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LEON-T

Low particle Emissions and lOw Noise Tyres

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Revision History

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Contents

Abbreviations and Units

6-PPD	N-(1,3-dimethylbutyl)-N0-phenyl-p-phenylenediamine
AFM-IR	Atomic Force Microscopy combined with Infrared Spectroscopy
Al	Aluminium
APS	Aerodynamic Particle Sizers
ATD	Automatic Thermal Desorption
BAM	Bundesanstalt für Materialforschung und -prüfung
BSE	Backscattered Electrons
BR	Polybutadiene
CEDR	Conference of European Directors of Roads
CL	Cathodoluminescence
CPC	Condensation Particle Counters
Cu	Copper
DCM	Dichloromethane
DMVCH	2,4-dimethyl-4-vinylcyclohexene
DP	Dipentene
DPG	1,3-diphenylguanidine
d-PB	Deuterated polybutadiene
d-PI	Deuterated polyisoprene
d-PS	Deuterated polystyrene
EC	External calibration
ECD	Equivalent circle diameter
EDX(MA)	Energy-dispersive X-ray spectroscopy (microanalysis)
ETRMA	European Tyre & Rubber Manufacturers Association
Fe	Iron
FFF	Field-flow fractionation
FIB	Focussed Ion Beam
GC	Gas chromatograph
GCMS	Gas Chromatography-Mass Spectrometry
GC-MSMS	Gas Chromatography with tandem mass spectrometry
H_2	Hydrogen
HCl	Hydrochloric acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
ISTD	Internal pyrolysis standard
KOH	Potassium hydroxide
LC-MSMS	Liquid Chromatography with tandem mass spectrometry
LDV	Light duty vehicle
Mg	Magnesium
ML	Machine Learning
MPs	Microplastics
MS	Mass spectrometer
N_2	Nitrogen
NaCl	Sodium chloride
NaClO	Sodium hypochlorite
NaI	Sodium iodide
NaOH	Sodium hydroxide
NR	Natural rubber
OPs	Optical Particle Sizers

PET	Polyethylene terephthalate
ppb	Parts per billion
PS	Polystyrene
PSD	Particle size distribution
Pyr-GCMS	Pyrolysis-Gas Chromatography-Mass Spectrometry
RSD	Relative standard deviations
S	Sulphur
SA	Standard addition
SB	Cyclohexenylbenzene
SBB	Phenyl-[4.4.0]bicyclodecene
SBR	Styrene-butadiene rubber
SE	Secondary electrons
SEM	Scanning electron microscope
SEM-EDX-CL	Scanning electron microscopy with energy-dispersice x-ray spectroscopy and cathodoluminescence
Si	Silicon
SMPS	Scanning Mobility Particle Sizer
SPT	Sodium Polytungstate
TED-GCMS	Thermal Extraction and Desorption combined with Gas Chromatography-Mass Spectrometry
TGA	Thermogravimetric analysis
Ti	Titanium
TOHA	9-tetradecyl-1,2,3,4,5,6,7,8-octahydro anthracene
TSP	Total Suspended Particles
TRWPs	Tyre and road wear particles
TWPs	Tyre wear particles
4VCH	4-vinylcyclohexene
VCI	The Swedish National Road and Transport Research Institute
Zn	Zinc
$ZnCl_2$	Zinc chloride
ZnO	Zinc oxide
µ-FTIR	Micro-Fourier Transform Infrared Spectroscopy

1 Introduction

1.1 Background

In the past decade, microplastics have drawn much attention. They have been found in every environment of the world, including Antarctica, the Alps and all the world's oceans.[1–4] Tyre wear particles (TWPs) are estimated to be the largest source of microplastics (MPs) emission,[5] with some sources estimating that they account for more than 90% of all MP emissions.[5] They are also referred to as tyre and road wear particles (TRWPs) when encountered in the environment due to ubiquitous road wear encrustations they contain. With the increasing use of electric cars, which are heavier and with high instant torque, these emissions are also expected to grow.[6] Although little is reported over the health effects of TRWPs specifically, it has been shown that particulate matter (PM) increases hospital admissions for cardiovascular and respiratory diseases.[7] The contribution of TRWPs to ambient PM10 and PM2.5 is estimated to vary between 0.8-8.5% and 0.25-3.0% by mass, respectively.[8] The European Tyre & Rubber Manufacturers Association (ETRMA) has recently reported contributions at the lower range of the above intervals. For better estimates its essential to distinguish TRWPs from other sources. Extensive research has been carried out on physical characterisation (e.g. shape, size distributions) of emitted TWPs in laboratory settings, however on-road measurements during different driving conditions are limited.[8–10] To fully understand the negative health effects and environmental impact of TRWPs accurate information on tyre wear generation, particle sizes and organic constituents including chemical transformations is essential. Although some studies performed chemical characterisation there is still limited information regarding organic constituents and the chemical composition of airborne TRWPs. The generation of submicron and ultrafine particles, which seem to originate from organic constituent due to heating of the tyres, is also not well understood.[11]

In the LEON-T project, we aim to address the lack of real-world measurements and provide much-needed data on which emission and fate models can be based. This will involve performing measurements of TRWPs in the air, water and soil at varying distances from highways, urban and rural roads. The samples collected in this measurement campaign (T3.1.1) will contain TRWPs at sometimes very low concentrations in complex environmental matrices. This necessitates the development and implementation of improved pre-treatment techniques to remove as much of the matrix as possible while leaving the desired particles unaffected for analysis. To provide information which can be used for emission modelling and fate modelling, as well as provide potential information for future risk assessments, comprehensive physicochemical characterisation will be required which can deliver data on such diverse properties as size and morphology, mass and number concentration, and chemical composition for both the inorganic and organic components. Such comprehensive characterisation requires that a complimentary toolbox of techniques is utilized, including some which have been underutilized due to their time limitations (scanning electron microscopy (SEM)), some which require continuing development due to the constantly changing automotive landscape (pyrolysis gas-chromatography mass spectrometry (Pyr-GCMS)) and some which have never been utilized previously in tyre wear emission research (cathodoluminescence (CL)).

1.2 Environmental Sampling Techniques

In order to build up a comprehensive picture of how TRWPs are transported through the environment and provide robust data for the emission and fate modelling, all environmental compartments need to be sampled, including the air, soil, water, sediment and atmospheric deposition. There are multiple ways to sample each of these compartments, the most relevant techniques are summarised in

Figure 1 and briefly described in this section.

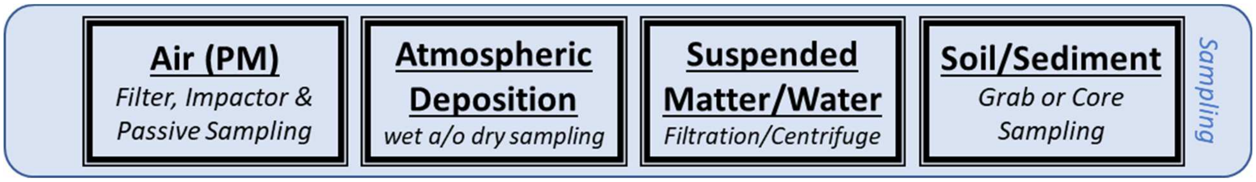

Figure 1: Summary of environmental compartments with appropriate sampling techniques.

Air & atmospheric deposition

For sampling particles in air there are two options: active and passive samplers.[12] Active samplers are so-called because they actively draw air into the sampling head through the use of a pump. The sampled air can then be analysed in multiple ways. In-line particle counting techniques analyse the sampled air on-location and in real-time and include techniques such as Optical and Aerodynamic Particle Sizers (OPS/APS), Condensation Particle Counters (CPC) and Scanning Mobility Particle Sizer (SMPS).[13,14] With these techniques the total particle concentration (number and mass) and particle size distribution in the air is determined for particle from ~3nm to ~30 µm. It's also possible to collect the measured particles for further analysis in the lab. With off-line analysis, sampling is carried out in the desired location for a certain period of time and then analysed afterwards. Typical sampling techniques include Total Suspended Particles (TSP), PM10 and PM2.5 sampling which collect all suspended particles, coarse particulate matter (<10 µm) and fine particulate matter (2.5 µm), respectively, on a filter.[15] Different filters can be used depending on the analysis technique, for example Teflon, Quartz and coated Glass Fibre filters. It is also possible to sample using a multi stage cascade impactor which fractions and collects suspended particles on the basis of their aerodynamic diameter. Passive samplers rely on the natural flow of suspended particles towards a sampler where they then settle or attach on a sampling plate due to electrostatic forces.[16]

It is also possible to sample particles that are deposited out of the air (atmospheric deposition) through deposition sampling. These particles can also be larger than typical particles that are suspended in the air and will include particles that can be transported through resuspension from the road surface, splashing and spray. Deposition samplers are typically simple apparatus consisting of a collection vessel that is open to the air, and optionally a collection funnel. This funnel serves to increase the collection area, protect the collection vessel and can act to keep larger particles out. More advanced deposition samplers work in tandem with a moisture sensor to only collect during episodes of rain or during only dry periods to investigate the effect of precipitation on particle dispersion and deposition.

Water, runoff and suspended matter

In the last decade the reporting of water sampling techniques has drastically increased due to the increasing research into the presence of microplastics in the aquatic environment. An overview of the most reported water sampling techniques is given in Figure 2a.[17] There are two main sampling philosophies for water sampling: single-moment sampling and continuous sampling. In single-moment sampling a volume of water is taken that represents only the short moment in time from which the sample was taken. With continuous sampling, collection occurs over a much longer period of time, therefore giving data over a much wider time period. The main techniques for water sampling are trawls, filtration samplers, centrifugation samplers and grab (bottle/jar) samplers. Trawl sampling is typically performed for microplastics by pulling a net, such as a manta, neuston or plankton net, behind a boat that travels a predetermined course. This has the advantage of being able to sample very large volumes of water from large areas in a relatively short period of time and to concentrate the particles on the net.[18] However, the minimum particle size is determined by the net and small particles, such as TWP, may not be collected. With filtration and centrifugation samplers water is pumped through the sampler (filter or centrifuge) and in such a way suspended matter from a large volume of water is concentrated. For filtration several techniques exist, cascade filtration, ultrafiltration, crossflow filtration (see paragraph 1.3.3 "Size fractionation"). The area from which the water comes is limited, however it has the advantage that different depths of the water column can be sampled and that smaller microplastics can be retained. For long term continuous sampling of surface water, the Umwelt Bundesambt developed a sampling method based on a sedimentation box. With this device the sampling period can be extended to 1 month, in which 200.000 L can be sampled. Finally, grab sampling is carried out manually or automatically by collecting a fixed volume with a bottle, jar or bucket. Automatic sampling is perform with an ISCO sampler, which collects composite or sequential samples based on flow rate, time or storm conditions. Grab sampling is only suitable for samples where the expected concentration of particles is high.

Soil, sediment and road dust

Finally, solid sampling techniques are used to analyse environmental compartments such as road dust, soil and sediment. Road dust is expected to contain a high concentration of TRWPs as this is closest to the source. Road dust can be sampled simply by sweeping and collecting a sample from the surface, however, there are also methods to collect more exposure relevant samples. Road dust PM10 samplers simulate the effects of traffic and gusting wind and collect the resuspended PM10 in a cyclone separator and filter.[19] Wet dust sampling is a technique which allows the collection of total road dust. It works through flushing a defined surface of road with high-pressure water and collecting the resulting dust-water suspension.[20] Soil and sediment both represent important compartments for the fate of TRWPs. It is estimated that 74% TWPs will end up in soils (roadside and agricultural)[21] and since TWPs have a relatively high density, those that make their way to waters will likely end up in the sediment. An overview of sediment sampling techniques is given in Figure 2b,[17] however similar techniques can also be used for soil. Shovels, trowels and spades can be used to collect surface samples, however, for deeper sampling, to investigate environmental transport of particles, it is advantageous to use a grab sampler such as the Van Veen or Petite Ponar grabbers. Sediment corers, such as the Beeker sampler, can also be used for deeper soil and sediment sampling.[17]

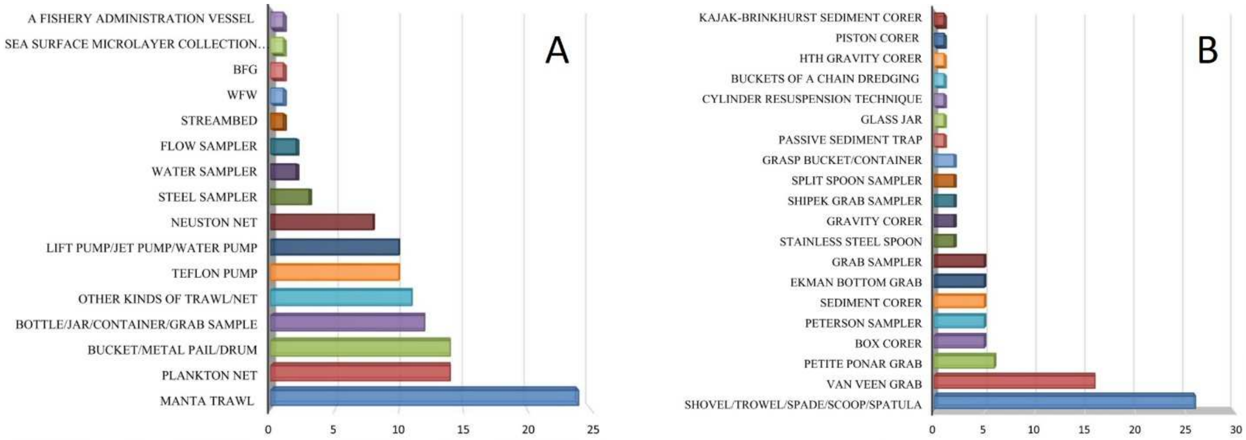

Figure 2: Various sampling techniques for A) Water; and B) Sediment ranked by how many publications they are used in. Figure adapted from literature.[17]

1.3 Sample Pre-treatment techniques

The number of pre-treatment steps that are required depend on the type of sample that needs to be processed and the analysis technique that will be applied. In general, sample pre-treatment can be split into three steps: concentration, digestion and separation. Concentration techniques are required for water-based samples where the aim is to remove liquid and concentrate the particles of interest. Digestion is used to chemically remove interfering matrix material through treatment with reagents such as acids, bases, oxidisers and enzymes, and is mainly used to remove natural organic matter. Separation techniques are then used to remove insoluble and chemically inert inorganic matter such as minerals and clays. With the use of salt solutions, particles with different densities sink or float, enabling the separation of particles of interest with other particles such as clay or mineral salts. Often a combination of density and digestion approaches is required to remove and separate TRWP from mineral and organic matter.[22] The extent to which pre-treatment methods need to be improved and utilised depends on the matrix from which the sample is taken. For example, suspended matter samples are expected to contain a far higher organic matter content, whereas soil and sediment samples will contain a much higher concentration of inorganic matter. An overview of the available pre-treatment methods is presented in Table 1 and described in detail below.

1.3.1 Digestion

As stated above, a pre-treatment step based on digestion can be used for the removal of organic matter present in, for example, water of suspended matter samples. By using a digestion agent organic matter is removed, ideally while leaving the particles of interest untouched. An overview of the most applied digestion reagents used during the pre-treatment of environmental samples is visualised in Table 1. Commonly used reagents are either acidic (hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and nitric acid (HNO_3)), basic (potassium hydroxide (KOH), sodium hydroxide

(NaOH)), enzymatic, or oxidative (hydrogen peroxide (H_2O_2), Fenton's reagent and sodium hypochlorite (NaClO)). Here, Fenton's reagent consists of H_2O_2 with the addition of a ferrous iron that serves as catalyst.

Currently, there is no consensus for the preferred method for environmental samples containing TRWPs, although some are preferred for other sample matrices types. For example, enzymatic digestion has often been recommended for the digestion of tissue, but less for other matrices. The reason for this could be that effective proteases, which cleave proteins, are readily available, but enzymes specialised in the cleavage of other biogenic materials are not.[23] Still, Löder et al. recommended the use of a cascade of enzymes for the digestion of various environmental samples with the use of protease, lipase, cellulase, amylase and chitinase, which act on proteins, fats, cellulose, starch and chitin.[24] Yet, these enzymes are highly specific and will not degrade all biogenic material present in samples such as soil. Furthermore, digestion methods employing enzymes are often associated with high costs as they are time consuming, taking up to 2 weeks per sample.[23] Acidic treatments using hydrochloric acid and nitric acid have been shown to be highly effective in the digestion of organic matter in various matrices, especially in the digestion of biological samples such as fish, however they also impact other particles present (e.g. PS and nylon).[25,26] Thus indicating that acidic methods might be too harsh as sample pre-treatment for TWP.

Table 1: Overview of sample pre-treatment reagents used in microplastics and TWP research.

	Reagent	Advantages	Notes	Ref.
Acidic Digestion	HNO_3	Highly effective in the digestion of organic content in various matrices	Procedures may be too harsh, resulting in deterioration of analytes (*e.g.* polystyrene, nylon)	25,27
	HCl			25,26
	H_2SO_4			28
Oxidative Digestion	H_2O_2	Simple, effective and affordable techniques for removal of organic content in organic rich freshwater, soil and sediment samples	Effectiveness depends on reaction temperature	22,25,27,29,30
	Fenton's Reagent		Lowers energy barrier with ferrous catalyst	22,25,26,30,31
	NaClO		High digestion rates for vegetal organic matter	22,25,30
Basic Digestion	KOH	Effective for protein-rich samples	Ineffective in dissolving humins	22,25
	NaOH			25
Enzymatic Digestion	Proteases	Highly specific for protein-rich samples	Time consuming, will not degrade all biogenic material	23
	Cascade of enzymes	Possible to combine a mixture of enzymes per sample type		23,24
Density Separation	NaCl	Readily available, cheap and easy to use	Separate organics and lighter plastics	22,32
	NaI	separate TRWPs from minerals and clays	Toxic, unstable in solution	22,33
	$ZnCl_2$	Non-toxic, easy to use	Not dense enough for all types of TRWP	22,28, 34,35
	Sodium Polytungstate (SPT)	Non-toxic, separate TRWPs from minerals and clays	Expensive, possible to reuse	27,28,36

Basic approaches using KOH and NaOH have also been shown to be effective for digesting protein-rich samples, much like proteases, but are less effective in the destruction of chitinous and cellulosic material.[25] Additionally, alkaline solutions are unable to dissolve humins (carbon-based macromolecular substances often found in soil samples).[26] With oxidative digestion using hydrogen peroxide, the efficiency of the method is highly dependent on the temperature of the reaction. At higher temperatures, degradation of particles may take place, similar to what has been observed under acidic conditions.[29] By adding Fenton's reagent (a ferrous catalyst), the reaction can be performed at lower temperatures, whereby an effective removal of at least 80% of the organic components is feasible.[26] In the presence of the catalyst, hydroxyl and hydroperoxyl radicals are formed *in-situ*, lowering the energy barrier of the reaction making the reaction exothermic.[26,31]

Fenton's reagent is often mentioned in microplastic research as a simple and affordable technique to remove organic matter that preserves the desired particles while also effectively reducing the organic content in soils and sludges.[26] Yet, research from Pfeiffer *et al.* showed higher digestion rates for vegetal organic matter (92% for wood, 88% for leaves) while using 7% NaClO when compared to peroxide-based methods, including Fenton's reagent.[25] This was later confirmed by Monteiro *et al.*, where treatment with NaClO showed the highest rates of organic matter removal in organic rich freshwater samples compared to Fenton's reagent and 10% KOH.[22] In recent work done by Goßman *et al.* it was also demonstrated that after an oxidative sample clean-up with H_2O_2 and Fenton's reagent the use of certain decomposition products (dimers and oligomers of isoprene and dipentene) as indicators in Pyr-GCMS measurements for natural rubbers was enabled, effectively removing disruptive signals from the matrix.[30] However, it was not confirmed whether the TRWP were impacted by the use of these reagents, thus there was no validation of this approach for TRWP analysis. Furthermore, the results by Pfeiffer *et al.* are in contradiction with Hurley *et al.,* who stated that Fenton's reaction was the only available method capable of digesting a soil matrix while simultaneously leaving particles unscathed.[26]

Clearly, there is an ongoing debate about which technique is most fit for the digestion of organic matter for the analysis of TWP in complex environmental samples. Inconsistencies in current available literature highlight the importance of thorough investigation into the effect of digestion methods on both sample matrix and the particles of interest. For the digestion of soil and sediment samples oxidative treatments utilizing H_2O_2, Fenton and NaClO are most promising, as these offer effective treatment towards removal of interfering matrix while still leaving analytes unharmed. It is emphasized that for finding the right digestion approach specific pre-tests are encouraged in order to optimize and validate digestion methods depending on sample type and analyte.[25]

1.3.2 Density Separation

Density separation is a pre-treatment technique that is used to separate an analyte from other particles present in a matrix. Higher density particles will sink, while particles with a lower density rise, allowing the separation between particles with varying densities. The density of the fluid is key to the success of the separation. When the goal is to collect TRWPs in the buoyant fraction, the density of the fluid should be higher than that of the TRWPs. However, if the density is too high the TRWPs will not be separated from higher density materials such as clay, minerals and metal oxides. This is where the challenge lies; rubber has a density of around 1.2 g/ml, however the density of TRWPs is modified by the presence of fillers, zinc oxide (ZnO) and other encrustations such as road wear minerals. This leads to TRWPs having a broad range of densities with the exact value highly debated between 1.2-2.2 g/ml.[27,28,37]

Solutions with a slightly higher density than the density of that of TRWPs should, in theory, provide the most optimal separation, keeping the highest amount of TRWPs while removing the higher density materials. As reference material for TRWP are not readily available for method optimalisation, sedimentation and flotation rates using a density solution of 1.9 g/ml on various relevant environmental contaminants can be calculated using Stokes' law, as done by Klöckner *et al.* and visualised in Figure 3.[28] It should be noted that these predictions are based on a spherical model, and most TRWP exhibit an elongated shape.

