Generally society is benefiting from plastic production, however there is a downside to large-scale plastic production, this being the accumulation of the product in natural habitats and its effect on the environment (Wagner et al., 2014).

Environmental concerns have been raised, mainly due to the resistance of plastic to degradation. Potential adverse effects of macroplastics have been highlighted decades ago. Quite recently more attention is being given to microplastics (Cole et al., 2011).

Macroplastics are defined as plastic particles in the size range above 5mm. Microplastics are plastic particles below the size limit of 5mm (Fendall et Sewell, 2009).

Accidental release and indiscriminate discards led to the accumulation of plastics in the environment. The occurrence of plastic waste in natural habitats reached a huge amount (Wright et al., 2013).

Scientific research shows that plastic pollution is not only a problem in areas close to human residences, but also in remote areas as well as the open oceans, where oceanographic features like global sea currents lead to an accumulation of plastics in concentrated areas (Fendall et Sewell, 2009).

There are different pathways of plastic waste entering the environment, specifically the aquatic systems. Plastic can be transported by wind or direct surface washout after heavy rain. According to Dris et al. (2015a) apart from large-size plastic debris this kind of distribution is particularly true for microplastics. The main source of microplastics entering aquatic systems is linked to insufficient end of life treatment of plastics. Along with water run-off and storm water, wastewater treatment plants serve as another pathway for plastic particles ending up in the freshwater system where they will further accumulate (Dris et al., 2015a).

Previous investigations into plastic pollution in aqueous environments focused on marine systems (Lechner et al., 2014). Not only are marine ecosystems affected by microplastics but studies have shown that they also occur in freshwater systems (Eerkes-Medrano et al., 2015; Faure et al. 2015; Lechner et al., 2014).

A systematic research into microplastics in freshwater systems basically started five years ago. However, there are still many unanswered questions. Experts are still aiming at the development of appropriate analytical methods and sampling procedures, further understanding of the impact of microplastics on aquatic organisms living in freshwater systems, its effect on ecosystem services and the assessment of possible consequences affecting human health, among other scientific questions (Eerkes-Medrano et al., 2015).

According to Eerkes-Medrano et al. (2015) the density of microplastics in waters as well as sediments reached high densities over the last couple of years. Initial studies on freshwater systems show, that the presence and interactions of microplastics with the environment are comparable to those in marine habitats.

The interaction of microplastics with the environment and organisms happens in different ways. The ingestion of microplastics via biota may result in physical damage to respiratory or digestive organs. Another possible impact is related to concentration and transfer of chemicals as well as persistent, bio accumulative and toxic substances (PBTs). The uptake of microplastics by freshwater organisms has been proven for Europe, Asia and North America (Eerkes-Medrano et al., 2015).

Even though aquatic organisms ingest microplastics, most of the time it is excreted within a couple of hours or days. Laboratory studies show that the uptake of high quantities of microplastics may decrease food uptake, diminishes energy levels and influences other physiological functions of the organisms observed. The studies also show that microplastics can enter the food chain by trophic transfer. Moreover further analysis will be needed to provide evidence of bioaccumulation and biomagnification of microplastics (Duis and Coors, 2016).

Plastic pollution became an increasing environmental concern on a global scale. Whilst macroplastics and microplastics in both, marine and freshwater systems are addressed by scientists, the contribution of wastewater plants to microplastic pollution has not been determined until now.

Different research design and measuring methods make the studies difficult to compare (Filella, 2015). However, some pilot projects give a first hint about the prevalence of microplastics in treated wastewater.

The effluents of wastewater treatment plants are a known pathway for household chemicals, influencing the quality of surface waters. This is why effluents of wastewater treatment plants are expected to be a major source of microplastics emitted to the environment (Van Wezel et al., 2015).
Consumer products like toothpaste and exfoliators, products used for air blasting, as well as pharmaceuticals are contributing to environmental pollution as they may contain microplastics. Scientists assume that 2.4mg of microplastics per person daily reach the environment due to the use of consumer products (Nerland et al., 2014).
According to Nerland et al. (2014), 70% of households are connected to sewage treatment within the European Union. The amount of microplastic particles in treated wastewater can reach up to 100 particles per liter (Nerland et al., 2014).
Duis and Coors (2016) state that wastewater of nearly 80% of the general public within OECD countries is treated in wastewater treatment plants. Even so, only 15-20% of wastewater is discharged to WWTPs on a global scale. At present, little is known about the effectiveness of WWTPs in the removal of microplastics in wastewater, the amount of microplastic particles in the effluent, as well as the amount of microplastic particles in sewage sludge (Duis and Coors, 2016).
Experts are addressing the problem of microplastic pollution in different ecosystems on an international scale. Apart from studies on microplastics in the river Danube (Lechner et al., 2014, Umweltbundesamt, 2015), little data is available for Austria. So far no studies are addressing a possible contribution of wastewater treatment plants to the microplastic pollution of freshwater systems in Austria.
Adverse effects of microplastics are widely unknown. While some scientists emphasise the growing environmental risk due to harmful chemical characteristics, others rank it as a minor risk (Faure et al., 2015). However, the application of the precautionary principle requires further observations and surveillance on possible negative impacts.
Therefore the amount of microplastics discharged into freshwater systems by the effluent of wastewater treatment plants as a possible source of microplastic pollution has to be studied in more detail.

2. Aims and objectives of the study

Microplastic pollution is an environmental concern of today and will continue to be addressed not only by scientists in the near future.
As mentioned in the introduction microplastic research is still lacking basic scientific requirements in terms of standardized procedures for measurements as well as reporting (Filella, 2015).
The aim of this study is to get information/data about the presence of microplastics in the effluent of a typical Austrian wastewater treatment plant. Therefore it compares two different approaches for determining the effluent quantities; visual inspection and elemental analysis.
As research design differs widely between international studies, a common approach to measure these plastic particles of microscopic size is essential.
Visual inspection is a frequently used methodology for microplastic studies (Filella, 2015), its effectiveness and possible downsides will be discussed in the course of this study. In order to test visual inspection against a second approach, suitable for measurement of microplastics, the performed elemental analysis of the samples is evaluated and described in detail.
The amount of microplastics in 10 samples, taken at the chosen study site, will be considered and then become the fundamental dataset for the study. It has been scientifically proven that microplastic does indeed occur in freshwater systems. Little is known about microplastics in wastewater, the removal efficiency of wastewater treatment plants and the role of wastewater treatment plants as a pathway of microplastics into freshwater systems as scientific studies are almost non-existent. In order to elaborate on the environmental concern and the sources of microplastic entering natural habitats, the existence of microplastics in the effluent of wastewater treatment plants has to be scientifically proven and quantified. Based on that information, further steps to rate the risk and minimize pollution should be undertaken.
The central research questions clarify the following assumptions:

- Treated wastewater is a potential source of microplastic pollution of freshwater systems in Austria. A remarkable number of microplastic particles (>1mm, <5mm) are expected to occur in all the samples taken.

- Consumer products contribute to the amount of microplastics in wastewater. Therefore microbeads represent the majority of microplastic particles found in the course of visual inspection.

- In comparison to visual inspection, elementary analysis provides more accurate results on microplastics occurring in wastewater samples, as the elemental analysis of C, N, S, O and H for sorted microplastics differ significantly compared to the elemental analysis of the remaining samples.

3. Definitions

To assist the reader, the following chapter provides definitions of the most frequently used terms related to microplastic research.

Microplastics are generally defined as plastic particles <5 mm in size. Plastic particles > 5 mm are defined as macroplastics (Fendall et Sewell, 2009).

The Marine Strategy Framework Directive (MSFD) provides an alternative scale. They suggest a further subdivision of microplastics regarding the “visible size fraction”, meaning particles bigger than 1mm in size.

MSFD recommends the following categories:

- large microplastics in the size range between 1 and 5mm,
- mesoplastics, from 5 to 25 mm
- and macroplastics, plastic particles larger than 25mm (Filella, 2015).

3.1 Macroplastics

Most of the studies define macroplastics as plastic particles > 5mm in size (Pettipas et al., 2016, Faure et al., 2015, Fendall et Sewell, 2009). However, some experts use the above-mentioned scale and consider only plastic particles bigger than 25mm as macroplastics (Li et al., 2016, Korrespondenz Abwasser, Abfall, 2016).

Due to fragmentation of larger plastic debris, macroplastic serves as source of microplastics (Duis and Coors, 2016).

3.2 Microplastics

Microplastics were detected for the first time in marine environments in the 1970. (Gallagher et al., 2015, Free et al., 2014). Several scientists specify them as plastic pieces < 5mm in size (Gallagher et al., 2015, Faure et al., 2015, Chang, 2015, Eerkes-Medrano et al., 2015, Free et al., 2014).

According to Filella (2015) microplastics are particles that are defined exclusively by their size. However, her review dealing with microplastics shows that there is no consistency referring to a fixed upper size limit for microplastics. Nevertheless most authors use the 5 mm limit.

Microplastics are particles of diverse origin and vary referring to their chemical composition. Experts differ between two categories, primary and secondary microplastics (Filella, 2015).

3.2.1 Primary Microplastics

Primary microplastics are manufactured plastic particles like resin pellets, microbeads and fibres and are produced for different applications in consumer products like toothpaste and peelings (Filella, 2015). Those manufactured plastics serve as scrubbers in cosmetic products and for cleaning utensils. More importantly, primary sources include manufactured plastic pellets that are used for different purposes such as raw material for plastic production and feedstock (Eerkes - Medrano et al., 2015). Plastic pellets vary in shape, colour and size. Their size range usually is between 1 and 5 mm in diameter. Frequently they are made out of polyethylene (PE) or polypropylene (PP). Plastic pellets were the first microplastics discovered and quantified in marine environments. They can enter the environment during the process of manufacturing or transport (Filella, 2015).

Microbeads

Small plastic particles in the shape of round pellets of generally about 4.5mm in diameter are called microbeads. Microbeads when melted down represent the raw material by which other plastic products are obtained (Gallagher et al., 2015).
Microbeads are also used for personal care products as their surface is smooth and therefore does not damage the skin like other substances. These polyethylene beads are usually between 4 µm and 1mm in size. Due to their appearance, microbeads from consumer products are very likely to be mistaken for plankton by diverse fish species which might cause physiological damage (Chang, 2015).
Microplastics used in cosmetic products vary depending on the product. A single product might contain up to three different types of plastics, like polyethylene, polypropylene and polystyrene (Li et al., 2016).
Microbeads are detected in marine environments and along beaches. Beyond that these particulates are predominant in rivers (Gallagher et al., 2015).

There are studies proving the existence of microbeads in freshwater environments, for instance in the Great Lakes in North America. Microbeads in cosmetic products are used less frequently nowadays. Public awareness and pressure on companies and governments has led to regulations and restrictions (Murphy et al., 2016).

3.2.2 Secondary Microplastics

Secondary microplastics are fragments that are derived from the breakdown of plastic items of larger size. They originate from various sources such as fishing nets, household items, raw material and consumer products (Eerkes - Medrano et al., 2015).
Large plastic debris can break down due to degradation, chemical processes or physical action (Gallagher et al., 2015). In general, plastic debris that ends up in the environment is highly resistant to degradation. According to experts the complete degradation process may take from decades up to centuries. Besides oxidative mechanisms, degradative mechanisms like hydrolysis and biodegradation lead to the breakdown of plastic debris. The latter takes much more time in comparison to the oxidative mechanisms. Plastic debris that is highly degraded may turn into brittle as well as disintegrated fragments that end up as particles of microscopic size, microplastics (Weinstein et al., 2016).

