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Evidence Project Final Report

Department for Environment Food & Rural Affairs

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Project identification

1. Defra Project code: WT15135

2. Project title:

   Measurement and Characterisation of Microplastics in English River Catchment Waters and Sediments

3. Contractor organisation(s):

   UK Centre for Ecology and Hydrology

   Suez Advanced Solutions Ltd. T/A Aqua Enviro

4. Total Defra project costs (agreed fixed price): £ 79,096.24

5. Project: start date 01/10/2021

   end date 30/03/2023

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Objectives

The objective of the project was to carry out a pilot study developing sampling and analytical protocols to determine the quantities, loads and types of microplastics (MP), in surface waters and sediments. Approaches to sampling surface waters and sediments would be reviewed and a selected approach tested on catchments agreed in consultation with the Environment Agency. From this, a provisional standard operating procedure (SOP) guideline is presented that may be used for sampling, processing and analysis of microplastics in river waters and sediments. In addition, it was investigated whether sediment samples collected in this manner could be analysed for the presence of vehicle tyre wear, through quantitative analysis of a common additive of tyre rubber, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). This compound has been identified as a Priority 2 substance for freshwaters and groundwaters (high risk, low certainty) under the Environment Agency's Prioritisation and Early Warning System (PEWS), flagging it for further consideration in sediments as it meets the toxicity criterion according to available ecotoxicity data and is not currently monitored (Environment Agency, 2023). The method is believed to be the first to be published to quantify this chemical marker in sediments using Gas Chromatography Mass Spectrometry.

Approach

We identified two key areas in need of development within the project: representative sampling and reproducible extraction procedures for microplastics, especially from sediment samples. Through technical and field replication, we have estimated the contributions of different sources of variation to the final quantification of microplastic particles. These sources of variability include the repeatability of the extraction method, within sample variation (technical replicates) and between sample variation (replicates from the field). Understanding these influences allows us to tailor the sampling approach to generate more consistent and representative samples of both surface waters and sediments. For the analysis of 6PPD, extraction methods were investigated involving microwave extraction followed by clean-up using size exclusion chromatography to ensure efficient recovery of a commercial reference standard using gas chromatography mass spectrometry-selected ion monitoring (GC-MS SIM) for optimal limits of detection of the target compound.

Conclusions

Microplastics represent a diverse class of contaminant for which quantification is operationally defined by the analytical technique, sampling strategy and extraction procedure employed. No single technique can measure microplastics across the entire size continuum, and so representative sampling must be tailored to the region of the microplastic continuum targeted in the analysis. Guidance towards estimating representative sample volumes was defined, based on concepts of the size region of microplastics targeted in the analysis, predicted concentrations in the environment monitored, data resolution and statistically derived minimum sample volumes. This approach to estimating representative sample volumes was refined and evaluated during the project to generate processed samples within the analytical window of the commonly used technique, micro-Fourier Transform Infrared Spectroscopy (µ-FTIR – quantifying microplastics >25 µm in diameter). On this basis, we recommend a minimum of 50 L to be representative of surface waters and recommend 15-30 g wet weight of sample (taken from a larger, homogenised sample ~200 g in the lab) for analysis of microplastics in sediments. To estimate representative sample volumes for larger or smaller microplastics, the guidance constructed in this report should be followed for each specific assessment. For both river waters and sediments, it was possible, through replication, to evaluate the underlying variability of the systems and compare this to the variability expected through assessment of the repeatability of the procedure as evaluated from spike recovery assessment (coefficient of variance of recovered known microplastics ~25%). We propose that differences smaller than this should not be interpreted between samples as this is the variability inherent to analytical repeatability. All reproducibility analysis was performed on the MP count data but estimates of mean total mass of particles at each field/study site were also calculated.

The microplastic load in surface waters appears to be highly heterogeneous, even when sampling concurrently from the same location. In all central channel sampling locations in the field campaign, the variation between simultaneous triplicates ranged from 30 to 136%, and was greater than the achievable accuracy of the method (i.e. >25%). This indicates that even at a single location, variability in local hydrodynamics mean that quantification of microplastics can vary. This effect may be more pronounced in high turbidity streams, locations where the microplastic contamination is high or locations where a greater diversity of polymers, but lower concentrations are found.

When ordered by catchment type, rural locations, with a total of 0.44 MP L^{-1}, were less contaminated by microplastics than either intermediate sites, 3.28 MP L^{-1}, or urban sites downstream of wastewater treatment works (WwTWs), 5.08 MP L^{-1}. Estimates of mean total mass of MPs were equivalent to 0.22, 0.02 and 0.26 mg L^{-1} in rural, intermediate and urban locations, respectively. These estimates of mass

should be treated with caution as they are based on assumptions of three-dimensional shape from two dimensional images and so can overestimate mass of films and flakes, which may make up a high proportion of suspended microplastic litter suspended in the water column. Other mass-based techniques outside of the scope for development in this report, such as GC-MS for microplastic polymers would be complementary in providing more confidence around the estimated polymer mass concentrations. The sampling protocol proposed in this report would be suitable for other analytical methods to complement the µ-FTIR if desired. For GC-MS based methods, there is still significant development work required. If using solvents to extract the polymer for quantification, much development is needed to extend these extractions to apply for more than just a few select polymers, particularly to be quantitative of multiple polymers simultaneously. Pyrolysis methods overcome some of these hurdles, but still require significant method development to concentrate plastics sufficiently in a sample to be detectable in the small total mass that can be introduced to the GC through pyrolysis.

No significant difference between total microplastic number concentrations was found between intermediate sites and urban sites downstream of WwTWs, but closer inspection of the polymer types detected did indicate some unique sources of certain polymers in different rivers. Ethylene-vinyl-acetate (EVA) and polypropylene (PP) were almost ubiquitous in the replicate samples from all sites. Polystyrene (PS) was only detected at one urban site, whilst polyvinylchloride (PVC) in surface waters was almost exclusively associated with sites downstream of WwTWs. Even with triplicate measurements concurrently of ~50 L at each site, statistical differences between individual sites were difficult to establish due to the high variability even between triplicates. Increasing sample volumes analysed may reduce the sampling error, however, this must be balanced against overloading of filters for analysis with recalcitrant interfering material. Classification of locations according to catchment type found microplastics were over 10-fold more numerous in urban catchments than rural. Considering this, careful consideration of the number of locations, the level of replication (concurrent and temporal) and representative sample volumes should be considered for any future monitoring survey of microplastics in rivers.

In sediments, a wider diversity of polymers was detected than in surface waters. Once more, PP and EVA were commonly detected across locations. With only three study sites possible to sample, generalisations are difficult to conclude. The difficulty in sampling in urban catchments, where artificial manipulation of the channels means often there is no/very little fine sediment material will be a problem for any future survey that aims to monitor microplastics accumulating in sediments within highly engineered catchments. However, the design of this pilot programme allowed for important insights into the repeatability of the method and has allowed us to estimate the heterogeneity of sediment samples in different catchment types. Triplicate field samples from an intermediate and urban site, alongside technical triplicates from each of the samples allow us to estimate the repeatability and reproducibility of the extraction and analysis workflow we propose in the provisional SOP.

At the intermediate site, microplastic contamination of sediments was quite homogenous. The repeatability relative standard deviation (the variation within technical triplicates from a single field replicate) was 28%, similar to the coefficient of variation when recovering a known concentration of PS from sediments (24%). We conclude that the extraction of microplastics from sediment samples following this provisional SOP allows for an achievable accuracy of ~25%. Between replicates in the field, microplastic concentrations at the intermediate site were more variable, with a reproducibility RSD_R of 33%. However, this difference is only marginally greater the repeatability within a single field replicate, indicating this sampling strategy delivered results representative of the microplastic sediment contamination. For the urban site downstream of an WwTW, sediment contamination was found to be more heterogeneous. Within sample variation in the urban sediment was 55% indicating that the overall level of variation was high, but still lower than the maximum variability between concurrent replicates in the water column of 135%. Sediments, whilst experiencing patchy spatial distribution at some locations, still appear a promising matrix to monitor plastic pollution in rivers, smoothing out the very high stochasticity observed in some waters. This makes them a good candidate for monitoring longer-term trends in plastic pollution and dynamics in rivers. Total indicative masses of MPs in intermediate and urban sites respectively were 40.5 mg kg^{-1} at IR4, 2.0 mg kg^{-1} at IU2 and 104 mg kg^{-1} at MU4.

Whilst the homogeneity of microplastic contamination in sediment samples was location- specific, differences in excess of 14-fold in total MP number concentrations were found between sites. Differences of up to 8-fold were observed between rural and urban surface waters. However, the high variability in surface waters make even these high differences difficult to disentangle statistically. Greater sampling volumes, frequency, locations and replication may improve the precision with which microplastics can be quantified in surface waters at particular locations, however the greater costs and resources associated with increased field sample replication need to be considered.

In addition to the use of FTIR for analysis of microplastics in river surface waters and sediments, a similar exercise was undertaken to develop a method for analysis of sediment samples for the chemical marker of tyre wear, 6PPD. Recovery of an analogous recovery reference standard was on average 95.8% with a

coefficient of variance of 11%. This analytical variation is accounted for in the analysis as the standard is analysed in each sample and so it is possible to recovery-correct all data. In this way, variation within subsamples from a single homogenised field sample tells us about the success of this homogenisation to guide further possible improvements; whereas differences between replicated field samples represents the patchiness and spatial heterogeneity of this chemical in sediments. Two sites were investigated, a river-bank profile of the Thames (Wallingford, Oxfordshire), and triplicate samples from the intermediate urban site IR4 on the Irk, Greater Manchester. Concentrations were 10-fold higher in the intermediate urban site than the more rural location on the Thames in Wallingford. The repeatability relative standard deviation within homogenised replicates was ~44%.

Recommendations

The objective of any monitoring campaign targeting microplastics in riverine environments must be clearly defined at the outset. No single sampling approach or analytical technique can quantify all particles that fall under the umbrella term “microplastics”. The definition of “microplastics” within any given study will be operationally defined. Here, we propose an SOP for sampling, extracting and quantifying microplastics in river surface waters and sediments, based on the spectroscopic technique µ-FTIR. We selected this technique for its common use reported in the literature, its ability to identify the polymer and quantify plastic particle counts. As well as size, it also provides qualitative information on other properties, colour (from cross-comparison of the visible scans) and shape (in two dimensions), making it a technique which can quantify multiple endpoints simultaneously in a sample in a non-destructive manner. We demonstrate repeatable results with this workflow when quantifying particle number-based concentrations and sufficient throughput to make this approach practical for wider monitoring programmes. Estimates of mass concentrations from spectroscopic techniques are currently based on assumptions that have known limitations. Therefore, we recommend that estimates of mass generated through i.e., µ-FTIR are only interpreted as indicative estimates, not quantitative results, until such validation can be made. Additional research should be prioritised to validate these assumptions, e.g., using mass-based methods such as Pyr-GC-MS.

The SOP detailed in this report, whilst optimised for this analytical workflow, can in principle be applied to other mass-based analytical techniques (e.g. GC-MS), if mass concentrations are required by the objective of a field sampling campaign. A series of cascade filters would be required if size classes are also desired when using mass-based analytical approaches. Specific details of the approach may have to be optimised, e.g., optimal sample volumes will have to be determined to make sure that they are representative based on the sensitivity of the analytical technique and the level of contamination in specific samples. As part of this SOP, we provide guidance on calculating minimum sample volumes based on the size fraction targeted in the sampling. In addition, the general considerations over sample representativeness, replication and different sources of variation are applicable no matter the analytical technique used. As such, the guidance in this report on how to optimise sampling and extraction approaches, and quality assurance and control measures to generate repeatable and robust measurements will be a useful resource for any such optimisation.

Alongside the development of the SOP for sampling rivers and sediments for analysis of MP through spectrometry, an additional component to the report demonstrates an extraction and analysis protocol for a proposed chemical marker for TWP, 6PPD. This compound is also of interest in its own right, being identified as a Priority 2 (high risk, low certainty) substance by the Environment Agency and flagged for consideration in sediments where it is not currently monitored. The sampling method for sediments used for spectrometry analysis of MPs was broadly applicable to the analysis of 6PPD, with minor refinement such as 2 mm sieving samples to remove small stones which are a potential source of the variability observed in sediments, where they can make up a significant proportion of sediment mass. The analytical procedure described resulted in excellent recoveries and good repeatability in the analysis of 6PPD. The concentration of 6PPD in contaminated sediments will be a function of the mass of TWP input into in the sediment, kinetics of migration of the 6PPD diffusing out from the tyre rubber and degradation rates of the chemical through oxidation in the sediment environment. Each of these rates must be established and considered in the future, to extrapolate from concentrations of the 6PPD chemical marker, to estimates of total microparticle TWP mass in sediments.

Project Report to Defra

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Meeting the objectives of the project

The objective of the project was to carry out a pilot study to develop sampling and analytical protocols to determine the quantities, loads and types of microplastics, in surface waters and sediments. Our approach consisted of development in four key areas to propose a draft standard operating procedure (SOP) to sample, extract and characterise microplastics in surface waters and sediments:

- Demonstrate a robust approach to sample collection of surface waters and sediments;
- Optimise extraction of microplastics from these two environmental matrices;
- Analytical measurement and polymer identification of microplastic down to a minimum 25 µm size; and
- Field testing of the complete workflow to evaluate its success at scale for routine monitoring of microplastics in surface waters and sediments

The project was structured in agreement with the Project Board and the Project Steering Group in two phases: a development phase to explore options for sampling and to define the draft SOP, and a field campaign to test the reproducibility of the proposed SOP.

Sample replication in the field campaign is designed to allow the sources of variation from sampling, extraction and analysis to be quantified. This will improve our understanding of the reproducibility of the SOPs developed within this project and quantify the natural heterogeneity expected in these samples. In doing so, our understanding of what a “meaningful difference” between sampling occasions, or locations is improved, with important implications for any future monitoring programme for microplastics in rivers. Finally, a proposed provisional SOP for sampling, extraction and analysis of microplastics using spectroscopic approaches is presented. As per the project specification, each of these elements contributes to our understanding of indicative quantities, estimated loads and types of microplastics in the surface waters and sediments of rural and urban sites in lower English river catchments. The draft SOP also provides guidance on how to generate representative samples more generically to target specific regions of the microplastic size continuum, details

the quality assurance protocols required for inclusion in future monitoring programmes for microplastics and highlights areas for further development.

Therefore, this report meets the objectives of the contract providing:

- Quantification of microplastic particle number and estimates of mass concentrations by polymer type in surface waters and sediments of three lower catchment rivers in England (the Roch, Irk and Medlock in the Greater Manchester region) in both urban and rural locations;
- Assess the reproducibility of measurement for both surface water and sediments through sample replication to understand the achievable accuracy of the proposed methodological approach to evaluate its fitness for purpose; and
- Provide recommendations for a provisional, fit-for-purpose SOP for sampling, extracting and analysing microplastics in river surface waters and sediments.

Review of approaches to sampling and quantification of microplastics in riverine surface waters and sediments

Regulators in the UK do not currently monitor rivers systematically for microplastics (House of Commons Environmental Audit Committee, 2022). Whilst product legislation such as REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals regulation) Restriction on intentionally added microplastics (EC 1907/2006), the Single Use Plastics (SUPs) and Fishing Gear (COM(2018)340) and the Marine Strategy Framework Directive (2008/56/EC) (including the amending Directive 2017/845/EC and Commission Decision 2017/848/EC) explicitly target microplastic pollution, monitoring of microplastics in surface waters and sediments is still not specifically required at present under such legislation (SAPEA, 2019). It has been argued that restrictions on the intentional use of primary microplastics should be more precise in focus, so that measurements can be used to evidence breaches of the regulation and thus ensure that the regulation is enforceable (Mitrano and Wohlleben, 2020). The ability to monitor microplastic contamination in particular environments or hotspots of concern is one mechanism through which the success of such legislation may be measured.

The objective of monitoring microplastics in river waters and sediments must be clearly defined so that appropriate sampling strategies can be designed. No single analytical technique can quantify the entire range of polymers and particle sizes that fall under the “microplastic” umbrella. Indeed, until recently there was little consensus in the literature even over how to categorise microplastics, though efforts to remedy this have been useful in providing a framework within which to classify plastic pollution (Hartmann et al., 2019). Each analytical method has limits of detection specific to the approach. This can be thought of as the analytical window in which microplastics can be quantified and is most commonly specific in terms of size and polymer composition. Appropriate sampling and processing of samples can also be specific to the analytical technique used for quantification. However, general rules to consider when sampling and quality assurance procedures to enable robust data to be generated irrespective of the analytical approach can and must be generalised as part of efforts to develop systematic protocols for monitoring microplastics in rivers. The region of the size continuum of microplastics (generally pragmatically defined as synthetic plastic material <5 mm, ECHA, 2019) targeted by any measurement is set by the analytical window of the technique used to measure and quantify microplastics. In this study we use micro-Fourier transform Infrared spectroscopy (µ-FTIR) to quantify microplastics to a lower size range of 25 µm. This is one of the most used chemically specific analytical techniques for quantifying microplastic particle number concentrations (e.g. Belz et al., 2021) and so a suitable candidate for refining and demonstrating a reproducible workflow for sampling, extraction and analysis of microplastics in rivers and sediments in the United Kingdom. However, many analytical techniques are available and so generic guidance is also needed to allow for lessons to be applied in different research contexts. To this end, below we describe generic guidance to estimate representative sample volumes based on the region in the size continuum targeted.

Calculating representative sample volumes based on the targeted region of the microplastic size continuum

Two key components allow the estimation of representative sample volumes: an estimate of the expected concentration of microplastics in the size region targeted by your analysis and an estimate of the expected differences you wish to interpret between sites or locations. These two components describe the criteria for acceptable data. The probability of capturing a single particle in a given volume of sample can be described statistically and used as a first estimate of minimum sample volume.

Bannick et al., 2019 provide a useful equation to predict the minimum volume required to detect 1 particle with 90% probability given an expected concentration of microplastics based on knowledge of the range of microplastic contamination expected in your sample. This statistical approach was based on a binomial distribution, which only allows two outcomes, like when repeatedly flipping a (biased) coin. This would assume that each time you take a unit of water (e.g. 1 litre) it will contain either 0 or 1 particle but not more than 1.

They then calculated how many times one would need to take one unit to have a 90% probability of at least one of those units containing a particle. When the concentration is very low the probability of catching 1 particle in a unit is approximately equal to the concentration and the probability of catching more than 1 particle in one unit is essentially zero and thus this binomial approach yields the correct answer. However, given that at higher concentrations a given volume could also contain more than one particle, the correct statistical approach to use is based on the Poisson distribution which is used to model rare events such as the random distribution of particles in dispersion. As such we have updated the statistical approach to be based on this more representative Poisson distribution (Equation 1).

