Czech University of Life Sciences

Faculty of Environmental Sciences

Department of Applied Ecology

Diploma thesis

Water pollution by Microplastics generated by Washing machine

Julio Leonardo García Viruez

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

Julio Leonardo Garcia Viruez

Engineering Ecology
Nature Conservation

Thesis title

Water pollution by micro-plastics generated by washmashine

Objectives of thesis

The purpose of this research is to contribute on a bigger project related to microplastics, made by the Department of Land Use and Improvement at the Faculty of Environmental Sciences of the Czech University of Life Sciences in Prague on the analysis of micro-fiber, (a subcategory of microplastics) detachment from 100% polyester garments after laundry to learn how to determine microplastics from other materials, and attempt to estimate how much pollution is generated by microfibers in domestic use.

Methodology

Microplastics are plastic particles smaller than 5mm long of mixed shape that are present in the air, soil, freshwater, seas, in biota, and in several components of our diet. Because of fragmentation and degradation of larger plastic item, smaller particles are formed. One of the sources of their release to the environment is domestic laundry. A simple method consists on taking water samples from a washing cycle of a 100% polyester T-shirt, analyze it by a microscope, fluorescent imaging, counting software and statistics to estimate the number of Microplastics that are potentially released in the water, a serious environmental issue that might be controlled by citizens and other stakeholders effectively.

The proposed extent of the thesis

40 p

Keywords

Microplastics, Polyester, Microfiber detatchment, domestic laundry

---

Recommended information sources

BOSSIO, D. – GEHEB, K. *Conserving land, protecting water.* Wallingford: CABI, 2008. ISBN 978-1-84593-387-6.

Ha, J., Yeo, M.-K., 2018. The environmental effects of microplastics on aquatic ecosystems. Mol. Cell. Toxicol. 14 (4), 353–359.

HILL, M K. *Understanding environmental pollution.* Cambridge: Cambridge University Press, 2010. ISBN 978-0-521-73669-5.

WANIELISTA, M P. *Hydrology and water quantity control.* New York: Wiley, 1990. ISBN 0471624047.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.

Expected date of thesis defence

2019/20 SS – FES

The Diploma Thesis Supervisor

Dr. Ing. et Ing. Miroslav Kravka

Supervising department

Department of Land Use and Improvement

Electronic approval: 3. 3. 2020

prof. Ing. Petr Sklenička, CSc.

Head of department

Electronic approval: 4. 3. 2020

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 21. 06. 2020

I hereby declare that I have independently elaborated the diploma/final thesis with the topic of:
Water pollution by Microplastics generated by washing machine *and that I have cited all the information sources that I used in the thesis and that are also listed at the end of the thesis in the list of used information sources.*
I am aware that my diploma/final thesis is subject to Act No. 121/2000 Coll., on copyright, on rights related to copyright and on amendment of some acts, as amended by later regulations, particularly the provisions of Section 35(3) of the act on the use of the thesis. I am aware that by submitting the diploma/final thesis I agree with its publication under Act No. 111/1998 Coll., on universities and on the change and amendments of some acts, as amended, regardless of the result of its defense. With my own signature, I also declare that the electronic version is identical to the printed version and the data stated in the thesis has been processed in relation to the GDPR.

In Prague on date: 21. 06.2020 _____

Acknowledgements

I would like to thank my supervisor Miroslav Kravka for all the guidance provided during the making of this research, as well as Mrs. Lenka Wimmerova for the assistance in the Laboratory and Martin Lexa for the support with the image processing.

Abstract

Microplastics (synthetic polymers <5mm) which can be found in many types, shapes and forms are an increasing problematic because of their ubiquity in high amounts, they may represent a high risk to ecosystems and biota. Microfibers of synthetic textiles, which are considered a subgroup and a main source of microplastics are detached from domestic laundry in almost every home in the developed world. Many studies have been made in this topic, however there is no standard methodology on the quantitative data. In consequence this research attempts to test one simple methodology by software counting and compare the results with the available literature. A mean of 1474 Microfibers were found in each wash for one garment, meaning 0.093 Mg in weight or 0.003% of the garment mass. This numbers were adjusted to previous estimations and converted to the population of Czech Republic in approximately 2 tons of Microplastic release into the water systems only by washing our clothes.

Keywords

Microplastics, Polyester, Microfiber detachment, Domestic laundry

Table of Content

Index of Figures

Index of Tables

Glossary

DDT= Dichlorodiphenyltrichloroethane

FTIR= Fourier-transform infrared spectroscopy

MP= Micro plastic

MF= Microfiber

MP-VAT= Microplastic visual analysis tool

NOAA= National Oceanic and Atmospheric Administration

PE=Polyethylene

PET= Polyethylene terephthalate

POPs= Persistent Organic Pollutants

PP= Polypropylene

PS= Polyester

PVC= Polyvinyl Chloride

RPM= Revolutions per minute

WTP= Water treatment plant

WWTP= Waste water treatment plant

1. Introduction

Plastic pollution has been an environmental issue of increasing concern in the past decades. Since its mass production, consumption and of course its waste has increased dramatically (Plastics—The Facts 2016). Nowadays a new issue is a matter of concern, the presence of small polymer debris of less than 5mm of longitude (Wright et al, 2013), including fragments, particles and fibers are found in vast quantities almost in every corner of the earth. Originated intentionally (primary production) or by degradation from larger plastics (secondary production), (Ha and Yeo, 2018), The accumulation of MPs is followed by high environmental risk and eventually the impact towards living organisms when confused by food, damaging and or obstructing the digestive system and after death by starvation (Browne 2013), at the same time, they can accumulate toxic substances such as Persistent Organic Compounds (COPs) and organochlorine pesticides such as DDT, In addition, from all of MPs that ends up in the ocean, approx. 80% of it originates from continental sources (Andrady, 2011), recent studies identify rivers as major pathways of land-based microplastics (Castaneda et al., 2014; McCormick et al., 2014). In this matter Textile microfibers (MFs) are a primary microplastics sub-group, which along products of personal care are the main source of primary microplastics in water ecosystems. MFs are mostly shed when laundry of any garment takes place, making these fibers a potential main source of pollution.

Increasing Concern in this matter has been taking place in the last years, many studies have been made towards the analysis and counting of microfibers detachment by domestic laundry (Browne et al., 2011; Salvador Cesa et al., 2017), However, given the high number of variables that could take place when trying to analyze quantitative data of synthetic MFs after laundry, many different methodologies can be applied and even now a days there is no standard, nevertheless many methods coincide in taking the sample directly after the washing cycle, dry it, reduce the organic matter and impurities and analyzing it for later counting, then estimating the amount of microfibers obtained by a spectroscope or a microscope and finally

estimating the amount of microfibers detached (Hernandez, 2013; Belzagui et al., 2019). In 2019 Prata et al. have developed a plugin in ImageJ that counts particles from images taken by spectroscope to samples stained with fluorescent ink, making the quantitative analysis easier for future research work. This research project attempts to provide a simple methodology with the software counting of fluorescent stained MFs and compare the results with available literature.

2. Aims of the study

To evaluate the fiber detachment in quantity from a 100% polyester garment after several domestic laundry cycles by testing lipophilic staining with Nile red and software counting MP-VAT and compare the results with available literature

2.1. Specific Aims

- To use Nile red staining on the detached material with luminescence under 365nm UV light for imaging samples for posterior software counting
- To count microfiber detachment with a specific plugin for ImageJ of one 100% polyester made T-shirt after washing it in order to observe any trend in increase or decrease of number of microfibers release.
- To read, understand and compare the results with available literature.

2.2. Hypothesis

- Less than 5mm Microfibers are detached in high quantities from 100% polyester garments after domestic laundry when new
- Microfiber detachment trend should decrease until the fifth washing cycle
- MP-VAT plugin for Imagej software is an accurate tool for counting microfibers after Nile red staining and Ultra Violet light picturing.

3. Literature review:

3.1. Pollution:

Pollution is defined by the excess of concentration of a substance in an ecosystem, which produces a toxic effect on living beings. A pollutant is any substance or form of energy which can produce harm or unbalance (reversible or not) in an ecosystem or the environment of a living being (Bermudez 2010). This toxic substances can cause damage to the air, water, land and bio accumulate in living organisms at the same time, causing damage to their organisms.

3.1.1. Sources of pollution:

The sources of pollution could be natural or caused by human activity, in general the natural sources of pollution are related with the composition of water and soils, components of food, volcanic emanations, and others. This type of natural pollution is low incidence on public health in comparison with the human caused pollution, nevertheless, volcanic eruptions produces high amounts of pollutants to the air, land and even to the water in short periods of time, putting both public health and ecosystems at risk (Albert, 1997).

Anthropogenic sources of pollution are ironically the main problem for us humans and environmental health. Many sources and types of pollutants exists they can be classified by the activity that originates them (Albert, 1997):

• Industrial

• Mining

• Agricultural

• Craft

• Domestic

On the other hand, in the particular case of air pollution they are further classified in (Albert, 1997):

• Fixed

• Mobile

Finally, depending on their location, they are occasionally also classified as scattered and localized sources, which in some documents are called point sources of pollution.

3.1.2. Types of pollution

According to its origin, pollution can be caused by natural events or by the activity of man. The pollution produced by man includes the emission of gases from industries and automobiles, the elimination of household and industrial waste, oil spills into the sea, etc. However, it can be affirmed that pollution of natural origin is not as important as that caused by man since, in fact, it is directly or indirectly responsible for any type of environmental alteration (Spiegel, 2001).

3.2. Water pollution

According to the World Health Organization (2004), water is polluted "when its composition has been modified so that it does not meet the necessary conditions for use, which would have been destined in its natural state." The water that comes from rivers, lakes and streams is subject to severe pollution, often the product of human activities. Also the World Wide Found of Nature states that water pollution can be caused in many ways, from the large sewage of a big city, the leak of fertilizers and/or pesticides in ground water because of agricultural activities, or the simply use of a plastic bottle in the shores of a touristic place. There are a large number of water pollutants that can be classified in very different ways. A widely used possibility is to group them into the following eight groups (Echarri, 2007):

• Pathogenic microorganisms

• Organic waste

• Inorganic chemicals

• Inorganic plant nutrients

• Organic compounds

• Sediments and suspended materials

• Radioactive substances

• Thermal pollution

3.2.1. Surface water pollution

Covering 70% of the surface of earth, surface water pollution is the most common type of pollution, happening in freshwater (rivers or lakes) and in oceans or seas. According to the UN every day, 2 million tons of sewage and industrial and agricultural waste are discharged into the world's water (UN WWAP 2003), this amount is the equivalent of the weight of the entire human population of 6.8 billion people.

Figure 1 The Great Pacific Garbage Patch located between Hawaii and California. It covers an approximate surface area of 1.6 million square kilometers. An area 3 times bigger than France (Source: Forbes magazine https://www.forbes.com)

Because of numbers like this and many more facts, ocean and sea pollution concern has been increasing during the last decade, but also it is good to mention that more than 80% of the ocean pollution comes from land sources where the main pollutant is plastic. Plastic that ends up in freshwater like rivers, in this matter because of the high biodiversity loss and the very slow

decaying of this material, it is estimated that by 2050 there will be more plastic than fish in the oceans. (Galafassi, 2019)

3.3. Plastics

Plastic in history is the term that means “pliable and easily shape” but, generally this term is the name of a category of materials called “polymers”. These polymers are from organic origin because they come from carbon, cellulose, natural gas and last but not least crude oil, but coming from an organic source does not need to mean that it can be degraded easily, p In the present time, plastic is one of the most used materials in the world, for its durability, malleability, impermeability, and its low cost of production. Its making process starts during the distillation of oil in a refinery, then it is separated into hydrocarbon chains of different molecules forming different polymers, the key fraction for the production of plastics is diesel. Approximately 4% of the world's oil production is used for the manufacture of plastics (Barnes et al., 2009; Thompson et al., 2009). Plastic Europe, the leading trade association for the plastics industry in Europe, describes plastics as a wide range of synthetic or semi-synthetic materials that are used in an increasingly growing range of applications. (Plasticeurope.org, 2018).

3.3.1. Early plastics

During the 19th century, because of their hardness, ivory and shells were used to make hard and durable products like keyboards of piano and billiard balls, although they had a high price and their obtaining was difficult (Knight, 2012). In the middle of that century, some scientists sought to replicate the consistency of ivory and shells and that it could also be done quickly at a low cost. In 1856, the British Alexander Parkes introduced the park (Brydson, 1999), registered years later as a celluloid. This is the trade name of cellulose nitrate, considered to be the first invented thermoplastic (Gilbert, 2017; Bensaude Vincent, 2013). Despite its malleability, the celluloid did not have the expected impact and its production was incomparable with that of iron, glass and cotton, probably because it was always seen as an imitation (Bensaude Vincent, 2013). At the end of that century, he began to

experiment with casein and formaldehyde. Until then, the raw material of plastic was natural (Knight, 2012; Brydson, 1999).

The research led to the production of new plastic materials, then by the beginning of the 20th century, shellac, gutta-percha, ebonite and celluloid were already available (Gilbert, 2017). Soon, it was discovered that formaldehyde can form resinous substances (Brydson, 1999). In 1909, Leo Baekeland developed and patented bakelite. This is considered the first synthetic plastic and was made from formaldehyde and phenol, a carbon waste (Harper, 2000). Materials like this one were the first commercially successful synthetic plastics, nevertheless it is not as versatile as those formulated in later years (Gilbert, 2017).

Figure 2 Old rotary telephone made from Bakelite. (Source: https://rubytuesdaysvintagehome.com)

Plastic polymers that are used in the present are totally synthetic and relatively recent, nowadays there are many types of plastics, and we can classify them into two main groups:

Thermoplastics: That are the most commonly used, they can be repeatedly rendered ductile as a function of temperature (polyethylene, polyvinyl chloride, polypropylene and polystyrene)

Thermosetting: Cannot be reshaped after curing (polyurethane or phenol-formaldehyde)

Plastics include materials whose principal constituent is polymers. In addition to these organic macromolecular substances, there are other additives present in the plastics, which are there to modify the properties of the plastic. During the making process, plastic undergoes at least one plastic state (Plasticeurope.org, 2018). All plastics are made of polymers, but not all polymers can be classified in plastics (Verschoor, 2015).

The production began to grow around the decade of 1930s (Knight, 2012). In this decade and the next the industrial development of the four most common thermoplastics occurred today (Gilbert, 2017)

The popularization of plastic is attributed to the successful marketing campaigns of chemical companies in the United States. The feeling towards plastic as a cheap imitation was changed to an innovative source of social stability (Bensaude, 2013). Its introduction to the market gradually replaced the use of other materials and allowed the creation of new products (Brydson, 1999).

One of the fundamental characteristics of plastic is that it is created and molded simultaneously, this action represents a saving of time and resources (Bensaude, 2013). Since the popularization of the use of plastics after the Second World War, the possibility and ease of creating new components revolutionized the life of the human being. The plasticity of these polymers, that gave its commercial name, has been a source of empowerment for industrial designers, leading to the creation of new types of artifacts, buildings and even textile fabrics (Bensaude, 2013). In 1951 polyester was introduced, an innovation in the textile industry, (it will be discussed more in deep in this document) (Knight, 2012). Ten years later in 1961 strong and light materials were made for spacecraft (Bryson, 1999). Also during the 60s there was a change in the use of coal to oil, that is plastic raw material, which is still used nowadays (Brydson, 1999). Currently, the plastics industry is closely linked with the oil industry (Gilbert, 2017), which has been shown to be one of the main sources of negative effects on the environment (Wake, 2005).

3.4. Microplastics

Every material degrades depending on their inner structure and the environment where they are, the time of degradation can be more or less. Plastic is known to be one of the commonly used materials that last longer in degrade, ranging from some months for a party balloon up to hundreds of years for a PET bottle. But eventually plastic will degrade. Once plastic becomes waste the degradation process starts, big particles of plastic are broken into smaller pieces either by mechanical movement, by temperature or other environmental factors that can affect the composition of plastic, the released smaller particles can be from many sizes and they are now the new big concern in the world.

This synthetic polymers debris with a size less than 5 mm, generally are defined as microplastics (MPs) by the National Oceanic and Atmospheric Administration (NOAA), their abundance and the high environmental risk they represent, mainly towards the marine ecosystems are the reasons why more attention has been paid to this matter. (Andrady, 2011). MPs occurrence is not a new topic, concern about it started in the last decades of the past century, as the first reports of MPs in marine environments were published in the early 70's (Andrady. 2011). However the attention towards MPs began to grow when studies started to show the environmental impact they can cause, mainly in biota.

