



NO MICROPLASTICS, JUST WAVES.

Deliverable ACTION B3

ANALYTICAL PROTOCOL FOR PROCESS CONTROL

MPs IN DRINKING AND WASTEWATER TREATMENT PLANTS

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Executive summary

Both drinking and wastewater treatment plants (DWTPs and WWTPs) are dealing with large volumes of water and wastewater. The amount of microplastics that pass through these facilities has several implications, both for DWTPs providing water for people and for WWTP effluents being either discharged into the environment or used as agriculture fertiliser or irrigation. Also, the final disposed sludge needs to consider this type of emerging pollutants. Therefore, there is a growing necessity to better understand the concentration, typologies and fate of microplastics in water treatment plants and to prevent the release of microplastics into household taps and the environment.

The present "Analytical protocol for process control: microplastics (MPs) in drinking and wastewater treatment plants" was developed by the Polytechnic University of Marche as deliverable of the Action B3 of Blue Lakes project.

In this document there will be presented results of a pilot study carried out on full-scale water services infrastructures applying sampling methods and analytical procedures developed and optimised for the collection and characterisation of microplastics in raw, treated waters and sludge. These were described in the "Technical report and operative manual regarding the improvement of the treatment process", previously produced within the same Action B3.

Data will be discussed in terms of MPs abundance and of the most frequently found shape, size class and polymer typologies, focusing on the individual water treatment units/processes to trace step by step possible changes in the number and characteristics of these contaminants.

Findings obtained from the study will be furthermore compared and integrated with the current available information in the technical-scientific literature on the presence of MPs in DWTPs and WWTPs and on the efficiency of different configuration schemes to reduce the presence of MPs in the final effluents.

The results will be shared with the water services managers and operators of the selected plants to highlight the most critical treatment steps for MPs control and to identify targeted intervention strategies and/or improvement actions.

This "Analytical Protocol" along with the "Technical report" offer guidelines for microplastics monitoring in water treatment plants and they highlight processes towards which efforts should be focused to reduce these contaminants in treated waters and sludge.



1. Water treatment facilities selected for the pilot study and description of treatment units/processes

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 chosen 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 identified for their different sizes (From 180000 to 330000 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.

2.2 Castreccioni Lake

In the following paragraphs the location, the context and the infrastructures serving the territory connected to Castreccioini Lake are presented.

2.2.1 Context

The water utility Acquambiente serves the municipalities of Cingoli, Filottrano, Osimo, Castelfidardo, Numana and Sirolo. Moreover, it provides drinking water also to other nearby territories, even if they are managed by other water utilities, such as Osimo, Castelfidardo (ATO 3) and Camerano (ATO 2) (**Figure 1**).

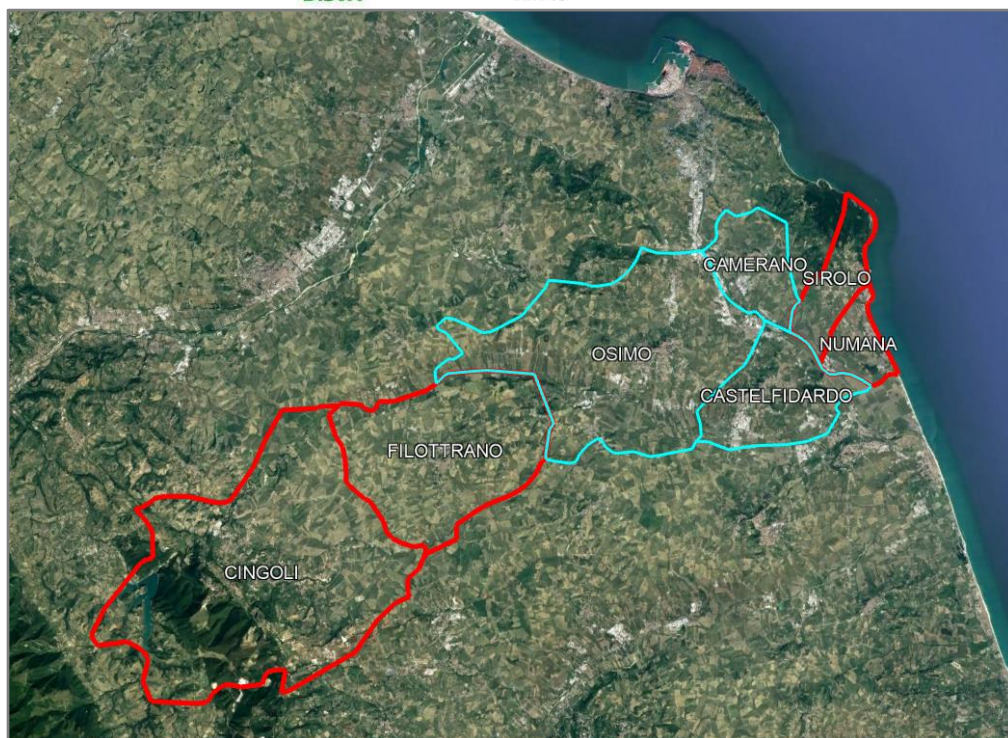


Figure 1: Territory managed by Acquambiente Marche

Water management from Acquambiente has a decisive impact on a wide part of the territory in the drainage basin of the Musone river, which extends from the hinterland (Cingoli) to the Conero Riviera (Numana, Sirolo). In relation to the served area, the importance of touristic seasonality and consumption variability play a decisive role, since in summer the need for drinking water supply can also be doubled in the coastal territories.

The final users consist mainly of domestic inhabitants, even if small amount of water is supplied for industrial purposes.

The infrastructure network managed by the Company consists of a single main supply line, whose main element is the artificial reservoir of Castreccioni, built close to Monte San Vicino, at about 70 km from the coast, in the territory of the Municipality of Cingoli.

The Castreccioni water reservoir, called "Lake Castreccioni" is located in the municipality of Cingoli (MC), in Italy (coordinates in the WGS84 reference system: Latitude = 43.383355°; Longitude = 13.161841°; Intake Heights = 314-324-334 m), and it has a total Surface of about 2.4 Km². The lake was artificially formed by the dam on the Musone river at the Petrella bridge, built in 1981-1987.



Figure 2: Castreccioni dam and intake

Today Lake Castreccioni represents the largest artificial basin in the Marche Region. The dam is 67 meters high and about 280 meters wide and supplies water for both irrigation and drinking purposes. The volume that can be stored by the lake at maximum altitude is 50 million cubic meters. The intake infrastructure that conveys the water to the treatment plant consists in three holes on the reinforced concrete structure, placed at different quotes and regulated by gate valves, which are opened to bring the flow rates necessary for the plant of potabilization. The presence of three uptake quotes is due to the need to catch the raw water that presents the best quality characteristics in the different periods of the year. In fact, based on the meteorological, environmental and microbiological conditions, variations occur in the quality characteristics of the raw water influent to the plant. Generally, only the first two holes are active, located at 314 m m.s.l. and 324 m m.s.l., respectively (**Figures 2 and 3**).

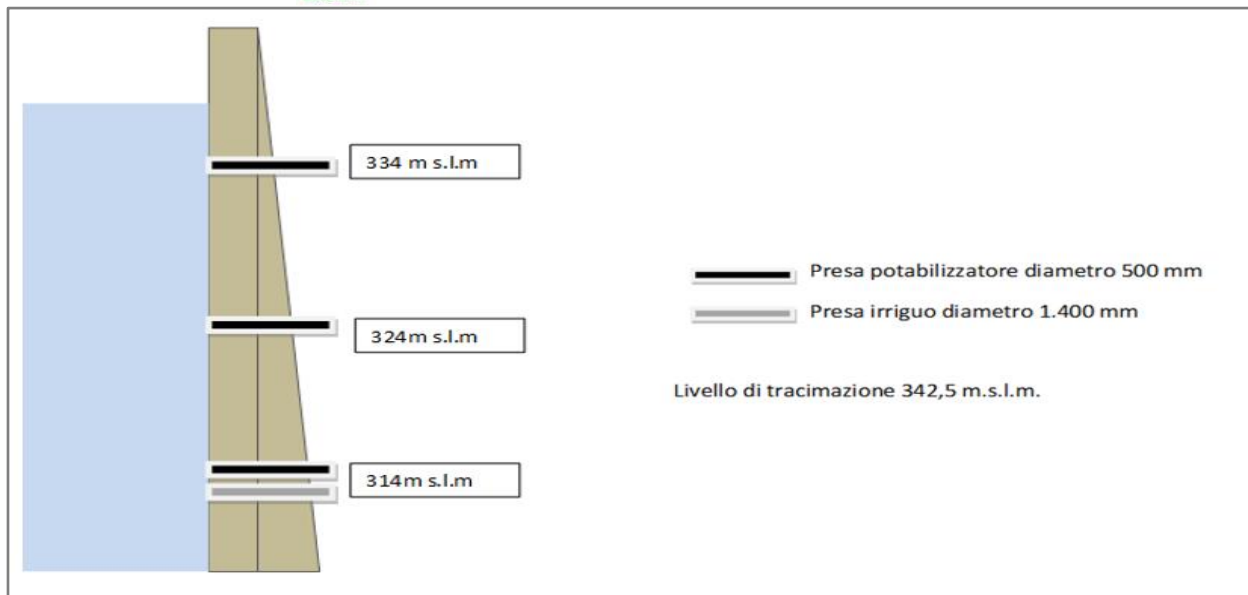


Figure 3: intake quotes at Castreccioni dam

The drinking water supply takes place mainly by gravity to the municipalities, through a steel pipeline with a diameter ranging between 800 and 400 mm, which extends over a length of more than 50 km, along the Musone valley. For distribution to users, the aqueduct system uses a series of tanks also located in the served area, in order to guarantee the provision of the service even to the small villages. The tanks represent the junction points between the supply network and the distribution to the end users guaranteeing the flexibility of distribution even in the case of extraordinary maintenance / breakage of the pipes. Along the network, there are pumping stations to provide the hydraulic load necessary to supply users. The distribution is characterized by the presence of sampling points, where water can be periodically collected and subsequently analysed.

In the distribution network, drinking water from intake wells is also conveyed and mixed with the effluent from the DWTP.

2.2.2 Castreccioni DWTP

The DWTP in Castreccioni Lake treats an average flow rate of 500 l/s and the layout includes:

- pre-ozonation pre-treatment
- flocculation phase with PAC dosage
- sand filtration
- post-ozonation unit
- activated carbon filtration.

Once passed through all the different units, the treated water is sent to storage before the final effluent point.

Pre-ozonation tank has a volume of 150 m³, works with an HRT of 517 sec and 20 Nm³/h of air. After pre-



ozonation, flow is fed to two flocculation tanks, each one with a volume of 1620 m³, characterised by an HRT of 186 min and an average PAC dosage of 13g/h. The next unit consists in six sand filters, five working in continuous and one reserve. Each unit has a volume of 48m³ and operates with an HRT of 14min, backwash air equal to 760L/sec and backwash water of 100L/sec. After sand filters, post-ozonation process is carried out in two tanks of 150m³ for each, with HRT equal to 517 sec and supplied air of 35 Nm³/h. Effluent to the post-ozonation is sent to GAC filtration, characterised by four units (plus two as reserve), with 48m³ of volume each and HRT of 11 min. Last unit is disinfection with chlorine, where 500 g ClO₂/h are dosed, and HRT is 15 min. Once water has passed through disinfection, it is stored in two accumulation tanks with effective volume of 7800 m³ and HRT of 15 h.

The influent is sampled on average once a month to carry out laboratory analyses for the chemical-physical characterization, while the effluent is analysed approximately every 15 days.

The following **Table 1** reports the characterisation of the three performed sampling campaigns, and the main functional parameters of the treatment units.

Table 1: Characteristics of Castreccioni DWTP

INFLUENT		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
Sampling date				07/07/2020	01/12/2020	04/05/2021
Reference intake quote for samplings	m m.s.l.	314-324	314-324	314	314	314
Average influent flowrate	l/s	225	227	290	210	220
Average influent flowrate	m ³ /h	810	820	1044	756	792
Temperature	°C			13.9	11.6	11.2
pH				7.8	7.8	7.8
Conductivity	μS/cm			452	460	469
Turbidity	NTU				0.21	
Total suspended solids	mg/l			< 5		< 5
Dissolved oxygen	mg/l				3.8	
TOC	mg/l			3.7	5.1	< 3
Ammonium	mg/l			< 0.1	< 0.1	< 0.1
Nitrates	mg/l			< 5	< 5	< 5
Nitrites	mg/l			0.08		< 0.2
Hardness	°F				18	
Calcium	mg/l				51	
Magnesium	mg/l				8.8	
Sodium	mg/l				18	
Potassium	mg/l				3.9	
Sulphates	mg/l				64	
Chlorides	mg/l				25	
Alkalinity	mgCaCO ₃ /l				147	
Bicarbonate	mg/l			240		234
Bromide	mg/l			< 0.5		< 0.5
Iron	μg/l			< 20		52



Manganese	µg/l			< 5	9	< 5
Oxidizability	mg/l			0.7		< 0.5
Zinc	mg/l				< 0.05	
Lead	µg/l				< 2	
Copper	µg/l				< 10	
Barium	mg/l				< 0.1	
Total hydrocarbons	µg/l				< 25	
Pesticides	µg/l				< 0.05	
Surfactants	mg/l				< 0.2	
Phosphates	mg/l				< 3	
Aluminium	µg/l			< 20	23	48
Coliforms	UFC/100 ml			2300	43	37
Escherichia coli	UFC/100 ml			2	1	< 1
Enterococci	UFC/100 ml				4	
Clostridium perfringens	UFC/100 ml				3	
PRE-OZONATION		AVERAGE 2020	AVERAGE 2021	1st SAMPLING	2nd SAMPLING	3rd SAMPLING
N units	n	1	1	1	1	1
Volume single unit	m ³	150	150	150	150	150
Air dosage	Nm ³ /h	20	20	20	20	20
Yield O ₃	gO ₃ /m ³	5.4				
Dosage O ₃	gO ₃ /h	600	600	600	600	600
Contact time	s	666	658	517	756	681
CLARI-FLOCCULATION		AVERAGE 2020	AVERAGE 2021	1st SAMPLING	2nd SAMPLING	3rd SAMPLING
N units	n	2	2	2	2	2
Volume single unit	m ³	1620	1620	1620	1620	1620
Surface single unit (trasversal)	m ²	360	360	360	360	360
Chemical dosage (PAC)	g/h	13	13	13	13	13
Superficial Hydraulic Load	m ³ /m ² /h	1.13	1.14	1.45	1.05	1.10
Contact time	min	240	237	186	257	245
SAND FILTERS		AVERAGE 2020	AVERAGE 2021	1st SAMPLING	2nd SAMPLING	3rd SAMPLING
N units	n	6	6	6	6	6
N working units	n	5	5	5	5	5
Volume single unit	m ³	48	48	48	48	48
Surface single unit (trasversal)	m ²	48	48	48	48	48
Superficial Hydraulic Load	m ³ /m ² /h	3.4	3.4	4.4	3.2	3.3
Contact time	min	0.89	0.88	0.69	0.95	0.91
Granulometry	mm	0.7-1.2	0.7-1.1	0.7-1.0	0.7-1.1	0.7-1.2
Q backwash air	l/s	760	760	760	760	760
Q backwash water	l/s	100	100	100	100	100
Frequency	n/time	1/24h	1/24h	1/24h	1/24h	1/24h



POST-OZONATION		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
N units	n	2	2	2	2	2
Volume single unit	m ³	150	150	150	150	150
Air dosage	N m ³ /h	90	90	90	90	90
Yield O ₃	gO ₃ /m ³	5.4				
Dosage O ₃	gO ₃ /h	600	600	600	600	600
Superficial Hydraulic Load	m ³ /m ² /h	3.38	3.42	4.35	3.15	3.30
O ₃ residual	mgO ₃ /l	0.15	0.15	0.15	0.15	0.15
Contact time	min	22	22	17	24	23
ACTIVATED CARBON ABSORPTION		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
N units	n	6	6	6	6	6
N working units	n	4	4	4	4	4
Carbon typology		granular	granular	granular	granular	granular
Density	kg/m ³	450-470	450-470	450-470	450-470	450-470
Volume single unit	m ³	48	48	48	48	48
Surface (trasversal)	m ²	48	48	48	48	48
Superficial Hydraulic Load	m ³ /m ² /h	8.44	8.54	10.88	7.88	8.25
Contact time	min	43	42	33	46	44
DISINFECTION		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
Chemical		ClO ₂	ClO ₂	ClO ₂	ClO ₂	ClO ₂
Contact time (storage tanks)	h	20	19	15	21	20
Dosage	gClO ₂ /h	380	400	500	360	380
STORAGE		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
N units	n	2	2	2	2	2
Volume single unit	m ³	7800	7800	7800	7800	7800
Storage time	h	15	15	15	15	15
FINAL EFFLUENT		AVERAGE 2020	AVERAGE 2021	1 st SAMPLING	2 nd SAMPLING	3 rd SAMPLING
Temperature	°C			9.4	11.4	8.8
pH				7.7	7.8	7.7
Conductivity	μS/cm			468	465	476
Redox	mV			419	418	490
Turbidity	NTU			0.22	0.2	0.2
Residual free Chlorine	μg/l			0.11	0.15	0.23
Chlorite	μg/l			300	390	420
Chlorate	μg/l			< 100	< 100	150
Chloroform	μg/l			< 1	< 1	< 1
Bromodichloromethane	μg/l			< 1	< 1	< 1
Dibromochloromethane	μg/l			< 1	< 1	< 1
Bromoform	μg/l			< 1	< 1	< 1
Total trihalomethanes	μg/l			< 5	< 5	< 5
Iron	μg/l			< 20	< 20	< 20
Manganese	μg/l			< 5	< 5	< 5



Aluminum	µg/l			< 20	< 20	< 20
Escherichia coli	UFC/100 ml			< 1	< 1	< 1
Clostridium perfringens	UFC/100 ml			< 1	< 1	< 1
Intestinal enterococci	UFC/100 ml			< 1	< 1	< 1

2.3 Garda Lake

Lake Garda is the largest Italian lake (65 m m.s.l., S= 368 km², V= 49 km³, Depth=346 m) and is a strategic drinking water basin, even subject to very high anthropogenic stresses (e.g., tourism): in most of its municipalities (**Figure 4**), catchment drinking water is provided by several DWTPs that collect and treat lake water. Specifically, in the East Coast of the Lake (107000 inhabitants in winter and >220000 inhabitants in touristic season). In this sensitive scenario, understanding the occurrence and fate of MPs in the urban water infrastructure of Lake Garda is needed.

2.3.1 DWTPs in Garda Lake

To ensure the quality standards of the water supplied, the drinking water system of the municipalities of Lake Garda in Verona are subjected to constant controls by the environmental and health protection agencies, to verify the compliance with the indications provided by the legislation and guarantee safe consumption.

In addition to these samplings, AGS carries out 3 routine monthly analyses in representative points of the network and a complete verification of the supply and main tanks, for more than 90 parameters. In addition, the efficiency of the disinfection systems and residual chlorine in the network are weekly controlled.



Figure 4: Municipalities served in Garda Lake. Brenzone Castelletto and Garda Molinet DWTPs are localised with a red dot, while Limone Tremosine and Peschiera del Garda WWTPs are identified with a blue dot.

2.3.1.1 Garda Molinet DWTP

In Garda territory, the infrastructure GAA01 located in Cavalla, in the south of the city, supply lake water to two DWTPs, called “La Rocca” and “Molinet”, before being distributed.

In particular, Molinet was built and activated a few years ago. Its maximum treatment capacity is 50 l/s and its layout consists of:

- Pre-oxidation with ozone, for influent disinfection, removal of any cyanotoxins, oxidation of organic matter and micro flocculation of algae and colloids present in lake water
- Rapid multilayer filtration with sand and granular activated carbon, for the abatement of micro flakes and suspended solids, in addition to the removal of any residual ozone and other micropollutants
- Chlorine disinfection for residual removal of pathogens in the distribution network.

The ozonation process is characterized by an average dose of about 42 gO₃/h. After the ozonation, water is treated into a combined filter composed by sand filtration integrated with GAC adsorption, with granule size of about 0.6 – 2.36 mm.

The following **Table 2** reports the main functional parameters of the treatment units.

Table 2: Characteristics of Molinet DWTP

INFLUENT	Unit	Average period summer	Average period winter
Flowrate	l/s	25	11

pH	-	7.1	
Turbidity	NTU	< 1.0	
Temperature	°C	12.9	11.1
Conductivity	μS/cm	216	
Alcalinity	mg/l CaCO ₃	137	
Fixed residue	mg/L	162	
TOC	mg/L	1.1	
OZONATION	Unit		
N° units	N°	2	
N° working units	N°	1	
Dosage (average)	Nm ³ /h air	1.5	
	yeld gO ₃ /m ³ air	1.05	
	gO ₃ /h	42	
SAND FILTRATION - ACTIVATED CARBON ABSORPTION	Unit		
Carbon typology		GAC	
Granule size	mm	0.6 – 2.36	
EFFLUENT	Unit	Average summer period	Average winter period
pH	-	8	7.5
Conductivity	μS/cm	229	221
Residual chlorine	mg/L	0.13	0.11
Turbidity	mg/L	0.7	< 1.0
Temperature	°C	16.2	10.4
Hardness	°F	13	12
Nitrates	mg/L	< 3	< 3
Ammonium	mg/L	< 0.05	< 0.05
Sulphates	mg/L	11	11

2.3.1.2 Castelletto di Brenzone DWTP

The DWTP located in Castelletto di Brenzone is characterised by a flowrate of 10 l/s and treats lake water, collected by a polyethylene pipeline. Treatment layout consists in:

- gross filtration
- membrane ultrafiltration unit.

Coarse filtration is characterized by a mesh size of 100 μm, while ultrafiltration unit reaches 0.02 μm. Ultrafiltration operates with an average flux of 90 l/m²/h under a pressure of 2 – 5 bar.

Before being finally sent to distribution, water is stored in a tank, where emergency disinfection may take place if required. After treatment, lake water is mixed with well water and sent to the final users.

The following **Table 3** reports the main functional parameters of the treatment units.

Table 3: Characteristics of Brenzone Castelletto DWTP

INFLUENT	Unit	Value
Flowrate (maximum)	l/s	10

pH	-	8.6
Turbidity	NTU	4.7
Temperature	°C	20
Conductivity	μS/cm	204
Alkalinity	mg/l CaCO ₃	161
TOC	mg/l	1.2
Fixed residue	mg/l	1153
COARSE FILTRATION	Unit	Value
N° units	N°	1
N° working units	N°	1
Mesh size	μm	100
Surface	m ²	2.2
ULTRAFILTRATION	Unit	Value
N° units	N	8
N° working units	N	8
Mesh size	μm	0.02
Membrane material		MULTIBORE fibers in PES
Flux J	l/m ² /h	90
Operating pressure	bar	2-5
Backwash frequency	minutes	15
CHEMICAL DISINFECTION	Unit	Value
Chemical	-	Sodium Hypochlorite
Purezza	%	12%
EFFLUENT	Unit	Value
Conductivity	μS/cm	390
Residual chlorine	mg/L	0.43
Turbidity	mg/L	1.6
Alkalinity	mg/l CaCO ₃	227
TOC	mg/l	292
Fixed residue	mg/l	0.6

2.3.2 WWTPs on Garda Lake district

2.3.2.1 Limone Tremosine WWTP

The Limone Tremosine WWTP is characterised by a maximum treatment capacity of about 188680 AE, in the summer period of maximum load. Wastewater from the municipalities of Limone and Tremosine is conveyed into the plant by external pumping stations. The layout is composed of:

- Coarse screening
- Fine screening
- Sand degritting
- Biological treatments with attached biomass
- Flotation unit



- Filtration
- UV disinfection.

Pre-treatments are characterized by coarse screening, followed by fine screening at 3 mm and by 2 circular units for sand removal. The configuration of the biological treatment is characterized by 3 lines, which can work in a modular way, to maximise the flexibility of the plant to the influent fluctuations. The height of the tanks is about 6 - 7 m. Biological treatments are based on attached biomass, with different types of carriers. For each line there are two pre-denitrification tanks and two oxidation tanks. Downstream of the biological treatments, there is a flotation process. Aluminium oxides are dosed, for the chemical precipitation of phosphorus, in order to comply with the limit of 1 mg/l. Moreover, polyelectrolyte and polyamine are dosed for flotation. The effluent from the flotation unit is sent to the tertiary filtration, characterized by a rotary filter. In case of a hydraulic overload, the excess flow bypasses the tertiary treatment and is conveyed together with the effluent. The effluent from the flotation unit is however characterized by a solids content of less than 35 mg / l. Before the final discharge there is a UV disinfection unit. The floated sludge is conveyed to an aerobic stabilization process, followed by an accumulation tank and a final dewatering unit.

Official samplings for chemical-physical analyses are carried out twice a month by the control authority, and once a week by the company for the internal procedures. The sampler is located after the screening unit.

2.3.2.2 Peschiera del Garda WWTP

The Peschiera del Garda WWTP treats wastewater from the Brescia and Verona territories of Lake Garda, excluding Limone and the Trentino Surface. The plant has a project treatment capacity of 330000 PE, with high variability in the summer period. Plant layout (**Figure 5**) consists of:

- Coarse screening
- Fine screening
- Sand and oil removal
- Biological treatments
- Secondary sedimentation
- Coagulation
- Lamellar sedimentation
- Sand filtration
- UV disinfection.

Wastewater is conveyed to the plant through external pumping stations managed by AGS and Acque Bresciane and arrive in two separate lines, which are connected to the two parallel pre-treatment units (approximately height of 4 - 5 m). Currently, the influent is partially fed under pressure and partially by gravity. Each of the two pumping station is connected to its own pre-treatment line, consisting of screening and sand removal. The two lines are then conveyed together to the 6 biological treatment units.

The primary settlers are not currently in use and work as off-line equalization tanks. The configuration of the biological processes is characterised by circular plug-flow, in which pre denitrification and nitrification processes take place. Denitrification is performed in the external part of the crown, oxidation in four sectors inside. The effluent flow, after secondary settling, is partially sent to final tertiary treatments and partially directly discharged. The tertiary treatments consist of coagulation units, lamellar pack sedimentation and sand filtration, before UV disinfection. Aluminium sulphate is dosed on the stream effluent from the secondary treatments for the chemical precipitation of phosphorus. Polyelectrolyte is added in the sedimentation tank. The sludge line is made up by thickening and dewatering units. Dynamic thickeners allow to reach a dry content of 3 - 4% TS. Final sludge is disposed for recovery purposes. Sampling for internal controls of the main chemical-physical parameters in the water line is carried out every week.

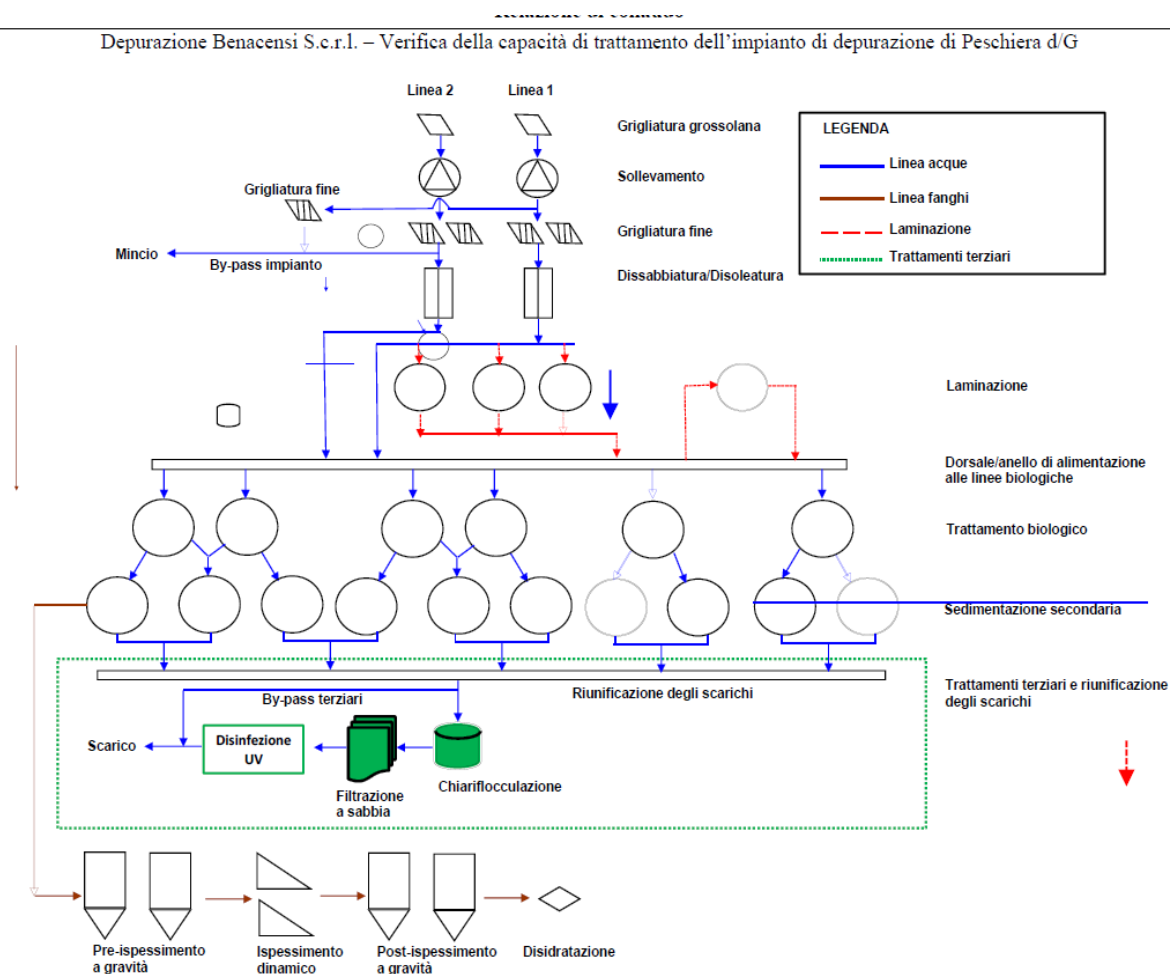


Figure 5: Peschiera del Garda WWTP Layout

The main characteristics of the treatment units of Peschiera del Garda WWTP are summarised in the following **Table 4**.

