SI Supplementary information

Title: *When every particle matters: a QuEChERS approach to extract microplastics from environmental samples*

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SI.1 – Starting principles and a purpose-built MP laboratory

Our own observations and published literature (e.g. reviewed in Dehaut et al. (2019) or Zarfl (2019)) agree that sample contamination is the crux of environmental MP research, especially when small MP sizes are targeted (i.e. < 500 µm). The omnipresence of plastic materials in a standard laboratory, but also in clothing, airborne dust and laboratory equipment enables the abrasion and intrusion of contaminating MP into the samples. Also, other non-plastic particles such as hair or skin ablation, cotton fragments or wear of glass or metal parts can contribute to the pool of contaminating particles. Together they have negative impacts on MP detection limits and analysis time.
Optimal working conditions for MP sample treatment are not easily, nor cheaply established in a typical research laboratory environment. The following list can be seen as an inexhaustible collection of the most impactful infrastructural adaptations of a purpose-built MP lab:

- dedicated clean room facility with no avoidable plastic devices (especially mechanical parts)
- fine particulate filters on any supplies of air, gases, water
- non-circulating laminar flow bench connected to lab air exhaust
- supply of external vacuum to avoid pump operation inside the clean room
- unavoidable plastic parts all made of the same (non-abrasive) non-target polymer (e.g. PTFE)
- wet lab capabilities (floor drain, large washing / rinsing area)
- muffle oven with exhaust system connection for pyrolysis of contaminating MP
- water ultra-purification system with a plastic-free dispenser unit
- working surfaces and equipment made of hard and smooth materials, e.g. polished stainless steel and borosilicate glass.

SI.2 – QA / QC measures

Precautions have to be taken at every protocol step to mitigate MP contamination, for example, from the laboratory air, tap water or the chemicals required for processing. A range of good practices on MP extraction is suggested by MP researchers working on diverse topics (e.g. Dehaut et al., 2019), and we also strongly encourage these measures to be a routine for MP studies. Firstly, a clean bench was installed in the lab creating a microplastic free (MPF) environment where samples can be safely manipulated and are protected from airborne MP, especially fibres. The clean-bench was set up with an assembly for the parallel mounting of separation funnels (up to 16) for a more efficient density separation. The installation of the clean bench per se is not enough. It is necessary to clean and rinse all the materials that eventually enter the clean bench (see also Specifications table and General module m0 in the associated paper). To minimise and monitor levels of fibres and other MP particles in laboratory air, an air filter equipped with a fine particulate sensor was installed allowing researchers to take decisions on whether to close the clean bench in a specific event (e.g. doors or windows of the lab being opened or cleaning of lab surfaces). All above measures aim to assure that quality standards required for particle-based MP analyses are met. However, those measures can only minimise and not eliminate particle loss or contamination. Quality control measures of regular parallel blind samples (negatives) and artificially spiked recovery samples (positives) further help to reify both what fraction of the environmental MP pool can be accounted for by a particular study as well as how large the error margins are likely to be.

SI.3 Simple and mobile tap water filters

In many cleaning and/or sample handling steps in our protocols, flushing, resuspending or soaking is necessary. Here the usage of filtered (for the minimum MP size range of interest, i.e. > 10 µm) normal tap water has many objective advantages, i.e. being quick, rugged and effective. This can be achieved by equipping an existing water tap with a filtration unit that only allows passage for particulates substantially smaller than the lower detection size limit. Depending on available funds and required amount of water, this may be constructed out of standard sanitary metal fittings with the insertion of a fine-meshed stainless steel sieve disk or as a larger compounded filtration device like a stainless steel cartridge filter (Figure SI.1). In any case, it is important that the non-targeted plastic polymers are used in or past the filter. Besides the supply of particle free water, a tap water MPF-filtration setup provides the advantage of a high-pressure rinsing possibility for cleaning procedures of glassware.