3.2.3 Fibres

Fibres can, depending upon their source be either be primary microplastics or part of the secondary microplastic segment (Korrespondenz Abwasser, Abfall 2016). Thin plastic fibres can be released from synthetic clothing during washing due to mechanical forces in the washing machine and finally end up in aquatic systems (Imhof et al., 2016).
Besides particulates fibres easily can enter the aquatic environment in high amounts as well, because both of them can pass through wastewater treatment facilities due to their small size and density (Gallagher et al., 2015).
Clothes made of synthetic fibres serve as a source of microplastics. During washing, clothes release fibres in the size range of millimetre and sub millimetre. Those fibres are categorized as secondary microplastic (Dris et al., 2015).

3.2.4 Plastic particles in the micrometre size range

The smaller fraction microplastics contribute to environmental pollution and is considered to be a potential ecological risk. Even so, there is a lack of studies, focusing on particles below 500 micrometre due to suitable identification methods. The fact that a qualitative and quantitative approach has not yet been found in order to analyse microplastics in the environment, is caused by a lack of methods identifying microplastics. So far only two technologies, Raman microspectroscopy and micro-FTIR support the detection of microparticles between 20 micrometre and 1 micrometre. The majority of studies concerning microplastics in aquatic systems focuses on particles bigger than 1mm in size as well as bigger than 300 micrometre. As a result the amount of studies taking into consideration smaller particles decreases with the size of the particles sampled (Imhof et al., 2016).

4. State of the Art

This study is focusing on the identification of a suitable methodological approach to detect and analyse microplastics and determine the quantities of microplastics being discharged into the aquatic system via the effluent of wastewater treatment plants. In order to examine this issue and address the problem also marine environments as well as freshwater systems will be covered in this chapter, as all of those compartments are affected.

4.1 Plastics

Plastic is an invention of the 20th century. It is a multifunctional material, light weighted, strong, durable and cheap to produce. Furthermore plastics serve as thermal and electrical insulators (Gallagher et al., 2015). Plastics have turned into an omnipresent material in everyday life, from food packaging to household items (Fendall and Sewell, 2009). Beyond that, the wide scope of application includes the automotive industry, the aviation sector, building and construction, equipment for electrical and electronic use, farming, sports as well as leisure equipment and the medical sector (Plastics Europe, 2016).

Plastic is a man-made polymer, produced via polymerisation of monomers. The monomer can be made from oil, gas, coal, cellulose, natural gas as well as latex extracted from trees. Hydrocarbons together with other compounds, that are naturally occurring, built the molecular backbone of plastic polymers. Additives such as plasticisers, are used to reach favoured properties like increasing the polymer's softness (Nerland et al., 2014). By the amendment of pigments, the polymer blend obtains the desired colouration (Imhof et al., 2016).

This combination of different polymers and several additives results in a material stream, that is very complex. Plastic production and its use are related to environmental concerns. The basis for plastic production is constructed from 90% non-renewable fossil resources. In total 4% of petroleum produced is used for plastic production each year. Furthermore energy consumption during the production phase accounts for additional 3 to 4% of the annual petroleum production (Van Eygen et al., 2015).

4.1.1 Different types of plastic

The most common types of plastics being produced are polyethylene (PE), polypropylene (PP) as well as polyvinyl chloride (PVC) (Nerland et al., 2014). According to Li et al. (2016) high-density polyethylene, low-density polyethylene, polyvinyl chloride, polystyrene, polypropylene as well as polyethylene terephthalate make up 90% of the world's plastic production. Hence these polymers can be detected more frequently in the environment. Most plastics are resistant to corrosion. The larger part of plastics are "hard to degrade" materials. Therefore they will persist in environmental compartments for up to 100 years (Li et al., 2016).

4.1.2 Production and demand (World, EU, Austria)

The amount of plastics produced worldwide in 1950 was 1.7 million tonnes. In 2009, 230 million tonnes of plastic were manufactured (Cole et al., 2011). Production reached 269 million tons of plastic materials (thermoplastics and polyurethanes) and 322 million tonnes including thermosets, adhesives, sealants, and coatings internationally in 2015. China is the world's biggest plastic producer (thermoplastics and polyurethanes), Europe ranks second, followed by the North Atlantic Free Trade Agreement (NAFTA) (Plastics Europe, 2016). Annually between 75 and 80 million tons of plastics were consumed worldwide (Umweltbundesamt, 2015).

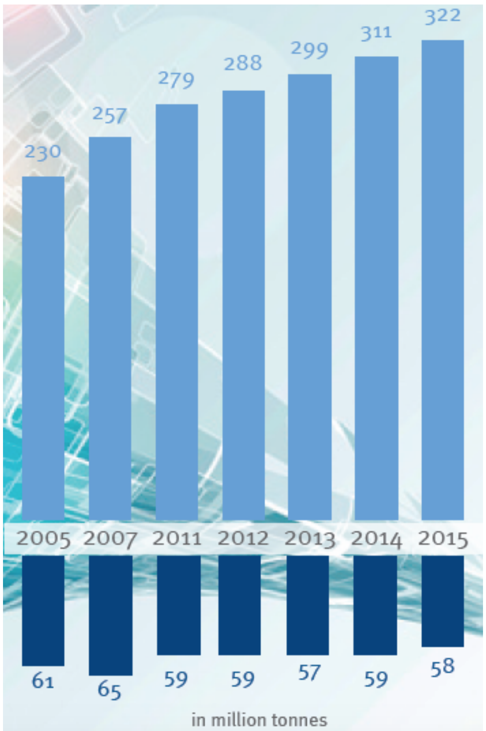


Figure 1: Plastic production data World/EU
World (light blue), EU (dark blue)
Plastics: Thermoplastics, polyurethanes, thermosets, adhesives, sealants, coatings.
Without taking into account PET-, PP-, PA- and polyacryl-fibres.
EU: EU 28+Norway+Switzerland
Source: Plastics Europe (PEMRG) / Consultic
(Plastics Europe, 2016,12)

As mentioned in the introduction, world plastic production is still increasing. This is not true for the plastic production within Europe as it has been constant over the last couple of years (Duis and Coors, 2016). As shown in Figure 1, 57 million tons of plastics were produced in 2013, 59 million tons in 2014 and 58 million tons in 2015 (Plastics Europe, 2016).

The demand for plastic within Europe is concentrated in six countries, Germany, Italy, France, Spain, The United Kingdom and Poland, as these countries hold 70 % of the overall plastic demand in Europe. In the year 2015 plastic demand for Europe added up to 49 million tons of plastic. The most frequently used plastic materials in 2015 were Polypropylene (PP), Polyethylene (PP) and Polyvinyl chloride (PVC). Plastic packaging counts for 39.9% of the annual demand of plastics within the 28 EU member states, Norway and Switzerland (Plastics Europe, 2016).

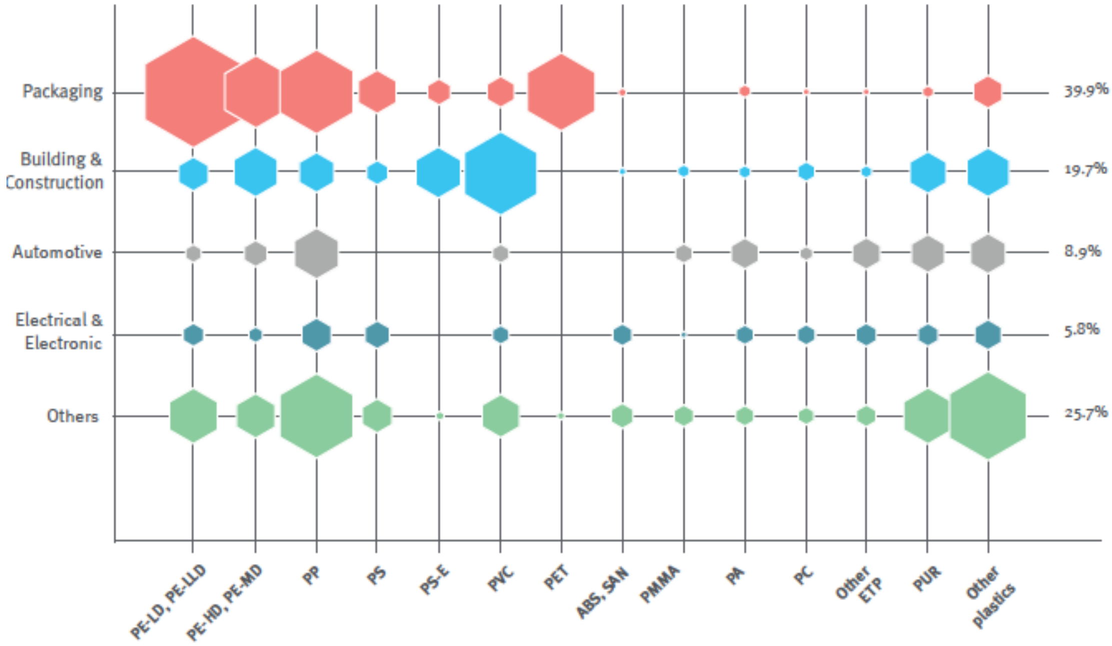


Figure 2: EU plastic demand including polymer type and market sectors, 2015
EU: EU 28+Norway+Switzerland
Source: Plastic Europe (PEMRG, Consultic, myCeppi)
(Plastics Europe, 2016, 20)

In Austria 1,112 kilotons of primary plastics were produced by the chemical industry in 2010. 877 kilotons polyolefins, 175 kilotons polystyrenes as well as 60 kilotons resins. In total 1,167 kilotons of plastic products were available on the Austrian consumption market in 2010 (Van Eygen et al., 2015).

4.1.3 Recycling

The process of recycling is split in four different tasks: collection, separation, reprocessing and marketing. Recycling reduces waste and furthermore leads to a decrease in the consumption of fossil resources. Recycled polymers can reach high market value in different sectors. Nowadays recycling of polymers is a common practice as it helps to cope with the amount of waste being produced. Apart from packaging, recycling is done in various sectors like building and construction, the automotive industry and agriculture (Singh et al., 2016) and basically across all sectors, where larger amounts of homogenous material occurs.

Plastic waste builds a highly complex waste stream. The durability of the polymer leads to accumulation in natural environments. Recycling is reducing the impact on the environment on the one hand and diminishes the consumption of resources involved in the life cycle of products made out of plastics on the other (Van Eygen, 2015).

Around half of the plastic production is meant to be for single use (Nerland et al., 2014). In most EU countries, plastic waste is still dumped in landfill. Nevertheless some countries, including Austria, have banned landfilling and achieve higher recycling rates of plastics. The official waste stream was 25.8 million tons of plastic waste (post consumption) for the 28 EU member states, Norway and Switzerland in 2014. 69.2% of that amount was recovered, including recycling rates and energy recovery. 30.8% was dumped at landfills. The recycling rate for plastic packaging was 39.5% and hence reached the highest rate compared to other plastic applications. More than 80% of the total quantity recycled is based on plastic packaging. In Europe, in 2014 more than 7.5 million tons of plastics were recycled (Plastics Europe, 2016).

4.2 Sources and pathways of microplastics into the environment

There are different sources and pathways of microplastics entering the environment. Apart from littering, the production, loading and transport of primary microplastics contributes to the microplastic pollution. Plastics from littering are washed off in urban areas and reach the sewer system or might directly enter surface waters via wind (Umweltbundesamt, 2015).

The aquatic system can be reached via the sewer system (combined or separated) or, entered directly due to littering (Dris et al., 2015).

The effluents of municipal wastewater treatment plants have been proposed to contribute to microplastic pollution of aquatic environments. Apart from treated wastewater discharge, other pathways have been identified for plastics entering receiving waters. Among them are storm water runoffs, atmospheric fallout and wind advection (Mason et al., 2016).

There is evidence that the abundance of microplastics positively correlates with the density of human population (Li et al., 2016). According to Dris et al. (2015) catchments exposed to intensive anthropogenic impact, in particular in urban areas might serve as an important source of plastic. The study on microplastic contamination in France (Greater Paris) found microplastics in all environmental compartments, surface water, the atmosphere, in sewage as well as treated water.

