

NO MICROPLASTICS, JUST WAVES.

Deliverable ACTION B3

TECHNICAL REPORT AND OPERATIVE MANUAL REGARDING THE IMPROVEMENT OF THE TREATMENT PROCESS

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Executive summary

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The following Report provides a protocol developed at Water and Wastewater Environmental Engineering Lab (WWEELab_UNIVPM) for the samples collection and at Ecotoxicology and Environmental Chemistry lab (DISVA_UNIVPM) for the extraction and identification of microplastics (MPs) and microfibers (MFs) from water and sludge samples collected from different treatment steps of WWTPs/DWTPs.

The protocol includes methodologies already described in scientific literature and directlyexperienced with analysis of MPs/MFs in those matrices (Magni et al., 2019; Pittura et al., 2021).

The protocol was developed and followed during the sampling activities performed under the LIFE BLUE LAKES project, concerning the pilots in the wastewater and drinking water treatment plants located in Garda and Castreccioni lake districts.



MPs and MFs extracted by water and sludge collected in WWTPs



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Cover photo: Microplastics extracted from Wastewater Treatment Plant, Italy

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1. Definitions

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Microplastics (MPs): any synthetic solid particle or polymeric matrix, with size ranging from 1 μm to 5 mm, consisting of either items that are manufactured to be of microscopic dimensions (primary) or that are formed from the weathering and fragmentation of larger plastic waste items, which are insoluble in water at20^oC (Bessa et al., 2019).

Microfibers (MFs): any natural or artificial fibrous materials of threadlike structure with a diameter less than 50 μ m, length ranging from 1 μ m to 5 mm, and length to diameter ratio greater than 100. Microfibers are released or shed to the environment from all kinds of fibrous materials, such as clothes, agricultural, industrial, and home textiles, and some textile products, semi-manufactured goods, or accessories used in other fields, during production, use, and end-of-life disposal (Liu et al., 2019).

For the classification of extracted items, elaboration and presentation of results, we'll consider MPs and MFs separately, given the definitions that highlight different origins and fate; consequently, they could require different mitigation strategies.

2. Materials

Consumables

- Steel container
- Glass Beakers
- Laboratory wash bottles
- Pump-up spray bottles
- Tanks (20-30L)
- Glass petri dishes
- 8 μm nitrate cellulose membrane
- 0.45 µm acetate cellulose membrane
- Glass laboratory cylinders (250 ml)
- Stirrer plates
- Magnetic stir bars

Reagents

- Distilled water
- Saturated solution of sodium bromide¹ (NaBr -1.4 g/cm³): 650 g/L of deionized water. Due to the elevated cost and of the salt, the solution might be reused, after filtration on a 0.45 μm acetate cellulose membrane: density is checked measuring the weight of the solution, and eventually readjusted adding salt.
- 15% hydrogen peroxide solution: it is prepared by diluting 30% hydrogen peroxide SIGMA-ALDRICH,cod. H1009) in deionized water (1:1, v:v).

¹ From the wide range of density separation solutions described in literature (Figure 1) we are using NaBr as a good compromise among price, toxicity, density of solution. We are moving for using NaI for a denser solution (1.8 g/cm3) inorder to improve extraction.



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All the solutions must be vacuum filtered on a 0.45 µm acetate cellulose membrane to reduce external contamination.

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Figure 1: Common density separation solutions (Frias et al., 2018)

Equipments

- Metal sieves with mesh size of 5 and 0.05 mm
- Cartridge-filter system with mesh size of 0.05 mm _
- Automatic sampler -
- **Pumping equipment** _
- Copper/brass connections _
- Filtration apparatus:
- Stereomicroscope (GZ808/810 Optech with Optech IS 4K-8 digital camera and Image -View softwarefor image analysis)
- Oven (work temperature 50°C)
- FTIR Microscope System (PerkinElmer Spotlight 200i



Figure 2: Components of the filtration apparatus:1) filtration ramp (speedflow); 2) glass filter holder (diam 47 mm) + Max volume 500 ml; 3) vacuum tube HW/55 diam mm 8 x 15; 4) non-return PP valve; 5) vacuum trap 2L; 6) vacuum tube HW/55 diam mm 8 x 15; 7) second Vacuum trap 2L (to protect the pump); 8) additional filter to protect pump (optional); 9) vacuum generator RCK400 34L/min



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3. Sampling method

3.1. Sampling methodology assessment

Different sampling protocols were defined depending on the water matrix to be analyzed: a) Drinking water,

b) Wastewater or c) Combined Sewer Overflows.

Literature research was carried out to evaluate the most appropriate ranges of sampling volumes, choose between grab or composite samples and select the sampling points.

For the investigation of MPs in drinking water treatment plants (DWTPs) higher volumes (e.g., 1000 l) of watershould be sampled compared to wastewater. Composite sampling is recommended using steel buckets and sieves (e.g., 50 µm mesh size).

As concern wastewater matrix, best practices suggest continuous composite sampling with as much volumeof wastewater as possible, using steel buckets and sieves (50 μ m mesh size). Volumes usually range between 30-300 l.

For Combined Sewer Overflows (CSOs) the same methodology as the one applied for wastewater can be followed.

A list of sampling procedures collected from literature is reported in Annex A.

For the detection of seasonal variability, for each water sector different sampling campaigns must be planned in different periods of the year (3 minimum number).

The sampling procedures for different water matrix are schematized in Table 1.



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Table 1: Sampling procedures depending on the water sector

Sector	Plant	Unit	Type of sample	Min. volume	Min. number of samples	Notes
		Influent	Min 1-2 h average sampling	1000 l	3*	*Min. number of sampling campaigns defined to detect seasonal variability.
		Effluent from each operative unit	Min 1-2 h average sampling	1000	3*	*Min. number of Sampling campaigns defined to detect seasonal variability.
Drinking	Potabilization Plant	Final Effluent	Min 1-2 h average sampling	1000	3*	*Min. number of Sampling campaigns defined to detect seasonal variability
water supply		Sludge**	Grab	51	3*	 *Min. number of Sampling campaigns defined to detect seasonal variability. **Sludge is considered as liquid at maximum TS% of about 5%TS.
	Distribution	Final Distribution**	Min 1-2 h average sampling	1000	3*	 *Min. number of Sampling campaigns defined to detect seasonal variability. **Min. Number of Sampling points has to be set according to the distribution network complexity.
Sewage system	Combined Sewer Overflow	cso	Grab or Average sampling	50 **	3*	*Min. number of Sampling campaigns defined to detect seasonal variability. **Min volume could be very variable depending on the quantity overflowed.