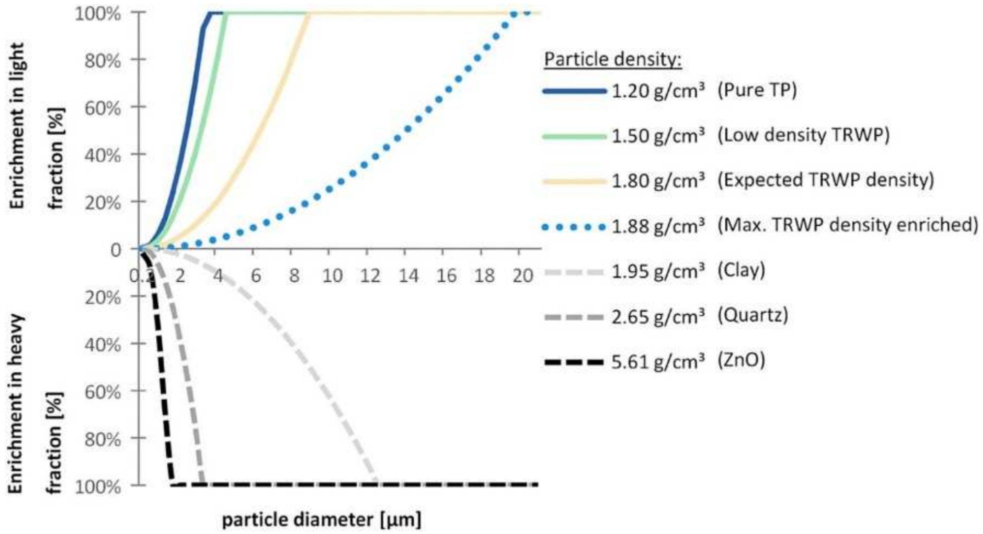

Figure 3: Enrichment of particles after 23 hours density separation in the light and heavy fraction for a density separation with a solution of 1.9 g/ml. Figure taken from reference.[28]

Based on these predictions, it can be concluded that in order to concentrate TRWP in the light fraction, high density solutions are required. Examples of salts that can be used to obtain higher density solutions are sodium iodide (NaI)[33], zinc chloride ($ZnCl_2$) and sodium polytungstate (SPT)[5]. Lange *et al.* for example used a NaI solution with a density of 1.85 g/mL to separate rubber, bitumen and microplastics from the heavier particles to determine the amount of those particles remaining in a stormwater treatment train.[33] Similarly, Klöckner *et al.* used a solution of SPT dissolved in MiliQ water with a density of 1.9 g/mL to separate TRWP from the matrix prior to further analysis.[27,28] The use of $ZnCl_2$ salts for the separation of TRWP is however discouraged, as the hypersaline solution is not dense enough to collect all the TRWP in the buoyant fraction, reaching only maximum density of 1.6 g/mL. SPT, on the other hand, has significant advantages when compared to $ZnCl_2$ and other toxic salts (such as NaI) since its non-toxic, reusable and has a low viscosity.[38,39] Due to its high water solubility, reaching maximum densities of 3.1 g/mL, it can easily be tuned for the desired application.[38] With its high costs as only drawback, the possibility for reuse makes SPT an interesting candidate for use in density separation approaches for TRWP in environmental samples.[40]

Although it is generally accepted that a hypersaline sodium chloride (NaCl, ~1.2 g/mL) solution is not applicable for the separation of TRWP, it might prove to be useful in the separation of light organic contaminants present in the matrix from TRWP. As TRWP have a relatively high density, it is possible to separate both organic and inorganic matter in a two-step density separation starting with a hypersaline NaCl solution (~1.2 g/mL) to remove lighter organic particles (including most microplastics), followed by a second separation using a SPT solution (~2.2 g/mL) to remove heavier inorganic particles, as suggested by Klöckner *et al.*[28] To ensure full recovery of the TRWP present, it is encouraged to use a high density solution in the second step of this process. To the best of our knowledge, this approach has not been tested and validated on real-world samples.

Although much research has already been done regarding the density separation of TRWP, there is still a need to evaluate and further develop current methodologies for their capability of concentrating analyte content, solving matrix interferences and achieving high and reproducible sample recoveries.

1.3.3 Size fractionation

For information on the size of TRWPs, fractionation techniques can be used. For fractionation of coarse TRWPs in solid matrices (soil, road dust), dry sieving techniques can be used. Müller et. al used a dry sieving technique for soil with a shaking plate with 5 stainless steel sieves of 2, 1, 0.5, 0.1 and 0.05 mm.[41] Due to the clay content soil aggregates can become stuck together. To avoid this, samples can be freeze-dried to decompose soil aggregates, but small aggregates will remain. For TRWPs in the smaller size ranges, wet sieving techniques normally perform better as there is less aggregation and clogging. For instance, Klockner et. al used a vibratory sieve shaker with five stainless steel sieves with mesh sizes of 20, 50, 100, 250, 500 and 1000 µm for the fractionation of TRWPs in highway tunnel dust.[27] For microplastics in runoff samples Eisentraut et. al used a fractional wet filtration technique with five stainless steel microsieves of 1000, 500, 100, 50 and 10µm. After sieving MPs were resuspended from the micro-sieves in ultrapure water and filtered through a glass fibre filter for further analysis.[42]

For the size fractionation of TWP below 10 - 20µm conventional sieving techniques can no longer be used. For particles below 20 µm crossflow-ultrafiltration and field-flow fractionation techniques (FFF) can be used.[43] Mintenig et al. used fibrous crossflow ultrafiltration membranes with an inner diameter of approximately 200 µm for the pre-concentration of microplastics < 100µm. Because the recovery of 50nm polystyrene (PS) spheres was not yet at its fully potential it was suggested that membranes could be damaged; crossflow ultrafilters are normally used as dialysis equipment and are made to retain proteins. For pre-concentration the crossflow-ultrafiltration technique has been used successfully, with concentration factors between 200 – 1580.[43] The particle size separation with field-flow fractionation (FFF) is not concentration dependent but is based on *a-priori* determined elution times of the target particles. So, the disadvantage of this technique is that for every type of particles a separate elution pattern has to be determined.

Fractional sieving techniques can be used in the laboratory as part of a sample pre-treatment procedure, but these techniques can also be used on-site as part of the sampling procedure. Fischer & Scholz-Böttcher used an on-board deck-wash system with 125µm and 20µm stainless steel sieves.[44] Bannick et. al developed and tested fractionated filtration for sampling of microplastics in water. The sampling system consists of three barrels with sieves of different mesh sizes. The water is pumped through a cascade of two sieves of 500 and 100 µm and subsequently through a single sieve of 50 µm; in the last stage the water is pumped under low pressure through a sieve of 10 µm.[45] Mintenig et. al developed a cascade sieving procedure with three stacked stainless sieves with mesh sizes of 300, 100 and 20 µm. The sampling was performed in two stages, with a flowrate of 2 m^3/h water was filtered over the 300 and 100 µm sieves after which the flowrate was lowered and the 20 µm sieve was placed underneath to collect the smaller micronsized particles.[8]

1.4 Analytical Techniques

As mentioned previously, TRWPs are heterogeneous samples that consist not only of tyre tread but also encrustations of minerals from the road surface and metals from brake wear. This, in combination with the high concentration of foreign particles in environmental samples and the comprehensive characterisation required, leads to a unique analytical challenge. Multiple techniques will be required in order to quantify the mass of TRWPs present in a sample, give information on the physical and chemical properties of the particles. This is complicated by the fact that not all techniques are able to measure the same size scale. A summary of available analytical techniques typically used in microplastic research, the information they give and the size range they cover is presented in Figure 4. From this it is evident that optical and fluorescence microscopy are unsuitable for TRWP analysis as they don't offer identification possibilities necessary to determine between the TRWPs and environmental matrix particles. Micro-Fourier Transform Infrared Spectroscopy (µ-FTIR) and µ-Raman do offer identification possibilities, however these are complicated by the fact that TRWPs are black (this is further elaborated on in section 1.4.2) and don't allow for the measurement of the smallest particles <1 µm. Therefore, for the environmental measurements carried out in task 3.1.1 we will use a combination of scanning electron microscopy with energy-dispersive x-ray spectroscopy and cathodoluminescence (SEM-EDX-CL) for single particle characterisation and Thermal Extraction and Desorption combined with Gas Chromatography-Mass Spectrometry (TED-GCMS) for bulk quantification, with Liquid Chromatography with tandem Mass-Spectrometry (LC-MSMS), Gas Chromatography with tandem Mass-Spectrometry (GC-MSMS), Inductively Coupled Plasma Mass-Spectrometry (ICP-MS) and carbon analysis providing correlative quantitative information based on other markers. In this chapter we discuss the state-of-the-art for SEM-EDX-CL and TED-GCMS to lay the foundation upon we will build our research. For LC-MSMS, GC-MSMS, ICP-MS and carbon analysis the working principles will be explained as will their use in previous studies as well as their limitations.

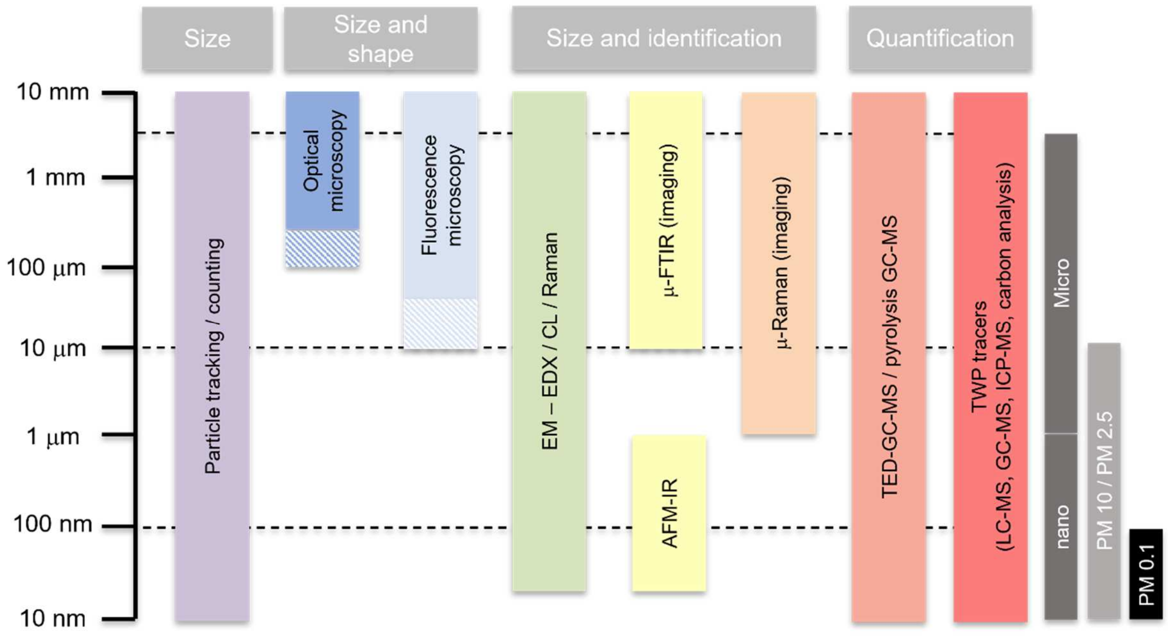

Figure 4: Summary of available analytical techniques, the size ranges they can measure and the information they deliver.

1.4.1 Thermo-analytical techniques (Pyr-GC-MS and TED-GC-MS)

One of the most promising ways to quantify the mass of TWP in a sample is by using pyrolysis-based gas chromatography mass spectrometry techniques. These methods have been widely used to analyse polymers and microplastics and progress has been made to also analyse TWPs.[42] In Pyr-GC-MS the sample is pyrolyzed by (rapid) heating to temperatures above the decomposition temperature in an inert atmosphere. This forms gaseous decomposition products which are then separated using a gas chromatograph (GC) and analysed by mass spectrometer (MS). This is illustrated in Figure 5. Pyrolysis products that are unique to a polymer or compound can be used as a marker. By using a calibration curve from a reference sample, the original concentration of the polymer or compound can be calculated from the signal of the marker.[46] TED-GC-MS is similar to Pyr-GC-MS, but instead of directly leading the gaseous products to the GC, they are concentrated on a sorbent material. The sorbent material is then transferred to a thermal desorption GC-MS system.[42]

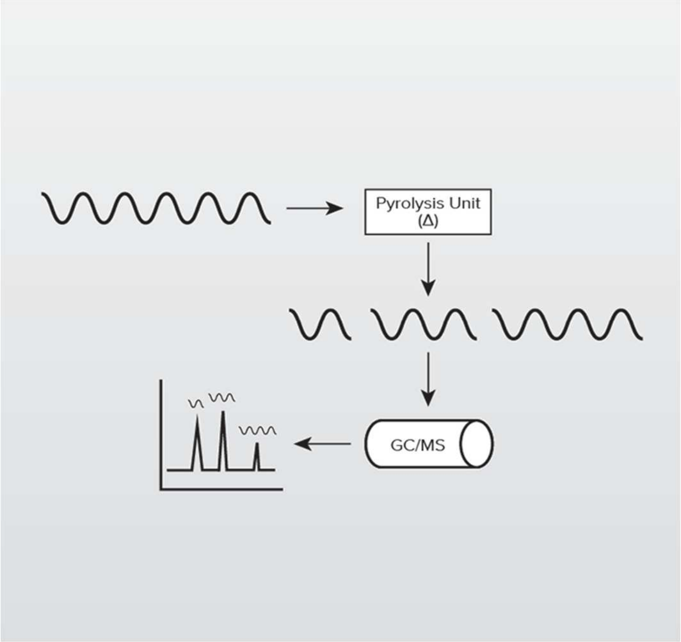

Figure 5: Illustration of the working principle of Pyr-GCMS.

Various markers have been identified for the quantification of TRWPs with Pyr-GC-MS. For instance, 4-vinylcyclohexene (4VCH) was used to compare the amount of TWPs in residential and industrial areas in Seoul.[47] Eisentraut et al. employed Pyr-GC-MS for the simultaneous quantification of TRWPs and other microplastics using cyclohexenylbenzene (SB) as marker for TRWPs and vinyl benzoate as a marker for polyethylene terephthalate (PET).[42] Markers for natural rubber (NR) include dipentene (DP), isoprene trimer and isoprene tetramer. Additionally, Goßmann et al. identified 2,4-dimethyl-4- vinylcyclohexene as a NR marker to distinguish between car and truck tyre wear particles.[48] The International Organization for Standardization (ISO) has published two technical specifications for the determination of mass concentration of and road wear particles in soil or sediments and air, respectively.[49,50] The ISO method monitors 4VCH as a marker for styrene-butadiene rubber and polybutadiene (SBR + BR) and requires the preparation of a calibration curve using SBR 1500 as a surrogate for SBR + BR and using deuterated polybutadiene (d-PB) as an internal standard. A correction of 0.9 is applied to compensate for the difference in styrene contents between SBR 1500 and the SBR applied in the industry which are 23.5% and 15%, respectively.

However, the chemical composition of the rubber used in tyres differs dramatically, not only between brands, but also between different tyres from the same brand. The ISO technical specifications assume that the rubber content of tread is 50%, that the car tyres contain 44% SBR+BR and truck tyres contain 45% NR.[49,50] However, in a study of 39 car and truck tyres from 13 brands (including 8 worn tyres), the SBR+BR content ranged from <0.05% – 28% when calculated from the 4VCH marker using the ISO procedure.[51] It has also been shown that the 4VCH signal is also strongly influenced by the microstructure of the SBR used in the tread, which can vary depending on synthesis procedure and manufacturer.[52] Both these studies demonstrate that the ISO technical specification may lead to over- or under-estimation of the TWP mass in environmental samples, therefore a wider analysis of tyres currently on the market is required to develop suitable calibration factors taking into account the variable nature of tyres expected to be found in environmental sample. Furthermore, as the composition of tyres varies over time, it may be necessary to periodically repeat these investigations to ensure that the calibration factors remain market-representative.

1.4.2 Scanning Electron Microscopy (SEM) Based Techniques

While the previously described techniques allow for the detection and quantification of TRWPs in a sample, they don't provide information on the structural and morphological characteristics of TRWPs. Such information is of great importance for emission and fate modelling and risk assessments as size plays an important role in whether a particle will stay airborne, how far it can be dispersed and whether it can be taken up by cells.

In order to positively identify TRWPs as well as get size and shape information, a micro- spectroscopic method is necessary. For many microplastics µ-FTIR and µ-Raman are used, however, these have a number of disadvantages. Due to the diffraction limit, the lower size limit for these techniques are 10 and 1 µm, respectively, meaning the smallest (and possibly most harmful, furthest spreading) nanoparticles are not analysed.[53] Due to these being light-based techniques, they also struggle with the analysis of dark and black coloured samples such as TRWPs.[54,55] Atomic Force Microscopy combined with Infrared Spectroscopy (AFM-IR) can detect nanoparticles <20 nm, however larger micron-sized particles cannot be analysed with this technique.[56] In addition, it is extremely time intensive. Hence, to advance our understanding of release and fate of tyre particles, robust, reliable and higher-throughput analytical methods need to be developed to detect and characterize TWP in environmental matrices.

By using electrons instead of photons (light) as an illumination source, a much smaller resolution can be achieved. This is because the wavelength of electrons is much smaller than that of light; at typically scanning electron microscopy (SEM) acceleration voltages the wavelength of an electron is 0.007-0.012 nm as opposed to 400-700 nm of visible light. Scanning electron microscopes produce images by scanning an electron beam across the surface to be imaged. When the electrons interact with the surface, numerous processes occur which lead to the release of low-energy secondary electrons (SE), high-energy backscattered electrons (BSE), CL and EDX. These are shown in Figure 6. By measuring the intensity of these signals as the electron beam moves across the surface an image can be built up which gives information on topology (SE), composition based on atomic number (BSE), electronic composition (CL) or elemental composition (EDX). Most frequently, a number of these techniques will be combined to give multiple insights into the same sample region.

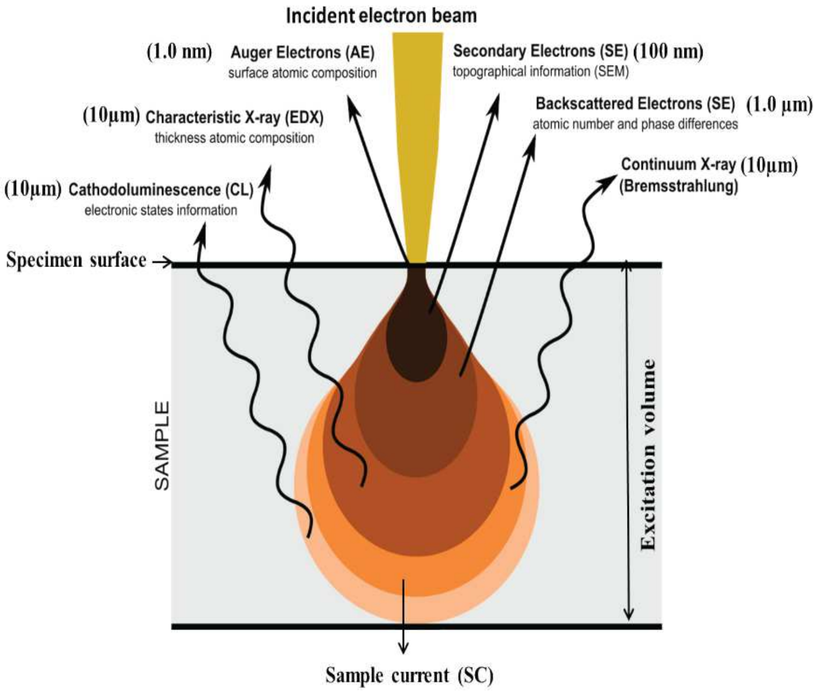

Figure 6: Interaction of an electron beam with the surface of interest and the process that occur.[57]

SEM allows for the analysis of TRWPs on a single particle level. For instance, Klöckner et al. examined the circularity of and road wear particles from different size and density fractions using SEM.[27] Moreover, SEM coupled with energy dispersive X-ray spectroscopy (EDX) can be used to simultaneously characterize the morphology and chemical composition of TRWPs. SEM-EDX has been used by to investigate the size and composition of airborne coarse particles (>10 µm) in Germany as well as to map the distribution of elements in single TRWPs.[58] Furthermore, SEM-EDX has been coupled with Focussed Ion Beam (FIB) milling to show how mineral encrustations from the road surface are not only present on the surface of particles but also inside TRWPs.[59]

While SEM provides a lot of information, the analysis itself can be time consuming, especially if large amounts of particles need to be analysed for statistical relevance. However, developments in data analysis and artificial intelligence are starting to facilitate automated single particle analysis with SEM. Rausch *et al.* used a computer controlled SEM, recognizing particles by simple grey value thresholding to characterise thousands of particles. Particles were collected on Boron substrates allowing quantitative EDX spectroscopy for carbon while also increasing the contrast between particles and the substrate. Additionally, they developed a machine-learning model to then classify the characterized particles as TWP, metals, minerals or biogenic/organic.[60]

1.4.3 Other Analytical Techniques

These techniques are used to determine the concentration of other TRWP components such as additives and vulcanization components. These techniques include GC-MS, LC-MS and ICP-MS and are described in further detail below.

GC-MS and LC-MS are used to measure the organic compounds, often referred to as molecular markers, present in tyre tread. These include additives such as antioxidants and vulcanization accelerators. Molecular markers are extracted from samples, often using a Soxhlet extraction, using organic solvents such as methanol, dichloromethane (DCM), and toluene.[40] The solution is then analysed with chromatography and mass spectrometry techniques such as GC-MS(-MS) or LC-MS(-MS) for separation, identification and quantification of the extracted components.[61,62] One of the most commonly analysed molecular markers are benzothiazoles, a class of organic compounds commonly found in tyres due to their use as vulcanization accelerators.[61,62] Other molecular markers include 1,3-diphenylguanidine (DPG), another vulcanization accelerator, and N-(1,3-dimethylbutyl)-N0-phenyl-p-phenylenediamine (6-PPD), an antioxidant.[63] While they are useful markers for identification due to their specificity towards TRWPs, quantification based on these marker concentrations is now discouraged due to their susceptibility to leaching and degradation in the environment.[40]

As well as organic compounds, TRWPs contain many inorganic components. These are measured through elemental analysis techniques and are referred to as elemental markers. The most commonly used elemental analysis technique for TRWP investigation is ICP-MS. In ICP-MS, elemental markers from a sample are brought into solution through digestion with strong acids and then ionized in an inductively coupled plasma. These ions are then detected using a mass spectrometer. ICP-MS has the advantage of incredibly high sensitivity, being able to detect elements down to 1 parts per billion (ppb) in environmental samples. Zinc (Zn), a vulcanization catalyst, and Sulphur (S), the cross-linking agent used for vulcanization, are the most commonly used elemental markers.[63,64] Elemental markers are more stable than the aforementioned molecular markers, however, they are less specific. Zn, for example, may be present in environmental samples due to galvanized steel and road salt. However, recent work by Klockner *et al.* have demonstrated that by first performing a density separation on the environmental samples, organic Zn (Zn contained in the organic fraction of rubber) could be separated from Zn present in insoluble metal and metal form (which sinks) and soluble Zn, such as the Zn present in salts.[28,65]

Whilst molecular and environmental markers are not as robust markers as the polymeric markers detected using Pyr-GCMS due to their instability (molecular) and lack of specificity (elemental), it is still advantageous to quantify them in environment. Klockner *et al.* have also recently shown that, when using suitable pre-treatment steps, a combination of ICP-MS (elemental), LC-MS (molecular) and TED-GCMS (polymeric) analysis can a correlated quantification of TRWPs in the environment.[28] Such a combinatorial approach is much more robust than only quantification through the use of one marker and gives more information on the transport and transformation of individual components in the environment, possibly giving more information on their environmental risks.