The city comprises a closed system. Primary plastics and microplastics can enter this system. Within the system, secondary plastics and hence more secondary microplastics are produced. Therefore, affecting three environmental compartments: soil, water and the atmosphere (Dris et al., 2015).

Dumping, poorly managed landfill and waste collection highly contribute to the amount of plastic ending up in the environment. Synthetic fibres, as a source of microplastics end up in the environment due to washing of fleeces, blankets etc. Furthermore the abrasion of car tyres contributes to microplastics in natural habitats (Duis and Coors, 2016).

Scientists also claim that the amount of microplastics occurring in the environment are higher close to plastic producing plants, serving as a point source (Duis and Coors, 2016; Lechner and Ramler, 2015) for instance the Danube river, Austria (Lechner and Ramler, 2015).

The following list by Duis and Coors (2016) provides an overview of sources of primary and secondary microplastics into the environment:

"Primary microplastics
-Specific personal care products containing microplastics as exfoliants/abrasives
-Specific medical applications (e.g. dentist tooth polish)
-Drilling fluids for oil and gas exploration
-Industrial abrasives
-Pre-production plastics, production scrap, plastic regranulate: accidental losses, run-off from processing facilities

Secondary microplastics
-General littering, dumping of plastic waste
-Losses of waste during waste collection, from landfill sites and recycling facilities
-Losses of plastic materials during natural disasters
-Plastic mulching
-Synthetic polymer particles used to improve soil quality and as composting additive
-Abrasion/release of fibres from synthetic textiles
-Release of fibres from hygiene products
-Abrasion from car tyres
-Paints based on synthetic polymers (ship paints, other protective paints, house paint,

road paint): abrasion during use and paint removal, spills, illegal dumping
-Abrasion from other plastic materials (e.g. household plastics)
-Plastic items in organic waste
-Plastic coated or laminated paper: losses in paper recycling facilities
-Material lost or discarded from fishing vessels and aquaculture facilities
-Material lost or discarded from merchant ships (including lost cargo), recreational boats, oil and gas platforms"
(Duis and Coors, 2016, 5).

In order to provide detailed information about microplastics in different environments, the following section covers the issue of microplastics in the aquatic system, more precisely marine environments, freshwater environments and wastewater.

4.2.1 Microplastics in marine environments

Not only scientists have recognized the increasing amount of plastic in the world oceans. The issue and its adverse effects have been highly discussed in the media. More than forty years ago, plastic debris have been found in perished sea birds. At the same time, the first studies trying to quantify the amount of floating plastic litter in the western North Atlantic Ocean were made (Filella, 2015).
Plastic production changed the composition, not only of household rubbish but also waste from industrial use. As a result a steady increase of plastic litter in marine environments was observed. Generally speaking plastic debris enter marine habitats as macroplastics where they continue their life cycle as microplastics after different forms of degradation (Imhof et al., 2016).
Experts estimate that between 75 - and 90% of plastic in marine environments comes from land based sources and between 10 - and 25% originate from ocean-based sources such as fishery (vessels), cargo shipping and gas as well as oil platforms located offshore (Duis and Coors, 2016).
Microplastics became an environmental concern due to the fact that large amounts of plastic are deposited in the ocean. According to Free et al (2014) about 10% of the yearly global plastic production contaminates the world´s oceans (Free et al., 2014).

Scientists claim that about 267 marine species ingest plastic particles, including microplastics and macroplastics. Among those species are turtles, birds and marine mammals (Dris et al., 2015).
Microplastics can be found throughout the world´s oceans. Adverse effects of contamination by microplastics are emerging. Due to their small size they are bioavailable and can enter the food web. The uptake via biota as invertebrates, fish, mammals and seabirds ingesting microplastics, may lead to health consequences like reduced feeding behaviour or lowering ecophysiological functions (Free et al., 2014).
Studies addressing the issue of plastic ingestion have been focusing on vertebrates. Also, smaller organisms have to be taken into account, as larger plastic particles break down and hence can be ingested by a wide range of biota. This has been proven for barnacles, polychaete worms and sea cucumbers. Translocation to the circular systems of tiny particles of about <10 µm was detected in bivalves. However, significant adverse effects could not be proven in the course of the experiment concerning ingestion as well as translocation (Claessens et al., 2013).
Toxicity is an environmental concern due to absorption and contamination of organic substances. Whether microplastics serve as a vector of pollutants to marine wildlife remains problematic and might be according to the authors of minor importance as fate and contaminants are barely understood. The determination of data gaps, missing supporting surveys and a lack of appropriate techniques to extract microplastics from organic tissue are necessary in order to clarify the affect of microplastic on marine organisms (Claessens et al., 2013).
Several studies show that larger quantities of microplastics in marine environments occur close to areas with high population density due to a larger input of industrial and domestic sources. It is a fact that this is highly influenced by oceanographic conditions, which determine spatial distribution of microplastics in marine environments (Gallagher et al., 2015).
A study covering the total amount of plastic debris in the world´s oceans (Northern and Southern Hemisphere) including four different size classes (small and large microplastics, mesoplastics, as well as macroplastics) suggests that currently there are 5.25 trillion particles drifting in the sea. This is a total amount of 268,940 tons. The occurrence of the four different size ranges for every ocean region was proven. Furthermore, it was confirmed that plastic debris disappears during the process of fragmentation. Stranding of particles as well as sinking and degradation leads to the fact

that the amount of plastic loads in the oceans of the northern hemisphere is lower than expected, taking into account international input scenarios (Eriksen et al., 2014).

4.2.2 Microplastics in freshwater environments

As mentioned before, microplastics and its impact on marine ecosystems became a matter of great importance for environmental studies decades ago. As a result a huge amount of studies and scientific papers is presently available. The same is not true for microplastics in freshwater systems. This area has been neglected and further research has to be done. Only a limited number of studies are available at the moment. Scientists are now focused on microplastics in lakeshore sediments, the effects on freshwater fauna that is ingesting microplastics, as well as the occurrence of pelagic microplastics, not only in rivers but also in lakes. The results of the studies proved the occurrence of microplastics in freshwater systems. Abundance is varying, but for some cases the occurrence of microplastic in freshwater systems can be compared to the amount of microplastics that was found in marine ecosystems. Furthermore, the uptake of microplastics by freshwater fauna was confirmed (Free et al., 2014).

Adverse effects due to ingestion of microplastics by biota in freshwater system are one of the major environmental concerns. Nevertheless up to now there is a lack of documentation. Microplastics can be excreted, retained within the body and also be translocated. Like that they reach other body tissues and fluids (Dris et al., 2015a).

According to Gallagher et al., (2015) microplastics are a source of so-called Persistent Organic Pollutants (POPs), which might lead to pollution with several associated chemicals (Gallagher et al., 2015). Hence, ingested microplastics may serve as a medium to concentrate and transfer harmful substances like chemicals and persistent bio accumulative and toxic substances (PBTs) to organisms. Microplastics can carry chemicals absorbed into the surface from the surrounding environment like Polychlorinated biphenyls (PCBs) or Dichlorodiphenyldichloroethylene (DDEs) as well as chemicals, added to the polymer during production like plasticizers (Eerkes - Medrano et al., 2015).

As mentioned before little is known about the occurrence of microplastics in freshwater systems (Free et al., 2014). Until now continental areas were neglected. There is a gap of knowledge concerning fate, transfer and sources of microplastics in continental areas. Preliminary studies show contamination of freshwater systems for both lentic and lotic water bodies for instance the Great Laurentian, Lake Geneva and Lake Garda, the Danube River and the Thames River among others (Dris et al., 2015).

A survey at the Austrian Danube found about 900 plastic items in 2009 as well as 50 in 2012 in the size range between 0.5 and 50mm for 1000m^3 of Danube water. The study suggests worst case the Danube might discharge 4.2t of plastics per day or 1500t of plastics each year into the Black Sea (Wagner et al. 2014).

As rivers are transporting a significant amount of not only microplastics but also larger plastic items they seem to play a crucial role in the contamination of marine habitats (Wagner et al. 2014).

Studies highlight the abundance of plastics in lakes and rivers whilst their sources as well as dynamics are largely unexplored (Dris et al., 2015).

Freshwater systems in undeveloped areas are more likely to be polluted by microplastics through degradation or fragmentation of macroplastics which reach rivers and lakes as they are transported by wind. Another possibility of microplastic entering freshwater systems is via input from shore (Free et al., 2014).

As research has focused on marine environments in past years, a large amount of literature is available addressing the problem of marine plastic waste pollution. Just a few studies are covering the issue in rivers and lakes. While lakeshore sediments and riverbanks have been studied, no data are available for lake bottom sediments (Dris et al., 2015a).

Defining the sources and pathways of microplastics entering aquatic systems as well as the development of a common methodology to sample, separate and identify microplastics will be a future research challenge. Hence, it will be the central focus in order to establish suitable management strategies to tackle adverse environmental impacts caused by microplastics (Dris et al., 2015a).

4.2.3 Microplastics in wastewater treatment plants

Microplastic particles can be found in various personal care products like toothpaste, soap and lotions, used on a daily base. Via the wastewater stream they enter municipal wastewater treatment plants (Carr et al., 2016). Furthermore wastewater contains synthetic fibres, abundant in domestic wastewater due to the washing of clothes (Filella, 2015). Scientists estimate that up to 1900 fibres are released during a single washing process. Even though wastewater treatment plants contribute to the elimination of plastic fibres, sewage sludge used for agricultural purposes might further distribute those fibres into the environment. However sufficient documentation of this possible pathway is needed (Dris et al., 2015a). In addition the improper disposal of hygiene products may increase the amount of fibres in wastewater (Duis and Coors, 2016).

Whether WWTPs are one of the major sources of discharging microplastics into the freshwater system or not, has yet not been clarified, as studies are contradictive. Scientific papers cite municipal wastewater treatment plants as a pathway of microplastics into the freshwater system. However, scientists have not yet confirmed a link between microplastic pollution in aquatic systems and wastewater treatment plants. The debate about discharged effluents and their impact on a potential pollution of rivers by microplastics is still on going. At the moment the behaviour of microplastics within the process of wastewater treatment is widely unknown. Moreover the fate and different possible pathways of this pollutant is of interest, not only for plant design engineers but also for environmental experts, in order to improve existing treatment processes to eliminate microplastics during the wastewater treatment system (Carr et al., 2016).

There are different forms of wastewater treatment. Usually physical, chemical and biological processes are applied in order to eliminate large objects and reduce nutrient concentration as well as organic matter. Some wastewater treatment plants include membranes as solid separation technique. However, some wastewater treatment plants lack this kind of treatment. Hence the effluent water contains more particulate material, as this final step of treatment contributes to a reduction (Magnusson and Norén, 2014).

Sieves and screens remove solids from the wastewater during primary treatment. The openings of coarse screens are between 20 and 50mm as well as 10-20mm for intermediate screens. The openings of fine screens are between 2 and 10mm.

Whilst screens capture macroplastics, they are not capable of capturing the smaller fraction of microplastics due to their opening size. Experts state that the grease-separating phase allows separating floating microplastics from wastewater. Furthermore high density of particles such as polyurethane (PU) leads to sedimentation, hence microplastics will be deposited in sand traps or in sludge. Nevertheless at the moment there are only little data available addressing the effectiveness of WWTPs referring to the removal of microplastics as well as to the level of microplastics remaining in sludge and the effluent (Duis and Coors, 2016).

It is a fact that the different sources of microplastics that occur in the environment are still not completely comprehensible. Wastewater treatment could theoretically significantly contribute to the amount of microplastics in the natural environment. Microbeads used in personal care products are an example as those tiny particles that bypass the screens within the plant. This is true for the coarse as well as the fine screens of the plant. Microbeads used in facial cleaner are made out of polyethylene. It behaves positively buoyant and therefore can be found in the upper layers of the wastewater where it is easy to skim off during the grease removing process. Murphy et al. (2016) therefore couldn't provide evidence of microbeads in the final effluent of an observed plant in Scotland. According to the authors, the small sample size has to be considered, but this is a strong indication that appropriate purification of wastewater can reduce the amount of microplastics ending up in the natural environment drastically by efficient wastewater treatment plants (Murphy et al., 2016).