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	Wastewater Treatment Plant (WWTP)	Influent	Average sampling	30-300 I**	3*	*Min. number of Sampling campaigns defined to detect seasonal variability. **Min. volume could be very variable depending on water characteristic.
Wastewater	Wastewater Treatment Plant	Effluent from each operative unit	Average sampling	30-300 **	3*	 *Min. number of Sampling campaigns defined to detect seasonal variability. **Min. volume could be very variable depending on water characteristic.
Treatment	Wastewater Treatment Plant	Final Effluent	Average sampling	30-300 **	3*	 *Min. number of Sampling campaigns defined to detect seasonal variability. **Min. volume could be very variable depending on water characteristic.
	Wastewater Treatment Plant	Sludge**	Grab	51	3*	 *Min. number of Sampling campaigns defined to detect seasonal variability. **Sludge is considered as liquid at maximum TS% of about 5%TS.



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3.2. Sampling apparatus

Depending on water characteristics and operative plant conditions, different sampling system can be used:

a) sieves battery, b) cartridge filters or c) automatic system (prototype).

3.2.1. Sieves battery

Sieves Batteries are made up by steel sieves of 5 mm, 2 mm and 50 μ m mesh size (ISO 3310-1:2000). They are used in case of grab samplings, eg., for wastewater, since this procedure is feasible only for limited amounts of volumes. A pumping system is required to convey the water flow to the sieves battery. The pumping system is made in non-plastic components, to avoid plastic contamination. In particular, the pump is in steel, and the connections are made in copper or brass.

Method description and sampling procedure

Filter the wastewater sample, e.g., 25 liters, through the steel sieves battery of 5 mm, 2 mm and 50 μ m meshsize (ISO 3310-1:2000): rinse the particles on 2 mm and 50 μ m sieves into glass jars with deionized water and subsequently filter onto cellulose nitrate filters (Sartorius Stedim Biotech, Ø 47 mm, 8 μ m pore size) using a vacuum pump. After filtering, remove the 5 mm mesh sieve: this sieve is used in battery with the 0.05 mm to exclude any plastic larger than 5 mm that may be present in the sampling phase. The material held on the5 mm sieve must not be recovered. Use the spray bottles and / or manual pressure pump with deionized water to collect the material held on the 0.05mm mesh sieve. The recovered material must be stored in glassjars closed with a lid and then with parafilm. If the smaller glass jars are sufficient to hold the sample, they can be used in place of the 1 L jars. Identify the sample by writing directly on the jar or on white tape / packs attached to the container. The preserved sample will then be processed later in the laboratory. To control any contamination, place a small jar and fill with deionized water up to $\frac{3}{4}$ of the volume. Leave the jar open during all the time in which the water is passed through the sieves and during the recovery operations of thematerial from the sieve. Close the jar with the lid and then with the parafilm.



Figure 3: Procedure for recovering samples collected by pumping and sieving battery

Sludge samples

In case of sludge samples, grab measurements are performed and thus the sieving battery is used. Before the filtration, sludge samples are collected using automatic samplers or steel bucket and



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maintained in tanksuntil the laboratory processing. Samples are stored at -20°C if not immediately processed.

3.2.2. Cartridge filter

Cartridge filters are preferably used when there is the possibility to connect the filtering system directly to the plant pipes. Direct filtration allows to obtain composite samples during the filtration time and can processbigger amounts of volumes.

The filtering device consists of a metal container inside in which there is a cylindrical filter with a mesh of $50\mu m$. The filtering cartridge is made of stainless steel micro-stretched sheet. The filter area is 0.034 m².

The connections between the cartridge filter and the plant pipeline are made with copper tubes to avoid anyplastic contamination.



Figure 4: Cartridge filter

Table 2: Technical specifications of cartridge filter



Method description and sampling procedure

Connect the filter to sampling tap of the DWTP (made of copper) via specific piping and connections made ofbrass. Adjust the flow rate of the water and record it manually. Calculate the necessary time to filter, e.g., 1000 L water, through 50µm steel stainless filter *in situ*. Once the filtration is completed, place the filter in asteel bowl or in glass beakers and rinse the filter with deionized water to recover the particles retained on the filter. If necessary, make use of washers and/or manual pressure pumps that could facilitate / optimize the operation. Place an empty jar filled



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with deionized water and leave open during the washing step for each sample. This will be used as control to represent any contamination from air. Once the filter is completely cleaned, pour the washed water into a glass jar and rinse the bowl into the jar to recover all particles. Close the jar with its lid and then using parafilm. Name the samples and each control jar, then bringto the laboratory for processing.



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Figure 5: Cartridge filter application

3.2.3. Prototype of automatic sampler for MPs

For a better quantification of MPs in wastewater, a prototype of autosampler (Figure 6) has been designed and built, in order to optimize sampling procedures.



Figure 6: Prototype of automatic sampler for MPs

The automatic sampling system, designed and developed under the project LIFE BLUE LAKES,



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consists of a timed sampling pump, a cartridge filtration system (mesh size up to 25 μ m, filtering area 1000 cm²), an electromagnetic flow meter and control and data recording plc. The device allows to acquire a composite sample (24h) and to filter higher amount of water, until the filter is not completed clogged. The flowrate measurements will permit to knowexactly how much volume is filtered. Moreover, the automatic system is designed to be upgraded with an additional cartridge filter of 25 μ m to detect lower sizes MPs.





Flowrate and pressure can be set by the regulation of the apposite valves. The device can pump up to 50 liters/minute in normal working conditions, with a pressure on the filter of 1-2 bar.

The logic of the sampler is schematized in Figure 8. The device can be set to work under time or combined time and flowrate controls. When it is set on time control, the pump is activated only at specific time intervals, with cycles of filtering and cycles of pause. In this way it is possible to perform averaged samples. On the other side, when the device works under time and flowrate controls, the pump automatically stops its filtering/pause cycles once the flowrate reaches a pre-defined lower threshold, meaning that the filter reached a definite clogging level.



