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Master Degree

Environmental Engineering

TRACING MICROPLASTICS IN DRINKING WATER TREATMENT PLANTS AND DATA ANALYSIS FOR WATER REUSE RISK MANAGEMENT PLANTS

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ABSTRACT (ENGLISH)

Microplastics (MPs) are emerging globally distributed pollutants of aquatic environments and today little is known about their fate at drinking water treatment plants (DWTPs). Essentially potential hazards associated with microplastics in drinking-water could be expressed in three forms: particles presenting a physical hazard; chemicals, including additives and absorbed substances; biofilms. Generally, health effects depend on concentrations of microplastics at which a subject is exposed to. The scope of this study has been to analyse the presence of MPs in the DWTP of Castreccioni. Sampling campaign has been done in two months: July and December 2020. MPs have been sampled filtering almost 1000L of effluent water from each treatment process of the DWTP. Then samples have been characterized in laboratory using µFT-IR in terms of: concentrations, shapes, sizes and polymers. Also a sample of flocculation sludge and one of backwash have been analysed. In Summer campaign MPs ranges from 0,0039n°MPs/L in the effluent to 0,012n°MPs/L in the influent. Synthetic MFs ranges from 0n°MFs/L in the effluent to 0,003n°MFs/L in the influent. Most of MPs are fragments included in size class 0,5-0,15mm. In general could be noticed that most present polymers in water samples are: polyethylene, polyester resin, polyurethane, polypropylene, polyester and in second distribution point styrene-butadiene. The biggest removal efficiency happens in pre-ozonation. In Winter campaign MPs ranges from 0,00939n°MPs/L in the effluent to 0,012n°MPs/Lin the influent. Synthetic MFs ranges from 0n°MFs/L in the effluent to 0,003n°MFs/L in the flocculation effluent. Also in this case most of MPs are fragments in size class 0,5-0,15mm. Microplastics most frequent polymers are: polyvinyl chloride, polyester resin and polypropylene, polyester, polyethylene, polystyrene, polyvinylidene fluoride, polyacryate, polyacrylic rubber, polyurethane, polytetrafluoroethylene, polyvinyl chloride+polyvinyl alcohol, silicone, thermoplastic elastomer, epoxide resin, polyacrylate and acrylonitrile butadiene styrene. The biggest removal efficiency happens in sand filters. QMRA is a mathematical quantitative approach for estimating risk caused by pathogens to human health. (V. Zhiteneva et al, 2020) and could be used to support water safety management decisions. The numerical output of QMRA could be compared with national level targets to assess the risk management question. In particular in this study, starting from outlet concentrations of E.coli from Peschiera Borromeo WWTP, the possible risk connected to a hypothetical reuse of the water to irrigate tomato crops has been obtained. Two periods have been analysed: first one in which the plant worked without a reuse-logic and second one in which the plant improved disinfection to obtain lower E.coli concentrations effluent. Exposure subject are fieldworkers, local communities and consumers of final products. While reference pathogens are E.coli, Campylobacter, Cryptosporidium and Rotavirus. Calculating maximum risk in both non-reuse and reuse scenario, considering results in terms of DALYs only Rotavirus has been the pathogen at risk, while considering them in terms of probability of risk all pathogens are a possible hazard for human health. Applying a first level of barriers aimed to obtain a raw final product, in all scenarios considered all the pathogen risks were below the maximum target levels. Another level of barriers has been applied aimed to obtain a processed final product but they turned out to be useless because first level of barrier was enough to guarantee safety. The analysis carried out was deterministic by adopting an average value for each input data. An example of stochastic analysis using the Monte Carlo simulation is provided to show how important the distribution of data in the period is.

ABSTRACT (ITALIAN)

Le microplastiche (MP) sono inquinanti emergenti distribuiti a livello globale negli ambienti acquatici e oggi si sa poco sul loro destino negli impianti di trattamento di acqua potabile (DWTP). Essenzialmente i rischi potenziali associati alle microplastiche nell'acqua potabile potrebbero essere

espressi in tre forme: particelle che presentano un pericolo fisico; prodotti chimici, inclusi additivi e sostanze assorbite; biofilm. In generale, gli effetti sulla salute dipendono dalle concentrazioni di microplastiche a cui è esposto un soggetto. Lo scopo di questo studio è stato quello di analizzare la presenza di microplastiche nell'impianto di potabilizzazione situato a Castreccioni. La campagna di campionamento è stata condotta in due mesi: luglio e dicembre 2020. Le microplastiche sono state campionate filtrando circa 1000 litri di acqua effluente da ogni processo di trattamento della DWTP più da due punti della distribuzione. Quindi i campioni sono stati caratterizzati in laboratorio utilizzando µFT-IR in termini di: concentrazioni, forme, dimensioni e polimeri. Sono stati analizzati anche un campione di fanghi di flocculazione e uno di controlavaggio. Nella campagna estiva le microplastiche variano da 0,0039 n°MPs/L nell'effluente a 0,012n°MPs/L nell'influente. Gli MF sintetici variano da 0n°MFs/L nell'effluente a 0,003n°MFs/L nell'influente. La maggior parte delle MP sono frammenti inclusi nella classe di dimensioni 0,5-0,15 mm. In generale si può notare che i polimeri più presenti nei campioni di acqua sono: polietilene, resina poliestere, poliuretano, polipropilene, poliestere e nel secondo punto di distribuzione stirene-butadiene. La più grande efficienza di rimozione si ha nella pre-ozonizzazione. Nella campagna invernale le microplastiche variano da 0,00939n°MPs/L nell'effluente a 0,012n°MPs/L nell'influente. Le microfibre sintetiche variano da 0n°MFS/L nell'effluente a 0,003n°MFs/L nell'effluente di flocculazione. Anche in questo caso la maggior parte dei parlamentari sono frammenti nella classe di grandezza 0,5-0,15mm. I polimeri più frequenti delle microplastiche sono: polivinilcloruro, resina poliestere e polipropilene, poliestere, polietilene, polistirene, polivinilidene fluoruro, poliacrilato, gomma poliacrilica, poliuretano, politetrafluoroetilene, polivinilcloruro + polivinil alcol, silicone, elastomero termoplastico, resina epossidica, poliacrilato butadiilonitrile stirene. La maggiore efficienza di rimozione si ha nei filtri a sabbia. La QMRA (quantitative microbial risk assessment) è un approccio quantitativo matematico per la stima del rischio causato da patogeni per la salute umana. (V. Zhiteneva et al, 2020) e potrebbe essere utilizzato per supportare le decisioni di gestione della sicurezza dell'acqua. L'output numerico della QMRA dev' essere confrontato con gli obiettivi a livello nazionale per valutare la questione della gestione del rischio. In particolare in questo studio, a partire dalle concentrazioni in uscita di E.coli dall'impianto di depurazione di Peschiera Borromeo, si è ottenuto il possibile rischio connesso ad un ipotetico riutilizzo dell'acqua per irrigare le colture di pomodoro. Sono stati analizzati due periodi: il primo in cui l'impianto ha funzionato senza una logica di riutilizzo e il secondo in cui l'impianto ha migliorato la disinfezione per ottenere minori concentrazioni di E.coli effluenti. I soggetti dell'esposizione sono contadini che lavorano le colture, le comunità locali e i consumatori dei prodotti finali. Mentre i patogeni di riferimento sono E. coli, Campylobacter, Cryptosporidium e Rotavirus. Calcolando il rischio massimo sia nello scenario di non riutilizzo che in quello di riutilizzo, considerando i risultati in termini di DALY solo il Rotavirus è risultato essere un patogeno a rischio, mentre considerandoli in termini di probabilità di rischio tutti i patogeni sono un possibile pericolo per la salute umana. Applicando un primo livello di barriere finalizzate all'ottenimento di un prodotto finale grezzo, in tutti gli scenari considerati tutti i rischi patogeni erano al di sotto dei livelli massimi ammissibili. Un altro livello di barriere è stato applicato per ottenere un prodotto finale lavorato ma si è rivelato inutile perché il primo livello di barriere era sufficiente per garantire la sicurezza. L'analisi effettuata è stata di tipo deterministico adottando un valore medio per ogni dato di input. Un'esempio di analisi stocastica usando la simulazione Monte Carlo è fornito per mostrare quanto la distribuzione di dati nel periodo sia di fondamentale importanza.

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1 INTRODUCTION

Microplastics (MPs) are defined as plastic particles lower than 5 mm of size, under this limit particles are defined nanoplastics. There are several studies on MPs presence in the environment and today they have been detected in several number of water bodies and even in potable water. The potential toxicological effects of MPs are still largely unknown and need more detailed studies, however they are classified as emerging compounds. Drinking water treatment plants (DWTPs) pose a barrier for MPs to enter drinking water; thus, the fate of MPs at DWTPs is of great interest. (Novotna et al., 2019) In this study presence and fate of microplastics in DWTPs have been detected with particular attention to sampling and characterization methods currently applied and their accuracy to represent real situations in plants. A sampling campaign in two sessions has been done in the DWTP situated near Castreccioni Lake, Marche Region. Sampled microplastics have been divided into particles (MPs) and synthetic microfibers (MFs) and have been characterized using µFT-IR spectroscopy in terms of concentration, shape, size and polymers. Microplastics represent a hazard to human health because of its toxicity when enter in contact with exposed subjects. In this context, a research on possible effect has been conducted. Human health consequences are several and not clear. The risk could be due to particles, to chemicals which are present and to biolfilms.

In this context, it is important to define the methodology to carry out water risk assessment. Since an important environmental issue regards water scarcity and water reuse for different purposes, Water Reuse Risk Management Plan (WRRMP) is going to be more and more important. One of most discussed and regulated purpose by European Commission is water reuse for irrigation. Water should be reused in a responsible, sustainable manner, so ensuring that no additional risks for human health and the environment are introduced. This results in ensuring the microbial safety of water and sanitation services. (WHO, 2016) WHO water quality guidelines recommend a preventive, risk-based approach to water quality management. The purpose of the risk assessment is to identify and evaluate the health risks associated with the water supply, to determine if the health hazards are adequately controlled, to inform operation and management of the water supply and to identify necessary improvements and upgrades to ensure the delivery of safe drinking-water. (WHO, 2016) This is traduced in the identification of risks that are critical for the safety of a specific water supply system and to help to select the best steps to improve the safety of the system. Risk has to be classified or quantified in terms of health impact. Risk assessment is therefore a decision support tool that provides the risk manager with an objective and rational picture of what is known about the risks associated with the water supply. Approaches to conduct a risk assessment are: sanitary inspection, risk matrix or quantitative microbial risk assessment. QMRA could be defined as a formal, quantitative risk assessment approach that combines scientific knowledge about the presence and nature of pathogens, their potential fate and transport in the water cycle, the routes of exposure of humans and the health effects that may result from this exposure, as well as the effect of natural and engineered barriers and hygiene measures. (WHO, 2016) In this study has been developed a QMRA risk assessment for the reuse of water effluent from Peschiera Borromeo WWTP. The purpose of reuse is irrigation of tomatoes crops. Different scenarios have been studied using E.coli effluent concentrations from the plant between the years 2018-2020. Risk posed by reference pathogens (E.coli, Campylobacter, Cryptosporidium and Rotavirus) chosen using conventions applied in literature has been evaluated and then compared with level targets adopted by WHO and U.S. EPA. Conducted analysis is deterministic but an additional comparison with a stochastic analysis improved with Monte Carlo simulation has been provided for one scenario.

2 STATE OF ART

2.1 FATE OF MICROPLASTICS IN DRINKING WATER TREATMENT PLANTS

2.1.1 Sampling and characterization methods

Today microplastics are present in the majority of surface freshwater sources (like river or lakes and groundwater) because they are easily contaminated by agricultural and human activities in different quantities depending on some parameters like pollution, location, hight, proximity to urban centres, etc. Some of these water bodies are catchment for drinking water treatment plants (DWTP) which constitute an obstacle to the entry of microplastics from raw water into daily drinking water (M.Shen et al., 2020).

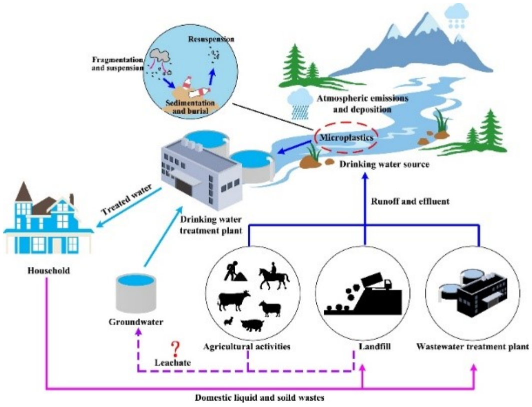


Figure 1 Sources and transport of microplastics in raw water and treated drinking water (tap water or bottled water). Source: M.Shen et al.,2020

The fate of microplastics in DWTPs has been the subject of some studies conducted in recent years and these is important to understand the efficiency of removal that current plants could have in front of these new category of micropollutant. But these studies concern all MPs in freshwater bodies, the research on MPs in groundwater is lacking. (K. Novotna et al., 2019). In the article *"Microplastics in drinking water treatment – Current knowledge and research needs" (K.Novotna et al, 2019)* all the most relevant studies are reported to compare their results in terms of efficiency of removal and concentration of microplastics (MPs) detected in all the stages of different processes of DWTPs. The results of the different studies vary significantly, ranging from zero or very few (< 10) to > 4000 microplastic particles per liter. (K. Novotna et al., 2019). These differences can be related to different reasons like:

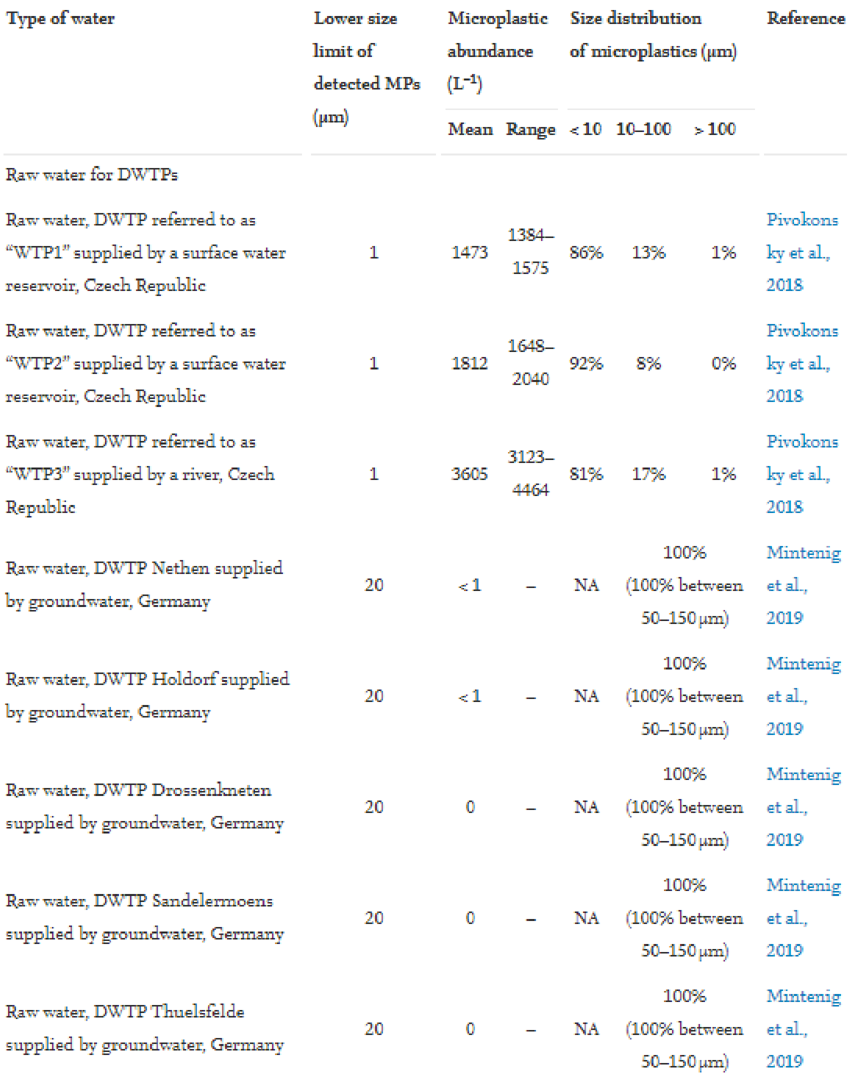
- Sampling location: water body conditions are very important because for example a very polluted site near an urbanized area could have tonnes of extra particles respect for example to a lake at high altitude.

- Dissimilarities in sampling: has been noticed that the thing that could bring the biggest differences in concentrations is the sampling method. In particular the main size of the used filter: the more the filter size is small, the more will be the number of retained microplastics

- Water treatment technologies

Another important challenge in the considered studies about MPs in DWTPs is the size distribution of MPs. In particular has been noticed that in all cases the majority of particles (81–92%) falls in the size range <10μm (K. Novotna et al., 2019). In the following table all this characterization has been summarized.

Table 1. The abundance and size distribution of microplastics in raw water for drinking water treatment plants. Source: K.Novotna et al.,2019



Type of water	Lower size limit of detected MPs (μm)	Microplastic abundance (L^{-1})		Size distribution of microplastics (μm)			Reference
		Mean	Range	< 10	10–100	> 100	
Raw water for DWTPs							
Raw water, DWTP referred to as "WTP1" supplied by a surface water reservoir, Czech Republic	1	1473	1384–1575	86%	13%	1%	Pivokonsky et al., 2018
Raw water, DWTP referred to as "WTP2" supplied by a surface water reservoir, Czech Republic	1	1812	1648–2040	92%	8%	0%	Pivokonsky et al., 2018
Raw water, DWTP referred to as "WTP3" supplied by a river, Czech Republic	1	3605	3123–4464	81%	17%	1%	Pivokonsky et al., 2018
Raw water, DWTP Nethen supplied by groundwater, Germany	20	< 1	–	NA	100% (100% between 50–150 μm)		Mintenig et al., 2019
Raw water, DWTP Holdorf supplied by groundwater, Germany	20	< 1	–	NA	100% (100% between 50–150 μm)		Mintenig et al., 2019
Raw water, DWTP Drossenkneten supplied by groundwater, Germany	20	0	–	NA	100% (100% between 50–150 μm)		Mintenig et al., 2019
Raw water, DWTP Sandelermoens supplied by groundwater, Germany	20	0	–	NA	100% (100% between 50–150 μm)		Mintenig et al., 2019
Raw water, DWTP Thuelsfelde supplied by groundwater, Germany	20	0	–	NA	100% (100% between 50–150 μm)		Mintenig et al., 2019

Another parameter object of study is the material composition. Main MPs particles are made of Polyethylene (PE) and polypropylene (PP) because are commonly used in a lot of products. In addition surface freshwater containes polyethylene terephthalate (PET), polystyrene (PS), polyvinylchloride (PVC), polyester (PES), polyamide (PA), polyacrylate (PAC), polytetrafluorethylene (PTFE) or rayon. In raw water for DWTPs, many other materials besides PE and PP were also identified, namely, PET, PS, PVC, polyacrylamide (PAM), polybutylacrylate (PBA), polymethyl methacrylate (PMMA), *p*-phenylene terephthalamide (PPTA), polytrimethylene terephthalate (PTT), di (2-ethylhexyl) phthalate (DEHP) and polyoxybenzylmethylenglycolanhydride (Bakelite) in raw water originating from surface waters and PVC, PES and epoxy resin in raw water originating from groundwater (K. Novotna et al., 2019).

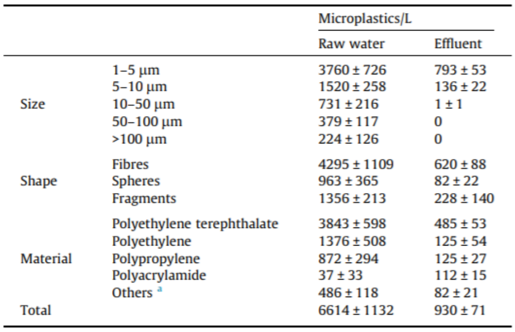
Some of these studies have been analysed to understand efficiencies of different processes in a DWTP.

2.1.2 Microplastics fate and removals in drinking water treatment plants

The study conducted by Z.Wang et al in 2019 called *“Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP)”* is focused on the presence of MPs in raw water from Yangtze River that is an important water source in China. The DWTP considered is one of the largest advanced drinking water treatment plants of China (its usual and maximum capacity are 1288/1736 L/sec). The processes included: coagulation/flocculation, sedimentation, sand filtration and ozonation combined with GAC filtration. Sampling points were at the inlet and downstream of each process and have been taken instantaneously during three days in winter. In this study, water samples were collected in 1 L brown glass bottles (pre-cleaned) from the raw water and effluents from each treatment process. Downstream samples have been digested with 30% hydrogen peroxide (H2O2) for 24 h and then filtered through a series of 5 µm membrane filters (PTFE) followed by a pore size of 0.22 µm. DXR2 micro-Raman imaging microscope system (Thermo Fisher Scientific, USA) has been used for qualitative analysis of particles. Collected spectra were processed by Omnic software and identified by comparing to a database (Z. Wang et al., 2019).

Three days concentrations have been averaged to obtain a single value per sampling point. Of each stage has been calculated the efficiency of removal. In this study has been also done an analysis about size distribution, shape and material of the particles. These characterizations have been done on influent and effluent of the plant and are resumed in the table below taken from the literature.

Table 2 Microplastic concentration in raw water and effluent in the ADWTP. Source: Z.Wang, 2019



		Microplastics/L	
		Raw water	Effluent
Size	1–5 µm	3760 ± 726	793 ± 53
	5–10 µm	1520 ± 258	136 ± 22
	10–50 µm	731 ± 216	1 ± 1
	50–100 µm	379 ± 117	0
	>100 µm	224 ± 126	0
Shape	Fibres	4295 ± 1109	620 ± 88
	Spheres	963 ± 365	82 ± 22
	Fragments	1356 ± 213	228 ± 140
Material	Polyethylene terephthalate	3843 ± 598	485 ± 53
	Polyethylene	1376 ± 508	125 ± 54
	Polypropylene	872 ± 294	125 ± 27
	Polyacrylamide	37 ± 33	112 ± 15
	Others [a]	486 ± 118	82 ± 21
Total		6614 ± 1132	930 ± 71

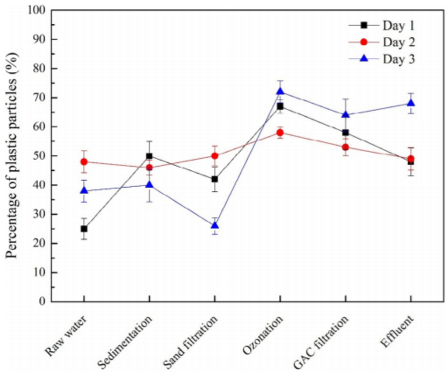
Efficiency of removal of each stage is resumed in table below.

Table 3 Efficiencies of removal in Z.Wang,2019

Reference	Unit treatment	MPs	Removal efficiency	Removal efficiency	Overall removal efficiency
		n/L	n/L	%	%
Z. Wang, 2020	Infuent	6614,0			
	Sedimentation	3466,7	3147,3	47,6	
	Sand filtration	2066,7	1400,0	40,4	
	Ozonization	2066,7	0,0	0,0	
	GAC	900,0	1166,7	56,5	
	Effluent	930,0	-30,0	-3,3	85,9

In the coagulation/sedimentation process, has been found that the larger size microplastics had a higher removal efficiency. MPs > 10 µm were almost completely removed, followed by the removal efficiency of 44.9–75.0% for 5–10 µm in this process. This probably happened because larger-sized microplastics have been more easily attached to floccules in the coagulation, having good sedimentation properties. While there was a poor removal efficiency (about 28.3–47.5%) for 1–10 mm microplastics. The number of microplastics in the effluent of ozonation has slightly increased, mainly due to the negative removal of small particles and fibrous microplastics. In the table this is reported as 0% removal efficiency because the result is an averaged value. The rising of microplastics has happened because they has been broken under the action of the shearing force of the water flow rising the number of MPs. The abundance of 1–5 µm MPs from the effluent of ozonation increased by 2.8–16.0%, resulting in a negative removal effect.

Table 4 Percentage of plastic particles detected in the effluent of each treatment unit. Source: Z.Wang,2019



The overall removal efficiency of microplastics is around 86%. By comparing the raw water with the effluent in the ADWTP, the removal efficiencies of larger size particles were higher than small particles. About the variations of microplastics with different shapes the removal efficiencies were: 82.9–87.5% for fibres; 89.1–92.7% for spheres and 73.1–88.9% for fragments.

In the study *“Occurrence of microplastics in raw and treated drinking water”* conducted by M.Pivokonský et al. in 2018 **,** samples of raw and treated water have been taken from three drinking water treatment plants all located in urban areas of the Czech Republic.

- WTP1 (usual/maximum capacity: 3700/7000 L/sec) takes water from a large valley water reservoir and the technologies of the process are: coagulation/flocculation and sand filtration.
- The water source for WTP2 (usual/max capacity: 100/200 L/sec) is a smaller water reservoir and process is composed by: coagulation/flocculation, sedimentation, sand and granular activated carbon filtration.
- WTP3 (usual/max capacity: 90/150 L s−1) uses water from a river and the treatment process includes: coagulation-flocculation, flotation, sand filtration and granular activated carbon filtration.

Samples has been collected in the winter period (November 2017– January 2018) for three times and each time every 8h in a 24h period. One sample of raw water and one sample of treated water (volume of 1 L each) has been taken into autoclavable borosilicate glass bottles. The samples has been stored at 4 °C. Wet peroxide oxidation has been conducted and the pre-treated samples has been passed through a series of polytetrafluoroethylene (PTFE) membrane filters of 5 µm and subsequently 0.2 µm pore sizes for SEM quantitative analysis and Al_2O_3 filters for filtration of the samples for qualitative analysis to assure no matrix interference. The obtained filters has served for the quantitative analysis of the retained particles, using a Vega high resolution scanning electron microscope. For qualitative analysis particles >10 µm has been analysed by using FTIR spectrometer Nicolet 6700 complemented by microscope Continuum while for the analysis of particles in the size range 1–10 µm DXR2xi microRaman imaging microscope system has been used.

Results about concentrations of microplastics and efficiencies are resumed in Table 5. (M. Pivokonsky et al., 2018)

Table 5 MPs concentrations and removal efficiencies from M. Pivokonsky, 2018

Reference	Unit treatment	MPs	Removal efficiency	Removal efficiency
		n/L	n/L	%
M. Pivokonsky, 2018	**DWTP 1 (from a large water basin)**			
	Influent	1473		
	Effluent	443	1030	69,9
	DWTP 2 (from a smaller water basin)			
	Influent	1812		
	Effluent	338	1474	81,3
	DWTP 3 (from a river)			
	Influent	3605		
	Effluent	328	3277	90,9

Microplastics has been divided into five categories according to their size (1–5 µm; 5–10 µm; 10–50 µm; 50–100 µm; >100 µm) and into three groups depending on their shape (fibres, spherical and fragments). Among the categories of MP distribution in raw water microplastics of 1–5 µm has prevailed in all the samples from any WTP, accounting for approximately 40–60% of the total MPs ,followed by the category 5–10 µm. About the treated water samples, our study has revealed no microplastics bigger than 100 µm and the prevailing size category has been again 1–5 µm, comprising approximately 25–60% of the microplastics. The second most abundant size group has been that of 5–10 µm (around 30–50% of MPs). MPs <50 µm seem to be almost completely removed from water at the treatment plants. Regarding the shape of microplastics, fragments have been by far the most abundant morphotype in the raw water supplying WTP1 and WTP2. WTP3 raw water contained fragments in substantial amounts as well (42–48%), but the proportion of fibres was also important (37–61%). A relative increase in the proportion of fibres to the exclusion of fragments appeared in WTP1 treated water, maybe related to the low removal of fibres and these results suggest that there might be some relationship between the shape of microplastics and their removability by various water treatment technologies. Regarding qualitative analysis Polyethylene terephthalate (PET), polypropylene (PP) and polyethylene (PE) particles have been the most abundant in all raw water samples. Within the treated water samples PET has been again the prevailing material followed by PP. (M. Pivokonsky et al., 2018)

Another study called “Occurrence and fate of microplastics at two different drinking water treatment plants within a river catchment” always by M.Pivokonský in 2020 analyses the occurrence of MPs at two different DWTPs that both lie on the Úhlava River (Czech Republic) river, separated by a distance of approximately 90 km by water: DWTP of Milence and DWTP of Plzeň. The DWTPs operate different water treatment trains and they have diverse capacities: usual/maximum capacities of the DWTP Milence and DWTP Plzeň are 180/400 L/sec and 400/1000 L/sec, respectively.

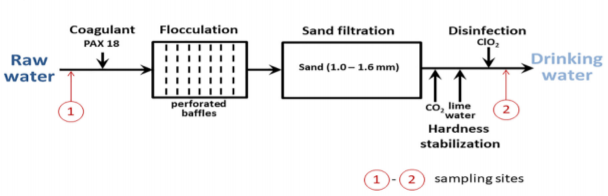


Figure 2 Milence water treatment train. Source: M. Pivokonský; 2020

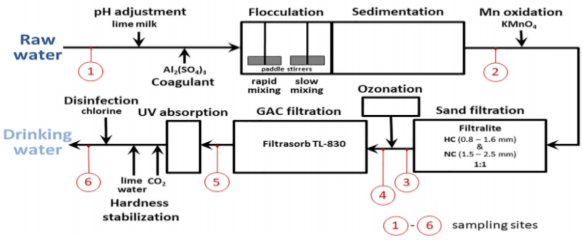
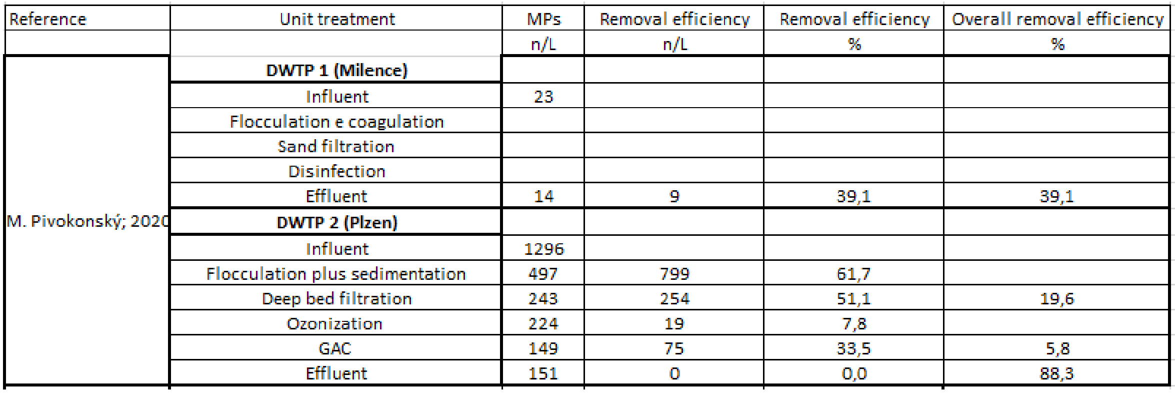


Figure 3 Plzeň water treatment train. Source: M. Pivokonský; 2020

At each DWTP, samples of raw water (at the DWTP inlet) and final treated water (at the outflow to water accumulation) has been collected. In DWTP of Milence, which has simple treatment technology, these has been the only sampling points; in the case of the DWTP of Plzeň, which involves more technological steps, has been collected water from each step (four more sampling points). Sampling has been performed during winter 2019/ 2020. Sampling has been conducted three times on a sampling day, i.e. every 8 h within a 24-h period. At each sampling occasion, 2 L of water has been filled into borosilicate glass bottles (pre-cleaned). The samples have then been stored in the dark at 4 ° C. Any contact of the samples with plastic materials has been avoided during sampling, sample preparation, filtration and analysis. Then the samples have been acidified to pH 3.5 by the addition of 1 M H2SO4.Then they have been passed through filters to retain particles: for SEM, polytetrafluoroethylene (PTFE) membrane filters with a 0.2 µm pore size has been used while for µRaman Al2O3 filters with a 0.2 µm pore size were utilized to assure no interference. Quantitative analysis of particles and their size and shape determination have been conducted by scanning electron microscopy (SEM) and qualitative analysis by micro-Raman spectroscopy. (M. Pivokonský et al., 2020)

Detected MPs concentration in both DWTPs and respective efficiency of removal have been resumed in Table 6.

Table 6 MPs concentrations and removal efficiencies in M. Pivokonský; 2020



Reference	Unit treatment	MPs	Removal efficiency	Removal efficiency	Overall removal efficiency
		n/L	n/L	%	%
M. Pivokonský; 2020	**DWTP 1 (Milence)**				
	Influent	23			
	Flocculation e coagulation				
	Sand filtration				
	Disinfection				
	Effluent	14	9	39,1	39,1
	DWTP 2 (Plzen)				
	Influent	1296			
	Flocculation plus sedimentation	497	799	61,7	
	Deep bed filtration	243	254	51,1	19,6
	Ozonization	224	19	7,8	
	GAC	149	75	33,5	5,8
	Effluent	151	0	0,0	88,3

In Plzeň there were 1296 ± 35 MPs/L in raw water on average, more than in the raw water of the DWTP of Milence. This may be explained by the fact that the DWTP of Milence is supplied by a water reservoir that lays on an upper flow of the Úhlava and anthropogenic impacts in the location are minimal. By contrast, the DWTP of Plzeň is supplied by water from a lower flow of the Úhlava River, while the river flows through several towns. Observing Plzeň concentrations can be noticed that a considerable portion of MPs has been removed by coagulation-flocculation with sedimentation (around 62%). Subsequent filtration also significantly contributed to MPs removal. A similar number has been observed after ozonation (additional 20%). Another decrease appeared as a result of GAC filtration (additional 6%). Finally, the number of MPs in treated water has been very close to that after GAC. At the DWTP of Milence, approximately 40% removal of MPs has happened and a decreased removal efficiency with decreasing size can be noticed. While the overall removal efficiency at the DWTP of Plzeň has been around 88%. Can also be noticed that in all the removing stages larger MPs particles have been removed more easily. (M. Pivokonský et al., 2020)

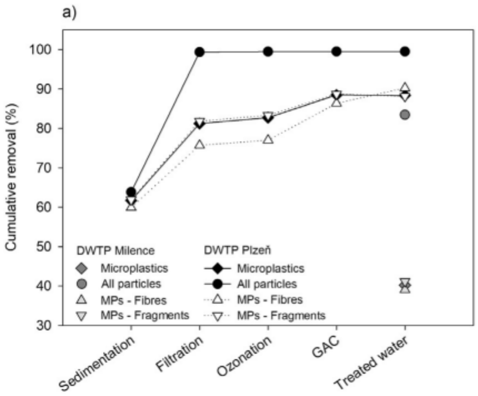


Figure 4 Cumulative removal of microplastics (MPs). Source: M. Pivokonský; 2020

Further analysis has been done on size and shape of MPs. MPs has beendivided into five size categories: ≥1 to <5 µm, ≥5 to <10 µm, ≥10 to <50 µm, ≥50 to <100 µm, and ≥100 µm. Further, three shape categories were distinguished: fibres, fragments, and spheres (which are not present in any sample). In the case of the DWTP of Milence, the content of fibres has been very low and most of them has been ≥50 µm. The remaining 80% have been fragments within the size category of ≥1 to <5 µm. Fragments also has prevailed at the DWTP of Plzeň, accounting for 87–92% in dependence of the sample type and fibres comprised the remaining 8–13% of all MPs. Fibres sized ≥5 to <10 µm and all larger-size fractions always appeared and the majority of fragments (>50%) were always within the smallest-size category of ≥1 to <5 µm. (M. Pivokonský et al., 2020)

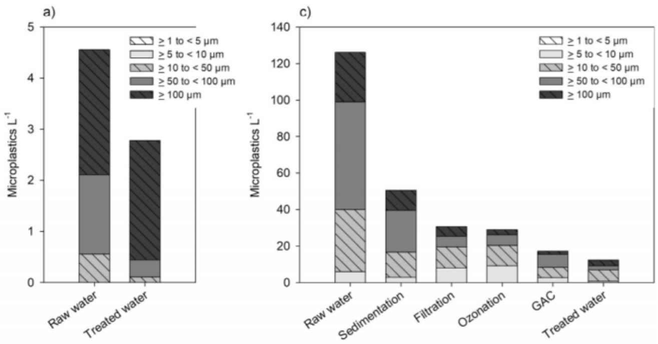


Figure 5 Size distribution for microplastics fibers. Source:M. Pivokonský; 2020

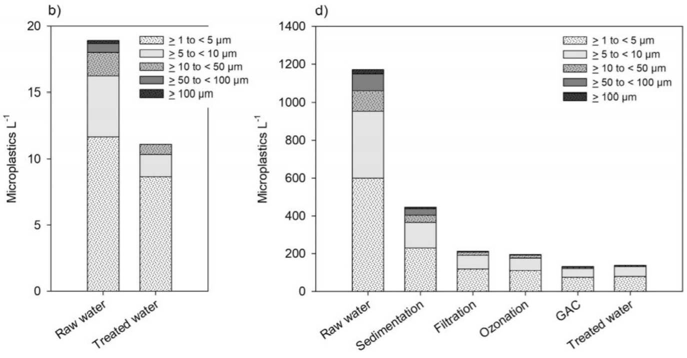


Figure 6 Size distribution for microplastics fragments. Source:M. Pivokonský; 2020

In general, 13 different plastic materials has been found in the samples as MPs. At the DWTP of Milence, raw water contained, in addition to CA, PET, PVC, PE, and PP, ethylene vinyl acetate copolymer (EVA), poly(butyl acrylate) (PBA), and polytrimethylene terephthalate (PTT). CA has prevailed over other materials. MPs in treated water have been mostly composed of the same materials as those in raw water. In the case of the DWTP of Plzeň, more different materials forming MPs have been identified. In raw water, some materials have been coincident with those observed at the DWTP of Milence, i.e., CA, PET, PVC, PE, PP, and EVA. However, raw water at the DWTP of Plzeň also contained polystyrene (PS), polyamide – nylon 6 (PA6), polyethylene oxide + polyethylene glycol (PEO + PEG), vinyl chloride/vinyl acetate copolymer (VC/VAC), PTT, and PTFE.. Only polytetrafluoroethylene (PTFE) has occurred in treated water but not in raw water. The most abundant plastics were CA, PET, PVC, PE, and PP, together comprising approximately 80% of all MPs in raw water . It can be seen that the material composition slightly varied after the treatment, but CA, PET, PVC, PE, and PP together always comprised >80%, and they constituted 90% in treated water. (M. Pivokonský et al., 2020)

In the study *"Low numbers of microplastics detected in drinking water from ground water sources"* conducted by Mintenig et al. in 2018 The sampling has taken place between August 13th and 20th 2014. The DWTPs in Nethen, Holdorf, Grossenkneten, Sandelermoens and Thuelsfelde have been chosen, here the raw water at the DWTP inlet and the drinking water at the plant outlet has been sampled. Additionally, one consumer household in the distribution system of each DWTP has been selected to sample at the water meter and at a conventional water tap. The raw water and drinking water samples have been filtered through 3 µm stainless steel cartridge filter. Between 300 and 1000 L of raw water and 1200 to 2500 L of drinking water have been filtered. Then the filter units have been stored refrigerated at 4 °C. The units have been filled again with diluted hydrochloric acid to dissolve calcium carbonate and iron precipitates. After 24 h the filter units have been emptied, the cartridge filters removed from the units and rinsed with Milli-Q and ethanol. The retentate has been collected on 3 µm stainless steel filters (47 mm in diameter) that were subsequently transferred into glass bottles and covered with 30 mL hydrogen peroxide. Qualitative analysis has been performed with FTIR microscope. Results of detected concentrations are represented in the Figure 7. (Mintenig S.M. , 2018)

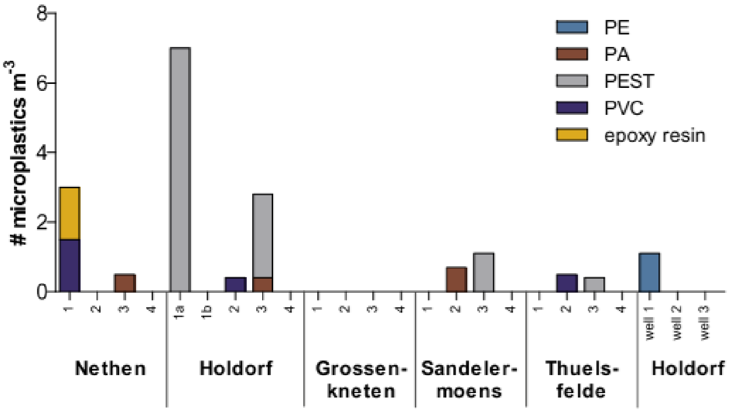


Figure 7 Microplastic particles identified in (1) raw water, (2) outlet, e (3) water meter and (4) a conventional water tap in a selected household. Source: Mintenig S.M., 2018

All particles have been characterized as small fragments of 50 to 150 µm and were made of five different polymer types, namely PEST, PVC, PE, PA and epoxy resin. (Mintenig S.M. , 2018)

To summary all the scientific literature detailed above, it can be concluded that:

- Wang et al.,2020 and Pivokonski et al.,2018 are the only studies that characterize MPs <10 µm by observing them at SEM and then with µRAMAN and using µFTIR to analyse microparticles >10 µm (withµFTIR it is not possible to go below 10-15 µM). This type of analysis has the drawback to be expensive and long lasting. In fact not whole filters have been analysed but only a representative part of them. This is a common approach due to the demanding and time-consuming methods applied. Pivokonski et al.'s studies concluded that MPs resulting in treated water are low but however not negligible constituting an important source of MPs to humans.
- Mintenig et al.,2018 finds only particles <150 µm. They used FTIR microscope and with this instrument they analysed all filter surface.Concentrations that they have found are really low compared with Pivokonski and Wang studies, to the point that they conclude that the drinking water does not constitute a significant risk of ingestion of MP compared to other food routes. To have a good representation of the small fraction of MPs could be useful to use polytetrafluoroethylene (PTFE) membrane filters and Al2O3 filters.
- K. Novotna et al., 2019 which makes a review of current knowledge about MPs in drinking water treatment plants concluded that concentrations in treated water are really variable between studies and so they couldn't be neglect and ignored as potential risk for human health. Moreover, it is still unclear which treatment step is

responsible for the majority of removal. However, there is no legislative limit for MP content in drinking water, and it is not possible to assess the residual concentrations as too high or sufficiently low. (K. Novotna et al., 2019)

Moreover, in Table 7 is summarized the MPs removal efficiencies of the different treatment processes applied in conventional DWT.

