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# PREPARATION OF PLASTIC MICRO- AND NANOFIBERS BY ELECTRO- SPINNING FOR CELL CULTURE

Master's Thesis  
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# ABSTRACT

Emmi Anttola: Preparation of plastic micro- and nanofibers by electrospinning for cell culture  
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Plastic production has increased significantly over the decades and due to lack of proper and efficient waste management, it is estimated that millions of tons plastic waste ends up in oceans annually. Besides aquatic environments, plastic wastes are found in soils and uninhabited areas around the world. In nature, plastic is exposed to multiple factors that degrade plastic into the smaller pieces, micro- and nanoplastics. Plastic particles with diameter less than 5 mm are defined as microplastics and particles with diameter less than 1  $\mu\text{m}$  as nanoplastics. Microplastics can be also manufactured originally to millimetric size for example to act as abrasives in skin care products.

Environmental degradation of plastic is commonly divided in two categories: abiotic and biotic degradation. Microorganisms degrade plastic in biotic degradation. Other processes, such as thermal and mechanical degradations are classified as abiotic degradation. Micro- and nanoplastics are proven to end up in our food chain through seafood, but plastic particles are also found in other consumers as table salts and tap water. Globally contamination of food has raised public health concerns. Moreover, airborne microplastic increases the number of particles humans are exposed daily. Inhalation is notable exposure route besides ingestion.

Methods to investigate microplastic are developed within the years and new imaging systems are developed to identify particles from different samples, such as sediment and biological samples. However, the field of microplastic research is still lacking the standardization, which complicates the comparison between different studies. At worst, results of the studies can be distorted due to contamination of the samples, when the sampling and separation is not performed with extra care. Microplastic exposure tests have been performed during the latest decade in laboratories and scientists are trying to understand all the mechanisms how micro- and nanoplastics might disturb systems in our body. Many studies have been performed by using commercially available polystyrene microspheres with different sizes. However, the range of polymeric materials and shapes are more complex in our environments. Notable part of microplastics is in the shape of fibers, which are usually detached from the textiles.

The aim of this thesis was to investigate the possibility to manufacture cut and separated nanofibers from three common plastics: polyamide, polyethylene terephthalate, and polystyrene, for cell exposure studies. Fibers were produced by using electrospinning, and all the fiber samples were imaged using scanning electron microscopy. Also, the imaging parameters and the effect of the coating were studied. A mortar and cryomicrotome were used for cutting and breaking the fiber film to obtain separation of the fibers. Both methods were performed in frozen conditions. Liquid nitrogen was used to achieve brittle behavior of the plastic. The operating temperature of cryomicrotome was  $-20^{\circ}\text{C}$ . Mortar was suitable to cut and separate fibers from the film, but due to inefficiency of the method, the process needs improvements. Results were verified with scanning electron microscope imaging. Fibers were packed together as chunks when film was crushed in the mortar. Only a few fibers were cut and detached from the film. Cutting the film with cryomicrotome was challenging because the orientation of the film on the sample holder was impossible due to electrostatic forces and fragileness of the film. Moreover, the freezing of the sample with liquid nitrogen made the film bulge uncontrollable. Also, the distinguishing of the fiber film from the surrounding encapsulation compound was challenging due to similar color. Cut sections were imaged using SEM, but fibers were not identified from the samples and therefore the cut and separation of the fibers by using cryomicrotome could not be demonstrated.

Keywords: micro- and nanoplastics, plastic degradation, electrospinning, scanning electron microscope imaging, cryomicrotome, mortar, coating

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# TIIVISTELMÄ

Emmi Anttola: Mikro- ja nanomuovikuitujen valmistus sähkökehruulla soluviljelykokeita varten  
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Muovin tuotanto on noussut merkittävästi vuosikymmenten aikana ja sen takia myös muovijätettä syntyy maailmassa valtavia määriä vuosittain. Huonosta jätteenkäsittelystä johtuen on arvoitu, että vuosittain jopa kahdeksan miljoona tonnia muovijätettä päätyy meriin. Lisäksi muovijätettä päätyy maaperään ympäri maapalloa. Asutettujen alueiden lisäksi teollisesti tuotettua muovia on löytynyt syrjäisistä ja asuttamattomista paikoista, kuten jäätiköiltä ja vuoristoista. Luonnossa muovijäte altistuu erilaisille kemikaaleille, auringon valolle, lämmölle, mekaaniselle rasitukselle ja mikroeliöille, jotka hajottavat muovia mikro- ja nanomuoviksi. Mikromuoviksi luokitellaan partikkelit, joiden halkaisija on alle viisi millimetriä ja nanomuoviksi partikkelit, joiden halkaisija on alle yksi mikrometri. Mikromuovi voi olla myös alun perin valmistettu tähän kokoluokkaan. Esimerkiksi kuorivissa ihonhoitotuotteissa voidaan käyttää mikromuovirakeita.

Luonnossa muovi hajoaa abiottisesti ja bioottisesti. Bioottinen hajoaminen tapahtuu mikro-organismien avulla. Abioottiseen lukeutuvat muut hajoamisprosessit, kuten mekaaninen ja kemiallinen hajoaminen. Tutkimuksissa on selvinnyt, että mikro- ja nanomuovia päätyy mereneläviin, kuten kaloihin ja simpukoihin, joita ihmiset syövät. Mereneläinten lisäksi, muovia on löydetty myös esimerkiksi suolasta sekä hana- ja pullovesistä. Tutkimukset ovat osoittaneet, että ihmiset altistuvat mikromuoveille myös hengitysteitse. Koska altistumisesta aiheutuvia mahdollisia haittoja ja pitkäaikaisvaikutuksia ei vielä kunnolla tunneta, tämä huolestuttaa tutkijoita ympäri maapalloa.

Mikro- ja nanomuovien tutkimus- ja kuvantamismenetelmät erilaisista näytteistä ovat kehittyneet vuosien saatossa. Kuitenkin tutkimusalalta puuttuvat yleiset standardit, joten eri tutkimusten vertailu keskenään on haasteellista. Pahimmillaan standardien puuttuminen voi johtaa tulosten vääristymiseen, jos esimerkiksi näytteiden käsittelyssä ei noudateta erityistä huolellisuutta ja näytteet pääsevät kontaminoitumaan. Tutkijat ovat yrittäneet selvittää viime vuosien aikana, kuinka mikro- ja nanomuovi mahdollisesti häiritsee solujemme toimintaa.

Mikromuoviin keskittyvissä altistuskokeissa käytetään usein polystyreeni palloja, joita on kaupallisesti saatavilla eri kokoisina. On kuitenkin huomioitavaa, että muoveja on hyvin erilaisia ja muodot vaihtelevat epäsäännöllisen muotoisista säännöllisiin. Erityisesti tekstiileistä irtoavat kuidut ovat merkittävä osuus mikromuoveista, joille altistumme päivittäin ilmasteitse.

Työssäni oli tavoitteena valmistaa katkottuja mikro- ja nanokuituja kolmesta yleismuovista solu- ja kudostutkimuksia varten. Muoveiksi valittiin polyamidi, polyetylenitereftalaatti ja polystyreeni. Kuidut valmistettiin sähkökehruulla ja kuidut kuvannettiin pyyhkäisyelektronimikroskooppilla. Kuidut jäädettiin nestetyllä ennen kuin niitä katkottiin morttelissa ja kryomikrotomilla. Kryomikrotomilla työskennellessä käyttölämpötila oli  $-20^{\circ}\text{C}$ . Morttelilla saatiin kuitufilmistä irrotettua ja katkottua kuituja, mutta saatujen kuitujen määrä oli melko vähäinen. Nestetyypin nopea haihtuminen sai muovikalvon lämpenemään nopeasti, jolloin suurin osa kuiduista vain pakkaantui tiiviisti yhteen, kun kuitukalvoa hierrettiin morttelissa. Kryomikrotomilla jäädetyistä näytteistä pystyttiin leikkaamaan leikkeitä, mutta kuitujen leikkaantuvuutta ei pystytty todistamaan, koska pyyhkäisyelektronimikroskooppinäytteestä ei löytynyt yhtään muovikuitua.

Avainsanat: mikro- ja nanomuovit, muovien hajoaminen, sähkökehruu, pyyhkäisyelektronimikroskooppikuvantaminen, kryomikrotomi, mortteli, pinnoite

Tämän julkaisun alkuperäisyys on tarkastettu Turnitin Originality Check –ohjelmalla.

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# ABBREVIATIONS

AA	acetic acid
AChE	acetylcholinesterase
ATP	adenosine triphosphate
BI	beam intensity
BSE	back scattered electrons
DCM	dichloromethane
DMF	dimethyl formamide
FA	formic acid
FEG	field emission gun
FTIR	Fourier Transform Infrared
IR	infrared
MNP	micro- and nanoplastic
MP	microplastic
NaCl	sodium chloride
NaI	sodium iodide
NP	nanoplastic
OCT	optimal cutting temperature
PA	polyamide
PE	polyethylene
PET	polyethylene terephthalate
PMA	polymethyl acrylate
POP	persistent organic pollutant
PP	polypropylene
PU	polyurethane
PVA	polyvinyl alcohol
PVC	polyvinyl chloride
SD	standard deviation
SE	secondary electrons
SEM	scanning electron microscopy
TFA	trifluoroacetic acid
ZnBr <sub>2</sub>	zinc bromide
$n$	sample size
$\nu_0$	frequency
$\nu_m$	molecular vibrational frequency
$x$	diameter
$\bar{x}$	mean diameter

# 1. INTRODUCTION

After discovery of the first synthetic plastic called Bakelite in the early 1900's, the consumption of plastics has increased globally year after year. (Frias *et al.* 2019) In 2018 plastic production worldwide almost reached 360 million tonnes and in Europe, the production was almost 62 million tonnes (Plastics Europe 2019). Plastics are versatile material with multiple great properties. Also, diversity of different plastics is wide and new compositions are developed daily. For example, different plastics can be used in multiple components instead of metals, which can decrease the weight of the multicomponent system due to smaller density. (Plastic Europe)

However, during the last decades, global concern has risen due to plastic rafts floating in the oceans and plastic findings from the uninhabited and remote areas such as South Pole and mountains. According to the National Oceanic and Atmospheric Administration, even 8 million metric tonnes of plastic end up to oceans annually (NOAA, 2019). In the oceans, plastics degrade to smaller pieces by different mechanisms. It is noted, that microplastics, that are plastic particles with diameter under 5 mm, end up inside of marine biota via ingestion (Arthur *et al.* 2009). Human consumption of seafood is one way of human exposure to microplastics. Besides ingestion, inhalation has been discovered to be also notable route to exposure to micro- and nanoplastics (MNPs). For example, textile fibers are notable sources of MNPs, especially indoors and air of cities (Boucher and Friot 2017; Prata 2018b).

When plastic particles end up inside of human body, the interactions between the cells and plastic particles are still quite unknown (Wright and Kelly 2017). Furthermore, plastics often include more than one additive, such as phthalates, colorants, and other chemicals to improve the properties of the end product. Also, plastic can absorb toxic compound from environment and concentration of pollutants can increase to high level in plastic particle (Hidalgo-Ruz *et al.* 2012).

Detection size of the plastic particles is usually limited in the studies. Often it is impossible to collect nanosized particles from the sediments or water samples because the minimum size of the collected particles is connected to the mesh size and pore size of sieves, that are used in sample collection. Also, the analyzation tools, such as microscopes, have limitations in the detection. (da Costa *et al.* 2016; Lindeque *et al.* 2020; Cai *et al.* 2021)



Laboratory studies, animal, or cell exposure studies are often performed with using commercially available microplastics in a shape of sphere (Stock *et al.*, 2021). Polystyrene as a microsphere is one of the commonly used materials since the material is easily available. However, the usage of polystyrene microspheres almost exclusively might give limited view of the results of the microplastic exposure cell studies.

The aim of this thesis was to investigate the possibility to produce micro- and nanofibers from common plastics by electrospinning and to cut them into smaller pieces for studies of cell interactions with micro- and nanofibers in the future. Microplastic and its interactions with environment and humans are presented as a literature review. Chapter 2 is focused on the background of micro- and nanoplastics and degradation processes in nature. Different studies are presented how microplastic affects to our environment and animals and how humans and animals are exposed to it. Chapter 3 is focused on characterization techniques of MNPs and their sampling methods on site. In the experimental part, covered in Chapter 4, materials and methods are presented. Polyamide, polystyrene, and polyethylene terephthalate were chosen as materials used in fiber manufacturing, because there were suitable solvents for them in room temperature. In addition, characterization of plastic fibers and optimization of parameters for nanosized fiber imaging using scanning electron microscope is also studied. Results and discussion are covered in Chapters 5 and 6 and finally the future aspects of this topic are concluded in Chapter 7.

## 2. MICRO- AND NANOPLASTICS

Global plastic production has grown annually (Plastics Europe 2019). Different polymers are produced for different solutions and new plastic materials are developed continuously. Plastics are excellent material for multiple solutions, for example in food packaging industry (Brandsch and Piringer 2008). With suitable processing methods, material compounds, and additives, properties can be modified and specified. However, the disadvantage of majority of common plastic types is that they do not decompose easily. Persistence and ubiquity of the plastics are threat to different ecosystems (Menéndez-Pedriz *et al.* 2020).

Plastic fragments appear in multiple sizes, colors, and shapes, but according to Arthur *et al.* (2009), microplastics are classified small plastics pieces with diameter smaller than 5 millimeters (Arthur *et al.* 2009; Rocha-Santos *et al.* 2019). In this work microplastic (MP) is defined as plastic particles with diameter 1  $\mu\text{m}$  to 5 mm and nanoplastic (NP) as particle with diameter  $< 1 \mu\text{m}$ . Abbreviation MNPs is used when particle size covers the total range from nanoplastics to microplastics.

Here the main sources of micro- and nanoplastics and their typical classification are introduced. Since microplastics often result from breakage of polymer chains, different degradation processes of plastics are discussed. In addition, environmental aspect is considered and how humans, animals, and environment are exposed to the micro- and nanoplastics.

### 2.1 Classification of polymers

Polymers are produced via polymerization process, in which small molecules called monomers are chemically combined to one another with different bonds to create a polymer. The main elements of the polymers are carbon and hydrogen, but also oxygen and nitrogen are common elements. Polymers can be categorized in multiple ways according to their properties or physical structure, but the primary classification of the most polymers is related to characteristics of the reactions in which the polymer is formed. The main categories of the polymers, depending on the type of polymer synthesis, are addition polymers and condensation polymers. Polymer chain is formed by adding of monomers to the growing chain without any by-products in addition polymer polymerization process. This means, that all the atoms in every monomer are retained. Normally this process is proceeded in the presence of catalysts. Typical addition

polymers are polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC) and polystyrene (PS). (Speight 2011; Yousif *et al.* 2013)

In condensation polymerization, also called as step-growth polymerization, small molecules are formed from the atoms of monomers during the process, when two functional group react with each other. In this linking process a small molecule is eliminated as a by-product. Typical by-product molecule is water. Common condensation polymers are polyamide (PA), polyethylene terephthalate (PET) and polyester (PES). (Speight 2011; Yousif *et al.* 2013)

Polymers are the base of the plastics, but it should be kept in mind that all the polymers are not plastics (Speight 2011). Plasticity is their apparent property, and they can be reshaped, or they undergo reshaping under elevated temperature and/or pressure. Compounding is a process where polymers are formed to plastics by blending them with specific additives, such as plasticizers, stabilizers, and antioxidants.

## **2.2 Sources of micro- and nanoplastics**

Microplastics often result from plastic degradation to smaller fragments within time. These type of microplastics are classified as secondary microplastic. On the other hand, some plastics are already produced as micro- and nanosized for the application and this type of microplastics are classified as primary microplastics. The classification of MNPs is introduced in more detail in Chapters 2.2.1 and 2.2.2.