3.4.1. Occurrence

The occurrence of MPs was studied mainly in marine environments, estimations have reported 15 to 51 trillion suspended MPs in the oceans, and at the same time statistics models predict that 21% and 54% of global MPs, equivalent of 5% and 10% of the global mass are located in the Mediterranean sea. (van Sebille et al., 2015), nevertheless these estimations are believed to be only the "tip of the iceberg". As there is a lack of data of microplastic counts in the southern hemisphere like in the coasts of America and Africa. (UNEP and GRID, 2016). See (Figure 3) Below.

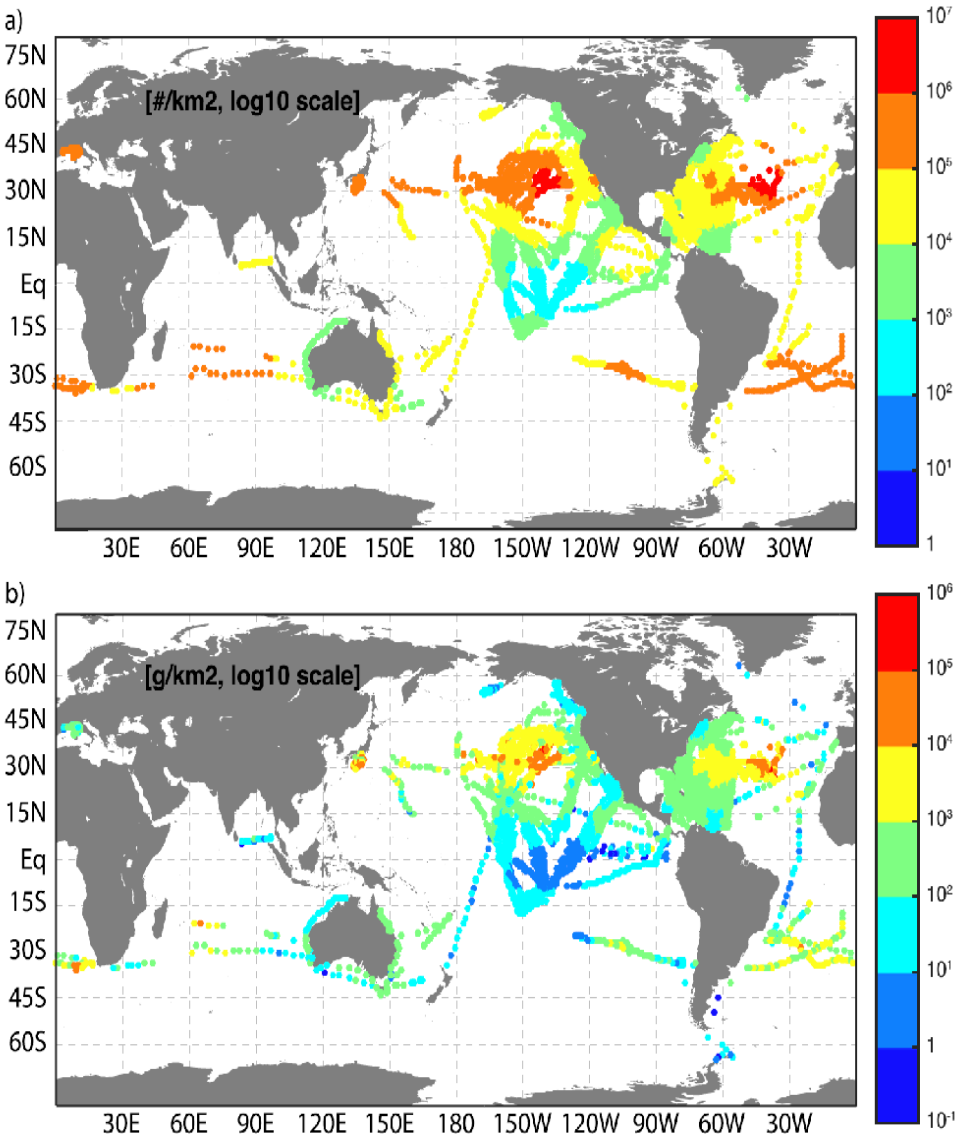

Figure 3) Location and standardized microplastic count (b) microplastic mass of all surface trawl data used in Van Sebille analysis, on a log10 scale. Standardization is done with respect to year of study, geographic location, and wind speed. (van Sebille et. al. 2015)

However it has been proven that the amount of MPs is increasingly growing in freshwaters, terrestrial and atmospheric ecosystems (Leslie et al., 2017; Dris et al., 2016), besides the high amount of microplastics that is present near coast lines around the world, there are studies that suggest MPs have even reached remote places far from anthropogenic influence from the bottom of the Marianas Trench, the top of the Everest mountain (C'ozar et al., 2015; Woodall et al., 2014), and even microplastic has been found in the snow and ice of artic and antartic glaciers. (Imhof et al., 2017; Peeken et al., 2018).

A study made by Pivokonsky et al. (2018) in Prague, showed the ubiquity of microplastics even in drinking water (Figure 4), after they found microplastics of less than 10µm of size in all samples of three different water treatment plants (WTPs) in Czech Republic. Fragments and fibers were predominant in one of the 3 WTP, and the materials that were most common were PET (polyethylene terephthalate), PP (polypropylene) and PE (polyethylene).

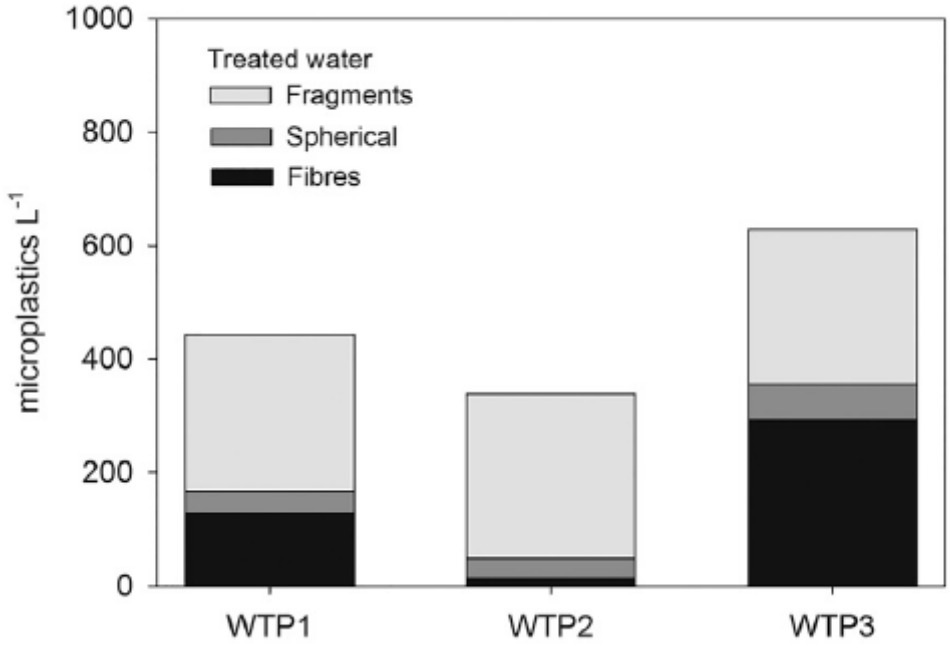

Figure 4 Number and proportion of Microplastics in drinking water from 3 water treatment plants (WTP) in Czech Republic (Pivokonsky et. al., 2018)

3.4.2. The problem with microplastics

The ubiquity of microplastics in every place on earth makes it a very big and important problem itself, but it has been found high risks in the environment regarding the presence and the accumulation of MPs. The ingestion of these plastic particles by biota is well registered (GESAMP, 2015), especially in marine organisms, where MPs have been identified in all levels of the trophic chain (Collignon et al., 2012; Fossi et al., 2014). However, it also extends to organisms of other ecosystems (Zhu et al., 2018; Huerta Lwanga et al., 2017).

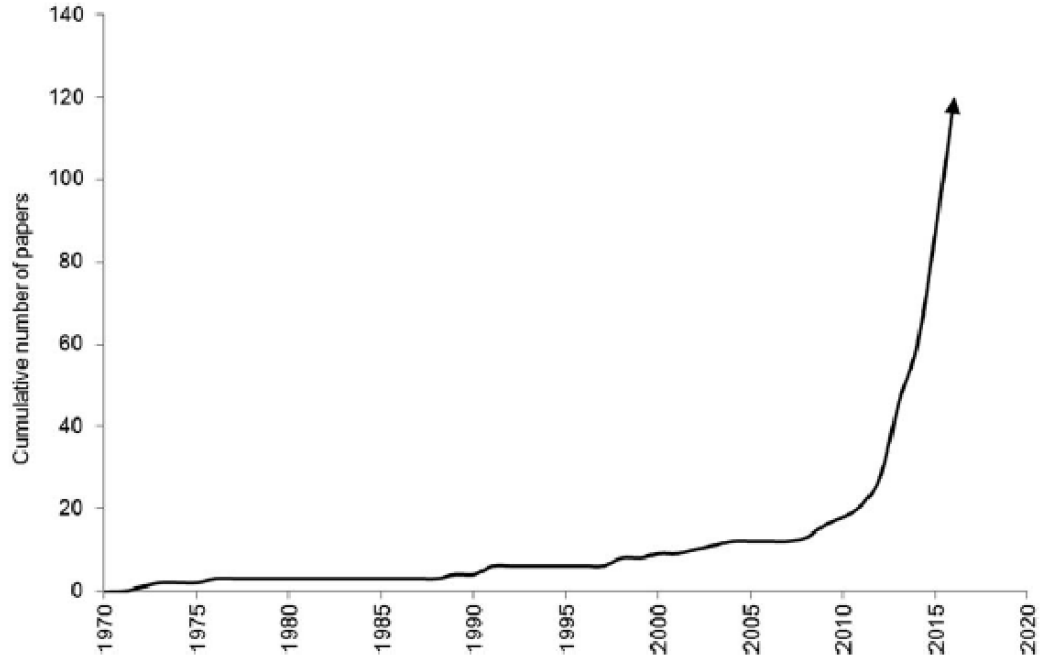

Figure 5 Number of publications regarding microplastics and biota (Lusher et. al. 2017)

Until now the possible documented impacts of MPs in biota are when they are retained in the living organisms body (Welden and Cowie, 2016) and by being retained in small animals MPs are transferred through the trophic chain (Nelms et al., 2018), at the same time retained MPs can reduce vital functions capacity and metabolism (Jin et al., 2018; Lei et al., 2018; Murphy and Quinn, 2018), but also MPs can be translocate to other organs and accumulate (Brennecke et al., 2016), produce gene exchange in unicellular organisms. (Arias-Andres et al., 2018), endocrine disruption (Rochman et al., 2014), increased mortality (Jemec et al., 2016), bioaccumulation of toxic chemicals (Rochman et al., 2013), altered sinking rates for fecal material (Cole, 2016) among many other impacts, MPs are also known to act as vectors for alien species and for hydrophobic contaminants (either added in the plastic manufacturing process or adsorbed once in the environment) (Rochman, 2013; Browne et al., 2013), and to alter physical properties of beach sediments.

3.4.3. Classification of microplastics

All literature agrees that exists two main groups of MPs:

Primary Microplastics: Are polymers that are manufactured as MPs, for cosmetics or beauty purposes mainly, besides cosmetic purposes, primary microplastics can be found in sprays, also added on abrasive materials, or microfibers that are part of the textile industry. (Andrady, 2011)

Secondary Microplastics: Larger plastic debris can degrade into micro-sized particles over time with exposure of high temperatures, oxygen, water, other substances and physical contact with harder materials. The appearance and shape of the secondary MPs vary widely, making it difficult to quantify and separate MPs from natural particles. There are three primary categories of MPs (Hernandez et al. 2017):

- **Microfibers**, usually the most common type of microplastics, are derived from synthetic textiles and slough off during daily use and machine washing of clothing (e.g., fleece jackets). Most microfibers released into water are between 0.1–0.8 mm in size.
- **Fragments** form as a result of physical breakage of macro-plastics.
- **Microbeads** are common in personal care products (Hernandez et al. 2017).

3.4.4. Sources of microplastics

According to (Galafassi, 2019) From all the microplastics that are being found in the marine ecosystems, at least 80% of the come from a land sources, like shorelines, rivers or air transportation, therefore the remaining 20% comes from sea-based human activities. From all the land-based sources there are 4 main it can be discussed in this literature review. (Figure 6)

3.4.4.1. Personal care products

The intense use of plastics that surged during the last quarter of 20th century involved all industrial sectors, including personal care products more commonly called cosmetics. Creams, body lotions, skin peeling, make ups, eyeliner, etc. appeared and started to be used everywhere during the decades of 60s and 70s.

Plastics began to replace natural ingredients in scrub and body peeling formulations, because of better dermatologic properties (Chang, 2015). Microbeads used in body peeling formulations are mainly made from polyethylene, they show great variety of shapes such as smooth and spherical to completely irregular fragments (Fendall and Sewell, 2009). The Microbeads used in personal care products have a roughly standard size, which is not greater than 0.5 mm and frequently closer to 0.1–0.2 mm (Chang, 2015; Fendall and Sewell, 2009) and the concentration varies in depending on the type of product that they are found. From 0.4% to 10.5% of the formulation (Strand, 2014).

The products where Microbeads can be found are not only soap and body peeling products, toothpaste, shower gel, shampoo, eye shadow, deodorant, blush powder, skin cream, liquid makeup, mascara, shaving cream, facial cleanser, bubble bath, lotions, hair coloring, nail polish and sunscreen have been reported to be another major sources (Conkle et al., 2018; Hintersteiner et al., 2015; UNEP, 2015). Another use for the incorporation of MPs as ingredients in cosmetic formulas is because they regulate viscosity, opacify color, bind liquid absorbents, and glitters. (UNEP, 2015).

Cosmetics Europe or also called European Cosmetic Industry Association in collaboration with Euromonitor International made a survey in 2015 calculating the total annual use of MP beads of the countries within the European Union, plus Norway and Switzerland, resulting in an amount of 4130 tons of plastic material used only in soap. (Gouin et al., 2015). Another analysis made for German women habits estimated a total of 6.2 g/year per capita meaning the emission of approximately 514 tons yearly only on the

use of shower gel and soap. But of course every country has different approaches and consumption habits, nevertheless MPs deriving from personal care products and cosmetics are one of the most abundant materials in Waste water treatment plants effluents (Carr et al., 2016). A good point from all this numbers is that because of the social and media concern about this topic resulting from the scientific reports and environmental associations work, many countries governments are taking action on banning Microbeads from cosmetics and other products, (Lam et al., 2018)

3.4.4.2. Washing synthetic fabrics

Along Microbeads from personal care and cosmetic products, people around the globe are also contributing with another significant type and very high amount of microplastics almost every day. These huge microplastic release are microfibers (MFs) from synthetic textiles that are detached in every wash. Depending on the type of synthetic textile (Napper and Thompson, 2016), the age of the garment (Hartline et al., 2016), the length of the fiber and the type of detergent used (De Falco et al., 2018), a single garment can detach more than 1900 fibers per washing cycle (Browne et al., 2011).

Taking in account that this number represents the MF detachment per garment, a common load of 6kg of laundry can release from 138,000 up to 6,000,000 fibers (De Falco et al., 2018; Napper and Thompson, 2016), meaning an average loss of 0.0012% of the garment mass per wash, calculating this number into an annual release per person could be approximately in 70mg/year. In a country like Czech Republic represents the emission of 745.5 kg/year of microplastics, meaning the equivalent of 215,884m^2 of synthetic surface (Pirc et al., 2016), the experimental set ups often does not involve the use of detergents, which vary significantly on the formulation and the chemical formulation in every country and leads to 30 times lower loss of fiber (De Falco et al., 2018).

3.4.4.3. Waste water treatment plants

Wastewater treatment plants (WWTPs) have to take in the water from all domestic and industrial sewage, also the particles from the daily deterioration of vehicle tires and all the finally also thy take all big and small suspended materials that get washed by the rain runoffs, WWTPs are generally the first contact that MPs have to get filtered and not reaching further areas or accumulate in more quantity, studies demonstrated the efficacy of WWTPs on removing MPs emissions to up of 99% of the debris. (Magni et al., 2019; Lares et al., 2018; Leslie et al., 2017; Murphy et al., 2016; Magnusson and Norén, 2014; Browne et al., 2011), nevertheless, considering the volume and quantity of debris that enter these facilities, the leakage of only 1% of these particles can mean a huge number of MPs released into the environment every day.