Table 7 Removal efficiencies of different DWTP

Reference	Unit treatment	MPs	Removal efficiency	Removal efficiency	Overall removal efficiency
		n/L	n/L	%	%
Z. Wang, 2020	Infuent	6614,0			
	Sedimentation	3466,7	3147,3	47,6	
	Sand filtration	2066,7	1400,0	40,4	
	Ozonization	2066,7	0,0	0,0	
	GAC	900,0	1166,7	56,5	
	Effluent	930,0	-30,0	-3,3	85,9
M. Pivokonský; 2020	**DWTP 1 (Milence)**				
	Influent	23			
	Flocculation e coagulation				
	Sand filtration				
	Disinfection				
	Effluent	14	9	39,1	39,1
	DWTP 2 (Plzen)				
	Influent	1296			
	Flocculation plus sedimentation	497	799	61,7	
	Deep bed filtration	243	254	51,1	19,6
	Ozonization	224	19	7,8	
	GAC	149	75	33,5	5,8
	Effluent	151	0	0,0	88,3
M. Pivokonsky, 2018	**DWTP 1 (from a large water basin)**				
	Influent	1473			
	Effluent	443	1030	69,9	
	DWTP 2 (from a smaller water basin)				
	Influent	1812			
	Effluent	338	1474	81,3	
	DWTP 3 (from a river)				
	Influent	3605			
	Effluent	328	3277	90,9	

As could be seen from the table above, the general efficiency of DWTPs in microplastics removal is quite high, ranging from 39,1% to 90,9%. This means that the majority of MPs could be removed using conventional treatment processes.

In particular from the analysis conducted by *Z.Wang et al.* the average removal efficiency of sedimentation is 47,6% and of sand filtration is 40,4%, while *M. Pivokonský,2020* concluded that they are 61,7% and 5,1% respectively. These values represent the biggest efficiencies between all single processes. Indeed, some studies have been conducted about a possible correlation between other pollutants and microplastic removal in primary treatments.

2.1.3 Microplastics characterization from literature

Microplastic concentrations in Z.Wang et al.,2019 are higher respect to other studies, for example compared with M. Pivokonsky,2020 results in Plzen. The difference could be due to sampling method (filters size) and location. For what concerns shape distribution in Wang et al. study most of MPs in raw water are fibers, followed by parcels and fragments. In M. Pivokonský; 2020 and 2018 most are fragments and the rest fibers. In treated water in Wang et al.,2019 most MPs has become fibers. While in M. Pivokonsky,2020 and 2018 fragments remained the most abundant shape.

Table 8 MPs characterization from literature (concentration and shapes)

	n°MPs/L						**MPs shape distribution (%)**					
	Z. Wang, 2019	M. Pivokonský; 2020 (Milence)	M. Pivokonsky, 2020 Plzen	M. Pivokonský; 2018 (1)	M. Pivokonský; 2018 (2)	M. Pivokonský; 2018 (3)	Z. Wang, 2019	M. Pivokonský; 2020 (Milence)	M. Pivokonsky, 2020 Plzen	M. Pivokonský; 2018 (1)	M. Pivokonský; 2018 (2)	M. Pivokonský; 2018 (3)
Influent	6614,0	23,0	1296,0	1473	1812	3605	53.9–73.9% fibers; 8.6–20.6% parcels; 17.6–25.5% fragments	5 fiber/L; 19 fragments/L (20%-80%)	126 fibers/L ;1170 fragments/L	fragments 71-76%	fragments 71-76%	fragments 42-48%; fibers 37-61%
flocculation/coagulation												
sedimentation	3466,7		497,0						51 fibers/L ; 446 fragments/L			
sand or deep bed filtration	2066,7		243,0						31 fibers/L ; 213 fragments/L			
ozonation	2066,7		224,0									
GAC	900,0		149,0									
disinfection												
effluent	930,0	14,0	151,0	443	338	328	51.6–78.9% fibers; 6.7–10.1% particles; 14.4–38.3% fragments	3 fibers /L; 11 fragments/L (20%-80%)	12 fibers/L ; 139 fragments/L	fragments 42-48%		

3 In Table 9 have been resumed MPs distributions by size and polymers. For what concerns size distribution in M. Pivokonský; 2020 (Milence) most of particles are comprised in range 1-5μm both in raw and treated water. In M. Pivokonský; 2018 (1) in Raw water 1–5 μm MPs are 40-60% followed by the 5-10 μm category, finally MPs larger than 10 μm did not exceed 10%. MPs polymers present in raw water in Z. Wang, 2019 are PET (Polyethylene terephthalate), PE (Polyethylene) and PP (Polypropylene);

PET remains in the effluent and is the most present. In M. Pivokonsky,2020 (Plzen) a polymer which is present both in influent and effluent is cellulose acetate (CA). Finally, in Milence common polymers in raw and treated water are: CA, PET, PVC, PP, PE.

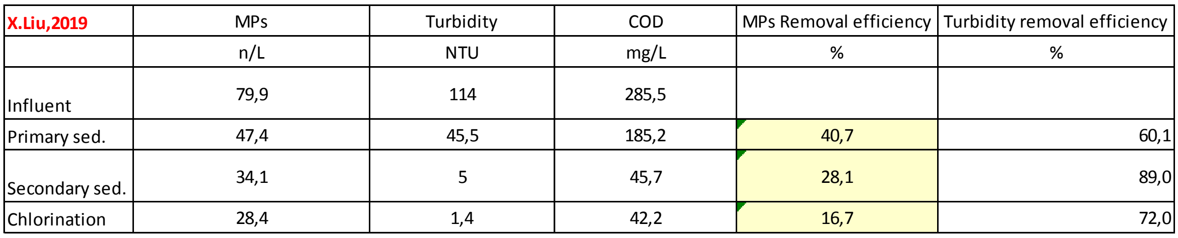
Table 9 MPs characterization from literature (size and polymers)

	Particles subdivision by size (%)						**Polymers distribution (%)**					
	Z. Wang, 2019	M. Pivokonský; 2020 (Milence)	M. Pivokonsky,2020 Plzen	M. Pivokonský; 2018 (1)	M. Pivokonský; 2018 (2)	M. Pivokonský; 2018 (3)	Z. Wang, 2019	M. Pivokonský; 2020 (Milence)	M. Pivokonsky,2020 Plzen	M. Pivokonský; 2018 (1)	M. Pivokonský; 2018 (2)	M. Pivokonský; 2018 (3)
Influent		FMPs between 1-5µm are between 50 in the influent and 60% in the effluent .		Raw water:1–5 µm 40-60% ,followed by the 5-10 µm category, about 30-40% of 5-10 µm. larger than 10 µm did not exceed a 10% .			55.4–63.1% PET; PE (almost 15.1–23.8%) e PP (almost 8.4–18.2%)	cellulose acetate, polyethylene terephthalate, polyvinyl chloride, polyethylene, or polypropylene , ethylene vinyl acetate copolymer, poly(butyl acrylate), polytrimethylene terephthalate	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/coagulation												
sedimentation												
sand or deep bed filtration												
ozonation												
GAC												
disinfection												
effluent				Treated water: no microplastics larger than 100 µm and only a minimum MP content of between 50 and 100 µm was observed. The prevailing size 1–5 µm 25–60%, The second largest group was that of 5-10 µm (approximately 30-50%).			PET 47.2–58.8% di MPs; PAM increases in effluent 10.1–14.7%	PTFE; CA=42%; no PP, EVA and PTT	CA, PET, PVC, PE, and PP>90% - no EVA, PA6, PEO + PEG, and PTT			

3.1 CORRELATION BETWEEN MICROPLASTIC REMOVAL AND OTHER POLLUTANTS

A study conducted by Xiaoning Liu et al. published in 2019 and called "*Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China*" has analysed a wastewater treatment plant in Wuhan,China using four sampling points: inlet of coarse grid (Influent, marked as W1), outlet of the primary sedimentation tank (marked as W2), outlet of secondary sedimentary tank (marked as W3), and outlet of chlorination disinfection (Effluent, marked as W4). Analysing removal efficiencies, particle size and shape distribution has been noticed that turbidity had a closer relationship with the removal of MPs than other parameters; small MPs particles could be adsorbed on the surface of hard and suspended particles in wastewater because of its large surface area and small size. The removal of MPs in WWTP has been accompanied with the decline of other pollutants in wastewater (Xiaoning Liu et al., 2019). In fact the study of Carr et al. and Lares et al. both demonstrated that the majority of MPs could be removed during the primary treatment stages through mainly skimming and settling processes. High reduction in this stage might be caused by the fact that many MPs particles were prone to adhere to suspended solids in wastewater.

Table 10 Characteristics of MPs and wastewater at various sampling sites and removal efficiencies elaborated on Xiaoning Liu, 2019 data

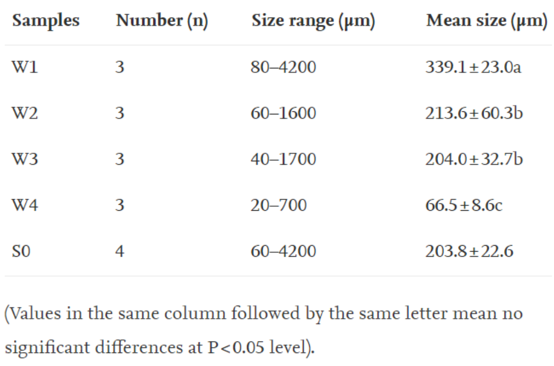


X.Liu,2019	MPs	Turbidity	COD	MPs Removal efficiency	Turbidity removal efficiency
	n/L	NTU	mg/L	%	%
Influent	79,9	114	285,5		
Primary sed.	47,4	45,5	185,2	40,7	60,1
Secondary sed.	34,1	5	45,7	28,1	89,0
Chlorination	28,4	1,4	42,2	16,7	72,0

From the resuming table above the highest removal efficiency takes place in primary sedimentation (40,7%) and it's accompanied by a turbidity removal od 60,1%. While biological treatment with activated sludge has removed MPs of an additional 16,6% and it seems that its function on the removal rate of MPs is not comparative with the primary treatment process. For what concerns MPs in activated sludge the study "*The removal of microplastics in the wastewater treatment process and their potential impact on anaerobic digestion due to pollutants association*" by *X.Zhang* noticed that degradation of MPs by microorganisms is generally not occurred to MPs. Hence, the main removal mechanism of MPs in activated sludge process would be adsorption and aggregation with sludge flocs. (X.Zhang et al., 2020)

Talking about primary treatments larger MPs particle are prone to be absorbed by suspended matter and was stored into primary and secondary sludge, resulting in the reduction of mean size in primary sedimentation, secondary sedimentation and chlorination. Meanwhile, the mean size of MPs is significantly smaller in sludge than in wastewater. This concept could be demonstrated in the table below where can be noticed that the mean size of MPs decreases.

Table 11 Change in size of fragment particles at various sampling sites. Source: Xiaoning Liu, 2019



Samples	Number (n)	Size range (μm)	Mean size (μm)
W1	3	80–4200	339.1 ± 23.0a
W2	3	60–1600	213.6 ± 60.3b
W3	3	40–1700	204.0 ± 32.7b
W4	3	20–700	66.5 ± 8.6c
S0	4	60–4200	203.8 ± 22.6

(Values in the same column followed by the same letter mean no significant differences at $P < 0.05$ level).

The disappearance of larger fragment might be caused by mechanical erosion by embrittlement and fracturing, chemical and biological degradation and is also associated with the fact that heavy fragment particles with larger size are prone to be adsorbed and settled into sludge (Xiaoning Liu et al., 2019).

The same conclusion has been inferred by *M.Lares et. al* in the *study "Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology"* in 2018. Has been stated that a fraction of the microplastic flux seems to be trapped within the WWTP. This could be related to their possible entrapment with solid materials from grit separation and grease from primary clarification. (M.Lares et al., 2018) The study of Lares et al. demonstrated that the majority of MPs could be removed during the primary treatment stages through mainly skimming and settling processes. High reduction in this stage might be caused by the fact that many MPs particles were prone to adhere to suspended solids in wastewater. (X.Liu et al., 2019)

3.2 RISK TO HUMAN HEALTH FROM MPS PRESENCE IN DRINKING WATER

Humans might be exposed to microplastics through ingestion of contaminated food and water, inhalation of air, and by direct dermal contact of particles (A. Rahman et al., 2020). Has been studied that ingestion is the primary route of human exposure.

Microplastics have different shapes, size and composition. These characteristics could influence their interaction with biological matrices during ingestion in human bodies, in particular their size. They could also contain additives which could be leached out and become bioavailable. Plastic particles could sorb chemicals from the environment and these could be toxic. Essentially potential hazards associated with microplastics in drinking-water could be expressed in three forms:

- particles presenting a physical hazard;
- chemicals, including additives and absorbed substances;
- biofilms.

(WHO, 2019)

Generally, health effects depend on concentrations of microplastics at which a subject is exposed to. Currently, due to a gap of studies and data, hasn't been estimated an accurate amount of microplastics assimilated by humans through exposure pathways (A.W. Verla et al., 2019). Some researchers believe that MPs exposure is an issue due to their potential toxicity and to the chemical hazards associated with the substances found in the plastic polymers or which they could adsorb. For others the expected exposure concentrations are low in drinking water and this made the risks negligible respect to other contaminants (Y. Li et al., 2020).

3.2.1 Toxicity associated with particles

The particle toxicity occurs when the critical mass of microplastics is localized and cause immune response from the body. Until now there are no studies about particle toxicity of microplastics to human maybe because humans are able to dispose > 90% of ingested plastics via faeces (A.W. Verla et al., 2019).

Toxicity posed by particles depends on different characteristics: shape, size and surface area for example. Until now there are a lot of gaps in the study of fate and transport of particles through ingestion. No epidemiological or human studies on ingested microplastics have been identified. The current database of information on plastic particle toxicity is limited to a few studies on laboratory animals but some of them are not even reliable.

In an OECD-compliant 90-day dietary study, Sprague-Dawley rats were administered a PE/PET polymer fabric for a minimum of 13 weeks at target concentrations of 0.5%, 2.5%, and 5%. No treatment-related adverse effects on blood parameters, organ weights or histopathology as well as mutagenicity, were seen when finely ground PET powder was mixed into the diet. (J.A. Merksi et al., 2018)

In the single identified study that evaluated oral toxicity of nano-plastics in a mammalian test species, the results show that the uptake of pristine nanoparticles did not affect behaviour of adult rats but the results need to be confirmed on a larger population due to the small sample size and study design limitations. The present study aimed to analyse potential neurobehavioral effects of polystyrene nanoparticles (PS-NPs) after long-term exposure on rats using four test dosages (1, 3, 6, and 10 mg PS-NPs/kg of body weight/day) administrated orally with adult Wistar male rats for five weeks. (M Rafiee et al., 2017)

In vitro assays in human cell lines were limited to a study in which PS and PE microplastics in two human cell lines showed oxidative stress for PS but not for PE and only at the highest concentration of 10 mg/L. No effects were seen at the lower concentrations of 0.05, 0.1 or 1 mg/L. (G.F. Schirinzi et al., 2018)

Other studies have been made on release of microplastics by surgical materials but they are of limited utility because of the very different exposure scenario. These studies have reported changes in gene expression, DNA damage, oxidative stress, cellular proliferation, tissue necrosis and inflammation. (WHO, 2019)

To understand the behaviour of microplastics when they are ingested is important to know where they could be absorbed in the gastrointestinal tract (GI). The EFSA panel on Contaminants in the Food Chain concluded that the largest fraction of ingested microplastics (>150 µm) are not absorbed and are excreted directly through faeces. (EFSA, 2016) Similar conclusions were reached by FAO: improbable absorption for microplastics >150 µm and limited absorption and uptake into organs for particles <20 µm. It is possible that absorption and distribution may be more significant for nano-plastics than microplastics (FAO, 2017).

3.2.2 Possible effects in human organ tissues

Because the gut is the first tissue with which microplastics enter in contact, it could have the greatest effects. Oral exposure to particles in general, also at very high levels, has been associated with mild intestinal irritation and inflammation. Particles could also alter the gut microbiome (L.Lu et al., 2018). Another study conducted using chickens suggests that exposure to nano-plastics may affect the barrier properties of the gut epithelium (G.J.Mahler et al., 2012). Several mechanisms for the entry of MPs through gut mucosa were proposed. Some researchers reported that MPs can be overwhelmed by some cell of the intestinal lymphoid tissue or directly absorbed, depending on the extent of the adherence to the gastrointestinal mucus membrane. Another group of researchers demonstrated that MPs might directly penetrate the intestinal mucosa when they are mixed with other intestinal contents (J.J. Powell et al., 2007).

Also after ingested by humans, microplastics could reduce lipid digestion through the formation of microplastics-oil droplet and inhibit the digestive enzymatic activities (H. Tan et al., 2020).

3.2.3 Particle properties and potential toxicity

As particle size decreases, its surface-area-to-volume ratio increases, and so smaller particles might be more susceptible to adsorbing biologically significant or toxic molecules. But an increased surface-area-to-volume ratio also means that microplastics degrade faster and it hasn’t been understood if conditions in GI tract are favorable to plastic degradation or not. Particles could also be uptake in the lung. Another consideration has been done on different properties of particles depending if they have been weathered: reduced hydrophobicity for example, which could reduce their ability to sorb hydrophobic substances (S. Endo et al, 2005) but increase potential for sorption of hydrophilic organic pollutants (G.Liu et al., 2018).

Microplastics could cause inflammation, obstruction and accumulation in organs (J. Wang et al., 2015). But this study has been conducted on marine organisms. The MPs remain intact inside the living organisms for a long time. Thus, the organisms get prolonged exposure to MPs, which may lead to chronic irritation, resulting in inflammation, cellular proliferation, and necrosis and may compromise immune cells (M. Smith et al., 2018).

Some studies conducted on organisms which live in marine environment concluded that microplastics migh affect metabolism influencing metabolic enzymes or indirectly by disrupting the energy balance. In humans MPs may have similar metabolic effects increasing or decreasing energy expenditure, lowering nutrient intake, and/or modulating metabolic enzymes. However, humans have high energy needs and complex metabolic activities compared to tested organisms (A. Rahman et al., 2020).

Other studies have reported that after exposure, MPs might translocate to distant tissues through the circulatory system causing a systemic inflammatory response, blood cell cytotoxicity through internalization, vascular inflammation, occlusions and pulmonary hypertension (A. Rahman et al., 2020).

A review by (J. Prata et al., 2018) has mentioned that chronic inflammation and irritation due to MPs intake might promote cancer due to DNA damage.

3.2.4 Potential hazards associated with monomers, additives and sorbed chemicals

Biodegradation and weathering of plastics could produce monomers starting from polymers. Some of them like acrylamide, 1,3-butadiene, ethylene oxide and vinyl chloride are considered hazardous. The risk associated with these monomers varies significantly, depending on factors, including the level and route of exposure. WHO Guidelines for Drinking-water Quality (WHO, 2017) established reference values for five substances (acrylamide, epichlorohydrin, 1,4-dichlorobenzene, styrene, and vinyl chloride) ranging from 0.3 (for vinyl chloride) to 300 µg/L (for 1,4-dichlorobenzene). These reference values generally represent concentrations in drinking-water that do not result in any significant health risk over a lifetime of consumption. (WHO, 2019)

Additives are added to plastics to confer specific properties. They could leach in the surrounding environment: low molecular weight molecules could migrate at a faster rate than larger additives.

The hydrophobic nature of microplastics implies that they have the potential to accumulate hydrophobic substances as persistent organic pollutants (POPs). Has been noticed that they are mainly present near urban areas. But in addition to accumulating in microplastics, POPs are sorbed by organic carbon which is in the environment. It could be for example in sediment, algae and in the lipid fraction of biological organisms. They would sorb a bigger fraction of POPs respect to microplastics. Therefore, the importance of microplastics in this sense could be negligible. Anyway, the potential for POPs to leach from microplastics will depend on a variety of factors like the size of the particle, mass of chemical accumulated, relative level of contamination within the gut, and the GI residence time of the particle. (WHO, 2019)

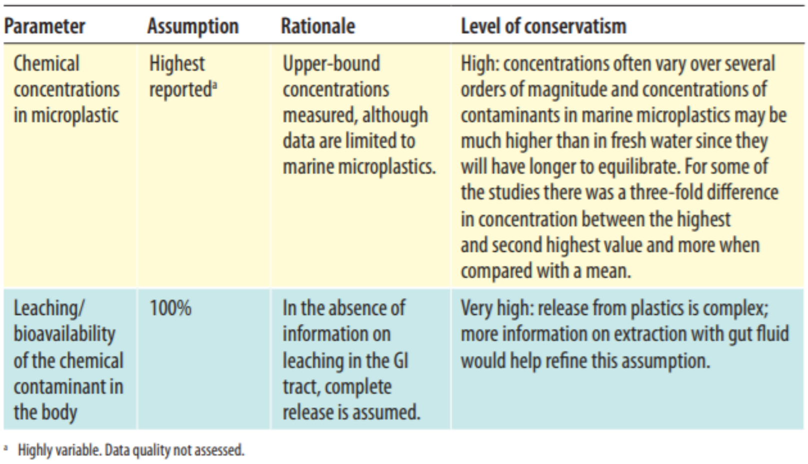
Currently we have little information to assess a potential risk associated with exposure to microplastics. However it is known that exposure to high levels of particles overwhelm biological mechanisms used to expel them.

Microplastic particles could contain monomers and additives, such as stabilizers and colourants, which may leach out. Regulations do not directly limit human consumption of microplastic particles but they rule the additives and monomer content that could be included. To estimate potential human health risk, the first step is to determine if very high exposures to these potential hazards pose a risk to consumers.

To calculate a hypothetical exposure in drinking water, due to limited data availability, is used an extremely conservative approach. If there is no apparent risk in an extreme exposure scenario there is no need to refine the assumptions. The estimated exposure in drinking-water could be compared to conservative levels at which adverse biological effects could be observed (the toxicological point of departure, or POD) to determine if there is a sufficiently large margin of exposure. This methodology is called screening level margin of exposure (MOE) approach. MOEs of at least 100 when based on animal data and 10 when based on human data are an indication for low health concern for effects with an apparent threshold. (WHO, 2019)

Chemicals have to be included in a risk assessment if: they have been detected in microplastics, are of toxicological concern and if have an acceptable POD to calculate a MOE. In the following table from WHO have been resumed the exposure assumptions to assess microplastics intake in drinking-water. (WHO, 2019)

Table 12 Exposure assumptions to assess microplastic intake in drinking-water, along with rationale (WHO,2019)



Parameter	Assumption	Rationale	Level of conservatism
Chemical concentrations in microplastic	Highest reported[a]	Upper-bound concentrations measured, although data are limited to marine microplastics.	High: concentrations often vary over several orders of magnitude and concentrations of contaminants in marine microplastics may be much higher than in fresh water since they will have longer to equilibrate. For some of the studies there was a three-fold difference in concentration between the highest and second highest value and more when compared with a mean.
Leaching/ bioavailability of the chemical contaminant in the body	100%	In the absence of information on leaching in the GI tract, complete release is assumed.	Very high: release from plastics is complex; more information on extraction with gut fluid would help refine this assumption.

[a] Highly variable. Data quality not assessed.

There is significant uncertainty related to exposure to smaller plastic particles.

It is possible that some small plastic particles might be able to pass through the gut wall and translocate to tissues remote from the mucosa, but this might not necessarily be a health risk. In addition is suggested that a big part of microplastics pass through the GI tract into the faeces. Therefore, it is not possible to draw any firm conclusions on toxicity related to microplastic exposure through drinking-water, particularly for the smallest particles. (WHO, 2019)

3.2.5 Possible human health risks associated with microplastics in drinking-water: biofilms

Biofilms in drinking-water are the result of the growth of microorganisms through pipes and other surfaces. In drinking-water distribution systems, biofilms could detach from the pipe walls into the water. Little is known about the presence of microplastic-associated biofilms in drinking-water and if there are any related possible human health risks. Microplastics and other materials provide a surface for biofilm-forming organisms. In particular MPs (>50 μm) could serve as a possible substrate on which biofilms may grow in aquatic environments (Y. Li et al., 2020). Their hydrophobicity and high surface area to volume ratio makes them favourable for the attached growth of microorganisms. These plastic-associated communities are sometimes referred to as “plastispheres”. Biofilm-forming organisms attach faster to hydrophobic nonpolar surfaces, such as plastics, than to hydrophilic surfaces, such as stainless steel. An increasing number of microbes that are capable of degrading MPs have been discovered, including fungi and bacteria. So plastic could provide energy for biofilms to grow on (Y. Li et al., 2020). Moreover, environmental conditions, including high nutrient concentrations (nitrogen and phosphorus), salinity, temperature, high UV radiation and oxygen content also influence microplastics-biofilm formation. Current evidence suggests that microplastics might be able to transport and disperse plastisphere communities over long distances. Microplastics may also serve as vectors for harmful organisms, including enteric viruses and protozoa because these organisms could accumulate in biofilms. A study conducted in nine rivers in Illinois, USA, found high presence of Pseudomonas spp., Burkholderiales incertae sedis, and Campylobacteraceae on microplastics in water (A.R. McCormick et al., 2016). It is unclear how long the pathogens will persist in transport by microplastics. Heavy metals and organic pollutants may also accumulate in biofilms on the surface of MPs (Y. Li et al., 2020).

Finally there isn’t currently evidence to suggest a human health risk from microplastic associated biofilms in drinking-water. (WHO, 2019)

3.3 RISK ASSESSMENT AND MANAGEMENT IN WATER SECTOR

During the last 60 years, there has been an acceleration of population growth, land-use changes, use of fertilizers, and increased demand for water. This has led to water quality degradation due to recalcitrant chemical contamination, increased eutrophication, hazardous algal blooms and fecal contamination associated with microbial hazards and antibiotic resistance. These environmental impacts are exacerbated by climate change and extreme precipitation events, which directly affect water quantity. Today has become more important than ever to implement risk-based and evidenced based approaches in order to effectively and efficiently mitigate the impacts of water contamination.

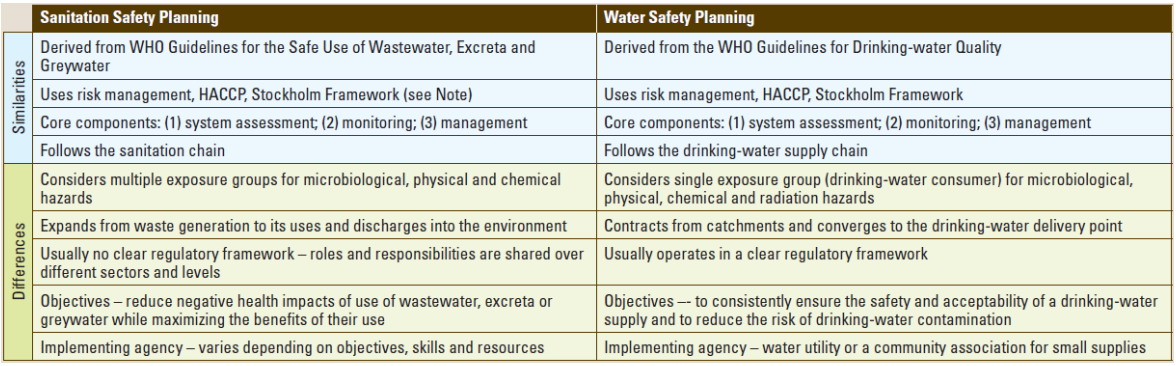
Formal risk assessment as a process has been designed to bring data and facts together to evaluate the hazards and to provide this information to stakeholders and decision makers for policy purposes. It is often used to examine quantitative probabilities of risk. (Global water pathogen project, s.d.)

"Risk (R) is a function of the probability (P) of an adverse health effect and the severity (S) of that effect, consequential to a hazard". (Seis, 2012) So:

$$R(P, S) = P * S$$

Every risk analysis is formed by: risk assessment, risk management, and risk communication. Risk assessment evaluate risks deriving from a specific hazard; it has to be written and published in a clear way (risk communication); the purpose of the last one is to plan and monitor risk reduction measures. A part of it is to decide if additional risk reduction measures are necessary. So in risk assessment, risks have to be quantified and compared to a level of risk which is acceptable or tolerable.

Well established examples in the water sector are Sanitation Safety Planning (SSP) and Water Safety Plan (WSP) providing a structure for risk assessment and management in the stepwise process.



	Sanitation Safety Planning	Water Safety Planning
Similarities	Derived from WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater	Derived from the WHO Guidelines for Drinking-water Quality
	Uses risk management, HACCP, Stockholm Framework (see Note)	Uses risk management, HACCP, Stockholm Framework
	Core components: (1) system assessment; (2) monitoring; (3) management	Core components: (1) system assessment; (2) monitoring; (3) management
	Follows the sanitation chain	Follows the drinking-water supply chain
Differences	Considers multiple exposure groups for microbiological, physical and chemical hazards	Considers single exposure group (drinking-water consumer) for microbiological, physical, chemical and radiation hazards
	Expands from waste generation to its uses and discharges into the environment	Contracts from catchments and converges to the drinking-water delivery point
	Usually no clear regulatory framework – roles and responsibilities are shared over different sectors and levels	Usually operates in a clear regulatory framework
	Objectives – reduce negative health impacts of use of wastewater, excreta or greywater while maximizing the benefits of their use	Objectives –- to consistently ensure the safety and acceptability of a drinking-water supply and to reduce the risk of drinking-water contamination
	Implementing agency – varies depending on objectives, skills and resources	Implementing agency – water utility or a community association for small supplies

Figure 8 Similarities and differences between WSP and SSP

Both SSP and WSP are derived from WHO Guidelines and are mainly composed by three parts: system assessment, monitoring and management. But SSP considers groups of multiple exposure microbiological, physical and chemical hazards while WSF considers single exposure group for the three types of hazard. The SSP is focused on waste from its generation to its disposal to reduce negative health impact. WSP is focused on drinking water from catchment to delivery point to ensure its safety and reduce contamination risk. Moreover WSP offers a clear regulatory framework.

3.3.1 Water Safety Plan Manual – Step-by-step risk management for drinking-water suppliers, WHO 2009

The Water Safety Plan scope is to ensure the safety and acceptability of a drinking water supply but it has to be implemented and revised continuously. It is structured in 11 modules. The first part is the "*Preparation*" and it is formed by:

- *the Module 1: Assemble the WSP team.*

This step consists in assembling a team of individuals from the utility, and also from a wider group of stakeholders, with the collective responsibility for understanding the water supply system and identifying hazards that can affect water quality and safety throughout the water supply chain.

The second part is the "*System assessment"* and it is formed by:

- *Module 2*: *Describe the water supply system*

  A detailed description of the water supply system is required to support the subsequent risk assessment process consisting in a validated flow diagram with a clear identification of the users and uses of water.

- *Module 3*: *Identify hazards and hazardous events and assess the risks*

For each step of the validated process flow diagram, the WSP team is required to assess what could go wrong at what point in the water supply system in terms of biological, physical and chemical hazards and hazardous events. So they are required to identify the hazards supported by historic information and events. The risk associated with each hazard may be described by identifying the likelihood of occurrence and evaluating the severity of consequences if the hazard occurred.

- *Module 4: Determine and validate control measures, reassess, and prioritize the risks*

  Existing control measures should be determined for each of the identified hazards and hazardous events. Missing controls need to be documented and addressed. Control measures ("barriers") have to ensure that the water meets water quality targets. They are activities and processes applied to reduce or mitigate risks.

  The team should consider whether the existing controls are effective (validation phase). The risks should then be recalculated in terms of likelihood and consequence, taking into account all existing control measures.

- *Module 5: Develop, implement, and maintain an improvement/upgrade plan*

  When the existing controls are not effective or absent, an improvement/upgrade plan should be drawn up. It can include short-, medium- or long-term programmes. Its implementation should be monitored.

Third part is the "*Operational monitoring"* and includes:

- *Module 6*: *Define monitoring of the control measures*

  WSP should define the assessment of the performance of control measures at appropriate time intervals and establish corrective actions for deviations that may occur.

- *Module 7*: *Verify the effectiveness of the WSP*

  Verification involves compliance monitoring, internal and external auditing of operational activities and consumer satisfaction. The outputs of this module should be the confirmation that the WSP is appropriate and It's working effectively. At the same time, it should confirm that water quality meets defined targets.

Fourth part is "*Management and communication*":

- *Module 8*: *Prepare management procedures*
  The WSP team should draw up: the management procedures for normal and incident/emergency conditions; the operational monitoring, and responsibilities of the utility and other stakeholders; communication protocols and strategies; a plan to alert and inform users of the supply and other stakeholders should be established and a programme with how and when to review and revise the documentation.

- *Module 9: Develop supporting programmes*
  Supporting programmes are activities that support the development of people's skills and knowledge, commitment to the WSP approach, and the capacity to manage systems to deliver safe water.

Last part is the "*Feedback*":

- *Module 10*: *Plan and carry out a periodic review of the WSP*
  The WSP team should periodically meet and review the overall plan and learn from experiences and new procedures.

- *Module 11*: *Revise the WSP following an incident*
  This step should include a comprehensive and transparent review of why the incident occurred and the adequacy of the utility's response.

3.3.2 Sanitation Safety Planning – manual for safe use and disposal of wastewater, greywater and excreta, WHO 2016

SSP process follows six main steps, called modules.

- Module 1: *"Prepare for SSP"*

The first module consists of the preparation phase to establish priorities and objectives, define the components to include in the plan and set up the working group.

- Module 2: *"Describe the sanitation system"*

SSP requires a detailed description of the sanitation system, to identify vulnerable points and evaluate performance requirements.

- flow diagrams are used to schematize the system and available quantitative information on flows and waste streams are collected;
- Characterization of the waste fractions;
- Identification of potential exposure groups;
- Collection of data on demographics, land use patterns, quality standards, certifications or requirements, information about system management and performances, variabilities, existing epidemiological and environmental data.
- Validation of the system description.

- Module 3: "*Identify hazards, assessing existing controls and assess exposure risks*"

The third module consists of the identification of hazardous events, risks evaluation and their prioritization, considering the effectiveness of the existing control measures.

- Module 4: "*Develop and implement an incremental improvement plan*"

Aim of the fourth module is to provide flexible solutions to improve the protection of all exposure groups along the sanitation chain and define the priority between selected control measure according to financial and resource limitations.

- Module 5: *"Monitor control measures and verify performance"*

It develops a monitoring plan that regularly checks that system is operating as intended and defines what to do if it so not. The outputs developed in this module generate system-specific evidence to justify existing operations or the need for ongoing improvements.

- Module 6: "*Develop supporting programmes and review plans*"

It's aim is to support the development of people's skills and knowledge, and an organization's ability and capacity to meet SSP commitments.

- Identify and implement supporting programmes and management procedures;
- Periodically review and update the SSP outputs.

Figure 9 Sanitation Safety plan modules, by WHO,2015

3.3.3 Regulation (EU) 2020/741 of the European Parliament and of the council of 25 May 2020 on minimum requirements for water reuse

The European Regulation 2020/741 establishes minimum requirements for water quality and monitoring and provisions on risk management, for the safe use of reclaimed water in the context of integrated water management.

Quality requirements for the reuse of water are specified in ANNEX I Synthesis of literature on MPs. Water quality classes are defined depending on crop category to be irrigated and the irrigation technique.

Table 13 Classes of reclaimed water quality and permitted agricultural use and irrigation method

Minimum reclaimed water quality class	**Crop category***	**Irrigation method**
A	All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	All irrigation methods
B	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	All irrigation methods
C	Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	Drip irrigation** or other irrigation method that avoids direct contact with the edible part of the crop
D	Industrial, energy, and seeded crops	All irrigation methods**

(*) If the same type of irrigated crop falls under multiple categories of Table 1, the requirements of the most stringent category shall apply.

(**) Drip irrigation (also called trickle irrigation) is a micro-irrigation system capable of delivering water drops or tiny streams to the plants and involves dripping water onto the soil or directly under its surface at very low rates (2-20 litres/hour) from a system of small-diameter plastic pipes fitted with outlets called emitters or drippers.

(***) In the case of irrigation methods which imitate rain, special attention should be paid to the protection of the health of workers or bystanders. For this purpose, appropriate preventive measures shall be applied.

In Table 14 have been summarized the water quality requirements for agricultural irrigation to be respected by reclaimed water and the relative belonging class.

Table 14 Reclaimed water quality requirements for agricultural irrigation

Reclaimed water quality class	Indicative technology target	Quality requirements				
		E. coli (number/ 100 ml)	BOD$_5$ (mg/l)	TSS (mg/l)	Turbidity (NTU)	Other
A	Secondary treatment, filtration, and disinfection	≤10	≤10	≤10	≤5	*Legionella* spp.: <1 000 cfu/l where there is a risk of aerosolization

B	Secondary treatment, and disinfection	≤100	In accordance with Directive 91/271/EEC (Annex I, Table 1)	In accordance with Directive 91/271/EEC (Annex I, Table 1)	-	Intestinal nematodes (helminth eggs): ≤1 egg/l for irrigation of pastures or forage
C	Secondary treatment, and disinfection	≤1 000			-	
D	Secondary treatment, and disinfection	≤10 000			-	

Table 15 Minimum frequencies for routine monitoring of reclaimed water for agricultural irrigation

Reclaimed water quality class	*E. coli*	BOD5	TSS	Turbidity	*Legionella* spp. (when applicable)	Intestinal nematodes (when applicable)
A	Once a week	Once a week	Once a week	Continuous	Twice a month	Twice a month or as determined by the reclamation facility operator according to the number of eggs in wastewater entering the reclamation facility
B	Once a week	In accordance with Directive 91/271/EEC (Annex I, Section D)	In accordance with Directive 91/271/EEC (Annex I, Section D)	-		
C	Twice a month			-		
D	Twice a month			-		

The indicator microorganisms selected are *E. coli* for pathogenic bacteria, F-specific coliphages, somatic coliphages or coliphages for pathogenic viruses, and *Clostridium perfringens* spores or spore-forming sulfate-reducing bacteria for protozoa. At least 90 % of validation samples shall reach or exceed the performance targets.