Figure 8: Automatic sampling system for MPs

In order to test the automatic sampler for real applications, sampling campaigns were first performed in a full-scale WWTP. Different short tests with wastewater were carried out to understand the minimum representative volume for microplastics quantification in WWTPs. In particular, from 25 L to 500 L of wastewater influent and from 25L to 12000 L of treated effluent were sampled (Table 3 and Table 4). The results of MPs count show that increasing the sampling volume the microplastics concentrations decrease, until they reach a stable level. Comparable values (Figure 9 and





Table 3: Sampling of different volumes of wastewater influent and effluent and MPs detection – influent

Sampled Volume (L)	MPps/L	TSS mg/L
25	1.800	~200
119	0.580	
310	0.610	

INFLUENT





385	0.160	
521	0.146	197



Figure 9: MPs concentration in influent wastewater related to different sampling volumes

Table 4: Sampling of different volumes of wastewater influent and effluent and MPs detection – effluent

Sampled Volume (L)	MPps/L	TSS mg/L
25	0.560	<5
88	n.d.	3.6
1898	0.047	2
4828	0.009	3
11758	0.037	2.2

EFFLUENT



Figure 10: MPs concentration in treated effluent related to different sampling volumes

Moreover, the clogging phenomena of the cartridge filters was related to the influent concentrations of total suspended solids (TSS). The graph in Figure 11 shows the maximum filtered volume at different solids concentrations, both by using 50-micron and 25-micron filters.



Figure 11: Maximum filtered volume at different solids concentrations



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4. Treatment of sample for MPs and MFs extraction

4.1. Water sample

After the recovery phase, sample is filtered onto cellulose nitrate filter (\emptyset 47 mm, 8 μ m pore size) using a vacuum pump. Obtained filter is recovered in a petri dish, covered with 15% H2O2 and maintained at 50 °C overnight to remove organic matter. If necessary, filtration of a single sample is performed onto more than one filter.

A direct vacuum filtration is preferred; however, a density separation can be applied for complex samples: it is carried out in 250 mL cylinders, stirring the samples with saturated NaBr salt solution (density 1.4 g cm3) for 30 min and leaving to settle the mixture overnight.

4.2. Sludge sample

A minimum of 4 litres is usually processed for analyses of MPs and MFs.

Sample, distributed in glass beakers, is dried in stove at 50 °C and subjected to a first organic matter digestionwith a 15% H2O2 solution at a temperature of 50 °C. Time of this first treatments depends on nature of sludge(e.g., content of water). Then, a density separation procedure is carried out in 250 mL cylinders, stirring the samples with saturated NaBr salt solution (1.4 g cm3) for 30 min (Frias et al., 2018) and leaving to settle the mixture overnight. The supernatant was then vacuum filtered onto cellulose nitrate filter (\emptyset 47 mm, 8 µm pore size) and filters were treated with 15% H2O2 solution for removing the residual organic matter:



Figure 12: Schematic representation of sludge sampling and treatment for microplastic and microfiber extraction



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5. Quality Assurance/ Quality Control

During all phases of sampling and processing, appropriates measures are taken to prevent and check the external contamination by MPs and MFs.

The operators wear nitrile gloves, cotton clothes and lab coats. Glass and metal equipment are used whenever possible and, before use, they are rinsed with ultrapure water, additionally cleaned with compressed air and covered with aluminium foils, which are also kept during stirring, decantation and filtration operations.

Steel sheaves and cartridge filters are carefully washed first with tap water and then three times with pre- filtered deionized water, to avoid cross-contamination.

The extraction and characterization procedures for MPs and MFs analysis are performed in a dedicated laboratory, where the presence of staff is limited to a maximum of two people at the same time. Work benches are cleaned with ethanol pure grade before starting the activities and between each processing steps. One blank is run for each sample: controls consist of filtered water that follow the same treatments assamples.

The yield of the density separation procedure, using NaBr salt for sludge fraction, has been evaluated testingdifferent kind of sludges: excess sludge (I SLUDGE), activated sludge (AerWAS), sludge after dewatering (DEWAT SLUD), granular sludge (AnaEXC SLUD). A total of 12 particles, two for each representative polymer (polyethylene, polypropylene, polystyrene, polyethylene terephthalate, nylon, polyisoprene rubber) in the size range of 0.5-1.5 mm, were spiked into samples and blanks, starting from the first organic matter digestion step. The particles of polyethylene, polypropylene and polystyrene were standard materialspurchased from a plastic company, while those of polyethylene terephthalate, nylon and polyisoprene wereobtained by cutting a plastic bottle, a fishing wire and an elastic band, respectively. All of them were photographed and measured, and IR spectra were acquired before and after the test, showing no appreciablechanging in shape, size and polymer characteristics of recovered particles. The resulted extraction yield of spiked MPs was 100% in blank samples, 95% in I SLUDGE, 92% in AerWAS, 96% in DEWAT SLUD and 98% in AnaEXC SLUD.



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6. Identification of MPs and MFs

Filters resulted from the extraction procedure is visually examined using a stereomicroscope (GZ808/810 Optech with Optech IS 4K-8 digital camera). All items resembling plastic and fibers were manually collected using a tweezer and transferred onto a clean cellulose acetate membrane (0.45 μ m pore size) located on a microscope slide that is subsequently used as support for the μ FT-IR analyses (Fig. 6).



Figure 13: Slide with the items isolated from the sample and analyzed by μ FT-IR in ATR mode. Area where items are located is delimited by a circle to facilitate the searching during characterization

Criteria taken in consideration for identification and classification of MPs and MFs include physical (size, shape, color) and chemical properties.

6.1. Physical properties

6.1.1. Shape

Sorted items are categorized in:

Fiber-shaped (MFs): thread-like structures with not regular diameter and frayed ends (Magni et al., 2019)that can assume trilobal, ribbon and L-shapes (Cesa et al., 2017).

Particle-shaped (MPs) including five main typologies:

- 1. Line: particle with regular diameter throughout the length and not frayed ends in respect to fibers(Magni et al., 2019)
- 2. Fragment: rigid, thick particle of irregular shape (Lusher et al., 2017)
- 3. Film: planar and flexible particle, considerably smaller in one than in the other dimensions (Hartmannet al., 2019)
- 4. Sphere: particle with every point on the surface having the same distance from the center (Hartmannet al., 2019)
- 5. Glitter: hexagonal geometry disc, remanence of metallic foil and iridescence can be observed(Yurtsever et al., 2019)



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Figure 14: Example of items (A) fibers-shaped and particle-shaped (B-F) (B) lines, C) fragments, D) films, E) sphere, F) glitters).

