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Analysis of studies and research projects regarding the detection of nanomaterials in different environmental compartments and deduction of need for action regarding method development

Final Report

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Analysis of studies and research projects regarding the detection of nanomaterials in different environmental compartments and deduction of need for action regarding method development

Final Report

by

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Abstract: „Analysis of studies and research projects regarding the detection of nanomaterials in different environmental compartments and deduction of need for action regarding method development"

The aim of the present report was to obtain an overview of current strategies and methods for the detection of (manufactured) nanomaterials (NMs) in the environmental compartments surface water, soil, sediment, air, biota and sewage sludge. Based on this several recommendations for future needs of action in the short to long term are derived in order to establish a standardized detection of NMs in the environment that is necessary in order to check the pollution in the environment, to check whether or not potential risk management measures take the intended effect and to validate NM release models with real data.

A literature review was performed using predominantly "Web of Science" and screening for literature, such as review articles summarising the state of the art of NM detection techniques for environmental samples. More than 160 scientific publications were evaluated concerning NM detection methods. Results of the literature survey clearly show that a combination of detection techniques is necessary in order to detect and identify NMs, and to differentiate between natural NMs and manufactured NMs. The crucial step is accurate sample preparation for the selected detection method which means in most cases complete removal of the (disturbing) matrix and transfer of the NM in appropriate media for measurement. So far field studies in terms of detection of unknown amounts of unspecific engineered NMs in natural samples are rare and only existing for a few compartments, mainly surface waters.

Hence, it is concluded that the need of action is focused on the development, standardization and validation of existing methods in a combinatory approach.

Kurzbeschreibung: „Nachweis von Nanomaterialien in den verschiedenen Umweltkompartimenten: Auswertung von Studien und Projekten zum Nachweis von Nanomaterialien in den verschiedenen Umweltkompartimenten und Ableitung eines Handlungsbedarfs bezüglich der Methodenentwicklung

Das Ziel der vorliegenden Studie war die Erstellung eines Überblicks verschiedener Strategien und Techniken, die bei der Detektion von (technischen) Nanomaterialien in den Umweltkompartimenten Oberflächenwasser, Boden, Sediment, Luft, Biota und Klärschlamm zum Einsatz kommen. Darauf basierend werden kurz- bis langfristige Handlungsempfehlungen für die Etablierung von standardisierten Messprotokollen für die Detektion von NM in der Umwelt abgeleitet. Diese Messungen dienen der Überprüfung der Belastungssituation in der Umwelt, können helfen zu prüfen, ob mögliche regulative Maßnahmen greifen und unterstützen die Anwendbarkeit von Resultaten aus Expositionsmodellen anhand des Vergleichs mit Realdaten.

Vor allem durch Verwendung des „Web of Science" wurde eine Literaturrecherche nach Übersichtsarbeiten, die den gegenwärtigen Stand der Technik der NM-Detektion in Umweltmedien zusammenfassen, vorgenommen. Über 160 wissenschaftliche Publikationen wurden im Hinblick auf NM-Detektion evaluiert. Die Ergebnisse dieser Literaturstudie zeigen deutlich, dass eine Methoden-Kombination zur Detektion und Identifizierung von NM nötig ist und welche insbesondere die Unterscheidung von natürlichen NM und technischen NM zulässt. Der wichtigste Schritt ist hierbei die Probenvorbereitung für die gewählte Detektionsmethode, die in den meisten Fällen die komplette Entfernung der (störenden) Matrix und einen Transfer der NM in ein für die Messung geeignetes Medium einschließt. Gegenwärtig herrscht ein Mangel an Feldstudien zur Detektion unbekannter Konzentrationen von nicht weiter spezifizierten technischen NM in Umweltproben. Feldstudien existieren nur für wenige Umweltkompartimente, hauptsächlich Oberflächenwässer.

Die Schlussfolgerungen identifizieren den Handlungsbedarf dementsprechend mit dem Hauptaugenmerk auf der Entwicklung, Standardisierung und Validierung eines Kombinationsansatzes existierender Methoden.

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List of abbreviations

AF^4	asymmetrical flow field-flow fractionation
AFM	atomic force microscopy
APCI	atmospheric pressure chemical ionization
AUC	analytical ultracentrifugation
autoSEM	automated scanning electron microscopy
CBED	convergent-beam electron diffraction
CCD	charge coupled device
CE	capillary electrophoresis
CFM	confocal fluorescence microscopy
CNT	carbon nanotubes
CPE	cloud point extraction
DiSC	diffusion size classifier
DLS	dynamic light scattering
EDTA	ethylenediaminetetraacetic acid
EDX/EDS	energy dispersive X-ray spectroscopy
EELS	electron energy loss spectroscopy
ELPI+^{TM}	electrical low pressure impactor
ELS	electrophoretic light scattering
ENPs	engineered nanoparticles
ESEM	environmental scanning electron microscopy
ESI	electrospray ionization
ETEM	environmental transmission electron microscopy
FESEM	field emission scanning electron microscopy
FFF	field flow fractionation
FMPS^{TM}	fast mobility particle sizer
GC-MS	gas chromatography mass spectrometry
HDC	hydrodynamic chromatography
HPLC	high performance liquid chromatography
HEDFM	hyperspectral-enhanced dark field microscopy
ICP-AES	inductively coupled plasma atom emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
ICP-TOF-MS	inductively coupled plasma time of flight mass spectrometry
LC	liquid chromatography
LOD	limit of detection
MALDI	matrix-assisted laser desorption/ionization
MALS	multi angle light scattering