A typical vision of microplastics is plastic fragments in all shapes and colors. However, microfibers manufactured from different types of plastics are also microplastics. Microfibers are widely used in cleaning industry and household equipment. Synthetic microfibers are also used in clothing industry and for example in different technical sportswear clothes due to anti-wetting properties. It is estimated, that more than 30 % of microplastic pollution is from synthetic fibers detached from textiles during the laundry. (Boucher and Friot 2017; Prata 2018a)

### **2.2.1 Primary micro- and nanoplastics**

Some plastics are manufactured with diameter < 5 mm. Those are called primary MNPs. Due to their large surface area, micro- and nanosized plastics have unique properties and therefore they are used in multiple applications in different fields of industries (Crawford & Quinn 2017, pp. 104-108). For example, microbeads found in personal hygiene products for scrubbing, plastic microfibers in clothes, and microfibers in cleaning textiles are classified as primary particles. The shape of the primary MNPs is often

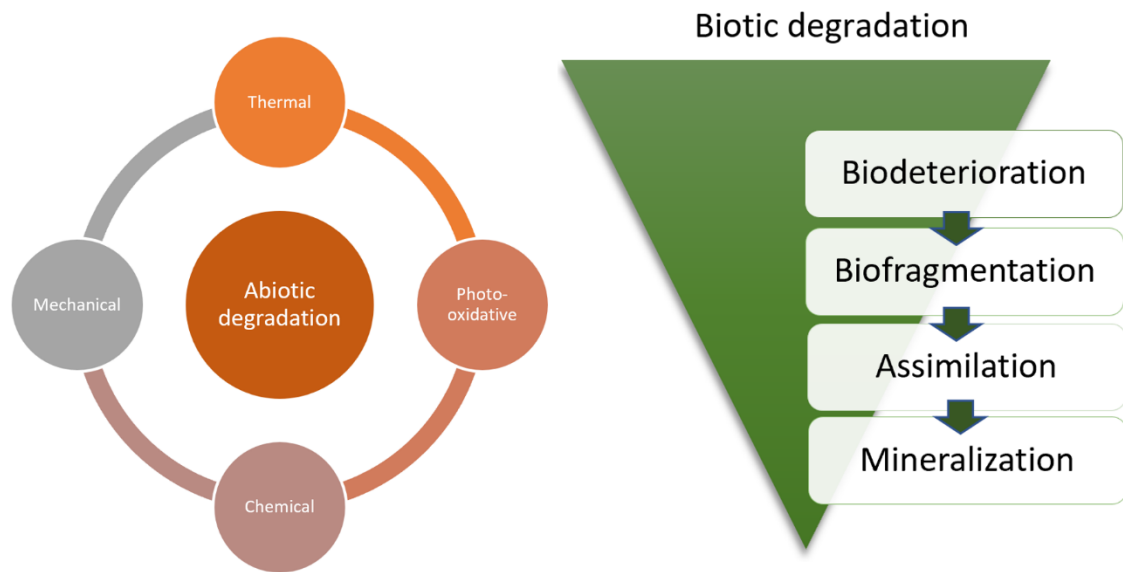
symmetrical, spherical shape or fiber form. Their usage is based on their size. However, these small particles end up to seas because wastewater treatment plant cannot filtrate all the small particles. Size range in diameters of plastic spheres used in personal hygiene products, for example as abrasives, is normally from 1  $\mu\text{m}$  to 1 mm (da Costa *et al.* 2017; Crawford & Quinn 2017).

### **2.2.2 Secondary micro- and nanoplastics**

Secondary MNPs result from breakage of bigger plastic pieces (da Costa *et al.* 2017). Plastic fragment particle size can decrease because of multiple factors, such as exposure to physical and chemical processes in environment. Fragmentation of the bigger particles produces often asymmetrical shaped particles in different sizes.

## **2.3 Degradation of plastics**

Environmental degradation in common plastics usually takes several years, even decades (Crawford & Quinn 2017; Lambert and Wagner 2018). The fragmentation of plastic to the micro- and nanosized is a complex process and several environmental factors such as temperature and oxygen level effect to the breakdown of the plastic (Pickett 2018). Degradation rate of the certain plastic is strongly bound to the structure of the polymer, such as molecular weight of the polymer, degree of crystallization, and additives. Mechanisms of common polymer degradations in environment can be divided in two categories: biotic and abiotic degradation (Ali *et al.* 2021). Figure 1 presents the processes of abiotic and biotic degradations. Abiotic degradation processes are thermal, photo-oxidative, chemical, and mechanical degradations. Biotic degradation processes are biodeterioration, biofragmentation, assimilation, and mineralization. Biotic degradation includes microorganisms and abiotic degradation includes all the other degradation mechanisms. However, plastic degradation usually occurs in both ways as a combination of multiple processes in environment.



**Figure 1. Difference between abiotic and biotic degradation**

### 2.3.1 Biotic degradation

In biotic degradation, biological organisms cause deterioration of plastics (Alshehrei 2017; Crawford & Quinn 2017, pp. 78-86). Some bacteria and fungi can cause biotic degradation of common plastics. Though, some plastics are protected with antimicrobial additives and many plastics are rather resistant to microbial attack due to durable structure and therefore biodegradation of synthetic plastics is very slow process. Biodegradation can be divided in two categories: aerobic and anaerobic. Aerobic process is microbial decomposition in the presence of atmospheric oxygen. Final products of the chemical reactions are typically water, carbon dioxide, carbon residuals and microbial biomass. Anaerobic process is the opposite to aerobic process. Since there are no atmospheric oxygen present, methane is typical end-product besides carbon dioxide and water. Anaerobic degradation is possible for example in sediments.

Biotic degradation is usually a four-stage process, that was presented on the right side in the Figure 1. Normally before biotic degradation, plastic undergoes abiotic degradation processes (Ali *et al.* 2021). In first phase, called biodeterioration, plastic is fragmented due to breakage of the carbon-carbon bonds. Energy to the breakage is usually result from external source, such as light or heat. After that, biodegradation, replaces the process. Specific microorganisms are able to attach to the hydrophilic surface of the polymer. They excrete extracellular enzymes that cause superficial degradation of the plastic. Also, the biofilm formation on the surface of the plastic by the microbes is a common phenomenon. Biofilm often enhances the biodegradation process. Next step in the biotic degradation is biofragmentation, in which extracellular enzymes excreted by

the microbes cause depolymerization of the molecule chains. Catalytic cleavage of the polymer chain produces oligomers, dimers, and monomers. These smaller molecules with low molecular weight are able to go through the cell walls of the microbes and some of them are transported into the cytoplasm of the microbe. Microorganisms use the carbon of the polymer chains as a source of energy. Transportation process of carbon through the microbe's cell wall is called assimilation. Final process of biodegradation is mineralization in which multiple enzymatic processes accomplish the degradation of the plastic. End products are often inorganic product, such as different salts and small molecules such as water and carbon dioxide, that are excreted by the microorganisms. (Crawford & Quinn 2017; Alshehrei 2017; Fotopoulou and Karapanagioti 2019; Amobonye *et al.* 2021)

Besides common plastics, there are also biodegradable plastics that are used in multiple purposes from medical applications to packaging solutions (Lenz and Marchessault 2005). They are designed to biodegrade in certain time according to their purpose. The degradation process is normally faster compared to common plastics, but biodegradable plastics can cause similar problems as common plastic when plastic is misused, and degradation takes place in conditions in which plastic is not designed to biodegrade. For example, some biodegradable plastic may biodegrade in waters with temperature over 20 °C, but in colder water plastic fragments to microplastic as common plastics (Wei *et al.* 2021).

### **2.3.2 Abiotic degradation**

Abiotic degradation can be divided into two categories: physical and chemical (Chamas *et al.* 2020). Physical includes mechanical breakdown of the bulk material for example due to abrasion of the sand, whereas chemical includes molecular level changes, such as breakdown of the polymer chains due to external UV radiation. Abiotic processes were presented on the right side of the Figure 1, and they are thermal, photo-oxidative, chemical, that includes atmospheric oxidation and hydrolytic degradations, and mechanical degradations (Crawford & Quinn 2017, pp. 87-98).

#### **Thermal degradation**

Thermal degradation of the polymer occurs by the change in temperature (Crawford & Quinn 2017). To initiate the breakage of the polymer chains, external energy in the form of heat must exceed the energy barrier of the chemical bonds in the polymer chain to complete the degradation process (Zhu *et al.* 2018). Chain-scission reaction is usually a three step -process, that includes initiation, propagation, and termination steps (Ray *et*

*al.* 2018; Polymer Database). In initiation process, chain-scission process results in the formation of small reactive molecules, called radicals. In the propagation step, multiple decomposition reactions induce the degradation of the main-chain of the polymer through for example intermolecular hydrogen transfer. If air is presence in the process, radicals formed in initiation process, can react with oxygen and they can abstract hydrogen atoms from the polymer chains. Excited hydrogen abstraction can lead to unsaturation of the main polymer chain. Also cross-link formation is possible between degraded chains. Finally, in termination step, two radicals can combine, or hydrogen atoms are transferred from radical to different radical. Reactions between molecules and radicals result in the deterioration of the mechanical properties of the plastic. Process will be terminated due to discontinue of the initial input energy or oxygen supply.

### **Photo-oxidative degradation**

Many common plastics are sensitive to the sunlight (Sørensen *et al.* 2021). Sensitiveness can be seen as a yellowing or cracking of the plastic. Oxidation of the polymer is a common reaction of photodegradation. Therefore, photodegradation is often photo-oxidative degradation due to presence of oxygen in environment. For example, plastic litter on the beaches are exposed to intense sunlight for long time periods. Photodegradation is a process in which photons are absorbed by the polymer molecules and cause the breakage of the polymer chains (Yousif *et al.* 2013). This can happen in multiple wave lengths, especially at the wave lengths in the UVB region, that is between 280 to 315 nm (Crawford & Quinn 2017). Susceptibleness to UV light is often result from chromophores, that are light absorbing part of the molecule. They are photo-reactive groups in the macromolecules in multiple common plastics and they are required for the initiation of photochemical reaction.

### **Chemical degradation**

Environmental chemical degradation of the polymers consists of oxidation and hydrolysis reactions (Crawford & Quinn 2017). Of course, plastics are exposed to multiple chemicals and pollutants, such as sulfur dioxide, in urban environments. Therefore, environmental degradation of the polymer is a sum of multiple processes. For example, oxygen is often involved in degradative reactions, and it can act as an initiator of plastic breakdown. More hazardous is the highly reactive ozone in atmosphere. Reaction with O<sub>3</sub> can weaken the plastic by breaking the bonds between atoms. In hydrolysis, water molecules penetrate to the polymer structure, especially to the amorphous regions and this phenomenon can cause the cleavage of the chemical bonds. Therefore, the ability to absorb water can define the plastic susceptibleness to chemical degradation. For

example, due to chemical structure, polyamide can absorb more water compared to polytetrafluoroethylene, that is resistant to hydrolysis due to its hydrophobic nature.

### **Mechanical degradation**

All the plastics are exposed to various kinds of mechanical stresses during their lifespan. For example, in the oceans the physical movement of the water and sand induces abrasion, that cuts polymer into smaller pieces (Crawford & Quinn 2017). Mechanical properties of the plastic often define the durability of the plastic against stresses. In mechanical degradation, continued stress results in breakup of the carbon-carbon bonds in polymer backbone. This phenomenon causes the fracture of the plastic and fragmentation into smaller pieces.

## **2.4 Microplastics in nature**

Nowadays microplastics are found all over the world (Wright and Kelly 2017). Water systems are often the most common environments. Plastic pollutants are found for example from freshwater environments, sea surface and deep seafloor. Tons of plastic enter the nature due to lack of proper waste management. Multiple rivers across the world are typical routes for plastic litter to the oceans. It is estimated that every year 1.15 to 2.41 tonnes of plastic waste end up to oceans from the rivers (Lebreton *et al.* 2017).

Household waters from washing machines are one notable source of synthetic fibers into the oceans and freshwater systems (Crawford & Quinn 2017). Small size of microplastic enables to pass through the wastewater systems. It is estimated that even more than 1900 fibers are detached from one synthetic garment during one wash cycle (Browne *et al.* 2011). Due to the limitations of the municipal wastewater treatment plant filtration system, excessive number of fibers end up rivers and seas every day worldwide.

Also, aerial transport is possible route for light particles, but the number of studies atmospheric fallout of MNPs are quite limited. Also, study methods vary, which make the comparison of the studies challenging. Zhang *et al.* (2020) have collected from different studies a review about current status of atmospheric microplastics in different locations around the world. Two European cities, Paris (France) and Hamburg (Germany), were studied. The average amount of particles in m<sup>2</sup> per day was 118 in Paris and more than 90 % of the particles were fibers. Corresponding number in Hamburg was 275, but the main type of particles were fragments (> 90 %). In Yantai (China) maximum deposition of atmospheric microplastics was 602 per m<sup>2</sup> per day. However, sampling methods were not similar, so the results are not fully comparable. (Zhang *et al.* 2020)

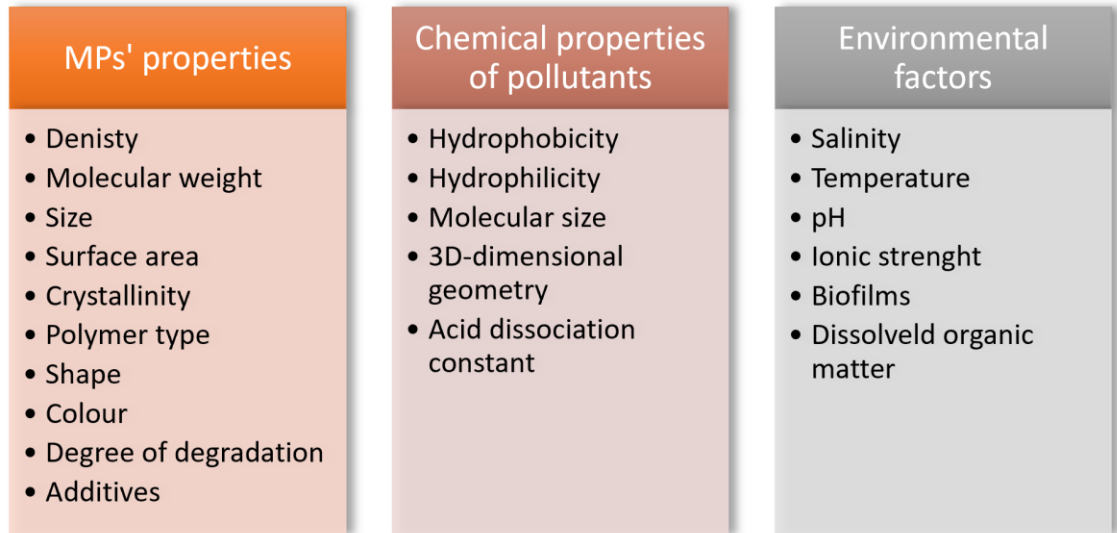


Discoveries are not limited only close of urban areas. Bergmann *et al.* (2019) found significant concentrations of MNPs in surface water in Polar regions. Arctic snow samples contained variety of different polymers, but varnish, rubber and PE were the most common types. According to the Arctic snow study, the route of the MNPs still remain unknown, but the atmospheric transport could be possible. (Bergmann *et al.* 2019) In laboratory studies, Ganguly *et al.* (2019) have shown the potential of MNPs to act as a nucleus for ice formation. This phenomenon stands with the discoveries, that in snow areas microplastic deposition can be significantly high, even the area is remote. (Ganguly *et al.* 2019)

## **2.5 Accumulation of pollutants**

Microplastics are spread all over the world and are proven to travel long distances in water systems (Horton *et al.* 2018). Researchers are interested in MNPs potential toxic impact to ecosystems, which is connected to the properties of microplastics. Besides physical spread and accumulation of plastic particles, microplastics are found to accumulate different pollutants and chemicals, such as persistent organic pollutants POPs, heavy metals, and toxins, that are entered to the environment within different waste streams. Trace metals have been found from the plastic pellets from the beaches and soils, and there is discussion about plastic acting as a vector to transport metals in different ecosystems (Holmes *et al.* 2012; Zhou *et al.* 2019). Moreover, microplastics are found to adsorb antibiotics in the aquatic environment in laboratory tests (Li *et al.*, 2018; Lambert and Wagner 2018).