Table 16 Validation monitoring of reclaimed water for agricultural irrigation

Reclaimed water quality class	Indicator microorganisms(*)	Performance targets for the treatment chain ($\log_{10}$ reduction)
A	*E. coli*	≥ 5,0
	Total coliphages/F-specific coliphages/somatic coliphages/coliphages(**)	≥ 6,0
	Clostridium perfringens spores/spore-forming sulfate-reducing bacteria(***)	≥ 4,0 (in case of *Clostridium perfringens* spores)

		≥ 5,0 (in case of spore-forming sulfate-reducing bacteria)

(*) The reference pathogens *Campylobacter*, Rotavirus and *Cryptosporidium* may also be used for validation monitoring purposes instead of the proposed indicator microorganisms. The following log10 reduction performance targets shall then apply: *Campylobacter* (≥ 5,0), Rotavirus (≥ 6,0) and *Cryptosporidium* (≥ 5,0).

(**) Total coliphages is selected as the most appropriate viral indicator. However, if analysis of total coliphages is not feasible, at least one of them (F-specific or somatic coliphages) shall be analysed.

(***) *Clostridium perfringens* spores is selected as the most appropriate protozoa indicator. However, spore-forming sulfate-reducing bacteria are an alternative if the concentration of *Clostridium perfringens* spores does not make it possible to validate the requested log10 removal.

In Annex II is specified that according to Article 5, the competent authority shall ensure that a water reuse risk management plan is established for water reuse supply and use. The plan shall be prepared by the reclamation facility operator, together with other responsible parties and end-users. Risks should be identified and managed in a proactive way in order to ensure a safe use of reclaimed water and minimize the risks to the environment and to human or animal health.

In particular, the WRRMP shall be based on all the key elements of risk management set out in Annex II. They can be summarized in:

1. Detailed and critical description of the entire water reuse system, from the source of wastewater to the irrigation method and crop type.
2. Identification of all parties involved in the water reuse system, focusing on roles and responsibilities.
3. Identification of potential hazards and hazardous events (such as treatment failures, accidental leakages or contamination of the water reuse system).
4. Identification of the environment and populations at risk and the relative exposure routes, also considering specific environmental factors such as hydrogeology, topology, farming and irrigation practices.
5. Assessment of risks to the environment and human health including confirmation of the nature of the hazards, the potential range of exposure or dose and the characterisation of the risks.
6. Identification of preventive measures that are already in place or that should be taken to minimize risks. They can include, for instance:
    a. access control;

    b. additional disinfection or pollutant removal measures;
    c. specific irrigation technology mitigating the risk of aerosol formation (e.g. drip irrigation);
    d. specific requirements for sprinkler irrigation (e.g. maximum wind speed, distances between the sprinkler and sensitive areas);
    e. specific requirements for agricultural fields (e.g. slope inclination, field water saturation and karstic areas);
    f. pathogen die-off support before harvest;
    g. establishment of minimum safety distances (e.g. from surface water, including sources for livestock, or activities such as aquaculture, fish farming, shellfish aquaculture, swimming and other aquatic activities);
    h. signage at irrigation sites, indicating that reclaimed water is being used and is not suitable for drinking

7. Establishment of adequate quality control systems and procedures as well as adequate maintenance programmes for equipment.
8. Definition of an environmental monitoring system.
9. Definition of an appropriate procedures and protocols to manage incidents and emergencies.

Risk assessment may be carried out at different levels of detail and complexity, depending on the specific objectives and data availability. A qualitative or semi-quantitative approach can be used. The quantitative risk assessment shall be applied when there are sufficient supporting data or in projects having a potentially high risk for the environment or public health.

Furthermore, depending on the outcome of the risk assessment, additional requirements shall be required, as specified in Annex I. They may concern heavy metals, pesticides, disinfection by-products, pharmaceuticals and other substances of emerging concern, including micropollutants and microplastics, and anti-microbial resistance. In any case, risk management should consider also other aspects such as pollution from nitrates, protected areas, environmental objective and quality standards, groundwater and soil protection, hygiene measures, maximum levels of contaminants and pesticides and animal health.

Table 17 shows the specific preventive measures set out in Annex II. More in general, preventive measures also include quality control systems and procedures, monitoring the reclaimed water for relevant parameters, maintenance programmes for equipment, environmental monitoring systems, procedures to manage incidents and emergencies, to inform all relevant parties of such events and regular update of the emergency response plan.

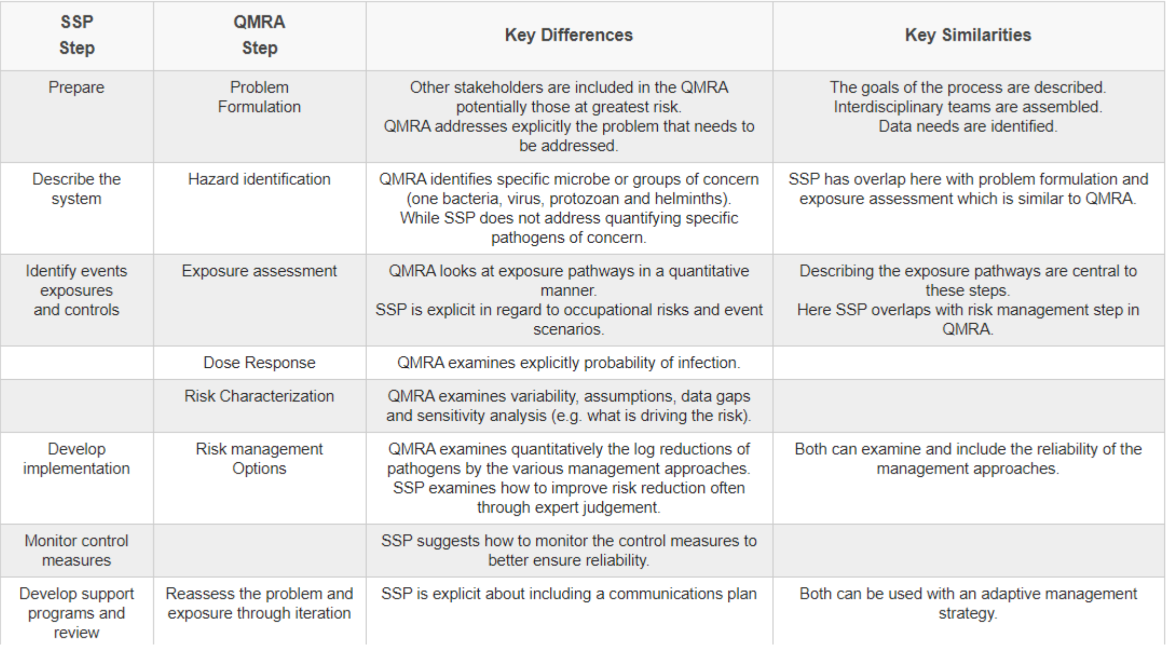
Table 17 Specific preventive measures

Reclaimed water quality class	**Specific preventive measures**
A	• Pigs must not be exposed to fodder irrigated with reclaimed water unless there is sufficient data to indicate that the risks for a specific case can be managed.
B	• Prohibition of harvesting of wet irrigated or dropped produce. • Exclude lactating dairy cattle from pasture until the pasture is dry. • Fodder has to be dried or ensiled before packaging. • Pigs must not be exposed to fodder irrigated with reclaimed water unless there is sufficient data to indicate that the risks for a specific case can be managed.
C	• Prohibition of harvesting of wet irrigated or dropped produce. • Exclude grazing animals from pasture for five days after last irrigation. • Fodder has to be dried or ensiled before packaging. • Pigs must not be exposed to fodder irrigated with reclaimed water unless there is sufficient data to indicate that the risks for a specific case can be managed.
D	• Prohibition of harvesting of wet irrigated or dropped produce.

3.4 GENERIC METHODOLOGY OF QMRA

QMRA is a mathematical quantitative approach for estimating risk caused by pathogens to human health. (V. Zhiteneva et al, 2020). QMRA methodology could be used to support water safety management decisions on a utility or regulatory level analyzing scientific quantitative data. The numerical output of QMRA could be compared with national level targets to assess the risk management question. World Health Organization (WHO) published detailed guidelines for conducting QMRAs (WHO, 2016). A detailed description of procedure and data availability has been also conducted in Australian National guidelines for water recycling (Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council & the Australian Health Ministers, 2006). This document has been considered despite it refers to Australian situation and parameters because of many lacking data in European legislation and current studies.

The SSP and QMRA are quite compatible and synergistic despite some of the differences. The SSP's manual is a step by step process to address options for improving sanitation. Some of the limitations of the SSP are that it treats all microbes as they have same risks and concentrations, and quantitative approaches are only briefly mentioned. SSP also includes occupational risks and communication plans. QMRA is more data intensive and is a quantitative approach. However, the health outcomes could be used as support for political decision making.



SSP Step	QMRA Step	Key Differences	Key Similarities
Prepare	Problem Formulation	Other stakeholders are included in the QMRA potentially those at greatest risk. QMRA addresses explicitly the problem that needs to be addressed.	The goals of the process are described. Interdisciplinary teams are assembled. Data needs are identified.
Describe the system	Hazard identification	QMRA identifies specific microbe or groups of concern (one bacteria, virus, protozoan and helminths). While SSP does not address quantifying specific pathogens of concern.	SSP has overlap here with problem formulation and exposure assessment which is similar to QMRA.
Identify events exposures and controls	Exposure assessment	QMRA looks at exposure pathways in a quantitative manner. SSP is explicit in regard to occupational risks and event scenarios.	Describing the exposure pathways are central to these steps. Here SSP overlaps with risk management step in QMRA.
	Dose Response	QMRA examines explicitly probability of infection.	
	Risk Characterization	QMRA examines variability, assumptions, data gaps and sensitivity analysis (e.g. what is driving the risk).	
Develop implementation	Risk management Options	QMRA examines quantitatively the log reductions of pathogens by the various management approaches. SSP examines how to improve risk reduction often through expert judgement.	Both can examine and include the reliability of the management approaches.
Monitor control measures		SSP suggests how to monitor the control measures to better ensure reliability.	
Develop support programs and review	Reassess the problem and exposure through iteration	SSP is explicit about including a communications plan	Both can be used with an adaptive management strategy.

Figure 10 Comparison of SSP and QMRA frameworks and approaches, by Global Water Pathogen project

QMRA is a formal risk assessment process where each component of the assessment is explicitly quantified. (WHO, 2016) These steps are:

1. Problem formulation and hazard identification
2. Exposure assessment
3. Dose-response
4. Health effects assessment
5. Risk characterization

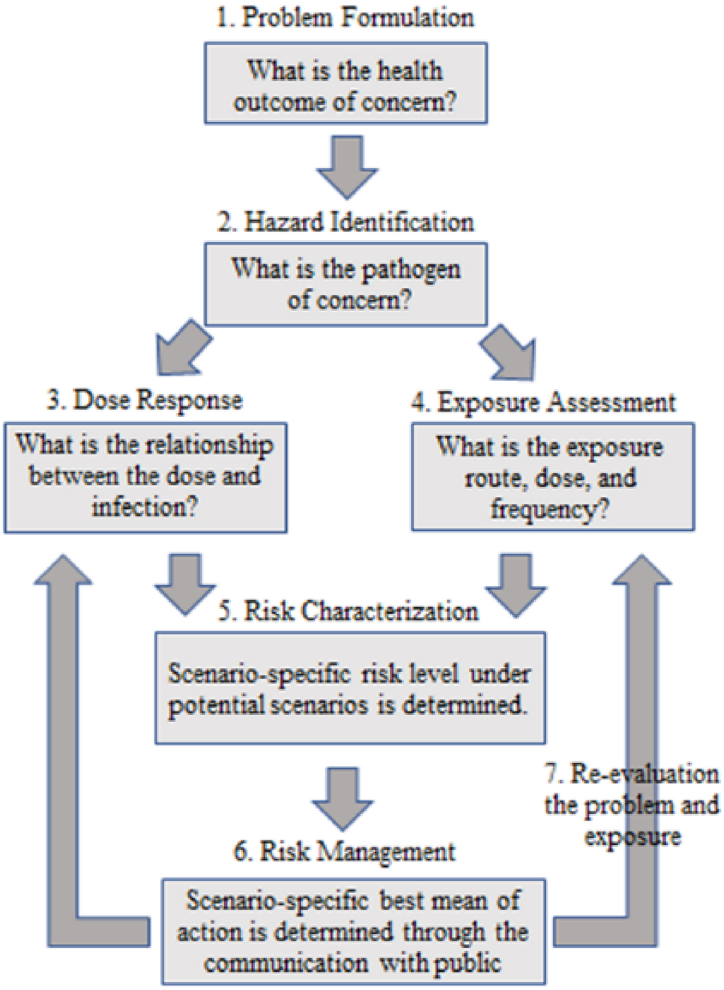


Figure 11 QMRA framework, by Global water pathogen project

3.4.1 Problem formulation and hazard identification

The scope and purpose of the risk assessment are defined usually by a team. Questions to be addressed concerns:

- the risk management decision required;
- the level of detail;
- which hazards (pathogens) and health outcomes should be considered
- which exposure pathways and hazardous events should be included.

The conclusion is to have a detailed description of the mechanisms and the cause of the actual adverse effect. Depending on the scope of the risk assessment different levels of sophistication of QMRA can be applied.

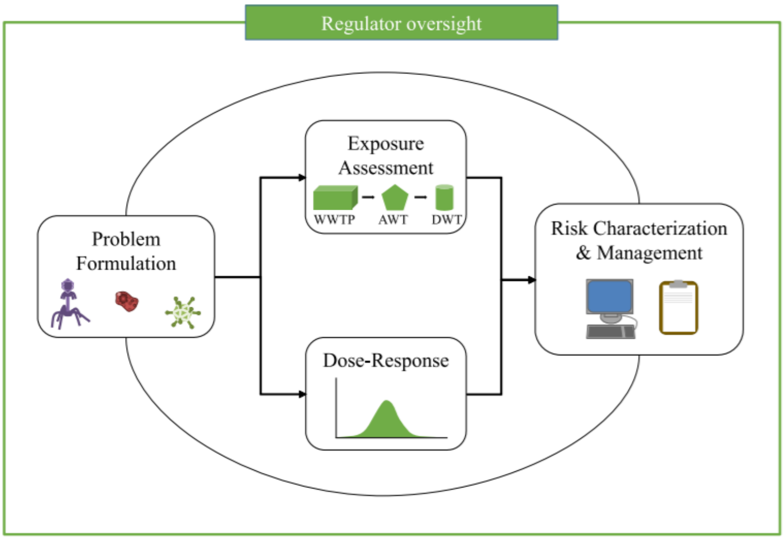


Figure 12 QMRA framework, where regulator oversight informs updates and additions to the process. (WHO guidelines, 2016)

It is impractical to identify health-based targets for all microorganisms, particularly since this would require information on concentrations present in source waters, dose responses and disease burdens that is often not available. A more practical approach is to identify reference pathogens for which this type of information is available. Reference pathogens representing each of the major groups of organisms (i.e. bacteria, viruses, protozoa and helminths) is required, due to variations in characteristics, behaviours and susceptibilities of each group to treatment processes. Rotaviruses are a good candidate for risk assessment because they pose a major threat of viral gastroenteritis worldwide, they have a relatively high infectivity compared with other waterborne viruses and a dose–response model has been established (A.H. Havelaar et J.M. Melse, 2003). Cryptosporidium parvum is a good candidate for a reference organism for protozoa, because it is reasonably infective is resistant to chlorination and is one of the most important waterborne human pathogens in developed countries (Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council & the Australian Health Ministers, 2006) . Campylobacter is by the most common cause of bacterial gastroenteritis in Australia therefore has been selected as the bacterial reference pathogen. (National Guidelines for Water Recycling:Managing Health and Environmental Risks , 2006)

3.4.2 Exposure assessment

The aim of this step is to determine the frequency and magnitude of exposure to pathogens via the pathways and hazardous events defined during the problem formulation. In this step quantitative information are needed: pathogen concentrations in water sources and fate of pathogens in barriers. Is important to determine dose-response relations.

3.4.3 Dose-response

Dose-response relations of pathogens consist in a mathematical functional relationship between the number of pathogens to which someone is exposed to and the probability of the specific adverse effect (between 0 and 1). A fraction of the infected people may develop different health outcomes. The simplest dose-response relation is an exponential relationship:

$$P_{inf} = 1 - e^{-r*d}$$

P_{inf} =probability of infection

r= infectivity constant

d= dose

The exponential model assumes that the probability of infection is constant for all pathogens of the same kind. (C.N. Haas et al, 1999). In reality not all pathogens of the same species are equally infective and not all human have the same health outcomes. To consider that other relationship are used like usually the BetaPoisson-model which can be approximated to:

$$P_{inf} = 1 - (1 - \frac{d}{\beta})^{-\alpha}$$

Or:

$$P_{inf} = 1 - (1 + \frac{d}{N50} * (2^{\frac{1}{\alpha}} - 1)^{-\alpha}$$

N50,β,α =model parameters

3.4.4 Health effects assessment

In this step, the health impact data for the identified hazards and the specific study population are compiled. This includes the type of health effects, the severity and duration of a disease or illness that might occur after ingestion of the pathogen and available information on the relationship between ingested dose and the probability that health effects occur (dose–response relationship). Also, the fraction and vulnerability of the population exposed might need to be considered.

3.4.5 Risk characterization

The final step of the risk assessment combines the information from the previous steps to estimate the likelihood of an adverse consequence. (Haas et al., 2014) The health impact data for the identified hazards and the specific study population have to be reported. They include: the type of health effects, the severity and duration of a disease or illness that may occur after ingestion of the pathogen and the relationship between ingested dose and the probability that health effects (infection, illness, sequelae) occur. (WHO, 2016)

Point estimates means that one value is chosen to represent each variable and the risk is calculated. Mean values of variables are chosen to calculate the average risk while extreme values, such as the 99-percentile, could give an idea of the worst-case situation. Such an approach does not give a comprehensive picture nor appropriate weight of all combinations. Stochastic modelling could be used to have a more realistic representation of the distribution of data. Monte Carlo methods is used to obtain the output risk distribution using random samples of each distribution. In Figure 13 is represented the logical sequence of events when calculating the risks of infection, for example from Cryptosporidium in drinking water. Arrows indicate random sampling from distributions with Monte Carlo methods. The distributions could be presented either as probability density curves, as shown for the exposure, or as cumulative density curves, as shown for the risk of infection. (Westrell, 2004)

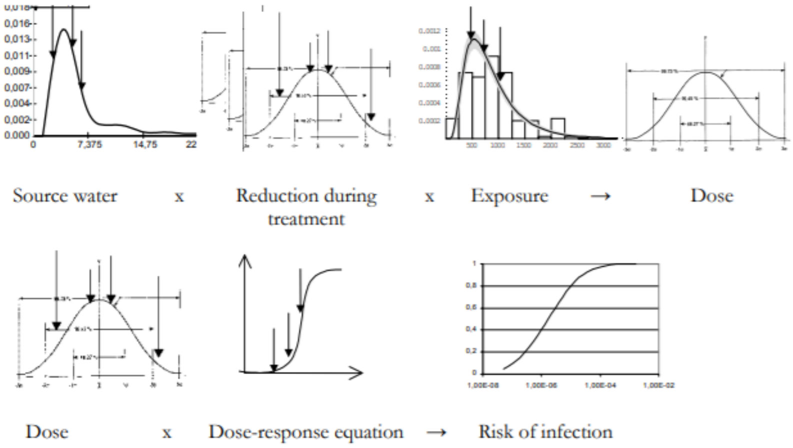


Figure 13 Schematic picture on the logical sequence of events when calculating the risk of infection using Monte Carlo, by T. Westrell 2004

3.4.6 Monte Carlo stochastic modelling

Monte Carlo simulations are used to model the probability of different outcomes in a process that cannot easily be predicted due to the intervention of random variables. It is a technique used to understand the impact of risk and uncertainty in prediction and forecasting models. When each model input is described by a probability distribution, quantifying the exact mathematical distribution for the risk output, like probability of infection, could be complicated and not reliable if the simulation is done assuming point parameters. Monte Carlo simulation is a random sampling technique that allows the outputs of the risk model to be quantified by sampling. Random samples are selected from each input variable distribution, and the risk output is quantified. The process is repeated thousands of times to obtain a random sample of the risk output. The frequency distribution of output samples is assumed to represent the probability distribution of risk. (WHO, 2016) In Figure 14 could be seen how in each step of QMRA input data and final output are represented by distributions of values.

Monte Carlo Simulations (MCS) is a sophisticated probabilistic modeling technique. (Seis, 2012) The name Monte Carlo Simulation refers to the extensive use of random variables. The whole distribution of the respective model parameter is used for calculating risk: a full risk distribution is obtained. Another important characteristic of this method is that uncertainties and variability are taken into account. In Monte Carlo simulation, uncertain inputs in a model are represented using ranges of possible values known as probability distributions. By using probability distributions, variables could have different probabilities of different outcomes occurring and they could better represent uncertainty in variables of a risk analysis. Depending upon the number of uncertainties and the ranges specified for them, a Monte Carlo simulation could involve thousands or tens of thousands of recalculations before it is complete. During a Monte Carlo simulation, values are sampled at random from the input probability distributions. Each set of samples is called iteration, and the resulting outcome from that sample is recorded. Monte Carlo simulation does these hundreds or thousands of times, and the result is a probability distribution of possible outcomes.

Therefore a probability distribution could be used to describe how a model input will vary: the range of expected values, and the probability that the input is equal to or less than those values. The probability distribution goes beyond simple descriptors (mean, median or upper 95th percentile) and characterizes the entire distribution. The cumulative distribution function (CDF) or probability distribution function (PDF) is the mathematical equation that describes the probability that a variable X is less than or equal to x.

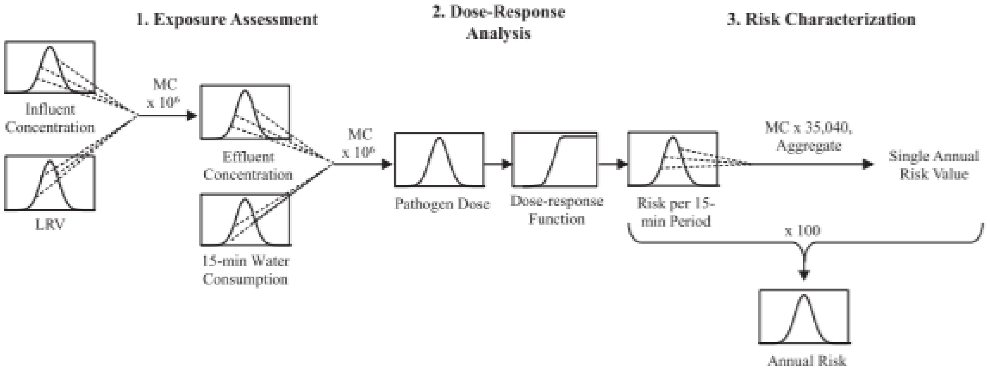


Figure 14 Example of data distribution in each step of a Monte Carlo simulation, by M.Pecson et al.,2017

3.5 HEALTH TARGETS (DALY, RISK OF INFECTION)

Calculated risks could be compared against health targets. Two different types of targets could be used from two different approaches: from WHO guidelines and from U.S. EPA. The United States Environmental Protection Agency stated that the calculated risks have to be compared with a tolerable level of risk which is less than 1 infection × 10^{-4} per year (less than one infection per 10.000 people per year), this is the target used for carcinogens in drinking water. In WHO Guidelines, the tolerable level of risk is defined as $<10^{-6}$ disability adjusted life years (DALYs) per person per year (pppy). Compared with the USEPA approach, this method requires the input of information on infection–illness ratios and on the impact or burden of illness.

DALYs in general measure the time lost because of disability or death from a disease caused by a pathogen compared with a life free of disability in the absence of the disease. DALYs are calculated by adding the years of life lost to premature death (YLL) to the years lived with a disability (YLD).

$$DALY = YLL + YLD$$

Years of life lost are calculated from age-specific mortality rates and the standard life expectancies of a given population while YLD are calculated from the number of cases multiplied by the average duration of the disease and a severity factor ranging from 1 (death) to 0 (perfect health) based on the disease. (D.Mara, 2008). So DALYs permit to compare different types of health outcomes both acute and chronic due to different pathogens. The tolerable additional disease of 10^{-6} DALY loss pppy adopted in the Guidelines means that a city of one million people collectively suffers the loss of one DALY per year.

4 MATERIALS AND METHODS

4.1 MICROPLASTICS

4.1.1 Drinking water plant description

4.1.1.1 Description of the drinking water network

The considered drinking water supply chain is managed by the company "Acquambiente Marche srl", which operates as manager of the integrated water service in the municipalities of:

- Cingoli
- Filottrano
- Numana
- Sirolo

Furthermore, being able to dispose of an important quantity of water supplied and to promote a correct and functional use of the captured water resource, the Company exports part of the water entering the aqueduct system to other municipalities within the ATO 3 (Osimo, Castelfidardo) and the A.T.O. 2 (Camerano). In the Figure 15 the municipalities directly served are shown in red and those which use part or all of the water sold by it in blue.

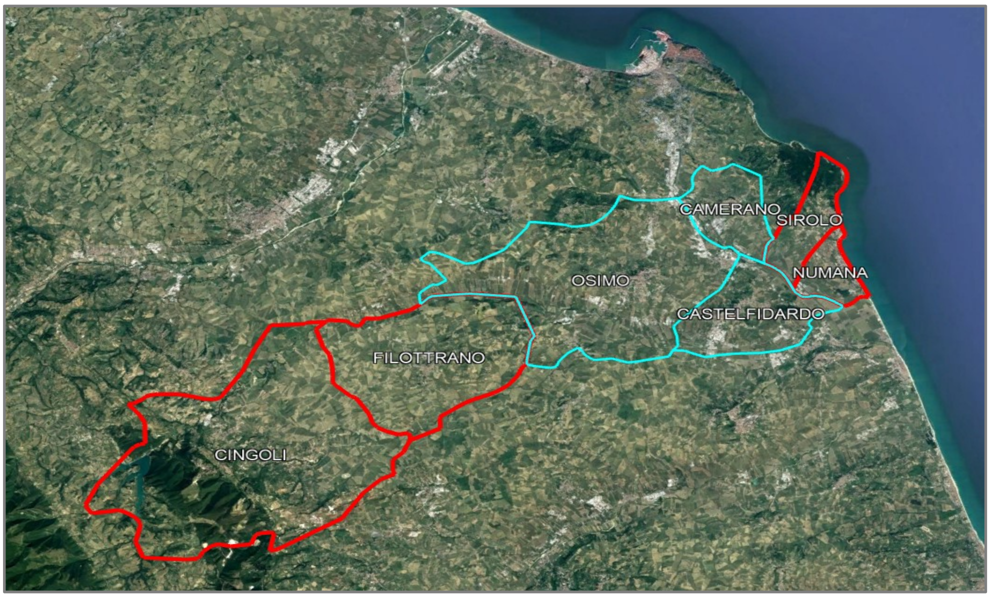


Figure 15 Municipalities served

In relation to the territory served is very relevant the seasonal consumption because in summer the need for drinking water supply could also double due to tourist inflows in coastal areas served, therefore it is necessary for the Company to have a flexible network and to adapt it to seasonal fluctuations.
The receivers of the water managed by Acquambiente Marche are mainly residential user; to a lesser extent water is supplied for industrial purposes.
The water network consists of a single main supply line, which finds its fundamental apex in the artificial reservoir of Castreccioni (maximum capacity of reservoir 55 Mil. mc), built close to Monte San Vicino.

At a distance of about 1.5 km from the reservoir, the water captured by the intake structure is conveyed to a water treatment plant (maximum capacity of 500 l / s), also located in Castreccioni in Cingoli, inside of which the raw waters are treated in order to achieve chemical and microbiological characteristics in compliance with the law. This plant represents the real heart of the supply network, as on the one hand the set of treatments guarantees the quality of the water supplied to the users, on the other it allows the control of the volumes introduced into the network, depending on the actual request of the users.

The drinking water supply of the municipalities managed takes place mainly by gravity, through a steel pipeline (called "Castreccioni") 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 makes use of a series of tanks located even peripherally in the area, in order to guarantee the provision of the service even to the small fractions of the aforementioned municipalities. The tanks represent the junction point between the supply network referred to in the "Castreccioni" pipeline and the distribution network of the resource to the end users in order to guarantee the flexibility of distribution in case of maintenance / breakage of the pipes.

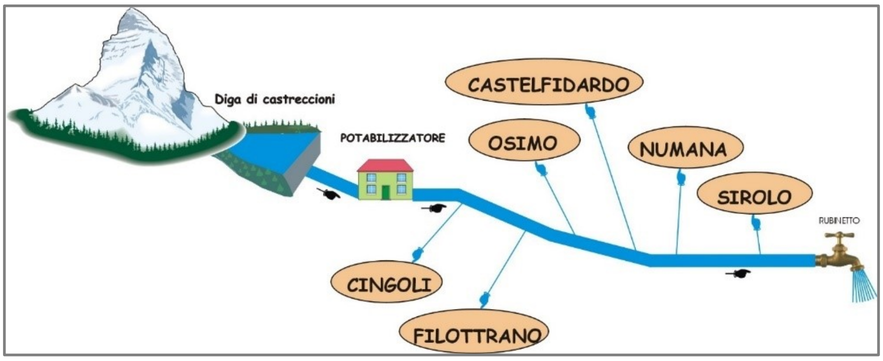


Figure 16 Drinking water network

Distribution pipelines are characterized by the presence of sampling points, in which the water samples and the consequent periodic analysis are carried out. Finally, along the network there is the presence of lifting systems to provide the water resource with the hydraulic load necessary to supply users with adequate pressure.

4.1.1.2 Description of the supply water body: Castreccioni Lake

The Castreccioni reservoir, called "Lake Castreccioni" with a total surface of about 2.4 Km^2, is located mainly in the municipality of Cingoli (MC), in the Castreccioni area. The lake was created due to the construction of a dam on the Musone river at the Petrella bridge (Cingoli). Today it represents the largest artificial basin in the Marche.

Figure 17 Castreccioni reservoir

The purpose of the construction of the dam, 67 meters high and about 280 long, was to respond to various needs, including the regulation of the floods of the Musone river, irrigation use and drinking water. The volume that could be invaded by the lake at maximum altitude is 50 million cubic meters. The intake work from the water intended to be treated in the company water purifier consists of three holes on the reinforced concrete structure, placed at different heights, regulated by gates (normally closed), which are opened to bring together the flow rates necessary for the plant of potabilization. The intake structure has the following coordinates in the WGS84 reference system: Latitude: 43.383355 °; Longitude 13.161841 °; H = 314 ÷ 334 m asl

Figure 18 Irrigation pipeline of larger diameter

Figure 19 Intake work of the dam

The presence of three withdrawal quotas is due to the need to capture the raw water which periodically presents the best quality characteristics: based on the weather, environmental and microbiological conditions, there may be variations in the quality characteristics of the raw water arriving at the plant. The three height are: 334 a.s.l., 324 a.s.l. and 314 a.s.l..

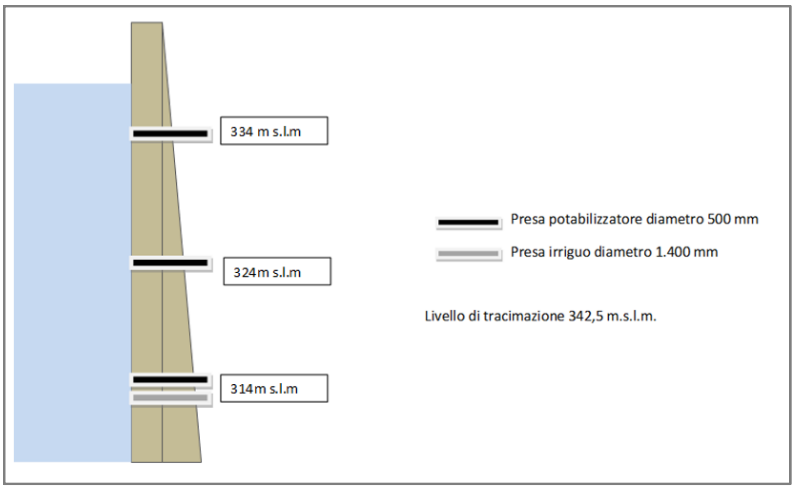


Figure 20 Withdrawal heights

Water of Castreccioni Lake has been classified as Category A2 (Water Protection Plan of the Marche Region, on a 2004 basis), ie it has to be subjected to normal physical and chemical treatment and disinfection. Castreccioni Lake has been the only surface water body to have been classified in A2 category while all the others present in the region have been classified as category A3 (they have to be treated with physical and chemical processes, refining and disinfection). Obviously, the quality of the raw water is linked both to the presence of atmospheric agents (rain, temperature, etc.), and to the presence of upstream drains, and to additional site-specific phenomena that may occur in particular conditions. Over the years, the development of analytical techniques, together with the increase in the selectivity and sensitivity of the instrumentation and the refinement of the methods, has made it possible to characterize the water bodies also in relation to previously undetectable substances, due to the low presence in the environment. At the same time, an increase in anthropogenic pressures on water bodies has been developed and so increasing attention must be paid to emerging contaminants, for the detection and mitigation of the related health effects. At the time of construction of the treatment plant for drinking water, this could have been considered "oversized" as it carried out treatments beyond what is strictly necessary by law; in recent years the water quality of the lake has been downgraded to A3, especially due to the problem linked to the presence of the so-called "red alga" (Plankthotrix Rubescens). The P. Rubescens algae can produce numerous types of toxins (microcystins) with hepatotoxic, gastrointestinal and carcinogenic values. Since one of the possible means of migration of these toxins is drinking water, the Company must monitor, through periodic analyzes, the concentrations of these substances in the distributed water.

The area near the intake structure is made up exclusively of the dam infrastructure body, in accordance with art. 94 co. 3 of Legislative Decree 152/06 and subsequent amendments, according to which the area of absolute protection has to be used exclusively for collection works and service infrastructures.

4.1.1.3 Drinking water treatment plant

The drinking water treatment plant (DWTP) of Castreccioni serves the municipalities of Cingoli, Filottrano, Osimo, Castelfidardo, Numana and Sirolo. The block chain of the drinking water treatment plant is schematized in Figure 21. The influent undergoes a pre-ozonation pre-treatment, a flocculation phase with PAC dosage, a sand filtration, a subsequent post-ozonation phase and an activated carbon filtration. After which it is sent to storage tanks and finally sent as a final effluent leaving the plant.

Influent to the plant is taken from two different points which are at 314m and 324m of height. The DWTP works with only one influent per time alternating it during the day. Average discharge sent to the plant is 19,5m^3/sec. Pre-ozonation tank has a volume of 150m^3 , works with an HRT of 517sec and 20Nm3/h of insufflated air. After pre-ozonation two flocculation tanks have been installed, each one of 1620m^3. They works with an HRT of 186min with PAC dosage of 13g/h. Six sand filter have been installed, five of them work and one as reserve. Each unit has a volume of $48m^3$, works with an HRT of 14min, backwashed air equal to 760L/sec and backwashed water to 100L/sec. Next unit to sand filters are post-ozonation tanks (two units) of $150m^3$ of volume, HRT equal to 517sec and insufflated air equal to $35Nm^3/h$. Outlet to the post-ozonation is sent to GAC filtration: four units plus two as reserve have been installed. They work with granulated carbon, have $48m^3$ of volume and HRT of 11min. Last unit is disinfection with chlorine: 500 $gClO_2/h$ are dosed and HRT is 15min. Once water has passed through disinfection is stored in two accumulation tanks with effective volume of $7800m^3$ and HRT of 15h.

Influent to the plant is sampled on average once a month to carry out laboratory analysis for the chemical-physical characterization, while the effluent is analyzed approximately every 15 days.

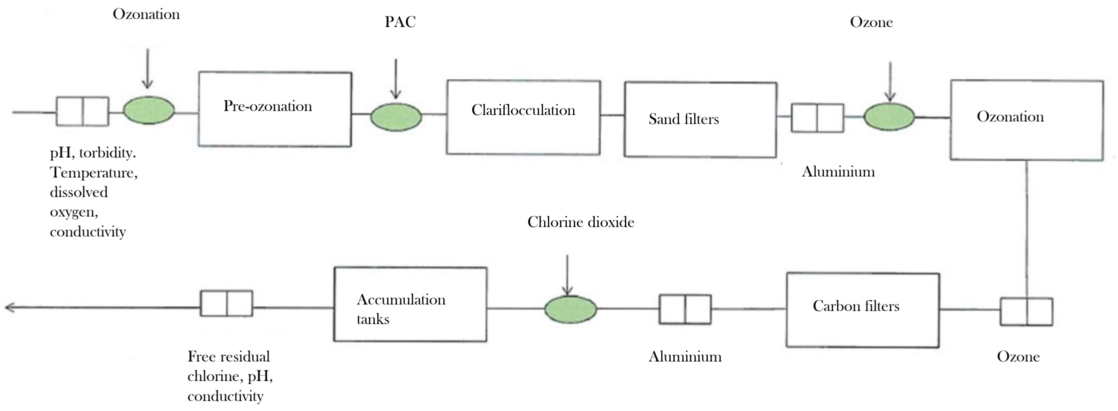


Figure 21 Block chain of drinking water treatment plant on Castreccioni

4.1.1.4 Sampling method

The scope of this study is to analyse the presence of MPs in the DWTP of Castreccioni. First of all has been done a sampling campaign. Sampling points have been decided after a site inspection and they are in proximity of: inlet to the plant at the two different heights of the dam, pre-ozonation effluent, flocculation effluent plus a sample of flocculation sludge, sand filters effluent, ozonation effluent, activated carbon effluent, final effluent and two points on distribution network: Montoro and Imbrecciata. In all the selected points around 1000L of water have been filtered installing a copper filter with a system of pipes also made of copper to convey water into samplers to each tap. Has been decided to use copper equipment instead of plastic one to avoid altering the final results in terms of microplastics concentration. The discharge at each point has been first calculated, then with it has been found the time necessary to filter 1000L of water. After having passed the calculated time necessary the filters have been uninstalled to be taken to the laboratory and the real volume filtered has been found taking the time. In the laboratory characterization of microplastics has been done. In particular have been found number of MPs in each sample, concentration of MPs per liter of filtered water, number and concentration of microfibers, shapes of MPs, material and size classes.

Two sampling campaigns with same sampling points and modalities have been made. First one during summer between 7/07/2020 and 9/07/2020; second one during winter in 1/12/2020.

Inlet to the two different heights of the dam

The influent to the plant is captured at two different altitudes by the dam. The altitude located at 324 m a.s.l. could be sampled directly near the dam, from a special tap with a diameter of DN 15 ,located inside a building used for the maintenance of the work, at which will be connected a filtration system with stainless steel cartridge-filter.

Figure 22 Dam tap (1)

Figure 23 Dam tap (2)

The second intake point, located at 314 m a.s.l., could be sampled directly inside the purification system, by a special 3/8 inch (316) diameter tap. The filtration system with stainless steel cartridge-filter will be connected to the tap.

Figure 24 Intake at drinking water plant inlet (1)

Figure 25 Intake at drinking water plant inlet (2)

Pre-ozonation effluent

The influent from the DWTP is sent to a pre-ozonation pre-treatment. Even the exit from the pre-ozonation compartment could be sampled from a tap, placed outside the compartment and having a diameter of ½ inches. The filtration system with stainless steel cartridge-filter has been connected to the tap.