MALLS	multi angle laser light scattering
MC-ICP-MS	multi collector inductively coupled plasma mass spectrometry
miniDISC	miniature diffusion size classifier
Nano-ID^{TM}	nanoparticle spectrometer model NPS500
NMs	manufactured nanomaterials
NP	nanoparticle
NTA	nanoparticle tracking analysis
PAH	polycyclic aromatic hydrocarbons
PCC	particle collision coulometry
PEC	predicted environmental concentration
PM	particulate matter
SAED	selected-area electron diffraction
SEC	size exclusion chromatography
SEM	scanning electron microscopy
SMPS^{TM}	scanning mobility particle sizer
SLS	static light scattering
STEM	scanning transmission electron microscopy
STP	sewage treatment plant
sp-ICP-MS	single particle inductively coupled plasma mass spectrometry
sp-ICP-TOF-MS	single particle inductively coupled plasma time of flight mass spectrometry
STEM	scanning transmission electron microscopy
STXM	scanning transmission X-ray microscopy
SV-AUC	sedimentation-velocity analytical ultracentrifugation
TEM	transmission electron microscopy
TOC	total organic carbon
UASE	ultrasound assisted extraction
UV/VIS	ultraviolet–visible spectroscopy
VIP	voltammetry of immobilized particles
WWTP	waste water treatment plant
XANES	x-ray absorption near-edge structure spectroscopy
XAS	x-ray absorption spectroscopy
XRF	x-ray fluorescence spectroscopy

1 Introduction

Manufactured nanomaterials (NMs) are materials in which 50% or more of the particles have one or more dimensions between 1 nm and 100 nm (European Commission, 2011). Due to their small size, these particles may have different properties from coarser particles of the same material. This may be a direct result of this small size, however; also their comparatively large surface area, shape, solubility, chemical composition, surface functionalization and surface treatment can influence their properties. Their new and enhanced properties make NMs interesting for many applications giving rise to the field of nanotechnology which gained much interest during the preceding decades. As a result of this, many NMs left the world of research laboratories and paved their way into new applications and consumer products. The classes of NMs most discussed in the scientific literature are carbon nanotubes (CNTs), fullerenes, nanowires, TiO2, ZnO, CeO2, silica NMs; Fe, Ag, Au, and dendrimers (Zänker and Schierz, 2012). The most prominent examples already in commercial applications are TiO2 as paint pigments/photo catalytically active paint additive or UV-reflectant in sunscreen, Ag nanoparticles in biocidal applications, CeO2 as a fuel additive or UV-reflectant, etc.. However, the same properties that motivate their use are also reason for concern, as NMs can cause toxic reactions and have mobilities in the environment different from bulk materials of the same elements (with the same chemical composition but bigger size fractions). In order to evaluate the risk associated with the widespread usage of manufactured NMs, a considerable scientific effort has been directed to study their toxicity and environmental mobility. Despite these efforts, the selective detection of engineered NMs in environmental compartments is still a very complex and challenging task and a huge number of researchers made use of very different approaches to meet this challenge. Most of the work published is concerned with laboratory studies with rather well-defined system components. A recent publication of Gondikas et al. (2018) describes this dilemma very well: "Data on engineered NPs (nanoparticles) concentrations in different environmental compartments are currently based only on model predictions, which have not been validated with field data, as no suitable analytical methods have been available for quantifying engineered NPs in complex matrixes."

The present study provides an overview of the various methods employed by researchers to detect manufactured nanomaterials in the different relevant environmental compartments of water, soil, air, sediment, biota and sewage sludge. Detection of NMs is always accompanied with characterization and may include e.g. information about primary particle size and shape, but also size distribution, degree of aggregation, surface charge and surface area. Some measurement techniques used in material characterization are very useful to identify NMs in natural samples after appropriate sample pre-treatment. Characterization techniques for NMs were summarized in a recent review of Mourdikoudis et al. (2018).