6.1.2. Size

Items are measured using an image analysis software (Image View): diameter and length are recorded for fibers, instead particles are measured on the basis of the largest dimension following the most widespread criterion (Hartmann et al., 2019).

Once single measurements were recorded, items are categorized in size classes that are identified in the range between 5 mm (i.e., the maximum dimension of microplastics for definition) and 20 μ m (i.e., the limit size for identification using the FTIR microscope system Spotlight 200i).

6.1.3. Color

The color categories are those suggested by Bessa et al., (2019). Colors such as purple, pink, grey, yellow or brown should be placed under the nearest color described or in the category *Others*.

- 1. Black ■
- 2. Blue
- 3. White
- 4. Transparent



6.2. Chemical properties

6.2.1. Polymer

Identification of polymer is performed by μ FTIR spectroscopy in attenuated total reflectance mode, using a Spotlight 200i FT-IR microscope system (PerkinElmer) equipped with Spectrum Two and driven by Spectrum10 software. After background scans, each sample spectrum is recorded performing 32 accumulations, ranging from 600 to 4000 cm⁻¹ with the resolution at 4 cm⁻¹. When the spectrum is not resolved at first acquisition, more than one measurement is conducted per



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samples. IR spectrum of the cellulose acetate membrane is aquired and substracted to that of each sample in order to avoid the overlay of spectra. The output spectra are subsequently subjected to a spectral search against reference libraries of polymer spectra represented by PerkinElmer database (ATRPolymer, polyATR, FIBERS3, plast1, RP, POLIMERI, PIGMENTI, resin and PERKIN1 libraries is usually selected), by the database compiled within the framework of the JPI-OCEANS project BASEMAN (Primpke et al., 2018) and by personal created ones. For accurate identification, the matchfactor threshold was calculated as 0.70 and a lower level (0.60-0.70) was accepted after careful examination peaks characteristics. Synthetic polymers (petroleum-based, biobased and hybrid polymers), copolymers and composites are considered as plastic.



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7. Quantification and reporting information

In the quantification of MPs only those of synthetic nature are considered; instead, data on quantification of MFs include both the natural and synthetic ones.

Results on quantification and characterization are expressed as follow:

- the average number (± standard deviation) of MPs and MFs extracted from sample. Concentration is related to L of sample (or mm³) or gram of sample, it depends on the typology of analysed matrix.
- percentage contribution of each shape, size class, color, polymer type on the total MPs and MFsextracted.
- in particular, for MFs the percentage of those natural and synthetic on the total are highlight.



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8. Application of the protocol in wastewater and drinking water treatment plants

For the pilot actions foreseen in the activity B3 of LIFE BLUE LAKES project, 3 drinking water treatment plants (DWTP) and 2 wastewater treatment plants (WWTP) were selected.

The first DWTP was located in central Italy, near Lake Castreccioni, and is managed by the water utility ACQUAMBIENTE. The other 2 DWTPs, respectively Garda Molinet and Castelletto di Brenzone, were selected in Lake Garda district and are managed by the water utility AGS. The plants were selected because they are characterized by different configurations of treatment units and processes, including ozonation, sand filtration, activated carbon adsorption, membrane filtration and chemical disinfection.

For WWTPs, 2 plants were selected in Garda district, respectively Limone Tremosine and Peschiera del Garda WWTPs, managed by the water utility Acque Bresciane. They were selected for their different size (From 180000 to 330 000 Population Equivalent) and configurations, respectively: pretreatments, attached-growth biological unit, flotation and rotary tertiary filtration for Limone WWTP and sand removal, conventional activated sludge, coagulation, lamellar sedimentation, sand filtration, UV disinfection in Peschiera del Garda. For all the sites, the responsible water utilities were contacted to plan the sampling campaigns and technical visits were performed to organize all the requirements.

Three sampling campaigns in Castreccioni DWTP (Figure 15) were carried out in different periods (summer, winter and spring season).



Figure 15: Sampling in Castreccioni DWTP

For each campaign, the following points were sampled (Figure 16): 1) influent from dam at 2 different quotes; 2) effluent from the pre-ozonation, 3) effluent from flocculation, 4) flocculated sludge, 5) effluent from sand filtration, 6) backwash of sand filters, 7) effluent from post-ozonation, 8) effluent from GAC absorption, 9) final effluent and 10) two different points in the distribution system.

For each sample, 1000 L were filtered with a mesh size of 50 μ m. Stainless-steel cartridge filters were used for every liquid sample, except for the effluent from flocculation, where pump and sieves were used. Differently, for sludge, grab sample of 20 were collected.





Figure 16: Castreccioni DWTP layout

Three sampling campaigns were performed in Garda Molinet and Castelletto di Brenzone DWTPs in different seasons (November, June-July, September).



Figure 17: Sampling in Garda Molinet and Brenzone Castelletto DWTPs

In particular, for Garda Molinet (Figure 18) were sampled: 1) influent, 2) effluent from ozonation, 3) effluent from filtration, 4) final effluent and 5) one point in the distribution network.



Figure 18: Garda Molinet layout

In Brenzone were sampled: 1) influent, 2) backwash of filtration, 3) backwash of membrane filtration, 4) effluent, 5) 1 point in the distribution network.



Figure 19: Brenzone Castelletto DWTP layout

For each sample, 1000 L were filtered with stainless-steel cartridge filters with a mesh size of 50 μ m. Grab sample of 20 L of sludge was collected.

For wastewater plants, 1 sampling campaign was performed using the prototype of autosampler.

At Limone Tremosine WWTP (Figure 20) were sampled: 1) influent, 2) pre-treatment effluent, 3) flotation effluent, 4) tertiary filtration effluent, 5) backwash and 6) sludge.



Figure 20: Limone Tremosine WWTP





At Peschiera del Garda (Figure 22)were collected: 1) influent, 2) clarified effluent, 3) effluent from



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lamellar settlers, 4) final effluent and 5) sludge. For each sample from 450 L to 100 L were filtered, according to the solid content of the water matrix. Differently, grab samples of 20 L of sludge were collected.

