Microplastics in different water samples (seawater, freshwater, and wastewater): Methodology approach for characterization using micro-FTIR spectroscopy

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Microplastics in different water samples (sea, fresh and wastewater) - Part I: methodology approach for characterization using micro-FTIR spectroscopy

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Abstract:

Microplastics of millimeter dimensions have been widely investigated in environmental compartments and today, studies are mainly focused on particles of smaller dimensions (µm-nm). However, as there are no relevant standards or policies for the preparation and analysis of water samples containing such particles, the results may be questionable. Therefore, a methodology approach for 10 µm to 500 µm microplastics analysis was developed using µ-FTIR spectroscopy coupled with the siMPle analytical software. This was undertaken on different water samples (sea, fresh and wastewater) considering rinsing water, digestion and filtration step and sample characteristics. Ultrapure water reveals to be the optimal rinsing water and ethanol was also proposed with a mandatory previous filtration. This improved quantitative and qualitative analytical methodology for microplastics detection by µ-FTIR spectroscopy will be used in Part II to assess the removal efficiency of conventional and membrane treatment processes in different water treatment plants.

List of abbreviations

Abbreviation	Full name
ABS	Acrylonitrile Butadiene Styrene
ATR	Attenuated total reflection
DWTP	Drinking water treatment plant

EVA	Ethylene Vinyl Acetate
FTIR	Fourier-transform infrared
IC	Inorganic carbon
MCT	Mercuric cadmium telluride
MP	Microplastic particle
PA	Polyamide (nylon)
PE	Polyethylene
PES	Polyether Sulfone
PET	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
SWTP	Seawater treatment plant
TOC	Total organic carbon
TC	Total carbon
TP	Total particles
UF	Ultrafiltration
UP	Ultrapure
WWTP	Wastewater treatment plant

1. Introduction

Researches on microplastics sources, pollution, transport, harmfulness, retention, recovery and analytical methods have been increasingly developed for the last 10 years. Microplastic particles (MPs) (0.1 μm ~ 5 mm) are originated either from primary manufactured or secondary degraded plastic objects (Frias and Nash, 2019). Annually in Europe, polymers production was increased greatly from 0.35 million tons in the 1950s to 55 million tons in 2020, while 63,000 to 430,000 tons of microplastics enter farmland through compost/sludges application (Nizzetto et al., 2016), and around 2,461~8,627 tons of microplastics enter in marine environments (Jambeck et al., 2015; Lebreton et al., 2017; C. Sherrington et al., 2016). Massive MP caused increasing adverse effects on humans and environments: (1) MPs can be swallowed by organisms and transported through food chains (Rillig, 2012). For instance, Ragusa et al., (2021) discovered the presence of

microplastics (5~10 μm) in human placenta, and Sussarellu et al., (2016) found polystyrene (PS) particles (2 ~ 6 μm) translocated in blood circulation and cause reproductive disruption for marine filter feeders; (2) Some MPs could release toxic compounds such as dioxins, phthalates, vinyl chloride, etc. Some additives added by manufacturers such as plasticizers, stabilizers and pigments to plastics, are mostly hazardous substances (Nobre et al., 2015); (3) MPs could induce chronic toxicity which was considered as a key issue for long-term exposure (Campanale et al., 2020; Prata et al., 2019); (4) MPs could act as vectors for toxic organic substances and microorganisms (Nobre et al., 2015; Yang et al., 2022); (5) Moreover, old microplastics could be furtherly degraded into smaller or even nanoparticles (Jambeck et al., 2015). Currently, public perception of the risks induced by microplastics is low, and few countries or regions have issued relevant detection method standards and production/limitation policies.

Among present analytical methods, micro-FTIR (μ-FTIR) spectroscopy is an advanced analytical approach for testing MPs. It is a non-destructive analytical technique and can produce IR absorption spectra for both thick and opaque materials (Hong et al., 2021; Shim et al., 2017). Generally, the requirement for sample pretreatment is necessary for avoiding shelter of microplastics by organic and inorganic materials and allow their indubitable identification by digestion. The common digestion protocols include oxidation, alkaline or acidic treatment, and enzymatic degradation (Stock et al., 2019; Sun et al., 2019). H_2O_2 and Fenton reagent are mostly used chemicals for oxidative digestion, which generates no or very few effects on microplastic properties (Hurley et al., 2018; A. Tagg et al., 2015). According to Prata et al. (2019), 95% of 20 studies in sediments used H_2O_2 and/or Fenton reagent for digestion, and >60% of 20 studies in water samples used H_2O_2 for digestion. Similar conclusion was also inferred in the survey of Table S1 (supplementary information, SI). In comparison, Fenton reagent is able to accelerate digestion rates compared to H_2O_2 (Hurley et al., 2018). Alkaline digestion with 10% KOH solution is highly recommended for sea animals or sea water digestion since it can break down soft tissue and bivalve tissues in marine samples (Thiele et al., 2019). KOH is also useful on the digestion of natural organic matters, feathers, and fat, etc. Enzymatic digestion is a rapid reaction for complete degradation of organics, while it is considered as an expensive, complex, and sometimes destructive method on MPs extraction (Prata et al., 2019). Density separation with salt of NaCl, NaI, and $ZnCl_2$ could be coupled with other digestion processes to isolate MPs (J. Li et al., 2018), but showed lower recovery on smaller and denser MPs (Radford et al., 2021). Currently, the

digestion protocols on microplastics recovery are mostly based on experiments from literature, the preparation methods are not yet standardized. In view of rinsing process, most studies used ultrapure water with or without ethanol (66.7% of 30 studies), secondly used distilled water by investigation in SI Table S2. Since particles from rinsing could easily stick to surfaces and remain, thus would lead to contamination of the sample, certain criteria need to be met for rinsing: 1) evaluating the MPs existence in potential rinsing water/solution and potentially filtrate it before using; 2) determining the reliable rinsing water/solution with minimum MPs before experiments. However, fewer studies completed the above steps, thus it is necessary to investigate the MPs in rinsing water/solution to avoid external invasion. Additionally, considerering the expression of MPs concentration, fewer researchers filtrated 1 L of samples for analysis while others, particularly for organic-rich samples, maybe filtrated only several milliliters (Simon et al., 2018). Most of them calculated MPs concentration by assuming the proportional relationship between the filtrated volume and the MPs counts but without evidencing it.

Therefore, current difficulties on MPs analysis include the incomplete recovery of MPs from samples, the selection of appropriate digestion method *vs.* nature of samples, the limitation on quantification and identification of MPs, and the global accuracy of analytical results. Therefore, this study aims to develop a method for identification (type and size) and quantification of MPs in different water samples. Efforts have been made on the improvement of detection accuracy: selection of purified rinsing water, suitable sampling and digestion methods depending on the water type, rigorous detection process, and high-precision analytical methods to obtain the counts, dimensions, and type of microplastics by μ-FTIR in reflectance mode.

2. Material and methods

2.1. Sample sources

Three types of water were analyzed and their location was shown in Figure 1: (i) samples from a seawater treatment plant (SWTP) which aims to treat seawater before shellfish farms to produce purified seawater; (ii) samples from four different wastewater treatment plants (WWTP); and (iii) samples from 3 drinking water treatment plants (DWTP). The detailed information of each site is

shown in Table 1. To avoid external pollution, all samples were collected and transported via 1 L glass bottles and frozen up until analysis.