The absorption behavior of microplastics is strongly connected to the structure of the polymer, especially to the density and crystallinity of the material (Holmes *et al.* 2012; Sørensen *et al.* 2021). Other important factors are polymer type, chemical properties of pollutants, and environmental factors. Environmental degradation also changes polymer properties which can have notable effect to the absorption behavior of the polymer (Ali *et al.* 2021). Moreover, the presence of biofilm and chemical precipitates might enhance the reactivity of the polymer's surface. Summary of the factors affecting to the interactions between microplastics and environmental pollutants are presented in Figure 2.



**Figure 2. Factors that affect to the interactions between MPs and pollutants.**

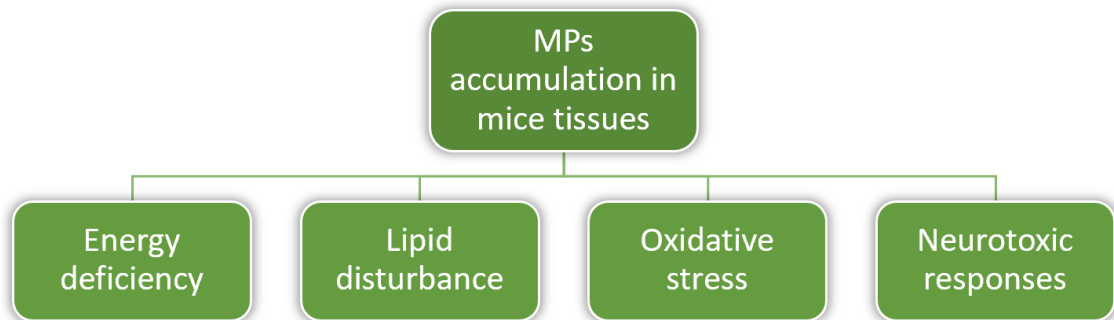
Saturated particles could act as a vector for several pollutants in environment and they can be severe to several organisms. However, there are discussion how easily absorbed pollutants are released from the polymer matrix after entering in for example marine biota and what is the real threat of polluted particles. (Jovanovi 2017; Lohmann 2017; Wang *et al.* 2018; Menéndez-Pedriza *et al.* 2020)

## 2.6 Influence of micro- and nanoplastics to organisms

Many plastic products are designed to be durable and long-lasting. Therefore, the persistence of plastic is the major concern, especially in the marine environment. (Lebreton *et al.* 2017). Plastic causes multiple problems to the marine animals, such as fishes, shellfishes and whales (Guzzetti *et al.* 2018). Accumulation of plastics into the marine animals could lead to starvation of the creature when some animals can mistakenly regard plastic fragments as food. In addition, microplastic can cause toxicological effect to marine biota. For example, microalgae have been found to be disturbed by the presence of microplastics (Liu *et al.* 2019; Ribeiro *et al.* 2019). Sorption of microplastics result in reduction of photosynthesis of microalgae.

Plastic exposure tests are performed in laboratories to study accumulation of MPs to different organs and how they affect to the animal. Studies are mainly focused on different marine organisms (Guzzetti *et al.* 2018). However, some land mammal studies are performed in laboratories (Walczak *et al.* 2015; Lu *et al.* 2018). For example, Deng *et al.* (2017) exposed mice to fluorescent polystyrene particles in size 5  $\mu\text{m}$  and 20  $\mu\text{m}$  to study how particles distribute and accumulate. Microplastic particles were dispersed to water, that was given to mice daily. Enzymatic biomarkers and metabolomic profiles

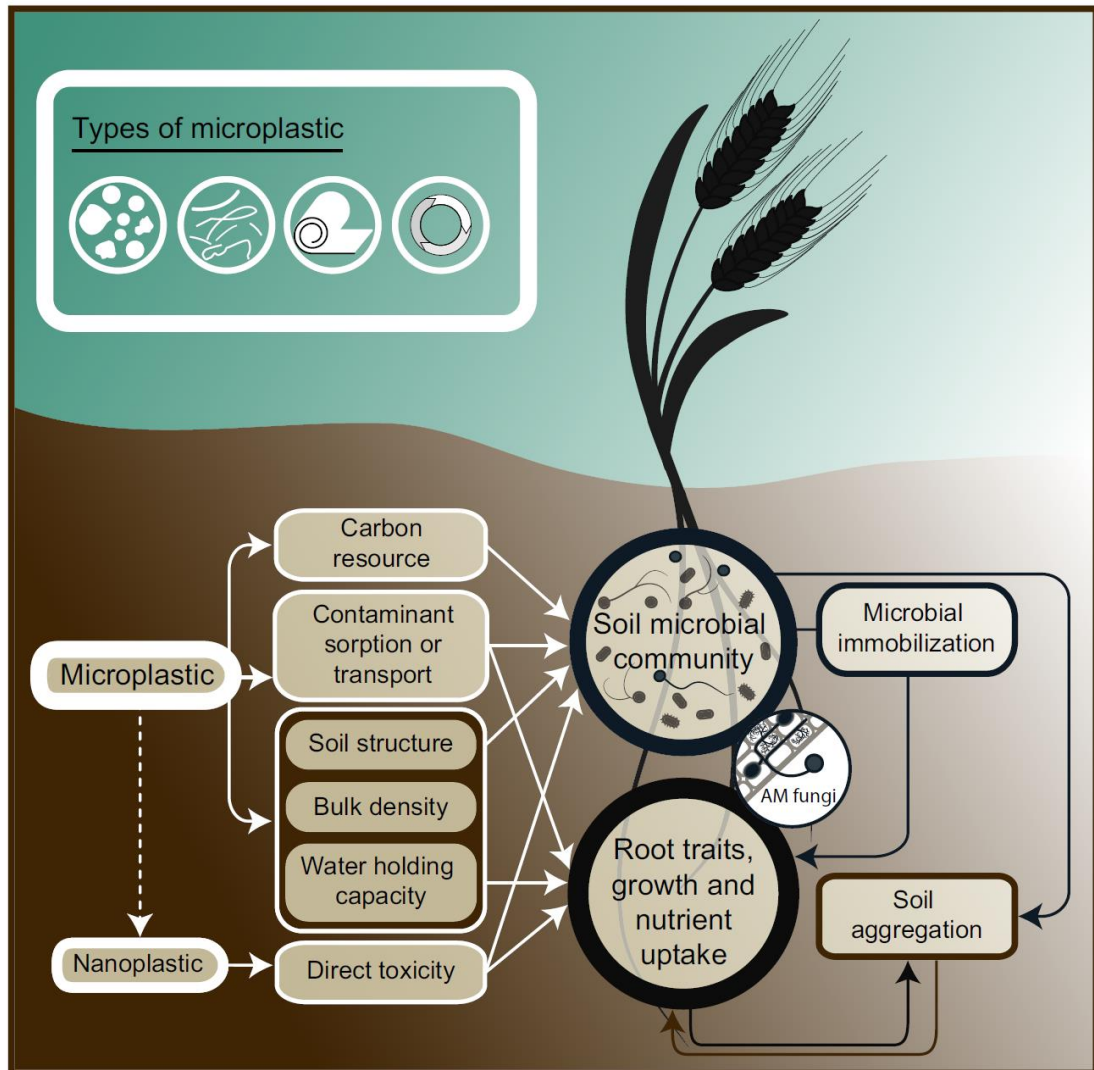
were used for investigating toxicological responses. It was noted that microplastics accumulated in liver, kidney, and gut. Figure 3 presents the summary of multiple effects of MPs exposure on mice.



**Figure 3. Summary of MPs exposure effects to mice.**

Energy deficiency was observed as a collapse of adenosine triphosphate (ATP) concentration and lactate dehydrogenase (LDH) in liver. Those biomarkers were used for evaluation of the energy metabolism. Lipid metabolism markers were total cholesterol and triglycerides. Notable decrease of the markers expressed lipid disturbance. Fluctuations in oxidative stress-related biomarker (catalase, glutathione peroxidase and superoxide dismutase) values were suggested to express imbalance in the antioxidant defense system. MPs also discovered to have an impact to neurotoxic responses. The activity of acetylcholinesterase (AChE) was increased in liver, which could represent the neurotoxic influence of MPs. (Deng *et al.* 2017)

Though MNPs studies are focused on aquatic environments and ecosystems, there are also discussions about microplastic effect on plants and soils (Rillig *et al.* 2019). Figure 4 presents possible effects of micro- and nanoplastics to plants. MNPs can affect to the water holding capacity and structure of the soil which can affect to roots and growth of the plant. Furthermore, MNPs can also affect to the microbial community of the soil.



**Figure 4. Possible mechanisms how MNPs could possibly affect to soil and plants (Rillig et al. 2019). Copyright (2021) Elsevier B.V.**

Almost all the possible effects are hypothetical since there are no comprehensive studies about MNPs effects on plants performed. However, the growth of the MNPs concentrations on the soils is possible hazard to the plants since plastic and its additives are foreign materials to the ecosystem.

## 2.7 Human exposure to microplastics

Humans are exposed to microplastics in everyday life (Wright and Kelly 2017). Ingestion and inhalation are typical exposure routes to microplastics to enter human body. It is discovered that air we breathe indoors contains single fibers detached from household textiles and clothes we wear. Also, some food we eat is proved to contain microplastics and therefore it is almost impossible to avoid being contacted with MNPs. Multiple industrial sectors, such as automobile, cosmetics and textile industry apply microplastics

in several applications (Rocha-Santos *et al.* 2019). Furthermore, polymeric microbeads are used in medical applications. Small size of the particles enables some polymers to cross cell membrane. From polymeric materials, polystyrene is widely used material in different medical applications as a drug carrier (Piskin *et al.* 1994). Medical grade of the material proves the safety and purity of the material but environmental plastic fragments and airborne microplastic can be contaminated from other pollutants. As it was presented in the Chapter 2.5, aged MNPs particles often contain other additives or pollutants due to capability of sorption of hydrophobic and hydrophilic organic pollutants (Liu *et al.* 2019). Furthermore, the leakage of additives used in polymer production are also concern when greater number of particles enter human body. The possibility of leakage of additives and degradation products of plastics at 37 °C in human body also creates health hazard (Melgert 2021).

According to the studies, estimation of human consumption of microplastic is around 121,000 particles annually (Cox *et al.* 2019). Other estimation was 553 particles per day (child) and 883 particles per day (adult) (Nor *et al.* 2021). Annually it is 201,845 particles (child) and 332,295 (adult). Estimations includes ingestion and inhalation. Here are presented the two main exposure routes for humans.

### 2.7.1 Ingestion

Consumption of seafood is one of the main routes of human exposure to microplastics (Käppler *et al.* 2016). Discoveries of plastic debris in oceans and accumulation of plastic particles to marine biota have risen the concern of safety of seafood. Especially the accumulation of microplastic to mussels, that are consumed entirely, has risen the concern of food safety and possible threat to human health. It is estimated that human consumption of microplastic via ingestion is from 39,000 to 52,000 particles annually (Cox *et al.* 2019). Researchers Senathirajah *et al.* (2021) have estimated that human ingest microplastics 0.1 to 5 grams per one week (Senathirajah *et al.* (2021).

Besides sea food, microplastic particles have been found from variety of other consumables, such as tap and bottled water, salt, and sugar (Cox *et al.* 2019). Moreover, packaging material can also be the source of contamination because bottled beverages have been found to contain clearly more MNPs compared to tap water.

Different studies present significantly varying results. For example, Karami *et al.* (2017) extracted number of plastic particles was 1 to 10 per kg of table sea salt. Minimum size of the particles was 147 µm and two main polymers found were polypropylene and polyethylene, while Iñiguez *et al.* (2017) found 50 to 280 microplastic particles from one

kg of salt and the most common polymer type was polyethylene terephthalate (PET (Karami *et al.* 2017; Iñiquez *et al.* 2017). Differences in the results are usually sum of multiple factors (Zantis *et al.* 2021). Pore size of the used filters has possibly the most notable effect, but also the sampling methods and analyzation tools could have a notable impact to the results.

### **2.7.2 Inhalation**

Exposure to different air pollutants happens day-to-day. Synthetic fibers and plastic particles have been found from human lungs, despite of effective clearance mechanisms of human respiratory system, including mechanical methods, mucociliary escalator, phagocytosis, and lymphatic transport (Prata 2018b). According to Prata (2018b), it is estimated that human might be exposed to 26–130 microparticles via inhalation a day. Exposure to MNPs in indoor is notable due to textiles, household textiles such as furniture and bed sheets. Detached fibers float in the air and part of the end up to human respiratory system (Prata 2018b). In other study, Vianello *et al.* (2019) used a Breathing Thermal Manikin to mimic human breathing in indoor location, showed that average number of the particles per one cubic meter was  $9.3 \pm 5.8$ . This study showed that 272 microplastic particles were inhaled in 24 hours. Polyester seemed to be one of the most common synthetic polymers in indoor air. Polyester covered 59–92 % from all the synthetic polymer particles (Vianello *et al.* 2019).

## **2.8 Bioaccumulation of micro- and nanoplastics in humans**

Accumulation of different materials in human body is studied several decades. Breathable microfibers are known to accumulate especially in lung tissues (Pott *et al.* 1974). Naturally occurring fibrous silicate mineral asbestos is one of the well-known materials, that is considered to be highly carcinogenic. Scientists have discovered that the size and shape of the asbestos fibers are significantly associated with increased risk of lung cancer. It is proved that thinner and longer fibers are more carcinogenic compared to shorter and thicker fibers. Therefore, breathable plastic micro- and nanofibers with their complex compositions have also risen the concern of their possible effects to the lung tissues because exposure to them is inevitable (Loomis *et al.* 2012; Vianello *et al.* 2019).

Microplastics studies have mainly focused of marine biota and marine organisms (Lambert and Wagner 2018). Only few studies of synthetic polymers found from human organs are published to this date (Amato-Lourenço *et al.* 2021; Wright and Kelly 2017; Ragusa *et al.* 2021). Studies prove that microplastics are capable to enter human body

and circulatory system. Microplastic particles are found from tissue samples of lungs, liver, spleen and kidneys. Even the particles were found in organs, the possible route into the bloodstreams is still unknown.

One of the recent studies have found microplastic bioaccumulation in human placentas. Ragusa *et al.* (2021) found 12 pigmented microplastic particles in four human placentas. Four of the particles were identified as PP. Other 8 polymer matrixes were not able to identify. However, composition of the pigments of the particles were identified and all of them were used for in different applications, such as paintings, cosmetics, adhesives, and personal care products (Ragusa *et al.* 2021).

While the plastic particles are found from human organs, the mechanisms to cross protective membranes of the cells are still unclear. Fleury *et al.* (2021) provides information about mechanical interactions between polymeric particles and blood cells. Particles between size range of 1 to 10  $\mu\text{m}$  were able to attach to lipid bilayer of the blood cells in vitro tests and caused significant stretching of the membrane. This phenomenon might be one of the mechanisms of microplastic cellular uptake. Stretching might also cause dysfunction of the cell (Fleury *et al.* 2021). Other researchers, Stock *et al.* (2021) were concentrated to investigate cellular uptake of PE, PP, PET and PVC particles in vitro. According to the results, the amount of PE particles with diameter 1–4  $\mu\text{m}$ , that were transported through the intestinal epithelium, was notable higher compared to PS cellular uptake. Polystyrene is the dominant material in cell test and therefore it is suggested that material selection in microplastics–cell studies should be more wide-ranging, because intestinal exposure to plastic microparticles seems to be material- and size-dependent (Stock *et al.* 2021).

Crossing of the intestinal barrier by plastic consists multiple variables from plastic properties, such as size, surface charge, protein corona and functionalization of the surface, to biological circumstances (Wright and Kelly 2017). Therefore, the exact knowledge of the crossing mechanisms remains still unknown. Besides mechanical stretching, endocytosis and paracellular persorption might also be possible pathways of microplastic uptake.