Table 4: Treatment units characteristics in Peschiera del Garda WWTP

GROSS SCREENING	UdM	Value
N° units	N°	2
Typology		manual, vertical bars



Mesh size	cm	10 – 13
FINE SCREENING	UdM	Value
N° units	N°	4
Typology		automatic steps
Mesh size	mm	3
SAND REMOVAL	UdM	Value
N° units	N°	2+2
Surface	m ²	351
H	mm	2.9
Volume	m ³	853
HRT	min	10
BIOLOGIC TREATMENTS	UdM	Value
Process		activated sludge
Configuration		circular plug-flow
N° units	N°	6
Anoxic volume	m ³	9890
Aerated volume	m ³	18435
Facultative volume	m ³	991
Q air max	m ³ /h	34420
Q recycle	m ³ /d	1354
TSS internal recycle	g/l	3.8
TSS recycle	g/l	8.1
SECONDARY SEDIMENTATION	UdM	Value
N° units	N°	10
H	m	2 – 4
D	m	31.9 – 35.4
Surface	m ²	7611
Volume	m ³	20550
COAGULATION/FLOCCULATION	UdM	Value
N° units	N°	3
Surface	m ²	120.08
Volume	m ³	1062.5
H	m	5.4
Dosage	-	Al ₂ (SO ₄) ₃ and poly
LAMELLAR SEDIMENTATION	UdM	Value
N° units	N°	4
Surface	m ²	489.6
Volume	m ³	2720
H	m	5.4
Surface lamellar	m ²	7132
SAND FILTRATION	UdM	Value
N° units	N°	8
Surface (horizontal)	m ²	576
Volume	m ³	1987
Volume filtering	m ³	777

H filtering bed	m	1.35
H hydraulic	m	3.45
UV DISINFECTION	UdM	Value
N° units	N°	2
Lamps typology		Mercury and Indium
N° lamps	N°	168
Nominal power	kW	52.9
Power UV-C emitted (new)	kW	25.5

The mean characterisation of the influent and effluent flows, distinguished between the low-season and high-season periods, are reported in terms of average values and related standard deviations, in the following **Table 5**.

Table 5: Characteristics of Peschiera Del Garda WWTP influent and effluent flows

INFLUENT	Average Jan-Apr / Ott-Dec	Dev.St. Jan-Apr / Ott-Dec	Average May-Sep	Dev.St. May-Sep	Average 2020	Dev.St. 2020
Flowrate [m3/d]	124186	19824	131572	18798	127274	19716
Temperature [°C]	14.0	2.7	21.0	2.0	16.9	4.2
pH	7.7	0.1	7.8	0.1	7.8	0.1
Conductivity [μS/cm]	737	70	782	87	756	80
Suspended solids [mg/l]	96	41	110	33	101	38
Solids sed. [mg/l]	6	2	6	2	6	2
BOD ₅ [mg/l]	75	30	90	25	82	29
COD [mg/l]	181	62	191	57	185	60
N-NH ₄ [mg/l]	10	3	15	4	12	4
N-NO ₂ [mg/l]	0	0	0	0	0	0
N-NO ₃ [mg/l]	2	0	2	0	2	0
TKN [mg/l]	18	5	24	7	20	6
TN [mg/l]	19	5	26	8	22	7
TP [mg/l]	3	1	3	1	3	1
P-PO ₄ [mg/l]	2	0	2	1	2	1
Escherichia coli Line 1 [UCF/100ml]	1652941	1985708	2755556	2885897	2034615	2339830
Escherichia coli Line 2 [UCF/100ml]	1230769	658451	1809091	925424	1495833	827768
EFFLUENT	Average Jan-Apr / Ott-Dec	Dev.St. Jan-Apr / Ott-Dec	Average May-Sep	Dev.St. May-Sep	Average 2020	Dev.St. 2020
Flowrate [m3/d]	124186	19824	131572	18798	127274	19716
Temperature [°C]	14.5	2.8	21.3	2.0	17.3	4.2
pH	8.1	0.1	8.1	0.1	8.1	0.1
Conductivity [μS/cm]	674	63	696	68	683	66
Suspended solids [mg/l]	5.3	0.8	5.5	1.0	5.4	0.9
Solids sed. [mg/l]	0.1	0.0	0.1	0.0	0.1	0.0
BOD ₅ [mg/l]	5.5	1.8	8.3	4.1	6.7	3.3
COD [mg/l]	11.3	3.3	14.1	4.9	12.5	4.3
N-NH ₄ [mg/l]	0.8	0.0	1.0	0.5	0.9	0.3
N-NO ₂ [mg/l]	0.01	0.01	0.02	0.03	0.02	0.02
N-NO ₃ [mg/l]	5.6	1.4	5.5	1.0	5.5	1.3

TKN [mg/l]	2.0	0.1	2.3	0.7	2.1	0.5
TN [mg/l]	7.4	1.3	7.8	1.3	7.6	1.3
TP [mg/l]	0.8	0.2	0.9	0.1	0.8	0.2
P-PO ₄ [mg/l]	0.7	0.2	0.8	0.1	0.8	0.2
Escherichia coli [UCF/100ml]	1660	1353	1623	1488	1645	1394
SLUDGE	Average Jan-Apr / Ott-Dec	Dev.St. Jan-Apr / Ott-Dec	Average May-Sep	Dev.St. May-Sep	Total 2020 [ton/year]	
Dewatered sludge [ton/month]	1046	228	1132	344	12977	
% TS	23.6	0.5	25.1	0.7	24.3	
CHEMICALS	Average Jan-Apr / Ott-Dec	Dev.St. Jan-Apr / Ott-Dec	Average May-Sep	Dev.St. May-Sep	Total 2020 [ton/year]	
Poly in powder [kg/month]	410	406	599	283	5869	
Dephosphating [ton/month]	31	25	56	20	500	

2. Sampling plan for the pilot study on MPs in DWTPs and WWTPS

For the pilot actions foreseen in the activity B3 of LIFE BLUE LAKES project, in each facility, 3 sampling campaigns during different seasons were performed, in order to improve sampling representativeness and possibly detect seasonal variabilities.

Information about the sampling procedures and details on sampling methods can be found in the previous Deliverable of Action B3 “Technical report and operative manual regarding the improvement of the treatment process”.

2.1 Sampling campaign in Castreccioni DWTP

Three sampling campaigns in Castreccioni DWTP (**Figure 6**) were carried out in three different periods (summer, winter and spring season, **Table 7**).



Figure 6: Sampling in Castreccioni DWTP

For each campaign, the following points were sampled (**Figure 7** and **Table 6**):

- influent from dam at 2 different quotes (Influent_324m and Influent_314m);

- effluent from the pre-ozonation (Out pre-ozonation)
- effluent from flocculation (Out flocculation)
- flocculated sludge
- effluent from sand filtration (Out sand filtration)
- backwash of sand filters
- effluent from post-ozonation (Out post-ozonation)
- effluent from GAC absorption (Out GAC absorption)
- final effluent (Effluent)

In addition to the points already identified within the DWTP, samples were defined also along the distribution network. Sampling points were identified in correspondence with Imbrecciata (Distribution 1) and Montoro (Distribution 2) (**Figure 7** and **Table 6**). The selected points have been identified in order to exclude the presence of water from the wells.

Along the network there are cabins with apposite taps and drainage systems, which were used for sampling, by installing the filtration system directly on the tap (**Figure 8**).

The system used for the sampling campaign and the protocol applied were detailed in the previous Deliverable “Technical report and operative manual regarding the improvement of the treatment process”.

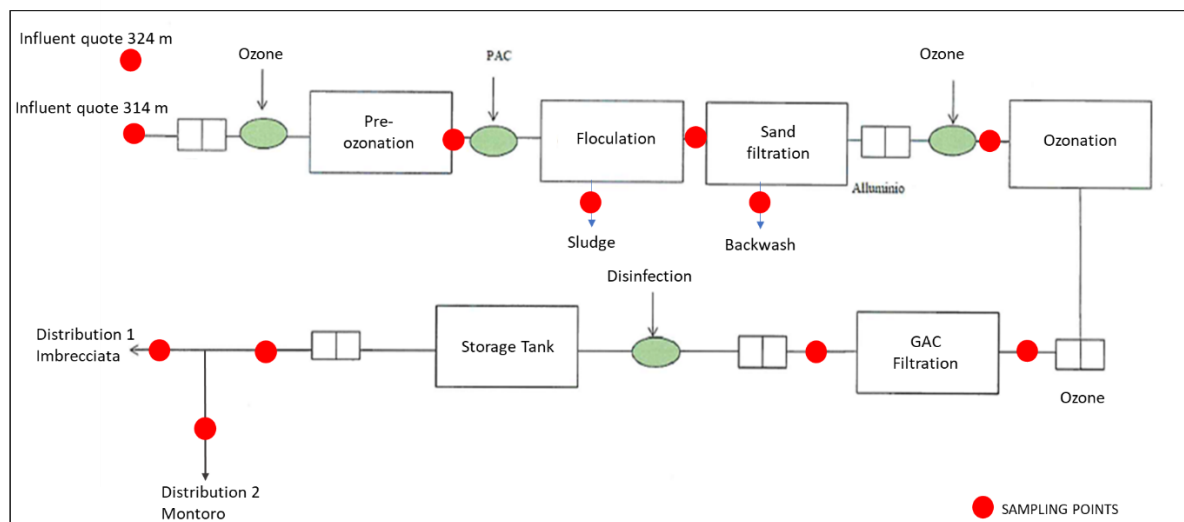


Figure 7: Castreccioni DWTP layout



Figure 8: Sampling in the distribution network

Table 6: Sampling points defined for Castreccioni DWTP

Sampling points	Sampling method
Influent_324m	cartridge steel stainless filter 50 μ m
Influent_314m	cartridge steel stainless filter 50 μ m
Out pre-ozonation	cartridge steel stainless filter 50 μ m
Out flocculation	Pumping and sieving
Out sand filter	cartridge steel stainless filter 50 μ m
Out post-ozonation	cartridge steel stainless filter 50 μ m
Out activated carbon	cartridge steel stainless filter 50 μ m
Effluent	cartridge steel stainless filter 50 μ m
Distribution 1	cartridge steel stainless filter 50 μ m
Distribution 2	cartridge steel stainless filter 50 μ m
Sludge	Grab sample
Backwash	Grab sample

Phases carried out for analysis and characterisation of MPs in water and sludge samples are reported in the following **Table 7**.

Table 7: Phases of MPs characterisation for Castreccioni DWTP

Sampling campaigns_Castreccioni	MPs extraction	Sorting MPs	μFTIR characterization
I: July 2020			
Water samples	✓	✓	✓
Sludge samples	✓	✓	✓
II: December 2020			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓
III: May 2021			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓

For each sample, about 1000 L were filtered with a mesh size of 50 μm, as reported in **Table 8**. 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 liters were collected.

Table 8: Sampled volumes in Castreccioni DWTP

	Sampled Volume (Liters)		
POINT	<i>I campaign</i>	<i>II campaign</i>	<i>III campaign</i>
Influent_324m	997	1128	1004
Influent_314m	1095	1011	1000
Out pre-ozonation	1001	1000	1002
Out flocculation	1022	1003	999
Out sand filter	1115	1085	1005
Out post-ozonation	998	1000	998
Out activated carbon	995	1001	996
Effluent	999	1262	999

Distribution 1	996	996	1000
Distribution 2	1000	995	1004
Sludge	4	4	5
Backwash	26	17	20

2.2 Sampling campaign in Garda Molinet DWTP

Three sampling campaigns were performed in Garda Molinet DWTP in different season periods (November, June, September) (**Table 10**).

As reported in **Figure 9** and **Table 9**, in the DWTP were sampled:

- Influent
- effluent from ozonation
- effluent from filtration
- final effluent
- one point in the distribution network.

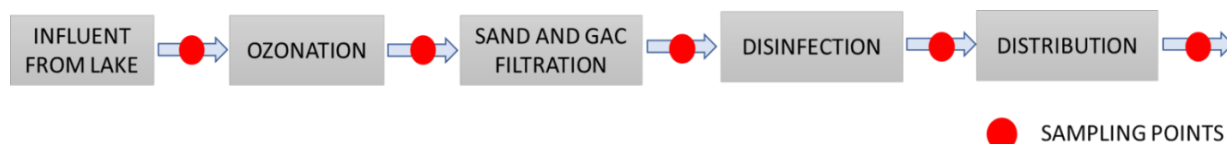


Figure 9: Molinet DWTP layout

All the samples were collected in Molinet DWTP using the cartridge steel stainless filter at 50 μm as summarised in **Table 9**.

The system used for the sampling campaign and the protocol applied were detailed in the previous Deliverable “Technical report and operative manual regarding the improvement of the treatment process”.

Table 9: Sampling points defined for Molinet DWTP

Sampling points	Sampling method
Influent	cartridge steel stainless filter 50 μm
Effluent ozonation (Out ozonation)	cartridge steel stainless filter 50 μm
Effluent filtration (Out filtration)	cartridge steel stainless filter 50 μm
Effluent	cartridge steel stainless filter 50 μm
Distribution	cartridge steel stainless filter 50 μm

Phases carried out for analysis and characterisation of MPs in water and sludge samples are reported in



the following **Table 10**.

Table 10: Phases of MPs characterisation for Garda Molinet DWTP

Sampling campaigns_MOLINET	MPs extraction	Visual Sorting	μFTIR characterization
I: November 2020	✓	✓	✓
II: June 2021	✓	✓	✓
III: September 2021	✓	✓	✓

For each sample, about 100 liters of water were sampled, as reported in **Table 11**.

Table 11: Sampled volumes in Garda Molinet DWTP

	Sampled Volume (Liters)		
POINT	<i>I campaign</i>	<i>II campaign</i>	<i>III campaign</i>
Influent	1029	972	996
Out ozonation	1000	1037	1008
Out filtration	1000	1000	1018
Effluent	1015	970	1016
Distribution	1011	1000	998

2.3 Sampling campaign in Brenzone Castelletto DWTP

Three sampling campaigns were performed in Brenzone Castelletto DWTP in different season periods (November, July, September, **Table 13**).

In the DWTP were sampled (**Figure 10**): influent, backwash from coarse filtration, backwash from ultrafiltration, final effluent and one point in the distribution network.

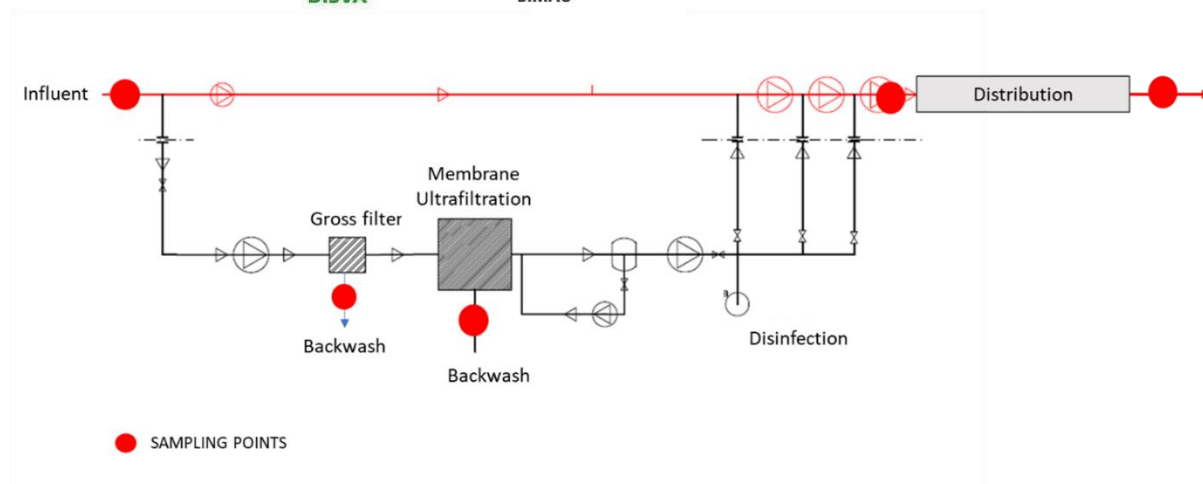


Figure 10: Brenzone Castelletto DWTP layout

Influent, backwash from ultrafiltration and effluent samples were performed using the cartridge steel stainless filter at 50 μm , while backwash from coarse filtration was sampled with a grab sample (**Table 12**).

The system used for the sampling campaign and the protocol applied were detailed in the previous Deliverable “Technical report and operative manual regarding the improvement of the treatment process”.

Table 12: Sampling points defined for Brenzone Castelletto DWTP

Sampling points	Sampling method
Influent	cartridge steel stainless filter 50 μm
Backwash coarse filter	grab sample 20 l
Backwash Ultrafiltration	cartridge steel stainless filter 50 μm
Effluent	cartridge steel stainless filter 50 μm
Distribution	cartridge steel stainless filter 50 μm

Phases carried out for analysis and characterisation of MPs in water and sludge samples are reported in the following **Table 13**.

Table 13: Phases of MPs characterisation for Brenzone Castelletto DWTP

Sampling campaigns_BRENZONE	MPs extraction	Visual Sorting	μFTIR characterization
I: November 2020	✓	✓	✓
II: July 2021	✓	✓	✓
III: September 2021	✓	✓	✓

For each sample, about 100 liters of water were sampled, as reported in **Table 14**.

Table 14: Sampled volumes in Brenzone Castelletto DWTP

	Sampled Volumes (Liters)		
POINT	<i>I campaign</i>	<i>II campaign</i>	<i>III campaign</i>
Influent	1050	996	994
Effluent	1400	999	1002
Distribution	1000	1010	996
Backwash Coarse filter	26	20	20
Backwash Ultrafiltration	75	49	155

2.3 Sampling campaign in Limone Tremosine WWTP

The sampling campaign in Limone Tremosine WWTP (**Figure 11**) was carried out during three different seasons (Summer, Winter and Spring, **Table 16**), and in different points of the plant (**Tables 15** and **17**).



Figure 11: Sampling performed at Limone Tremosine WWTP

The selected sampling points were the influent, the effluent from the flotation unit and the final effluent from the tertiary filtration. As concern sludge matrix, samples of floated sludge and aerobic stabilised sludge were taken.

The system used for the sampling campaign and the protocol applied were detailed in the previous Deliverable “Technical report and operative manual regarding the improvement of the treatment process”.



The identified sampling points are summarized in Table 15. For all the samplings collected along the mainstream wastewater treatment, the equipment used was the automatic sampler, with a filtration size of 50 μm . On the other hand, for sludge matrix, grab samples were considered.

Table 15: Sampling points defined for Limone Tremosine WWTP

POINT	SAMPLING METHOD
Influent before pre-treatments	Automatic sampler
Effluent from flotation unit	Automatic sampler
Effluent from tertiary filtration	Automatic sampler
Flotated sludge	Grab sample 25 l
Aerobically stabilized secondary sludge	Grab sample 25 l

Phases carried out for analysis and characterisation of MPs in water and sludge samples are reported in the following Table 16.

Table 16: Phases of MPs characterisation for Limone Tremosine WWTP

Sampling campaigns_LIMONE	MPs extraction	Sorting MPs	μFTIR characterization
I: July 2021			
Water samples	✓	✓	✓
Sludge samples	✓	✓	✓
II: November 2021			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓
III: May 2022			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓

The volumes sampled at each point, both for wastewater and for sludge samples, are showed in Table 17.

Table 16: Sampled volumes in Limone Tremosine WWTP

	Sampled Volumes (Liters)
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POINT	I campaign	II campaign	III campaign
Influent before pre-treatments	471	90	151
Effluent from flotation unit	873	1001	nd
Effluent from tertiary filtration	546	426	1627
Floated sludge	10	nd	5
Aerobically stabilized secondary sludge	10	10	5

2.4 Sampling campaign in Peschiera del Garda WWTP

The sampling campaign in Peschiera del Garda WWTP (**Figure 12**) was carried out during three different seasons (Summer, Winter and Spring, **Table 19**), and in different points.



Figure 12: Sampling performed at Peschiera del Garda WWTP

The selected sampling point were the influent, the effluent from secondary settlers, the effluent from lamellar pack units and the effluent from tertiary filtration. Sludge samples included the influent sludge to the thickeners and the chemical sludge from tertiary treatments (**Table 18**).

For all the samplings collected in the mainstream wastewater treatment, the equipment used was the automatic sampler, with a filtration size of 50 μm . On the other hand, for sludge matrix, grab samples were taken (**Table 18**).

The system used for the sampling campaign and the protocol applied were detailed in the previous Deliverable “Technical report and operative manual regarding the improvement of the treatment process”.

Table 18: Sampling points in Peschiera del Garda WWTP

	POINT	SAMPLING METHOD
1.	Influent	Automatic sampler
2.	Effluent from secondary settlers	Automatic sampler
3.	Effluent from lamellar packs unit	Automatic sampler
4.	Effluent from sand filtration	Automatic sampler
5.	Sludge to the thickeners	Grab sample 25 l



6.	Chemical sludge from lamellar packs unit	Grab sample 25 l
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Phases carried out for analysis and characterisation of MPs in water and sludge samples are reported in the following **Table 19**.

Table 17: Phases of MPs characterisation for Peschiera del Garda WWTP

Sampling campaigns_PESCHIERA	MPs extraction	Sorting MPs	μFTIR characterization
I: July 2021			
Water samples	✓	✓	✓
Sludge samples	✓	✓	✓
II: November 2021			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓
III: May 2022			
Water samples	✓	✓	✓
Sludge sample	✓	✓	✓

The volumes sampled at each point, both for wastewater and for sludge matrix, are showed in **Table 20**.

Table 18: Phases of MPs characterisation for Peschiera del Garda WWTP

	Sampled Volumes (Liters)		
POINT	<i>I campaign</i>	<i>II campaign</i>	<i>III campaign</i>
Influent	1043	49	70
Effluent from secondary settlers	1036	1124	1669
Effluent from lamellar packs unit	1001	829	14
Effluent from sand filtration	1201	1254	1299
Sludge to the thickeners	20	10	5



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Chemical sludge from lamellar packs unit	20	5	5
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3. Presence and physico-chemical characterisation of MPs in DWTPs

The results of the sampling campaigns carried out for each one of the drinking water treatment plants selected for the Blue Lakes project are reported in the following paragraphs.

Data are showed as MPs, representing the sum of the microparticles and microfibres made of plastic polymers (MPPs and MPFs, respectively). Furthermore, in the Annex A it is reported the relative contribution of synthetic and natural microfibers on the total extracted and characterised during the present study given the increasing attention on artificial microfibers of natural origin (produce from animal- or plant-based materials) as emerging contaminants beyond MPFs.

Information about the characterisation procedures and results reporting can be found in the previous Deliverable of Action B3 “Technical report and operative manual regarding the improvement of the treatment process”.

3.1 Castreccioni DWTP

Results of the sampling campaigns performed for Castreccioni DWTP are summarised and discussed in the following figures.

MPs concentration for the three sampling campaigns were shown in **Figure 13**, for the different units of the DWTP layout and in **Figure 14** for backwash and sludge.

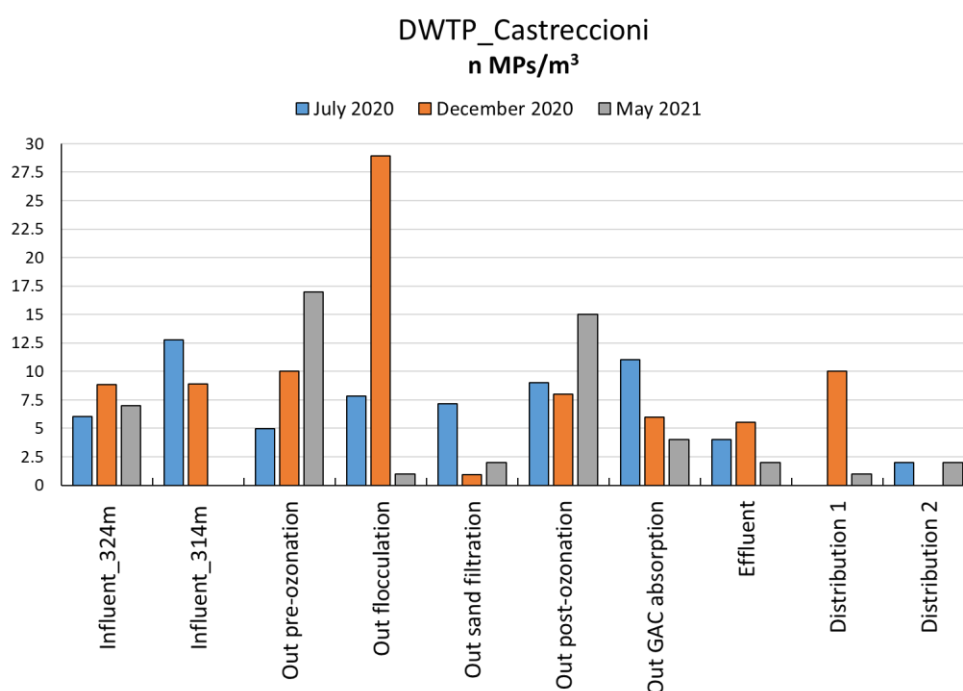


Figure 13: MPs abundance in waste and treated waters of Castreccioni drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

Concentrations measured in the Influent are on average in the range of 0 – 13 MPs/m³, specifically around 7 ± 1 MPs/m³ for the influent at 324 m and about 7 ± 7 MPs/m³ for the quote at 314 m, showing a higher

variability for the lower quote, since no MPs were found in Influent_314 sampled during May 2021.

After the pre-ozonation process, an increase in the MPs concentration could be observed, with mean values of 11 ± 6 MPs/m³. These results are in line with the literature research, as detailed in Chapter 6.1, since the ozonation process was found to be a possible cause of MPs release. In fact, the ozonation is characterised by an elevated oxidation effect on the organic matter. Generally, MPs could be attached to solid particles and therefore are more easily removed in the settled sludge, residual screening and degritting. Differently, some chemical processes enhancing the solid oxidation, determine release phenomena of MPs in the main water line. Possible correlations between the ozone dose and the indirect effect on MPs are probably expected and need to be furtherly investigated.

The following flocculation presented variable trend of 13 ± 15 MPs/m³, with higher concentration for the first two campaigns and significant reduction of MPs during the last sampling of May 2021. This finding is in line with some of the literature works analysed in Chapter 6, which observed an increase in MPs concentration after coagulation-flocculation processes. Possible correlations between the type of dosed reagents and the indirect effect on MPs are probably expected and need to be furtherly investigated.

The effluent from sand filtration was characterised by a decrease in MPs, with mean concentrations of 3 ± 3 MPs/m³, even if after the post-ozonation process a new increase was observed, with values in the range of 11 ± 4 MPs/m³.

However, the effluent from the following GAC adsorption process decreased to the final effluent values of about 4 ± 2 MPs/m³.

Similar concentrations were measured in the two distribution points, each of one was characterised by values in the range of 4 ± 6 MPs/m³ and 3 ± 3 MPs/m³, respectively. Notwithstanding the really low detected value, the final data in the distribution network were found to be comparable or lower than the effluent from the DWTP, suggesting possible further settling phenomena in the distribution system.

