Provisional SOP for processing river and sediment samples to extract microplastics for analysis by µ-FTIR

To minimize background contamination of microplastics during processing, the following controls were observed (

Table 1).

Table 1: contamination control measures recommended when sampling and processing samples for

Contamination control method **Contamination control method**	**Description**
Limiting contamination during sampling	Cross-contamination from clothing is minimised by standing downwind from the sample and keeping lids on the buckets and bottles whenever possible. Field blanks for each sampling campaign are run as part of the QA/QC to check for contamination
Limiting contamination from equipment	Where possible, non-plastic or uncommon plastic substitutes are used in sampling and processing equipment, including natural fibre brushes, glass Pasteur pipettes, stainless steel buckets, stainless-steel or aluminium filter rigs, stainless steel or pure silver filters, FEP/ETFE wash bottles and glass bottles with PTFE lined lids and ETFE pouring rings for all sampling and processing vessels
Limiting contamination from airborne microplastics in the laboratory environment	HEPA filter removes 99.999% of particles >0.3 µm in size. All processing steps in the laboratory are performed in this safety cabinet. When outside of the safety cabinet, all equipment/samples are covered with clean aluminium foil.
Limiting contamination from reagents	All reagents used in microplastic processing are filtered through a 1.2 µm glass fibre filter to remove any particulates prior to use. A systematic use of procedural blanks allows all data to be corrected for any unavoidable background contamination.
Limiting contamination from synthetic fibres by operators	All laboratory processing is performed by operators wearing 100% cotton lab-coats.
Limit contamination arising from re-using stainless steel disc filters used in processing	All stainless-steel disc filters are washed between samples with detergent, RO water and filtered RO water. Absence of carry over between samples is demonstrated as part of the QA/QC for the project.
Limiting contamination during washing of equipment	All equipment and glassware is washed using only natural fibre scouring brushes to prevent contamination during washing and rinsed repeatedly with filtered RO water before air drying under foil to prevent airborne contamination

microplastics

River water sample processing

All samples arrive from the field as stainless-steel filter cartridges. Excess water is released from the base of the cartridge and the filter is removed for further processing. Solids were removed from the filter by thorough rinsing with 0.7 µm GF/F filtered DI water and natural hair brush. Approximately 1 L of

sample was collected from the filter and stored in a glass beaker. The sample then underwent a Fenton's reaction to break down any organic matter. The Fenton's reaction was left to exhaust for 20 hrs, before being acidified. The samples were then concentrated onto a 5 µm mesh steel filter and submerged in GF/F Filtered 2% HCl for 24 hrs before being 100% deposited on 3 µm silver nitrate filters for µ-FTIR analysis. The use of the Fenton's reaction proved to be effective on the river water samples, however the following issues were observed; 1. A significant fine mineral residue appeared to overload the silver nitrate filter during deposition, 2. The 1 L sample was difficult to work with and reduced the effectiveness of the Fenton's reaction by diluting the reagents. To improve the efficiency of the process and the standard of the final deposited sample, the processing method was refined for trial two. Samples from trial two were removed from the filter using the same method as trial one, however the sample was immediately concentrated on a 5 µm filter and transferred to a 150ml glass beaker. The samples then underwent the Fenton's reaction, which was much more vigorous than Trial 1 due to the concentration of the reagents. The Fenton's reaction was then acidified and the sample was once again concentrated on the same 5 µm filter before being submerged in 2% HCl for 24hrs. After submersion in 2% HCl, an acid washing stage was added to the process. Samples were concentrated on a 5 µm steel filter to remove the acid and new clean 2 % HCl was flushed through the filter, washing any mineral particles < 5 µm through the steel filter. This washed sample was then washed from the steel filter with 0.7 µm GF/F DI water and 100% deposited.

Sediment sample processing

Wet river sediment was stored frozen at -20 °C in glass Kilner jars until processed. Once thawed, sediments were mixed thoroughly with a clean stainless steel spatula for sub-sampling. 30 g of wet river sediment underwent a Fenton's reaction following the same protocol as for water. The sample was then suspended in $ZnCl_2$ at 1.7 g cm^{-3} in 100 mL cylinders for density separation to remove more dense mineral colloids, isolating the microplastics at the surface of the suspension. The $ZnCl_2$ column was mixed to homogenise then allowed to settle for 24 hours. The supernatant was decanted, then additional $ZnCl_2$ was added to perform a second separation on remaining solids to minimise the potential for microplastics to be trapped within the sedimented layer during the first separation. After this second density separation, the combined supernatants were filtered and a final Fenton's reaction performed to further degrade natural organic matter that had been concentrated by the density separation. For sediments, a final size separation into a coarse (>198 µm fraction) and fine (<198 µm fraction) is necessary due to the presence in most river sediment samples of large dark fragments in the sample after extraction. Samples were then stored in 50% ethanol before depositing and analysis.