Since MNPs have been found from different environments from soils and water systems to human organs, different methods of sampling and particle extraction are demanded (Fu *et al.* 2020). Moreover, the identification of particle to specific plastic type requires knowledge of chemical structure of the sample (Ribeiro-Claro *et al.* 2017). Next are presented commonly used characterization methods of MNPs from sampling to imaging instruments.

## 3. CHARACTERIZATION OF MICRO- AND NANOPLASTICS

Wide variety of different plastics with different characteristics end up into the nature and manual identification of plastics is challenging. Distribution and impact of the microplastics are determined by their characteristics, such as density and crystallinity. Therefore, different characterization methods are extensively used. With different microscopy techniques it is possible to study morphology, structure, and properties of the MNPs (Hidalgo-Ruz 2012; Bergmann *et al.* 2019; Cai *et al.* 2021).

Since the examined particles are very small, sample contamination during examination is also possible. Usage of plastic instruments might affect to the final results of the sample. For example, fibers from the clothes might end up to the sample on the windy outdoor area and distort the results of the study. Due to lack of standardization of sampling of microplastics, every step from sampling to characterization must be planned to minimize the possibility of contamination from other plastic sources (Xu *et al.* 2019).

Microplastic samples from the soils, aquatic environments and organic samples consist often lot of other materials such as sand and organic materials. Isolation of plastic from the matrix is important step before further investigation and identification of the plastics (Cai *et al.* 2021). Next are presented different separation techniques and their advantages and limitations more detailed. Moreover, the common identification techniques are presented later.

### 3.1 Separation techniques

Normally environmental samples from soil and water consist of a lot of other materials besides studied plastic particles (Fu *et al.* 2020). Quantification and characterization are often processes that are challenging to proceed without the separation of MNPs from other particles. Complex matrices of the samples and the size distribution of the MNPs set limitations for different separation techniques. Suitable technique must be considered individually in different cases. In this section are presented typical separation techniques and some of their advantages and disadvantages.

#### 3.1.1 Manual separation

When samples are collected, the aim is to collect representative sample (Xu *et al.* 2019). Often the first step is to manually collect and separate plastic pieces out of the other



material. Visual separation of the plastics is normally done in the beginning before other more detailed separation methods. Visual inspections always include human-error and therefore other methods are always needed for further inspections.

Sieving is common technique to perform separation on-site (Hanvey *et al.* 2017). Different sieves with range of mesh sizes and filters are used for performing separation of microplastics from the matrix. Technique is quick and easy to perform, but it is only particle size dependent. Therefore, other materials are normally present after sieving. (Fu *et al.* 2020) In water environment, different nets and pumps are used for collecting solid particles from the water. Typical nets are for example plankton and neuston nets. (Prata *et al.* 2019) Naturally the mesh size has significant effect to the reported concentrations of collected plastics. For example, Vermaire *et al.* (2017) revealed that concentration of plastic particles with a net with mesh size 100  $\mu\text{m}$  was almost 100 times higher than the concentration of particles collected by using a net with mesh size 333  $\mu\text{m}$  (Vermaire *et al.* 2017).

### 3.1.2 Density based separation

Density based separation of microplastic fragments from denser sediment is one of the most common ways to separate particles for further studies (Prata *et al.* 2019; Fu *et al.* 2020). Two common density-based methods are flotation and elutriation.

In flotation process, the aim of the solution is to render the plastic buoyant, and the particles float passively to the surface (Hidalgo-Ruz *et al.* 2012). Sodium chloride (NaCl) is often used due to its availability and cheapness, but it has its limitations due to relatively low density ( $1.2 \text{ g}\times\text{cm}^{-3}$ ) (Prata *et al.* 2019). Sodium iodide (NaI) and zinc bromide ( $\text{ZnBr}_2$ ) are also commonly used chemicals in microplastic separations due to higher density, but  $\text{ZnBr}_2$  is also hazardous chemical. Water is also used in some cases, but it is suitable only for polymers with lower density than water ( $1 \text{ g}\times\text{cm}^{-3}$ ). Flotation process is easy to perform and cheap, when plastic particles are separated from the sediment samples. First the sample is mixed with saturated solution. Solution is then stirred or shaken for suitable time. In this procedure, the heavier component such as sand, sink to the bottom of the container and lighter element, such as polymers float to the surface.

Table 1 presents different polymer types and common solutions that are used in density separation. Separation is defined as “+” and no separation is defined as “-“. As it is noted, the solution with higher density compared to polymer density, is possible to use in separation (Prata *et al.* 2019; Hidalgo-Ruz *et al.* 2012).

**Table 1. Polymer types and solution used in density separation. (Adapted from Prata *et al.* 2019)**

Polymer	Density [g/cm <sup>-3</sup> ]	Water 1 g/ cm <sup>-3</sup>	NaCl 1.2 g/ cm <sup>-3</sup>	NaI 1.6 g/ cm <sup>-3</sup>	ZnBr <sub>2</sub> 1.7 g/ cm <sup>-3</sup>
PP	0.9–0.91	+	+	+	+
PE	0.92–0.97	+	+	+	+
PA	1.02–1.05	-	+	+	+
PS	1.04–1.1	-	+	+	+
PMA	1.17–1.20	-	+	+	+
PU	1.2	-	+	+	+
PES	1.24–2.3	-	-	+	+
PET	1.37–1.45	-	-	+	+

Elutriation is a process for separating lighter particles from heavier ones in an elutriation column by the stream of liquid or gas. Separation of the particles is based on their terminal falling velocities. Properties of the plastic, such as density, size, and form, affect to falling velocities (Kedzierski *et al.* 2016). After flotation or elutriation, the samples are usually further filtrated.

### 3.1.3 Purification

Even the microplastic samples are extracted from matrices, such as sediment, there can still excess organic and inorganic particles that can distort chemical analyses of the sample (Gewert *et al.* 2017; Xu *et al.* 2019). Especially organic samples, such as biopsies, must be chemically treated before spectroscopic analyses. The treatment term is chemical degradation when sample is treated with different chemicals for removal of extra material. Typical chemical is for example 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution to digest organic material. Besides oxidative digestion, also acids, alkalis and enzymatic methods are used.

## 3.2 Identification of micro- and nanoplastics

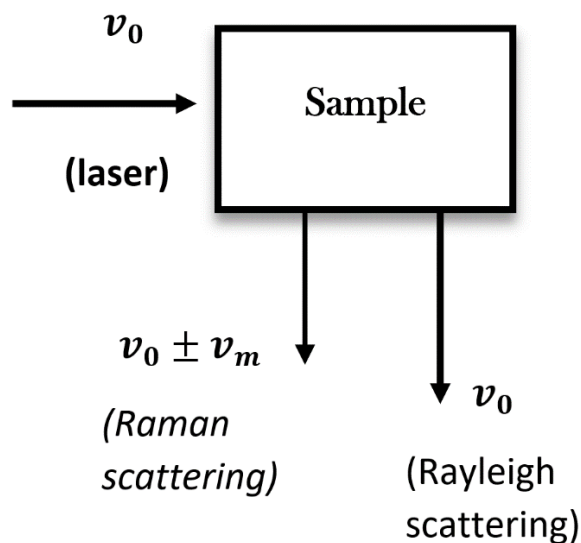
Different microscopy imaging methods are widely used in microplastic studies. The imaging field is evolving, and new methods are continuously developed (Arajuo *et al.* 2018; Chen *et al.* 2020). Optical microscopy is suitable for coarse imaging of microplastics, such as shape and color. However, for chemical analysis, morphology and/or nano-scale imaging, more sophisticated methods are on demand. Especially

nano-scale is still very challenging in MNPs researches, because the detection and separation of nanoparticles from the samples is difficult (da Costa *et al.* 2016; Al-Thawadi 2020). Therefore, studies of environmental nanoplastics are highly limited. Some recent studies have focused on nanoplastics and some of them provide new methods for characterization (Mintenić *et al.* 2018; Schwaferts *et al.* 2019; Materić *et al.* 2020, Cai *et al.* 2021). For example, Materić *et al.* (2020) present thermal desorption–proton transfer reaction–mass spectrometry for identification of nanoplastics. In their study, the identification of 10 ng of PS was successfully performed from the Austrian Alp snow samples. The method was based on ion detection and the identification was possible even the snow consisted other dissolved organic compounds (Materić *et al.* 2020). Next are presented commonly used basic methods for MNPs identification: Raman spectroscopy, FTIR and SEM.

### 3.2.1 Raman spectroscopy

Raman spectroscopy is one method to analyze micro- and nanosized samples and the spectrum is result from polarizability of chemical bonds (Prata *et al.* 2019). It is based on Raman scattering, that is inelastic scattering of photons. The sample is irradiated with monochromatic light, that is usually a laser. The interaction between light and sample changes a small part of the light's wavelength. The scattered light is analyzed with a spectrograph and the result is a Raman spectrum, that provides characteristic signals of the sample (Ferraro *et al.* 2003). The spectrum is a result from interactions between molecular vibrations and electromagnetic field. Vibrational transitions are individual to every molecule and therefore they provide a fingerprint of the chemical structure. This fingerprint enables the identification of the elements and further the sample material (Ribeiro-Claro *et al.* 2017).

Figure 5 presents the principle of the light scattering in Raman spectroscopy. The method is based on the comparison of molecular vibration frequency  $\nu_m$  to original laser frequency  $\nu_0$ . The scattered light consists of two components: Rayleigh scattering, that has the same frequency than the incident beam  $\nu_0$ , and Raman scattering, that is combination  $\nu_0 \pm \nu_m$  frequencies (Wolverson 2008). The sum factor ( $\nu_0 + \nu_m$ ) is called anti-Stokes and difference ( $\nu_0 - \nu_m$ ) is called Stokes frequencies.



**Figure 5. Principle of light scattering in Raman (adapted from Ferraro et al. 2003)**

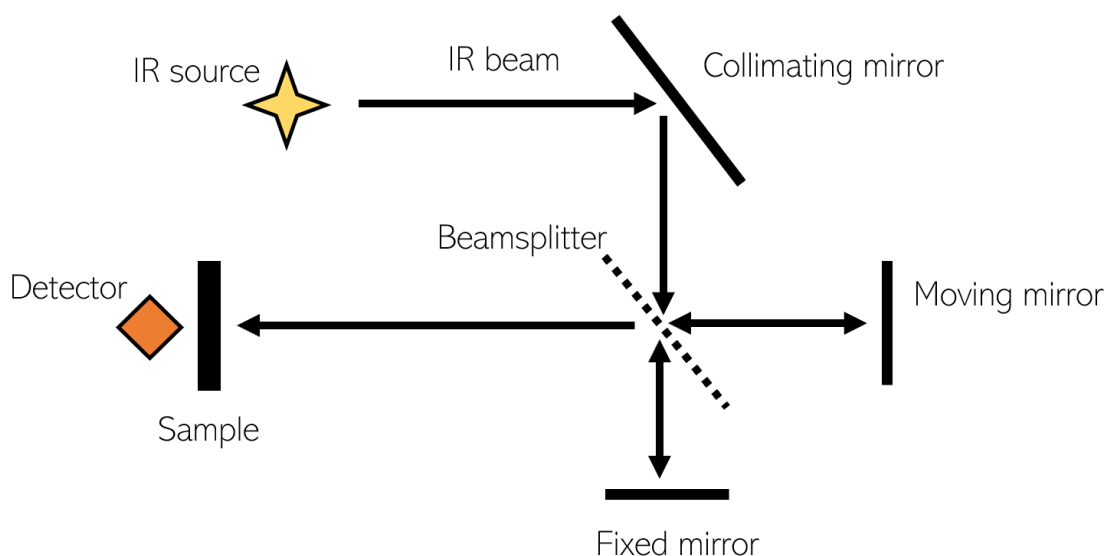
The spectrum of the sample is usually compared automatically to chemical database. Due to high resolution, Raman microscopy is suitable tool for nanoscale imaging with chemical analysis of MNPs (Ribeiro-Claro *et al.* 2017; Araujo *et al.* 2018). Raman spectroscopy is mainly nondestructive characterization method. However, with sensitive samples, laser can cause photodegradation or heating of the sample.

### 3.2.2 Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is chemical analysis technique, that is based on the interaction of infrared (IR) light with matter (Smith 2011). When infrared reaches the analyzed sample, some part of the radiation is absorbed by the sample and part of the IR is passed through the sample. When light hits the detector, it is transformed to signal and the processed to spectrum of the sample. Data conversion to actual spectrum is based on mathematical Fourier transform process. The excitation of vibrational modes of the sample molecules are seen as peaks in IR spectrum (Ismail *et al.* 1997). Obtained spectrum is individual to material and therefore FTIR is an excellent method for chemical analysis of unknown material.

The interferometer is the base device of FTIR spectrometer. Michelson interferometer is one of the oldest and the most used type of interferometer. The concept of Michelson interferometer is presented in Figure 6. Simplified, IR beam travels from the source to collimator mirror and then to the beamsplitter, which divides the beam into two parts. Transmitted beam travels to fixed mirror and reflected beam to the moving mirror. Then the beams are reflected from the mirrors back to the beamsplitter and they are recombined to produce a constructive/destructive interference pattern. Changes in the

amplitude of the light is result from difference between the travelled path of the two components of the beam. Recomposed beam travels then through the sample and hits the detector. (Ismail *et al.* 1997; Smith 2011)



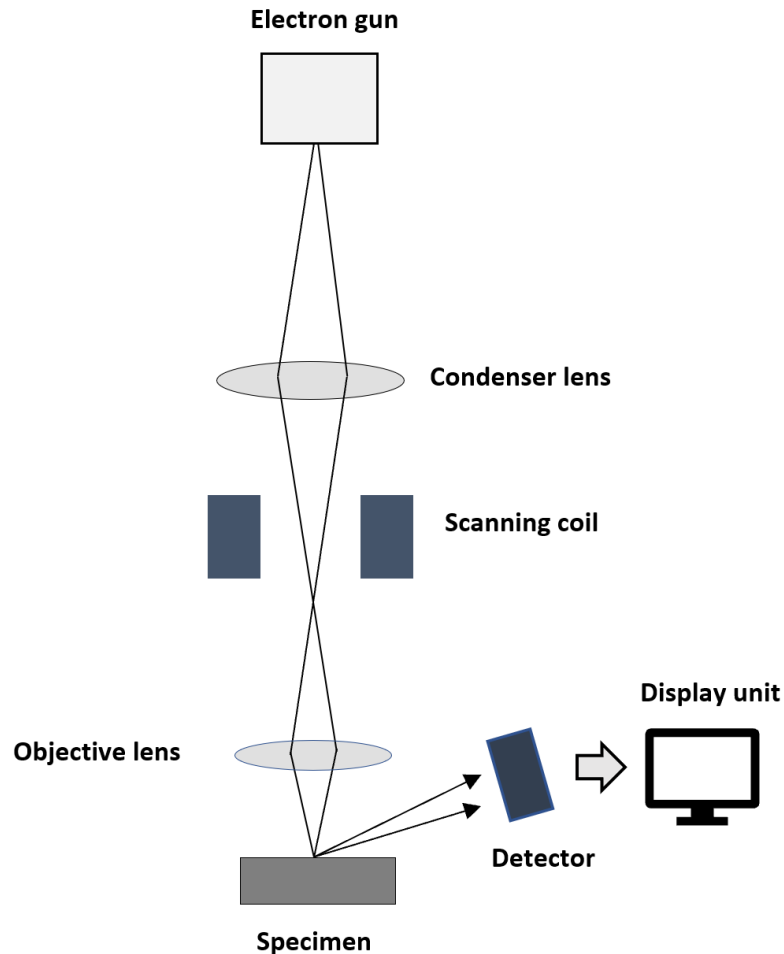
**Figure 6. A concept diagram of Michelson interferometer (adapted from Smith 2011)**

There are four major FTIR techniques to upgrade the performance of the basic instrument: transmission, attenuated total reflection, specular reflection, and diffuse reflectance (Thermo Fisher). Sampling method is usually selected by the demands of the material under investigation and what information is needed from the sample.