Equation 1

$$v = \frac{-ln\,(1-a)}{c}$$

Here, *v* is the minimum sample volume required given a concentration in the environment of *c* to have *α* probability of finding at least one particle or a (1- *α)* probability of a false negative result (catching no particles at all in the collected volume despite there being some in the environment). This should be thought of as the absolute minimum volume required to detect microplastics in surface waters. A representative sample volume to estimate a concentration rather than just presence or absence will always be greater than this minimum required volume. Indeed, existing standards such as the ASTM D8332-20 or ISO 5667-17 recommend greater volumes that would be predicted from this equation as do we in this report, to account for the heterogeneity we expect in the field and which this report aims to quantify. However, this statistical approach importantly allows minimum sample volumes to be calculated for any target size range of microplastics, given some knowledge or estimate of the expected concentration in this size range. This statistical approach is therefore generic and can be applied to any analytical method which counts particle number concentrations.

It is important to collect sufficient sample to allow for expected differences to be detected between locations. To do so, sufficient volumes must be sampled and analysed to allow the resolution in your data to be finer than these expected differences.

To calculate this data resolution, we can consider the following. The minimum detectable number of particles in a sample using spectroscopic techniques is a single particle. However, the volume represented in a sample often differs from sample to sample and is a function of the original volume sampled in the field and the proportion of that sample that could be deposited and quantified in the final analysis (Horton et al., 2021). In this way, each sample has a minimum "data resolution" to which particles can be quantified. One particle detected in a 1 L sample gives a data resolution of 1 particle L^{-1}, whilst if 1 particle were detected in a 100 ml sample, this would give you a resolution in your data of 10 particles L^{-1}. For each additional particle detected in 100 mL sample, your final concentration would increase in increments of 10. Therefore, sufficient volumes should be sampled in the field that when processed provide a data resolution that is "fine" enough to detect expected differences between sites. In the absence of estimates of expected differences between locations (which can be based on previous publicly available literature for example), a recent paper by Tanaka *et al.* (2023) provides additional criteria which should be met when sampling and quantifying microplastic number concentrations. Using a similar statistical Poisson point process as we demonstrate in Equation 1, Tanaka *et al.* recommend a minimum of 10 particles to be detected in a sample for predictions of the sampling error to be estimated. Capturing 50 particles in a sample reduces the predicted sampling error to a 95% confidence interval of +/- 30%.

Together, the minimum sample volume estimate from Equation 1, the concept of the desired data resolution and the guidance on minimum total particle detections from Tanaka *et al.* 2023 provide tools that can be applied to estimate representative sample volumes in environmental matrices for methods which count microplastic number concentrations. These estimates should be performed individually for each target size region of the microplastic continuum and/or analytical technique used.

Selecting an appropriate sampling strategy for surface waters

As larger plastic items degrade and fragment into ever smaller secondary microplastics, there appears a logarithmic increase in particle number concentration as size decreases (e.g. Jones et al., 2019), although recent work into the mechanistic understanding of the formation of smaller micro and nanoplastic fragments from surface ablation from larger plastic items complicates this picture (Pfohl et al., 2022). As no single analytical technique can quantify microplastics along the entire size continuum, the analytical technique, and the window which it offers into this continuum of microplastic pollution must be considered in the context of the purpose of the study.

Monitoring should be hypothesis driven and the purpose of monitoring must be clearly defined so that the appropriate region of the plastic pollution continuum can be investigated. To answer questions of total material flows of plastic through the environment, capturing these larger microplastics and indeed macroplastic litter will be essential to adequately parameterise material flow models. This information can help identify where plastic is "leaking" out of production and use phases of the products life cycle, into our environment and

improve our understanding of major sources and pathways of plastic pollution in UK rivers. However, when it comes to describing the exposure of freshwater species to microplastics in UK rivers, there is an argument for characterisation of the smaller microplastic region to be quantified. The relationship between physiology of organisms, size and shape characteristics of microplastics and the role these two factors play in determining bio-accessibility of microplastics has been documented (Porter et al., 2023), and it follows that under non-selective feeding, smaller particles will be available for ingestion by a wider range of species than larger particles (where the particles may simply be too large to ingest) and so may present a greater risk to the ecosystem as a whole. Just as targeting the smaller size of microplastics may result in missing the larger items that may contribute more to total material flows, targeting only the larger plastics can miss this biologically important region in the microplastic continuum.

Monitoring river surface waters may have a different objective to monitoring of sediments. River surface waters will be dynamic and represent "snap shots in time" of microplastics loads in the river. Particle size distribution and polymer composition of microplastics at a given site will be a function of the morphology of the river, the properties of the microplastics (dispersion stability, buoyancy) and the hydrodynamics of that particular moment in time. Whilst the location of sampling sites (both along a river catchment, but also the specific sampling point within the cross sectional profile of the river being monitored) can be designed to meet the specific requirements of monitoring, one universal consideration is the minimum sample volume, following the guidance outlined previously.

The number of particles expected in river surface waters may be estimated from existing knowledge, however, data from the literature should be limited to studies which had a similar analytical window (in terms of size range and polymers quantified) for the analysis, as the frequency of microplastics increases significantly as the minimum size detected decreases. For example, sampling with 80 μm instead of 330 μm mesh nets resulted in 250 times higher concentrations detected in the river Seine, France (Dris et al., 2015). Therefore, we take a conservative approach and use the concentrations of microplastics in sewage treatment effluents released to rivers, measured in-house 5.45 MP L^{-1} (Horton et al., 2021), which used the same sampling and analytical approach to predict the minimum sample volume using Equation 1. The minimum sample volume in this case to detect a single particle would be 422 mL. However, it is not just the chance of detecting a single particle that should be considered when calculating the required sample volume needed for a monitoring programme of river surface waters. As discussed, the required data resolution should also be considered.

If we were to sample only 0.42 L at each location, we would have a data resolution of 2.4 particles L^{-1}. When considering catchment-wide modelling of microplastic flows, we would only be able to distinguish between locations that differed by more than 2,400 MPs/m^3. As can be seen, this sample volume of 0.42 L, whilst it may be sufficient as a minimum to detect microplastics with confidence, it gives a relatively poor resolution in the final data (each additional particle detected between samples is equal to an increment of 2,400 MPs m^{-3}) which could preclude differences from being detected along the river course, or between catchments. Therefore, we recommend a target volume of 50 L when sampling river surface waters, sufficient to detect microplastics >25 μm in size with >90% probability and results in a resolution of 0.02 MP L^{-1} or 20 MP m^{-3}. Based on the estimate of ~5 MP L^{-1}, this should result in >50 particles detected per sample, with an estimated 95% confidence interval of +/-30% (Tanaka et al., 2023). It is important to remember that this recommended representative sample volume is specific to the size of microplastics investigated and to the expected concentrations in these environments. Using the statistical approach of the minimum sample volume calculation, and the concepts of data resolution and expected concentrations, provides a generic framework with which to calculate representative sample volumes for any study into microplastic concentrations in the environment.

To capture this volume, we use pumped filtration based on established methods for sampling WwTW effluents (Horton et al., 2021), which is a practical solution to sampling such high volumes in the field as it avoids the need for transporting large volumes of water. The expectation is that pumped filtration of this volume of water will also reduce the within site variability, as well as improving limits of detection and resolution in the data, as the final sample is a composite of water pumped from a point within the river flow over 15 to 30 minutes, rather than a single snapshot grab bucket sample for example, which would be more susceptible to local temporal patchiness in microplastics passing down along the stream, particularly in turbulent flows.

Calculating the required volume and appropriate sampling strategy for sediments

Similar considerations should be made for sediment samples. The minimum sample volume can be estimated following the same approach as above, informed by the minimum predicted sample volume and the desired resolution in the data. Sediments represent a more challenging media in which to quantify microplastic contamination due to the overwhelming presence of interfering particulate material in the same size range as the microplastics you wish to quantify. Density separation and oxidation of organic material are therefore necessary to clean up samples sufficiently to quantify microplastics amongst the remaining recalcitrant interfering natural material. A recent systematic assessment of approaches to the extraction of microplastics from marine sediments concluded that 50 g dry weight of sediment is an optimum, reducing the variability in repeat measures of the same sample compared to extraction from a larger 100 g of dry weight sediment at a

ratio of 1:10 sample to density separation solution volume (Filgueiras et al., 2021). The sediment composition and physical characteristics are likely to influence this optimal mass, but this recommendation can be seen as a ballpark region for targeting for analysis of microplastics through spectroscopic methods. A study analysing particles down to a size of ~50 µm using optical microscopy and µ-FTIR found a mean number of MPs of 6350 MP kg^{-1} in sediments in the Greater Manchester area (Hurley et al., 2018). As this study sampled using a sediment resuspension technique it is uncertain how concentrations from this sampling method would scale to sampling methods which take the entire bulk sediment (e.g. trowel sampling, van Veen grab sampling etc.). Based on the above, the estimated minimum sample mass required to detect a single particle would be 0.36 g. Therefore, we suggest targeting 50 g sediment for analysis, with a minimum of 10 g wet weight sediment which would give a resolution of 0.1 MPs g^{-1} or 100 MP kg^{-1} dry weight of sediment.

Sampling by hand using stainless steel trowels is possible in low order streams and in intertidal estuarine areas of higher order streams. The British Geological Survey monitors stream sediments as part of the G-BASE survey programme, for the distribution of trace elements in stream sediments to establish a geochemical baseline across the United Kingdom (Johnson, 2005). This programme collects wet sieved sediments <150 µm from low order streams for routine analysis by X-ray fluorescence spectroscopy. This method requires people to physically enter the stream to collect kilogrammes of material using shovelling and wet sieving. Whilst applicable to low order streams where such an approach is practically viable, it cannot be applied in deeper streams and so would have to be combined with other methods for catchment wide monitoring of microplastics in sediments. This does not preclude samples generated in long term monitoring studies like the G-BASE survey from being a useful resource for historic monitoring of the baseline in microplastic contamination in these low order streams. It would be valuable to have the same sediment sampling method applied throughout the river course to ensure that results from lower order to higher order streams are consistent and comparable.

With this in mind, the suitability of Van Veen grab samplers for sampling river sediments was selected for trial in this project. These samplers collect the top consolidated layer of sediment up to a depth of 15 cm, where it is expected that microplastics in the size range we are analysing will be concentrated. The infiltration depth of microplastics is a function of the plastic size and shape as well as the grain size of the sediments and even for the smallest microplastic particles <10 µm the average infiltration depth into fluvial sediments has been suggested as 13 cm (Waldschläger and Schüttrumpf, 2020). This depth of 15 cm is also considered biologically relevant for exposure assessment, as this is within what may be termed the “biologically relevant sampling depth”, in which 80% of species (by abundance) resides. In river environments, this 80th percentile depth of species abundance is estimated to range from 15 to 35 cm (U.S. EPA, 2015).

When the claw of the Van Veen sampler hits the riverbed, the securing pin on the sampler is released and as the sample is retrieved, the claw closes, capturing the surficial sediment layer. Whilst an exact depth to which sediment is taken cannot be known from sample to sample (the depth of sediment collected will depend on how soft or hard the riverbed is), the surface area sampled is known based on the size of the sampler. This Van Veen grab sampler provides advantages for consistent sampling of sediments: it can be manually operated, it samples surficial sediments of a known surface area and it does not require physical entry into the stream, so can be operated along the course of the river, from low order streams all the way to estuaries and marine sediments, with deployment from boats for example. Van Veen grab samples have also been demonstrated to be similarly effective in recovering microplastics down to a size of 1 mm as freeze cores (~80% recovery from medium sand under flow conditions representing low flow conditions near a shore of a riverine or lake environment), and performed better than shovel sampling which recovered ~50% particles 1 – 5 mm under these conditions (Adomat et al., 2022). No systematic analysis of recovery of microplastics <1 mm in size from sediments under simulated flow conditions is publicly available at the time of reporting, and such an assessment was beyond the scope of this proposal.

Selecting an appropriate chemical marker for tyre wear particles (TWP) in sediments

The high content of carbon black in tyre rubber means that infra-red is strongly absorbed, impairing this techniques ability to detect and identify tyre rubber in environmental samples. The use of a common chemical marker as a surrogate to quantify tyre wear particles (TWPs) in environmental samples is therefore an interesting alternative analytical approach for this challenging material. At the outset of this project, 2-(4-morpholinyl) benzothiazole (24MoBT) was the selected compound to be used as a marker for vehicle tyre wear, given it had been previously analysed and suggested to be a suitable marker (Kumata et al., 1997). However, this compound was not commercially available to develop a method and another potential marker had to be identified. A literature review was undertaken to find a suitable alternative to be a representative marker of tyre particle presence. The compound selected was N-(1,3-Dimethylbutyl)-N’-phenyl-p-phenylenediamine (6PPD). 6PPD is most commonly used as a protective agent in vehicle tyre rubber at concentrations ranging between 1-2% as an antioxidant and anti-ozonat (OSPAR Commission, 2006). 6PPD is also a good candidate as it was found to be more stable and less prone to leaching from the tyre rubber than some of the alternative benzothiazoles and so is gaining interest as a relevant marker for tyre wear particles (Klöckner et al., 2021; Wagner et al., 2018). With few other applications, abrasion of tyres during

use, and end-of-life recycling processes are relevant direct pathways for this chemical into the environment (California Department of Toxic Substances Control, 2022).

This compound has also been identified as a Priority 2 (high risk, low certainty) substance by the Environment Agency in freshwaters and groundwaters (Environment Agency, 2021, PEWS Screen 6PPD, 01/02/2021) and flagged for consideration in sediments as it meets the toxicity criterion according to available ecotoxicity data and is not currently monitored. The degradation product of 6PPD in water, 6PPD-quinone (6PPD-q) has also been implicated in urban runoff mortality syndrome in Pacific Northwest coho salmon (Tian *et al.*, 2021). The high K_{oc} value of 69 700 L/kg of 6PPD means it is likely to bind to organic matter and so have sediments have been recommended for monitoring (OSPAR Commission, 2006). The density of TWP and in particular the abrasion products that can be a composite of tyre rubber and road abrasion material are reported in the range of 1.26 – 2.2 g cm^{-3}, suggesting sedimentation will be significant of this material in aquatic systems (Baensch-Baltruschat et al., 2020), again indicating the importance of monitoring sediments for TWP and 6PPD. Therefore, there is an interest in development of a method for quantifying this compound and its degradation products in sediments in their own right as contaminants, as well as being a marker for tyre wear particle presence.

Phase 1: River profiling and method development

The aim of this development phase was to road-test the sampling method for both surface waters and sediments to construct the draft sampling SOP to be used in the field campaign. In addition, some optimisation of sampling volumes and the location of sampling points along a length profile of the river were made to further inform the sampling SOP and variability at the location. To give context to the site, a satellite map of the rural market town of Wallingford is presented with a detailed inset of the sampling locations close to the road bridge that crosses the River Thames, entering the town (Figure 1).

Figure 1: Map showing the land use context surrounding the sampling locations on the Thames in the rural market town of Wallingford, Oxfordshire. Inset in the white box shows details of the location of the river profile trials. The white star is the sampling location for sediment and water samples from the central channel off the road bridge The Street that crosses the Thames River. The white dotted line represents the approximate region of sampling along the bank of the river for sediments as part of the sediment profile of the site. The white box marker is the what3words (*///*) location of the concurrent bank pumped water samples taken in the river profiling phase of the project. The white arrow signifies the direction of drainage of surface water from the bridge towards the west bank (Wallingford) and the two red triangles represent the approximate locations of the outlet of these gutter drains into the Thames.

Methods

Two sampling and processing trials were conducted on the River Thames at Wallingford, Oxfordshire, with the ambition of optimising the techniques both in terms of reproducibility and efficiency. All samples were run with corresponding process blanks representing any contamination arising through the act of extracting MPs from the sample and this accounted for in the final data. Controls were in place to limit background MP contamination, detailed in the final SOP. The trials aimed to answer the following questions:

- What volume of river water could be passed through the sampling filter before clogging, and what flow rates could be achieved and sustained?
- What matrix do river water and sediment samples consist of, and what processes would be required to extract the microplastics?
- How much variation in sample matrix type and microplastic concentration occurs between samples taken in replicate?
- Is sampling from the riverbank with the proposed methods feasible, and how?
- Is bridge sampling with the proposed methods feasible, and how?

Water samples

Sampling

Trial 1 was conducted to assess the feasibility of using the equipment at the sampling sites, and to determine whether representative volumes of river water could be efficiently cleaned and how. Four water samples of 5, 10, 20 and 30 L were collected from the bank of the River Thames (location latitude and longitude 51.600946, -1.1199, what3words ///shoppers.contemplate.blurts, white box marker in Figure 1), using auto samplers that comprise peristaltic pumps filtering river water through 5µm steel filters. Water samples were collected from the bank by submerging a silicone hose 50 cm below the water surface using a weight. A depth of 50 cm was selected to be consistent between samples as we wished to sample from the faster surface currents of the river, thus monitoring plastic debris that is being transported downstream. Vertical or steep banks make better sampling location as it allows the hose end to be positioned in the flow and well above the riverbed. The aim of trial one was to collect samples ranging from 20L to 100L, however, filters quickly became blocked with particles and so volumes were adjusted. The final results in this report evaluate the suitability of 50 L as a representative sample for analysis. Sampling from the bridge was attempted, however we discovered that the 12 mm diameter hose was unable to pull the water up to the filter rig on the bridge, 7 m above the water's surface. To resolve this issue, a smaller diameter hose of 6 mm was used in the second trial to allow for bridge sampling from height.

Trial 2 aimed to road test repeat sampling of the selected optimum volume, and explore the variation between replicates. Triplicate samples were collected from both the bank and the central channel (the white star maker in Figure 1, this time using a thinner diameter hose of 6 mm), and flow rates were recorded every five minutes to monitor the samplers. To enable samples to be drawn 7 m vertically upwards, the diameter of the silicone hose was reduced from 12 mm to 6 mm, however flow rates were reduced in comparison to bank samples (starting flow rates of ~3 L/min from the bank versus <1 L/min from a height of 7 m above the central channel and so the period integrated into a single sample was longer from the central channel than the bank.