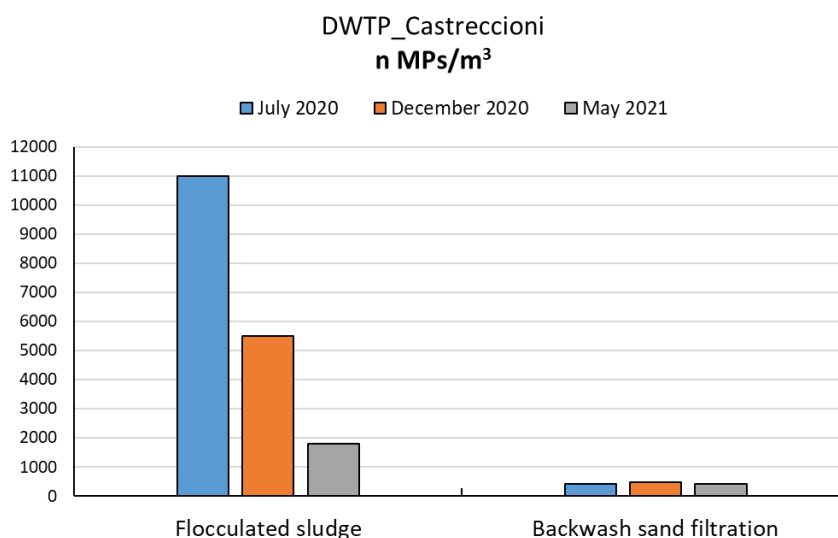


Figure 14: MPs abundance in sludge and backwash water of Castreccioni drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

Concentrations of MPs measured in Flocculated sludge and Backwash sand filtration are significantly higher than those found in the water line. In fact, levels are in the range of 6100 ± 4629 MPs/m³ for sludge

samples and about 431 ± 36 MPs/m³ for backwash samples.

Flocculated sludge is furthermore characterized by high variations in MPs concentration comparing the 3 sampling periods, with the highest levels found in Summer (July 2020) and the lowest in Spring (May 2021). On the contrary, no evidence was found about the influence of seasonality on MPs concentration in backwash samples.

In addition, statistical indexes were obtained for each sampling point, from the aggregation of the data from the three sampling campaigns. Results are graphically reported in **Figure 15**. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes.

It can be generally observed that the mean values in the influent are lower than 10 MPs/m³. Highest variations could be detected in the different operative units, especially for ozonation and flocculation processes. However, the DWTP is able to provide good water quality in the effluent, with mean values below 5 MPs/m³ and limited variability.

Error! Reference source not found. confirms that backwash and sludge are generally characterised by expected higher concentrations.

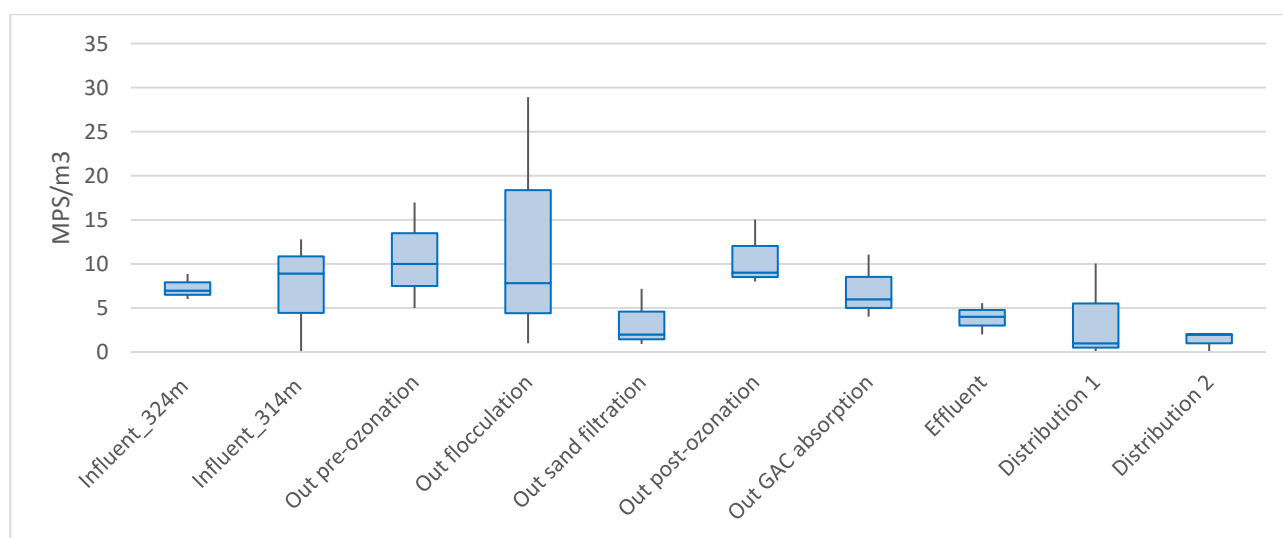


Figure 15: Distribution of MPs occurrence in Castreccioni DWTP

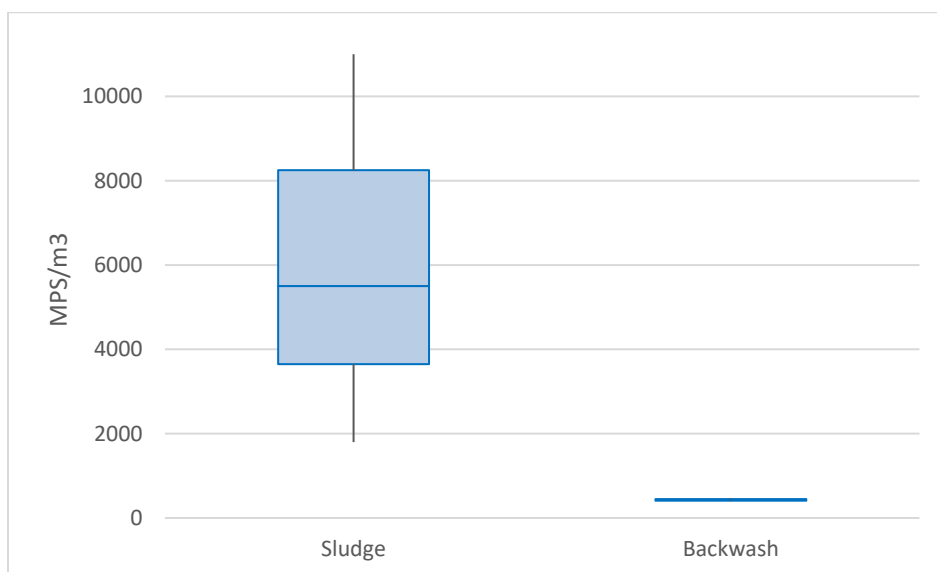


Figure 16: Distribution of MPs occurrence in sludge (left) and backwash (right) of Castreccioni DWTP

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (**Figure 17**Figure 29), their size (**Figure 18**) and their polymer composition (**Figure 19**), at the different steps of Castreccioni DWTP (A), and observed in the backwash and in the sludge samples (B).

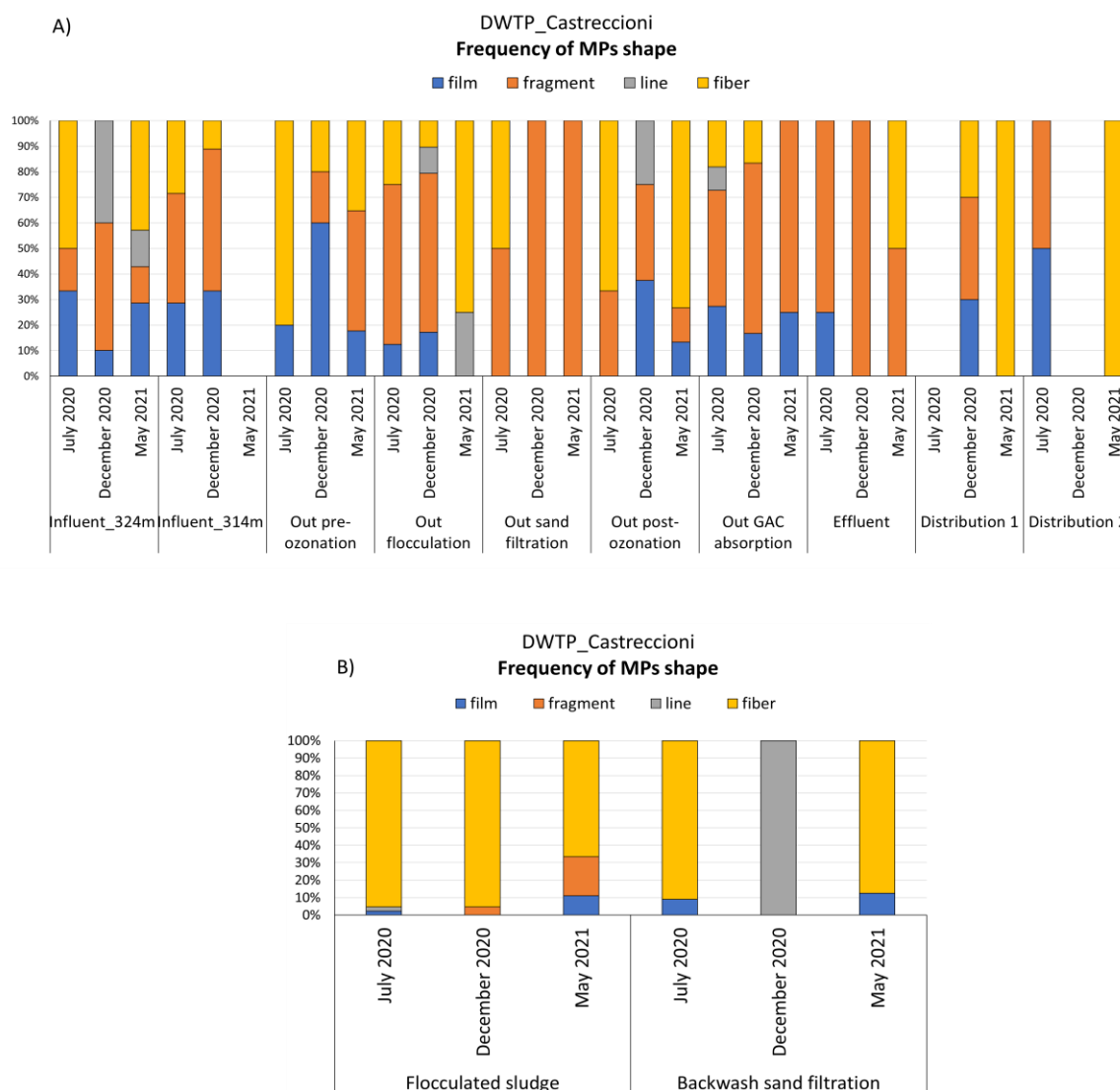


Figure 17: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castreccioni drinking water treatment plant during the three-sampling campaigns.

As concern particles shape, MPs are distributed between film, fragments, lines and fibres along the treatment line, while in backwash and flocculated sludge fibres are the most present.

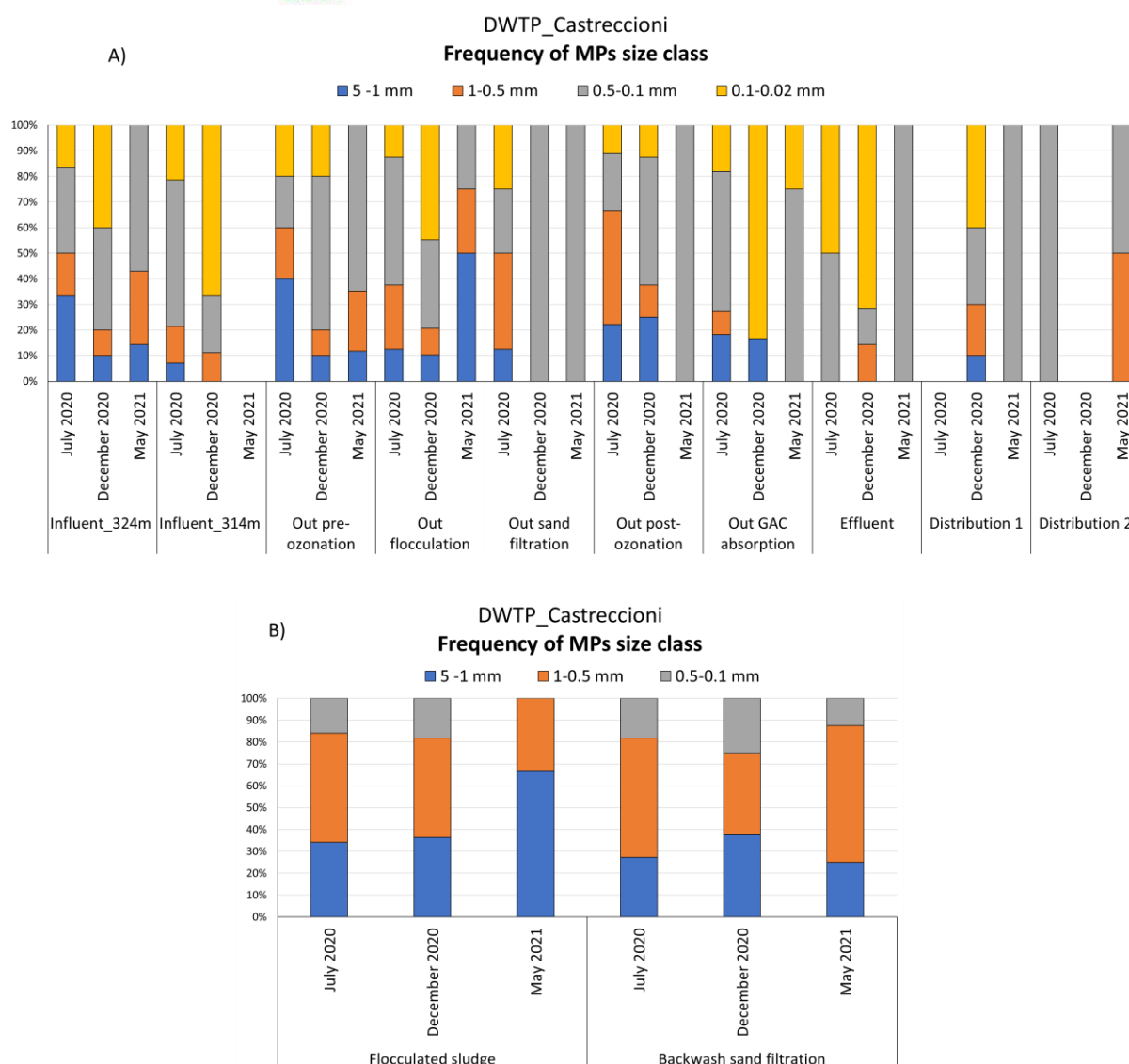


Figure 18: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castrecconi drinking water treatment plant during the three-sampling campaigns.

Regarding MPs dimensions, size classes were differently distributed in the influent, while in the effluent the presence of lower particles (0.02 – 0.1 mm) increased, mainly due to the removal of the bigger size MPs by the treatment units.

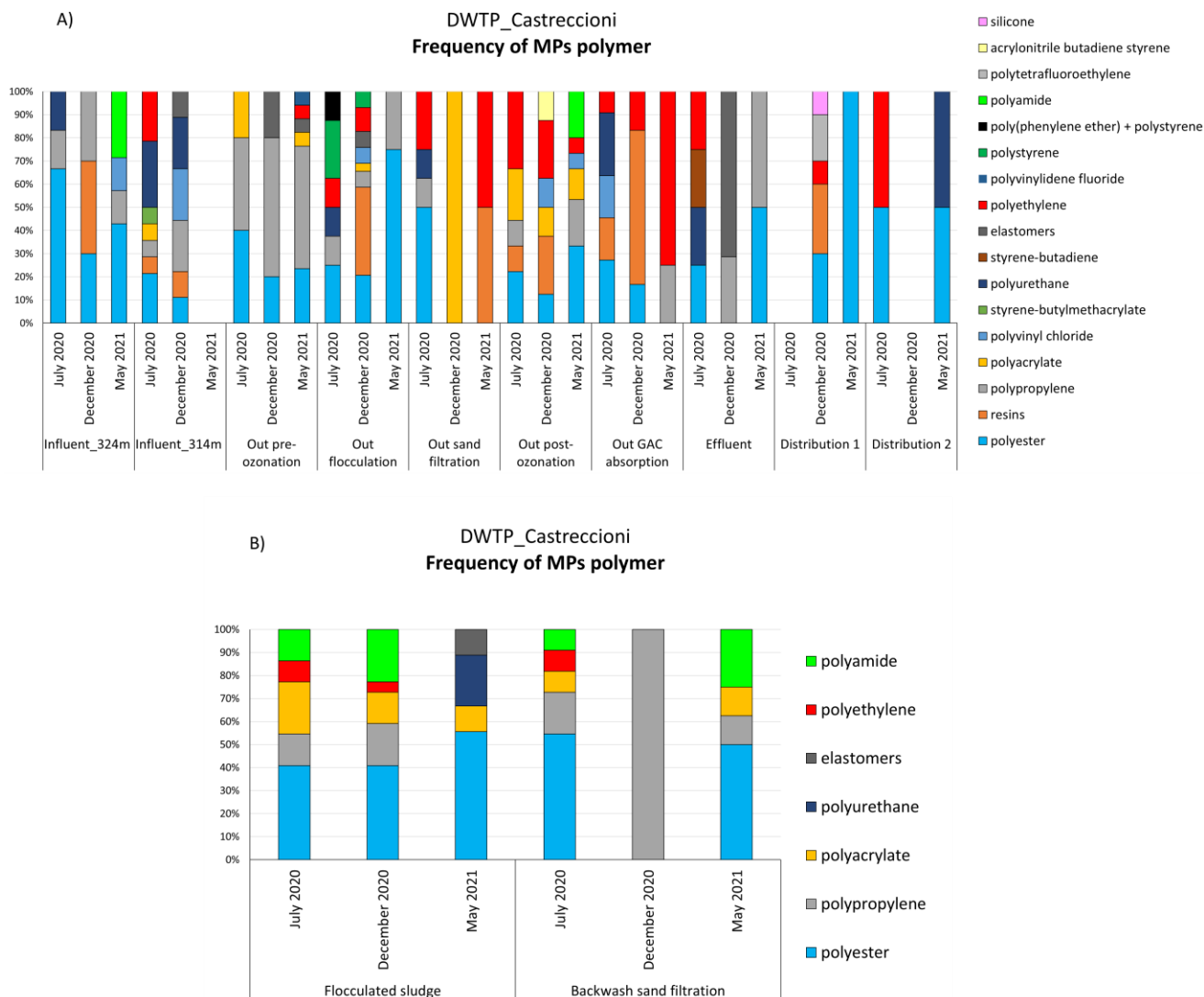


Figure 19: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) sludge line and backwash waters of Castrecconi drinking water treatment plant during the three-sampling campaign.

Considering chemical characterisation, MPs were constituted by a wide variety of material types.

Influent MPs mainly presented polystyrene, resins, polypropylene and polyurethane.

The number of total typologies detected in the different sampling periods for each sampling point are reported in **Table 21**.

Table 19: Number of different plastic typologies detected during the sampling campaigns in Castreccioni DWTP.

	July 2020	December 2020	May 2021
Influent_324m	3	3	4
Influent_314m	7	6	0
Out pre-ozonation	3	3	6
Out flocculation	6	8	2
Out sand filter	4	1	2
Out post-ozonation	5	6	6
Out activated carbon	5	3	2
Effluent	4	2	2
Distribution 1	1	5	1
Distribution 2	2	0	2
Backwash	5	1	4
Sludge	5	5	4

The influent at the lower quote of 314 m was characterised by the highest variations, suggesting a correlation between particle density.

Figure 20 Figure 24 shows the relative decrement and increment of MPs also considering the size classes, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of units' role in MPs fate.

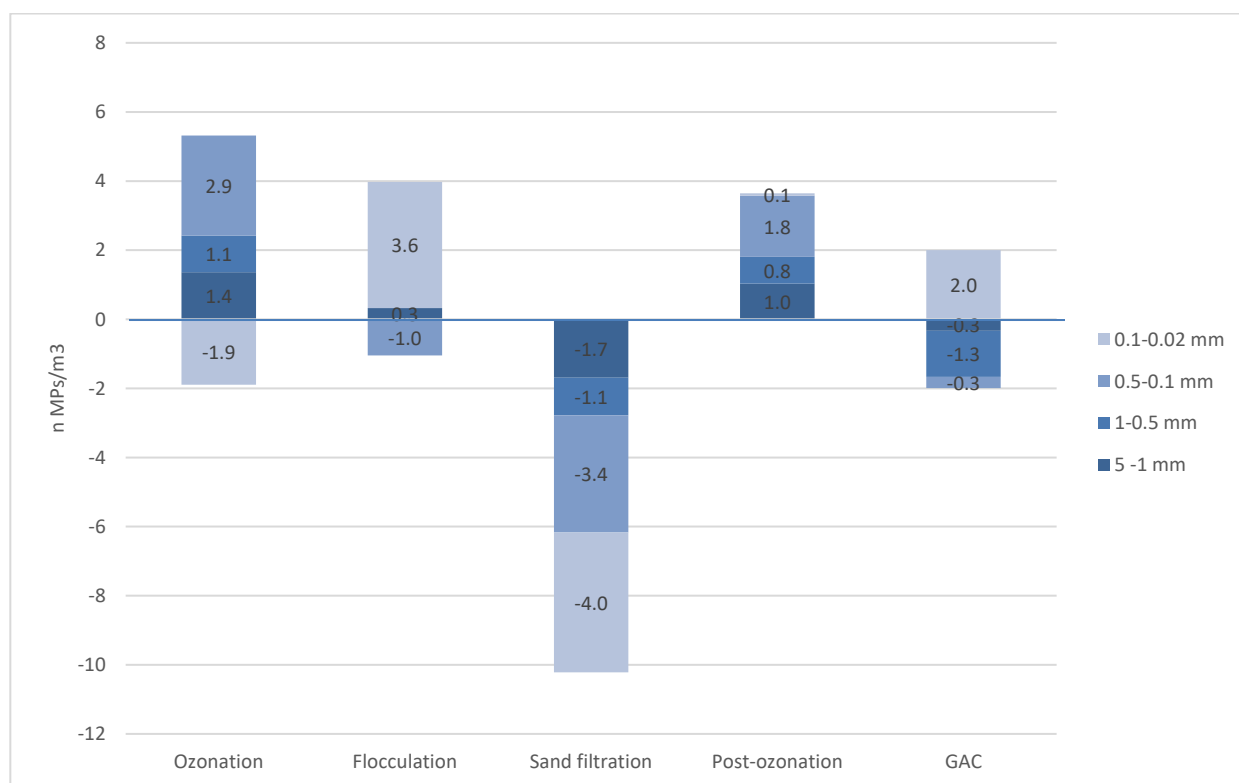


Figure 20: Decrement or increment of MPs in the treatment units of Castreccioni DWTP

It can be observed that the pre-ozonation unit was characterised by an increment on medium-high size particles (0.1 – 5 mm).



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On the other hand, sand filtration accounted for the reduction of all the class-sizes of MPs.

Post-ozonation unit was affected by a slight increase in MPs, mainly of medium-big size.

Finally, GAC adsorption was able to reduce the medium-high sizes (0.1 – 5 mm), while an increase was observed for the MPs of lowest dimensions.

This semi-qualitative evaluation highlights the higher effect in MPs reduction linked to the physical processes, more than the chemical ones.

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

3.2 Garda Molinet DWTP

Results of the sampling campaigns performed for Molinet DWTP are schematised in the following figures.

Figure 21 shows MPs concentrations along the different treatment units for the three sampling campaigns performed.

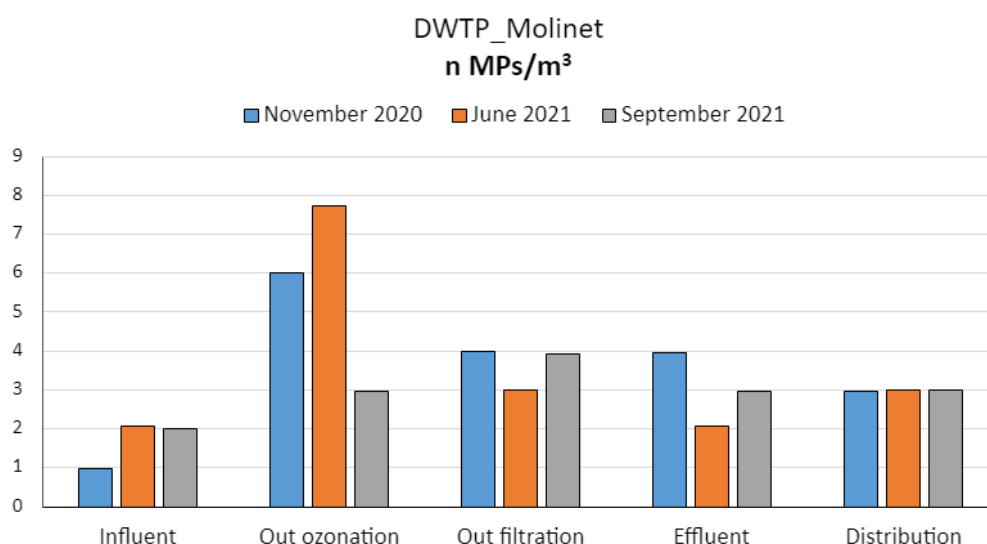


Figure 21: MPs abundance in waste and treated waters of Molinet drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

It can be observed that the MPs concentrations in the different treatment units were quite similar in all the different sampling periods, except for the ozonation unit, which presented the highest variability.

Influent MPs concentration is about 1 – 2 MPs/m³ and due to microparticles with shape of film or fragment (Figure 18A).

Similar to Castreccioni DWTP, in the effluent from the ozonation process, an increase in MPs concentration was observed, with values of about 6 ± 2 MPs/m³. The highest increment was identified in June, while in the last sampling campaign of September 2021 only a slight increase was detected.

These results are in line with the findings already discussed for Castreccioni DWTP (see Chapter 3.1) and in the literature research, as detailed in Chapter 6, since the ozonation process was found to be a possible cause of MPs release. Possible correlations between the ozone dose and the indirect effect on MPs are probably expected and need to be furtherly investigated.

However, after the filtration unit, a reduction of MPs can be observed, with average effluent concentration values around 4 ± 1 MPs/m³.

In the final effluent, low concentration data were detected, of about 3 ± 1 MPs/m³.

Accordingly, the concentrations measured in the distribution point had an average of 3 MPs/m³.

Figure 22 shows the distributions of MPs concentration for each point monitored in Molinet DWTP during the three sampling campaigns performed. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and

the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes. It can be observed that on average, values are almost always less than 7 MP_s/m³. Influent and effluent concentrations are mainly characterized by low concentrations of MPs, even if an increase can be detected after the ozonation unit. Moreover, it must be highlighted that the effluent from the ozonation process is the point affected by the highest variability.

However, globally the DWTP can provide a final effluent and distributed flow with MPs values lower than 3 MP_s/m³, with very limited variations, independently to the variability of the influent and the previous steps.

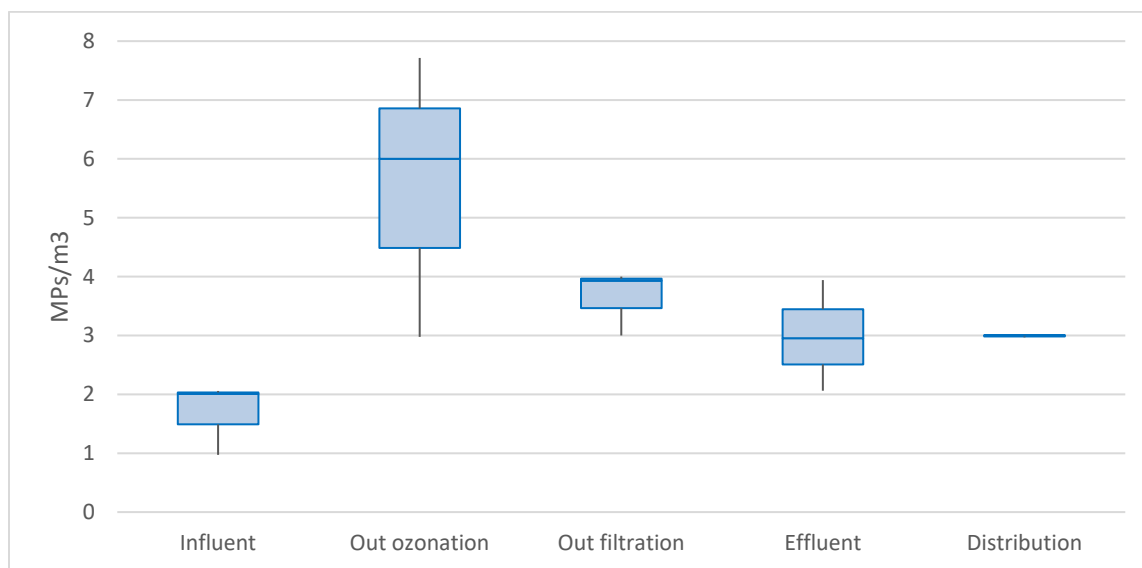


Figure 22: Distribution of MPs occurrence in Molinet DWTP

Graphs reported in **Figure 23** show the distribution of MPs characteristics, depending on their shape (A), size (B) and polymer composition (C).