Figure 26 Pre-ozonation outlet (1)

Figure 27 Pre-ozonation outlet (2)

Figure 28 Pre-ozonation outlet (3)

Flocculation effluent (sand filters inlet) + flocculation sludge

From the pre-ozonation pre-treatment, the flow is sent to the flocculation compartment, where it is mixed with PAC solution. After the flocculation phase, the water is sent to the sand filtration unit. The entrance to the sand filtration can be sampled by extraction from the tanks with a volumetric pump without plastic elements and filtered on site with a battery of sieves. The filtered water could be directed to a drain.

The chemical sludge coming from the flocculation treatment could be sampled from the point in the Figure through an instant sampling of about 5-10 liters.

Figure 29 Inlet to filtration sampling point

Figure 30 Chemical sludge from flocculation sampling point

Sand filters effluent

The flow is conducted out of the sand filtration towards the ozonation compartment. The flow can be sampled by installing the filtration system with stainless steel cartridge-filter to a tap, placed at the outlet from the filtration unit and having a diameter of DN 15.

Figure 31 Sand filters effluent sampling point (1)

Figure 32 Sand filters effluent sampling point (2)

Ozonation effluent

At the outlet from the post-ozonation compartment there is a tap with a diameter of DN 15, to which the filtration system with stainless steel cartridge-filter could be installed. After the ozonation treatment, the flow is sent to the activated carbon filtration system.

Figure 33 Ozonation effluent sampling point (1)

Figure 34 Ozonation effluent sampling point (2)

Activated carbon effluent

From the activated carbon compartment the flow is conveyed towards the storage tanks. The flow can be sampled by connecting the filtration system with stainless steel cartridge-filter to a 3/8 inch diameter tap.

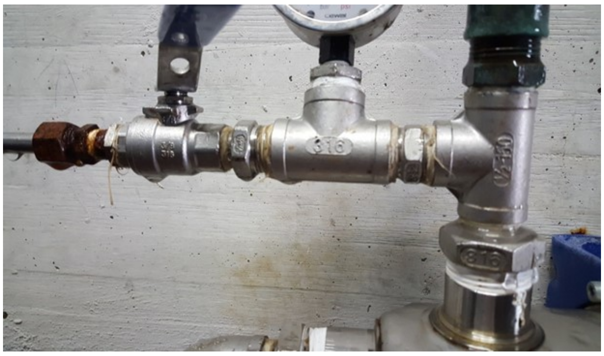


Figure 35 Activated carbon effluent sampling point (1)

Figure 36 Activated carbon effluent sampling point (2)

Final effluent

The final effluent can be sampled by installing the filtration system with stainless steel cartridge-filter to the appropriate 3/8 inch tap.

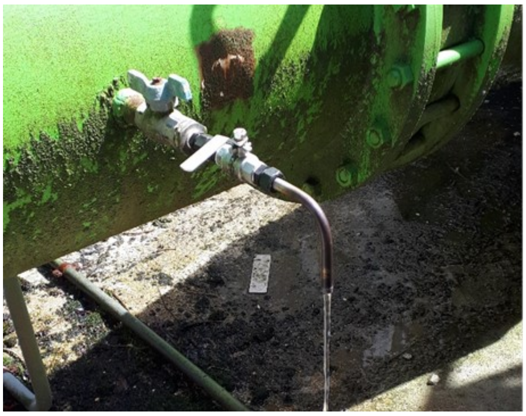


Figure 37 Final effluent sampling point (1)

Figure 38 Finale effluent sampling point (2)

Points on distribution network: Montoro (in line) and Imbrecciata (tank)

In addition to the points already identified within the drinking water treatment plant, sampling along the distribution network could be prepared. Water from intake wells also flow along the network. Along the network there are booths with special taps and drain wells, which could be used for sampling by installing the filtration system with stainless steel cartridge-filter on the tap. Potential areas for sampling have been identified, in correspondence with Montoro, in line, and Imbrecciata, from the reservoir. The localities have been identified in such a way as not to compromise samplings with the water coming from the wells.

4.1.2 Microplastics characterization

4.1.2.1 Microplastics extraction from wastewater

Collected wastewater samples have been passed through a steel sieves battery of 5 mm, 2 mm and 63 mm mesh size (ISO 3310- 1:2000): solids retained on 2 mm and 63 mm sieves were rinsed into glass jars with ultrapure water and subsequently filtered onto cellulose nitrate filters (Sartorius Stedim Biotech, Ø 47 mm, 8 mm pore size) using a vacuum pump. Filters have been recovered in petri dishes, covered with 15% H_2O_2 and maintained at 50°C overnight to remove organics.

4.1.2.2 Microplastics extraction from sludge

After collection the first step has been a first organic matter digestion performed in glass beakers adding 15% H_2O_2 and maintaining sludge samples in stove at 50°C for two days. Second step has been the density separation procedure, carried out in 250 mL cylinders, stirring the samples with high-density saturated NaBr salt solution (1.4 g $cm3^{-1}$) for 30 min (Frias et al., 2018) and leaving to settle the mixture overnight. The supernatant was then vacuum filtered and filters have been treated with 15% H_2O_2. To evaluate the potential for loss during the density separation procedure for sludge fraction and to calculate the extraction yield of microplastics, a total of 12 particles, two for each representative polymer (polyethylene, polypropylene, polystyrene, polyethylene terephthalate, nylon, polyisoprene rubber) in the size range of 0.5e1.5 mm, have been spiked into samples and blanks, starting from the first organic matter digestion step. The particles of polyethylene, polypropylene and polystyrene have been standard materials purchased from a plastic company (Fainplast, Italy), while those of polyethylene terephthalate, nylon and polyisoprene have been obtained by cutting a plastic bottle, a fishing wire and an elastic band, respectively. All of them have been photographed and measured, and IR spectra have been acquired before and after the test, showing no appreciable changing in shape, size and polymer characteristics of recovered particles.

4.1.2.3 Microplastics quantification and characterization

Wastewater filters resulted from the extraction procedure have been analysed using a stereomicroscope (Optika SZM-D equipped with OPTIKAMB5 digital camera), with maximum magnification of 45°. All items which resembling plastic and fibers have been manually collected using a tweezer, transferred onto a clean cellulose acetate membrane (Sartorius Stedim Biotech, Ø 47 mm, 0.45 mm pore size) located on a microscope slide (subsequently used as support for the mFT-IR analyses), quantified and categorized in base of their shape, size and polymer type. In terms of their shape, MPs were categorized in fiber-shaped (MPFs) according to the definition proposed elsewhere (Liu et al., 2019) and particle-shaped (MPPs), which included five main typologies: lines, fragments, films, spheres, glitters identified according to characteristics given in (Hartmann et al., 2019; Lusher et al., 2017; Magni et al., 2019; Yurtsever, 2019). MPPs were measured on the basis of the largest dimension (Hartmann et al., 2019), using an image analysis software (Optika Vision Lite 2.1) and classified in four size classes in the range of 1-5 mm, 0.5-1 mm, 0.1-0.5 mm and 0.03-0.1 mm. Then all the collected particles and fibers have been characterized by mFTIR **spectroscopy** in attenuated total reflectance mode, using a Spotlight 200i FT-IR microscope system (PerkinElmer) equipped with Spectrum Two and driven by Spectrum 10 software. After background scans, each sample spectrum was recorded performing 32 accumulations, ranging from 600 to 4000 cm^{-1} with the resolution at 4 cm^{-1}. IR spectrum of the cellulose acetate membrane has been aquired and substracted to that of each sample in order to avoid the overlay of spectra. The output spectra have been subsequently subjected to a spectral search against reference libraries of polymer spectra represented by PerkinElmer database (ATRPolymer, polyATR, FIBERS3, plast1, RP, POLIMERI, PIGMENTI, resin and PERKIN1 libraries were selected), by the database compiled within the framework of the JPI-OCEANS project BASEMAN (Primpke et al., 2018 Synthetic polymers (petroleum-based, biobased and hybrid polymers), modified natural ones (e.g. rayon), copolymers and composites have been considered as plastic.

4.2 QMRA FOR WATER REUSE IN IRRIGATION

The reuse of wastewater in agriculture might be a good option to face with water scarcity. But it is necessary to individuate the possible hazards and evaluate the relative risks implementing a risk assessment. The purpose of the risk assessment is to identify and evaluate the health risks associated with the water reuse, to determine if the health hazards are adequately controlled, to inform operation and management of the water reclamation and to identify necessary improvements and upgrades to ensure the delivery of safe reclaimed-water. The procedure could be done with different approaches and in this case quantitative microbial risk assessment has been used. QMRA is commonly used for assessing microbial risks in recycled water systems because it is a powerful tool for estimating order-of-magnitude risks within a community following exposure to pathogens associated with specific scenarios.

4.2.1 Peschiera Borromeo wastewater treatment plant description

Water reuse in agriculture is assumed to be applied to the full-scale plant of Peschiera Borromeo WWTP. In particular in this study, starting from outlet concentrations of pathogen in Peschiera Borromeo, the possible risk connected to a hypothetical reuse of the water to irrigate tomato crops has been calculated. The final aim is to identify and quantify the risk connected to different exposure scenarios with related barriers applied.

The WWTP selected is in the municipality of Peschiera Borromeo, in Via Roma - Cascina Brusada. The plant serves a large urban area (Milan and neighbouring municipalities) and the Lambro River acts as water body receptor, located in the spring-line, near a few quarries. The plant has a treatment capacity of 566.000 P.E. and an average flow rate of 216.000 m^3/day. The plant consists of two water lines receiving wastewater from different urban areas:

- Line 1: Municipalities of Brugherio (MB), Carugate, Cassina de' Pecchi, Cernusco sul Naviglio, Cologno Monzese, Peschiera Borromeo, Pioltello, Segrate e Vimodrone.
- Line 2: Municipality of Milan and Linate district of Peschiera Borromeo

Figure 39 Localization of Peschiera Borromeo WWTP

Line 1 treatment process consists of:

- Coarse screening, fine screening and deodorization;
- Grit removal system;
- Primary Sedimentation: two circular settlers. After this section is located the emergency by-pass;
- Activated sludge oxidation;
- Secondary Sedimentation: 4 circular settlers;

- Tertiary treatment: 2 stages up flow biological filtration with nitrification and denitrification;
- Final disinfection with peracetic acid.

Line 2 treatment process consists of:

- Grit chamber: located in deodorized local; -
- Initial drainage and fine grit, with cover and deodorization;
- Sand separation and oil extraction: with odour cover and suction;
- Primary decanting;
- Denitrification / Nitrification: with a biological up flow filtration system, it includes a first predenitrification stage and a second nitrification stage, with an automatic backwash system;
- Final disinfection: disinfection by UV rays.

Sludge treatment line consists of:

- Pre-thickening: Covered and connected to the intake unit and air deodorization;
- Dynamic thickening;
- Primary anaerobic digestion;
- Secondary anaerobic digestion;
- Post-thickening;
- Dewatering with centrifuges;
- Dewatered sludge is stored in removable pallets in a covered and deodorized building.

The plant is also equipped with deodorization facilities applied to pre-treatments, primary treatments and to sludge line and with biogas recovery system used to produce electricity and to heat the digesters.

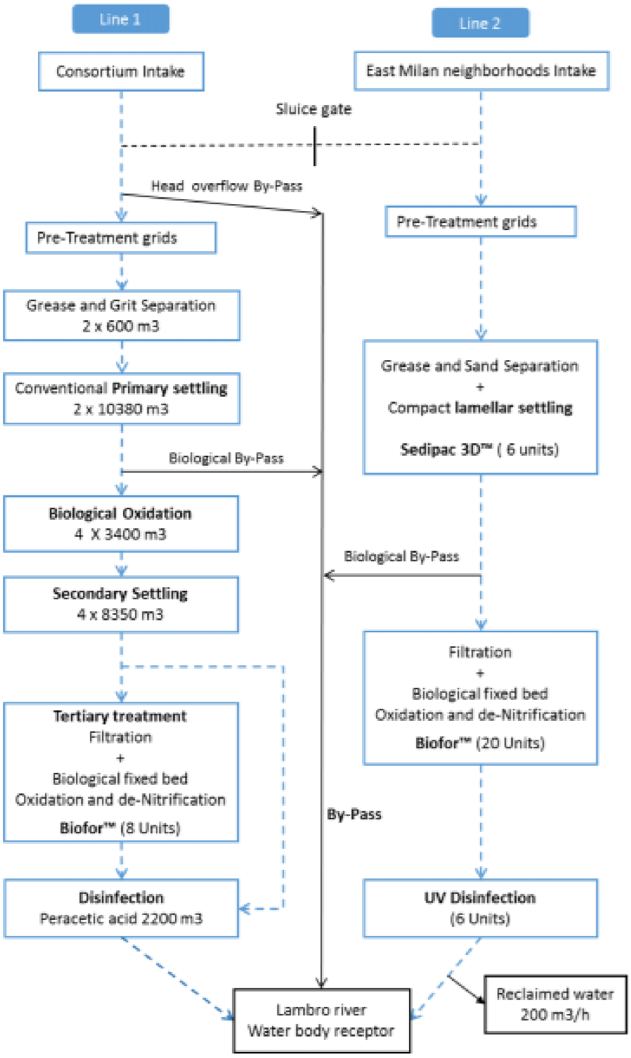


Figure 40 Block-flow diagram of Peschiera Borromeo WWTP

The water treatment lines are equipped with 47 instruments to monitor the performance of the plant, divided into Flow Meters, Probes and Samplers. Also sludge line is equipped with flow meters to monitor different parameters.

Daily average samples are taken on a weekly basis:

- Inlet wastewater (both lines):

COD (chemical oxygen demand), BOD5 (Biological oxygen demand), Ammonia (N basis and NH4 basis), total nitrogen, nitrate (NO3 basis), total phosphorous, TSS (total suspended solid), metals (Al; As; Cd; Cr; Mn; Ni; Pb; Cu; Zn; Fe), pH, conductivity, chlorides, phosphate, sulphate;

- Outlet treated water (both lines):

COD (chemical oxygen demand), BOD5 (Biological oxygen demand), Ammonia (N basis and NH4 basis), total nitrogen, nitrate (NO3 basis), total phosphorous, TSS (total suspended solid), metals (Al; As; Cd; Cr; Mn; Ni; Pb; Cu; Zn; Fe), pH, conductivity, chlorides, phosphate, sulphate, E. coli; - At the inlet of the biological treatment (Biofor line 2): pH, conductivity; TSS (total suspended solid), COD (chemical oxygen demand), total nitrogen, total phosphorous.

4.2.2 Risk assessment through QMRA

QMRA is a proccess formed by four steps which are:

- Hazard identification: This step involves deciding which microorganisms are of interest in the study and finding out what diseases these microorganisms cause. Hazard ID comprises general information about the microbial agent (pathogens) and the adverse consequences to the host from infection. Characteristics that may be included are: % without asymptomatic rates, latency, incubation times, duration of infectiousness and disease, % of cases with various symptomology, excretion rates and immunity.
- Dose response: the risk of a response is estimated given a known dose of a pathogen. Dose response models are mathematical functions that describe the dose response relationship for specific pathogens, transmission routes, and hosts.
- Exposure assessment: the dose of the pathogen that an individual ingests, inhales, or comes in contact with are calculated. This number feeds into the dose-response models to predict the probability of infection. In most cases exposure can be viewed as a pathway from the source of the pathogen to the actual exposure (consume of products). So, understanding the transport and survival of the microbe are fundamental.
- Risk characterization: is the integration of the Exposure Assessment with how much risk is associated with different doses so the Dose Response Assessment to estimate a risk.
- Risk characterizations range from a "point estimate" of risk to more sophisticated efforts that consider uncertainty in model input parameters and variability across individuals and subpopulations. These more sophisticated methods are known as probabilistic risk assessment.
- Local available data

Available data are daily average samples taken on a weekly basis of E.coli concentrations in the effluent stream from the WWTP of Peschiera Borromeo. The investigated interval time goes from 16/01/2018 to 18/12/20. It has been divided in two different periods: from 16/01/2018 to 12/03/2019, and from 19/03/2019 to 18/12/2020, respectively.

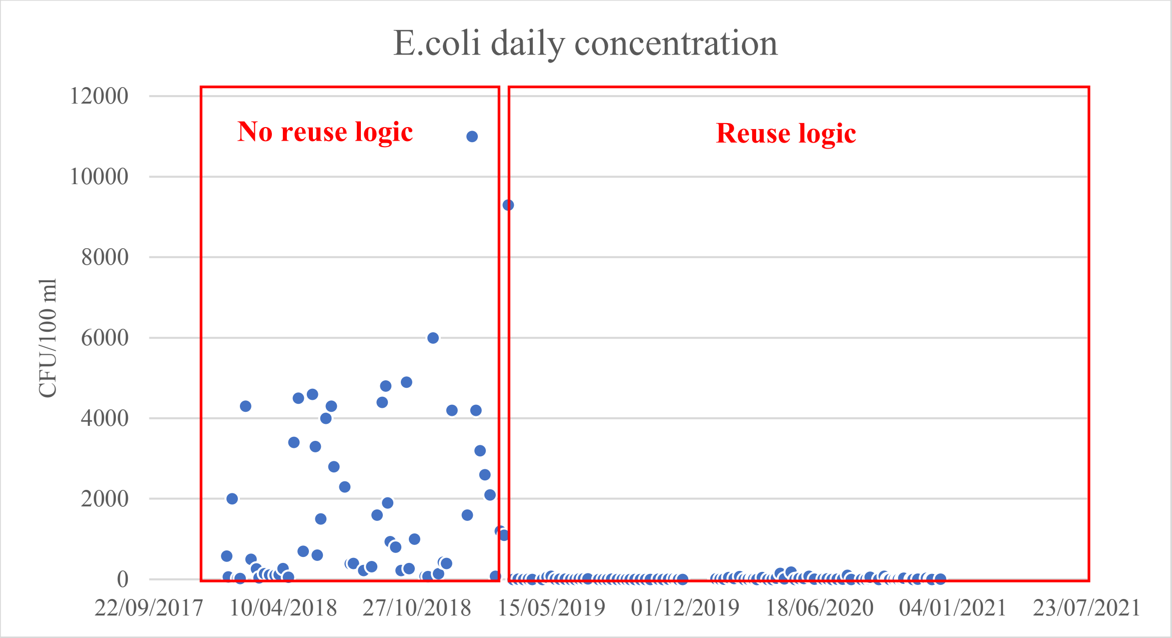


Figure 41 E.coli daily concentrations with periods division

In the first period the plant worked normally without consider the possibility to re-use effluent water, as visible by the high E.coli concentrations. According to the Italian law (D.Lgs 152/2006) the maximum E.coli effluent concentration is set to 5.000 CFU/100 ml. In the second period, a different pattern is identified. The WWTP has been managing the UV disinfection to comply with the stricter water reuse limit (<10 CFU/100 ml – D.lgs 185/2003). It was easily achieved increasing the UV dose consuming more electricity. Data distribution has been divided in a “Non-reuse” and in a “Reuse” seasons. Two different QMRA risk assessments have been performed for the two periods. Mainly deterministic analysis has been used, this means that for each period a single representative concentration value of all the distribution has been taken. In particular the mean value has been used which in Non-reuse period is 1937 CFU/100mL, in Reuse period it is 16 CFU/100mL.

Table 18 Characteristic values of No-reuse and Reuse periods

	“No reuse”	“Reuse”
Operation time	16/01/2018-12/03/2019	19/03/2019-18/12/2020

Mean	1937	16
Median	940	5
Trend	20	0
Standard deviation	2335	31
Interval	10980	180
Minimum	20	0
Maximum	11000	180
Count	57	90

As concerns the minimum requirements reported in Table 2 (Table 13 in this study) of the new European Regulation (EU) 2020/741, the Peschiera Borromeo effluent complies with class D and class B reclaimed water quality, in the first and second period respectively. In first period E.coli concentrations (CFU/100mL) have to be lower than 10000, indeed for example mean value of distribution is 1937 CFU/100mL, the median 940 CFU/100mL and the 75th percentile is 3300 CFU/100mL. In second period E.coli concentrations have to be lower than 1000 CFU/100mL and indeed mean value is 16 CFU/100mL, the median 5 CFU/100mL and the 75th percentile is 13,74 CFU/100mL.

Table 19 Classes of reclaimed water quality and permitted agricultural use and irrigation method

EU 2020/741		**No Reuse**		**Reuse**	
	Limit (CFU/100 ml	n. of sample lower or equal	compliance	n. of sample lower or equal	compliance
Class D	10000	56	98%	90	100%
Class C	1000	30	53%	90	100%
Class B	100	9	16%	88	98%
Class A	10	0	0%	62	69%

In this study has been considered to use reclaimed water to irrigate tomatoes crop.

4.2.2.1 *Hazard characterization*

The first step to assess a QMRA procedure is to identify the possible hazards in the real scenario of exposure, so hazardous pathogens have been identified. In addition to E.coli concentration other reference pathogens have been chosen to have a more complete view of final risk. In particular reference pathogens considered are the same used in Australian and WHO Guidelines: Campylobacter for bacteria, Cryptosporidum for protozoas and Rotavirus for viruses. Pathogens concentrations have been calculated starting from E.coli effluent concentration assuming typical ratios from literature (in particular from tab. C6 of WHO guidelines 2006 and R.F.Goncalves et al.,2020) between it and pathogens; so how much Cryptosporidium, Campylobacter and Rotavirus could be present in water respect to a known E.coli concentration. Used ratios referred to influent wastewater in a WWTP and correspond to: 10^{-6} for Rotavirus, 10^{-6} for Cryptosporidium and 10^{-5} for Campylobacter. Since available E.coli concentration is referred to the effluent of the plant, first of all has been calculated its possible value before primary treatments adding to it the quantity of pathogens that has been removed from UV disinfection, secondary treatment and primary treatment. Reference values of log reductions applied by the above-mentioned treatment processes have been taken from Australian Guidelines and resumed in Table 20. Considered values are averages between maximum and minimum of each reference pathogen.

Table 20 log reductions of activities in a DWTP from Australian Guidelines

Reference	*E.coli*		*Campylobacter*		*Cryptosporidium*		*Rotavirus*	
	min	max	min	max	min	max	min	max
Primary Sedmentation	0	0,5	0	0,5	0	0,5	0	1
Secondary Sedimentation	1	3	1	3	0,5	1	0,5	2
UV disinfection	2	>4	2	>4	>3		>1	

Then, indicator to reference ratios have been applied to obtained influent concentration of reference pathogens. Finally, log reductions of the different process treatments have been applied to obtain effluent values. Each treatment step, indeed, has a different removal efficiency against the three types of pathogens (bacteria, protozoa and viruses).

4.2.2.2 *Exposure assessment*

The purpose of exposure assessment in quantitative risk analysis is to predict the fate of a hazard from its source to the endpoint of interest and the quantity this endpoint is exposed to. In this study the main objective is to determine the dose of the respective agents, which people are exposed to. Different groups of people could be exposed to hazards through different pathways and they are: fieldworkers, local communities and final consumers of the products.

Routes of exposure

Each people category enters in contact with pathogens doing some activities called intended use or routes of exposure. To each activity is associated a volume (mL) with which they enter in contact and a frequency per person per year (pppy) calculated from empirical data. Due to lack of data and impossibility to obtain local information, have been taken into account default data from *Australian Guidelines,2006*. The main route of exposure to microbial hazards from recycled water is ingestion, including ingestion of droplets produced by sprays (inhalation). Dermal exposure is also possible, but there is a lack of evidence of health impacts through this route and it is considered unlikely to cause significant levels of infection or illness in the normal population. (NWQMS, 2006) Examples of exposure volumes and frequencies of exposures per person are provided in Table 21. In general, the volumes provided are considered to be conservative.

Table 21 Activities of exposure

Activity	**Route of exposure**	**Activity + exposure**	**Volume (mL)**	**Frequency/ person/year**	**Comments**
	NWQMS, 2006				
		NONE	**-**	**-**	
Garden irrigation	organisms	Garden irrigation (Ingestion of sprays)	0,1	90	Garden watering estimated to typically occur every second day during dry months (half year). Exposure to aerosols occurs during watering.
Garden irrigation	Routine ingestion	Garden irrigation (Routine ingestion)	1	90	Routine exposure results from indirect ingestion via contact with plants, lawns, etc. Infrequent event.
	Accidental ingestion	Garden irrigation (Accidental ingestion)	100	1	Frequencies moderate as most people use municipal areas sparingly (estimate 1/2–3 weeks). People are unlikely to be directly exposed to large amounts of spray and therefore exposure is from indirect ingestion via contact with lawns, etc. Likely to be higher when used to irrigate facilities such as sports grounds and golf courses (estimate 1/week).
Municipal irrigation	Ingestion	Municipal irrigation (Ingestion)	1	50	100 g of lettuce leaves hold 10.8 mL water and cucumbers 0.4 mL at worst case (immediately post watering).a A serve of lettuce (40 g) might hold 5 mL of recycled water and other produce might hold up to 1 mL per serve. Calculated frequencies are based on ABS data.b
Food crop consumption (home grown)	Ingestion of lettuce	Food crop consumption (home grown) (Ingestion of lettuce)	5	7	100 g of lettuce leaves hold 10.8 mL water and cucumbers 0.4 mL at worst case (immediately post watering).a A serve of lettuce (40 g) might hold 5 mL of recycled water and other produce

	Ingestion of other raw products	Food crop consumption (home grown) (Ingestion of other raw products)	1	50	might hold up to 1 mL per serve. Calculated frequencies are based on ABS data.b
Food crop consumption (commercial)	Ingestion of lettuce	Food crop consumption (commercial) (Ingestion of lettuce)	5	70	100 g of lettuce leaves hold 10.8 mL water and cucumbers 0.4 mL at worst case (immediately post watering).a A serve of lettuce (40 g) might hold 5 mL of recycled water and other produce might hold up to 1 mL per serve. Calculated frequencies are based on ABS data.
	Ingestion of other raw products	Food crop consumption (commercial) (Ingestion of other raw products)	1	140	
Toilet flushing	Ingestion of sprays	Toilet flushing (Ingestion of sprays)	0,01	1100	Frequency based on three uses of home toilet per day. Aerosol volumes are less than those produced by garden irrigation.
Washing machine use	Ingestion of sprays	Washing machine use (Ingestion of sprays)	0,01	100	Assumes one member of household exposed. Calculated frequency based on ABS data.d Aerosol volumes are less than those produced by garden irrigation (machines usually closed during operation).
Fire fighting	Ingestion of water and sprays	Fire fighting (Ingestion of water and sprays)	20	50	Median ingestion for firefighters estimated at 20 mL per fire with a maximum number of fires fought within area served by recycled water of 50 per year.
	Ingestion	Cross-connection of dual-reticulation systems with drinking water mains (Ingestion)	1000	1/1000	Total consumption is assumed to be 2 litres per day, of which 1 litre is consumed cold.f Affected individuals may consume water 365 days per year. A conservative estimate of 1/1000 houses has been considered.
Municipal irrigation	Inhalation	Inhalation for local communities (min)	0,0045	365	The average wind speed for the region of Braunschweig is set to 3m/s (DWD 2004). For this wind speed and distance range Viau et al. published an inhalation dose of PM10 particles produces by biosolid land application form 4.5-6.9 µg per application event (Viau et al. 2011b). W.Seis et al. 2012
	Inhalation	Inhalation for local communities (max)	0,0069	365	

In this study, according to three different exposure pathways analysed, have been considered three activities:

- Municipal irrigation (ingestion),
- food crop consumption (commercial)
- inhalation deriving from municipal irrigation.

Preventive measures

Preventive measures could be traduced in “safe use of recycled water”. Exposure to hazard have to be reduced with strategies as:

- preventing hazards from entering recycled water;
- using treatment processes;

- reducing exposure, either by using preventive measures at the site of use or by restricting uses.

Therefore it’s possible to apply preventive measures, also called barriers, directly in the WWTP with treatment processes, or reducing the exposure to pathogens both in situ and after restricting the use of products, for example processing them.

Each applied barrier is expressed as a log reduction value. In this study, since the outlet value of pathogens concentrations have been considered, only barriers after the WWTP have been applied. It means only in the fields or even after the harvest of products. Characteristic values of log reduction have been taken from *NWQMS,2006* and *WHO Guidelines* and are summarized in Table 22. In particular have been chosen both maximum and minimum values.

Table 22 Log reductions applied to each barrier

Reference	*NWQMS, 2006*		WHO guidelines for safe use of wastewater, excreta and greywater	
	min	max	*min*	max
Cooking or processing of produce (eg cereal, wine grapes)	5	6	6	7
Removal of skins from produce before consumption	2		2	
Drip irrigation of crops	2			
Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	3			
Drip irrigation of raised crops with no ground contact (eg apples, apricots, grapes)	5			
Subsurface irrigation of above ground crops	4			
Withholding periods — produce (decay rate)	0,5		4	>6
Withholding periods for irrigation of parks/sports grounds (1–4 hours)	1			
Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	1			
Drip irrigation of plants/shrubs	4			

Subsurface irrigation of plants/shrubs or grassed areas	5	6		
No public access during irrigation	2			
No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	3			
Buffer zones (25–30 m)	1		1	
Washing with water			1	
Natural die-off			0,5	2

Each activity done by the subject of exposure will have its proper series of log removals applied, so at the end the value to consider to calculate the dose will be the sum of all the barriers.

Once the defined the exposure groups and related route, as well as the possible preventive measures, the dose of pathogens with which the exposed group enter in contact has to be calculated with the following equation:

$$d = \frac{c * exposure/event}{\log reduction}$$

Where:

- c=concentration of pathogen
- log reduction=reductions required to achieve a residual risk coming from preventive measures or barriers
- exposure/event= volume (mL) with which people enter in contact in a single event of exposure with a certain activity

For each activity, there will be one related dose.

If the calculated doses are at low levels some simplifications could be done on the following calculation steps. Low doses concentrations to take as reference levels have been taken from Australian Guidelines (NWQMS, 2006) and they are: $1*10^{-1}$ for Campylobacter and Cryptosporidium, $1*10^{-2}$ for Rotavirus.

Table 23 Low doses concentrations of pathogens

		E.coli	**Campylobacter**	**Cryptosporidium**	**Rotavirus**

References			*NWQMS, 2006*		
Low dose concentration	organisms	-	1,00E-01	1,00E-01	1,00E-02

4.2.2.3 Dose-response relationship

Next step has been to identify dose-response relations which are mathematical functional relationship between the number of pathogens someone is exposed to and the probability of the specific adverse effect. The functional relation is pathogen specific. The values for probability lie between zero (no adverse effect) and one (adverse effect is certain) (Seis, 2012). According to (Haas et al., 2014), a Beta-Poisson model has been assigned to Campylobacter, Rotavirus and E.coli and an exponential model to Cryptosporidium. Inside these mathematical functions are present some constants which are fundamental to differentiate one pathogen from the other. They are in fact specific of each category. Due to lack of data and the impossibility to calculate them, they have been taken from literature studies. In particular constant values are: "α", "β" and "N_{50}" for Beta-Poisson relationship and "r" for Exponential relationship. To choose better ones has been done a comparison between different sources of literature and the final values are resumed in Table 24. Sources of literature are first of all Australian guidelines, then also QMRA Wiki community portal (a web data collection created by researchers of the Center for Advancing Microbial Risk Assessment (CAMRA) OF Michigan State University) and Aquanes tool (a portal to calculate QMRA risk assessment) have been taken into account.

Table 24 Dose-response constants of pathogens

		E.coli	**Campylobacter**	**Cryptosporidium**	**Rotavirus**
References			QMRA wiki community portal / Aquanes tool/ NWQMS, 2006		
Dose-response constants	α	1,55E-01	1,45E-01		2,53E-01
	N50	2,11E+06			
	β	2,44E+04	7,58		4,26E-01
	r			5,90E-02	

Dose-response model is used to calculate the Probability of infection (Pinf) which comes from each event of exposure to the pathogens.

$P_{inf} = 1 - e^{-r*d}$	$P_{inf} = 1 - (1 - \frac{d}{\beta})^{-\alpha}$

A probability of infection for each chosen activity has to be calculated.

If there are low dose conditions, a simplified calculation of probability of infection could be applied, as as follow:

$$P_{inf}(\text{low doses}) = d * \frac{\alpha}{\beta}(\text{or r})$$

Where:

- d = dose per event
- α, β, r = dose response constants

4.2.2.4 *Risk characterization*

Next step has been to calculate the total probability of infection in a year by multiplying Pinf per single event per the frequency of activity. To combine the different chosen routes of exposure the following equation could be uses:

$$P_{inf}\ \text{combined final} = 1 - \prod_{1}^{n}(1 - P_{inf\,i})^{Ni}$$

Where:

- n= number of activities
- Pinf i= probability of infection of the i^{th} activity
- Ni= frequency/person/year of i^{th} activity

Final probability of infection of each pathogen could be compared with the value established by USEPA of $1*10^{-4}$ .If they are lower than maximum level, the risk would be acceptable.

Once P_{inf} combined final has been calculated the probability of illness has to be found. This is obtained multiplying Pinf combined final for the ratio illness/infection. Of this last data have been assumed values from *NWQMS,2006*.

Table 21 Ratio illness/infection of pathogens

		E.coli	Campylobacter	Cryptosporidium	Rotavirus
References	NWQMS, 2006				
Ratio illness/infection	-		0,3	0,7	0,88

Probability of illness has been found as:

$$\text{Pill} = P_{inf}\ \text{combined final} * \text{ratio illness/infection}$$

Last value to obtain are DALYs/year for each pathogen.

$$\text{DALY per year} = \text{Pill} * \text{DALYd} * \text{susceptibily fraction}$$

Where:

- Pill= probability of illness per year
- DALYd= DALY per case

Susceptibily fraction values are once again taken from literature, in particular from NWQMS,2006.

Table 25 Susceptibility fraction from NWQMS,2006

		E.coli	**Campylobacter**	**Cryptosporidium**	**Rotavirus**
References	NWQMS, 2006				
Susceptibility fraction	-		100%	100%	6%

Daly per case of each pathogen has been obtained by *A.H.Havelaar, J.M. Melse, 2003* and *NWQMS,2006* and are reported in Table 26.

Table 26 DALYd for each pathogen

		E.coli	**Campylobacter**	**Cryptosporidium**	**Rotavirus**
References	A.H.Havelaar, J.M. Melse, 2003 / NWQMS,2006				
DALYd (per case)			4,60E-03	1,50E-03	1,40E-02

Once also DALYs per year have been calculated, they could be compared with the tolerable level of risk ($1*10^{-6}$) in order to understand if they are acceptable or not.

4.2.2.5 Different alternative scenario comparison

Fieldworkers and local communities might be exposed through direct contact with wastewater or contaminated soil or crops. These groups might inhale or ingest wastewater when sprinkler irrigation is used. Concerning local communities, children playing on agricultural areas have to be considered as well. The third group of people are consumers of food which has been grown on wastewater irrigated fields.

Each group of people could enter in contact with contaminated wastewater or products in several ways or route of exposure. To each route of exposure could be applied different barriers or log removals to reduce the risk.

For all the groups three different level of risk have been analysed. In the first case a maximum risk has been calculated considering no barriers; in the second case a situation with barriers applied in situ and raw products consumed has been considered; in the third case a situation with barriers applied in situ and processed products has been considered. The risk for all three groups have been analysed considering in one case drip irrigation and in a second case spray irrigation.

First of all, exposure pathways of each group have been identified. In drip irrigation case fieldworkers could enter in contact with contaminated water through ingestion during all their working days (supposed to be 100 in a year according to Seis et al.,2012) and consuming products of that crops. In spray irrigation case fieldworkers could be also exposed to inhalation during working days. Due to lack of local data has been considered that they inahlate $6,9*10^{-3}$ mL of wastewater for each event using as reference Seis et al.,2012. In Table 71 and Table 72 for each activity in the three scenarios have been summarized the applied barriers. In the last two lines of the table are summarized the log removals of each case and number of microorganisms per event.

Local communities in drip irrigation case activities could also enter in contact with contaminated water through ingestion but for a lower number of times (10 per year) and consuming products of that crops for 140 times per year. In spray irrigation case local communities could be also exposed to inhalation during all the year. Due to lack of local data has been considered that they inhalate $6,9*10^{-3}$ mL of wastewater for each event using as reference Seis et al.,2012. In Table 73 and Table 74 for each activity in the three scenarios have been summarized the applied barriers.

Last group of exposed people could be final consumers of tomatoes. In this case they could enter in contact with pathogens only consuming products in raw or processed mode.

Another scenario has been simulated: the case in which fieldworkers and local communities don’t consume the products. In this case they could only accidentally ingest or inhale pathogens during work or because they are in proximity of the irrigation site.

4.2.3 Risk characterization approach

Risk characterization comprise for sure a certain level of variability and uncertainties within its estimation. The terms variability and uncertainty refer to the problem of imprecise or not reliable data, which might lead to errors in the overall result. (Seis, 2012) Because of a lack of data assumptions have to be made for different scenarios. Lack of data could be due to the impossibility to further investigate human behaviour doing certain activities for example or impossibility to extrapolate data like ratio illness/infection or susceptibility fraction with new studies. Risk characterization could be done with different methods: point or stochastic estimation. A quantitative method to discuss and evaluate uncertainties is Monte Carlo Simulation.

4.2.3.1 Point estimation (deterministic)

Early approaches of quantitative microbial risk assessment (QRMA) were based on point estimates and thus resulted in a single value of risk. This type of analysis has been done using a single averaged value as input for the E.coli concentration. In this way a lot of uncertainties are connected with the results. For example in this study there is a series of effluent concentrations of the pathogen in three years. In the first months values were very high respect to the rest of the years. So the final values could be defined as not representative of the series.

Moreover all the inputs for DALYs estimation have been taken as single values and not as a range to evaluate possible random variations.

4.2.3.2 Stochastic estimation (Monte Carlo Simulation)

In this study, Monte Carlo simulation is applied to the case of risk assessment for consumers, considering both the "*No reuse*" and "*Reuse*" periods.

First of all, the data of both periods needed to be represented by a probability density function (PDF). The raw data series of E.coli concentration in *No reuse* period are represented in Figure 42. Maximum value is 11000 CFU/100mL and minimum one 20 CFU/100mL.

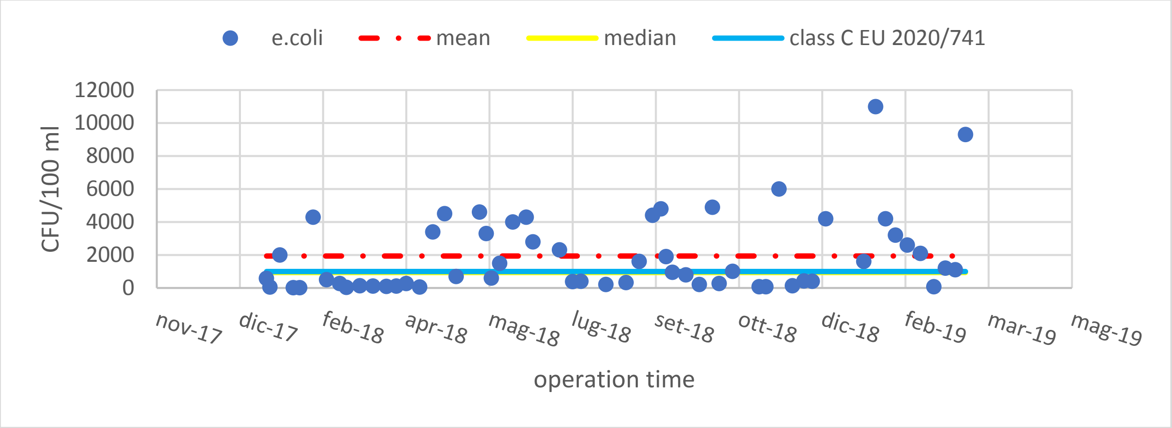


Figure 42 E.coli concentration series in No reuse period

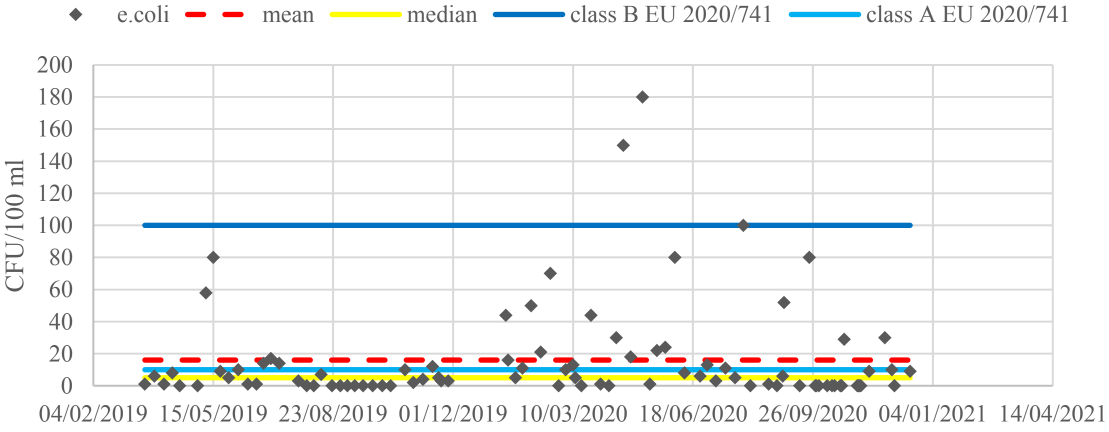


Figure 43E.coli concentration series in Reuse period

The best fitting distribution resulted to be the Weibull distribution. The cumulate density probability is shown in Figure 44.

