Supplementary Materials of:

Intra-Laboratory Calibration Exercise for Quantification of Microplastic Particles in Fine-Grained Sediment Samples: Special Focus on the Influence of User Experience

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1. Protocol of Extraction

Briefly, thaw samples stored at -20°C by placing them in an oven at 40°C for at least 2 hours. Then homogenize the samples manually with a metal spoon and add 50 ml of sediment (equivalent to about 75-100 g) to a 250 ml glass jar that has been previously placed on the balance and calibrated. After noting the wet weight, immediately cover it with aluminum foil. The determination of the dry weight, although irrelevant for calibration exercise, is essential in the context of the application of the protocol for environmental monitoring, since the contamination of the plastics is conventionally expressed in terms of the number of particles per g or kg of dry sediment. Continue with the other samples. Place everything in the oven at 40°C for at least 48 hours (times can be extended depending on water content). For environmental monitoring, it is recommended to prepare 3 aliquots of 50 ml for each sediment sample (so the final result is the sum of the three aliquots).

Remove the dried samples from the oven and reweigh them for dry weight determination. Add the set of internal standards to the beaker (only to the sediments, not to the control) and make sure that all particles have been transferred. Then proceed to pretreat the sample in hydrogen peroxide to digest the organics that would otherwise form flocs and prevent separation by the density gradient of the next phase. Then add 50 ml of 6% H_2O_2, cover and place on the stirrer for 1 minute (speed 6). Let the solution sit on the counter for 5 minutes. Repeat stirring a few times, if necessary, to promote diffusion of the peroxide throughout the sample and to reduce the foam produced. Allow the mixture to sit for 72 hours.

Then proceed with filtration of the supernatant using a vacuum pump and 3 µm filters of nitrocellulose (NC). Pick up the liquid from the edges of the beaker and stay on the surface (this is where the plastics accumulate). When all the liquid has been absorbed, rinse the tip inside and out with water using a spray bottle in the filtration funnel. Then, always using the spray bottle, rinse the walls of the funnel: all the plastics adhering to the glass will thus be collected on the filter. Place the filter (called POST-DIGESTION) in a glass petri dish and cover immediately. For the controls, take 50 ml. Then proceed to the first density gradient separation step: add 200 ml of saline solution to each beaker and mix on a magnetic stirrer for 10 minutes. Then transfer them to the counter and let them sit for 24 hours. Shake the controls for 5 minutes. Place the filters in the oven at 40°C for a few hours or leave them on the counter until evaporation of water.

Remove the saline solution by proceeding with filtration of the supernatant at 3 µm in NC (FILTER 1) and then place the filters in the oven. Perform a second extraction with another 200 ml of brine (wait 24 hours). Continue with the final filtration of the supernatant (FILTER 2). Allow the filters to dry. View the filters under the microscope.