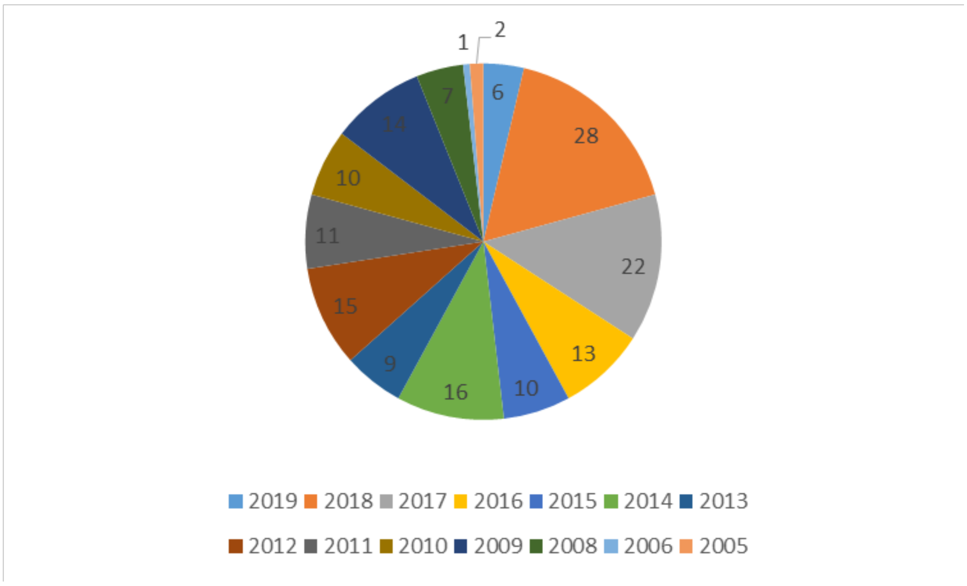
Evaluating the relevant literature quickly led to the conclusion that while some detection methods will lend themselves more easily towards detection of NMs in a specific compartment, there is no strictly compartment specific method. NMs can be detected with any of the different methods after application of suitable sample preparation techniques. Accordingly, the results of this literature review study will be presented as follows: First, the methodology will be introduced. Second, the detection methods will be presented on their own with a short explanation of their working principle, their specifications (detection limit, etc.) and identification of advantages and disadvantages. Third, a compartment-specific overview of the detection strategies employed for water, soil, air, sediment, biota and sewage sludge including the specifics of sampling and sample preparations will be given. Fourth, the results will be discussed and summarized in their totality, which is then followed by the outline of a need for action and recommendations extracted from the preceding discussion.

1.1 Methodology

To obtain a general overview about the most relevant detection techniques and strategies for the environmental compartments surface water, soil, air, sediment, biota and sewage sludge, a literature review was performed using predominantly “Web of Science” and screening for peer-reviewed literature, such as review articles summarising the state of the art of NMs detection techniques for environmental samples. More than 150 scientific publications up to May of 2019 which themselves refer to more than 10000 sources were evaluated concerning nanoparticle detection methods (figure 1). Results from several projects such as nanoMONITOR and nanoFASE, were analysed and contributions to the UBA Scientific Stakeholder Meeting on Nanomaterials in the Environment (10-11th October 2017, UBA, Dessau-Rosslau) were considered. Furthermore expert opinion was sought via interview and input inquiry from Dr. Stephan Wagner (Helmholtz Centre for Environmental Research – UFZ, Department of Analytical Chemistry) and Dr. Marina Maier (Bavarian Environment Agency (LfU)), concerning sp-ICP-MS and NM extraction techniques. The techniques identified through the literature review and expert advice were evaluated for their capability to detect the relevant NM-related properties such as size, concentration, composition, shape, etc. of arbitrary NMs in environmental samples. This puts major constrains on the detection methods as they need to be suitable for a wide variety of different NMs/NM properties and be adaptable for a monitoring application where the user wants to detect manufactured NMs in environmental samples at environmentally relevant concentrations without prior knowledge about what exactly to detect, in terms of material, size and shape. In general, there is a huge number of detection and characterisation methods described in the literature that were developed for pristine NMs from research or production processes. An overview of those methods is given in table 14 in the Appendix. However, only few methods lend themselves to the challenging task of detecting unspecified NMs in complex matrices.

Figure 1: Overview over the evaluated literature

Number of evaluated recent literature by year



1.2 Predicted environmental concentrations of manufactured nanomaterials

So far actual measured data of environmental NM concentrations is largely lacking and for the most part only modelled PECs are available. These are based on mass flow calculations with the main input parameter being the amount of NM production. A method that serves the purpose of monitoring and validating these modelled PECs needs to be suitable for these concentration ranges.

A review of modelling and analytical studies of environmental concentrations of engineered NMs was presented by Gottschalk et al. (2013). Briefly, predicted environmental concentrations for e.g. TiO2 NM range from 3 ng L-1 to 1.6 µg L-1 for surface water (rivers) and about 5 µg L-1 for effluents from wastewater treatment plants. The low predicted concentrations and analytical difficulties lead Gottschalk et al. to the conclusion that “Further development of analytical procedures and methods that are able to identify and quantify specifically ENM is needed in order for the models to be validated” and “therefore, to ensure we do not compound pronounced uncertainties in concentration (and risk) assessments – and to calibrate/restructure the available models – we have to very critically evaluate the sparse measurements that are currently available”.

Table 1: Predicted environmental concentrations of manufactured NMs

Ranges of PECs of NMs TiO_2, ZnO, Ag, fullerenes, CNT and CeO_2 as presented by Gottschalk et al. (2013) based on the available modelling and experimental data.