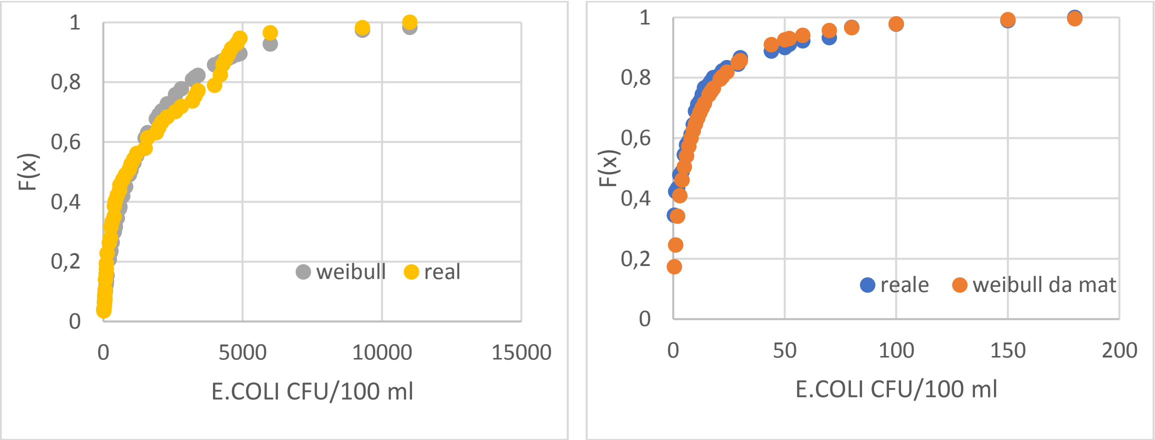


Figure 44 Overlapping of real cumulate density probability and the simulated one by Weibull distribution.: a) "No Reuse", b) "Reuse" period

The resulting distribution parameters for *No reuse* and *Reuse* scenarios have been resumed in Table 27.

Table 27 Parameters used in Weibull distributions

	Peschiera DATA FITTING	
E.coli concentration	REUSE	NO REUSE
distribution	weibull	weibull
α	9,34	1607,60
β	0,57	0,73

The same parameters describedd in the previous section that are needed for the risk characterization, are in that case considered no more as point values but with their variability. In the following, the values considered in this study are listed. They are common to the *No reuse* and *Reuse periods*.

Table 28 UV disinfection distributions and parameters

UV disinfection log reduction			
Reference	Ayuso-Gabella et al, 2011		
Pathogen	**campylobacter**	**cryptosporidium**	**rotavirus**
Distribution	**triangular**	**triangular**	**triangular**
min	2,0E+00	2,0E+00	1,0E+00
max	4,0E+00	3,5E+00	2,0E+00
mode	3,0E+00	3,0E+00	3,5E+00

In Table 29 have been resumed selected distributions and characteristics for E.coli reference ratios.

Table 29 Distribution and parameters for E.coli reference ratios

E.coli - reference ratio			
pathogen	**campylobacter**	**cryptosporidium**	**rotavirus**
distribution	**uniform**	**uniform**	**uniform**
min	1,00E-06	1,00E-07	1,00E-06
max	1,00E-05	1,00E-06	1,00E-05

For what concerns log reductions applied in the crops and to final product, exposure volume and infection/illness ratio has been mantained a single value except for infection/illness ratio of Rotavirus for which uniform distribution has been chosen.

Table 30 Distribution and parameters for innless/infection ratio of Rotavirus

infection:illness ratio	
pathogen	**rotavirus**
Reference	*Verbyla et al, 2016*

distribution	**uniform**
min	3,50E-01
max	0,9000

Daly per case of reference pathogens have been represented using uniform distributions as showed in Table 31.

Table 31 Distributions and parameters for DALY per case

Daly per case			
Reference	*Verbyla et al 2016: Managing Microbial Risks from Indirect Wastewater Reuse for Irrigation in Urbanizing Watersheds*		
pathogen	**campylobacter**	**cryptosporidium**	**rotavirus**
distribution	**uniform**	**uniform**	**uniform**
min	4,60E-03	1,20E-04	1,50E-02
max	0,0410	0,0015	0,0260

5 RESULTS AND DISCUSSIONS

5.1 MICROPLASTICS RESULTS

5.1.1 Microplastic quantification in drinking water treatment plant (Summer campaign)

In Table 32 has been resumed a part of the sampling campaign results done in July 2020 (summer campaign), in particular to what concerns particles concentrations. In first column sampled volumes have been reported, they are around 1000L for water samples while are 18L and 26L for flocculation sludge and backwash samples respectively. Inlet water to the DWTP is taken from two different points of the dam located at two different heights: 324m and 314m. Number of MPs in the inlet point at 324m of height of the dam is really different to the number at 314m: first one is 3n°MPs and second one is 10n°MPs. The drinking water treatment plant always work with water which comes from a single inlet point and they are alternated during the day. In particular when samples for this study have been taken the plant was working with the influent from the 314m point. Microplastics concentrations are quite similar in all the points except for effluent from activated carbons which increases from 3 to 8n°MPs. The final effluent has 4n°MPs. In sludge samples and in the single distribution point analysed MPs are quite low. But numbers of microplastics are not objective because they are referred to the filtered volume, so to refer them to 1L and calculate the concentration they have to be divided for the volume itself. From concentrations it's possible to confirm a higher value in influent from 314m of height respect to 324m point. Then it's possible to observe a rising of MPs in flocculation and activated carbon effluents. In distribution point 2 the concentration is lower (0,0020n°MPs) respect to the effluent. In flocculated sludge microparticles per liter are 0,1111 and in backwash 0,0385n°MPs/L.

For what concerns microfibers could be observed that numbers are similar (15 and 19n°MFs) in the inlet points; in sand filters effluent there is an increasing from 3 to 11n°MFs. Also in this case has been possible to calculate microfibers concentrations relating n°MFs to the corresponding sampled volume. Concentrations confirm that there are similar values in influent points and that sand filters effluent is equal to 0,0099 n°MFs/L. higher respect to the influent to that process. In final effluent it's possible to note an abrupt decrease. In distribution point 2 microplastics are absent. Sludge samples haven't been analysed in terms of microfibers.

Table 32 MPs and MFs concentrations in Summer campaign's samples

Sampling point	**Sampling volume**	**N°MPs**	**N°MPs/ L**	**N°MFs**	**N°MFs/ L**
	L				
Inlet point 324m (Dam)	997,34	3	0,0030	15	0,0150
Inlet point 314m	1095	10	0,0091	19	0,0174
Pre-ozonation effluent	1000,5	1	0,0010	5	0,0050
Flocculation effluent	1022	6	0,0059	3	0,0029
Sand filters effluent	1115,2	4	0,0036	11	0,0099
Post-ozonation effluent	998,14	3	0,0030	0	0,0000
Activated carbons effluent	994,84	8	0,0080	5	0,0050
Final effluent	998,76	4	0,0040	0	0,0000
Distribution point 1 (Imbrecciata)	995,6				
Distribution point 2 (Montoro)	1000	2	0,0020	0	0,0000
Flocculation sludge	18	2	0,1111		

Backwash	26	1	0,0385		

In Figure 45 could be observed MPs trend in each water sample (so flocculation sludge and backwash have been excluded), remarking an increase of concentration in activated carbon's effluent.

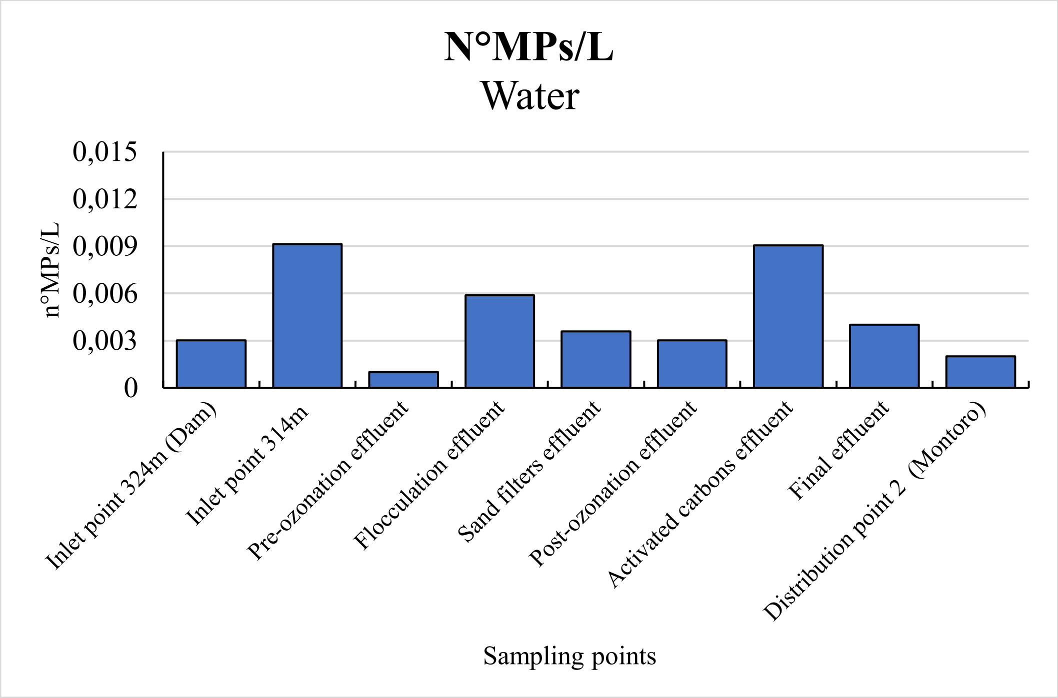


Figure 45 MPs concentrations in each sampling point (Summer campaign)

In Figure 46 MPs concentration in sludge sample and backwash have been represented.

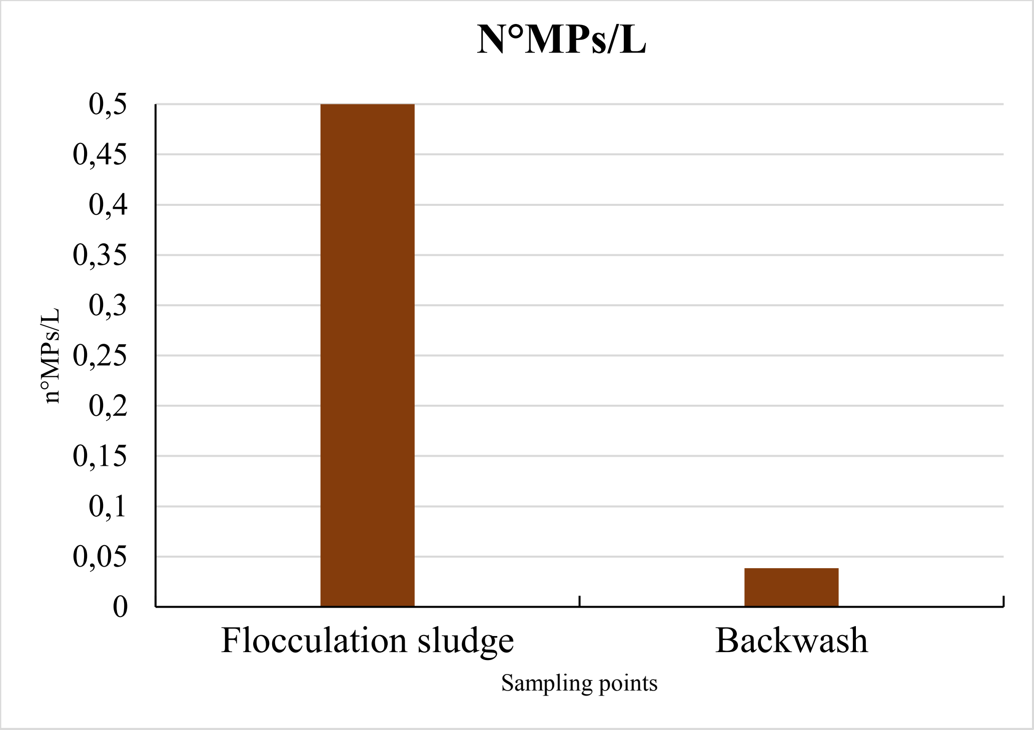


Figure 46 MPs concentrations in sludge sample and backwash (Summer campaign)

Shape,size and polymers analysis have been done only for microplastics. In Table 33 have been resumed microplastics' shapes in terms of frequency (%) of occurrence in each sampling point. They could be divided into film, fragment and line shape. In the influent from 324m of height the majority of MPs are in form of film (66,7%) and the rest are fragments (33,3%). While in influent from 314m of height the majority of MPs are in form of fragments (60%) and the rest are films (40%). In pre-ozonation effluent film shape is the only present shape. Going through final effluent more and more particles are present in the form of fragments. Line shaped MPs are the less present (only in activated carbons effluent and flocculation sludge). In distribution point 1 all MPs are in form of fragments and in point 2 a half is in fragment and other half in film shape. Flocculation sludge has for the 50% MPs in form of film and 50% in form of line. Finally, in backwash sample only film shaped MPs have been found.

Table 33 MPs shapes in each sampling point (Summer campaign)

Sampling point	**MPs Shape (frequency %)**		
	film	**fragment**	**line**
Inlet point 324m (Dam)	66,7	33,3	0
Inlet point 314m	40,0	60,0	0

Pre-ozonation effluent	100,0	0	0
Flocculation effluent	16,7	83,3	0
Sand filters effluent	0,0	100,0	0
Post-ozonation effluent	0,0	100,0	0
Activated carbons effluent	33,3	55,6	11,1
Final effluent	25	75	0
Distribution point 1 (Imbrecciata)	0	100	0
Distribution point 2 (Montoro)	50	50	0
Flocculation sludge	50	0	50
Backwash	100	0	0

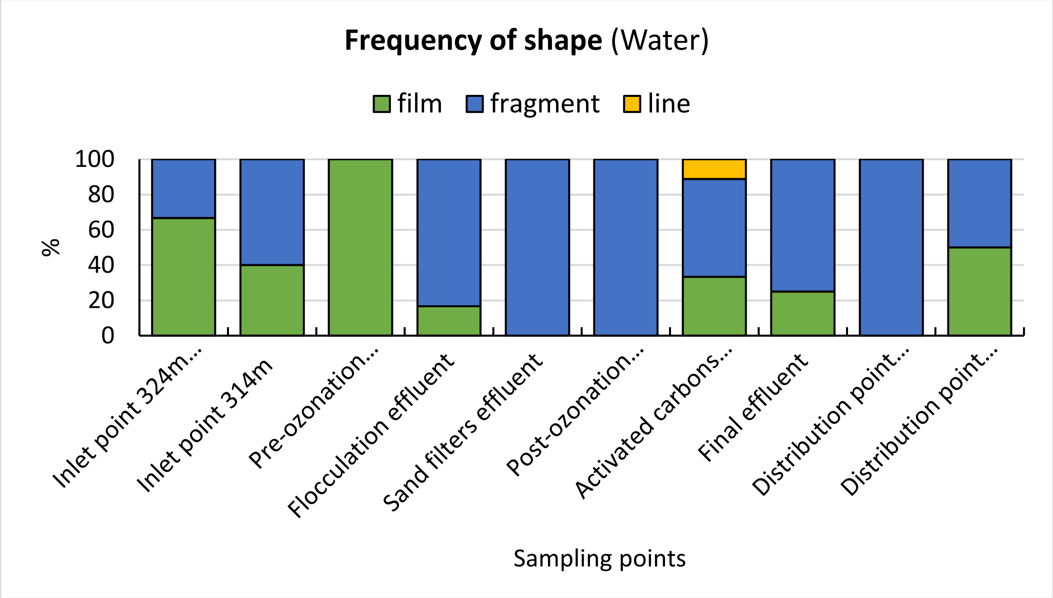


Figure 47 Frequency of shapes presence in water samples (Summer campaign)

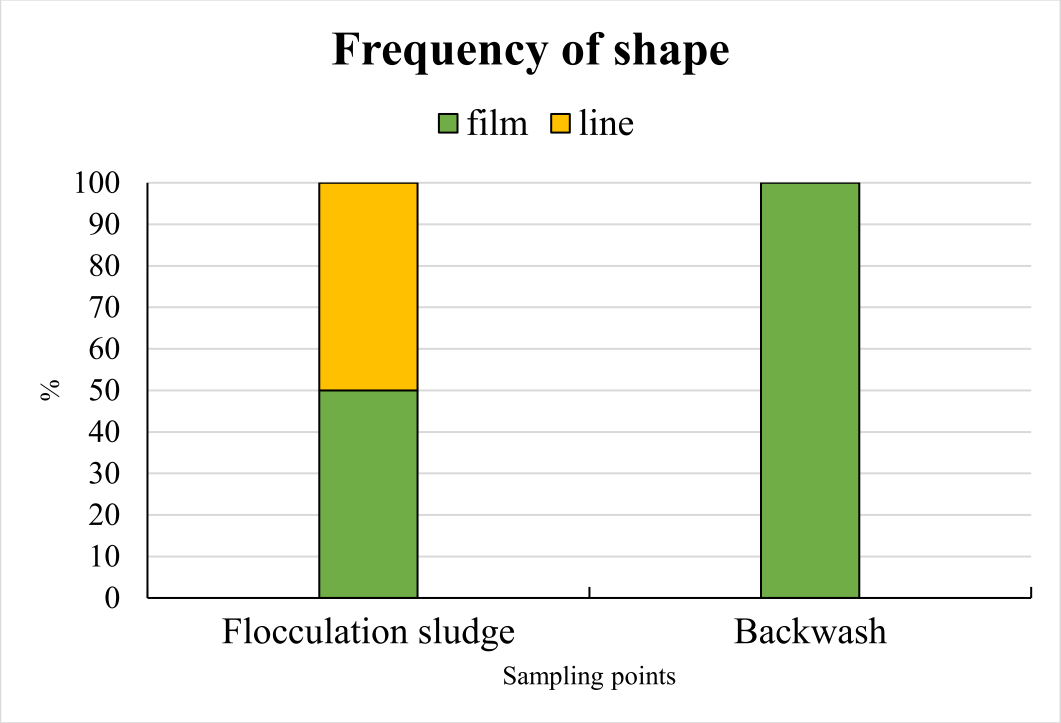


Figure 48 Frequency of shape in sludge and backwash samples (Summer)

In Table 34 have been resumed MPs size classes in terms of frequency (%) of occurrence in each sampling point. In both inlet points the majority of MPs are included in size classes: 0,5-0,15mm and 0,15-0,05mm, in particular they prevail in the first one. This trend remains up to the activated carbons effluent because in the final effluent the fraction of MPs in 0,5-0,15mm increases to the 50%. Bigger fractions of particles (2-1mm and 1-0,5mm) are less present; it's possible to find them in flocculation effluent, sand filters effluent and in the first distribution point. In flocculated sludge sample particles size is comprised in 2-1mm and 0,5-0,15mm classes. In backwash sample all MPs are in the class 0,5-0,15mm.

Table 34 MPs size classes frequency (Summer campaign)

Sampling point	**MPs Size classes (frequency %)**			
	2-1mm	**1-0.5mm**	**0.5-0.15 mm**	**0.15-0.05 mm**
Inlet point 324m (Dam)	0	0	66,67	33,33
Inlet point 314m	0	0	70	30
Pre-ozonation effluent	0	0	100	100
Flocculation effluent	16,67	0	66,67	16,67
Sand filters effluent	0	25	25	50
Post-ozonation effluent	0	0	66,67	33,33

Activated carbons effluent	0	11,11	66,67	22,22
Final effluent	0	0	50	50
Distribution point 1 (Imbrecciata)	16,67	30	33,33	20
Distribution point 2 (Montoro)	0	0	100	0
Flocculation sludge	50	0	50	0
Backwash	0	0	100	0

In Figure 49 could be noticed that MPs in size class 2-1mm (the biggest one) are only present in flocculation effluent and in the 1st distribution point.

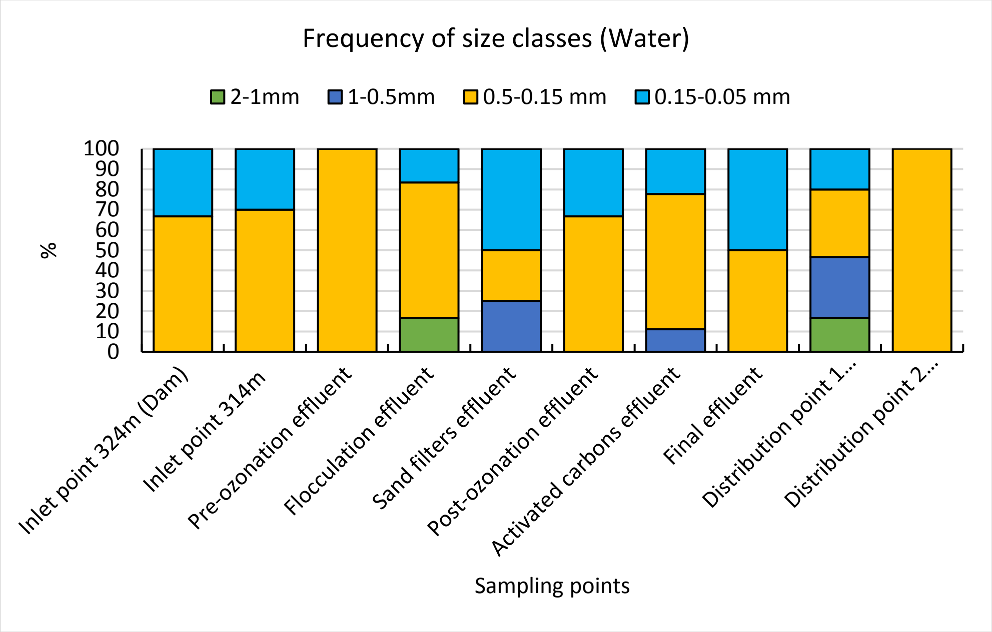


Figure 49 Frequency of size classes in water samples (Summer campaign)

In Figure 50 have been plotted size classes present in flocculation sludge and in backwash.

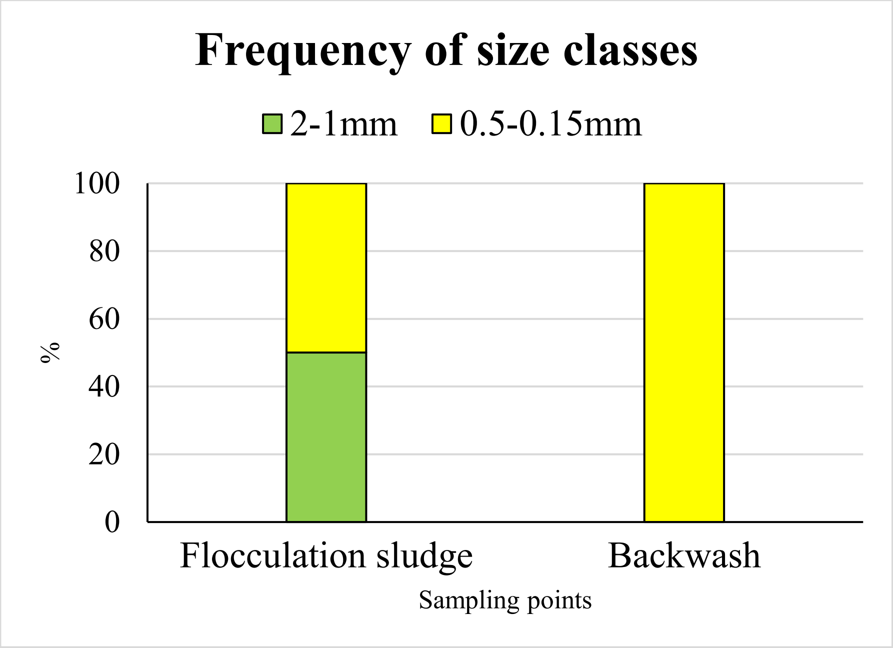


Figure 50 Frequency of size classes in flocculation sludge and backwash samples (Summer campaign)

In Table 35 have been summarized polymers of which each sample is composed. In inlet point at 324m of height MPs are made for 66,7% of polyester and 33,3% of polyurethane, while in second inlet point only polyurethane is still present and polyethylene, polyester resin, polypropylene and styrene-butadiene are introduced. Polypropylene is the only material of MPs in pre-ozonation effluent. In general could be noticed that most present polymers in water samples are: polyethylene, polyester resin, polyurethane, polypropylene, polyester and in second distribution point styrene-butadiene. Flocculation sludge contains polyester and polypropylene MPs in equal measure, while backwash samples only polyethylene.

Table 35 MPs material frequency in each sampling point in Summer campaign

Sampling point	MPs Material (frequency %)
Inlet point 324m (Dam)	66,7% polyester, 33,3% polyurethane
Inlet point 314m	10% polyester resin, 40% poluyrethane, 10% polypropylene, 30% polyethylene, 10% styrene-butadiene
Pre-ozonation effluent	100% polypropylene
Flocculation effluent	16,7% polyurethane ,16,7% polypropylene ,16,7% polyethylene ,33,3% polystyrene ,16,7% polyphenyl ethere + polystyrene

Sand filters effluent	25% polyurethane ,25% polypropylene ,50% polyethylene ,
Post-ozonation effluent	33,3% polyester resin ,66,7% polyethylene
Activated carbons effluent	22,2% polyester resin ,11,1% polyester ,33,3% polyurethane ,11,1 polyethylene ,22,2 polyvinyl chloride
Final effluent	25% polyester ,25% polyurethane ,25% polyethylene ,25% styrene-butadiene
Distribution point 1 (Imbrecciata)	100% polyvinylidene fluoride
Distribution point 2 (Montoro)	50% polyethylene,50% polytetrafluoroethylene
Flocculation sludge	50% polyester,50% polypropylene
Backwash	100% polyethilene

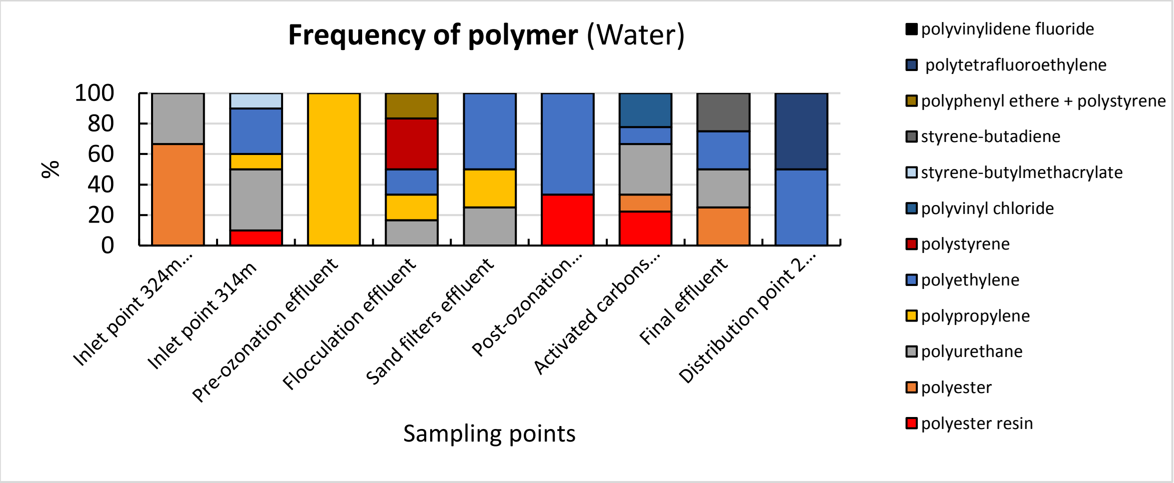


Figure 51 Frequency of polymers in water samples (Summer campaign)

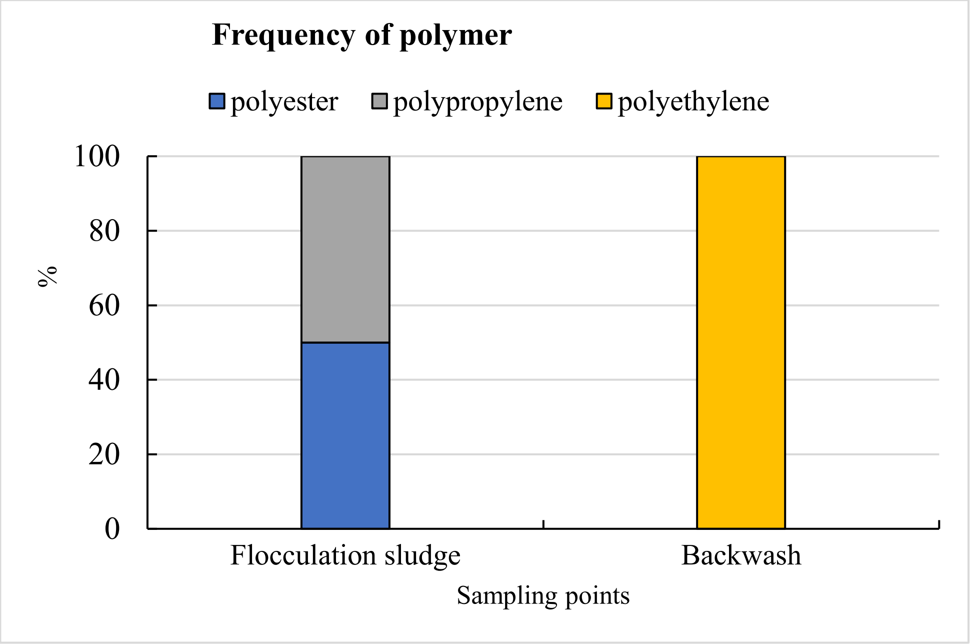


Figure 52 Frequency of polymers presence in flocculation sludge and backwash samples (Summer)

5.1.2 Microplastic quantification in drinking water treatment plant (Winter campaign)

Winter campaign has been done in December 2nd and same points of Summer campaign have been sampled. Also this time even if both inlet points have been sampled (at 314m and 324m of height), the DWTP was working with influent water coming from the point at 314m of height during all the campaign. An exception has been done for the final effluent which corresponds to the treated water coming from second point at 324m of height.

First characteristics that could be observed are microplastics and synthetic microfibers concentrations in water and sludge samples. Numbers of particles have been obtained in laboratory, then they have been divided for the filtered volume to obtain particles and fibers concentrations (n°/L).

Concentrations of MPs are quite variable and fluctuate between 0,001 and 0,026 in water samples. Considering as influent value 0,008 n°MPs/L, it remains the same after pre-ozonation treatment. After flocculation it increases to 0,026 n°MPs/L to then fluctuate up to 0,039 n°MPs/L in the effluent. In distribution point MPs are low and near zero.

For what concerns synthetic microfibers they also fluctuate in water samples in treatment processes, but values are lower than MPs and oscillate between 0 and 0,003 n°MFs/L.

Table 36 MPs and MFs concentrations in Winter campaign

Sampling point	Sampling volume	N°MPs	N°MPs/L	N°MFs	N°MFs/L
	L				
Inlet point 324m (Dam)	1127,76	14	0,012	0	0,0000
Inlet point 314m	1010,8	8	0,008	1	0,0010
Pre-ozonation effluent	999,6	8	0,008	2	0,0020
Flocculation effluent	1003,2	26	0,026	3	0,0030
Sand filters effluent	1085,4	1	0,001	0	0,0000
Post-ozonation effluent	999,6	8	0,008	0	0,0000
Activated carbons effluent	1000,5	6	0,006	1	0,0010
Final effluent (324m)	1000	4	0,0039	0	0,0000
Distribution point 1 (Imbrecciata)	996	7	0,007	3	0,0030
Distribution point 2 (Montoro)	995,4	0	0	0	0,0000
Flocculation sludge	20				
Backwash	30				

In Figure 53 could be seen MPs trend in water samples only. Could be noted that there has been an increasing after flocculation.

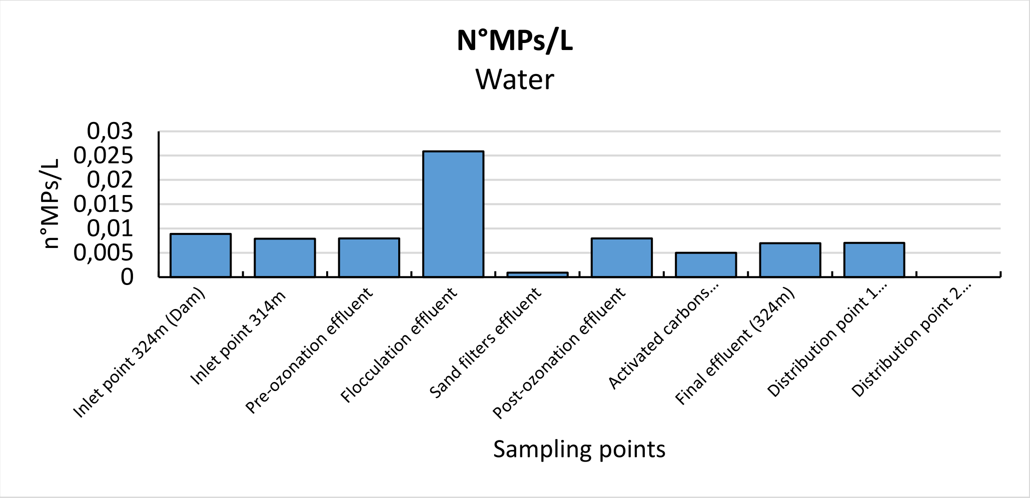


Figure 53 MPs concentrations in Winter campaign

Shape,size and polymers analysis have been done only for microplastics. In Table 37 have been summarized shapes frequency expressed in %. Both in inlet at 324m and 314m of height fragments is the prevalent shape; in first inlet point (324m) fragments are followed by line and then film shape, while in second inlet point (314m) lines are not present. Except for pre-ozonation effluent, in which film shape is the most present (75%), fragments are the prevailing form of microparticles in water samples followed by film and line.

Table 37 MPs shapes in each sampling point (Winter campaign)

Sampling point	**MPs Shape (frequency %)**		
	film	**fragment**	**line**
Inlet point 324m (Dam)	7,7	61,5	31
Inlet point 314m	37,5	62,5	0
Pre-ozonation effluent	75,0	25	0
Flocculation effluent	19,2	69,2	12
Sand filters effluent	0,0	100,0	0
Post-ozonation effluent	25,0	50,0	25
Activated carbons effluent	16,7	83,3	0,0
Final effluent (324m)	0	100	0
Distribution point 1 (Imbrecciata)	2	98	0
Distribution point 2 (Montoro)	43	57	0
Flocculation sludge			
Backwash			

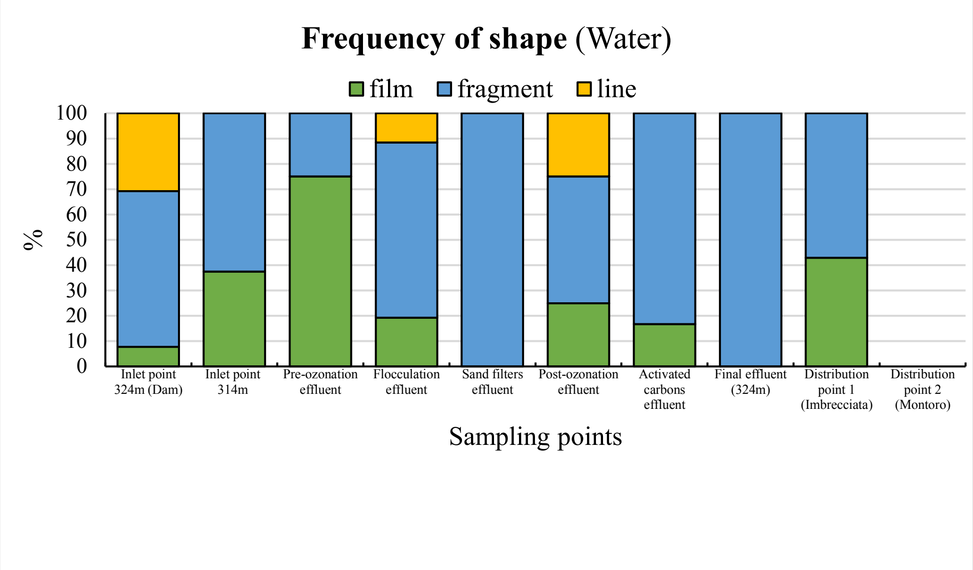


Figure 54 MPs frequency of shapes trend in Winter campaign

For what concerns size classes MPs in inlet point at 324m are distributed between: 1-0,5mm , 0,5-0,15mm , 0,15-0,05mm and 0,05-0,02mm. While in second point they are only distributed between 0,5-0,15mm, 0,15-0,05mm and 0,05-0,02mm. In both sampling points most of the particles are in the range of 0,15-0,05mm. In final effluent most of the microplastics are in the range of size 0,15-0,05 mm.

Table 38 MPs frequency of size classes in Winter campaign

Sampling point	MPs Size classes (frequency %)					
	5-2 mm	2-1mm	1-0.5mm	0.5-0.15 mm	0.15-0.05 mm	0.05-0.02 mm
Inlet point 324m (Dam)	0,0	7,7	7,7	30,8	53,8	0,0
Inlet point 314m	0,0	0,0	0,0	25,0	50,0	25,0
Pre-ozonation effluent	0,0	0,0	0,0	75,0	25,0	0,0
Flocculation effluent	0,0	3,8	7,7	38,5	42,3	7,7
Sand filters effluent	0,0	0,0	0,0	100,0	0,0	0,0
Post-ozonation effluent	12,5	12,5	12,5	50,0	12,5	0,0
Activated carbons effluent	0,0	0,0	0,0	16,7	50,0	33,3
Final effluent (324m)	2,6	0,0	2,6	23,1	66,7	5,1
Distribution point 1 (Imbrecciata)	0,0	3,1	17,6	29,0	39,7	10,7
Distribution point 2 (Montoro)	0,0	0,0	0,0	42,9	57,1	0,0
Flocculation sludge						
Backwash						

From Figure 55 could be noticed that prevailing classes in water samples are: 0,5-0,15mm and 0,15-0,05mm.