Processing and extraction

Solids from Trial 1 were recovered from the filters by thorough rinsing with 0.7 µm GF/F filtered DI water and non-synthetic fibre (natural hair) brush. Approximately 1 L of filtered DI was used to collect the solids from the filter and stored in a glass beaker. The sample then underwent a Fenton's reaction to break down any organic matter, 10 mL Fe(II) 0.05 M solution (> 98% purity) and 20 mL >30% H_2O_2 (reagents: Fisher Scientific, USA) (Horton et al., 2021). The Fenton's reaction was left to exhaust for 20 h, before being acidified. Samples were concentrated onto a 5 µm steel filter and submerged in GF/F filtered 2% HCl for 24 h before being 100% deposited on 3 µm silver filters for µ-FTIR analysis. The use of the Fenton's reaction proved to be effective in reducing interfering organic material in river water samples, however the following issues were observed: 1. A significant fine mineral residue appeared to overload the silver filter during deposition, 2. The 1 L sample was difficult to work with and reduced the efficiency of the Fenton's reaction by diluting the reagents. To improve the efficiency of the process and the condition of the final deposited sample, the processing method was refined for Trial 2. Samples from Trial 2 were removed from the filter using the same method as Trial 1, however the sample was immediately concentrated on a 5 µm stainless steel filter and transferred to a 150 mL glass beaker. The samples then underwent the Fenton's reaction, which was more vigorous than Trial 1 as these reagents were no longer diluted, but simply the neat 10 mL 0.05 M Fe(II) and 20 mL >30% H_2O_2. 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 final

collected material on the filter was then washed from the steel filter with 0.7 µm GF/F DI water and 100% deposited for analysis.

Sediment Samples

Sampling

Sediment samples were collected using a 250 cm² Van Veen sediment grab from four locations along the River Thames in Wallingford (approximate transect of samples is overlayed as a white dotted line in Figure 1). One sample was taken 10 m upstream of the bridge and triplicates were taken down stream of the bridge at 3, 6 and 10 m. Samples were taken by manually lowering the grab over the concrete wall that provide the bank in this section of the river. It proved to be difficult to collect a sample in areas where the bank had a shallow slope, so locations with vertical banks were chosen to easily reach the riverbed for the purpose of this trial. Sediment sampling from the central channel was also trialled for feasibility, with deployment of the Van Veen grab from a central position of the bridge span. The location of sediment sampling should be defined during the problem formulation stage of any monitoring campaign to select appropriate sampling locations according to the objective of monitoring. Feasibility of sampling will be highly location specific and should be tested prior to any sampling campaign during a pilot trial of each site. The samples were transferred into 250 ml glass Kilner jars using a metal funnel and a metal spoon.

Processing and extraction

Microplastic extraction followed an in-house UKCEH SOP for sediments. 30 g of wet river sediment underwent two Fenton's reactions and two density separations in $ZnCl_2$ (density 1.7 g cm^{-3}, > 98% purity) before being stored in 50% ethanol or deposited on 3 µm silver filters. The coarse grain size and low organic matter content of river sediments in trial 2, resulted in no refining of the method for river sediments being necessary, as the sample matrix was broken down or separated easily. Fractioning the final sample into coarse (>198 µm) and fine (>5 µm and <198 µm) was found to be necessary due to large charcoal-like fragments present in the sample after extraction.

Results

Water samples

Increasing the volume of sample captured and analysed reduced the variability in microplastic concentration on a per volume basis between samples (Figure 2A). Sample volumes greater than 25 L resulted in more consistent concentrations (highlighted in green region) than sample volumes <25 L which were highly variable (CoV 66%). The apparent inflation in particle concentrations on a per volume basis when <20 L was sampled may be in part due to differences in local conditions at the time of sampling, as the <20 L samples formed part of Trial 1, whilst samples >20 L were collected a week later in Trial 2. Alternatively, it may be that lower sample volumes can inflate particle number counts where total numbers of plastic particles are quite low, for example, if only one particle is detected, the final concentration is highly sensitive to the volume sampled. Therefore, we recommend maximising the volume taken to capture as representative sample as possible, reducing the stochasticity associated with the measurement of the rarer polymers in water samples.

Concurrent sampling at the bank resulted in greater precision in the measurement, with lower variation observed between triplicates of this sample, CoV 48%, compared to sequential triplicates taken from the central channel, CoV 68% (Figure 2B). Whilst the CoV of 48% is not low, it is closer to the inherent variability we see in a known standard of polystyrene spheres in water of 24% when recovered from the process in Phase 2 of the project (full details are provided later and in Figure 6). Mean results from either location were comparable (ANOVA, $F(1,4) = 2.01$, $p=0.23$) and so the exact position of sampling within the river channel should be informed by the objectives of the monitoring survey, rather than being set as a specific requirement within any survey. For example, concurrent sampling rather than sequential seems effective in reducing interference of very localised temporal variation, whilst composite sampling over time may integrate and smooth out these temporal differences observed between sequential samples.

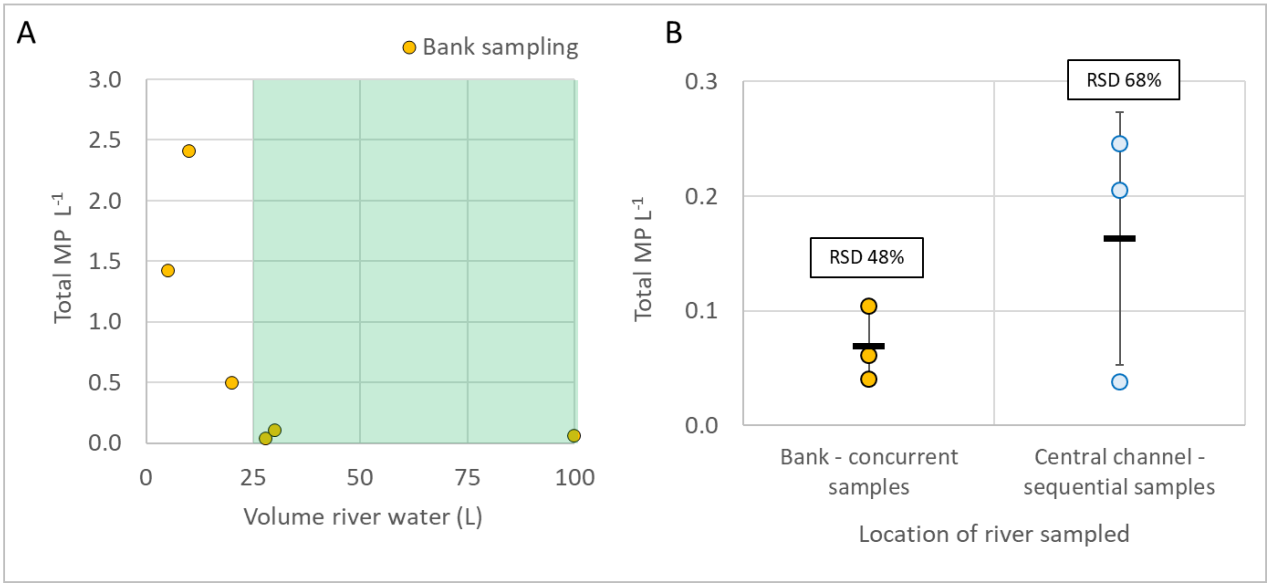


Figure 2: (A) Selection of representative sampling volumes based on the number of microplastic particles detected in increasing volumes of river water from Trial 1 and 2 and (B) exploring the variability in sampling different locations within the river cross sectional profile in Trial 2.

Pumped filtration does present challenges, with filters clogging during sampling in some, but not all cases. This occurred even when replicates were taken simultaneously, with inlet pipes tied together to ensure that replicates demonstrate as closely as possible the same conditions and location of sampling.

Figure 3 shows the variation in flow rate through the filters of samples collected during trial 2. Samples collected from the bank were started exactly synchronously, with the hose ends all within 10 cm of each other. Surprisingly, bank 01 replicate did not experience any clogging issues and the flow rate remained constant throughout sampling, despite pumping from the same position in the water column as bank 02 and 03. It is thought that the filters clogging is initiated by plumes of suspended particles being pumped onto the filter decreasing the pore size, this then allows a gradual build up to occur. It should be noted that the volume taken is designed to collect sufficient material on the filter to consistently measure microplastics in the sample. Even though each replicate from the riverbank sampling represented different volumes from 35 to 100 L (Figure 3), the microplastic counts normalised per L were quite consistent (Figure 2B), with a relative standard deviation (RSD) of 48%. Therefore, the variability even within concurrent samples in the volume that could be sampled does not appear to preclude consistent quantification of microplastics, suggesting microplastic loads may scale in some way similarly to suspended solids in a similar size range.

Sampling from the bridge in the central channel significantly reduced flow rate through the filters to <1 L minute^{-1}, resulting in slower sampling. It was also found that samplers needed to be monitored to ensure air bubbles did not make their way into the tubing and stop the sample reaching the filter. Due to the time intensive monitoring required for central channel samples, sampling was concluded at 30 L, taking ~30 – 45 minutes per sample.

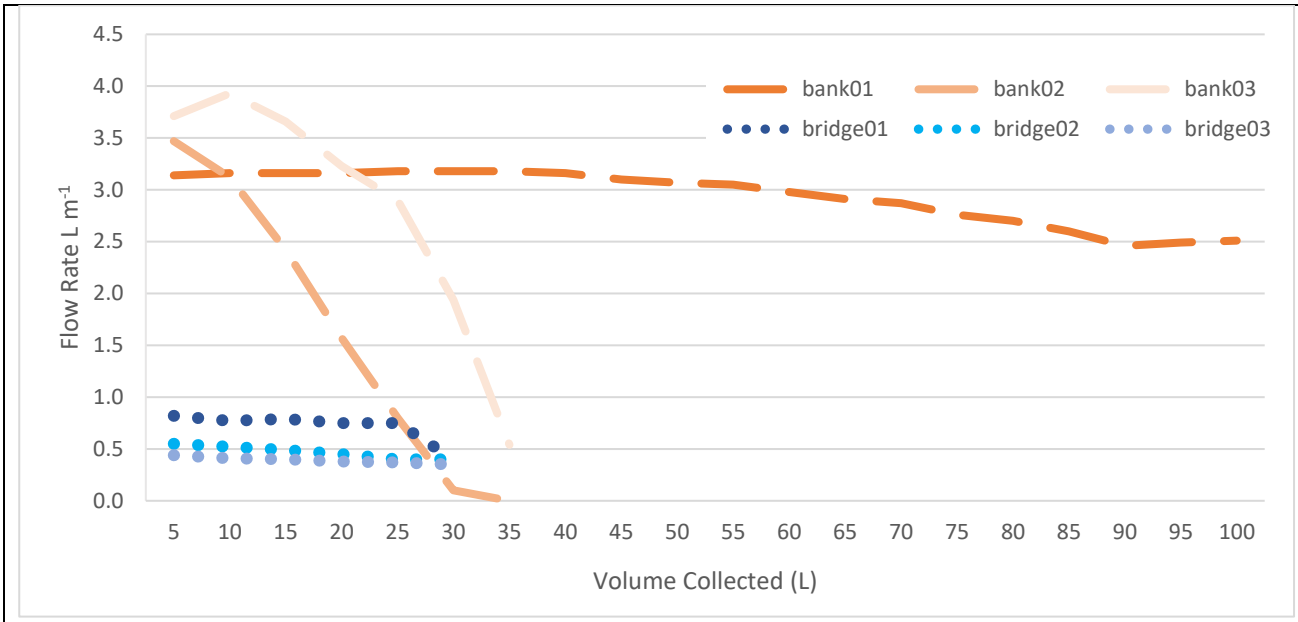


Figure 3: Changing flow rates pumped from bank (dashed orange lines) and bridge (dotted blue lines) locations. Bank samples were collected simultaneously and from the same location. A slowing in the flow rate indicates blocking of the filter pores.

Processing trials demonstrated that that extraction techniques could accommodate samples up to 100 L, and ease of extraction was related to how loaded the filter cartridge is with suspended solids rather than the volume sampled. Additionally, 30 L samples were demonstrated to contain measurable microplastic concentrations >LOD. No difference in the total microplastic counts per L were found between samples 30 L in volume (0.04 and 0.1 MP L^{-1}) and the replicate that managed 100L (0.06 MP L^{-1}). To balance feasibility with capturing sufficient sample to be representative, 50 L was recommended as the target volume for sampling to meet the needs of a representative sample for the analytical window we are targeting in our analysis.

Sediment Samples

Trial 2 sediment sampling along the bank of the River Thames quantified microplastic concentrations ranging from 314 MP kg^{-1} to 2393 MP kg^{-1} (Figure 3). Variation between replicate samples was found to be highest closest to the bridge where, river flow rate appeared highest. Variation between replicates decreased below and above the bridge. This decrease corresponded to the flow rate of the river water at the sample location, with decreased variance at locations where the river moved more slowly. The mean microplastic concentrations at each location along the riverbank were shown to be significantly different. Results show that sampling location along the riverbank should be carefully considered and hypothesis driven when collecting samples. This is demonstrated by the close to 7-fold difference between the replicates taken 3 m below the bridge and 10 m below the bridge.

Sampling from the bridge in the central channel was attempted during this trial phase, however no sediment was collected as the riverbed was found to be scoured, likely by the high flow rate, leaving a rocky bed and large detritus such as larger sticks and branches. It has yet to be established how microplastic concentrations relate to riverbed composition such as composition of the benthic substrate and sediment particle size distributions. Monitoring of river sediments may require specific sampling methods depending on the substrate that were beyond the scope of this study to evaluate, but should be considered in future efforts to monitor for microplastics in rivers.

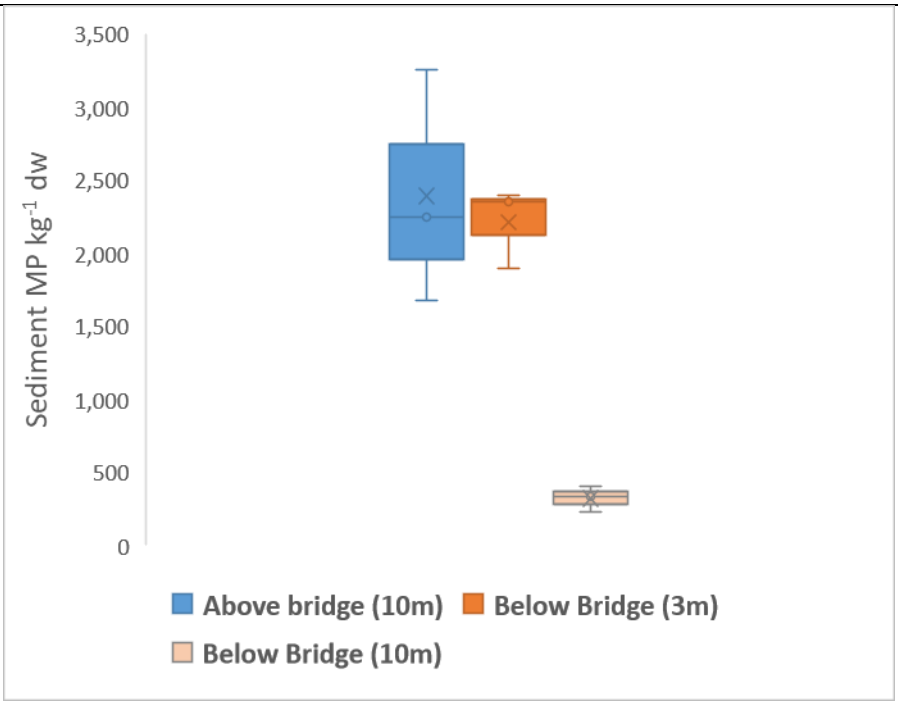


Figure 4: Box plot showing the changing microplastic concentration (MP kg^{-1} dw) between sediment sampling locations on the Thames at Wallingford Bridge, and the variation between triplicates at each location.

Conclusions

Results of the river profiling pilot study highlighted necessary adjustments and recommendations for the sampling and processing procedures to capture microplastics >5 µm in size.

- A representative sample volume should be sufficient to detect microplastics with the required accuracy (i.e. achieve an acceptable resolution in the data for the purpose of the monitoring) and should be sufficient that concentrations per L measured are no longer a function of the volume sampled.
- A sample volume of 30 L is considered the minimum required for river water sampling. This is based on knowledge that this is sufficient to detect microplastics with >90% confidence, that this volume could consistently be sampled by the applied pump and filtration technique and that differences in total number of microplastics per L did not change if higher volumes (100 L) were processed and analysed. On the assumption that increased sample volume reduces the variability, particularly for rarer polymers that are not so consistently quantified at volumes <30 L, we recommend a target volume of 50 L in our provisional sampling SOP.
- Bridge sampling allows for monitoring plastics in the central flow of the channel, useful for establishing microplastic concentrations and transport downstream for instance as inputs to reach-based river models as part of exposure assessment for microplastics. However, bridge sampling can face challenges of access for example on road bridges, or in lower reaches of rivers where height can limit deployment of pumped sampling. A practical solution to this was demonstrated with the use of a small diameter tubing (<6 mm), making bridge sampling from a height of up to 7 meters feasible.
- Sediment sampling location is a significant factor in microplastic concentration, and hydrodynamic properties at sampling locations should be carefully considered when selecting sites for monitoring according to the objective of the survey.
- When processing, washing samples through a 5 µm steel filter after Fenton's reaction and submersion in acid further reduces non-polymer matrix from samples, allowing 100% deposition.
- The analytical window could be extended or reduced to smaller or larger sizes of plastic through use of additional or alternative physical filters to analyse other regions of the microplastic continuum, from 5 mm to 1 µm and below as new analytical capabilities become available, beyond the current state-of-the-art. Calculations for representative sample volumes must be specific to the minimum size detectable and the expected concentrations in this size range, following the rationale outlined in this report.