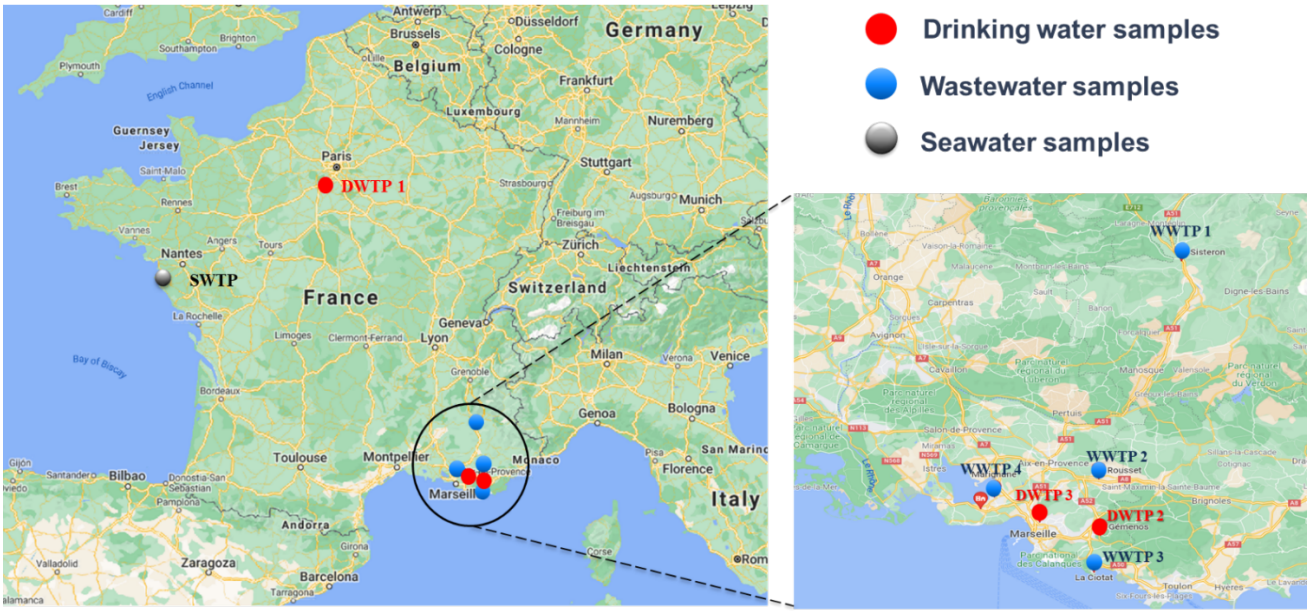


Figure 1 Locations and types of sampling in France (background map from Google Map © 2022)

Table 1 Information of water treatment plants

Types	Names	Samples	Main process (studied in Part II)	Location in France (French department)	Sampling date
Wastewater	WWTP 1	Pharmaceutical membrane bioreactor (MBR) feed and effluent	MBR (0.02 μm UF)	Alpes-de-Haute-Provence	April 2021
	WWTP 2	Municipal wastewater and secondary effluents	MBR (0.1 μm UF)	Bouches-du-Rhône	October 2021
	WWTP 3		Physico-chemical with biofiltration	Bouches-du-Rhône	October 2021
	WWTP 4		Activated sludge, 200 kDa UF	Bouches-du-Rhône	October 2021
Seawater	SWTP	Seawater and secondary/tertiary effluents	Zeolite, filters, 200 kDa UF	Vendée	April 2021
Drinking water	DWTP 1	Underground water and effluents	Sieves, 200 kDa UF	Paris	September 2021
	DWTP 2	Surface water and effluents	200 kDa UF	Bouches-du-Rhône	October 2021
	DWTP 3	Surface water and effluents	Sedimentation, sand filtration	Bouches-du-Rhône	October 2021

2.2. Sample pretreatment

The commonly used rinsing water/solutions tested in this study included Evian water, ultrapure water (UP water) (LaboStar TWF7 Siemens), distilled water (DI), tap water, HPLC water (34877-2.5L-M, Sigma Aldrich), and ethanol both in PE and glass bottles (ethanol absolute, VWR, USA). The digestion chemicals used for samples included 30% (w/v) H_2O_2 (Fisher Chemical), 10% (w/v) KOH (Fischer Chemicals), 1–10% H_2SO_4 (>95%, Fisher Scientific), and Fenton reaction (0.05 M $FeSO_4 \cdot 7H_2O$ with 30% H_2O_2 at volume ratio of 1:1). $FeSO_4 \cdot 7H_2O$ solution was made by dissolving 2.5 g of $FeSO_4 \cdot 7H_2O$ (Fisher Scientific) in 165 mL UP water and acidified with 1 mL of concentrated H_2SO_4. All operations were conducted in cleaned glass devices and covered with aluminum foil to prevent airborne contamination. Digestion processes were all operated at room temperature (25 °C). The handling of samples was carried out under controlled air conditions in cleaned fume hood, and operators wore cotton laboratory coats throughout the experiment.

Turbidity of water samples was measured using WTW Turb 550 IR in Nephelometric Turbidity Unit (NTU). Total Organic Carbon (TOC) was measured using Shimadzu TOCL-LCSH/CSN TOC Analyzer. Detailed information was shown in SI Section 1.1.

2.3. MPs collection

The final step before μ-FTIR imaging was the filtration of samples. Three types of filters were used: 3 or 5 μm gold-coated polyester membranes (i3 TrackPor P, Germany), and 25 μm stainless-steel filters. In reflectance mode, the gold material and stainless-steel slide were both good choices as background as they reflected IR radiation with less absorption (Gonzalez-Torres et al., 2017). The samples were filtrated through a vacuum Büchner funnel apparatus (Fisher Scientific, Sweden), shown in Figure S1. The filtration masks were round metal discs with a square hole in the center to regulate the sedimentation area on filters. Square holes with side dimension of 5,500 and 10,000 μm, were respectively used on gold-coated filters and stainless steel filter. For samples with visible particles and solids, a two-stage filtration was performed: Step 1 by stainless-steel filter, step 2 by gold filter. For purified water samples, the filtration was directly achieved on a gold filter. Before and after analysis by μ-FTIR spectroscopy, the filter with collected sample was stored in a glass Petri dish to avoid any external pollution.

2.4. μ-FTIR spectroscopy

The detection of microplastics was achieved with the μ-FTIR imaging system Spotlight 400 M-FTIR microscope (PerkinElmer, USA) with high sensitivity on smaller particles (<10 μm). The detailed information was described in SI section 1.2. In this study, the spectrometer was set up to reflectance mode with a focal-plane-array detector which assembly enabled rapid analysis of thick and opaque samples such as microplastics (A. S. Tagg et al., 2015). A 16 cm^{-1} spectral resolution was used as the best compromise between spectral quality and acquisition rapidity (Zheng et al., 2021). A background spectrum imaging was collected from the gold filter both at 6.25 μm or 25 μm pixels. The other identification parameters of μ-FTIR are: 2 scans per pixel, an interferometer velocity of 2.2 $cm \cdot s^{-1}$, IR spectral range of 4,000 cm^{-1}–690 cm^{-1}, and imaging resolution of 6.25 μm or 25 μm was selected depending on the filters. For each sample, the μ-FTIR generated an absorbance image (<1.5h duration per filter) with an infrared spectrum information on each pixel.

2.5. siMPle for Rapid Identification and Quantification of Microplastics

To identify microplastics' structures in this study, a freeware, siMPle, developed by Aalborg University, Denmark and Alfred Wegener Institute, Germany (https://simple-plastics.eu/), was adopted. The reference database contains most polymers and natural materials such as protein and cellulose, a total of 23 material types. Primpke et al. (2020) verified the high sensitivity and high accuracy for microplastic identification by siMPle, with > 95% correct assignment rates on spectra. siMPle provided polymer types, range of abscissa and ordinates, number of pixels, minor/major dimensions, surface area, and estimated volume and mass of each particle. In this study, MPs concentrations were expressed ($MP \cdot L^{-1}$) and surface area ($\mu m^2 \cdot L^{-1}$) was also provided to better understand the 2D structure of particles. The estimated 3D data in the detection system, such as mass ($mg \cdot L^{-1}$) and volume ($\mu m^3 \cdot L^{-1}$), were not used because the thickness and shapes of particles were both calculated by empirical assumption in previous studies (Mintenig et al., 2020; Simon et al., 2018).

2.6. Verification of type and dimensions of polyethylene microspheres and of proportionality between quantity of MP and water volume

Synthetic polyethylene (PE) microspheres (0.96 $g.cm^{-3}$; 10 µm-150 µm) from Cospheric, USA were used as referred MPs. Moreover, due to their hydrophobic properties, PE particles were oxidized under UV light for 5 days (400 W, 60 °C in SEPAP 12–24, Atlas), and their spectra were not changed by FTIR-ATR verification, to evenly distributed in UP water. The µ-FTIR images of PE microspheres were shown in Figure 2. Statistical data analysis was performed using Microsoft Office Excel 2016 and SPSS (version 22, SPSS Inc.). The test for normal distribution uses Shapiro Wilk test on distribution of particle dimensions and showed that both minor dimensions (d_{min}) and major dimensions (d_{maj}) of PE fitted with a normal distribution (normality test, $p > 0.05$), resulting in $50 < d_{min} < 100$ µm and $100 < d_{maj} < 150$ µm, respectively.