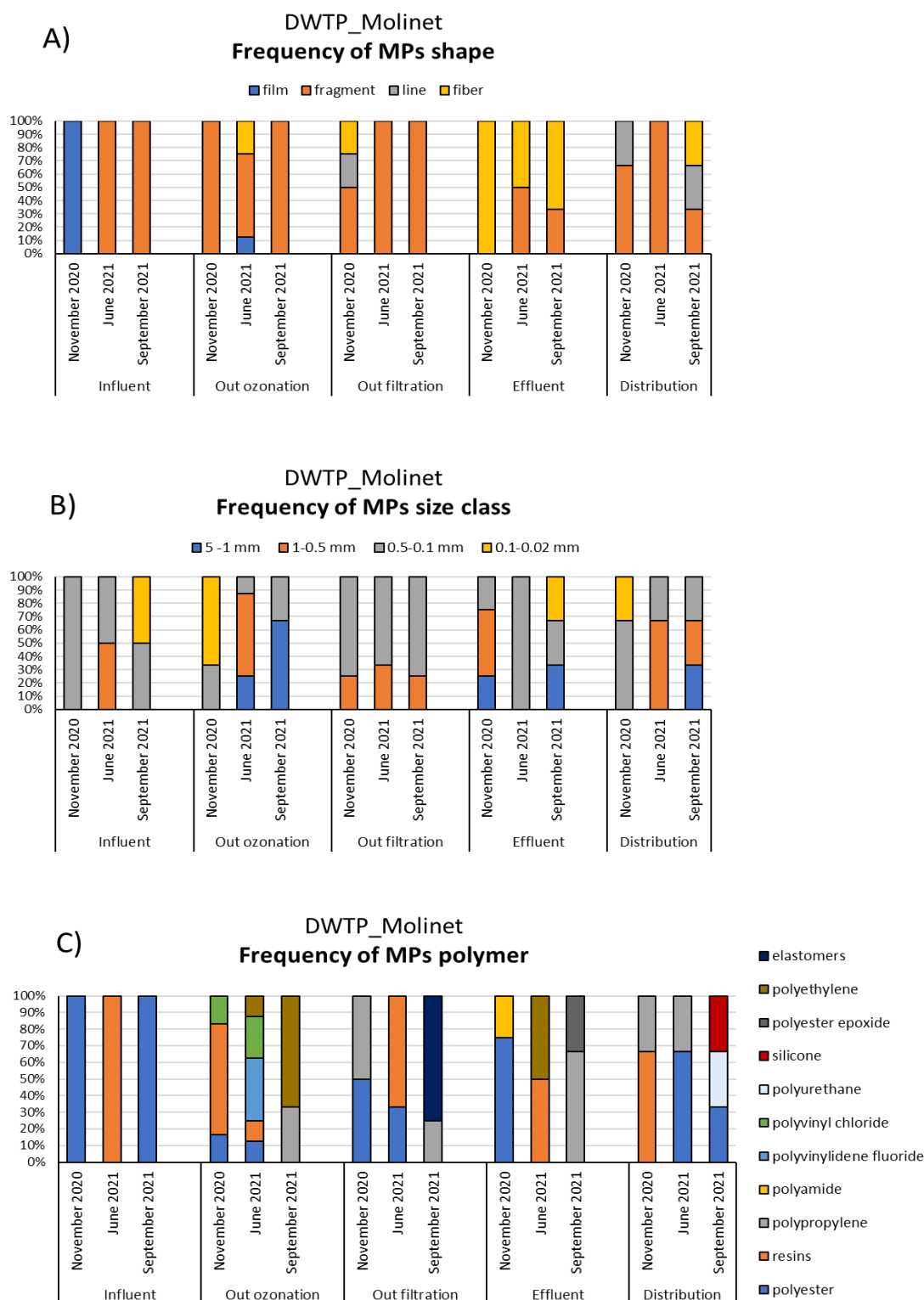


Figure 23: Characteristics of MPs extracted from waste and treated waters of Molinet drinking water treatment plant during the three-sampling campaign: A) relative contribution of shapes (%), B) relative contribution of size classes (%), C) relative contribution of polymers.

It can be observed that in the effluent a higher percentage of fibres was detected compared to the other steps where MPs are mainly in form of particles (i.e. fragments, films and lines)

As concern MPs size, it can be observed that in the influent were found low-medium particles, while after the ozonation unit, particles of 1 – 5 mm were observed. This is in line with the high presence of fibres that was analysed, which are generally longer than fragments and films.

It can also be noted that MPs detected in the influent were mainly made of polyester and resin. However, along the treatment layout, polyvinyl chloride, polyvinylidene fluoride, polyethylene, polypropylene, elastomers, polyamide and polyester epoxide were also found.

The number of total typologies detected in the different sampling periods for each sampling point are reported in **Table 20**.

Table 20: Number of different plastic typologies detected during the sampling campaigns in Molinet DWTP.

	November 2020	June 2021	September 2021
Influent	1	1	1
Out ozonation	3	5	2
Out filtration	2	2	2
Effluent	2	2	2
Distribution	2	2	3

Figure 24 shows the relative decrement and increment of MPs also considering the size classes, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the different units in MPs fate.

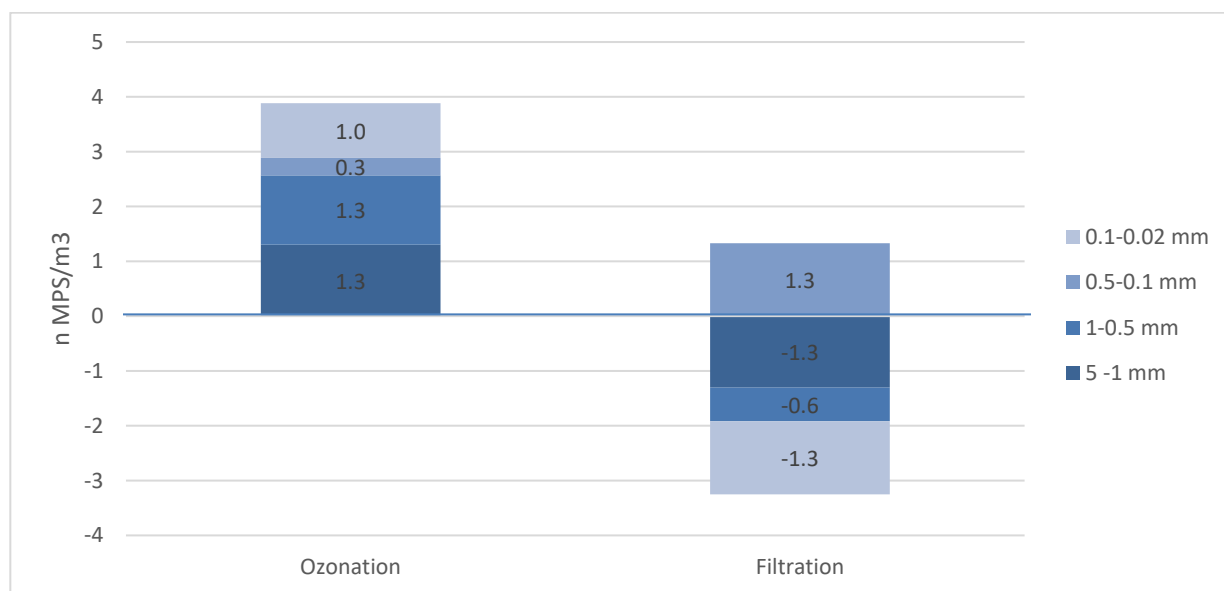


Figure 24: Decrement or increment of MPs in the treatment units of Molinet DWTP

For all the three sampling campaigns, an increase of MPs can be observed after the ozonation treatment, mainly due to medium-big size.

For the filtration unit, a general decrement of MPs can be identified, even if particles in the range of 0.1 – 0.5 mm seem to slightly increase. Generally, best performances were related to particles removal, rather than to microfibrs (see Annex A).



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Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

3.3 Brenzone Castelletto DWTP

Results of the sampling campaigns performed for Brenzone Castelletto DWTP are summarised in the following figures.

Figure 25 shows MPs concentrations in the different units of Brenzone DWTP for the three sampling campaigns performed.

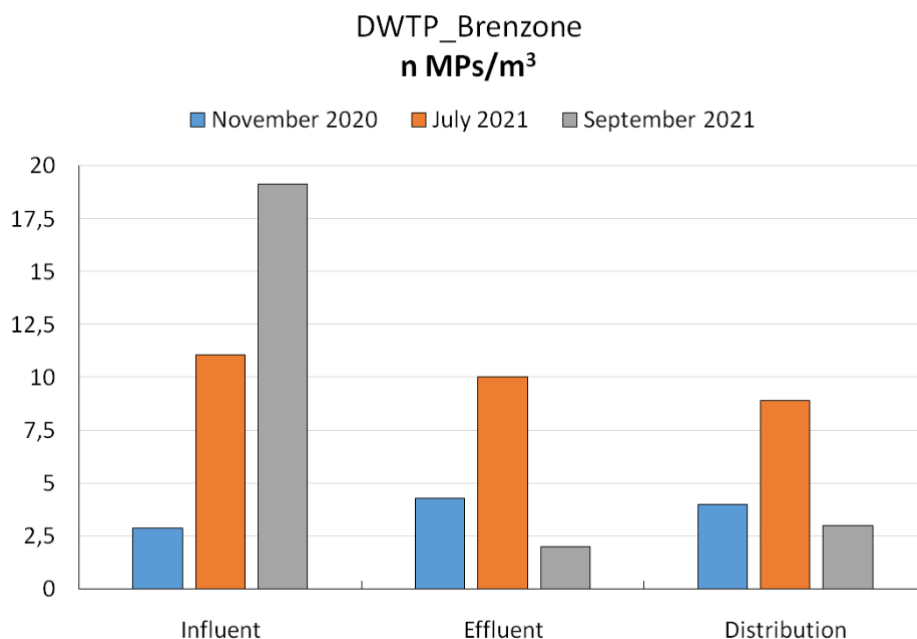


Figure 25. MPs abundance in waste and treated waters of Brenzone drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

Influent concentrations were affected by high variability (11 ± 8 MPs/m³). Accordingly, the effluent showed values of about 5 – 4 MPs/m³, which were also similar to the ones detected in the distribution (about 5 – 3 MPs/m³). The sampling campaign performed in the summer period (July 2021) was characterised by constant concentrations a from the influent to the distribution, while the sampling campaign of September 2021 showed the highest influent values, with a reduction of MPs in effluent and in the distribution point.

It must be noticed that in the period 26 – 28 September 2021 a rainy event occurred (https://www.arpa.veneto.it/bollettini/storico/2021/0118_2021_PREC.htm). In fact, it is known that rain events may cause run-off in the catchment and overflows from sewer system, collecting and convey materials to the basin.

Figure 26 shows concentrations measured in the backwash fluxes of the coarse filter and the ultrafiltration units. Concentrations are significantly higher (177 – 110 MPs/m³ for the backwash of the coarse filter and about 68 – 19 MPs/m³ for the backwash of the ultrafiltration), since backwash removes all the material that was accumulated during the operative working conditions of the filters.

DWTP_Brenzone
n MPs/m³

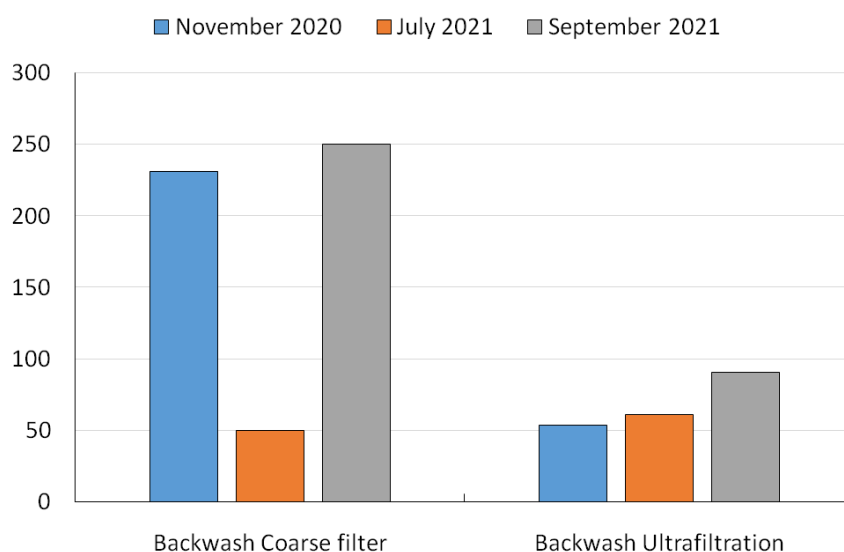


Figure 26: MPs abundance in backwash waters of Brenzone drinking water treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

Figure 27 and **Figure 28** report the distributions of MPs concentration for each monitored point in Brenzone Castelletto DWTP during the three sampling campaigns performed. The boxes represent the variability of the measures at each point in the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes.

It can be observed that on average, values are almost always less than 15 MPs/m³. Moreover, it must be highlighted that a net decrement can be observed in the DWTP. Influent and effluent concentrations are mainly characterized by low concentrations of MPs.

Backwash fluxes are characterized in general by higher distributions, since their effect is to remove the solid particles retained by the filtration units.

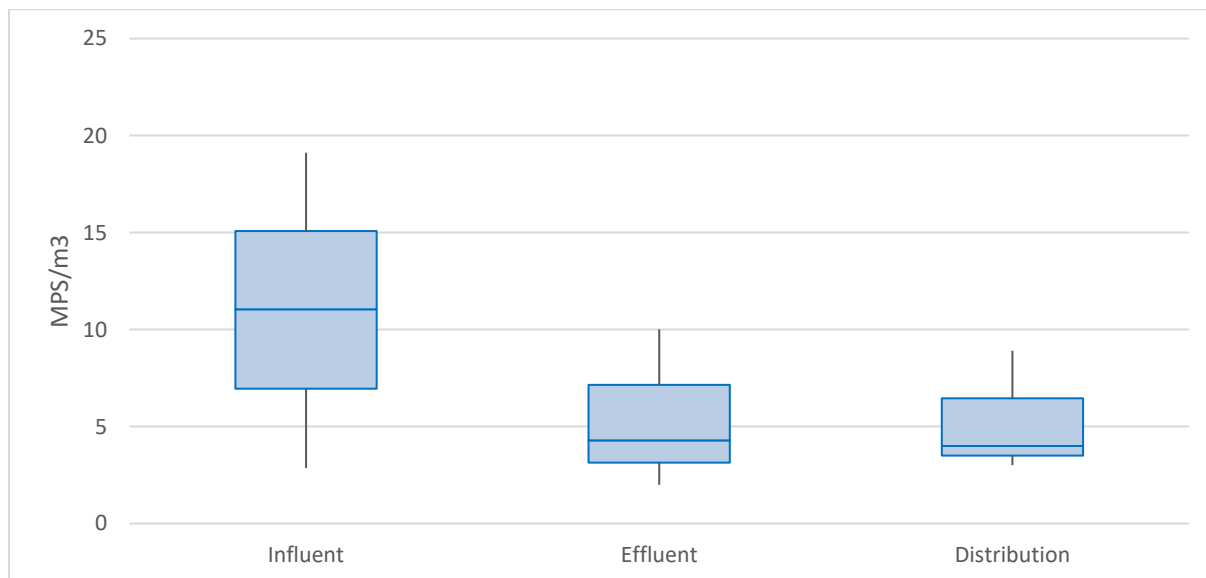


Figure 27: Distribution of MPs occurrence in Brenzone Castelletto DWTP

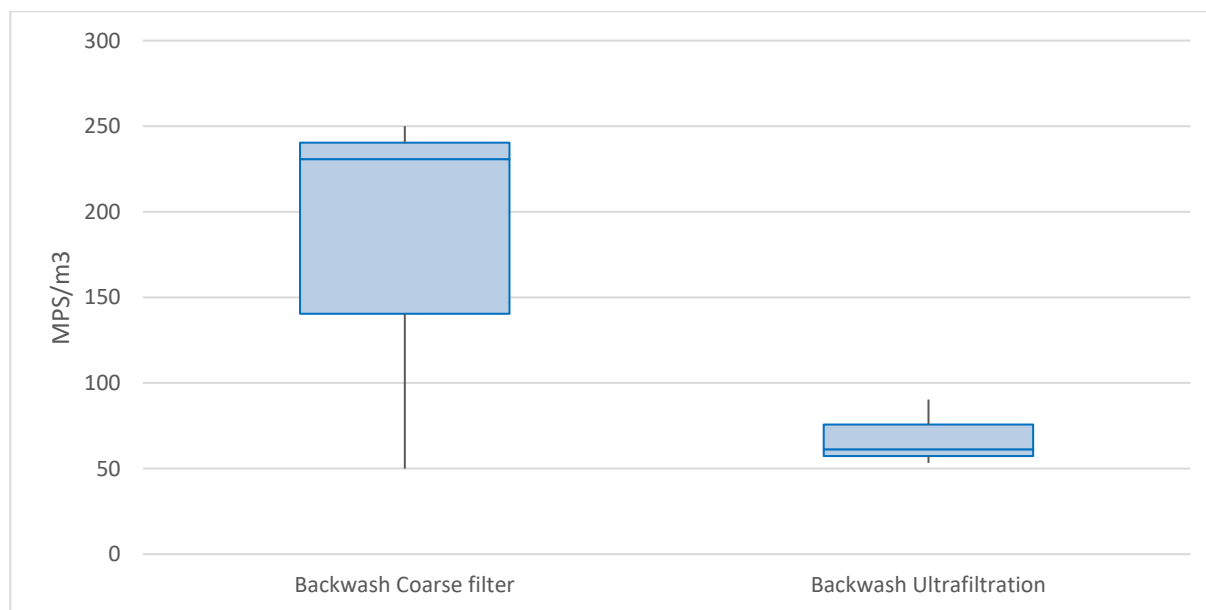


Figure 28: Distribution of MPs occurrence in backwash fluxes of Brenzone Castelletto DWTP

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (**Figure 29**), their size (**Figure 30**) and their polymer composition (**Figure 31**), at the different steps of Brenzone Castelletto DWTP (A), and at the backwash fluxes (B).

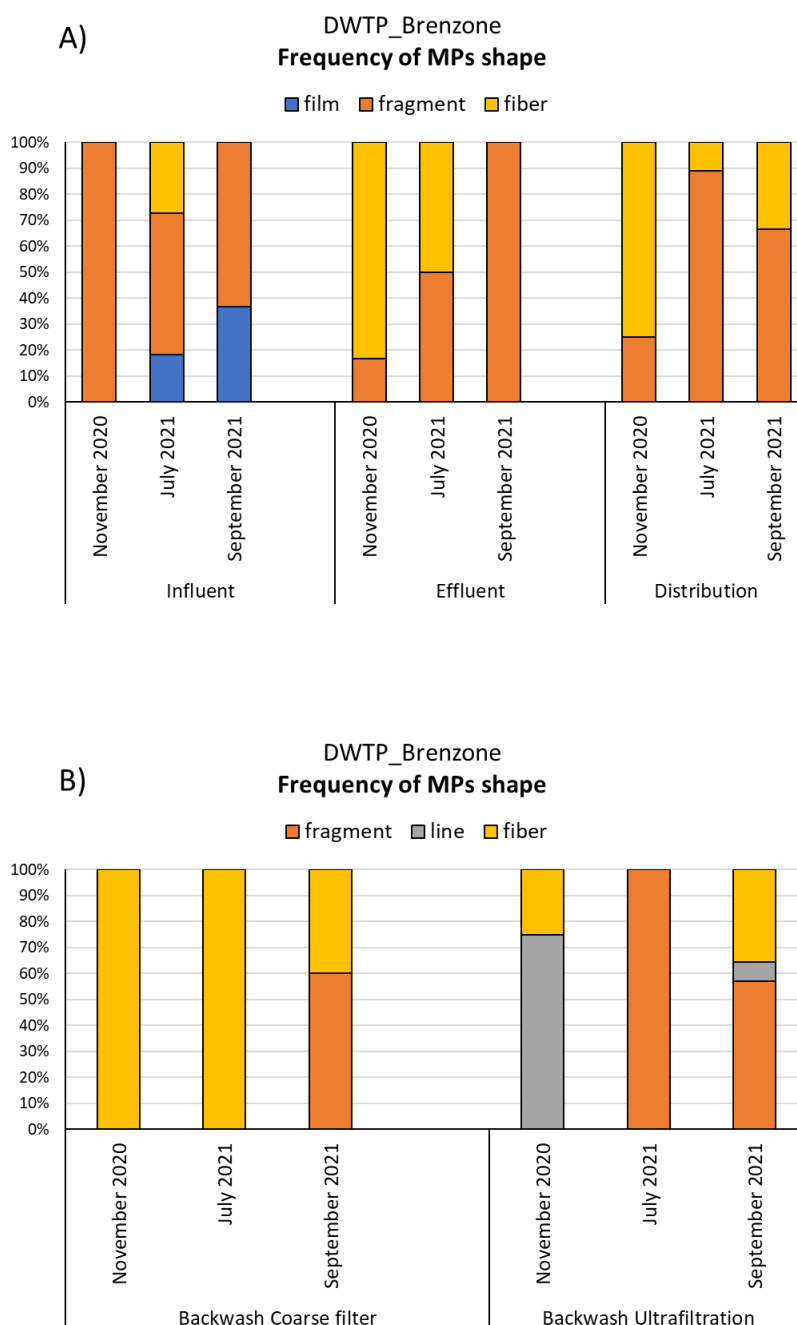


Figure 29: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

It can be noticed that influent is mainly characterized by fragments, while fibers could be detected at the effluent.

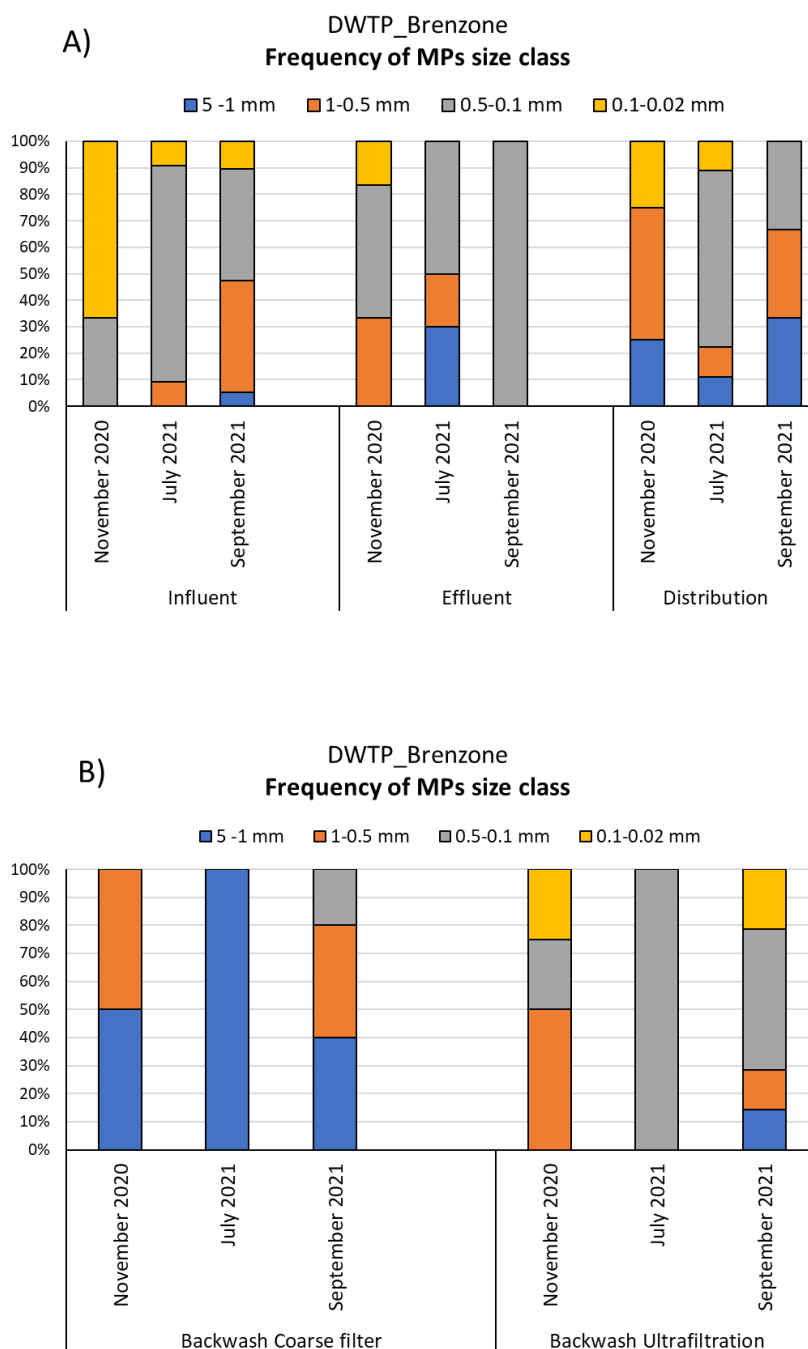


Figure 30: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

Influent size distribution is mainly characterized by low-medium dimensions, even if on September 2021 sampling campaign, bigger size particles were detected also in the distribution.

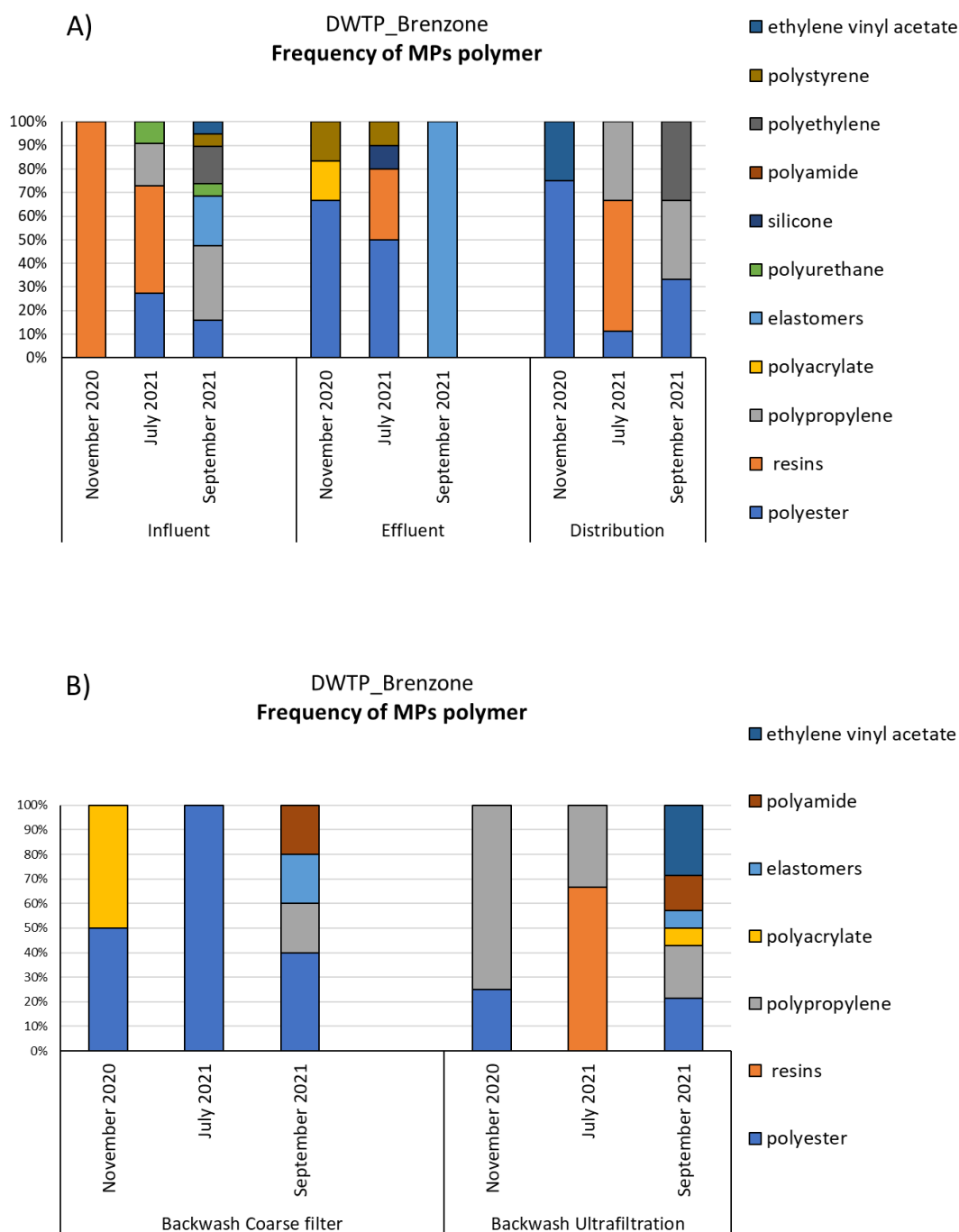


Figure 31: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) backwash waters of Brenzone drinking water treatment plant during the three-sampling campaigns.