Phase 2: Field testing and reproducibility assessment of microplastic sampling surface waters and sediments in rural and urban catchments

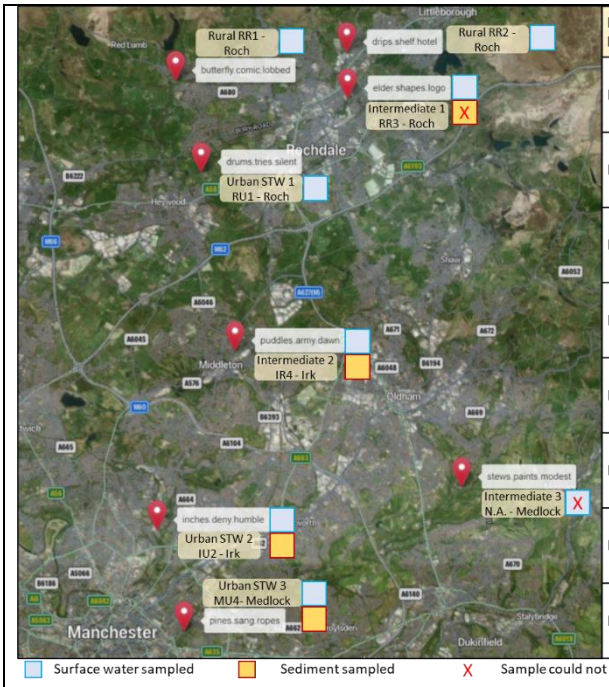
The field sampling campaign of the project was conducted between 31st January and 10th February 2022. The objective of this component of the project was to road-test the methods selected during the river profiling development phase, providing SOPs for sampling both surface waters and sediments to the sub-contractors AquaEnviro. These samples were then processed at the UKCEH Wallingford laboratories to extract microplastic particles for analysis following draft SOPs specific to surface water and sediment processing. The design of this phase of the project allows the performance of the draft SOPs for sampling, extraction and analysis to be evaluated. Lessons learned from the experiences in this field campaign are included in the final draft SOPs, a key deliverable of the project.

Methods

Site selection

Given the considerable preliminary research carried out by UKCEH on a mixed urban/rural catchment for the Manchester microplastics project (GMCA 226/DN 549718), it was considered beneficial to adopt these locations for this study. There would be potential mutual benefit to both projects and indeed the wider scientific understanding of microplastic origins, fate and behaviour from allowing replicate sampling at these locations to occur. The 8 sites selected consisted of 3 urban locations down stream of wastewater treatment works, point sources of microplastics (urban WwTW: RU1, IU2, MU4), 3 urban locations upstream of WwTW reflecting more diffuse sources of litter and microplastics pollution such as road run-off (intermediate: RR3, IR3 and a site not monitored as part of the GMCA project, on the Medlock) and 2 sites upstream of urban locations (rural: RR1 and RR2). These sites are on the Rivers Roch, Irk and Medlock in the Greater Manchester area (Figure 4). Note that to keep naming consistent across the project we use the previously defined IDs when reporting results. For each location, multiple backup sampling sites were identified using satellite images from what3words (https://what3words.com/) and Google Street View. Selection of these sites was based on practical access for simultaneous sampling of water and sediments, and the need to select sufficient sampling sites to investigate the three different catchment characteristics. The suitability of sites for concurrent surface water and sediment sampling could only be known when visited in person. Therefore, longlisting multiple sampling points for each prospective location along the river course is necessary. Of 26 provisional sampling points identified in this desk exercise, only 4 of these locations were suitable for concurrent sampling of surface waters and sediments. The most common reason was that no fine sediment material could be collected from the channel bed. Often only stony substrate was captured by the grab sampler or the course of the river had been modified and diverted along artificial concrete channels that were continuously scoured of fine sediment material. When conducting such a scoping exercise in the future it would be useful to include considerations such as channel straightness for example, which may be an indicator of modification of the river course and so less chance of sediment to sample. The suitability of different sampling devices depending on the sediment constitution, whilst not the focus of this study, has also been the focus of other reports and should be considered (Adomat et al., 2022). Most importantly, the selection of sites for sediment sampling should be hypothesis driven and consider what local dynamics would drive plastic accumulation in these sediments. This problem formulation phase can inform on how best to design the sampling strategy around the needs of the hypothesis and purpose of monitoring for microplastics.

Of these 4 suitable sites, the intermediate site on the Roch could not be sampled on the day, as high rainfall had scoured the sediment material that was present during the preliminary site visit, meaning no suitable sample could be captured. The final samples with concurrent sediment and surface water sampled were, therefore, the Irk intermediate site and the two urban WwTW downstream of the Rochdale on the Roch and Castleton and Oldham WwTWs on the Irk. This exercise has highlighted the difficulty with monitoring plastic contamination in sediments within urban catchments. These lessons are summarised in Conclusion and Recommendations. A complete description of the sites and their suitability for sampling as determined from the preliminary risk assessment and scoping site visits is provided in Appendix 1.



Defra Site ID	GMNC site ID	Site type	Catchment	What3Words	Samples taken
DEFRA-R1	RR2	Rural	Roch	drips.shelf.hotel	Surface water
DEFRA-R2	RR1	Rural	Roch	butterfly.comic.lobbed	Surface water
DEFRA-I1	RR3	Intermediate	Roch	elder.shapes.logo	Surface water and sediment*
DEFRA-I2	IR4	Intermediate	Irk	puddles.army.dawn	Surface water and sediment
DEFRA-I3	N.A.	Intermediate	Medlock	stews.paints.modest	Surface water*
DEFRA-U1	RU1	Urban downstream of Rochdale STW	Roch	drums.tries.silent	Surface water
DEFRA-U2	IU2	Urban downstream of Castleton and Oldham STW	Irk	inches.deny.humble	Surface water and sediment
DEFRA-U3	MU4	Urban downstream of Failsworth STW	Medlock	pines.sang.ropes	Surface water and sediment

Figure 5: Overview of sampling sites in the Greater Manchester area on the Roch, Irk and Medlock. A summary of the site identifiers, site type and the samples taken at each are given in the summary table. On the map, surface water sampling sites are denoted by a blue box, whilst sediment sampling sites are marked with a yellow box. Note that the GMNC IDs are used in this report for consistency. The first letter denotes the catchment whilst the second letter denotes the site type according to the GMNC classification. It should be noted that we introduce a third "intermediate" classification in this project for the two semi-rural sites RR3 and IR4.

Updated sampling SOP for the field campaign

The sampling teams were provided with the provisional SOP for sampling river surface waters and sediments (Appendix 2). Updates to the original proposed method were:

- Simultaneous sampling in triplicate with inlet pipes tied together 50 cm below river surface for water sampling;
- 6 mm internal diameter inlet pipe to be used if sampling from a height to allow for peristaltic pump to draw water;
- 50 L is the target volume for sampling surface waters;
- For sediment sampling, supernatant allowed to clarify then decanted before taking solids sample; and
- Multiple grab attempts can be made to generate sufficient field sample per replicate in the field as in some locations, sample volume captured with each grab is variable.

Updated SOPs for the extraction of microplastics from surface waters and sediments

The provisional SOP for processing and extracting microplastics from water and sediment samples is provided in Appendix 3. Key adjustments to the original protocol included:

- When processing, washing samples through a 5 µm steel filter after Fenton's reaction and submersion in acid further reduces non-polymer matrix from samples, allowing 100% deposition of surface water samples; and
- For some surface water samples with high mineral load, an optional density separation step may be desirable to remove this interfering material that could not be degraded through Fenton's nor acidification. This may be performed using $ZnCl_2$ at 1.7 g/cm^3 density in a glass density separation funnel and is described as an optional step in the final provisional SOP.

Contamination control measures are also detailed in Appendix 3. Briefly these included:

- Limiting contamination during sampling and processing, minimising cross contamination from clothing by wearing 100% cotton where possible (exemptions for high-vis requirements for safety when sampling for example) and running field blanks for each sampling campaign

- Where possible replacing common plastic components with non-plastic or uncommon plastic materials for all sampling and processing equipment.
- Limiting airborne contamination through covering all samples with clean aluminium foil unless working with the biological safety cabinet fitted with HEPA filter.
- Limiting contamination from reagents through pre-filtering all reagents and water with 0.7 µm glass fibre filter.
- Ensuring no contamination from equipment or re-using stainless steel filters through comprehensive washing protocol using only natural fibre scouring brushes and filtered water for washing.

µ-FTIR image analysis

Detection of microplastics and identification of the polymer composition is performed by spectroscopic µ-FTIR analysis. The processed sample, suspended in 50% ethanol for storage is deposited onto a 2.5 cm diameter 3 µm pore size silver membrane filter. This pore size was selected as the minimum spot resolution of the µ-FTIR is 6.25 µm. Whilst we only run the µ-FTIR at a spot size of 25 µm in this study, these samples could in future be analysed at the finer resolution of 6.25 µm. This sets a physical lower limit for the size of particles captured and possible to analyse in these samples of 3 µm. If the minimum spot size of 25 µm is considered acceptable for future monitoring, then a larger pore size filter could be used and would reduce clogging and so could offer some gains in the amount of sample that can be deposited and analysed. For the cleaner water samples the ambition is for the complete sample to be deposited, however if this results in overloading of the filter, a subsample may be deposited, or the sample may be deposited across several filters. For the sediment samples it is expected that only a sub-sample may be deposited. The proportion of sample represented under the FTIR is calculated from the weighed mass before and after depositing for analysis. The analysis using the Perkin Elmer Spotlight 400 µ-FTIR spectrometer were conducted over a 11 x 11 mm area at an 8 cm^{-1} resolution using 2 accumulations (i.e., four scans per spectra) at 25 µm pixel resolution in reflectance mode, and an interferometer speed of 2.2 cm/s. Scanning at this resolution gives a trade-off between mapping time and spectral quality. This 11 x 11 mm area captures the entire deposition area (10 mm diameter) and so maps MPs in the entire deposited sample. Under these settings, a single sample takes ~1.5 hours to analyse. Scans from 4000 cm^{-1} to 700 cm^{-1} wavenumbers, cover the main diagnostic areas within the FTIR spectrum. All the generated spectra are analysed using the freely available siMPle software (http://simple-plastics.eu). Spectra are matched against an expanded polymer database of Primpke *et al.* 2018. The full list of manufactured and natural polymer targets, as well as some common 'contaminants', that can be identified by siMPle are listed in Table 2. Note that in the siMPle library, false positive detection of the class of polymer acrylates/polyurethanes/varnish (A/P/V) was found to be prevalent, with natural plant material wrongly identified as this synthetic polymer. As such, this polymer class was discounted from all further analysis.

Operationally defining a "microplastic"

It is important we try to harmonise the framework with which we describe microplastics to allow for better longevity of data and improve our ability to compare data from different studies. Using the framework described in (Hartmann et al., 2019), we can describe the applicability domain of the analysis recommended in this provisional SOP, and thus the operational definition of "microplastics" in this report.

The SOP is suited to monitoring solid phase microplastic fragments, with a major dimension greater than 6.25 µm in size (the lower theoretical size limit of detection for µ-FTIR). For this particular study, we operate at a pixel resolution in the µ-FTIR of 25 µm. This analytical technique is chemically specific (microplastic particles are identified by their polymer type and so is restricted to those polymers within your library) and is quantitative of particle number concentrations and two-dimensional descriptions of size. This sets a theoretical lower size limit of detection. Microplastics with a thickness much greater than 200 µm can absorb infra-red significantly, making matching of spectra from the µ-FTIR challenging. In these cases, chemical identity can be confirmed using attenuated total reflectance Fourier transform infrared (ATR-FTIR) where the plastic fragment is in direct contact with an optically dense crystal, a technique that is insensitive to the thickness of the material, unlike the µ-FTIR image analysis. Note that the upper limits of the applicability window for µ-FTIR image analysis is limited only by the field of view that can be captured and the thickness of the particle. For example, a 5 mm square of microplastic film would be detectable by µ-FTIR image analysis, provided the thickness of the film did not absorb completely the infra-red beam. Were this flake sufficiently thick, and polymer identification impaired, ATR-FTIR analysis would allow for identification of the polymer.

All analytical techniques are constrained to quantifying microplastics only within the optimum analytical window of the technique. As µ-FTIR does not require visual identification of microplastics it is not limited to identifying microplastics by aspects such as shape or colour which are subject to both false negative and false positive user biases. This makes it sensitive to small fragments and particles that otherwise are difficult to distinguish from naturally occurring particles. On the other hand, in our analysis we perform the spectral

scanning at 25 µm pixel size, which may limit the sensitivity of the technique to synthetic fibres, for example a recent study found almost all synthetic textile fibres in river waters and sediments from a sampling campaign in Columbia were <20 µm in width (Silva and Nanny, 2020). Fibres with a width smaller than 25 µm can still be detected under the 25 µm spot size but their thickness may be overestimated. Systematic comparison between µ-FTIR and other analytical approaches are needed to evaluate the sensitivity of different techniques to different shapes of microplastic (from fibres to films to fragments) but is not the focus of this study.

Data treatment, statistical analysis and reproducibility assessment

The raw data from the µ-FTIR image analysis consists of microplastic particle counts per polymer in each sample. Our previous work has highlighted the need for careful blank correction on a polymer-by-polymer basis (Johnson et al., 2020). Even with the stringent contamination controls listed in (Appendix 3), some background is unavoidable particularly of common polymers in the laboratory, such as polypropylene. Quality assurance procedures such as blank correction and recovery assessment are essential. These provide confidence in the validity of the analysis and allow the setting of robust and reliable detection limits that avoid misrepresenting background ambient plastic that cannot be removed from contaminating samples during their processing, as true environmental microplastics (which would be the case if controls are not included). A summary of the final corrected data on a polymer-by-polymer basis, the blank data and calculation of the limits of detection is provided in Annex 4.

Conversion of count data to mass data:

The conversion of data from counts to the total mass of each polymer in the samples is performed automatically in the siMPle software. The conversion from a two-dimensional area to a volume-based estimate of mass makes some assumptions about the shape and relative dimensions of the particles.

To estimate a mass for each particle detected by the µFTIR, the longest dimension is calculated as the longest distance between pixels of the particle. The minor dimension is calculated by the software assuming that the particle is an ellipse and knowing the two-dimensional area of the particle. The third and final dimension to be calculated, the thickness, is assumed to be 0.67 times the minor dimension. From these dimensions, the volume of the particle is estimated assuming the microplastic particle is ellipsoidal, and the estimated mass is calculated from the volume and the density of the identified plastic polymer. Unfortunately, until an effort is made to corroborate predicted mass given by µFTIR and siMPle with a mass based chemical analysis, such as by Pyr-GC-MS, it is unclear how close this estimating method is to reality. Therefore, we only report indicative total mass of MPs in samples in this report and do not use this data for the reproducibility assessment or in statistical analysis between sites, as it is unknown what variability violations of these assumptions may make to the estimated masses.

Blank correction:

The mean concentration of each polymer (expressed as either particle number or mass per sample) is calculated from the replicate blank samples. These blank samples are processed alongside the sample batches, following the same extraction procedure specific to the surface water and sediment processing. This value is subtracted for each polymer from all samples (blank correction). The limit of detection (LOD) for each polymer is then defined as 3.3x the standard deviation of the blank. This gives 95% confidence that any detected value >LOD is not a false positive result. The limit of quantification (LOQ) is a more stringent criterion and is defined as 10x standard deviation in the blank. Blank correction at the level of the individual polymer is performed for both the number and mass concentration data for all samples. The LOD and LOQ are calculated for surface waters and sediments independently (as the extraction protocol differs between these sample types and so blanks specific to these extraction protocols are required). Data which passes the LOD is interpreted in the subsequent analysis. Data which is <LOD is expressed in terms of a “concentration < x per L or per kg”. Whilst this is not quantitative, it can provide important information on the overall limits of detection in the field, i.e. what level of contamination is required to confidently detect and quantify microplastics. This can help inform future sampling, as suitable adjustments to the method can be made (for example sampling more water for particularly clean sites), making the approach responsive and flexible to the requirements of the reality of monitoring in the real environment.

Spike recovery:

We adopt an approach using certified standard polystyrene 90 µm beads (Polysciences, GmBH Germany, Lot #A794079) which we know to be stable and consistent in terms of particle number concentrations as a standard. These were spiked into our two matrices, water and sediment (taken from the Thames in Wallingford and used as a matrix representative of sediments). Triplicates for each matrix were processed alongside the sample batches in exactly the same manner as the field samples and the recovery of the spiked microplastic from these samples is used as indicative of the reproducibility of the extraction and analysis. The recovery is calculated as C1/C0, with C0 being the starting concentration spiked to each sample, whilst C1 the final quantified number of particles after extraction and analysis. This is expressed as a percentage.

Correction or adjustment of microplastic counts according to the spike recovery results is not yet possible. Single polymers may be used as representative materials for spike recoveries and this can infer the performance of the extraction and analysis of microplastics in real samples. However, the recovery of a material is a combination of the physical recovery across the sampling and extraction process (losses to equipment, degradation and destruction of fragile polymers during processing) and technical or analytical recovery of the analytical instrument (matching spectra for weathered plastic against virgin polymer libraries, interferences with spectra matching by other material not completely eliminated during the extraction, impaired spectral quality due to the extraction, small size of plastics or weathering in the environment). Therefore, whilst recoveries of a representative known plastic can infer qualitatively the success of the analysis, validation that this test material is truly representative of the recovery of all detected environmental microplastics is very technically challenging and the generation and testing of such materials is the focus of international inter-laboratory comparisons (Belz et al., 2021; van Mourik et al., 2021).

Statistical analysis:

The basic statistical model is a one-way analysis of variance (ANOVA), from which the between-group variance (or model mean squares MS_b) and the within-group variance (or residual mean squares MS_w) can be determined.

A statistical F-test can be used to determine if between-group variability is significantly larger than the within-group variability, i.e., if the results from the different sampling locations for surface waters and sediments are significantly different. The F variable is calculated from:

Equation 2

$$F = \frac{MS_b}{MS_w}$$

If F is smaller than the critical F value (Fcrit) for the degrees of freedom, the difference between locations is not statistically different. If assumptions of normality and homogeneity of variance are not met for the dataset, Welch's F-ratio adjustment was used. If the assumptions of ANOVA are met and a significant difference is observed between sampling locations, Tukey's HSD is used to identify which pairs of sampling locations differed significantly. If the assumptions for ANOVA are not met and Welch's F-ratio finds a significant difference between sampling locations, the Games-Howell *post hoc* test is used to identify which sampling locations differed significantly from each other, based on Welch's degrees of freedom correction with adjustment for multiple testing at a confidence level of 0.95.

We also evaluate the in-house reproducibility of the method and underlying heterogeneity of microplastics in these waters, through analysis of the replicate samples taken concurrently at each site. The purpose of this is to estimate the precision of our protocols and so the accuracy that can be achieved in the measured environmental concentrations. It is useful to consider the achievable accuracy as the level of difference that corresponds to the minimum meaningful difference between sampling locations or occasions that should be interpreted. A difference between samples should only be interpreted as such if it is greater than the precision of the method. Often, monitoring studies do not replicate simultaneous sampling of the same location. Such studies cannot test whether differences between two sites are statistically significant as they are limited to a single data point per sampling location. Estimating the achievable accuracy of a method is, therefore, an extremely useful tool for interpreting existing data and future studies that are designed in this way. If two sites do not differ by more than the achievable accuracy of the analytical method, differences between the two should not be interpreted.