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Figure 22: Peschiera del Garda WWTP layout



Figure 23: Sampling in Peschiera del Garda WWTP

The prototype allowed to carry out action B3 - Phase 1 (Sampling in a real environment) with more adequate methods and more reliable results. This aspect was supported also from the literature review presented in Action A2 in the first phase of the project. Also considering the COVID emergency, the automatic system permitted to reduce the direct contact with water and wastewater, comparing with other conventional methods. Moreover, the new system has ensured less cross-contamination of MPs from external environmental conditions.

The sampling campaigns for DWTPs were concluded in September 2021. First results on MPs concentration in the different treatment units of DWTPs show: 1) the importance of the filtered amounts, 2) the seasonal variability in terms of MPs concentration and 3) the MPs removal efficiencies achieved by the treatment processes. Also, similar evidence was detected for WWTPs. The automatic sampling system was successfully tested in the two full-scale plants.

The analytical results will allow to develop and define a specific "Analytical protocol for process control", that will be reported in the next Deliverable.



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Annex A – Literature review on sampling methods

Drinking water

Drinking water has been suspected as a potential source of microplastics to human according to recent limited researches. The investigation of microplastics in drinking water treatment plants (DWTPs) are much more limited and only few studies exist as given below. Sampling methods are similar to wastewater. However, there have been questions regarding the quality of these occurrence studies since there are no standard sampling, extraction and identification methods for microplastics.

Most of the sampling campaigns conducted grab sampling, while many studies reported sampling volumes between 1 and 5 Liters (Wang, Lin, & Chen, 2020; Eerkes-Medrano, Leslie et al. 2017).

The range volumes sampled on site from literature is very variable and the data changes from 1 l up to 2500 l. In terms of number of particles with dimensions between 50 and 150 microns, the values averaged between 0.001 and 0.007 MPPs /L in the considered DWTP.

<u>SAMPLING</u>	VOLUME	TYPE of	METHODS OF SAMPLING AND DETENCTION	FREQUENCY or	<u>Ref.</u>
<u>POINTS</u>		<u>SAMPLE</u>		<u>num[°] of samples</u>	
Raw and treated	1L	Grab samples	Digestion with 30% hydrogen peroxide (H_2O2) for 24 h.	3 times /winter	(Wang, Lin, and
drinking water			Filtration through a series of 5 μ m (PTFE) membrane filters		Chen 2020)
(after each			followed by a 0.22 μm pore sizes. The purpose of this two-		
process)			filtration was to descend mesh size to pass the entire sample		
			through the filter without clogging. These filters were used for		
			scanning electron microscope (SEM) analysis of retained		
			particles. For each sample, a volume of 250 ml was separately		
			filtered for quantitative and qualitative analysis of particles.		
			The filters after drying in an oven at 30°C for 30 min were stored		
			in covered glass petri dishes for subsequent analysis. DXR2		
			micro-Raman imaging microscope system (Thermo Fisher		
			Scientific, USA) was employed (532 nm laser, laser spot size		

Table A1: List of sampling methods for Drinking water



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				around 0.5 μ m, Raman shift 50–3550 cm_1, spectral resolution of 5 cm ⁻¹) for qualitative analysis of particles.		
Raw and treated drinking water	27L sample	each	Average daily samples	Wet peroxide oxidation was conducted to remove organic material, Filtration through a series of 5 μ m (PTFE) membrane filters followed by a 0.22 μ m pore sizes. The purpose of this two-filtration was to descend mesh size to pass the entire sample through the filter without clogging. These filters were used for scanning electron microscope (SEM) analysis of retained particles. For each sample, a volume of 250 ml was separately filtered for quantitative and qualitative analysis of particles. The filters after drying in an oven at 30°C for 30 min were stored in covered glass petri dishes for subsequent analysis	3 times within a 24-hour period (every 8 h) and repeated three times in winter period	(Pivokonsky et al. 2018)
Raw and treated drinking water	9-27 L			Scanning electron microscopy analysis for particle counts; both micro-Raman spectroscopy and μ -FT-IR were used for identification of particles with size of 1e10mm and>10mm		(Eerkes- Medrano, Leslie, and Quinn 2019)
Raw and treated drinking water	1000 L		Grab samples	Samples directly sieved, tap water require no digestion.		(Koelmans et al. 2019)



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Raw and treated	300-2500 L	3μm stainless	Residual raw water and drinking water was removed from t e	24 samples	(Mintenig et al.
drinking water		steel cartridge	filter units by using filtered (0.2 μ m) compressed air. Then, the		2017)
		filters	units were filled again with diluted hydrochloric acid (Carl Roth		
		4 7/8",	GmbH & Co. KG, Germany, 0.2 µm filtered, pH=2) to dissolve		
		Wolftechnik,	calcium carbonate and iron precipitates. After 24 h the filter		
		Germany	units were emptied, the cartridge filters removed from the		
			units and rinsed with Milli-Q and ethanol (30%, Carl Roth GmbH		
			& Co. KG, Germany, filtered over 0.2 μ m). The retentate was		
			collected on 3 μ m stainless steel filters (47mm in diameter) that		
			were subsequently transferred into glass bottles and covered		
			with 30 mL hydrogen peroxide (35%, Carl Roth GmbH & Co. KG,		
			Germany). The bottles were closed using aluminium foil and		
			incubated for 24 h at 40 °C. Finally, each sample was enriched		



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	onto a 0.2 µm aluminium oxide filter (Anodisc 25 mm,	
	Whatman, U.K.) by using an in-house fabricated filter-funnel	
	with an inner diameter of 11 mm. The filters were dried at 40	
	°C in half closed glass petri dishes for subsequent analysis.	