As concern MPs characterization, in the influent polyester, resins, polypropylene, polyacrylate, elastomers, polyurethane, silicone, polyamide, polyethylene, polystyrene, ethylene and vinyl acetate were detected. It must be noticed that the widest variability occurred in correspondence of the September 2021 sampling campaign, that was probably affected by the rain events of the previous days. The number of total typologies detected in the different sampling periods for each sampling point are

reported in **Table 20**.

Table 21: Number of different plastic typologies detected during the sampling campaigns in Brenzone Castelletto DWTP.

	November 2020	July 2021	September 2021
Influent	1	4	7
Effluent	3	4	1
Distribution	2	3	3
Backwash Coarse filter	2	1	4
Backwash Ultrafiltration	2	2	6

Figure 32 shows the relative decrement and increment of MPs also considering the size classes, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the treatment units in MPs fate.

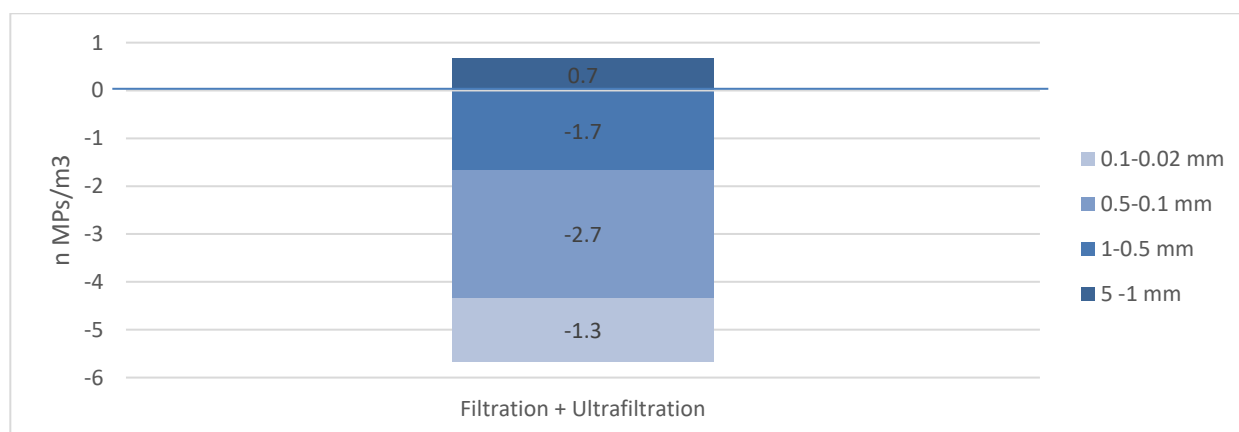


Figure 32: Decrement or increment of MPs in the treatment units of Brenzone Castelletto DWTP

Generally, decrements in the filtration units were observed for almost all the range sizes, even if some medium big size particles (0.1 – 5 mm) were still detected.

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

4. Presence and physic-chemical characterisation of MPs in WWTPs

The results of the sampling campaigns carried out for each one of the wastewater treatment plants selected for the Blue Lakes project are reported in the following paragraphs.

Data are showed as MPs, representing the sum of the microparticles and microfibres made of plastic polymers (MPPs and PMFs, respectively). Furthermore, in the Annex A it is reported the relative contribution of synthetic and natural microfibers on the total extracted and characterised during the present study given the increasing attention on artificial microfibers of natural origin (produce from animal- or plant-based materials) as emerging contaminants beyond MPFs.

Information about the characterisation procedures and results reporting can be found in the previous Deliverable of Action B3 “Technical report and operative manual regarding the improvement of the treatment process”.

4.1 Limone Tremosine WWTP

Results of the sampling campaigns performed for Limone Tremosine WWTP are summarised in the following figures.

Figure 13 and **Figure 14** show MPs concentrations in the treatment units for the three sampling campaigns performed, respectively, for the units of the WWTP layout and for the sludge grab samples analysed.

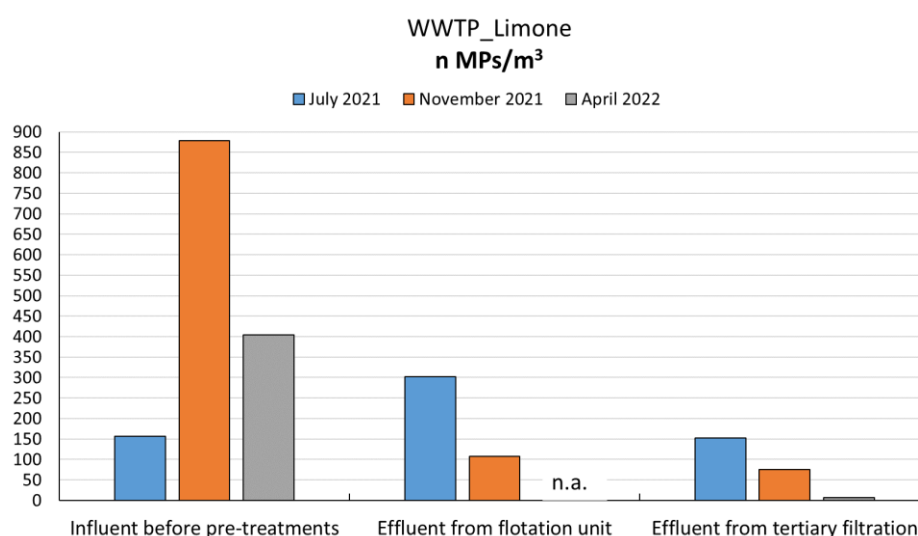


Figure 33: MPs abundance in waste and treated waters of Limone Tremosine wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m³). n.a.= sample not available for that sampling campaign (April 2022).

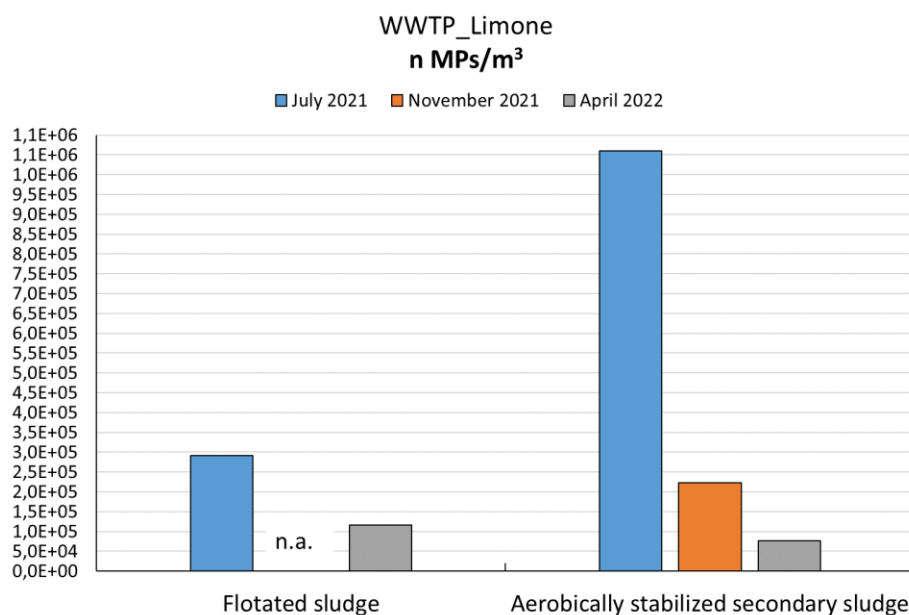


Figure 34: MPs abundance in sludge samples of Limone Tremosine wastewater treatment plant detected during the three-sampling campaign. Data are given as number (n) of microplastics (MPs) per volume of sample (m³). n.a.= sample not available for that sampling campaign (November 2021).

It can be observed that the influent is characterised by high variability of MPs concentration, in the range of 408 ± 367 MPs/m³, with the highest values during the winter campaign and the lowest in summer.

It must be underlined that during the winter campaign very low influent flows were observed in the WWTP, affected by touristic seasonality. Therefore, the influent collection was probably affected by the elevated presence of settled material on the bottom of the sampling point.

In the first sampling campaign an increase was observed at the effluent from the flotation unit, after biologic treatments; however, concentrations decreased again at the final effluent after the tertiary filtration.

On average, concentrations after the flotation units were measured of 205 ± 137 MPs/m³, while in the final effluent mean values were of about 78 ± 72 MPs/m³.

MPs concentrations in sludge samples are generally higher (respectively, 203500 ± 123744 MPs/m³ in the flotated sludge and 453083 ± 530540 MPs/m³ in the stabilised sludge), due to the sedimentation of solid matter and accumulation effects.

Figure 22 show the distributions of MPs concentration for each point monitored in Limone WWTP during the three sampling campaigns performed. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes.

It can be observed that the average number of MPs/m³ entering through the influent is halved after each process step, highlighting the effectiveness of the treatments applied in reducing the levels of microplastics in the effluent. However, the high concentrations of MPs/m³ found in the sludge samples compared to the water ones show that MPs are subtracted from the water line, but they were concentrated in the sludge line. This evidence is currently widely shared by studies on the subject, but it strengthens the need to develop ad-hoc management strategies to prevent release of microplastics in

environment through for this complex matrix.

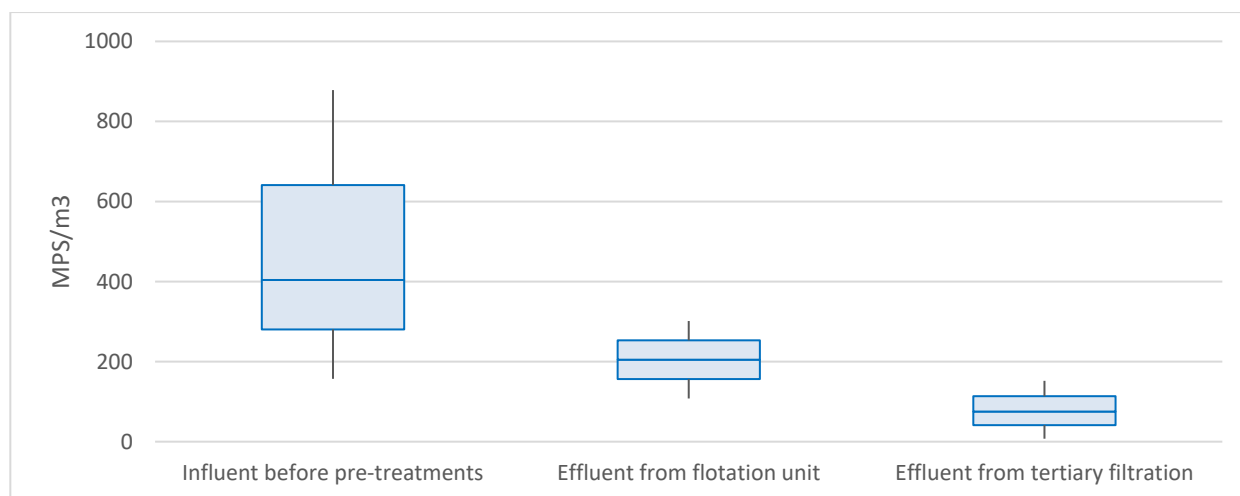


Figure 35: Distribution of MPs occurrence in Limone Tremosine WWTP

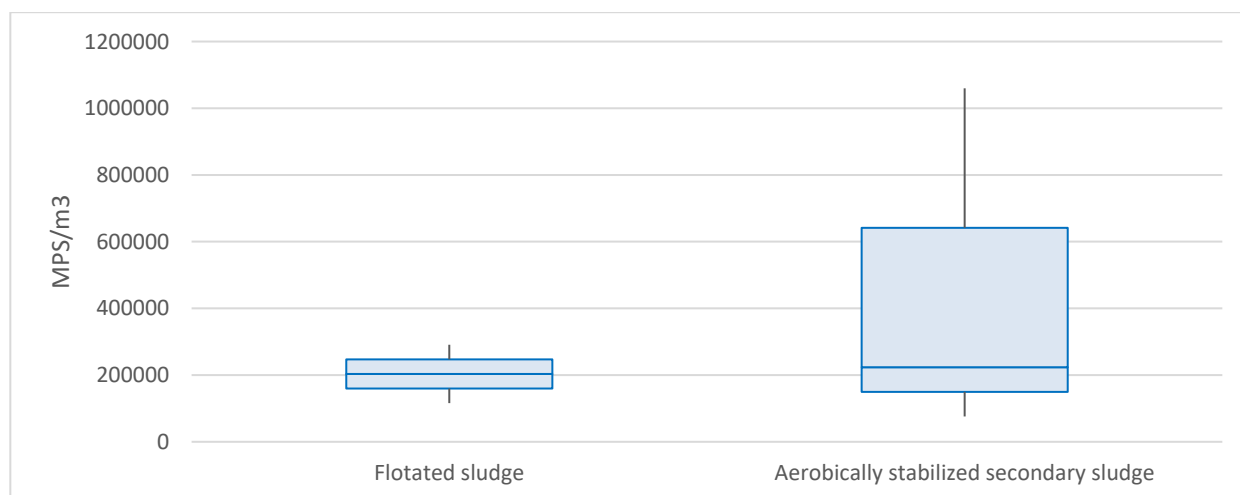
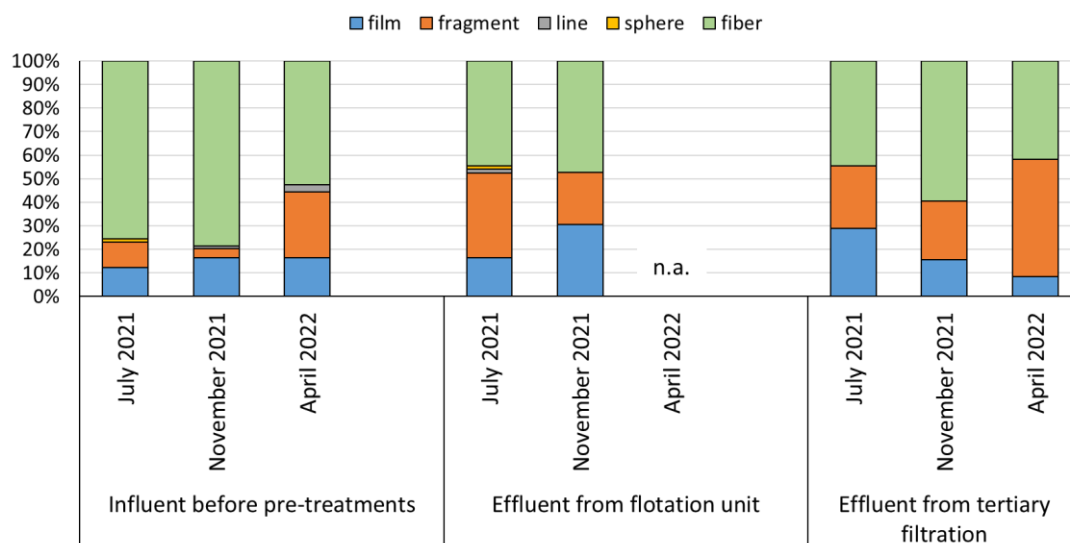


Figure 36: Distribution of MPs occurrence in Limone Tremosine WWTP

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (**Figure 29**), size (**Figure 30**) and polymer composition (**Figure 31**), at the different steps of Limone Tremosine WWTP (A), and measured in the sludge (B).

A)

WWTP_Limone Tremosine
Frequency of MPs shape



B)

WWTP_Limone Tremosine
Frequency of MPs shape

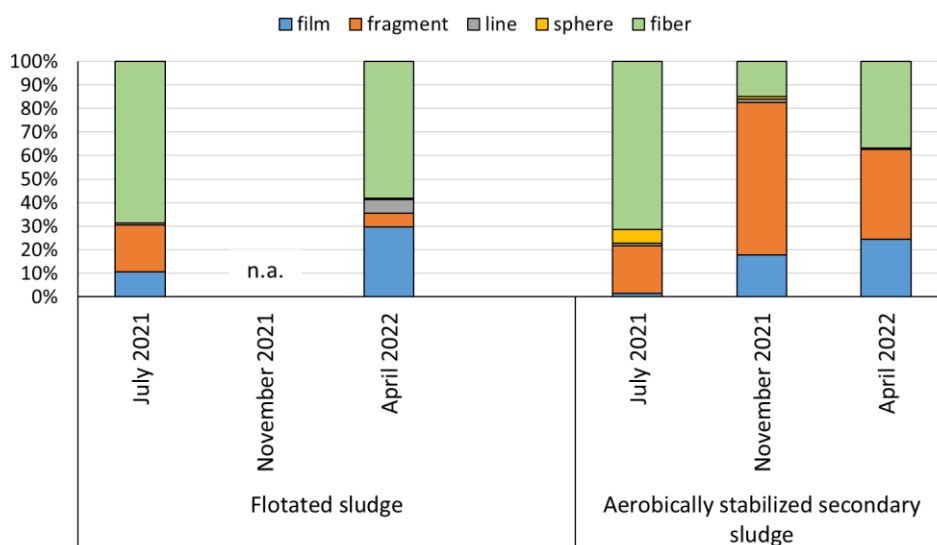
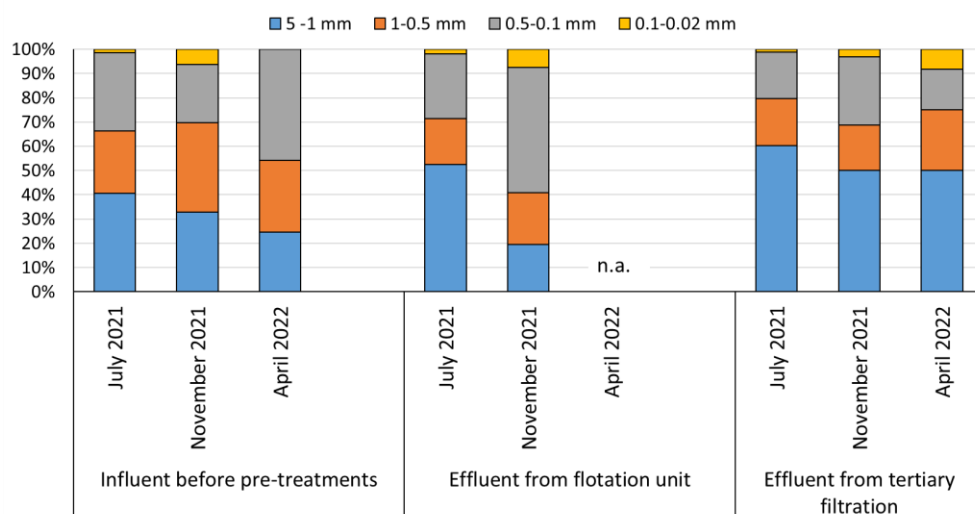


Figure 37: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

It can be observed that fibres are the most frequently detected shape, followed by fragments and films. The relative presence of the fragments, expressed in percentage, increased along the treatment line.

A)

WWTP_Limone Tremosine
Frequency of MPs size class



B)

WWTP_Limone Tremosine
Frequency of MPs size class

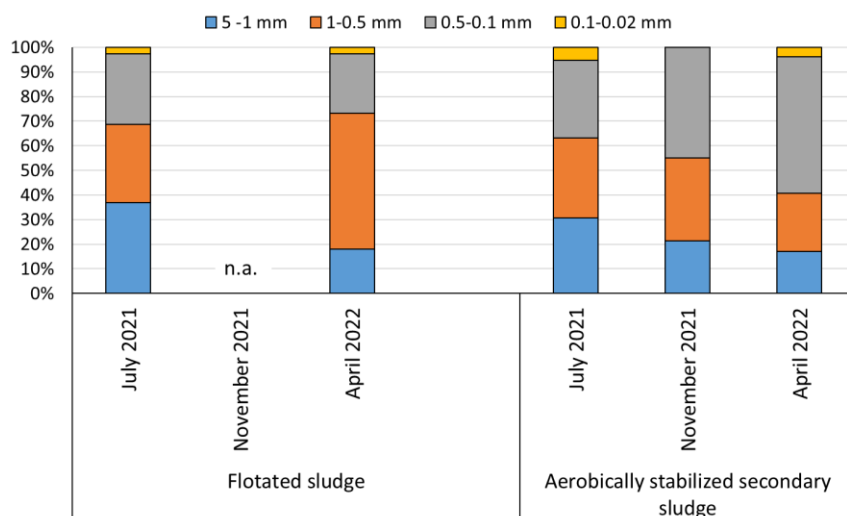


Figure 38: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

Considering particles dimension, it can be noted that medium-big size is the most frequent, according to the findings obtained for shape analysis, since fibres are usually characterised by the biggest sizes.

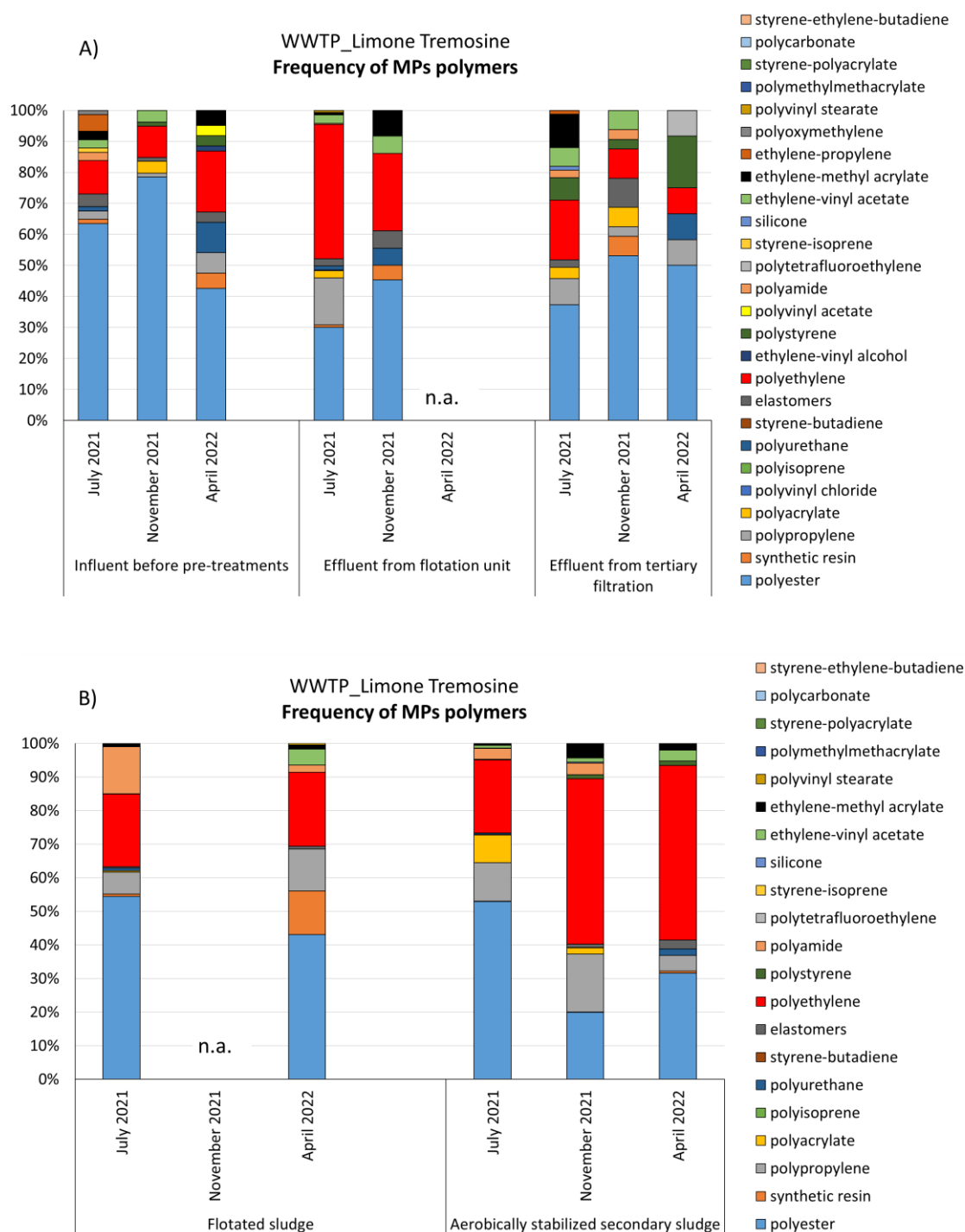


Figure 39: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) the sludge line of Limone Tremosine wastewater treatment plant during the three-sampling campaigns. n.a.= sample not available for that sampling campaign.

Analysing chemical characterisation, the most frequent particles are of polyester and polyethylene. Usually, polyester can be associated to synthetic fibres, while polyethylene can be found in fragments or films. Moreover, the relative presence of polyethylene, expressed as percentage, increased along the

treatment line after secondary treatments. The presence of polyethylene is also detected in the sludge line. However, significant variations were observed in the treatment units, as the typologies and the relative percentages varied along the treatment steps.

To better investigate the possible origins of the MPs detected, a chemical characterisation was carried out on the carriers that are used as supporting material to the attached-growth biomass in the biologic treatment. It was found that the IR spectra can be attributed to high density polyethylene, as shown in **Figure 40**.

**Infrared Spectre (IR) of carriers in Limone WWTP compared with library spectres IR standard:
Polyethylene (high density), percentage of similarity: 96%**

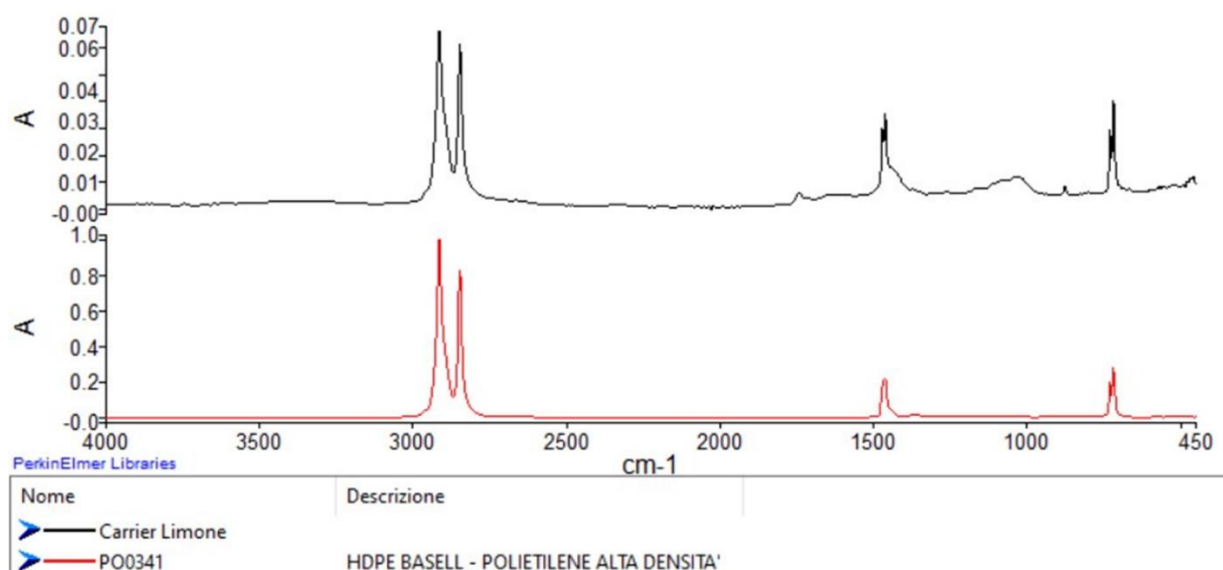


Figure 40: Characterization of the carriers in the biologic unit of Limone Tremosine WWTP

The number of total typologies detected in the different sampling periods for each sampling point are reported in **Table 20**.