According to Li et al. (2016) latest wastewater treatment plants are able to eliminate up to 99% of microplastics from the wastewater. Nevertheless the amount of microplastics discharged via the effluent is still significant.

Most experts claim a positive correlation between the density of urban population and the occurrence of microplastics. Therefore three major pathways of pollution have been identified in these areas:

1. Wastewater treatment plants
2. Sewage overflow (Storm water overflows in combined sewerage)
3. Sewage-based fertilizer used in agriculture or on public land (runoff).

(Free. et al., 2014).

Microplastics are likely to pass the treatment screens of wastewater treatment facilities and therefore end up in the oceans (Fendall and Sewell, 2009) as well as freshwater systems (Dris et al., 2015). According to Fendall and Sewell (2009) the typically coarse is >6mm, whilst fine screens are in the size range between 1.5 and 6mm.

Due to technical adjustments over the past couple of years usually fine screens >3mm are installed or have been changed over.

According to Wagner et al. (2014) between 70 and 80% of litter, primarily plastics, in the ocean are transported by rivers from inland sources. However, inland sources of microplastics still have to be identified. The main contributors most probably will include wastewater treatment plants, run off from anthropogenic influenced areas as well as sewage sludge being used for landfilling purposes and agriculture.

In Austria 17% of sludge produced is used in agriculture. This comes up to an amount of 250,000t dry matter annually. In Europe 39% of sludge are used for farming. Due to the drying process, microplastic particles become volatile and can be further transported by wind (Umweltbundesamt, 2015).

4.3 Case studies - The role of wastewater treatment plants

Scientists are investigating the amounts of microplastics entering the environmental system via wastewater effluents. Studies show different results for wastewater treatment in several countries. Some case studies from the United States, France, Great Britain Sweden and Scotland have been selected and will be elaborated in more detail.

A study conducted by Carr et al. (2016) examined the amount of microplastics in seven tertiary WWTPs and one secondary WWTP in California, USA. The study suggests that effluent filters play a minor role when it comes to the removal of microplastics from wastewater. Most microplastics were eliminated in the primary treatment zone, as during sludge settling. Moreover, the results show that the contribution of the tested WWTPs to the contamination of oceans and surface waters via microplastics is minimal. Most microplastics identified were similar to polyethylene particles found in toothpaste.

The microplastics occurring in wastewater differ from the microplastics found along beaches, in rivers and lakes. This debris are mostly a result from consumer packaging as well as garbage from industrial purposes undergoing mechanical and physical processes like photodegratation. All these factors leading to embrittlement and the breakdown of larger plastic debris are neglectable during wastewater treatment. In the United States cosmetic industry is now trying to replace microplastics in cosmetically products by environmental friendly alternatives in order to minimize environmental threats by this pollutant. However, the study suggests that present wastewater treatment processes are very effective according to the elimination of microplastics entering the wastewater system (Carr et al., 2016).

A study investigating microplastic contamination in Greater Paris found a high amount of particles in the inflow of the plant located at the river Seine. Most of the particles were of fibrous nature. This might be due to fibres from clothing, set free whilst washing. Nevertheless the amount of fibres decreased drastically towards the final effluent. Therefore the obtained result for removal of microplastics ranges between 83% and 95%. The author's hypothesis suggests that those particles go to the sludge. Observations showed that there were fibres present in the sludge. The plant was most efficient removing long fibres. They could not be detected in treated water at all (Dris et al., 2015).

A study conducted by Gallagher et al. (2015) focused on microplastics in different estuaries in the UK showed that wastewater treatment plants and plastic industry plants serve as main local sources for the input of microplastics.

A Swedish study suggests that 99% of microplastics were eliminated. The study was conducted in a small wastewater treatment plant for only 12,000 people. Furthermore the identification methods on specific polymers were limited. The study further suggests that about 2,000 microplastics enter the effluent of this plant on a single day (Murphy et al., 2016).

Considering the size of some wastewater treatment plants and the volume of wastewater that passes through the plant, the small number of microplastics per liter of wastewater will significantly contribute to the total amount of particles discharged into the natural environment (Murphy et al., 2016).

A study conducted in Scotland investigating a secondary WWTP with a population equivalent of 650,000 measured an average of 15.7 microplastic particles per liter of wastewater entering the plant. For the final effluent they measured a number of 0.25 particles. This means the amount of microplastics from the inflow of the plant until the final effluent decreases by 98.41%. However, taking into account the volume of water treated, the calculations for the amount of microparticles discharged into the receiving water is 65 million in one day.

Also, flow rates and their variability as well as changes in the concentration of pollutants should be considered. The same is true for other environmental factors that might change or vary. Weather conditions can also influence the samples obtained. Moreover frequent sampling several times a day might help to obtain better results than just sample a snapshot (Murphy et al., 2016).

4.4 Legal background

While the European Marine Strategy Framework Directive (MSFD, 2008/56/EC) refers to the issue of litter in marine environments including plastics, the Water Framework Directive (WFD,20/60/EC) for European inland waters doesn't address plastic litter. Nevertheless anthropogenic pressures have to be measured by the Member States of the European Union.

Other directives are linked to the problem of microplastics in freshwater systems including packaging waste (2004/12/EC), waste (2008/98/EC), urban wastewater (91/271/EEC) and sewage sludge (86/278/EEC) among others. Quite recently a green paper on a European strategy for plastic waste in the environment was published. Besides mitigation strategies of plastic litter at its source microplastics have been identified as a particular concern (Wagner et al, 2014).

Whilst the Marine Strategy Framework Directive wants to reach good environmental status for marine environments within the European Union until 2020, the Water Framework Directive is aiming at reaching good chemical and ecological status for all water bodies within the EU. The fact that the contamination of freshwaters with micro and macroplastics has not yet been considered is most probably linked to the lack of data as well as absent adequate methodologies to measure concentrations of this emerging environmental issue (Dris et al., 2015a).

5. Material and Methods

The study in hand combines a literature review with data collection and two different methodologies of data analysis for the determination of quantities of microplastic in wastewater effluents. This chapter provides an overview of material used and methods applied. The study was conducted following a predetermined gradual defined structure, including the following steps:

- Literature Review
- Establishment of the research design
- Data Collection
- Data Analysis Method I -Visual Inspection
- Data Analysis Method II - Elemental Analysis

5.1 Literature Review

In the introduction we were presented with the current state of knowledge concerning plastic pollution with a strong focus on microplastics. This information, based on a literature review, takes into account different environments, namely freshwater systems, the marine environment and in particular wastewater systems. The review covers environmental concerns and potential problems related to the occurrence of microplastics in natural habitats. The main focus of attention is the investigation into the role of wastewater treatment plants as a potential contributor to microplastic pollution in freshwater systems. This represents a possible pathway for further distribution into the aquatic system. The analysis elaborates on the developments in microplastic research, its progress, current findings and states the background of information on which the rest of the study is built.

The information provided is based upon literature from scientific papers, journals and reports. Comprehensive online research has been conducted, mainly via the online databank SCOPUS, in order to access peer reviewed studies about microplastic research, as well as common data sampling methods and in particular analytic models. Other sources of data, such as reports from governmental organisations were also included. In order to avoid common mistakes during sampling and data analysis the methodology for the quantification of microplastics was developed based on literature review.

5.2 Data collection

To substantiate the possible contributions of wastewater as a main polluter of aquatic systems in Austria, one must quantify the amount of microplastics being discharged into the freshwater system after wastewater treatment. Therefore the lack of data in this recent field of research has to be addressed.

The collected data are supposed to be a spot image to have an indication of the presence or absence of microplastics in the effluent of the wastewater treatment plant, to identify an appropriate methodology to sample microplastics in wastewater and to provide information about the suitability of the two different analytical methods applied.

In order to sample microplastics in the effluent of the chosen plant, an appropriate measurement device has been developed as a first step.

The analysis was conducted between June 7th and June 29th in 2016. All samples were taken at the final effluent of the chosen study site, a wastewater treatment plant based in Austria. In total 10 samples where taken. The first sample was assessed in two steps listed as Ia and Ib. For the purpose of visual inspection and elemental analysis it was considered as sample I. Data were further analysed at the Research Center for Water Quality Management at the Institute for Water Quality, Resource and Waste Management, TU Wien (Visual Inspection). Subsequently analysis took place at the laboratory of Waste and Resource Management at TU Wien (Elemental Analysis). The data were collected during the morning on five different days in June 2016. To get scattered data, the samples were taken on different days, where there was a minimum of one day between two data collections. Sampling always included two samples taken from the effluent on the same day.

5.3 Sampling procedure

As mentioned in the introductive part, there is still no common approach to quantify the amount of microplastics in aquatic surroundings.

At the moment there are two common sampling methods used for microplastics in environmental testing. Sampling of solid matter (terrestrical, semiterrestrical and subhydrical) on the one hand and sampling in the aqueous phase on the other (Korrespondenz Abwasser, Abfall, 2016). The method used for this study was sampling in the aqueous phase. In order to filter the microplastic particles from the effluent, the following equipment has been used:

- Sieves in decreasing mesh sizes (5mm, 1mm, 630µm, 500µm, 63µm, Ø:200mm)
- 1 sieve serving as a final catchment (200µm, Ø:400mm)
- 1 water flow meter
- 2 water hoses
- Receptacles

According to Fillela (2015) sieves used for microplastic studies are generally made out of woven wire cloths with square apertures. The same is true for the sieves used for this study. Three sieves in decreasing mesh sizes were clamped in the purpose-built frame and fixed to the water hose. The water was pumped through the sieves, filtering particles of organic and non-organic origin. During the test stage of the sampling device, sieves with varying mesh sizes were applied until the best arrangement of sieves was obtained. At the beginning of the sieving process it was found that the sieve in the size range of 63 microns was too small at mesh for the filtration, as the pressure of the water pumped through the measuring device was too high and caused damage to the filter element. The sieve had to be changed for a larger mesh size and was replaced by a 630 micron sieve after the first four samples (including sample 1a and 1b). Also the sieve in the size range of 500 microns was changed for a larger mesh.
The most effective way to sample was the following arrangement of sieves (Figure 3):

- 5mm,
- 1mm
- 630 µm

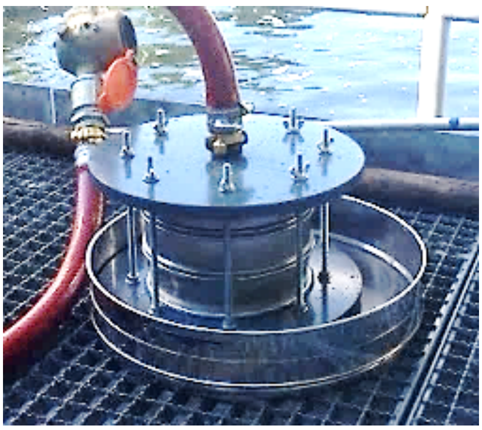


Figure 3: Most suitable sampling procedure (5mm, 1mm, 630 µm, 200 µm)

The water hose was attached to a pump, located at the wastewater treatment plant's tailrace pool. The installation of the water flow meter as a connector of the two water hoses allowed measuring the amount of water pumped through the measuring device.