### 3.2.3 Scanning electron microscopy

For surface studies of MNPs, scanning electron microscopy (SEM) is commonly used microscope. It is an electron microscope, in which sample under investigation is scanned with focused beam of electrons (Goldstein *et al.* 1992; Goodhew *et al.* 2000). Electron beam interacts with sample atoms by exciting the atoms. The acceleration voltage of electrons is typically between 1 to 30 keV. The excitation result in the emission of different electrons and electromagnetic waves, that are detected using specified detectors and converted to signal. The signal is processed to image that provides topographical and elemental information about the specimen. A type of the detector determines the information type, that can be received from the sample. SEM image is formed by a scanning action of the beam (Goldstein *et al.* 2003, pp. 99-103). The beam is moved by current strength alterations of the scanning coils resulting in the scanning of the sample and detectors detect specimen-sample interactions in sequence points.

Figure 7 presents the main components of SEM. As simplified, the main components are electron gun, condenser lens, scanning coils, objective lens, detector, and display unit.

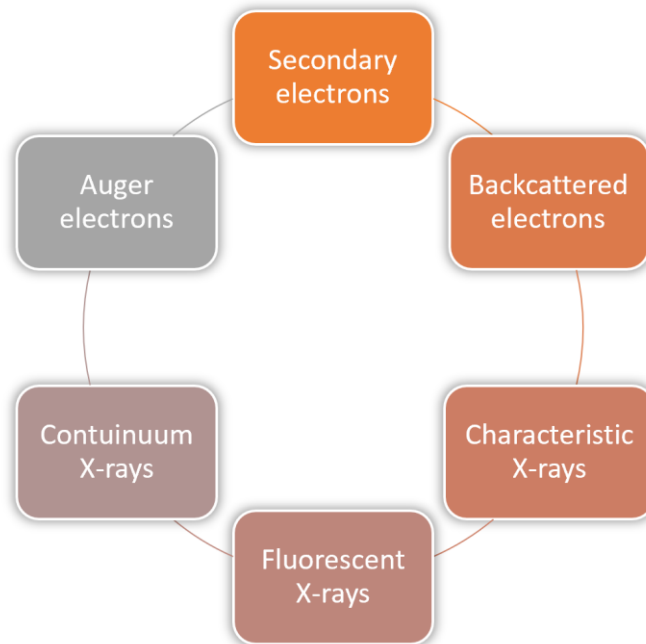


**Figure 7. The main components of SEM**

Typical electron gun is Tungsten filament, that is heated to create electron beam, that travels through the column to the specimen. Other commonly used electron sources are solid state crystal (for example Lanthanum hexaboride) and field emission gun (FEG) (Goldstein *et al.* 2003, pp. 29-40). The electron beam is demagnified to the required spot size with magnetic condenser lens. Diameter of the electron beam is usually around 2 to 10 nm when it hits the sample. After condenser lenses, objective lens is used for focus the beam onto the specimen surface. It also defines the final spot size of the beam when the beam hits the specimen surface. Between the lens system are located the scanning coils. Coils deflect the electron beam to scan the specimen's surface in raster form. The deflect of the beam is executed in the directions of X and Y axes (Goldstein *et al.* 2003).

Figure 8 presents various electron-types and electromagnetic waves that are emitted from the sample due to beam electron bombardment. Acceleration voltage of the electron

beam and sample material properties are the main characters that determine received information.



**Figure 8. Emitted electrons and electromagnetic waves from the interaction volume of the sample.**

The sample can emit Auger electrons, secondary electrons (SE), backscattered electrons (BSE), characteristic x-rays, fluorescent x-rays, and continuum x-rays (Goodhew *et al.* 2000). The information is received from the interaction volume of the sample. The interaction volume is the volume in which beam electrons interact with the sample electrons and the size of the interaction volume is directly connected to the voltage of electron beam and molecular weight of the material (Goldstein *et al.* 2003 pp. 61-71). Therefore, the penetration depth of the electron beam has the major influence of the received signals. Auger electrons come from the very surface of the sample, whereas X-rays come from the deepest parts of the interaction volume.

Polymeric materials, such as common plastics are considered as insulators which creates own challenges in SEM imaging. Nonconductive materials tend to charge negatively under electron beam because of the electron accumulation to the specimen area under investigation (Sabbatini 2014, pp. 183-186). Normally with insulating materials, the number of electrons penetrating to the specimen is higher than the electrons (SE and BSE) scattered from the specimen. The buildup of negative charge lowers the kinetic energy of the beam electrons and results in higher emission of SE and BSE (Goldstein *et al.* 2003). Changes in electron behavior creates artifacts and this is usually seen as brighter areas in images. Coating the specimen with conductive material,

such as noble metals or carbon, is simple and commonly used method to avoid charging of the specimen (Jeol). Noble metals have high yield of SE and moreover they stay stable under electron beam. Desired thickness of the coating is typically from 1 to 10 nm. Too thick film could cover detailed structure of the specimen and on the other hand, if the coating is too thin, coating could lose its constancy resulting in charging of the sample. Therefore, controllability of the coating thickness during the coating process is desired feature.



## 4. EXPERIMENTAL METHODS

The aim of the experimental part was study suitable parameters for micro- and nanofiber production by using electrospinning and for imaging plastic micro- and nanofibers. Plastic micro- and nanofibers from common polymer solution were manufactured by electrospinning and then the film surface area was decreased by crushing the film. Imaging was performed using SEM.

### 4.1 Polymer solutions preparation

Polystyrene (BASF), polyamide 66 (DuPont) and polyethylene terephthalate (Indorama) pellets were used for fiber production in different concentrations. Solutions were prepared in room temperature. Polystyrene was dissolved in dimethylformamide (DMF) (VWR Chemicals) and tetrahydrofuran (THF) (VWR Chemicals) with ratio 1:1. Polyamide was dissolved in formic acid (FA) (J.T.Baker) and acetic acid (AA) (Sigma-Aldrich) with ratio 4:2. Moreover, a one PET solution was prepared by dissolving 1 gram of the PET pellets to dichloromethane (DCM) (Fisher Scientific) and trifluoroacetic acid (TFA) (Sigma-Aldrich) with ratio 9:5. Table 2 presents polymer solutions. Solutions were prepared by mixing solvents 10 ml as total volume and adding 1, 1.5, or 2 grams solid plastic pellets depending on the solution. Since the PET solution contained 9 ml of DCM and 5 ml of TFA, the volume of the PET solutions differed from the other solutions. Solution with number 10 contains 1 g, number 15 contains 1.5 g and number 20 contains 2 g of solid pellets.

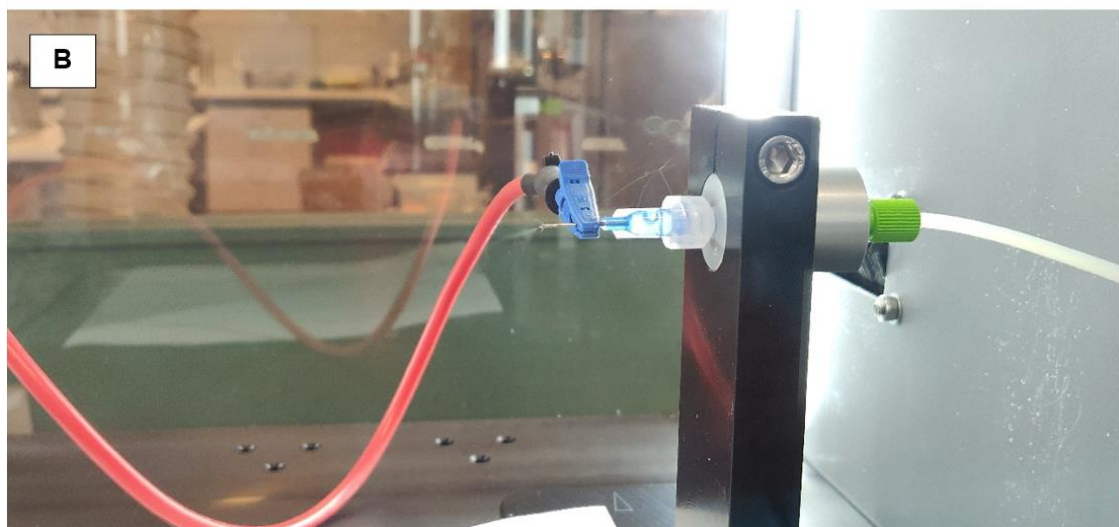
**Table 2. Polymer solutions and weight per volume percent**

<b>Weight per volume %w/vol [g/ml]</b>	<b>Polystyrene</b>	<b>Polyamide</b>	<b>Polyethylene terephthalate</b>
<b>10</b>	PS10	PA10	PET10
<b>15</b>	PS15	PA15	-
<b>20</b>	PS20	PA20	-

### 4.2 Fabrication of micro- and nanofibers by electrospinning

Electrospinner (Spinbox Electrospinning by Bioincia, Spain) was used for fabricating micro- and nanofibers. Before electrospinning, the polymer solutions were ultrasonicated

at least 10 minutes in order to achieve as uniform composition as possible. Then the solution was inserted in the syringe with volume of 24 ml. Then the syringe was connected to the pump station and pipe. Solution was pumped with suitable pump rate through the pipe and needle (Bioincia), that was connected to the high voltage. The inner diameter of the needle was 0.718 mm. Needle tip to collector plate distance was 19 cm. Created electric force drew the polymer melts towards the collector and created the spray of polymer fibers. During the spray, the solvent of the polymer solution evaporated, and plastic fibers solidified on the collector. Figure 9 presents the setup of the electrospinning.



**Figure 9. A: Setup of the electrospinning and B: high voltage attached to the needle. Polymer fibers are spraying from the tip of the needle**

Polymer fibers were collected of the collector plate, that was covered with Teflon coated foil (Reynolds). Table 3 presents the parameters for electrospinning.

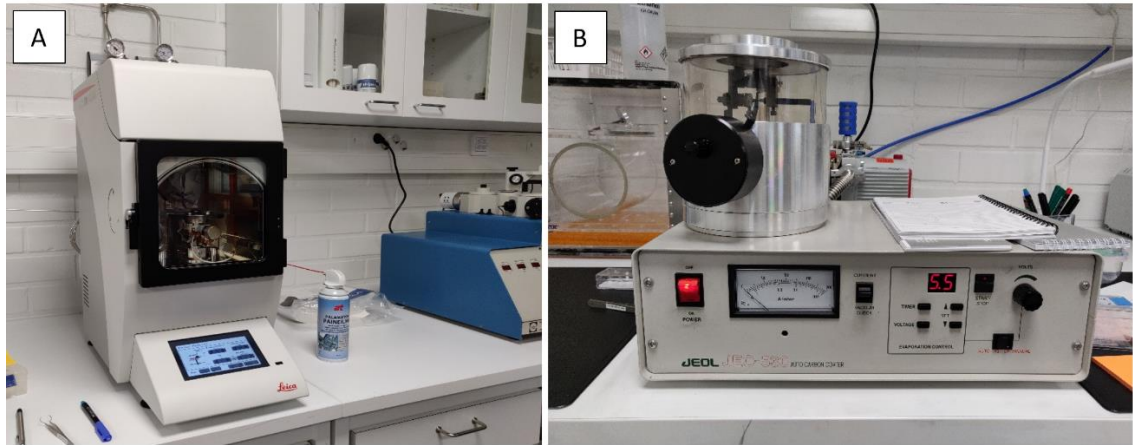
**Table 3. Parameters for electrospinning**

Polymer solution	Pump rate [ml/h]	Voltage [kV]
PA10	1	14–15
PA15	0.5	24–25
PA20	1	26
PS10	8	8–9
PS15	3	8.7–9.7
PS20	1–3	10–12
PET10	1	16.8–17.2

After formation of peelable film of fibers, fabrication was finished, and the foil covered with fibers was collected to imaging.

### 4.3 Sample preparation and microscopy imaging

All the fibers were imaged using SEM to verify the formation of fibers. Imaging of this work made use of Tampere Microscopy Center facilities at Tampere University. The samples were mounted on an aluminum stub and carbon tape (Agar scientific). Prior to the analysis, the samples were made electrically conducting by vacuum sputter coating. Two different coatings were compared: carbon (JEC-530 Auto Carbon Coater, JEOL) and platinum-palladium (Pt-Pd) mixture with ratio 80/20 coating (ACE600 EM, Leica). The thickness of the Pt-Pd coating was 2.5 nm. Figure 10 presents the coating instruments: A is Pt-Pd coater ACE600 and B is carbon coater JEC-530.



**Figure 10. A: ACE600 EM, Leica, B: JEC-530 Auto Carbon Coater, JEOL**

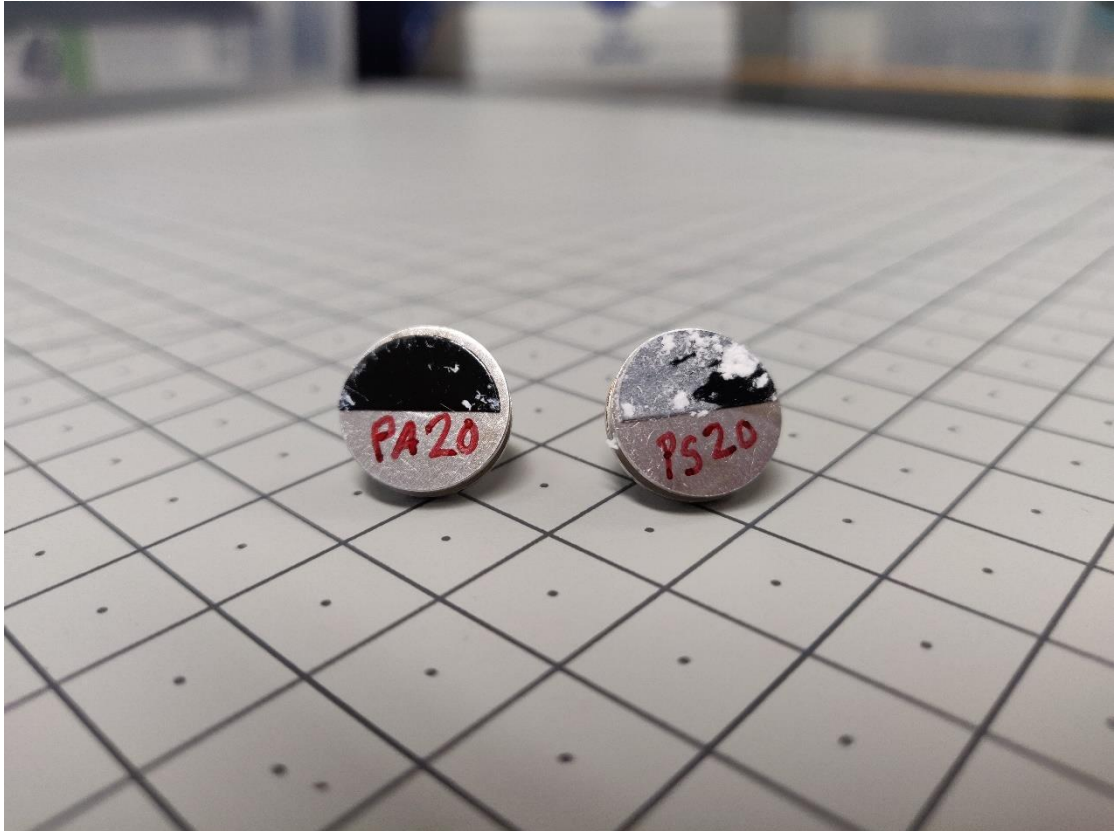
Imaging of PA and PS was performed using a SEM (Tescan Vega, Tescan) and PET imaging with other SEM (Jeol IT-500). Acceleration voltage was 5 kV and beam intensities were 4 to 8 depending on the sensitivity of the sample to charge. Magnifications 1000, 3000 and 8000 were taken from every sample except PET10 sample. Mean diameter of fibers was determined by measuring thickness of 10 different fiber per one sample from SEM image and calculating the mean in Excel. Standard deviation (SD) of diameters was also calculated in Excel with sum function

$$SD = \sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}} \quad (1)$$

in which  $x$  is diameter,  $\bar{x}$  is mean diameter and  $n$  is sample size. Standard deviations are seen as error bars in bar charts of mean diameter of different fibers.