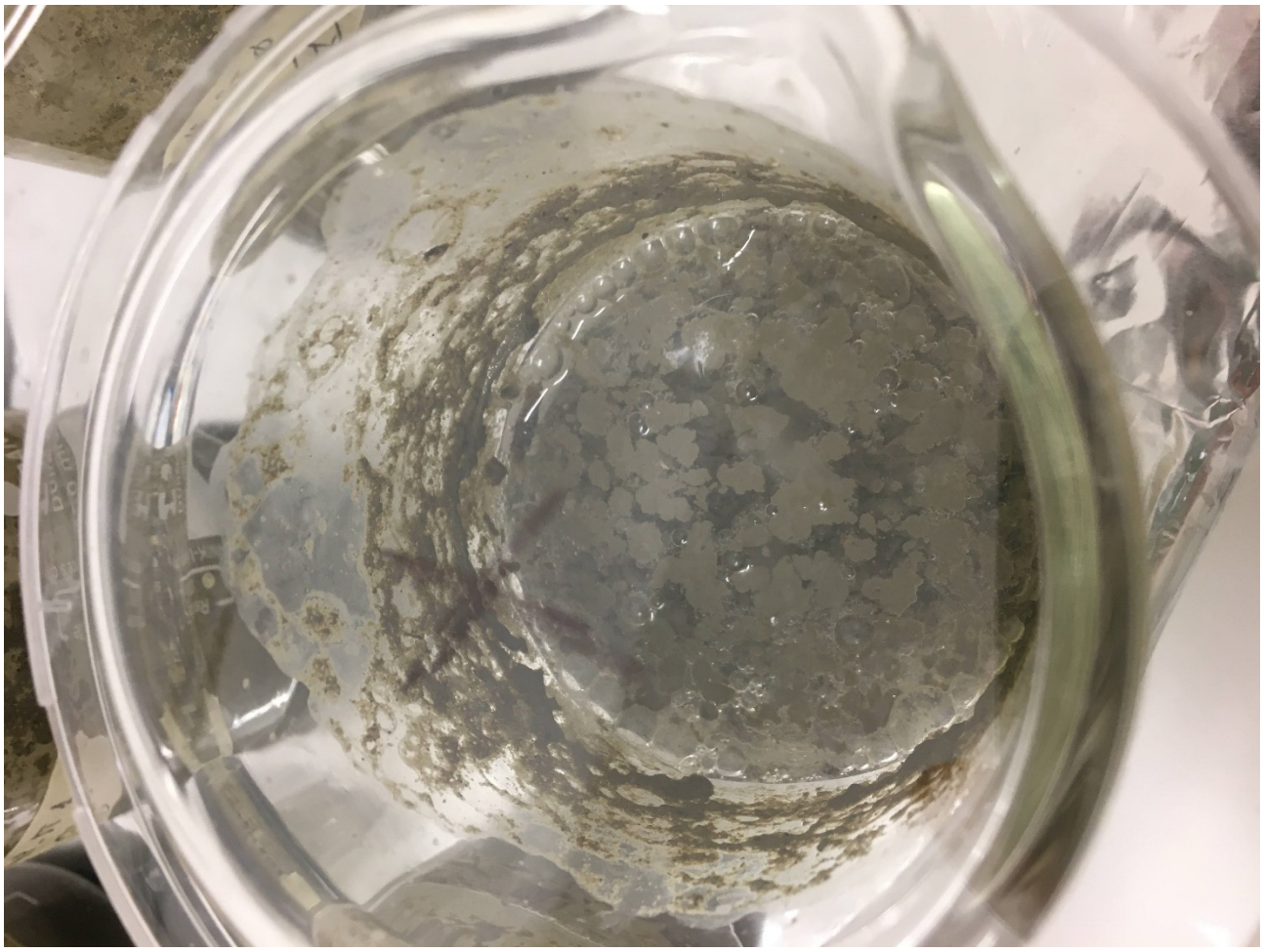

Figure S1. A sample not digested with hydrogen peroxide exhibits flocculation that prevents proper density-based separation.

2. Microplastic Alterations

Further details on the percentage of agreement between the spectra of 6% peroxide treated and untreated particles are given below.

Table S1. Percentage of agreement between treated and untreated particles. For an understanding of the meaning of a-n, the reader is referred to the main text.

type of Particle	% Matching with No Treated
a	99.93
b	75.06
c	78.08
d	97.40
e	95.73
f	96.36
g	99.77
h	98.53
i	98.29
l	99.70
m	99.99
n	95.90

3. Cost estimation

Below is the cost estimate for a sediment sample (in triplicate), which is for consumables only. The cost of labor and chemical analysis is not included.