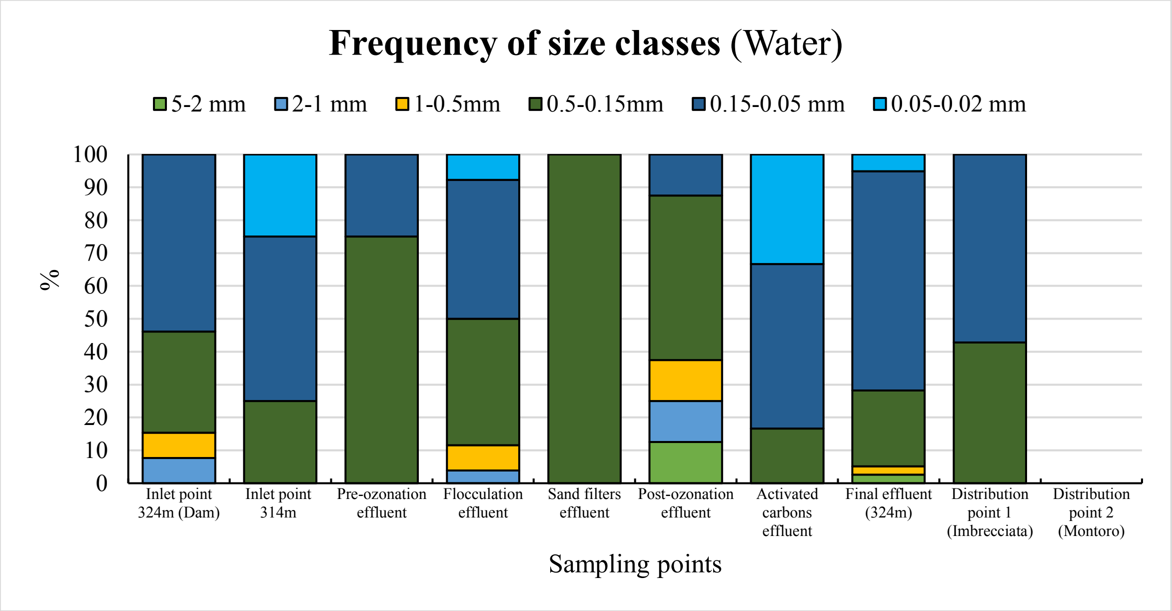


Figure 55 Frequency of size classes in water samples (Winter campaign)

Microplastics most frequent polymers are: polyvinyl chloride, polyester resin and polypropylene. But have also been found: polyester, polyethylene, polystyrene, polyvinylidene fluoride, polyacryate (particularly in sand filters effluent), polyacrylic rubber, polyurethane, polytetrafluoroethylene, polyvinyl chloride+polyvinyl alcohol, silicone, thermoplastic elastomer (PEST based), epoxide resin, polyacrylate and acrylonitrile butadiene styrene.

Table 39 Frequency of polymers in water samples (Winter campaign)

Sampling point	MPs Material (frequency %)
Inlet point 324m (Dam)	23,1% polyester, 30,8% polyester resin, 23,1%polypropylene, 23,1% polyvinylidene fluoride
Inlet point 314m	12,5% polyester resin, 25% polypropilene, 25% polyvinyl chloride, 25% polyacrylic rubber, 255 polyurethane
Pre-ozonation effluent	75% polypropylene, 25% polyacrylic rubber
Flocculation effluent	15,4% polyester, 38,5 polyester resin, 7,7% polypropilene, 11,5% polyethylene, 7,7%

	polystyrene, 3,8% polyvinyl chloride, 3,8% polyvinyl chloride+polyvinyl alcohol, 7,7% thermoplastic elastomer (PEST based), 3,8% epoxide resin
Sand filters effluent	100% polyacrylate
Post-ozonation effluent	12,5% polyester, 12,5% polyester resin, 25% polyethylene, 12,5% polyvinyl chloride, 12,5% epoxide resin, 12,5% polyacrylate, 12,5%
Activated carbons effluent	66,7% polyester resin,16,7% polyethylene ,16,7% polyvinylidene fluoride
Final effluent (324m)	5,1% polypropylene,82,1% polyvinylidene fluoride,12,8% polyacrylic rubber
Distribution point 1 (Imbrecciata)	42.9% polyester resin,14,3% polyethylene ,28,6% polytetrafluoroethylene ,14,3% silicone
Distribution point 2 (Montoro)	
Flocculation sludge	
Backwash	

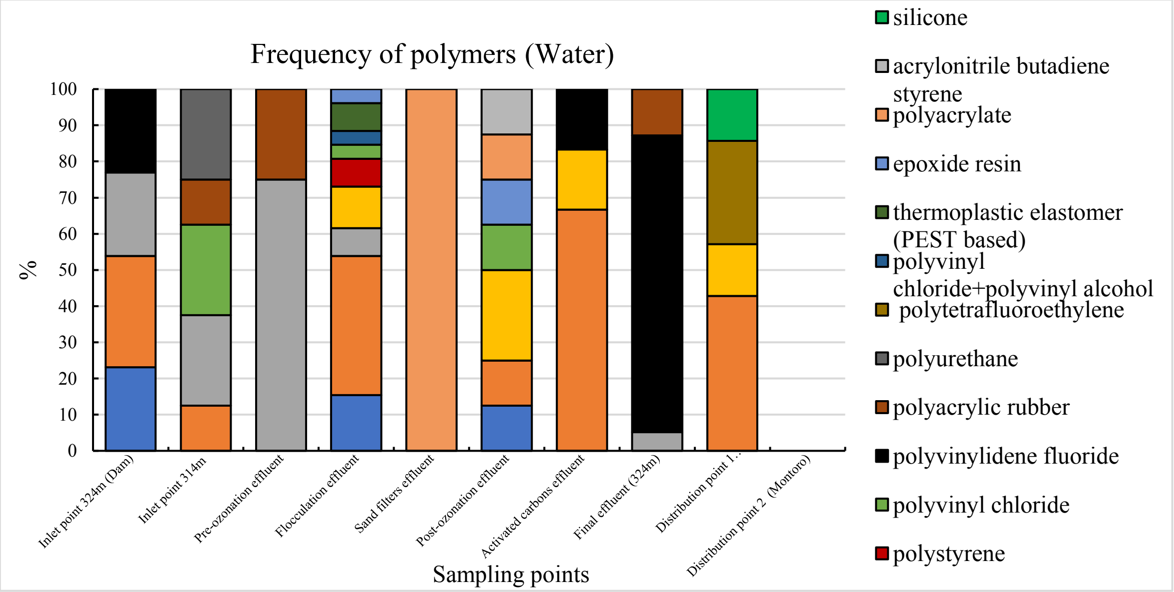


Figure 56 Frequency of polymers in water samples (Winter campaign)

5.1.3 Removal efficiencies of different treatment processes (Summer campaign)

Removal efficiencies of each treatment has been calculated as follows:

$$Removal\ efficiency\ (\%) = \frac{n°MPs/L(in)\ - n°MPs/L(out)}{n°MPs/L(in)} * 100$$

The presence of some negative values could be due to a particles release in that process and could be seen an increase on MPs load. Release point in Summer campaign are: flocculation and activated carbons. The biggest part of MPs has been removed in pre-ozonation process, followed by sand filters. While the littlest part of MPs is removed in post-ozonation. From Table 40 could be also noticed that the overall removal efficiency, so the removal efficiency of all the DWTP is 56,19%.

Table 40 Removal efficiencies in Summer campaign

July 2020	**Q**	**N°MPs/L**	**MPs Load**	**Removal efficiency**	**Removal efficiency**	**Overall removal efficiency**
	m3/d		**n°/d**	**n°/d**	**%**	**%**
Influent 314	25.056	9,13E-03	2,29E+05			
Pre-ozonation	25.056	1,00E-03	2,51E+04	2,04E+05	89,05	
Flocculation	25.056	5,87E-03	1,47E+05	-1,22E+05	0	
Backwash	8640	3,85E-02	3,33E+05			
Sand filters	25056	3,59E-03	9,00E+04	5,71E+04	38,84	
Post-ozonation	25056	3,01E-03	7,54E+04	1,45E+04	16,16	
Activated carbons	25056	8,04E-03	2,01E+05	-1,26E+05	0	
Effluent	25056	4,00E-03	1,00E+05			56,19

5.1.4 Removal efficiencies of different treatment processes (Winter campaign)

Table 41 reports the removal efficiencies during the Winter campaign. Pre-ozonation efficiency is zero. Flocculation and post-ozonation are release points. Sand filters have an efficiency of 96,15%. The overall efficiency of the plant is 51,25%.

Table 41 Removal efficiencies (Winter campaign)

December 2020	**Q**	**N°MPs/L**	**MPs Load**	**Removal efficiency**	**Removal efficiency**	**Overall removal efficiency**

	m3/d		n°/d	n°/d	%	%
Influent 314	25.056	8,00E-03	2,00E+05			
Pre-ozonation	25.056	8,00E-03	2,00E+05	0,00E+00	0,00	
Flocculation	25.056	2,60E-02	6,51E+05	-4,51E+05	0	
Sand filters	25.056	1,00E-03	2,51E+04	6,26E+05	96,15	
Post-ozonation	25.056	8,00E-03	2,00E+05	-1,75E+05	0	
Activated carbons	25.056	6,00E-03	1,50E+05	5,01E+04	25,00	
Effluent	25.056	3,90E-03	9,77E+04			51,25

5.1.5 Differences between Summer, Winter campaign and data from literature (Water samples)

- **Concentrations**

Mintenig et al.,2018 is the only study from literature which have concentrations comparable with Castreccioni sampling campaign. From Table 42 and Figure 57 it’s possible to compare Summer and Winter concentrations in water samples. Inlet concentration from Mintenig et al.,2018 is quite similar to inlet concentration in Summer at 324m; other influent values of Castreccioni are all higher than literature. Both in Summer and Winter there is an increase of concentrations in flocculation effluent. Other rising point in Summer is activated carbon effluent, while in Winter post-ozonation effluent. In both campaigns final effluent concentrations are similar and around 0,004 n°MPs/L while from literature concentration is 0,001 n°MPs/L. In distributions points values are in both cases lower respect to water line and in Summer concentration is equal to literature value.

Table 42 Differences between MPs concentrations

Sampling point	**Microplastics concentration (Summer) n°/MPs/L**	**Microplastics concentration (Winter) n°/MPs/L**	**Literature n°/MPs/L**
			(Mintenig et al., 2019)
Inlet point 324 m	0,003	0,012	0.004
Inlet point 314 m	0,009	0,008	
Pre-ozonation effluent	0,001	0,008	-
Flocculation effluent	0,006	0,026	
Sand filters effluent	0,004	0,001	

Post-ozonation effluent	0,003	0,008	
Activated carbons effluent	0,008	0,006	
Final effluent (324m)	0,004	0,0039	0.001
Distribution point 1 (Imbrecciata)		0,007	0.002
Distribution point 2 (Montoro)	0,002	0	
Flocculation sludge	0,111		-
Backwash	0,038		-

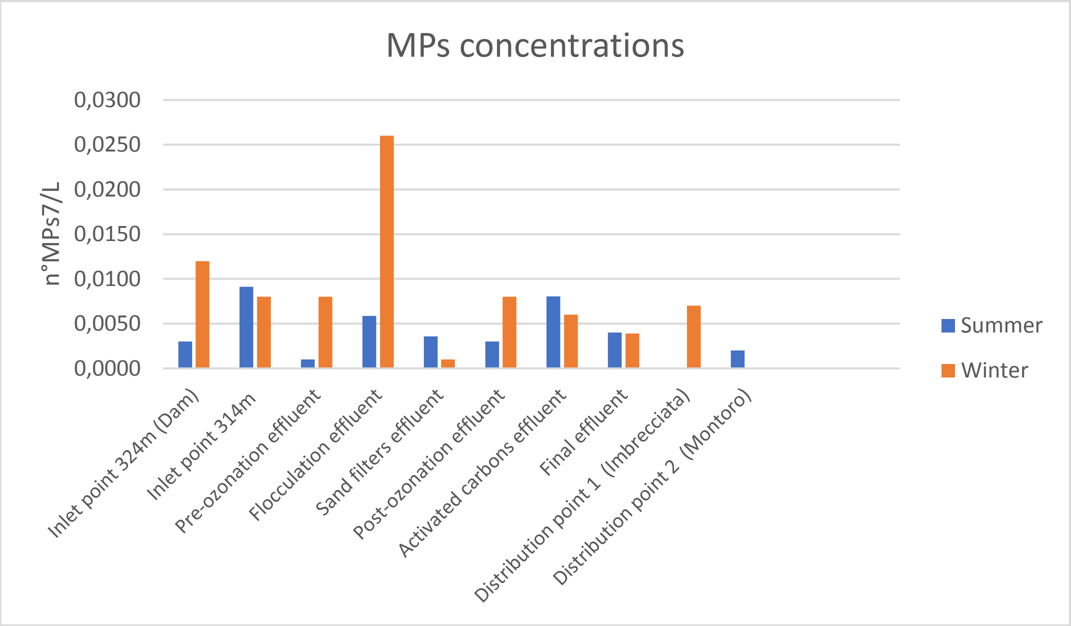


Figure 57 MPs concentrations comparison in water samples

- **Shapes distribution**

A similar comparison could be also done for microplastics' shapes distribution in water samples.

Table 43 Differences between shapes

Sampling point	MPs Shape (frequency %)					
	film Summer	film Winter	fragment Summer	fragment Winter	line Summer	line Winter
Inlet point 324m (Dam)	66,7	7,7	33,3	61,5	0,0	30,8
Inlet point 314m	40,0	37,5	60,0	62,5	0,0	0,0

Pre-ozonation effluent	100,0	75,0	0,0	25,0	0,0	0,0
Flocculation effluent	16,7	19,2	83,3	69,2	0,0	11,5
Sand filters effluent	0,0	0,0	100,0	100,0	0,0	0,0
Post-ozonation effluent	0,0	25,0	100,0	50,0	0,0	25,0
Activated carbons effluent	33,3	16,7	55,6	83,3	11,1	0,0
Final effluent	25,0	0,0	75,0	100,0	0,0	0,0
Distribution point 1 (Imbrecciata)	0,0	1,5	100,0	98,5	0,0	0,0
Distribution point 2 (Montoro)	50,0	42,9	50,0	57,1	0,0	0,0

Film shaped MPs increase in pre-ozonation effluent, then decrease in Summer up to post-ozonation effluent and in Winter up to sand filters effluent. In distribution points frequency of occurrence is similar and near 50%.

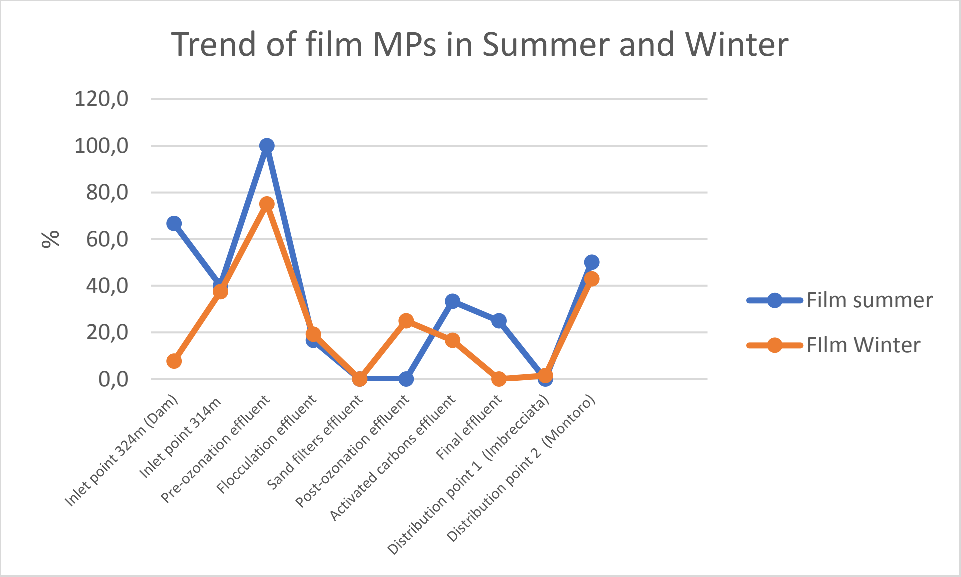


Figure 58 Trend of film MPs in Summer and Winter

In both Summer and Winter campaigns fragments frequency of occurrence decreases in pre-ozonation effluent, while it has an oscillatory trend up to the effluent where they are present with a frequency of 75% in Summer and 100% in Winter. From Wang et.al.,2019 raw water contains from 17.6 to 25.5% of fragments, so values more similar to Summer. While from

M. Pivokonsky et al. studies result that most present shape is fragment itself with values between 42% and 76%. Final effluent both in Summer and Winter is formed by fragments which increase in Wang et.al and remains high in M. Pivokonsky et al. In first distribution point (Imbrecciata) in Castreccioni fragments are both near 100%, in second one (Montoro) they decrease up to 50%.

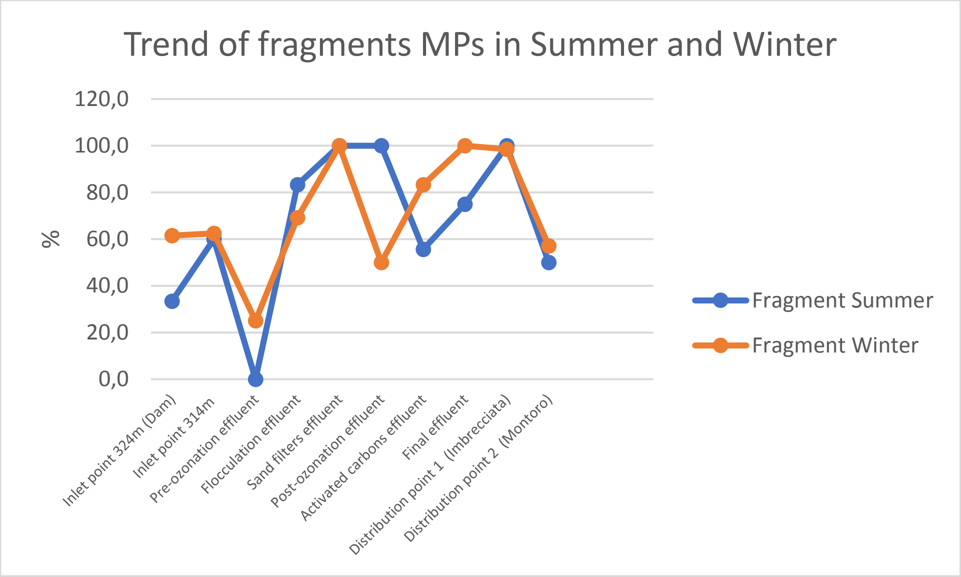


Figure 59 Trend of fragments MPs in Summer and Winter

Line shaped MPs are less present type. An exception is done for inlet point a 324m, flocculation effluent and post-ozonation effluent in Winter campaign. In Summer they are only present in activated carbons effluent.

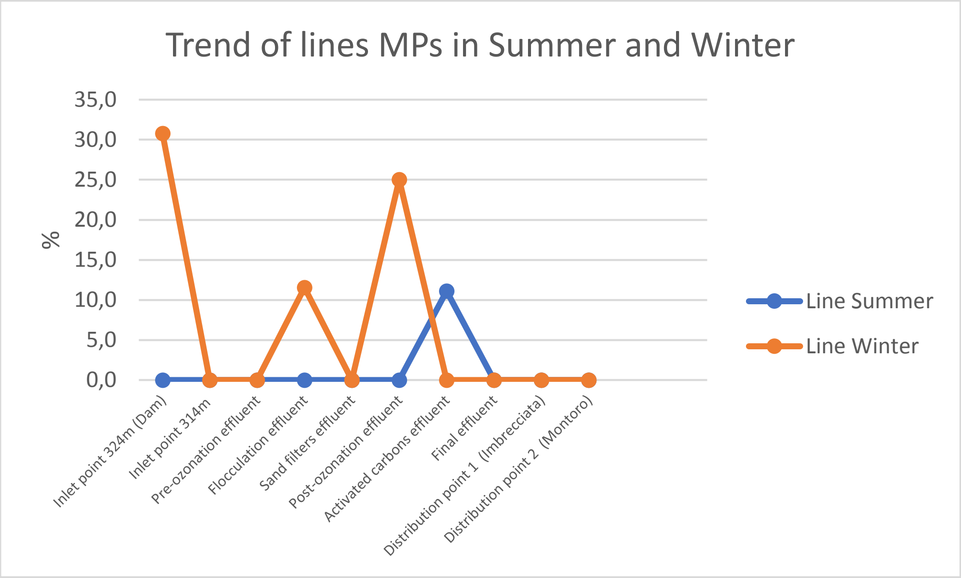


Figure 60 Trend of lines MPs in Summer and Winter

- **Size distribution**

In both campaigns size classes in which MPs are classified are: 2-1mm, 1-0,5mm, 0,5-0,15mm, 0,15-0,05mm. In addition, in Winter classes MPs are also in the ranges: 5-2mm and 0,05-0,02mm. In both campaigns biggest microplastics are present not in the influent to the plant but in effluents from treatment processes: in Summer 2-1mm particles are present in flocculation effluent, in Winter 5-2mm particles are present in post-ozonation effluent. In both situations most present particles ranges are 0,5-0,15mm and 0,15-0,05mm. From literature could be noticed that most of MPs belong to classes 0,001-0,005mm and 0,005-0,1mm because analysed studies (by Wang et al. and M. Pivokonsky et al.) have used filters with smaller dimensions for water samples.

Table 44 Size classes distribution comparison (1)

Sampling point	MPs Size classes (frequency %)					
	5-2mm Summer	5-2mm Winter	2-1mm Summer	2-1mm Winter	1-0,5mm Summer	1-0,5mm Winter
Inlet point 324m (Dam)	0,0	0,0	0,0	7,7	0,0	7,7
Inlet point 314m	0,0	0,0	0,0	0,0	0,0	0,0
Pre-ozonation effluent	0,0	0,0	0,0	0,0	0,0	0,0
Flocculation effluent	0,0	0,0	16,7	3,8	0,0	7,7
Sand filters effluent	0,0	0,0	0,0	0,0	25,0	0,0
Post-ozonation effluent	0,0	12,5	0,0	12,5	0,0	12,5
Activated carbons effluent	0,0	0,0	0,0	0,0	11,1	0,0
Final effluent	0,0	2,6	0,0	0,0	0,0	2,6
Distribution point 1 (Imbrecciata)	0,0	0,0	16,7	3,1	30,0	17,6
Distribution point 2 (Montoro)	0,0	0,0	0,0	0,0	0,0	0,0

Table 45 Size classes distribution comparison (2)

Sampling point	MPs Material (frequency %)

	0,5-0,15mm Summer	0,5-0,15mm Winter	0,15-0,05mm Winter	0,15-0,05mm Summer	0,05-0,02mm Summer	0,05-0,02mm Winter
Inlet point 324m (Dam)	66,7	30,8	33,3	53,8	0,0	0,0
Inlet point 314m	70,0	25,0	30,0	50,0	0,0	25,0
Pre-ozonation effluent	100,0	75,0	100,0	25,0	0,0	0,0
Flocculation effluent	66,7	38,5	16,7	42,3	0,0	7,7
Sand filters effluent	25,0	100,0	50,0	0,0	0,0	0,0
Post-ozonation effluent	66,7	50,0	33,3	12,5	0,0	0,0
Activated carbons effluent	66,7	16,7	22,2	50,0	0,0	33,3
Final effluent	50,0	23,1	50,0	66,7	0,0	5,1
Distribution point 1 (Imbrecciata)	33,3	29,0	20,0	39,7	0,0	10,7
Distribution point 2 (Montoro)	100,0	42,9	0,0	57,1	0,0	0,0

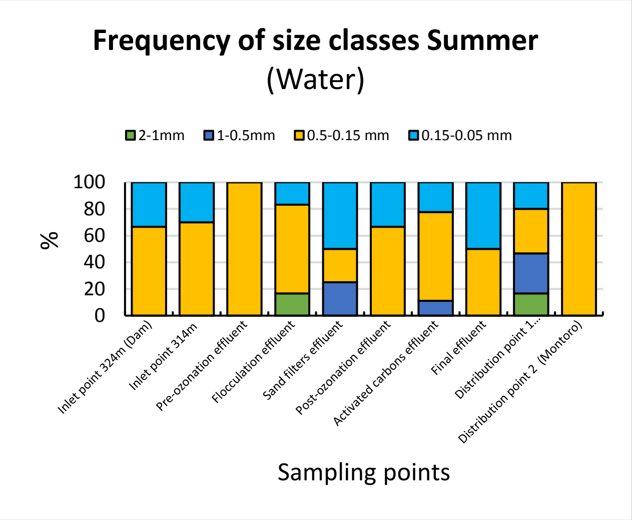


Figure 61 Frequency of size classes in water samples (Summer)

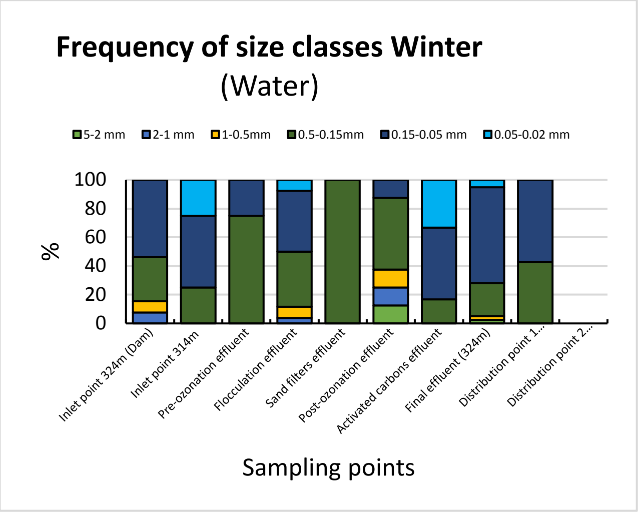


Figure 62 Frequency of size classes in water samples (Winter)

- **Polymers distribution**

Most present polymers during Summer campaign are polyester, polyester resin polyurethane, polyethylene and polypropylene. In Winter campaign most presents are: polyester resin, polypropylene, acrylonitrile butadiene styrene and polyvinylidene fluoride. In the inlet point at 324m common polymer is polyester. In inlet point at 314m common polymers are polyester resin and polypropylene. In Wang et al., 2019 in raw water most present polymers are: PET (55.4–63.1%); PE (almost 15.1–23.8%) e PP (almost 8.4–18.2%) ,so only polypropylene which has been found in winter is a common polymer. While according to M. Pivokonsky,2020 (Milence) also polyethylene and polypropylene are common polymers. In pre-ozonation effluent main part of MPs is formed by polypropylene. In flocculation effluent common polymer is polystyrene. In post-ozonation and activated carbons effluent common polymers are polyester resin and polyethylene. In Winter campaign in activated carbons effluent appears polyvinylidene fluoride which remains also in final effluent. Post-ozonation and flocculation are in Winter two point of particles rising, indeed in Table 46 is possible to see an increased number of new polymers. Same thing could be noticed also in Summer where MPs increasing happens in flocculation and GAC effluent. Final effluent polymers are different between Summer and Winter campaigns, and could be noticed that they are also different from polymers in literature's studies (Table 9)

Table 46 Polymers comparison

Sampling point	MPs Material (frequency %) Summer	MPs Material (frequency %) Winter

Inlet point 324m (Dam)	66,7% polyester, 33,3% polyurethane	23,1% polyester, 30,8% polyester resin, 23,1% polypropylene, 23,1% polyvinylidene fluoride
Inlet point 314m	10% polyester resin, 40% poluyrethane, 10% polypropylene, 30% polyethylene, 10% styrene-butadiene	12,5% polyester resin, 25% polypropylene, 25% polyvinyl chloride, 25% polyacrylic rubber, 25,5 polyurethane
Pre-ozonation effluent	100% polypropylene	75% polypropylene, 25% polyacrylic rubber
Flocculation effluent	16,7% polyurethane , 16,7% polypropylene , 16,7% polyethylene , 33,3% polystyrene , 16,7% polyphenyl ethere + polystyrene	15,4% polyester, 38,5 polyester resin, 7,7% polypropilene, 11,5% polyethylene, 7,7% polystyrene, 3,8% polyvinyl chloride, 3,8% polyvinyl chloride+polyvinyl alcohol, 7,7% thermoplastic elastomer (PEST based), 3,8% epoxide resin
Sand filters effluent	25% polyurethane , 25% polypropylene , 50% polyethylene ,	100% polyacrylate
Post-ozonation effluent	33,3% polyester resin , 66,7% polyethylene	12,5% polyester, 12,5% polyester resin, 25% polyethylene, 12,5% polyvinyl chloride, 12,5% epoxide resin, 12,5% polyacrylate,
Activated carbons effluent	22,2% polyester resin , 11,1% polyester , 33,3% polyurethane , 11,1% polyethylene , 22,2 % polyvinyl chloride	66,7% polyester resin, 16,7% polyethylene , 16,7% polyvinylidene fluoride
Final effluent	25% polyester , 25% polyurethane , 25% polyethylene , 25% styrene-butadiene	5,1% polypropylene, 82,1% polyvinylidene fluoride, 12,8% polyacrylic rubber
Distribution point 1 (Imbrecciata)	100% polyvinylidene fluoride	42.9% polyester resin, 14,3% polyethylene , 28,6% polytetrafluoroethylene , 14,3% silicone
Distribution point 2 (Montoro)	50% polyethylene, 50% polytetrafluoroethylene	-

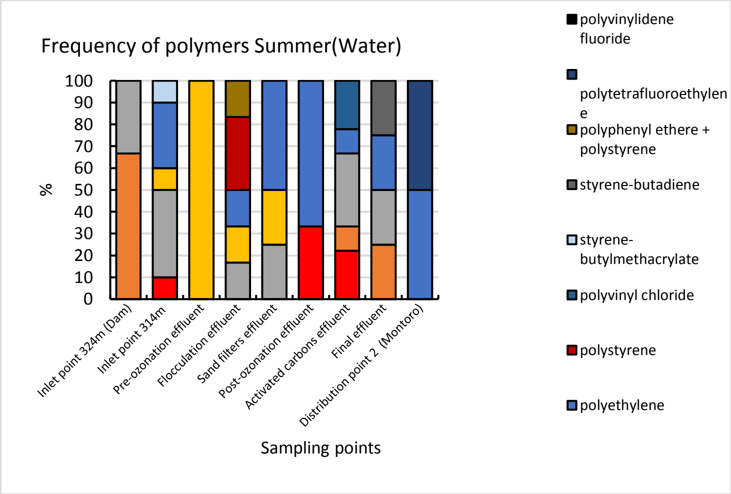


Figure 63 Frequency of polymers Summer(Water))

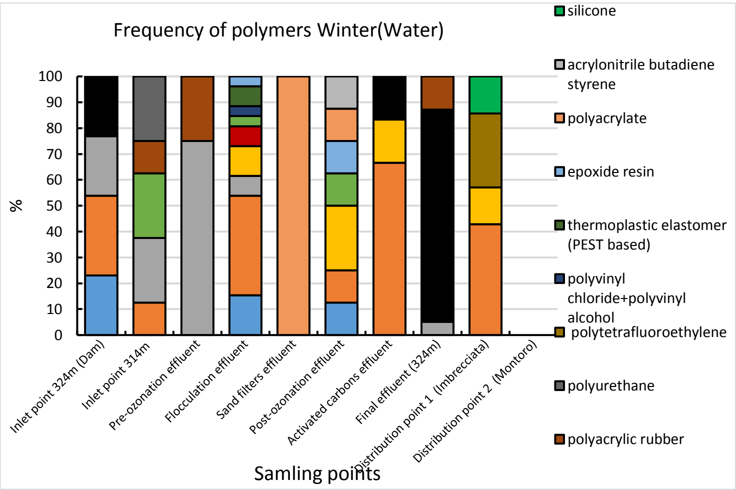


Figure 64 Frequency of polymers Winter(Water)

- **Removal efficiencies**

For what concerns removal efficiency while in pre-ozonation in Summer is 89%, in Winter it is of 0%, so more similar to study Z.Wang and M. Pivokonský results. Flocculation has quite strange results because there is an increase of particles and so it's not possible to check effective removal efficiency, which according to M. Pivokonský et al., 2020 is almost 62% and 41,7% for Wang.et al. Sand filtration efficiency is 38,8% in Summer, near to Z. Wang et al., 2019 value, while in Winter it increases to 96,2%. Post-ozonation has little values of efficiency: 16,2% in Summer and negative in Winter because of an increase in MPs concentration. GAC efficiency is negative in Summer and 25% in Winter, near M. Pivokonský value of 33,5%.

Table 47% Comparison between Summer and Winter removals

	Removal/Release efficiency during Winter campaign	Removal/Release efficiency during Summer campaign	Z. Wang, 2020	M. Pivokonský; 2020 (Plzen)
	%	%	%	%
Influent 314				
Pre-ozonation	89,0	0,0	0	7,8
Flocculation	0,0	0,0	47,6	61,7
Sand filters	38,8	96,2	40,4	
Post-ozonation	16,2	0,0	0	7,8
Activated carbons	0,0	25,0	56,5	33,5
Effluent				

5.2 QMRA MODEL RESULTS (PESCHIERA BORROMEO CASE STUDY)

Results about E.coli have been only presented in terms of risk of infection since was not possible to calculate DALYs due to lacking data (infection:illness ratio, DALY per case and susceptibility).

5.2.1 Worst scenario: Influence of E.coli concentration

The worst scenario is representing by applying no barrier in situ and to the final products. It means the E.coli effluent from Peschiera Borromeo WWTP is the same of that in ingested water.

5.2.1.1 No-reuse scenario

No-reuse scenario point analysis assumed an E.coli input concentration of 1937 CFU/100mL. Results have been calculated in terms of DALYs and of Risk of infection and they have been resumed in Table 48 (drip irrigation) and Table 49 (spray irrigation).

Table 48 DRIP irrigation results in no-reuse scenario without barriers

E.Coli	Campylobacter	Cryptosporidium	Rotavirus

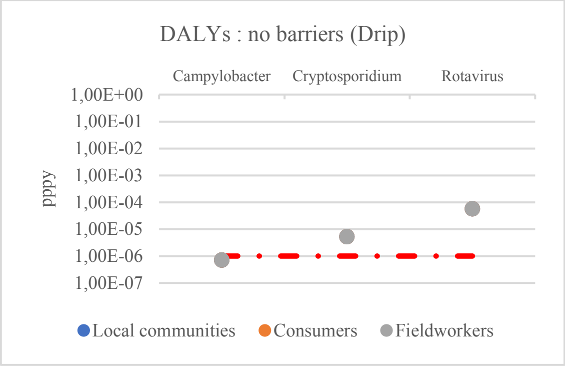
Drip irrigation		Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
Local communities	no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
Consumers	no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
Fieldworkers	no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,74E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded

Table 49 SPRAY irrigation results in no-reuse scenario without barriers

		E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Spray irrigation		Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
Local communities	no barriers	1,74E-02	5,28E-04	7,29E-07	5,14E-03	5,39E-06	7,89E-02	5,83E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
Consumers	no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
Fieldworkers	no barriers	1,72E-02	5,22E-04	7,20E-07	5,08E-03	5,33E-06	7,80E-02	5,76E-05
		Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded

DALYs calculated without barriers are comparable for fieldworkers, local communities and consumers for all three pathogens because consume is assumed to be the same for all categories: 140 times per year and 1mL per event. But comparing the two irrigation methods, the spray scenario shows slightly higher level of risk because of the additional possibility of particles inhalation (which is not present using drip irrigation). It is valid for fieldworkers and local communities, while the consumers are not affected since the amount of consumed products and the number of events per year is not influenced by the agronomic procedure.

The red line in Figure 65 and Figure 66 represents the DALY limit of $1*10^{-6}$ (the health target suggested by the WHO Guidelines). It could be noticed that in both irrigation scenarios Cryptosporidium and Rotavirus pose a higher risk than the acceptable one.



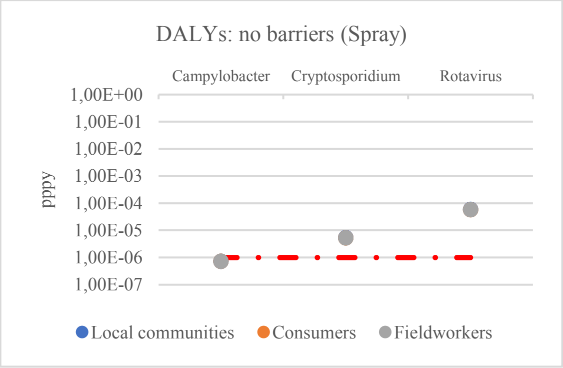


Figure 65 DALYs in no barriers drip case (no reuse)

Figure 66 DALYs in no barriers spray case (no reuse)

Risk could be evaluated also in terms of risk of infection (Pinf) which is the probability that one person can be infected in one year (pppy). In this case results for drip and spray irrigation have been both resumed in Figure 67. Also, in this case the final risk for consumers, fieldworkers and local communities is almost comparable for each pathogen, since the inhalation or ingestion of soil/water is negligible compared to the consumption of final products.

The main difference with DALYs outcomes is that all the values are above the acceptable limit of $1*10^{-4}$ suggested by the US.EPA even if there is the same trend: higher risk for Rotavirus, in this case followed by E.coli, Cryptosporidium and Campylobacter.

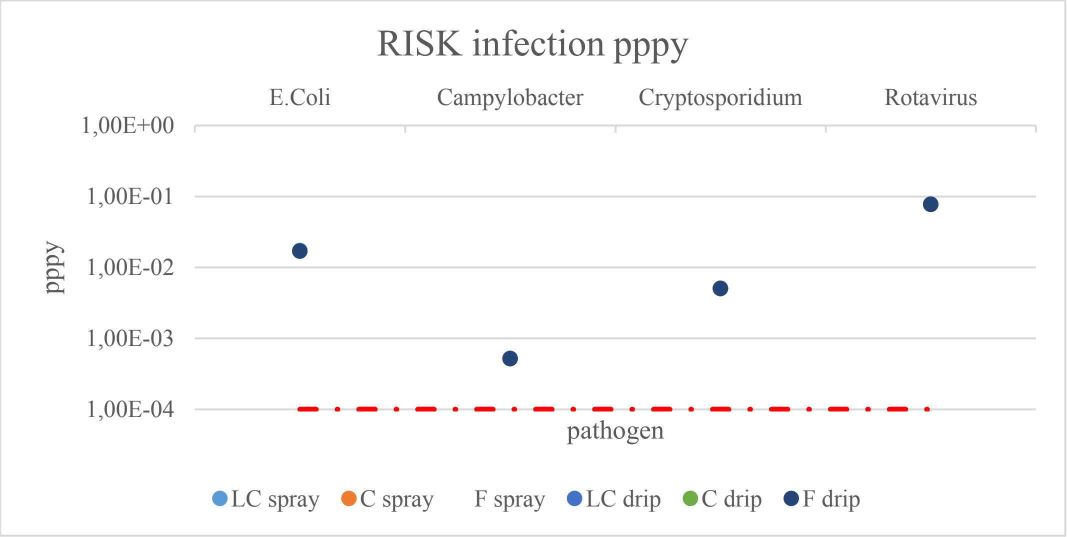


Figure 67 Risk of infection without barriers in drip and spray cases (no-reuse)

Where:

- LC spray= local communities (spray)
- C spray=consumers (spray)
- F spray= filedworkers (spray)
- LC drip= local communities (drip)

- C drip=consumers (drip)
- F drip= filedworkers (drip)

5.2.1.2 Reuse scenario

When considering the Reuse period, the only difference in the risk calculation is about the E.coli input concentration. It was assumed the mean value of 16 CFU/100mL.

Results have been calculated in terms of DALYs and of Risk of infection and they have been resumed in Table 50**Errore. L'autoriferimento non è valido per un segnalibro.** (drip irrigation) and Table 51 (spray irrigation).