1.5 Aims

The aim of this research is to develop improved sample pre-treatment, thermo-analytical and microscopic methods for determination of TWP the environmental samples gathered during the measurement campaign (T3.1.1). These samples will be include: air, water, suspended matter, deposition, soil and road dust samples. To optimise these methods and investigate the influence of each sample component on the various methods, a series of samples will be used which will allow for a logical build-up in sample complexity. These are: pristine TWPs generated through cryogenically milling tyres, simulated TRWPs prepared on a circular road simulator and finally environmental deposition, soil and sediment samples.

Pre-treatment techniques will focus on digestion and separation techniques with the aim to remove organic and inorganic matter, respectively. We will investigate the extent to which oxidative digestion reagents H_2O_2, Fenton's reagent and sodium hypochlorite remove organic matter from environmental sample without damaging the TRWPs of interest. NaCl and SPT solutions with different densities will be investigated for their potential to separate TRWPs from environmental matter. This is likely to be difficult due to the varying range of densities of both TRWPs and soil components which may also overlap. Therefore, our aim is not the complete removal of environmental matter which risks also losing TRWPs, but the enrichment of all TRWPs in one fraction by removing as much environmental matter as possible whilst reliably maintaining 100% TRWP recovery.

Thermo-analytical techniques will be developed to give an accurate quantification of TWP concentration in a sample based on the measured pyrolysis products. By performing a thorough literature review we will identify the limitations in the state-of-the-art and where progress can be made. Based on this, we will combine literature data with our own measurements to produce the largest tyre pyrolysis dataset to date. This will allow us to determine the most accurate and reliable TWP markers and their conversion factors for TWP quantification. We will then demonstrate the effect by comparing quantification using our method to the same samples quantified using current state-of-the-art methods.

By developing a sequential filtration system to couple with TED-GCMS it will be possible to create a mass-size distribution. We will first demonstrate a proof-of-concept using model PS beads with low polydispersity and highly regular shape before testing more relevant conditions by increasing the sample complexity to contain mineral particles, simulated TRWPs and environmental organic matter. We aim to demonstrate that the method can fractionate a sample to fractions of >190, 25, 10, 5, 2, 0.8 and 0.4 µm without overlap in the size distribution of fractions. This will be confirmed using SEM imaging.

Finally, an automated SEM-EDX procedure will be developed and validated to increase the efficiency of the technique. By automating particle identification, measurement, chemical analysis and subsequent data analysis, particle size distribution information can be acquired for more samples. We will achieve this by comparing automated measurements with manual measurements to validate the accuracy of particle recognition, size measurement and classification based on elemental composition. Another SEM-based technique, cathodoluminescence, which has been recently demonstrated for the identification of other common microplastics, will also be investigated for its applicability for TRWP research.

Through this work we aim to create an optimised pre-treatment and analysis framework that will allow for efficient and accurate quantification and physicochemical characterisation of TWPs in different environmental matrices. Such a workflow will enable us to collect as much information as possible from the measurement campaign providing the most comprehensive data for emission and fate modelling and allowing for most in-depth understanding of TWP in the environment to date.

2 Results and Discussion

2.1 Samples Used

In this work, a number of samples were used to allow research to be initiated from model TWPs, through simulated TRWPs and finally to real-world environmental samples. A number of suitable control materials were also used. These are summarised in Table 2. The methods used to acquire these samples are described in more detail below.

Table 2: Overview of samples used in this research.

Sample Name	Description	Country of Origin	Notes
Pristine TWPs (Budget)	Linglong	NL	Tyre purchased from retailer. Cryomilled to produce particles.
Pristine TWPs (Executive)	Continental	NL	Tyre purchased from retailer. Cryomilled to produce particles.
Simulated TRWPs	Pirelli	Sweden	Sample of simulated TRWPs from road testing facility at project partner VTI. Contains also contains road wear
Deposition	Mix	NL	Deposition sample collected next to A27 Utrecht during winter 2021/22.
Run-off Sediment	Mix	Sweden	Sediment sample taken from storm drain next to the E18 Sweden. Previously used as part of the Microproof project.[1]
Run-off water	Mix	Germany	Run-off water taken from A61 Germany. Previously used as part of the Microproof[1] project.
Soil (10 m)	Mix	Germany	Soil sample taken from A61 Germany. Distance to road is 10 m. Previously used as part of the Microproof[1] project.
Soil (1 m)	Mix	Germany	Soil sample taken from A61 Germany. Distance to road is 1 m. Previously used as part of the Microproof[1] project.

Pristine TWPs

Pristine TWPs are an important start point for research into pre-treatment methods as they offer insight into how reagents interact with TWPs without the influence of road encrustations of other environmental matrix factors. The composition of tyres on the market varies dramatically between brands and also between models from the same brand. In order to ensure the influence of this was taken into account, pristine TWPs were prepared from different market segments, including budget and executive brands. SEM-EDX analysis of the pristine tyres (shown in Appendix 5.3) showed that the executive tyres use silicon dioxide (SiO_2) as a filler whereas the budget tyres contained carbon black as a filler with only a low concentration of SiO_2 present. TED-GCMS analysis of the tyres (Appendix 5.4) showed that the executive tyres contain more synthetic rubber whereas the budget tyres contain more natural rubber. Tread sections of the pristine tyres were removed with a jigsaw and cryogenically milled in a Retsch rotary mill with screen size of 250 µm to produce pristine TWPs with a comparable size to environmental TRWPs.

Simulated TRWPs

Simulated TRWPs are a key step towards optimising both pre-treatment and analysis methods as the TRWPs generated are comparable to those formed under real-world conditions, containing road wear encrustations which lead to properties such as varying density and elemental composition. However, they are free from most disturbing matrix components such as soil and organic matter. The sample was prepared using the circular road simulator at project partner The Swedish National Road and Transport Research Institute (VTI) in Sweden using a Pirelli tyre (exact model unknown). Through TED-GCMS analysis it has been determined that this sample contains relatively high concentration of natural rubber (Shown in Appendix 5.4). The sample contains ~10% TRWPs with the rest being small road wear particles.

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[1] Microproof: Microproof - Home (microproof-cedr.nl)

Deposition

Deposition samplers will be used at multiple locations throughout the Netherlands during the measurement campaign (Task 3.1.1) to investigate the TRWPs that settle out of the air in different locations. Deposition samplers consist of a large glass funnel (~15 L) that feeds into a glass collection funnel (10 L). The samplers are replaced monthly. In the current research, deposition samples were collected ~10 m from the A27/28 splitting in Utrecht, NL.

Soil

Soil samples will be collected next to various types of road (highway, urban and rural) and at various distances to understand how far TRWPs travel after they are produced. Soil is sampled by using a trowel to collect the top 2-5 cm of the soil. In the current research, soil samples were collected at both 1 and 10 m from the roadside next to the A61 near Goldene Meile, Germany. The soil sample at 1 m contains a high concentration of TWPs (5.9 g/kg) and is used as an environmental test sample whereas the 10 m sample is known to contain a low concentration of TWP (0.7 g/kg) and is used as a soil control sample.

Run-off

During rain events, many TRWPs are washed from the road surface onto surrounding soils, surface waters and storm drains. This likely presents an important transport route for TRWPs to aquatic environments. This water with washed-away TRWPs is called run-off and can be collected in samplers next to the road. In this research, run-off samples were collected next to the A61 near Goldene Meile, Germany. These samples were collected as part of the Microproof project.

Run-off sediment

At some locations, storm drains are present to catch run-off water containing TRWPs. From here some of which are captured in the sediment traps and the rest are carried further to WWTPs or further into surface waters. In this research, sediment from a storm water drain were collected next to the E18 highway in Sweden. These samples were collected as part of the Microproof project.

2.2 Pre-treatment Methods

In order to understand the emission and fate of TRWPs it is necessary to investigate their presence in all environmental compartments: the air, water, soil and sediment. Such environmental samples typically contain high concentrations of contaminants such as minerals, clays, biogenic material and other anthropogenic pollutants. In order to facilitate analysis of the TRWPs in these samples it is necessary to pre-treat them in order to reduce the contaminants and increase the relative concentration of TRWPs in the sample. Depending on the sample matrix, different steps may need to be taken, including concentration, digestion and separation.

Figure 7 illustrates schematically which environmental samples can benefit from which pre-treatment pathway and what the desired outcome of each step is.

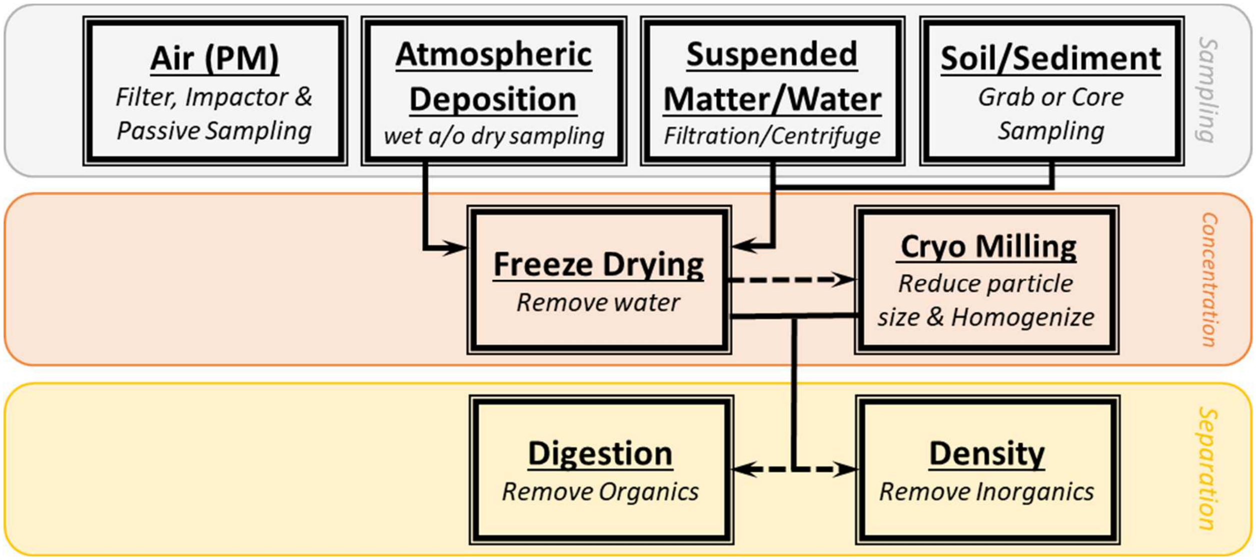


Figure 7: Scheme illustrating a workflow for the pre-treatment of samples in various environmental matrices

2.2.1 Drying and Milling

From aqueous environmental samples (e.g. surface water, runoff, wet/bulk deposition samples) first the solid matter must be separated from the water matrix. Multiple methods are possible, such as (crossflow/ultra)filtration, centrifugation, (vacuum)evaporation and freeze-drying and some of them can already be performed in the field. A full assessment of these methods is out of the scope of this research. For this work, similar to soil and sediment samples, freeze-drying was utilised to remove the water matrix because it does not affect the integrity of the sample. Especially for SEM analyses, the agglomeration state of the particles is important and is least affected by freeze-drying. After freeze-drying a solid powder sample is acquired which can be further processed. To obtain a homogeneous sample, for bulk TED-GCMS, LCMS and GCMS analyses the solid material is treated by cryogenic milling, where liquid nitrogen is added to keep the temperature of the TWP as low as possible. This is necessary to avoid evaporation of the semi-volatile additives in TWP. Also it hardens the rubber matrix and prevents smearing effects during milling.

2.2.2 Digestion

In order to facilitate analysis without sample matrix interference, chemical treatments are used to remove the organic contaminants present. As alkaline and acidic treatments are often found to be harsh procedures that result in the degradation of common polymers, [25,26] it was investigated whether oxidative digestion methods would be suitable for the removal of organic contaminants in soil matrices. A screening was performed by applying Fenton's reagent,[31] H_2O_2 and NaClO[22] treatments to pristine Budget and Executive TWPs, simulated TRWPs, and environmental samples from run-off sediment and soil (1 m). The method was applied on the pristine TWP samples in order to investigate the influence of the procedure on tyre material solely. As a control, all samples were treated in the same way using MilliQ water. Results of these tests are shown in Figure 8.

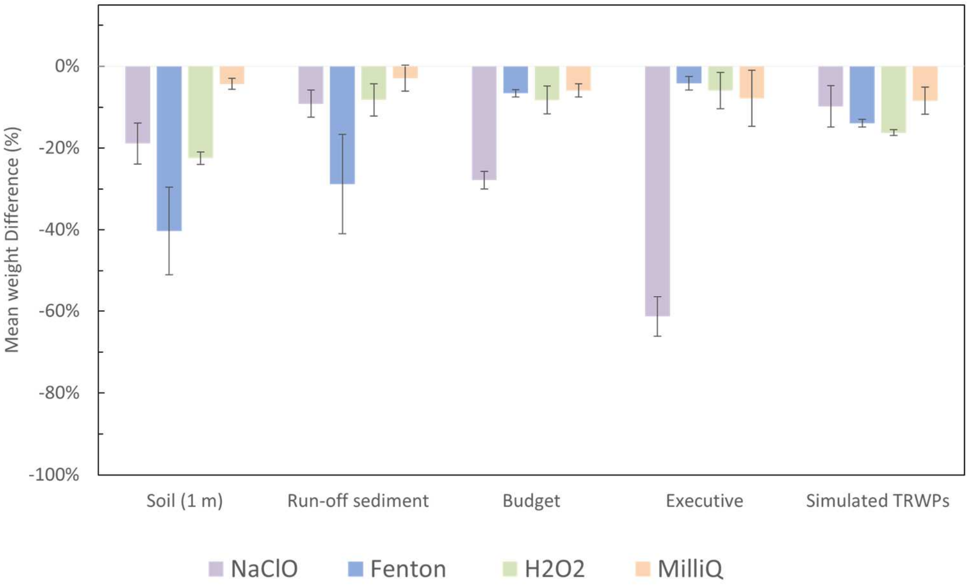


Figure 8: Mean weight difference on Soil (1 m), Run-off sediment, Budget, Executive and Simulated TRWPs samples after treatment with NaClO, Fenton, H_2O_2 and MilliQ.

As was expected, treatment with MilliQ water resulted in limited mass loss after digestion, obtaining high recovery rates of >92 % for all sample types. The small amount of sample lost can be attributed to mass losses from the procedure itself, such as the filtration step, or by dissolving salts that were present in the sample. For the tests on the Budget and Executive tyres, H_2O_2 and Fenton's reagent showed comparable mass loss to treatment with MilliQ water. This is important as it shows that these treatments have no ill effects on the tyre rubber material of both types. The simulated TRWPs showed slightly higher mass loss for H_2O_2 (16%) and Fenton's (14%) than for the MilliQ (8%), however it is not certain whether this is due to digestion of the TWPs or salts and road particles that may be present. The simulated TRWPs contain a high concentration of natural rubber, thus suggesting that these digestion reagents may be more aggressive towards natural rubber than towards synthetic rubber, however this was not observed for the pristine Budget sample, which also consists of natural rubber. The difference between the Budget and Simulated TRWPs sample is that next to tyre material the simulated sample also contains mineral particles originating from road wear. These results indicate that the presence of road wear might influence the reactivity of H_2O_2 and Fenton's digestion methods towards tyre material.

Interestingly, treatment with NaClO resulted in 28% and 61% mass loss for the budget and executive tyres, respectively. It was also observed during these experiments that the solution in which the particles were suspended turned black during digestion, visually suggesting that the tyre particles were also being dissolved. This clearly demonstrates that NaClO is unsuitable for treatment of these samples as it has a negative impact on the analyte. The difference in mass loss between the Budget and Executive tyres was large and may be due to the mass and type of fillers present, as well as the composition of synthetic and natural rubber used in the tyre. The simulated TRWPs, which are mostly natural rubber suffered no weight loss, suggesting that natural rubber is more resistant to treatment with NaClO than synthetic rubber is.

The three digestion methods were also applied to two environmental samples, soil (1 m) and run-off sediment. After treatment with Fenton's reagent these samples lost 48% and 35% mass, respectively. This suggests that it is highly effective at removing environmental matrix components. NaClO and H_2O_2 showed comparable results for both samples. However, as we have seen NaClO has a strong effect on TRWPs it may be that the loss of mass in this case is due to digestion of the particles of interest with minimal effect on matrix components. H_2O_2, on the other hand, showed minimal effect on tyre particles so it is likely that the mass loss in this case is due to removal of matrix, as desired.

To gain more insight into the (matrix) components that are digested with these treatments, the environmental samples were analysed by TED-GCMS. As reference, these samples were also analysed without any prior treatment. Based on these measurements, the mean recovery of TWP markers was calculated for each treatment, as visualised in Figure 9.

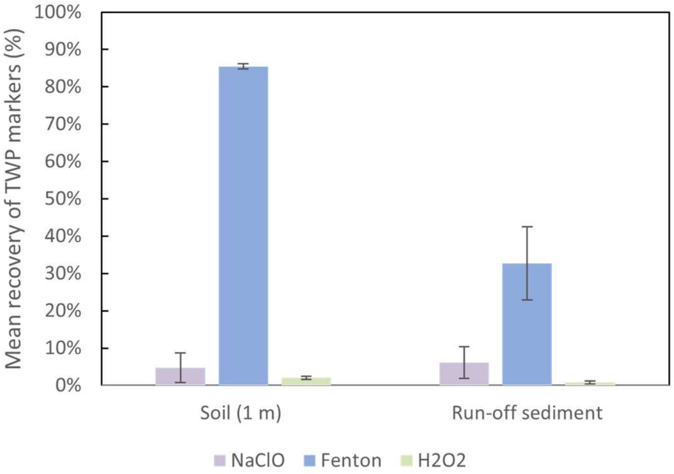


Figure 9: Mean recovery of TWP markers on environmental Soil (1 m) and Run-off sediment samples after treatment with NaClO, Fenton and H_2O_2.

After treatment with NaClO and H_2O_2, very low recoveries were obtained with mean values below 10%. These results indicate that NaClO and H_2O_2 digest TWPs, thus clearly showing that the analyte is affected by these pre-treatments. For Fenton, higher recoveries of 85% ± 1 and 33% ± 10 were obtained, however still indicating loss of TWPs after treatment.

From this screening we can conclude that the use of NaClO and H_2O_2 digestion methods for TRWPs in environmental soil and sediment samples is undesirable as it attacks tyre material while showing sub-optimal performance against other environmental matrix components. While H_2O_2 digestion shows promise as it digests matrix matter while leaving tyre particles intact, Fenton's reagent has a stronger effect on matrix matter whilst also showing the least effect on tyre rubber with the additional advantage that the procedure itself is less time consuming. However, TED-GCMS results still indicate some loss of TWPs through pre-treatment with Fenton's, demonstrating the importance of further optimising and validation of this method. Therefore, future work is focussed on further development and optimisation of the oxidative digestion using Fenton's reagent looking more into the specific reaction conditions (*e.g.* reaction time, H_2O_2 concentration *etc.*) in order to apply this pre-treatment procedure in the measurement campaign in the Leon-T project.

2.2.3 Density Separation

First, parameters of the density separation method, including mass of sample, volume of solution, addition of surfactant, size of separatory funnel and duration of the density separation were optimised based on sample recoveries. Initial tests were carried out varying these parameters on soil (10 m) sample and using a 1.2 g/mL NaCl solution. Once satisfactory recoveries were obtained on the soil samples, the method was tested on combusted soil (10 m), simulated TRWPs and a model sample containing a mixture of combusted soil (10 m) with simulated TRWP (50/50 w/w%). The results of the optimalisation of the density separation method are summarised in Figure 10.