Environmental compartment	NM	PEC
Surface water	TiO_2	ng L^{-1} to µg L^{-1}
	ZnO,	pg L^{-1} to ng L^{-1}
	Ag	pg L^{-1} to ng L^{-1}
	Fullerenes	pg L^{-1}
	CNT	pg L^{-1}
	CeO_2	ng L^{-1}
Cleared waste water	TiO_2	ng L^{-1} to µg L^{-1}
	ZnO,	µg L^{-1}
	Ag	ng L^{-1} to µg L^{-1}
	Fullerenes	ng L^{-1} to µg L^{-1}
	CNT	ng L^{-1}
	CeO_2	pg L^{-1}
Sewage sludge	TiO_2	ng g^{-1} to mg g^{-1}
	ZnO,	µg g^{-1}
	Ag	pg g^{-1} to µg g^{-1}
	Fullerenes	ng g^{-1}
	CNT	ng g^{-1}
	CeO_2	pg g^{-1}
Sediment	TiO_2	mg kg^{-1}
	ZnO,	µg kg^{-1}
	Ag	µg kg^{-1}
	Fullerenes	ng kg^{-1}
	CNT	µg kg^{-1} to mg kg^{-1}
Soils/soils treated with sewage sludge	TiO_2	pg kg^{-1} to mg kg^{-1}
	ZnO,	µg kg^{-1}
	Ag	pg kg^{-1} to µg kg^{-1}
	Fullerenes	ng kg^{-1}

Environmental compartment	NM	PEC
	CNT CeO_2	ng kg^{-1} to µg kg^{-1} µg g^{-1}
Air	TiO_2 ZnO, Ag Fullerenes CNT CeO_2	ng m^{-3} pg m^{-3} pg m^{-3} to ng m^{-3} fg m^{-3} to ng m^{-3} pg m^{-3} to ng m^{-3} pg m^{-3} to ng m^{-3}

2 Detection Methods

For general detection and characterization of NMs, a multitude of methods are described in the literature. In some cases, these methods have only academic relevance and are only suitable for pure NM samples or NMs with little environmental relevance such as Au-NMs in well-defined matrices. An overview of those methods is given in table 14 in the Appendix. In this chapter, detection methods that are relevant for the detection of NMs in or from environmental samples are shortly described and advantages and disadvantages are pointed out. For further reading, a comprehensive overview about feasible methods for the detection, characterization and quantification of inorganic engineered nanomaterials in complex samples is also presented in the review of Laborda et al. (2016).

2.1 Electron Microscopy

Electron microscopy is considered one of the most powerful techniques for the analysis of NMs because of its capability to visualise NMs, and hence to obtain information about their size, shape and aggregation/agglomeration state, as well as to guide the interpretation of results from other techniques (Laborda et al., 2016). These microscopic techniques make use of electron beams that are directed onto the sample with the image being produced by either the electrons transmitted by the sample (transmission electron microscopes (TEM)) or secondary electrons emitted by the sample or electrons backscattered by the sample (scanning electron microscopy (SEM)). A very good overview of current electron microscope techniques including tables that list special detector systems is provided by Lapresta-Fernandez et al. (2014).

In the literature, transmission electron microscopes are described with resolutions up to 0.047 nm (high resolution TEM) by using double aberration correction (Rose and Wan, 2005) while scanning electron microscopy allows typical spatial resolutions in the range of 1 nm (high resolution) to 3 nm (conventional) (Dudkiewicz et al., 2011). However, these resolutions are valid for optimal samples under vacuum conditions. Electron microscopy can be coupled with energy dispersive x-ray spectroscopy (EDX) which measures the X-rays emitted by atoms exited by the electron beam. As the emitted x-rays are characteristic of the element of the atom they are emitted from EDX allows the measurement of the elemental composition of a sample. Independent of the sample origin, typically pre-treatment steps are necessary to use these techniques as the electron beams scan the material surface predominantly under vacuum conditions and a strong contrast of the NM chemistry compared to the background, for example Ag or Au NMs in a light-element matrix (e.g. organic tissue), leads to better detection of smaller NMs (Liu, 2005).

For dry samples and bulk material, SEM is a good option to obtain information about the presence of NMs in individual samples. Typically, small amounts of sample material are fixed on a grid for analysis. Samples have to be conductive or shall be covered with a conductive layer. Scanning of the sample and searching for individual NMs in a certain matrix is a time consuming method, especially, if the sample is highly complex and the NMs do not possess a significant contrast compared to the background as often the case for soil, sediment or sludge material.

Some common drawbacks for the use of TEM or SEM are (Lapresta-Fernandez et al., 2014):

(i) Typically electron microscopy is a destructive technique either due to sample preparation or electron beam damage. Sample preparation steps (such as dehydration, staining, embedding in epoxy resins and cutting of the samples) in particular can significantly change the structure of a sample.

(ii) The sample can potentially only be analysed once (particularly in the case where the sample may be degraded by the beam, with the implication that it may be necessary to adjust beam energy (and hence resolution) to mitigate damage.

(iii) Contrast is only good for materials with high electron density which impairs the application of electron microscopy techniques for low atomic weight NMs detection in biological samples.

(iv) TEM, as well as, SEM work under vacuum conditions, so wet samples or those involving hydration artefacts, are not suitable.

