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MICROPLASTICS IN WASTE WATER TREATMENT PLANTS AND SEPARATION TECHNIQUES

Diploma Thesis
Master Water Management and Environmental Engineering

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TABLE CONTENT

Abstract

Microplastics (MP) are emitted to the environment, nevertheless the possible impacts in the ecosystem are still under investigation and the knowledge about them in the environment is poor. This research focused on investigating the behavior of commercial used and defined green-fluorescent MP (ø 50-1000 µm) in waste water treatment plant (WWTP) simulation tests, the collection efficiency of different techniques (change in density and addition of chemicals) for separation of MP, the presence of commercial MP in different units of a WWTP with a capacity of 55000 p.e. and the changes in the shape of fluorescent MP after their digestion by mice.

The WWTP simulation tests were carried out in beakers. It was observed that the commercial and fluorescent MP had a different density, the colour of the commercial MP samples changed from white to grey but the size of the MP did not change. Various collection methods such as NaCl addition and separation in separatory funnels without (SF) and plus centrifugation (SF+C), adapted separatory dispenser (ASD), adapted separatory column (ASC), chemical methods (CHEM) were tested; density techniques with NaCl plus centrifugation (SF+C) or separation by the addition of solvents (CHEM) proved to be the best collection methods with a mean collection efficiency of 94 % and 86 % respectively. In all units of the WWTP, using the SF+C separation method, commercial MP were identified and the amount of microparticles in the influent and effluent discharged into the river or surface water were approximately 6667 and 8667 particles/m³ respectively; about 50 % of the microparticles could be identified as commercial MP. In the case of digestion it was observed that 22.6 % of large fluorescent MP particles (ø 425-500 µm) were reduced (deformed) from their original size after digestion in a mouse. On the other hand, the digested small fluorescent MP (ø 50-75 µm) kept their size in the average range.

Zusammenfassung

Mikroplastikpartikel (MP) können aus verschiedenen Quellen in die Umwelt gelangen, allerdings ist der Wissenstand über mögliche Auswirkungen auf Ökosysteme eher gering. Diese Forschung konzentrierte sich auf die Untersuchung des Verhaltens von definierten grün-fluoreszierenden und kommerziell verwendeten MP (ø 50-1000 µm) in Fed-Batch Testsystemen, der Ermittlung der Effizienz von verschiedenen Trenntechniken durch Veränderung der Dichte und Zugabe von Lösungsmittel zur Separation von MP aus Abwasser. Zusätzlich wurde das Vorhandensein von kommerziellen MP in den verschiedenen Reinigungsstufen einer Abwasserreinigungsanlage (ARA; Kapazität 55000 EW) und die Veränderung der Form von fluoreszierenden MP nach Passieren des Verdauungstraktes von Mäusen ermittelt.

Die Fed-Batchtests wurden in Bechergläsern durchgeführt. Dabei konnte festgestellt werden, dass die kommerziellen und fluoreszierenden MP verschiedene Dichten aufwiesen. Die kommerziellen MP haben ihre Farbe von weiß nach grau verändert, die Größe der MP blieb unverändert. Verschiedene Sammlungsmethoden wie NACl Zusatz und Trennung im Scheidetrichter ohne (SF) und mit Zentrifugation (SF+C), Mikroseparator (ASD), adaptierte Trennsäule (ASC) und diverse Lösungsmittelzusätze (CHEM) wurden untersucht. Die Erhöhung der Dichte mit NaCl plus Zentrifugation (SF+C) und die Trennung durch Zugabe von Lösungsmitteln (CHEM) waren die besten Verfahren mit mittleren Abscheidegraden von 94 % und 86 %. In allen Reinigungstufen der ARA wurden mittels SF+C-Trennverfahren kommerzielle MP identifiziert, der Ein- bzw. Austrag beträgt 6667 bzw. 8667 Mikropartikel pro m³ allerdings konnten davon nur ca. 50 % als kommerzielle MP identifiziert werden. Die Untersuchungen von MP Partikel nach Passieren des Verdauungstraktes von Mäusen zeigen, dass 22,6 % der größeren fluoreszierenden MP (ø 425-500µm) in ihrer Größe reduziert bzw. deformiert waren, jedoch die kleineren fluoreszierenden MP (ø 50-75µm) eher unverändert ausgeschieden wurden.

1. INTRODUCTION

The pollution caused by microplastics in aquatic environments has recently had wide discussions in the media due to recent publications about the interaction of microplastics within ecosystems and possible impacts. Most of the knowledge about this theme or subject is still under investigation, for instance the understanding of its behaviour in water, alternatives to separate from mixed samples, techniques to identify the composition, amount of discharge in a water body and so on.

It is well known that microplastics are widely used by society for different purposes, for example in “cleaning products or cosmetics“, and most have a size smaller than five millimetres (Roex et al., 2009). Due to its lightweight and durable nature, microplastics have become prevalent in waste water from households, and eliminated through the sewage system, which finally reach a water body. Also, most microplastic is produced with polyethylene as a main ingredient, and it is present in forms variously described as ‘‘micro-beads”, ‘‘microbead formula” or ‘‘micro exfoliates”(Fendall and Sewell, 2009).

As mentioned above, microplastics are generated in households and through the sewage system are transported to waste water treatment plants. These plants then become the main source of this kind of microparticle which will reach a body water. Information about the amount of microplastics discharged is still negligible; therefore the challenge to supply new valuable data from different treatment plants is very important.

The topic of study is very recent, as there is not enough information available about the real effects of microplastics in nature and defined standard techniques to work with this type of material. Under this context the research of this work is divided into three main components where the first part describes basic information in regards to microplastics: definition, types, sources of production, impacts in nature, recent techniques for separations and identification. The second part includes the materials used for the different tests and the methodology applied for each activity: simulation of waste water treatment, new separation techniques, collection of microplastics from waste water treatment and digestion of microplastics by mice; and finally the third part is focused on the results obtained through the different tests undertaken to understand the behaviour of microplastics in waste water treatment, the best technique to recover most of the particles from waste water samples the amount of microplastics collected per cubic meter and changes on the surface of microplastics after the digestion in mice.

2. OBJECTIVES

The present thesis developed new alternatives or options to improve the collection efficiency of microplastics and knowledge about their behaviour in different environments. For that purpose round fluorescent microspheres of polyethylene were used due to their easiness to identify and notice changes on the surface under the different treatments.

This research about microplastics aims to:

- Evaluate the behaviour of microplastics in simulated waste water treatment processes to determine possible surface damages, size, and colour changes in microplastics after the degradation process.
- Evaluate the collection efficiency of different techniques tested for separation of microplastics from sewage sludge.
- Identify the presence of microplastics in different units and calculate the amount microplastics generated per cubic meter of waste water from a municipal waste water treatment plant (WWTP) with a capacity of 55000 p.e.
- Observe the changes in microplastics after their digestion by mice.

3. FUNDAMENTALS

This chapter includes a general background and outstanding information in regards to water quality, microplastics and environment, and separation techniques for microplastics.

3.1. General background

Demand of plastics is considerable; in the last 60 years the annual world plastic production has increased dramatically from 1.7 million tonnes in the 1950s to approximately 288 million tonnes in 2012; however in Europe the amount decreased from 57 in 2011 to 56 million tonnes (Plastics Europe, 2013). After the use of this material the discarded plastic accumulates, particularly in marine habitats where contamination stretches from shorelines to the open-ocean and deep-sea. (Browne et al., 2011).

Plastics are synthetic organic polymers, which are derived from the polymerisation of monomers extracted from oil or gas. Since the development of the first modern plastic; 'Bakelite', in 1907, a number of inexpensive manufacturing techniques have been optimised, resulting in the mass production of a plethora of lightweight, durable, inert and corrosion-resistant plastics (Cole et al., 2011).

The concern about plastic hazards started with the discovery of high concentrations of plastic litter in the northern path of the Pacific Ocean in the late nineties and recently caused a lot of commotion around the group of microplastics, which may eventually end up in the marine food chain via sewage treatment plants and riverine transport (Roex et al., 2011). Nowadays the research efforts are focusing on microplastic (<5 mm) fractions (Fendall and Sewell, 2009).

As mentioned before, in the nineties it was recognized that a minor source of microplastic pollution was derived from liquid hand-cleansers that would have been rarely used by the average consumer. In 2009, however, the average consumer is likely to be using microplastic-containing products on a daily basis, as the majority of facial cleansers now contain polyethylene microplastics which are not captured by wastewater plants and will enter the oceans (Fendall and Sewell, 2009). An important source of microplastic in this case is represented by fibers from washing clothes. Forensic evaluation of microplastic from sediments showed that the proportions of polyester and acrylic fibers used in clothing resembled those found in habitats that receive sewage-discharges and sewage-effluent itself. Experiments sampling wastewater from domestic washing machines demonstrated that a single garment can produce >1900 fibers per wash (Browne et al., 2011).

Particular consideration has been placed on plastic debris at the micro-scale, as it is widespread in the environment and these microplastics have accumulated in oceans and sediments worldwide in recent years, with maximum concentrations reaching 100 000 particles m^3. Due to their small size, microplastics may be ingested by low trophic fauna; with uncertain consequences for the health of the organism (Wright et al., 2013). In all cases ingestion of microplastics provides a potential pathway for the transfer of pollutants, monomers, and plastic-additives to organisms with uncertain consequences for their health (Browne et al., 2011), due to the fact that they are considered bioavailable to organisms throughout the food-web (Cole et al., 2011).

For further investigations, accurate assessment of quantity and type of microplastic is needed. Cleassens et al. (2013) proposed new techniques for extracting microplastics from sediment. The method developed for sediments involves a volume reduction of the sample by elutriation, followed by density separation using a high density NaI (Sodium iodide) solution; this new method has considerably higher extraction efficiency. Also Imhof et al. (2012) improved the density separation approach by the design of a separator called Munich Plastic Sediment Separator (MPSS), which obtained higher rates of recovery for large and small microplastic particles with values up to 95.5%.

3.2. Water quality and water regulations

In the world each state or country has different procedures to generate their own laws; for example in the United States In general, different federal statutes or regulations, such as water quality regulations, pass by Congress and afterwards they are signed into law by the President (EPA, 2013).

In the case of water regulation, it is important to know the meaning of different technical parameters and its application in different countries.

3.2.1. Definition of water quality

Water quality is commonly defined by its physical, chemical, biological and aesthetic (appearance and smell) characteristics. A healthy environment is one in which the water quality supports a rich and varied community of organisms and protects public health (NSWG, 2012).

Alternative definitions of the term water quality are used to describe the condition of the water, including its chemical, physical and biological characteristics, usually with respect to its suitability for a particular purpose (i.e., drinking, swimming or fishing). Water quality is also affected by substances like pesticides or fertilizers that can negatively affect marine life when present in certain concentrations (Diersing, 2009).

3.2.2. Physical parameters in surface water

The main physical parameters are described as follows:

3.2.2.1. Total suspended solids

Total suspended solids (TSS) are particles that are larger than 2 microns found in the water column. Anything smaller than 2 microns (average filter size) is considered a dissolved solid. Most suspended solids are made up of inorganic materials, though bacteria and algae can also contribute to the total solids concentration. Total suspended solids, as a measurement of mass are reported in milligrams of solids per litre of water (mg/L) (FEI, 2014).

3.2.2.2. Dissolved oxygen

Water molecules contain an oxygen atom. This oxygen is not what is needed by aquatic organisms living in natural waters. A small amount of oxygen, up to about ten molecules of oxygen per million of water, is actually dissolved in water. Oxygen enters a stream mainly from the atmosphere, in areas where ground-water discharge into streams has a large portion of streamflow, and from groundwater discharge. This dissolved oxygen is breathed by fish and zooplankton and is needed by them to survive (Perlman, 2014).

Dissolved oxygen is usually reported in milligrams per litre (mg/L) or as a percent of air saturation (FEI, 2014).

3.2.2.3. Turbidity

Turbidity is an physical or optical determination of water clarity. Turbid water will appear cloudy, murky, or otherwise coloured, affecting the physical look of the water. Suspended solids and dissolved coloured material reduce water clarity by creating an opaque, hazy or muddy appearance. Turbidity measurements are often used as an indicator of water quality based on clarity and estimated total suspended solids in water. Turbidity is reported in units called Nephelometric Turbidity Unit (NTU), or a Jackson Turbidity Unit (JTU) (FEI, 2014).

3.2.3. Chemical parameters in surface water

The main physical parameters are described as follows:

3.2.3.1. pH

The pH is a determined value based on a defined scale, similar to temperature. This means that pH of water is not a physical parameter that can be measured as a concentration or in a quantity. Instead, it is a figure between 0 and 14 defining how acidic or basic a body of water is along a logarithmic scale. The lower the number, the more acidic the water is. The higher the number, the more basic it is. A pH of 7 is considered neutral (FEI, 2014).

A measurement of pH is usually determined by electrochemical measurements, in which the potential of a pH electrode immersed in the test solution is measured. The pH electrode responds quantitatively and specifically to hydrogen ions even in the presence of other ions. The potential of the pH electrode is measured with respect to a second, reference, electrode (also in contact with the test solution) using the pH meter (Prichard, 2003).

3.2.3.2. Electric conductivity

Electrical conductivity is the proportionality factor relating to the current that flows in a medium to the electric force field that is applied. It is a measure of the ability of the material to conduct an electrical current to move through the material. The units of conductivity are Siemens per meter (S/m). The practical unit is milliSiemens per meter (mS/m) (Environmental Geophysics, 2011).

Conductivity is a measure of water's capability to pass electrical flow. This ability is directly related to the concentration of ions in the water (FEI, 2014).
The probe consists of two metal electrodes spaced 1 cm apart (thus the unit of measurement is milliSiemens per centimeter). A constant voltage is applied across the electrodes resulting in an electrical current flowing through the aqueous sample. Since the current flowing through the water is proportional to the concentration of dissolved ions in the water, the electrical conductivity can be measured. The higher the dissolved salt/ion concentration, the more conductive the sample and hence the higher the conductivity reading (Bruckner, 2012).

3.2.3.3. Redox potential

The redox potential of water is a measure of electrochemical potential or electron availability within the system. Electrons are essential to all organic and inorganic chemical reactions.

Redox potential is determined from the concentration of oxidants and reductants in the environment. The unit for redox potential used is millivolts (mV) (De Laune and Redy, 2005).

For the redox-potential, the methods are based on measuring the potential of a platinum electrode against the silver/silver-chloride reference electrode immersed in the medium under investigation. In other words, the phenomenon used here is that of a shift in the potential of the platinum electrode placed in an aqueous solution due to adsorption of oxygen on surface of platinum (Goldin et al. 2007)

3.2.3.4. Chemical oxygen demand

Chemical oxygen demand (COD) is often used to measure organic matter in wastewater, treated effluent, and receiving waters. Although COD measures more than organic constituents, the organic fraction usually predominates and is the constituent of interest. The unit for COD is milligram per liter (mg/L) (Stromwaterx, 2014).

3.2.3.5. Total nitrogen

Total Nitrogen (TN) is the measure of the complete nitrogen content in water. In water, the forms of nitrogen of greatest interest are nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia-nitrogen (NH_3-N) and organically bonded nitrogen. Total Nitrogen (TN) content of water can be determined by measuring nitrite, nitrate, ammonia, and Kjeldahl nitrogen. The unit for TN is mg/L (McTigue and Symons, 2010).

3.2.3.6. Total organic carbon

TOC is defined as the amount of carbon covalently bound in organic compounds in a water sample. The TOC is a more suitable and direct expression of total organics than BOD or COD, but it does not provide the same kind of information. The common unit for TOC is mg C/L (Nollet, 2007).

3.2.4. Water quality regulation in Austria

In Austria, water is mainly protected under the Water Act (BGBl. Nr. 215/1959 i.d.F.) and corresponding regulations on waste water disposal (Schmelz and Rajal, 2012).

The Water Act differentiates between publicly owned water and privately owned water. Publicly owned water may be used by everyone without special authorisation, provided that the utilisation does not exceed general use. Basically the same applies to privately owned water. Water utilisation exceeding general use is prohibited unless the user holds a water use permit.

The Austrian regulatory regime for water pollution is further characterised by the implementation of the (EU) Water Framework Directive 2000/60/EC into national law. The Water Act has adopted most of the Directives' provisions concerning the protection and improvement of waters. In 2011, the Water Act was substantially amended. In order to reach the water quality goals under the Water Framework Directive 2000/60/EC, the Water Act now provides for a specific water restoration mechanism applying to existing installations.

The Water Act sets out two provisions concerning the responsibility for contamination of water bodies. One provision regulates the liability for endangering water bodies, while the second provision applies to soil and groundwater contaminations.

In addition, the Federal Environmental Liability Act (BGBl. Nr. 55/2009) applies to direct or indirect damage and imminent threat of damage to the aquatic environment resulting from occupational activities, where a causal link can be established between the damage and the activity. If there is an imminent threat of damage to the aquatic environment, the competent authority will require the operator (the potential polluter) to take the necessary preventive measures, or will take such measures itself and recover the costs incurred. The Federal Environmental Liability Act is aimed at restoring the environment to how it was before it was damaged.

3.3. Microplastics and environment

This chapter describes all information in regards to microplastics and their potentials effects in the environment when it is not treated properly.

3.3.1. Definition of microplastic

Plastics is a common term for a broad family of organic materials of typically high molecular weight suitable for the manufacture of industrial products. This term is often interchangeably used with the term polymers (Goricka, 2009). For Cole et al. (2011) Plastics are synthetic organic polymers, which are derived from the polymerisation of monomers extracted from oil or gas.

The term “microplastics” has been used since the threat of microscopic plastic debris was recognized, however unspecified description was causing confusion of the actual dimension of the problems the microplastics brought. Only recently this term was properly defined. The International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris defined microplastics as “plastic particles smaller than 5 mm” (Goricka, 2009). Thereon Sahil et al. (2011) mention microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm); microspheres are sometimes referred to as microparticles. Finally the National Oceanic and Atmospheric Administration (NOAA) of the UK defined microplastic as less than 5 mm in size (Wright et al., 2013).

Microplastics have been attributed with numerous size-ranges, varying from study to study, with diameters of <10 mm, <5 mm, 2–6 mm, <2 mm and <1 mm (Cole et al. 2011). For research and classification of microplastics in the environment there is not still any uniform method or standard; and common is the classification in the following size classes (Burkard, 2014):

Macroparticle:	>5 mm
Microparticle:	50 µm to 5 mm
Nanoparticle:	< 5 µm

Nowadays in the world the macroparticles or macroplastics for example plastic bags have been widely discussed, so far microplastics have not been properly discussed considering that this particles are potentially more hazardous to the environment.

3.3.2. Types of microplastic

Forensic techniques that compare the size, shape, and type of polymers found that the major types of macroplastic in the habitats are polyethylene, polystyrene, polypropylene (Browne et al., 2011). Below the most common types of microplastics are described according Burkard (2014):

- **Polyethylene (PE)**

  Polyethylene defined with the formula C_2H_4 (Fig. 1), is the most produced material for microplastics with approximately 29% of the total world production. In 2011 52 million tons is produced. Most of the production (67%) is used for packing and approximately 68000 tons is used as plastic bag.

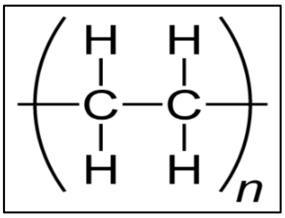

Fig. 1 Chemical structure of polyethylene

- **Polypropylene (PP)**

  Polypropylene is chemically defined as C_3H_6 (Fig. 2). The global production is around 45 million tons. Part of the total is used in activities which as vehicles industry, packing and so on. The main characteristics for their use are: the tolerance by the skin and odourless. These properties allow to use this material in the area of foods and pharmacy; in general it is harmless.

  This material is frequently used instead of polyethylene due to its higher rigidity, hardness and strength.

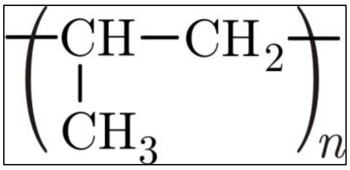

Fig. 2 Chemical structure of polypropylene

- **Polyamide (PA)**

  Polyamide is chemically a complex chain linked by amide bonds (Fig. 3). This material can be made through production of nylons or aramids. Polyamides are frequently used in the textile industry also as seam-material in surgery; the main reason is its resistance, as well as consistence and smooth surface.

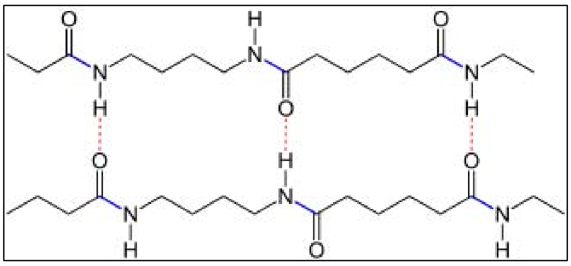

Fig. 3 Chemical structure of polyamide

- **Polystyrol (PS)**

  Polystyrol is chemically defined as C_8H_8 (Fig. 4). It is a kind of white foam material usually used as soft material for packing and insulation material. Approximately 20% of the production is used in electronic applications, 39% in packing materials. Also 15% is used in construction activities as insulation material. This material is physiological harmless and unrestricted used in packing of foods.

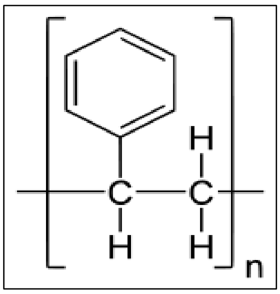

Fig. 4 Chemical structure of polystyrol

- **Polyvinylchloride (PVC)**

  Polyvinylchloride chemically is defined as C_2H_3Cl (Figure 5). It is a thermoplastic material with brittle and hard properties. PVC is widely used in different activities as construction, when is combined with additives; they improve its physical properties as the resistance and malleability.

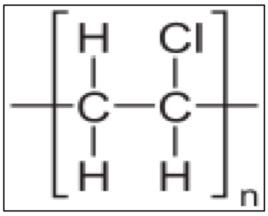

Fig. 5 Chemical structure of polyvinylchloride

3.3.3. Impacts of microplastics

The plastic particles that eventually end up in the surface water are not easily biodegradable under normal environmental conditions. The degradation rate of synthetic polymers is extremely low (depending on the type of plastic and environmental conditions probably ranging from a few decades to several centuries), resulting in an accumulation of microplastics in the aquatic environment for the time coming. The risk of microplastics is primarily caused by the combination of persistency of these materials and the potential accumulation in food chains (Roex et al., 2011).

Wright et al. (2013) mention that microplastics occupy the same size fraction as sediments and some planktonic organisms; they are potentially bioavailable to a wide range of organisms. Microplastics can be ingested by low trophic suspension, filter and deposit feeders, detritivores and planktivores.

Also Wright et al. (2013) describe below the factors which affect the bioavailability of microplastics.

- **Size**

  A key factor contributing to the bioavailability of microplastics is their small size, making them available to lower trophic organisms. Many of these organisms exert limited selectivity between particles and capture anything of appropriate size. Alternatively, higher trophic planktivores could passively ingest microplastics during normal feeding behaviour or mistake particles for natural prey. For example on the Mediterranean fin whale Balaenoptera physalus, one of the largest filter feeders in the world. B. physalus can engulf approximately 70 000 L of water at one time, potentially risking microplastic ingestion both directly and indirectly from the water and plankton, respectively.