To establish the reproducibility of the method to estimate the achievable accuracy we follow the guidance in the Eurachem Guide: The fitness for purpose of analytical methods (Magnusson & Örnemark, 2014). Note that we use this approach to estimate the repeatability and reproducibility relative standard deviations for "total microplastic particle contamination" within samples, not for each individual polymer.

Briefly, the repeatability standard deviation (S_r) is calculated from the MS_w, the residual mean squares within groups (i.e. the standard deviation within repeat measurements on a single sample) using Equation 3.

Equation 3

$$S_r = \sqrt{MS_w}$$

The between-groups standard deviation (S_b, standard deviation between concurrent samples from the same location) is then calculated using Equation 4, where MS_b is the mean squares between groups (i.e. between replicate samples taken concurrently from the same location), and n is the mean number of replicate samples.

Equation 4

$$S_b = \sqrt{\frac{MS_b - MS_w}{n}}$$

From these two equations, the intermediate precision (S_I, also known as the reproducibility standard deviation) can be calculated, Equation 5.

Equation 5

$$S_I = \sqrt{S_r^2 + S_b^2}$$

The intermediate precision (S_I) represents the sum of within-group and between-group variance. Whilst true intermediate precision is most accurately calculated from a full interlaboratory comparison study, this pilot study estimates S_I under conditions mimicking those under which the method would be used routinely, but within a single laboratory.

From S_r and S_I the relative standard deviation (%RSD) may be calculated for both the repeatability (%RSD_r, RSD explained by within sample variation) and reproducibility (%RSD_R, RSD explained by the variation associated with simultaneous sampling at a single location) demonstrated in Equation 6 and Equation 7, where $\bar{y}$ is the grand mean across all data.

Equation 6

$$\%RSD_r = \frac{S_r}{\bar{y}} * 100$$

Equation 7

$$\%RSD_R = \frac{S_I}{\bar{y}} * 100$$

Results

µ-FTIR analysis of microplastics

A summary of the data is available as an Appendix to this report (Appendix 5).

Recovery assessment:

Recovery assessment performed using 90 µm polystyrene (PS) spheres demonstrated complete recovery of particles from the extraction process for both surface waters and sediments run in parallel to the samples (Figure 6). The apparent recovery >100% can in part be due to the variation in particle numbers in the stock suspension of PS that was prepared to perform this recovery assessment. Whilst the PS stock is quite consistent (coefficient of variation, CoV 14%), by its very nature, the starting number of PS introduced to the recovery samples is not a single number, but a range due to this heterogeneity in the stock. Whilst the recovery cannot be used to quantitatively adjust the data, having demonstrated ~100% physical recovery of this representative microplastic from the processing workflows, we can at least be confident that any physical losses from the extraction process (such as adhesion to equipment surfaces, disintegration of particles due to chemical degradation of the polymers etc.) are likely to be negligible. It is also interesting to note that the CoV grew from 14% in the stock to 24% in the recovered samples. This suggests there is some variation attributable to the process of extraction itself, which should be compared to the RSD_r of field collected samples.

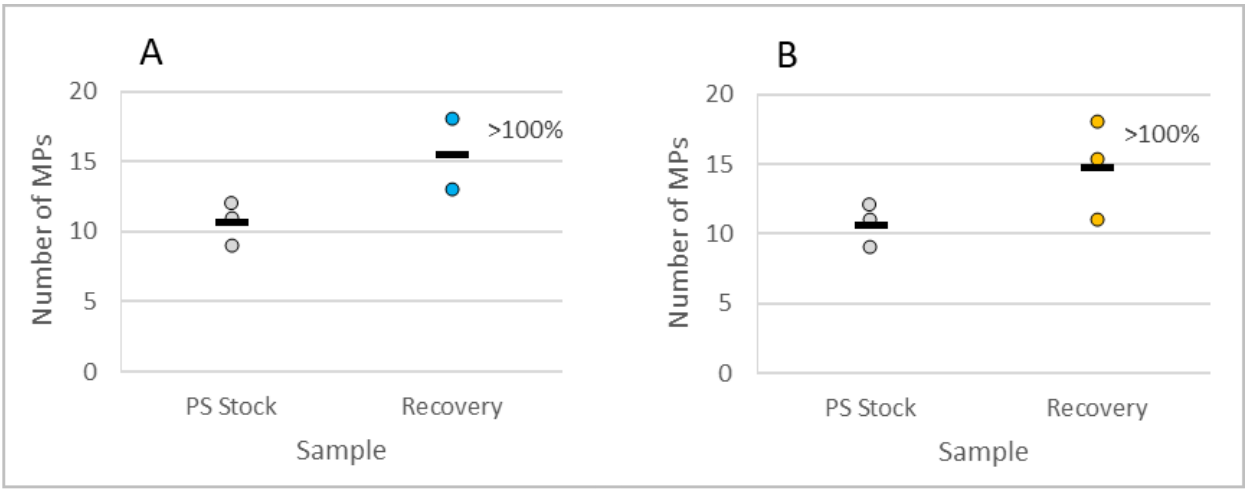


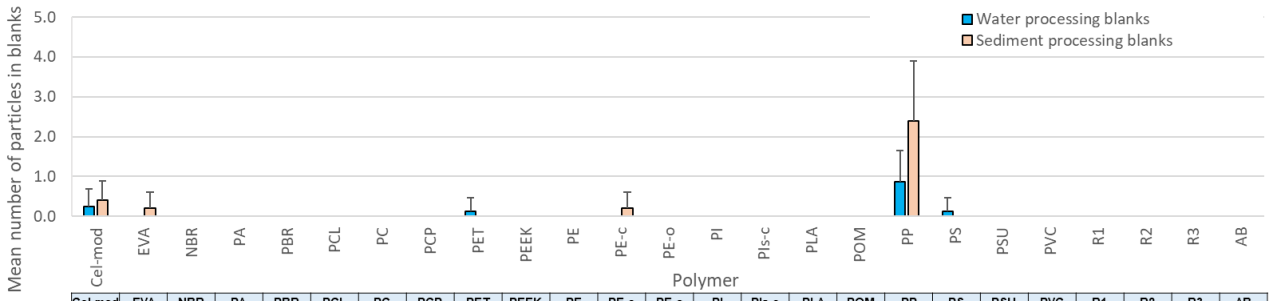
Figure 6: Process recovery of polystyrene standard after A) the complete processing workflow for surface waters and B) the processing workflow for sediments.

Background contamination of plastics during processing of samples

Blanks (negative controls) were run alongside the sample batches, taking clean equipment and running the full process SOP as if for a real sample. Contamination was low from both processes, though the range of polymers and concentrations were slightly higher for the more complex sediment extraction than the surface water process, as would be expected (Table 1). From the processing workflow for surface waters, background contamination was limited to artificially modified cellulose (Cell-mod), polyester (PET), polypropylene (PP) and polystyrene (PS). For Cell-mod, PET and PS, only one or two blank replicates contained these polymers at very low numbers, whilst even PP (the most pervasive background polymer) was only detected in 5 out of 8 blank samples. Few studies routinely report on blanks that represent the process undertaken for samples, with 32 of 50 peer reviewed publications prior to 2019 reviewed by Koelmans et al., 2019 reporting no procedural blanks as part of their study. We recommend procedural blanks as a minimum reporting requirement for any investigation of microplastics. Of those peer reviewed articles which do report blanks, it is rare for the raw data to be available. However, there are examples that demonstrate the background contamination observed in this report is in line with what can be expected from the state-of-the-art. For example, a recent study by the Institute for Environmental and Process Engineering, Germany found similar concentrations of polymers in their blanks of between 0.2 and 2.8 MP on average (Weber et al., 2021). This would result in similar LOD and LOQs as we describe here. It should be noted that this study was focused on drinking water and used only a single acidification step during processing, so to achieve similar background contamination from the more complex processing steps required for river water and sediments demonstrates very effective contamination control of microplastics in this size range in keeping with the state-of-the-art.

The sediment processing was contaminated by PP in every blank replicate. as was ethylene-vinyl-acetate (EVA) and chlorinated polyethylene (PE-c). For those polymers detected in the blanks, the LODs were mostly 2 particles per sample or lower, with the exception of PP which had a LOD in sediment samples of 7.3 particles. This for example would require more than 7.3 particles of PP to have been detected in a sediment sample (after blank correction) to consider the microplastic polymer detected in the sample with confidence.

Table 1: Summary of the blank data for waters and sediments. Mean, standard deviation, LOD and LOQ in terms of particle numbers per sample are reported. Polymers that were not detected in the blanks are highlighted in green. Low contaminating polymers are highlighted in yellow and the most contaminating polymer (PP) in red.



	Cel-mod	EVA	NBR	PA	PBR	PCL	PC	PCP	PET	PEEK	PE	PE-c	PE-o	PI	PIs-c	PLA	POM	PP	PS	PSU	PVC	R1	R2	R3	AB
Water processing blanks																									
Average	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Stdev	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.3	0.0	0.0	0.0	0.0	0.0	0.0
LOD (#)	1.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.6	1.1	1.0	1.0	1.0	1.0	1.0	1.0
LOQ (#)	4.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	7.8	3.3	1.0	1.0	1.0	1.0	1.0	1.0
Sediment processing blanks																									
Average	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stdev	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LOD (#)	1.6	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.0	1.0	4.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0
LOQ (#)	4.9	4.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	4.0	1.0	1.0	1.0	1.0	1.0	15.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Inter-site differences in microplastic numbers and polymer types between rural, intermediate and urban catchments:

Two sites were successfully sampled for surface waters at both rural and intermediate locations, whilst three were captured in urban locations, downstream of WwTWs. Data was normally distributed at all sites bar RR2, but the assumption of homoscedasticity was not met and therefore Welch's F adjustment was needed for ANOVA. The statistical significance was around the alpha of 0.05 (Welch's $F(6,5.93) = 4.23$, $p = 0.052$) indicating that the high variation in samples makes it difficult to attribute significance to differences at this level of replication when comparing sites. The variation in particle numbers was generally much greater in intermediate and urban sites than in rural sites (Figure 7). The coefficient of variation in the rural site RR1 for example was 43% whilst in urban site IU2, downstream of Castleton and Oldham WwTWs, the CoV was 136%, meaning even simultaneous samples could result in over two-fold differences depending on the stochasticity of the heterogeneous mix of plastic fragments within the flow.

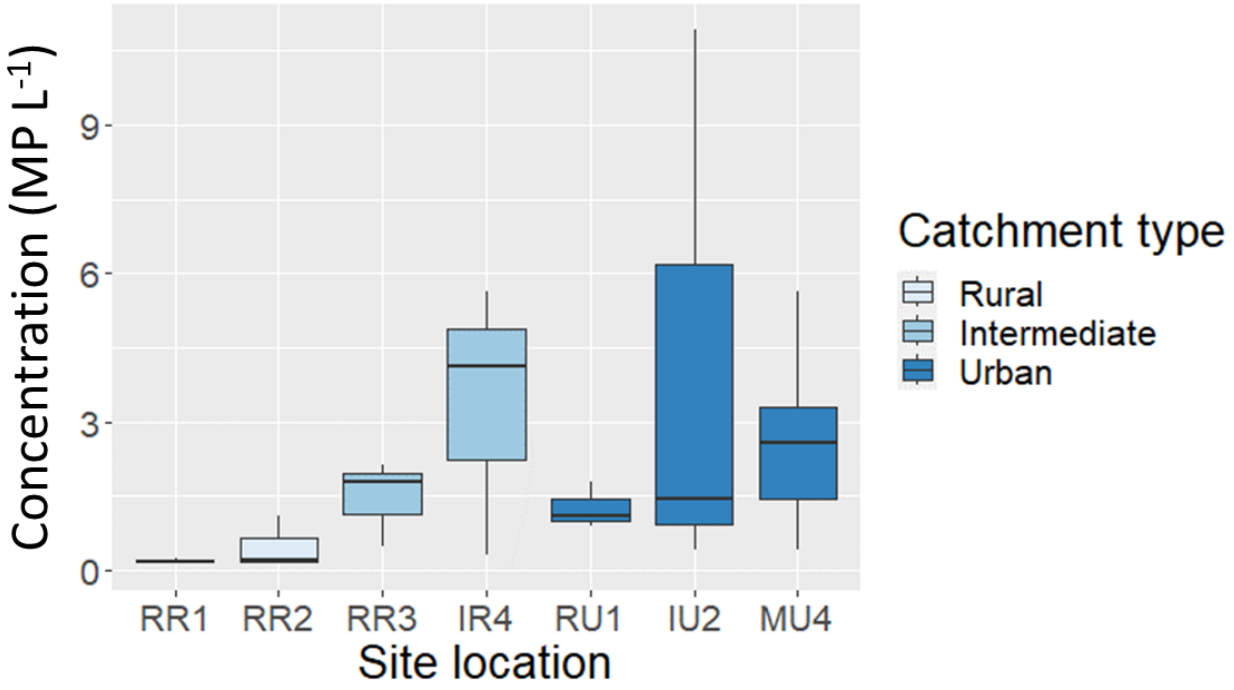


Figure 7: Total number concentration of microplastics detected >LOD in river water at each site. Rural, intermediate and urban sites are denoted by the scaled colour gradient in the legend. Box plots represent median, interquartile ranges and the minimum and maximum for triplicate simultaneous samples taken at each location in the field, with the exception of MU4 where 5 replicates were taken due to blocking of the first sampler and so the exercise was repeated.

It is interesting to consider whether the baseline microplastic loads in the higher order streams closer to the head of the catchments differ significantly from those intermediate and urban locations, to start to diagnose possible inputs of microplastics into the river course. Grouping site locations according to whether they are rural, intermediate or urban downstream of a WwTW, we find significant differences can be found between these catchment types (Figure 8). Data grouped in this way was non-normal and homoscedastic ($F(2,20) = 2.6$, $p = 0.1$). One way ANOVA, therefore used Welch's F adjustment. A significant difference between catchment types was found (Welch's $F(2,9.39) = 5.45$, $p = 0.027$). The fold difference in means between intermediate and urban catchments was only 1.1-fold, whilst between the rural and the urban sites, urban sites downstream of WwTWs had 8.3-fold higher total numbers of MP L^{-1}. Games-Howell post hoc analysis found no statistical significance in the difference between intermediate and urban locations (estimate 1.79, p adj. = 0.68), whilst the 11.4-fold difference between rural and urban locations was close to significance (estimate 0.3, p adj. = 0.96). The mean total concentration of MPs in rural sites was 0.33 MP L^{-1} (CoV 114%), at intermediate sites was 2.4 MP L^{-1} (CoV 87%) and in urban sites downstream of WwTWs was 2.71 MP L^{-1} (CoV 115%).

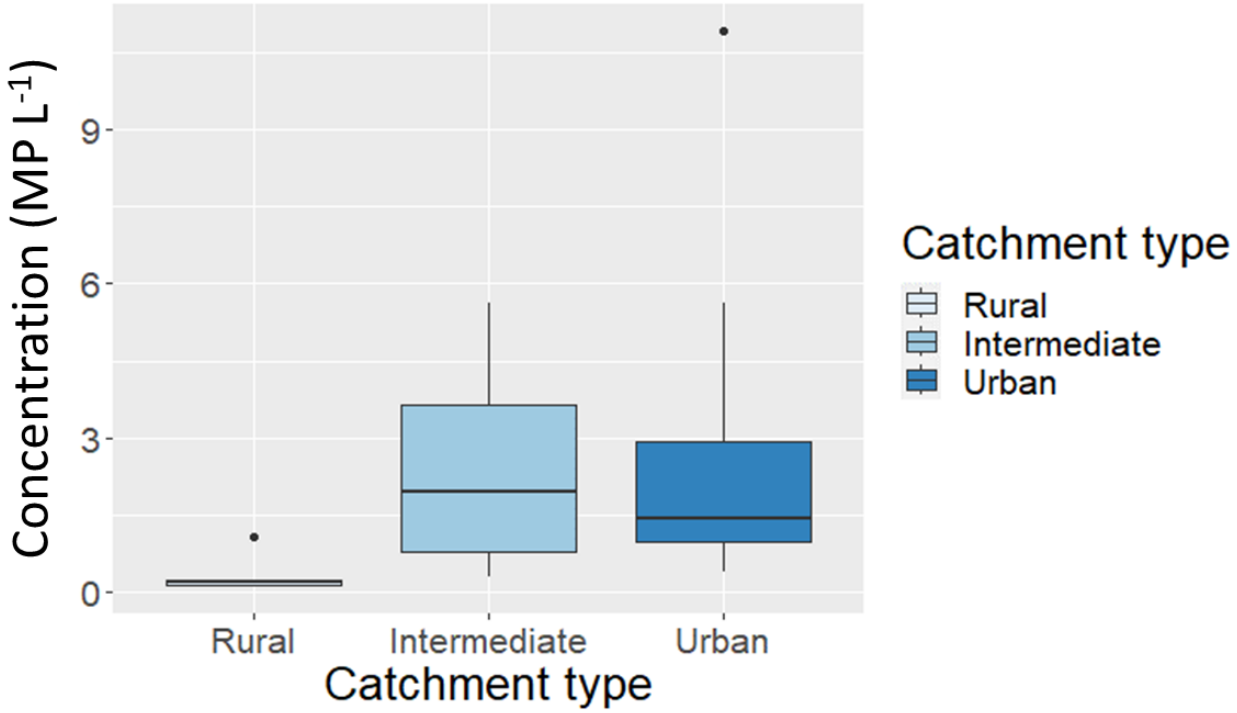


Figure 8: Total number concentrations of microplastics detected >LOD in river water at sites representing rural, intermediate and urban catchments. Box plots represent median, interquartile ranges and the minimum and maximum for samples taken from 2 rural sites, 2 intermediate and 3 urban sites downstream of a WwTW.

Ethylene-vinyl-acetate (EVA) and polypropylene (PP) polymer classes were the most consistently detected polymers in surface waters in the majority of replicate samples from rural, intermediate and urban sites. Where these polymers were not detected >LOD in individual replicates, this was mainly associated with sites expected to have lower inputs of microplastics.

Modified cellulose, chlorinated polyethylene (PE-c), and polyvinylchloride (PVC) were never detected in rural catchments (with the exception of 0.2 MP L^{-1} in one replicate at RR2), but were routinely found in intermediate and urban settings, suggesting an urban source of these microplastics in rivers. Polystyrene was relatively consistently detected only at the urban site downstream of the Castleton and Oldham WwTWs, whilst PVC was almost exclusively detected at urban sites downstream of WwTWs (bar one replicate at the intermediate site IR4 where 0.33 MP L^{-1} were detected).