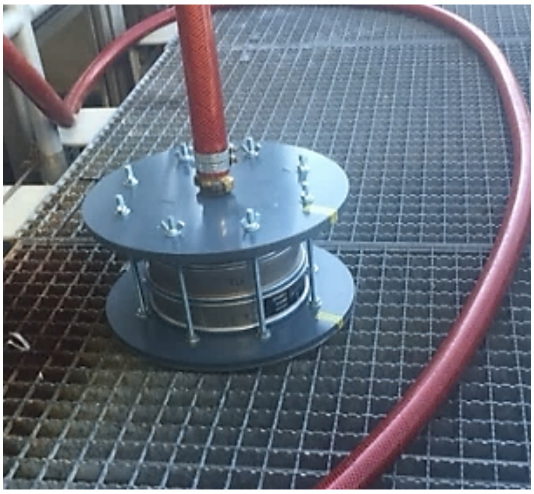


Figure 4: Measurement device (frame, sieves, water hose)

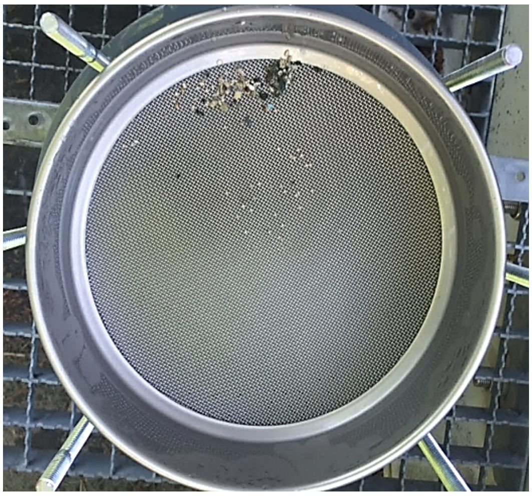


Figure 5: Filtered particles (organic, non-organic origin)

Due to the use of different sieves at the initial phase of the experiment the amount of water filtered differs between the samples taken.

To standardize the methodology, the same amount of water (1,5 m^3) as well as the repetitive constellation of sieves (5mm, 1mm, 630 µm) was applied for sample number

V, VI, VII, VIII, IX and X. The amount of time for 1,5 m^3 was between 58 and 62 minutes for the different samples (V-X). For sample I, III and IV the experiment was stopped earlier, as the screen was clogged before 1,5 m^3 of water could pass the pumping system. After the amount of water sampled passed the water flow meter, the obtained particles from the sieves in decreasing mesh sizes were washed into the final catchment (200 micrometres) to be collected and filled into receptacles.

The obtained material (organic and non-organic matter plus water) was stored in the fridge until further analysis took place.

Additional information on the samples (raw data set) and the equipment used will be provided in the Table 1.

Table 1: Samples taken at the effluent of the wastewater treatment plant

Sample	Date	Amount of water/m^3	Time	Screen	Final catch	Weather
Probe I	07.06.16	2,15	no data	1mm, 500µm, 63µm	200µm	sunny
Probe Ia	07.06.16	1	8:42-9:20	1mm, 500µm, 63µm	200µm	sunny
Probe Ib	07.06.16	1,15	no data	1mm, 500µm, 63µm	200µm	sunny
Probe II	07.06.16	1,29	no data	1mm, 500µm	200µm	sunny
Probe III	21.06.16	1,16	09:05-09:45	1mm, 500µm, 63µm	200µm	sunny
Probe IV	21.06.16	1,05	09:55-10:36	1mm, 500µm, 63µm	200µm	sunny
Probe V	23.06.16	1,5	09:17-10:17	5mm, 1mm, 630µm, 200µm	200µm	sunny
Probe VI	23.06.16	1,5	10:30-11:29	5mm, 1mm, 630µm, 200µm	200µm	sunny
Probe VII	27.06.16	1,5	09:06-10:08	5mm, 1mm, 630µm, 200µm	200µm	heavy rain
Probe VIII	27.06.16	1,5	10:26-11:25	5mm, 1mm, 630µm, 200µm	200µm	heavy rain
Probe IX	29.06.16	1,5	08:40-09:42	5mm, 1mm, 630µm, 200µm	200µm	sunny
Probe X	29.06.16	1,5	09:54-10:52	5mm, 1mm, 630µm, 200µm	200µm	sunny

5.4 Data analysis - Visual Inspection

The samples were analysed under a light optical microscope (Wild Heerbrugg M3Z). The material obtained from the filtration process elaborated in chapter 5.3 was diluted with deionized water.

Experts claim that due to organic matter, the identification process of microplastics is hampered. Methodologies to remove organic matter from the samples do exist. Nevertheless only a few studies apply prior treatment before analysing the sample (Dris et al., 2015a).

Organic matter might cause interferences when it comes to the analysis of the samples. In order to get rid of organic matter, acids, bases or enzymes were applied. The elimination of carbon is much more difficult in the raw effluent or the effluent of the primary treatment of the wastewater treatment plant (Korrespondenz Abwasser, Abfall, 2016).

In this study the samples were invariably taken at the final effluent of the plant. So no further treatment was applied before the counting was started. Microplastic particles were counted and stored separately. After counting, the sorted microplastic particles were photographed. Microplastics in wastewater are usually given in particles per volume (liter) or mass per volume (Korrespondenz Abwasser, Abfall, 2016). In this case the obtained results were given in particles (n) per liter (l).

Some of the microplastics were easy to identify, mainly due to their colour and specific shape. Whereas organic matter is characterized by a typical cell form, that becomes visible under the microscope. Nevertheless for other particles it was not possible to assign them to either organic matter or microplastics without double-checking under a microscope with a higher resolution (Leica DMRE).

Following the visual inspection, the samples were handed over to the laboratory to conduct an elemental analysis, which will be explained in more detail in the following chapter.

During the whole process of visual inspection, disposable gloves were used to avoid contamination of the samples.

The statistics software R as well as Microsoft Excel were used for data analysis and graphical representation of the obtained results.

5.5 Data analysis - Elemental analysis

The elemental analysis was conducted at the laboratory of Waste and Resource Management at TU Wien after completion of the visual inspection. Carbon (C), Hydrogen (H), Nitrogen (N) Sulfur (S) and Oxygen (O) contents for 10 samples taken at the effluent as well as the sorted microplastics (visual inspection) were determined. In total 11 samples have been analysed, 10 samples free from microplastics, selected during the visual inspection and one additional sample, concentrating all the microplastics found during the primary step of analysis. The idea behind this approach was to investigate, if a comparable higher amount of C within the microplastics, separated during visual inspection can be found. This was done in order to detect microplastic pollution in wastewater samples via controlling a threshold value for C. The applied methodology serves as a comparison between the elementary composition (C, H, N, O and S) of the total amount of microplastic found and the elementary composition of the remaining organic material (activated sludge flocks) of the 10 samples taken at the study site.

In order to determine the parameter for the elementary composition of the 10 samples plus the sorted microplastics, the analytical method was as follows:

As an initial step, the samples were pulverized using a disc vibrating mill (Fritsch Pulverisette 9) provided with a grinding set of agate, as well as an ultra-centrifugal mill (Retsch UZM 200) until a grain size <200 µm was reached. Afterwards the material was dried in a drying cabinet at 40 °C and stored in an exsiccator using silica gel as a desiccant.

Further analysis was conducted using an ELEMENTAR Vario Macro elemental analyser (CHNS mode, WLD) and an ELEMENTAR Vario EL (O_2 mode, WLD) elemental analyser. The procedure was repeated twice.

The obtained results for the elementary composition parameters expressed as a percentage are arithmetic mean values. The given values for both, the microplastics and the remaining material will be compared to elementary composition content of polymers (PET etc.), serving as a benchmark for elemental composition of plastic particles.

For data analysis, the Statistical Software R was used. The results obtained from elemental analysis were accomplished through a hypothesis test (One sample t-test) in order to determine the p-value to demonstrate the probability of a derivation of the results measured for microplastics (taken as a reference value) from the elemental analysis of sorted samples. The significance level α has been defined at 0.05 ($p \leq 0.05$).

Furthermore hierarchical cluster analysis (average linkage) has been used for data analysis. Apart from 10 samples taken at the effluent of the WWTP, as well as the sorted microplastics, selected fibres have been analysed in order to compare the obtained results to the elementary composition (C,N,S,O,H) content of polymers.

6. Results

The results are split into results obtained from the visual inspection and results from the elemental analysis. The two procedures have been chosen in order to provide evidence of microplastics present in the samples taken at the effluent of the wastewater treatment plant. The outcome from two different methodologies applied, is considered as an essential finding in the course of this study. Both methodologies, visual inspection and elemental analysis, are based on a completely different approach. Hence their results can not be directly compared to each other, but based on the survey and the findings, their suitability as an analytic process for general microplastic research, and not solely in wastewater samples.

6.1 Visual inspection of microplastics

The following parameters are accessed regarding the results obtained from visual inspection:

- the number of particles (n) detected within each sample
- the amount of water (l) pumped through the sampling device for each sample
- the amount of microplastic particles per liter of effluent water (n/l)
- the amount of water containing 1 microplastic particle for each sample, depending on the amount of particles detected within the sample, as well as the amount of water tested

Furthermore the results of visual inspection involve some information about different types of microplastic particles found.

6.1.1 Number of microplastics found (n)

The 10 samples taken at the effluent of an Austrian wastewater treatment plant differ in the amount of microplastics found. In 10 samples a total number of 126 particles were detected.

Microplastics occurred in all samples investigated. The highest amount of microplastics (n) occurred in sample I (33), and VIII (26) followed by sample VII (23).

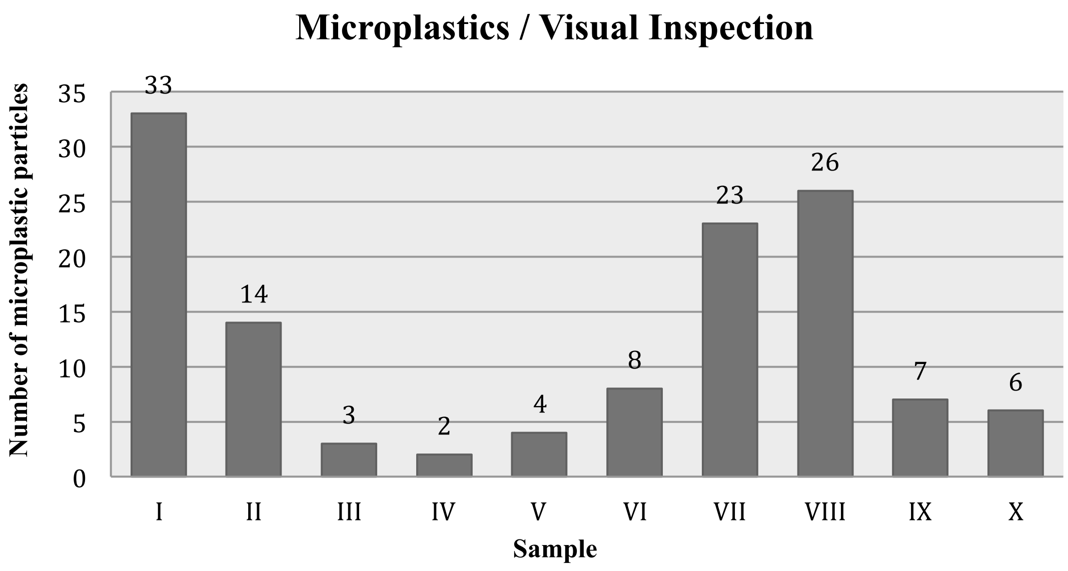


Figure 6: Microcplastics (n) found in sample I to X

6.1.2 Volume of water sampled (l)

Figure 7 shows that the amount of water investigated differs between the samples. The same amount of water has been tested for sample V to X. The mean value lies at 1,451.5 liter. The maximum value is 2,015 liter tested for sample I, the minimum value is 1,050 liter of water filtered for sample IV. In total a number of 14,515 liter of water has been filtered.