- **Density**

  The density of the plastic particles will determine bioavailability in the water column; therefore the type of plastic ingested may vary between organisms. Planktivores, filter feeders and suspension feeders inhabiting the upper water column are likely to encounter positively buoyant, low-density plastics, such as PE (specific gravity 0.91-0.94), on the sea surface. The buoyancy of plastic is influenced by biofouling, for example, PE food bags (20 - 28 cm) displayed a well-developed biofilm within one week, which continued to increase throughout a three week exposure period. By the third week, the PE food bags had started to sink below the sea surface, indicating neutral buoyancy. De-fouling in the water column by foraging organisms is a potential pathway for microplastic particles to return to the sea-air interface. Alternatively, fouled microplastics could continue to sink, as would high density plastics such as PVC (specific gravity 1.38). Such particles will become available to benthic suspension and deposit feeders and detritivores as they sink, eventually reaching the benthos.

- **Abundance**

  An increase in the abundance of microplastics in the marine environment will also affect its bioavailability, as the chance an organism will encounter a microplastic particle is enhanced. Therefore the progressive fragmentation of macroplastic items is likely to increase the amount of particles available for ingestion to a wider range of organisms.

- **Colour**

  The colour of microplastics may potentially contribute to the likelihood of ingestion, due to prey item resemblance. Studies reported that plastic particles sampled from the North Pacific exhibited size variation related to colour; white plastic particles consistently decreased in abundance with decreasing size class. Some commercially important fish and their larvae are visual predators, preying on small zooplankton, and may feed on microplastics which most resemble their prey. Microplastic ingestion due to food resemblance may also be applicable to pelagic invertebrates, which are visual raptorial predators.

Apparently microplastics have become both widespread and ubiquitous, information on the biological impact of this pollutant on organisms in the marine environment is only just emerging (Cole et al., 2011). The main impacts are explained below:

- **Intoxication**

  Toxicity of microplastics is connected with intrinsic toxicity of microplastics (or more specifically of its additives and its monomers) and their large surface area. Released, desorbed monomers of microplastics can be highly carcinogenic, reproducing abnormities in humans, invertebrates and in rodents.

  Plastics usually are enriched by different additives already during the manufacturing process, including organotin (a catalyst), nonylphenol (an antioxidant), polybrominated diphenyl ethers (flame retardants), triclosan (an antimicrobial). Furthermore, plastics are able to act like sponges by absorbing and concentrating hydrophobic contaminants, including the majority of persistent organic pollutants (POPs), like polychlorinated biphenyls (PCBs), at concentrations with magnitudes several orders higher than of surrounding marine environment the contaminated plastic particles may serve as toxins source to marine organisms ingesting them. Furthermore, toxic effects are suggested to compound damage resulted from the impacts related to the plastic ingestion by marine organisms (Gorycka, 2009).

- **Accumulation**

  Another potential danger to marine environments exists from the accumulation of plastic debris on the sea floor. According to UNEP report (2005) as much as 70 percent of the total marine litter input sinks to the sea bed in both shallow and deep parts of the oceans. Majority of consumer plastics are neutrally buoyant, hence grains of sand trapped in their fouling matter or seams make many plastics (including microplastics) sink to the sea floor.

  The vertical organic and inorganic particulate sinking fluxes may be compromised by microplastics settling in the water column. This process can slow down the gas exchange and sequestration between the overlying waters and the pore waters of the sediments, and by doing so – disrupting or smothering inhabitants of the benthos (causing hypoxia or anoxia) and generally altering the normal functioning of the marine ecosystem and life on the sea floor. It is also believed, that benthic debris is interfering with carbon cycling in waters (Gorycka, 2009).

  Also when plastic continues to fragment, the potential for it to accumulate within the circulatory fluid and phagocytic cells of an organism is likely to increase, as the smaller the microplastics, the greater the abundance available for translocation (Wright et al., 2013).

- **Physical damages**

  Another impact of plastic litter on marine organisms concerns the physical hazard. The harmful effects concern the ingestion of plastics followed by the possible mechanical blockage of intestinal tract, gastric enzyme secretion, diminished feeding stimulus, lowered steroid hormone levels, delayed ovulation and reproductive failure. Those worsening physical conditions can eventually cause a serious internal injury or even death. Several studies however, also indicate that some organisms may eventually regurgitate plastic particles together with other indigestible matter, hence reducing the harmful effects. Concerning amounts of ingested plastic items and wide range of organisms found to ingest plastic particles, especially many seabirds and fish species this type of hazard seems rather significant (Gorycka, 2013).

Blockage of the digestive systems, transfer of items to the circulating system and their retaining in the blood system however, are also speculated to occur due to the presence of microplastics within organisms of marine species. However, to investigate this suspicion, more research needs to be done and such impact would probably depend on, among the others, the type and densities of microplastics (Wright et al., 2013; Gorycka, 2009).

- **Effects in food chain**

  Some additives and POPs are hazardous and if sorbed to microplastics the toxicological effects on the organisms which have ingested those particles would largely depend on the concentrations of the contaminants; however, it is still not known to what extend the microplastics can transport those pollutants and whether such transport would increase the threat to the environment (Gorycka, 2009)

  According to Richard Thompson (2007) (cited by Gorycka, 2009), exposure to wide variety of organisms is facilitated by microplastics distribution combined with their small size. Also it is documented transmission of microplastics up the food chain. Fish and mussels are widely sought after as a food source for humans, hence the suggestion can be drawn, that microplastic pollution may also be up-taken to the human organism. Cole et al. (2011) mention the presence of nanoplastics in the marine environment is likely to be of increasing significance in the years to come, and researchers, have already begun to speculate on the impact that such a pollutant might have on the base of the marine food web.

  Roex et al. (2011) mention that a desk study performed in the Netherlands by the Institute for Environmental Studies (IVM) shows that both humans and animals are capable of taking up microplastics in their body tissues and/or fluids, causing adverse health effects (particle-toxicity). In marine organisms like lugworms, barnacles, mussels, lobsters, petrels and seals microplastics have already been reported (Roex et al., 2011). In Germany after a recent research, Almut Kottwitz, Permanent Secretary in the Lower Saxony Ministry of the Environment, Energy and Climate Protection, mentioned his worries and suggest the need of a nationwide study on the input of microplastics into the food chain (Gerdts and Mehrtens, 2014).

3.3.4. Source of microplastic in nature

To manage the environmental problems of microplastic it is important to understand and target the major pathways of microplastic into habitats with mitigation-measures. While sewage waste provides one potential route for entry of microplastics, others have been identified including fragmentation of larger items, introduction of small particles that are used as abrasives in cleaning products, and spillage of plastic powders and pellets (Browne et al., 2011).

Microplastics can be divided in primary and secondary microplastics (Roex et al., 2011). Plastics that are manufactured to be of a microscopic size are defined as primary microplastics. They are typically used in facial-cleansers and cosmetics, or as air-blasting media, whilst their use in medicine as vectors for drugs is increasingly reported. Under the broader size definitions of a microplastic, virgin plastic production pellets (typically 2–5 mm in diameter) can also be considered as primary microplastics, although their inclusion within this category has been criticised (Cole et al., 2011).

Secondary microplastics describe tiny plastic fragments derived from the breakdown of larger plastic debris, both at sea and on land. Over time a culmination of physical, biological and chemical processes can reduce the structural integrity of plastic debris, resulting in fragmentation.

Over prolonged periods, exposure to sunlight can result in photo-degradation of plastics; ultraviolet (UV) radiation in sunlight causes oxidation of the polymer matrix, leading to bond cleavage. Such degradation may result in additives, designed to enhance durability and corrosion resistance leaching out of the plastics. The cold, haline conditions of the marine environment are likely to prohibit this photo-oxidation; plastic debris on beaches, however, have high oxygen availability and direct exposure to sunlight so will degrade rapidly, in time turning brittle, forming cracks and ‘‘yellowing’’. With a loss of structural integrity, these plastics are increasingly susceptible to fragmentation resulting from abrasion, wave-action and turbulence. This process is ongoing, with fragments becoming smaller over time until they become microplastic in size. It is considered that microplastics might further degrade to be nanoplastic in size, although the smallest microparticle reportedly detected in the oceans at present is 1.6 μm in diameter (Cole et al., 2011).

3.4. Microplastic in waste water

In waste water treatment plants at the moment little is known about the removal efficiency of microplastics in an STP. Because of the low density a substantial part of the plastic particles will remain floating on the water surface microplastics. Sedimentation tanks are not designed to remove light particles as polyethylene and nylon, whenever these particles are not captured in the flakes produced by the activated sludge or will end up in de drift layer. Also in the primary settlement these particles will not be removed, and for the time being they are also not intentional removed by the addition of flocculants. A pilot study performed by the Vrije Universiteit in cooperation with the TU Delft en Deltares showed that approximately 90% of the plastic particles present in the influent were removed in the STP, finally resulting in 20 particles per litre effluent (Roex et al., 2011).

3.4.1. Source of microplastics in sewage systems

Microplastic ‘‘scrubbers’’, used in exfoliating hand cleansers and facial scrubs, have replaced traditionally used natural ingredients, including ground almonds, oatmeal and pumice. Since the patenting of microplastic scrubbers within cosmetics in the 1980s, the use of exfoliating cleansers containing plastics has risen dramatically. Typically marketed as ‘‘micro-beads’’ or ‘‘micro-exfoliates’’, these plastics can vary in shape, size and composition depending upon the product. For example, the presence of polyethylene and polypropylene granules (<5 mm) and polystyrene spheres (<2 mm) in one cosmetic product was reported (Cole et al., 2011).

Microplastics used both in cosmetics and as air-blastingmedia can enter waterways via domestic or industrial drainage systems (Fendall and Shewell, 2009; Cole et al., 2011); whilst waste-water treatment plants will trap macroplastics and some small plastic debris within oxidation ponds or sewage sludge, a large proportion of microplastics will pass through such filtration systems, because of their small size are likely to escape capture by the preliminary treatment screens on waste water plants (typically coarse, >6 mm, and fine screens, 1.5–6 mm).

3.4.2. Characteristics of microplastics in sewage systems

Sewage waste provides one potential route for entry of microplastics, one of he more important are the microplastics in scrubbers in cleaning products, we would expect most of the material to consist of fragments and spheres of polyethylene. However many researches do not account for the occurrence of microplastic fibers in sludge and effluent taken from sewage treatment plants and soil from terrestrial habitats where sewage sludge had been applied, the source of which is more likely explained by fibers shed from clothes/textiles during washing (Browne et al., 2011).

In Germany, the typical or most common microstructures found in the sewage water were classified as follow (Burkard, 2014):

- Textile: fibre from washed clothes.
- Detergents: pearls in cleaning material, shower gel, care products, etc.
- Cosmetics: peelings, creams, powder
- Weathered particulates
- Others

Also Burkard (2014) mentions that until 2000 synthetic fibre, mainly from polyester or polyacryl, is produced pro wash cycle and eliminated by the sewage treatment system. However other study has shown that one single piece of garment may produce up to 1900 plastic fibres per wash (Browne et. al., 2011).

3.4.3. Cleaning strategies in waste water treatment plants

The pathway of the sewage water plays an important role in the spread of the microplastics (Burkard, 2014). But at the moment little is known about the removal efficiency of microplastics in a STP. Because of the low density a substantial part of the plastic particles will remain floating on the water surface (Roex, 2011).

Browne et al. (2011) mention that although larger debris is removed in sewage treatment plants, filters are not specifically designed to retain microplastic and terrestrial soils that have received sewage sludge do contain microplastic fibers.

In studies carried out in Germany determined that treatment plants cannot completely keep microplastics out of waste water by conventional means. This is one of the results of a pilot study commissioned by the regional water association of Oldenburg and Ostfriesland, Germany (OOWV – Oldenburg-Ostfriesischer Wasserverband) and the Lower Saxony Water Management, Coastal Defence and Nature Conservation Agency (NLWKN - Niedersächsischer Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz) (Gerdts and Mehrtens, 2014). They also reported that a tertiary filtration to collect the microplastics is important. However the input of microparticles has to be avoided much earlier, i.e. during the production process. Burkard (2014) mentions that in the waste water treatment plant in Nürnberg as last treatment step, a sand filter is used to retain all microparticles.

3.5. Separation techniques and identification of microplastics

The process of separation for microplastics is still in development, basically two main methods exist; density technique and froth flotation. For identification of microplastics tools as optic microscopy and Raman microspectroscopy are used.

3.5.1. Density technique

The specific density of plastic particles can vary considerably depending on the type of polymer and the manufacturing process. Density values for plastics range from 0.8 to 1.4 $g \cdot cm^{-3}$, specifically for polypropylene from 0.85 to 0.94 $g \cdot cm^{-3}$, polyethylene from 0.92 to 0.97 $g \cdot cm^{-3}$, and for polystyrene from <0.05 to 1.00 $g \cdot cm^{-3}$. Typical densities for sand or other sediments are 2.65 $g \cdot cm^{-3}$. This difference is exploited to separate the lighter plastic particles from the heavier sediment grains by mixing a sediment sample with a saturated solution (sodium chloride NaCl) and shaking it for a certain amount of time. After mixing, the sediment is expected to rapidly settle to the bottom, while the low density particles remain in suspension or float to the surface of the solution. Subsequently, the supernatant with the plastic particles is extracted for further processing (Hidalgo-Ruz et al., 2012).

The most applied product for this separation technique is a concentrated saline NaCl solution (1.2 $g \cdot cm^{-3}$). Other solution used is a sodium polytungstate solution with a density of 1.4 $g \cdot cm^{-3}$, tapwater, and seawater (Hidalgo-Ruz et al. 2012). Another method uses the principle of elutriation. Elutriation is a process that separates lighter particles from heavier ones using an upward stream of gas or liquid. This principle has, for example, been used extensively in marine biology for separating meiofauna from sand with an apparatus called “Barnett’s fluidized sand-bath” (Claessens et al., 2013).

However plastics such as polyvinylchloride (density 1.14-1.56 $g \cdot cm^{-3}$) or polyethylene terephthalate (density 1.32-1.41 $g \cdot cm^{-3}$) will not float in this concentrated NaCl solution. These two polymers, however, represent 18% of the european plastic demand and as such could represent an important proportion of the microplastics present in the marine environment (Claessens et al., 2013).

3.5.2. Sieving

Microplastics can be separated from samples using sieves of variable mesh sizes. Materials retained in the sieve are collected (and sorted), while those that pass through are usually discarded. The use of sieves with different mesh sizes allows distinguishing size categories of microplastics (Hidalgo-Ruz et al., 2012).

3.5.3. Froth flotation

Froth flotation relies heavily on the physical properties, such as bulk density, particle size, shape, surface energy, and surface roughness (Shent et al., 1999). Herein the most important feature is the wettability of the plastic. To facilitate the segregation between various plastic types, it is possible to modulate the wettability by altering the surface tension of the medium and/or chemical conditioning. Novel plastic particle separation methods of the particles are by hydrophobic modifications or the adsorption of chemicals (Imhof et al., 2012).

3.5.4. Optic microscopy

The optical microscope has been a standard tool in life science as well as material science for more than one and a half centuries now. Basically the optical microscope magnifies an object in two steps. In both steps optical systems acting like converging lenses are used (Rühl, 2012):

The first step is to place the object (specimen) between the single and double focal point. The result is a magnified, real image. This microscope lens (in reality an optical system consisting of several lenses) is called the objective.

Then a second lens is used to pick up this image exactly in its front focal point. As a result we generate a beam of parallel rays, but not a real image. This optical element is called eyepiece. The human eye is able to handle this parallel beam and generates an image onto its retina.

Finally, this is what one can expect from a microscope: objects can be observed on a magnified scale with details undetectable by the naked eye (Fig. 6).

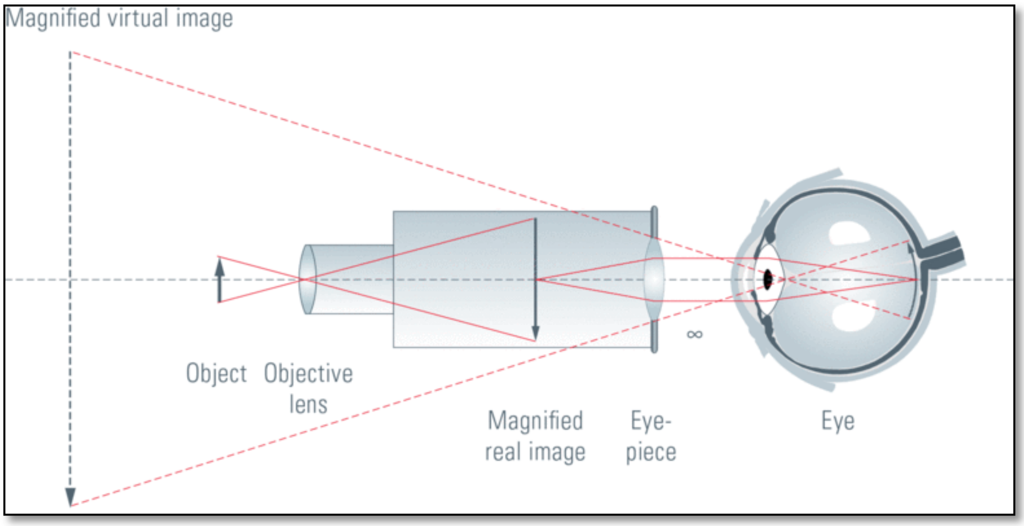

Fig. 6 Schematic compound of microscope (Source: Rühl, 2012)

In a modern optical microscope, when light from the microscope lamp passes through the condenser and then through the specimen (assuming the specimen is a light absorbing specimen), some of the light passes both around and through the specimen undisturbed in its path. Such light is called direct light or undeviated light. The background light (often called the surround) passing around the specimen is also undeviated light. Some of the light passing through the specimen is deviated when it encounters parts of the specimen. Such deviated light (is called diffracted light) is rendered one-half wavelength or 180 degrees out of step (more commonly, out of phase) with the direct light that has passed through undeviated. The one-half wavelength out of phase, caused by the specimen itself, enables this light to cause destructive interference with the direct light when both arrive at the intermediate image plane located at the fixed diaphragm of the eyepiece. The eye lens of the eyepiece further magnifies this image which finally is projected onto the retina, the film plane of a camera, or the surface of a light-sensitive computer chip (Davidson and Abramowitz, 2009).

3.5.5. Raman microspectroscopy (RM)

Raman spectroscopy is a type of molecular spectroscopy that involves the dispersion of electromagnetic radiation by molecules or atoms. It measures the rotational, vibrational, and other low-frequency modes of molecules. This technique is nondestructive and needs little or sample preparation. In fact, Raman analysis can be conducted directly via glasses, jars, plastic bags, cuvettes, and other transparent containers. Moreover, Raman can be used for qualitative as well as quantitative analysis. Also this technique is highly selective, which means that it can easily differentiate molecules in chemicals that are very similar (B&W, 2013).

In recent years, this technique is being extensively used in the characterization of polymers and plastics for both qualitative and quantitative analysis. Earlier, these materials were examined using burn tests, chromatographic separation, and wet chemical techniques which tend to damage the sample (B&W, 2013). Nowadays Raman imaging has recently gained significant attention in analysis of polymer blends. There are a number of papers discussing structure, morphology, composition and/or crystallinity of polymer blends by means of Raman mapping or global imaging (Sasic et al., 2003).

The components of the equipment (Figure 7), would include holographic gratings, for improved excitation light rejection, a set of monochromators and a liquid nitrogen- or Peltier effect-cooled CCD (charge-coupled device) mosaic for detection. The laser source is often built-in but light coming from an external excitation source can also be used. In the "macro"-configuration, the beam section is ~1 mm^2 but the laser spot can be reduced to ~1 µm diameter by using the high-magnification microscope objectives with which most commercial Raman spectrometers are equipped. The technique involved is known as micro- Raman Spectroscopy (µRS). The main additional options are motorized stages for XY(Z) mappings and optical fibre plugs for connection to remote optical heads equipped with microscope objectives (Gouadec and Colomban, 2007).

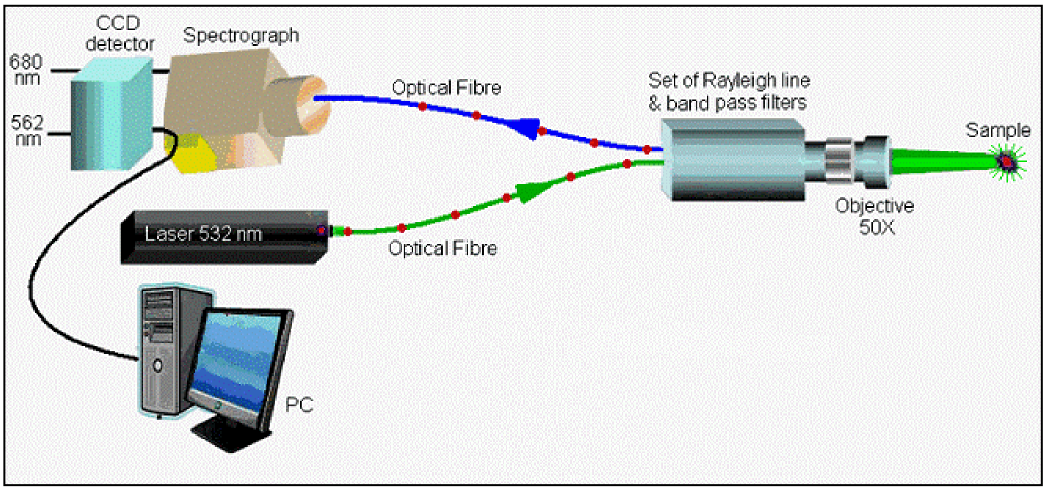

Fig. 7 Components of Raman spectroscope (Source: Stromatias, 2012)

The Raman scattering light is displayed in a form called the Raman spectrum that is used for analysis. The horizontal axis of the Raman spectrum indicates the difference between the input laser light and scattering light energy, while the vertical axis indicates the intensity of the Raman scattering light (Fig. 8) (Left coast instruments, 2011).

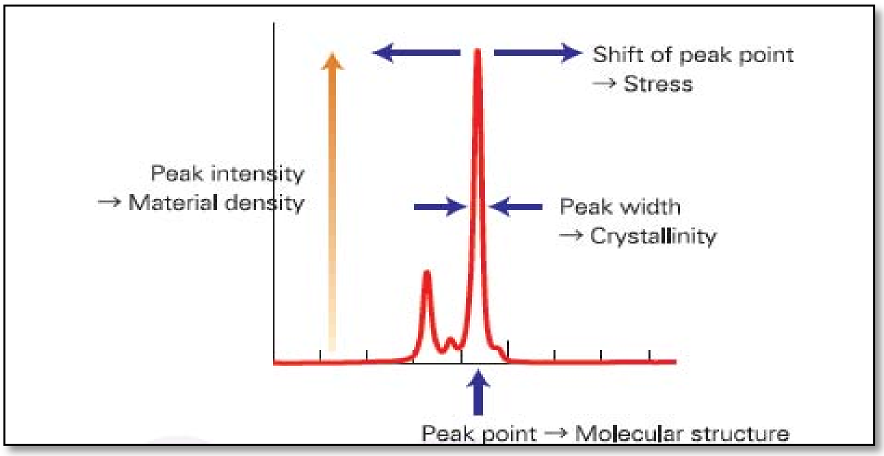

Fig 8 Raman spectrum (Source: Left coast instruments, 2011)

Different types of information can be obtained from a Raman spectrum. Every molecule has its own unique Raman spectrum, which can be correlated to a molecular fingerprint. This helps in developing databases of known standards that can be subsequently utilized for verification of unknown standards. Through this technique, slight differences in the matrices can be utilized for instant analysis which otherwise would not be possible using conventional univariate techniques. Figure 9 illustrates the basic principles of a Raman analyser, along with Raman spectra of five similar molecules. These Raman spectra can be clearly distinguished (B&W, 2013).

