

Analysis report: Microplastic Detection | Sample XXXX | Client XXXX

For detailed inspection of the data and findings, see the attached certificate of analysis (COA), available in .xlsx format, including details on the blank- and recovery subtraction performed.

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1. Sample matrix

'Anonymous brand' mineral water stored in 1.5 L polyethylene terephthalate (PET) bottles with green polyethylene (PE) screwcap (Fig. 5).



Figure 1 - Photograph of the untreated sample matrix of 'Sample_XXXX' *i.e.* Bottled mineral water in a PET container. The bottle in this photograph is an example and does not represent the true analyzed, anonymous brand.

2. Results | Sample_XXXX

2.1. Microplastic concentration | Sample_XXXX

Sample_XXXX was analyzed for microplastic content, including particles down to 1 μm in diameter. The sample matrix *i.e.* bottled mineral water, was determined to contain 504 microplastics ($\geq 1 \mu\text{m}$) (n/L), of which polypropylene (PP) constituted more than 65% of all detected microplastics. In terms of mass, the microplastic concentration was determined at 0.045 $\mu\text{g/L}$ (Fig. 1). Only microplastics of fragment-type morphology were detected.

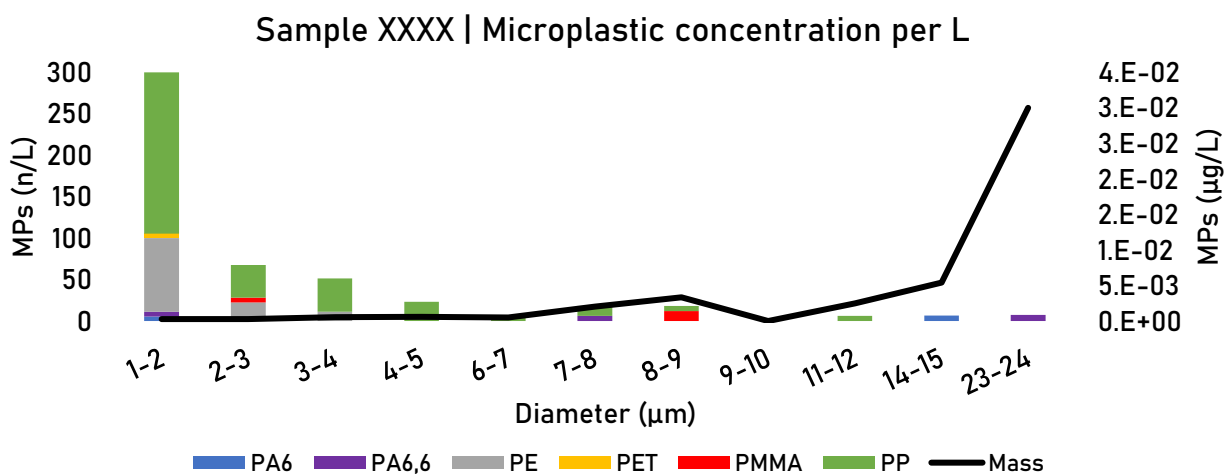


Figure 2 - Comprehensive figure displaying number of individual polymer types within size ranges on the order of 1 μm , as well as total mass of microplastics detected in Sample_XXXX. Data is normalized to 1L of sample, as well as blank- and recovery corrected.

2.2. Particle size distribution (PSD) | Sample_XXXX

Microplastics in the 1-2 µm range constituted 59.5% of all detected microplastics and the largest detected microplastic measured less than 24 µm in diameter (Fig. 2).

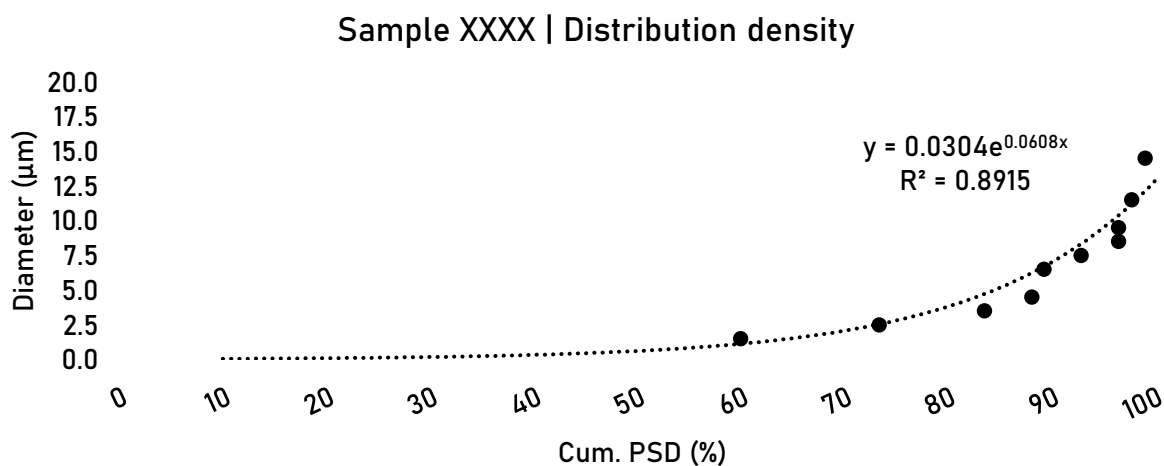


Figure 3 - Cumulated distribution density of detected microplastics. Particle size distribution (PSD).