Table 24: Number of different plastic typologies detected during the sampling campaigns in Limone Tremosine WWTP

	July 2021	November 2021	April 2022
Influent before pre-treatments	12	7	10
Effluent from flotation unit	12	7	n.a.
Effluent from tertiary filtration	11	9	6
Flotated sludge	12	n.a.	9
Aerobically stabilized secondary sludge	16	15	9

Figure 41 shows the relative decrement and increment of MPs considering also size classes, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the treatment units in MPs fate.

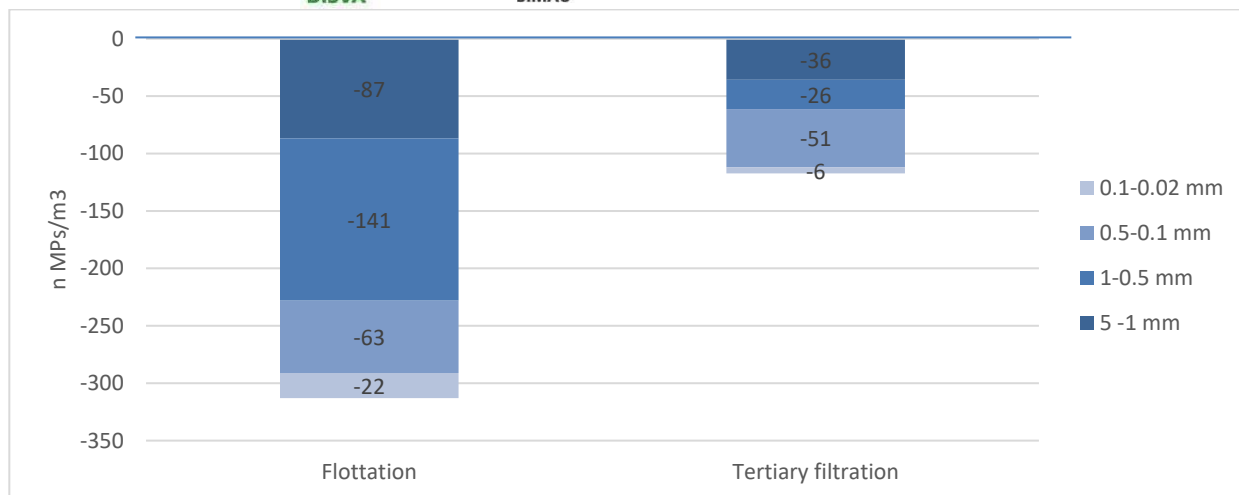


Figure 41: Decrement or increment of MPs in the treatment units of Limone Tremosine WWTP

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

4.2 Peschiera del Garda WWTP

Results of the sampling campaigns performed for Peschiera del Garda WWTP are schematised in the following figures.

Figure 42 and **Figure 43** show MPs concentrations along the different treatment units for the three sampling campaigns performed, respectively, for the different units of the WWTP layout and for the sludge grab samples analysed. It must be noticed that results are plotted using a logarithmic scale, because of the very high measure detected at the influent on November 2021 sampling campaign.

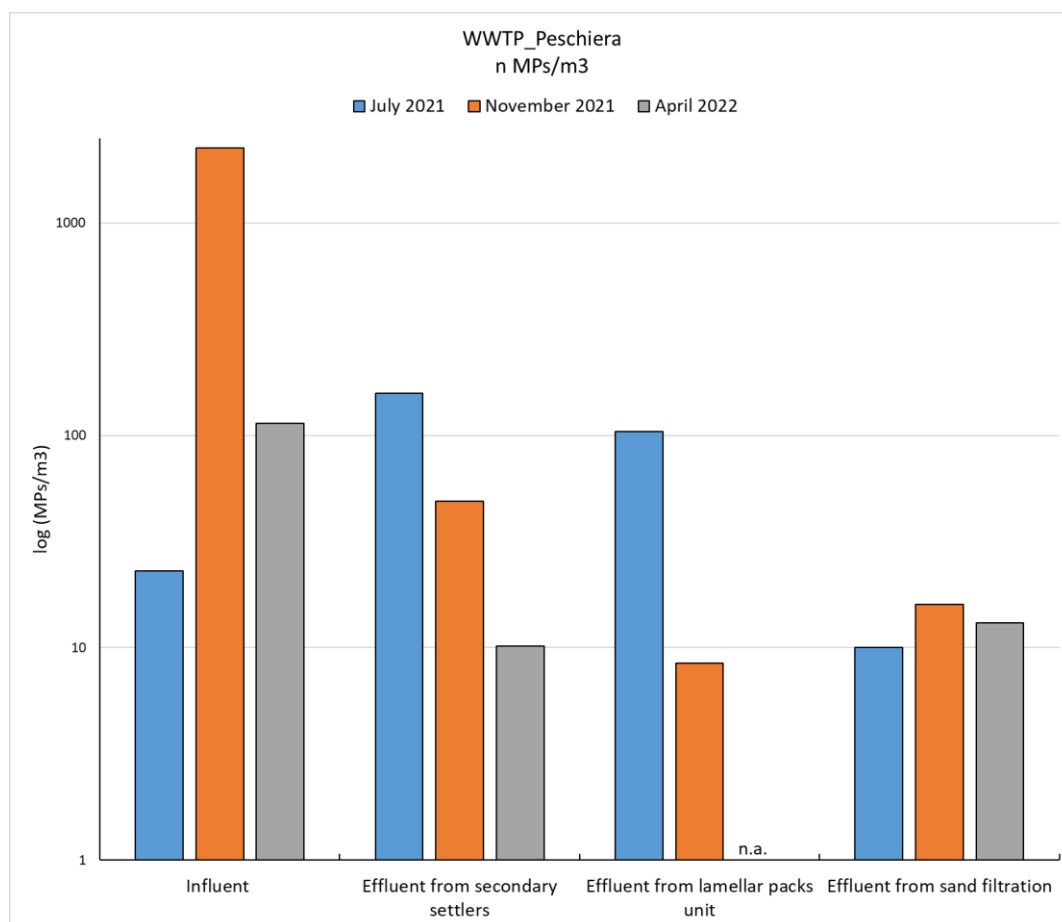


Figure 42: MPs abundance in waste and treated waters of Peschiera del Garda wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

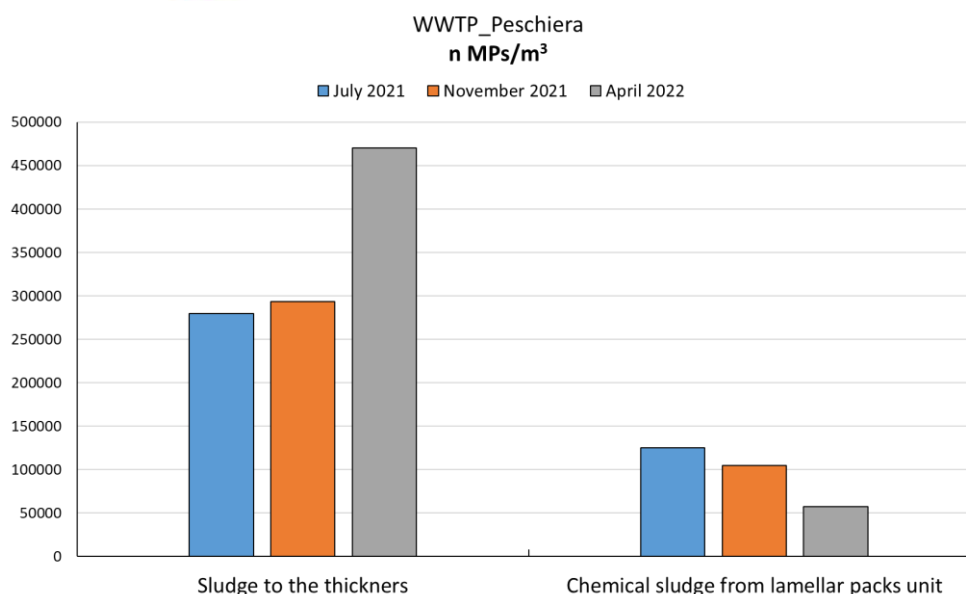


Figure 43: MPs abundance in sludge samples of Peschiera del Garda wastewater treatment plant detected during the three-sampling campaigns. Data are given as number (n) of microplastics (MPs) per volume of sample (m³).

As concern samples collected in the water treatment line, a very high peak was detected in the influent on November campaign, while other concentrations were generally below 150 MPs/m³.

It must be underlined that during the winter campaign very low influent flows were observed in the WWTP, affected by touristic seasonality. Therefore, the influent collection was probably affected by the elevated presence of settled material on the bottom of the sampling point.

It must be noted that the influent seems to be particularly diluted and thus the impact from the anthropic contribution could be smoothed by this dilution.

This hypothesis is in line with the low values of ammonia nitrogen found in the influent, which were on average at about 12 mg N-NH₄/l.

Generally, a decreasing trend can be observed along the treatment train, with effluent concentrations lower than 10-15 MPs/m³. However, only during the first sampling campaign of July, an increase was observed after the secondary treatments.

As concern sludge line, sludge samples before the thickening units were generally characterised by higher concentrations and variability, in respect to chemical sludge.

Figure 44 and **Figure 45** show the distributions of MPs concentration for each point monitored in Peschiera del Garda WWTP during the three sampling campaigns performed. The boxes represent the variability of the measurements in each point during the three different sampling periods. In particular, the extremes of the boxes correspond to the first and third quantiles, while the vertical lines reach, respectively, the minimum and the maximum values detected. The median values along the three samplings are reported as the blue lines into the boxes.

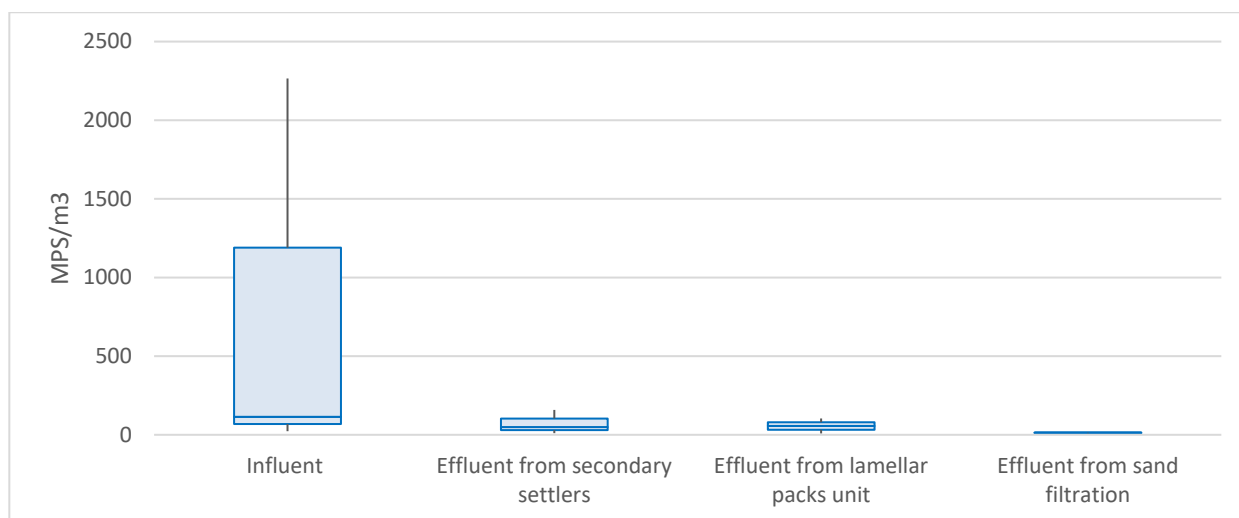


Figure 44: Distribution of MPs occurrence in Peschiera del Garda WWTP

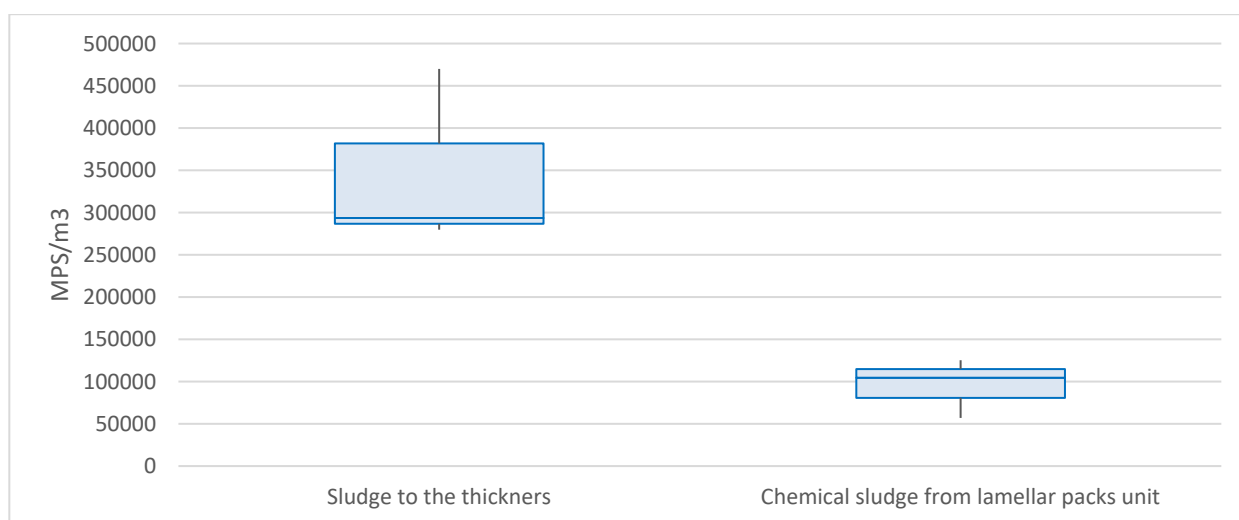


Figure 45: Distribution of MPs occurrence in Peschiera del Garda WWTP

Graphs reported in the Figures below show the distribution of MPs characteristics depending on their shape (**Figure 29**), their size (**Figure 30**) and their polymer composition (**Figure 31**), at the different steps of Peschiera del Garda WWTP (A), and measured at the sludge samples (B).

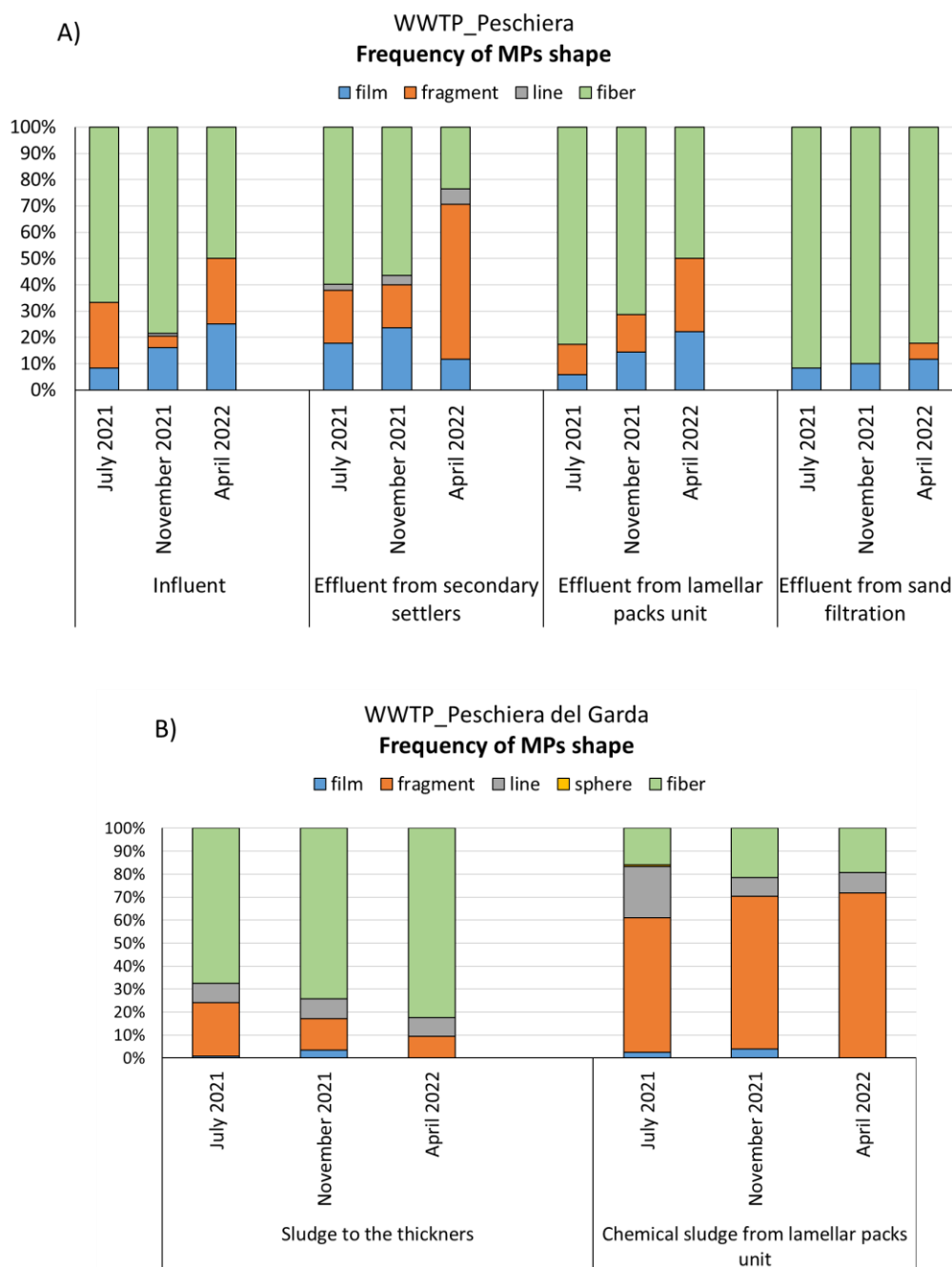


Figure 46: relative contribution of shapes (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

It can be observed that fibres are the most frequent particles shape in all the water samples and in the sludge before the thickening unit. Chemical sludge is, on the other hand, characterised by the predominant presence of fragments.

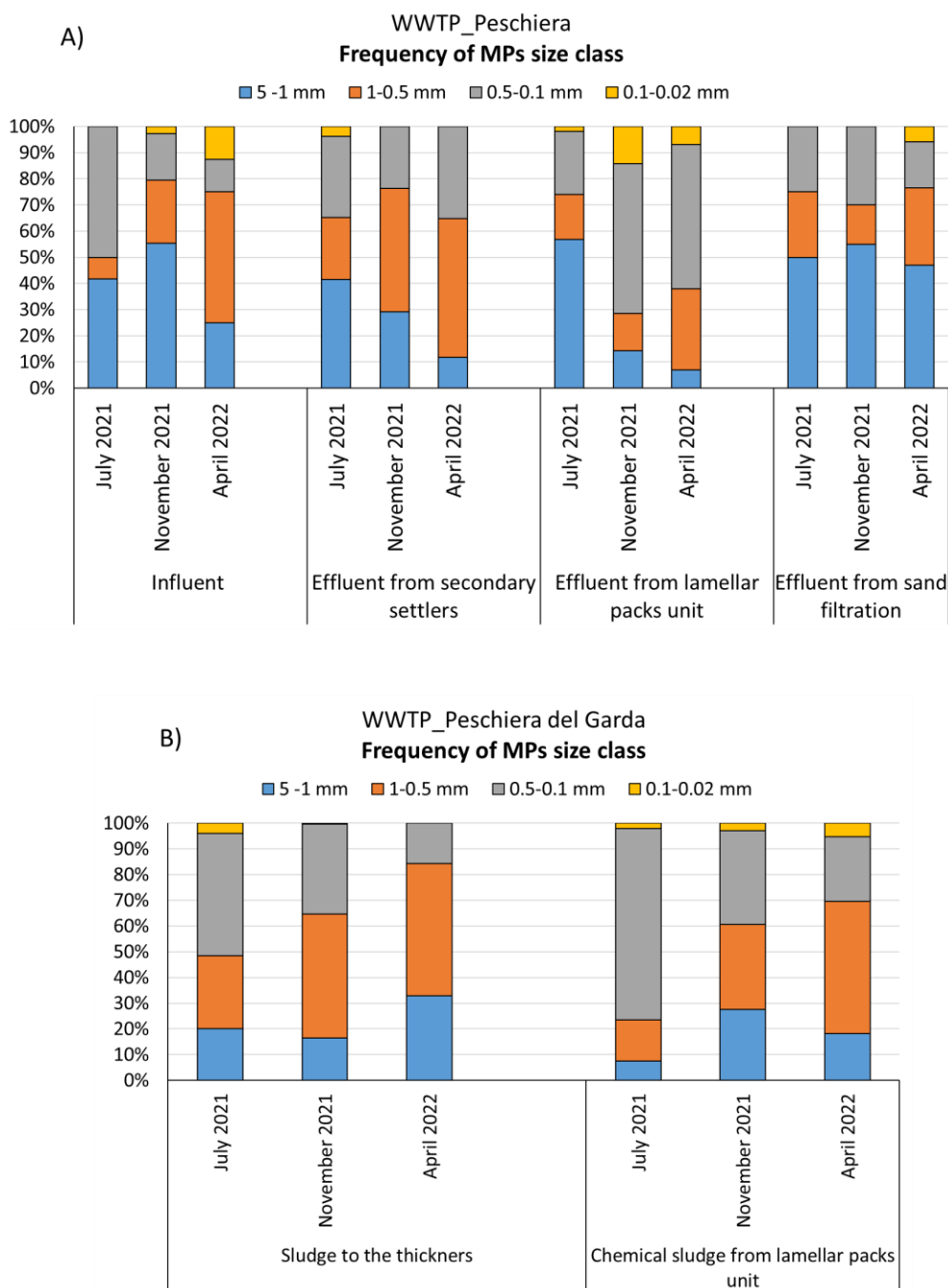
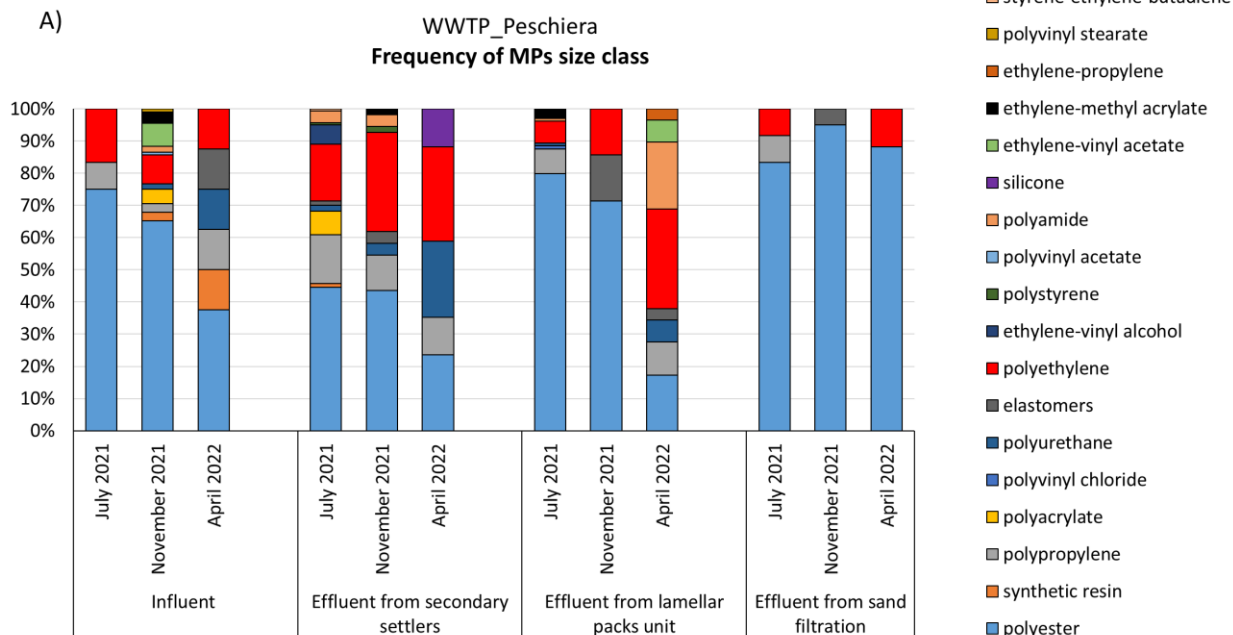


Figure 47: relative contribution of size class (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

Dimensions are quite well distributed, except for the lower size, which were less frequent.

A)



B)

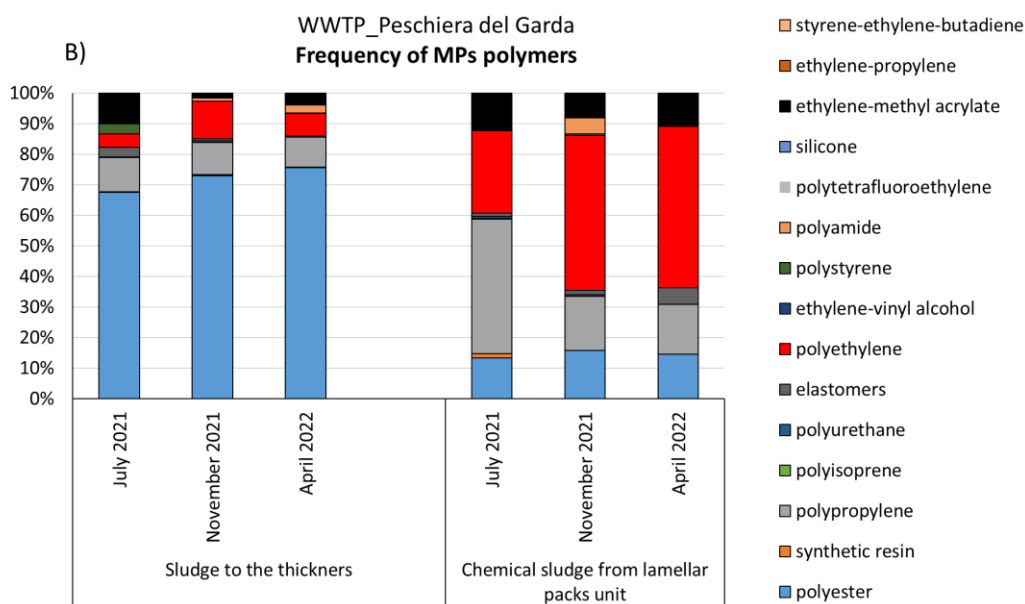


Figure 48: relative contribution of polymers (%) to the total MPs extracted from A) the water line and from B) the sludge line of Peschiera del Garda wastewater treatment plant during the three-sampling campaigns.

The chemical characterisation is in line with the physical analysis of MPs shape. In fact, usually, polyester can be associated to synthetic fibres, while polyethylene can be found in fragments or films.

Comparing these results with the characterisation obtained for Limone Tremosine WWTP, it can be noted that in Peschiera del Garda the percentage of polyester is generally higher and remains almost constant in all the different treatment steps. However, at the effluent a low amount of different plastic types was detected, mainly characterised by polyester and polyethylene.

The number of total typologies detected in the different sampling periods for each sampling point are reported in **Table 20**.

Table 22: Number of different plastic typologies detected during the sampling campaigns in Peschiera del Garda WWTP

	July 2021	November 2021	April 2022
Influent	3	11	6
Effluent from secondary settlers	11	8	5
Effluent from lamellar packs unit	7	3	n.a.
Effluent from sand filtration	3	2	2
Sludge to the thickeners	10	10	11
Chemical sludge from lamellar packs unit	10	9	6

Figure 49 shows the relative decrement and increment of MPs, averaged in the three sampling periods, observed for the different treatment processes, in order to suggest semi-qualitative indication of the role of the treatment units in MPs fate.