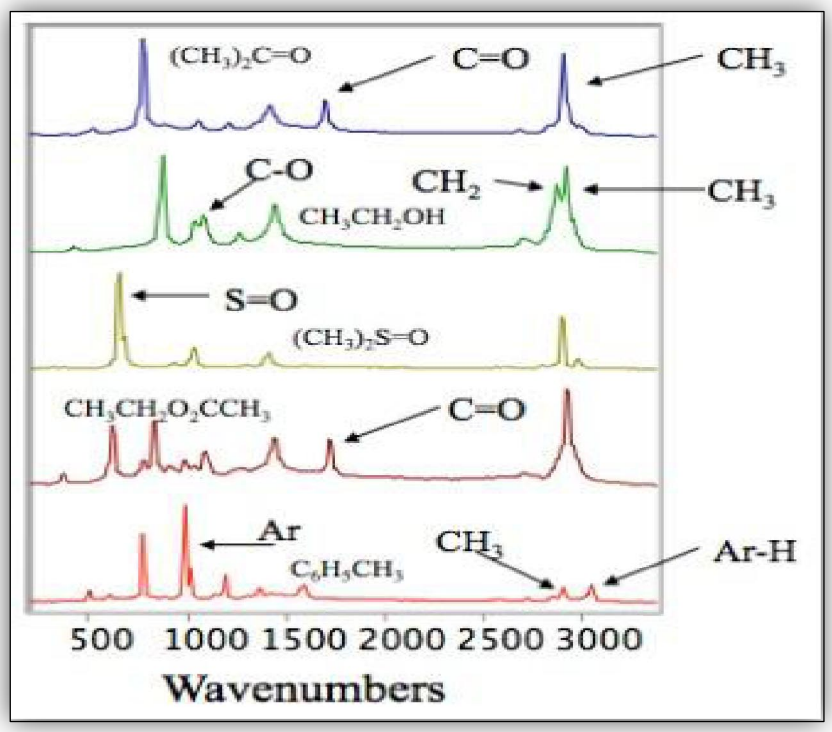

Fig 9 Spectra of five similar molecules: acetone, ethanol, dimethyl sulfoxide, ethyl acetate, and toluene (Source: B&W, 2013)

4. MATERIAL AND METHODS

The locations, materials and methods used in the experimental part of the thesis are described below; and for this research, the pictures and graphics were produced by the author.

4.1. Location

The experiment was carried out in the following facilities of the University of Natural Resources and Life Sciences Vienna:

- Technikum of the Institute of Sanitary Engineering and Water Pollution Control (SIG)
  Responsible: Mr. Friedrich Kropitz
- Analytical and microbiological laboratory of the Institute of Sanitary Engineering and Water Pollution Control (SIG)
  Responsible: Dipl.-Ing. Dr.techn. Roza Allabashi
- Analytical laboratory of the Department of Biotechnology (DBT)
  Responsible: Ao.Univ.Prof. Dipl.-Ing. MBA Peter Holubar
- Laboratory of the Institute of Hydraulics and Rural Water Management (IHLW)
  Responsible: Dipl.-Ing.(FH) Martina Faulhammer
- Laboratory of Institute Institute of Physics and Materials Science (IPM)
  Responsible: Ass.Prof. Dr.rer.nat Notburga Gierlinger

The experiments were executed from May 9th to 31st of October 2014.

4.2. Materials and equipment

For the research, each objective was developed using specific materials and equipment.

4.2.1. Simulation of waste water treatment

The following materials and equipment were used for the simulation part:

- Sludge and domestic waste water from the technikum
- Fluorescent microplastics (fluorescent MP) with Ø 425-500 µm of the company COSPHERIC LLC
- Commercial microplastics (commercial MP) of beauty care: Clean and clear (150 ml), Body scrub pineapple Grace Cole (238 ml), Palmolive thermal spa (250 ml) and My Face sanftes peeling (150 ml).
- Multifunction equipment with pH meter, conductimeter and redox electrode
- TOC equipment Shimadzu

4.2.2. Mechanical separation

In this method, the materials used were sieves with different sized mesh openings, in our case the 3 dimensions: 650, 250 and 125 µm.

4.2.3. Separation by density technique

For this technique, the following materials and equipment were used:

- Separatory funnels of 500 ml, 1000 ml
- Centrifuge Oppenheimer RPM max 5000

- Sludge and domestic waste water from the technikum
- Sodium chloride (NaCl)
- Fluorescent microplastics (fluorescent MP) with Ø 425-500 µm of the company COSPHERIC LLC
- Adapted aerated separatory column 6,3 L
- Adapted separatory injector or dispenser 1 L, built with a glass microseparator
- Vacuum pump
- Optic Microscope
- Raman spectrometer
- UV light chamber
- Vacuum oven chamber dryer

4.2.4. Separation by chemical technique

For this technique, the following materials and equipment were used:

- Separatory funnels of 500 ml, 1000 ml
- Centrifuge Oppenheimer RPM max 5000
- Vacuum pump
- Fluorescent microplastics (fluorescent MP) with Ø 425-500 µm of the company COSPHERIC LLC
- Vacuum pump
- Optic microscope
- Raman spectrometer
- Chemical products: toluene, hexane, benzene, cyclohexane. All of them per analysis of quality.
- Biosorb NAINTSCH
- UV light chamber
- Vacuum oven chamber dryer

4.2.5. Sampling of waste water and sludge in a waste water treatment plant

Samples of waste water to be treated by the best separation techniques were taken from the different units of a waste water treatment plant (WWTP) with a capacity of 55000 p.e., located approximately 10 km from Vienna. The materials used were:

- Glass bottles of 5L
- Funnel
- Beaker for waste water collection

The collected samples were stored in the cooling room of the laboratory of the Institute of Sanitary Engineering and Water Pollution Control.

4.2.6. Digestion of microplastic in mice

The materials used for this test were: fluorescent microplastics COSPHERIC (fluorescent MP) with Ø range of 425-500 µm and Ø 50-75 µm, and 2 white laboratory mice of the species *Mus musculus*.

This experimental part was executed in the laboratories of the Department of Pharmacology and Toxicology at the University of Vienna.

4.3. Methodology for the simulation of waste water treatment

The simulation process was developed in 2 steps: pre-test and simulation test, both of them are described as follows:

4.3.1. Waste water treatment pre-test

At the beginning of this step the microplastics were separated from the cleaning products (Fig. 10). The products were dispersed in water and their microplastics separated using a sieve with a mesh of 63 µm. The collection was carried out in the laboratory of the Institute of IHLW. The retained microplastics were dried in the open air and collected in a glass flask. The collected sample was used for all the experimental treatments.

Fig. 10 Commercial cleaners with microplastics

The microplastics used in the experiments had the following characteristics (Fig. 11and Table 1):

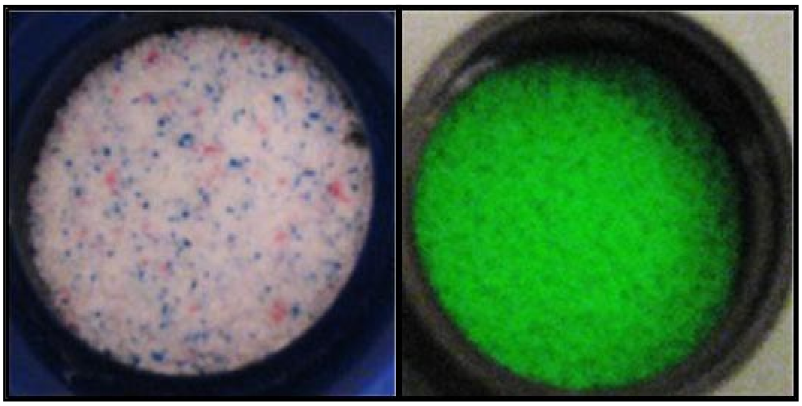

Fig. 11 Commercial MP and fluorescent MP

Table 1 Characteristic of microplastics

Property	Fluorescent MP	Commercial MP
Colour	Green	White, blue and pink
Shape	Round	Irregular
Size range (µm)	Ø 425-500	45-1090

For the pre-test, three 250 ml sized beakers were prepared as is described in Table 2.

Table 2 Treatments of the pre-test

NAME OF TEST	TREATMENT
Control I	200 ml sludge Technikum
Commercial MP I	200 ml sludge Technikum + 109 mg of commercial microplastics
Fluorescent MP I	200 ml sludge Technikum + 113.8 mg of fluorescent microspheres

All of the treatments, as a batch process, were aerated with an interval of 3 hours during the day and for at least 3 weeks. Every two days the beakers were supplied with 50 ml of diluted waste water. In this pre-test the behaviour of both microplastics during the aeration and non aeration was observed; and at the end of the test possible changes in size, colour and damages on the surface of the microplastics were evaluated (Fig. 12).

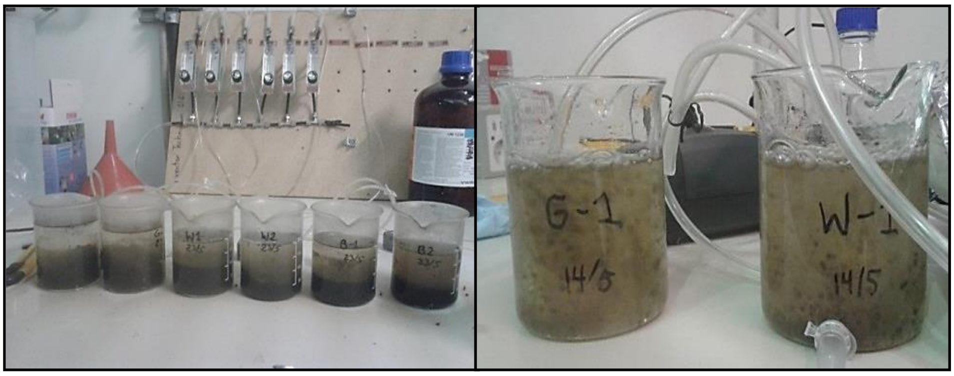

Fig. 12 WWTP simulation test with commercial MP (W-1) and fluorescent MP (G-1)

4.3.2. Waste water treatment test

After the evaluation of the pre-test, a new round of experiments was prepared as is described in Table 3.

Table 3 Treatments of the simulation test

NAME OF TEST	REP	TREATMENT
Control II	2	150 ml sludge WWTP + 50 ml filtrate waste water
Commercial MP II	2	150 ml sludge WWTP + 50 ml filtrate waste water + 100 mg of commercial microplastic
Fluorescent MP II	2	150 ml sludge WWTP + 50 ml filtrate waste water + 100 mg of fluorescent microspheres

During the treatment process, the behaviour of the microplastics was observed and at the same time the following parameters were measured:

a. **pH, electric conductivity and redox potential .-** These parameters were measured using calibrated multifunction equipment (Fig. 13) from the laboratory of SIG. Each electrode was introduced directly into the beakers with the different treatments and recorded.

Fig. 13 Multifunction equipment

b. **Total organic carbon and total nitrogen.-** These parameters were obtained using the TOC equipment Shimadzu (Fig. 14) which was calibrated previous to the measurement by the equipment, approximately 20 ml of each sample was filled in a glass flask and covered with a special cap.

The equipment to determine TOC and TN adopts the 680°C combustion catalytic oxidation method. This method achieves total combustion of samples by heating them to 680°C in an oxygen-rich environment inside TC combustion tubes filled with a platinum catalyst. Since this utilizes the simple principle of oxidation through heating and combustion, pre-treatment and post-treatment using oxidizing agents are unnecessary, which enhances operability. The carbon dioxide generated by oxidation is detected using an infrared gas analyzer (NDIR).

The process starts when a small amount of hydrochloric acid is added to acidify the sample and the sample is sparged with sparge gas. After that the sample is injected into the combustion tube, where the TN decomposes and the TOC (NPOC) of the sample simultaneously is oxidized to form CO2. This carbon dioxide is detected by an NDIR connected in series with the chemiluminescence detector. The series connection between the NDIR (non-dispersive infrared detector) and the chemiluminescence detector allows the nitrogen monoxide and carbon dioxide generated from a single sample injection to be simultaneously detected (Shimadzu, 2014).

Fig. 14 TOC equipment Shimadzu

NPOC is a measurement which corresponds to the organic carbon that cannot be purged by using gas sparging under specific conditions. The purgable organic carbon (POC) value is usually less than 10% of the NPOC concentration and is commonly neglected, assigning the NPOC value as the TOC value (Leo and Nollet, 2007)

At the end of the treatment or experiment, microplastic samples from each treatment were taken using sieves to be evaluated for colour, size and surface damages. To evaluate these aspects, it was necessary to use an optic microscope (Fig 15); by means of the equipment the particles were compared visually between the original microplastics and the collected microplastics from the treatment.

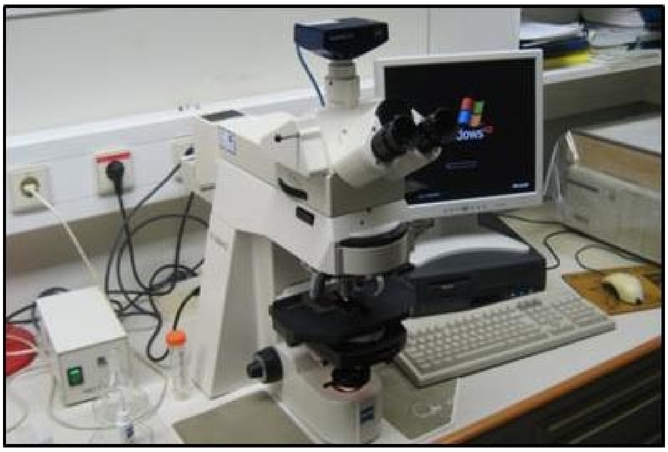

Fig. 15 Optic Microscope

4.3.3. Statistical analysis for waste water treatment simulation

Statistically the mean size of the fluorescent MP with the original fluorescent MP using the analysis of variance (ANOVA) and Tukey test, with an error rate at 95% (α=5%) was evaluated. To perform this analysis the statistical program R was used.

ANOVA is a statistical method used to test differences between two or more means. However, since the ANOVA does not reveal which means are different from which, it offers less specific information; the Tukey test is therefore preferable to ANOVA (Lane, 2013).

The null and alternative hypotheses for the ANOVA were designed as follows:

H_0: $\mu_1 = \mu_2 = \ldots = \mu_k$ the mean size of fluorescent microplastic is statistically equal across the different treatments

H_1: At least one mean size is not statistically equal

To reject the null hypothesis is only possible if: F (observed value) > FCV (critical value) or when our p-value (Pr(>F)) is less than 0.05.

In the comparison of treatments by the Tukey test, the difference between two treatments is accepted when zero is not included in the range value of comparison tests.

4.4. Methodology for mechanical separation

In this technique as a first step, a solution was prepared composed of 500 ml of domestic waste water and 100 units of fluorescent MP with Ø 425-500 µm and density of 1.005 $g \cdot cm^{-3}$.

The process of separation was executed using sieves with different mesh sizes: 650, 250 and 125 µm, and with help of water from the public supply, they were cleaned as much as possible to eliminate the impurities. After the process of separation, the efficiency of collection expressed in percentage (%) was calculated. The process was repeated 2 times.

4.5. Methodology for density technique

In the density technique, the main product used was a saturated solution of sodium chloride (NaCl) up to 26% of salt in water (Fig. 16), with a density of 1.164 $g \cdot cm^{-3}$ (Lide. 2005). However, Imhof et al. (2012) mention that saturated saltwater reaches a density of 1.2 $g \cdot cm^{-3}$. By use of this solution, the sewage water increases its density in values higher than 1 g/cm^3.

The change of the density allows the light microplastics to float, for example from the cleanser the main component is low density polyethylene 0.92 $g \cdot cm^{-3}$or other polymers as polystyrene 1.04 $g \cdot cm^{-3}$ (Claessens et al., 2013), therefore the collection of these micro materials is feasible.

Similar to the mechanical separation, a solution was prepared consisting of 500 ml domestic waste water and 100 units of fluorescent MP with Ø 425-500 µm and density of 1.005 $g \cdot cm^{-3}$. For this technique four strategies for the separation of microplastics were developed:

Fig. 16 Saturated solution of sodium chloride (NaCl)

4.5.1. Separatory funnels (SF)

For all strategies, the waste water sample was pre-treated in a sieve with a mesh of 650 µm with the goal of eliminating large sized impurities. After this process, 500 ml of the sample with fluorescent MP was filled in a separatory funnel (1L) and 500 ml of the saturated salty solution was added in two steps of 250 ml for each step (Fig. 17).

Fig. 17 Density technique by separatory funnels

After each addition of the salty solution the funnel was mixed, and after the homogenization, the sediment at the bottom of the separatory funnel was gravitationally extracted, and the floating microplastics were finally collected in a glass bottle.

To count the number of microplastic collected, a UV light chamber was used. Finally the sample was filtrated using a 0.45 µm filter by a vacuum pump and dried in a vacuum chamber oven dryer to get the clean and pure microplastic particles. The efficiency of collection expressed in percentage (%) was calculated. This process was repeated three times

4.5.2. Combination of separatory funnels and centrifugation (SF+C)

After the pre-treatment process, 500 ml of the sample with microplastics was filled in a separatory funnel (1L) and 500 ml of the saturated salty solution was added in two steps of 250 ml each. The process of collection was similar to the technique with the separatory funnels. However one step was added; the sediments with the retained microplastics were centrifuged to get additional microplastic units (Fig. 18).

Fig. 18 Density technique by separatory funnels and centrifugation

In the sedimentation process, particles of different densities or sizes in a suspension will drop out of suspension at different rates, with the larger and denser particles dropping out faster. These sedimentation rates can be increased by using centrifugal force (Frei, 2011). Considering this information, this process was executed as follows:

- The sediment was filled in centrifuge tubes (50 ml)
- The fully filled tubes were set in the centrifuge
- The centrifuge ran in two velocities: first 2 minute with 1000 rpm and the second 2 minutes with 4000 rpm.
- Once the solid and liquid parts were separated the liquid part with floating microplastics was collected in a glass bottle.

Finally the collected particles were counted and dried. The collection efficiency expressed in percentage (%) was calculated. This process was repeated three times.

4.5.3. Adapted aerated separatory column (ASC)

For this test, the sample prepared with microplastics (500 ml waste water plus 100 fluorescent MP) was used and filled in an adapted aerated separatory column (6.3 L) with 1500 ml of saturated salty solution added in 3 steps, each step with 500 ml of this solution.

The column was prepared as shown in Fig. 19; the main components were an air supply system at the bottom part of the column and 3 outlets in the column to collect the water with microplastics.

Fig. 19 Density technique by adapted aerated separatory column

The process of collection was as follows:

- Addition of 500 ml of saturated salty solution in the column filled with the sample
- Aeration for 2 minutes
- After homogenization, collection of the liquid part with floating microplastics
- Second addition of 500 ml of saturated salty solution
- Again aeration for 2 minutes
- Second collection of water and microplastics
- Third addition of 500 ml of saturated salty solution
- Again aeration for 2 minutes
- Third collection of water with floating microplastics

The goal of the aeration was to improve the mix of waste water and salty water and avoid the retention of the microplastics in the process of sedimentation by the large sized sediments and keep the majority of particles floating.

Finally the collected samples were counted and dried as in the separatory funnels method. The collection efficiency expressed in percentage (%) was calculated. This process was repeated three times.

4.5.4. Adapted separatory dispenser (ASD)

For this trial, the sample was prepared with microplastics (500 ml waste water plus 100 fluorescent MP), with 250 ml of it filled in an adapted separatory dispenser (500 ml) and 400 ml of saturated salty solution was added in 2 steps.

The adapted separatory dispenser was prepared as shown in Fig. 20; it was featured by a glass connector with an inlet and outlet, an Erlenmeyer flask (500ml) and with a stir magnet. The process of collection was as follows:

- Filling of the Erlenmeyer flask (500 ml) with 250 of sample
- Switch on of the stir magnet for a soft homogenization
- Continuous addition of 400 ml of saturated salty solution
- Collection of water with floating microplastics
-

Fig. 20 Density technique by adapted separatory dispenser

With this strategy, the goal was to improve the homogenization of the saturated salty solution and get a continuous collection of water with floating microplastics with the help of the stirrer to avoid the retention of microplastics by the sediments.

Finally the collected samples were counted and dried in the vacuum chamber oven dryer. The efficiency of collection expressed in percentage (%) was calculated. This process was repeated three times.

4.6. Methodology for chemical technique (CHEM)

In this technique as a first step a solution composed by 500 ml of domestic waste water and 100 units of fluorescent MP with Ø 425-500 µm and density of 1.005 g·cm^{-3} was prepared.

For the chemical technique, a pre-treatment of the sample was done with a sieve with mesh size of 650 µm The goal was to eliminate large size impurities; and at the same time, dissolved Biosorb, which is a coagulant of organic polymers with anionic or cationic functional groups, was prepared with 50 g of Biosorb NAINTSCH and 500 ml of reverse osmosis water. The goal of this product was to precipitate suspended particles held in suspension by electrostatic surface charges; and with appropriate mixing to promote contact between the particles, allowing particle growth through the sedimentation phenomenon called flocculant settling (FRTR, 2014).

After this process, the sample with microplastics was filled in a separatory funnel (1L); after that 400 ml of dissolved Biosorb and 75 ml of hexane was added (Fig. 21). It is important to mention that previously in a preliminary evaluation for recovery of fluorescent MP using organic solvents: toluene, benzene, cyclohexane and hexane; hexane had the best performance and it was used in this research.

Fig. 21 Chemical technique with Biosorb and hexane

Polyethylene or polyfluoroethylene are nonpolar polymers, but as valence electrons move around the nuclei, they can become temporarily imbalanced. For a brief moment of time one part of a molecule will be negative, another part positive; and it is temporarily polar (Mihalick, 2014). The use of a solvent such as hexane, cyclohexane, benzene, or toluene; which are non-polar (Ashenhurst, 2012); had the goal to attract the units of microplastics from the liquid part to the solvent part by means of London disperse forces (attraction between two non-polar molecules).

Once all the mixtures in the separatory funnel were executed, the number of microplastics collected were counted using the UV light chamber. This process was replicated three times.

Finally the collected microplastics were filtrated using a 0.45 µm filter by a vacuum pump, dried in a vacuum chamber oven dryer and washed in a sieve with a mesh of 120 µm to get the clean and pure microplastics. Additionally, many particles obtained after the separation process were evaluated by the Raman spectroscope using an excitation wavelength of 780 nm.

4.7. Statistical analysis for separation of microplastics efficiency

To compare the mean for collection efficiency of the different techniques of collection, statistical evaluation using analysis of variance (ANOVA) and the Tukey test, with an error rate at 95% (α=5%) were used. To perform this analysis the statistical program R was used.

The null and alternative hypotheses for the ANOVA were designed as follows:

H_0: $\mu_1 = \mu_2 = ….. = \mu_k$ The mean of the collection efficiency of fluorescent microplastic is statistically equal across the different separation methods.

H_1: At least one mean of the collection efficiency is not statistically equal

Where, to reject the null hypothesis is possible if: F (observed value) > FCV (critical value) or when our p-value (Pr(>F)) is less than 0.05.

In the comparison of the mean of collection efficiency by the Tukey test, the difference between two separation methods is accepted when zero is not included in the range value of comparisons tests.

4.8. Methodology for analysis of microplastic content in waste water samples

For this preliminary analysis of microplastic content, samples of waste water (1.5 L) from the different treatment steps of a municipal Waste Water Treatment Plant with a capacity of 55 000 p.e. were used:

- Influent
- Biological Treatment
- First Clarifiers
- Second Clarifiers
- Effluent

The samples passed through the best or most efficient separation technique and the collected samples with microplastics and impurities were observed, counted, weighted and dimensioned by the optic microscope and identified or checked by the Raman spectroscope.