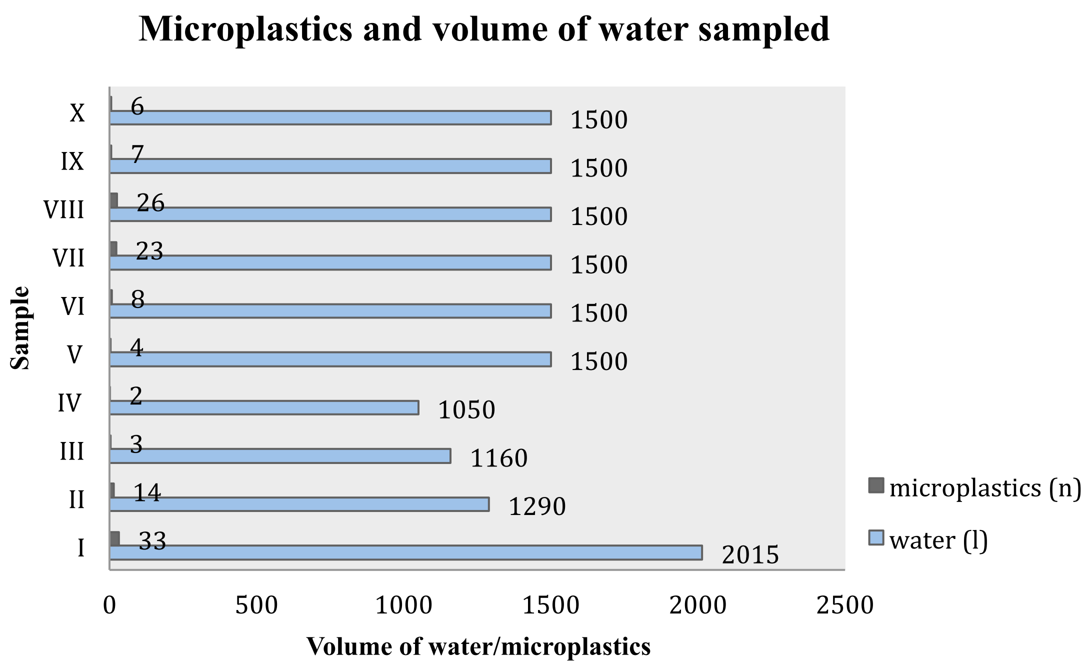
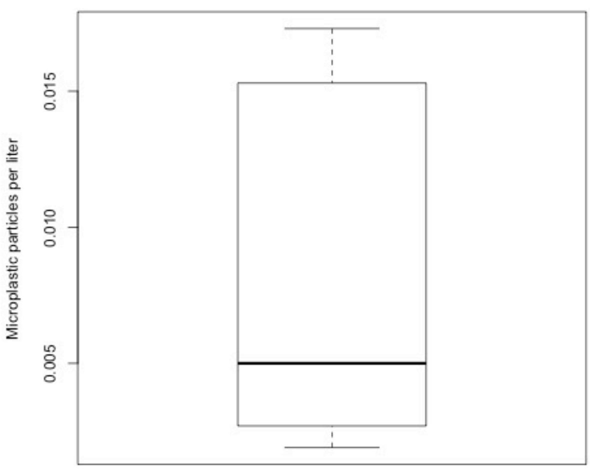


Figure 7: Microplastics (n) and volume of water sampled (l)

6.1.3 Microplastics per liter of water sampled (n/l)

The following graph shows the data obtained for the amount of microplastic particles per liter (n/l) of sampled water occurring in sample I to X presented in a Box Plot diagram including minimum and maximum value of the data, median as well as lower and upper quartiles. The calculations are based on data provided in Table 2.



	n/l
min	0,0019
lower quartile	0.0027
median	0.005
upper quartile	0.0153
max	0.0173
average mean value	0.00811

Figure 8: Microplastics found (n) per liter of tested effluent water (l) taking into account sample I to X

6.1.4 Overall data - Visual inspection

Table 2 merges all the data obtained from the visual inspection. Microplastic particles found (n), amount of water sampled (l), the amount of microplastic particles per liter of sampled water (n/l) and the amount of water containing 1 single microplastic particle. The highest number of microplastics per liter occurs in sample VIII (0.0173), I (0.0164) and VII (0.0153). An average value of 12.6 microplastic particles occurred in the total amount of samples taken. The average amount of water filtered for one sample was 1,451.5 liter of water, containing 0.00811 microplastic particles per liter. From the total amount of effluent water tested, 221 liter contain 1 microplastic particle.

Table 2: Data surveyed in the course of visual inspection

Sample	I	II	III	IV	V	VI	VII	VIII	IX	X	Mean
Number of MP particles (n)	33	14	3	2	4	8	23	26	7	6	12.6
Amount of water (l)	2015	1290	1160	1050	1500	1500	1500	1500	1500	1500	1451.5
MP particle per liter (n/l)	0.0164	0.0109	0.0026	0.0019	0.0027	0.0053	0.0153	0.0173	0.0047	0.004	0.00811
Amount of water (l) containing 1 MP particle	60.9756	91.7431	384.6154	526.3158	370.3704	188.6792	65.3595	57.8035	212.766	250	220.8629

6.1.5 Types of microplastics found

It has to be mentioned that for some particles occurring in the samples it was rather difficult to see with the naked eye whether the particle has to be categorized as microplastic or not. For those particles a high magnification (Leica DMRE) was used. Most of the plastic debris detected in the samples were coloured (blue, green, yellow, red) with sharp edges and therefore easy to assign.

The samples contained various types of organic matter such as leaves, seeds, amoeba, and clutch, making the detection of microplastics more difficult in some cases. Surprisingly no synthetic fibres were detected in any of the samples during visual inspection. Particles that appeared to be of a fibrous nature have been checked under a higher resolution. However, they were found do be of organic origin as all of them were algae. For a minor amount of microplastic particles it was possible to draw conclusions from their origin. Some plastics found were presumed to originate from nets, covering fruits or vegetables in supermarkets (Figure 9 left). In total 4 elements were found within 3 samples. Also, microbeads (Figure 9 right) were found in 6 out of 10 samples. Due to their round shape and colour they are easily differentiated from the organic matter. Results found that out of 126 particles, 7 were considered microbeads.

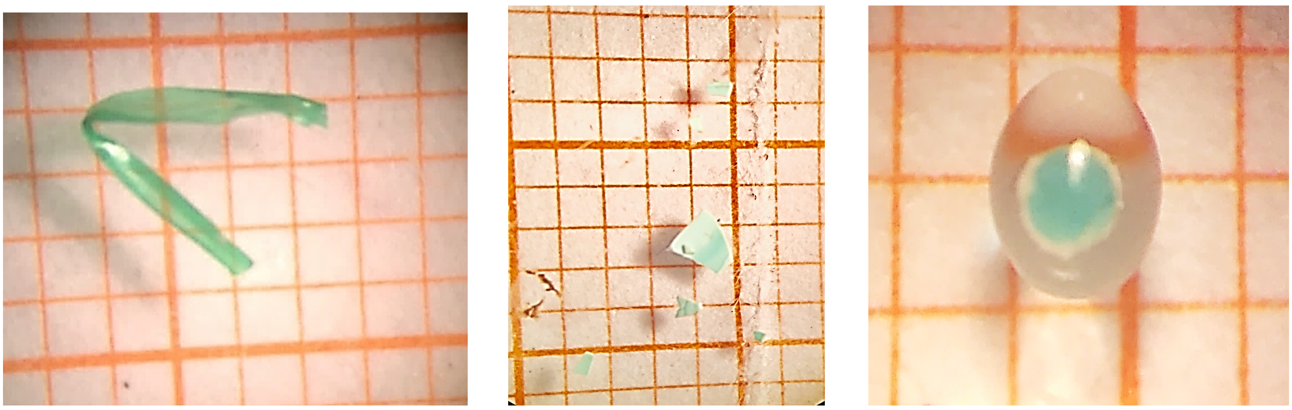


Figure 9:	left:	Secondary microplastic particle, derived from packaging, sample I
	middle:	Polygonal microplastic particles, sample I
	right:	Microbead, sample IX

6.2 Elemental analysis

The outcome from the elemental analysis showed similar results for C, S and H content in all the samples taken (Table 3). Sorted microplastics tested, resulted in 64.99% Carbon, 1.83% Sulphur and 9.77% Hydrogen values, that did not differ remarkably from other samples visually inspected before components were analysed. The same is not true for N content. The t-test shows that the obtained mean value for microplastics derivates significantly from the mean value of sample I to X for Nitrogen (p =0,0006132). Whereas the null hypothesis has not to be rejected for C (0.5485), S (0.3907) and H (0.8877). O does not allow a calculation, as it was not possible to measure the content of O within the microplastic sample as well as for sample I, II and III.

Table 3: Data obtained from the elemental analysis, sample I-X plus sorted microplastics
C: Carbon; N: Nitrogen S: Sulphur; O: Oxygen H: Hydrogen;
in % of total elemental composition

LAB ID	C (%)	N (%)	S (%)	O (%)	H (%)
I_mean	57.67	2.85	1.65		7.94
II_mean	60.41	2.27	1.13		8.63
III_mean	63.33	1.53	1.11		8.54
IV_mean	66.49	0.90	2.26	17.32	9.77
V_mean	64.65	1.28	2.66	15.30	11.16
VI_mean	62.05	1.44	2.21	17.86	9.86
VII_mean	64.73	1.31	2.12	15.17	10.80
VIII_mean	62.80	1.65	2.61	16.39	9.85
IX_mean	67.09	0.99	2.14	14.05	11.08
X_mean	63.86	1.43	1.95	15.48	10.59
Microplastics	64.99	0.61	1.83		9.77
t-test: p-value	0.5485	0.0006132	0.3907		0.8877

The results obtained from the cluster analysis including sample I to X as well as sorted microplastics are demonstrated in Figure 10. For data analysis Carbon, Nitrogen, Sulphur and Hydrogen content (%) of the samples have been used (Table 3).

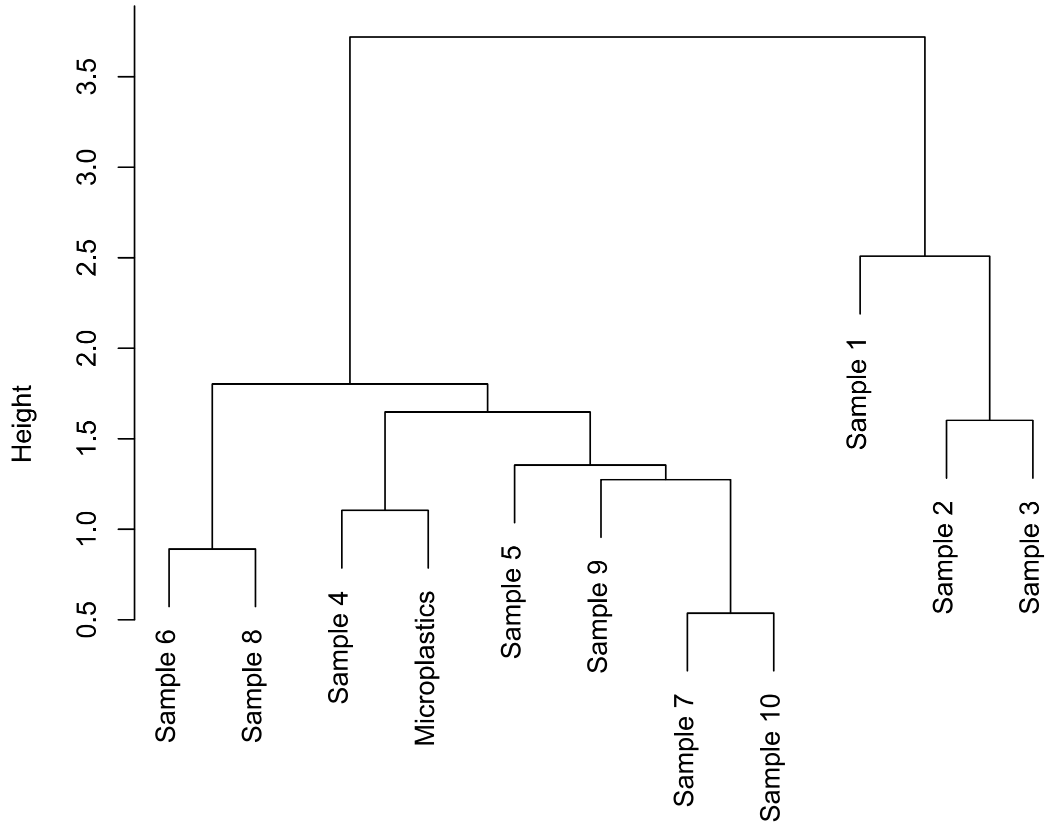


Figure 10: Cluster Analysis 1: Sample I to X including sorted microplastics
Included parameters: C, N, S, H

7. Discussion

As microplastic research in freshwater systems is a recent area of research, scientists face a lack of data and knowledge on the one side, and an urgent need for a common approach towards a standardised sampling design as well as analytical procedures on the other.