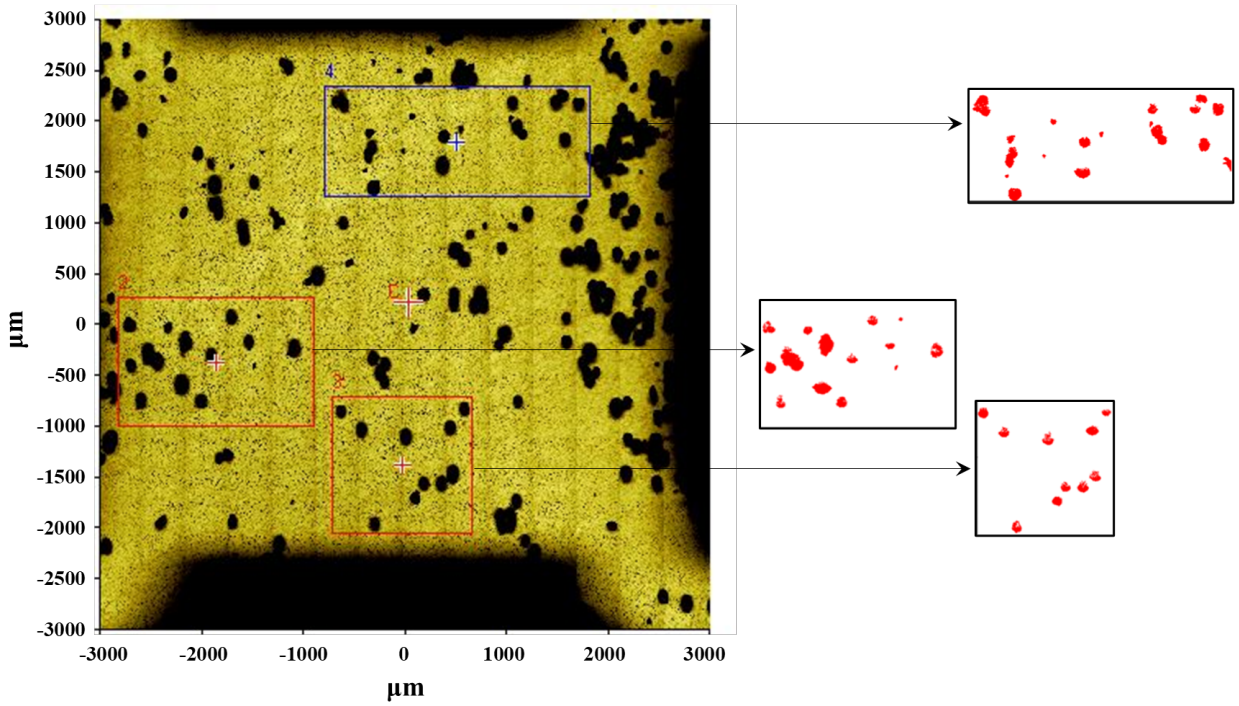


Figure 2 Visible survey and image of PE microspheres under µ-FTIR

To confirm whether the filtrated volume and the MPs counts is positively related, two experiments were designed to investigate: 1) MPs concentration in different volumes (250, 500, 1,000 and 2,000 mL) of UP water and 2) PE_MPs concentration in different volumes (250, 500, 1,000 and 2,000 mL) of synthetic PE suspension (0.1 $mg \cdot L^{-1}$). 0.1 $mg \cdot L^{-1}$ PE suspension was made

from a 10 mg·L^{-1} PE stock solution which has been stirred ⩾1 h before sampling. The synthetic PE suspensions were also stirred for 1 h and sonicated for 15 min before filtration. Each sample was detected in 3 replicates, and all filtrated through the 5μm gold-coated filters. The PE_MPs concentration in synthetic PE suspensions excluded the number of PE in UP water. The coefficient of MPs concentration in different volumes was calculated based on Eq(1):

$$E=(1-C_{MPs} / C_{MPs\text{-}Ave}) \times 100\% \qquad Eq(1)$$

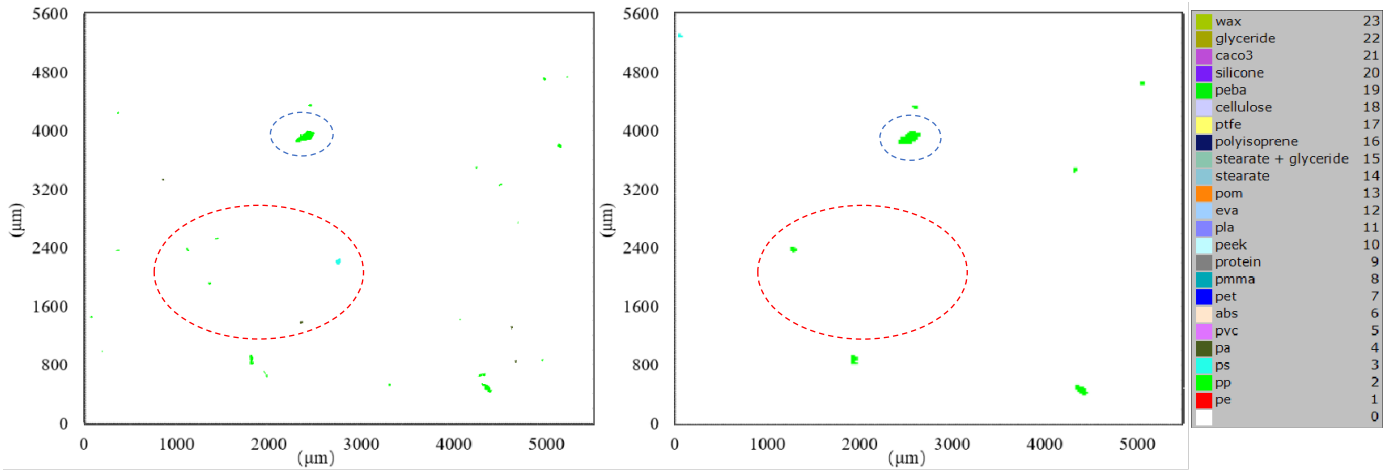
Where C_{MPs} (MP·L^{-1}) represents the tested MPs concentration in samples; $C_{MPs\text{-}Ave}$ (MP·L^{-1}) represents average MPs concentration in effective samples. A one-way analysis of variance (ANOVA) was conducted for linear regression test on particle numbers. All tests were statistically verified with $p<0.05$.

3. Results

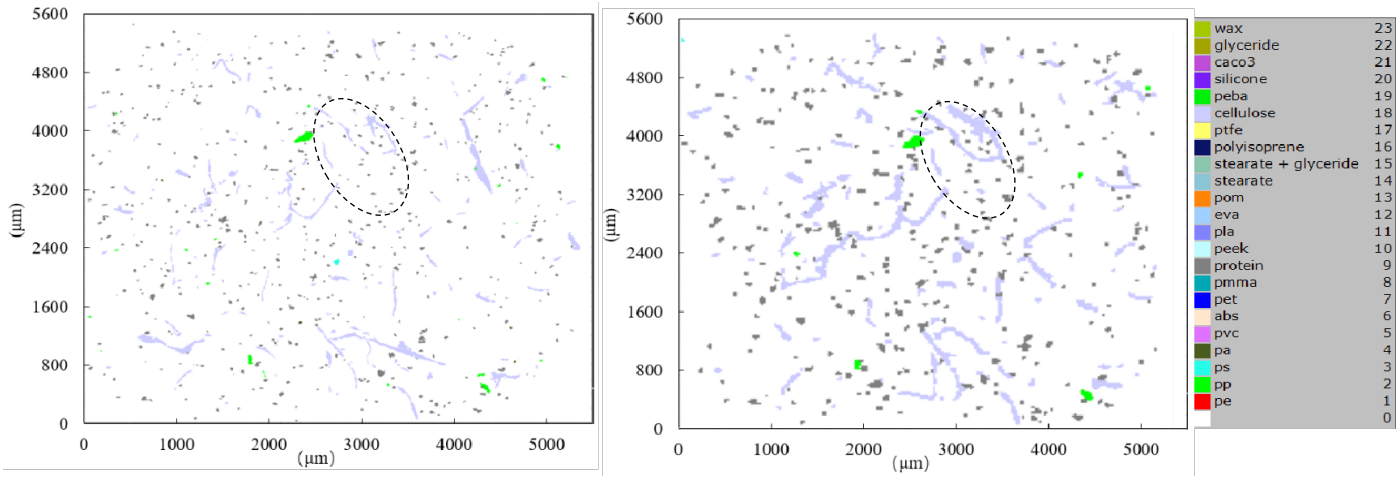
3.1. Identification and quantification of microplastic particles by μ-FTIR and siMPle