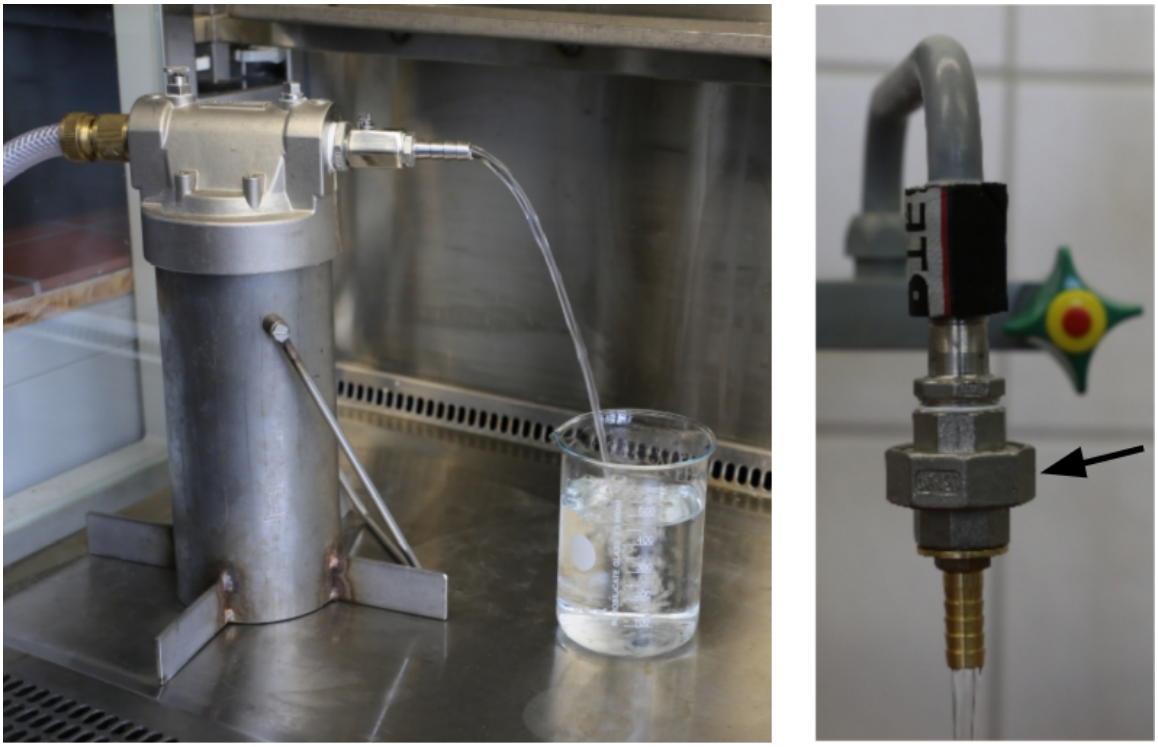

Figure SI.1: Demonstration of two quickly-constructed and mobile tap water filtration devices for the provision of ample MPF water as required for many tasks in a MP laboratory. (A) A stainless steel cartridge filter is used (pore size below the minimum MP size limit, here: 5 µm) and is portable by means of a custom-built stand with a handle. The water is delivered through a standard PVC water hose and dispensed after passing the filtration device either directly through the nozzle or by use of a hose piece of a non-target material (e.g. silicone rubber or PTFE, if they are excluded during analysis). (B) A much simpler version can be built from sanitary pipe fittings, where a hand-cut circle of stainless steel mesh can be inserted and is held in place by screwing the pieces together (arrow). It can then be mounted to the water tap directly or through a short hose piece. The drawback of the much smaller filter is that it will clog much faster.

SI.4 Mass reduction rates

Table SI.1: *On a set of 5 sediment samples the mass reduction efficiency was estimated by comparing initial sample dry weights with the reduced dry weights which were submitted to spectroscopic MP analysis after completion of the treatment pipeline. Sediment samples vary in composition, from low to high organic matter (OM) contents and fine to coarse sediment grain size. Masses in italics are estimates by calculation from particle numbers, others are measured.*

Prior to Treatment		**After Treatment**			**Reduction Ratios**	
Sample (matrix type)	**Dry weight [g]**	**Particle amounts on 50 µm filter**	**Dry weight on 50 µm filter [g]**	**Dry weight on 10 µm filters [g]**	**Mass % remaining**	**Mass % reduced**
A (high OM, fine sediment)	66	16926	0.0011	0.0044	0.0067	99.993
B (high OM, fine sediment)	93	91777	*0.0060*	*0.0239*	0.0257	99.974
C (low OM, coarse sediment)	250	29918	*0.0019*	*0.0078*	0.0031	99.997
D (low OM, coarse sediment)	250	11374	*0.0007*	*0.0030*	0.0012	99.999
E (low OM, coarse sediment)	250	94545	*0.0061*	*0.0246*	0.0098	99.990
F (low OM, coarse sediment)	30	96084	*0.0062*	*0.0250*	0.0833	99.917
					Average:	99.978
					Standard dev.:	0.031

SI.5 Process time

Depending on the sample type, hence the pathway taken through the decision tree, time estimates vary. The total time period needed for a sample treatment is usually in the region of 6 – 17 days, however, as up to 8 samples can be treated in parallel, one has to calculate with approximately 1 – 3 days per sample on average. The largest time fraction is due to waiting / processing time. Thus, the actual working time for a person per sample amounts to roughly 2 h or up to 12 – 19 h for 8 samples in parallel, depending on sample composition (see example 1 and 2 in Table SI.2). Not included are laboratory preparation time and cleaning (see m0 in associated paper). Some samples require less predictable procedural measures, e.g. the manual elimination of large organic matter fraction or the visual pre-analysis of large MP fraction, which essentially scales with the amount of total material in the sample (see example 3 in Table SI.2).