Table A2: Literature references for drinking water

REFERENCE	Plant	Place	Sample	Sampling point	Volume (L)	n° MPS/l	Size	Plastic Typology
				Influent	300-1000	0.003	50-150 μm	
	DWTP 1	Nethen	grab	Effluent	1200-2500		50-150 μm	
				Distribution		0.001	50-150 μm	
			grab	Influent	300-1000	0.007	50-150 μm	
	DWTP 2	Holdorf		Effluent	1200-2500	0.001	50-150 μm	
				Distribution		0.003	50-150 μm	
(Mintonia et al	DWTP 3	Grossenkneten	grab	Influent	300-1000		50-150 μm	PEST, PVC, PE, PA
2019)				Effluent	1200-2500		50-150 μm	and epoxy resin
			grab	Influent	300-1000		50-150 μm	
	DWTP 4	Sandelermoens		Effluent	1200-2500	0.001	50-150 μm	
				Distribution		0.0015	50-150 μm	
			grab	Influent	300-1000	0.001	50-150 μm	
	DWTP 5	Thuelsfelde		Effluent	1200-2500		50-150 μm	
				Distribution			50-150 μm	



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Wastewater sampling

Even though the fate and effects of microplastics in the marine environment has been studied quite extensively for the last few decades (Hidalgo-Ruz et al. 2012), the special case of wastewater treatment plants (WWTP) as a sink of microplastics, have started to gain more attention only during recent years (Carr, Liu, and Tesoro 2016; Dris et al. 2015; Magnusson and Norén 2014; Mahon et al. 2017; Mason et al. 2016; Michielssen et al. 2016; Mintenig et al. 2017; Murphy et al. 2016; Talvitie et al. 2015, 2017). Biggest concern regarding the determination of the MPs occurance in WTTPs is that the comparison between the concentrations is difficult because of the variable sampling techniques and identification methods employed (Murphy et al. 2016). The detection of microplastics in WWTPs usually contains three steps as follows: sample collection, sample pretreatment and microplastics can be present in both wastewater and sewage sludge (Sun et al. 2019). Most of the researchers have based their results on wastewater or sludge samples collected only for a short period of time.

SOURCE	PROCESS	VOLUME	METHOD	REFERENCE
Municipal wastewater	Grit-grease Primary clarifier Activated sludge Secondary clarifier effluent	60.1 L 59.3 L 103.4 L 143 L	 Grabbed in glass bottles, both in the morning and in the afternoon Filter through diameter 110 mm, pore size 0.45 mm 	(Bayo, Olmos, and López-Castellanos 2020)
Municipal wastewater	Influent (after 6mm screen) After the primary clarification After the disinfection	4-30 L	Collected with a 10-L stainless steel bucket attached to a metal wire and poured to a cascade of two test sieves with mesh sizes of 0.25 and 5.0 mm	(Lares et al. 2018)
Municipal wastewater	Influent After the settler	30 L	 In the morning (9-11 am) Filtered in loco with a suite of steel sieves with a mesh of 5 mm, 2 mm and 63 μm. 	(Magni et al. 2019)

Table A3: List of sampling methods for wastewater



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	Effluent				
Municipal wastewater	Effluent	500-21,000 L	• Filtered through a set of Tyler sieves at a flow rate of 12-18 L per minute for a period of 2-24 h	(Mason et al. 2016)	
			 A 0.355 mm-mesh sieve was stacked atop a 0.125 mm mesh sieve for the shorter (2 h) sampling times, while the 0.355 mm-mesh sieve was used in isolation for the longer sampling periods 		
Municipal	Influent	1-2 L	Grab samples were collected in plastic containers	(Michielssen et a	ıl.
wastewater	Pretreated influent	1-6 L		2016)	
	Primary effluent	10-20 L			
	Secondary effluent	10-20 L			
	Final effluent	34-38 L			
Municipal (+industrial) wastewater	Effluent from different configurations of WWTPs	390-1,000 L	 Custom made mobile pumping device with a filter housing containing a 10 mm stainless steel cartridge filter 	(Mintenig et a 2017)	al.
Municipal	Influent	30-50 L	• n=303	(Murphy et a	эl.
wastewater	Grit&grease effluent		• First passed through steel sieves (65 μm), then vacuum filtered through	2016)	
	Primary effluent		Whatman No. 1 qualitative circles, 90 mm filter paper, with a pore size of 11		
	Final effluent		μm.		
Municipal	Disc filter	Different	Custom made filtering device with in-situ fractionation	(Talvitie et a	эl.
wastewater		volumes for	• The mesh-sizes of the filters were 300, 100 and 20 mm, giving particle size	2017)	
	Rapid sand filter	different filter size and	 fractions of >300 mm, 100-300 mm and 20-100 mm Additional composite samples for 24 h 		
	Dissolved ait floatation	(2-1,000L) (see the			
	MBR	paper)			



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	CAS			
Municipal wastewater	Post primary treatment	3-200		
	Post primary and secondary treatment		Each sampling event took approximately 1 h with a maximum flow rate of 10 L/min The compliant device consists of four removable steinless steel much screenes	(Ziajahromi, Kumar, et al.
	Post primary, tertiary and RO treatment		 The sampling device consists of four removable stainless-steel mesh screens (plain Dutch weave) with sizes of 500, 190, 100 and 25 mm with a diameter of 12 cm. 	Neale, et al. 2017)
Raw wastewater		11	Retsch AS 200 vibratory sieve shaker through 2 mm, 1mm and 500 mm sieve meshes. Sodium dodecyl sulfate (SDS) as a surfactant added to a final concentration of 0.15 g/L before sieving to detach adhered MP particles from the larger solids. 200mL of the pre-sieved wastewater was incubated with cellulase enzyme (Aspergillus sp., Sigma-Aldrich, CAS no. 9012-54-8) for 48 h at 40 °C to degrade cellulose fibers deriving mainly from toilet paper. Organic material was oxidized with hydrogen peroxide where iron (II) was added to catalyze the reaction (Fenton reaction). Peroxide was added to a final concentration of 250 g/L and iron (II) sulfate to 2.5 g/L. The pH of the mixture was adjusted to approximately 3 with sodium hydroxide. The oxidized sample was wet-sieved (demineralized water with 0.15 g/L SDS) into two size fractions through an 80 μ m sieve mesh. The effluent containing particles <80 μ m was collected into a glass beaker. Particles >80 μ m were removed from the sieve mesh into filtered demineralized water containing 0.15 g/L SDS by treatment in an Elma S50R ultrasonic bath. Particles from this liquid and the collected effluent were gathered on separate 10 μ m steel meshes. Particles were removed from the filters into 25mL HPLC grade ethanol by ultrasonic treatment. The resulting particle-ethanol suspensions of the two size fractions were transferred into glass vials where their final volume was set to 5mL by evaporation with nitrogen gas. The chemical composition of the extracted particles was determined with an FPA-based FT-IR imaging technique.	(Simon, van Alst, and Vollertsen 2018)