50 ml H_2O_2 6%	=		0.56 euro
400 ml brine (NaCl pure)	=		56.16 euro
Sartorius, cellulose nitrate filter, 3 µm pore size	=		4.74 euro
total		**=**	**61.5 euro**

400 ml brine (table salt)	=		0.58 euro
total		**=**	**5.9 euro**

4. Contamination in Controls

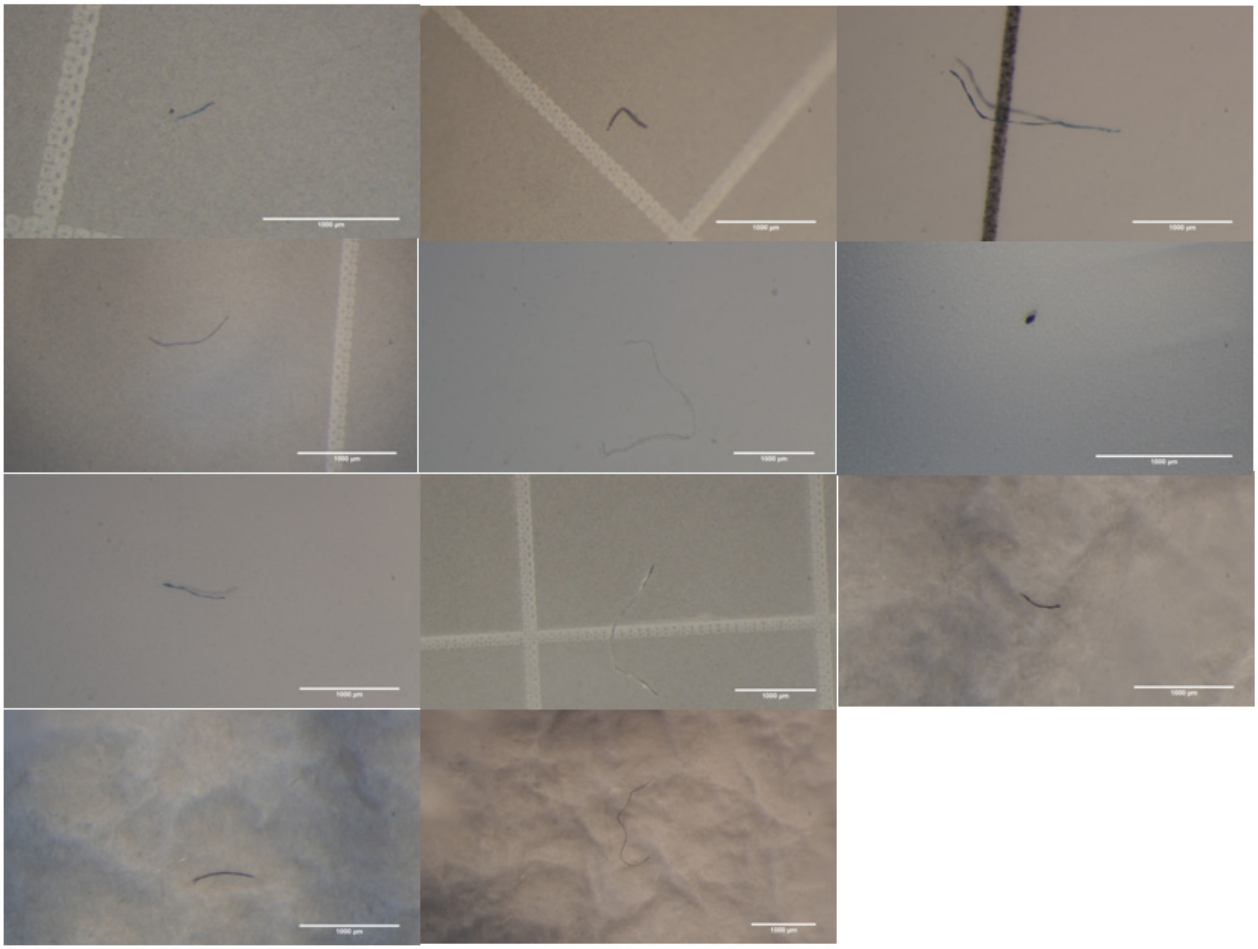

Figure S2. Example of particles found in controls (water) during the execution of the calibration exercise. The average contamination level was of 1.5 fibers/lines for samples, 0.5 fragments for samples (although chemical analysis of the particles is lacking as confirmation).