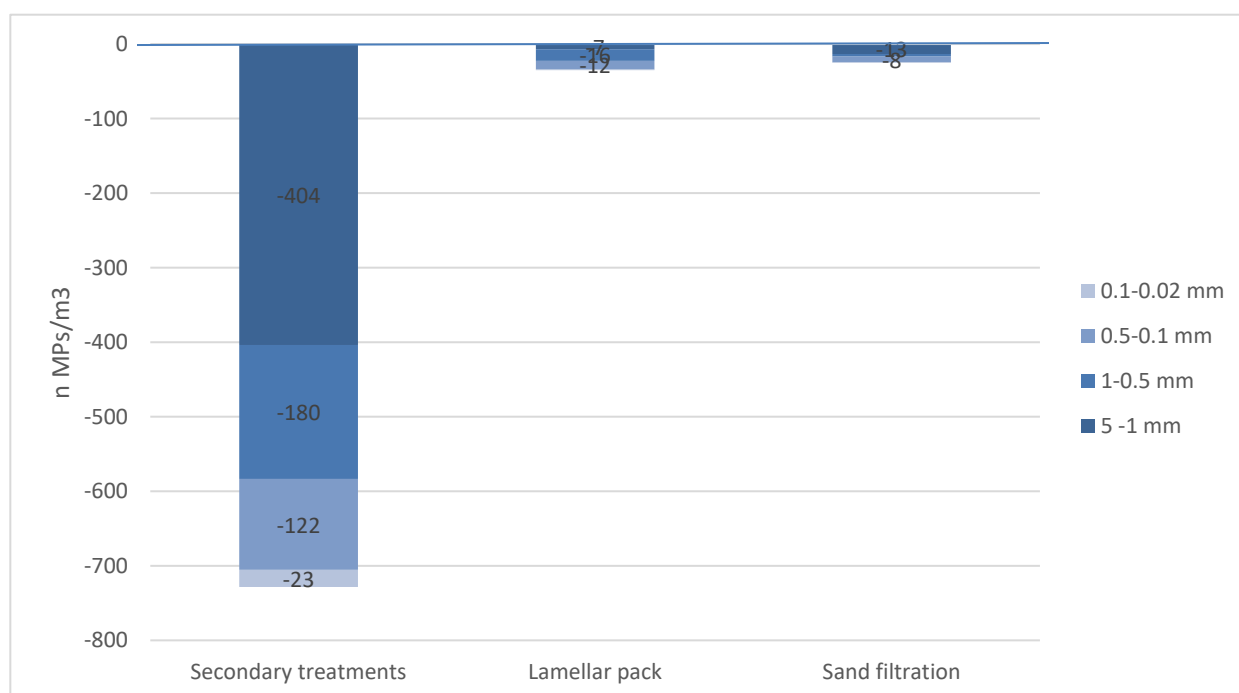


Figure 49: Decrement or increment of MPs in the treatment units of Peschiera del Garda WWTP

Considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plant, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.



5. Critical comparison with scientific-technical data and benchmarks

The fate of microplastics in water treatment facilities has been the subject of many studies conducted in recent years by scientific research to understand the efficiency of removal of water treatment units in respect of this new category of micropollutant (K. Novotna et al, 2019).

The results of the different studies about the occurrence of MPs in water treatment plants vary significantly, ranging from zero or very few (< 10) to thousands microplastic particles per litre.

It must be underlined that it is essential to keep separated drinking water from wastewater treatment plants, due to their different functions and objectives and to the different quality of the influent water to be treated. Accordingly, also the results must be analysed and discussed separately.

These differences can be related to several reasons, such as:

- Sampling location: water body conditions, pollution and level of urbanization of the surrounding area could have significant impact on MPs occurrence.
- Dissimilarities in sampling: size of filters, volumes sampled, frequency, sampling methods and characterisation techniques varied in different literature research, due to the lack of a standardised method.
- Water treatment technologies.

In particular, the comparison between different studies may be not always appropriate because of the different methodologies applied. Filtered volumes, mesh size and environmental conditions may impact significantly on the results. Moreover, sampling equipment may be affected by external contamination, especially in case the equipment is not sealed, or the blank test is not performed.

Moreover, other than sampling methods and analytical procedures used, some authors may have reported the results including all the fibres (as well as particles in other cases), independently from their natural or synthetic origin.

Below a summary of the main literature findings is reported for a comparison with the results obtained by the Blue Lakes sampling campaigns. It has to be noticed that the differences in the sampling methods and characterization may lead to significant discrepancies and thus results are not always properly comparable.

5.1 Occurrence of MPs in DWTPs

Literature research of DWTPs shows a wide variety of results, mainly depending on sampling method and DWTP location, other than site-specific conditions.

It can be observed that MPs concentrations found by Wang et al. (2020), related to Chinese DWTPs and obtained by grab samples of about 1-3 litres, are much higher than the ones observed by Mintenig et al. (2018), which refer to German treatment facilities and are obtained filtering a volume of about 300-2500 litres.

As explained above, this wide difference on literature findings may also be attributed to the different sampling methods and analytical procedures used, as well as on the reporting of the results considering fibres and particles of various origin.

Table 26: MPs occurrence in literature DWTPs

Author	Treatment unit	Volume sampled	n°MPs/L	Shape	Typology
(Wang et al., 2020)	Influent	1L x 3 samples	6614 ± 1132	53.9–73.9% fibres; 8.6–20.6% particles; 17.6–25.5% fragments	55.4–63.1% PET; PE (about 15.1–23.8%) e PP (about 8.4–18.2%)
	Sedimentation	1L x 3 samples	3473		
	Sand filtration	1L x 3 samples	2221		
	Ozonation	1L x 3 samples	2348		
	GAC	1L x 3 samples	970		
	Effluent	1L x 3 samples	930 ± 71	51.6–78.9% fibres; r 6.7–10.1% particles; 14.4–38.3% fragments	PET 47.2–58.8% di MPs; PAM about 10.1–14.7%
(Pivokonský et al., 2020)	Influent	2L x 3 times/day	23±2	5 fibres/L; 19 fragments/L (20%; 80%)	cellulose acetate (CA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), or polypropylene (PP), ethylene vinyl acetate copolymer (EVA), poly(butyl acrylate) (PBA), and polytrimethylene terephthalate (PTT)
	Effluent	2L x 3 times/day	14±1	3 fibres /L; 11 fragments/L (20%; 80%)	PTFE; CA=42%; no PP, EVA and PTT
	Influent	2L x 3 times/day	1296±35	126 fibres/L; 1170 fragments/L	CA, PET, PVC, PE, PP, EVA, polystyrene (PS), polyamide – nylon 6 (PA6), polyethylene oxide + polyethylene glycol (PEO + PEG), vinyl chloride/vinyl acetate copolymer (VC/VAC), PTT, and PTFE. CA, PET, PVC, PE, and PP=80%
	Flocculation+ Sedimentation	2L x 3 times/day	497± 44	51 fibres/L; 446 fragments/L	
	Filtration deep bed	2L x 3 times/day	243 ± 17	31 fibres/L; 213 fragments /L	
	Ozonation	2L x 3 times/day	224 ± 3		
	GAC	2L x 3 times/day	149 ± 1		
	Effluent	2L x 3 times/day	151 ± 4	12 fibres/L; 139 fragments/L	CA, PET, PVC, PE, and PP>90% - no EVA, PA6, PEO + PEG, and PTT
(Pivokonsky et al., 2018)	Influent	1L x 3 samples/day x 3 times x 3 days	1473 ± 34	fragments 71-76%	

	Effluent	1L x 3 samples/ day x 3 times x 3 days	443 ± 10	fragments 42- 48%	
	Influent	1L x 3 samples/ day x 3 times x 3 days	1812 ± 35	fragments 71- 76%	
	Effluent	1L x 3 samples/ day x 3 times x 3 days	338 ± 76		
	Influent	1L x 3 samples/day x 3 times x 3 days	3605 ± 497	fragments 42- 48%; fibres 37-61%	
	Effluent	1L x 3 samples/day x 3 times x 3 days	628 ± 28		
(Mintenig et al., 2019)	Influent	300-1000 L	0.003		PEST, PVC, PE, PA and epoxy resin
	Distribution	1200-2500 L	<0.001		
	Influent	300-1000 L	0.007		
	Effluent	1200-2500 L	<0.001		
	Distribution	1200-2500 L	0.003		
	Influent	300-1000 L	0.001		
	Effluent	1200-2500 L	0.002		
	Influent	300-1000 L	0.001		
(Cherniak et al., 2022)	Influent	10 L *2 duplicates	42 ± 18 particles/L	89% fibres	cellulosic fibers (40–53 %), 1–6 % PET-PEST
	Sand filtration	10 L *2 duplicates	11.2 ± 1.3 particles/L		
	Clearwell	10 L *2 duplicates	31.0 ± 8.2 particles/L		
	Effluent	10 L *2 duplicates	20 ± 8 particles/L		
	Distribution	10 L *2 duplicates	20.5 ± 7.6 particles/L		
(Jung et al., 2022)	Influent	10-100 L *12 monthly samples	2.2 ± 1.3		PE, PP, PET, PMMA, PS, PA, PU, PVC-u
	Pre-ozonation		2.3 ± 0.8		
	Sedimentation		1.4 ± 0.15		
	Sand filtration		0.2 ± 0.15		
	Effluent		0.02 ± 0.02		
Shi et al., 2021	Raw water		6614 ± 1132		
	Sedimentation		~3000		
	Sand filtration		~2000		
	Ozonation		~2000		
	GAC filtration		<1000		
	Treated water		930 ± 71		
(Yuan et al., 2022)	Raw water		17.88		
	Pre-disinfection		17.53		
	Flocculation		17.11		

	Pulse clarification		6.99		
	Sand filtration		11.17		
	Treated water		2.75		
(Johnson et al., 2020)	Raw water		21.09 ± 20.49		
	Treated water		0.001-0.024		
(Leslie et al., 2017)	Raw water		1385 (dry season); 1796.6 (wet season)		
	Screen-outlet		1298.5 (dry season); 1669.2 (wet season)		
	Clarifier-outlet		823.2 (dry season); 122.9 (wet season)		
	Filtration		536.7 (dry season); 844.6 (wet season)		
	Treated water		448.7 (dry season); 769.4 (wet season)		
This study, Castreccioni DWTP	Influent_324m	About 1000 l, 3 sampling campaigns in different seasons	7 ± 1	Fragments (38%) > fibres (28%) > films (15%) > lines (4%)	mainly poly(phenylene ether) and polystyrene, resins, polypropylene and polyurethane
	Influent_314m		7 ± 7		
	Out pre-ozonation		11 ± 6		
	Out flocculation		13 ± 15		
	Out sand filter		3 ± 3		
	Out post-ozonation		11 ± 4		
	Out activated carbon		7 ± 4		
	Effluent		4 ± 2		
	Distribution 1		4 ± 6		
	Distribution 2		1 ± 1		
This study, Molinet DWTP	Influent	About 1000 l, 3 sampling campaigns in different seasons	2 ± 1	Fragments (66%) > fibres (20%) > films (8%) > lines (4%)	mainly polyester and resin (polyvinylchloride, polyvinylidene fluoride, polyethylene, polypropylene, elastomers, polyamide and polyester epoxide were also found)
	Out ozonation		6 ± 2		
	Out filtration		4 ± 1		
	Effluent		3 ± 1		
	Distribution		3 ± 0.02		
This study, Brenzone Castelletto DWTP	Influent	About 1000 l, 3 sampling campaigns in different seasons	11 ± 8	Fragments (63%) > fibres (31%) > films (6%)	polyester, resins, polypropylene, polyacrylate, elastomers, polyurethane, silicone, polyamide, polyethylene, polystyrene, ethylene and vinyl acetate
	Effluent		5 ± 4		
	Distribution		5 ± 3		

5.2 Comparison with bottled and tap water

Literature research also analysed MPs occurrence in bottled and tap water, as reported in the Table below, which has been adapted from WHO Report on Microplastics in drinking water (2019). It must be observed that differences in sampling methods and characterisation led also to different ways on

reporting results. In particular, some studies expressed their results as microplastic-like particles and thus particular attention must be paid when comparing their findings.

Table 27: Summary of reported microplastic or microplastic-like particle numbers and particle characteristics from drinking-water studies (modified from WHO, 2019. Microplastics in drinking-water)*

Author	Water type	Lower size bound (µm)	Particles/L in sample (average)	Particles/L in blanks (average)	Particle size (µm)	Particle shape	Predominant polymer type
(Oßmann et al., 2018)	Bottled (mineral water) • Glass • Single use PET • Reusable PET	1	3074–6292 2649 4889	384	Most particles smaller than 5 (>75% in glass and >95% in plastic bottles),	No discussion of shapes	PET in plastic bottles, PE, and styrene butadiene copolymer in glass
Schymanski et al. (2018)	Bottled • Single use • Returnable • Glass • Beverage carton	5–20	14 118 50 11	14 ± 13	40–50% in 5–10 range; over 80% <20	No discussion on shape; described as fragments	PET but also PP, PE
(Mason et al., 2016)	Bottled	6.5–100 lower bound based on microscope and software	315	23.5	Not specified	Not specified	No characterization
		>100	10.4	4.15	Not specified	Fragments (66%), fibres (13%), films (12%)	PP (54%)
Strand et al. (2018)	Tap from ground-water sources	10–100	0.2, 0.8 and 0.0 (LoD = 0.3)**	Unknown	Mainly 20–100.	Fragments	PET, PP, PS, acrylonitrile butadiene styrene, PUR
		>100 (10 µm sieve size)	0.312 (LoD = 0.58)**	0.26	Not specified	Fibres (82%), fragments (14%), films (4%)	PET, PP, PS
(Mintenig et al., 2019)	Tap from ground-water sources	20	0.0007	0.67 particles/L 0.3 fibres/L	In the range 50–150. Fragments	Fragments	Polyester, PVC, PE, PA, epoxy resin

Uhl, Eftekhari, and Svendsen (2018)	Tap from 24 sources	60	Average not reported since only a single result above LoQ (that result was 5.5)	0.5 (LoQ = 4.1 LoD** = 0.9)	Not specified	Not specified	No characterization
Kosuth, Mason and Wattenberg (2018)	Tap from unspecified sources	100 lowest reported	5.45	0.33 (based on 5 particles in 30 blanks (ea. 500 mL)	Fibre lengths 100–5000	Mainly fibres (98.3%).	No characterization

* For details on whether particles identified were confirmed to be microplastics, see WHO, 2019.

** LoD/LoQ = Limit of detection/Limit of quantification.

5.3 Occurrence of MPs in WWTPs

Wastewater treatment plants (WWTPs) are considered to be important receptors and processors for microplastics generated through urban drainage, and from municipal and industrial effluents (Lusher et al., 2017).

As well as for DWTPs, occurrence of MPs in WWTPs found in literature research varied, depending on site-specific conditions and sampling and characterisation methods. The main results are reported in **Table 28**, adapted from Koelmans et al., 2019.

As explained above, this wide difference on literature findings may also be attributed to the different sampling methods and analytical procedures used, as well as on the reporting of the results considering fibres and particles of various origin.

Table 28: Literature occurrence of MPs in WWTPs – modified from (Koelmans et al., 2019)

Reference	Treatment	Size, shape	Polymers, chemicals	Value	Sampling method	Analysis method
Browne et al. 2011	3° treatment	-	PEST, PMMA and PA	EF mean: 1 #/L	Samples collected in glass bottles with metal caps.	Filtered and identified with Transmittance FT-IR
(Carr et al., 2016)	2° & 3°	Size: (20), 45, 180, 400 µm.	-	(1) Tertiary EF: 3-23 MP in 9.46-9.57 × 10 ⁶ L; (2) Secondary EF: 1 MP in 5.68 × 10 ⁴ L; (3) Final EF: 0 MP in 1.89 × 10 ⁵ L	Method 1: EF sieved through stacked stainless steel sieves (400, 180, 45 and only 2 events used 20 µm). Flows-11.4-22.7 L min ⁻¹ .	Tertiary EF: Centrifuging at 4000 RPM for 20 min.
		Shape: spheres, fragments and fibres.			Method 2: Skimmed final effluent outfall with surface filtering assembly.	Secondary EF: subsamples of 5mL in gridded petri dish, 20% of total sample. Skimming:

					Collected sample until clogging.	digestion with bleach. All samples were examined under microscope and checked with a micro-spatula. Some MPs analysed with ATR- FTIR.
Dris et al. 2015	2° treatment	Size: 100-500 µm, 500-1000 µm, 1000-5000 µm.	-	IF: mean 293 (range: 260-320) #/L	Collected with automatic sampler and 24-h average samples analysed. A 0.05L aliquot was analysed.	Samples filtered on filter (1.6 µm) and particles counted with stereomicroscope (16x).
		Shape: Fibre		EF: mean 35(range: 14-50) #/L		
Dyachenko et al. 2017	2° treatment	Size: 5-1 mm, 0.355 - 1mm, 0.125-0.355 mm;	Polyacrylic, PP, PE	Max: 24-hour sampling- 0.02 #/L; 2-hour sampling- 0.17 #/L.	Effluent flow filtered through 5, 1, 0.355 and 0.125 mm stacked sieves. Flow of 1 gal/min for 24 hours. or 2-hour composites at peak flow. Sieve contents transferred with DI water into glass jars and stored at 4°C.	WPO with FeSO ₄ catalyst at 70°C. WPO solution filtered through 0.8 µm. Examined with dissecting microscope (45X). Micro- FTIR for most commonly observed particles.
		Shape: Fibre, film, foam, fragment, pellet.				
Gündoğdu et al., 2018	2° treatment	from <100 µm to 5000 µm		IF: 12–36 particles/L. EF: 2–9 particles/L		
Lares et al. 2018	3° treatment	Size: <0.25mm, 0.25-5.0mm, >5.0mm	PE, PA, PP	Mean IF: 57.6 ± 12.4 (S.E.) #/L	Grab sampled 4.0-30.0 L of IF and EF with 10-L stainless steel bucket and poured over 2 sieves (0.25 and 5.0 mm). Residues transferred with DI water in beakers and sealed with aluminium foil and rubber band for transfer to lab. Stored at 4°C in the dark.	Samples dried at 75°C in oven for at least 40h until dryness. WPO heated to 75°C. IF samples treated with cellulase for 24h at 40°C with 160 rpm shaking. Samples were vacuum filtrated with cellulose nitrate filter, porosity (0.8 µm) and glass fibre filters (1.5 µm) at the bottom. Filters dried for 24h at room
		Shape: Fibres and particles.		Mean EF: 1.0 ± 0.4 (S.E.) #/L.		

		Surface: Dull				temperature covered with aluminium foil. Samples examined under digital optical microscope and classified rep samples (1.3-1.4% of overall particles) using micro-FITR/Raman spectroscopy.
(Leslie et al., 2017)	-	Size: 300-5000 μm and <300 μm .	-	IF: 68-910 #/L (mean range)	Samples collected in 2L glass jars and stored in dark until analysis.	Samples were homogenized and 100 g aliquots were extracted. Sodium chloride solution was added to sample to saturation point (1.2 kg L ⁻¹) before filtration.
		Shape: Fibres, spheres, foils.		EF= 51-81 #/L (mean range). Median EF: 52 #/L. Range: 9-91#/L		
(Magni et al., 2019)	screening, grit and grease removal stages, biological treatment, sedimentation, sand filter treatment and disinfection.	Size: 0.5–0.1 mm (36%). Shape: film, fragments and lines		IF: 2.5 \pm 0.3 MP/L; Secondary treatment: 0.9 \pm 0.3 MP/L; EF: 0.4 \pm 0.1 MP/L		
(Magnusson & Norén, 2014)	Tertiary treatment	Shape: Fibre, fragment and flake.	PE, PP, thermoset plastic based on aliphatic polyester resin.	IF=15.1 \pm 0.89 (SE) #/L	Used a Ruttner sampler for influent and filter holder with tube for effluent. Filter over 300 μm mesh to collect 2 L of IF water per sample (triplicate) and	Identification with stereo microscope (50x) Suspect fibres were placed on an object glass and heated over the flame of an alcohol burner. Subset of particles were picked out for ATR- FTIR analysis.
				EF= 8.25 \pm 0.85 (SE) 10 ⁻³ #/L	1000 L of EF per sample (quadruplicate)	
(Mason et al., 2016)	2° and 3° treatment	Size: 125-355, >355 μm ;	-	Mean: 0.05 #/L; Range: 0.004-0.195 #/L; 95% CI: 0.050-0.024 #/L.	Pumped effluent through 0.355 mm and 0.125 mm (12-18 L/min, for 2-24	WPO with Fe (II) catalyst. Sieved through 0.125 mm and transferred to petri dish.

		Shape: Fragments, pellet, line/fibre, film and foam			hours). Preservation in 70% isopropyl alcohol.	Microscopic inspection (40x).
(Michielssen et al., 2016)	2° and 3° treatment	Shape: Fragments, fibres, paint chips, micro- beads	-	Detroit: IF=133.0 ± 35.6 #/L; Final EF=5.9 SAL * L ⁻¹ ; Northfield: Final effluent = 2.6 SAL L ⁻¹ AnMBR system Final effluent = 0.5 SAL L ⁻¹	Grab sample in plastic containers cleaned with DI and air dried. Stored at 4°C.	Sieved (4.74, 0.85, 0.3, 0.106 and 0.02 mm). Stereo- microscope
(Mintenig et al., 2017)	2° treatment (n=8), 3° treatment (n=4)	Size: <500, >500 µm;	PE, PP, PA, PVC, PS, PUR, silicone, paint, SAN, PEST, PET, EVA, PVAL, ABS, PLA.	Range >500mm: 0 – 40 x 10 ⁻³ #/L; Range < 500mm: 10 – 9000 x 10 ⁻³ #/L	Pumped with filtration (10 µm SS filter) and flowmeter, 10 cm below water surface with pre- rinsing. Filtration unit sealed and stored at 4 °C.,	Enzymatic maceration, SDS at 70 °C for 24 h, enzymatic digestion at 40- 50 °C up to 6 d. Sonication in MQ for 3 mins. Filtration (500 µm). <500 µm: WPO at 50 °C for 24 h and chitinase at 37 °C for 48 h and repeat WPO. Density separation with ZnCl ₂ (1.6 g/cm ³), filtered (0.2 µm) and dried at 40 °C. FTIR imaging analysis (25%). >500mm: Microscopic inspection and ATR-FTIR analysis for all particles. 60 fibres/sample analysed with FTIR imaging.
		Shape: Fibres				
(Murphy et al., 2016)	3° treatment	Size: 0.598 ± 0.089 mm. Shape: Flakes, fibres, film, beads and foam.	PMMA, alkyd, PET, PA, polyaryl ether, PEST, PE, PP, PS, PUR, polyvinylfluoride , PS acrylic, PVA, PVC, PVE	Mean (#/L): (1) IF: 15.70 ± 5.23; (2) Grit and grease: 8.70 ± 1.56; (3) Primary EF: 3.40 ± 0.28; (4) final EF: 0.25 ± 0.04.	Grab sampling with 10 L steel buckets and sieved with 65 µm mesh. Vol. sampled: (1) IF- 30 L; (2) EF-50 L.	Vacuum filtration with 11 µm filter paper. Subset (4/24 th) of each filter paper analysed for particle count. Subset polymer identification using micro-FTIR.

(Simon et al., 2018)	2° treatment	Size: Up to 600 µm	Acrylate, SAN, VAC-PMMA copolymer, PE, PP, PE-PP co polymer, PEST, PS, PUR, PVC, EVA, PA, PVA	Raw wastewater median: 7216 #/L or 250 ug/L	Sampled with auto samplers. Raw WW samples filtered on-site through 10 µm stainless steel meshes.	Raw wastewater was wet-sieved with SDS. Sample incubated with cellulase enzyme for 48 h at 40°C then WPO. Reactor was kept in an ice-bath and temperature maintained between 15 and 30°C. 2-6% of homogenized sample transferred on transmission/reflectance window, all analysed with FTIR- imaging.
				Treated wastewater median: 54 #/L or 4.2 ug/L		
				Recovery efficiency=99.3 %		
(Talvitie et al., 2015)	Bar screening, grit removal, pre-aeration, primary sedimentation , activated sludge treatment, secondary sedimentation and tertiary biological filtration	Size: 200, 100 and 20mm;	-	IF = Mean fibres: 180 #/L; Mean particles: 430 #/L.	Pump, flow rate of 1.0 ml/min. Transparent plastic tubes (60 mm diameter), with 200, 100 and 20 µm nets plasticized between connectors of tubes. Sample size: 0.3 - 285L.	Stereomicroscope (x50), identified and counted. particles and fibres. Blanks processed simultaneously
				Primary sedimentation = Mean fibres: 14.2 (± 0.7) #/L; Mean particles: 290.7 (±28.2) #/L		
		Shape: fibres and particles.		After secondary sedimentation = Mean fibres: 12.8 (± 1.6) #/L; Mean particles: 68.6 (± 6.3) #/L		
				EF = Mean fibres: 4.9 (± 1.4) #/L; Mean particles: 8.6 (± 2.5) #/L		
(Talvitie et al., 2017)	Coarse screening, grit removal, chemical treatment and primary sedimentation , active sludge method.	Size: 20-100mm, 100-300mm, > 300mm;	PES, polyacryl, PE, PS, PP	EF (general): Range: 0.006 – 0.651 #/L (for different days), or 1.7E6 - 1.4E8 #/day.	1. Grab samples: three replicates, pumping through tubes with 300, 100 and 20µm filter mesh. Sampling volume 0.1 l - 1 m³. IF: beaker because of clogging filters. 2. 24-h composite sample - 15	Stereomicroscope (50x). Particles counted, categorized in shapes. FTIR for 3 EF samples. In total 752 particles, but 18% success rate.
		Shape: fibres, fragments, flakes, films and spheres.		Grab sample: Range IF: 380 (± 52.2) - 686.7 (±155.0) Range after pre-treatment: 9.9 (± 1.0) - 14.2 (± 4.0) Range after AS: 1.0 (± 0.6) - 2.0 (± 0.2)		

				<p>Range EF: 0.7 (\pm 0.6) - 3.5 (\pm 1.3).</p> <p>24-hour composite sample: Range IF: 390.0-900 Range after pre-treatment: 4.1-23.8 Range after AS: 1.5-2.8, EF: 1.4-2.8, blank: 0.4-0.8.</p>	min intervals over 24-h period, for 3 days in a week. Sampling volume: 0.1 L - 14.5 L. 3. Sequential sampling: 1-h interval samples for 24 hours, pooled per 3 hours with automated samplers.	
(Talvitie et al., 2017)	3°: micro-screen filtration with disc filters, rapid sand filters, dissolved air flotation, membrane bioreactor.	Shape: Fragments, flakes, films and spheres	<p>PES, PE, PP, PS, PU, PVC, PA, acrylamide, poly-acrylate, alkyd resin, polyphenylene oxides, ethylene vinyl acetates.</p>	<p>Range before treatment: 6.9 (\pm 1.0) - 0.5 (\pm 0.2) #/L,</p> <p>Range after treatment: 0.3 (\pm 0.1) - 0.005 (\pm 0.004) #/L.</p>	Three replicates, filter over 300, 100 and 20 μ m sieves with pump. Also 24-h composite samples. Water volume: 0.4 - 1000L.	Visual inspection, followed by an analysis using FTIR imaging for all pre-sorted particles. Blanks included.
The Danish Environmental Protection Agency, 2017	-	<p>Size IF, median: 50mm</p> <p>Size EF, median: 51.5mm.</p>	Nylon, PE, PE-PP copolymer, PP, and PVC.	<p>IF: Median: 5.9; Mean: 8.0mg/L.</p> <p>EF: Median: 0.016, Mean: 0.034mg/L.</p> <p>IF: Median: 86000, Mean: 127000#/L.</p> <p>EF: Median: 6400, Mean: 5800 #/L.</p>	<p>IF: 3 times 24h auto sampler. 1L stored in glass jar. EF: 3 times, 10μm filters until clogging of 3 filters (0.5 - 108 L per filter). Sludge: 2 times, 1 kg.</p>	<p>IF: 1mL sodium dodecyl sulphate addition, then 500mm pre-sieved. Cellulose digesting enzyme to 200 mL subsample. Incubation for 48h at 40°C, hydrolysed with H₂O₂. Fractions sieved: > and < than 80mm. From sieves to water + SDS. Filtered over 10mm mesh. Filters in ethanol, sonicated, scraped. 5mL ethanol. EF: the 3 10mm filters were hydrolysed and oxidized like IF. All samples: Micro-FT-IR.</p>
Vermaire et al. 2017	-	Shape: Microfibers, microbeads, unidentified fragments	-	Median EF: 0.07 #/L	100 L EF, triplicate. ISCO peristaltic pump, 100 μ m nylon mesh	WPO at 80°C for 7h. 100mm filter, Leica stereomicroscope 40x.