To obtain high-qualified results, differences between two imaging resolutions of μ-FTIR (6.25 μm and 25 μm) was investigated. As the maximum pore size of filters used was 25 μm, the oversized resolution of 50 μm was not considered in this study. Figure 3 showed the spectra maps of MPs and total particles (TPs), including MPs and natural particles, on the same sample (1L DI water collected on 3 μm filter) under 6.25 μm and 25 μm resolutions imaging. The results showed that: imaging at 6.25 μm resolution quantified 713 TPs, 31 MPs with types of PP, PA and PS; while imaging at 25μm resolution quantified only 407 TPs, 9 MPs with types of PP and PS on the filter. In Figure 3, the position, shape, and material types of most particles at 25 μm resolution were consistent to that at 6.25 μm resolution. While, higher resolution, 6.25 μm, exhibited more precise identification and quantification of MPs by μ-FTIR compared to lower resolution (25 μm): 1) lower resolution showed larger dimensions/surface area than higher resolution (blue dotted circle); 2) lower resolution might fail to capture smaller particles (red dotted circle); 3) lower resolution showed weaker quantification of closer particles which may be considered as one big particle (black dotted circle); 4) lower resolution identified less materials of particles. In contrary, higher resolution usually consumed longer detection time and larger space for storage, imaging at

6.25μm took almost 16 times longer than at 25 μm. Similar results were also exhibited in other samples, such as Tap water (SI Figure S2).



(a) MP_6.25 μm (b) MP_ 25 μm



(c) TPs_6.25 μm (d)TPs_25 μm

Figure 3 Spectra map of only MPs and TPs in distilled water from siMPle: (a) (b) represents MPs image by μ-FTIR at 6.25 μm and 25 μm resolution, respectively; (c) (d) represents TPs image by μ-FTIR at 6.25 μm and 25 μm resolution, respectively.

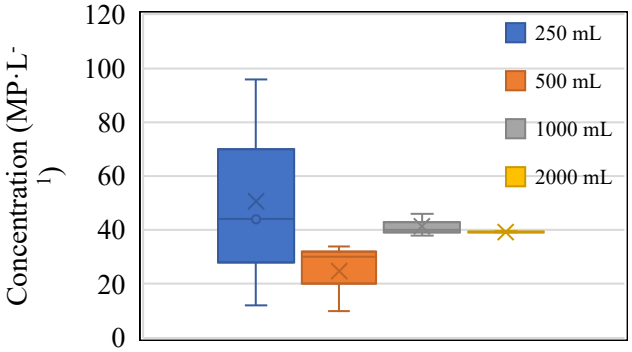
Considering the pros and cons, resolution of 6.25 μm was the dominant detection for all samples, and imaging at 25 μm was just recommended when the pore size of filters were ⩾25 μm, with

shorter detection time and larger storage space. In this study, the image on 25 μm filters used the 25 μm resolution, and the image on 3 or 5 μm filters used 6.25 μm resolution.

3.2. Filtration apparatus efficiency and rinsing selection.

Table 2 showed the abundances, types, and dimensions of MPs and TPs in different types of rinsing water by 6.25 μm imaging. Air quality was tested by filtrating air by vaccum pump for 30 min at 80 kPa, with 46 TPs and 2 PE MPs detected, confirming the ignorable influence of air on MPs results. The filtration of all water samples (1 L) through the gold-coated filter (5μm) was rapid (< 30 s) and replicated ($n \geqslant 3$).

In result, ethanol either in glass or PE bottles contains hundreds of MPs (most in PE) thus was not recommended for rinsing directly. DI water was secondly ranked in MPs concentration, while HPLC water exhibited the lowest concentration. The MPs concentrations in Evian, tap water, and UP water were ranged in the middle, while UP water contained the lowest TPs. Since DI and UP water were both generated from tap water, the increase MPs concentration and MPs types (PET, silicone, or EVA) might be related to process contamination by polymer pipes, taps, or filters, and the purified process was contributed to TPs removal. Considering the pros and cons, the following water/solution was not applied for rinsing in this study: tap and DI water excluded due to large amount of TPs, untreated ethanol due to abundant MPs; HPLC water due to expensive cost.; and Evian water due to the considerable cost and the presence of large particles (d_{maj}:20-200μm; d_{min}:16-100μm). In this study, UP water and purified ethanol (<5MPs filtrated by 0.22 μm membrane) were used for rising. As UP water was selected due to acceptable MPs concentration, the lowest quantity of TP, and smaller dimensions of MPs; filtrated ethanol was recommended due to with its merits on both hydrophilicity and lipophilicity. Ethanol was suggested to be prefiltered through a <1μm of non-plastic membranes to control the microplastics abundance before use. Additionally, the volume of rinsing water was controlled within <50mL ($\approx 1.0 \pm 0.4$ MPs) which caused ignorable influences on MPs results.



' in bold and TPs in italic), types and minor and major dimensions
MP in different rinsing solutions

		Types	Major dimension of MP (µm)	Minor dimension of MP (µm)
		PE, PP, PS, PA, PET, PVC, Silicone	20-150	10-60
DI Water	**26 ± 10** *558 ± 239*	PA, PP, PS, PET, PVC, ABS, PE, EVA, Silicone	20-250	10-120
Evian	**8 ± 7** *350 ± 146*	PA, PE, PP, PS, PET	20-200	16-100
Tap water	**6 ± 3** *1410 ± 156*	PS, PA, PP, PVC	45–80	10–40
HPLC grade water	**1** *361*	PP	25–64	13–28
Ethanol in glass bottle	**202** *526*	PE, PP, PET, PA, PS	20–304	13–112
Ethanol in PE bottle	**304 ± 70** *1317 ± 445*	PA, PE, PET, PP, PS	15–488	7–177

3.3. Abundance and Distribution of MPs *vs.* Volume Analyzed

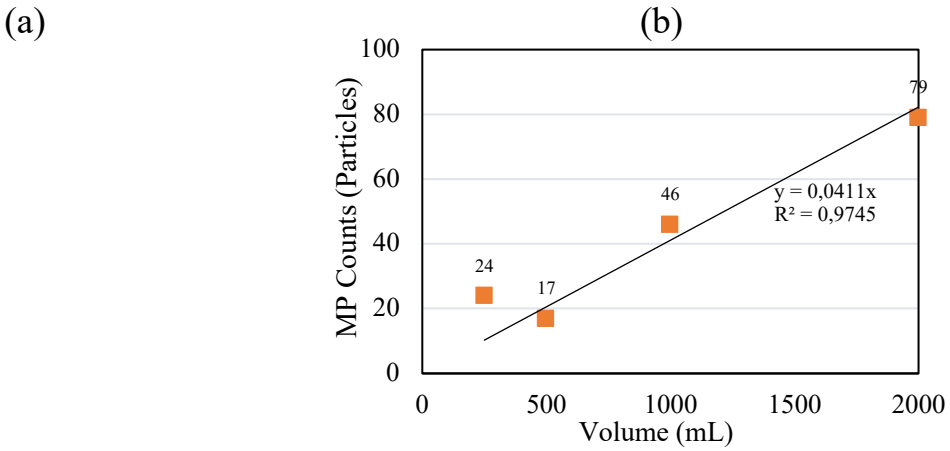


Figure 4 (a) MPs concentration (theoretically constant) versus different volumes of UP water in Box Plot and (b) linear regression of MPs counts versus UP volume