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Treated wastewater			10 μ m steel filters, ultrasonic treatment, collection in filtered demineralized water containing 0.15 g/L SDS. Incubation in a serum flask for 48 h at 40 °C with cellulase enzyme. Samples oxidized in 180 g/L hydrogen peroxide catalyzed by 1.8 g/L iron (II) sulfate and pH adjusted to 3 by sodium hydroxide. Size fractionation by wetsieving and transferring the particle-ethanol suspension into glass vials.	
Municipal wastewater effluent	Screening Grit and grease removal Settling tank Aeration basin Clarifier	30-50 L	Steel buckets and sieve	(Murphy et al. 2016)
Municipal wastewater effluent			Fractionated filtering	(Triebskorn et al. 2019)
Municipal wastewater effluent			Sieving and filtering method	
Municipal wastewater effluent			Custom made pump +stainless steel cartridge filter	
Municipal wastewater effluent		2 L	Effluent: grab samples	



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Wastewater,	Influent wastewater,	From 100 ml	Filter device consists of three transparent plastic tubes (diameter 60 mm) and	(Talvitie et al.
24-hour	after	(incoming	screw-on plastic connectors attaching the tubes to one another. Round (diameter	2015, 2017)
composite	mechanical	wastewater)	80 mm) filters are placed into the filter device between the connectors and tubes	
samples	purification and after	to 8 liters	are screwed tightly together with rubber o-rings. Round filters are cut from	
	the process from	(purified)	different mesh size plankton nets. The largest mesh size filter 300 μ m is placed on	
	discharged	wastewater).	the top of the device, 100 μ m filter in the middle and 20 μ m filter at the bottom.	
	wastewater.	50 liters of	All equipment has to be rinsed thoroughly prior to sampling.	
		purified		
		wastewater		
		were filtered		
		through 300		
		and 100 µm		
		filters and 1		
		liter through		
		the 20 µm		
		filter.		
Sewage	Anaerobic digestion	2 kg	Composite over one day in each month	(Xu et al. 2019)
sludge	-	_	• Suspended, pre-washed and then filtered through 5 mm stainless steel	
Sewage	Activated sludge	150-200 mL	Poured in glass flasks with metal funnel, kept in dark	(Lares et al. 2018)
sludge	MBR sludge			
	Anaerobic digestion			
Sewage	Drained	500 g	Taken by shovel, stored in dark	(Mintenig et al.
sludge		C C		2017)
_				,
Sewage	Anaerobic digestion	30 g	Three replicates	(Mahon et al.
sludge	Thermal drying	_	• Pellets of TD sludge were placed in water for 1 week to induce softening,	2017)
			transferred to a water bath (30 °C) for 24 h, and placed in a shaker for 12	
	Lime stabilization		h.	

There is a huge differentiation in the volume of wastewater and sludge samples taken from the influent and/or effluent as well as from different processes



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of theWWTPs. The microplastics in wastewater can be collected in different ways, mainly including container collection (Magnusson and Nore n, 2014; Murphy et al., 2016; Tagg et al., 2015), autosampler collection (Michielssen et al., 2016; Talvitie et al., 2016), separate pumping and filtration (Mason et al., 2016; Mintenig et al., 2017; Talvitie et al., 2015; Ziajahromi et al., 2017) and surface filtration (Carr et al., 2016). Collecting microplastics with containers or autosamplers is easy for practicing; however, have limited volumes. In this regard, filtration devices are more preferred (Sun et al. 2019).

Due to relatively low concentrations of microplastics as well as their uneven temporal and spatial distributions in wastewater, the representativeness of the sample should be considered during the collection (Sun et al., 2019). Most of these studies were conducted by grab samples using steel buckets and sieves. For instance, Murphy et al. (2016) collected water and sludge samples from different stages of a large secondary WWTP within couple of days. Mintenig et al. (2017) studied effluents and sludge from several WWTPs in Germany and each sample was collected during one day. Carr et al. (2016) studied wastewater and sludge samples from different stages of several WWTPs and samples were collected for maximum 12 days during less than one and a half months. Talvitie et al. (2017a) collected sludge and water samples for microlitter studies either once or thrice during one week from different stages of WWTP using a filter device. Custom- made filter devises came forward in the recent years that can provide composite sampling. Wastewater sampling is comparatively more defined than sludge. Sludge sampling in the early studies was around 30 g (Mahon et al., 2017) or 150-200 mL (Lares et al., 2018). In a recent study conducted by (Xu et al., 2020), 2 kg sludge composite samples were taken over one day in each month. Recent researches highlighted the necessity to analyze microplastic pollution in WWTPs for a longer time period to reveal the temporal variation in microplastic concentrations.



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Combined Sewer Overflows

The occurrence and fate of microplastics in CSOs have not been fully recognized up to date. Existing literature reported the microplastics in stormwater runoff and pond as summarized below.

Table A4: List of sampling methods for CSOs

SAMPLING POINTS	VOLUME	TYPE OF	METHODS OF SAMPLING AND DETENCTION	FREQUENCY or	<u>Ref.</u>
		<u>SAMPLE</u>		<u>num° of samples</u>	
stormwater runoff	1L per each sample	Grab sample	Iron bucket that was rinsed three times with the runoff before taking the samples. The samples were a combination of sediment and water due to the strength of the runoff streams in the streets or storm drains. These samples were collected at the beginning of the rain event and at approx 10 min and 30 min after the first samples were collected.	94 samples	(Piñon-Colin et al. 2020)
Stormwater pond	up to several thousand liters for the bigger mesh size, 10-70 liters with a mesh size of 20 μm.	Grab sample	Sampling method consisted of a gasoline pump, hoses, filter holder and filter. Two types of filters were tested: plankton net (mesh size 300 μ m) manually cut into circles and prefabricated polycarbonate filters (mesh size 10 μ m). A mechanical volumeter was attached to the outlet hose to measure the volume of water filtered. The filter holder consisted of stainless-steel pipes, gaskets, and a clamp. The inner diameter		(Coalition Clean Baltic 2017)



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	of the stainless-steel pipes was in this case 2 inches. The inlet	
	and outlet hoses chosen had inner diameters of 1,5 and 1 inch.	
	The inlet hose was of sturdier material, not to deflate due to the	
	suction pressure of the pump. Polyester plankton nets (Sefar	
	Petex), where cut into circles to fit the filter holder. Two mesh	
	sizes were used. The mesh size of 300 μm was used to allow for	
	comparison of results with most studies conducted thus far.	
	Quantification performed manually by counting MPs using a	
	microscope. Some of the detected MPs were analyzed	
	with FTIR spectroscopy.	