The cumulated distribution density (Cum. PSD) can be described by an exponential function:

$$\text{Cum. PSD} = 0.0304e^{0.0608 \cdot \text{Diameter}}$$

3. Negative quality control (blank correction)

5 L of ultrapure grade-A milliQ water was filtered through 0.2 μm , 47 mm aluminium oxide filter membranes (AnoDisc, Whatman) into individual kiln sterilized 1 L glass bottles, to remove potential microplastic contaminants, producing a microplastic-free solution to be used in the procedural blank experiment. The blank was treated according to the same protocol as the true sample(s) to estimate and correct for procedural contamination during sample treatment.

On the 2x2 mm grid subsample investigated, a total of 20,307 individual particles were analyzed by Raman microspectroscopy, under the same conditions as the true sample. Out of these, 9 microplastics were detected (Fig. 3). The detected microplastics were subtracted from the true sample(s), based on polymer type and size (see details for blank subtraction <https://www.microplasticsolution.com/microplastic-detection>). The case-specific subtraction is available in the COA.

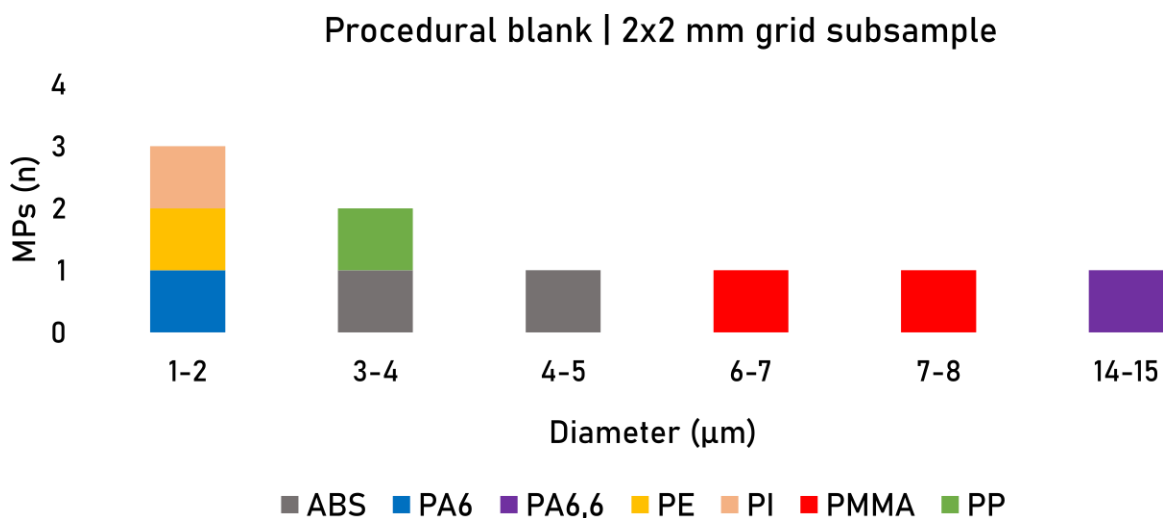


Figure 4 - Figure displaying number of individual polymer types, within size ranges on the order of 1 μm , detected in the 2x2 mm grid subsample of the procedural blank. A total of 9 microplastics were detected.

4. Positive quality control (recovery correction)

To correct for unintentional microplastic particle loss during sample treatment ^[1,2], a procedural recovery experiment using a precise number of red polyethylene (PE) fragments in the 10-100 µm range (PrecisionMP™, microplasticsolution.com, France), was conducted.

In three individual samples of 5 L of grade-A milliQ water, a total of n = 1,004, 1,095 and 1,003 microplastic fragments were intentionally added and the samples were treated according to the same protocol as the true sample(s). Following the full protocol, the remaining number of spiked microplastics was evaluated. Here a total of n = 936, 856 and 809 MPs were recovered.

The recovery experiment demonstrated an increase in analytical microplastic recovery with decreasing size, leading to positive recovery, possibly caused by particle break-up during the sample treatment (Fig. 4). The recovery rate (RR) can be described by an exponential function:

$$RR = 141.277e^{-0.013 \cdot \text{Diameter}}$$

RR was corrected for within size ranges on the order of 1 µm, calculated using the-above formula.