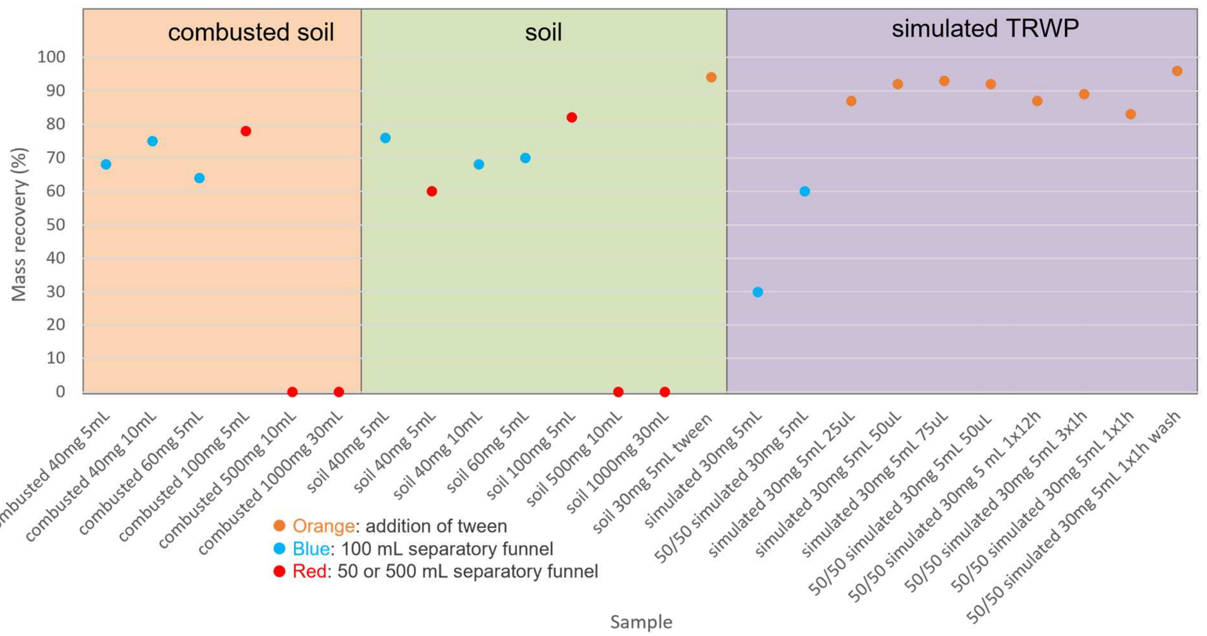


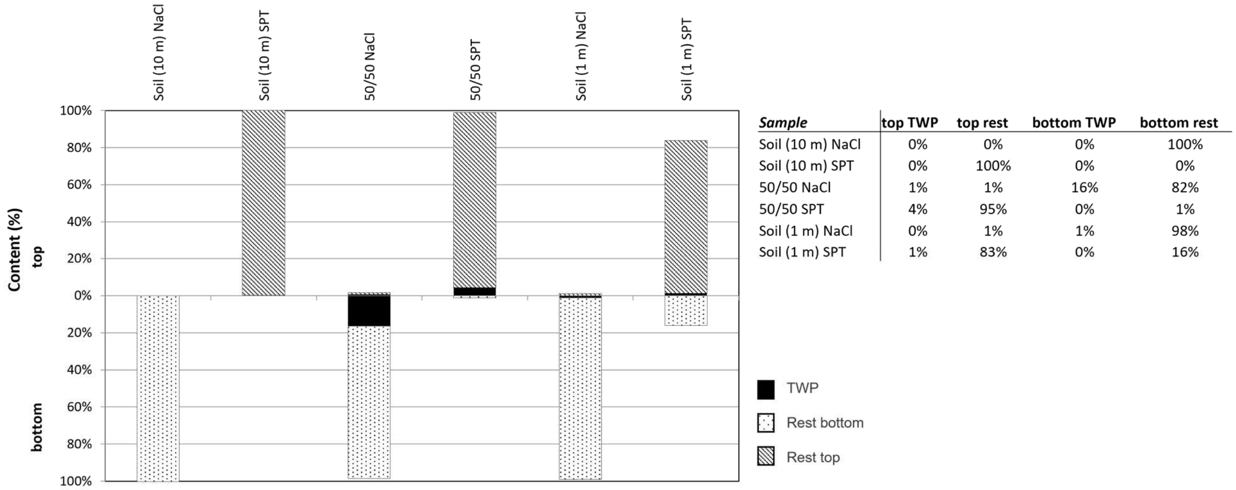
Figure 10: Optimalisation of density separation method tested on samples with varying sample amount, solution volume, size of separatory funnel and duration of the density separation. Samples and the method tested are described on the x-axis (combusted = combusted soil (10 m), soil = soil (10 m), simulated = simulated TRWPs, 50/50 simulated = mixture of combusted soil (10 m) with simulated TRWP (50/50 w/w%)).

The use of large amount of sample material (*e.g.* 500 mg or higher) resulted in low mass recoveries of 0%, indicated by red points in Figure 10. This is caused by clogging of the sample material in the outlet of the separatory funnel, obstructing recovery of sample material. As similar problems were observed for samples above 60 mg, it was recommended to use a sample amount between 30 – 60 mg. Densities can vary between sample types, and since 30 mg sample is sufficient for most applications (*e.g.* SEM and TED-GCMS), 30 mg was chosen as an optimal amount of sample. To optimise the volume of solution used in the separation method, two different solution volumes were investigated, 5 ml and 10 ml. The recoveries for 10 mL were lower (75%) compared to using 5 mL (82%). This combined with the overall cost of the method in mind, especially as more expensive solutions such as SPT will be applied, a solution volume of 5 mL was chosen as optimal. Although similar recoveries were obtained by comparing the 50 mL separatory funnel with the 100 mL separatory funnel, the latter was chosen for practical reasons. The smaller opening of the 50 mL separatory funnel could lead to clogging, thus preventing optimal sample recovery. Therefore a separatory funnel of 100 mL was used for the separation of the samples.

While optimising the method on the simulated TRWPs, limited solvent interaction between sample and solution was observed, thus complicating sample recovery. To overcome this problem, a small amount of surfactant (Tween 20) was added. This helped break the solution-sample barrier and made it possible for the particles to mix with the solution. The addition of surfactant significantly increased mass recoveries, from 30% to 87% for the simulated TRWPs. The amount of surfactant was further optimised, wherein the 25, 50 and 75 µL were compared to each other. 75 µL provided a recovery of 93% compared to 25 and 50 µL (87 and 92%), but also caused additional foaming in the separatory funnel. Therefore an amount of 50 µL was used in further experiments. To test the influence of settling time on the method, settling times of 1 h and 12 h were compared. Next to settling time it was investigated whether additional washing steps could improve recoveries. A comparison between 1x 12 hours, 1x 1 hour and 3x 1 hour with additional washing steps was made, shown in the most right samples in Figure 10. The highest recovery (96%) was obtained by applying a settling time of 1 hour with an additional washing step.

Based on these results, the optimised parameters chosen for the density separation of soil samples were 30 mg sample, 5 mL solution, a separatory funnel of 100 mL, the addition of 50 µL Tween 20 and a settling time of 1 hour with a washing step after the first separation.

Using these parameters, density separation was applied with two different density solutions on three samples: soil (10 m), soil (1 m) and a model sample (containing 50/50 w/w% simulated TRWP and combusted soil (10 m)). This was done in order to test the separation of organic and inorganic matter for a two-step density separation utilising low density (~1.2 g/mL) and high density (~2.2 g/ml) solutions. As TRWP have a relatively high density with its upper limit still highly debated, a 2.4 g/mL SPT solution was selected as high density solution. This was done to ensure high recovery of the TRWP present whilst still allowing the separation between undesired particles such as clay, silt and sand that have reported mean particle densities of 2.5 g/mL.[66] As a low density solution, hypersaline NaCl was chosen with a density of 1.2 g/mL. After separation, the top and bottom fractions were collected, weighed and the TWP content was determined by TED-GCMS analysis using the TNO method with 4VCH marker for TWP quantification. For more details on the TED-GCMS method see section 2.3.1. With these results the distribution of TWPs in the top and bottom fractions for each sample could be calculated, as shown in Figure 11.



Sample	top TWP	top rest	bottom TWP	bottom rest
Soil (10 m) NaCl	0%	0%	0%	100%
Soil (10 m) SPT	0%	100%	0%	0%
50/50 NaCl	1%	1%	16%	82%
50/50 SPT	4%	95%	0%	1%
Soil (1 m) NaCl	0%	1%	1%	98%
Soil (1 m) SPT	1%	83%	0%	16%

Figure 11: Distribution of TWP and other matrix material contents in the top and bottom layer of NaCl (1.2 g/mL) and SPT (2.4 g/mL) solutions. Black blocks indicate TWP contents determined by TED-GCMS and striped and dotted bars indicate other matrix material (rest). Percentages of the distribution are shown in table on the top right.

Here, it can be seen that for the soil sample (10 m), with limited TWP present, matrix material is fully distributed over the bottom (NaCl) and top (SPT) layers. This is also observed for the model sample (indicated by 50/50), with the total recovered sample mass being wholly in the bottom and top layers of the 1.2 and 2.4 g/mL solutions. While it is expected that the soil sample may be denser than 1.2 g/mL, separation was expected in the 2.4 g/mL solution as many soil particles such as clay, silt and sand should have mean particle densities of ~2.5 g/mL.[66] This could indicate that the composition of the soil has fewer siliceous minerals and clays such as kaolin and therefore exhibits a lighter mean density. The soil (1 m) sample, however, did show separation with the 2.4 g/mL solution with ~84% sample recovered in the top layer and ~16% sample recovered in the bottom layer. This indicates that the procedure was successful for soil sampled close to the roadside, enriching the sample by a factor of 1.2.

No significant separation between matrix material and TWP is observed for all three samples with the NaCl solution, thus questioning the applicability of a two-step density separation method for the separation of both inorganic and organic material. For the 50/50 sample, 16% TWPs content was recovered with the NaCl separation, whereas only ~5% TWPs content was recovered with SPT. As this sample was prepared with a specific concentration TWPs, one would expect similar contents for both experiments. These results indicate that either TWPs are not homogeneously distributed throughout the sample, or that they are not fully recovered from the experimental setup. Although the procedure was optimised for soil samples using NaCl obtaining high total recoveries, lower recoveries were obtained using the same conditions with the use of SPT (67 – 82% compared to >90 %). This indicates that sample material, and possibly also TWPs, is lost during the procedure with the use of SPT. This may be due to varying particle sizes of the matrix components, inter particle interactions, particle-solution interactions or complex soil composition. As one of the objectives in this approach is to enrich the sample in TWP concentration as a sample-pre-treatment, further research is needed to ensure full TWP recovery with higher total sample recoveries by further method optimalisation testing lower density solutions (*e.g.* 2.2 g/mL).

Given these results, further investigation is required on refining the solution density for optimal separation between TWPs and other matrix particles present in soil. Although the initial results for soil (1 m) look promising, due to the varying soil composition (and therefore varying soil densities) at each measurement location, this optimisation may be required for each sample which would not be feasible.[67] Due to the high costs of SPT, it is not feasible to use for such a large scale measurement campaign without reuse of the solvent. This recovery and purification process is also time consuming which adds to the unsuitability of the method. We therefore foresee a pragmatic approach to applying density separation as a pre-treatment step and taking into account the pros (*e.g.* slight TWP enrichment) and cons (*e.g.* time consuming, expensive, possibility of introducing lab contamination), we conclude that this method is not suitable for use during large-scale measurement campaigns.

2.2.4 Summary pre-treatment methods

Sample pre-treatment optimisation efforts have focused on two methods: digestion to remove organic components and density separation to remove inorganic components. Oxidative reagents NaClO, H_2O_2 and Fenton’s reagent were investigated with a first screening for the their efficiency to remove matrix material without damaging TWPs. It was found that NaClO is unsuitable as it has low activity towards matrix material while destroying TWPs. H_2O_2 showed medium activity towards matrix and was inactive towards TWPs, however Fenton’s reagent was demonstrated to be the most promising digestion as it showed both high activity towards matrix removal and low effect on TWPs. Further research should be focussed on further optimising the Fenton’s digestion procedure whilst also investigating which specific components it removes and any potential effect on the TWPs markers used in TED-GCMS.

Density separation was optimised in relation to the recovery of sample from the experimental setup, however, an optimal solution density for enrichment of TRWP from inorganic components has not yet been found. Further research should investigate SPT solutions with densities varying from 1.7 – 2.2 g/ml. However, due to the complexity of optimising this procedure, along with it being highly time consuming and expensive, we conclude that the method is not suitable for its use in the follow-up phase (measurement campaign) of this project.

2.3 Analytical Methods

Sample analysis can be performed in two different ways: bulk analysis for information on environmental TRWP concentrations with a coarse size indication and single particle analysis for detailed information on the particle size distribution and particle morphology. In this project we will be developing two methods beyond the state-of-the-art: TED-GCMS, a bulk technique which can be used for quantitative determination of TRWP mass in environmental samples; and SEM-EDX(-CL), an electron microscopy based single particle analysis method that gives information on size, morphology, number of particles and elemental composition. We will also optimise a cascade filtration technique to give coarse size information in combination with TED-GCMS quantification. An overview of these techniques and with which samples they will be used is shown in

Figure 12. An extended scheme detailing all techniques that will be utilised in the LEON-T measurement campaign (Task 3.1.1) (including those not optimised in this report) can be found in Appendix 5.2.

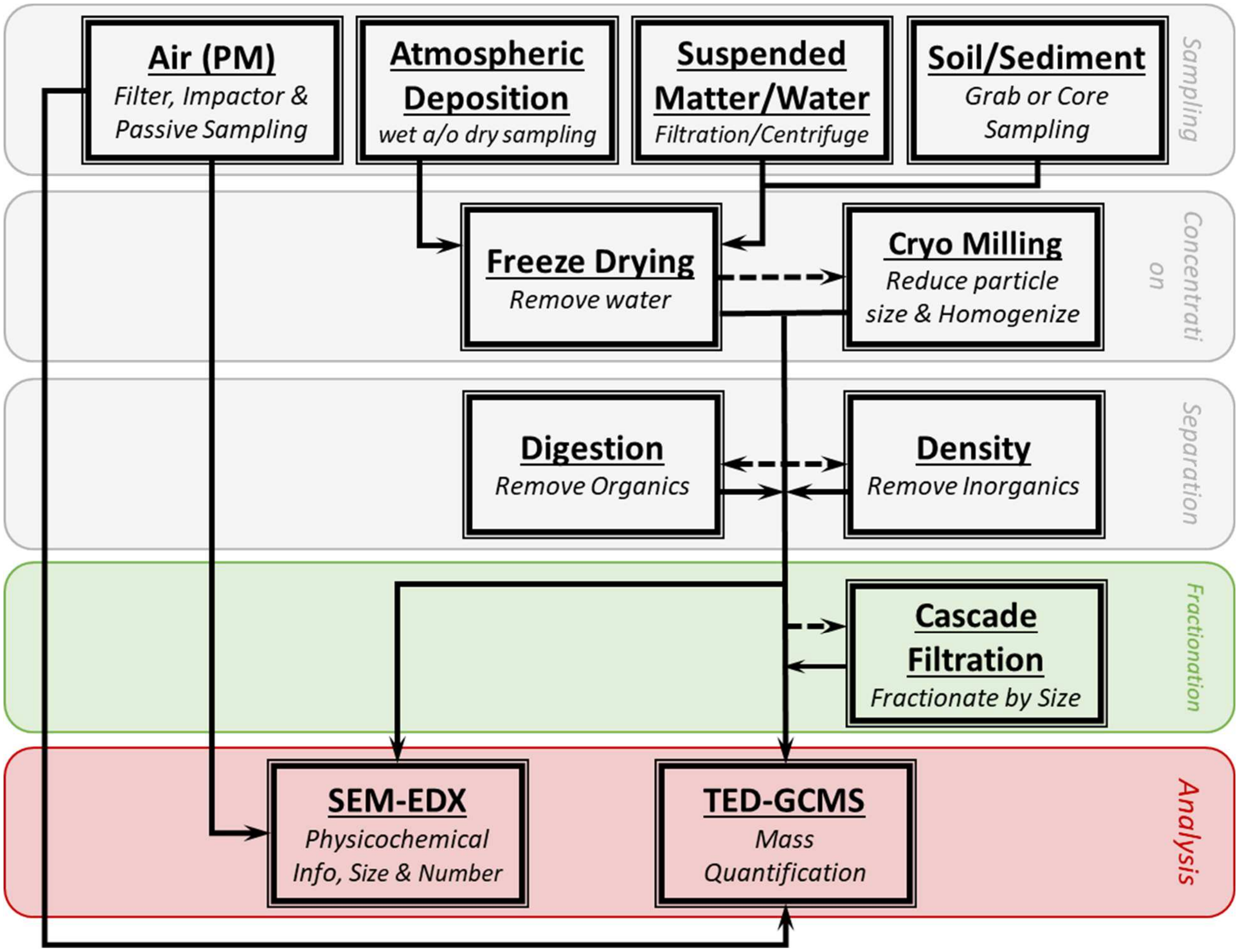


Figure 12: Workflow for the analysis of environment TRWPs with the analysis steps highlighted.

2.3.1 Thermo-analytical techniques

2.3.1.1 Overview thermo-analytical techniques

Thermal techniques combined with GCMS, such as Pyr-GCMS and TED-GCMS, are among the most promising techniques for the quantification of TRWP and MPs in environmental samples. Thermo-analytical techniques have already been used in numerous studies to quantify the mass of TWPs in ambient air as well as in soil, sediment and water. As discussed in more detail in the introduction, thermo-analytical methods begin with a pyrolysis step where the sample is heated to high temperatures under an inert atmosphere. This produces a mix of decomposition products, some of which are indicative of the polymer that they are derived from. This mix of pyrolysis decomposition products is then passed through a gas chromatograph and mass spectrometer for separation, identification and quantification. Despite the widespread use of thermo-analytical methods, reliable quantification is still a challenge because polymer composition and microstructure of TWP all have an effect on the measured concentration of pyrolysis decomposition products. Furthermore, methodologies lack harmonization and standardization. For example, existing methodologies use different decomposition products as markers and different quantification techniques (e.g. conversion factors) which could result in different results.

Table 3 shows an overview of four of the most commonly used procedures in literature and the current measurement method of TNO. These use two subtly different thermo-analytical methods: Pyr-GCMS and TED-GCMS. In Pyr-GCMS the pyrolysis products are directly fed to the GCMS system, whereas in TED-GCMS the pyrolysis products are first captured by an absorbent and then thermal desorbed from this absorbent. The ISO have published two technical specifications for the quantification of TRWP in ambient air, soil and sediment samples: ISO/TS 21396 and ISO/TS 20593.[49,50] These are based upon prior research carried out by Unice and Panko.[68–71] This is a Pyr-GCMS technique where the sample is rapidly heated to 670 °C with the use of a Curie-point pyrolyser under hydrogen (H_2) atmosphere. As this is a Pyr-GCMS technique no gas adsorption medium is needed. Subsequently, 4-vinylcyclohexene (4VCH) is used to quantify styrene-butadiene rubber (SBR) and butadiene rubber (BR) and dipentene (DP) is used to quantify natural rubber (NR). The method is using deuterated polyisoprene (d-PI) and deuterated polybutadiene (d-PB) as internal pyrolysis standards and decomposition products styrene, isoprene and butadiene are used as additional identifiers for the presence of SBR, NR and BR, respectively. Calibration curves for NR and SBR+BR are prepared through the pyrolysis of IR2200 and SBR1500, respectively, dissolved in chloroform. Rauert *et al.* have reported that by following this method the standards for analysis of NR are not soluble so it cannot be reliably used for the analysis of NR.[51]

To calculate the concentration of SBR+BR from 4VCH concentration, first a correction factor of 0.9 was applied. This is suggested to correct for the difference in styrene content between SBR 1500 used in the calibration curve of 23.5% and the suggested SBR formulation used in the industry of 15%. Based on characterisation studies of TRWP by Unice *et al.* to convert marker concentration into TRWP concentration, the method assumes that the tyre tread content in TRWP is approximately 50% and the polymer content (SBR+BR+NR) in tyre tread is also 50%.[70,72] This is in agreement with Baensch-Baltruschat *et al*. and Wagner *et al.* who published contributions of 40 - 50% and 40 - 60% of rubbers and polymers.[72,73] Other studies reported slightly smaller contributions of rubber in tyres: 41% rubber with 30% SBR and 20% BR (Grigoratos and Martini, 2014), 20–30% SBR+BR with a 60:40 ratio between SBR and BR (Vogelsang et al., 2018) and 31.9 ±12,7 % SBR+BR.[74] Tromp *et al.* have performed organic carbon and elemental carbon analysis on approximately 35 tyre tread samples.[75,76] Based on these analyses an average organic carbon content of 57 ± 3% was calculated. Approximately 70% of the organic carbon content consist of rubbers and polymers, the rest of the organic carbon consist of softeners (mineral oils) and additives (preservatives, anti-oxidants, desiccants and plasticizers).[72,73] Based on these findings a rubber/polymer content (SBR+BR+NR) of approximately 40% is calculated. It has been shown by Unice *et al.* that the microstructure of the SBR has a large effect on the concentration of the quantification marker 4VCH, which can lead to a possible over- or underestimation of the TRWP content.[52]

Table 3: Comparison of three of the most commonly used literature methods and the current TNO method.

Description	TNO (2012)	ISO/TS 21396/20593, Unice (2012, 2013), Panko (2013, 2019)	Eisentraut (2019), Muller (2022)	Gossmann (2021)
Analysis technique	Pyrolysis + TD-GCMS	Pyr-GCMS	TGA-TED-GCMS	Pyr-GCMS
Pyrolysis conditions	550 °C, 15 min, N_2	670 °C, 5 sec, H_2, induction time <0.2 sec	25-600 °C , 10 °C/min, N_2	590 °C, 1 min, He
Pyrolysis system	tube furnace sample with stainless steel tube	micro-furnace with quartz tube, Curie-point with holder, Pt-filament with holder	TGA with 150 µL alumina crucibles	micro-furnace pyrolyzer with evolved gas analyser
Adsorption medium	Tenax-TA	-	SPE Sorbstar / PDMS Twister	-
Sample pre-treatment	-	-	pre-heating 25-290 °C (optional)	oxidative sample clean-up
Pyrolysis markers :				
Styrene (SBR)	qualifier	qualifier	-	-

Description	TNO (2012)	ISO/TS 21396/20593, Unice (2012, 2013), Panko (2013, 2019)	Eisentraut (2019), Muller (2022)	Gossmann (2021)
Isoprene (NR)	-	qualifier	-	-
Butadiene (SBR+BR)	-	qualifier	-	-
Dipentene (NR)	qualifier/quantifier	quantifier	qualifier	qualifier
Iso3/Isi4 (NR)	-	-	qualifier/quantifier	-
DMVCH (NR)	-	-	-	quantifier
4VCH (SBR+BR)	quantifier	quantifier	-	-
SB (SBR)	quantifier	-	quantifier	quantifier
SBB (SBR)	-	-	qualifier	-
Pyrolysis internal standard	4-fluorostyrene (dissolved in DCM)	d-PI and d-PB (dissolved in chloroform)	d-PS (dissolved in DCM)	anthracene-d + TOHA (dissolved in hexane) and d-PS (dissolved in DCM)
Injection internal standard	toluene-d8 / ethylbenzene-d10	-	-	-
Quantification method	external 5 points calibration with decomposition products spiked on Tenax-TA	matrix-free external 5 points calibration by pyrolysis of IR2200 and SBR1500 (dissolved in chloroform)	standard addition with SBR / matrix-free external 6 points calibration by pyrolysis of SBR (dissolved in toluene)	matrix-free external 5 points calibration by pyrolysis of typical truck tread and car tread (particles)
Quantification result	TWP	SBR+BR and NR	SBR	truck TWP and car TWP
Conversion T(R)WP	average concentration of pyrolysis markers in car- and truck tyres, with correction for freight traffic (6% in NL); conversion factors VCH, PCH and dipentene are *ca.* 1.0, 0.17 and 1.2 mg/g TWP; no conversion to TRWP	rubber content (SBR+BR+NR) in tyre tread is *ca.* 50% (SBR in passenger tread is 44%, NR in truck tread is 45%); correction factor of 0.9 for styrene content: styrene content in SBR+BR in tyre tread is 15% (reflecting 65% SBR and 35% BR) and styrene content in SBR1500 is 23.5%; content tyre wear in TRWP is 50%	average content SBR in tyre tread is ca. 11.3%; content tyre wear in TRWP is 50%	concentration of pyrolysis markers in a typical truck tread (DMVCH) and car tread (DP) from 15 car/truck tyres; correction for truck TWP: DMVCH truck = DMVCH total - (SB * 0.25)

The Bundesanstalt für Materialforschung und -prüfung (BAM) have also developed a thermo-analytical method using a thermogravimetric analyser (TGA) coupled with TED-GCMS.[77,78] In this method, samples in 150 µL alumina crucibles are heated from 25 to 600 °C at a heating rate of 10 °C/min under N_2 atmosphere. The advantage of using a TGA for the pyrolysis is that also information is obtained about the mass loss during heating. As the TED-GCMS method contains an added adsorption/desorption step that is absent in Pyr-GCMS, volatile decomposition products are less suitable to be used as tyre specific markers. By testing four pure SBR polymer materials Dümichen et al. established that cyclohexenylbenzene (SB) and phenyl-[4.4.0]bicyclodecene (SBB) had comparable concentrations and were suitable as quantification and qualifying markers, respectively. Interestingly, they deemed the 4VCH marker used in the ISO methods too unspecific to be used, as 4VCH is a marker of both SBR and BR rubber.[79] For quantification a calibration curve for SBR is prepared through the pyrolysis of pure SBR rubber, dissolved in toluene. Müller *et al.* used dissolved SBR to spike the environmental samples for quantification using the standard addition technique.[78] Deuterated polystyrene (d-PS) is used as an internal pyrolysis standard. None of the decomposition products of BR were deemed to be specific enough to be used as markers. For NR, isomers of dimers, trimers and tetramers of isoprene (iso2/iso3/iso4) were shown to be highly comparable in pure polymer tests and were used for quantification of NR. However, when testing the influence of environmental matrices it was shown that plant matter also decomposed to the same iso2/iso3/iso4 decomposition products and it was concluded that quantification of NR is not possible for environmental samples where plant matter may be present. Therefore, with this method, only SBR is quantified using SB as the only quantification marker. From the SBR mass concentration a conversion factor of 8.9 is used to determine TWP mass from SBR. The SBR content in tyre materials was estimated to be 11.3% based on literature data of the SBR content in different tyre materials. This is much lower than the 44% SBR in passenger tyre tread mentioned by Unice et al.[70] and is close to the average SBR content (9.3%) measured by Rauert *et al.* when using 4VCH for quantification;[51] in this study also was shown that tyre tread formulations are highly variable and contain between <0.05 to 28% synthetic rubber (SBR+BR).