Just as an example the WWTP of Glasgow-UK, that treats the waste water of an approximate of 650,000 people has a 98,41% of MPs removal efficiency, this number could sound very relieving to the water pollution by MPs, still, that result in the releasing of 65 million MPs every day. (Galafassi, 2019). The amount of grows higher with less capable MPs removal WWTPs in more populated areas can result with hundreds of millions of MPs released every day (Magni et al., 2019), at the same it has to be considered also that during high rain season, water flow can surpass the handling capacity of WWTPs (Gatidou et al., 2019; Sun et al., 2019). And result in the direct discharge of polluted water into rivers, lakes and shores across the globe, these events can have low probability to happen, however they do, and very hardly quantifiable they might greatly impact on the total amount of plastic released to environment (Gallafassi, 2019)

3.4.4.4. Wastewater treatment plants sewage sludge reuse

Even with high efficient WWTPs there is still a risk of a huge amount of MPs leaking through the effluent of the treated water. But where the 98% of the retained MPs are kept? The answer is in the WWTP sewage sludge, all MPs that have more density than water are retained almost completely in the sewage sludge during primary and secondary treatment (Galafassi, 2019),

and it is also known that sewage sludge is used after as a fertilizer in agricultural applications, because it represents lower costs for both farmers and water treatment facilities.

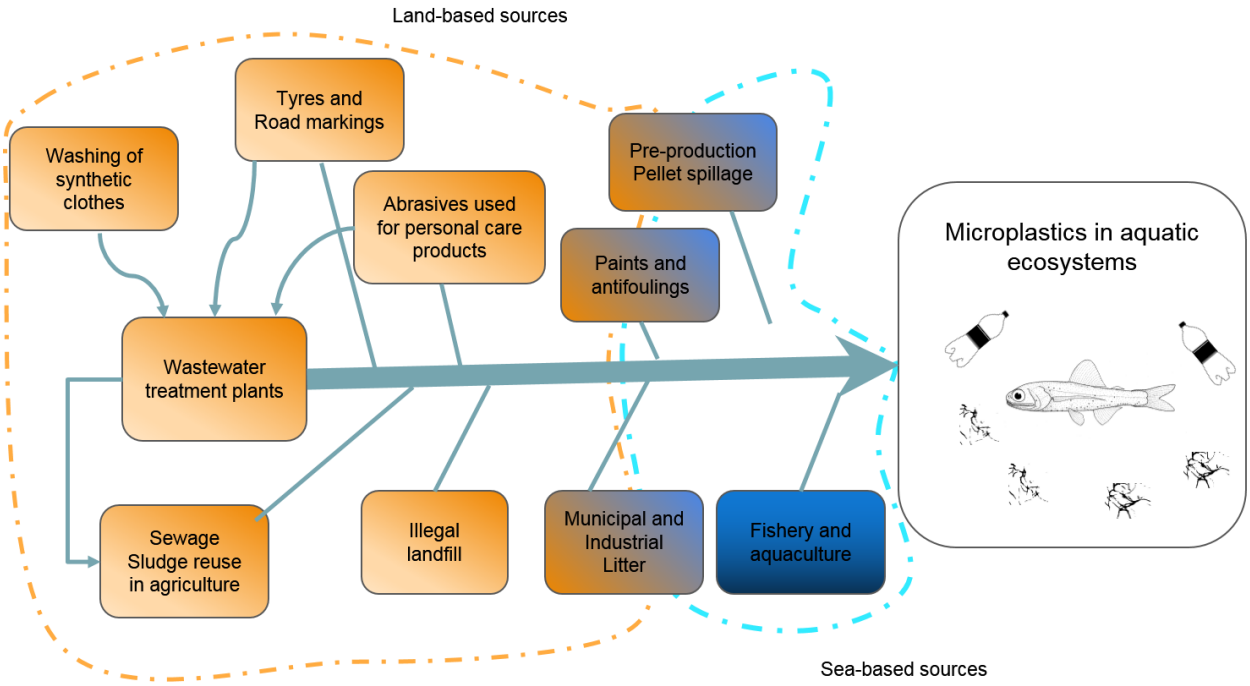

Figure 6 Graphical Representation of Microplastic pollution sources in water ecosystems (Source: own graph based on Galafassi 2019)

According to the data of the national data farm areas, population and sewage sludge fate (Eurostat) about 50% of sewage sludge is used in agricultural activities in Europe, Nizzetto et al. (2016) estimated that 125 and 850 tons of MPs per million inhabitants are added annually to European agricultural soils. Scaling to European population a total yearly input of 63,000–430,000 tons of MPs for European farmlands were calculated. This is a high input if compared to the 93,000–236,000 tons MPs estimated to be present in the surface water of the globe (van Sebille et al., 2015).

3.5. Microfibers

Textile microfibers (MF) are a Microplastic sub-group. These are mostly shed by daily use of garments, and when put in a washing machine to make the laundry. Special attention has been put onto them, as high concentrations have been found in products for human consumption as shellfish and tap water, as it is pointed one of the main sources microplastics found in fresh water and marine environments (Essel et al, 2015), mostly enter the oceans through these drainage systems. Some of the particles are trapped in the sewage sludge in wastewater treatment plants, while a large proportion of microplastics will pass through such systems (Magnusson, 2014). Both natural textile fibers (wool, linen and cotton), and synthetic textile fibers (polyester, nylon) found in oceans are thought to be from washing machines. Earlier studies showed fiber losses of 100-300 fibers per liter effluent from washing machines. The garment shedding the most amounts of fibers was fleece, shedding around 1900 fibers every wash (Browne et al, 2011). After washing clothes or using cosmetics containing microplastics, plastic particles and fibers are passing via the draining system to the wastewater treatment plants. Via the domestic drainage system 10 000 to 100 000 microplastic particles per cubic meter was found in the incoming water. 70-100% of these particles were retained through wastewater treatment but still many particles could be found in the outgoing effluent. Most of the particles leaving the wastewater treatment plant was of a smaller size (<300 μm) in the range. Non-synthetic particles were removed to a greater extent than synthetic particles (Magnusson, Wahlberg, 2014). Altogether, a normal load of laundry of about 5–6 kg can release from 137,951 up to 6,000,000 fibers

The amount of microplastics on shores around the world has been studied and published in many reports. Scientists have found greater amounts on shores near the big cities compared to remote locations. The different fractions of plastic on shores near cities resemble with the fractions found in effluent from the waste-water treatment plants. (Browne et al, 2011) suggest these microplastic fibers were mainly derived from sewage by the washing of clothes. This is showing that the washing of clothes made of

synthetic fabrics indirectly contributes to add a considerable amount of microplastic fibers to marine habitats. The different fractions of these plastics are shown in table below:

Table 1 Proportion of different types of plastic polymers found in waste water based on Browne et al. 2011

Material	*Percentage*
Polyester:	***56%***
Acrylic:	***23%***
Polypropylene:	***7%***
Polyethylene:	***6%***
Polyamide:	***3%***

The most abundant microplastic material found in waste water is polyester with a proportion of more than half compared with other materials.

3.5.1. Polyester properties

Polyester is the most used materials for textile fabrics cause of its elasticity, wrinkle resistance, shape retention and durability and easy manufacture at a low cost. However, polyester fiber is poor in moisture absorption, making the wearer feel hot and sticky when wearing it for long periods of time, also can attract dust and cling to the body because it produces static electricity easily with friction. Info available in (www.textile-school.com)

3.5.1.1. Manufacturing of Polyester

Polyester is widely used in the whole globe. DuPont Company introduced its Dacron brand of polyester in 1951, but the material itself was patented earlier in 1941. Before that, textile fabrics where mainly made from natural fibers such as cotton or wool, but due to the consequences of World War II industries started to experiment with new organic materials and chemicals, in this way polyester polymer was found. The trend of synthetic textiles outplaced natural fibers during the decades of 60s, 70s and 80s where the total consumption of synthetic textiles only in the United States such as

nylon and polyester was about of 3.1 billion kilograms 70% of the whole use of fabrics. (Hardin, 1990).

Polyester polymers are made by reacting dicarboxylic acid with a dihydric alcohol. This base material produces polyethylene terephthalate (PET), at this point in the present everybody knows that this material is widely used from soda bottles to clothing fibers. Like nylon, polyester is melt-spun. This process allows the fibers to be made in different shapes and sizes for specific applications. In the past years, chemists found that by altering the size and shape of polyester fibers in ultra-thin microfibers can give polyester a smooth and feel more like natural fibers. Softer feel than the polyester of twenty years ago.

3.5.1.2. Chemical properties of Polyester

Polyester fibers have a good resistance to weak alkalis like ammonium (NH_3) at high temperatures. It exhibits only moderate resistance to strong alkalis such as bleach ($NaClO_3$) at room temperature. Polyester is degraded at temperatures above 95 °C.

Weak acids, even at the boiling point, have no effect on polyester fibers unless the fibers are exposed for several days. Polyester fibers have good resistance to strong acids at room temperature. Prolonged exposure to boiling hydrochloric acid (HCl) destroys the fibers, and 96% sulfuric acid (H_2SO_4) causes disintegration of the fibers.

Polyester fibers are generally resistant to organic solvents. Chemicals used in cleaning and stain removal do not damage it, but hot m-cresol ($CH_3C_6H_4$) destroys the fibers, and certain mixtures of phenol (C_6H_5OH) with trichloromethane ($CHCl_3$) dissolve polyester fibers. Oxidizing agents do not damage polyester fibers.

Polyester fibers are resistant to direct sunlight, and it also resists abrasion very well. Soaps, synthetic detergents, and other laundry aids do not damage it. However one of the only faults with polyester is its oleophilic capacity as it absorbs oily materials easily and holds the oil tenaciously.

3.5.1.3. Physical properties of Polyester

Polyester moisture absorption is low, it ranges between 0.2 to 0.8 per cent. Although polyesters are non-absorbent, moisture can be carried on the surface of the fiber without absorption.

Polyester fibers have a density greater than polyamide fibers and lower than rayon. Fabrics made from polyester fibers have a density of 1.1 – 1.2 (g/cm3). (www.textile-school.com)

The melting point of polyester is close to that of polyamide, ranging from 250 to 300°C. Polyester fibers shrink from flame and melt, leaving a hard black residue. The fabric burns with a strong, pungent smell. Heat setting of polyester fibers, not only stabilizes size and shape but also enhances wrinkle resistance of the fibers. (www.textile-school.com)

3.5.1.4. Mechanical properties of Polyester

Polyester fibers can be more elastic, tensile, or stronger depending on the manufacturer method, usually fibers can't have all this mechanical properties as a high parameter, generally if a fiber is more elastic, it losses tensile strength, the same happens with the shrinkage, if the fibers have relaxation in stress and strain, shrinkage decreases, but initial modulus may be also reduced. (www.textile-school.com)

3.6. Existing methods of sampling and analyzing microplastics

One of the problematic regarding the research in microplastics is the methodology for evaluating MPs is in classifying shape, material, and either counting the number or the mass of the particle, this questions are always in doubt as there is no standardized methodology for microplastics, mainly as said before with all the variables that secondary MPs face for their degradation, there is no common consensus of how to measure and sample microplastics. (Hermandez et. al. 2017).

This problem is also present when sampling microfibers after washing cycles, the type of washing machine, the temperature of the program, the length of the washing cycle, the detergent, are variables that can be different in almost every different country. Also preparing the samples and their

following analysis can play a major role, depending on what kind of synthetic fiber is being tested and if several materials would be tested and researchers want to know the ratio of them with spectroscope analysis (e.g. Raman analysis), they would interfere this analysis if they want at the same time to calculate the number of microfibers with poliphylic stains.

A point that is specifically missing involves systematic studies under controlled conditions to better understand the mechanisms behind fiber shedding through use, especially considering variables such as different fiber production (extruded filaments or wound staples), fabric structure (woven, knitted or nonwoven), washing conditions (temperature, detergent/surfactant, length of washing, multiple washes), and specific size distributions and masses of fibers shed during this process. (Hernandez, 2017)

3.6.1. Water sampling

3.6.1.1. NOAA laboratory method

In 2015 Masura, et al. from the National Oceanic and Atmospheric Administration (NOAA) published a manual on how to analyze samples of microplastics mainly in water samples. Their methodology consists on taking the samples from water using a 0.355mm cone shaped surface net. On laboratory takes place the separation of the organic matter in wet peroxide oxidation in presence of Fe (II) as catalyst for making the organic matter faster, once the organic matter is separated the microplastics are isolated by density with NaCl, this way is easier to separate plastics from denser minerals or particles, as also separate different types of polymers, they suggest to use a 0.3mm filter air-dried density separator.

The method is widely used and it is applicable for the determination of many common polymers such as polyethylene (0.91-0.97 g/mL), polypropylene (0.94 g/mL), polyvinyl chloride (1.4 g/mL), and polystyrene (1.05 g/mL). The analysis is applicable for sizes of microplastics between 5mm to 0.3mm, as solid particles ranging this sizes are resistant to wet peroxide oxidation and have good visuals under a 40x microscope. Unfortunately for debris under

0.3mm and for fibers wet peroxide oxidation is not recommended as the fibers can be damaged along the process.

3.6.2. Identification of Microplastics

3.6.2.1. Visual Identification

According to Hidalgo-Ruz et al. (2012) visual sorting to separate potential microplastics from other organic or inorganic material in the sample residues is a mandatory step for MPs identification. Visual inspection is better if large microplastics (more than 500 µm) are the target of a study (Morét-Ferguson et al. 2010). Whereas smaller microplastic particles should generally be sorted out under a dissection microscope (Doyle et al. 2011).

Sorting MPs in water samples can be facilitated by the use of sorting chambers like “Bogorov counting chamber”, but generally, if there is no possibility to use more accurate methods such as Fourier-transform infrared spectroscopy (FTIR) or Raman spectroscopy (Methods discussed below) that are used to verify the material of synthetic microplastic particles, visual identification should not be applied to particles smaller than 500 µm as the probability of a misidentification is very high (Löder, 2015). Hidalgo-Ruz et al. (2012) thus suggest an even higher size limit of 1 mm for visual identification.

Norén (2007) have four suggestions on how to reduce the possibility of misidentification of particles:

- No structures of organic origin should be visible in the plastic particle or fiber
- Fibers should be equally thick and have a three-dimensional bending to exclude a biological origin
- Particles should be clear and homogeneously colored,
- Transparent or whitish particles must be examined under high magnification and with the help of fluorescence microscopy to exclude a biological origin

General aspects that are used to describe visually sorted microplastics are source, type, shape, degradation stage, and color of the particles (Hidalgo-Ruz et al. 2012). Also it is strongly advised to analyze subsequently the sorted particles by techniques which facilitate a proper identification of plastics (Dekiff et al. 2014), as the visual sorting depends very much on the counting person, the quality and magnification power of the microscope and the sample matrix of other materials like wood, plankton, sediment or gut content. And the most important as it was said above the target size of the research, because smaller particles simply can't be sorted visually from other material, furthermore, the time that takes to complete several samples in visual sorting can be extremely long. In summary, even a person with experience could misidentify potential MPs with other particles, thus the error rate of visual sorting reported in literature ranges from 20% up to 70% (Eriksen et al. 2013) and increases with the decreasing particle size. On the other hand visual sorting could be the only option when there is no much economic resources and it could improve significantly if there is a counting software to help in the research.

3.6.2.2. Identification of Microplastics by Their Chemical composition

The molecular composition of plastic polymers has a repetitive pattern almost like a finger print, by knowing this, it allows a clear assignment of a sample to a certain polymer origin. In the past years, methods have been developed for polymer identification until reaching to the present used FTIR and Raman analyses of microplastics.

Morét-Ferguson et al. (2010) used the specific densities of particles to identify the polymer origin of visually sorted microplastics. For this purpose, the sample was placed in distilled water and, depending on the density of the sample, either ethanol or concentrated solutions of calcium or strontium chloride were added until the sample was neutrally favorable. Then the density of the particles was evaluated by weighting of a specific volume of the solution, this procedure facilitated the determination of density with high

precision. Different polymer densities were expressed in the NOAA method in the beginning of this topic.

Other groups of polymers have a characteristic elemental composition, that is used to identify the plastic origin of a particle by the subsequent C:H:N chain analysis, By comparison with the densities and C:H:N ratios of virgin-polymer samples the particle could be assessed as either plastic or not and assigned to a group of potential polymers (Morét-Ferguson et al. 2010).

This approach represents an approximation to the identification of micro-plastic particles by narrowing the search for the potential polymer type instead of a rigorous chemical analysis and it is useful when there are samples of many types of MPs polymers present. Further drawbacks are the relatively high time effort, which hampers a sample with many counts throughput and that this technique is not applicable to smaller particles.