### 4.3.1 Film breakage with mortar

Breakage of polymer film was performed by mortar. A piece of film was placed in the ceramic mortar and liquid nitrogen was poured into the mortar. Film was quickly crushed before warming and remains of the film were stamped on the aluminum stub covered by carbon tape. Figure 11 presents crushed PA20 and PS20 fiber films on carbon tape on SEM stubs.



**Figure 11. Polyamide (PA20) and polystyrene (PS20) fibers on the carbon stubs after breakage of polymer film using mortar.**

Crushed samples were imaged by using SEM to investigate the effect of breakage of frozen film. Because polystyrene films were so delicate, only PS20 was chosen to test crushing. PS20 was easiest to handle from other PS films. PA10 was very thin and delicate to handle and PA15 and PA20 were so similar between them, so therefore PA20 was chosen to test crushing of polyamide.

### 4.3.2 Cryomicrotome

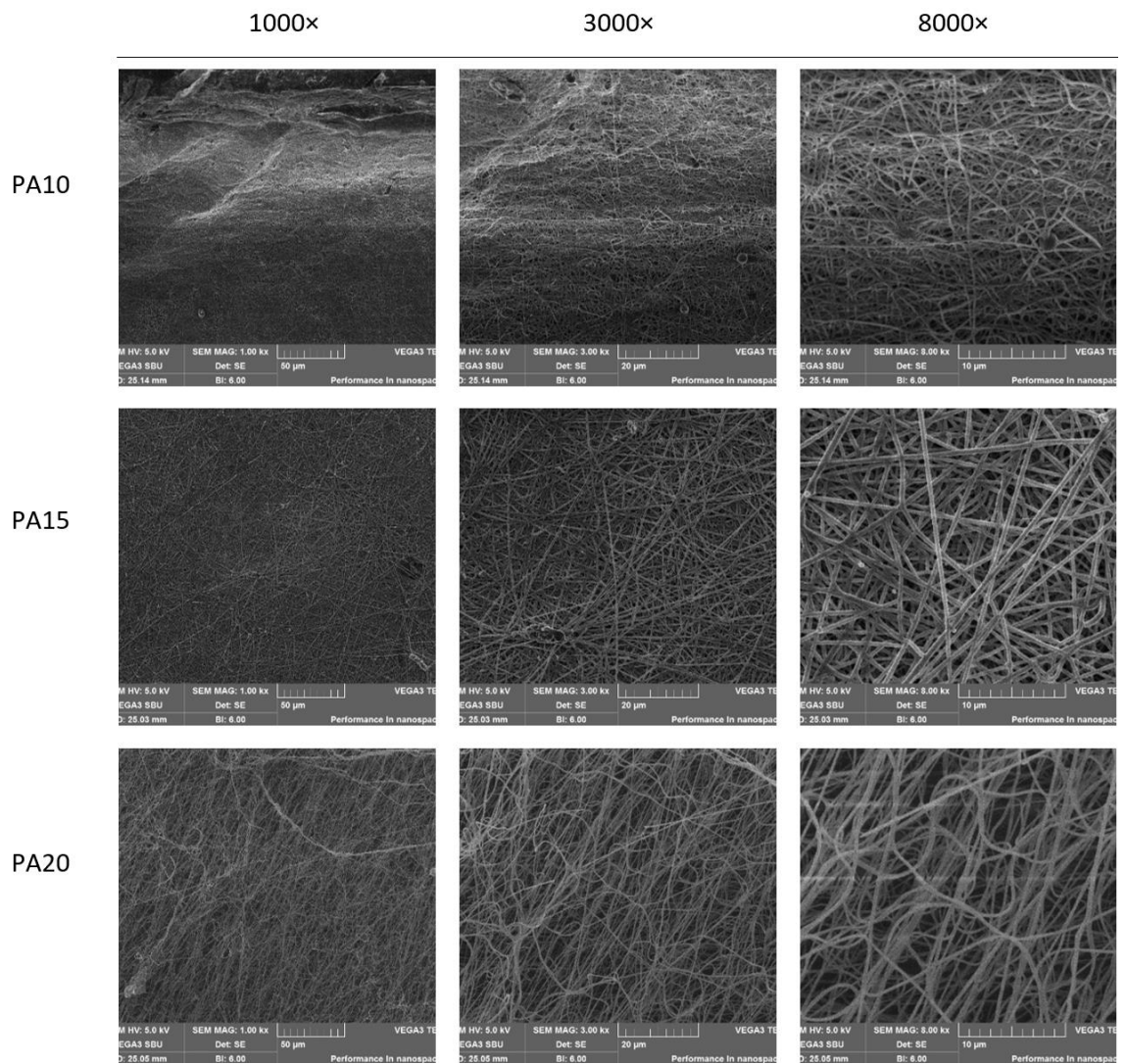
Cryomicrotome (SLEE MEV+) was also tested to cut plastic fibers. Piece of PA20 film was placed on the holder and encapsulated in OCT compound (Tissue-Tek®, Sakura) and then the holder was sunk into liquid nitrogen to freeze the sample. After freezing, the sample was taken out from the holder and glued with OCT compound on the stub. Operating temperature in cryomicrotome was - 20 °C. The stub was vertically in front of the blade and the blade was manually turn towards the sample resulting in thin sliced sections of the sample. Sections were moved on the mount (SuperFrost+) to verify plastic fibers from the OCT compound sample was imaged with optical microscope (Olympus B×4, Japan). Frozen section was also placed on the stub for further SEM imaging.

Furthermore, the sample was colored (Hematoxylin, Mayer's) after moved on the mount for fiber identification using optical microscope.

## 5. RESULTS

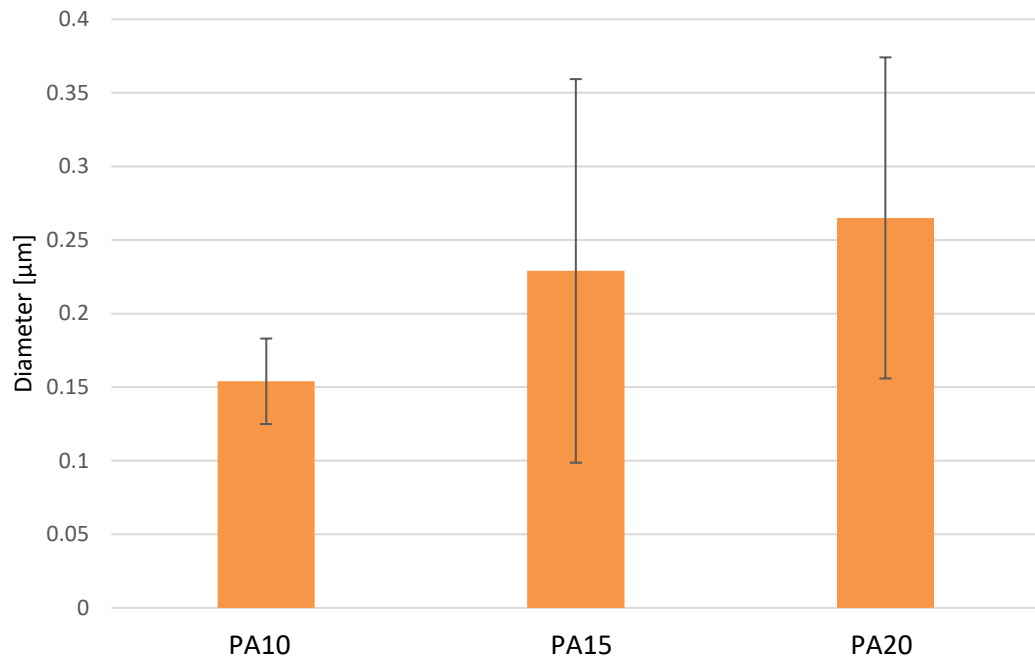
### 5.1 Polyamide fibers

Polyamide pellets dissolved in FA-AA solvent system within 24 h on stirrer in room temperature. Fibers were uniform and bead-free. Figure 12 presents the SEM images of polyamide fibers three different magnifications (1000×, 3000×, 8000×) Beam intensity was 6.



**Figure 12. SEM images of polyamide fibers PA10, PA15 and PA20 with magnifications 1000, 3000 and 8000.**

Figure 13 presents the mean diameter of PA10, PA15 and PA20 fibers. Standard deviations are on top of the bars. Nanosized fibers were obtained with all the solutions. Mean diameter of PA10 fibers was 0.15  $\mu\text{m}$ , PA15 0.23  $\mu\text{m}$  and PA20 0,27  $\mu\text{m}$ .



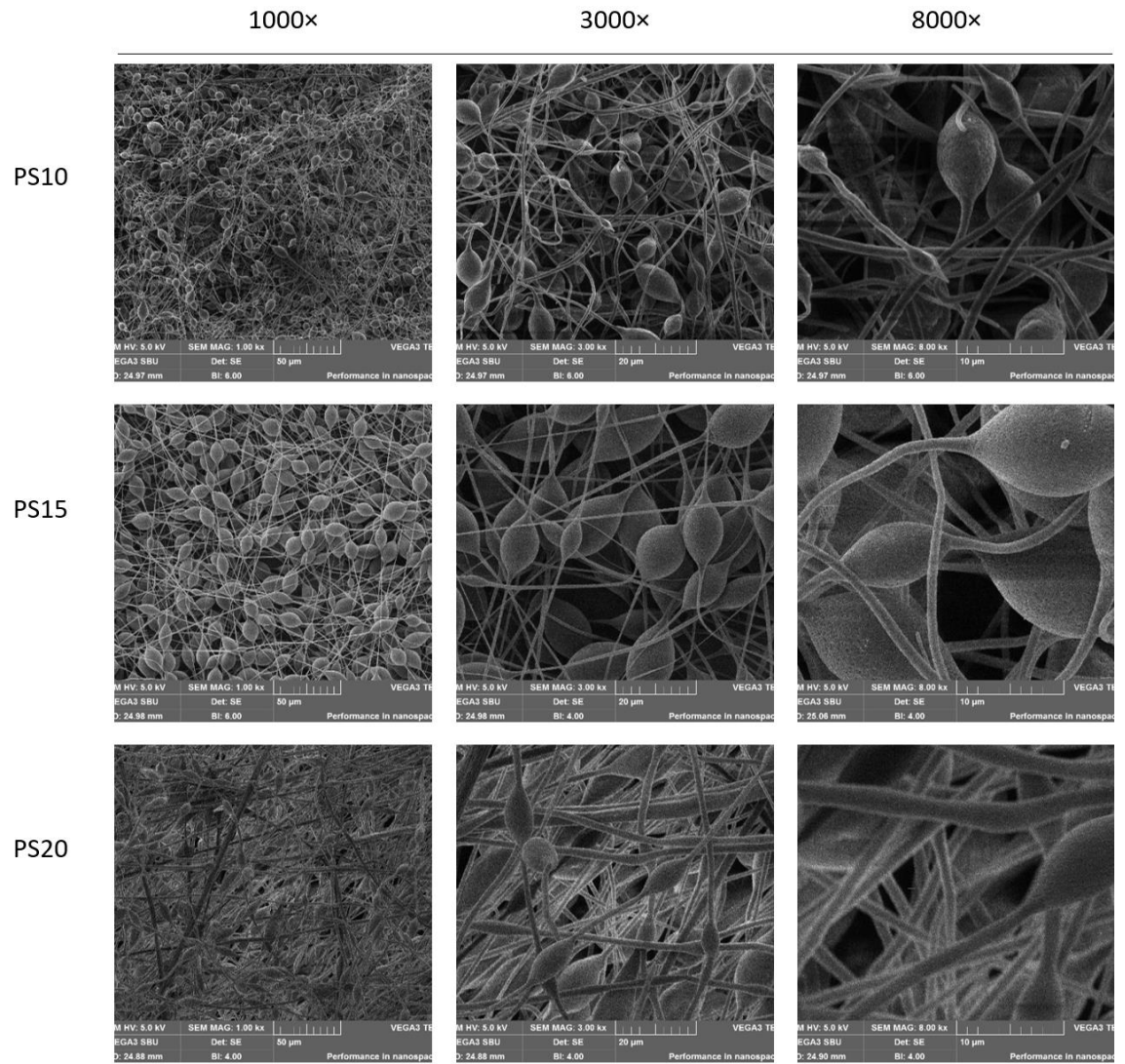
**Figure 13. Mean diameter of PA fibers and standard deviations**

As noted, fiber diameter increases when the amount of polymer is added to the solution. However, there was significant fluctuations in fibers thicknesses, as it is noted from the error bars.

## 5.2 Polystyrene fibers

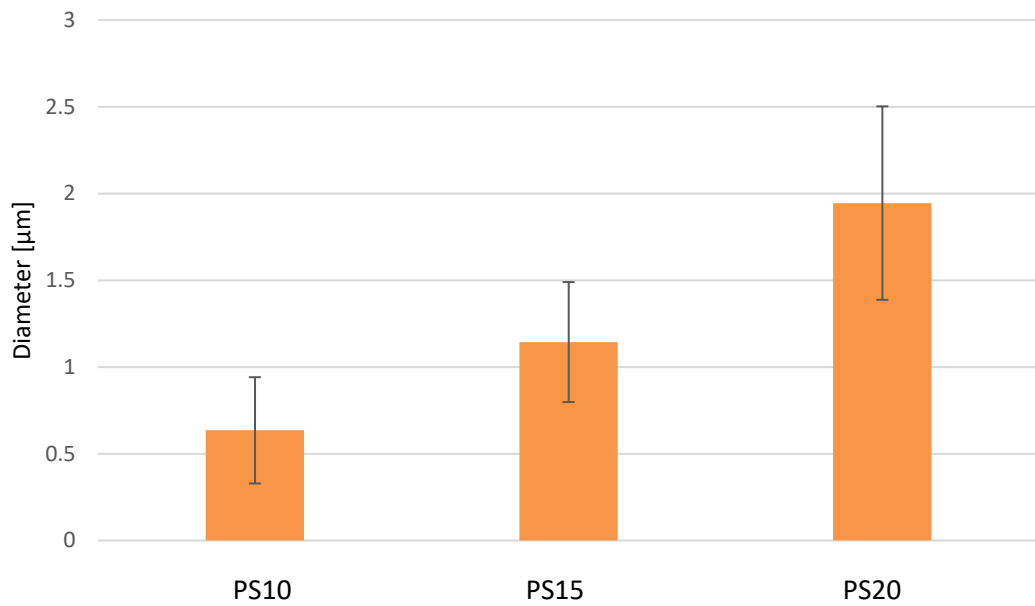
Uniform PS fibers were not obtained with solvent system DCM-THF. Fibers were formed, but they were full of beads. SEM microscopy images are presented in Figure 14 with same magnifications as PA fiber images. All the PA10 and PA15 1000 $\times$  were taken with beam intensity 6, and PS15 3000 $\times$  and 8000 $\times$ , and all PS20 images are taken with beam intensity 4.





**Figure 14. SEM images of polystyrene fibers PS10, PS15 and PS20 with magnifications 1000×, 3000× and 8000×.**

Figure 15 presents the development of mean diameters of PS fibers when amount of plastic is added to the solution. Also, standard deviations are presented on top of the bars. Mean diameter of PS10 was 0.64  $\mu\text{m}$ , PS15 was 1.14  $\mu\text{m}$  and PA20 1.95  $\mu\text{m}$ .