Gossman *et al.* also used Pyr-GCMS to develop a method for the simultaneous determination of car- and truck-derived TRWP and other microplastics in environmental samples.[48] In this method, pyrolysis occurs at 590 °C under He atmosphere in a micro-furnace pyrolyzer. Suitable markers for qualification and quantification were determined through the pyrolysis of 15 commercial car and truck tyres, also taking into account the varying types of seasonal tyres: summer, winter and all-season. Referring to the BAM method previously described, SBB and SB were chosen for the qualification and quantification of SBR, respectively. Using the comprehensive pyrolytic data collected by Tsuge *et al.*, 2,4-dimethyl-4-vinylcyclohexene (DMVCH) was chosen as a quantifier for NR.[80] It is also noted that due to the sample clean-up steps used in this method, interference with plant matter in the determination of NR, as described by Eisentraut *et al.*, are negligible.[42] 4VCH was deemed unsuitable for quantification due to its presence in both car and truck tyres. Quantification was performed by using tyre tread particles of the “most average” car and truck tyres for an external calibration curve; that is the car and truck tyre whose composition is the closest to that of the average of the 15 tested tyres. The disadvantage of this approach is that it is assumed that the “most average” tyres used for calibration are assumed to be representative for the year-round car and truck fleets, however they are chosen based on relatively small sample sizes of 12 and three tyres, respectively. As internal pyrolysis standards anthracene-d, 9-tetradecyl-1,2,3,4,5,6,7,8-octahydro anthracene (TOHA) and d-PS were used.

Tromp et al.[75] are using a modified method of the TED-GCMS technique developed by Duemichen *et al.*.[77] With a separate tube furnace, samples are pyrolyzed at a temperature of 550°C during a period of 15 minutes. During heating nitrogen (N_2) is passed over the sample and decomposition products are collected by a thermal desorption tube containing Tenax-TA. After sampling, the Tenax tubes are analysed with an automatic thermal desorption (ATD) GCMS. With the external tube furnace samples up to 1 g can be analysed. As an internal pyrolysis standard a non-deuterated standard: 4-fluorostyrene dissolved in DCM was used. The deuterated standard d-PS has frequently been used as internal standard for MP quantification, but can suffer from H–D exchange during pyrolysis, which is catalyzed by inorganic components of the sample matrix.[81] For quantification, calibration curves of selected markers were used, dissolved in methanol and spiked on Tenax-TA. Markers were derived from Unice *et al.* (DP and 4VCH) and Eisentraut *et al.* (SB) and tested on 30 market-representative car and truck tyres in the Netherlands. For 4VCH and DP average concentrations of 0.98 mg/g and 1.32 mg/g were found, with RSDs of 52% and 102%. For 4VCH there was no significant difference between car and truck tyres; on the other hand, for DP the average concentration in truck tyres was much higher (4.90 mg/g) than in car tyres (1.01 mg/g). This makes sense given the higher proportion of NR in truck tyres. For the European CEDR project Microproof, based on the analysis data of these 30 tyres conversion factors for 4VCH (0.97 mg/g) and DP (1.18 mg/g) were developed taking into account the market percentage of these tyres and the percentage of freight traffic (6%) in the Netherlands.[82] Due to the presence of plant material in the environmental samples DP could not be used and only 4VCH was selected for quantification.

2.3.1.2 Variability in rubber composition of tyres

In LEON-T the main approach would be to quantify TWP accurately and consistently whilst taking into account the difference in composition between car tyres and truck tyres and the changing composition across multiple years. The advantage of this method is a direct quantification of TWP using real-world marker conversion factors based on a large dataset of market-representative tyres. The disadvantage would be that the method cannot differentiate between SBR, BR and NR. A correction for the proportion of truck tyres is made, using official transport figures. The current freight traffic contribution in NL as 6%; this is comparable with the environmental measurements of Gossman *et al.* who estimated contribution from truck tyres to be between 6-9% and with the UMSICHT who estimated it to be 9% based on production and consumption data.[83]

It has been noted by multiple sources that the microstructure of the rubber polymer, varying tyre compositions and ever-changing combination of tyres on the market all play an influential role on the measured concentration of tyre wear markers using Pyr-GCMS. The dataset of 30 tyres in Tromp et. al were tested in 2012, as a result of which the data can no longer be valid for the current generation of car and truck tyres. To investigate this, the database of Tromp et. al is compared with the database of Rauert et. al, representing 39 market-representative car and truck tyres for the year 2020 in Australia and Norway with information on the markers 4VCH, SB and SBB. DP was not analysed because NR could not be sufficiently dissolved in chloroform as prescribed in ISO/TS 21396/20593. For LEON-T an additional amount of light duty vehicle (LDV) tyres were analysed in 2022 for 4VCH, SB and DP which are going to be used for the assessment and characterisation of tyre emissions in WP2. Concentrations of 4VCH (SBR+BR marker), SB (SBR marker) and DP (NR marker) for both databases and the LEON-T tyres are shown in Figure 13.

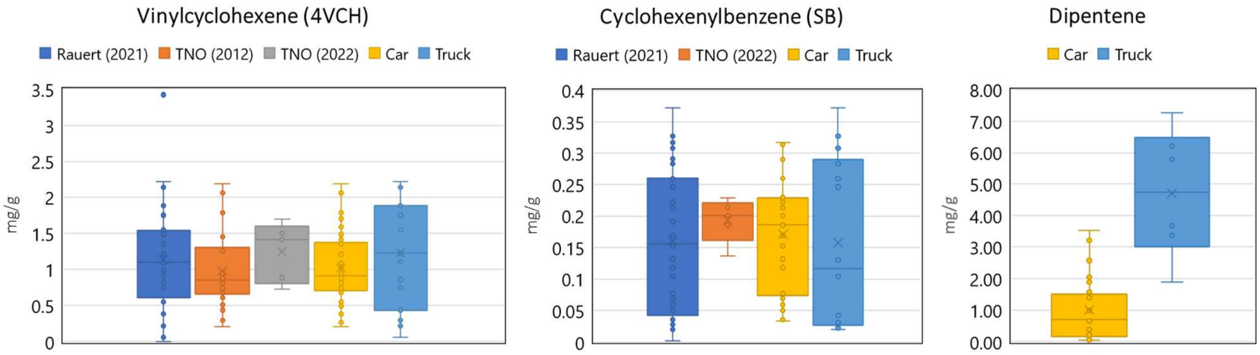


Figure 13: Concentration of common TRWP markers from different tyre datasets, as calculated following the TNO method (2019). Each dot represents a tyre, the cross represents the arithmetic mean, the line represents the median value, the box represents the 25th and 75th percentiles and the error bars indicate the lower and upper values. Dots outside the error bars are outliers.

As has been previously reported, there is a wide variation in 4VCH concentration dependent on tyre composition. This is also evident here, with all three datasets having a broad distribution in concentrations and showing relative standard deviations (RSD) of 54% (Rauert 2020), 52% (TNO 2012) and 33% (TNO 2022). It has previously been suggested that due to the ever-changing composition of tyres, periodic testing of a market representative set of tyres should be carried out to ensure that conversion factors stay relevant to actual conditions. However, our work shows that the average concentration of 4VCH has stayed comparable over the past 10 years, from 0.98 ±0.51 mg/g in 2012, to 1.08 ±0.58 mg/g in 2020 and 1.25 ±0.42 mg/g in 2022 for LDV tyres; there is no significant difference in 4VCH concentration between the databases ($p > 0.1$). From these databases combined, 4VCH concentrations were calculated for 57 car (including personal cars and vans) and 15 truck tyres. It is widely reported that 4VCH is a marker for SBR+BR, synthetic rubbers that aren't usually present in truck tyres (which are mainly composed of NR). However, we show that 4VCH is measured in every truck tyre studied and that concentrations of 4 VCH in truck tyres are not significantly different from car tyres ($p > 0.1$).

In the methods of BAM and Gossling, SB is used as a marker for SBR due to its specificity for SBR and comparable concentration through various tyre compositions. However, in this work, we show that the SB concentration is far more variable than 4VCH, with a RSD of 70% for the Rauert 2020 dataset, which may lead to less reliable calculation of environmental concentrations. Remarkably, a comparison between car and truck tyres, shows not only that SB is detected in every truck tyre but also that no significant difference in SB concentration between car tyres (0.17 ±0.09 mg/g) and truck tyres (0.16 ±0.13 mg/g) could be demonstrated ($p > 0.1$). This suggests that SB may not be so indicative of car tyres as previously reported. Dipentene has also been suggested as a specific marker for NR, which as previously discussed is usually present in higher concentrations in truck tyres than car tyres. When comparing the DP concentrations for the 27 cars and 6 trucks in the TNO datasets, despite the large variances for car tyres (RSD = 97%) and truck tyres (RSD = 43%) it is demonstrated that the DP concentration is significantly higher ($p < 0.01$) for truck tyres (4.9 ±2.2 mg/g) than car tyres (1.01 ±0.97 mg/g).

Using all datasets, with the assumption of 6% of freight traffic, conversion factors are 1.0 (±0.4), 0.17 (±0.09) and 1.2 (±1.0) mg/g for 4VCH, SB and DP, respectively. Using these conversion factors to directly calculate TWP mass from environmental measurements has numerous advantages over the other techniques:

1. There is a large variation in polymer composition between tyres (RSDs: 4VCH 45%, SB 54% and DP 84%). However results show that mean concentrations of polymer markers didn't change significantly over time, even though completely different tyres (types, brands, models) were tested by Tromp et. al in 2012 and Rauert et. al in 2020. So conversion factors can be applied reliably to calculate TWP concentration in environmental matrices. For more accurate results, country-specific conversion factors can be derived, using market-representative tyres for specific countries.

2. As these markers were measured from pure TWPs, a direct calculation for TWP mass can be made on the basis of marker concentration. This is as opposed to the ISO and BAM methods where SBR (and NR) is calculated and then using assumed concentrations of these polymers in TWP converted.

2.3.1.3 Quantitative comparison thermo-analytical methods

As reported in 2.3.1.1, different thermo-analytical techniques and methodologies are developed for identification and quantification of TWP in environmental samples. For a quantitative comparison of the three most commonly used thermo-analytical methods, three environmental samples were analysed following the procedures as published by Tromp et. al (TNO), Eisentraut et. al (BAM) and ISO/TS 21396 & 20593 (ISO) that are summarized in Table 3. Environmental samples of different origin were selected: road side soil at highway A61 in Germany, deposited dust at the road side of highway A27/A28 in the Netherlands and runoff from a sedimentation tank at the road side of highway E18 in Sweden. All analyses were performed with TED-GCMS using a separate tube furnace (550°C – 15min) and collection of decomposition fragments with Tenax-TA. Results are summarized in Figure 14. The BAM method uses SB as a marker and assumes 11,3% SBR in TWP; only for this comparison TWP is also calculated using 44% SBR based on the ISO method and using 4VCH as a marker.

The average variability in the calculated concentrations of the three environmental samples with the three quantification methods are similar, with RSDs of 9.8% (TNO), 12,1% (BAM) and 10.5% (ISO). In general the ISO method produces lower TWP concentrations than the TNO and BAM methods; for soil (A61) the difference is the largest and for runoff (E18) the difference is the smallest. This can be explained by the DP concentration; for runoff the DP concentration was the largest and for soil the smallest. Because the ISO method uses both 4VCH and DP as equivalent markers, this affects the TWP quantification. Caution has to be taken when using DP as marker for NR. It has been previously reported that plant matter from environmental sample disturbs NR markers, such as DP. The lower TWP concentrations with the ISO method, can be explained by the assumptions made. In the ISO method an equal proportion of SBR+BR and NR in tyre tread has been assumed, while the majority of NR is present in truck tyres with a contribution of only 6-9% of the total amount of tyres on the road. Also 50% of rubber (SBR+BR+NR) in tyre tread has been assumed, which may be on the high side, considering the ~40% rubber calculated from the organic carbon content by Tromp et. al and the 41%, 20-30% and 31.9% reported by Grigoratos & Martini, Vogelsang et al and Rodland et. al. (see 2.3.1.1).

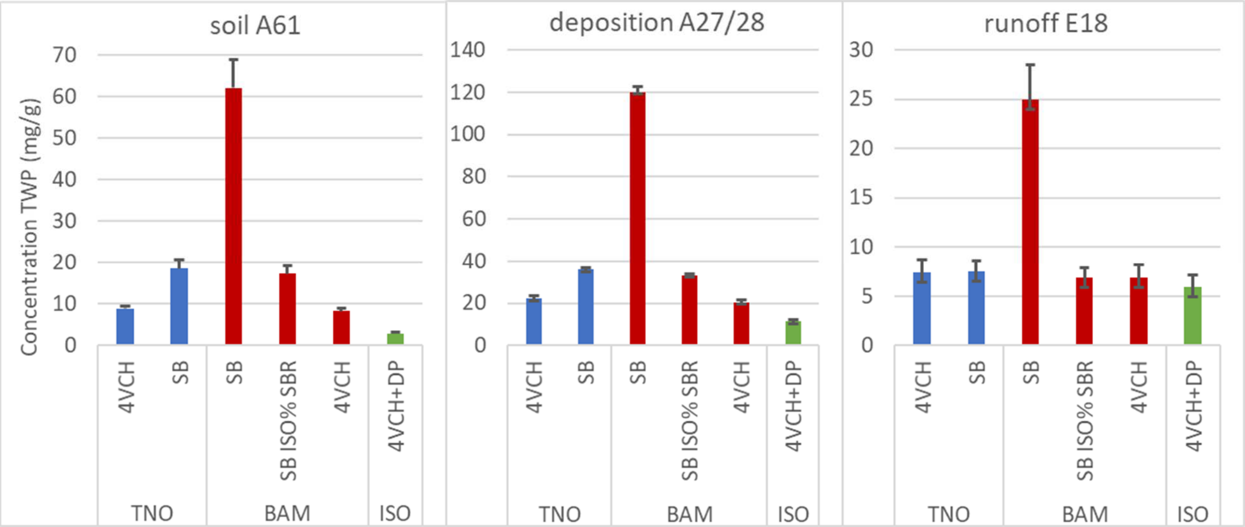


Figure 14: TWP concentration in soil (Germany, A61), deposition (NL, A27/28) and runoff (Sweden, E18) samples as determined by the TNO, BAM and ISO methods from 4VCH, SB and 4VCH+DP markers.

For both the TNO and BAM methods the results show that when SB is used for quantification a higher TWP concentration is calculated than when 4VCH is used. Apart from possible matrix effects, this difference cannot be explained. With the BAM method the calculated TWP concentrations were the highest. Mainly this is caused by the assumption of only 11.3% SBR in tyre tread, which is much lower than the assumed 44% SBR in car tyres reported in the ISO method. If the TWP concentration is calculated with 44% SBR then concentrations are a factor ~4 lower and similar to the TWP concentrations with SB using the TNO method, as can be seen in Figure 12 (SB ISO% SBR). The results show that using SBR and NR calibration standards for quantification, an accurate estimate of the amount of SBR and NR in tyre tread is essential for a solid quantification of TWP in environmental samples. Poorly substantiated assumptions for the amount of SBR and NR in tyre tread can lead to either under- or overestimation of the TWP concentration. This shows that a direct determination of TWP, based on marker concentrations in real tyres is a more accurate methodology for quantification, as already stated in 2.3.1.2.

For the quantification of TWP two procedures can be followed: external calibration and standard addition. In addition an internal pyrolysis standard can be used. The external calibration is the most common method where the analyte signal in the sample is compared to a multi-point matrix-free calibration curve of signal responses prepared from pure standards. Standard addition is typically used in samples where the sample matrix may affect analyte response. Here, the sample to be analysed is measured with and without being spiked with different and known amounts of the analyte. Finally, internal pyrolysis standards are used to account for changing pyrolysis conditions and possible loss of analyte during pyrolysis. A specific amount of a pyrolysis standard is added to each sample and the standard/analyte signal ratio can be used to calculated analyte concentrations.

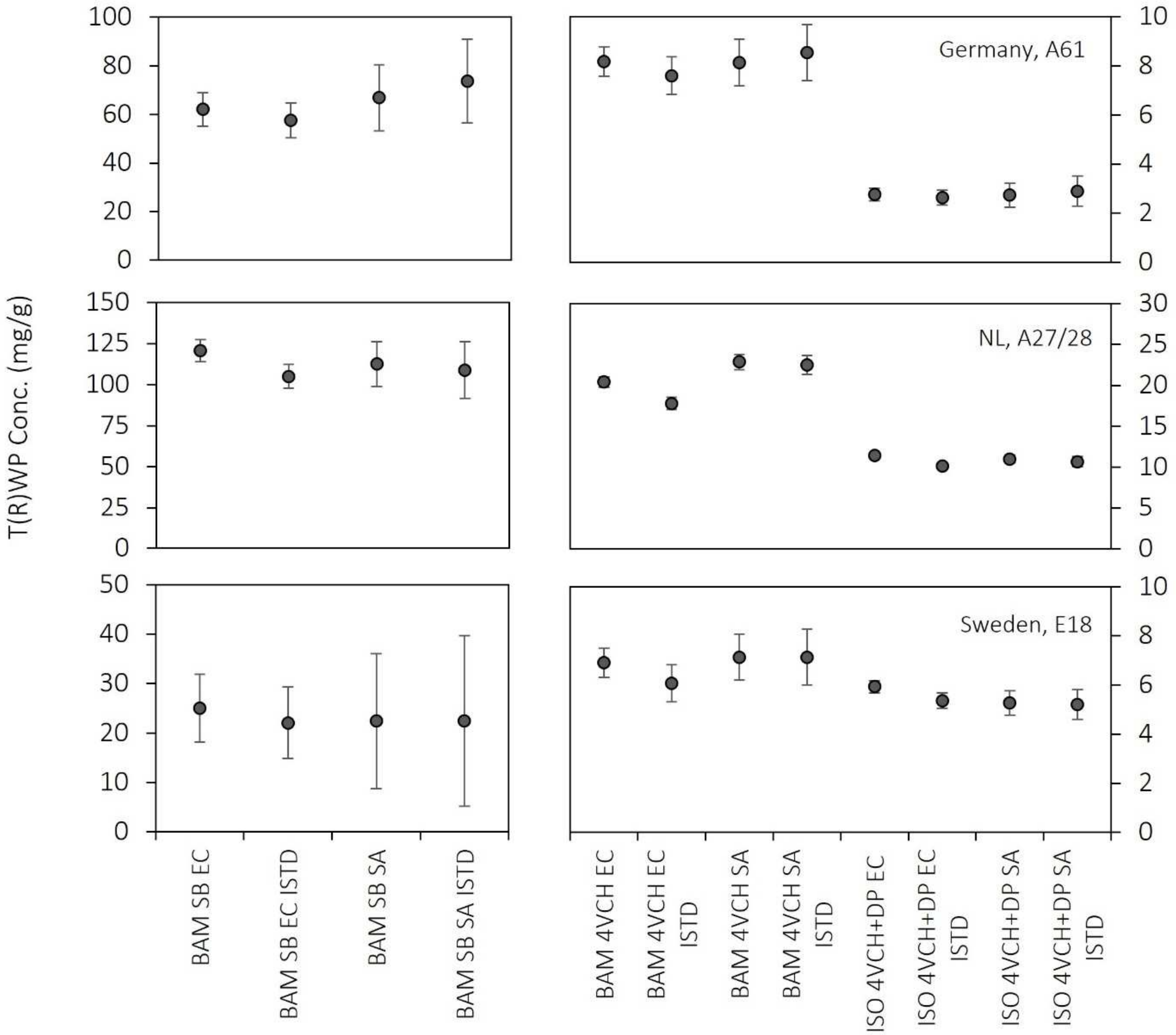


Figure 15: Influence of the use of external calibration (EC), standard addition (SA), and internal standard (ISTD) on the T(R)WP concentrations of environmental samples determined using the BAM and ISO methods from SB, 4VCH and 4VCH+DP markers.

To investigate the reliability of the different quantification techniques, the TWP concentration of the three environmental samples was calculated using the BAM method based on SB and 4VCH markers, and the ISO method using 4VCH+DP markers. TWP concentration was calculated using both matrix-free external calibration (EC) and standard addition (SA) of SBR and NR, both with and without 4-fluorostyrene as an internal pyrolysis standard (ISTD) for the three environmental samples. The results in Figure 15 show that there is no statistically significant variation in TWP concentration calculated by the two different calibration methods, with and without internal standard. The average variability (RSD) in the calculated concentrations of the three environmental samples with EC, SA and w/wo ISTD was 10.5%, 12.5%, 12.2% and 10.8% respectively. This means that for the investigated samples of different origin (soil, runoff, deposition) matrix effects do not play a role and pyrolysis conditions were stable.