- **Pyrolysis-GC/MS**

Pyrolysis-gaschromatography (GC) in combination with mass spectrometry (MS) could be a useful method to assess the chemical composition of potential MPs particles by analyzing their thermal degradation products (Fries et al. 2013).

This analytical approach is already used after extraction and visual sorting of microplastics from sediments. The pyrolysis of plastic polymers results in pyrograms, which facilitate an identification of the polymer type. The polymer origin of particles is then identified by comparing their characteristic combustion products with reference pyrograms of known virgin-polymer samples (Nuelle et al. 2014; Fries et al. 2013). If a thermal desorption step precedes the final pyrolysis organic plastic additives can be analyzed simultaneously during pyrolysis-GC/MS runs (Fries et al. 2013). Although the pyrolysis-GC/MS approach allows for a relatively good assignment of potential microplastics to polymer type it has the disadvantage that particles have to be manually placed into the pyrolysis tube (Löder, 2015). The application of this method can be done to a certain amount of particles that can be manipulated manually only one at the time, increasing dramatically

the time that requires when there is a high amount of particles, also depends on the size the particle, if the manipulability is too hard then the method is not possible. Nevertheless currently there are promising approaches for the analysis of microplastics by pyrolysis-GC/MS that are being developed in order to handle whole environmental samples. (Löder, 2015)

- **Raman Spectroscopy**

Raman spectroscopy is a very useful straightforward technique that identifies successfully diferent types of microplastic particles in different environmental samples with high reliability (Van Cauwenberghe et al. 2013; Cole et al. 2013; Murray and Cowie 2011; Imhof et al. 2012, 2013). It works by irradiating the sample with monochromatic laser in different wave lengths that ranges between 500nm and 800nm, the reaction of the molecules and atoms from the sample could be vibratory, rotational, or other low frequency reactions determines the type of material that the particle is made of, making a Raman spectra, since plastic polymers have specific Raman spectra, the technique can be applied to identify plastic polymers within minutes with the reference spectra.

Raman spectroscopy is a “surface technique” and the analyzed particles can be coupled with microscopy to sort plastic polymers and make the analysis much accurate this is called “micro-Raman spectroscopy” and it increases the size range of particles that can be analyzed from below 1 µm (Cole et al. 2013), and also this technique can be combined with spectral imaging to provide spatial chemical images based on the Raman spectra of the sample. The best quality of the use of this method is the possibility to detect even the smallest MPs but the applicability in environmental samples have to be tested. (Löder, 2015).

Raman spectroscopy can also be coupled with confocal laser-scanning microscopy to locate polymer particles within biological tissues with subcellular precision (Cole et al. 2013).

One of the only disadvantages of Raman spectroscopy is when analyzing fluorescent samples are excited by the laser and confuses the reaction,

misidentifying the Raman spectra, it is advisable to first analyze the polymer samples by Raman spectroscopy and then stain them with fluorescent ink, in addition environmental samples with biological residues can also be excited by the laser and cannot be measured transforming the low wave length of the laser into higher signal intensity. According to (Löder, 2015) the fluorescence can be minimized by using higher wave lengths laser light of more than 1000nm, however more research is necessary to find the optimum laser wave length for reaching a good result between the suppressed fluorescence and low signal intensity, that is why environmental samples that uses Raman spectroscopy have to get rid of the organic material and other pollutants before the analysis in order to identify the polymer type of MPs.

- **IR Spectroscopy**

Infrared or Fourier-transform infrared spectroscopy is similar to Raman spectroscopy, in this case the plastic polymers are identified by their characteristic infra-red spectra (Thompson et al. 2004; Ng and Obbard 2006; Vianello et al. 2013; Harrison et al. 2012; Frias et al. 2010; Reddy et al. 2006) FTIR and Raman spectroscopy are complementary techniques. Molecular vibrations, which are Raman inactive are IR active and vice versa and both can be used to provide more accurate information on MPs samples. IR radiation acts by exciting molecules with a specific wave length in a certain amount. Plastic polymers possess highly specific IR spectra with distinct band patterns that can analyze polymers with high accuracy in less than one minute, making IR spectroscopy an optimal technique for the identification of microplastics (Hidalgo-Ruz et al. 2012). There are two measuring modes in IR spectroscopy: reflectance and transmittance, while reflectance have the disadvantage of not being able to measure irregular shaped MPs because of the refractive error result in non-interpretable spectra (Harrison et al. 2012). The transmittance mode needs IR transparent filters (e.g. aluminum oxide) and is, owing to total absorption patterns, limited by a certain thickness of the microplastics sample. Another disadvantage of IR is that the samples have to be dry before the analysis as water absorbs IR radiation in high quantities. In summary IR and Raman

spectroscopy work better when complementing each other, of course not for all the research projects there is the time and resources to apply both analysis in each sample. It is also convenient to see if the analysis would give a reliable output for the target of the research.

4. Methodology

After looking at the available literature and comparing the different methodologies that exists for sampling, sorting and analyzing MPs, the methodology chosen for this research was the software counting after fluorescent staining of the detached fibers of a single polyester T-shirt after a quick laundry cycle without detergent, the reason behind the chosen methodology falls in the available resources for making the project. By choosing a single material and counting the fiber detachment by software the time in analyzing the samples should be relatively faster in comparison with other methodologies such as visual counting, which needs a selective pre-treatment for each sample, besides, the addition of more variables like other types of synthetic textiles would increase the error probability and the analysis time for each sample.

For this research a new 100% polyester new T-shirt was used, the color (Blue) of the garment was chosen for the easier differentiation of the MFs from other fibers and particles, the washing procedure and sampling was made in a similar way stated in Hernandez (2017) and Belzagui, (2019).

For the washing process. 5 washing cycles were set for a single garment, then analyze the number of material detached from each washing cycle separately, however the process done was different from the procedure done by Hernandez (2017), whose sampling was done under laboratory conditions, for this study, sampling was done under domestic environment instead of a laboratory, firstly because of the availability of the washing machine to make the samples and secondly because the willing to compare the results under domestic environment. See (Figure 7)

In literature, it is stated that new garments have higher detachment rate on the first and second washing cycles, then it decreases and normalizes until the 5^{th} wash. The main idea of the research was to analyze different garments and the relationship that has each into the other, but due to the time that took to find the washing machine necessary for the research, and further complications from the outbreak of Covid-19 in Europe, it was not possible to analyze more samples.

For the first Sample a washing machine Miele softtonic W2205 was used at a temperature of 30 degrees Celsius and 400 rpm and a time of 37 minutes of washing cycle, no detergent was used.

For the rest of the samples the Washing machine used was a device from the brand Gorenje “Senso care” Model W6403/S that has a load capacity up to 6 kg. For each washing cycle, it was set on the quick wash program of 17 minutes, at 30 degrees Celsius and 600 rpm. Each washing cycle used a water volume of 20 liters in total. No detergent was used for the samples.

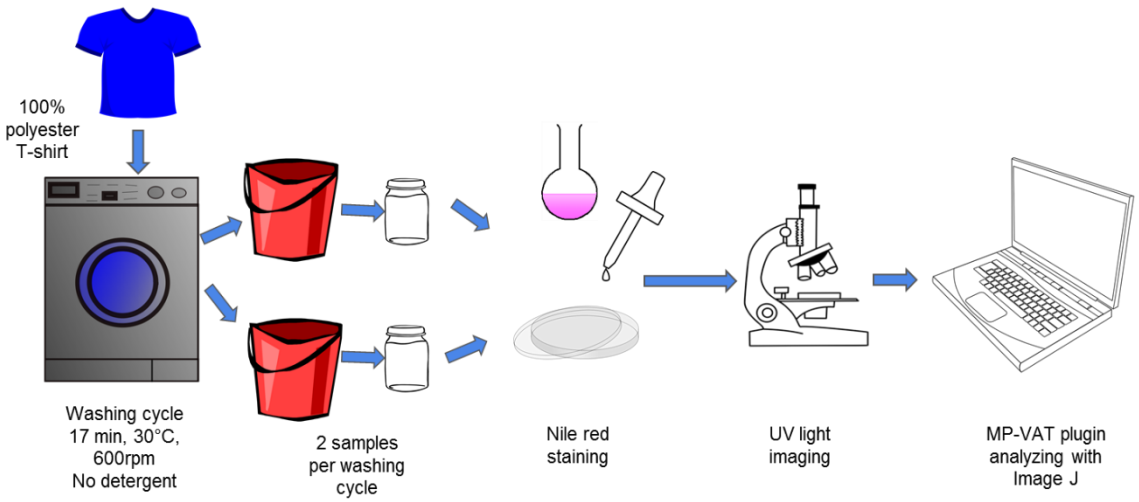

Figure 7 Graphical description of the sampling and analysis done for the microfiber counting

4.1. Sampling

In order to have a baseline to compare the results obtained, a control sample was taken after 2 prewashes in order to obtain the cleanest water possible to start, 10 samples of microfibers were taken and filtered each in a 25x25cm cotton fabric, as cotton should not interfere with posterior staining for fluorescence imaging, 5 different washing cycles were made for a single T-shirt, Every sample was taken on the following procedure (Figure 8):

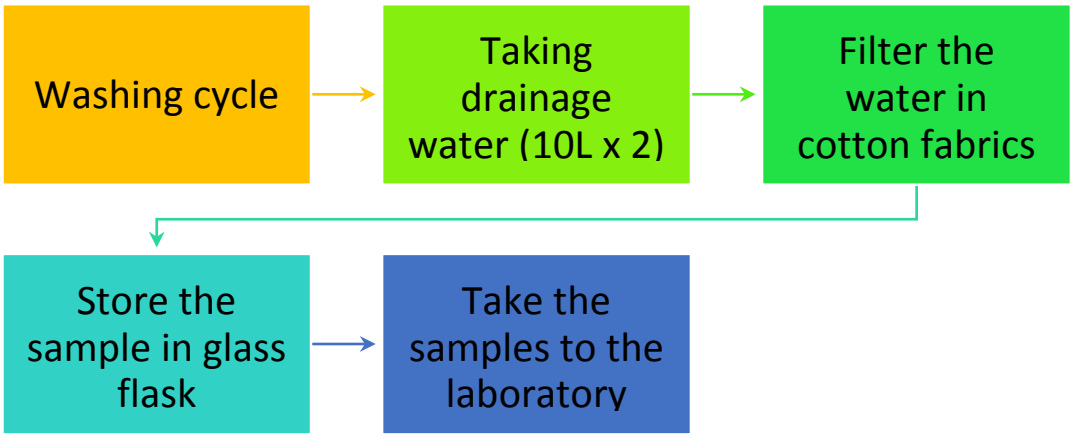

Figure 8 Sampling process

To watch the fiber detachment in first place, also a sample of another black 100% polyester T-shirt was used in order to evaluate the incidence of microfiber remains in subsequent wash cycles and finally a control sample without any garment to compare results with the water from the washing machine.

4.2. Post sampling and pretreatment for Analysis

After the samples were obtained, they were taken to a laboratory located in the Faculty of Environmental Sciences of the Czech University of Life Sciences in Prague, then cotton filters were washed with ultra-filtered water and the microfibers were extracted in sterilized glass flasks (Figure 8) and then set up to dry in a laboratory oven at 45°C, the cotton fabrics used to filter the microfibers were also dried in order to take any remaining of the microfibers of them after.

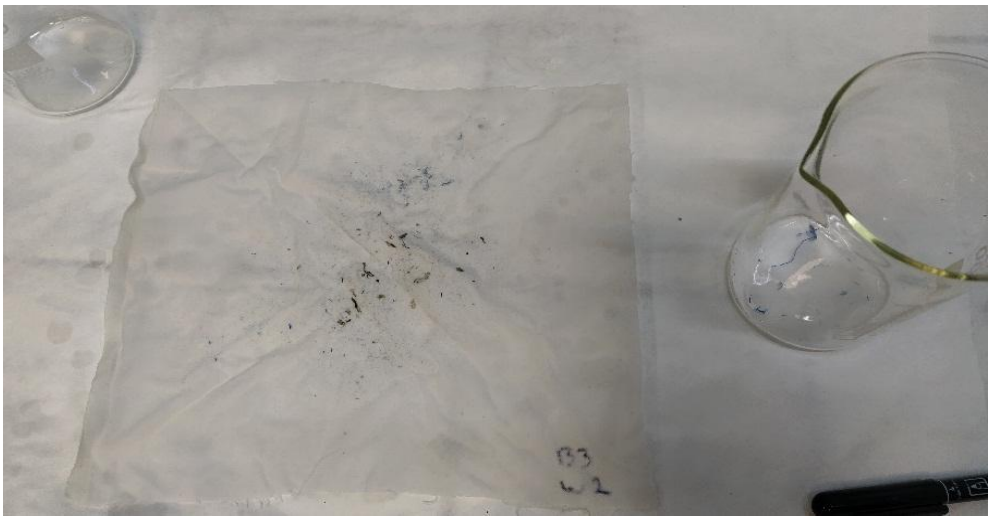

Figure 9 Sample preparing for analysis

Alongside the drying process of the samples, it was prepared a solution of 50ml of Nile red lipophilic stain on a concentration of 0.01mg/ml diluted in reagent grade ethanol which was used as fluorescent ink for the imaging (Figure 11). The staining was chosen in order to avoid the use of peroxide or other substance in order to separate the organic matter from the sample, it is known that Peroxide oxidation could damage polyester fibers and produce different output on the final counting (Search citation). Nile red staining works by making plastic polymers sensitive to UV light, by having only one type of polymer for this research (polyester), only the stained fibers should glow under ultraviolet light, facilitating the image taking and the software counting without damaging the sample.

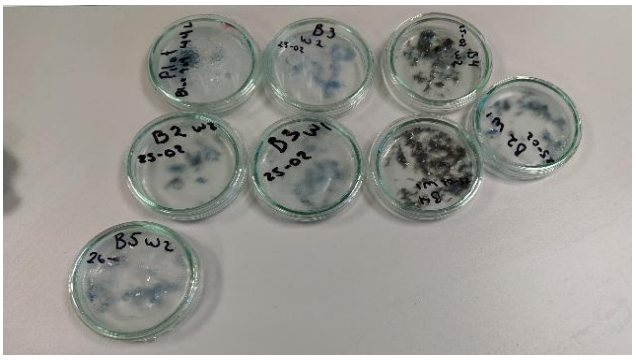

Figure 10 Dried samples ready for staining

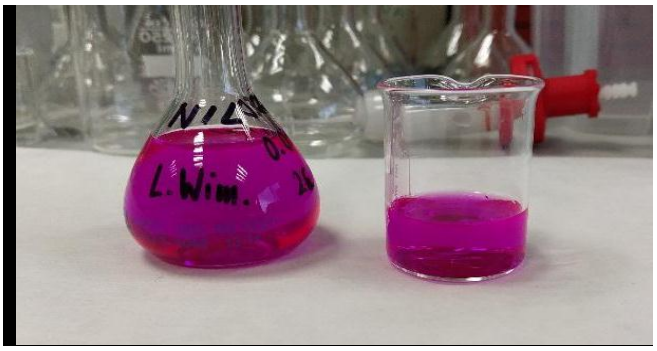

Figure 11 Nile red solution

Once the samples were dry, they were placed in a petri dish of 65mm of diameter, then 0.5ml of Nile red stain solution was placed in order to color the sample for the posterior microscope analysis (Figure 12).