For conventional SEM imaging, samples must be electrically conductive. For non-conductive samples, typically, a conductive coating needs to be applied.

To overcome the drawbacks of staining and dehydration additional techniques, such as scanning TEM (STEM), environmental SEM (ESEM), environmental TEM (ETEM), wet TEM, wet SEM and cryo-TEM have been developed (Lapresta-Fernandez et al., 2014). Especially ESEM, ETEM, wet TEM and wet SEM and cryo-TEM are extremely interesting techniques for environmental samples since sample preparation and thereby possible alteration of the sample is minimized and detection and localization of NMs in e.g. liquids, wet samples or biota (plant material, small organisms) is possible. However, the capability of NM size measurements using ESEM is low compared with the conventional approach where the sample is dehydrated before measurement. Images of Au NMs with a size of 30 nm can be barely obtained in aqueous media (Luo et. al., 2013). The images appear blurry and the periphery lacks definition. This is because the electron beam scattering is broadened in solution and the electrons are absorbed during the pathway to and from a particle in the sample (Luo et. al., 2013). For example, the smallest size TiO2 aggregates/agglomerates detected with ESEM in bovine serum albumin is 80 nm although conventional TEM imaging suggests that the TiO2 aggregates are down to ~30 nm. The detection limit might be improved through changes in the beam diameter and in sample preparation. While less destructive than for conventional electron microscopy sample preparation for ESEM measurements is also a challenge since NM movement should be at least minimized. Negative effects of NM movement can be somewhat mitigated by settling the NMs on the surface of a SiNx membrane which can be also charged to minimize the movement of the particles during examination.

While detection of Ag or Au NMs is rather easy compared to e.g. TiO2 or CeO2 a differentiation between natural and manufactured NMs is extremely challenging and dependent on the chemical composition of the NMs and the matrix. Recently, Dudkiewicz et al. (2015) investigated the uncertainty associated to the size measurements in electron microscopy characterisation of NMs in food samples. It could be shown, that the number of measured NMs was only a minor source of uncertainty, compared to the combined influence of sampling, sample preparation and image analysis. The authors found that expanded uncertainties around 21-27 % could be achieved, concluding that replications and matrix removal should be considered to improve uncertainty.

Electron microscopy is the most powerful technique in terms of detection of small particles in well prepared samples. The large variety of microscopy techniques allows analysis of samples from all environmental compartments, even under inert atmosphere and without dehydration (e.g. using ESEM). ESEM which is very interesting for wet environmental samples is not feasible for detection of very small NMs so far, but provides the unique opportunity to study NMs in natural matrices. Electron microscopy should be recognized as imaging method to proof the presence of nanoscale objects in environmental samples and to generate information about their size, shape and chemical composition (using EDX). However, since only a very small section of any sample is monitored measurements of NM concentration lack validity.

So far, the most sophisticated (analytical) electron microscopes are mainly available in research institutions due to high costs, complexity of the instruments and structural requirements. However, for routine analysis in analytical laboratory companies, smaller TEM and SEM devices may be affordable and automatic particle analysis routines may alleviate issues of low sample throughput.

Table 2: Analytical electron microscopy techniques

Analytical electron microscopy techniques with limits of detection (LOD) in complex systems and relevant analytical information adapted from Laborda et al. (2016), Luo et al. (2013) and Fairbairn et al. (2013)

Technique	Size LOD [nm]	Concen-tration LOD	Analytical information	Advantages (+)/ Disadvantages (-)
TEM	< 1 (up to 0.047)	-	- size (average and distribution) - shape - elemental composition (+EDX) - chemical structure (+EELS) - crystal structure (+SAED/ CBED)	+ high resolution + particle information + automated image analysis available - destructive method - sample preparation (dehydration) - vacuum conditions - number of analysed NMs is maybe not representative for the sample - no valid concentration data - time consuming, low throughput
FESEM	1	-	- size (average and distribution) - shape - elemental composition (+EDX)	+ clear, less electrostatically distorted images + no need for conducting coatings - high vacuum conditions - no valid concentration data
SEM	1…2	-	- size (average and distribution) - shape - elemental composition (+EDX)	+ sample remains mechanically stable - coating with conductive material (e.g. Au) - no valid concentration data
ESEM	30	$10^{12} L^{-1}$	- size (average and distribution) - shape	+ measurements in real matrix without high vacuum - low resolution - images may be blurry - EDX is restricted in validity - no valid concentration data

2.2 Optical Microscopy

Besides electron microscopy techniques, certain modified optical microscopy techniques have proven useful in the detection of NMs in bio-related disciplines. Here, the scattering or fluorescent properties of the NMs are used to reach resolutions below what is typically reached with optical microscopes. For example, quantitative spectral analysis and characterization (hyperspectral microscopy) of nano-objects at a spectral resolution of approximately 2 nm can be reached using hyperspectral-enhanced dark field microscopy (HEDFM). In dark-field microscopy the light scattered by the sample is used for image production so that scattering objects are displayed against a black background. Using this principle NMs can be detected through their scattering properties even below the optical resolution and tenuous information on NM concentration and size can be gained. A coupling of the microscope to a visible-near infrared spectrophotometer with mapping capabilities (i.e. the collection of all vis–near-infrared spectral data within each pixel of the scanned area) provides the possibility of NM type identification by matching of the found spectral profiles against a NM spectral library (Zamora-Perez et al., 2018). However, the technique so far has mainly been used in mechanistic studies concerning the interaction of NMs in biological samples with spiked concentrations of specific NMs. For further application of the technique in an environmental monitoring scenario, problems with the detection of a multitude of different NMs at low concentrations need to be overcome.