2.3.2 Size Fractionation (Cascade Filtration)

Whilst thermo-analytical techniques are relatively high-throughput techniques (in comparison with microscopy techniques) for the quantification of TWP in environmental samples, no information on the size of the TWP is gained. For fractionation of coarse TWPs conventional dry and wet sieving techniques can be used, either by using individual sieves or in a cascade setup. For the size fractionation of TWP below 10 - 20µm crossflow-ultrafiltration and field-flow fractionation are available but not straight forward and applicability of these techniques for TWPs has never been proven. For microscopic analyses Nuclepore filters are commonly used; these flat filters, usually made of polycarbonate, have uniform pore-sizes of any desired diameter, from ca. 50nm to 25µm. Using these filters in a cascade setup enables the fractionation of particles down to submicron and nano sizes and is beyond state-of-the-art. Furthermore, it’s an easy to use and low cost fractionation technique which can be used in a routine laboratory environment.

A cascade filtration setup was made of six interlinked Swin-Lok filter holders connected to a peristaltic pump to force a flow of liquid/suspension through the filters as shown in Figure 16. The 25 mm Swin-Lok holders are designed for microfiltration of small volumes of liquids using positive pressure. During use, the filter holders were placed in an ultrasonic bath to keep particles from blocking the pores and to prevent agglomeration of the particles.

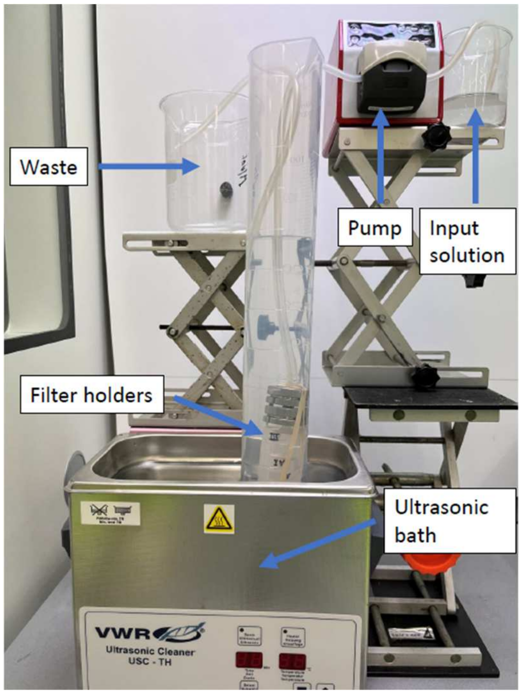


Figure 16: Annotated photo of the cascade filtration setup used in this research.

As an initial proof-of-concept study, suspensions of spherical polystyrene (PS) particles of a mixture of sizes in Milli-Q water were used. The well-defined shape and narrow particle size distribution of the PS spheres act as model particles with which the setup can be tested under ideal conditions. These were filtered through the cascade setup using Nuclepore filters with pore sizes of 2, 0.8, 0.4, 0.2, 0.1 and 0.05 µm. After filtration, the size distribution of particles present on each filter was determined using SEM, the results of which are shown in Figure 17. Most of the particles smaller than the pores passed through the filters. For most particle sizes less than 3% of the particles didn’t pass through, with the exception of 100nm particles (12%). So the majority of particles present at the surface of a certain filter were all larger than the pore size. There were two remarkable results: 35% of the 460nm particles passed through the 0.4µm filters and 40% of the 100nm particles passed through the 0.1µm filters. The reason for this behaviour could be well explained by the real pore size of the filters. SEM analyses of the filters showed that the majority of the pore sizes of the 0.4 and 0.1 µm filters were actually larger than stated, respectively 0.45 and 0.11µm.

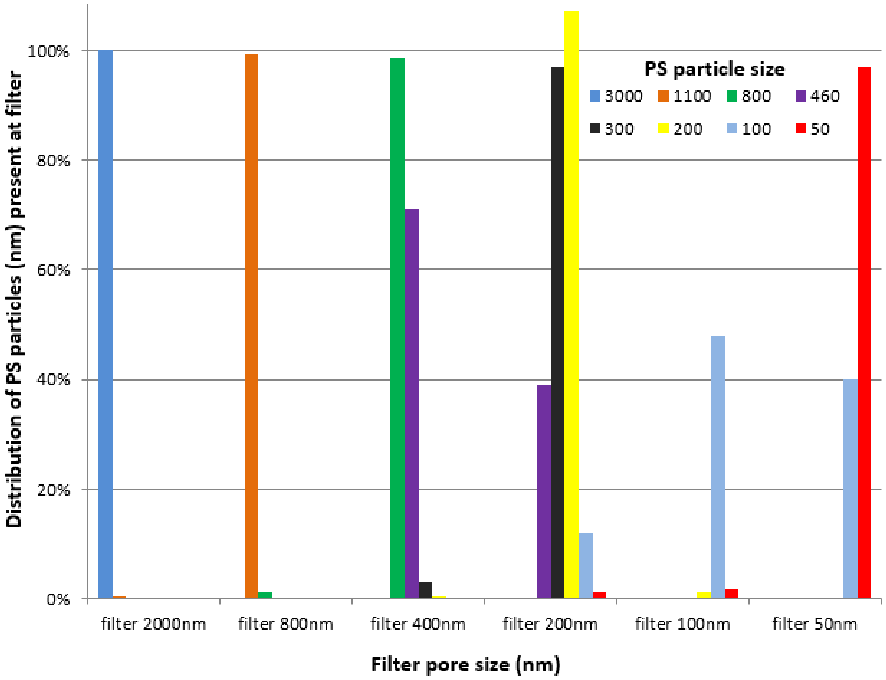


Figure 17: Size distribution of PS spheres on each filter after cascade filtration.

The initial proof of concept study shows promise with regards to achieving accurate size fractionation of a sample. However, the environmental samples consist of larger, often non-spherical particles which could complicate separation. According to Wagner et al the TRWP found in nature are in the range of 1-100 µm. In order to validate the use of the cascade filtration setup for TRWP three additional experiments were performed with increasing complexity of the sample:

1. Combusted soil, without organic material (plant matter, TWP)
2. Collected tyre wear from the road simulator at VTI
3. Road run-off from the A61 in Germany (the run-off sample was filtrated on a glass fibre filter)

All three samples were filtered through the cascade setup using pre-weighed filters with pore sizes of 25, 10, 5, 2, 0.8 and 0.4 µm. A stainless steel microsieve with mesh size of 200 µm was added to remove coarse particles, giving an additional fraction. For the filters with pore sizes of 10 and 25 µm stainless steel filter holders were used instead of Swin-Lok filter holders. With the steel filterholders a more homogeneous distribution of particles can be achieved, making it possible to run a larger sample through the cascade setup. For each experiment 25 - 200 mg of the sample was suspended in 100 mL of MilliQ water, after which a surfactant (TWEEN20) is added. Before running the cascade setup the suspension was sonicated in an ultrasonic bath for 15 minutes. After complete filtration of the suspension the cascade filtration setup was rinsed with 100 mL of MQ water.

To demonstrate the applicability for larger, non-spherical particles and to get insight into the maximum sample size, different amounts of combusted soil (25, 50, 100 and 200mg) were run through the cascade filtration setup. Figure 18 shows SEM images of the combusted soil particles on the filters with pore sizes 25, 10 and 5 µm. These show that the majority of the particles remaining on the filters are larger than the filter pore sizes. Additional particle image analysis with SEM shows that more than 95% of the particles on the filters are larger than the pore sizes, demonstrating that cascade filtration of non-spherical particles with larger dimensions also achieves the desired size fractionation. The maximum amount of sample is ≤100 mg; increasing the sample amount above 100 mg results in clogging of the filter.

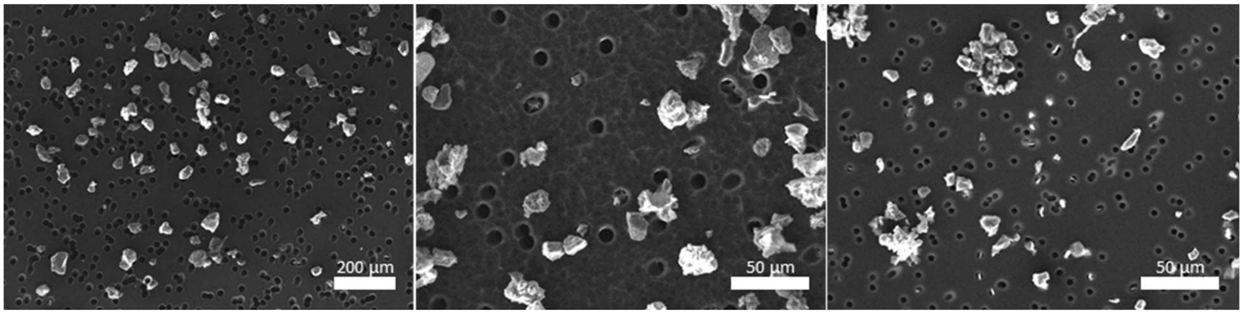


Figure 18: Combusted soil particles on filters with pore sizes 25 (left), 10 (centre) and 5 µm (right).

Following this, tyre wear dust from the road simulator at VTI was tested to investigate if TRWPs, which are often elongated with one small dimension and one long dimension, would also be well fractionated. SEM images from the filters 25 - 0.8 µm are displayed in Figure 19. Figure 19 shows the majority of particles on the filters are larger than the filter pore sizes. While the separation is less optimal than with combusted soil, with occasional smaller particles observed in larger fractions, image analysis with SEM shows that size fractionation is still largely effective with more than 90% of the particles larger than the pore sizes of the filters.

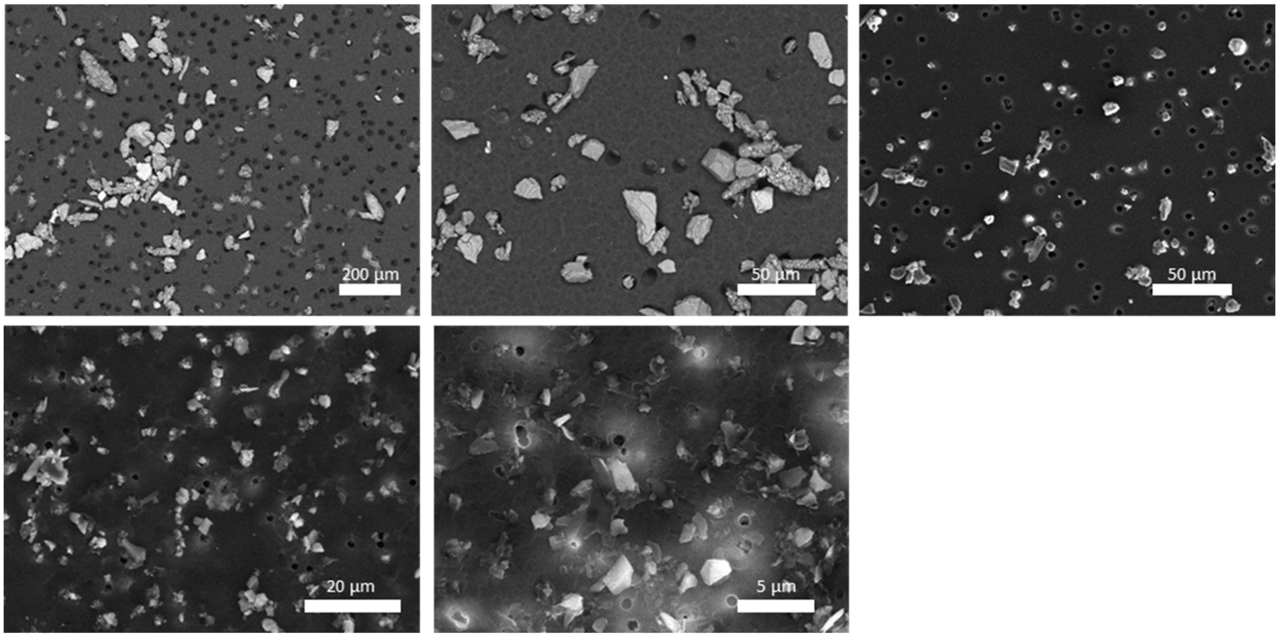


Figure 19: Tyre roadwear particles on filters with pore sizes 25 (upper left), 10 (upper centre), 5 (upper right), 2 (lower left) and 0.8 µm (lower centre).

The original simulated tyre dust sample was analysed using SEM, without undergoing cascade filtration to give a particle size distribution of the whole sample. The PSD determined with the SEM was transformed to a size distribution based on weight and compared with the mass fractions after cascade filtration. This is shown in Figure 20a. The mass distribution based on the cascade filtration correlates reasonably well with the size distribution of TRWP found by SEM analysis, demonstrating an accurate fractionation by cascade filtration. To confirm these observations the cascade fractions were also analysed using TED-GCMS, to determine the actual TWP concentration in the different size fractions. TWP concentrations were calculated using both 4VCH and DP markers and are displayed in Figure 20b, together with the size distribution of TRWP particles found by SEM analysis. Comparable concentrations of TWPs were determined using both markers. The highest concentration of TWPs is found in the 25-190 µm fraction (~60%) with the rest of the mass being found in the >190 µm (~30%) and 10-25 µm (~10%) fractions. Here the size distribution of TWP determined with TED-GCMS correlates even better with the TRWP PSD determined with SEM, indicating that not all of the tyre wear dust in the sample consists of TWP. With SEM this is confirmed as a major part of the tyre wear dust consist of mineral (simulated road wear) particles.

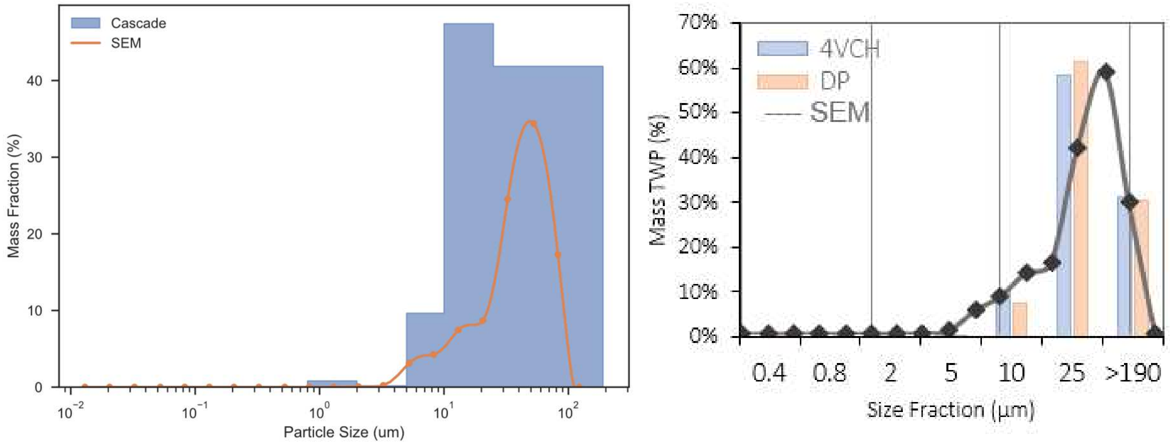


Figure 20: Results cascade filtration of tyre wear sample from the road simulator at VTI, with PSD of total particle mass with cascade setup (left) and PSD of TWP with TED-GCMS (right), both combined with PSD of TRWP determined with SEM.

Finally, in order to determine the effectiveness of the cascade filtration setup for real-world environmental samples, a run-off sample from the highway A61 in Germany was tested. After size fractionation, the filters were analysed using SEM to investigate the effectiveness of fractionation. Unlike the fractionations using combusted soil and simulated TRWPs, the environmental sample was less effectively fractionated. SEM images of the filters with pore sizes 25 - 0.8 µm are displayed in Figure 21. In Figure 21 (left) the particle size distribution of TRWP determined with SEM is compared with the TED-GCMS results of the cascade filtration.

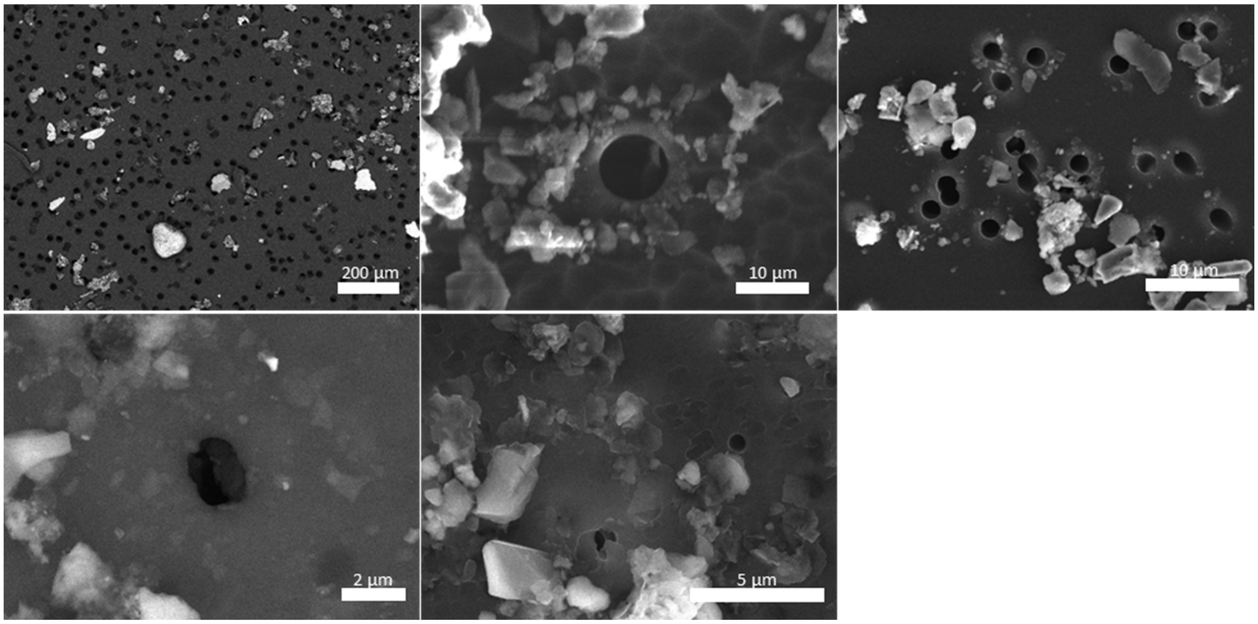


Figure 21: Environmental particles on filters with pore sizes 25 (upper left), 10 (upper centre), 5 (upper right), 2 (lower left) and 0.8 µm (lower centre).

For the environmental runoff sample many smaller particles are visible on the filters, which would be expected to pass through the pores. This is confirmed by the TED-GCMS results; compared with the PSD of TRWP determined with SEM the fractions 5-10 µm and 10-25 µm are underestimated. The lower efficiency of the separation may be caused by the high organic matter content and/or the high agglomeration state of the particles in the environmental sample. The latter may have been caused by the runoff sample being filtered through a glass fibre filter first (for mass determination), after which the material was re-suspended in water for cascade filtration. Also overloading of the filters can potentially hinder the separation. The cascade filtration was performed with 25 mg of runoff material. To investigate if a smaller sample amount gives better results a sample of 10 mg runoff material was run through the cascade filtration setup. The results in Figure 21 (right) show that with a smaller amount of sample the results are much better.

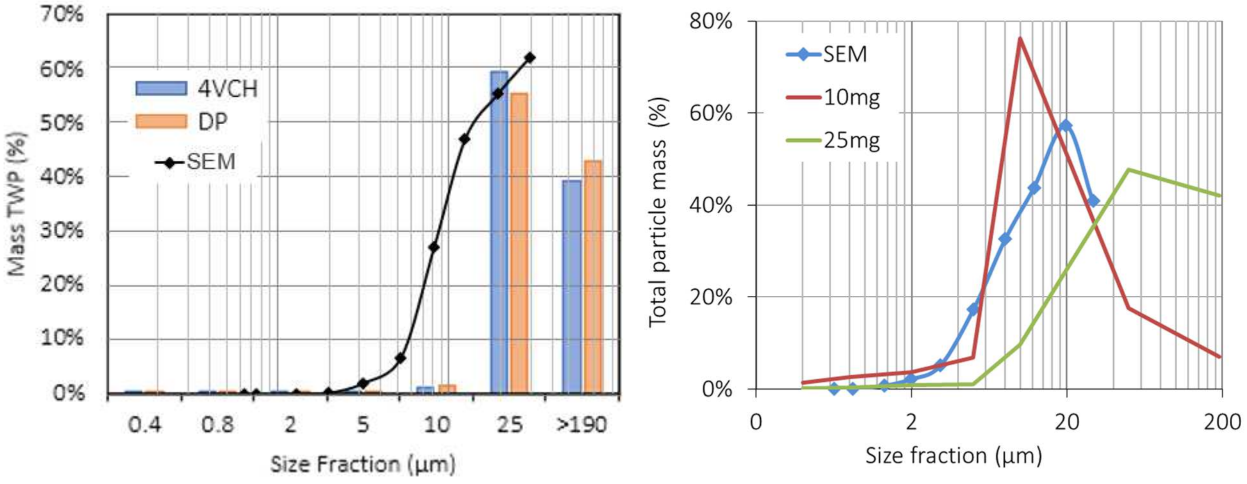


Figure 22: Results cascade filtration of road run-off sample from the A61 in Germany, with the TED-GCMS fractionation results compared PSD of TRWP determined with SEM (left) and the fractionation results of total particle mass with different sample amounts (10 and 25mg) combined with PSD of total particles determined with SEM (right).

Problems with overloading are probably caused by the limited amount of pores on the nucleopore filters. To overcome this problem, it is suggested to replace the filters with poresizes of 10 and 25 µm by stainless steel microsieves, which is expected to allow the material to flow more easily through the cascade filtration setup. It has been demonstrated for the simulated TRWP sample that the cascade filtration in combination with TED-GCMS can provide valuable size information of TWP. With some further optimisations for the fractionation of environmental samples, we believe this will be an easy to use and low cost tool for size information on TWP.