Once the numbers of microparticles were known, the amount of microparticles per cubic meter for each facility was calculated.

4.9. Methodology for the evaluation of the digestion of microplastic

This part of the investigation was done at the facilities of the Department of Pharmacology and Toxicology of the University of Vienna.

For this purpose, two laboratory mice were used; they were fed with a mixture of their normal food with a small amount of microplastics as follows:

Mouse I: 10 g of food + fluorescent MP with Ø 425-500 µm

Mouse II: 10 g of food + fluorescent MP with Ø 50-75 µm

This additional test was developed to observe the behaviour of the fluorescent MP after a digestion process in a mouse; as a first step the animal was fed with its usual food mixed with fluorescent MP and after 24 hours the faeces were collected. Once the microplastics were separated, they were observed by the optic microscope for detection of surface damages of randomly collected particles and compared with the original microplastics.

5. RESULTS AND DISCUSSION

5.1. Simulation of waste water treatment

During the time when the test was running, it was observed that the commercial MP were located on the surface of the water or attached to the wall of the glass beaker, while most of fluorescent MP were located at the bottom part of the beaker and a few of them were circulating due to the aeration (Fig 22).

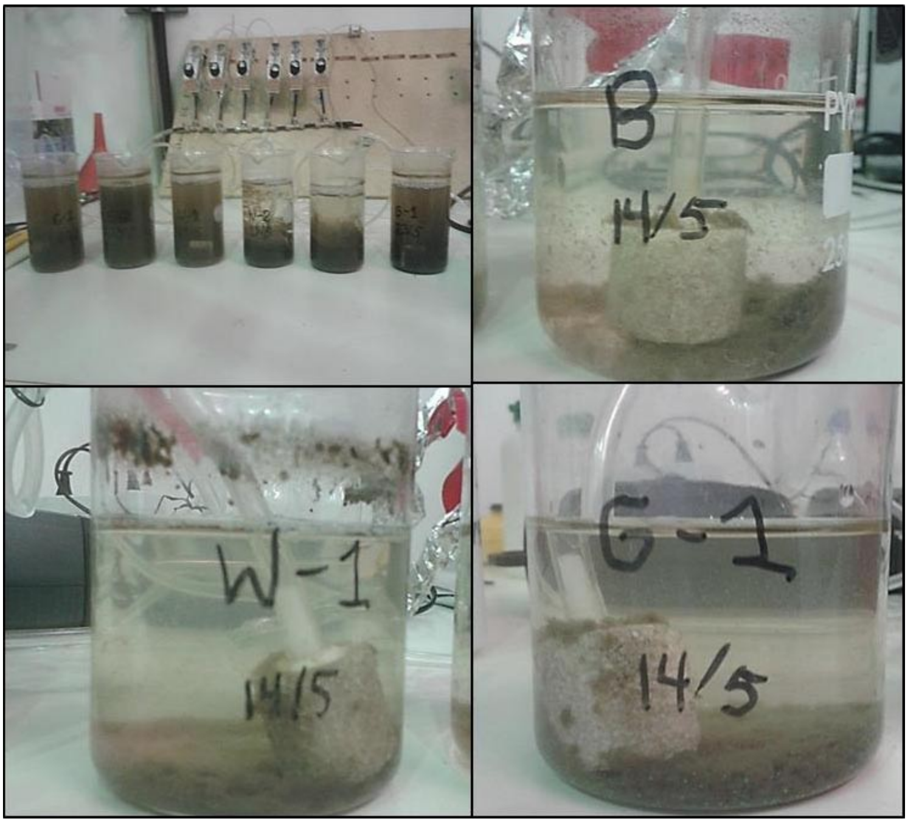

Fig. 22 Behaviour of control (B), commercial MP (W-1) and fluorescent MP (G-1) in WWTP simulation test

Before the WWTP simulation test, the original fluorescent and commercial MP were observed in the optic microscope (Fig. 23)

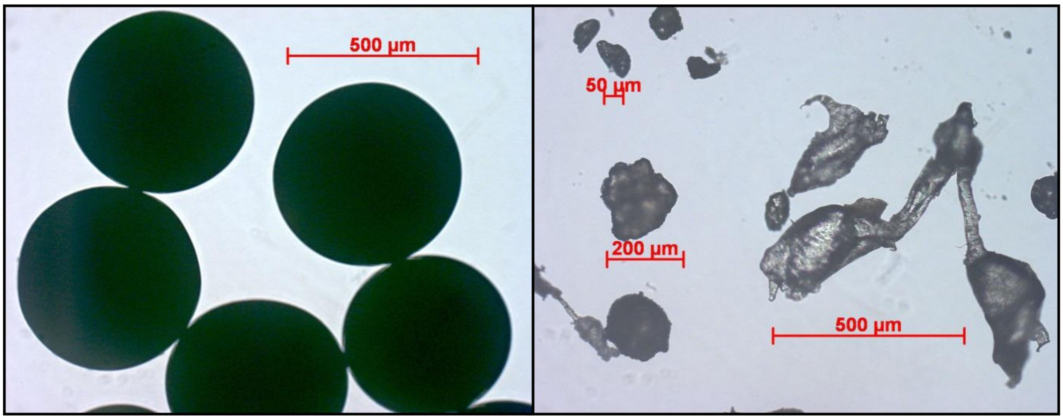

Fig. 23 Image of original fluorescent and commercial microplastics before the simulation test

Once the test ended, many fluorescent MP and commercial MP were observed in the optic microscope, where the size was measured and the colour changes observed (Fig. 24).

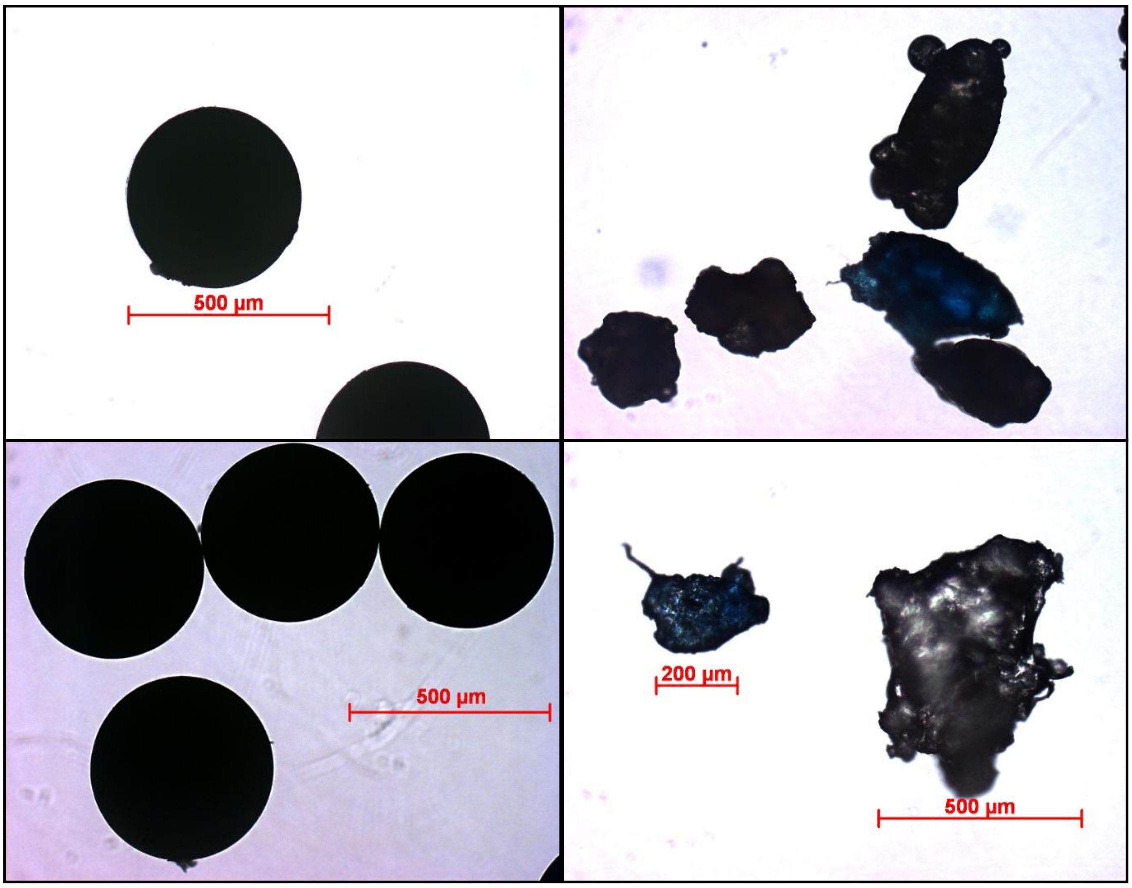

Fig. 24 Fluorescent MP (left side) and commercial MP (right side) after the WWTP simulation test

The measurements of sizes obtained from the randomly collected fluorescent MP and commercial MP are detailed in the Table 4.

Table 4 Sizes of collected microplastic units

	SIZE OF MICROPLASTIC UNITS (µm)											
TREATMENTS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Mean**
Original F MP	498	468	450	438	495	448	480	445	435	447		460
Fluorescent MP (pre test)	451	451	443	451	491	468	446	448	436	461		455
Fluorescent MP I	436	473	440	433	458	457	451	457	455	444		450
Fluorescent MP II	435	441	440	435	457	453	433	443	449	456		444
Commercial MP	596	1090	340	631	465	538	315	785	608	318	45	528
Commercial MP I	570	320	322	565	840	533	530	282	180	465	750	487
Commercial MP II	585	295	530	230	239	669	1005	568	680	362	585	523

During measurements, the smallest particle size found in the fluorescent MP treatments (F MP) was 433 µm and the maximum 491 µm; and in the commercial MP treatments (C MP) the smallest size was 45 µm and the maximum 1090 µm.

The distribution of the data is shown in a box plot (Fig. 25). Box plots are useful for identifying outliers and for comparing distributions; where the bottom of each box is the 25th percentile, the top is the 75th percentile, and the line in the middle is the 50th percentile (Lane. 2013). The IQR (Interquartile range) is used to build box plots, as simple graphical representations of a probability distribution.

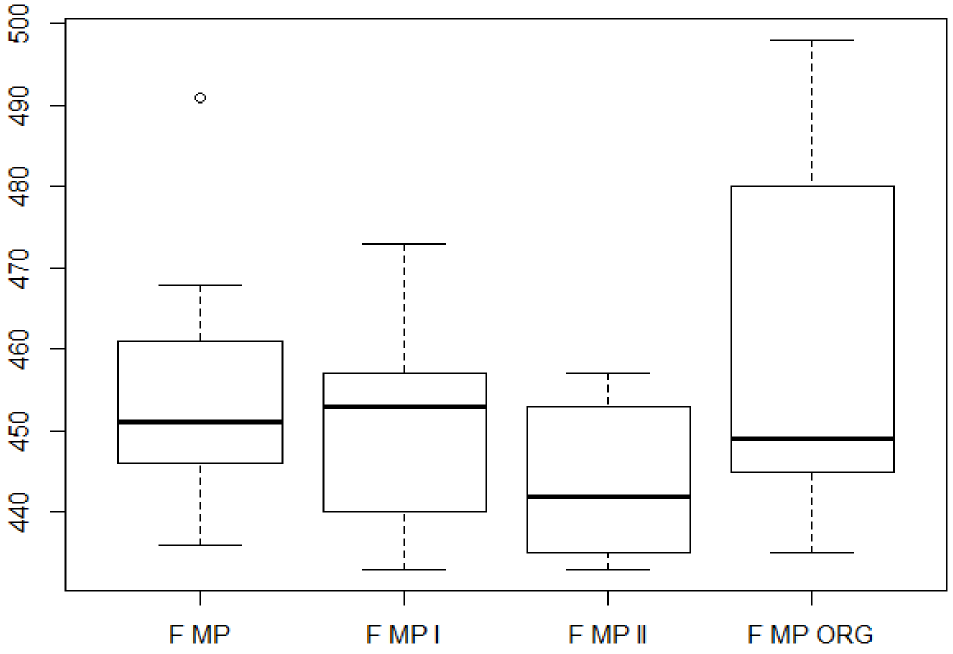

Fig. 25 Boxplot of the particle size in each F MP treatment and original particles

The boxplot shows higher IQR in the original fluorescent MP (F MP ORG) than the other treatments. The median of the treatments was around 450 µm and in the treatment fluorescent MP (F MP), an outlier was observed (490 µm).

Table 5 shows the ANOVA results, the p value (Pr(>F)) is equal to 15.7% (0,157) and has a higher value than α = 0.5% (0,05); therefore the H_0 is accepted and the mean size of fluorescent MP is statistically equal across the different treatments.

Table 5 ANOVA table of the fluorescent MP data

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Category FMP	3	1401	466.9	1.842	0.157
Residuals	36	9125	253.5		

In the evaluation of commercial MP, statistical evaluation was not possible due to the irregularity of the shape and size of the microparticles., For this reason a classification of particle sizes by classes was done to know the distribution size of the commercial microplastics. Table 6 shows the interval size ranges of commercial MP before the simulated WWTP test (n= 40 particles) and after the simulated WWTP test (n= 94). In both cases the minimun and maximun interval values were 45 µm and 1049 µm respectively.

Table 6 Interval size of commercial MP before and after the WWTP simulation test

N°	**Interval (µm)**			**Before**	**After**
	Lower bound	**Upper bound**	**Mid-point class**	**(n)**	**(n)**
1	45	245	145	27	16
2	246	446	346	25	12
3	447	647	547	29	5
4	648	848	748	10	4
5	849	1049	949	3	3

There for both cases, the mid-point class N° 1 has an interval of 145 ± 100 µm, N° 2 of 346 ±100 µm ; N° 3 of 547 ±100 µm, N° 4 of 748 ± 100 µm and N° 5 of 949 ± 100 µm. Also the amount of particles for each class before and after the WWTP simulation test is observed.

Furthermore, some quality parameters such as pH, electric conductivity (Table 7), total organic carbon TOC and total nitrogen TN (Table 8) in the waste water used for the different treatments were measured.

Table 7 Measurement of pH and electric conductivity in the different treatments

	Treatment					
Parameter	Control I	Control II	Commercial MP I	Commercial MP II	Fluorescent MP I	Fluorescent MP II
pH	8.0	7.9	7.6	7.9	8.2	8.2
Electric Conductivity (mS/m)	1417	1665	1454	1621	1329	1205

The pH in the different treatments remained stable, and the highest values were obtained from the treatments fluorescent MP I and fluorescent MP II with 8.2. The electric conductivity also remained stable and the highest value was obtained in the treatment control II with 1665 mS/m.

Table 8 Measurement of total nitrogen and total organic carbon in the different treatments

	Treatment						
Parameter	Waste water	Control 1	Control 2	Commercial MP 1	Commercial MP 2	Fluorescent MP 1	Fluorescent MP 2
TN (mg/L)	276	23.5	35.2	28.4	70.2	24.0	14.5
TOC (mg/L)	213	77.1	82.6	80.8	111	53.0	51.9

In the measurements of total nitrogen (TN) and total organic carbon (TOC), the sample of waste water used in the WWTP simulation tests had the highest values with 276 mg/L and 213 mg/L respectively.

5.2. Mechanical separation

In the case of mechanical separation, after the separation of fluorescent MP samples with the sieves (650, 250 and 125 µm), the collected microplastics were combined with organic impurities. This problem made it impossible to take out or isolate the particles and count them (Fig. 26).

Fig. 26 Separation process by mechanical technique with sieves

Due to the complexity in separating the microplastics from impurities, this method was not evaluated by the statistical comparison with the other treatments in regards collection efficiency.

5.3. Separation by density technique

The results of the different alternatives of this technique are described as follows:

In the case of separatory funnels, the collection efficiencies after the separation process were 40, 64 and 36% (Fig. 27).

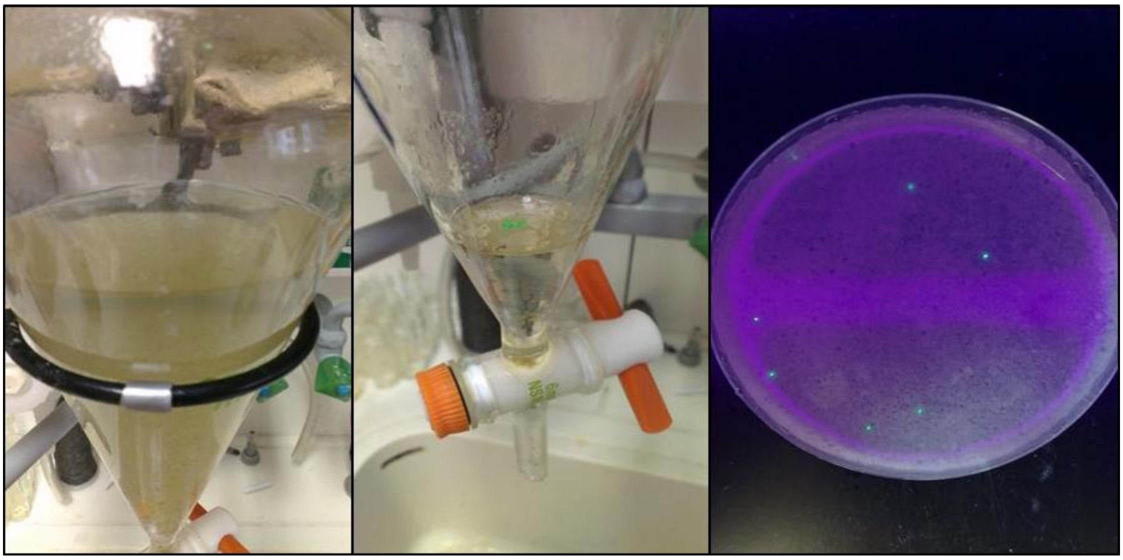

Fig. 27 Separation process by density technique (Separatory funnel) and counting the fluorescent MP in a petri dish in the UV light chamber

In Fig. 28 the isolation process using a vacuum pump and 0.45 µm filter paper is observed.

Fig. 28 Isolation process and collection of isolated microplastics in density technique

The technique using a combination of separatory funnels with centrifugation resulted in a collection efficiency of 94, 94 and 95% (Fig. 29).

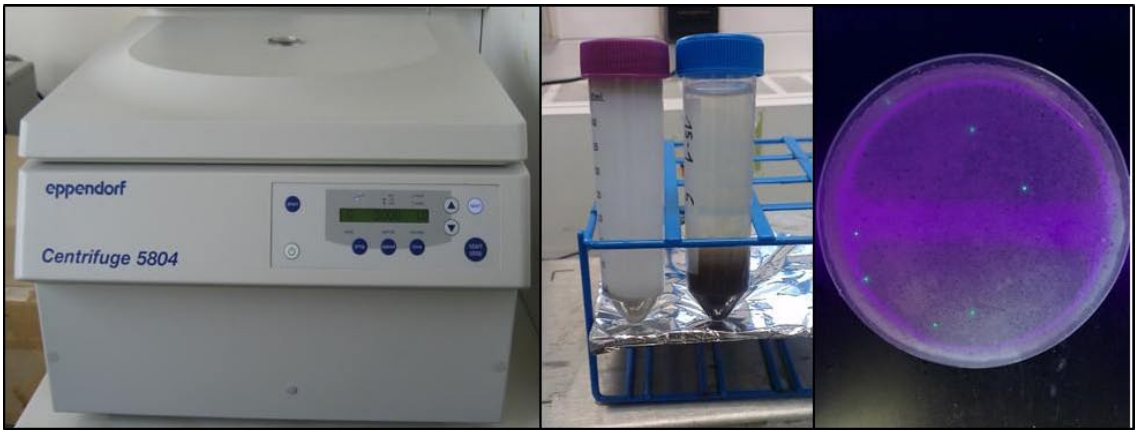

Fig. 29 Separation process by density technique: Separatory funnel and centrifugation and counting the fluorescent MP in a petri dish in the UV light chamber

While for the technique of adapted separatory column the efficiency results were 66, 41 and 54% (Fig. 30).

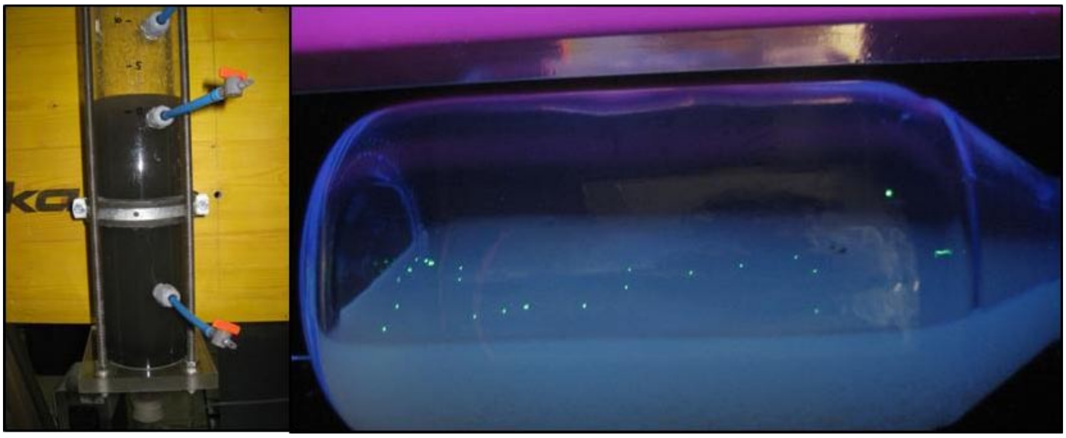

Fig. 30 Separation process by density technique: Adapted separatory column and centrifugation and counting the fluorescent MP in the UV light chamber

Finally for the adapted separatory dispenser (ASD), in the first collection an efficiency of 38, 6 and 0% was obtained (Fig. 31). In this technique, the main problem observed was that the most of the microplastics were attached to the surface of the flask and plastic pipe of the ASD or in the waste water sludge.

Fig. 31 Separation process by density technique: Adapted separatory dispenser

5.4. Separation by chemical technique

The collection efficiencies of the fluorescent MP, after the process of separation with hexane, were 88, 84 and 87 % (Fig. 32).

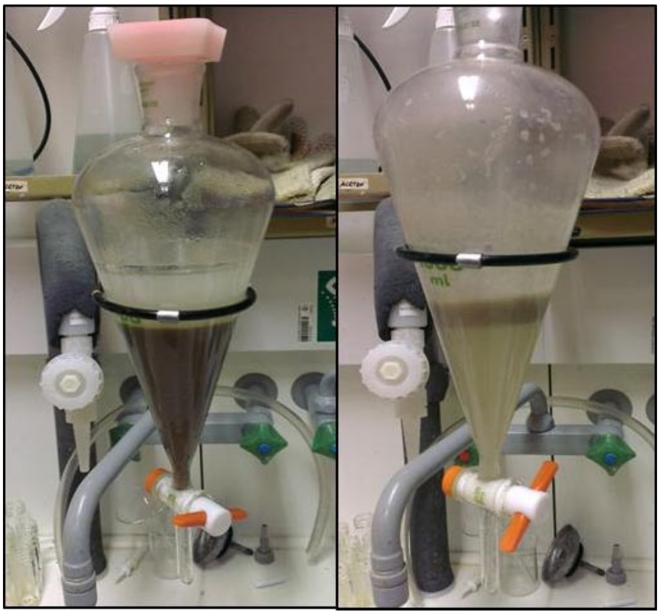

Fig. 32 Separation process by chemical technique before mixing (left side) and after the mixing (right side)

However for the final isolation of the collected units, the final product was a mixture of new impurities and the fluorescent MP (Fig. 33).