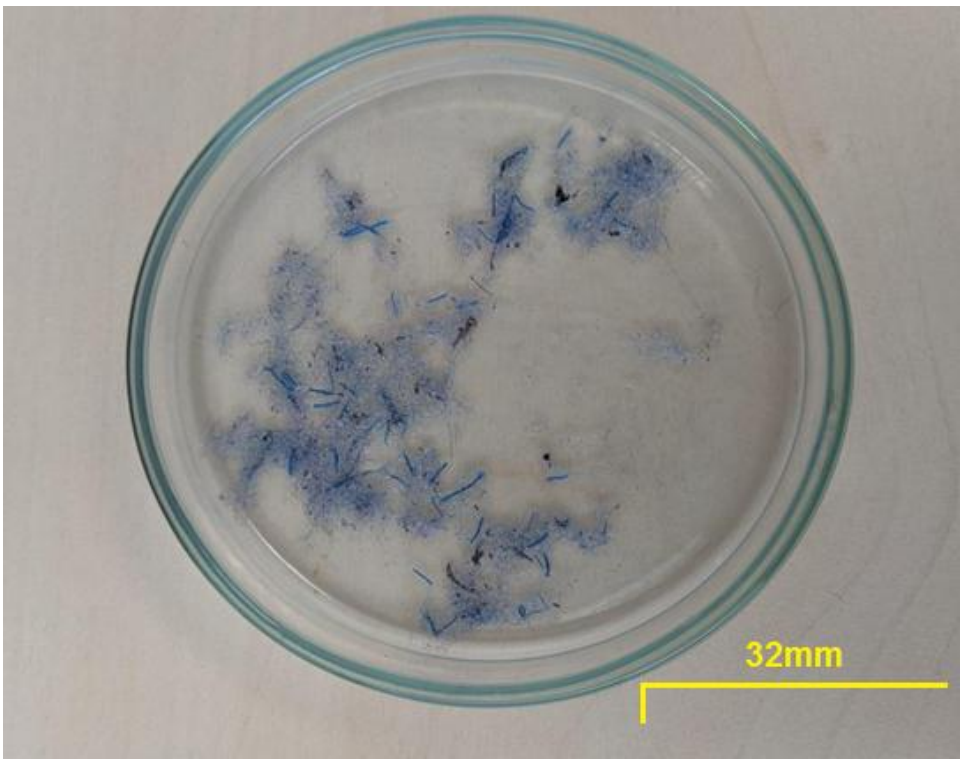

Figure 12 Sample after filtering, drying and Nile red staining ready for image taking

4.3. Picture taking and Image obtaining

The image quality plays a key part in the software analysis because MP-VAT analyzes the differences of pixels in each image, so if the pixels are blurred and/or if there is overlapping and not enough light, individual fibers could be interpreted as many or many fibers could be interpreted as a single big particle. The picture taking was made in a dark room with a smartphone camera (Xiaomi Mi A3) with 12 Mega pixels resolution, aperture of f/1.8,

shutter speed 1/10, a focal length of 4.71mm and automatic ISO under the Google camera application in 365nm UV light. The images showed satisfactory quality so the analysis process continued as normal. (See results and appendix)

4.4. Microfiber counting

For the microfiber counting the software used was Imagej, the software was created to count particles and it was added a plugin named MP-VAT (Microplastics Analysis Tool) to analyze microplastics, the script was developed by Prata et. al. (2019) after dying the microplastics with Nile red stain. The images were analyzed and the results taken in Microsoft Excel and R studio in order to obtain the statistics of the total fiber count, average size, and differences between different washing cycles, this numeric results, then, were used to estimate the possible MPs pollution for a city like Prague, Czech Republic.

4.5. Data processing

After processing the images in Imagej and getting the results, the data was transferred into spreadsheets, considering 2 samples for every washing cycle, each sample dataset was merged with its pair into a single dataset representing the total counting from a washing cycle, and then basic statistics analysis was performed in order to get the results. (See Appendix 3 for the garment weighting)

Once obtained the total count of MFs, the shape and average size length a relation between of the number of MFs and their mass was made (MF/mg) which can be calculated with the linear weight, usually named yarn count usually named yarn count C (dtex), and the MFs' average length, LMF (mm/MF), by using the following equation (Belzagui et al., 2019):

$$MF_W = \frac{1}{C \cdot \bar{L}_{MF}}$$

A decitex (dtex) is a unit of measurement of the linear weight of a filament textile fiber. It is expressed in grams of filament fiber per 10000 m. equation weight (C) is described as following (Belzagui et al., 2019):

$$C = \varnothing^2 \cdot \frac{\pi \cdot \gamma}{400}$$

Where: ø= Average diameter of a fiber (For polyester is usually 10µm)

γ= Specific Weight of the fiber (For polyester is usually 1.38 g/m^3)

Table 2 Relations between the linear density (*dtex*) and the length of a microfiber to estimate the quantity of microfibers in a milligram (Source: Supplementary Belzagui 2019).

	dtex = g/10000m					
L MF mm	**0.5**	**1**	**2**	**3**	**4**	**5**
	MF/mg					
0.1	200000	100000	50000	33333	25000	20000
0.5	40000	20000	10000	6667	5000	4000
1.0	20000	10000	5000	3333	2500	2000
1.5	13333	6667	3333	2222	1667	1333
2.0	10000	5000	2500	1667	1250	1000
2.5	8000	4000	2000	1333	1000	800
3.0	6667	3333	1667	1111	833	667
3.5	5714	2857	1429	952	714	571
4.0	5000	2500	1250	833	625	500
4.5	4444	2222	1111	741	556	444
5.0	4000	2000	1000	667	500	400

5. Results

5.1. Imaging

To achieve a reliable output of results the Nile red staining and the image processing has been followed as stated in Prata et al. (2019). On the counting of MFs, the staining of Nile red was very important because the software dependence from the image quality that will be discussed in more detail in the next point. After of the application of the Nile red solution and before the image taking the samples had to be taken in a dark room and put them in a glowing UV light In the Figures (13, 14) it is shown how the stained microfibers with Nile red solution glow under 365nm UV light, with small ruler it was measured the biggest fibers, which were approximately between 1mm and 3mm, these bigger fibers did not glow under the UV light, probably because of the concentration of Nile red solution and the density of this fibers, however, the most important number of microfibers had an average size of 0.3mm and 0.6mm and they glowed with good intensity.

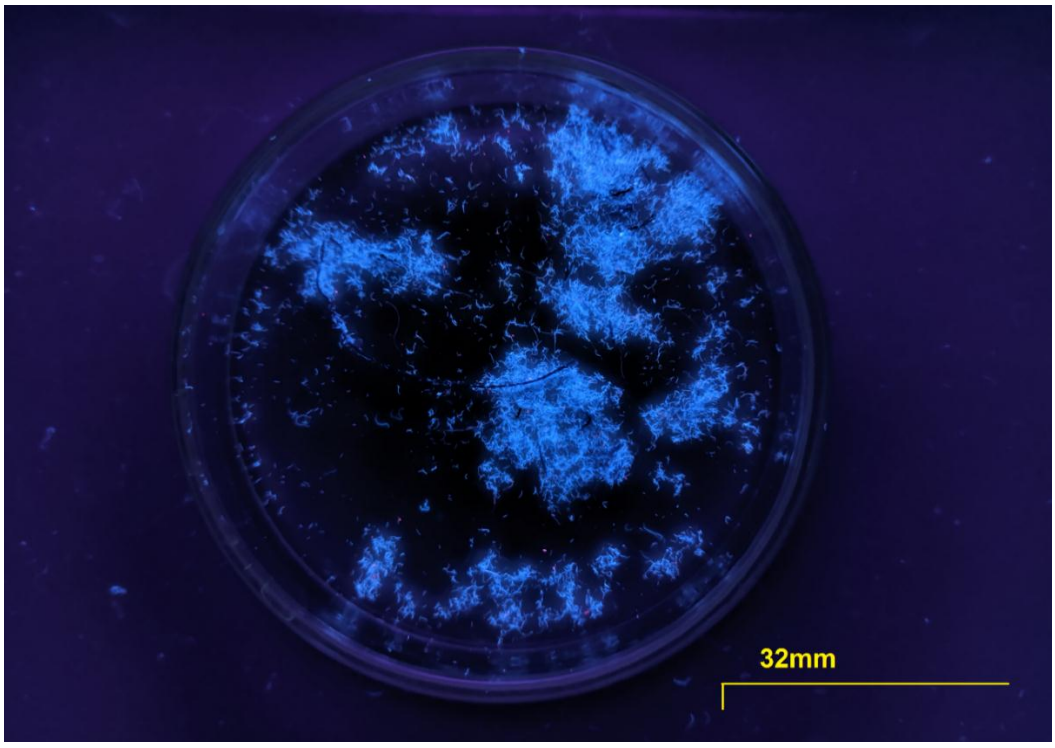

Figure 13 Sample under UV light 365nm wave length and its amplification, it is visible the smaller microfibers glowing

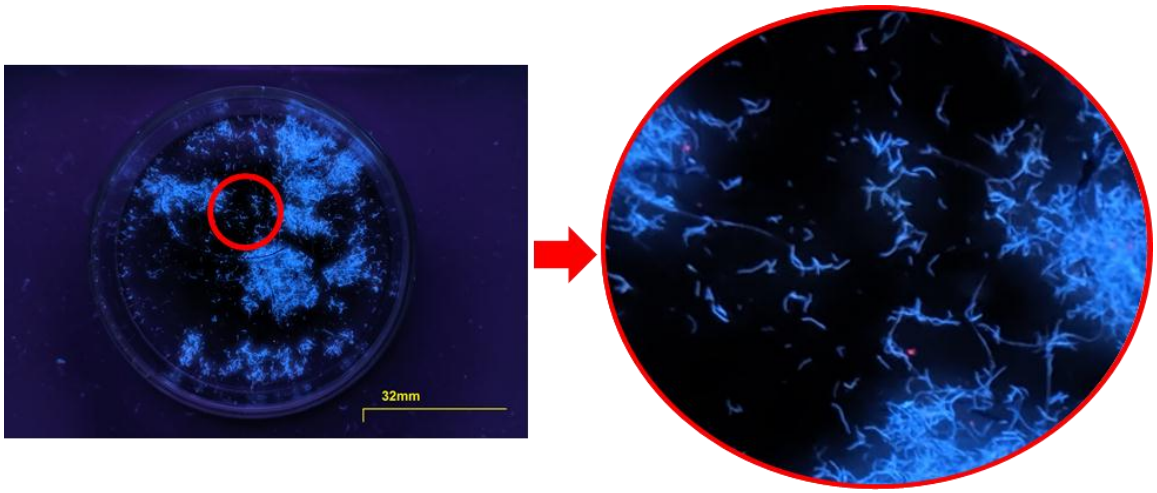

Figure 14 Zoom of the smaller particles under UV light

5.2. Image processing

For the image processing the bright pixels from a boundary we set using MP-scale, another plugin that comes with MP-VAT, MP-scale allows the user to set the boundary and the scale (in millimeters) of the sample that should be analyzed, but first, the user has to use the oval shape in Imagej and set the boundary manually, in this case, the boundary is the circumference of the petri dish, after the oval shape is set as boundary and the user click on the MP-scale plugin, the program will ask the user to set the diameter of the circumference, the petri dishes used for this research were of 65mm in diameter, the program is set by default to count the diameter in millimeters and it will set the scale of the distance in pixels automatically, the scale is set the program will be able to measure the approximate area and size of the particles counted by MP-VAT. subsequently the user have to run MP-VAT plugin it will make the sample an image of 8 bit with only black and white pixels, that will use to count the fibers found in the sample.

The program will throw a list of result with many variables that the user is able to save it as a .csv file, in which there is the total count of the MPs, the classification of the MPs in three categories (fibers, fragments and particles) according to their circularity, ranging between 0.3-0.6-1 respectively,

In the (Figure #) it is visible the black spots and the microfibers that MP-VAT uses for counting, there is also visible bigger black spots were a big amount

of fibers were overlapped among others, it was difficult to determine if the program counted them as single or many particles. In (Table 3) the total count of MFs had a similar number showed as in Browne et al. (2011) per washing cycle, so the accuracy of the program could be evaluated as satisfactory.

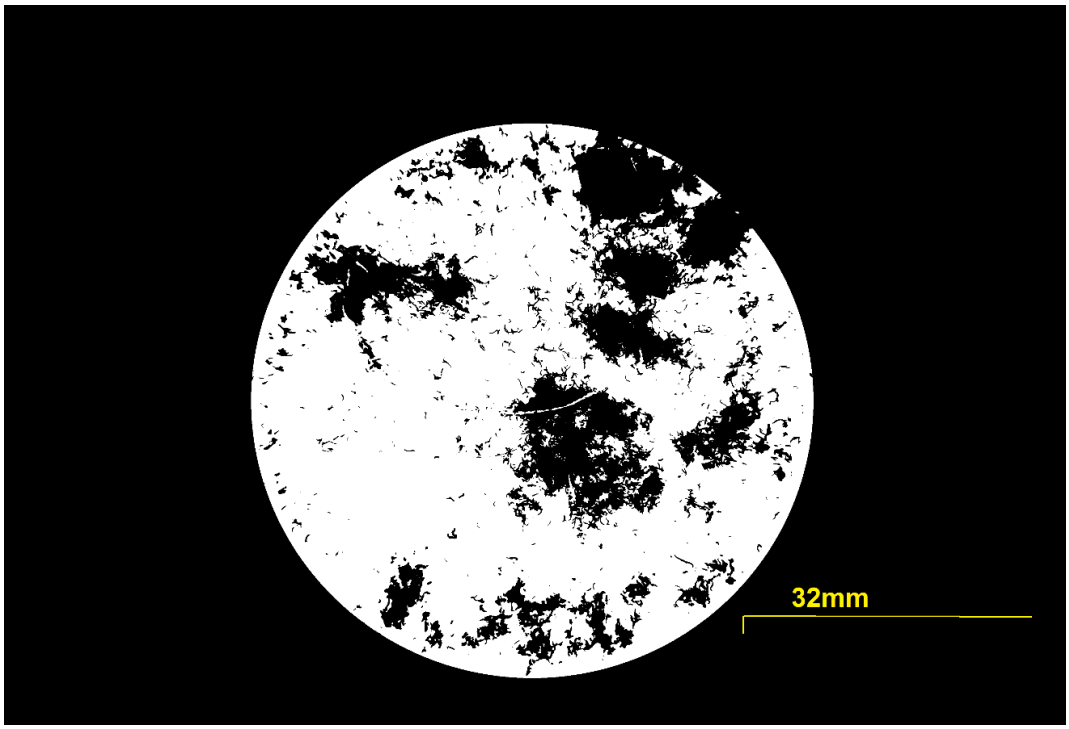

Figure 15 Binary image resulting from the software analysis

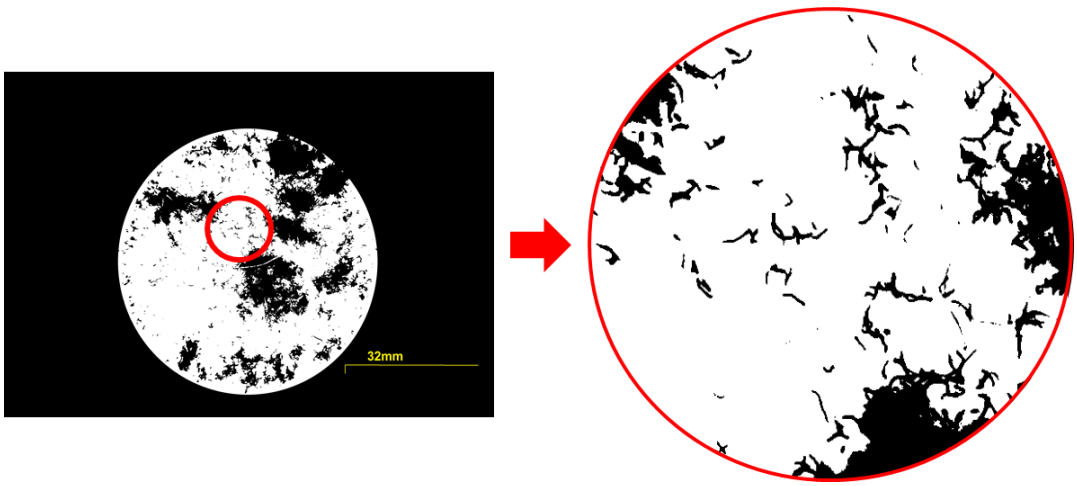

Figure 16 Zoom of the smaller MFs in the binary resulting image

5.3. Microfiber Counting

The control sample which was obtained after 2 pre-washing cycles got out with a heavy load of dust and sediment-like material, it got the same treatment as the rest of the samples in sampling, filtering, drying and staining stages, after the image taking and software analysis a total count of 206 particles mostly of a size below 0.6mm. This number was taken as the reference number for the rest of the samples (Figure 17). On the other hand the way how the plugin classifies the counted MPs into fibers, fragments and particles (Figure 17) depends on how circular the shape of the pixels are represented in the image taken (Figure 16), for achieving this it counts from 0 to 1 the circularity of the particle, meaning that close to 0 are elongated shapes and close to 1 are semi-circular or circular shapes. Taking in mind that the output from a washing cycle of a synthetic textile should be mainly fibers, meaning that the circularity counted by the software should be below 0.4. However there is always the chance of having different particles present at the moment of starting the wash.

Nevertheless, according to the plugin, the results of all samples showed a higher amount of particles than fibers. By comparing the control sample and the 3rd washing cycle. When making the statistics the average circularity in every washing cycle that ranged between 60% and 80% of particle circularity (Figure 18). See discussion chapter for more in detail, the possible outcomes from this results.