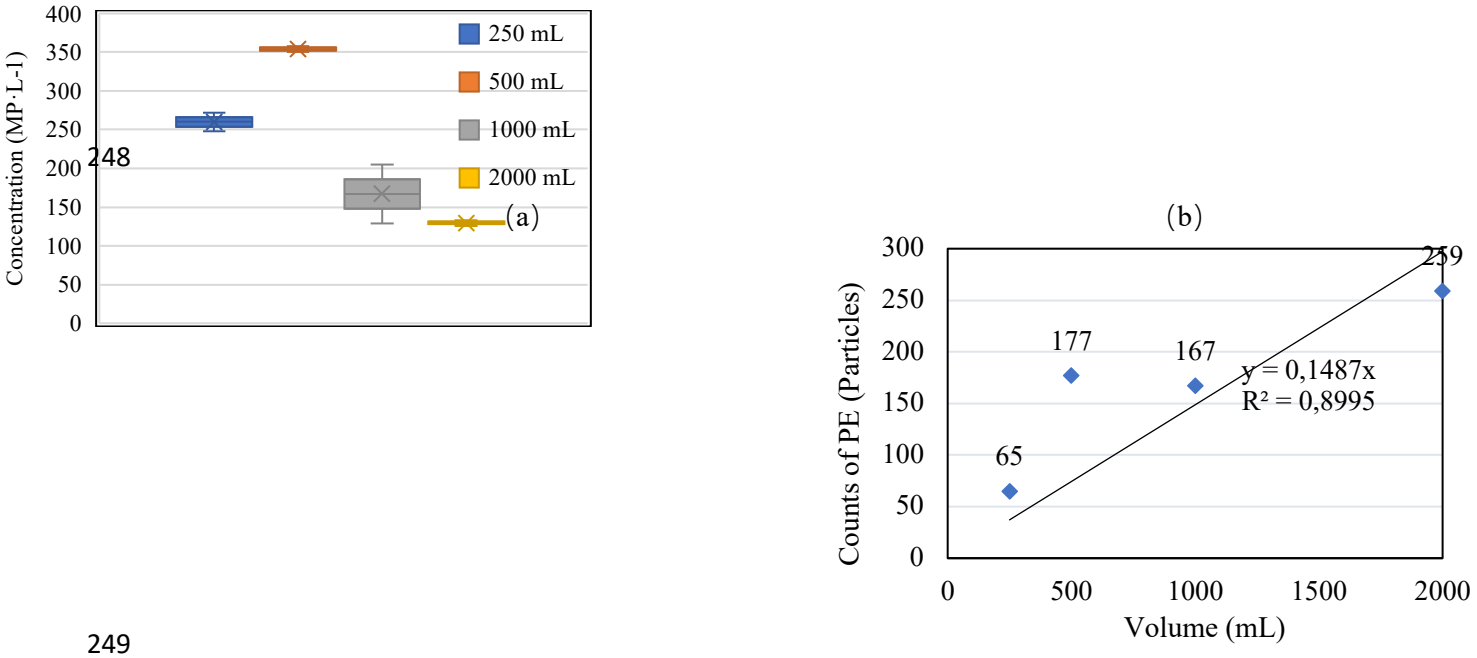


Figure 5 (a) PE_MPs concentration (theoretically constant) versus volumes of PE suspension (0.1 mg·L^{-1}) in Box Plot and (b) linear regression of PE counts versus filtrated volume

In UP water, the statistical test showed a positive correlation between MPs concentration and filtrated volume ($p = 0.004 < 0.05$, $R^2 = 0.9745$), shown in **Erreur ! Source du renvoi introuvable.**. In **Erreur ! Source du renvoi introuvable.**(a), MPs concentration tended to stabilize when filtering volume ≥500 mL which was consistent with the results obtained by Prata et al. (2020). The coefficient of variation decreased with increasing filtrated volume, resulting in 13.6% (500mL) → 11.9% (1000mL) → 3.8% (2000mL). In MPs-enriched suspension in Figure 5, PE_MPs concentration versus volumes of 0.1 mg·L^{-1} PE synthetic suspensions resulted in positive correlations ($p = 0.038 < 0.05$, $R^2 = 0.8995$), as expected. Moreover, PE_MPs concentration tended to stabilize when filtering volume ≥ 1000 mL. As a result, it can be inferred that MPs concentration was definitely positive-related with filtrated volumes. Particularly, both UP water and PE synthetic suspensions did not contain organic matteres, therefore, poor-organic samples with volumes ≥ 500 mL were considered as an optimum compromise between drawbacks and reliability of results.

However, samples in actual conditions may contain thousands or millions of MPs per liter and be rich in organics while impossible to complete by one-time detection. According to Anger et al. (2018) and Karlsson et al. (2020), MPs counts in subsamples fitted with continuous Gaussian distribution when samples with higher level of contamination, thus subsamples provided higher probability of accuracy. Being more precise, it is necessary to take subsamples with smaller volume (< 500mL) but more replicates (4~10 times) to improve the accuracy and reliability of

results. Accordingly, samples in this study were separated into two groups: poor-organic samples (once filtrated volume ⩾ 500mL) and rich-organic samples (once filtrated volume < 500mL). To be rigorous, poor-organic samples were tested with volume of 500~1000mL once and with 2-3 replicates; rich-organic samples were tested with the proper volume (<500mL) once and with 4-10 replicates to increase the reliability of samples (shown in Table 3).

3.4. Digestion protocol

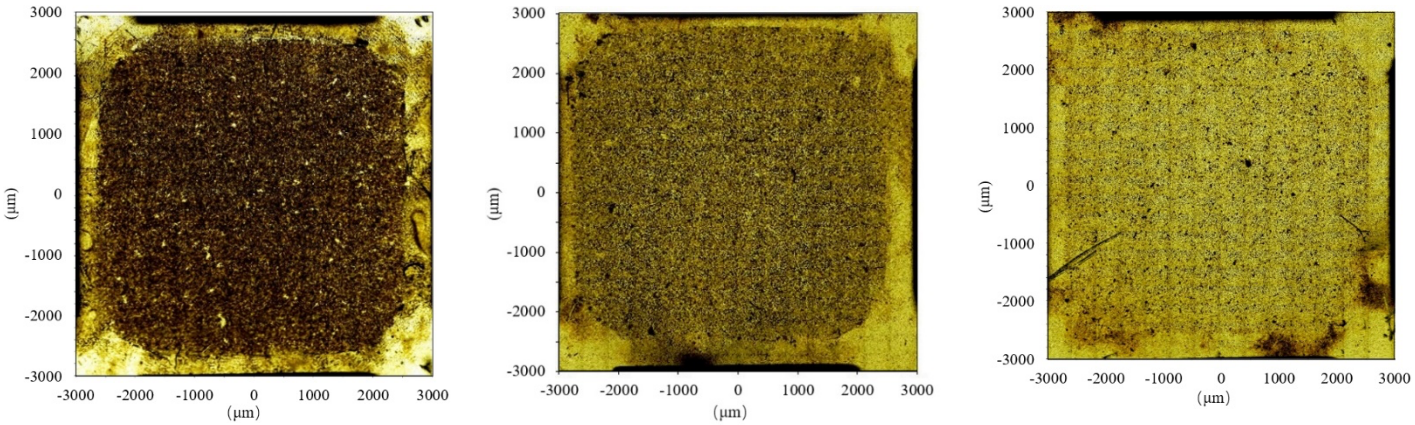
In this study, transparent samples with no visible suspended particles that were not digested and thus directly filtrated, including tap water, DI water, ultrapure water, Evian water, HPLC water, ethanol, tertiary treated effluents from DWTPs, WWTPs and SWTPs such as UF permeate, 1 and 0.2 μm outlets. Otherwise, for other samples (from DWTPs, WWTPs and SWTPs), a digestion process was necessary. The choice of digestion methods in different samples was explored and discussed in the following sections. The thoroughness of the digestion was closely related to temperature, dose of chemicals, and reaction duration. In this study, the dose of chemicals was added based on the literature experiments and testing in the lab.

3.4.1. Digestion of samples from WWTPs with H_2O_2 and Fenton

In view of secondary effluents from WWTPs, secondary effluents usually caused dense fouling on gold-coated filters which was unusable by IR spectroscopy.

Figure *6* (a) showed the filter image filtrated with 50 mL secondary effluent from WWTP 4, resulting in severe dark fouling cake digested by 1d H_2O_2. In following 7–10 d, the filter became cleaner with reaction time (

Figure *6* (b) (c)). Similar results were appeared on other WWTPs, digestion with 30% H_2O_2 (V_{sample}:V_{H2O2}=1:2) for 3-10d showed effective oxidation effects on all secondary effluents from WWTPs, as shown in Table 3.



(a) 50 mL - 1 day digestion (b) 50 mL - 7 days digestion (c) 50 mL - 10 days digestion

Figure 6 Visible survey of 50 mL secondary effluent (UF feed) from WWTP 4 on gold filter under (a) 1d digestion with H_2O_2 (b) 7d digestion with H_2O_2 (c) 10d digestion with H_2O_2.