These results indicate that the baseline contamination in rural headwaters, where the prevailing sources may be atmospheric deposition and agricultural sources, could be differentiated from urban settings where runoff and WwTWs may also be drivers of microplastic contamination in river surface waters. Polymers unique to intermediate and urban catchments may indicate an urban source of particular polymers, however, a wider monitoring scheme would be needed to infer whether the unique signal of polymers such as PS and PVC detected downstream of WwTWs are attributable to this source alone. Interestingly, the mean concentration of MP in urban sites downstream of WwTWs of 2.7 MP L^{-1} is quite similar to our previous finding of median concentrations of MPs in WwTW effluent of 5.45 MP L^{-1} when surveying effluents from 8 WwTWs across the UK (Horton et al., 2021). The estimated mass of MPs in surface waters was 0.001 mg L^{-1}, 0.014 mg L^{-1} and 0.005 mg L^{-1} for rural, intermediate and urban catchments respectively. Caution should be taken over these estimates as they are indicative only, due to the unknown influence that violations of assumptions used to estimate the mass may have. For example, rural locations had far lower total numbers of MPs than urban sites, but the estimate for mass is similar. It may be that thin films such as from packaging or agricultural films (Li et al., 2022) dominate rural locations where surface macroplastic litter may be prevalent (Billings et al., 2023) and blow off from litter on land or where atmospheric deposition may be the main contributors to contamination. The mass of thin films may be overestimated by the siMPle software from the two-dimensional area as the thickness of the particle is assumed to be proportional to the major dimension and the surface area. Further work is needed to refine these algorithms to calculate mass and to corroborate these against mass-based analytical techniques such as Pyr-GC-MS.

Only 3 sediments could be sampled in the campaign. These corresponded to one intermediate sample on the Irk (IR4) and two urban sites, one downstream of Castleton and Oldham WwTW on the Irk (IU2) and the other on the Medlock (MU4) downstream of Failsworth WwTW (Figure 9). Whilst IU2 was one of the most contaminated and variable surface waters monitored, the total number of microplastics entrained in the sediment at this location was significantly lower than at the other urban site MU4 (Games-Howell p adj. =

0.003). The mean total number of MPs at the intermediate site IR4 was 2,485 MP kg^{-1}, whilst at the urban sites IU2 and MU4 it was 1,005 MP kg^{-1} and 13,684 MP kg^{-1}, respectively. The indicative estimates of mass were equivalent to 40.5 mg kg^{-1} at the intermediate site IR4, whilst at the two urban sites IU2 and MU4 total mass of MPs was 2.0 and 103.4 mg kg^{-1}, respectively.

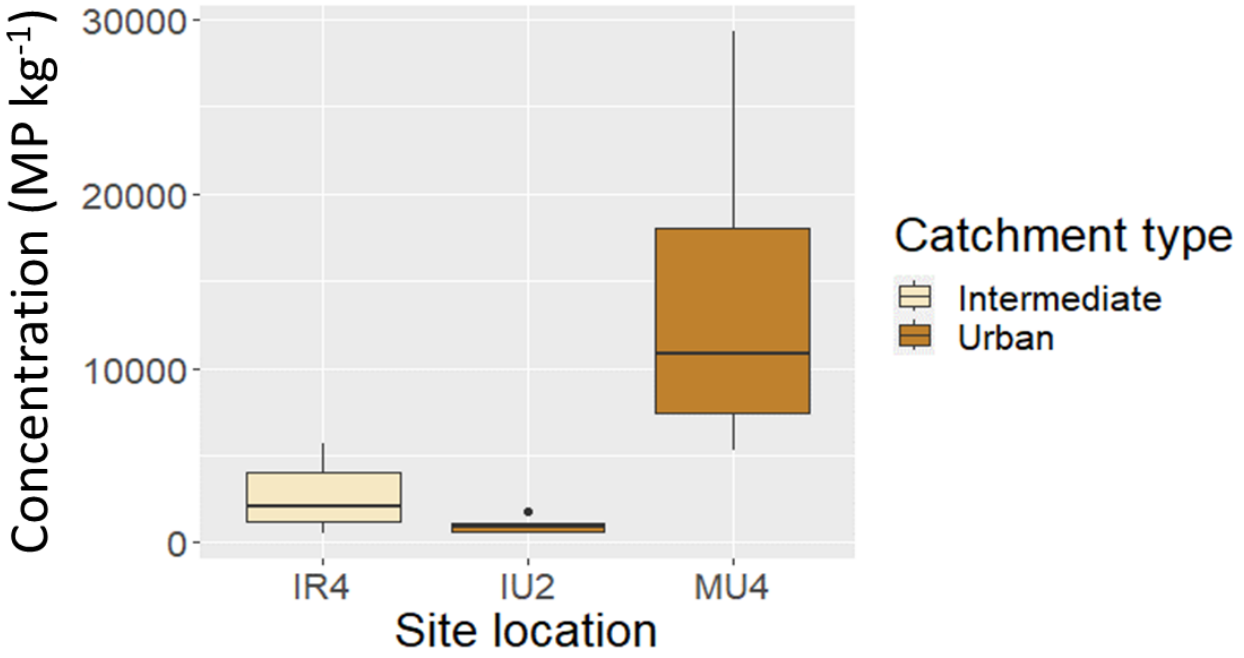


Figure 9: Total number concentrations of microplastics detected >LOD in sediments at three sites representing intermediate and urban catchments. Box plots represent median, and interquartile ranges. The data represents technical triplicates of three independent replicate samples from the field for both IR4 and MU4 (n=9) whilst IU2 is triplicate independent samples from the field plus replicates from one of these homogenised independent samples (total n = 5).

A wider range of polymers were detected in sediments than in surface waters, but it should be noted that the distribution of many polymers is patchy in the sediments, with only EVA, PE, PE-c, PP, PS and PVC, when detected at a site, being found in the majority of sub-samples analysed. Other polymers including polyoxymethlyene (POM), polylactic acid (PLA), polyetyhlene oxidized (PE-o), polyester (PET), polycarbonate (PC), polyamide (PA) and modified cellulose were detected sporadically across independent replicates from sites. Interestingly, PA and PET, two polymers largely associated with synthetic textiles were only detected at MU4, downstream of Failsworth WwTW. This site was also the only site in which macroplastic litter was identified in the 15 g subsamples of sediment analysed. Three large fragments, two of polyethylene and one of cellophane were identified using ATR.

EVA and PP were once again common in sediments. PE-c, and PVC were more common in the sediments, with PVC detected in all urban and most intermediate sediment replicates.

With such a small number of locations it is difficult to generalise the status of microplastic contamination in sediments. However, we can see differences in the total concentration of microplastic particles between the two urban sites, both of which are downstream of WwTWs, with the intermediate site with no WwTW input, falling somewhere within this range. IU2 was one of the more heavily contaminated surface waters and with the highest variability, however, the particle concentrations in the sediment were the lowest detected and relatively consistent (CoV = 50%). One might expect surface waters with higher loads of microplastics to correspond to sediments in which contamination is also correspondingly higher. However, this is to ignore the local dynamics of the river course. For example, the high heterogeneity of the surface waters suggests perhaps a dynamic, faster flowing section of river, in which microplastics are borne along, suspended by the current and thus sedimentation and accumulation of microplastics in the sediments is reduced. The objective of monitoring sediments must therefore be carefully considered when designing a sampling campaign as by their nature, contamination of the sediment by particulate pollutants such as microplastics integrate local sources of contamination, inputs from upstream and the dynamics of deposition and resuspension that complicate the source attribution of microplastics detected.

Reproducibility assessment of the detection and quantification of microplastics in surface waters and sediments

True reproducibility assessment requires testing of a representative test material under conditions representing routine use across multiple laboratories. For microplastics, there are no certified test materials representing known particle number or mass concentrations of microplastics within a relevant matrix, though efforts at the international scale are ongoing (Belz et al., 2021; van Mourik et al., 2021). In the meantime, the statistical approach used in reproducibility assessment can be a useful tool to understand the underlying sources of variation that may underpin a field measurement of microplastics in either river surface waters or sediments.

For surface waters, our sampling strategy of concurrent sampling at a single location all from almost identical locations within the channel of the stream aimed to control as much as possible for variation in local hydrodynamic conditions influencing the triplicate samples taken at each site. In this way, the assumption is that the variance attributed to replication at individual sites is due to the repeatability of the method. If this is the case, we would expect the coefficient of variation (CoV) within sites to be similar to the CoV we observe for our polystyrene (PS) standard that we know to be relatively stable and homogenously dispersed. However, this was not the case. CoV varied from 30 to 136% in RR1 and IU2 respectively which were the extremes of the range. When recovering PS standards of a known concentration from waters (Figure 6) the CoV was 23%. The PS standard itself we consider a proxy for a stable dispersion of microplastics in suspension that is as homogenous as we can achieve in our final sample (CoV 14% for the PS stock). Therefore, we consider the CoV of 23% to be indicative of the best-case variation one might expect between technical replicates on a representative freshwater sample, being based on a single, uniform and spherical pristine polymer rather than a heterogenous mix of environmentally weathered material. Variation greater than this is therefore not due to poor repeatability of the extraction and analytical technique, but rather more likely representative of real variation within the field samples. All surface waters measured in triplicate from independent replicates taken concurrently from the same location at the same time had a CoV >23%. Therefore, the data from the field collected surface waters cannot be used to calculate the relative repeatability standard deviation (RSD_r) or relative reproducibility standard deviation (RSD_R) of the analytical method. However, this does give us an important insight into the underlying variability when measuring microplastics in river surface waters. We expect that a minimum interpretable difference between sites might be ~20% (based on the repeatability of the recoveries of PS from water), but that within sampling location variation can be much higher than this, even when replicates are controlled as far as possible to account for local spatial and temporal variability by sampling concurrently and with sampling hoses tied together to give as close as possible the same sampling point within the river channel. On the basis of the highest CoV observed for IU2 (CoV 136%), one might require >2-fold differences in total microplastic number concentrations between sampling sites to be sufficient to interpret as meaningful differences between sites. However, it must be cautioned that differences smaller than this may still be significant, it is dependent on the underlying variability of the sampling point that determines what the achievable accuracy is with which a measurement can be made. In this way, it is recommended that replicate sampling will deliver a more robust conclusion than single grab samples, as this will allow the underlying variability of the sample to be known, and statistical differences to be inferred. Such replication is not commonly employed to-date. This means it is often difficult to interpret reported differences between sampling locations in the literature. Statistical approaches to estimating sampling error in single samples are under development (Tanaka et al., 2023), however they have yet to be fully validated. For example, of the 23 water samples analysed, only 3 did not pass the criteria set in Tanaka et al., (2023) of >10 particles detected in the sample, whilst 11 (48%) of the combined expected total in samples passed the criteria of >50 particles required to result in an estimated sampling error of +/- 30%. However, the variability in concurrent replicate samples was often >30%, indicating under the conditions during sampling, water column concentrations may be more stochastic than expected.

When sediment sampling however, we were able to take excess sample as part of each replicate in the field, allowing for technical replication as well as true replication to be investigated. For two sites (intermediate site IR4 on the Irk and the urban site MU4 on the Medlock, downstream of Failsworth WwTW), this sampling scheme was performed to allow for repeatability of the sediment extraction and reproducibility of assessment (within a single laboratory) at individual sites to be ascertained, using the reproducibility assessment equations as a tool (Equation 2 to Equation 7). The assumption of this assessment is that homogenisation of the sediments in the laboratory means that technical replicates (pseudo replicates taken from the same Kilner jar of sediment taken from the field) are analogous to a representative test material, for which variation should only arise from extraction and analysis itself. If this is the case for the sediment samples we have found, RSD_r should be equivalent in each field replicate as that found for PS standards recovered from sediment matrix (CoV 24%).

The data for both IR4 and MU4 conformed to the assumptions of normality and homogeneity of variance as required by ANOVA ($F(2,6) = 2.22$, $p = 0.19$ and $F(2,6) = 1.58$, $p = 0.28$ respectively). No statistically significant

difference between replicates taken in the field were observed (ANOVA F(2,6) = 1.78, p = 0.25 and F(2,6) = 1.37, p = 0.32 respectively). This indicates single samples from river sediments may be sufficiently representative to not require replicate sampling in the field. However, the variation within single field replicates can still be large (Figure 10).

For the intermediate location IR4, the repeatability RSD_r was excellent, at 23% this is what would be expected for the variability attributable to the method, as it is similar to the CoV we have observed for our known standard recovered from sediment matrix. The reproducibility RSD_R is only slightly higher at 32%. This is in good agreement with what would be expected from Tanaka et al., 2023. All sediment samples passed the minimum criteria of >10 particles detected per sample and when extrapolated to total particle numbers per processed sample, 61% of samples contained >50 particles, for which we would expect 95% CI within +/- 30% of the measured concentration. Our achievable accuracy for this type of sample therefore may be considered to be +/- 32%. Differences smaller than this should not be interpreted between sites.

For the urban location MU4, variability was higher both within technical replicates of the same sample (RSD_r 56%) and between replicates in the field (RSD_R 61%). This may represent the heterogeneity of more contaminated sites. Indeed, the higher variety of polymer types detected in MU4 was also associated with inconsistent detection of some of these rarer polymers amongst the replicates, testament to the heterogeneous nature of these sediments. Patchiness in sediments is a challenge that can only be overcome through increasing the mass of sediment extracted, whilst at the same time remaining within the analytical window of the chosen technique. Upper limits to detection can also exist, particularly for particle number-based techniques which can suffer from underestimation of particle numbers if the filter areas are overloaded with microplastics of the same polymer, after which the analysis cannot distinguish between touching particles. Therefore, it may be that for some polymers that are lower in frequency in these environments, data will always suffer from high variability as they will always be in the lower region of resolution due to their low particle numbers. This demonstrates the importance of optimising the extraction process to the samples analysed. For samples which were highly contaminated with interfering material, even after extraction, multiple depositions across more than one filter could mitigate for this. Alternatively,

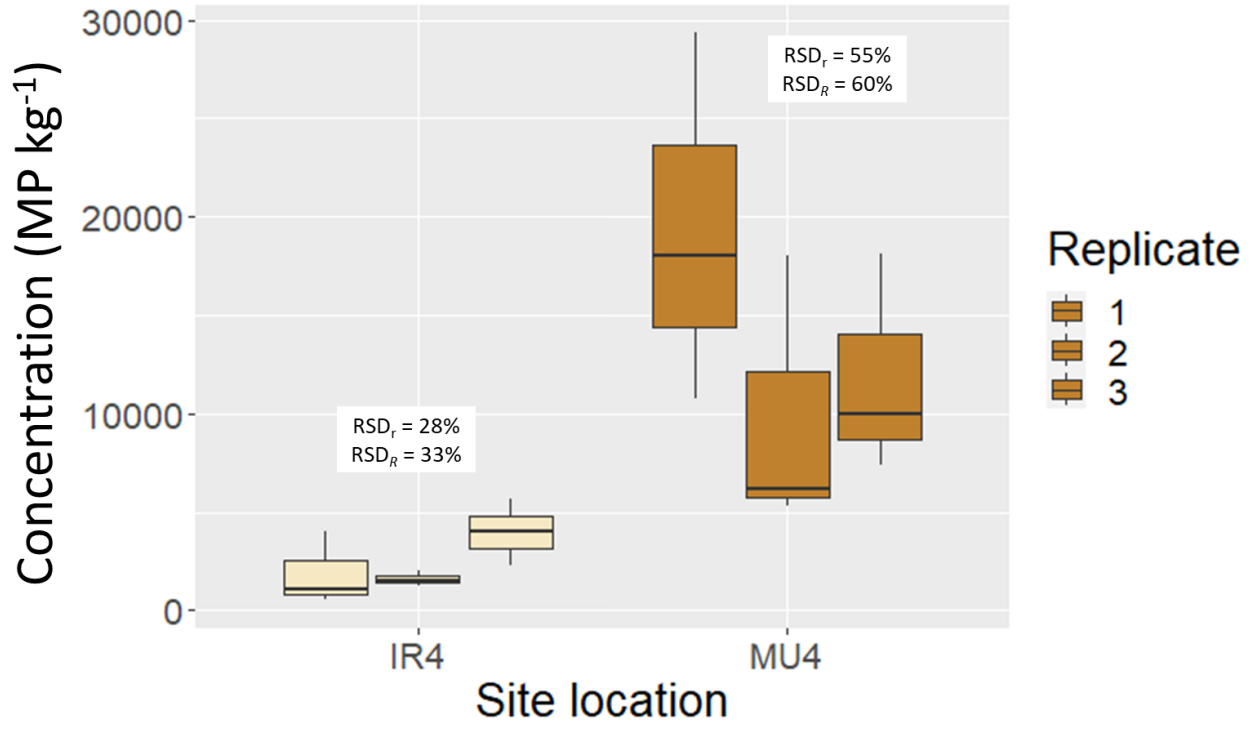


Figure 10: Analysis of replicate sediment samples from one intermediate (IR4) and one urban site (MU4) used for reproducibility assessment of the method. Replicates refer to the independent replicate samples taken in the field whilst the box plots for each replicate represent the median and interquartile ranges for technical triplicates processed and analysed for each independent field replicate.

Phase 3: Gas Chromatography-mass spectrometric (GC-MS) analysis of 6PPD as a marker for vehicle tyre wear particles (TWP)

Methods

Site selection and study design

To develop the method for extraction and quantification of 6PPD as a marker for TWP in sediments, we used two of the locations sampled in the project: the river-bank sediment profile of the Thames at Wallingford, Oxfordshire and the intermediate site in the Greater Manchester catchment on the river Irk, IR4 (Figure 11).