Table SI.2: Exemplary sample treatments (i.e. pathways through the protocol tree) were selected to calculate total treatment times. The actual working time that a person needs to spend on the preparation is given in black, the additional waiting time (due to machine or chemical process times) is given in red. Estimated times were calculated based on the minimum time needed for each step by an experienced person. Not included are the duration needed for preparation of MPF solutions, cleaning of equipment, note taking, etc. The abbreviation X_{DS} describes the number (X) of density separation (DS) cycles.

#1	**Particle-rich sample, predominantly inorganic (e.g. sediments)**	**#2**	**Particle-poor sample, predominantly organic (e.g. water, small MP)**	**#3**	**Particle-poor sample, predominantly organic (Manta net water, large MP)**
Freeze-drying	10 min + 84 h	**Filtration**	20 min + 1 h	**Filtration**	20 min
Splitting (Homogenisation + Split/ weighing)	6 min (5 min, 1 min)	**Freeze-drying**	10 min + 84 h	**Freeze-drying**	10 min + 84 h
Density separation (Transfer, shaking, wet sieving, stirrer installation, X_{DS} * DS cycles, filtration)	50 min + 15 h * X_{DS} (5 min, 5 min, 10 min, 5 min, 10 min * X_{DS}, 15 min)	**H_2O_2 Digestion** (Refill * X_{H2O2}, Filtration)	16 min + 24 h * $X_{H2O2,}$ (1 min * X_{H2O2}, 15 min)	**H_2O_2 Digestion** (Cut, Pick and rinse large organic matter pieces, Fill Filtration)	36 - 86 min + 24 h (10 min, 10 – 60 min, 1 min, 15 min)
H_2O_2 Digestion (Transfer, Refill * X_{H2O2}, Filtration, Final Transfer)	26 min + 24 h * X_{H2O2} (5 min, 1 min * X_{H2O2}, 10 min, 10 min)	**Density separation** (Transfer, shaking, X_{DS} * DS cycles, filtration, Final Transfer)	43 min + 15 h * X_{DS} (1 min, 12 min, 10 min * X_{DS}, 10 min, 10 min)	**Density separation** (Transfer, shaking, DS cycle, filtration, Final Transfer)	43 min + 15 h (1 min, 12 min, 10 min, 10 min, 10 min)
Total $_{min}$ (X_{DS}= 1, X_{H2O2} = 1; coarse sediments, low in organic matter)	**92 min + 123 h ~ 6 d** (if based on a 8 h working day)	**Total $_{min}$** (X_{DS}= 1, X_{H2O2} = 1)	**89 min + 124 h ~ 6 d** (if based on a 8 h working day)	**Visual sorting** > 1 mm particles	30 min – 40 h (depending on particle amounts)
Total $_{max}$ (X_{DS}= 5, X_{H2O2} = 10)	**141 min + 399 h ~ 17 d** (if based on a 8 h working day)	**Total $_{max}$** (X_{DS}= 2, X_{H2O2} = 3)	**101 min + 187 h ~ 6 d** (if based on a 8 h working day)	**Total $_{min}$** (X_{DS}= 1, X_{H2O2} = 1)	**139 – 2559 min + 133 h ~ 6 -11 d** (if based on a 8 h working day)
Total $_{min/\ parallel}$ (up to 8 samples treated in parallel)	**736 min + 123 h ~ 6 d** (if based on a 8 h working day)	**Total $_{min/\ parallel}$** (up to 8 samples treated in parallel)	**712 min + 124 h ~ 6 d** (if based on a 8 h working day)	**Total $_{min/\ parallel}$** (up to 8 samples treated in parallel)	**1112 – 20472 min + 133 h ~ 6 -20 d** (if based on a 8 h working day)
Total $_{max/\ parallel}$ (up to 8 samples treated in parallel)	**1128 min + 399 h ~ 17 d** (if based on a 8 h working day)	**Total $_{max/\ parallel}$** (up to 8 samples treated in parallel)	**808 min + 187 h ~ 8 d** (if based on a 8 h working day)		