Practices on CSO/runoff water are very limited up to date, including only grab samples. Composite samples can be conducted at different CSO points.



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Annex B – Literature review on treatment processes

Drinking water

There are a few researches related to the removals of microplastics in DWTPs and the information on the plastic contamination and removal effect in drinking water treatment processes is still limited. Mintenig et al. (2019) examined microplastics in groundwater and its treated water from five DWTPs to attain the highest concentration of 7 particles/m³ (size range 50–150 μm) in the raw water. Compared to the raw water, a significant decrease (by approximately 70– 80%) in microplastics numbers in the treated water was reported (Pivokonsky et al. 2018). However, it was unclear that which treatment unit was main process responsible for the removal effect due to no available data on the removal efficiency of MPs by each treatment process. A recent study by (Wang et al., 2020) investigated the microplastics in full-scale DWTPs. Overall, comparatively lower removal efficiencies were reported then wastewater treatment due to lower concentrations and detections in the influent. Coagulation-flocculation process was the most investigated unit in the treatment scheme and overall removal efficiencies ranged between 40-90% with respect to the coagulant used. In addition, some units, such as sand filters, can be a sink of microplastics, and especially backwash waters may contain high concentrations of microplastics.

Table A5: Removal efficiencies on DWTPs

Treatment unit	Removal efficiency	Reference
Sedimentation	48 %	Z. Wang, 2020
Sand Filtration	40 %	Z. Wang, 2020
Ozonation	8 %	M. Pivokonský; 2020
GAC	33-56 %	Z. Wang, 2020; M. Pivokonský; 2020
Floculation and sedimentation	62 %	M. Pivokonský; 2020
Deep bed filtration	51 %	M. Pivokonský; 2020



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Wastewater

Huge efforts have been given and still being given to determine the fate and removal of microplastics in WWTPs. The overall microplastics removal efficiencies of WWTPs without tertiary treatment are above 88% and the number increased to over 97% in the WWTPs with tertiary treatment. So far, physical treatment units (filtration, settling, mechanical units) have been reported to be the most effective section of the WWTPs in terms of microplastics removal. It was reported that approximately 35%~59% of the microplastics could be removed during the preliminary treatment and 50%e98% of the microplastics were removed after primary treatment. The secondary treatment (usually comprise of biological treatment and clarification) managed to further decrease the microplastics in the wastewater to 0.2%-14%. Overall, the microplastics in the wastewater further decreased to 0.2%e2% relative to the influent after the tertiary treatment (Sun et al., 2019). So, in a conventional treatment scheme (i.e., CAS), the removal efficiencies can reach up to 90%-95%. In case of using more advanced technologies, such as AeMBR, AnMBR, BAF or disc filters, up to 99.9% removal can be achieved. For instance, in the study of (Ngo, Pramanik, Shah, & Roychand, 2019), various units of different WWTPs were sampled and compared. Highest removal efficiency was achieved by AeMBR (99%), followed by rapid sand filter (97%) and DAF (95%).

Treatment units	Removal efficiency	Reference	
Grit and grease removal	44-58%	(Murphy et al., 2016; Ngo, Pramanik, Shah, & Roychand, 2019)	
Primary treatment	60%	(Murphy et al., 2016)	
A20	54%	(Ngo, Pramanik, Shah, & Roychand, 2019)	
Activated sludge	66%	(Ngo, Pramanik, Shah, & Roychand, 2019)	
Sedimentation	91%	(Ngo, Pramanik, Shah, & Roychand, 2019)	
Trickling filters	80%	(Ngo, Pramanik, Shah, & Roychand, 2019)	
Rapid sand filter	90-97%	(Ngo, Pramanik, Shah, & Roychand, 2019; Talvitie et al., 2017)	
Disc filter	40-98%	(Ngo, Pramanik, Shah, & Roychand, 2019)	

Table A5: Removal efficiencies on WWTPs



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RO	90%	(Ngo, Pramanik, Shah, & Roychand, 2019)
MBR	94-99%	(Sun, Dai, Wang, van Loosdrecht, & Ni, 2019; Ngo, Pramanik, Shah, & Roychand, 2019; Talvitie et al., 2017)
UASB and AnMBR	96%	(Pittura et al., 2020)
Dissolved air flotation	48-95%	(Ngo, Pramanik, Shah, & Roychand, 2019; Talvitie et al., 2017)
Chlorinate disinfection	17%	(Ngo, Pramanik, Shah, & Roychand, 2019)
Free water surface (FWS) wetlands	Close to 100 % for MPs > 20 μm	Jönsson, 2016

Combined Sewer Overflows

The existing studies only investigated the effects of stormwater ponds, while high removal efficiencies were achieved. In recent years, innovative technologies have been developed treat combined sewer overflows (CSOs) (Botturi et al., 2020). The effect of different treatment processes on the removal of microplastics from the effluent of CSO-treatment plants can be further investigated.

Table A6: Removal efficiencies on CSOs

TREATMENT	SCALE	REMOVAL EFFICIENCES	<u>Ref.</u>
Tibbledammen Stormwater Pond	5.7 ha,	- Microplastic 20-300 μm: 98%	Jönsson 2016
	4300 m3/d	- Microplastic >300 μm: 73%	
	Inlet: 5.4-10 MPs/L	 Red "potential" microplastics >20 μm: 99% 	
		- Black partickles > 20 μm: 89%	
Korsängen vattenpark Stormwater	9 ha,	- Microplastic 20-300 μm: 90%	Jönsson 2016
Pond	3440 m3/d	- Microplastic >300 μm: 100%	
	Inlet: 5.4-10 MPs/L	 Red "potential" microplastics >20 μm:100% 	
		- Black partickles > 20 μm: 99%	



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