The study in hand is addressing this lack of a suitable and standardized methodology to obtain data from WWTPs. In addition, the study contributes to the identification of wastewater treatment plants as a possible pathway for microplastics into freshwater by pointing at an indication of effluent quantities being discharged.

Municipal wastewater treatment plants are considered a point source of microplastic pollution to aquatic systems. Nevertheless there is a lack of evidence, as some researchers could not confirm a significant contribution of WWTPs to microplastic pollution in rivers. The debate remains controversial and on-going (Carr et al., 2016).

While some international studies show that WWTPs are highly efficient in eliminating microplastic particles during the different steps of wastewater treatment (Dris et al., 2015; Carr et al., 2016; Murphy et al., 2016), others state that the retention capacity of wastewater treatment facilities is only limited (Wagner et al., 2014).

Therefore it was assumed that WWTPs serve as a main source of microplastic pollution of freshwater systems in Austria, as a significant amount of microplastics is discharged via the effluent.

The present study detected microplastics in all the samples taken during the experiment, although the amount occurring in the samples was much lower than primarily expected. The data collection shows an average mean value of 0,008 microplastics per liter of effluent water, or recalculated into another unit 1 particle per 221 liter of tested water.

However other parameters have to be taken into account to rate the significance of this number. The following assumptions are based on surrogate values in order to establish a model of potential microplastic pollution based on the obtained snap image to get some first data on microplastic particles in the effluent water of WWTPs in Austria.

Assuming an average value of 200 liters of wastewater per capita per day, reaching the wastewater treatment plant, the value of microplastic particles per capita per day comes up to 1.622. Considering a population of 8,700,471 inhabitants (first quarter 2016 according to Statistik Austria, 2016), the total value of microplastic particles incurring across the country results in 14.1 x 10^6 particles every day, accounting for 5.2 x 10^9 microplastic particles every year.

The chosen wastewater treatment plant only discharges a minor fraction of microplastics per liter into the freshwater systems. Due to a large amount of water being purified on a single day, even this small fraction might result in a significant amount of microplastics on a daily base.

In order to obtain information about the plant's efficiency, concerning the elimination of microplastic, samples also have to be taken from the inflow of the plant. Despite not having been the focus of this study, this is of interest in order to optimize state of the art wastewater treatment.

This study provides data on the amount of microplastics occurring in the effluent of a wastewater treatment plant in Austria and can be seen as a pilot project. Further data will be needed in order to substantiate the amount of microplastics being discharged. Different scenarios have to been taken into account, for instance intense rain. The study indicates that more microplastics occur within the samples during/after rainfall. The author suggests that this is related to the flushing of the sewer system, resulting in a higher amount of microplastics ending up in the WWTP and subsequently in the effluent too. Furthermore sampling has to be done on weekdays and on weekends, to define whether there is a variation of detected microplastic particles along the week and identify a possible peak.

One of the main concerns of this study was to test two different methods to measure microplastics in wastewater, as the sampling designs differ among studies. Dris et al. (2015) for instance used an automatic sampler for raw wastewater, settled wastewater and treated wastewater. Therefore they could analyse 24 hour average samples. The same could have been an option for this study. Samples were only taken for about 1 hour each. Extended sampling could contribute in order to investigate a higher volume of water and to obtain more data over a longer period of time. The problem of integrated samples is, that the sampling devices that are used nowadays have their limitation in sampling of particulate material from the inflow. Grab samples from the effluent as undertaken in this study on the other hand are showing a more reliable situation, as the content of suspended solids in the effluent is usually rather constant in WWTP effluents, therefore a grab sample is representative for a longer period of time.

As the impact of microplastics on the environment is not completely understood to date, the contribution of WWTPs to microplastic pollution still has to be clarified under internationally standardized approaches to measure the amount of microplastic particles being discharged. After the determination of the effluent quantities, based on long-term scientific studies, further steps can be undertaken. Technical adjustments to the plant could be an option. Nevertheless technical needs have to be based on sound findings (long term studies) on the extent of microplastics in wastewater and possible further distribution to freshwater systems rather than on spot samples or pilot studies that take along the risk of an overinterpretation of results obtained.

Clogging of screens is a common problem during screening processes in wastewater treatment, as the capacity of screens might be reduced. At the moment, there is no evidence for a different behaviour between naturally occurring particles and microplastic particles, so an additional challenge regarding WWTPs has not yet been assessed. As the amount of microplastic occurring in wastewater is only a small fraction of particulates, a contribution of adverse impacts on the wastewater treatment process may be of minor importance. Further investigation is needed to prove the concerns about microplastics right. The awareness of the present extent of pollution and the amount of microplastic particles in aquatic systems needs to be taken into account when planning new WWTPs as well as during the development of policy initiatives to manage microplastic pollution and to maintain ecosystem services (Eerkes-Medrano et al., 2015).

Microplastic research is still at its very beginnings. Scientists emphasise that in order to obtain concordance between microplastic studies, standardised methods as well as a single definition for the term microplastics are needed (Dris et al., 2015a). Whilst the lower size range limit is classified via the mesh size of the sampling device, the upper size range limit remains often unclear for several scientific studies. The applied method for measurements of particles in microplastic studies is not always clear yet not mentioned and explained in detail in diverse studies. Sieving and again visual inspection seems to be most widely used in order to obtain the size of the particles. Sometimes a combination out of the two is applied (Filella, 2015).

In order to draw conclusions on microplastics and their impact on the environment, the reliability of the obtained results has to be assessed. There are certain limitations to present techniques used to detect microplastics during counting, measuring and sampling of microplastics. The results of studies focusing on microplastics depend on the methodology being used and are therefore descriptive (Filella, 2015).

Moreover microplastic in freshwater systems might have other sources than the effluent of wastewater treatment plants. Lechner and Ramel (2015) state that the rise in awareness of plastic pollution in the environment has to be answered with the identification and the regulation of the contributing sources. Dris et al. (2015) detected the occurrence of microplastics in total atmospheric decomposition for the very first time. If this will be confirmed by long-term studies, it might be proven another relevant pathway for microplastics into freshwater systems.

Also plastic producing plants serve as a significant point source of industrial microplastics into the freshwater system. This has been proven for a plant located in Austria discharging industrial microplastics into the river Danube (Lechner and Ramler, 2015).

The study of Filella (2015) shows that scientists tend to sample in areas where the amount of plastic concentration is higher. She stresses that this kind of collection of study sites might affect the obtained results and leads to an overestimation of the subject related to adverse environmental impact.

This has to be taken into consideration when sampling in the effluent of wastewater treatment plants. The amount of microplastics in sewage is expected to be significantly higher than in freshwater systems, as microplastics are used by humans on a daily base and therefore end up in the wastewater treatment plant. What is affecting the amount of microplastics found in the effluent is the efficiency of the plant in removing those particles during the different cleaning phases. However the particles that are not eliminated by the plant will end up in the environment. For that reason highly efficient wastewater treatment plants seem to be a prerequisite to reduce the risk of microplastics entering the environmental system.

The second research question is intended to clarify the contribution of consumer products, in particular microbeads to the amount of microplastic particles ending up in the effluent. With 7 particles identified as microbeads only ~ 6% of the total amount of microplastics found, belongs to this segment. Therefore microbeads do not represent the majority of microplastic particles found in the course of visual inspection, although they were detected in 6 out of 10 samples. However, in order to avoid primary microplastics ending up in the wastewater stream, avoiding those particles is reasonable.

In the United States of America, microbeads are already banned in the State of New York, California and Illinois. Apart from legislative action, consumers have to be aware of detrimental effects of microplastics on the environment. Products containing polyethylene therefore have to be labelled correctly and adequate alternatives have to be offered. Apart from microbeads, natural abrasives might form an appropriate alternative like for instance oatmeal, sugar and husks of ground walnut. Consumer behaviour can contribute to reduce the amount of microbeads in the wastewater stream and hence avoid further distribution into the environment (Chang, 2015).

A study conducted by Carr et al. (2016) investigating 9 WWTPs in the United States of America, found the most frequent particles detected in wastewater samples originating from toothpaste formulations. According to the authors this might be linked to the popularity of a product on the one side as well as use on a regular base on the other.
For this reason the use of selected products can indeed contribute to the heightened occurrence of primary microplastic particles found in effluents. Therefore the replacement with natural alternatives might contribute to lower input scenarios.

The third hypothesis intends to clarify if elemental analysis in comparison to visual inspection provides more accurate results on microplastics occurring in wastewater samples, as a higher share of Carbon or other elemental compounds within the separated microplastics can be found.
The results confirm a significant deviation of sorted microplastics from the organic matter of the activated sludge only for Nitrogen. The results for C, S and H content in all the samples taken did not differ significantly. This is why Carbon content in wastewater samples cannot serve as a reliable proof of microplastics present in the sample. The reason for an elevated carbon content within the 10 samples checked for microplastics may be related to the limitation of visual inspection as an analytical tool for the detection of microplastics.

According to Magnusson and Norén (2014) the differentiation between particles of anthropogenic origin and natural particles is easy, due to different morphological features.

However, the present study showed that some of the particles detected in the samples were difficult to assort to either the plastic fraction or to organic matter. As the samples were only checked by microscopy, the fraction of microplastics not accessible to the microscopic investigation cannot be taken into account. This might result in a higher value of C content of the total elemental components when conducting an elemental analysis of the remaining organic matter.

Scientists claim that visual inspection might lead to the missing or misidentification of microplastic particles. The obtained results rely on the observer and the outcome of the observation can vary between different examiners. One test resulted e.g. in three different numbers for microplastic particles detected in one sample, when observing the sample by three different observers (Filella, 2015).

Furthermore it has to be stated that no microplastic particles of a fibrous nature were found within the samples tested. According to Nerland et al. (2014) wastewater is a source of fibrous plastic particles entering the environment via the effluent.

Nevertheless particles of a fibrous nature are expected to be < 1mm in diameter and maximum a few mm in length. The experiment was focusing on microplastic particles in the size range between 1 and 5 mm.

A study conducted by Magnusson and Norén (2014) showed a very high retention capacity for all different shapes of microplastics including fibres. Fibres were most efficiently cleaned comparing data from the inflow and the effluent but still made up 49% of the microplastics (≥ 300mycrometer) found.

Studies show an indication of a significant contribution of laundry to the amount of fibres in wastewater treatment whereas data on the size in particular thickness and length of synthetic fibres are almost non-existent (Magnusson et al., 2016). The continuation of the fragmentation process of synthetic fibres to the nano scale is possible but yet has to be quantified (Nerland et al., 2014).

In order to compare the results obtained from elemental analysis to the elementary constitution of synthetic fibres, some selected fibres are listed in Table 4.