Table 50 DRIP irrigation results in reuse scenario without barriers

		E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Drip irrigation		Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
Local communities	no barriers	1,42E-04	4,29E-06	5,91E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
Consumers	no barriers	1,42E-04	4,28E-06	5,91E-09	4,18E-05	4,39E-08	2,37E-04	1,75E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
Fieldworkers	no barriers	1,42E-04	4,29E-06	5,92E-09	4,18E-05	4,39E-08	2,37E-04	1,75E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok

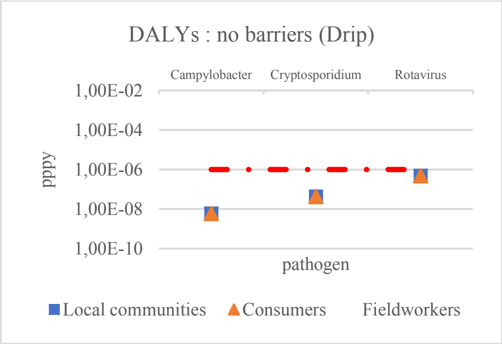
Table 51 SPRAY irrigation results in reuse scenario without barriers

		E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Spray irrigation		Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
Local communities	no barriers	1,45E-04	4,36E-06	6,02E-09	4,25E-05	4,47E-08	6,79E-04	5,02E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
Consumers	no barriers	1,42E-04	4,28E-06	5,91E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
Fieldworkers	no barriers	1,43E-04	4,31E-06	5,95E-09	4,20E-05	4,41E-08	6,70E-04	4,95E-07
		Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok

It is expected the same pattern of the previous section, but with a resulting lower level of risk. Indeed, once again, the DALYs and risk of infection for the three group of exposure are comparable and the spray irrigation results to be the more risk method due to the additional route of exposure for fieldworkers and local communities.

On the other hand, with a so high quality of reclaimed water (class B of new EU Regulation 202/741), no reference pathogens pose a risk to the human health if compared to the DALYs threshold. This is shown in Figure 68 and

Figure 69.



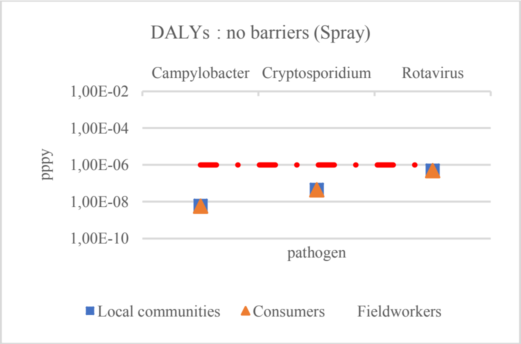


Figure 68 DALYs in no barrier drip case (reuse)

Figure 69 DALY in no barriers spray case (reuse)

Risk of infection's results for drip and spray irrigation have been both resumed in Figure 70. In that case, E.coli and Rotavirus still overcome the U.S. EPA target of $1*10^{-4}$, while Campylobacter and Cryptosporidium don't. Actually the risk of infection of E.coli is comparable to the health target ($1.42x10^{-6}$ vs $1x10^{-6}$). While the rotavirus is however decreased respect the no reuse period of 2 order of magnitude.

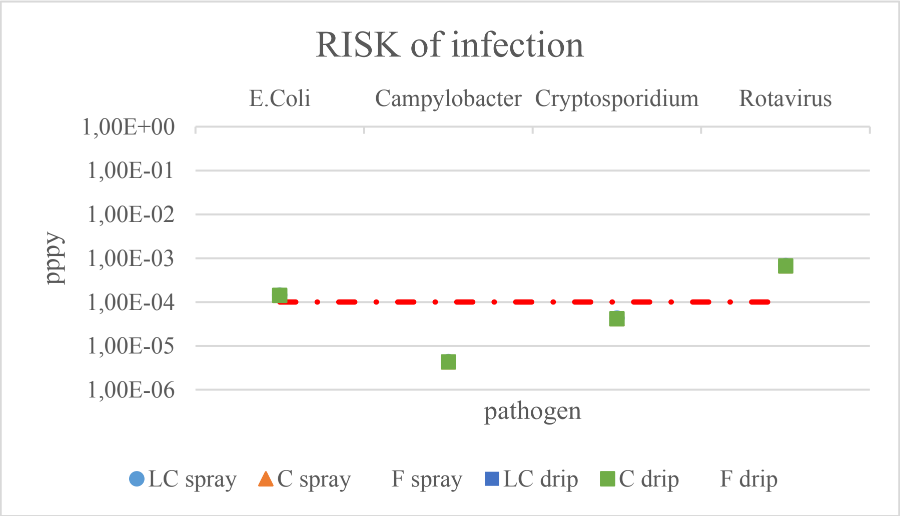


Figure 70 Risk of infection without barriers in drip and spray cases (reuse)

Where:

- LC spray= local communities (spray)
- C spray=consumers (spray)
- F spray= filedworkers (spray)
- LC drip= local communities (drip)

- C drip=consumers (drip)
- F drip= filedworkers (drip)

Main differences between No-Reuse and Reuse scenario without barriers are that in first one considering DALYs only Campylobacter is not at risk, while in second case all three pathogens are not at risk. Considering Risk of infection in No-Reuse scenario all four pathogens result to be at risk, while in Reuse scenario only E.coli and Rotarvirus are above the target.

5.2.2 Influence of on-site preventive measures

5.2.2.1 No-reuse scenario

- **Fieldworkers**

Fieldworkers are exposed to risk because they are in crops during irrigation in working days, they could ingest a bigger volume of contaminated water than local communities and they could be also consumers of products. Applied barriers could be divided in two levels: barriers to have raw products and barriers to have processed products. Common to both level of barriers is to take into account the log reduction due to the irrigation methods. The Australian Guidelines suggest a 3-log reduction for drip irrigation, while with spray irrigation only 1-log is achievable if control systems are adopted. In Table 52 and Table 53 have been resumed all risks in terms of DALY and Probability of infection per person per year in drip and spray irrigation case.

Table 52 Fieldworkers risks drip irrigation (no reuse)

Drip irrigation	**E.Coli**	**Campylobacter**		**Cryptosporidium**		**Rotavirus**	
Fieldworkers	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,74E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	1,72E-11	5,13E-13	7,08E-16	5,07E-12	7,08E-16	8,07E-11	5,97E-14
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00

	Ok	Ok	Ok	Ok	Ok	Ok	Ok

Table 53 Fieldworkers risks spray irrigation (no reuse)

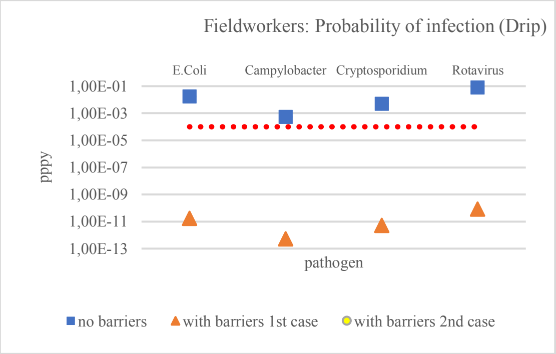
Spray irrigation	**E.Coli**	**Campylobacter**		**Cryptosporidium**		**Rotavirus**	
Fieldworkers	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,72E-02	5,22E-04	7,20E-07	5,08E-03	5,33E-06	7,80E-02	5,76E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	1,73E-08	5,21E-10	7,19E-13	5,08E-09	5,34E-12	8,11E-08	6,00E-11
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	8,50E-09	2,56E-10	3,53E-13	2,49E-09	2,62E-12	3,98E-08	2,94E-11
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

In

Figure 72 and Figure 71 results for fieldworkers in terms of probability of infection have been resumed. It could be noticed that even using first level of barriers all pathogens don't overcome the risk, so second level of barriers are not necessary. As expected, the trend of pathogens is the same: higher risk for Rotavirus, then E.coli, Cryptosporidium and Campylobacter. Indeed, the on-site preventive measures are assumed to have the same effect on all the three categories of pathogen (bacteria, viruses, protozoa). It is an easier way to reduce the quantity of water that the exposure group enters in contact with.

As mentioned above, in this analysis the irrigation methods are now considered also as on-site barrier. It is evident that using spray irrigation, risk is of almost 3-4 order of magnitude bigger than using drip because the latter causes more localized contact of crops with water.

The fieldworker is assumed to be exposed to a dose of $1*10^{-3}$ mL of E.coli for 100 times per year, if drip or spray irrigation is used, respectively.



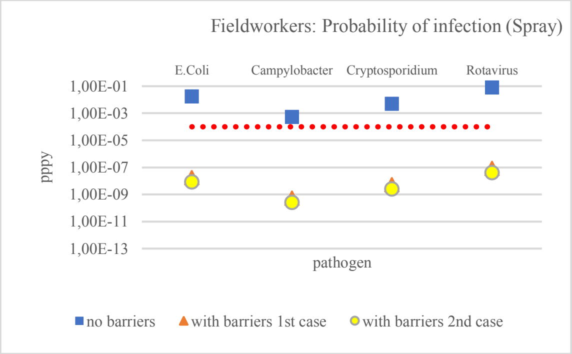


Figure 71 Fieldworkers Pinf no-reuse drip case

Figure 72 Fieldworkers Pinf no-reuse spray case

- **Local communities**

For the local communities, similar discussion to fieldworkers can be conducted. They live in the proximity of crops, so could be exposed both to accidental ingestion and to consumption of products. In Table 54and Table 55 have been resumed all risks in terms of DALY and Probability of infection per person per year in drip and spray irrigation case.

Table 54 Local communities risks drip irrigation (no reuse)

Drip irrigation	**E.Coli**	**Campylobacter**		**Cryptosporidium**		**Rotavirus**	
Local communities	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	1,72E-11	5,13E-13	7,08E-16	5,07E-12	7,08E-16	8,07E-11	5,97E-14
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

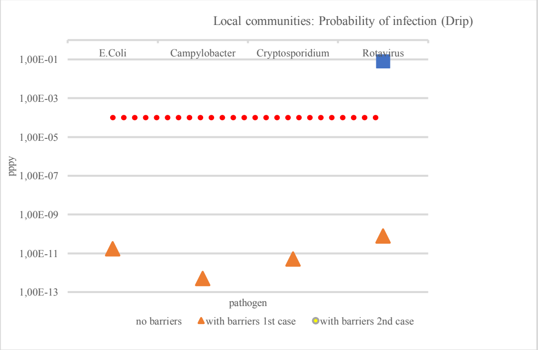
Table 55 Local communities risks spray irrigation (no reuse)

Spray irrigation	**E.Coli**	**Campylobacter**	**Cryptosporidium**	**Rotavirus**

Local communities	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,74E-02	5,28E-04	7,29E-07	5,14E-03	5,39E-06	7,89E-02	5,83E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	2,05E-08	6,16E-10	8,50E-13	6,01E-09	6,31E-12	9,58E-08	7,08E-11
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	3,22E-09	9,70E-11	1,34E-13	9,46E-10	9,94E-13	1,51E-08	1,12E-11
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

In Figure 73 and Figure 74 the results for local communities in terms of probability of infection have been resumed. Could be noticed that also in this case first level of barriers is enough to put in security all pathogens, so second level of barriers could be not used. In particular first level of barriers limit ground contact of tomatoes, limit the public access during irrigation, consider a natural die-off and washing of final products before consumption. Second level of barriers add only the cooking process and the removal of skins before consumption.

Also with barriers the trend of pathogens is the same: higher risk for E.coli, then Rotavirus, Cryptosporidium and Campylobacter. Using spray irrigation risk is of almost 3-4 order of magnitude bigger than using drip because fieldworkers could be also exposed to inhalation of sprays.



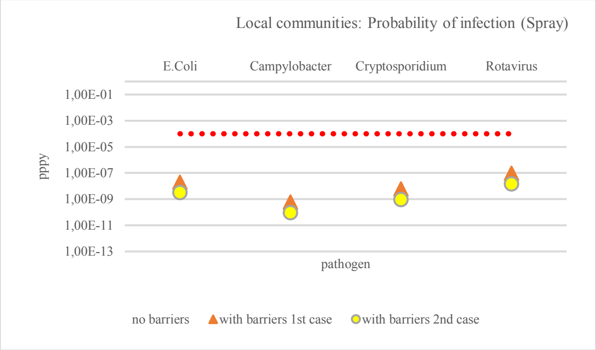


Figure 73 Local communities Pinf no-reuse drip case

Figure 74 Local communities Pinf no-reuse spray case

- **Consumers**

Consumers results have been summarized in Table 56and Table 57. The first level of barriers means to consume a raw product irrigated with a specific technique, while in the second one tomatoes are processed (9-log removal value for first level of barriers and 16-log removal value for second level) after being irrigated in the same way.

Results highlight that the use of drip irrigation or controlled spray method, guarantees a safe consumption of raw tomatoes even if class D reclaimed water is used. Actually, the EU Regulation 2020/741 does not permit the reuse of such water for food-crops irrigation.

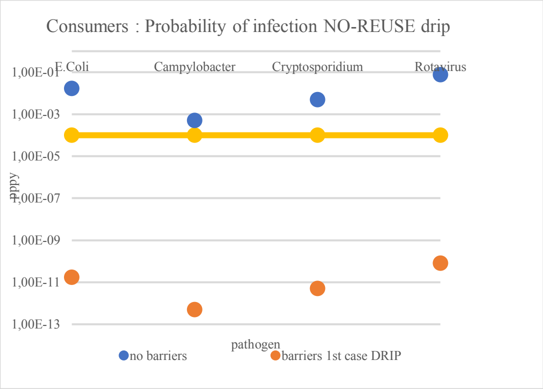
Table 56 Consumers risks drip irrigation (no reuse)

Drip irrigation	**E.Coli**	**Campylobacter**		**Cryptosporidium**		**Rotavirus**	
Consumers	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	1,72E-11	5,13E-13	7,08E-16	5,07E-12	5,32E-15	8,07E-11	5,97E-14
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

Table 57 Consumers risks spray irrigation (no reuse)

Spray irrigation	**E.Coli**	**Campylobacter**	**Cryptosporidium**	**Rotavirus**

Consumers	**Pinf (pppy)**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**	**Pinf (pppy)**	**DALY/y**
no barriers	1,71E-02	5,19E-04	7,16E-07	5,05E-03	5,30E-06	7,76E-02	5,73E-05
	Risk exceeded	Risk exceeded	Ok	Risk exceeded	Risk exceeded	Risk exceeded	Risk exceeded
with barriers 1st case	1,72E-08	5,19E-10	7,16E-13	5,06E-09	5,31E-12	8,07E-08	5,97E-11
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	1,55E-14	1,15E-17
	Ok	Ok	Ok	Ok	Ok	Ok	Ok



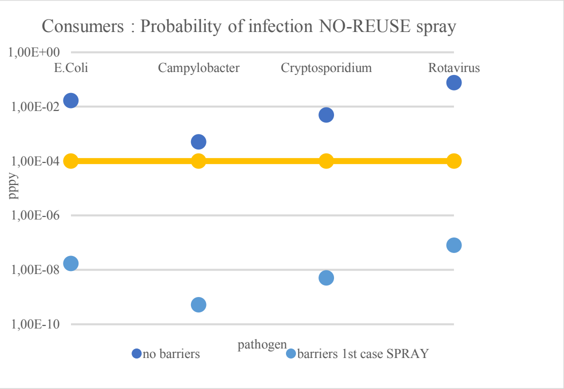


Figure 75 Consumers Pinf no-reuse drip case

Figure 76 Consumers Pinf no-reuse spray case

5.2.3 Reuse scenario

In reuse scenario input concentration of E.coli is much lower than previous case, so final risk would be of different orders of magnitude respect to non-reuse case. Considering that in the no reuse scenario the first level of barrier is sufficient to ensure a safe water reuse, in this reuse scenario is not relevant to analyse a second level of barriers. At the same time, we expect the first level insufficient to guarantee a highly safe management of irrigation.

- **Fieldworkers**

In this case risk in terms of both DALY and probability of infection would be much lower than target values.

Table 58 Fieldworkers risks drip irrigation (reuse)

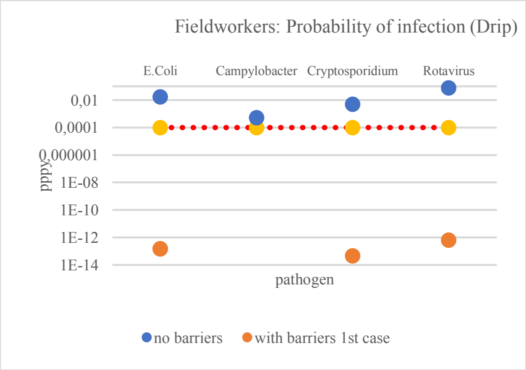
Drip irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Fieldworkers	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,42E-04	4,29E-06	5,92E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07

	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,55E-13	0,00E+00	0,00E+00	4,66E-14	0,00E+00	6,68E-13	4,94E-16
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

Table 59 Fieldworkers risks spray irrigation (reuse)

Spray irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Fieldworkers	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,43E-04	4,31E-06	5,95E-09	4,20E-05	4,41E-08	6,70E-04	4,95E-07
	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,43E-10	4,31E-12	5,95E-15	4,20E-11	4,41E-14	6,70E-10	4,95E-13
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	7,02E-11	2,11E-12	2,91E-15	2,06E-11	2,16E-14	3,29E-10	2,43E-13
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

As could be seen in Figure 77 and Figure 78 use of second level barrier is not useful and could be avoided.



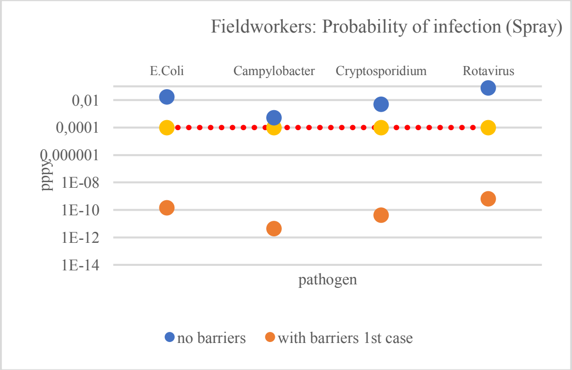


Figure 77 Fieldworkers Pinf reuse drip case

Figure 78Fieldworkers Pinf reuse spray case

- **Local communities**

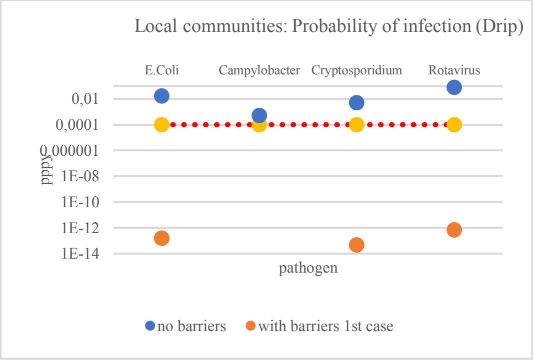
As for fieldworkers, local communities could be considered in safety respect to all considered pathogens. In particular second level barriers could be considered useless.

Table 60 Local communities risks drip irrigation (reuse)

Drip irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Local communities	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,42E-04	4,29E-06	5,91E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07
	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,55E-13	0,00E+00	0,00E+00	4,66E-14	0,00E+00	6,68E-13	4,94E-16
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

Table 61 Local commnities risks spray irrigation (reuse)

Spray irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Local communities	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,45E-04	4,36E-06	6,02E-09	4,25E-05	4,47E-08	6,79E-04	5,02E-07
	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,69E-10	5,09E-12	7,03E-15	4,96E-11	5,21E-14	7,91E-10	5,85E-13
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	2,66E-11	8,01E-13	1,11E-15	7,84E-12	8,23E-15	1,25E-10	9,22E-14
	Ok	Ok	Ok	Ok	Ok	Ok	Ok



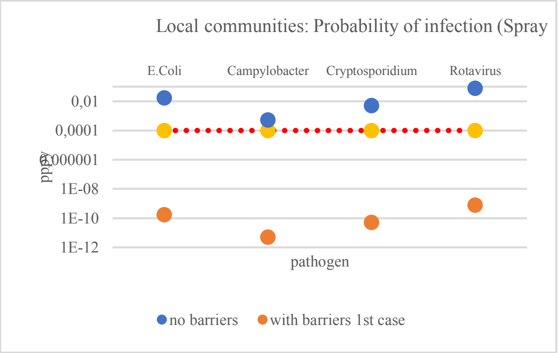


Figure 79 Local communities Pinf no-reuse drip case

Figure 80 Local communities Pinf no-reuse spray case

- **Consumers**

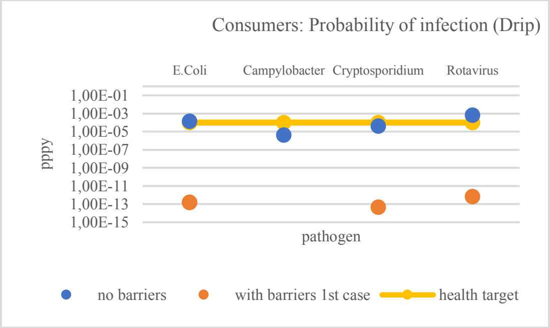
Consumers results have been summarized in Table 62 and Table 63. Also, in this case first level of barriers are enough to guarantee an acceptable level of risk.

Table 62 Consumers risks drip irrigation (reuse)

Drip irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Consumers	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,42E-04	4,28E-06	5,91E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07
	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,55E-13	0,00E+00	0,00E+00	4,66E-14	4,90E-17	6,68E-13	4,94E-16
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok

Table 63 Consumers risks spray irrigation (reuse)

Spray irrigation	E.Coli	Campylobacter		Cryptosporidium		Rotavirus	
Consumers	Pinf (pppy)	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y	Pinf (pppy)	DALY/y
no barriers	1,42E-04	4,28E-06	5,91E-09	4,18E-05	4,39E-08	6,67E-04	4,93E-07
	Risk exceeded	Ok	Ok	Ok	Ok	Risk exceeded	Ok
with barriers 1st case	1,42E-10	4,29E-12	5,92E-15	4,18E-11	4,39E-14	6,67E-10	4,93E-13
	Ok	Ok	Ok	Ok	Ok	Ok	Ok
with barriers 2nd case	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	Ok	Ok	Ok	Ok	Ok	Ok	Ok



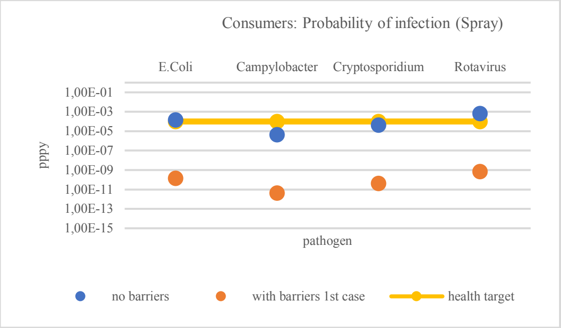


Figure 81 Consumers Pinf reuse drip case

Figure 82 Consumers Pinf reuse spray case

5.2.4 Influence of exposure assessment

A further consideration is presented about the exposure assessment. Up to now, both fieldworkers and local communities are exposed to risk even through the consumption of final products. In the following, the influence of not considering fieldworkers and local communities as consumers is assessed.

The results in Figure 83 and Figure 84 are related only to the worst scenario. From the previous section, indeed, we know that the application of the first level of barrier is sufficient to ensure a safe water reuse and therefore it is no more investigated.

The main differences between each exposure group are related to the activity they perform in the site. Obviously, the level of risk for local community and fieldworkers will decrease than the previous section, but a proper calculation is needed to define if it is acceptable or not. For instance, local communities are still at risk when spray irrigation is used with a reclaimed water of class D ("no reuse period"). It is due to the exposure to aerosol 365 days per year. On the other hand, if drip irrigation is applied, the risk is acceptable both for fieldworkers and local communities.

Using a reclaimed water with an average of 16 E.coli CFU/100 ml (reuse scenario), the risk is always below the acceptable value.

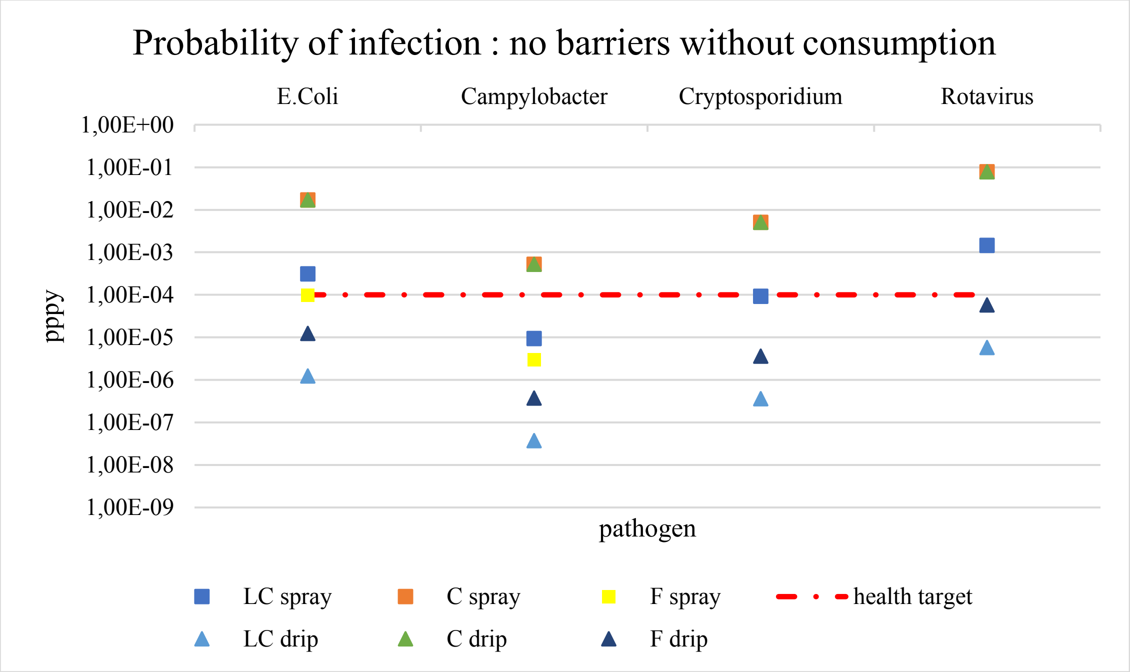


Figure 83 Pinf without barriers, not considering consumption (no reuse)

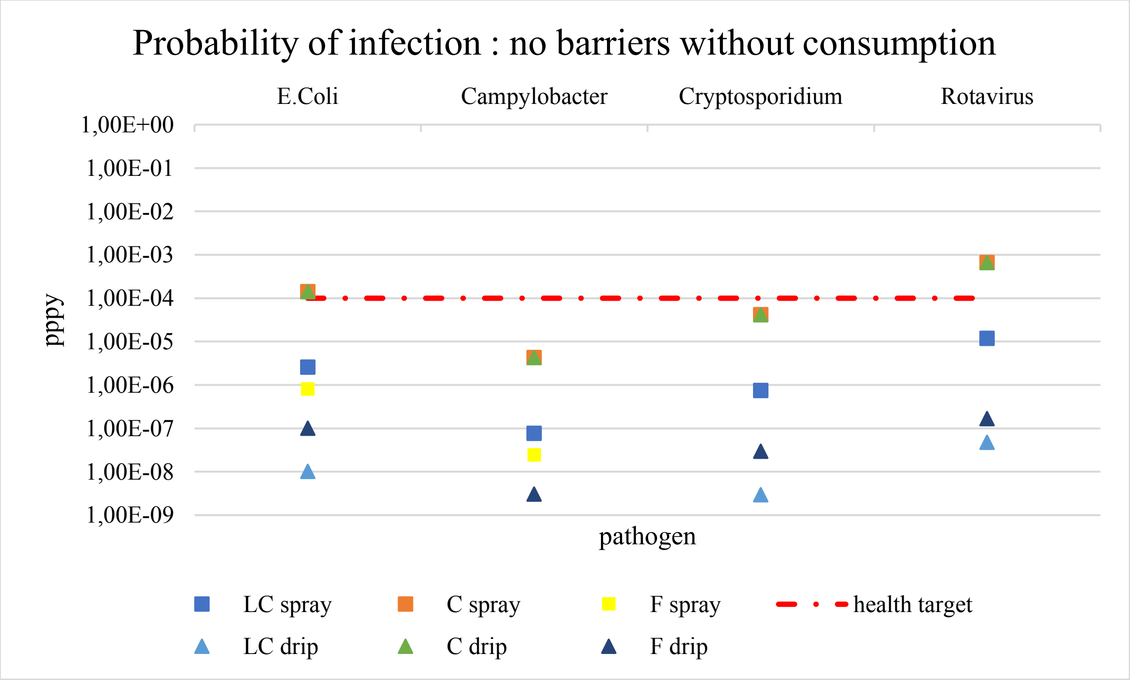


Figure 84 Pinf without barriers, not considering consumption (reuse)

5.2.5 Monte Carlo simulation: Consumers

Monte Carlo simulation has been used only to assess the risk of consumers in the worst scenario, both considering low and high E.coli concentration. The results are compared to the ones obtained through a point estimation approach.

In Figure 85 is showed the final plot of Monte Carlo simulation resulting from "No reuse" period. In this case, respect to point analysis in Figure 65 and Figure 66, also Campylobacter is at risk. This difference could be due to the fact that Monte Carlo considers a range of value in which mean value could be higher than the respective of point analysis.

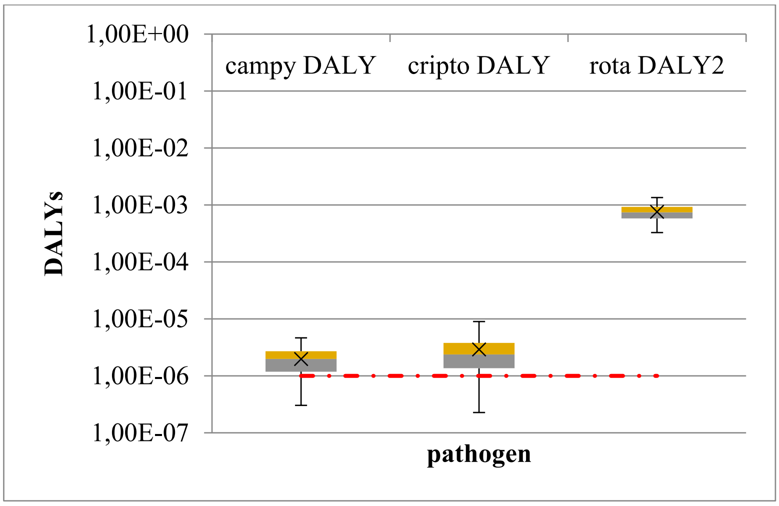


Figure 85 Monte Carlo final output in terms of DALYs without barriers (no reuse)

Probability of infection remains for all three pathogens over the U.S. EPA target of $1*10^{-4}$ as in point analysis.

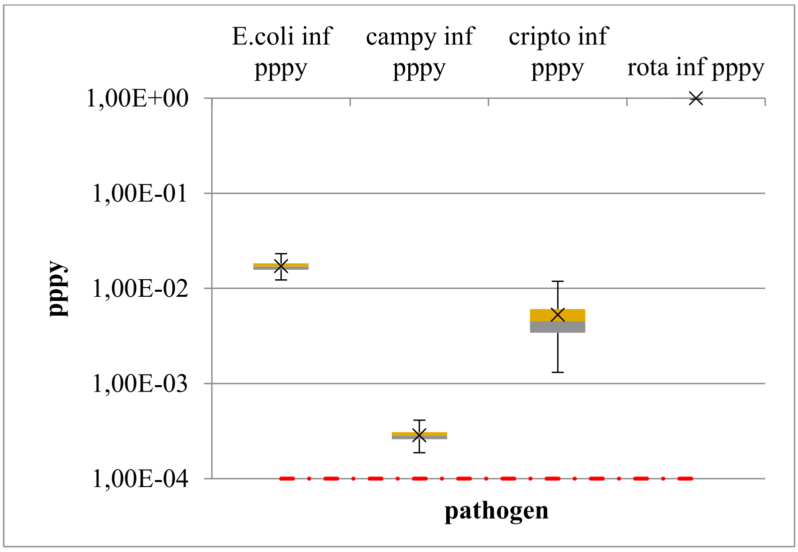


Figure 86 Monte Carlo final output in terms of risk of infection without barriers (no-reuse)

In Table 64 could be seen minimum, maximum, mean and the median values obtained using stochastic analysis. These values are compared with the mean value obtained in deterministic analysis. Effectively while in point analysis mean value was under the DALY target, in Monte Carlo it is higher than it. The risk per person per year of all pathogens are of the same order of magnitude between the two analysis; except for Rotavirus which using Monte Carlo is one order of magnitude higher, but it remains in any case at risk.

Table 64 Risk comparison in terms of DALYs and risk of infection for Consumers without barriers (no-reuse)

		DALYs "No-reuse" Consumers without barriers				**Risk of infection "No-reuse" Consumers without barriers**			
		Min	**Max**	**Median**	**Mean**	**Min**	**Max**	**Median**	**Mean**
E.COLI	MCA					1,23E-02	2,32E-02	1,70E-02	1,71E-02
	PA								1,71E-02
Campylobacter	MCA	3,03E-07	4,64E-06	1,99E-06	1,99E-06	1,87E-04	4,12E-04	2,83E-04	2,85E-04
	PA				7,16E-07				5,19E-04
Cryptosporidium	MCA	2,27E-07	8,99E-06	2,36E-06	2,91E-06	1,31E-03	1,19E-02	4,52E-03	5,26E-03
	PA				5,30E-06				5,05E-03
Rotavirus	MCA	3,28E-04	1,35E-03	7,46E-04	7,66E-04	9,77E-01	1,00E+00	9,96E-01	9,93E-01
	PA				5,73E-05				7,76E-02

MCA=Monte Carlo analysis, PA=point analysis

In Reuse scenario Campylobacter and Cryptosporidium remain under the maximum level like in Figure 68 and Figure 69, but Rotavirus in this case is higher than $1*10^{-6}$. The risk of infection has the same trend of point analysis.

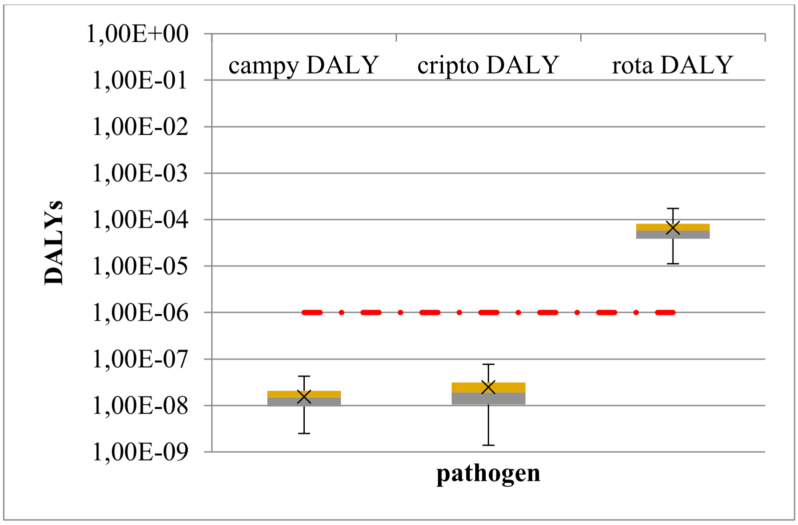


Figure 87 Monte Carlo final output in terms of DALYs without barriers (reuse)

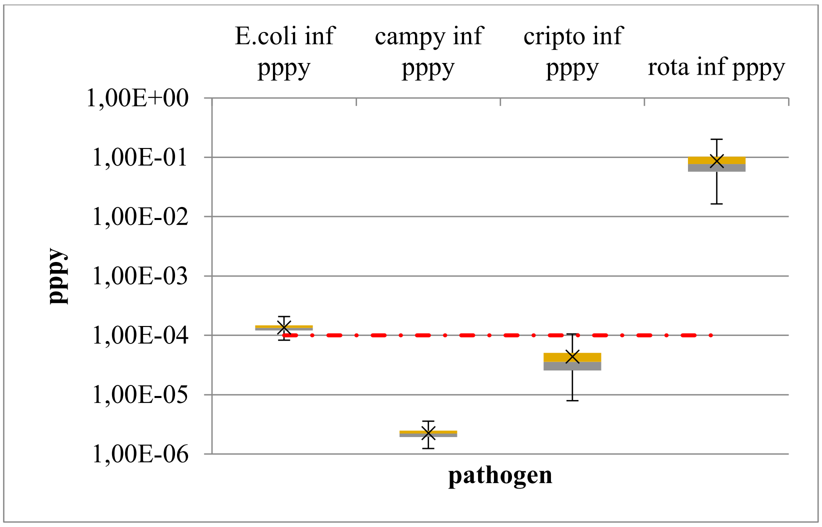


Figure 88 Monte Carlo final output in terms of risk of infection without barriers (reuse)

From Table 65 could be noticed that Rotavirus' DALY is two order of magnitude higher in Monte Carlo than in point analysis and this led to the fact that in this case is at risk. Also in this case only Rotavirus is two orders of magnitude higher using M.C. than point value.

Table 65 Risk comparison in terms of risk of infection and DALYs for Consumers without barriers (no-reuse)

		DALYs "Reuse" Consumers without barriers				**Risk of infection "Reuse" Consumers without barriers**			
		Min	**Max**	**Median**	**Mean**	**Min**	**Max**	**Median**	**Mean**
E.COLI	MCA					6,72E-05	2,11E-04	1,34E-04	1,35E-04
	PA								1,42E-04
Campylobacter	MCA	2,5E-09	4,3E-08	1,5E-08	1,5E-08	1,04E-06	3,55E-06	2,21E-06	2,25E-06
	PA				5,9E-09				4,28E-06
Cryptosporidium	MCA	1,4E-09	7,7E-08	1,9E-08	2,5E-08	8,30E-06	1,06E-04	3,46E-05	4,39E-05
	PA				4,4E-08				4,18E-05
Rotavirus	MCA	1,1E-05	1,7E-04	5,8E-05	6,6E-05	2,16E-02	2,03E-01	7,53E-02	8,39E-02
	PA				4,9E-07				6,67E-04

MCA=Monte Carlo analysis, PA=point analysis

Comparing the mean value of the probability density functions used in the Monte Carlo Analysis, with the average value assumed in the point analysis, only for reference ratios there is a considerable difference. This is the main reason that led to the different distribution of DALYs and risk of infection seen above.

Table 66 Reference ratios comparison between deterministic and stochastic analysis

Monte Carlo analysis	**Point analysis**

RATIO E.COLI-REFERENCE	campy/e.coli	cripto/e.coli	rota/e.coli	campy/e.coli	cripto/e.coli	rota/e.coli
median	5,5E-06	5,5E-07	5,5E-06			
MEAN	5,5E-06	5,5E-07	5,5E-06	1,00E-05	1,00E-06	1,00E-06

6 CONCLUSIONS

In this study microplastic particles in the DWTP of Castreccioni (Cingoli) have been analysed during two sampling campaigns to understand their characteristics in terms of concentrations, shapes, sizes, polymers and the amount that could be removed by treatment processes of the plant. Results have been compared with literature to verify the truthfulness of data to represent reality. Microplastic concentrations result to be similar to concentrations reported in Mintenig et al.,2019 study where similar sampling methods were used. Microplastic particles rise in two points during each season, probably due to infiltrations: in Summer in the flocculation and activated carbon sections, in Winter in the flocculation and post-ozonation sections. Most of the microparticles are in form of fragments, followed by films and lines. In particular fragments increase in the above-mentioned MPs rising points. Fragments are also the most present MPs in Wang et al. and M. Pivokonsky. Most of the particles from Castreccioni DWTP belong to ranges of size 0,5-0,15mm and 0,15-0,05mm. While from a literature overview most MPs in influent and effluent belong to classes 0,001-0,005mm and 0,005-0,1mm. Size classes usually found in literature are smaller than classes analysed in Castreccioni probably because of different sampling methods. Most common polymers in Summer and Winter are polyester, polyester resin, polypropylene and polystyrene. Between them polypropylene has been usually found in literature samples. For what concerns removal efficiencies in Summer, best removals have been done by pre-ozonation and sand filters, while in Winter by sand filters and activated carbons. Anyway because of rising points has been not possible to appreciate realistic removal efficiencies of some processes. Overall removal efficiency of the plant has been 56,1% in Summer and 51,3% in Winter. Higher removal efficiencies have been found in literature (near 80%) in Wang et al. and M. Pivokonsky studies.