In view of raw wastewater, it contained higher concentrations in organic matters and particles compared to treated effluents, digestion by H_2O_2 (V_{sample}:V_{H2O2} = 2:1) was insufficient to completely oxidize the organics, even after 14 d reaction→ opaque and turbid (Figure 7 (a), (b) and (c)). Imaged by μ-FTIR, the main components identified in raw wastewater from WWTP5 were proteins (Figure 7 (c.1)).As Fenton reagent could provide stronger digestion effects on organic-rich samples with less duration, and with fewer impacts on microplastic chemistry or size (Tagg et al., 2016), Fenton reagent (V_{sample}:V_{Fenton}=4:1) was added into the mixture after 14d H_2O_2 oxidation and the pH was kept at 2.5-3.5 to avoid the oxidation of iron and iron flocs (Pilli et al., 2015). Sample after another 24h digestion by Fenton became much cleaner and transparent (Figure 7 (d)), and the complete decomposition of proteins after 24h Fenton reaction was observed by μ-FTIR images, as shown in Figure 7 (d.1) and (d.2).

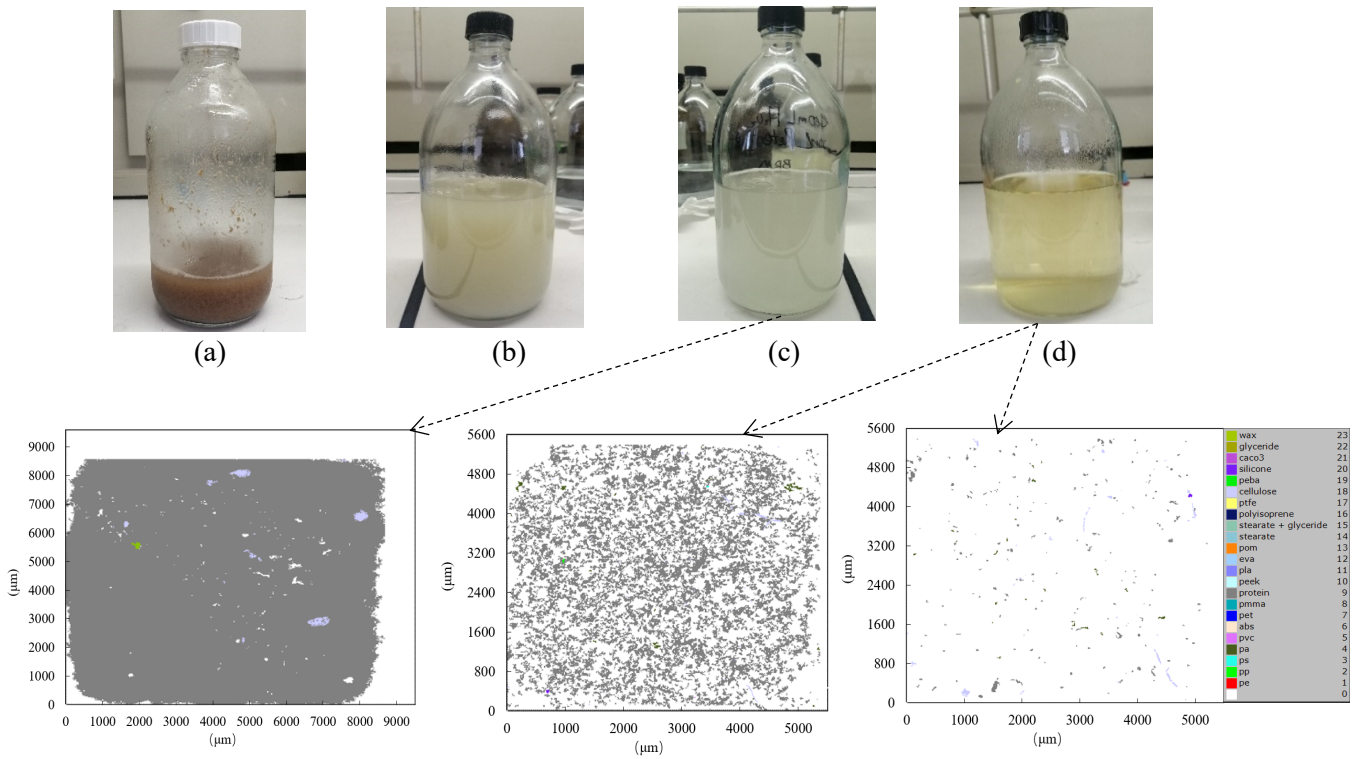


Figure 7 Digestion process of raw wastewater from WWTP5 and the related spectral map of TPs on gold filters (a) without digestion (b) after 6d digestion with 30% H_2O_2 (c) after 14 d digestion with 30% H_2O_2 (d) after 14 d digestion with H_2O_2 and 24h digestion with Fenton (c.1) 5 mL raw wastewater after 14d, H_2O_2 digestion (d.1) 5 mL raw wastewater after 14d H_2O_2 and 12 h Fenton digestion (d.2) 15 mL raw wastewater after 14d H_2O_2 and 24h Fenton digestion V_{sample}:V_{H2O2}=1:2; V_{sample}:V_{Fenton}=4:1.

Therefore, it could be inferred that H_2O_2 was able to digest secondary effluents (V_{sample}:V_{H2O2} =2:1) with availability to digest organic matters, but not proteins and cellulose. Fenton as a stronger oxidative reagent showed effective decomposition on protein/cellulose-rich samples such as raw wastewaters. Therefore, sequentially digestion by H_2O_2 and Fenton for raw wastewater samples was suggested: H_2O_2 was firstly added (V_{sample}:V_{H2O2} =1:1 to 1:2) to partially oxidize samples within 3-14d, then Fenton was applied (V_{sample}: V_{Fenton} =4:1 to 2:1) to finalize the samples digestion within 12-36h.

3.4.2. Digestion of Seawater From SWTP With H_2O_2 and KOH

Seawater samples (seawater, Zeo-A outlet, Zeo-B outlet) digested by one-step H_2O_2 (>10d) resulted in little improvement on filtration. 15 mL net samples could completely foul the gold

filters (Figure 8 a1,a2). Since seawater might contain shellfish and plant/algae tissues, 10% (w/v) KOH was added to samples which could break down soft tissue and bivalve tissues (Thiele et al., 2019). However, some calcium hydroxide and magnesium hydroxide were generated after 10% KOH applied, resulting in white and turbid solution. Therefore, 10% H_2SO_4 was added drop by drop into the solution to eliminate the insoluble alkaline precipitation after KOH digestion. Afterwards, the visible survey of seawater samples became much clear for identification and quantification (Figure 8 (b1,b2)).