Table 4: Elemental analysis of selected polymer fibres
C: Carbon; N: Nitrogen S: Sulphur; O: Oxygen; H: Hydrogen;
in % of total elemental composition

	C (%)	N (%)	S (%)	O (%)	H (%)
PET fibres	62.5	0	0	33.3	4.2
PCDT fibres	70.06	0	0	23.33	6.61
Nylon 6	63.69	12.38	0	14.14	9.8
Nylon 6.6	63.69	12.38	0	14.14	9.8

To detect potential similarities between the elemental analyses of sorted polymer fibres and the data obtained from the samples taken at the effluent, again a cluster analysis has been performed. Figure 11 shows the analysis including sample I to X, sorted microplastics (Table 3) as well as selected polymer fibres (Table 4). The data analysis was conducted, taking into account C, N, S and H content (%) of the elemental analysis.

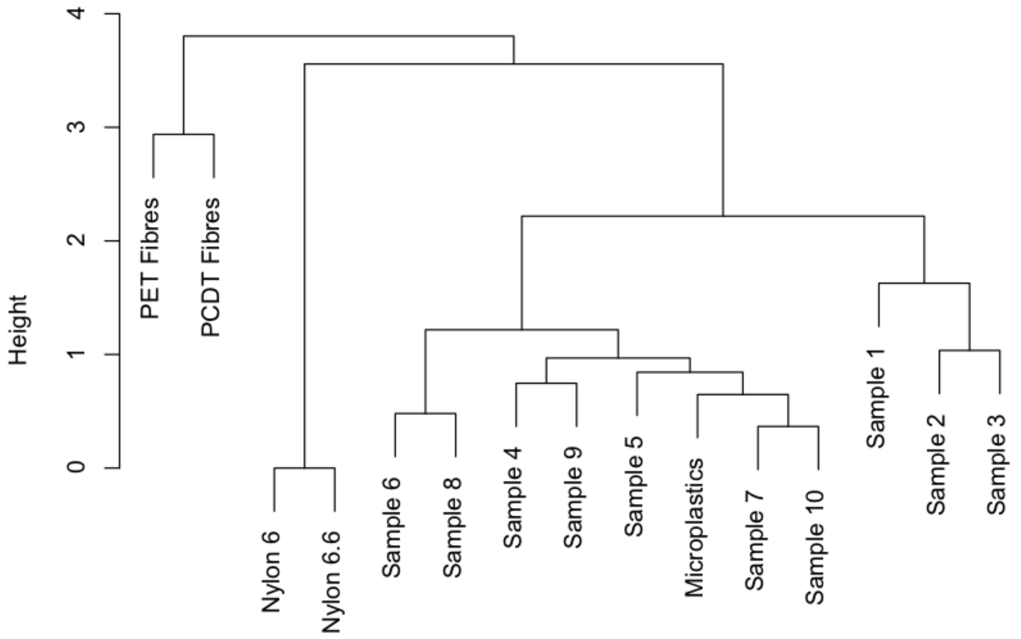


Figure 11: Cluster Analysis 2: Sample I to X, sorted microplastics and selected polymer fibres. Included parameters: C, N, S, H

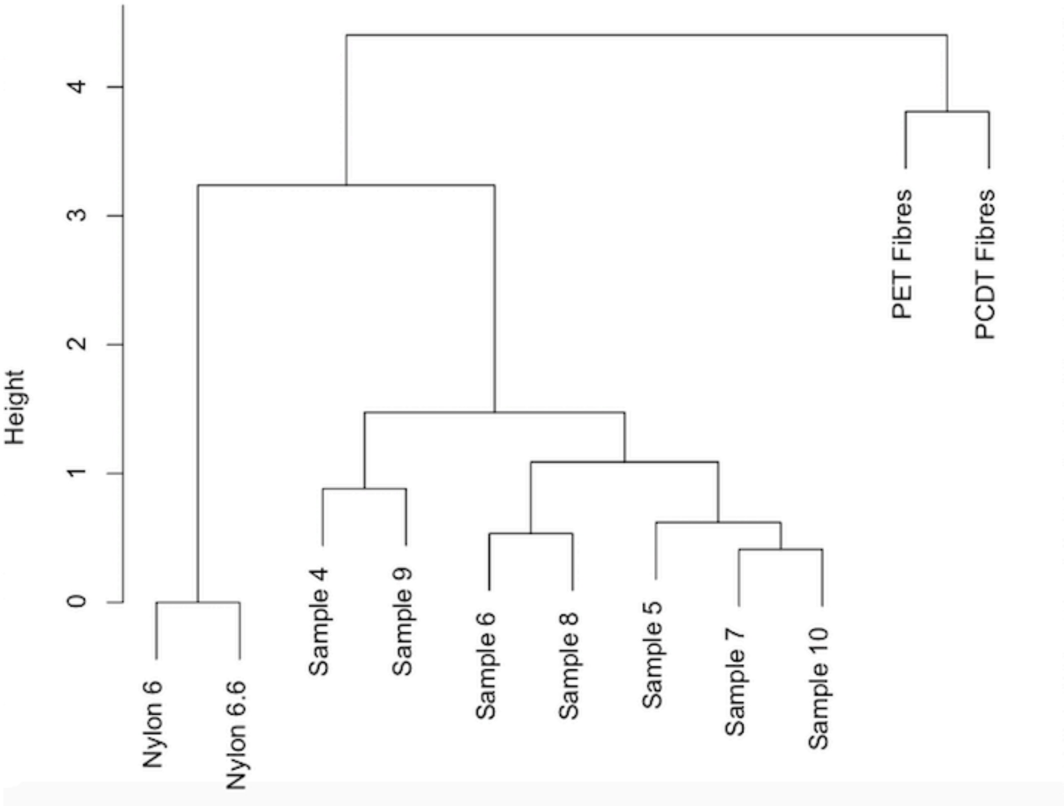


Figure 12: Cluster Analysis 3: Sample IV to X and selected polymer fibres
Included parameters: C, N, S, O, H

Figure 12 represents the cluster analysis including sample IV to X and selected polymer fibres. The following parameters have been taken into account: Carbon, Nitrogen, Sulphur, Oxygen and Hydrogen.

The results show that there is no indication of contamination with synthetic PET and PCDT fibres. However, the proximity of the data for Nylon and Nylon 6 is more related to the samples taken at the effluent, taking into account the outcome of elemental analysis. Having said that, it has to be mentioned that a minor contamination of samples is possible due to the limitations of the methodology (visual inspection) when it comes to the objective evidence of small-scale microplastic particles (<1mm).

Even if microplastics became a topic that received attention in media on a regular base, basic data on the status of contamination of freshwater systems is lacking. Initial research indicates that the dimension of pollution is comparable to marine environments. The identification of sources, fluxes and fate as well as impacts of microplastics and associated chemical substances on freshwater systems is of high priority. Microplastic research is an emerging scientific field. Most of the studies available in this area are pilot projects. While methodological heterogeneity is high, the comparability between the studies is low. Developing an adequate, automated and standardized methodology for sampling is a challenging task but will be of utmost importance in order to enhance comparability of studies (Dris et al., 2015a).

The link of microplastic pollution to wastewater treatment plants still has to be scientifically proven. Plastic particles present in wastewater generally differ from microplastics found in freshwater systems since primary sources differ. These kind of particles mostly originate from consumer packaging as well as industrial garbage undergone a breakdown due to chemical and physical processes. These factors are not influencing microplastics in wastewater treatment, as they are absent. Furthermore the cosmetics and beauty product industry is aware of environmental harm of products containing microplastics. Apart from proposing a ban for these products, microplastic particles have already started to be phased out and replaced by natural products (Carr et al., 2016).

From a global point of view, freshwater is a resource under pressure. Freshwater demand is rising. Ecological quality might be threatened due to pollution of freshwater systems. In Europe around 50 % of surface waters are rated of poor ecological quality, hence identifying sources of water pollution as well as surveillance will become even more important. The Water Framework Directive of the European Union aims at a control of water pollutants whereas this includes matter in suspension (Eerkes - Medrano et al., 2015).

According to Eerkes - Medrano et al. (2015) microplastic pollution in freshwaters is so far not substance to any regulation.

Nevertheless in Austria the Water Rights Act 1959 is addressing targets towards protection of surface water bodies as well as groundwater. For rivers the „good ecological and chemical status“ has to be reached. The water policy in Austria is following the European Water Framework Directive, aiming at reorientation according to water and its protection policy. Proper disposal of wastewater is mentioned explicitly as a key issue of the water protection policy in Austria. Referring to the precautionary principle, state of the art wastewater treatment is obligatory. When necessary more far reaching requirements on the performance of purification have to be applied in order to protect water bodies (BMLFUW, 2015).

8. Conclusion

Environmental concerns due to microplastic pollution are rising on an international scale. Not only marine systems but also freshwater environments are affected. As research has only started recently, more information and data are needed in order to answer open questions and to scientifically proof presumptions regarding the degree of pollution in freshwaters and the possible adverse environmental effects, especially highly discussed for biota and in consequence on humans. This is why the identification of all sources and their extent of contribution to microplastics ending up in the freshwater environment is an absolute necessity.

Further studies have to be conducted to prove whether wastewater treatment plants serve as a significant point source of microplastics being discharged into the environment. The study in hand could provide evidence of microplastic particles occurring in the effluent of the observed wastewater treatment plant. Even though the amount of particles per liter was low it was not negligible.

Summing up the main finding of the study, the following points have to be deduced:

- At the moment no adequate and standardized methodology to measure and analyse microplastics in wastewater samples exists. The development of adequate measurement devices, a repeatable measurement technique as well as consistent analytical procedure is a prerequisite to quantify the amount of microplastics in wastewater and will play a key role in further research. The research design has to be replicable and easy to apply, as microplastic sampling and its current methodologies, in particular visual inspection is very time consuming. As hardly any study on microplastics in wastewater as well as microplastics in Austrian freshwater systems is available by now, a comparison and accordingly an evaluation of the obtained results is not possible.

- The amount of microplastics being discharged per liter of effluent water (arithmetic mean value 0,008) seems marginal. Nevertheless the amount discharged on a single day might still contribute remarkably to microplastic pollution of freshwater systems due to high amounts of treated wastewater. Surveillance and expanded studies have to be conducted. Sampling in the inflow, sewage sludge as well as the natural aquatic system will contribute to the understanding of microplastic pollution, its effects and its extent. This has to be done for reasons of precaution, will ensure the prevention of adverse environmental effects and hence will support the protection and maintenance of the ecological status of freshwater systems not only in Austria.

- Visual inspection turned out to be the favoured analytical tool in this study. Nevertheless there are downsides of this method, experienced during the project. One has to be aware of the inaccuracy when it comes to identification of smaller particles (fibres, nanoparticles etc.) and the risk of misinterpretation. Whilst component analysis is not suitable for the detection of a threshold value of C in wastewater samples, further research is in progress in order to detect microplastic pollution with state of the art chemical analytical tools. This will contribute to more accuracy in microplastic research in the near future.

- Plastic pollution is an environmental concern of modern life. In order to tackle microplastic pollution, macroplastic pollution has to be faced too as this is a significant source of secondary microplastic. Apart from boosting recycling on a global scale, careless disposal of plastic waste has to be reduced. As most of the microplastic particles found within the plant were of the secondary microplastic segment, there is an indication of macroplastics contributing remarkably to microplastic contamination in wastewater.

- International cooperation could play a key role in achieving an effective network concerning microplastic research, also related to wastewater treatment. Initiatives have to be based on long run scientific studies, rather than pilot projects in order to obtain reliable results and subsequently find the most appropriate management tools for this pollutant. Data gaps and the lack of knowledge have to be closed before an effective management tool can be established.

The consequences of microplastic pollution in freshwater systems and adverse effects coming along with this newly established pollutant have not been fully investigated to this day. However the persistence of microplastics in natural habitats, the occurrence of high concentrations detected for some sites and the prospect of an increasing amount of microplastics ending up in the environment urges to minimize the release of plastics into natural habitats, even if environmental risks cannot be comprehensively assessed by now (Duis and Coors, 2016).

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List of tables

List of figures