Fig. 33 Isolation process of microplastics in chemical technique: Filtering (left side), drying (middle) and sieving (right side)

5.4.1. Characterization of microplastic particles

The collected particles in the chemical process were analysed by the Raman spectroscope and the final curve drawings or Raman spectra of the different particles are shown in the following graphics (Fig. 34-37). The reference curve for an original fluorescent MP is described in chapter 5.9.

In Fig. 34 the characteristic Raman spectrum of a fluorescent MP after the process of separation is observed.

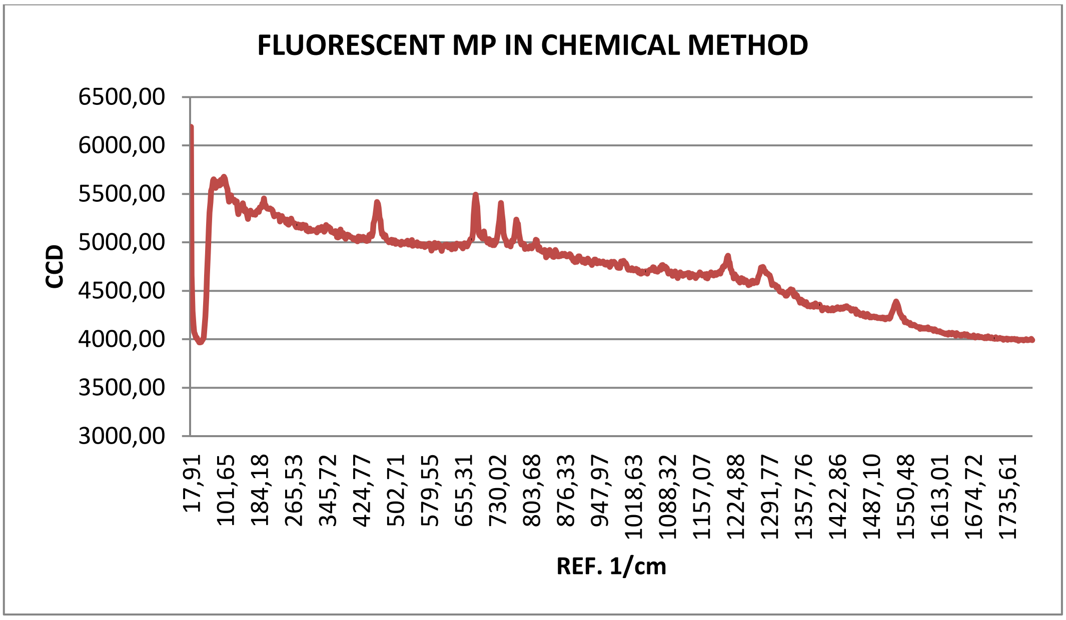

Fig 34 Raman spectrum of particle of fluorescent MP

In Fig. 35 Raman spectrum of an impurity after the process of separation is observed.

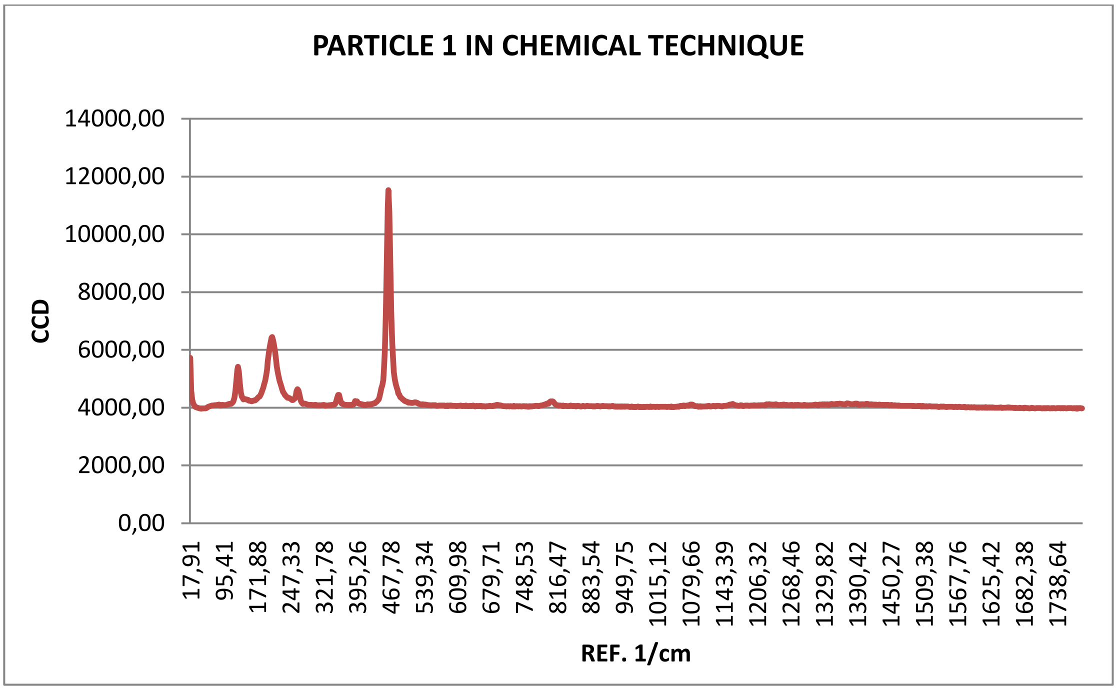

Fig. 35 Raman spectrum of impurity of chemical technique

In Fig. 36 Raman spectrum of an impurity after the process of separation is also observed.

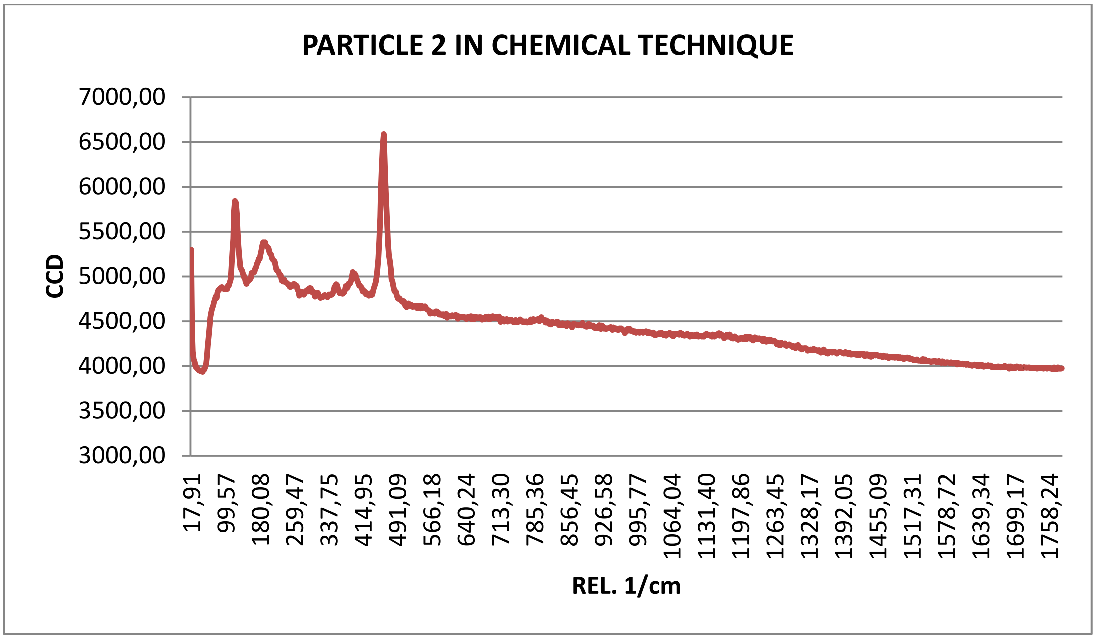

Fig. 36 Raman spectrum of impurity of chemical technique

In Fig. 37 Raman spectrum of an impurity after the process of separation is also observed. The Raman spectra of the impurities look different to the Raman spectrum of the fluorescent MP.

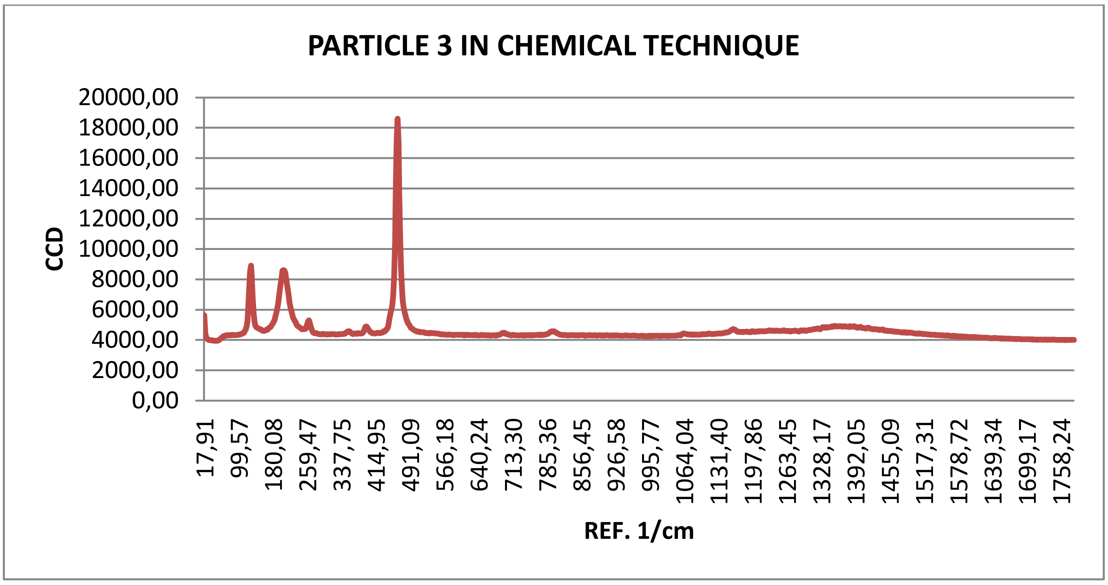

Fig. 37 Raman spectrum of impurity of chemical technique

5.5. Comparison of separation techniques

Table 9 shows the summary of the collection efficiency of fluorescent MP obtained by the evaluated techniques. The highest collection efficiency was obtained by the "combination of separatory funnels with centrifugation technique" with 95% in both replications and the lowest efficiency was by the "adapted separatory dispenser" with 0% in the second replication.

Table 9 Summary of collection efficiency of the different techniques

Technique	1° Replication	2° Replication	3° Replication
Mechanical separation	Discarded	Discarded	Discarded
Separatory funnels- **SF**	40%	36%	64%
Combination of separatory funnels with centrifugation- **SF+C**	94%	94%	95%
Adapted separatory column -**ASC**	66%	41%	54%
Adapted separatory dispenser -**ASD**	38%	0%	6%
Separation by chemical technique- **CHEM**	88%	84%	87%

A boxplot with data collection efficiency (%) of the different separation techniques is shown in Fig. 38. The graphic shows that adapted separatory dispenser technique (ASD) has a higher IQR than the other separation techniques.

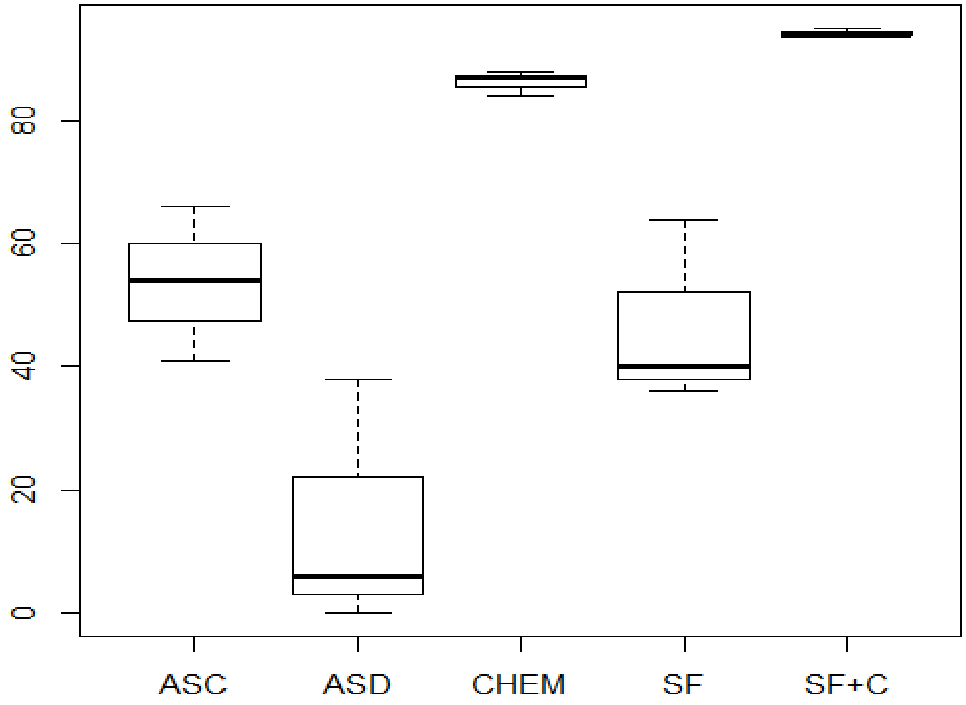

Fig. 38 Boxplot of collection efficiency for each separation method

Table 10 shows the ANOVA results, the p value (Pr(>F)) is equal to 0.01% (0,000109) and has a higher value than α = 0.5% (0.05); therefore the H_0 is rejected,and at least one of the means of the collection efficiency of fluorescent MP is not statistically equal across the different separation methods. .

Table 10 ANOVA table of collection efficiency

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
MP$Method	4	12424	3106.1	19.23	0.000109 ***
Residuals	10	1615	161.5		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

5.6. Sampling of a waste water treatment plant

In the samples of the WWTP with a capacity of 55 000 p.e., the collected microparticle sizes and amounts were measured. The results are shown in Table 11.

Table 11 Size and number of particles in WWTP

Facility	**Size (µm)**										**N° Particles (1.5 L)**
Influent	595	318	475	190	180	440	460	210	345	360	10
Biological Treatment	65	223	193	650	663	245	478	257	480	180	37
1° Clarifiers	540	297	388	170	487	595	460	745	335	292	22
2° Clarifiers	148	348	460	220	250	640	242	345	560	150	24
Efluent	240	300	160	420	350	175	170	368	545	320	13

The particles collected from the waste water were analysed by the Raman spectroscope. The Raman spectra are shown in the following graphics (Fig. 39-45). The reference curve for an original commercial MP is described in chapter 5.10.

Fig. 39 shows the spectrum of commercial MP found in the primary clarifier of the WWTP

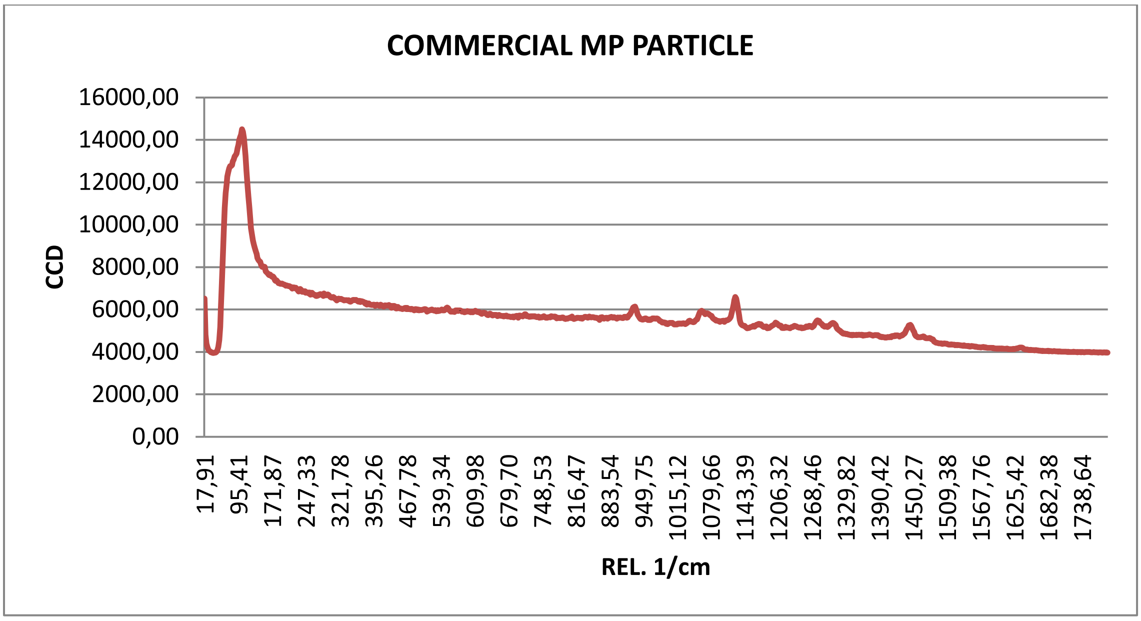

Fig. 39 Raman spectrum of a commercial MP particle in primary clarifier

Fig. 40 shows the spectrum of commercial MP found in the biological treatment step of the WWTP

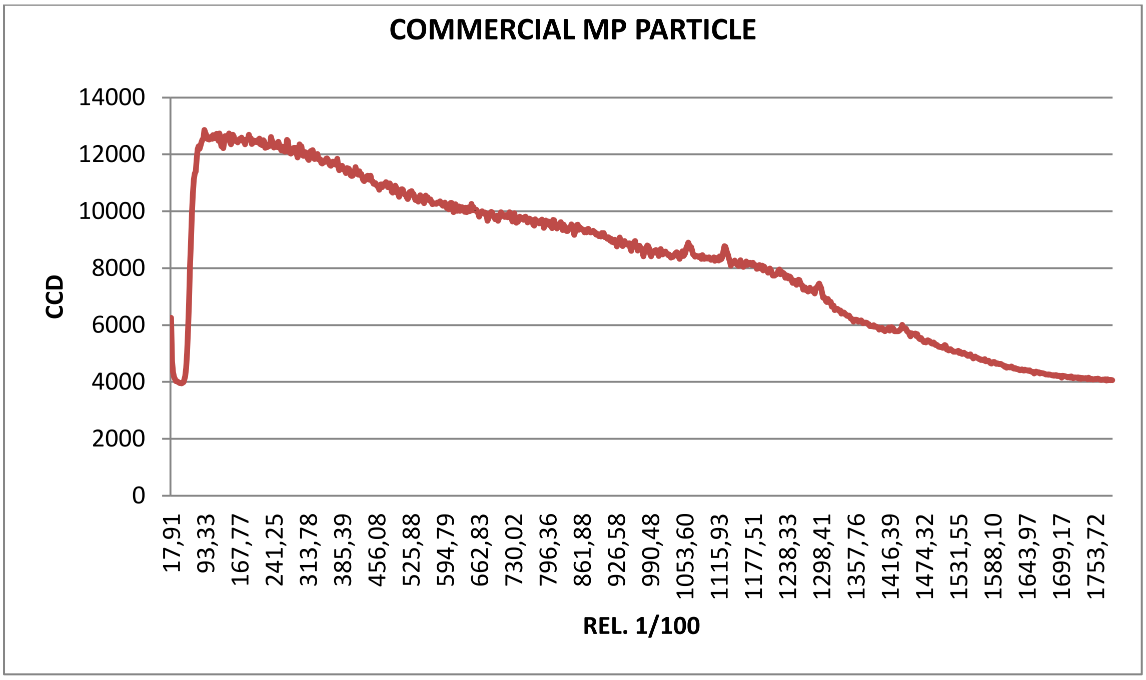

Fig. 40 Raman spectrum of a commercial MP particle from biological treatment

Fig. 41 shows the spectrum of an impurity found in the biological treatment of the WWTP

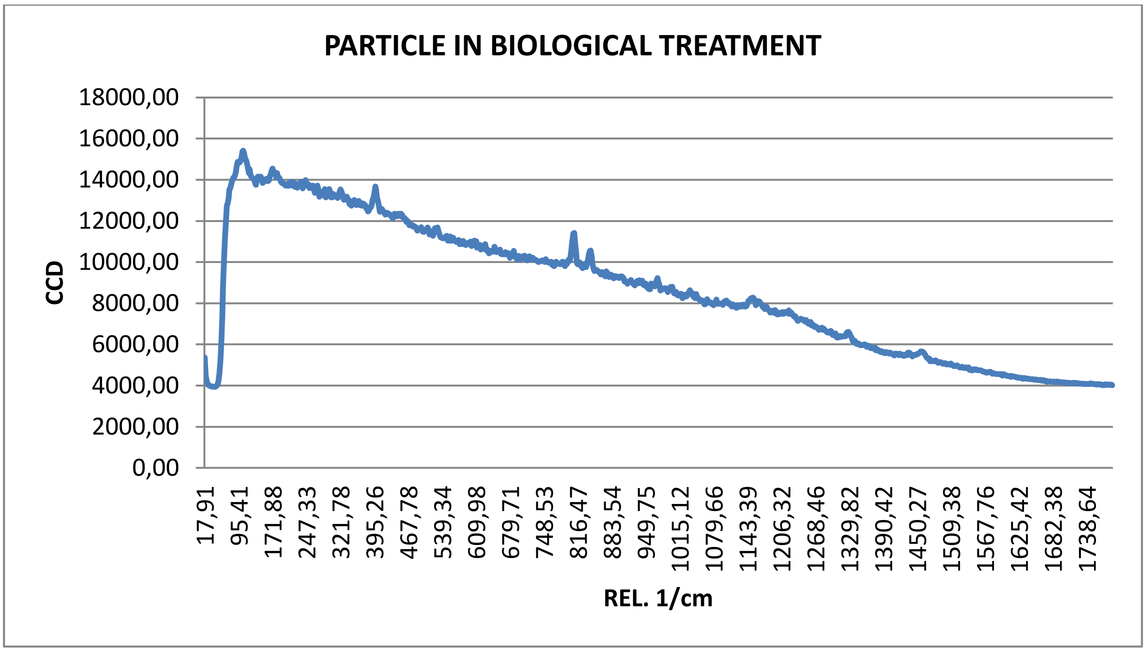

Fig. 41 Raman spectrum of an impurity from biological treatment

Fig. 42 shows the spectrum of commercial MP found in the secondary clarifier of the WWTP

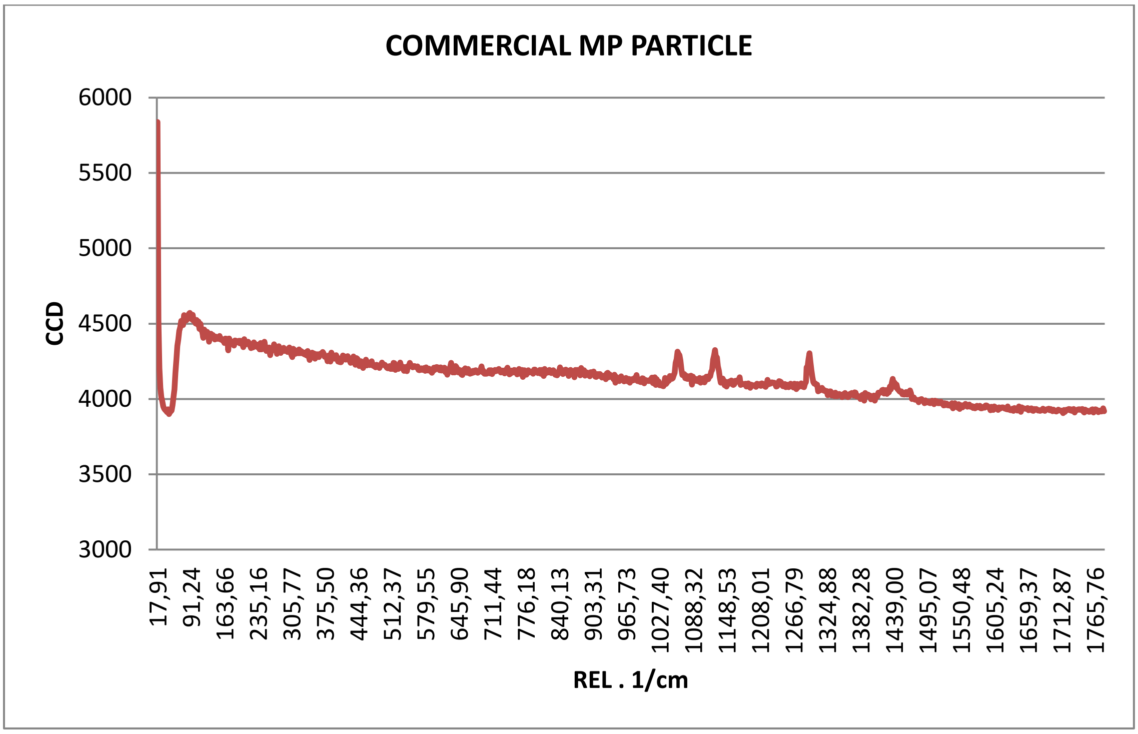

Fig. 42 Raman spectrum of a commercial MP from secondary clarifier

Fig. 43 shows the spectrum of cellulose particles found in the secondary clarifier of the WWTP