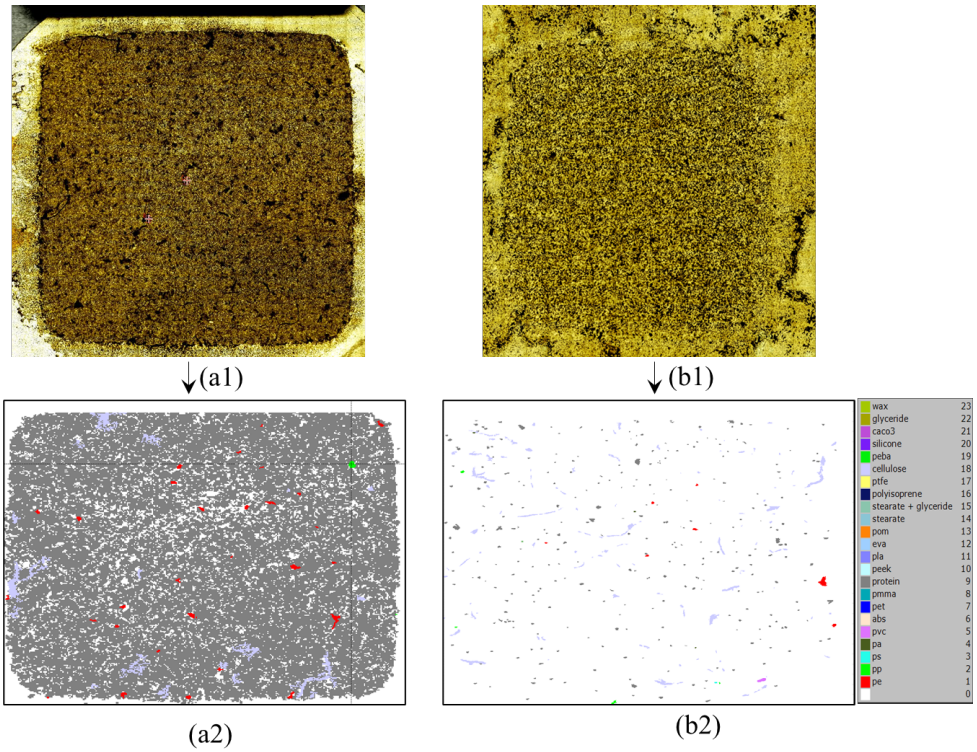
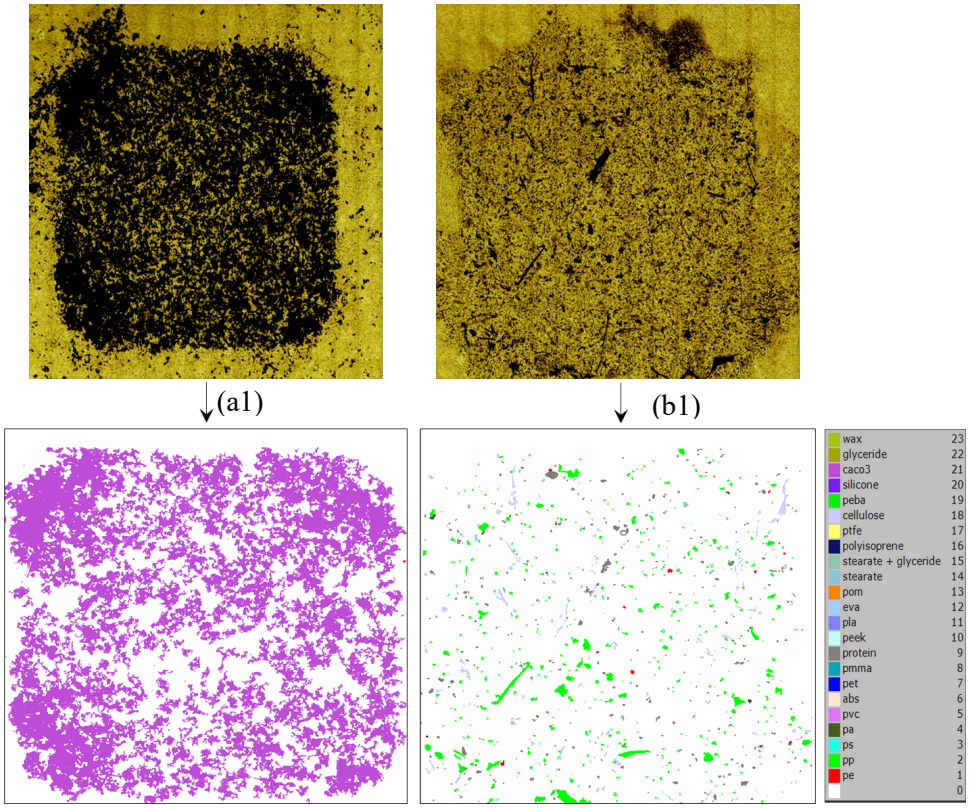


Figure 8 Visible survey and Spectral map of seawater: (a1) (a2) represents the visible survey and spectral map of 15 mL seawater without digestion, respectively; (b1) (b2) represents the visible survey and spectral map of 37.5 mL seawater after KOH digestion, respectively.

3.4.3. Digestion of surface and underground water with acid and H_2O_2

Surface water and groundwater quality were significantly better than the samples from WWTPs and SWTP with transparent and less visible particles. Slight or none digestion was needed for these samples due to their components: Underground water from DWTP 1 needed some acid (10% H_2SO_4) to dissolve the $CaCO_3$, which formed white cake on gold-coated filter and covered MPs, shown in Figure 9 (a2). The components of surface water seem to be a bit more complex than

underground water, which contained $CaCO_3$, proteins, and cellulose (Figure 9 (c1,c2,c3)). A combined treatment by alkali (10% KOH) and acid (10% H_2SO_4) was applied for surface water from DWTP 3. Samples from DWTP 2 were purified enough to be filtrated through 5μm filters thus with no digestion requirement.



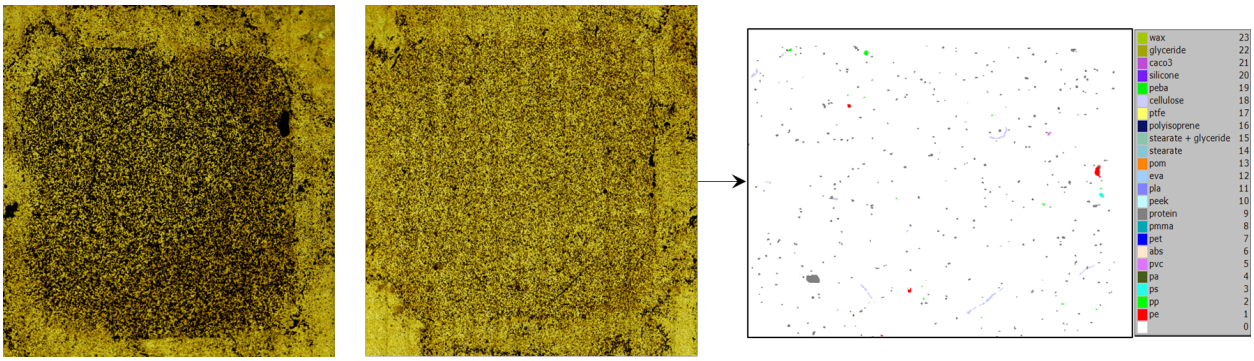


Figure 9 Visible survey and Spectral map of underground water (DWTP1) and surface water (DWTP3): (a1) (a2) represent the visible survey and spectral map with TPs of 225 mL underground water without digestion, respectively; (b1) (b2) represent the visible survey and spectral map with TPs of 485 mL sample after acidification, respectively; (c1) represent the visible survey 30mL surface water digested with 10d H_2O_2; (c2) (c3) represent the visible survey and spectral map in TPs of 30mL surface water digested with 10d H_2O_2 and 24h KOH.

3.4.4. Discussion on digestion improvement

Samples with digestion requirement were summarized in Table 3. Notably, samples without digestion requirement were excluded, such as the UF permeates and DWTP2 samples. In fact, the quality of samples without digestion were good enough to be filtrated directly all with turbidity <1.0 NTU, TOC <6.0 $mgC \cdot L^{-1}$, transparent, and non-visible suspended particles. Samples with digestion requirement were discussed: In WWTPs, the decreased TOC and turbidity in secondary effluents showed mild digesting method with 5-6 d H_2O_2, while raw wastewaters should be digested with 7-14 d H_2O_2 and 12-24h Fenton. In SWTP, samples contained lower TOC (2.0 - 3.0 $mgC \cdot L^{-1}$) but unignorable turbidity (1.3-6.3 NTU). The coupling of H_2O_2 and KOH were effective for digestion and without further oxidation by Fenton probably due to the dissolved/undissolved solids (salts, minerals, and tissues) (Al Dahaan et al., 2016). Samples from DWTPs had the best qualities with lowest TOC (0.5-1.3 $mgC \cdot L^{-1}$) and turbidity (0.34-1.2 NTU). DWTP2 samples need no digestion demand. While DWTP 1 samples need slight oxidation by H_2O_2, and DWTP3 samples need a further acidification, mainly due to the existences of proteins, cellulose, and $CaCO_3$. Therefore, it was inferred that water quality could, but not decisively, influence the selection of digestion especially on oxidative reactions; the composition of samples was considered as the main factor for digestion. Normally, samples with higher TOC (>10 $mgC \cdot L^{-1}$) and turbidity (>2 NTU) usually need further oxidation compared to samples of better quality, while these samples of better quality may still need slight oxidative, acidification, or alkalization process due to the dissolved or undissolved particles.

