D1.3-RE:
Report on an optimised or new procedure for purification and enzymatic digestion for environmental samples

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Objectives of WP1 - Marine Microplastics sampling & processing

Our hypotheses are that highly-populated areas (via estuaries etc.) and Baltic Sea lanes are the most relevant sources for microplastics (MP), and that beaches and coastal sediments are the major sinks of MP. On the other hand, besides beaches, vertical flux of MP towards the sediments is supposed to be one of the essential sinks in the Baltic Sea. To test this, the aim of this WP is to identify MP distribution:
- vertically, in the water column and associated sedimentation rates.
- spatially, in water, biota, sediment and beaches of relevant estuaries, shorelines and the open seas in the Baltic Sea.

This deliverable is a contribution to Task 1.2 Extraction, identification and quantification of MP from field samples, (A) Extraction.

The focus of this task lies on a quantitative extraction of MP from the environment. This requires adaptive purification measures which (1) efficiently remove non-targeted substances, both being of environmental and anthropogenic nature and (2) keep the synthetic polymer intact. Methods and efforts applied to yield sufficient sample quality for subsequent spectroscopic analysis are tailored to the specific sample matrices. Current best practises utilise the differences of specific gravity (density) and chemical resistance among the targeted vs. non-targeted particle fractions. However, previously applied methods were often based on either small sample amounts causing low statistical robustness, did not quantitatively account for MP down to 10 µm, did not extract all major synthetic polymers or were enormously time consuming. Polymer resistance towards the chemicals applied during purification has not yet been quantitatively tested for the smallest MP and paints.

Within the work in this task for WP1 we compiled the experiences with the previously used protocols of density separation and enzymatic organic matter digestion and describe our new efforts to increase sample quality and throughput.

Results

Digestion and chemical resistance testing

Protocols for digestion by enzymatic treatments have proven weak (low purification rates/high time consumption) to remove the organic matrix in some samples rendering spectroscopic analysis difficult. In an effort to confirm other potent and already published digestion methods for MP sample purification, test series were conducted on a large range of polymers in the smallest MP size range. The methods considered as potential promising candidates Fenton’s 1, and KOH 2 have each been tested by the original studies for their effectiveness and partially also possible destructive effects on polymers. However, to confirm their applicability for the targeted large range of polymers (including so far untested
paint resin polymers) and the small particle sizes a coherent test series was deemed necessary. In addition to the applied protocol for Fenton’s digestion a non-catalysed peroxidative treatment was applied for two reasons:

• the catalytic Fe2+ ions partially form Fe3+ precipitates which are of low solubility and coagulate to form new inorganic particles themselves, especially when FeSO4 is added in excess or not corresponding to the individual sample’s COD
• naturally present iron ions in a sample can already induce the catalysed Fenton’s oxidation in a mild manner

For these reasons Fenton’s reaction protocols can – with respect to MP purification – be regarded as an intensified and expedited hydrogen-peroxidation. Also most enzymatic treatments include a step of H2O2 digestion, which often may contribute the largest effect of material reduction in the complete digestion procedure 3 depending on the individual sample composition.

The results of the test series are yet to be published but a general overview is given here:

• The full polymer range has been tested for impairments of Raman and FTIR spectroscopic recognisability under the digestion conditions of the respective recommended protocol from 1 and 2 and pure H2O2 digestion.
• Spectral deviations after the treatment were never completely impairing the polymer identification when both Raman and FTIR were applied.
• Reductions in spectral quality (additional peaks, diminished or removed fingerprint bands, decreased SNR, fluorescence overlay, baseline effects) were mainly affecting Raman spectra, e. g. in KOH digestion of all paint and several ordinary polymers (e. g. ABS, PA 6, PA 12, PVC, TPU).
• FTIR results showed the importance of working with deionised water during filtration and rinsing steps in the purification procedure as inorganic deposition may otherwise contribute to a lower spectral quality and hindered polymer identification.
• Whether and how a possible reaction of the digestant with the polymer or its additives can be attributed to the observed spectral changes has not been included in the scope of the conducted tests.

The qualitative results confirm that the application of H2O2 and KOH / NaClO according to the conditions tested is generally suitable for further sample processing within the project. A quantitative study is being conducted to investigate the effect of the treatments on particle number, size, shape and surface morphology.

For water and sediment samples hydrogen peroxide is used as the method of first choice to eliminate organic materials. The successful and standardised application of hydrogen peroxide to eliminate organic matrix from sediment samples in geological analytics confirms their efficiency and universal applicability on largely variable sample compositions. A critical improvement of our digestion protocols was to initially freeze and lyophilize samples which led to a further reduced organic fraction after the subsequent digestion steps.
Density separation

In the recent years, we have been working on the development of (sampling and) extraction methods of MP from all environmental matrices. Exemplary, sediment samples represent the most complex environmental matrix and will be described to greater detail in the following.

Concerning the high number and variety of particles, sediment samples require enhanced purification measures. In order to reach statistically sound MP numbers large sample volumes are anticipated.

Recently developed instruments such as the Microplastic Sediment Separator (MPSS) 4, come with relatively long processing times, averagely 4 days per sample, which hinders large sample throughput. It is not applicable for other sample matrices and has been suspected for grinding and thus fragmenting MP.

The multitude of sample types to be investigated necessitated the development of procedures of a broader applicability and where possible simpler and faster sample turnover rates. In a clean bench setup of several separation funnels MP samples from marine or freshwater, waste water, sludge extracts, stomach contents, as well as soils and sediments can now be separated. For water samples from very dynamic environments, such as rivers, a density separation step was found necessary as usually fine suspended solids such as silt or clay are present in those samples.

Concerning matter-rich samples, such as sediment samples, a custom-made conical spiral conveyor is applied that ensures high extraction efficiencies and a gentle agitation to not fragment MP. The slow revolving and up-lifting effect continuously exposes buried material to the interface with the liquid phase allowing MP particles to rise into the supernatant.

Homogenised subsamples are used to extract MP down to 10 µm this way, which ensures statistically robust numbers for small MP. The remaining fraction (several kilos) is analysed for large MP (> 500 µm) via electrostatic separation. We could show that electrostatic separation reaches high recovery rates of particles of this size class. This way a high sample throughput and statistically sound numbers are guaranteed for the individual size fractions. Average purification efficiencies of one sample/ day are now feasible.

The separation liquid is a sodium polytungstate solution at 1.8 g/cm³ and pH 3. While being relatively expensive in acquisition costs, the liquid can be recycled to a high percentage and has the ultimate advantage of being non-toxic and reaching high densities, necessary to also extract high density polymers, such as PVCs (~1.4 g/cm³) and paint flakes (~ 1.6 g/cm³).

Internal standard particles of different sizes and polymer types are used to continuously quantify and monitor purification effectiveness during all steps of the treatment procedure. Reports on average recovery rates, which vary by instruments and labs, or the usage of internal standards is not well-established in MP studies yet. This bears the risk of low-quality data acquisition.

Crucial for the improved separation efficiency were several density separation cycles in which the surface tension and the wall-adhering particles are repeatedly disturbed and left to settle again.
References