QMRA risk assessment has been applied to Peschiera Borromeo WWTP to evaluate the risk level associated to the reuse of effluent water for irrigation. The only used input data from the plant have been E.coli effluent concentrations. Based on their trend, the data from 2018 to 2020 has been divided in “No-reuse” period and “Reuse” period characterized by an average effluent E.coli concentration of 1937 and 16 CFU/100 ml, respectively . Risk has also been calculated for three reference pathogens: Campylobacter, Cryptosporidium and Rotavirus. Three categories of people at risk have been considered: fieldworkers, local communities and consumers of products. Final risks have been compared with target values in terms of DALYs and risk of infection per person per year, suggested by WHO Guidelines and US.EPA, respectively. Deterministic analysis has been conducted using mean value of

E.coli concentrations of the two periods. In the “no reuse” season, assuming to not consider barriers between irrigation and final product, only DALY caused by Campylobacter result to not being over the risk level. On the other considering the risk of infection, all four pathogens result to pose a risk to all the exposure groups. In reuse scenario all DALYs are under the maximum acceptable level, while only Campylobacter and Cryptosporidium are in safe conditions if considering risk of infection. Two levels of barriers have been considered: the first one applies safety measures only on fields, the second one applies further precautions to final products (like cleaning or cooking the food). Both in no-reuse and reuse periods fieldworkers result in not being at risk, but in the specific the drip irrigation method confirms to be safer than spray avoiding inhalation of droplets. Same thing happens for local communities and consumers. Could be noticed that in all cases, the pathogen that poses the highest risk to the human health is Rotavirus, probably due to its dose-response model, followed by E.coli, Cryptosporidium and Campylobacter. As result, the first level of barriers is enough to ensure safety of exposed people and so second level is not necessary. Both fieldworkers and local communities are exposed to risk even through the consumption of final products, so the influence of not considering fieldworkers and local communities as consumers has been analysed. From this analysis could be seen that removing the ingestion of final products risk of infection decreases from two up to five orders of magnitude. In addition to deterministic analysis, an example of stochastic analysis using Monte Carlo simulation has also been made . In particular it has been applied to consumers assuming to not have barriers. Result are expressed as probability density and presented by box plot. Compared to point estimation, in the no-reuse period also Campylobacter presents a risk if expressed as DALY. The other results are comparable to the point estimation, demonstrating that mean values chosen are quite representative.

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ANNEX I Synthesis of literature on MPs

Table 67 MPs characterization from literature (1)

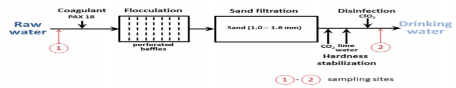
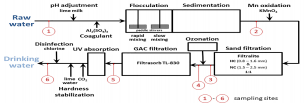
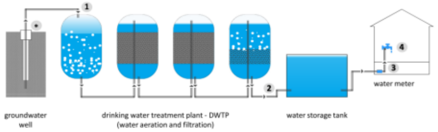
Reference	Type of plant	Location	Block diagram	Treated discharge	Sampling method
-	-	-	-	L/s	
Z. Wang, 2019	ADWTP	Yangtze River Delta,an important source of supply in China but highly polluted by plastics.	Processes include coagulation / flocculation, sedimentation, sand filtration and advanced treatment units, ozonation combined with GAC filtration	1388-1736	Water samples were collected in 1 L brown glass bottles (pre-cleaned) from the raw water and effluents from each treatment process.
M. Pivokonský; 2020	DWTP 1 (Milence)	Úhlava River (Czech Republic)		180-400	Two liters of water was filled into borosilicate glass bottles (pre-cleaned). The samples were then stored in the dark at 4 ° C. Any contact of the samples with plastic materials was avoided during sampling campaign.
	DWTP 2 (Plzen)	Úhlava River (Czech Republic)		400-1000	
M. Pivokonsky, 2018	DWTP 1 (from large valley water reservoir)	Czech Republic	coagulation / flocculation and sand filtration	3700-7000	A raw water sample and a treated water sample (1L of both) were taken in autoclavable (pre-cleaned) borosilicate glass bottles. The samples were stored at 4 ° C before analysis. Any contact of the samples with plastic materials was avoided during sampling campaign.
	DWTP 2 (from a smaller water reservoir)		coagulation / flocculation, sedimentation, filtration on sand and granular activated carbon	100-200	
	DWTP 3 (from a river)		coagulation-flocculation, flotation, sand filtration and granular activated carbon filtration.	90-150	
Mintenig S.M., 2018	DWTP 1	Nethen	The process units of the individual plants are not specified. 	-	-
	DWTP 2	Holdorf		-	-
	DWTP 3	Grossenkneten			
	DWTP 4	Sandelermoens			
	DWTP 5	Thuelsfelde		-	-

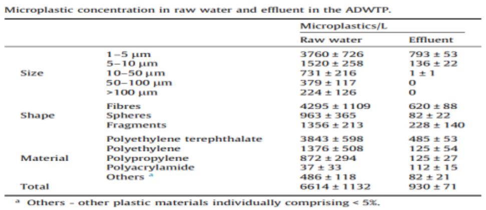
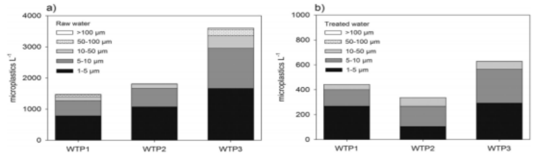
Table 68 MPs characterization from literature (2)

Reference	Downstream sample treatment	Frequency of sampling	Treatment unit	Sample type	NOTE
-	type		-		-
Z. Wang, 2019	Digestion with 30% hydrogen peroxide (H2O2) for 24 h. Filtration through a series of 5 µm membrane filters (PTFE) followed by a pore size of 0.22 µm.	3 days during winter	Infuent	instantaneous	The averaged data of MPs in the intermediate treatments are approximate because they are taken from a histogram. The actual data reported are those of the influent and effluent. On the other hand, the removals of the various process phases are exact because they are reported in the article and not calculated.
			Sedimentation	instantaneous	
			Sand filtration	instantaneous	
			Ozonization	instantaneous	
			GAC	instantaneous	
			Effluent	instantaneous	
M. Pivokonský; 2020	The samples were acidified to pH 3.5 by adding 1 M H2SO4 (Sigma-Aldrich, USA) to dissolve the aggregates. The samples were then passed through the filters to retain the particles. A glass vacuum filtration device and a stainless steel manifold connected to a vacuum pump were used. Before analysis, the filters were dried in an oven (30 ° C, 30 min) and stored in stoppered glass Petri dishes in a desiccator.	3 times a day, every 8h	Influent	Averaged on 24h	The quantities of microplastics are very low in this plant because it is served by a high-altitude reservoir with no anthropogenic impacts.
			Flocculation e coagulation		
			Sand filtration		
			Disinfection		
			Effluent		
			Influent		
			Flocculation plus sedimentation		
			Deep bed filtration		
			Ozonization		
			GAC		
			Effluent		
M. Pivokonsky, 2018	Initially, the oxidation of the wet peroxide was carried out to remove the organic from the water samples. The pretreated samples were passed through a series of 5 µm polytetrafluoroethylene (PTFE) membrane filters and subsequently 0.2 µm pore size.	3 times a day in 24 hours for three random days in winter	Influent	Averaged on 24h	Differences in MPs concentrations are due to various factors such as the type of water body and surrounding human activity.
		3 times a day in 24 hours for three random days in winter	Influent		
			Effluent		
		3 times a day in 24 hours for three random days in winter	Influent		
			Effluent		
Mintenig S.M., 2018	3µm cartridge filters were used. The residual raw water and drinking water were removed from the filter units using filtered compressed air (0.2 µm). Then, the units were refilled with dilute hydrochloric acid to dissolve the calcium carbonate and iron precipitates. After 24 hours the filter units were emptied, the cartridge filters were removed from the units and rinsed with ethanol. The retentate was collected on 3 µm (47 mm diameter) stainless steel filters which were subsequently transferred to glass bottles and coated with 30 mL of hydrogen peroxide. The bottles were closed using aluminum foil and incubated for 24 hours at 40 ° C. Finally, each sample was enriched on a 0.2 µm aluminum oxide filter using an in-house fabricated filter funnel with an internal diameter of 11 mm. The filters were dried at 40 ° C in semi-closed glass Petri dishes for subsequent analysis.	24 samples	Influent	istantaneous	Data are approximate because they are reported only in a histogram
			Effluent		
			Water meter		
			Influent		
			Effluent		
			Water meter		
			Influent		
			Effluent		
			Influent		
			Effluent		
			Water meter		
			Influent		
			Effluent		

Table 69 MPs characterization from literature (3)

Reference	Treatment unit	Unit specifications	Sampled volume	MPs
-	-		L	n°MPs/L
Z. Wang, 2019	Infuent	The averages of the influent and the effluent were already calculated in the text, while for the intermediate units the calculations are those in the table to the side.	1	6614,0
	Sedimentation		1	3466,7
	Sand filtration		1	2066,7
	Ozonization		1	2066,7
	GAC		1	900,0
	Effluent		1	930,0
M. Pivokonský; 2020	Influent	Coagulant PAX18	2	23
	Flocculation e coagulation	perforated deflectors	2	
	Sand filtration	1-1,6 mm	2	
	Disinfection	are dosed to stabilize the hardness of the water	2	
	Effluent		2	14
	Influent		2	1296
	Flocculation plus sedimentation	Coagulant: Al2(SO4)3	2	497
	Deep bed filtration	Dosage di KMnO4	2	243
	Ozonization	UV + Chlorine; CO2+calcium hydroxide	2	224
	GAC		2	149
	Effluent		2	151
M. Pivokonsky, 2018	Influent		1	1473
	Effluent		1	443
	Influent		1	1812
	Effluent		1	338
	Influent		1	3605
	Effluent		1	328
Mintenig S.M., 2018	Influent		300-1000 L influent, 1200-2500 Effluent	3
	Effluent			<1
	Water meter			
	Influent		300-1000 L influent,	7
	Effluent			<1
	Water meter			3
	Influent		300-1000 L influent,	
	Effluent			
	Influent		300-1000 L influent,	
	Effluent			1
	Water meter			1 2
	Influent		300-1000 L influent,	
	Effluent			1

Table 70 MPs characterization from literature (4)

Reference	Treatment unit	MPs typology	Plastic typology	Particles subdivision by size	Removals
-	-	particelle/fibre/frammenti	polietilene/poliestere		%
Z. Wang, 2019	Infuent	53.9–73.9% fibers; 8.6–20.6% parcels; 17.6–25.5% fragments	55.4–63.1% PET; PE (circa 15.1–23.8%) e PP (circa 8.4–18.2%)		
	Sedimentation				40.5–54.5
	Sand filtration				29.0–44.4
	Ozonization				The number of microplastics in the ozonation effluent is slightly increased, mainly due to the negative removal of small particles and fibrous microplastics
	GAC				
	Effluent	51.6–78.9% fibre; r 6.7–10.1% particelle; 14.4–38.3% frammenti	PET 47.2–58.8% di MPs; PAM aumentano nell'effluente, circa 10.1–14.7%		-3,3
M. Pivokonský; 2020	Influent	5 fiber/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)	Calcium hydroxide MPs were divided into five size categories: ≥1 to 5 µm, ≥5 to 10 µm, ≥10 to 50 µm, ≥50 to 100 µm, and ≥100 µm. FMPs between 1-5µm are between 50 in the influent and 60% in the effluent.	
	Flocculation e coagulation				
	Sand filtration				
	Disinfection				
	Effluent	3 fibers /L; 11 fragments/L (20%-80%)	PTFE; CA=42%; no PP, EVA and PTT		39,13
	Influent	126 fibers/L;1170 fragments/L	CA, PET, PVC, PE,		
	Flocculation plus sedimentation	51 fibers/L; 446 fragments/L			61,65
	Deep bed filtration	31 fibers/L; 213 fragments/L			51,11
	Ozonization				7,82
	GAC				33,48
	Effluent	12 fibers/L; 139 fragments/L	CA, PET, PVC, PE, and PP>90% - no EVA, PA6, PEO + PEG, and PTT		-1,34
		87-92%fragments; 8-13% fibers			
M. Pivokonsky, 2018	Influent	fragments 71-76%		MPs were divided into five size categories: ≥1 to 5 µm, ≥5 to 10 µm, ≥10 to 50 µm, ≥50 to 100 µm, and ≥100 µm. Among the MP distribution categories in raw water, microplastics of 1–5 µm prevailed in all samples from any WTP, accounting for approximately 40-60% of the total MP count, followed by the 5-10 µm category. A similar percentage (about 30-40%) of 5-10 µm. Particles larger than 10 µm did not exceed a 10% portion in any raw water sample. When focusing on treated (potable) water samples, the study revealed no microplastics larger than 100 µm and only a minimum MP content of between 50 and 100 µm was observed. The prevailing size of the category was again 1–5 µm, which comprises about 25–60% of the microplastics. The second largest group was that of 5-10 µm (approximately 30-50%).	70
	Effluent	fragments 42-48%			
	Influent	fragments 71-76%			81
	Effluent				
	Influent	fragments 42-48%; fibers 37-61%			83
	Effluent				
Mintenig S.M., 2018	Influent		PEST, PVC, PE, PA and epoxy resin	 **Fig. 2.** Size distribution of microplastics detected in raw (a) and treated water (b) at water treatment plants 1 (WTP1), 2 (WTP2) and 3 (WTP3).	
	Effluent				
	Water meter				
	Influent				
	Effluent				
	Water meter				
	Influent				
	Effluent				
	Influent				
	Effluent				
	Water meter				
	Influent				
	Effluent				

Microplastic concentration in raw water and effluent in the ADWTP.

		Microplastics/L	
		Raw water	Effluent
Size	1–5 µm	3760 ± 726	793 ± 53
	5–10 µm	1520 ± 258	136 ± 22
	10–50 µm	731 ± 216	1 ± 1
	50–100 µm	379 ± 117	0
	>100 µm	224 ± 126	0
Shape	Fibres	4295 ± 1109	620 ± 88
	Spheres	963 ± 365	82 ± 22
	Fragments	1356 ± 213	228 ± 140
Material	Polyethylene terephthalate	3843 ± 598	485 ± 53
	Polyethylene	1376 ± 508	125 ± 54
	Polypropylene	872 ± 294	125 ± 27
	Polyacrylamide	37 ± 33	112 ± 15
	Others [a]	486 ± 118	82 ± 21
Total		6614 ± 1132	930 ± 71

[a] Others - other plastic materials individually comprising < 5%.

ANNEX II QMRA COMPARISON OF SCENARIOS

Table 71 Fieldworkers drip irrigation

Irrigation type	Drip					
Fieldworkers	No barriers		With barriers 1st case		With barriers 2nd case	
exposure activity/route	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)
Volume per event (ml)	1,00E-03	1,00E+00	1,00E-03	1,00E+00	1,00E-03	1,00E+00
events/y	100	140	100	140	100	140
barriers	-	-	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Cooking or processing of produce (eg cereal, wine grapes)
	-	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Washing with water	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Removal of skins from produce before consumption
	-	-	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)
	-	-	-	-	-	Washing with water
	-	-	-	-	-	Natural die-off (Withholding time of one month)
tot log reduction	0	0	11	9	11	16
dose (microorganism/event) no reuse	1,94E-02	1,94E+01	1,94E-13	1,94E-08	1,94E-13	1,94E-15
dose (microorganism/event) reuse	1,60E-04	1,60E-01	1,60E-15	1,60E-10	1,60E-15	1,60E-17

Table 72 Fieldworkers spray irrigation

Irrigation type	Spray								
Fieldworkers	**No barriers**			**With barriers 1st case**			**With barriers 2nd case**		
exposure activity/route	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation
Volume per event (ml)	1,00E-03	1,00E+00	6,90E-03	1,00E-03	1,00E+00	6,90E-03	1,00E-03	1,00E+00	6,90E-03
events/y	100	140	100	100	140	100	100	140	100
barriers	-	-		Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Washing with water	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Cooking or processing of produce (eg cereal, wine grapes)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)
	-	-		No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Natural die-off (Withholding time of one month)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Removal of skins from produce before consumption	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-	-		Natural die-off (Withholding time of one month)	-	-	Natural die-off (Withholding time of one month)	Washing with water	-
	-	-		-	-	-	-	Natural die-off (Withholding	-
	-	-		-	-	-	-	-	-
tot log reduction	0	0	0	9	6	4	9	13	4
dose (microorganism/event) no reuse	1,94E-02	1,94E+01	1,34E-01	1,94E-11	1,94E-05	1,34E-05	1,94E-11	1,94E-12	1,34E-05
dose (microorganism/event) reuse	1,60E-04	1,60E-01	1,10E-03	1,60E-13	1,60E-07	1,10E-07	1,60E-13	1,60E-14	1,10E-07

Table 73 Local communities drip irrigation

Irrigation type	**Drip**					
Local communities	**No barriers**		**With barriers 1st case**		**With barriers 2nd case**	
exposure activity/route	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Municipal irrigation (Ingestion	Food crop consumption (commercial) (Ingestion of other raw products)
Volume per event (ml)	1,00E-03	1,00E+00	1,00E-03	1,00E+00	1,00E-03	1,00E+00
events/y	10	140	10	140	10	140
barriers	-	-	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Cooking or processing of produce (eg cereal, wine grapes)
	-	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Washing with water	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Removal of skins from produce before consumption
	-	-	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)
	-	-	-	-	-	Washing with water
	-	-	-	-	-	Natural die-off (Withholding time of one month)
tot log reduction	0	0	11	9	11	16
dose (microorganism/event) no-reuse	1,94E-02	1,94E+01	1,94E-13	1,94E-08	1,94E-13	1,94E-15
dose (microorganism/event) reuse	1,60E-04	1,60E-01	1,60E-15	1,60E-10	1,60E-15	1,60E-17

Table 74 Local communities spray irrigation

Irrigation type	Spray								
Local communities	No barriers			With barriers 1st case			With barriers 2nd case		
exposure activity/route	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation	Municipal irrigation (Ingestion)	Food crop consumption (commercial) (Ingestion of other raw products)	Inhalation
Volume per event (ml)	1,00E-03	1,00E+00	6,90E-03	1,00E-03	1,00E+00	6,90E-03	1,00E-03	1,00E+00	6,90E-03
events/y	10	140	365	10	140	365	10	140	365
barriers	-	-	-	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Washing with water	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Cooking or processing of produce (eg cereal, wine grapes)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)
	-	-	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	Natural die-off (Withholding time of one month)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	-	Removal of skins from produce before consumption	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-	-		-	-	Buffer zones (25–30 m)	-	Washing with water	Buffer zones (25–30 m)
	-	-		-	-	-	-	Natural die-off (Withholding	-
	-	-		-	-	-	-	-	-
tot log reduction	0	0	0	4	6	5	4	13	5
dose (microorganism/event) no reuse	1,94E-02	1,94E+01	1,34E-01	1,94E-06	1,94E-05	1,34E-06	1,94E-06	1,94E-12	1,34E-06
dose (microorganism/event) reuse	1,60E-04	1,60E-01	1,10E-03	1,60E-08	1,60E-07	1,10E-08	1,60E-08	1,60E-14	1,10E-08

Table 75 Consumers with drip irrigation

Irrigation type	Drip		
Consumers	**No barriers**	**With barriers 1st case**	**With barriers 2nd case**
exposure activity/route	Food crop consumption (commercial) (Ingestion of other raw products)	Food crop consumption (commercial) (Ingestion of other raw products)	Food crop consumption (commercial) (Ingestion of other raw products)
Volume per event (ml)	1,00E+00	1,00E+00	1,00E+00
events/y	140	140	140
barriers	-	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Cooking or processing of produce (eg cereal, wine grapes)
	-	Washing with water	Removal of skins from produce before consumption
	-	Natural die-off (Withholding time of one month)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)
	-	-	Washing with water
	-	-	Natural die-off (Withholding time of one month)
tot log reduction	0	9	16
dose (microorganism/event) no-reuse	1,94E+01	1,94E-08	1,94E-15
dose (microorganism/event) reuse	1,60E-01	1,60E-10	1,60E-17

Table 76 Consumers with spray irrigation

Irrigation type	Spray		
Consumers	**No barriers**	**With barriers 1st case**	**With barriers 2nd case**
exposure activity/route	Food crop consumption (commercial) (Ingestion of other raw products)	Food crop consumption (commercial) (Ingestion of other raw products)	Food crop consumption (commercial) (Ingestion of other raw products)
Volume per event (ml)	1,00E+00	1,00E+00	1,00E+00
events/y	140	140	140
barriers	-	Washing with water	Cooking or processing of produce (eg cereal, wine grapes)
	-	Natural die-off (Withholding time of one month)	Removal of skins from produce before consumption
	-	-	Washing with water
	-	-	-
	-	-	-
tot log reduction	0	6	13
dose (microorganism/event) no-reuse	1,94E+01	1,94E-05	1,94E-12
dose (microorganism/event) reuse	1,60E-01	1,60E-07	1,60E-14

Table 77 Fieldworkers with drip irrigation, no consumption case

Irrigation type	Drip		
Fieldworkers	No barriers	With barriers 1st case	With barriers 2nd case
exposure activity/route	Municipal irrigation (Ingestion)	Municipal irrigation (Ingestion)	Municipal irrigation (Ingestion)
Volume per event (ml)	1,00E-03	1,00E-03	1,00E-03
events/y	100	100	100
barriers	-	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)
	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)
	-	-	-
	-	-	-
tot log reduction	0	11	11
dose (microorganism/event) no-reuse	1,94E-02	1,94E-13	1,94E-13
dose (microorganism/event) reuse	1,60E-04	1,60E-15	1,60E-15

Table 78Fieldworkers with spray irrigation, no consumption case

Irrigation type	**Spray**					
Fieldworkers	**No barriers**		**With barriers 1st case**		**With barriers 2nd case**	
exposure activity/route	Municipal irrigation (Ingestion)	Inhalation	Municipal irrigation (Ingestion)	Inhalation	Municipal irrigation (Ingestion)	Inhalation
Volume per event (ml)	1,00E-03	6,90E-03	1,00E-03	6,90E-03	1,00E-03	6,90E-03
events/y	100	100	100	100	100	100
barriers	-		Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)
	-		No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-		Natural die-off (Withholding time of one month)	-	Natural die-off (Withholding time of one month)	-
	-		-	-	-	-
	-		-	-	-	-
tot log reduction	0	0	9	4	9	4
dose (microorganism/event) no-reuse	1,94E-02	1,34E-01	1,94E-11	1,34E-05	1,94E-11	1,34E-05
dose (microorganism/event) reuse	1,60E-04	1,10E-03	1,60E-13	1,10E-07	1,60E-13	1,10E-07

Table 79 Local communities with drip irrigation, no consumption case

Irrigation type	Drip		
Local communities	**No barriers**	**With barriers 1st case**	**With barriers 2nd case**
exposure activity/route	Municipal irrigation (Ingestion)	Municipal irrigation (Ingestion)	Municipal irrigation (Ingestion)
Volume per event (ml)	1,00E-03	1,00E-03	1,00E-03
events/y	10	10	10
barriers	-	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)	Drip irrigation of crops with limited to no ground contact (eg tomatoes, capsicums)
	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-	Natural die-off (Withholding time of one month)	Natural die-off (Withholding time of one month)
	-	-	-
	-	-	-
tot log reduction	0	11	11
dose (microorganism/event) no-reuse	1,94E-02	1,94E-13	1,94E-13
dose (microorganism/event) reuse	1,60E-04	1,60E-15	1,60E-15

Table 80 Local communities with spray irrigation, no consumption case

Irrigation type	**Spray**					
Local communities	**No barriers**		**With barriers 1st case**		**With barriers 2nd case**	
exposure activity/route	Municipal irrigation (Ingestion)	Inhalation	Municipal irrigation (Ingestion)	Inhalation	Municipal irrigation (Ingestion)	Inhalation
Volume per event (ml)	1,00E-03	6,90E-03	1,00E-03	6,90E-03	1,00E-03	6,90E-03
events/y	10	365	10	365	10	365
barriers	-	-	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)	Spray drift control (microsprinklers, anemometer systems, inward-throwing sprinklers, etc)
	-	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)	-	No public access during irrigation and limited contact after (non-grassed areas) (eg food crop irrigation)
	-		-	Buffer zones (25–30 m)	-	Buffer zones (25–30 m)
	-		-	-	-	-
	-		-	-	-	-
tot log reduction	0	0	4	5	4	5
dose (microorganism/event) no-reuse	1,94E-02	1,34E-01	1,94E-06	1,34E-06	1,94E-06	1,34E-06
dose (microorganism/event) reuse	1,60E-04	1,10E-03	1,60E-08	1,10E-08	1,60E-08	1,10E-08

ANNEX III CHARACTERISTIC PARAMETERS OF INPUT AND OUTPUT VALUES FROM MONTE CARLO SIMULATION

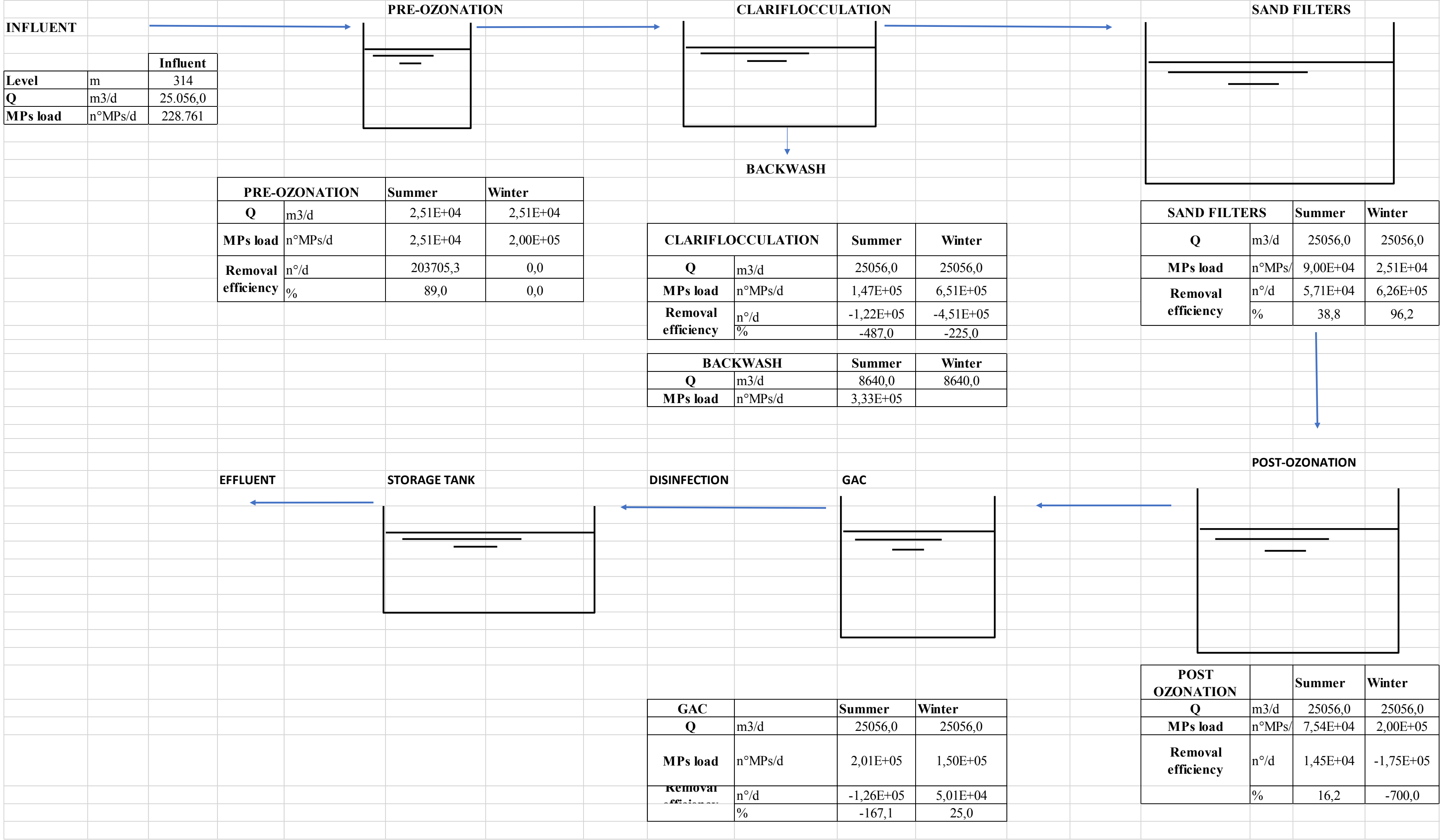
	LR PRIMARI			LR BIOLOGICO			LR UV			CONC. INFLUENTE				RATIO E.COLI-REFERENCE		
	I treat bacteria	I treat protozoa	I treat viruses	II treat bacteria	II treat protozoa	II treat viruses	LR UV campy	LR crypto	LR rota	e.coli in	campy in	cripto in	rota in	campy/e.coli	cripto/e.coli	rota/e.coli
median	**2,49E-01**	**2,50E-01**	**4,98E-02**	**2,00E+00**	**1,25E+00**	**7,51E-01**	**3,00E+00**	**2,87E+00**	**2,12E+00**	**7,54E+05**	**3,60E+00**	**3,54E-01**	**3,56E+00**	**5,51E-06**	**5,51E-07**	**5,50E-06**
MEAN	2,49E-01	2,50E-01	4,99E-02	2,00E+00	1,25E+00	7,50E-01	3,00E+00	2,83E+00	2,05E+00	9,42E+06	5,19E+01	5,14E+00	5,19E+01	5,51E-06	5,50E-07	5,50E-06
st. dev	1,44E-01	1,44E-01	2,88E-02	5,77E-01	4,34E-01	1,44E-01	4,08E-01	3,12E-01	3,72E-01	4,17E+07	2,59E+02	2,46E+01	2,53E+02	2,60E-06	2,60E-07	2,59E-06
min	3,91E-06	2,66E-07	2,27E-06	1,00E+00	5,00E-01	5,00E-01	2,00E+00	2,00E+00	1,00E+00	1,26E-03	6,86E-09	9,25E-10	4,47E-09	1,00E-06	1,00E-07	1,00E-06
max	5,00E-01	5,00E-01	1,00E-01	3,00E+00	2,00E+00	1,00E+00	4,00E+00	3,50E+00	2,58E+00	3,41E+09	3,04E+04	1,49E+03	1,61E+04	1,00E-05	1,00E-06	1,00E-05
range	5,00E-01	5,00E-01	1,00E-01	2,00E+00	1,50E+00	5,00E-01	1,99E+00	1,49E+00	1,58E+00	3,41E+09	3,04E+04	1,49E+03	1,61E+04	9,00E-06	9,00E-07	9,00E-06
conta.numeri	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000
25 percentile	1,24E-01	1,26E-01	2,50E-02	1,50E+00	8,73E-01	6,25E-01	2,71E+00	2,61E+00	1,79E+00	1,14E+05	5,18E-01	5,19E-02	5,19E-01	3,27E-06	3,25E-07	3,26E-06
75 percentile	3,75E-01	3,76E-01	7,49E-02	2,50E+00	1,62E+00	8,75E-01	3,29E+00	3,07E+00	2,37E+00	4,27E+06	2,13E+01	2,13E+00	2,12E+01	7,78E-06	7,76E-07	7,74E-06
95 percentile	4,75E-01	4,75E-01	9,50E-02	2,90E+00	1,93E+00	9,75E-01	3,69E+00	3,31E+00	2,54E+00	4,02E+07	2,15E+02	2,11E+01	2,13E+02	9,55E-06	9,56E-07	9,55E-06

	CONC. EFFLUENTE						DOSE			INFECTION RISK PER CASE						
	E.coli conc	campy conc	cripto conc	rota conc	Log reduction	ml water	E.coli dose	campy dose	cripto dose	rota dose	E.coli inf	campy inf	cripto inf	rota inf	E.COLI 1-P	CAMPY 1-P
median	**4,91E+00**	**2,28E-05**	**1,62E-05**	**4,87E-03**	**0,00E+00**	**1,00E+00**	**4,91E-02**	**2,28E-07**	**1,62E-07**	**4,87E-05**	**3,12E-07**	**4,37E-09**	**9,53E-09**	**2,89E-05**	**1,00E+00**	**1,00E+00**
MEAN	1,52E+01	8,39E-05	5,31E-04	1,11E-01	0,00E+00	1,00E+00	1,52E-01	8,39E-07	5,31E-06	1,11E-03	9,63E-07	1,60E-08	3,13E-07	6,25E-04	1,00E+00	1,00E+00
st. dev	2,86E+01	1,80E-04	4,97E-03	7,46E-01	0,00E+00	0,00E+00	2,86E-01	1,80E-06	4,97E-05	7,46E-03	1,82E-06	3,45E-08	2,93E-06	3,55E-03	1,82E-06	3,45E-08
min	3,77E-09	3,64E-14	8,21E-15	3,16E-12	0,00E+00	1,00E+00	3,77E-11	3,64E-16	8,21E-17	3,16E-14	#RIF!	6,97E-18	4,84E-18	1,88E-14	1,00E+00	1,00E+00
max	7,33E+02	6,69E-03	5,83E-01	6,92E+01	0,00E+00	1,00E+00	7,33E+00	6,69E-05	5,83E-03	6,92E-01	4,66E-05	1,28E-06	3,44E-04	2,17E-01	1,00E+00	1,00E+00
range	7,33E+02	6,69E-03	5,83E-01	6,92E+01	0,00E+00	0,00E+00	7,33E+00	6,69E-05	5,83E-03	6,92E-01	#RIF!	1,28E-06	3,44E-04	2,17E-01	4,66E-05	1,28E-06
conta.numeri	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000	140000
25 percentile	1,06E+00	4,72E-06	1,93E-06	6,44E-04	0,00E+00	1,00E+00	1,06E-02	4,72E-08	1,93E-08	6,44E-06	6,72E-08	9,03E-10	1,14E-09	3,82E-06	1,00E+00	1,00E+00
75 percentile	1,66E+01	8,36E-05	1,17E-04	3,23E-02	0,00E+00	1,00E+00	1,66E-01	8,36E-07	1,17E-06	3,23E-04	1,05E-06	1,60E-08	6,91E-08	1,92E-04	1,00E+00	1,00E+00
95 percentile	6,45E+01	3,66E-04	1,65E-03	3,95E-01	0,00E+00	1,00E+00	6,45E-01	3,66E-06	1,65E-05	3,95E-03	4,10E-06	7,01E-08	9,76E-07	2,35E-03	1,00E+00	1,00E+00

			ANNUAL INFECTION RISK			
	CRYPTO 1-P	ROTA 1-P	E.coli inf pppy	campy inf pppy	cripto inf pppy	rota inf pppy
median	**1,00E+00**	**1,00E+00**	**1,34E-04**	**2,21E-06**	**3,46E-05**	**7,53E-02**
MEAN	1,00E+00	9,99E-01	1,35E-04	2,25E-06	4,39E-05	8,39E-02
st. dev	2,93E-06	3,55E-03	2,19E-05	3,99E-07	3,50E-05	3,91E-02
min	1,00E+00	7,83E-01	6,72E-05	1,04E-06	8,30E-06	2,16E-02
max	1,00E+00	1,00E+00	2,26E-04	4,17E-06	3,81E-04	2,68E-01
range	3,44E-04	2,17E-01	1,59E-04	3,12E-06	3,73E-04	2,47E-01
conta.numeri	140000	140000	1000	1000	1000	1000
25 percentile	1,00E+00	1,00E+00	1,19E-04	1,98E-06	2,43E-05	5,63E-02
75 percentile	1,00E+00	1,00E+00	1,49E-04	2,50E-06	5,01E-05	1,02E-01
95 percentile	1,00E+00	1,00E+00	1,72E-04	2,92E-06	1,01E-04	1,62E-01

	ANNUAL INFECTION RISK				INF:ILL RATIO			ILLNESS RISK			DALY per case			ANNUAL DALYs		
	E.coli inf pppy	campy inf pppy	cripto inf pppy	rota inf pppy	campy inf:ill ratio	crypto inf:ill ratio	rota inf:ill ratio	campy ill	cripto ill	rota ill	py DALY per	to DALY per	a DALY per ca	campy DALY	cripto DALY	rota DALY
median	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**	**1,34E-04**
MEAN	1,35E-04	2,25E-06	4,39E-05	8,39E-02	3,00E-01	7,00E-01	6,27E-01	6,74E-07	3,07E-05	5,24E-02	2,26E-02	8,03E-04	2,04E-02	1,52E-08	2,45E-08	6,40E-05
st. dev	2,19E-05	3,99E-07	3,50E-05	3,91E-02	5,66E-15	6,44E-15	1,56E-01	1,20E-07	2,45E-05	2,81E-02	1,05E-02	3,96E-04	3,16E-03	7,61E-09	2,46E-08	3,58E-05
min	6,72E-05	1,04E-06	8,30E-06	2,16E-02	3,00E-01	7,00E-01	3,52E-01	3,13E-07	5,81E-06	8,16E-03	4,66E-03	1,20E-04	1,50E-02	2,62E-09	1,01E-09	1,07E-05
max	2,26E-04	4,17E-06	3,81E-04	2,68E-01	3,00E-01	7,00E-01	8,99E-01	1,25E-06	2,67E-04	2,19E-01	4,10E-02	1,50E-03	2,60E-02	4,17E-08	3,49E-07	2,47E-04
range	1,59E-04	3,12E-06	3,73E-04	2,47E-01	0,00E+00	0,00E+00	5,48E-01	9,37E-07	2,61E-04	2,11E-01	3,63E-02	1,38E-03	1,10E-02	3,91E-08	3,48E-07	2,37E-04
conta.numeri	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
25 percentile	1,19E-04	1,98E-06	2,43E-05	5,63E-02	3,00E-01	7,00E-01	4,89E-01	5,95E-07	1,70E-05	3,26E-02	1,33E-02	4,62E-04	1,76E-02	8,96E-09	1,02E-08	3,86E-05
75 percentile	1,49E-04	2,50E-06	5,01E-05	1,02E-01	3,00E-01	7,00E-01	7,58E-01	7,49E-07	3,51E-05	6,38E-02	3,14E-02	1,15E-03	2,32E-02	2,06E-08	3,14E-08	7,94E-05
95 percentile	1,72E-04	2,92E-06	1,01E-04	1,62E-01	3,00E-01	7,00E-01	8,74E-01	8,77E-07	7,09E-05	1,11E-01	3,94E-02	1,42E-03	2,54E-02	2,81E-08	6,46E-08	1,42E-04

ANNEX IV CASTRECCIONI FLOW DIAGRAM WITH MPS REMOVAL EFFICIENCIES



		Influent
Level	m	314
Q	m3/d	25.056,0
MPs load	n°MPs/d	228.761

PRE-OZONATION		Summer	Winter
Q	m3/d	2,51E+04	2,51E+04
MPs load	n°MPs/d	2,51E+04	2,00E+05
Removal efficiency	n°/d	203705,3	0,0
	%	89,0	0,0

CLARIFLOCCULATION		Summer	Winter
Q	m3/d	25056,0	25056,0
MPs load	n°MPs/d	1,47E+05	6,51E+05
Removal efficiency	n°/d	-1,22E+05	-4,51E+05
	%	-487,0	-225,0

BACKWASH		Summer	Winter
Q	m3/d	8640,0	8640,0
MPs load	n°MPs/d	3,33E+05	

SAND FILTERS		Summer	Winter
Q	m3/d	25056,0	25056,0
MPs load	n°MPs/	9,00E+04	2,51E+04
Removal efficiency	n°/d	5,71E+04	6,26E+05
	%	38,8	96,2

POST OZONATION		Summer	Winter
Q	m3/d	25056,0	25056,0
MPs load	n°MPs/	7,54E+04	2,00E+05
Removal efficiency	n°/d	1,45E+04	-1,75E+05
	%	16,2	-700,0

GAC		Summer	Winter
Q	m3/d	25056,0	25056,0
MPs load	n°MPs/d	2,01E+05	1,50E+05
Removal	n°/d	-1,26E+05	5,01E+04
	%	-167,1	25,0