2.3.3 Scanning Electron Microscope Methods

Whilst the development of cascade filtration in combination with thermo-GCMS allows for the generation of mass-size distributions, this also has limitations. The coarse bins don't give a full picture of the particle size distribution (PSD) of a sample, the smallest (and potentially furthest travelling and most harmful) nanoparticles have very little mass so may be under the limit of detection and other physical information such as morphology is lost. Scanning Electron Microscopy (SEM) and other electron microscope based chemical analysis techniques such as EDX and CL, can be used to compliment the thermo-GCMS techniques to fill in these gaps. SEM-EDX(-CL) has previously been used to investigate the morphology of real-world TRWPs compared to simulated tyres, the distribution of elements in a particle and the location of mineral encrustations. However, SEM has the disadvantage that analysis of a statistically-relevant number of particles is time consuming so it is not suitable for routine, high-throughput analysis. In the last few years, developments in artificial intelligence and data analysis have meant that automated SEM analyses are being developed which drastically decrease analysis time and open the door for using SEM for routine analysis. This field is still in its infancy, and we aim to improve the state-of-the-art by developing (semi-)automated SEM analysis procedures for environmental samples.

Depending on the detection mode utilised, SEM-EDX can give an array of information. When measuring secondary electrons (SE), topographic information of the sample surface is gathered whilst back scattered electrons (BSE) give information on sample composition based on relative atomic mass. EDX gives information on elemental composition which is often presented as either a "map" (in combination with one of the previously mentioned detection modes) detailing elemental distribution or as a spectrum. Figure shows a typical TRWP using these detection methods. From the SE image (A) it can be seen that the particle has the frequently reported elongated shape with mineral encrustations. From the BSE image (B) the differences in rubber (dark), mineral encrustations (light gray) and metal encrustations possibly due to brake wear (white) are made clearer. Finally, the EDX data (C+D) shows how TRWPs contain >10 different elements, with C, O and Si being the main constituents. The sulphur (S) observed is present in rubber as a cross-linker and is an important elemental marker confirming that the particle is a TRWP. Another important marker is zinc (Zn), which acts as an accelerator activator and can be found in low concentrations in TRWP, however in aqueous matrices Zn can leach out of the TRWP. None tyre wear elements such as alumina (Al), magnesium (Mg), Potassium (K), Sodium (Na), Titanium (Ti) and Iron (Fe) may be (but not always) present due to road dust (Al, Mg, K), road paint (Ti) and brake wear (Fe).

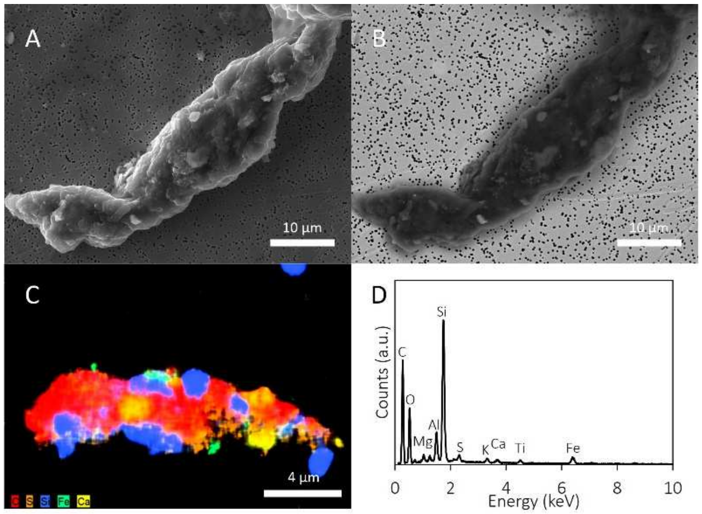


Figure 23: SEM-EDX analysis of a typical TRWP particle as imaged using A) SE detector; B) BSE detector and C) EDX detector. D) EDX spectrum of the whole particle.

To automate SEM-EDX measurements, the Feature analysis tool in the Bruker Esprit EDX software was used. This tool uses image processing and thresholding to generate a binary image from which particles can be recognised. These particles are then analysed with EDX to give full physicochemical characterisation on the single particle level. In order to implement an effective automated measurement system, we need to have confidence that particles are correctly identified and accurately. The first step to testing this was to compare the particle recognition and measuring capabilities of the Features software. For this, nine view fields of the simulated TRWP sample were recorded using SEM-EDX and either analysed automatically using Features, or manually with ImageJ. These results are shown in Figure 24. ImageJ was used as each image can be individually processed to ensure optimal particle recognition, whereas Features applies the same image processing to every view field. In this analysis, Features recognized and measured 924 particles as opposed to the 855 measured manually using ImageJ. This suggests that due to the application of generalized image processing parameters, Features overestimates the number of particles by ~7%. The particle size distributions generated from automated measurements correlated well with the manually measured results.

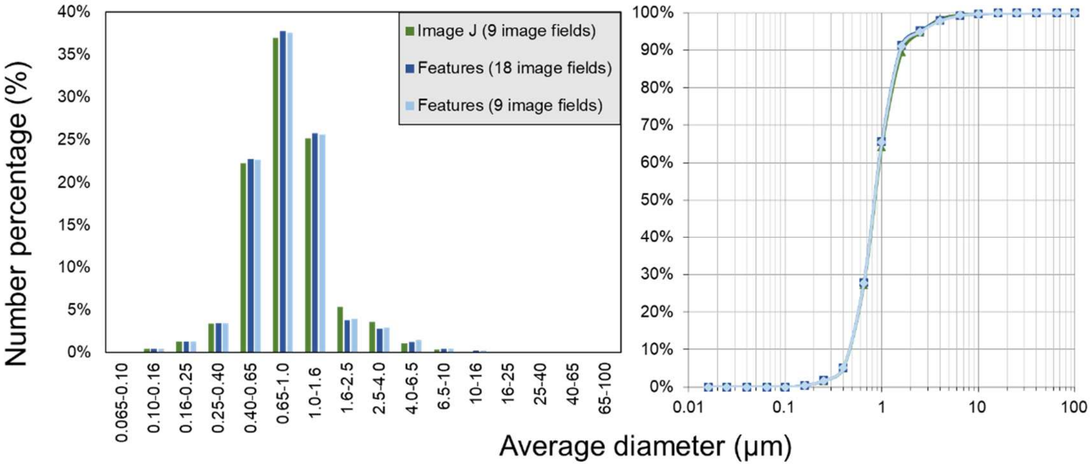


Figure 24: Comparison between manual (ImageJ) and automated (Features) recognition and measurement of particles with SEM-EDX.

Particle sizes measured through image processing are defined as the equivalent circle diameter (ECD): the diameter of a circle of the same area as the particle. As particles are not regular shapes it was desirable to compare the ECD with the manually measured sizes (which is often biased) as many previous microscopic studies have relied on manual measuring. In order to verify this, 50 particles from the deposition and soil samples were measured both manually and automatically. From these 50 particles, five distinct particle morphologies, square, irregular, elongated, oval and triangular, were observed. Examples of these five morphologies, along with a comparison of the automated ECD and manual size measurements are presented in Figure 25. The automatically measured sizes correlates well with the manually measured sizes, with almost all particles within 20% error. Spherical, irregular and triangular particles correlated very well, likely as these particles are most similar to a circle, whereas the size of square particles tends to be overestimated and that of elongated particles is generally underestimated.

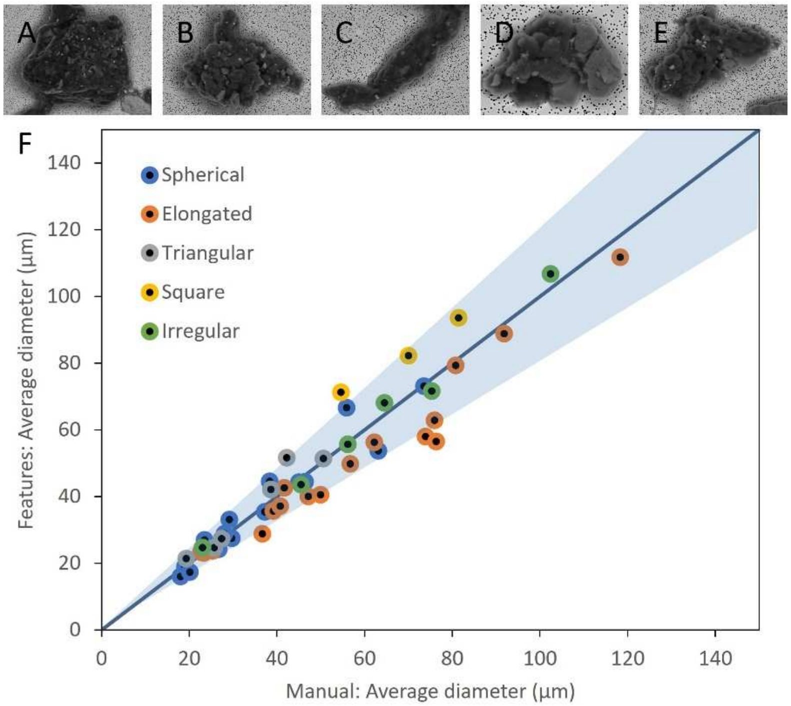


Figure 25: Examples of the differ TRWP morphologies observed (A-E) and a comparison of the particle size measured manually and automatically (F).

The final step in automated analysis is making sure the software can correctly classify particles as TRWPs or not, based on their physicochemical properties. Given the widely varying morphology of the particles observed, it was decided that classification based only on chemical composition would be the most effective approach. The same 50 particles were also measured using EDX to determine the elemental composition and this is shown in Table 4. The composition was mainly C and O, although the concentration of these varied widely; lower concentrations of Si, Al and Mg were always present due to encrustations of road wear. Zn and S, typical elemental markers, were always present in concentrations <2%. Numerous other metals were observed in trace amounts which are like due to road paint (Ti) and brake wear (Copper (Cu) and Fe).

Table 4: Composition of environmental TRWPs determined using SEM-EDX.

	Mass (%)												
	C	O	Na	Mg	Al	Si	S	K	Ca	Ti	Fe	Cu	Zn
Average	55,5 ±8,7	28,3 ±6,9	0,3 ±0,1	0,7 ±0,5	2,7 ±1,9	6,3 ±2,7	1,0 ±0,4	0,5 ±0,3	0,7 ±0,5	0,3 ±0,2	2,8 ±1,4	0,3 ±0,2	0,6 ±0,4
Min.	36,1	14,6	0,1	0,2	0,7	1,0	0,3	0,1	0,1	0,1	0,6	0,1	0,2
Max.	78,5	40,3	0,6	3,3	13,3	12,9	2,0	1,4	2,1	0,8	7,2	1,1	2,0

The elemental concentrations shown in Table 4 were used as boundary conditions for the classification of TRWPs automatically measured. To test the accuracy of classification, >2000 particles were measured using SEM-EDX and the concentration of particles classified as TRWPs was compared to the TRWP concentration of the same sample as determined with the TED-GCMS method. The concentrations determined by automated SEM-EDX and TED-GCMS were 9.3% and 13%, respectively, suggesting that the classification by SEM-EDX is accurately classifying TRWPs. Whilst these results are satisfactory, work on automated classification are ongoing with a machine learning (ML) classifier being trained to identify particles based on both physical and chemical information. It is hoped that in the future such a ML model will be able to identify not only TRWPs in environmental samples but also brake wear, microplastics and other environmental contaminants to provide more information from measurement campaigns.

Whilst SEM-EDX gives a plethora of physicochemical information, it has limitations. Many carbon-based materials cannot be distinguished from each other. For example many plastics such as polyethylene, polypropylene and polystyrene cannot be differentiated by SEM-EDX. Another electron microscope based spectroscopic technique that can be used for further chemical identification of particles is cathodoluminescence. Cathodoluminescence (CL) is the emission of light generated when a material is excited with an electron beam. By equipping an electron microscope with a cathodoluminescence detector, this spectral information can also be coupled with spatial information on the same length scale as the microscope. Materials are excited through the electron beam inside the microscope. As this energy is relatively high, this excitation will result in energy loss through a diverse number of processes among which fluorescence, recombination-induced luminescence and chemiluminescence are examples. The light is emitted in a wide spectral range ranging from the ultraviolet through the mid-infrared and is dependent on the optical properties of the underlying material. This luminescence is affected by factors such as electronic composition and microstructure.[84] It has recently been shown that using SEM-CL, (micro)plastics can be positively identified which would not normally be possible with other techniques due to size (µ-FTIR, µ-Raman) or chemical composition (SEM-EDX).

We would like to investigate whether SEM-CL can contribute to the advancement of TRWP characterisation, either through facilitating identification or elucidating new details on chemical composition, transformations and distribution. CL data, like EDX, can be used for spectral analysis of certain locations or to give information on distribution by mapping signal intensity in combination with different filters. This is shown in Figure 26. The spectrum shows a sharp peak at ~370 nm which, along with the smaller peak at ~770 nm is attributed to ZnO.[85] There is also a broad, weak peak centred around 600 nm which may be due to the organic rubber. Such a unique spectrum with definable peaks suggests that this spectrum may be used for identification of TRWPs, however, further measurements are necessary to investigate the variability in spectra due to the heterogeneous nature of the particles. It has been reported in literature that a peak *ca.* 415 nm is due to carbonyl (C=O) bonds and can be indicative of microplastic degradation. In the later parts of this project, tyre degradation will be investigated. With the generated degraded TRWPs we intend to investigate if this is also applicable to rubber particles to give more information on the chemical processes behind degradation.

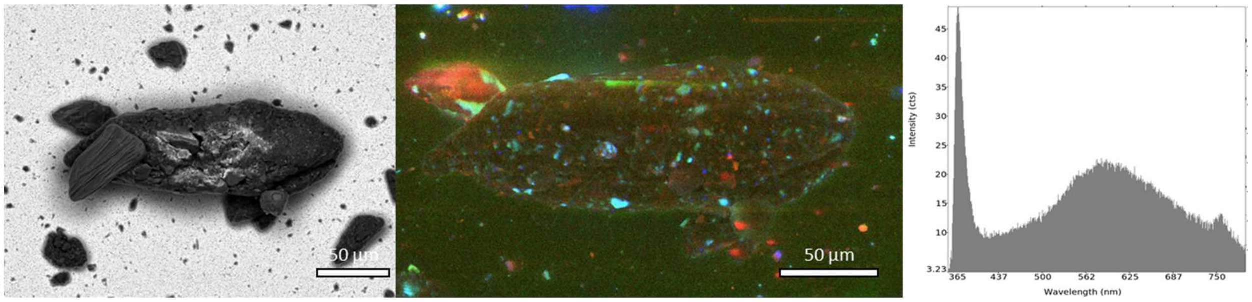


Figure 26: SEM BSE image (left), CL intensity map (middle) and CL spectrum (right) of a TRWP.

By filtering out certain wavelengths of emitted light, it is possible to map the distribution of components in a TRWP. For example, in Figure 26, light emitted at ~400, 550 and 700 nm are shown as dark blue, green and red, respectively. We discussed previously how ZnO exhibits strong emission at ~370 nm, based on this the small dark blue spots are likely due to ZnO.[85] This enables us to identify the distribution of key components in particles. This can also be complimentary to EDX mapping. EDX signal intensity is based on the elemental concentration, which for Zn is relatively low and so is not so easily measured. CL signal intensity, on the other hand, is dependent on the electronic transitions in the molecules which for inorganic compounds such as ZnO are much higher than for organic polymers. Therefore a combination of EDX- and CL-mapping may allow for a more detailed understanding of component distribution than is currently available.

In conclusion, efforts were made to automate TRWP classification and measurement in order to increase throughput and the applicability of SEM-EDX measurements for environmental samples. The accuracy of the automated SEM-EDX to both recognise and measure particles was tested by comparison with manual measurements and deemed to be satisfactory with a recognition error of 7% and good correlation between manual and automated measurement. The average elemental composition of TRWPs was determined through the measurement of 50 real-world particles and used as a classification tool. Classification accuracy was tested on an environmental sample of known concentration determined by TED-GCMS. The concentration determined by automated SEM-EDX classification (9.3%) was comparable to that determined by TED-GCMS (13%), indicating that such a system is suitable for automated analysis of TRWPs in environmental samples. Finally, analysis using SEM-CL was demonstrated for the first time. Initial results show that this technique is potentially advantageous for the identification of TRWPs and may be used in combination with SEM-EDX to give a more complete picture of component distribution through particles.

2.3.4 Summary

We have demonstrated improvements to the state-of-the-art for both the thermo-analytical method and single particle analysis methods using SEM-EDX(-CL). A new TED-GCMS method was developed in which TWP concentration is calculated directly from marker GC response. This direct conversion factor was determined based on a large tyre pyrolysis dataset, taking into account variations in tyre composition between countries, car and truck tyres and over a ten-year period. Analysis showed that the markers 4VCH and SB were most reliable but, depending on the matrix, can give different quantification results.

A cascade filtration system was tested to provide size fractionation of samples before TED-GCMS analysis. Whilst the sample was optimized for simulated TRWPs, showing an accurate size fractionation and working in tandem with TED-GCMS to give TRWP concentration for the different size fractions, it was less effective when used with a runoff sample, possibly due to the combination of high particle load, high agglomeration state of the particles and high concentrations of organic material. Lowering the amount of sample improved the fractionating considerable. It is recommended to continue to optimize this method by investigating other surfactants and the use of stainless steel microsieves in order to increase flow through the system in attempt to improve fractionation.

Finally, SEM-EDX analysis were improved to allow for the automatic classification and measurement of TRWP in environmental matrices. Tests of the accuracy of the recognition, measurement and classification were all satisfactory allowing this method to be used in the upcoming measurement campaign. We also demonstrated, for the first time, the use of CL spectroscopy for TRWP analysis. We demonstrate how it could be used to aid in identification of TRWPs through their unique spectrum as well as give information on the distribution of inorganic components that are usually of too low a concentration to be mapped by SEM-EDX.

3 Conclusions

Our research has been aimed at optimising pre-treatment and analysis methods for TRWPs in environmental samples and developing them beyond the state-of-the-art. Our novel approach to be used in this measurement campaign is summarised in Figure 27. Environmental samples will be collected using air, deposition, water and soil/sediment samplers. Depending on the collected sample matrix, different pre-treatment methods will be employed. Air samples require no pre-treatment and will be analysed directly. Water samples will first be concentrated through freeze-drying and then can be worked up as dry powder samples in the same way as soil/sediment samples.

Dried samples require two pre-treatment steps; a first to remove organic matrix components and a second to remove inorganic. To remove organic components, a digestion can be used. We have investigated three oxidation-based digestions using H_2O_2, Fenton's reagent and NaClO. NaClO was shown to be unsuitable due to low selectivity towards environmental matter and strong attack towards rubber, particularly synthetic rubber. While H_2O_2 did not digest TRWPs, it was also not so active against environmental matter. Fenton's reagent was shown to be the optimal digestion reagent as it had high activity against matrix matter whilst also not reacting with TRWPs. While the separation method has been optimised so far as recovery of the sample is concerned, further optimisation of the solution density is required. Whilst 2.4 g/ml seemed a logical choice based on literature values for the densities of TRWPs and soil components, our results suggest that this solution is too heavy. Investigation into the optimal solution densities should be conducted with a focus on investigating SPT solutions with densities of 1.7 – 2.2 g/ml.

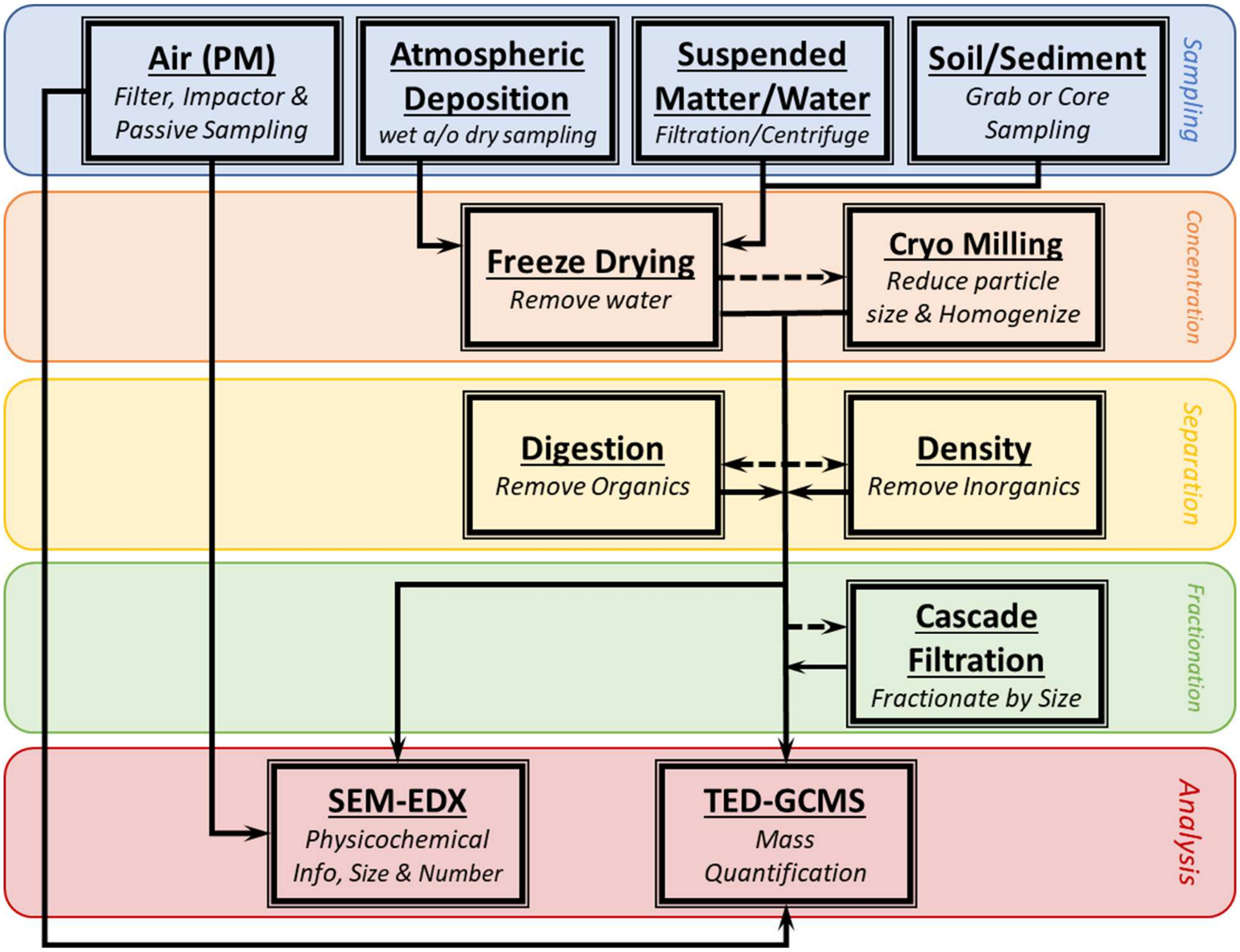


Figure 27: Flow diagram illustrating an optimised workflow for the sampling, pre-treatment and analysis of environmental TRWP samples.