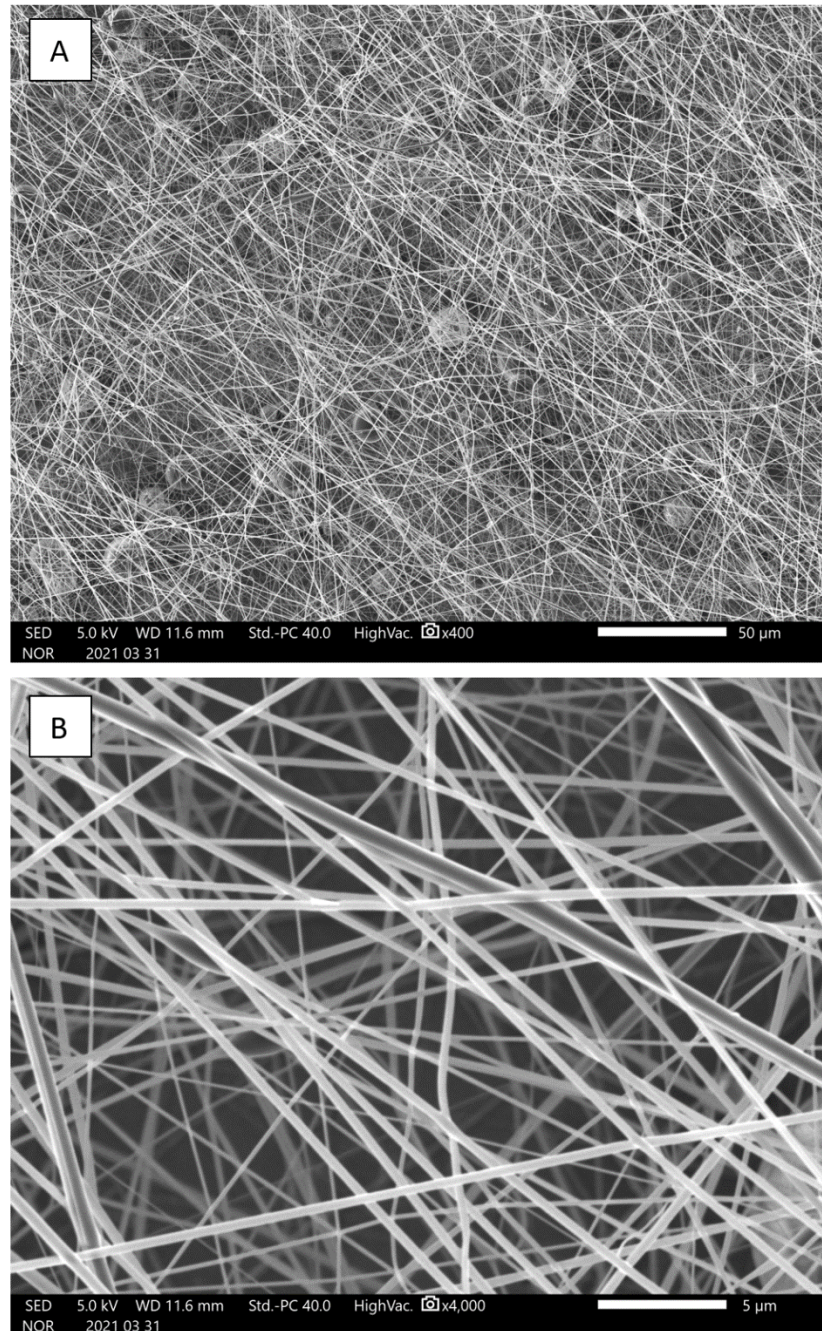


**Figure 15. Mean diameter of PS fibers and standard deviations.**

As it was seen in Figure 14, increasing the weight of the plastic in the solvent-system obtained thicker fibers. Only the mean diameter of PS10 fibers was nanosized. Other two PS fibers were classified as microfibers.

### 5.3 Polyethylene terephthalate fibers

The solvent, TFA, was crucial to achieve dissolution of the PET in room temperature and without it, PET did not dissolve. However, plastic parts of the electrospinner damaged when being contact with TFA. If more TFA containing solutions would have been used, the safety of the process would have not been able to guarantee in the presence of possible breakage of pipeline. Therefore, fibers were only imaged to investigate fiber formation in the presence of used electrospinning parameters. Figure 16 presents the PET fibers with magnifications 400 (A) and 4000 (B)

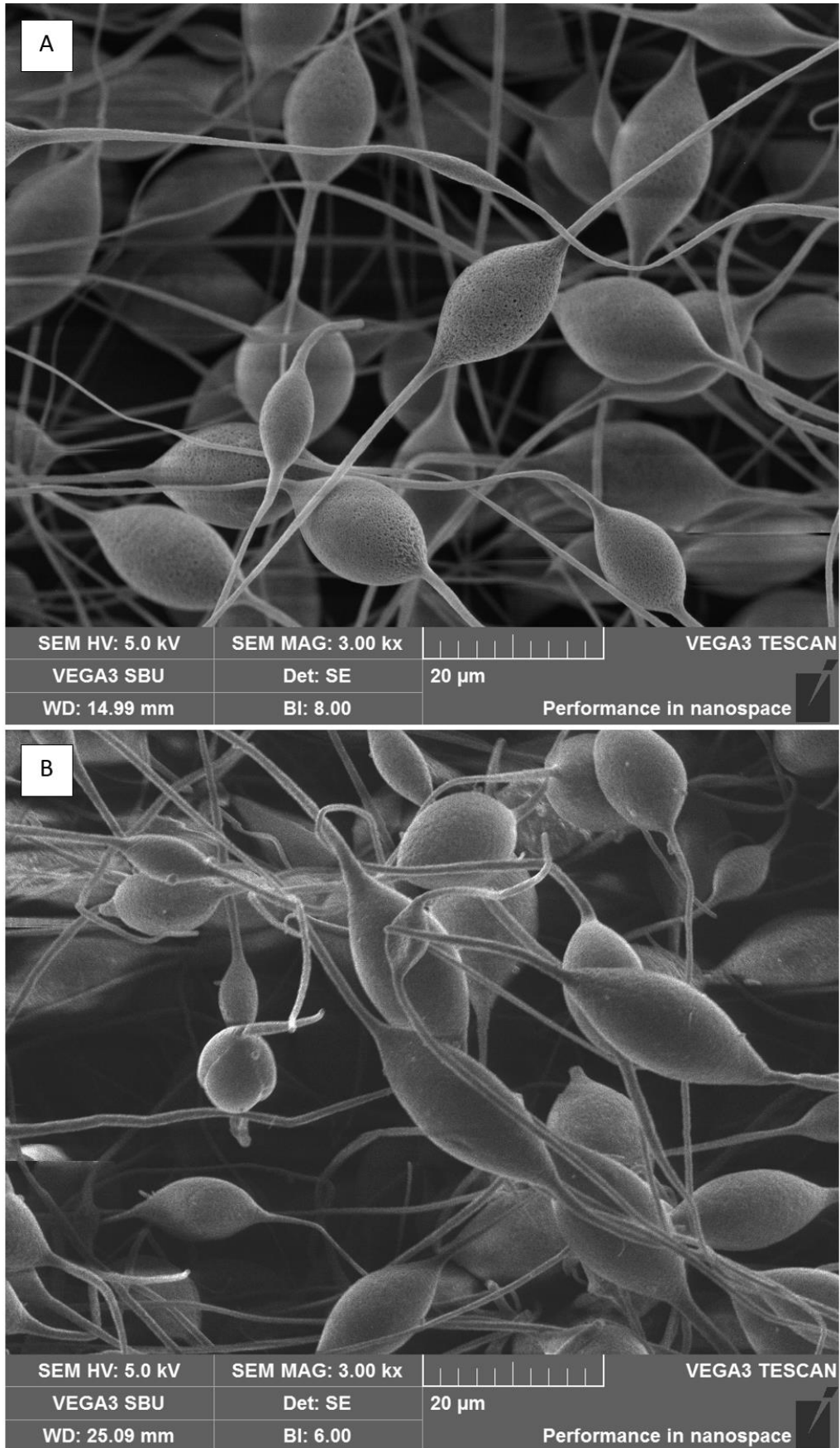


**Figure 16. PET10 fibers with magnification 400 (A) and 4000 (B)**

Fibers were coated with carbon. The mean diameter of the fibers was  $0.32 \mu\text{m}$  with SD value 0.092.

#### **5.4 The effect of the coating to the image quality**

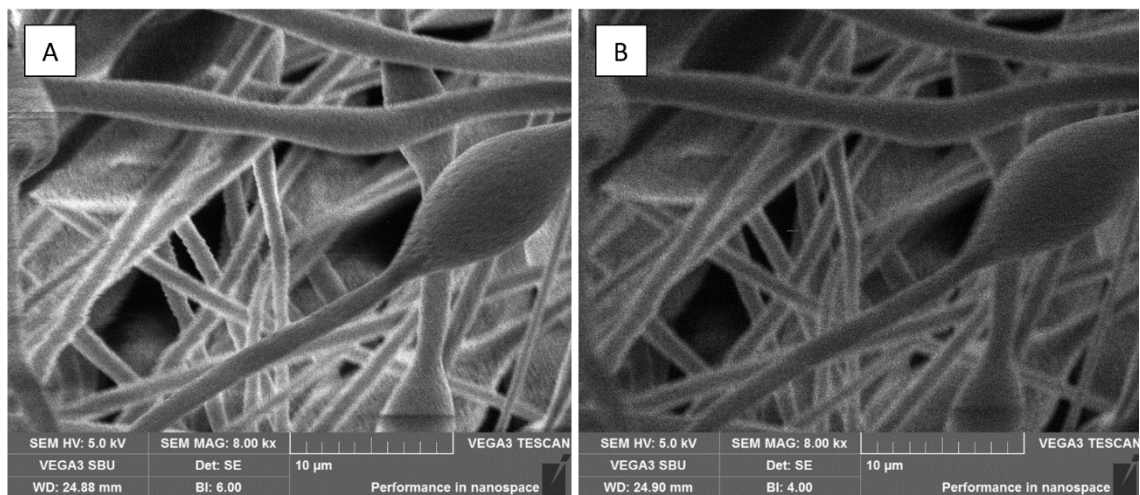
Figure 17 presents the effect of coating to the image quality. The image above presents PS15 fibers coated with platina-palladium mixture. Image below is PS15 fibers coated with carbon.



**Figure 17.** The effect of the coating material to image quality. Image A: Pd-Pt coating, image B: carbon coating

As it is noted, Pt-Pd coating enables more detailed imaging of the surface structure and topography of the fibers and beads compared the carbon coating. It was impossible to create smooth and dense carbon coating and the thickness of the coating could not be determined. The thickness of Pt-Pd coating was possible to determine before coating.

Usually, higher beam intensity enables more detailed information of the sample topography, but information is also gained from the larger interaction volume of the investigated area of the sample because the beam penetrates deeper to the surface. Figure 18 presents the effect of the beam intensity. On the left (A) is PS20 fibers imaged with BI value 6 and on the right (B) are same fibers with BI value 4. The sample was coated with carbon.



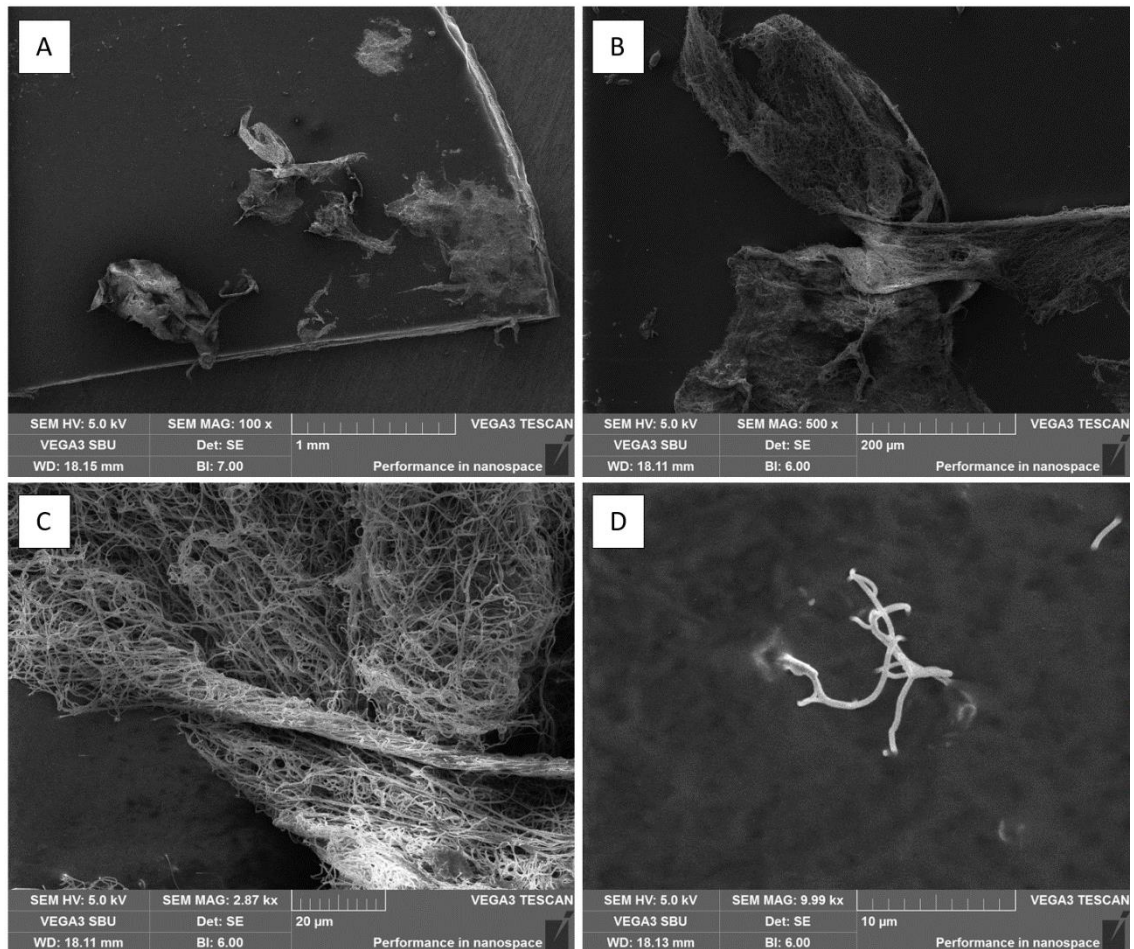
**Figure 18. The effect of the beam intensity. A: BI 6, B: BI 4**

As it is noted, higher BI gives more detailed information of the sample, but it distorts the image more easily. Distortion is seen clearly on the left side of both (A, B) images and charging effect due to high BI is seen in the figure A as white edges of fibers. Contrast of the image B is also decreased when only BI is lowered, and other parameters are kept constant. Higher emission of SE is noticeably seen in the first picture. Brighter areas distort the image and make it unusable. In Chapter 3.2.3 was presented more detailed the charging phenomenon of polymeric, nonconductive materials.