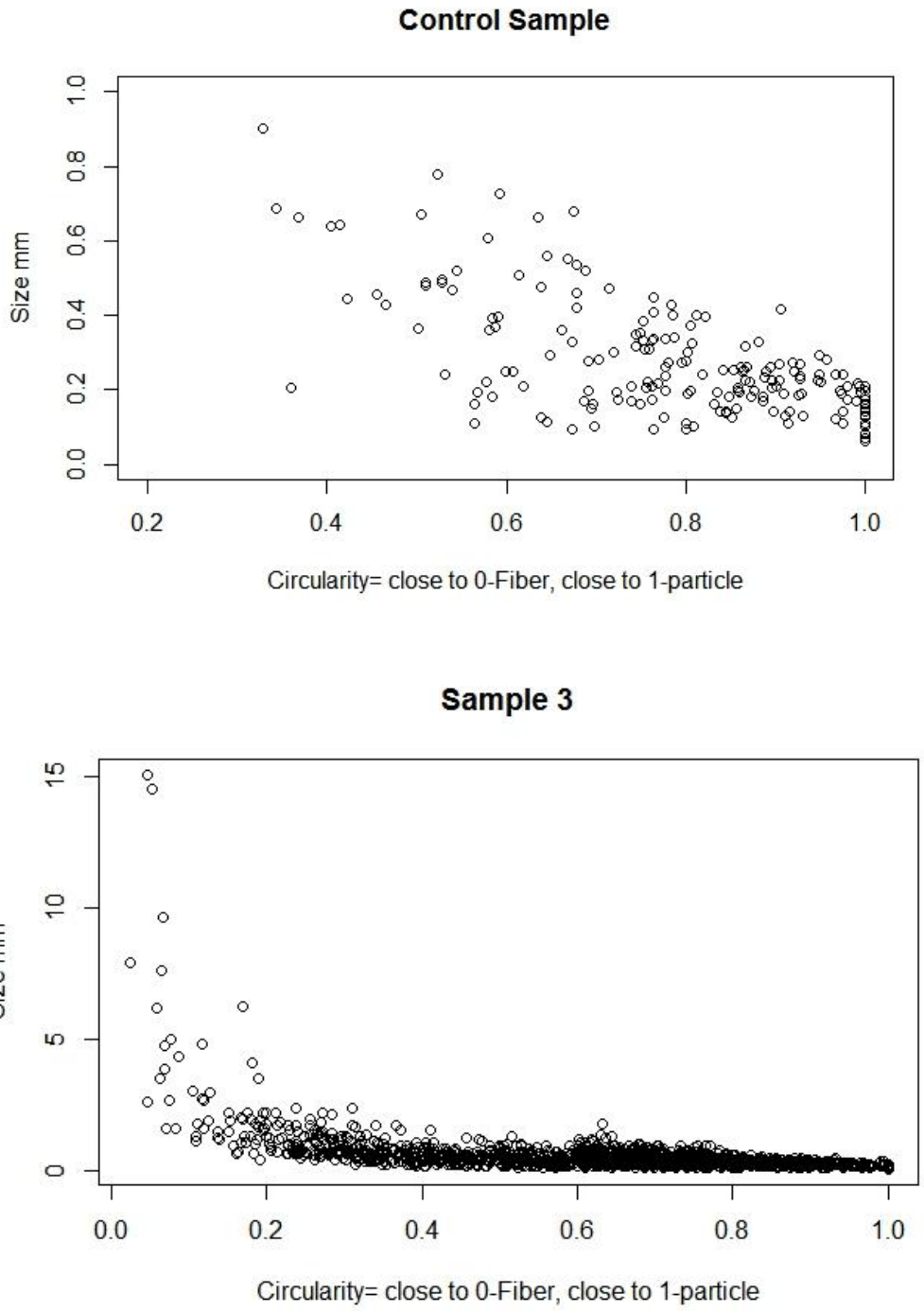

Figure 17 Microplastics distribution according the circularity counted by MP-VAT from approximately 206 counts for Control and 1919 counts for Sample 3

The final count result (Figure 19) showed an average count of 1478 MFs released per washing cycle. Having a counting of 206 particles released in the control sample there is a visible increase trend of particles from the 1st to the 5th washing cycle from 719 MFs to 2305 MFs with an only decrease between the 1st and 2nd washing cycle of 24.76% but then an increase of 71.81% from the 2nd and 3rd washing cycle. (See table 2 for more details). Taking in mind that the 1478 MFs in average from all the washing cycles were from 20 liters of water, it gives a number of 73.9 MFs per liter,

In addition, the Plugin is scripted in a way that by setting the scale it would be able to determine the size of each particle it gives a maximum and a minimum approximated size called “Feret”. By the tests done by (Prata et al, 2019) and personal tests the accuracy of the software is very close to the reality using the number given by the maximum Feret.

In (Figure 11) it is shown the average length of the MFs that oscillated from 0.3mm to 0.6mm, with an average length of 0.517mm, visual sorting is possible but given the amount of particles released is not advisable.

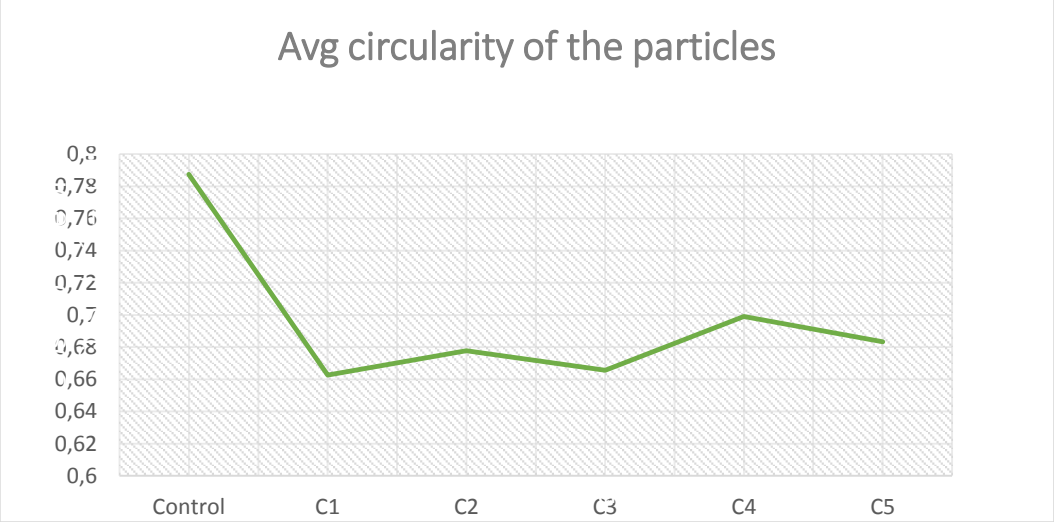

Figure 18 Average particle circularity result from the Software analysis in all the samples

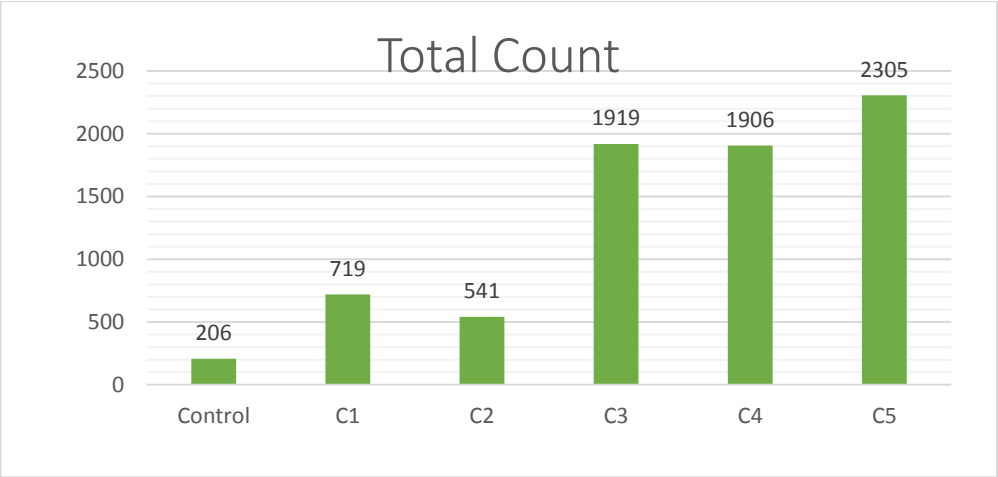

Figure 19 Total count of MFs during all 5 washing cycles, the Increasing trend of MFs is visually clear

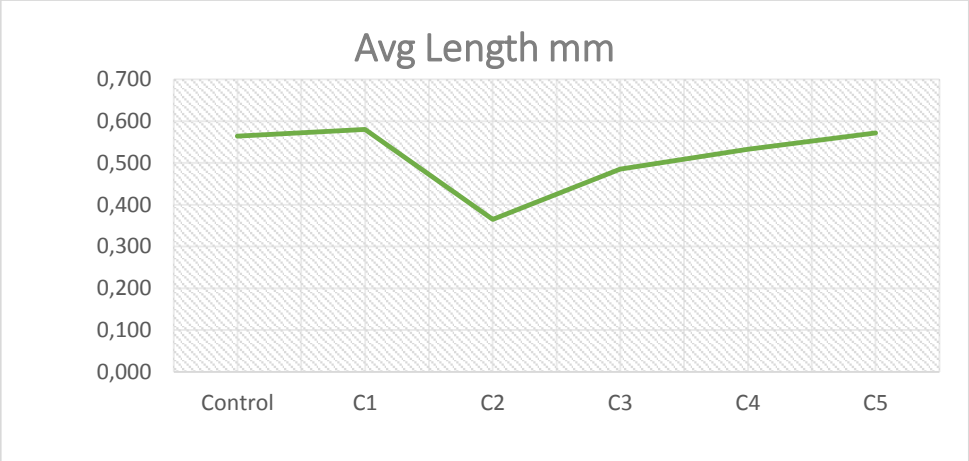

Figure 20 Average length of MFs in mm

Table 3 Results from MP-VAT Micro Fiber counting

Sample	Total MFs Count	Mean Circularity	Mean Length mm	Diff last %
Control	206	0,787	0,565	
Cycle 1	719	0,663	0,580	71,35
Cycle 2	541	0,678	0,365	24,76
Cycle 3	1919	0,666	0,485	71,81
Cycle 4	1906	0,699	0,533	0,68
Cycle 5	2305	0,683	0,572	17,31
Total Mean	1478	0.696	0.517	37.18

5.4. Weight loss of the garment

By making a relationship of the average length (L MF mm) of the microfibers per wash and the number of counted microfibers from the equations and Table (2) from methodological chapter, it is possible to calculate the weight loss of every wash cycle described down below.

Table 4 Mass loss per wash cycle based on data from Belzagui 2019

Sample	Avg. Length mm	Total Count	Length*n	Mg/MF
Control	0,565	206	116,294	0,01400697
C1	0,580	719	417,323422	0,05026429
C2	0,365	541	197,593237	0,02379901
C3	0,485	1919	931,020159	0,11213621
C4	0,533	1906	1015,971038	0,12236807
C5	0,572	2305	1318,349	0,15878781
Mean Mg/MF				0,09347108

Having the results of a range from 0.02mg/MF – 0.158mg/MF per wash. an average length of 0.517mm of MF, then calculating the relationship of MF/mass with the average diameter of 10µm and dtex of 1.08 a result of 0.093 mg/MF from the 100% polyester garment used for the experiment, considering that it weighted 242g, the fiber detachment means a 0.0034% of mass loss from the garment per wash.

6. Discussion

6.1. Sample making

It is good to mention that from the 11 samples that were taken, the very first sample was taken in different conditions, with a different washing machine, different time of washing and different rpm, this difference for that specific sample conditions took place because it was agreed in the beginning that the washing machine will be used for the whole research, but due to external problems with the water system in the place where the first washing edsd7another washing machine could be found with a controlled environment, however, the first sample had the same subsequent procedure with the staining, imaging and software counting as the rest of the samples.

In addition to the control sample, after discarding the possibility of comparing the detachment of many garments under the same conditions, an experimental sample between the 2nd and the 3rd cycle took place introducing a T-shirt of the same brand, material and type, but with a different color (black), the purpose of this experiment was to see if there were MFs remaining from previous washing cycles that could affect the counting on each sample. After mixing the washing cycles it was observed different color of fibers (blue) at least 5% of the total fibers were in the sample from the black washing and black microfibers that remained in the washing machine after the black T-shirt was removed. The number of alien fibers on the samples couldn't be counted and it was counted in the total MFs of 3 samples (2nd, 3rd and Black sample), but it could be a good point to take in mind in future research that MFs from previous washing cycles stay in the washing machine and are released in the next cycle.

6.2. Image Taking and data Processing

There were several failed attempts to take good quality images of the samples under UV light, the ideal case would be to have all samples images taken with a microscope in order to be able to appreciate smaller debris and have accurate results, unfortunately it was only possible to have the image of one sample with very low illumination. Then a second attempt was made with a semiprofessional camera trying to compensate the photographic parameters like ISO and shutter speed, but, because of the focusing capability of the camera the images where too blurry and they could not be processed properly by Imagej, Finally and surprisingly, the most satisfactory images, with acceptable quality were obtained with the mobile smartphone camera (already stated in Methodology chapter), as it was seen in the results chapter and be checked in more detail in the appendix all the data processing was made with mobile phone pictures, MP-VAT plugin was able to detect and count the MF detached from every sample and the research kept its course.

Nevertheless the whole details of each sample probably were not obtained by the smartphone camera, There was a final attempt of taking the images of the samples with a microscope Nikon Eclipse E100 in 100x, in this case the imaging would take place by picturing many fragments of one sample and then use the “Photo merge tool” in Photoshop in order to obtain a full size sample with all the merged fragments from one sample, Unfortunately probably because of the similar pattern of the fibers in each fragment image, Photoshop was not able to merge the complete picture, making impossible to use the image for the software counting.

To confirm that the microscope image quality could affect the results, a single comparison has been made by analyzing with MP-VAT two fragments of the same size in scale but one taken with the microscope and one with the smartphone camera (Figure 21) having a counting of 116 particles in the microscope picture and 35 in the smartphone camera, at first sight this could suggest that the results from the research are only 1/3 of the potential counting, even though the images are from the same sample, they are not

showing the same exact area, the comparison was made only to assess the quality of image in this scale.

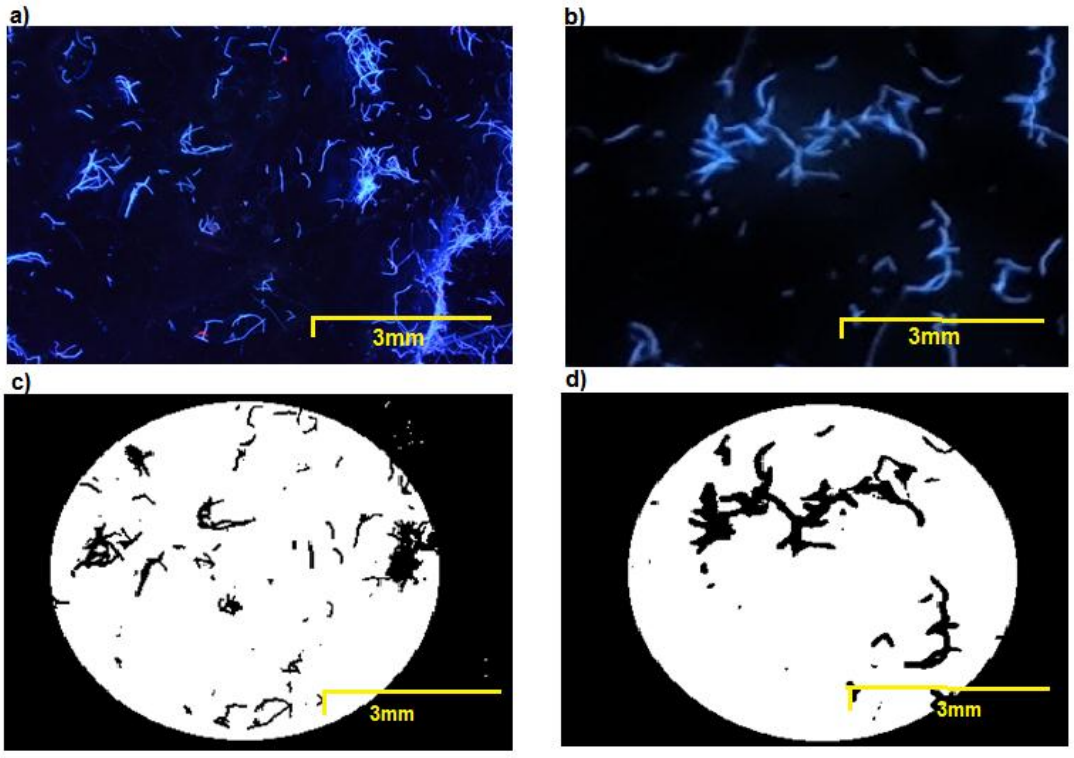

Figure 21 Counting comparison between a microscope image and a smartphone image of two fragments of the same size, a) & c) Microscope images with 116 counts, b) & d) smartphone images with 35 counts, resulting 1/3 of the potential counting of MFs

An indirect problem that affected the results from this research was the outbreak of Covid-19 that took place in Europe in the months of February and March of 2020. Due to the interruptions and mandatory quarantine, the obtaining of the necessary images for the posterior MF analysis was very difficult to take in a laboratory. After the release of the restrictions of the quarantine in Czech Republic it was possible to take some Microscope images which were compared with the ones used in for software counting (Figure 21).