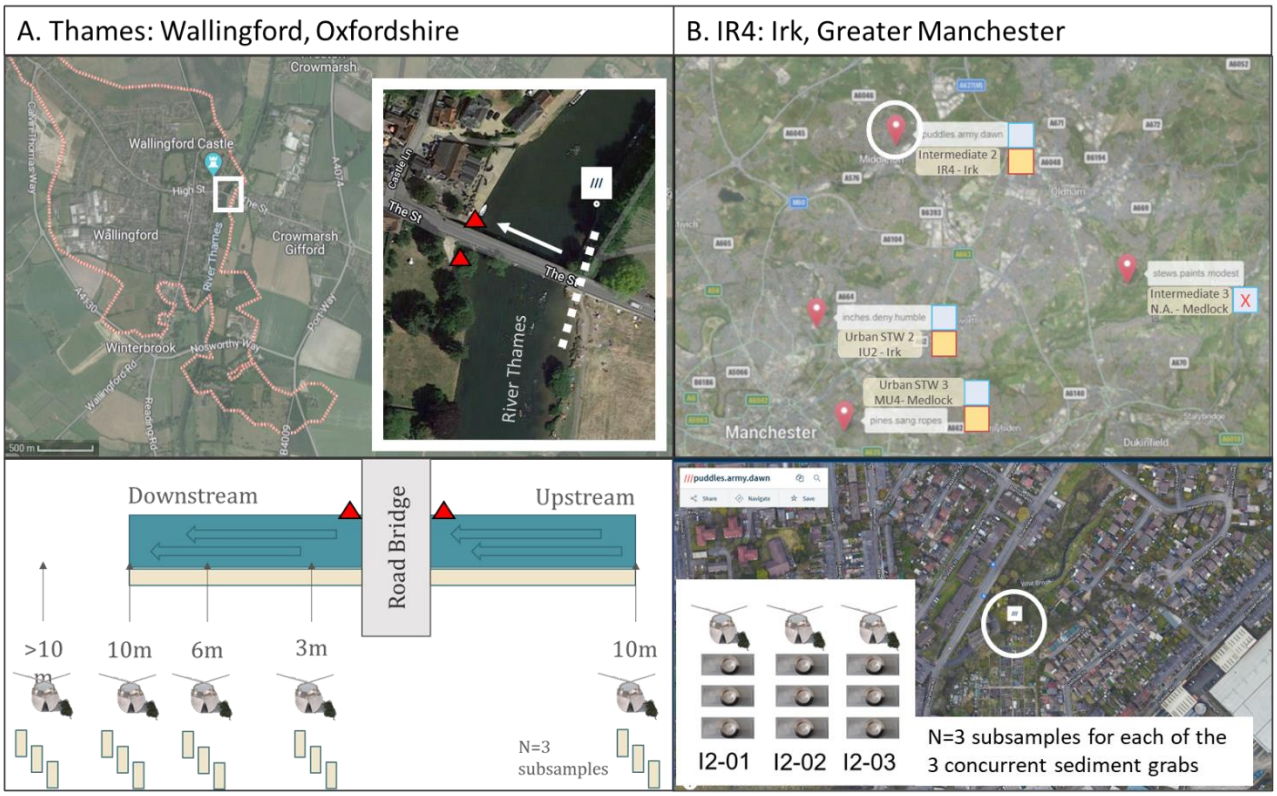


Figure 11: Overview of the different sampling strategies at the two sites A) the river-bank profile of the Thames at Wallingford, Oxfordshire and B) the single point location with concurrent triplicate sampling at site IR4 on the Irk, Greater Manchester.

The sampling strategies at these two sites differed slightly due to the different purpose of the original sampling. The river-bank profile on the Thames at Wallingford consisted of five different sampling locations with triplicate 200 g Van Veen grab samples from each location. Individual subsamples from these were taken to examine the distribution of 6PPD in independent triplicate samples along this profile. At the Irk site in Greater Manchester, triplicate grab samples were taken from the same launch location. These were then subsampled in triplicate to examine how successful homogenisation of the bulk 200 g sample is, whilst the variation between individual grab samples explores the heterogeneity inherent to sampling sediment from a single location.

GC-MS method development

Optimising GC-MS

The aim of the work was to establish a method to detect and quantify tyre particles in sediments, using 6PPD as a chemical marker. Using the literature as a basis, the GC and MS were set up to give an optimised identification and quantification of the 6PPD (SigmaAldrich) from a pure reference standard. A suitable solvent dichloromethane (DCM, Rathburn, HPLC grade >99.9%) to 'dissolve' the 6PPD was chosen to allow the compound to be injected into the GC. The dissolved standard was used to optimise the inlet and GC operating parameters. The MS settings were then switched from Scan mode (50 – 800 m/z), where all ions are detected to selected ion monitoring (SIM) mode to increase sensitivity, using m/z 211, 212 and 268 m/z as the relevant

fragments for 6PPD. This allowed the method to be set up with the lowest limit of detection (LOD) possible with the lowest detectable standard above the noise. A dilution series of 6PPD from 0.4ng mL^{-1} up to 161ng mL^{-1} was analysed to determine the LOD and to generate a calibration curve to allow calculation of 6PPD concentrations relative to sample sediment mass.

All samples were analysed using a 6890N Gas Chromatograph (Agilent, Santa Clara, CA, USA). Helium was used as a carrier gas, set at a constant flow of 1.5 mL min^{-1}. The GC was interfaced with a 5975B Mass Spectrometer (Agilent, Santa Clara, CA, USA) to detect the mass fragments. MassHunter 10 software was used to control the GC-MS conditions as well as identify and quantify the polymers (Figure 12).

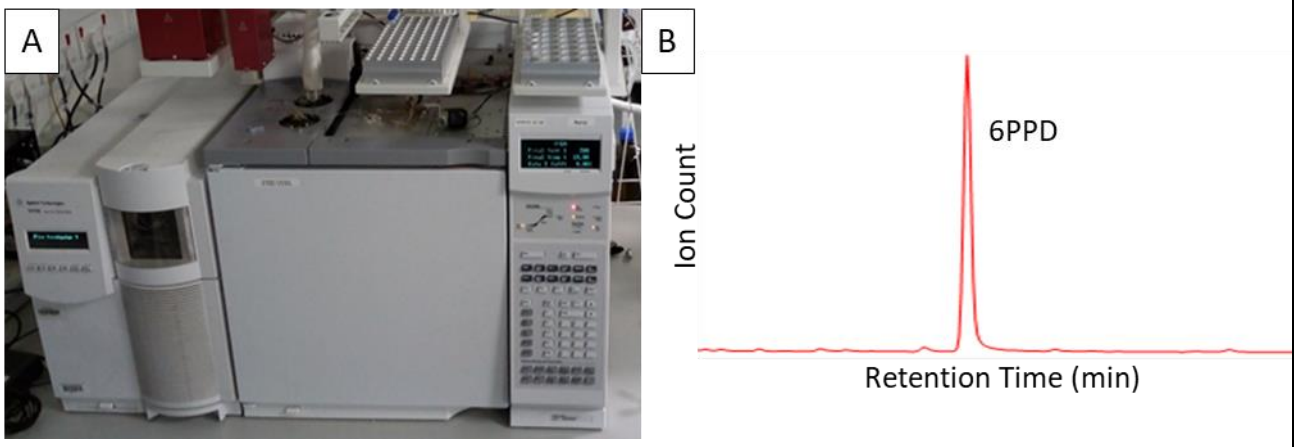


Figure 12: A) GC-MS instrument: 6890N Gas Chromatograph (Agilent, Santa Clara, CA, USA) interfaced with the 5975B Mass Spectrometer (Agilent, Santa Clara, CA, USA) and B) an example chromatogram of the 6PPD signal in the sediment sample.

A known standard of the target compound (6PPD, Sigma Aldrich) was analysed on the GC-MS. The samples were injected into the GC inlet set at 250°C in splitless mode and the column used was a HP-5MS (30 m, 0.25 mm, 0.25 µm; Agilent J&W GC Columns). On each run, the GC oven was programmed from an initial temperature of 50 °C (1.5 min) followed by a 30 °C min^{-1} increase to a final temperature of 260 °C, which was held for 10 minutes to allow all compounds to pass through the column, avoiding compound retention.

Optimising 6PPD extraction from sediments

To establish an effective and quantitative method for measurement of 6PPD in sediments, systematic evaluation of different combinations and ratios of solvent mixtures (DCM and acetone), temperatures (50°C – 90°C) was performed to identify the most efficient at recovering the 6PPD standard.

A variety of extraction tests with different solvent mixtures, DCM, Acetone and a variety of mixtures of those at different ratios, and various temperatures (50°C – 90°C) using a microwave extractor (Ethos X, Milestone). WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories) proficiency testing sediments were used to test the method, as a surrogate matrix for the real samples. Four grams dry weight of the sediment was spiked with 835 ng of the 6PPD (equivalent to 208.75 µg kg^{-1}) before extraction and then analysed on the GC-MS to assess the method recoveries. The tests found that a 1:1 DCM:Acetone mixture gave the best recoveries when at an extraction temperature of 80°C, with mean recoveries of 80% (s.d = 17.3, CoV = 21.7%) and 86% (s.d = 15.5, CoV = 18%) over two different extraction runs each containing eight replicates.

Sample clean-up and spiking

Once the extraction method was established, it was important to look at clean-up techniques that remove interferences that can affect instrument analyses and bias the detection and/or quantification of 6PDD once extracted. We used automated Size Exclusion Chromatography (SEC), in 100% DCM solvent at 5 mL min^{-1} with a full run lasting 23 minutes, a commonly used technique, which gave a mean recovery of non-extracted standard of 105% (s.d. = 10.5, CoV = 10%) in eight replicates.

The final development step to ensure a robust method was to find an analogous spike which we could use to assess the efficiency of the extraction and extract clean-up processes. Given that we could not obtain a labelled compound of 6PPD or another tyre marker, we used a D10 labelled fluoranthene (polyaromatic hydrocarbon) reference standard due to having similar properties to the 6PPD and its wide commercial availability (so it is a pragmatic recommendation for an analogue to use routinely), to avoid overestimations from its presence in the sediment samples. This standard was used only for recovery correction of the results.

For the development of the method and to understand method performance for the analysis of 6PPD as a tyre wear marker, over 300 samples a mixture of real samples and spiked samples, standards and blanks were analysed in total.

Standard Operating Procedure (SOP) for the extraction and GC-MS analysis of the vehicle tyre wear chemical 6PPD in riverine sediments

The following SOP is also provided as an Appendix to this report for reference (Appendix 4).

Sample Preparation

Field samples were stored frozen at -20 °C in cleaned glass jars with foil lining the lids (following the same washing and contamination control procedures for equipment as for the microplastic µ-FTIR analysis protocol) to protect the sample from contamination and defrosted only as required for the analysis. Defrosted samples were mixed thoroughly using a metal spatula, visually ensuring agitation of sediment in contact with the vessel and down from the top surface, to homogenise. Approximately 4 g of sediment was decanted into a clean beaker and the weight recorded accurately. The samples were then dried with sodium sulphate before being transferred to the microwave vessels. Each sample was spiked with labelled recovery standard (D10-Fluoranthene; Spex) and microwave extracted in dichloromethane:acetone (DCM:Acetone) for 0.5 h at 80°C. The extract was the concentrated in DCM only to 1.5 mL and cleaned using automated size exclusion chromatography (Agilent HPLC 1200). Post clean-up extracts in DCM were concentrated to 1 mL and transferred to GC vials for analysis.

GC-MS Analysis

1.7 µL of sample extract was injected into a GC-MS (Agilent, 6890N, 5975B) with splitless injection at 250 °C. The GC-MS had a 30 m HP5-MS column (0.25 mm diameter, 0.25 µm internal diameter, Agilent, Santa Clara, CA) and the carrier gas was helium (1.5 mL min^{-1}). On each run, the GC oven was programmed from an initial temperature of 50 °C (1.5 min) followed by a 30 °C min^{-1} increase to a final temperature of 260 °C, which was held for 10 minutes. 6PPD was quantified using a calibration curve of the 6PPD (Sigma Aldrich) and the samples were recovery corrected using D10-Fluoranthene. Mean recovery of the D10 was 95.8% (78% to 119% (n=24, s.d = 10.73, CoV = 11.2%) and the limit of detection (LOD) ranged from 0.26 – 0.4 µg kg^{-1} d.w.

Quality Assurance

Blank assessment and limits of detection

As with the microplastic extraction and analysis and for quality control and assurance purposes, a solvent blank sample was included in each batch which followed the entire extraction protocol. The performance of the method was assessed in terms of the limit of detection (LOD), which was calculated based on the lowest standard we were able to quantify above the noise. This resulted in excellent and consistent mean instrument method limits of detection equivalent to 0.34 µg kg^{-1} (CoV 15.7%) dry weight of sediment.

Recovery correction through spiked analogous chemical tracer

For identification and quantification purposes, each batch had its own set of calibration standards. Every sample, including blanks were spiked with an analogous chemical recovery standard (D10-Fluoranthene) which was used to assess and adjust for the recovery efficiency of the process. Recovery was excellent, with an overall mean recovery across samples of 95.8% (SD 10.73%, CoV 11.2%). All 6PPD concentration values were corrected for any loss in recovery from the D10 reference standard to give a 100% result in the sediment. Thus, all variability between replicates and sites is assumed to arise from heterogeneity in the samples, as any analytical or instrument variability is controlled for through this recovery correction procedure. In this way, the sampling and homogenisation method in the lab is evaluated through assessment of the variability of 6PPD to see whether further improvements through drying and sieving for example to homogenise before extraction could further improve repeatability of the method.

Statistical analysis

Analysis of variance was performed on each data set from each of the two river catchments to explore the within- and between-group variances. For the Wallingford river-bank profile, the within-group variance (Equation 3) represented the variation arising from triplicate independent samples taken from the same bank location along the profile. The between-group variance was from differences between each location along the profile. At the Irk site IR4, the within-group variance represents the variance between technical replicates from subsamples of the same independent grab sample from the field, and so is an indication of the success of homogenising these independent sediment samples. The between group variance is then the difference between independent field samples from the same location.

Results

Inter-site differences in 6PPD as an indicator of TWP in sediments

6PPD was consistently detected at the Irk site IR4 in the Greater Manchester catchment, with recovery adjusted concentrations averaging 12 ± 5.98 µg kg^{-1} dry weight (d.w.), n = 9. This site was considered an intermediate site, with a combination of residential and industrial land use in proximity of the sampling location. Concentrations of 6PPD in IR4 sediments were ten-fold higher than the mean concentration of 1.02 ± 0.37 µg kg^{-1} (n = 15) at the more rural location on the River Thames at Wallingford (Figure 13). These concentrations are within the reported range of a recent study looking at a number of PPD chemicals in sediments from the Pearl River Delta, China, using a different technique of liquid chromatography-MS, which reported a median concentration of 6PPD of 14.4 µg kg^{-1}, with a range between 0.585 and 468 µg kg^{-1} (Zeng et al., 2023). Taking a worst-case assumption that 6PPD does not degrade within sediments and that the concentration of 6PPD in tyres is a uniform 2%, these concentrations would suggest 599 and 51 µg kg^{-1} of TWP in the IR4 and Wallingford sediments respectively. These values should only be considered indicative until the rates of degradation, leaching and mobility of 6PPD are better understood.

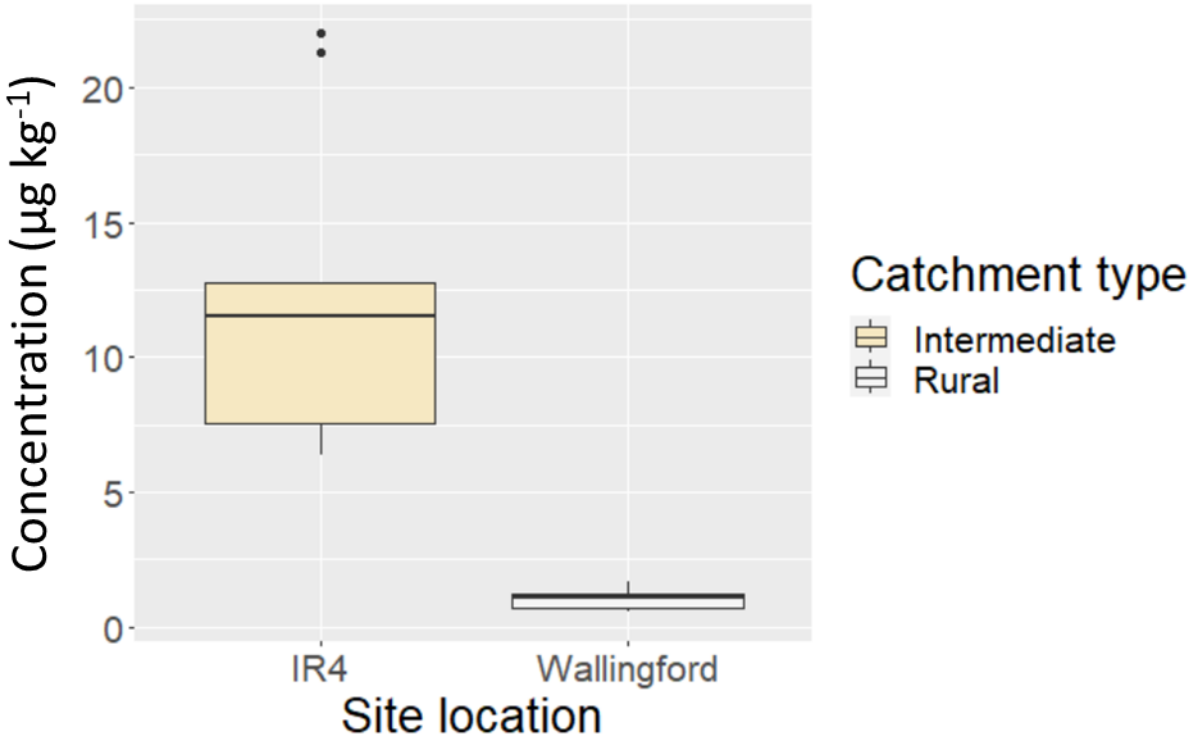


Figure 13: Summary of 6PPD concentrations (µg kg^{-1} d.w.) detected at a location on the River Irk, Manchester IR4 (n=9, technical triplicates from 3 independent samples) and in the River Thames at Wallingford, Oxfordshire (n=15, technical triplicates from 5 independent samples along a profile).