Sample analysis will be performed in two ways: bulk analysis for information on environmental TRWP concentrations with a coarse size indication and single particle analysis for detailed information on the particle size distribution and particle morphology. For bulk analysis, a novel TED-GCMS method has been developed on the basis of the largest tyre pyrolysis dataset to date. A survey of current literature methods was performed and it was determined that the current downfalls are due to insufficient datasets on which to based quantify SBR and NR from rubber markers and poor assumptions in conversion factors for indirect TRWP determination from SBR and NR concentration. With a wide-reaching dataset of 74 tyres from literature and our own experiments, spanning three countries, ten years and both cars and trucks we determined what we believe are the most accurate conversion factors to date. TWP concentration is also directly calculated from marker concentrations, avoiding the uncertainty of a second conversion factor. 4VCH was chosen as the optimal marker for quantification, as opposed to the commonly used SB and DP, as it showed the lowest variation between samples, leading to the most reliable quantification. We have also shown that the variation in tyre composition at any one time is far larger than the variation in tyres across different years, seasons and vehicle types, demonstrating the reliability of these conversions across different times and locations. Finally, a comparison of calibration methods and use of standards has shown that our combination of external calibration and internal standard gives a concentration comparable to that with standard addition but with a lower variance.

To add coarse size information to the bulk analysis, a sequential filtration system was investigated. Initial studies showed that this system fractionated model nanoplastics, relevantly sized environmental components, and simulated TRWPs successfully. However, when untreated environmental samples were tested it was shown that many small particles were retained with the larger fractions meaning the use of such a system is limited. Work to optimise the filtration set-up is recommended. If an optimal solution is found before the measurement campaign, this will also be applied.

Finally, single particle analysis is carried out using SEM-EDX(-CL). While this is a very powerful technique due to the detailed physicochemical information that can be gathered, it suffers in that analysis of statistically relevant numbers of particles is slow and often impractical in samples with a large contribution from matrix particles. In order to address these problems, we developed an automated analysis procedure which could positively classify and measure TRWPs in environmental matrices. The automated analyses were assessed for accuracy in recognition and measurement through comparison with manual measurements and for classification by comparison with TED-GCMS measurements. Recognition, measurement and classification were all deemed to be suitable for the implementation of this method for the automated analysis of environmental samples.

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5 Appendix

5.1 Experimental Methods

Quality control

During the experiments, a cotton lab coat was worn. All used glassware was rinsed prior to use with MilliQ water. Filtration experiments were conducted inside a laminar flow cabinet (Esco) to minimize contamination from the lab environment. Nitrile gloves were worn whenever the samples were handled. Beakers and Erlenmeyer were covered with aluminium foil to prevent airborne contamination. Use of plastic equipment was avoided as much as possible and otherwise accounted for by measuring blanks.

Freeze Drying

Approximately 600 ml sample was added to a 850 ml sample vial and frozen at -40 °C for 24 h. The frozen samples were then transferred to a Lablyo Mini freeze dryer where they were kept under vacuum at -50 °C until dry.

Digestion procedures

Preparation of iron sulphate buffer[31]

16 g Iron(II) sulphate heptahydrate was dissolved in 800 mL of MilliQ water. The solution was stirred under room temperature until the salt fully dissolved. The pH was adjusted to a pH between 3-5 using concentrated sulphuric acid (98 %, SIGMA ALDRICH). The solution was stored at 4°C. (Final concentration: 20 mg ml^{-1})

Fenton's reaction[31,86]

Ca. 30 mg of sample was added to a 50 mL Erlenmeyer flask. 1 mL of catalyst solution (iron sulphate buffer) was added. The mixture was shaken slightly to ensure good mixing of the sample with the solution. The flask was placed in an ice bath. While cooled, 2 mL of 30 % (v/v) H_2O_2 solution was added. Upon addition the solution turned orange. After ~4 hours, or until no bubbles were observed, concentrated sulphuric acid (XX %, SIGMA ALDRICH) was added to the solution, dissolving the formed iron precipitates. The solution was filtered and the mass was determined.

Hydrogen peroxide reaction[25]

Ca. 30 mg of sample was added to a 20 mL sample vial. 5 mL hydrogen peroxide solution (30 % (v/v)) was added and the vial was shaken slightly. The reaction mixture was heated for a period of 24 hours at elevated temperature (55 °C) using a water bath. After reaction, the solution was filtered and the mass was determined.

Hypochlorite reaction[25]

Ca. 30 mg of sample was added to a 20 mL sample vial. 5 mL hypochlorite solution (6-14 %) was added and the vial was shaken slightly. The reaction mixture was exposed to the hypochlorite solution for 24 hours, of which 16 hours on elevated temperature (55 °C) using a water bath. After reaction, the solution was filtered and the mass was determined.

Density Separation

Preparation of salt-solutions

For the sodium chloride (NaCl) solution 80 g NaCl was dissolved in 250 mL MilliQ obtaining a density of 1.18 g/cm^3. For the sodium polytungstate (SPT) solution 70 g of SPT was dissolved in 25 mL MilliQ and a density of 2.45 g/cm^3 was obtained. The solutions were stirred under room temperature until the salts were fully dissolved. The densities of the solutions were determined by transferring 100 µL of salt-solution onto an analytical balance. The solutions were kept in glass bottles (1 L and 250 mL) and stored at room temperature.

General protocol for optimized density separation method

A 50 mL separatory funnel was filled with 2.5 mL salt-solution and 50 µL Tween 20, before 30 mg sample was transferred into the separatory funnel. After the sample was added, an additional 2.5 mL salt-solution was added to the separatory funnel. The mixture was shaken and left to settle. After one hour, the bottom layer was separated from the top layer and poured inside a glass beaker. 2 mL salt-solution was added to the separatory funnel and the mixture was shaken and left for an additional ten minutes. The bottom layer was again separated. This step was repeated two times. Thereafter the top layer was removed from the separatory funnel as well by pouring it into another glass beaker. The separatory funnel was rinsed with salt-solution to ensure maximum recoveries.

Determination of organic content

The organic content of samples was determined by weighing samples before and after combustion inside muffle furnace. The oven program of the muffle furnace starts with a ramp of 3.0 °C/min to a temperature of 300°C, this was held for 2 hours. Followed by a ramp of 1.5 °C/min to increase the temperature to 800°C as final temperature. The final temperature was held for 10 hours, making the total run time 19 hours. After cooling down to room temperature, the samples were weighed using an analytical balance (CXXXX).

Cascade Filtration System

The cascade filtration setup consisted of six 25 mm Swin-Lok Filter holders stacked together. Figure 28 shows a schematic drawing of the Swin-Lok filter holder. Additionally, the setup was run with a combination of Sartorius Stedim stainless steel filter holders and Swin-Lok filter holders, where the stainless steel filter holders were used for the filters with the greatest pore sizes. The filter holders were placed in an ultrasonic bath (VWR ultrasonic cleaner USC-TH). Pre-weighed filters with pore sizes 25, 10, 5, 2, 0.8 and 0.4 µm were used in the cascade filtration. The 25 and 5 µm filters were ipPORE track-etched membrane polycarbonate filters, while the 10, 2, 0.8 and 0.4 µm filters were Nuclepore track-etch membrane filters. The fraction >190 µm was collected using a Swin-Lok filter holder with a 190 µm sieve.

The solution consisted of 100 mL of demi water with 1 drop of TWEEN20 (SIGMA ALDRICH) for every 25 mg of material. The solution was forced through the filter holders using a peristaltic pump, at an RPM of 60. Afterwards the setup was rinsed with an equal amount of MilliQ as the starting volume. The recovered material was determined gravimetrically using a Mettler Toledo AX205.

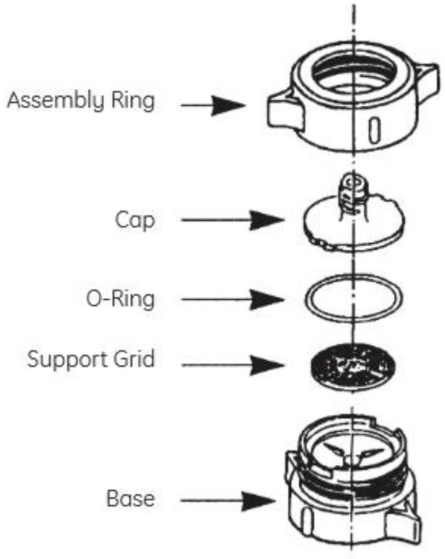


Figure 28: Schematic drawing of the Swin-Lok Filter holder. The filter is positioned between the O-ring and the support grid.

SEM-EDX (-CL) Measurements

Samples were prepared for SEM/EDX analysis either by filtration or by transferring a small amount of sample onto an aluminium SEM-stub covered with carbon coated tape. Filtration was done by pouring samples over 25 mm gold coated polycarbonate filters under reduced pressure (200 mbar) with pore sizes of 0.8 µm, 0.4 µm and 0.1 µm (TJ Environmental). After filtration, the filter was transferred onto an aluminium SEM-stub covered with a carbon coated tape. The samples were coated with a thin conductive surface film of carbon using a carbon evaporator (Quorum Q150 T carbon evaporator) to make the particles conductive for SEM/EDX analyses.

SEM analyses were performed with a Tescan MAIA 3 GMH field emission scanning electron microscope, equipped with a Bruker Quantax 30 mm^2 silicon drift detector for energy dispersive X-ray microanalyses (EDXMA) and a Delmic SPARC-Spectral system equipped with a high resolution CCD camera (Andor Newton DU920P) mounted on a SPARC ATM spectrograph for cathodoluminescence (CL) measurements. CL spectra were recorded using the hyperspectral imaging mode with the VIS-NIR optical module optimized for measurements in the λ = 400 – 1000 nm range. The measurement were performed using a 300 lines per mm ruled grating, blazed for a free space wavelength at λ = 500 nm with a resolution of 0.96 nm and centred at λ = 550 nm. To excite the particles, a beam energy of 15 keV was applied to the material. The entrance slit in front of the spectrograph was closed to 200 µm. Hardware binning of the CCD pixels (full vertical binning and 2 times horizontally) was applied to improve the signal-to-noise ratio (SNR). Typical integration times of 200 ms/pixel were applied.

SEM images were recorded in the so-called secondary electron (SE) (highlighting surface topography) and backscattered electron (BSE) (highlighting atomic number contrast) modes. The SEM was operated using an acceleration voltage of 15 kV.

TED-GCMS

Samples were prepared for thermal analysis by filtering samples onto a 25 mm glass-fibre filter under reduced pressure (200 mbar). After filtration, the filter was transferred into a glass petri dish and dried for overnight at 55 °C. Prior to the analysis the mass on the filters was determined.

The samples were pyrolysed with a tube oven (Naberteherm) at a temperature of 550°C for 15 minutes under a flow of 50 mL/min N_2. The thermal desorption took place at a temperature of 260°C for 10 minutes with a split flow of 40 mL/min. The GC temperature gradient started at 40°C held for 1 min, followed by a ramp of 8°C/min to 112°C, which was followed by a ramp of 12°C/min to 232°C, this was followed by a ramp of 16°C/min to 296°C. The final temperature was held for 6 minutes. For the MS the scan range was 29-300 amu with detector gain 0.5.

5.2 Overview Analytical techniques and Analysis Scheme for Environmental Samples

Table 5:Overview of analytical techniques to be used in the measurment campaign.

	Technique	Chemical compound / characteristics	Relevancy / remark
(Thermo)-analytical techniques	Thermal-optical EC-OC analyser (elemental/organic carbon)	Carbon black (additive) and total rubber content	EC in $PM_{2.5-10}$ can be used as an alternative marker for quantification of TWP in air
	Thermal extraction and desorption GCMS (TED-GCMS)	Rubber/bitumen (TWP-markers: 4-VCH, SB, dipentene)	Quantification of TWP in all environmental matrices
	Liquid chromatography in combination with tandem mass spectrometry (LCMSMS)	Benzothiazoles & derivatives (vulcanization accelerator)	Benzothiazoles can be used as a marker for quantification of TWP (may be released as small particles and volatiles when tyre gets hot)
		Amines (anti-oxidants)	Organic pollutants
		Oxygenated compounds	Chemical transformation products
	DNPH-derivatization and liquid chromatography in combination with UV spectrophotometry (LC-UV)	Carbonylcompounds (aldehydes & ketones)	Chemical transformation products
	Gas chromatography in combination with mass spectrometry (GCMS)	Polycyclic aromatic hydrocarbons (PAH) (extender oils)	Organic pollutants
	Gas chromatography in combination with mass spectrometry in negative ionisation mode (GC-NCI-MS)	Nitro and oxygenated PAH	Chemical transformation products of PAH
	Thermal desorption gas chromatography in combination with mass spectrometry (DTD/TD-GCMS)	Volatile and semi-volatile organic compounds (oils and resins)	Screening (S)VOCs in air
	High resolution inductively coupled plasma mass spectrometer (HR-ICP-MS)	Zinc oxide (accelerator activator) and other elements	Screening heavy metals, zinc was often used as a marker of TWP
Microscopic techniques	High resolution scanning electron microscopy with energy dispersive X-ray analysis and cathodeluminesense (FEG-SEM-EDX/CL) combined with automated image analysis	Counting and physico-chemical properties (PSD, elemental composition) particles	Identification and quantification tyre related submicron and ultrafine particles, differentiation TWP and RWP, particle size distribution (PSD)
	Infrared microscopy (µFTIR)	Identification of rubber/bitumen	Identification of rubber in tyre tread particles > 10 – 20 µm
	Light microscopy (in combination with visible-, fluorescence- and polarized light) combined with automated image analysis	Counting & physical properties particles	Quantification and size distribution of tyre tread particles > 10 – 20 µm

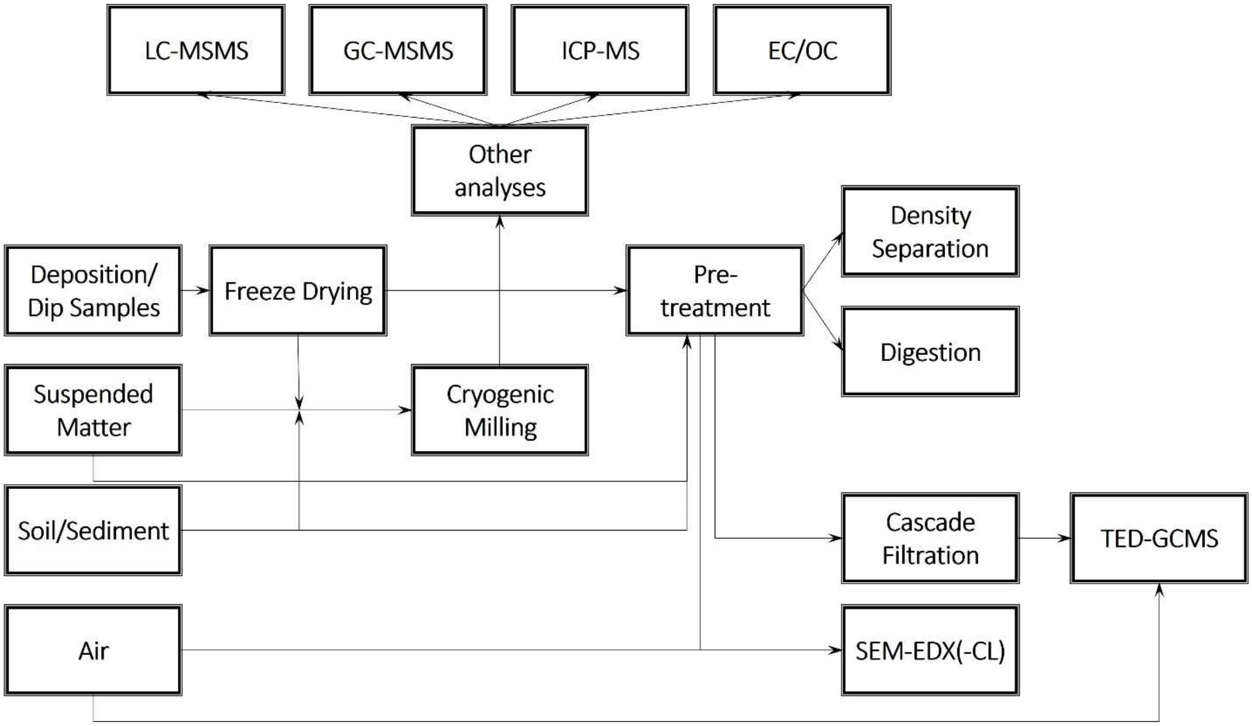


Figure 29: Scheme of analysis techniques to be used in the measurement campaign.

5.3 Identification of Filler Materials in Pristine Tyre Samples

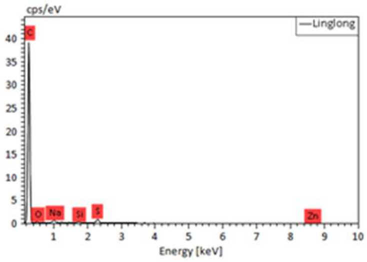
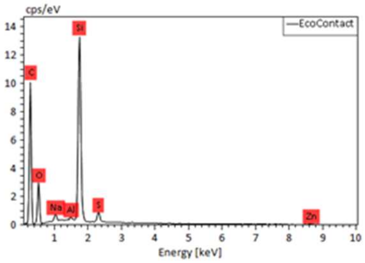
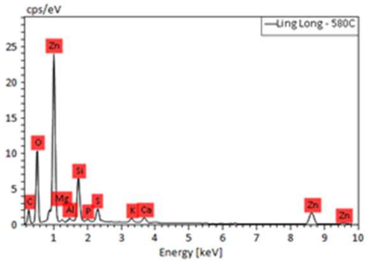
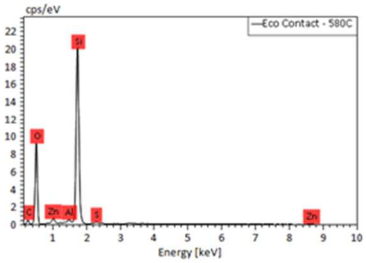
	Linglong	Continental
EDX Before		
Comp. Before	C: 96.3% O: 2.9% Na, Si, S, Zn: <0.5%	C: 73.3% O: 17.1% Si: 8.4% Na, Al, S, Zn: ≤ 0.5%
Loss on Ignition	95%	61%
EDX After		
Comp. After	O: 57.5% Zn: 26.3% Si 8.8% S: 3.8% Al: 0.9%	O: 67.1% Si: 31.4% Al: 0.6% S, Zn: ≤ 0.5%

Figure 30: Elemental analysis of the budget and executive tyres.

5.4 Total Ion Chromatograms of Budget and Executive Pristine TWPs and Simulated TRWPs

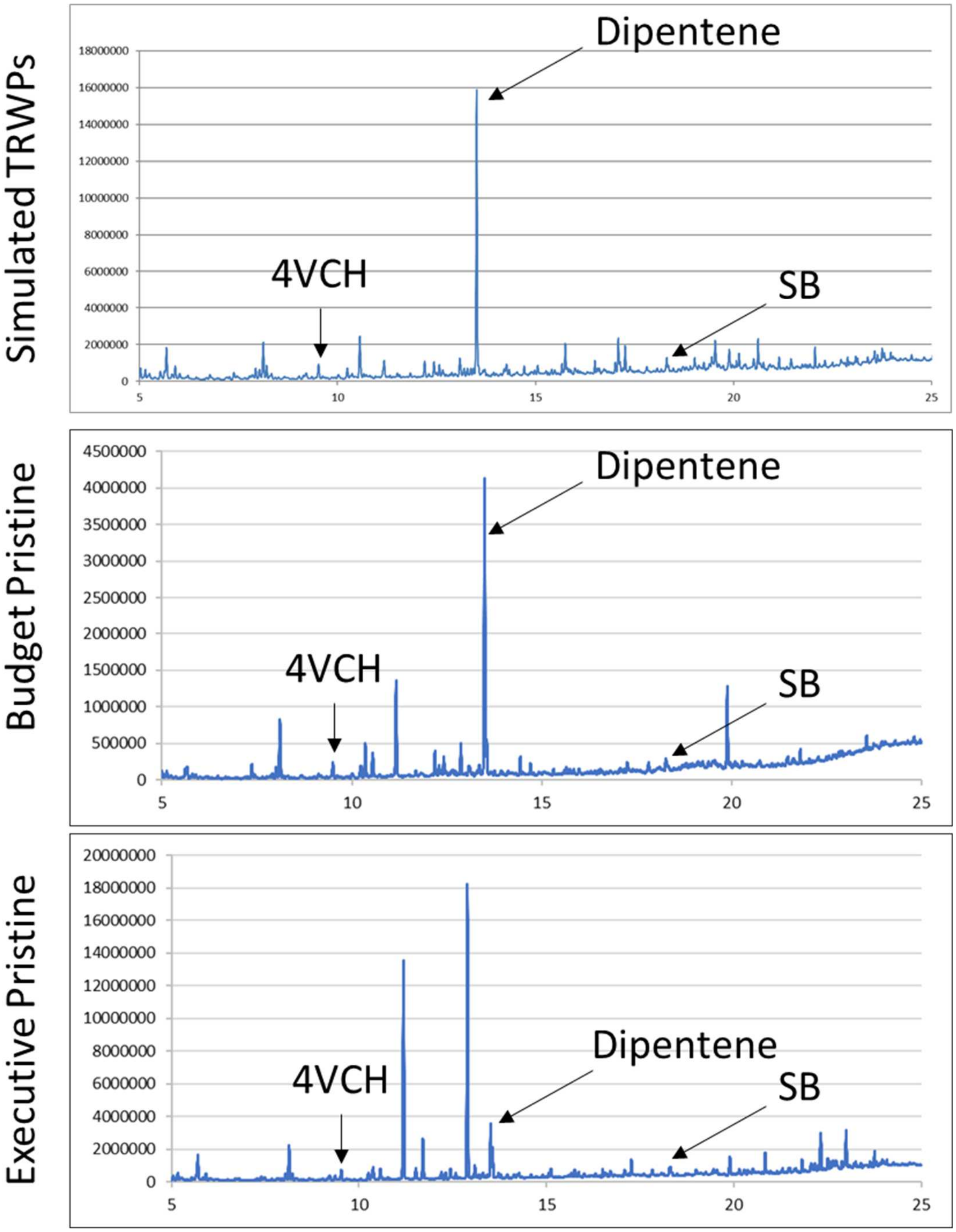


Figure 31: TED-GCMS analysis of the budget, executive and simulated T(R)WPs