6.3. Microfiber Counting

For the control sample having more particles than fibers makes sense as the synthetic garment was absent, and studies already made in Czech Republic already showed presence of MPs in tap water (Pivokonsky, 2018).

After analyzing the results, with the sampling, staining, image taking and software analysis of all 5 washing cycles. The quantity of MFs obtained per washing cycle was on average (1478 MFs) Taking in mind that the 1478 MFs in average from all the washing cycles were from 20 liters of water, it gives a number of 73.9 MFs per liter, similar to the number of MFs got by Browne et al. (2011), however not so close to the amount of MFs that Belzagui (2018) who had from 6,000 to 14,000 MFs per Liter (Figure 22), nevertheless, it is a high amount of MFs release only for one garment for one washing cycle, In addition, more attention have to be paid in the increasing release trend that was obtained, more than 50% increase from the first cycle to the fifth one, on the contrary of the expected from Belzagui (2019) who concluded that after the 5^{th} washing cycle new synthetic garments show higher MFs detachment, in this case, there was an increase of the MFs counted.

Another anomaly that can be related with the increasing trend of MFs, is the labeling of the plugin that depends on the circularity that showed more particles than fibers. This could be explained by the high overlapping of the fibers producing clusters of fibers in the petri dish and could be misinterpreted in the software analysis, maybe taking more samples of the same washing cycle and analyzing them separately and also with a better image quality could improve the results of the labeling and the counting results in general. This labeling of the particles probably, on the other hand needs software improvement to become more accurate and should be considered only with samples that have low amount of MPs. (<300 particles). In the end, several factors can determine this difference from the fact that the first sample was made in different conditions than the rest of the washing cycles, the manufacture of the textile, the way how the MFs

were placed into the petri dish at the moment of the picture taking. And also the hardware used for the image taking could affect the results.

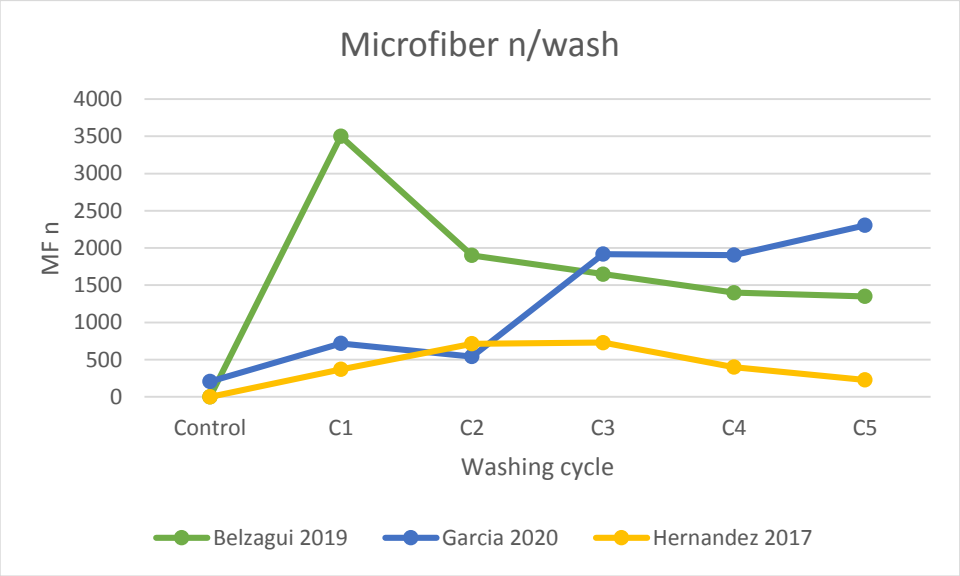

Figure 22 MF detachment trend after 5 washing cycles of a Polyester textiles and comparison with other studies

Finally when talking about the image processing, quality and light in the room, play a very important role in the final result, as the software depends entirely on how detailed and well illuminated are the images, so the plugin will be able to count all the bright spots with no misinterpretation.

Nevertheless, the fact that the image quality of a smartphone could be used for the software MPs counting opens up a good alternative for future research, as technology is moving forward almost every day, with help of a smartphone in field work, samples can be pictured and analyzed in situ. Avoiding extra steps with more software analysis, as always it is better to use it as a first reference for detailed results and not for ultimate data.

6.4. Weight loss

In Chapter 2.4.4.2. It was reviewed the estimate of weight loss per wash by Napper and Thompson (2016) were they had an average weight loss of a 2.21 Mg of polyester in a load of 6kg of laundry. Meaning a 70mg/year release per capita taking in mind EU population.

A comparison have been made between different researches on the same topic and based on the estimations made in 2016 by Napper & Thompson (Table 4). In Figure (24) it is shown that the mean mass loss per garment in this research are in between the values gotten by Hernandez et al. (2017) and Belzagui (2019). However the % of loss per garment is closer to the values obtained by Napper & Thompson (2016) and Belzagui (2019) highest in this research, even though the mass and the type of garments used in each research can vary widely.

Hernandez et al. (2017) had the highest mass loss per garment with 0.1mg/garment and the highest loss percentage per garment, but is important to remark they used smaller cut fabrics from a garment in combination of different detergents see Table (4) for more details on the methodology used in each research.

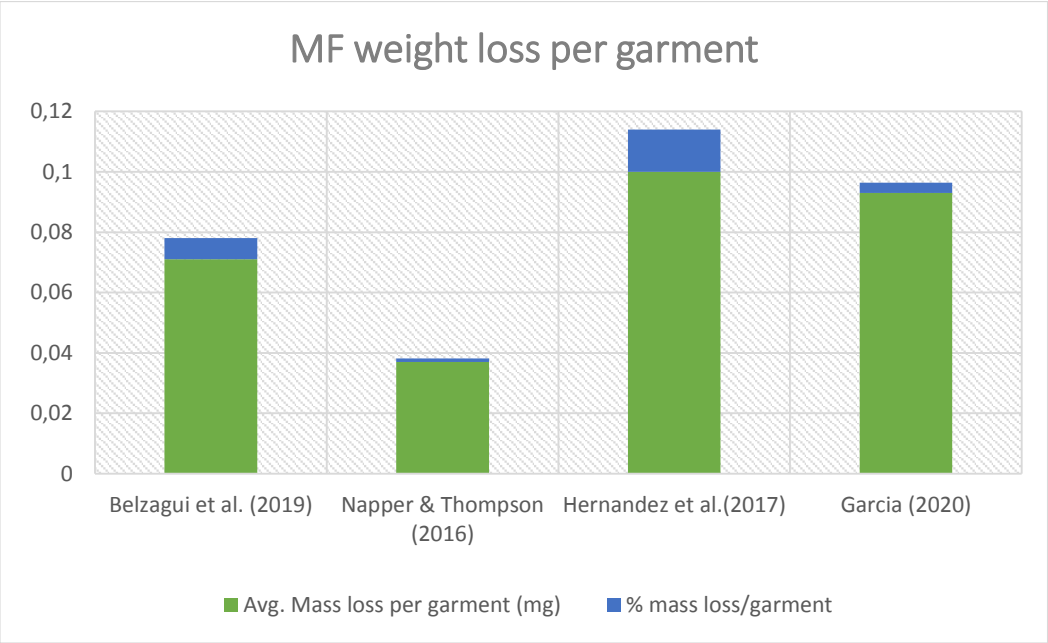

Figure 23 Weight loss in Mg (green) and % of mass loss per garment (Blue) of different researches on MF release after laundry

To have a better understanding of what the mass loss per garment represents, an estimation of weight release per capita and an adjustment to the Czech Republic population (Figure 25) has been made in order to make an idea of how much MFs can be released into the water systems from synthetic clothes. Nevertheless the estimated number have to be taken as a guide, because as shown in the graphic, variables like temperature and usage of detergent can make the total weight release much higher. Despite the high variability of the values, a range of approximately 750 Kg to 2000Kg of polyester MFs can be released into the environment only by an ordinary action such as laundry in a small country like Czech Republic.

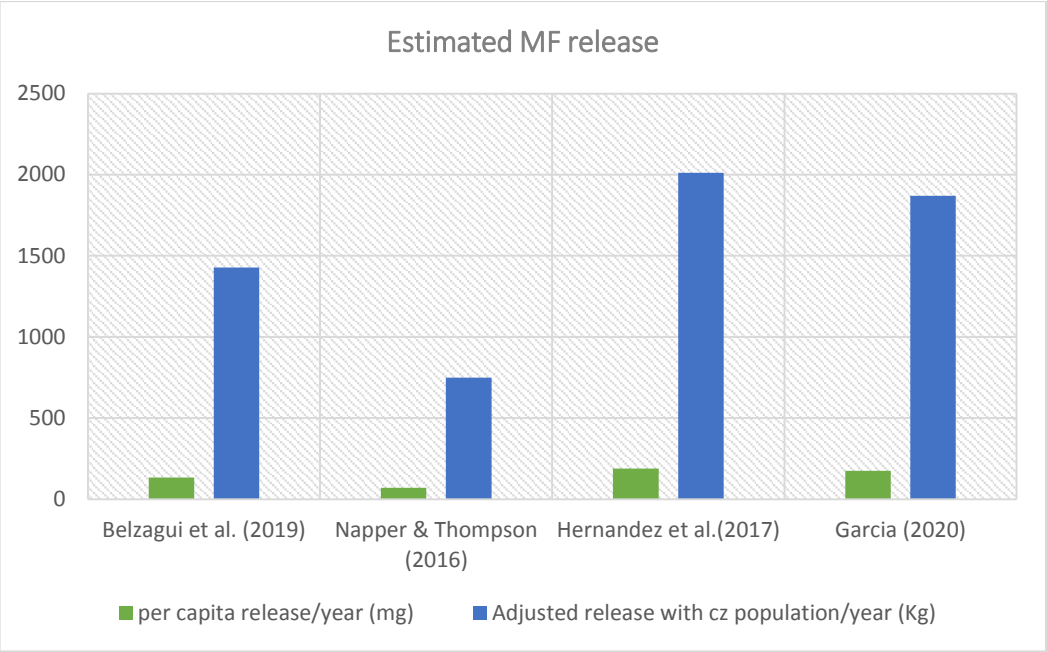

Figure 24 Estimations of Polyester release into the environment from domestic laundry from the perspective from different research studies, based on Napper & Thompson (2016) estimations

Table 5 Mass loss comparison between different studies in MF, based on Napper & Thompson (2016) estimations

	Avg. Mass loss per garment (mg)	% mass loss/garment	per capita release/year (mg)	Adjusted release with CR population/year (Kg)	Observations
Belzagui et al. (2019)	0,071	0,007	133,6	1428,184	Used 60`C washing cycles, No detergent
Napper & Thompson (2016)	0,037	0,0012	70	748,3	Used a combination of temperature and detergents
Hernandez et al.(2017)	0,1	0,014	188,17	2011,5373	Used 25x7cm fabrics, combination of liquid and powder detergent
Garcia (2020)	0,093	0,0034	174,99	1870,6431	30`C washing cycle, No detergent

6.5. Usage of filters as Microfiber reduction

In 2018 McIlwraith et al. made a study with already marketed technologies of filters that could be attached to the drain pipe of the washing machines in order to retain the MFs. In their results after washing a fleece blanket, they obtained a reduction of 87% of the MF counting from a control sample (Figure 25). Nevertheless their findings are subject to the length of the fibers, the filters are not able to catch microfibers smaller than 100µm, but if some technology like this is introduced in most of the washing machines, the reduce of MFs could be drastically decreased. It depends on the cost-benefit and the willing of every household to take the time of throw the MFs from the filters.

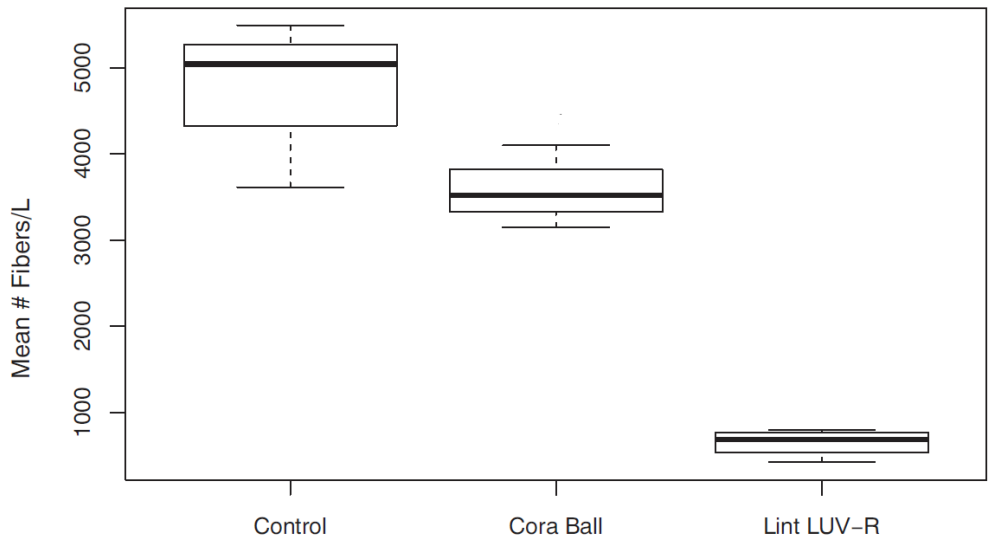

Figure 25 Microfiber Reduction using two different filters for washing machines (Source: McIlwraith et al. 2018)

7. Conclusions and Recommendations

7.1. Conclusions

After reviewing the problematic of synthetic Microfiber detachment from domestic laundry as one of the main sources of Microplastic accumulation in the environment and analyzing the Microfiber detachment after Nile red staining and Software counting under UV light from a 100% polyester T-shirt the conclusions are the following:

- Sample imaging with smartphone camera is possible for the counting of microfibers with MP-VAT plugin.
- Microfiber detachment from a 100% polyester garment was in average 1478 MFs per wash, with fibers of 0.517 mm in average.
- Decreasing trend of Microfibers release until the 5th wash hypothesis was shown opposite, having instead, an increase of 3 times more MFs from the first wash and the fifth wash.
- Similar results from reviewed literature have been obtained in Microfiber mass loss with 0.093 Mg release per garment. And probably representing a release of 175 mg/year per capita in a country like Czech Republic.

7.2. Recommendations

- Nile red staining and software counting are good tools for analyzing Microplastics, however another methodology have to be present where more types of plastics are involved and the researchers have to take in mind very carefully the image quality at the moment to analyze samples. It is advisable to analyze more samples with less overlap than few samples with high load of Microfibers
- Image quality is a Key process for this kind of research, it is recommended to avoid overlapping of the MF by taking more samples.
- The use of specific filters in the washing machine's drain pipe could contribute to reduce the microfiber release in more than 85%.

References:

Albert, L. 1997. Contaminación ambiental. Origen, clases, fuentes y efectos. Toxicology Mexican Society, A.C., Xalapa. México.

Arias-Andres Maria, Uli Klümper, Keilor Rojas-Jimenez, Hans-Peter Grossart. 2018, Microplastic pollution increases gene exchange in aquatic ecosystems, Environmental Pollution, Volume 237, Pages 253-261, ISSN 0269-7491.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605.

Barnes David K. A., Francois Galgani, Richard C. Thompson and Morton Barlaz, 2009, Accumulation and Fragmentation of plastic debris in global environment. Phil. Trans. R. Soc. B3641985–1998.