(Ziajahromi et al., 2017)	WWTP A: 1° treatment; WWTP B: 2° with UV; WWTP C: 3° with Cl, UF, RO	Size: 25-100, 100-190, 190-500, 500 µm;	PET, nylon, PE, PP, PS, PVC	Effluent WWTP A- 1.5 #/L;	Pumped 3 - 200 L through stacked sieves of 500, 190, 100 and 25 µm at max flow rate of 10 L/min. Mesh screens stored on petri dishes sealed in Al foil.	Rinsed from sieves with UP water, and concentrated to 100 mL by drying at 90°C. WPO at 60 °C and dried. Density separation with NaI (1.49 g/ml). Centrifugation for 5 min at 3500xg. Supernatant filtered over 25 µm mesh and stained with Rose-Bengal solution. Dried at 60 °C for 15 min and microscopic inspection, analysis with ATR FTIR.
		Shape: Irregular, granular and fibre.		Effluent WWTP B: 0.48 #/L.;		
				Effluent WWTP C- 0.28 #/L, (3° treatment),0.21 #/L.		
Pittura et al., 2021	Conventional WWTP (CAS)	Most frequent shapes of fibres and particles, ranging in 0.1 - 0.5 mm	Mostly polyethylene and polypropylene	PRE-TREATED IN: 3.64 MPs/l	Sieving battery 5mm, 2mm, 63 µm; about 25 l filtered for each sampling point.	Extraction procedure, analysis with stereomicroscope and µFT-IR analyses
				I EFF: 1.9 MPs/l		
				II EFF: 0.76 MPs/l		
	Innovative WWTP: UASB + AnMBR			UASB EFF: 1.72 MPs/l		
				Permeate: 0.2 MPs/l		
This study, Limone Tremosine WWTP	Screening, sand removal, biologic (attached biomass) unit, flotation, tertiary filtration, UV disinfection	Shape: fibres (53%) > fragments (27%) > films (18%) Size: mostly medium-big size: 5-0.1 mm	Mostly polyester and polyethylene	Influent: 480 ± 367 MPs/m³	Automatic sampler, filtered almost 1000 liters (when possible), according to Deliverable “Technical report and operative manual regarding the improvement of the treatment process”	According to Deliverable “Technical report and operative manual regarding the improvement of the treatment process”
				Effluent from flotation unit: 205 ± 137 MPs/m³		
				Effluent (from tertiary		

				filtration): 78 ± 72 MPs/m ³	of the treatment process"	
This study, Peschiera del Garda WWTP	Screening, biologic treatment, secondary sedimentation, coagulation, lamellar sedimentation, sand filtration, UV disinfection	Shape: fibres (61%) > fragments (25%) > films (11%) > lines (4%) Size: mostly medium-big size: 5-0.1 mm	Mostly polyester and polyethylene	Influent: 801 ± 1269 MPs/m ³ Effluent from secondary settlers: 72 ± 77 MPs/m ³ Effluent from lamellar packs unit: 56 ± 67 MPs/m ³ Effluent from sand filtration: 13 ± 3 MPs/m ³	Automatic sampler, filtered almost 1000 liters (when possible), according to Deliverable "Technical report and operative manual regarding the improvement of the treatment process"	According to Deliverable "Technical report and operative manual regarding the improvement of the treatment process"

* SAL = small anthropogenic litter.

5.4 Occurrence of MPs in sludge and waste streams

An important consideration for both wastewater and drinking-water treatment is that the plastics are usually not destroyed, but rather transferred from one phase to another. Since limited biodegradation of the microplastics removed from the liquid phase in WWTP processes can be expected, particles are expected to be mainly found in the final sludge. Before being delivered to its final disposal, however, sludge is treated through a series of processes in WWTP, mainly consisting of thickening, dewatering or stabilisation. Preliminary work suggests that biosolid treatment does not have a large influence on reducing microplastic concentrations; however, this needs to be further assessed. Sludge disposal methods must therefore be considered since sludge application to land is a probable route for re-contamination of the environment.

Data from a large Italian WWTP (400 million L/day) reported 113 ± 57 MPs/g in dewatered sludge (Magni et al., 2019). The shape of plastic microparticles (MPPs) were mainly constituted by films (51%), fragments (34%) and lines (15%), while the main size class was 0.5–0.1 mm (54%). Co-polymers of acrylonitrile-butadiene were the more abundant chemical typologies detected in the sludge (27%), followed by polyethylene (18%) and polyesters (15%). The 65% of the total microfibers collected in the sewage sludge was synthetic and represented only by polyesters.

In a study conducted in Norwegian WWTPs (A. L. Lusher et al., 2017), the average plastic abundance was 6 077 particles/kg (d.w.) (with a range of 1701 – 19 837) or 1 176 889 particles/m³ (with a range of 470 270 – 3 394 274). Particles from sludge consisted of beads (37.6 %), fragments (31.8 %) fibres (28.9 %) and glitter (1.7 %). Most of the particles were clear in colour (41 %). Polyethylene particles were the most common (30.5 %) followed by polyethylene terephthalate (26.7 %) and polypropylene (20.3 %).

For the current case studies analysed, MPs concentration in sludge samples varied in the range of 6100 ± 4629 MPs/m³ for sludge from DWTP, while as concern WWTPs, MPs were detected in the range of 203500 ± 123744 MPs/m³ in floated sludge, 453083 ± 530540 MPs/m³ in stabilised sludge, 347783 ± 106069 MPs/m³ in liquid sludge and 95583 ± 34988 MPs/m³ in chemical sludge. Generally, detected concentrations were lower than the ones reported in literature.



Where membrane cleaning or back-flushing of filters is practiced, waste streams may be returned directly to the aquatic environment. Although it is clear that use and/or disposal practices for waste products containing microplastics warrants special consideration, there are limited data available on the impact of such practices (WHO, 2019).

As concern measured concentrations in backwash water in the DWTPs analysed in the project activities, mean values ranged between 60 and 230 MPs/m³ for ultrafiltration and gross filtration systems, respectively, while for flocculation systems an average of about 400 MPs/m³ were detected.

6. Remarks on MPs fate in water treatment units

6.1 Drinking water treatments

Literature research found that DWTPs are effective to decrease the number of MPs from source water, reducing their quantities from raw water into drinking water supply (M.Shen et al., 2020).

In conventional DWTPs, the main processes for particle decrement include coagulation and filtration (Cherniak et al., 2022).

Coagulation, flocculation, and sedimentation processes accounted for the highest decrements, reaching up to 70%, according to (S.L. Cherniak et al., 2022). In the same way, Wang et al. (2020) reported that the larger size microplastics were reduced in the coagulation/sedimentation process. MPs > 10 µm decreased significantly in this process, followed by the 5–10 µm MPs, with a decrement of 45 – 75%. This probably happened because larger-sized microplastics were more easily attached to flocs during the coagulation phase, increasing their sedimentation properties.

It must be noted that coagulants are dosed to neutralize surface charges of organic matter and pathogens, but since the surface characteristics of microplastics are variable and treatment has not been specifically designed for their removal, they may not be optimal for microplastics (Poerio et al., 2019).

Since most plastic particles are hydrophobic, they may be attached to organic compounds and adopt the same characteristics of background organic matter, influencing their distribution in the solid-liquid separation processes. (WHO, 2019). Among the organic matter constituent, humic acids can stabilize particles in water, preventing their aggregation and subsequent sedimentation (Jarvis et al., 2005), especially as concern micro- and nano-plastics.

Hydraulic conditions can also influence the effective removal of particulate matter, including microplastics, during clarification processes. Flocs can be broken by shear forces or changes in pH, forming smaller particles more difficult to remove by clarification processes (Jarvis et al., 2005; Slavik et al., 2012).

It should be also noted that in some cases treatment processes may actually contribute to MPs increase in the treated water. Specifically, the use of polymer coagulant aid in the coagulation-flocculation process may conceivably result in higher concentrations of MPs in the treated water. Wang et al. (2020) found the coagulation-flocculation process resulted in a + 114% increase in PAM (commonly used coagulant aid) concentrations in the water (Xue et al., 2022).

Filtration units also contribute to MPs removal, with observed treatment efficiencies in the range of 33-86%.



As concern membrane filtration, cut-off size is generally below the size of microplastics that have been detected in drinking-water. Consequently, no microplastics above the membrane size ranges should be found in the effluent, unless the membranes are damaged. However, since many membranes are composed of polymeric materials, their use can impact on MPs presence in treated water (Xue et al., 2022).

Oxidative processes, such as ozonation, are often applied to break down organic matter into more biodegradable particles (Le Chevallier et al., 1992), improving the following bio-filtration removal efficiency. Nonetheless, the size, shape, and surface properties of MPs seem to be changed by ozonation, which may influence MPs removal also of subsequent treatment processes. It was observed that ozonation may alter the structures of PE, polypropylene (PP), polystyrene (PS), and polyamides (PA) (Gatenholm et al., 1997; Ozen et al., 2002), even if the impact of these reactions on microplastic removal is still unknown (S.L. Cherniak et al., 2022) and scarce information is available on how microplastics are transformed during oxidative processes used in water treatment, such as ozonation, chlorination or advanced oxidation. Ozonation efficiency on MP removal was found to be not significative in some literature studies (Pivokonsky et al., 2018; Pivokonsky et al., 2020). Wang et al. (2020) found an increase in MP concentration after ozonation process. The number of microplastics in the effluent of ozonation has slightly increased, mainly due to the presence of small particles and fibrous microplastics. The abundance of 1–5 μm MPs from the effluent of ozonation increased by 3–16%, resulting in a negative removal efficiency (Wang et al., 2020). This increase could be attributed to the breakdown of larger MPs, as well as to the reduction of organic matter that might was covering MPs particles, which thus became better detectability after ozonation process.

Dalmau-Soler et al. (2021) detected MP materials in the treatment plant, which had not been detected in the raw water, such as PTFE and epoxy resins.

Generally, high shear-rate processes used in both drinking-water and wastewater treatment, such as in mixing systems, may degrade plastic particles into smaller pieces, making them more challenging to remove. Water pipes composed of plastic materials could also be subject to abrasive processes.

Accordingly, results from the sampling campaigns carried out during the project activities showed an increment of MPs after oxidative processes, while reductions were detected after physical units such as filtration.

6.2 Wastewater treatment

WWTPs are a principal barrier to the direct discharge of waterborne microplastic pollution into the aquatic environment (WHO, 2019).

The main processes to achieve microplastic removal in wastewater treatment include solid-liquid separation, such as agglomeration into biological flocs followed by separation using sedimentation, flotation and filtration (Murphy et al., 2016; Talvitie et al., 2017). Due to their hydrophobic nature, many microplastics are expected to be removed together with fats, oils and greases.

According to literature data, conventional wastewater treatment can remove microplastics from wastewater with reported efficiencies of 90%. Results from a large Italian WWTP (400 million L/day) reported a removal efficiency of microplastics of 84% (Magni et al., 2019).

Nowadays, performances of wastewater treatment processes have been significantly improved to satisfy the stricter quality targets for surface water and thus the microplastic load originating from the WWTP is



expected to be significantly reduced. Innovative WWTPs with implemented tertiary treatment units, such as sand filtration membrane bioreactors and dissolved air flotation, could achieve between 95–99.9% of microplastics removal efficiencies (Talvitie et al., 2017; Lares et al., 2018). Removal efficiency can be influenced by the surface characteristics of the microplastic, such as roughness, hydrophobicity and surface charge, as well as the size of the particles being filtered.

7. Concluding highlights

MPs occurrence in drinking water and wastewater treatment plants varies in a wide range, depending on the specific site conditions, but also on the sampling procedures, the equipment used and the characterisation technique.

Drinking water and wastewater must be analysed separately, due to the different concentrations expected, the different aims and the different characteristics of the different treatment units.

Sampling methods, procedures to take into account the eventual environmental contamination, sampled volumes and mesh-size vary widely in literature research. Moreover, the analysis and the characterisation phases are essential to understand how many of the microparticles detected in the phase of screening were confirmed to be of synthetic origin. This is particularly essential for microfibres, since most of them can be attributed to a natural origin.

For these reasons, a harmonised Protocol was first established, as reported in the Deliverable “Technical report and operative manual regarding the improvement of the treatment process”, to define the minimum volumes, the equipment required, the procedures to take into account environmental contamination, the characterisation phases and the results presentation.

All the results presented in this Deliverable were consistent with the requirements of the “Technical report and operative manual”, since all the sampling campaigns performed under the LIFE Blue Lakes project for the DWTPs and the WWTPs followed the procedures reported in it. In particular, significant sampling volumes were considered according to the matrix and the characteristics, the mesh size was constant among all the sampling points, external contamination was eliminated by closed systems or by blank experiments, synthetic fibres were distinguished from natural fibres, and final results accounted only for plastic microparticles and plastic microfibres.

Nonetheless, some variations were observed, maybe due to the discrete nature of the MPs, influent fluctuations, site-specific characteristics of the infrastructures or of environmental conditions.

It is important to underline that, considering the discrete and not homogeneous presence of MPs in the water and the low concentration detected, the general variability observed in the plants, as number, shape, dimension and type of MPs, may be caused both by releases / decrement phenomena occurred in the different treatment stages and by influent fluctuation.

Observing MPs occurrence in drinking water (**Figure 50**), comparing literature research with the results obtained from the campaigns performed under LIFE Blue Lakes project, it can be noticed that the effluent quality from all the DWTPs analysed were on average lower than the ones detected in literature. Moreover, they were also lower than the concentrations detected in Bottle and tap water found in literature.

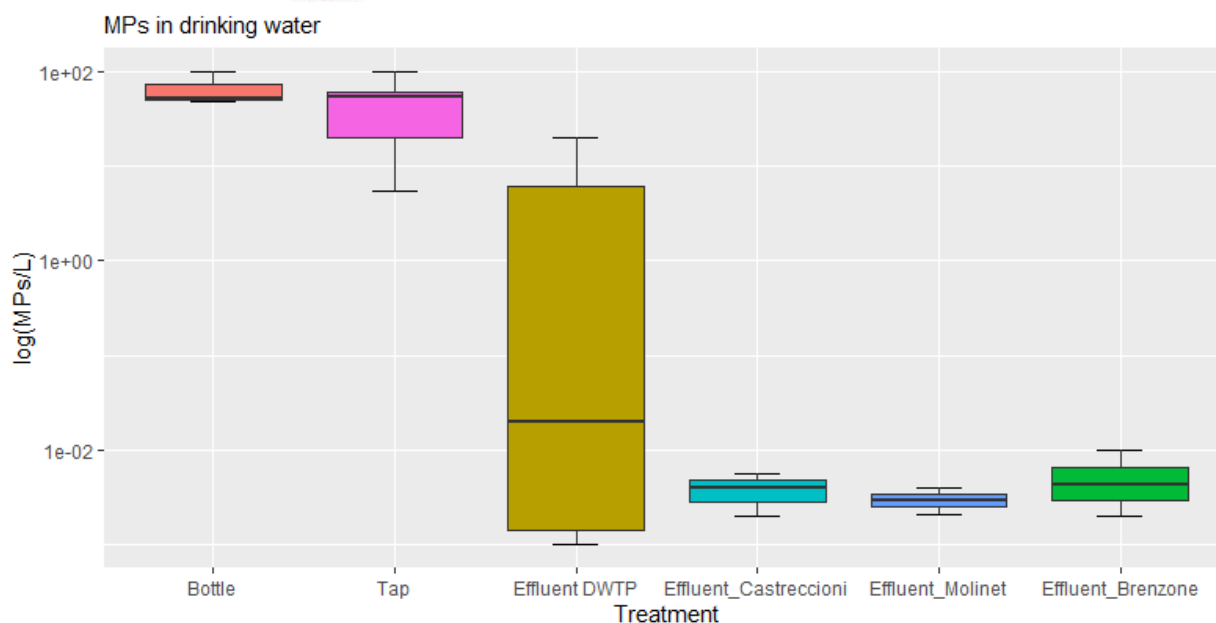


Figure 50: MPs occurrence in drinking water

Analysing the decrements/increments detected in the DWTPs, from **Figure 51** it can be observed that globally a general decrement of MPs can be observed after the DWTPs. It must be underlined that, for drinking water treatments, it is not feasible to properly define a removal efficiency, due to the low concentrations detected and the different objectives of the treatment units. The variations between the influent and the effluent concentrations are reported in **Figure 51**.

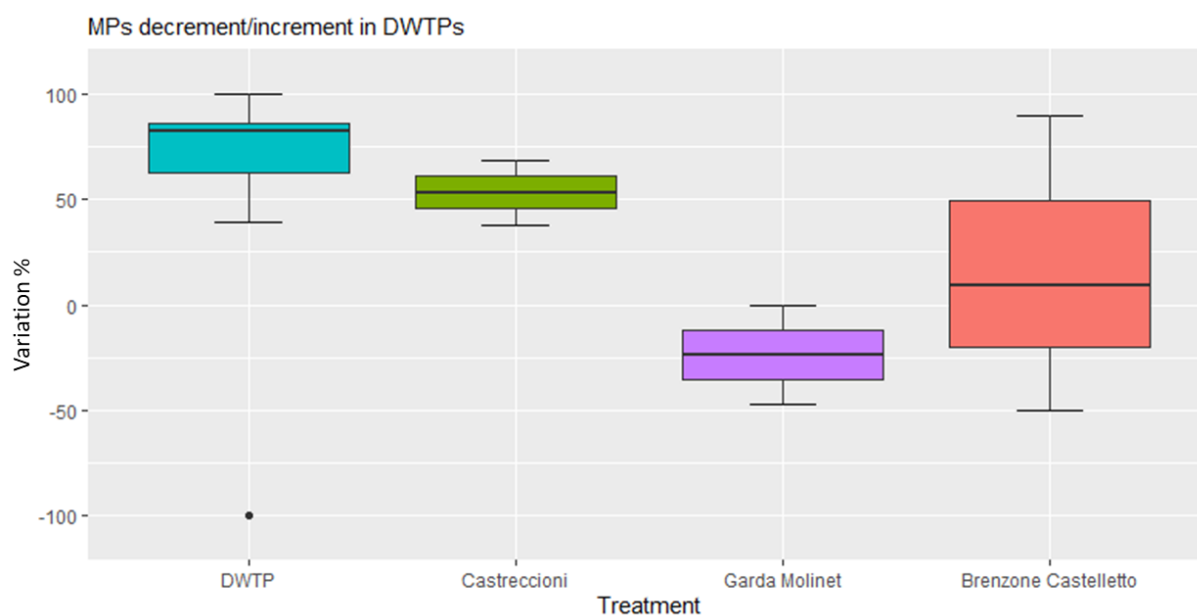


Figure 51: MPs decrement or increment in DWTPs

Figure 52 shows MPs occurrence at the effluent from WWTPs. Both Limone Tremosine and Peschiera del Garda WWTPs could reach lower concentrations than the ones observed in the literature research.

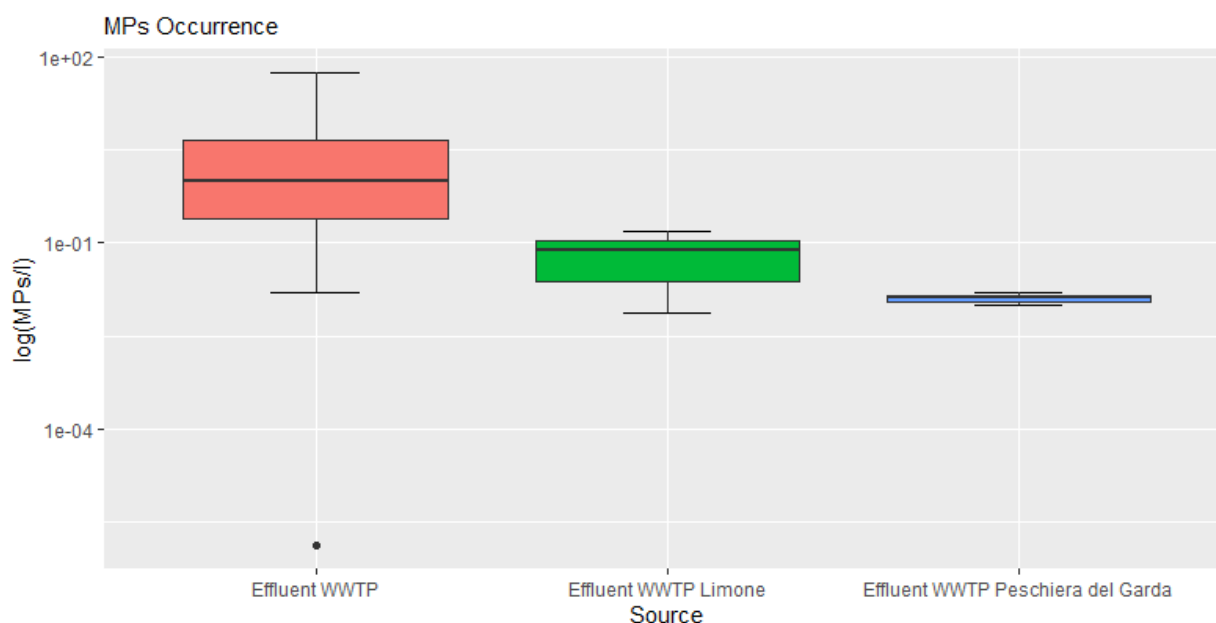


Figure 52: MPs occurrence in effluent wastewater

Analysing the decrements/increments detected in the WWTPs, from **Figure 53** it can be observed that globally a general decrement of MPs can be observed after the WWTPs. As for the DWTPs, due to the relatively low values detected in the influent flows, it is not feasible to properly define a constant removal efficiency. However, it can be observed that WWTPs can anyway decrease the MPs concentrations, with decrements from 50% to almost 75%, on average.

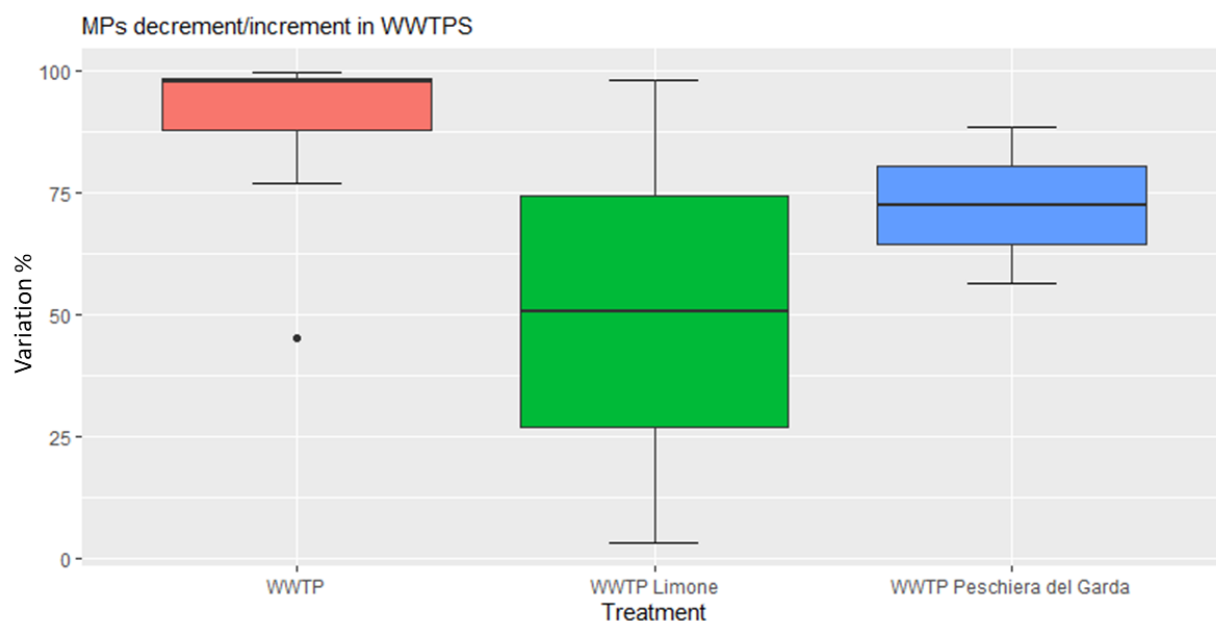


Figure 53: MPs decrement or increment in WWTPs



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Annex A – Data on microfibers (MFs) characterised in DWTPs and WWTPs selected for the present study

Table A1: quantification of microfibers (MFs) extracted from samples collected in Garda Molinet DWTP considering both those of synthetic (plastic) and natural origin and their relative contribution on the total.

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Garda Molinet (DWTP)	Influent	November 2020	0.0	0	0
		June 2021	2.06	0	100%
		September 2021	5.02	0	100%
	Out ozonation	November 2020	2.0	0	100%
		June 2021	4.82	40%	60%
		September 2021	0.99	0	100%
	Out filtration	November 2020	3.0	33%	67%
		June 2021	2.00	0	100%
		September 2021	2.95	0	100%
	Effluent	November 2020	3.9	100%	0%
		June 2021	3.09	33%	67%
		September 2021	8.86	22%	78%
	Distribution	November 2020	3.0	0	100%
		June 2021	1.00	0	100%
		September 2021	6.01	17%	83%



Table A2: quantification of microfibers (MFs) extracted from samples collected in Brenzone Castelletto DWTP considering both those of synthetic (plastic) and natural origin and their relative contribution on the total.

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Brenzone Castelletto (DWTP)	Influent	November 2020	0	0	0
		July 2021	3	100%	0
		September 2021	1	0	100%
	Effluent	November 2020	5	71%	29%
		June 2021	6	83%	17%
		September 2021	0	0	0
	Distribution	November 2020	6	50%	50%
		July 2021	1	100%	0
		September 2021	1	100%	0
	Backwash Coarse filter	November 2020	231	100%	0
		July 2021	50	100%	0
		September 2021	250	40%	60%
	Backwash Ultrafiltration	November 2020	40	33%	67%
		July 2021	0	0	0
		September 2021	181	18%	82%



Table A3: quantification of microfibers (MFs) extracted from samples collected in Castreccioni DWTP considering both those of synthetic (plastic) and natural origin and their relative contribution on the total.

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Castreccioni (DWTP)	Influent_324m	July 2020	15	20%	80%
		November 2020	0	0	0
		May 2021	18	17%	83%
	Influent_314m	July 2020	16	22%	78%
		November 2020	7	14%	86%
		May 2021	0	0	0
	Out pre-ozonation	July 2020	5	80%	20%
		November 2020	10	20%	80%
		May 2021	13	46%	54%
	Out flocculation	July 2020	3	67%	33%
		November 2020	3	100%	0
		May 2021	3	0	100%
	Out sand filtration	July 2020	10	36%	64%
		November 2020	1	0	100%
		May 2021	4	0	100%
	Out post-ozonation	July 2020	15	40%	60%
		November 2020	6	0	100%
		May 2021	29	38%	62%
	Out GAC absorption	July 2020	5	40%	60%
		November 2020	8	12%	88%
		May 2021	2	0	100%
	Effluent	July 2020	0	0	0
		November 2020	2	0	100%
		May 2021	4	25%	75%



Table A3 (continued)

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Castreccioni (DWTP)	Distribution 1	July 2020	1	0	100%
		November 2020	3	100%	0
		May 2021	5	20%	80%
	Distribution 2	July 2020	0	0	0
		November 2020	0	0	0
		May 2021	7	14%	86%
	Flocculated sludge	July 2020	26,500	40%	60%
		November 2020	23,250	23%	77%
		May 2021	2,000	60%	40%
	Backwash sand filtration	July 2020	962	40%	60%
		November 2020	529	0	100%
		May 2021	800	44%	56%



Table A4: quantification of microfibers (MFs) extracted from samples collected in Limone Tremosine WWTP considering both those of synthetic (plastic) and natural origin and their relative contribution on the total.

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Limone Tremosine (WWTP)	Influent before pre-treatments	July 2021	153	78%	2%
		November 2021	1056	65%	35%
		April 2022	384	55%	45%
	Effluent from flotation unit	July 2021	148	91%	9%
		November 2021	63	81%	19%
		April 2022 (Sample not available for this campaign)	-	-	-
	Effluent from tertiary filtration	July 2021	84	80%	20%
		November 2021	61	73%	27%
		April 2022	7	42%	58%
	Flotated sludge	July 2021	212,500	94%	6%
		November 2021 (Sample not available for this campaign)	-	-	-
		April 2022	101000	67%	33%
	Aerobically stabilized secondary sludge	July 2021	808,500	94%	6%
		November 2021	34,750	96%	4%
		April 2022	3100	90%	10%



Table A5: quantification of microfibers (MFs) extracted from samples collected in Peschiera del Garda WWTP considering both those of synthetic (plastic) and natural origin and their relative contribution on the total.

Selected facility	Sampling step	Sampling period	MFs/m ³	Synthetic MFs (plastic)	Natural MFs
Peschiera del Garda (WWTP)	Influent	July 2021	23	67%	33%
		November 2021	2,083	85%	15%
		April 2022	142	40%	60%
	Effluent from secondary settlers	July 2021	132	72%	28%
		November 2021	36	76%	24%
		April 2022	4	67%	33%
	Effluent from lamellar packs unit	July 2021	96	90%	10%
		November 2021	6	100%	0
		April 2022	1,259	61%	39%
	Effluent from sand filtration	July 2021	13	69%	31%
		November 2021	18	78%	22%
		April 2022	11	100%	0
	Sludge to the thickeners	July 2021	202,000	94%	6%
		November 2021	350,000	78%	22%
		April 2022	442,000	88%	12%
	Chemical sludge from lamellar packs unit	July 2021	26,500	75%	25%
		November 2021	28,000	80%	20%
		April 2022	13,000	85%	15%