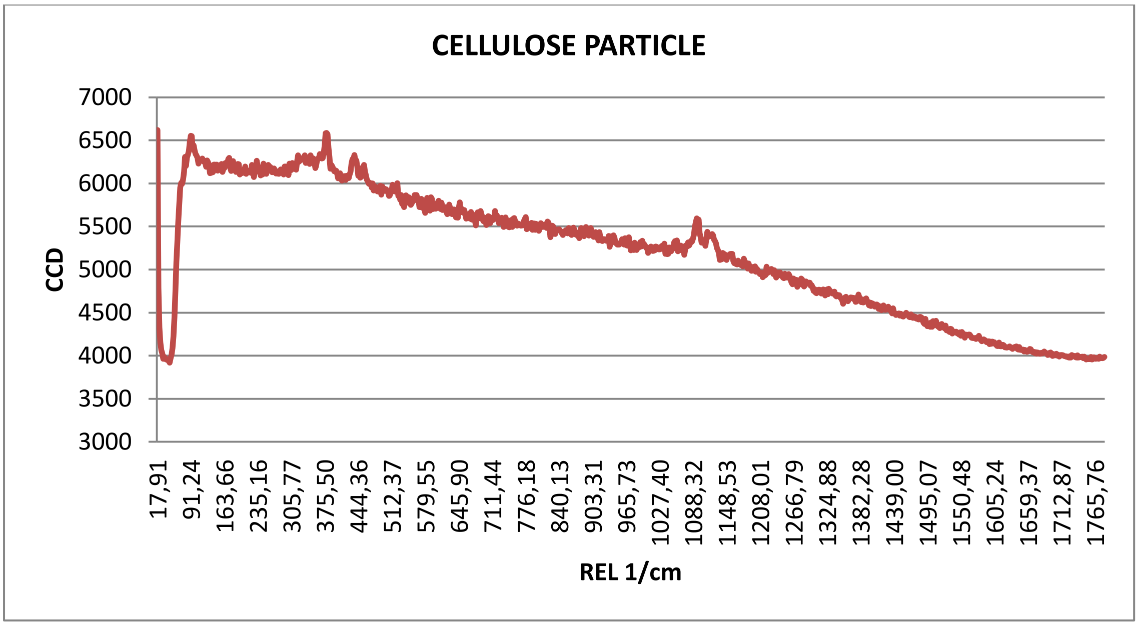

Fig. 43 Raman spectrum of a cellulose particle from secondary clarifier

Fig. 44 shows the spectrum of commercial MP found in dewatered sludge of the WWTP

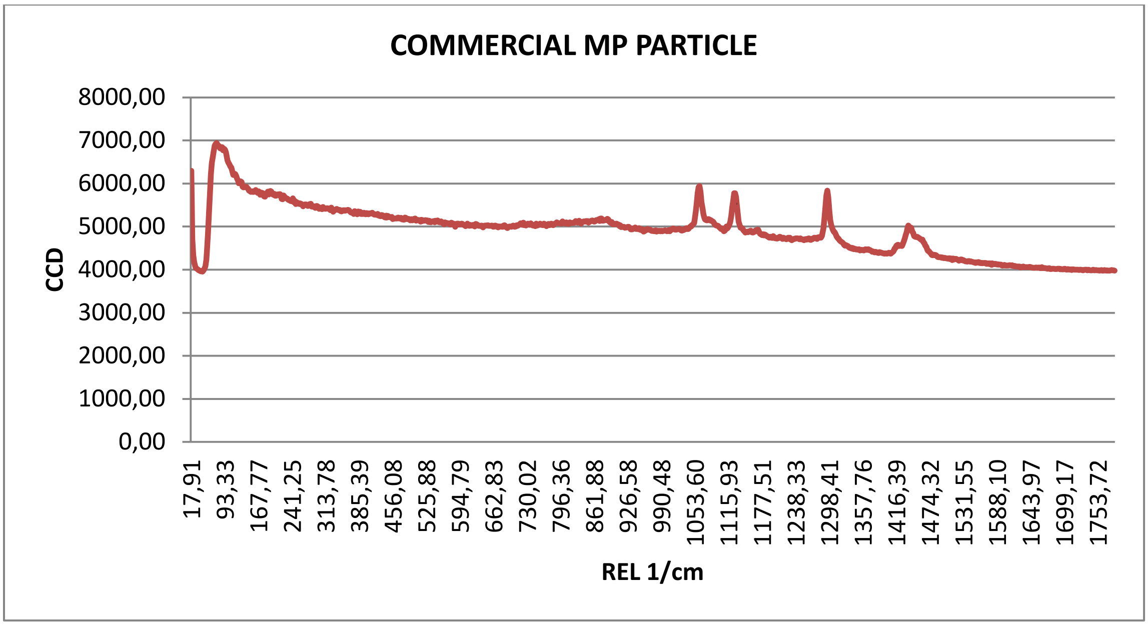

Fig. 44 Raman spectrum of a commercial MP particle from dewatered sludge

Fig. 45 shows the spectrum of an impurity found in dewatered sludge of the WWTP

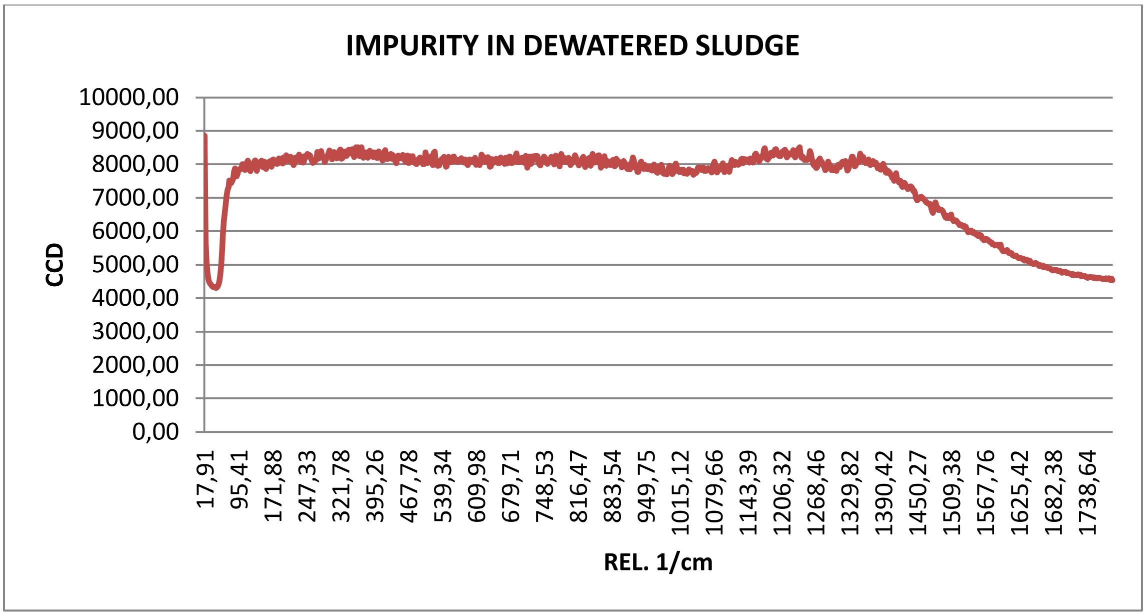

Fig. 45 Raman spectrum of an impurity from dewatered sludge

The collected number of particles in the range of 65 - 745 µm, where partly characterized in the Raman spectrometer and could be to about 50% identified as commercial MP (reference peaks took from the collected commercial products, chapter 4.2.1).

5.7. Digestion of microplastics in mice

In the case of digested large fluorescent MP (Ø 425 -500 μm) in the faeces of the mouse, deformations were observed (Fig. 46 and 47); sizes of the measured particles (n=126) were classified in interval groups as shown in Table 12. It was observed that in both the minimum and maximum interval values were 246 μm and 500 μm respectively.

The size of original fluorescent MP measured kept in their range (Ø 425 -500 μm).

Table 12 Size of digested fluorescent MP

Group 1	**Intervals (μm)**		
	Lower bound	**Upper bound**	**Middle point class**
1	246	296	271
2	297	347	322
3	348	398	373
4	399	449	424
5	450	500	475

Fig. 46 shows the shape and size of the original fluorescent MP (Ø 425 -500 μm), it is also possible to observe a small failure of the original particles.

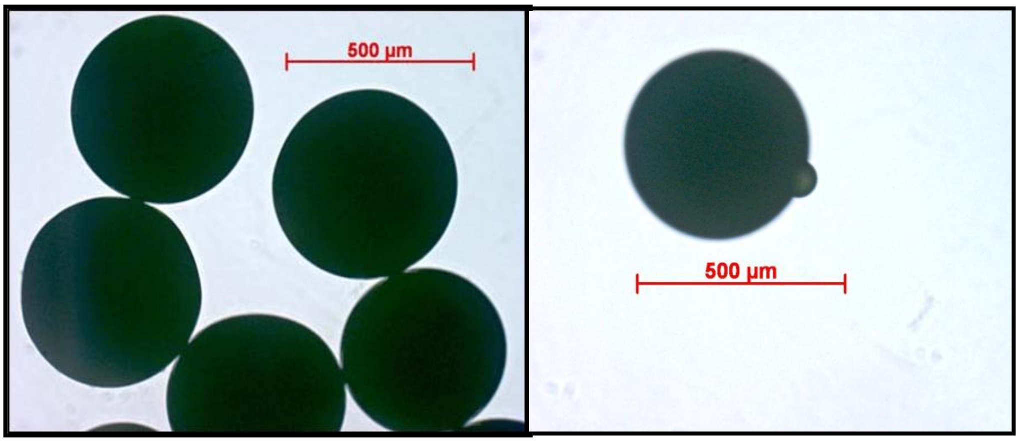

Fig. 46 Original fluorescent MP (Ø 425 -500 μm) before the digestion test

Fig. 47 shows the shape and size of the digested fluorescent MP and observed deformation of the microplastics.

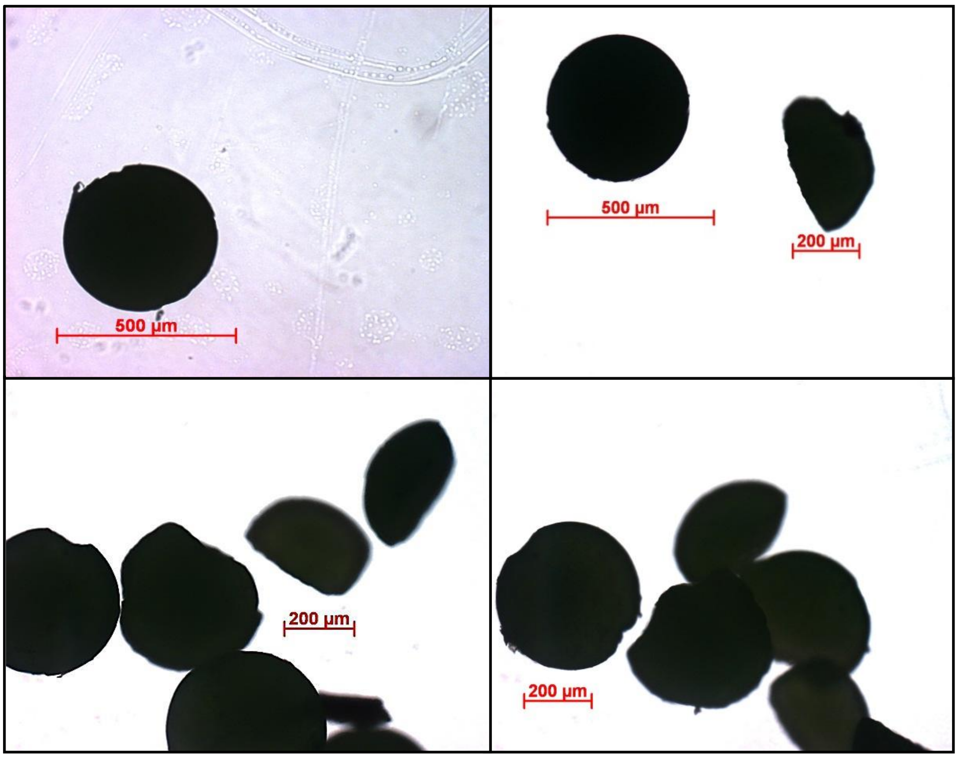

Fig. 47 Deformed fluorescent MP (Ø 425 -500 µm) after the digestion test

The digested small fluorescent MP (Ø 50 – 75 µm) kept their size in the average range. However, deformations were observed in both original fluorescent MP and digested fluorescent MP (Fig. 48 and 49); considering this observation, determination if the microplastics were damaged during the digestion process is not possible.

Fig. 48 shows the shape and size of the fluorescent MP (Ø 50 – 75 µm), where it is also possible to observe large failures in the original particles.

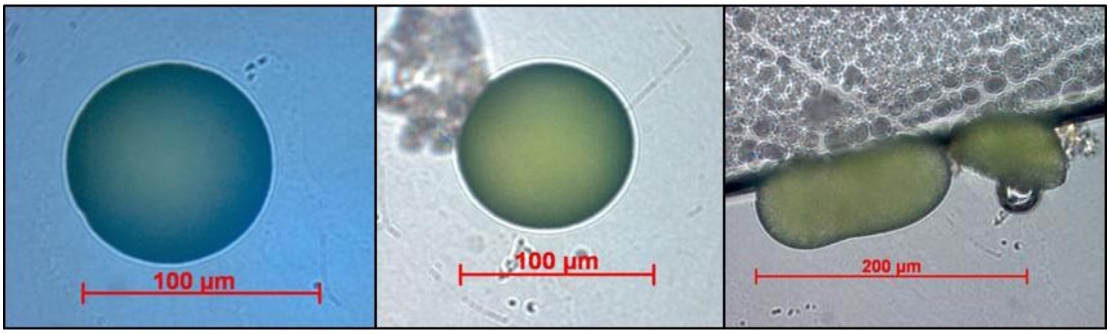

Fig. 48 Non deformed and deformed original fluorescent MP (Ø 50 – 75 µm)

Fig. 49 shows the shape and size of the digested small fluorescent MP. Different shapes of deformation of the particles can be observed, but as the originals are also deformed, it cannot be clearly assigned to the digestion.

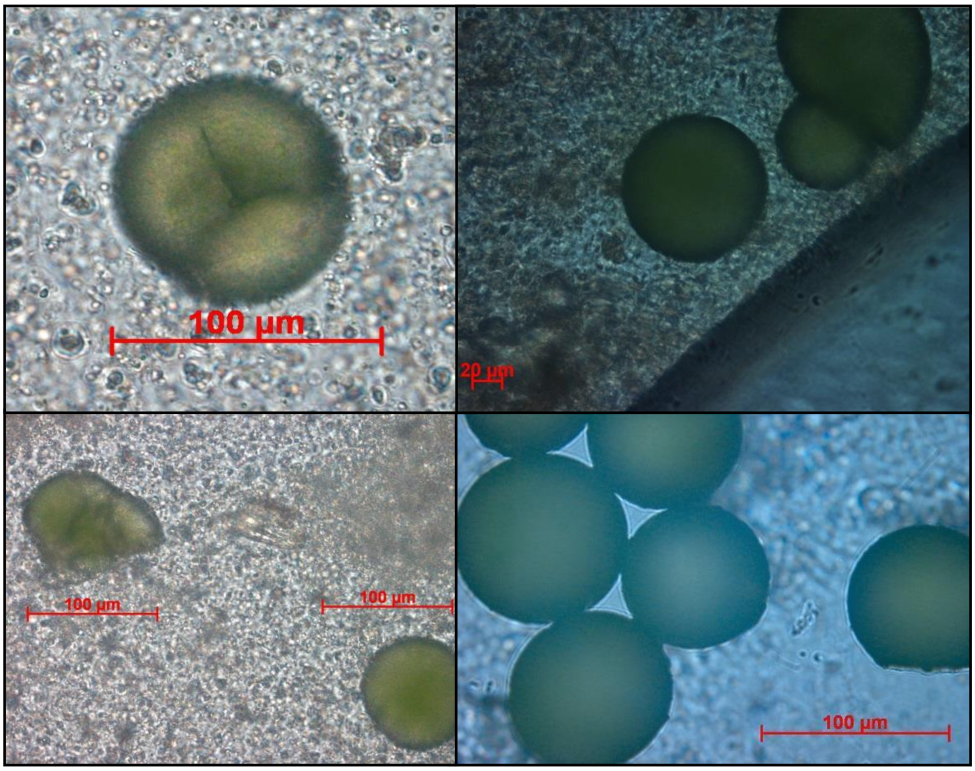

Fig. 49 Fluorescent MP (Ø 50 – 75 µm) after the digestion test

5.8. Discussion of simulation of waste water treatment

In the WWTP simulation test it was observed that the microplastic behaves according to its density. On the one hand, lighter particles always kept floating; the reason is because these particles have a lower density, from 0.91 to 0.94 kg/L (Wright et al., 2013) than the water 1.0 kg/L (Imhof et al., 2012), and on the other hand, the microplastics with higher density, as with the fluorescent MP with a density of 1,0005 kg/L, were located at the bottom of the glass beaker.

Using the optic microscope, it was observed that both types of microplastics changed their colour. This change could be caused by the growing of bacteria, from the waste water on the surface of the particles as biofilms (National Research Council, 2006).

In regards to the size changes for the treatments with fluorescent MP (F MP) with Ø 425 - 500 µm, after the statistical analysis and Tukey test of the results; it was found that there exists no difference in the average size of microplastics after different treatments in the WWTP simulation test: F MP, F MP I, F MP II, and original fluorescent MP (F MP ORIG). In Fig. 50 the range values of all comparison tests, where those for which zero is included, no differences were observed.

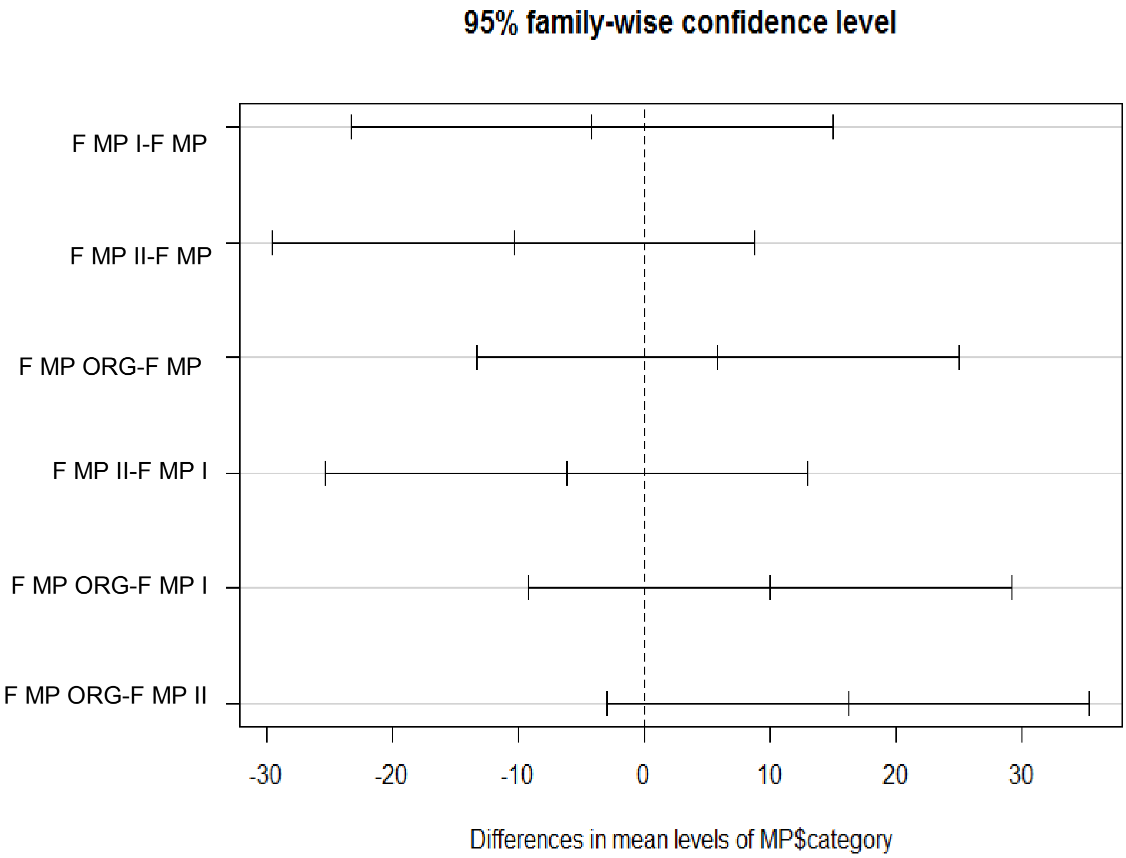

Fig. 50 Differences between fluorescent MP in different treatments by Tukey test

The reason for this zero change in the particles is the long time needed by the polyethylene to be degraded in natural environments or in a waste water treatment plant. The lifetime of polyethylene in a moderate and tropical climate is 15-20 and 2-5 years respectively (Klyosov, 2007).

In Fig. 51 the classification of the sizes of commercial MP before the simulation test is observed. There, 40% of the total particles were in the range of 145 ± 100 μm, 30% in the range of 346 ± 100 μm, 12,5 % in the range of 547± 100 μm and 7,5% were in 949 ± 100 μm range.

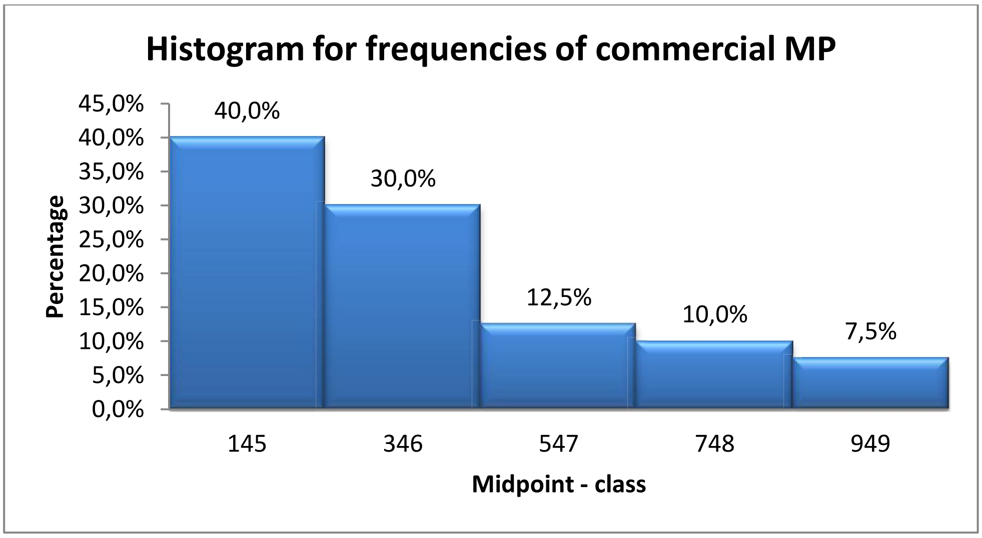

Fig. 51 Histogram of commercial MP before treatment

In regards to the classification of the size of commercial MP after the simulation test, 28.7 % of the total particles were in the range of 145 ± 100 µm, 26.6% in the range of 346 ± 100 µm, 30.9 % in the range of 547± 100 µm and only 3.2% were in 949 ± 100 µm range (Fig 52).