6PPD appeared quite patchy in distribution in sediments taken as part of a profile of the riverbank upstream and downstream of the main road bridge over the Thames in the centre of Wallingford (Figure 14). It is hypothesised that TWP may enter the river at this location through surface guttering that drains the bridge towards the west bank or through airborne abraded material. 6PPD was detected both upstream and downstream of the bridge, and when detected, was at relatively consistent concentrations averaging at 1.02 ± 0.37 µg kg^{-1} (d.w). However, the two sampling locations immediately downstream of the bridge found no presence at all of 6PPD. Each sampling location represented three independent grab samples from that point, and so complete absence of detection in these samples indicates that sediments at the bank immediately downstream of the bridge, were indeed free from 6PPD. Sediments were all visually categorised as similar, consisting of coarse sand with shell, some fine sands and clay. It is as yet unexplained what dynamics in the river result in this absence of detection closest downstream of the bridge, whilst 10 m upstream and 10 m downstream of the bridge, relatively consistent concentrations of 6PPD were found. The peak of the bridge's longitudinal section is inland on the east bank (Crowmarsh side) and so the bridge itself mostly drains towards the west bank and Wallingford. Two outlets are visible draining from the bridge gutters, one north of the bridge

and one south, both on the west bank (marked approximately as red triangles in Figure 14). This west bank is private land upstream and downstream of the bridge and so could not be sampled, whilst the bank is also modified as a jetty north of the bridge. Whilst the source of the 6PPD in sediments upstream and downstream of the bridge on the east bank could not be ascertained from this small pilot sampling campaign, it is an interesting finding that those sites closest downstream of the bridge consistently found no detection (not even a low signal but below the LOD) raises the question that local dynamics (e.g. the faster flow under the bridge where the channel width is restricted) may be very important in determining hotspots of TWP and associated additives in sediment. This could be the focus of further improvement to sampling design specifically for 6PPD as a marker for TWP.

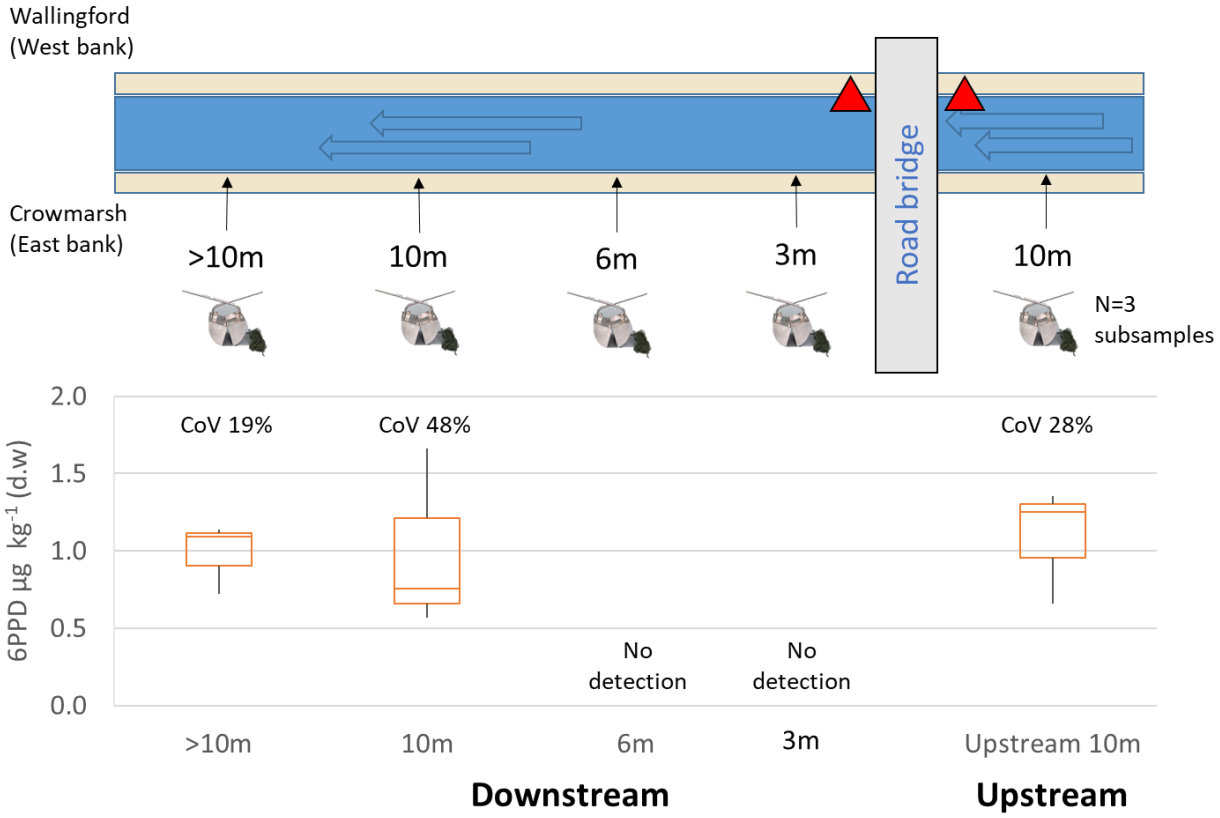


Figure 14: Schematic demonstrating the change in 6PPD concentration detected along a profile of the bank of the Thames close to a suspected point source of TWP, the road bridge at Wallingford, Oxfordshire. The schematic is not to scale but provides an indication of the site, with sampling taking place on the east bank (Crowmarsh side). The red triangles represent approximate locations of the discharge points of two gutters that drain from the bridge into the Thames. Box plots for each grab sample represent the median and interquartile ranges for technical triplicates homogenised, processed and analysed for each independent field sample taken along the profile.

Outlook for using 6PPD as a marker for TWP

This pilot study demonstrates the development of a reproducible method for the extraction and quantification of 6PPD from sediments using GC-MS as an indicator of the presence of TWP in selected English rivers. Other studies have developed liquid chromatography methods for detection of 6PPD in environmental samples, however these have either not been developed or demonstrated for detection in sediments (Chen et al., 2023) or were restricted in sample mass that could be extracted for example to 200 mg sediment (Zeng et al., 2023) as compared to several grams analysed following this provisional SOP.

The LOD for 6PPD was 0.26 – 0.4 µg kg^{-1} (dry weight) sediment, adequate for the detection of 6PPD in environmentally relevant concentrations in both rural and intermediate urban sites. The two demonstration sites, on the Rivers Irk and Thames could be statistically distinguished, with ten-fold greater concentrations of 6PPD found at the Irk site, representing a more urbanised location, compared with the Thames, Wallingford, representing a more rural reach of river. The concentration of 6PPD in contaminated sediments will be a function of the mass of TWP input into in the sediment, kinetics of migration of the 6PPD diffusing out from the tyre rubber and degradation rates of the chemical through oxidation in the sediment environment. Each of these rates must be established and considered in the future, to extrapolate from concentrations of the 6PPD chemical marker, to estimates of total TWP mass in sediments. It is currently unknown how much impact these processes will have on the stability of 6PPD concentrations over time in sediments and how the

dynamics of TWP accumulation in sediment can be understood from this chemical marker. This should be the focus for future research for this promising marker of TWP in the environment, particularly in relation to monitoring concentrations in the same locations over time.

Conclusions and recommendations

This section summarises the conclusions from our assessment of the repeatability of measurements of microplastics in river waters and sediments following our proposed SOP. These recommendations are focused on implementation of the draft SOP into future monitoring programmes for microplastics in river waters and sediments.

Pumped filter sampling can generate representative water samples

Pumping and filtering water on-site was demonstrated to generate highly repeatable results, suitable for quantification of microplastic particle number concentrations in river waters. In our approach we provide guidance on how to estimate representative sample volumes based on the minimum sample volume required to detect microplastics (size specific) and the desired resolution in the data. This approach is likely suitable for water sampling where high sample volumes (many litres) are required to generate a representative sample, irrespective of the source, provided safe and practical sampling of the water body can be carried out. There is no reason this method could not be applied beyond simply freshwater surface waters as demonstrated in this report, for example, in marine environments or groundwaters. Practically, we can recommend additional back-up filter cartridges be taken in any field sampling campaign in case of blockage by particulate plumes before the minimum sample volume is reached.

Requirements of a representative sample include:

- Sufficient volume to detect microplastics in the sample
- Sufficient volume to detect microplastics at concentrations that deliver the required resolution in the data
- Sufficient volume that concentration per L is not sensitive to the sampled volume
- Sufficient volume that less common polymers are also detected consistently

The SOP developed as part of this project specifically targets the size region of microplastics quantifiable using µ-FTIR. To extend the analysis to larger or smaller particle sizes, we would recommend in-situ filtering of different size fractions e.g. cascade filtering. It should be noted that the volume required to capture larger sized plastics (e.g. >1 mm) versus smaller plastics (e.g. <1 µm) will be specific to the size region of microplastics targeted and needs to be calculated following the same rationale for representative samples we detail above. The selection of analytical technique will determine the size region of the microplastic continuum described in any given study and should be hypothesis driven. Larger particles may contribute significantly to the overall load of microplastics by mass to rivers and so is also of interest for future assessment for example for material flow analysis and mass transport studies. Meanwhile, analysis of smaller microplastics such as those targeted in this study have relevance for the hazard component to any risk assessment as these may be more bio-accessible for internalisation by aquatic life and so a more relevant region of the microplastics continuum to quantify for risk assessment of these particles.

Concurrent replication is uncommon in the literature but provides important information on the underlying variation in these systems

We demonstrate high variability in some freshwater and sediment samples, particularly in more contaminated locations. Concurrent water sampling using the pumped filtration system is recommended where the underlying variation in the sample locations is important to capture. For example, to statistically compare overall microplastic concentrations between different locations in the catchment, this replication was necessary. Not all monitoring programmes may require replication at each site, depending on the purpose of the study. However, replication during a pilot phase before any monitoring campaign begins would provide useful insights into the variability across the catchment/ river system that will be invaluable when interpreting the findings of any monitoring programme. This would support approaches such as that proposed by Tanaka *et al.* 2023 where sampling error can be estimated for sites where no replication is performed based on statistical assessment when data meets specific criteria relating to representative sample volumes.

Considering where, when and why we should sample sediments?

Microplastic contamination was generally more consistent in sediments than in overlying waters, making sediments a promising candidate for monitoring longer-term trends in microplastic concentrations in rivers. However, careful consideration should be taken as to where and when to take sediment samples, driven by an understanding of what environmental fate processes are integrated in a sediment sample – they “why” in your problem formulation for any exposure assessment of microplastics.

In urban catchments it was difficult to find locations suitable for concurrent sediment and surface water sampling. Sediments were more transient than expected, with entire sediment substrates lost after rainfall in

the case of the intermediate site RR3 on the Roch. The selection of sites should be hypothesis-driven to allow for a more responsive approach to monitoring sediments for microplastic contamination. In this project, the desire was to identify sites where sediments could be sampled alongside surface waters. This was to allow the reproducibility to be explored for these two river compartments and to infer through polymer identification and quantification possible dynamics between these compartments. However, in our experience, the availability of sites where this combined sampling is possible was limited in urban catchments, with only 4 sites of 26 candidate locations being identified as suitable during initial site visits and one of these sites then being unsuitable on the day due to high rainfall and flows scouring of the riverbed of fine sediment prior to sampling. Sites in future could be specifically sought where there is known high deposition of sediments and quiescent river flows, identified by hydrodynamic modelling. However, these low-flow, lower energy locations may not be optimal for sampling surface waters as they may not represent the most important flows needed to model catchment-wide transport of plastics. For example, more turbulent streams may be more important to monitor for microplastic concentrations in the water column, as microplastics will most likely be suspended within the current in these locations. Hence, such locations may have higher loads, but also are most representative of the flows which lead to downstream transport of microplastics along the river course. Only sampling in sites of low flow and high deposition could in this instance underestimate material flows in the catchment whilst overestimating the loss of microplastics from the water phase into the sediment phase. Therefore, particular care should be taken when considering sampling locations to monitor microplastics along river courses and the selection of sites must be hypothesis-driven in this context. Indeed, it may be the case that uncoupled water and sediment sampling may be needed depending on the aims and intentions of any study. Some specific hypotheses that sediment monitoring allow us to evaluate, separately from the question of microplastics transported in the water column could be:

- long-term monitoring where sedimentation integrates inputs of microplastics over longer timescales,
- source apportionment for microplastics which may have lower transport within the water column,
- identification of hotspots of exposure to benthic dwelling/feeding species

Further optimisation of methods and responsiveness in extraction procedure should be encouraged, particularly for water or sediment samples with a high content of fine solids

Any SOP should have some flexibility to account for variation in the sample condition, particularly for waters where pump filtering the same volume of water may result in very different loading of interfering material that is location and time specific. No single extraction can be universal to all sample types, and indeed the extraction process followed may depend on the analytical technique used. Waters and sediments with higher content of fine solids were more challenging to analyse. We observed how introducing an optional additional density separation for higher suspended solids waters could allow quantification of microplastics in these samples. To avoid overloading of samples, depositions across multiple filters is also recommended. Other adaptations could be refined in future. For example, we performed a conservative density separation at 1.7 g cm^{-3} in order to extract all polymers we searched for in our spectral library. However, the density separation conditions could be tailored for specific hypotheses. For example, if packaging material was the target of monitoring, selecting a lower density separation say of 1.2 gcm^{-3} would still extract common packaging materials such as PET, PE, PS and PP. Larger volumes of sediment would also be possible to process as less interfering material would also be separated along with these plastics. However, you would not capture another common plastic, PVC, with a density of 1.38 g cm^{-3}. Therefore, a balance is always needed between increasing the sample volume you can analyse, whilst understanding and communicating how this might restrict your quantification of microplastics e.g. by reducing the number of polymers targeted in the analysis. We recommend that flexibility should be possible in the extraction process to allow tailoring and optimisation for different sample types and dependent on the hypothesis, but clear guidance on quality control and recoveries must be agreed that will allow for data from different workflows to be compared directly (see the following detailed recommendation below).

Good quality control guidance is essential for all future studies into microplastic detection and quantification

To allow data to be comparable across different sample types (water versus solid matrices, temporal and spatial) and extraction procedures, good guidance on quality controls and study validation criteria will be essential. The objective of quality controls and assurance procedures would be to allow the user to demonstrate that the analysed sample has fidelity to the real sample in the field and thus that any method that can demonstrate such fidelity can then be compared and the results interpreted with confidence. Here we demonstrate two elements of QA/QC which are essential for all microplastic studies, blank correction and assessment of recovery. These we would consider minimum requirements for any study to be interpreted.

Blank correction is necessary to avoid overestimation of particle number concentrations. Running full procedural blanks and correcting microplastic numbers on a polymer-by-polymer basis is essential to demonstrate that only those particles arising from the field sample are quantified, not any which arise inevitably from the handling and processing of the sample itself. We provide guidance on how to conduct an effective negative control assessment during the processing and analysis of microplastics in environmental samples.

We also provide step-by-step guidance on the data treatment that is required to correct for blank contamination on a polymer-by-polymer basis, allowing interpretation of the data in terms of LODs and LOQs.

Spike recoveries cannot yet be quantitative, due to lack of standards, incomplete understanding of the parameters which are most relevant in driving losses or variation in particular analytical workflows and the inherent complexity in quantitatively adjusting for recovery that may be based on multiple factors. In this way, to date, we can only be qualitative when assessing recovery. We have demonstrated an approach that allows for qualitative assessment of recovery using PS spheres as representative microplastics. Recovery must be performed in the relevant matrix (water or sediment) and must follow the entire extraction and analytical process in the same manner as the samples to be representative. It is unknown whether polymer type, size, shape, colour or aging could impair recovery relative to the successful recovery we demonstrate for a known uniform virgin polymer in this study. However, in lieu of this knowledge, demonstrating complete recovery using a known representative test material as we do here, still provides a robust demonstration that there are minimal physical losses of microplastics through the extraction process. It may be that the analytical detection of weathered, rough, smaller particles may be poorer than the recoveries we demonstrate here (Song et al., 2017; ter Halle et al., 2017) and this should be the focus of future investigations if a universal approach to recovery assessment is to be possible for microplastics using spectroscopic techniques. In this study we report on separate spike recovery samples run alongside batches of samples as they are processed. As standards or representative test materials become available for use as internal reference controls (such as those prepared for various international interlaboratory studies e.g. (Belz et al., 2021; van Mourik et al., 2021), we recommend that using these materials as internal tracers spiked to each sample may allow for recovery correction of data in the future, as was possible when quantifying 6PPD through use of the analogous chemical recovery standard D10-Fluoranthene.

μ-FTIR is not the only method to detect and quantify microplastics but benefits from the possibility to automate, relatively fast sample analysis and being a chemically specific analysis

Whilst μ-FTIR is not the only analytical technique which can quantify microplastic particle numbers, it benefits over optical methods due to the possibility of automation of the analysis, and in being chemically specific, able to determine polymer identity which will be essential in source appropriation and monitoring the success of policy or legislation at reducing contamination of the environment with microplastic litter. We demonstrate analysis at 25 μm resolution. Finer resolution in particle size can be achieved with FTIR, down to ~6.25 μm, and even further down to ~1 μm for Raman analysis, however, the increased amount of time and the data generated when pushing the analysis down to this scale is exponential. Analysis at 25 μm resolution offers a compromise between increasing the operational definition of a microplastics in the analysis (through lowering the minimum particle size that can be resolved) and the throughput of samples, with high throughput being a necessity of large or wide-reaching monitoring networks.

Validation of mass-based estimates of microplastic concentrations from spectroscopic techniques is needed

A key recommendation for future work is to validate and refine approaches to estimate mass-based concentrations from spectroscopic techniques, using for example pyrolysis gas chromatograph mass spectrometry (Pyr-GCMS) techniques. Spectroscopic techniques such as μ-FTIR benefit over the mass-based approaches as they can enumerate particle number concentrations and aspects of particle size and morphology. Particle size and morphology are important considerations, as well as the polymer chemistry, when considering the risk microplastics may pose to the environment, particularly when considering physical effects on organisms. Without more time-consuming sampling methods such as cascade filtering, mass-based methods cannot resolve particle sizes. However, the estimation of microplastic mass based on the two-dimensional data obtained from spectroscopic techniques (such as micro-FTIR or Raman) still needs validation against mass-based methodologies (such as Pyr-GCMS). A short-term priority would be to establish a workflow that can generate a filter sample that could be analysed by the μ-FTIR and the Pyr-GC-MS sequentially and would be above the LOD for both techniques and representative according to the principles we outline in this report for generating representative samples.

6PPD is a promising marker for TWP, additional information on degradation and leaching rates are needed to refine extrapolation from 6PPD concentrations to TWP concentrations

We have demonstrated an effective and repeatable method for the extraction and quantification of 6PPD an anti-degradant used universally in road vehicle tyres. Use of this marker is two-fold as it is both a representative marker for the presence of TWP but also a chemical that is gaining attention as an environmental contaminant in its own right. 6PPD has been identified as priority 2 (high risk, low certainty) for risks to both surface waters and groundwater and flagged for further consideration in both soil and sediment. The developed SOP for extraction and quantification of 6PPD fills an important need, allowing monitoring of this chemical in sediments which will enable us to reduce uncertainty about the environmental exposure to this contaminant. To refine estimates of TWP in sediments, it is necessary to understand the rates of release of 6PPD from the trye matrix and the rate at which 6PPD is degraded in sediment environments.

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