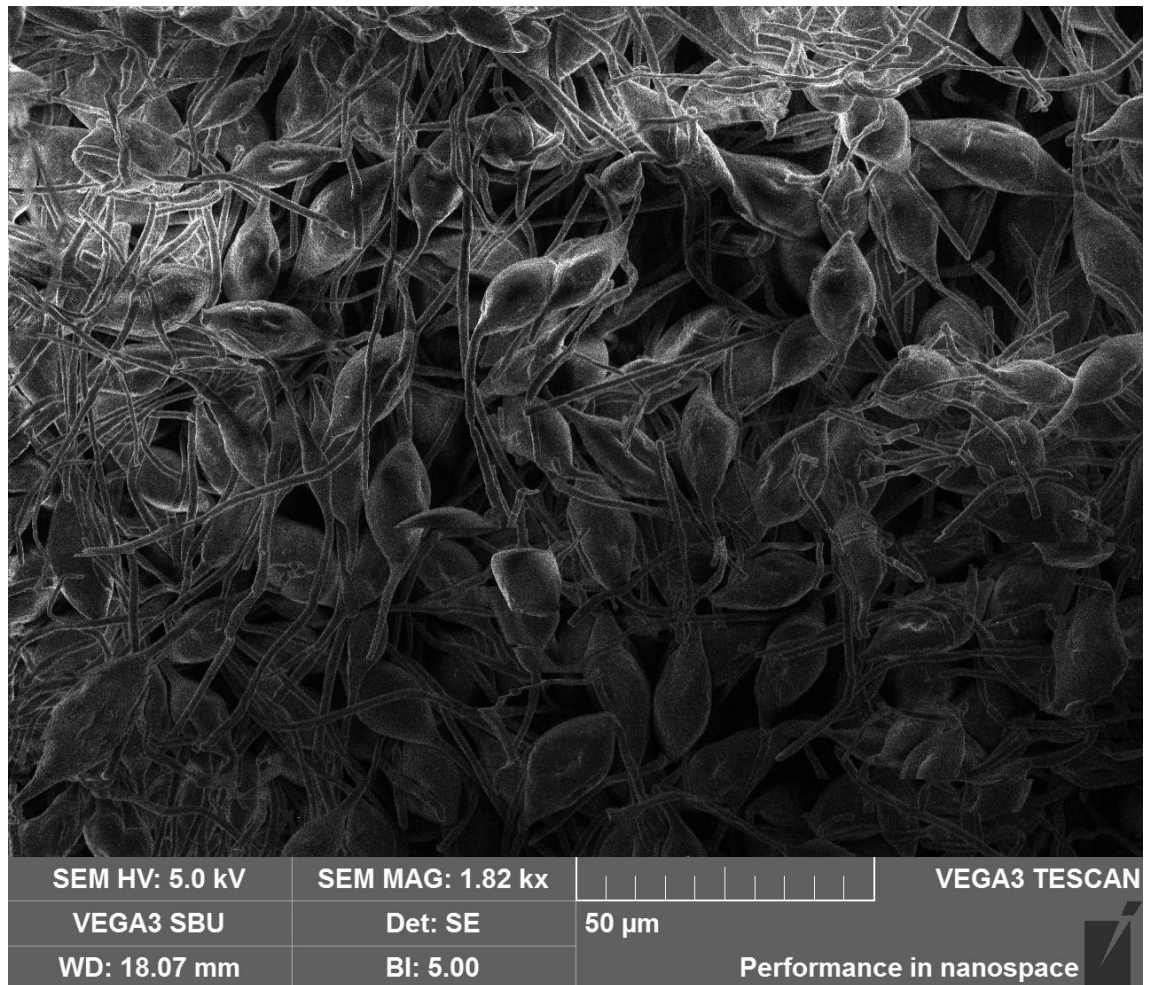
## 5.5 Fiber cutting

After fibers were prepared using electrospinning and imaged, films were crushed by mortar or cut by cryomicrotome in frozen conditions to result in separations of fibers. Frozen conditions were demanded to obtain brittle behavior of fibers, which could ease the cutting.

Figure 19 presents the result of breaking PA20 fiber film with mortar. The method worked partly. As it is seen, with this method was possible to separate single fibers, but there was very small amount of them to be efficient method. In images A, B, and C are seen SEM images of crushed fibers with different magnifications 100, 500 and 2870. As it is noted, most of the fibers were packed together to denser fiber mat. The image D (magnification 9990) presents successfully separated and cut PA20 fiber with diameter  $0.5 \mu\text{m}$ .



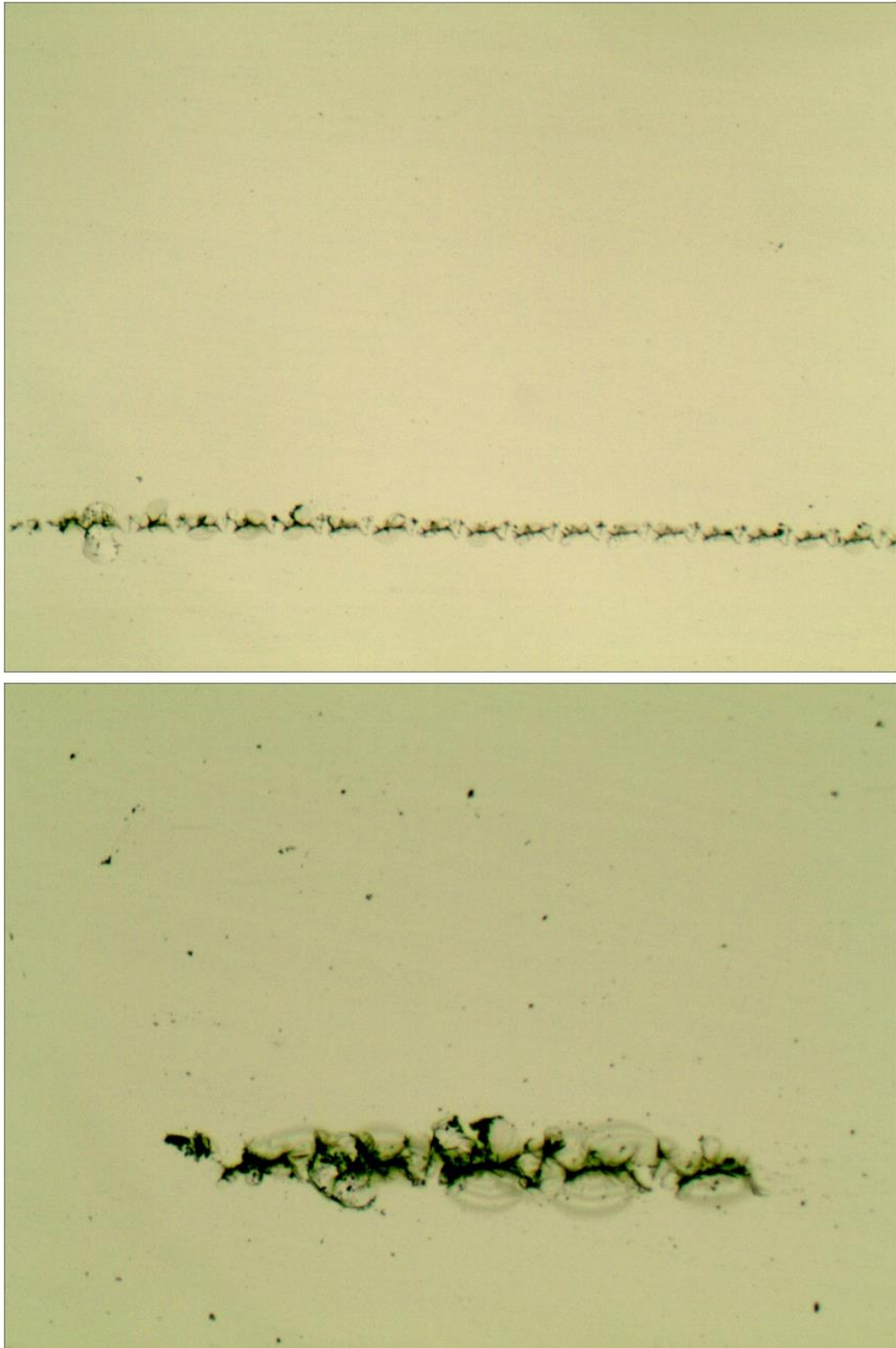
**Figure 19. PA20 fibers crushed by mortar. A: 100 $\times$ , B: 500 $\times$ , C: 2870 $\times$ , D: 9990 $\times$**   
 PA20 were classified as nanofibers. Because they are seen only with electron microscope, fiber identification from the mortar was not possible. Figure 20 presents PS20 microfibers crushed in mortar. As it is seen, also PS20 fibers were packed more closely together.



**Figure 20. PS20 fibers crushed using mortar**

As it is seen in the image, many of the fibers were cut and multiple ends of the fibers were detected, but the separation of the fibers was not achieved.

Cutting the plastic fiber film using cryomicrotome was also possible but challenging. Figure 21 presents sliced section of the sample PA20 with two different magnifications. Dark area in the images was not able to identify as PA20 fibers, since no fibers were found from the SEM sample taken from the same section area.



**Figure 21. Optical microscope image of cryomicrotome section of PA20.**

Optical microscope was used only to verify the existence of solid material on the mount. Therefore, the scale and magnification information are not included to the image. OCT compound transformed back to liquid when touching the mount that was in room temperature.



## 6. DISCUSSION

Humans are exposed to MNPs in everyday, but it is still unclear how they affect to humans (Wright and Kelly 2017). It is known that MNPs accumulate in organs and could disturb normal cell functions in laboratory tests. Materials used in laboratory tests should be comparable to plastic fragments, that we are exposed in everyday life. Material variety should also be wide, and shape of plastic particles should be versatile from fibers to irregular fragments. The mechanism of MNPs to enter for example into the blood stream and what are the possible long-term effects of MNPs exposure, are unknown and therefore more research is needed to understand their possible impacts to human health. Moreover, the identification of MNPs and the field of research would need standardizations, because with standardization the comparability of the studies would be easier, and the results could be more reliable (Oliveira and Almeida 2019).

Nanofiber manufacturing of PA with FA/AA solvent-system and PET with DCM/TFA solvent-system were possible by using electrospinning but DMF/THF solvent-system was not suitable for smooth fiber manufacturing of PS. PS fibers were full of swellings. Also, the mean diameter below 1  $\mu\text{m}$ , excluding beads, was obtained only with PS10 solution. However, all the samples included variations in fiber diameter. Fluctuations could be result from interruptions in the spinning process. During the spinning, all the fibers formed a build-up to the tip of the needle. To remove the build-up, the pumping of the solution was paused for a moment and the voltage was turned off. After cleaning, the process could continue. The alterations in voltage during the process could also affect to the fiber diameter.

Bead formation is usually result from low molecular weight, low viscosity, high surface tension, low concentration and/or low charge density. (Fong *et al.* 1999) In Figure 14, SEM images of PS fibers, was seen the effect of increasing the polymer concentration in solvent system especially when PS15 and PS20 with magnification 1000 were compared. In this comparison, higher concentration of plastic in the solvent system decreases the size of beads in fibers. However, if the concentration of the pellets would have been only variable in the process, then PS10 should have had the highest number of beads. Therefore, other solvent-system for PS should be used for to achieve bead-free fibers. Also, more specific analyzation of raw polymer pellets and steady electrospinning conditions could help to develop the process to obtain bead-free fibers.

PET pellets were tried to dissolve in solvent-system, that included 9 ml of DCM and 1 ml TFA. However, the pellets did not dissolve in 24 hours. The addition of 4 ml of TFA resulted in the complete dissolution of PET pellets within two hours. It was clear that usage of TFA was essential, but the electrospinner parts were not resistant to strong acid and therefore the electrospinning process was not safe. Solvents are needed when common plastics are dissolved in room temperature and typically used solvents are hazardous chemicals (Brown *et al.* 2016). To electrospin PET fibers, all the parts of the instrument contacted with polymer solution should be ensured to be resistant to strong acid like TFA. For example, glass could be one suitable material for the piping. Other option would be melt-electrospinning instrument, in which pellets are melted by heat. In melt process, the usage of hazardous solvents can be avoided (Brown *et al.* 2016).

With mortar it was possible to separate and cut fibers from the fiber film, but the method was rather inefficient. Only few fibers were detached and cut to short length with mortar. It can be assumed that some single fibers were left on the mortar due to geometry of the equipment and the conditions of the process. The bottom of the mortar was arched while the SEM stub was flat. Therefore, stub could not be pressed on the bottom of the mortar. Process conditions could also affect to the amount of the single fibers on the stub. Crushing was performed in the fume hood due to evaporation of the liquid nitrogen. Since the fibers were charged easily and they were drawn by the plastic gloves that were used, it was possible that single fibers were flew out of the mortar due to electric charge and airflow of the fume hood.

Cryomicrotome has been used to cut Nylon microfibers to specific length (Cole 2016). Microplastic fibers were prepared for exposure tests in laboratory. With cryomicrotome it was possible to slice fibers in film form below 10  $\mu\text{m}$  sections, but it was challenging to orientate the film due to electrostatic forces and cooling method that made the film bulge uncontrollable. The frozen sample was full of air bubbles and because OCT compound turned white when frozen, separation of film from the glue matrix was almost impossible with an eye. Therefore, thin sections were inspected with optical microscope to identify solid particles on the mount. Multiple unknow particles and thin, rough surfaced fibers were found from the SEM stub, but none of them were electrospun fibers. Isolation of the fibers on SEM stub and identification with SEM were not achieved with methods and equipment that were on use. In the future, breaking methods of the fiber film should be developed to achieve more single nano- and microfibers.

To overcome the challenges related to working with cryomicrotome, method and course of action would need improvements. The coloring of the fiber film before freezing would make it visible to human eye. Then it would be possible to identify the film from the OCT

compound matrix during the slicing. Sections could be then removed straight to the SEM stub without the inspection by using optical microscope. Slower cooling method might prevent the bulging effect of the film during freezing. With tweezers, the film might be possible to place straight on the glue. PA films were possible to handle with tweezers, but PS film was too fragile to handle. In a case of very fragile fibers, fibers could be scrape gently on the glue. Moreover, the cryomicrotome chamber should be cleaned properly before slicing. The stub especially with carbon tape was very easily contaminated from other particles in the chamber and air. Sealed transportation of SEM stub could also reduce the possible contamination of the sample.

Steady frozen conditions might be demanded, because in room temperature fibers are too soft to cut and liquid nitrogen evaporates too quickly and therefore the elevation of temperature is fast. Brittle behavior of the fibers makes them easier to cut. Also, the airflow and electric charge should be avoided. Other approach to overcome the separation problem of the fibers would be to manufacture single fiber for example by drawing. Then the fiber would only need to be cut with suitable method. However, the isolation of the nanofibers is one of the main challenges to overcome in the future since the fibers are only visible under electron microscope.

Imaging of the fibers with SEM was quite simple. However, fibers were easily charged which created defects to the images. Sensitiveness of the samples had to be taken into account. Imaging of plastic nano- and microfibers should be performed with low (5kV) acceleration voltage. Also, the beam intensity must be controlled, or it can cause distortions of the image. Suitable coating material and uniform coating prevent image distortions. Moreover, in the fiber film, fibers are not supported entirely even they are placed on the stub. There is free space around the fibers, which enables fibers to move under external stress. The electron beam can cause mechanical vibration of the sample, especially the area under the probe. Vibration complicates the achievement of high-quality images. Therefore, the probe current should be controlled. Platinum-palladium coat proved to be more suitable for nanofibers, compared to carbon coat, because it was possible to deposit dense and smooth layer with controlled thickness on the samples. In addition, the electrical conductivity of Pt and Pd are higher compared to carbon (Goldstein *et al.* 1992, pp.683). The high yield of SEs enabled high-resolution imaging and the elimination of charging effect were the advantages of Pt-Pd coating. With Pt-Pd coating, the imaging with higher BI was possible, which produced more detailed information of fibers and especially of the topography of the beads. However, the selection of suitable coating and imaging parameters is always dependent on the properties of the characterized sample.

## 7. CONCLUSION

Since the plastic particles are known to accumulate in humans and animals, the focus should be the prevention of plastics to enter environment. The main targets in the future are the development of the waste management and the prevention of the leakages of plastics production. Moreover, new degradable materials for example to the packaging industry are demanded. Changing the persistent and non-degrading plastic materials to new materials that degrade in specific conditions or materials that do not fragment to persistent and harmful micro- and nanoplastics could slow the increasing accumulation of plastic in our environment.

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## APPENDIX A: DATA OF FIBER MEASUREMENTS

Ten measurements of fiber thickness per fiber samples

PA10	PA15	PA20	PS10	PS15	PS20	PET10
0.14	0.10	0.13	1.3	1.13	1.8	0.42
0.16	0.12	0.23	0.87	1.12	1.64	0.25
0.14	0.08	0.28	0.68	1.66	1.94	0.23
0.18	0.10	0.41	0.48	0.66	2.62	0.34
0.19	0.14	0.15	0.93	0.94	2.91	0.3
0.13	0.37	0.18	0.56	1.57	1.19	0.31
0.12	0.36	0.22	0.26	1.06	1.37	0.18
0.15	0.45	0.20	0.25	1.63	2.33	0.52
0.12	0.25	0.41	0.48	0.97	1.31	0.34
0.21	0.32	0.44	0.54	0.7	2.34	0.29

Mean diameter of the samples

PA10	PA15	PA20	PS10	PS15	PS20	PET10
0.154	0.229	0.265	0.635	1.144	1.945	0.318

Standard deviations of the fibers

PA10 SD	PA15 SD	PA20 SD	PS10 SD	PS15 SD	PS20 SD	PET10 SD
0.029052	0.130342	0.10911	0.306276	0.345694	0.557589	0.092065