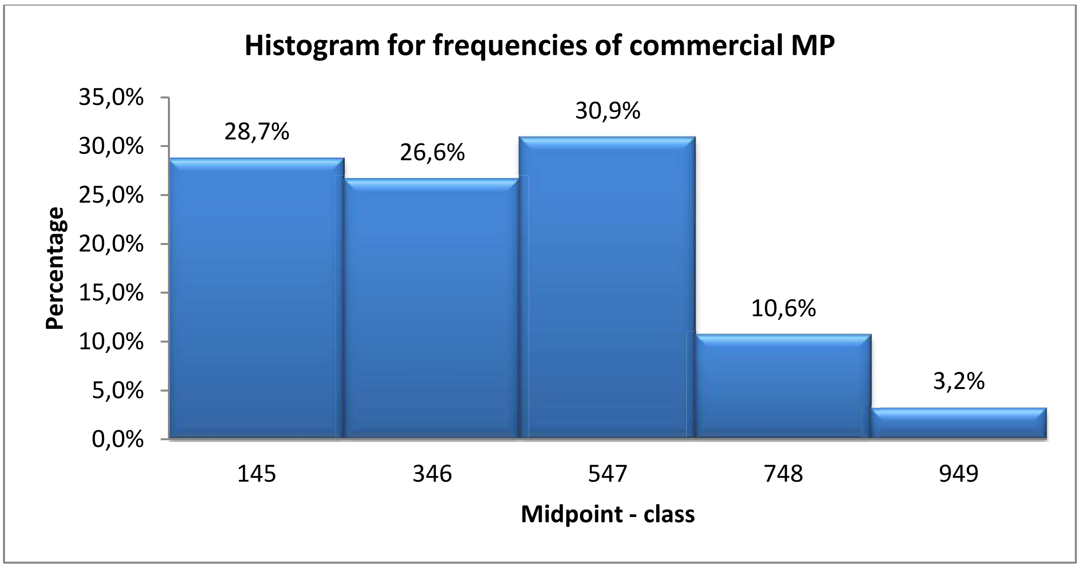

Fig. 52 Histogram of commercial MP after treatment

5.9. Discussion of separation techniques

For the separation techniques of microplastics, the best performance was obtained by using the density technique: “Combination of separatory funnels with centrifugation” with a mean of 94% in the collection efficiency. The second best efficiency was obtained by “separation by chemical technique”. And the poorest collection efficiency was obtained by the “adapted separatory dispenser” with a mean of 15% (Table 13).

The traditional technique of separation, “separation by funnels”, received a collection efficiency of 47%; this result is quite higher to that obtained by the test developed in Munich called “classical density separation”, using zinc chloride ($ZnCl_2$) as the solution, with a recovery of 39.8% for aquatic sediments (Imhof et al., 2012). However Claessens et al. (2013) reported an efficiency of 61% using saturated salt of NaI (sodium iodide) as a solution but in sandy sediments, this recovery is quite higher than in our “Adapted Separatory Column” (54%).

In other techniques performed, for instance the research carried out by the elutriation method (using an upwards stream of gas or liquid) with sodium iodide (NaI) solution obtained a collection efficiency of 100% for the spiked particles of PVC or polyethylene (Cleassens et al., 2013), but this method used sandy sediment as the sample. Finally the equipment called Munich Plastic Sediment Separator (MPSS), spiked in sediment with microplastics of polyamide, polyethylene, polyester and so on, obtained recovery rates of 95.5% for microplastics (< 1 mm) (Imhof et al., 2012).

In the case of separation by the “Chemical Technique”, collection efficiency was 86 %. This result was quite higher than the “froth flotation technique” using additives (surfactants) to separate the microparticles, with a recovery efficiency of 55% (Imhof et al., 2012).

Table 13 Mean of collection efficiency by each technique of separation

Technique	**1° Replication**	**2° Replication**	**3° Replication**	**Mean**
Separatory funnels **SF**	40%	36%	64%	47%
Combination of separatory funnels with centrifugation **SF+C**	94%	94%	95%	94%
Adapted separatory column **ASC**	66%	41%	54%	54%
Adapted separatory dispenser **ASD**	38%	0%	6%	15%
Separation by chemical technique **CHEM**	88%	84%	87%	86%

In comparisons between the means of the collection efficiency of the different techniques investigated, differences between SF+C technique with ASD, ASC and SF techniques were found. Additionally differences exist between CHEM technique with ASD and SF techniques; and finally differences also exist between ASD technique and ASC technique. No differences were found in comparisons between CHEM technique and SF+C technique. In Fig. 53 the range values of all comparisons tests, where those for which zero is included, no differences are observed.

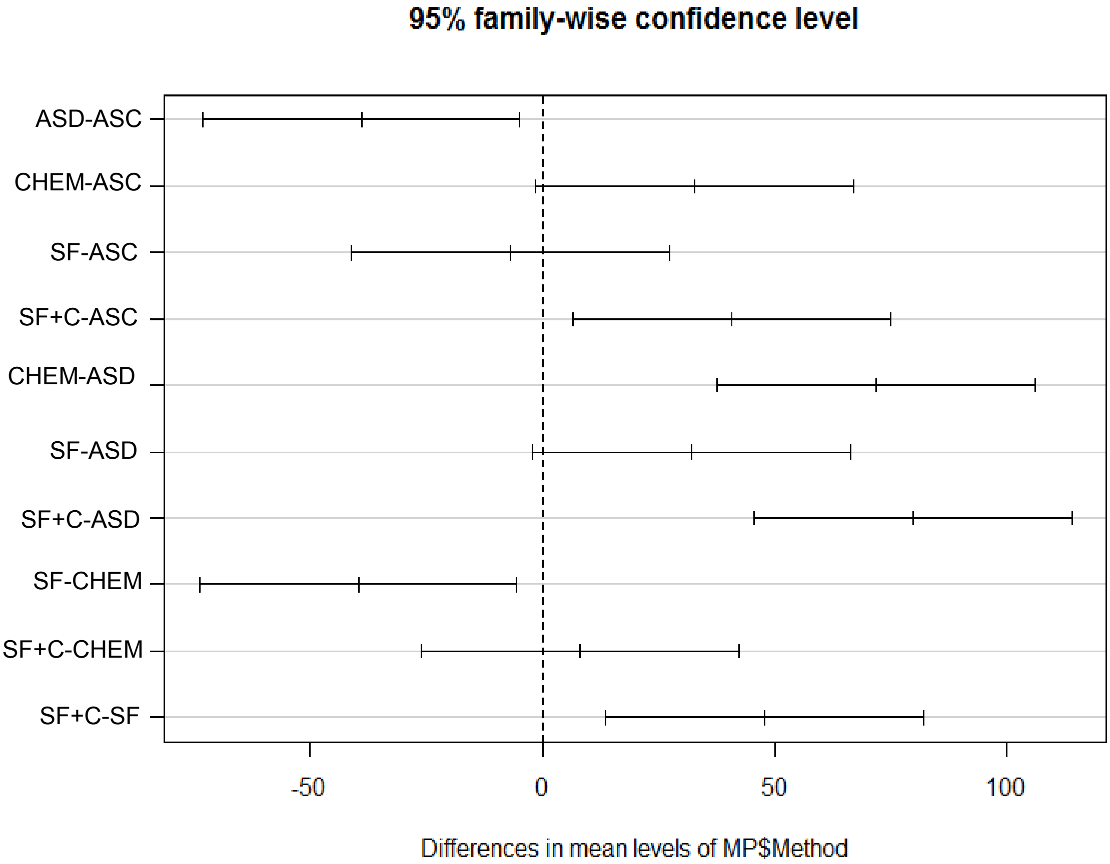

Fig. 53 Differences of mean of collection efficiency between the separation techniques

In the case of the chemical separation technique, during the isolating process of the fluorescent MP, it was not possible to separate the particles properly from the impurities, which were in the form of white powder; for that reason a sieve with a mesh of 125 µm was used to wash the sample and eliminate the impurities. However after drying, the collected fluorescent MP was mixed with small solid crystal impurities (the powder changed its structure).

The new mixture were analysed by the Raman spectrometer to evaluate if the solid impurities had a similar spectrum and compare with the spectrum curve of an original fluorescent MP. The Raman spectra are shown in the following graphics (Fig. 54-56).

For the original fluorescent MP (reference FMP), typical peaks at 681.58, 737.43, 770.66, 1209.7, 1275.13 and 1528.39 1/cm in the spectra curves were observed (Fig. 54).

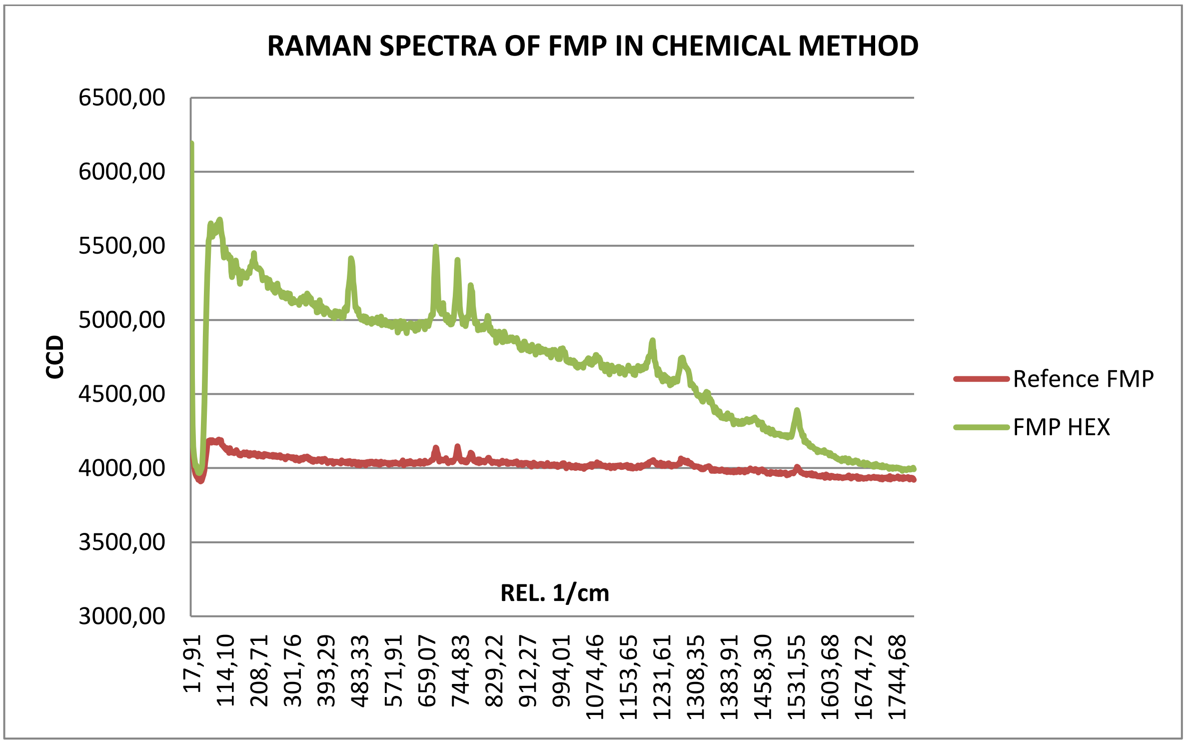

Fig 54 Raman spectra of collected and reference fluorescent MP in chemical method

In Fig. 55 the different spectra emitted by the impurities (P1 HEX, P2 HEX and P3 HEX) and fluorescent MP particles (Reference FMP and FMP HEX) are observed.

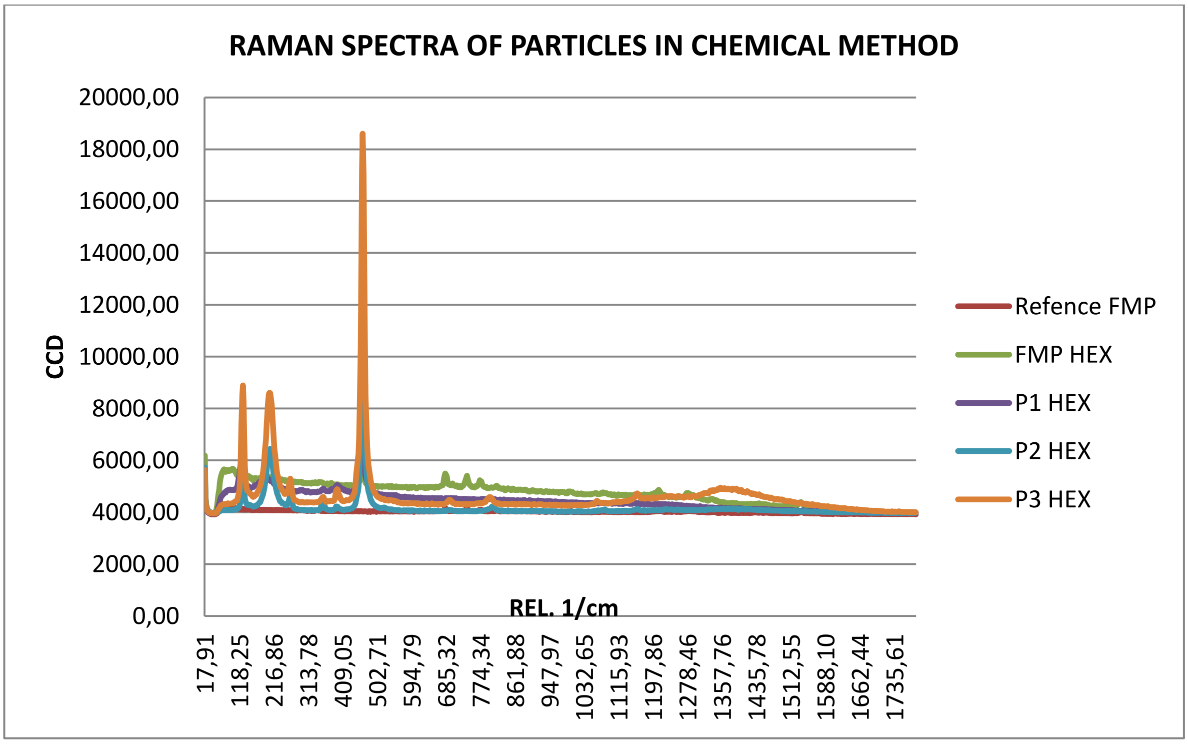

Fig. 55 Raman spectra of impurities and fluorescent MP in chemical method

Fig. 56 shows that in all of the impurities a peak at 463.88 1/cm exists; meaning that the impurities are similar or have the same composition.

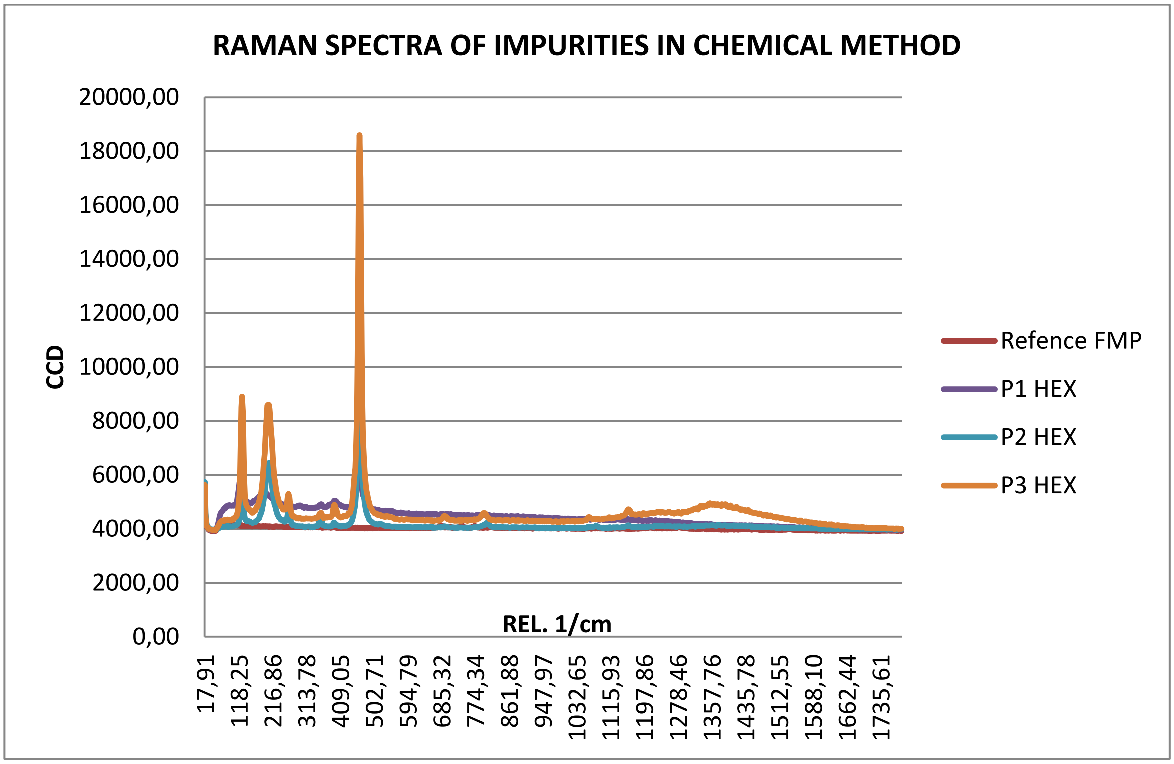

Fig. 56 Raman spectra of impurities in chemical method

5.10. Discussion of sampling of water of a waste water treatment plant

Once the collected particles were analysed by the Raman spectrometer, the spectra of each particle obtained from this analysis and from the different steps of the WWTP with a capacity of 55 000 p.e. were compared and identified with the reference spectrum of the commercial white MP. The Raman spectra are shown in the following graphics (Fig. 57-61).

Fig. 57 shows the Raman spectrum of the reference white colored commercial microplastic with characteristic peaks of 1060.56, 1126.25, 1291.77 and 1439 1/cm.

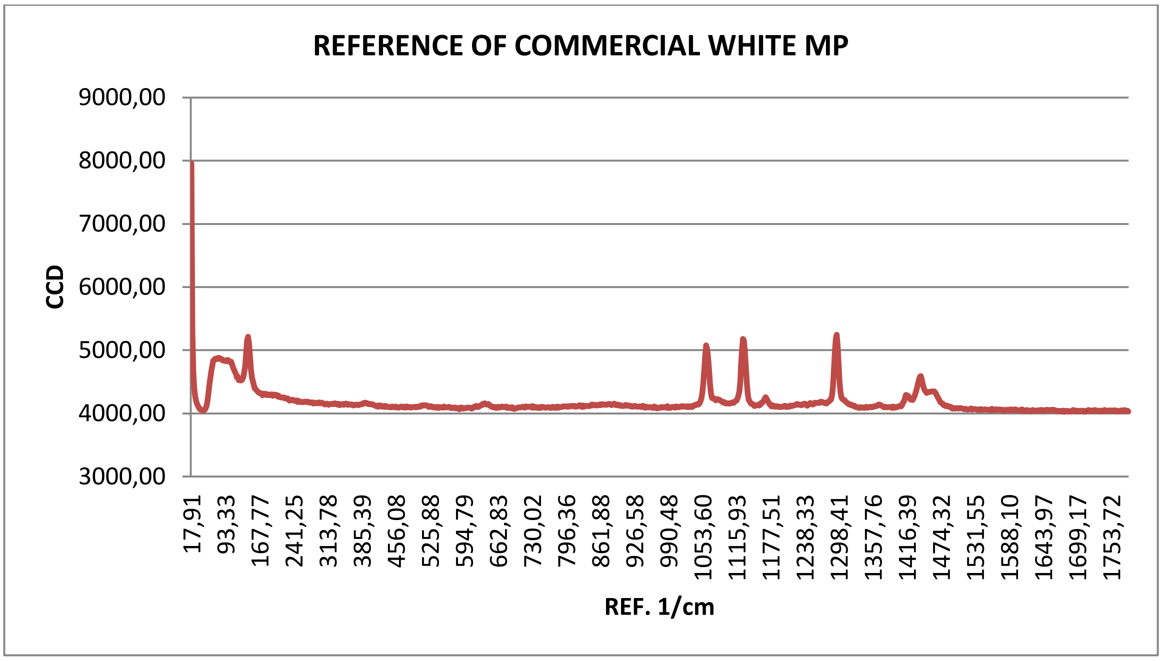

Fig. 57 Raman spectrum of reference commercial white MP

Fig. 58 shows the Raman spectrum of the blue colored reference commercial microplastic with characteristic peaks of 1060.56, 1126.25, 1291.77 and 1439 1/cm.

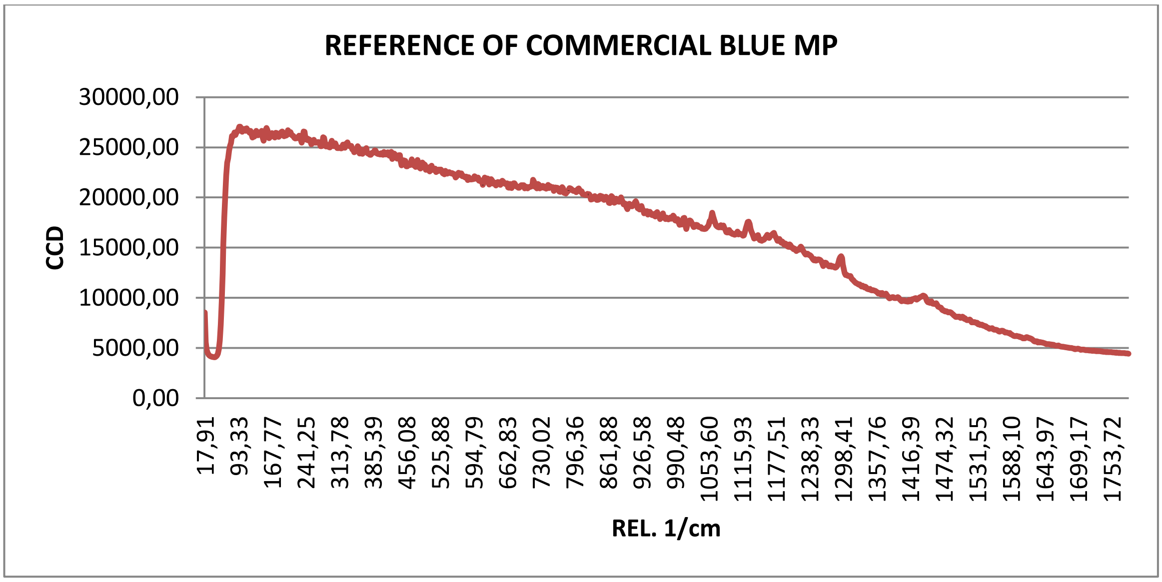

Fig. 58 Raman spectrum of reference commercial blue MP

Fig. 59 shows the spectra of all collected particles from the WWTP: commercial MP in the biological treatment (CMP BIO), first clarifiers (CMP 1°), second clarifiers (CMP 2°), dewatered sludge (CMP PRESS); as well as impurities in the biological treatment (IMP BIO) and dewatered sludge (IMP PRESS), white and blue reference commercial MP (Reference CMP and Ref Blue CMP) and cellulose in the second clarifiers (CELLULOSE 2°).