Belzagui Francisco, Martí Crespi, Antonio Álvarez, Carmen Gutiérrez-Bouzán, Mercedes Vilaseca, 2019. Microplastics' emissions: Microfibers' detachment from textile garments, Environmental Pollution, Volume 248, pages 1028-1035.

Bensaude Vincent, B. 2013. Plastics, materials and dreams of dematerialization. En J. Gabrys, G. Hawkins, & Michael, M. (Eds.) Accumulation: The material politics of plastic. UK: Routledge.

Bermúdez, M. 2010. Pollution and sustainable tourism. CETD SA.

Brennecke Dennis, Bernardo Duarte, Filipa Paiva, Isabel Caçador, João Canning-Clode. 2016,Microplastics as vector for heavy metal contamination from the marine environment, Estuarine, Coastal and Shelf Science, Volume 178, Pages 189-195, ISSN 0272-7714.

Browne, M.A., Crump, P., Niven, S.J. Galloway, Teuten, E., Tonkin, A., T., Thompson, R., 2011. Accumulation of microplastics on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179.

Browne, M.A., Niven, S. Galloway, T. Rowland, S. Thompson, R 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Current biology 23, 2388-2392. Doi:10.1016/j.cub.2013.10.012.

Brydson, J.A. 1999. Plastics materials. UK: Elsevier.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182.

Castaneda, R.A., Avlijas, S., Simard, M.A., Ricciardi, A., 2014. Microplastic pollution inSt. Lawrence River sediments. Can. J. Fish. Aquat. Sci. 71, 1767e1771.

Chang, M., 2015. Reducing microplastics from facial exfoliating cleansers in wastewater through treatment versus consumer product decisions. Mar. Pollut. Bull. 101, 330–333.

Cole, M., et al., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., et al. 2013. Microplastic ingestion by zooplankton. Environmental Science and Technology, 47(12), 6646–6655.

Cole, M. 2016. A novel method for preparing microplastic fibers. Sci Rep 6, 34519

Collignon Amandine, Jean-Henri Hecq, François Glagani, Pierre Voisin, France Collard, Anne Goffart, 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea, Marine Pollution Bulletin, Volume 64, Issue 4, Pages 861-864. ISSN 0025-326X,

Conkle, J.L., Del Valle, C.D.B., Turner, J.W., 2018. We under estimating microplastic contamination in aquatic environments? Environ. Manag. 61, 1–8.

Cózar et al, 2014. Pastic debris in the open ocean. PNAS, vol 111 no 28. ISBN: 10239-10244

De Falco, F., Gullo, M.P., Gentile, G., Di Pace, E., Cocca, M., Gelabert, L., Brouta-Agnésa, M., Rovira, A., Escudero, R., Villalba, R., et al., 2018. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. Environ. Pollut. 236, 916–925.

Dekiff, J. H., Remy, D., Klasmeier, J., & Fries, E. (2014). Occurrence and spatial distribution of microplastics in sediments from Norderney. Environmental Pollution, 186, 248–256.

Dris Rachid, Johnny Gasperi, Cécile Mirande, Corinne Mandin, Mohamed Guerrouache, Valérie Langlois, Bruno Tassin. 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments, Environmental Pollution, Volume 221, Pages 453-458, ISSN 0269-7491.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., et al. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Marine Pollution Bulletin, 77(1–2), 177–182.

Essel Roland, Linda Engel, Michael Carus, 2015. Sources of microplastics relevant to marine protection in Germany, Federal Environment Agency of Germany, ISSN 1862-4804.

Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar. Pollut. Bull. 58 (8), 1225–1228.

Fossi M. C., Daniele Coppola, Matteo Baini, Matteo Giannetti, Cristiana Guerranti, Letizia Marsili, Cristina Panti, Eleonora de Sabata, Simona Clò, 2014. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First eco-toxicological investigation using skin biopsies. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 199, 48–58.

Frias, J., Sobral, P., & Ferreira, A. M. 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Marine Pollution Bulletin, 60(11), 1988–1992.

Fries, E., Dekiff, J. H., Willmeyer, J., Nuelle, M. T., Ebert, M., & Remy, D. 2013. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environmental Science-Processes & Impacts, 15(10), 1949–1956.

Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (Cetorhinus maximus) and fin whale (Balaenoptera physalus), Marine Environmental Research, Volume 100, Pages 17-24, ISSN 0141-1136,

Galafassi Silvia, Luca Nizzetto, Pietro Volta. 2019, Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water, Science of The Total Environment, Volume 693, 133499, ISSN 0048-9697

Gatidou, G., Arvaniti, O.S., Stasinakis, A.S., 2019. Review on the occurrence and fate of microplastics in sewage treatment plants. J. Hazard. Mater. 367, 504–512.

GESAMP. 2015, “Sources, fate and effects of microplastics in the marine environment: a global assessment” (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p

Gilbert, M. (2017). Brydson’s plastics materials. UK: Elsevier.

Gouin, T., Avalos, J., Brunning, I., Brzuska, K., Graaf de, J., Kaumanns, J., Konong, T., Meyberg, M., Rettinger, K., Schlatter, H., Thomas, J., Welie van, R., Wolf, T., 2015. Use of micro-plastic beads in cosmetic products in Europe and their estimated emissions to the North Sea environment. SOFW J 141 (3), 40–46

Ha, J., Yeo, M.-K., 2018. The environmental effects of microplastics on aquatic ecosystems. Mol. Cell. Toxicol. 14 (4), 353–359.

Hardin Amy L., 1990, Industry Structure and the Marketing of. Synthetic Fibers. University of Delaware, Business and Economic History, 2d ser., 19 (1990): 213–22

Harper, C.A. 2000. Modern Plastics Handbook. The Mcgraw-Hill companies, Inc. ISBN: 9780070267145

Harrison, J. P., Ojeda, J. J., & Romero-Gonzalez, M. E. 2012. The applicability of reflectance micro-Fourier-transform infrared spectroscopy for the detection of synthetic microplastics in marine sediments. Science of the Total Environment, 416, 455–463.

Hartline, N.L., Bruce, N.J., Karba, S.N., Ruff, E.O., Sonar, S.U., Holden, P.A., 2016. Microfiber masses recovered from conventional machine washing of new or aged garments. Environ. Sci. Technol. 50 (21), 11532–11538.

Hayley K. McIlwraith, Jack Lin, Lisa M. Erdle, Nicholas Mallos, Miriam L. Diamond, Chelsea M. Rochman, Capturing microfibers – marketed technologies reduce microfiber emissions from washing machines, Marine Pollution Bulletin, Volume 139, 2019, Pages 40-45.

Hidalgo-Ruz, V., & Thiel, M. 2013. Distribution and abundance of small plastic debris on beaches in the SE Pacific (Chile): A study supported by a citizen science project. Marine Environmental Research, 87–88, 12–18.

Hintersteiner, I., Himmelsbach, M., Buchberger,W.W., 2015. Characterization and quantitation of polyolefin microplastics in personal-care products using high-temperature gel-permeation chromatography. Anal. Bioanal. 407 (4), 1253–1259.

Imhof, H. K., Schmid, J., Niessner, R., Ivleva, N. P., & Laforsch, C. 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnology and Oceanography-Methods, 10, 524–537.

Imhof, H. K., Ivleva, N. P., Schmid, J., Niessner, R., & Laforsch, C. 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Current Biology, 23(19), R867–R868.

Imhof H. K., Robert Sigl, Emilia Brauer, Sabine Feyl, Philipp Giesemann, Saskia Klink, Kathrin Leupolz, Martin G.J. Löder, Lena A. Löschel, Jan Missun, Sarah Muszynski, Anja F.R.M. Ramsperger, Isabella Schrank, Susan Speck, Sebastian Steibl, Benjamin Trotter, Isabel Winter, Christian Laforsch, 2017. Spatial and temporal variation of macro-, meso- and microplastic abundance on a remote coral island of the Maldives, Indian Ocean, Marine Pollution Bulletin, Volume 116, Issues 1–2, Pages 340-347, ISSN 0025-326X,

Jemec Anita, Petra Horvat, Urban Kunej, Marjan Bele, Andrej Kržan, 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna, Environmental Pollution, Volume 219, Pages 201-209, ISSN 0269-7491.

Jin Yuanxiang, Jizhou Xia, Zihong Pan, Jiajing Yang, Wenchao Wang, Zhengwei Fu. 2018, Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish, Environmental Pollution, Volume 235, Pages 322-329, ISSN 0269-7491.

Knight, G. 2012. Plastic pollution. UK: Raintree.

Lam, C.S., Ramanathan, S., Carbery, M., Gray, K., Swaroop Vanka, K., Maurin, C., Bush, R., Palanisami, T., 2018. A comprehensive analysis of plastics and microplastic legislation worldwide. Water Air Soil Pollut. 229, 345.

Lares, M., Chaker Ncibi, M., Sillanpää, M., Sillanpää, M., 2018. Occurrence, identification and removal ofmicroplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. 133, 236–246.

Lei Lili, Siyu Wu, Shibo Lu, Mengting Liu, Yang Song, Zhenhuan Fu, Huahong Shi, Kathleen M. Raley-Susman, Defu He. 2018, Microplastic

particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans, Science of The Total Environment, Volumes 619–620, Pages 1-8, ISSN 0048-9697.

Leslie, H. A., Brandsma, S. H., van Velzen, M. J. M., & Vethaak, A. D. 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environment International, 101, 133–142.

Löder M.G.J., Gerdts G. 2015 Methodology Used for the Detection and Identification of Microplastics — A Critical Appraisal. In: Bergmann M., Gutow L., Klages M. Marine Anthropogenic Litter. Springer, Cham, Print ISBN 978-3-319-16509-7

Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., Gorbi, S., Regoli, F., 2019. The fate of microplastics in an Italian wastewater treatment plant. Sci. Total Environ. 652, 602–610.

Magnusson, K., Norén, F., 2014. Screening of Microplastic Particles in and Down-Stream a Wastewater Treatment Plant, Report C55. Swedish Environmental Research Institute, Stockholm (22 pages).

Masura, J., Joel Baker, Gregory Foster, Courtney Arthur, 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. National Oceanic and Atmospheric Administration, Technical Memorandum NOS-OR&R-48.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci.Technol. 48, 11863e11871.

Morét-Ferguson, S., Law, K. L., Proskurowski, G., Murphy, E. K., Peacock, E. E., & Reddy, C. M. (2010). The size, mass, and composition of plastic debris in the Western North Atlantic Ocean. Marine Pollution Bulletin, 60(10), 1873–1878.

Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50 (11), 5800–5808.

Murphy F., Brian Quinn. 2018, The effects of microplastic on freshwater Hydra attenuata feeding, morphology & reproduction, Environmental Pollution, Volume 234, Pages 487-494, ISSN 0269-7491.

Murray, F., & Cowie, P. R. 2011. Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758). Marine Pollution Bulletin, 62(6), 1207–1217.

Napper, I.E., Thompson, R.C., 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. 112 (1–2), 39–45.

Nelms Sarah E., Tamara S. Galloway, Brendan J. Godley, Dan S. Jarvis, Penelope K. Lindeque. 2018, investigating microplastic trophic transfer in marine top predators, Environmental Pollution, Volume 238, Pages 999-1007, ISSN 0269-7491.

Ng, K. L., & Obbard, J. P. 2006. Prevalence of microplastics in Singapore's coastal marine environment. Marine Pollution Bulletin, 52(7), 761–767.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779.

Peeken, I., Primpke, S., Beyer, B. et al., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat Commun 9, 1505.

Pirc, U., Vidmar, M., Mozer, A. et al. Emissions of microplastic fibers from microfiber fleece during domestic washing. Environ Sci Pollut Res 23, 22206–22211 (2016).

Pivokonsky Martin, Lenka Cermakova, Katerina Novotna, Petra Peer, Tomas Cajthaml, Vaclav Janda. 2018, Occurrence of microplastics in raw and treated drinking water, Science of The Total Environment, Volume 643, Pages 1644-1651,

Prata Joana C., Vanessa Reis, João T.V. Matos, João P. da Costa, Armando C. Duarte, Teresa Rocha-Santos, 2019, A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT), Science of The Total Environment, Volume 690, Pages 1277-1283.

Reddy, M. S., Basha, S., Adimurthy, S., & Ramachandraiah, G. (2006). Description of the small plastics fragments in marine sediments along the Alang-Sosiya ship-breaking yard, India. Estuarine Coastal and Shelf Science, 68(3–4), 656–660.

Rochman C.M., M.A. Browne, B.S. Halpern, B.T. Hentschel, E. Hoh, H.K. Karapanagioti, L.M. Riosmendoza, H. Takada, S. Teh, R.C. Thompson 2013. Classify plastic waste as hazardous, Nature, 494 (2013), pp. 169-171

Salvador Cesa Flavia, Alexander Turra, Julia Baruque-Ramos, 2017. Synthetic fibers as microplastics in the marine environment: A review from textile perspective with a focus on domestic washings, Science of The Total Environment, Volume 598, Pages 1116-1129, ISSN 0048-9697,

Spiegel, J. 2001. Environmental pollution control. Health and work security. Spain.

Strand, J., 2014. Contents of polyethylene microplastic in some selected personal care products in Denmark. Poster, NMC Conference on Plastics in the Marine Environment, Reykjavík, Iceland.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., et al. 2004. Lost at sea: Where is all the plastic? Science, 304(5672), 838.

Thompson Richard C., Swan Shanna H., Moore Charles J. and vom Saal Frederick S, 2009. Our plastic age, Phil. Trans. R. Soc. B3641973–1976

Van Cauwenberghe, L., Vanreusel, A., Mees, J., & Janssen, C. R. 2013. Microplastic pollution in deep-sea sediments. Environmental Pollution, 182, 495–499.

Van Sebille Erick, Chris Wilcox, Laurent Lebreton, Nikolai Maximenko, Britta Denise Hardesty, Jan A van Franeker, Marcus Eriksen, David Siegel, Francois Galgani and Kara Lavender Law, 2015. A global inventory of small floating plastic debris, ARC Centre of Excellence for Climate System Science, Climate Change Research Centre, University of New South Wales, Sydney, Australia. Environ. Res. Lett. 10 (2015) 124006.

Verschoor A. J. 2015. Towards a definition of microplastics, National Institute for Public Health and the Environment, Bilthoven Netherlands.

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., et al. 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science, 130, 54–61.

Wake, H. (2005). Oil refineries: a review of their ecological impacts on the aquatic environment. Estuarine, Coastal and Shelf Science, 62: 131-140.

Welden Natalie A., Phillip R. Cowie. 2017, Degradation of common polymer ropes in a sublittoral marine environment, Marine Pollution Bulletin, Volume 118, Issues 1–2, Pages 248-253, ISSN 0025-326X.

Woodall Lucy C., Sanchez-Vidal Anna, Canals Miquel, Paterson Gordon L.J., Coppock Rachel, Sleight Victoria, Calafat Antonio, Rogers Alex D., Narayanaswamy Bhavani E. and Thompson Richard C, 2014. The deep sea is a major sink for microplastic debris, Royal Society Open Science, PubMed: 26064573

© World Health Organization, 2004, Guidelines for drinking water quality, third edition, volume 1, Geneva.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.

Zhu Dong, Qing-Lin Chen, Xin-Li An, Xiao-Ru Yang, Peter Christie, Xin Ke, Long-Hua Wu, Yong-Guan Zhu, Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition, 2018. Soil Biology and Biochemistry, Volume 116, Pages 302-310, ISSN 0038-0717,

Web pages:

Snowden Scott, 2019, “300-Mile Swim through the Great Pacific Garbage Patch Will Collect Data on Plastic Pollution, Forbes Media LLC. Available at:

https://www.forbes.com/sites/scottsnowden/2019/05/30/300-mile-swim-through-the-great-pacific-garbage-patch-will-collect-data-on-plastic-pollution/

© Textile school, 2019. Polyester Fiber and its uses. Available at:

https://www.textileschool.com/234/polyester-fiber-and-its-uses/

© Plastics Europe, 2016. An analysis of European plastics production, demand and waste data. Available at:

https://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2016.pdf

© Plastics Europe, 2018, the facts – 2018, an analysis of European plastics production, demand and waste data. Available at:

© UNEP (United Nations Environment Programme), 2015. Plastic in Cosmetics. 38 pages. Available at. http://apps.unep.org/publications/index.php?option=com_pub&task=download&file=011718_en.

© Eurostat. Sewage sludge use, 2010, available at:

https://ec.europa.eu/environment/waste/sludge/

© World wide found of nature: Water Pollution. Available at:

https://wwf.panda.org/knowledge_hub/teacher_resources/webfieldtrips/water_pollution/