For fluorescent NMs such as quantum dots or NMs loaded with fluorescent dyes the fluorescence of the particles when excited by light can be used for detection. For quantum dots, i.e. semiconductor nanocrystals such as CdSe, CdTe, InP, etc., or their core-shell variants such as CdSe/ZnS, etc., transitions between the valence and the conduction bands cause fluorescent light emissions while for dye loaded NMs transitions between single energy levels of the dye are responsible for the fluorescent emissions (Boschi and De Sanctis, (2017)). These emissions can be detected and localized using confocal fluorescence microscopy (CFM) using a confocal microscope coupled to a CCD camera. As only a very low amount of fluorophores need to be excited for detection (pico – nanomolar) potentially very low detection limits are possible. However, only in case of appropriate sample preparation or separation of NMs from highly complex environmental samples, the very low detection limit for fluorescent NMs may be utilized. Quenching of fluorescence and alteration of the fluorescence signal through aging of NMs and interaction with the complex matrix and fluorescent background substances pose major challenges for the controlled application of such techniques in environmental monitoring contexts. Consequently, similar to HEDFM, this technique so far has mainly been utilized in mechanistic biological uptake and toxicity studies where specific fluorescent NMs or dye-functionalized NMs were used (Brown et al., 2018).

The macroscopic variants of these techniques, i.e. UV/Vis, IR and fluorescence spectroscopy are also often used for the detection and characterisation of NMs but rely heavily on prior knowledge of the NM and their spectral profiles. For example, Ag NMs of a specific size may exhibit a specific UV/Vis spectra with a peak relating to their size but this can only be used reliably for NM detection if the spectra of the particles to detect is known before the actual analysis. The mere detection of a signal at the position of a potential Ag NM size peak in an unknown sample could stem from different NMs of various sizes or other background substances. Hence, the applicability of most spectroscopy methods is limited to situations where it is known what to detect beforehand and the extensive isolation of these particles of interest from any interfering substances. If this can be achieved for the matrix in question or when the NM of interest has a suitably unique spectral profile standard spectroscopy methods may be used with benefit in conjunction with information gained with other methods.

Table 3: Analytical optical microscopy techniques

Analytical electron microscopy techniques with limits of detection (LOD) in complex systems and relevant analytical information adapted from Laborda et al. (2016), Luo et al. (2013) and Fairbairn et al. (2013)

Technique	Size LOD [nm]	Concen-tration LOD	Analytical information	Advantages (+)/ Disadvantages (-)
HEDFM	<50	-	- size (average and distribution) - elemental information	+ in situ measurements in biological samples + elemental information - heating of the NM during measurement
CFM	<5	$10^{12} L^{-1}$ (of the fluorophore)	- presence of fluorescent NMs - size (size-dependent fluorescence)	+ potentially low detection limit - only suitable for fluorescent NMs - fluorescence quenching and alteration