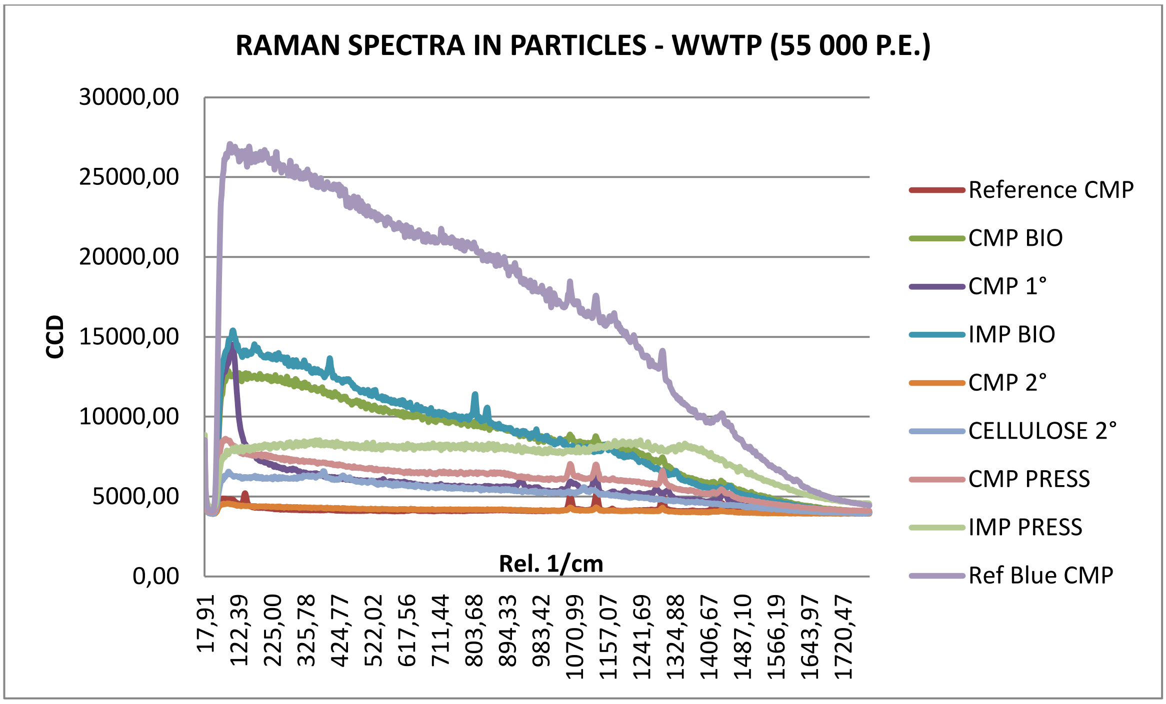

Fig. 59 Raman spectra of impurities and commercial white MP

In Fig. 60 it is possible to observe that many collected particles spectra show similar peaks at 1060.56, 1126.25, 1291.77 and 1439 1/cm. These are similar to the reference spectrum of the commercial MP. With this result the presence of commercial MP in all steps of the WWTP with capacity of 55 000 p.e. is confirmed, although not all the particles are commercial MP.

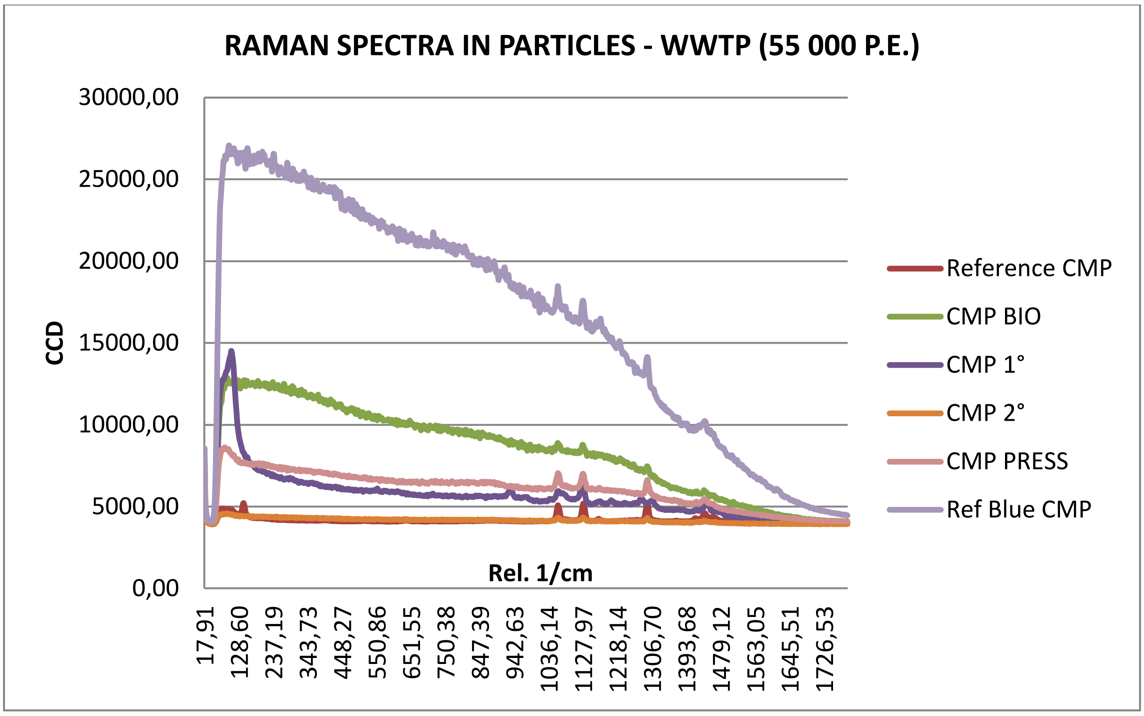

Fig. 60 Raman spectra of collected and reference commercial white MP

In the case of impurities (Fig.61), only a cellulose particle was possible to identify due to its Raman spectrum peak of 380, 1098 and 1120 1/cm, which is similar to that observed by Agarwal (2008); the other impurity particles were not identified.

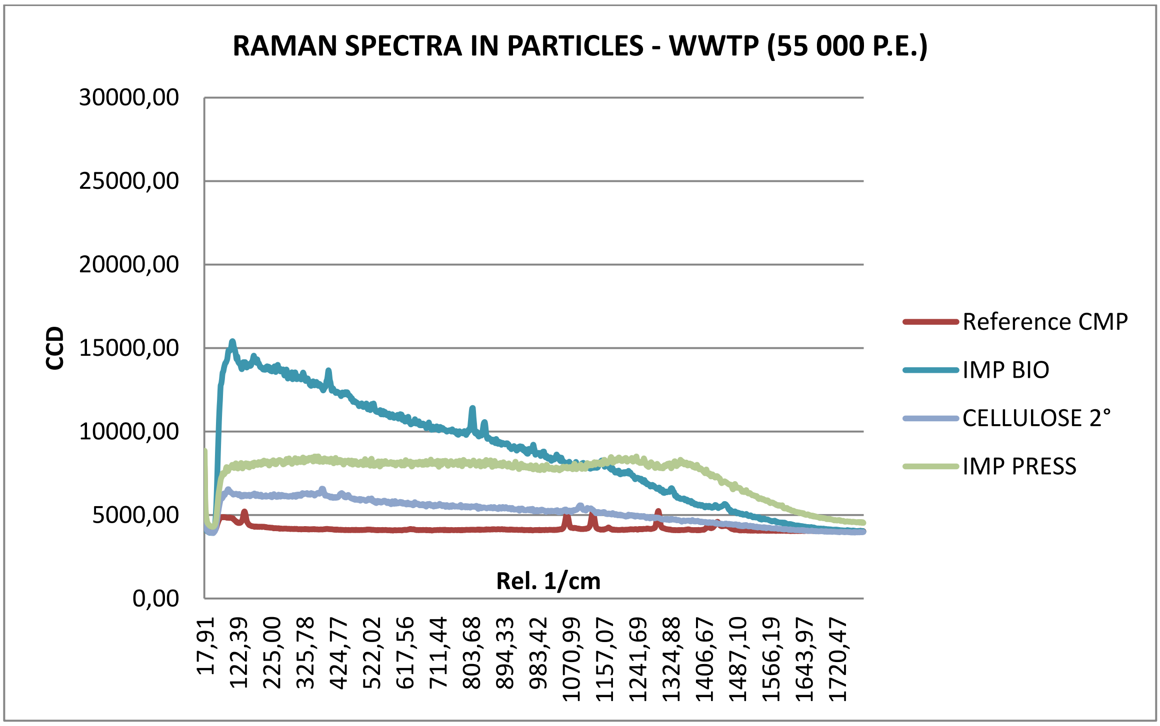

Fig. 61 Raman spectra of impurities in WWTP

Furthermore, the collected microparticles were weighted and counted. With this information the amount of microparticle content in waste water (WW) per cubic meter (m^3) was calculated; Table 14 includes the number of particles and weight in each treatment step and its subsequent calculation per cubic meter.

Table 14 Amount of microparticles in each unit of the WWTP with capacity of 55000 p.e.

Treatment step	**Weight (mg per 1.5 l)**	**N° Particles (per 1.5 l WW)**	**N° Particles (per m^3 WW)**
Influent	0.2	10	6667
Biological treatment	0.3	37	24667
1° Clarifier	0.3	22	14667
2° Clarifier	0.3	24	16000
Effluent	0.2	13	8667

It was observed that amount of microparticles (size of 50 – 745 µm) in the influent and effluent were in the same order of magnitude with 6667 and 8667 per m^3, respectively. The reason for the relative low amount of microparticles in the influent could be caused by the time of the collection of the sample (almost midday), where personal cleaning activities at home are less discharged.

Research carried out in Sweden at the WWTP Långeviksverket in Lysekil showed in the filtrated sample of incoming water, a mean concentration of 15 000 microplastic particles (plastic fibres, plastic fragments and plastic flakes from polyethylene, polyester resin) ≥300 µm per m^3 and in the effluent 8.25±0.85 particles per m^3 (Magnusson and Norén, 2014). Consequently a huge amount of microparticles are retained in the facilities of WWTP (> 99%). In Russia, an evaluation for microplastics in the Central WWTP of Vodokanal in San Petersburg, using a 24 hours composite sample, showed a high retention of microplastic with >95%; from 3787 microplastic particles per litre in the influent to 148 microplastic particles per litre in the effluent (Talvitie and Laurila, 2014).

In a recent study in Germany, an evaluation carried out at twelve waste water treatment plants from Lower Saxony showed that the number of microplastics in effluent depend on the process of treatment and ranged from 29 (WWTP Oldenburg) to a maximum of 13 700 microplastic particles per m^3 (WWTP Holdorf) (OOW, 2014).

5.11. Discussion of digestion of microplastics in mice

During digestion in the intestine, the size of the fluorescent MP particles changed. It could be shown that 43% are in the range of 424 ± 25 µm, 29.4% in the range of 475 ± 25 µm, 14.3% in the range of 373 ± 25 µm, 6.3% in the range of 322 ± 25 µm and 6.3% of the particles were in the range of 271± 25 µm and (Fig. 62). In the cases where a degradation in the particles was observed, the origin of this change is the possibly effect of the digestive fluids content from the gastrointestinal system of the mouse, on the surface of the particles, as the hydrochloric acid HCl (Foster et al., 1983) can catalyse the hydrolysis of the polyethylene (Kutz, 2011).

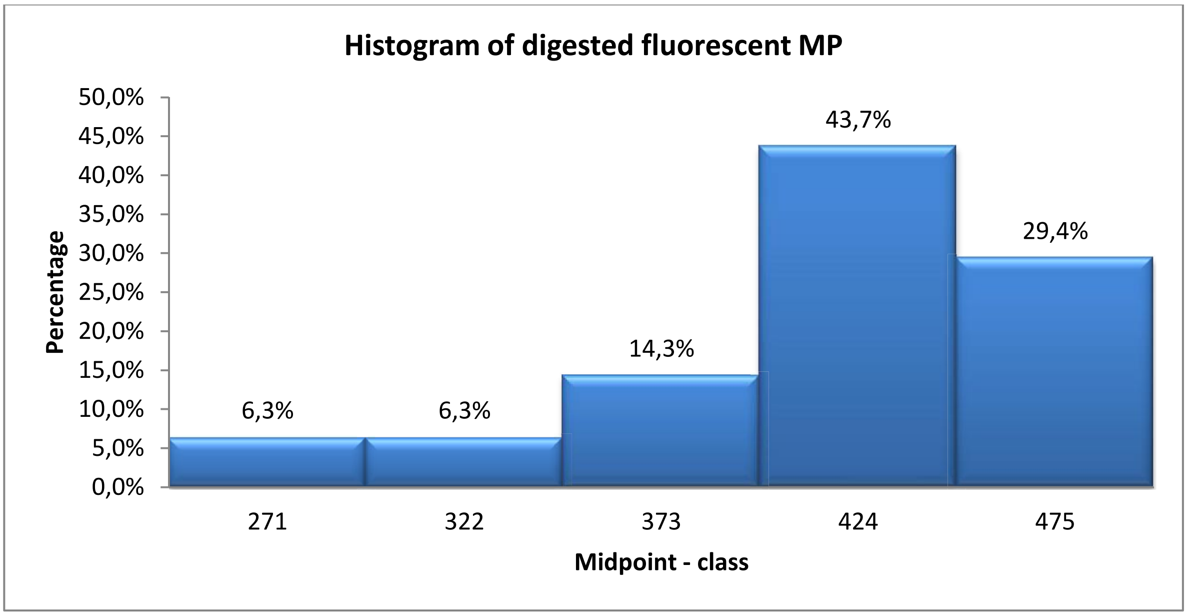

Fig. 62 Histogram of frequencies of digested fluorescent MP (Ø 425 – 500 µm)

In the case of the small digested fluorescent MP (Ø 50 – 75 µm), the original fluorescent MP was already deformed and would not be allocated to the digestion. Nevertheless, the observed little or no change of the particles could be caused by the low retention time during the digestion process. It is proofed that in species as chicken, fine particles of feed are likely to move through the gizzard more quickly and not be held back by the gizzard sphincter (Bundu, 2009).

6. CONCLUSION AND OUTLOOK

6.1. Conclusion

- In the WWTP simulation test the commercial MP kept floating in the beaker glass due to its lighter density 0.91 - 0.94 mg/cm^3. However the fluorescent MP (1.0005 mg/ cm^3) remained at the bottom of the glass.
- The colour of the microplastic samples changed from white to grey in colour, caused by the attached sediments or biofilms on the surface of the particles.
- Regarding the comparison of size between the different treatments in the WWTP simulation test with fluorescent MP and commercial MP; no significant difference was found in the mean size of the particles in all treatments.
- The best collection method was the combination of separatory funnel method with centrifugation with a mean collection efficiency of 94%; and the poorest collection efficiency was obtained by the adapted separatory dispenser with a mean of 15%.
- In the comparison of the collection efficiency, significant differences were found between separatory funnels plus centrifugation technique (SF+C) and separatory funnels (SF), adapted separatory dispenser (ASD) and adapted separatory column (ASC) techniques, respectively.
- Although there was a high performance of the chemical method (CHEM) with 86%, isolation of the particles collected from the remaining impurities was very difficult.
- The Raman spectroscope was useful to distinguish the presence of a specific type of particle, such as commercial MP or fluorescent MP from a mixed sample.
- The separatory funnels with centrifugation technique was used to separate the microparticles from the different facilities of the municipal waste water treatment plant (WWTP) where commercial MP were found in all the facilities of the plant.
- The amount of microparticles discharged from a municipal WWTP to the river or surface water body is approximately 8667 particles/m^3, with about of 50% of commercial MP.
- In a mouse which was fed with fluorescent MP (425 -500 µm), 22.6% of the particles were reduced (deformed) from their original size after digestion.
- In a mouse which was fed with fluorescent MP (50 -75 µm), the majority of particles seemed unchanged from their original size probably due to the low retention time of the particles in the digestive system.

6.2. Outlook

- Optimize the aeration system in the WWTP simulation treatments.
- Prove the efficiency of collection of microplastics in centrifuges with high capacity vials to work with higher amounts of waste water samples.
- For the adapted separatory dispenser technique, ASD; a test with equipment made completely of glass, without any plastic material in the structure, could improve the efficiency of collection of microplastics.
- In the chemical method, search for a technique to improve the separation of fluorescent MP from the interphase solution (hexane and the impurities) or to avoid the formation of impurities.
- Take additional samples from other waste water treatment plants to compare whether the amount of microplastics discharged per m^3 are similar.
- Repeat the digestion test of fluorescent MP in more laboratory mice to validate the deformation effects during digestion.

7. SUMMARY

The pollution in aquatic environments caused by microplastics smaller than five millimetres has recently had wide discussions in the media due to recent publications about interactions within ecosystems and possible impacts on biocoenoses. Most of the knowledge about this subject is still under investigation, for instance the understanding of its behaviour in water, alternatives to separate from mixed samples, techniques to identify the composition, amount of discharge in a water body and so on.

This research work is divided into three main components where the first part describes basic information in regards to microplastics; the second part includes the materials used for the different tests and the methodology applied for each activity; and finally the third part is focused on the results obtained through the different tests undertaken to understand the behaviour of microplastics in waste water treatment., The best technique to recover most of the particles from waste water samples, the amount of microplastics collected per cubic meter and changes on the surface of microplastics after the digestion in mice is investigated.

EXPERIMENTAL PART

The experiment was carried out in the different facilities of the University of Natural Resources and Life Sciences Vienna and the experiment was executed from 9th May 9th to May 31st of October 2014. The mice were provided and fed at the University of Vienna.

The simulation test of waste water treatment was developed in 2 steps: pre-test and simulation test, both of them used a solution (200 ml) of domestic waste water and extracted microplastics from commercially available cleaning products or fluorescent microplastics with Ø 425-500 µm from the company COSPHERIC. In all of the treatments, batch samples, were aerated and supplied with 50 ml of diluted waste water. The behaviour of both microplastics during the aeration and non aeration was observed; and at the end of the test possible changes in size, colour and damages to the surface of the microplastics were evaluated. Statistically the mean size of the fluorescent MP with the original fluorescent MP using the analysis of variance (ANOVA) and Tukey test, with an error rate at 95% (α=5%) was evaluated. To perform this analysis the statistical program R was used.

The separation techniques were developed in three main groups: mechanical separation, density technique (separatory funnels, separatory funnels plus centrifugation, adapted separatory column and adapted separatory dispenser) and chemical technique. In all techniques as a first step, a solution was prepared composed of 500 ml of domestic waste water and 100 units of fluorescent MP (density of 1.005 g/cm3). For the mechanical separation, the process of separation was executed using sieves with different mesh sizes (650, 250 and 125 µm). In the case of density technique, to separate the microplastics, the main product used was a saturated solution of sodium chloride (NaCl) up to 26% of salt in water; the floating microplastics were collected in a glass bottle and afterwards they were isolated and counted. The chemical technique used a solution of Biosorb and hexane with the microplastics; the microplastics trapped in the solvent were counted and isolated. Finally, to compare the collection efficiency of the different separation techniques, statistical evaluation using analysis of variance (ANOVA) and the Tukey test, with an error rate at 95% (α=5%) was undertaken. To perform this analysis the statistical program R was used.

For the analysis of microplastics content in waste water samples, all samples from the different treatment steps of a WWTP with a capacity of 55 000 p.e. passed through the best or most efficient separation technique (Combination of separatory funnels and

centrifugation) and the collected samples of microplastics and impurities were observed, counted and dimensioned by the optic microscope and identified or checked by the Raman spectroscope.
Regarding the evaluation of the digestion of microplastics, two laboratory mice were used; they were fed with a mixture of their normal feed with a small amount of fluorescent MP, after 24 hours the faeces were collected. Once the microplastics were separated from the faeces, they were observed by the optic microscope for detection of surface damages of randomly collected particles and compared with the original microplastics.

RESULTS AND DISCUSSION

For WWTP simulation test, during the time when the test was running, it was observed that the commercial MP was located on the surface of the water or attached to the wall of the glass beaker, while most of fluorescent MP were located at the bottom part of the beaker and a few of them were circulating due to the aeration. In the size characterization, the smallest size found in the fluorescent MP was 433 µm and the maximum 491 µm; and in the commercial MP the smallest size was 45 µm and the maximum 1090 µm. The colour of the microplastic samples changed from white to grey colour caused by the attached sediments and biofilms on the surface of the particles. Regarding the comparison of size between the different treatments with fluorescent MP and commercial MP; no significant difference was found in the mean size of the particles in all treatments.

In the case of the separation techniques, the mechanical separation due to the complexity in separating the microplastics from impurities was discarded. In the density and chemical techniques the efficiency of collection (%) is detailed in the Table 1.

Table 1 Summary of collection efficiency of the different techniques

Technique	1° Replication	2° Replication	3° Replication
Mechanical separation	Discarded	Discarded	Discarded
Separatory funnels- SF	40%	36%	64%
Combination of separatory funnels with centrifugation- SF+C	94%	94%	95%
Adapted separatory column -ASC	66%	41%	54%
Adapted separatory dispenser -ASD	38%	0%	6%
Separation by chemical technique- CHEM	88%	84%	87%

The best collection method was the combination of separatory funnels with centrifugation with a mean collection efficiency of 94%; and the poorest collection efficiency was obtained by the “adapted separatory dispenser” with a mean of 15%. In the comparison of the collection efficiencies, significant differences were found between separatory funnels plus centrifugation technique (SF+C) and separatory funnels (SF), adapted separatory dispenser (ASD) and adapted separatory column (ASC) techniques, respectively.

Regarding the sampling of a waste water treatment plant, the collected microparticle amounts were measured in 1.5 L of WW and calculated per cubic meter of sample (Table 2). Furthermore, by means of the Raman spectroscope, commercial MP were identified in all steps of the municipal WWTP with a capacity of 55000 p.e. The amount microparticles with a part of commercial MP discharged to the river or surface water body is approximately 8667 particles/m3.

Table 2 Amount of microparticles in each unit of the WWTP

Treatment step	Influent	Biological treatment	1° clarifiers	2° clarifiers	Efluent
N° Particles (per m3 WW)	6667	24667	14667	16000	8667

In the case of large fluorescent MP (425 -500 µm) digested in a mouse, deformations were observed and measured. It was observed that the smallest size of the particles was 246 µm and the biggest particle 500 µm; 22.6% of the particles were reduced (deformed) from their original size. On the other hand, the digested small fluorescent MP (50 – 75 µm) kept their size in the average range. However, deformations were observed in both original fluorescent MP (50 – 75 µm) and digested fluorescent MP (50 – 75 µm); considering this observation, determination if the microplastics were damaged during the digestion process is not certain and the majority of the particles were not reduced (deformed) from their original size due to the low retention time of the particles in the digestive system of the mouse.

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9. CURRICULUM VITAE

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10. AFFIRMATION

I certify, that the master thesis was written by me, not using sources and tools other than quoted and without use of any other illegitimate support.

Furthermore, I confirm that I have not submitted this master thesis either nationally or internationally in any form.

Vienna, 8th April 2015,

Miguel, Mucha Torre, *signature*