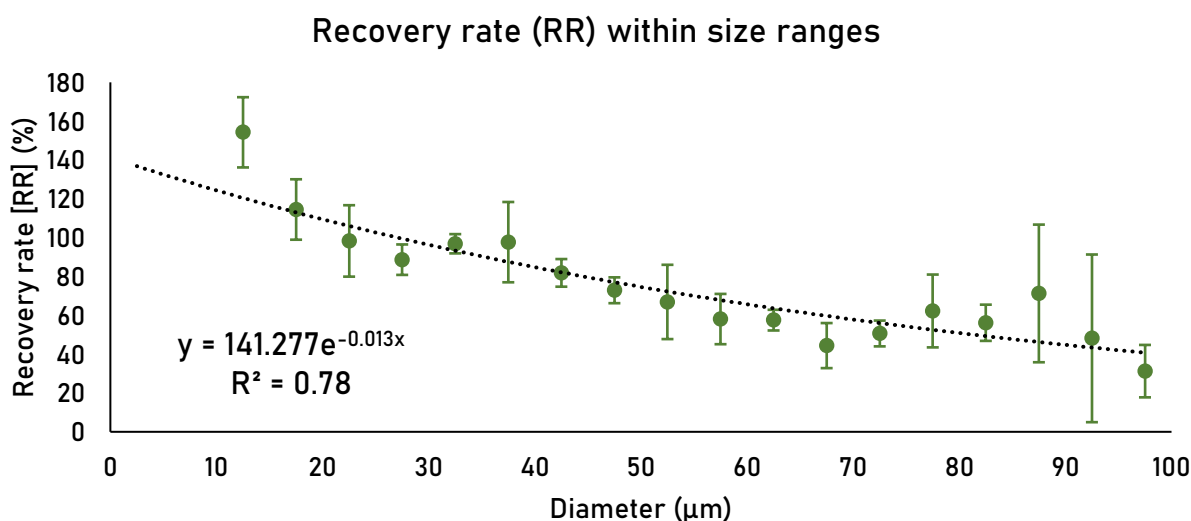


Figure 5 - Recovery rate (RR) was evaluated within size ranges on the order of 5 µm, from 10 µm, using PrecisionMP™ red polyethylene (PE) fragments.

5. Methods and materials

5.1. Sample pre-treatment

To realize the detection of microplastics down to 1 μm in diameter using Raman microspectroscopy, a total of 5L of sample (3 and 1/3 1.5L bottles) was filtered through hydrophilic 0.45 μm , 47 mm polyvinyl fluoride (PVDF) filter membranes (Durapore®, Merck KGaA, Germany), using a glass vacuum filtration device. Each filter membrane was transferred into 50 mL glass vials filled with 30 mL of 30 vol.% hydrogen peroxide (H_2O_2) (Fischer Scientific, Belgium). To improve the transfer from the filter membrane to the H_2O_2 -solution, each vial and its contents were ultrasonicated (BPAC, France) for 1 minute. Consequently, the filter was evacuated from the vial using a stainless-steel tweezer while being flushed with H_2O_2 (30 vol.%) to impede particles from sticking onto the filter membrane thus increasing microplastic recovery. H_2O_2 (30 vol.%) The filter was flushed until the vial held 40 mL. Following six continuous days of hotplate-induced digestion at 50°C, 5 mL of 5 vol.% hydrochloric acid (HCl) was added to each vial under a fume-hood, to increase the acidity of the solution and improve the digestion efficiency of carbonate minerals. The reagents together lead to a redox reaction that is weaker than Fenton's reaction, slowly forming hypochlorous acid (HOCl) and water (H_2O) ^[3]. Following 24 h of reaction, each solution was filtered through individual 0.2 μm , 25 mm aluminium oxide filter membranes (Whatman Anodisc, U.K.) and flushed with ultrapure grade-A milliQ water (18.2 M Ω -cm), leaving the desired particles on a flat surface suitable for microspectroscopic Raman analysis ^[4].

5.2. Raman microspectroscopy

In the center of the filter membrane, a 2x2 mm grid corresponding to 2.6% of the filtered area was analyzed by automated Raman microspectroscopy. A total of 7,821 individual particles were inspected. Raman measurements were carried out at 20°C using a Horiba (Jobin Yvon, France) LabRAM Soleil. The samples were excited at 8% (7.2 mW) power output with a high stability air-cooled He–Cd 532 nm laser diode utilizing a Nikon LV-NUd5 100x objective. The lateral resolution of the unpolarized confocal laser beam was on the order of 1 μm . Spectra were generated in the range of 200–3400 cm^{-1} using a 600 grooves/cm grating with a 100 μm slit. The spectral resolution was on the order of 1 cm^{-1} .

5.3. Spectral matching and verification

The processed spectra were cross-referenced for their entire spectral range, using our in-house library containing selected spectra from the SLoPP and SLoPP-E ^[5] and the Cabernard ^[6] spectral libraries, also including self-obtained in-house polymer spectra. Spectral matches were denominated by hit quality index (HQI)-values from 0 to 100% match. Spectra rated above 65% HQI were considered as microplastic candidates and were manually inspected and sorted by a trained interpreter to determine their validity. All analyzed spectra are available in Thermo Galactic GRAMS (.spc) format.

6. References

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6. Cabernard L, Roscher L, Lorenz C, Gerdts G, Primpke S. Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment. *Environ Sci Technol* 2018;52(22):13279–88.