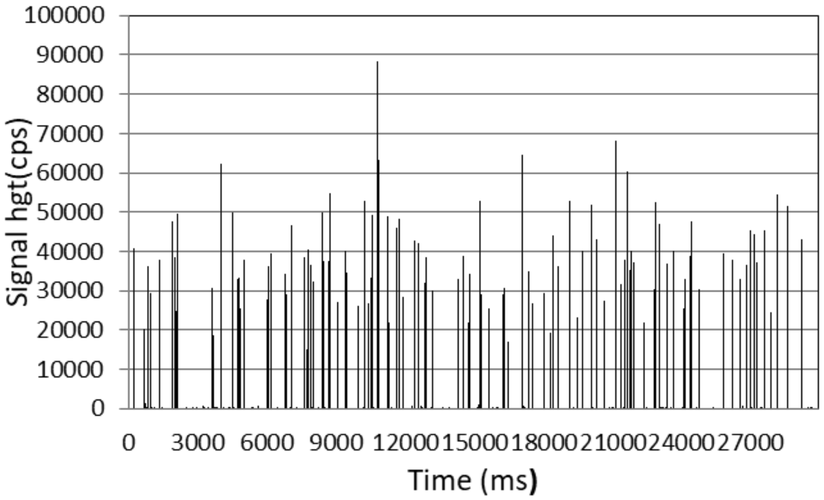
2.3 Atomic Spectrometry

Inductively coupled plasma atomic emission spectrometry (ICP-AES) or especially inductively coupled plasma mass spectrometry (ICP-MS) is considered as the most powerful analytical technique for determining trace elemental concentrations in liquid samples. Briefly, small amounts of sample solution get ionized by injection into a plasma and the constituents of the sample are analysed based on the radiation the excited atoms emit (ICP-AES) or their mass-to-charge ratio is measured by mass spectrometry. Hence, for measurement, environmental samples containing NMs would be pre-treated by chemical digestion and the dissolved samples (e.g. in diluted nitric acid) are analysed using ICP-AES or ICP-MS. Considering the low expected environmental concentrations of NMs, ICP-MS is the method of choice since it has significantly lower detection limits than the ICP-AES. As a prerequisite for its use in NM detection, the elemental composition of the NMs under study should significantly differ from the background matrix. This is usually the case as for Au NMs that are often used as model materials in environmental studies for this reason (and their chemical inertness), despite their relative environmental irrelevancy. The main data obtained by such a procedure is the determination of total elemental concentrations for mass balance purposes, for example to study extraction efficiencies from solid samples or to characterise monodisperse purified NM standards (Meermann and Nischwitz, 2018). Determination of total elemental contents of NMs is most reliable performed by digestion of the sample to minimize any possible interfering matrix compounds and to achieve dissolution of the particles. Validation of the digestion efficiency in environmental samples has to be done using pure NM batches and the identical procedure. Alternatively, an aqueous suspension of NMs can be directly introduced into the ICP-MS. Allabashi et al. (2009) reported the quantification of Au NMs of 5-20 nm size regarding their total elemental content by direct introduction into the ICP-MS. The results were not significantly different from those obtained after digestion of the NMs. However, quality control and validation of direct injection of NM suspensions are more challenging since it needs to be proven that the particles generate the same number of ions in the plasma leading to the same signal as the respective solution of the particle after digestion (Meermann and Nischwitz, 2018).

A special mode of ICP-MS that is particularly useful when it comes to the analysis of NMs is the so-called single particle ICP-MS (sp-ICP-MS). In sp-ICP-MS a very dilute suspension of NMs is injected into the ICP-MS, such that statistically only one single particle at a time (per dwell) enters the plasma. Within the ICP atomisation and ionisation of the NMs constituent takes place. Transient signals are recorded which are characterised by signal flashes. The intensity of the flash of ions is a function of the size (in case of solid spherical and pure NMs) or mass of the initial particle and the frequency of the flashes is a direct function of the number-concentration of the particles. Several crucial steps based on prior knowledge or assumptions (such as assuming a pure spherical NM) need to be undertaken to transfer the recorded data (figure 2) into a particle number versus size information histogram (Meermann and Nischwitz, 2018). Almost any commercial ICP-MS instrument can be utilised for sp-ICP-MS as no instrument modification is required apart from special software for data evaluation. Furthermore, sp-ICP-MS allows the superb detection capabilities of regular ICP-MS. In comparison to other nanoanalytical techniques unrivalled extremely low number concentrations are detectable (106 L-1 to 108 L-1).

Figure 2: Typical raw data of sp-ICP-MS

Typical signal record of sp-ICP-MS (curtesy of AUD Chemnitz). The sample is diluted to such a degree that every signal corresponds to one nanoparticle. From the signal height the size of each particle can be calculated. The sample concentration is derived from the signal frequency.



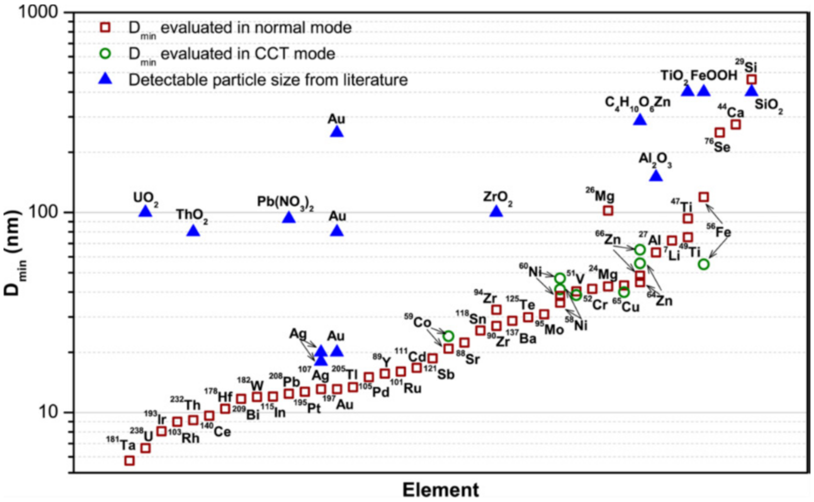
As evidenced by examining the technical programs of relevant international conferences of recent years the interest in sp-ICP-MS for NM detection is exploding globally. More and more laboratories are investing resources in developing and applying the technique, and ICP-MS instrument manufacturers are now, or soon will be, offering hardware modifications, dedicated software packages and application notes to enable easy sp-ICP-MS measurements (Bustos and Winchester, 2016; Liu et al., 2018; Montano et al., 2016; Navratilova et al., 2015; Peters et al., 2015; Stephan and Thomas, 2017; Witzler et al., 2016). Nevertheless, the technique also has some weaknesses:

(i) Typical mass analysers do only provide limited multi-element capabilities

(ii) Detection limits are strongly dependent on the background signal and so the detection of NM strongly depends on their chemical composition and size with detectable sizes in the range of 10–20 nm or even higher (Lee et al., 2014; Montoro Bustos and Winchester, 2016).