Table 3 Water quality of digestion-required samples and the related digestion processes

Sources	Water types	TOC ($mgC \cdot L^{-1}$)	Turbidity (NTU)	Digestion method	Digestion process and duration	Main components identified	*$V_{Net-max}$	Number of replicates
WWTP 1 (Pharmaceutical)	Raw wastewater (MBR feed)	170–620	323–864	H_2O_2, Fenton	14d H_2O_2, 12h Fenton	Proteins, cellulose	14 mL	10
	Secondary effluent (MBR permeate)	36.5	17.4	H_2O_2	6d		200 mL	5
WWTP 2	Raw wastewater	85.7	187	H_2O_2, Fenton	7d H_2O_2, 24h Fenton	Proteins, cellulose, stearate	40 mL	10
	Secondary effluent	4.9	0.18	H_2O_2	5d	Proteins, cellulose	500 mL	4
WWTP 3	Raw wastewater	125.1	180	H_2O_2, Fenton	7d H_2O_2, 36h Fenton	Stearate, proteins, cellulose	45 mL	10
	Secondary effluent	9.9	10.7	H_2O_2, Fenton	7d H_2O_2, 24h Fenton	Proteins, cellulose	300 mL	4
WWTP 4	Raw wastewater	121.1	204	Fenton	24 h Fenton	Proteins, cellulose	60 mL	10
	Secondary effluent (UF feed)	6,627	1.8	H_2O_2	5d		225 mL	4
Seawater treatment plant (SWTPs)	Seawater	-	-	H_2O_2, KOH (+acid)	5–6 d H_2O_2 1d KOH	Soft tissues (unidentified) and Minerals	37.5 mL	10
	Zeo-A outlet	2,322	1.3	-	-		200 mL	8
	Zeo-B outlet	2,096	4.8	H_2O_2, KOH (+acid)	5–6 d H_2O_2 1d KOH		42 mL	10
	Old UF feed	-	6.3	H_2O_2, KOH (+acid)	10d H_2O_2 1d KOH		14 mL	10
	New UF feed	2,896	3.4	H_2O_2	7d		70 mL	5
DWTP 1	Underground water	0.59	0.7	Acid	2h	$CaCO_3$	485 mL	3
	Primary effluent	0.62	1.2	H_2O_2	2d	Proteins	150 mL	4
DWTP3	Surface water	1.3	0.34	H_2O_2, Acid	14 d H_2O_2	Proteins, $CaCO_3$, cellulose	30 mL	10
	Sedimentation outlet	1.2	0.68	H_2O_2, Acid	7–14 d H_2O_2		100 mL	5

*$V_{Net-max}$ represents the max volume of the samples passing through the gold filters in this study

In view of digesting duration, oxidation by H_2O_2 needed the longest duration (2-14d) compared to Fenton oxidation (12-36h), alkalization ($\leqslant$ 24h), and acidification (<1h), thus 14 d by H_2O_2 was considered as a turning timepoint where stronger oxidation was necessary. To shorten digestion duration, larger dosage of chemicals, increased concentration, or higher temperature could be applied (Hurley et al., 2018; Prata et al., 2019). With samples enriched in organics, the

pre-digesting process by H_2O_2 could be partially shortened (to 5-7d) and followed by Fenton oxidation. Notably, improvements by heating and digestion duration, and higher concentration, particularly by alkalization and acidification, could increase the risks on damage of microplastic properties, such as decolorization, oxidation, or even degradation (Hurley et al., 2018; Schirinzi et al., 2020). Regarding negative effects of digestion, some studies demonstrated the partial degradation (<18% of recovery) of PC and PET by 10 % KOH when increasing temperature to 60 °C (Karami et al., 2017), and some polymers were founded to be damaged with concentrated acid (e.g. ⩾ 69 % HNO_3) under high temperatures (T ⩾ 50 °C) (Schirinzi et al., 2020). Actually, most plastic polymers were impervious to digestion by 10 % KOH under controlled temperature (⩽60 ◦C) and digestion duration (≤24h) (F. Li et al., 2018), and reducing acid concentration would protect most polymers (e.g. PA, PC, PE, PET, PP, PS and PVC) from breaking down at room temperature (Schirinzi et al., 2020). To minimize damage to microplastics, this study was conducted under room temperature throughout all digestion processes, and the digestion duration by alkalization were controlled within 24h, and acidification was usually applied before filtration immediately. More efforts on optimization of digesting duration can be made in recent future.

3.5. Reliability of the Method

To evaluate the reliability of the μ-FTIR coupled with siMPle detection method , nine criteria described by Koelmans et al. (2019), including sampling method, sample size, processing and storage, laboratory preparation and clean air conditions, negative and positive controls, sample treatment and polymer identification were self-assessed (Table 4). The details about each criteria were listed in Table S3 SI . The highest reliability is obtained for the highest score. On negative controls, MPs in air and in various types of rinsing water were detected for ≥3 replicates (section 3.2), and rinsing water (UP and filtrated ethanol) was evaluated to be ignorable (<2 MPs) both used for lab samples and field samples (DWTPs, WWTPs, and SWTP); The uncertainty was the control not always detected before each type of water. Therefore, the negative control should be scored at least for 1, and probably for 2. Relatively, the PE solution was detected as the positive control to evaluate μ-FTIR and siMPle method, resulted with qualified MP types, and with dimensions (10-150 μm) and shapes (micropheres) consistent with manufactures, while the recovery rate was undetectable, resulting in 1 score for positive control. Compared to Koelmans

et al. (2019) scoring 11.5 for treated tap water, 12.5 for DWTP water, 7.9 for surface water, and 7.3 for wastewater, this study obtained equivalent score for tap water (11-12), higher scores for surface waters (11-13) and wastewaters (13-14). To be more relevant, four other recent studies (<3 years) were also evaluated for comparison. The scores (11-14) in our study still ranked in front position. Particularly, the comparative detection on drinking water by Kirstein et al. (2021) and on potable water by Johnson et al. (2020) also used μ-FTIR for analysis. Therefore, the qualified scores in this study and the proves by studies using μ-FTIR both demonstrated the reliability of this proposed approach.

Table 4 Self-Assessment of microplastic identification and quantification method in this work and the recent studies (Johnson et al., 2020; Kirstein et al., 2021; Ourgaud et al., 2022; Primpke et al., 2020b)

Type of samples	**Criteria**									
	Sampling methods	Sample size	Sample process and storage	Lab preparation	Clean air conditions	Negative control	Positive control	Sample treatment	Polymer identification	Total score
Seawater (surface)	1	1	1	2	1	1-2	1	2	2	12-13
Wastewater	2	1	1	2	1	1-2	1	2	2	13-14
Surface water/ groundwater	1	0	1	2	1	1-2	1	2	2	11-12
Tap water	1	0	1	2	1	1-2	1	2	2	11-12
Marine water (Ourgaud et al., 2022)	2	1	2	1	1	1	1	1	1	11
Various waters (Primpke et al., 2020)	1	0	0	0	0	1	1	0	1	4
Drinking water (Kirstein et al., 2021)	2	2	1	1	1	2	1	1	2	13
Potable water (Johnson et al., 2020)	2	2	1	1	1	2	2	1	2	14

4. Conclusion

This study aims to develop an independent method approach for the identification and quantification of microplastics in different water samples (sea, fresh and wastewater). After comparison between different rinsing waters/solution, ultrapure water and filtrated ethanol were selected based on the lowest number of MPs found. Synthetic PE particles were used to verify the type and sizes obtained by μ-FTIR focal-plane-array coupled with SiMPle software. A proportional relationship was obtained between the number of PE particles and filtrated volume even if filtrating the same and highest volume possible of the sample is preferable. The pretreatment of samples (i.e., digestion) was demonstrated to be crucial. In summary, organic matters such as proteins and cellulose can be oxidized by H_2O_2 or Fenton, some salts such as $CaCO_3$, stearate can be digested by acids, and soft tissues or muscle can be digested with KOH. Normally, samples with higher TOC, turbidity, and higher suspended solids need stronger digestion. Some ranges of these parameters are given to estimate the digestion mode. The water quality can give some reference to the level of digestion, but it is not the decisive factor and there is no specific relationship between the water quality and digestion levels (method, duration, dosage). For examples, the TOC and turbidity among tertiary treated water, seawater, and surface water are very similar and all of them are in quite low values, but the tertiary treated effluents are all good enough to be filtrated more than 500 mL at once without digestion, while the others cannot. Therefore, it is necessary to know the real components in the samples and choose the effective methods. The concentration of chemicals and contact time are both controlled in acceptable ranges to have no or very few effects on microplastic properties, according to the research experiences and literature.

Finally, considering the recent literature, rigorous detection process and high-precision analytical methods to obtain the number, size and type of microplastics by μ-FTIR focal-plane-array imaging were developed in this Part I and will be used in Part II to assess the efficiency of (membrane) treatment processes to remove MPs in very different water treatment plants.

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