Figure 3: Detectable minimum particle sizes by sp-ICP-MS

Size detection limits *D*_{min} for different NMs as established by Lee et al. 2014



Although considerable progress has been made, sp-ICP-MS is still an emerging technique with numerous development opportunities. Today, despite its easy and fast applicability, sp-ICP-MS remains a screening tool (Peters et al. 2015) and a priori knowledge about particle composition and shape is mandatory (as the theoretical description is mostly based on the assumption of spherical shape) to draw conclusions about the true particle size. In combination with suitable sample preparation and electron microscopy techniques for particle size and shape information, sp-ICP-MS provides an appropriate detection method of NMs in natural samples which potentially also could be available for analytic laboratories and not only for research centres.

Furthermore, as sp-ICP-MS is still an emerging technique which has rapidly developed during the past few years, it is still evolving. Efforts have been made to overcome the limitation of only single-element analysis and to permit multi-element measurements of short transient signals. The detection of several elements within one short transient signal opens up new perspectives:

(i) Many manufactured NMs discussed for applications are not strictly pure compounds but are mixtures of several elements (e.g. core–shell particles) whose detection and identification relies on multi-element information.

(ii) The multi-element analysis revealing particle source specific elemental fingerprints potentially could allow the distinction between manufactured and natural nanomaterials in complex matrices.

The analysis of isotope ratios within single particles is available. Currently, multi collector (MC-ICP-MS) as well as time of flight (ICP-TOF-MS) instruments have been applied for multi-element sp-ICP-MS (Meermann and Nischwitz, 2018). Praetorius et al. (2017) described the use of sp-ICP-TOF-MS for the specific identification of engineered NMs (CeO2, <50 nm, spiked concentrations 0.04 to 400 mg kgSoil-1) against the elevated natural background in soils. Complete mass spectra were recorded every 300 µs, each mass spectrum was the average of 10 consecutive TOF extractions which were continuously performed at a rate of 33 kHz. The high temporal resolution maximised the signal-to-background ratio of NMs versus dissolved background because fewer background ions are integrated in a time frame of a measurement. In addition, the entire NM signal could be detected in one measurement because the duration of a single particle event is ~ 300 µs (Praetorius et al., 2017).

Table 4 provides an overview about the atomic spectrometry techniques proven to be suitable for analysis of complex systems.

Table 4: Analytical atomic spectrometry techniques

Analytical atomic spectrometry techniques with LOD in complex systems and relevant analytical information adapted from Laborda et al. (2016), Laborda et al. (2014), Lee et al. (2014) and Naasz et al. (2018)

Technique	Size LOD [nm]	Concentration LOD	Analytical information	Advantages (+) Disadvantages (-)
ICP-AES	-	µg L^{-1}	- Bulk element composition - Total mass concentration	- no direct particle information, only elemental content
ICP-MS	-	ng L^{-1}	- Bulk element composition - Total mass concentration	- no direct particle information, only elemental content
sp-ICP-MS	10…20	10^6 L^{-1} ng L^{-1}	- Detection of dissolved element/NM - Element mass per particle (average and distribution) - size (average and distribution) - number concentration - mass concentration	+ NM information + possible with almost any ICP-MS without big effort - only one element can be detected - no differentiation between engineered and natural NMs - no shape information
sp-ICP-TOF-MS	7 (Au), 13 (Ag), 14 (Mo), 29 (Ti)	ng L^{-1}	- size (average and distribution) - mass concentration - number concentration - simultaneous multi-element analysis	+ multi-element information + high mass resolution + precise isotope ratios + differentiating between natural and engineered NMs - high price of the instrument

The aforementioned ICP-based methods show great potential for the detection of metal and metal oxide NMs. However, carbon-based NMs such as fullerenes and carbon nanotubes or organic nanoparticles such as polymers (nanoplastics) pose a significant challenge, as ICP machines are typically open to air so that CO2 and oxygen would interfere with the carbon detection process. Additionally, significant carbon backgrounds in environmental samples hinder plasma formation. Therefore, generally carbon is not detected using ICP machines and is often excluded from measurements as it can interfere also with the detection of other elements. A complete disintegration of carbon NMs in the plasma would also lead to a significant loss of information concerning actual chemical structure of the NM in question which can be much more diverse in case of carbon-based NMs (fullerenes, CNTs, graphene, polymers) than for those of elements further down the periodic table.

However, this does not mean that mass spectrometry is not suitable to gain information about carbon nanoparticles in general. More mild ionization techniques (e.g. electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) or matrix-assisted laser desorption/ionization (MALDI)) that do not lead to a complete disintegration of the NPs are suitable to measure ionized carbon structures and fragmentations. These are most suitable to identify fullerenes due to their characteristic carbon cage structures (e.g. C60) (Issacson et al., 2009). Identifying organic molecules from their mass fragmentation after ionization is a complex endeavour and in practice is therefore mainly reached by coupling the mass spectrometer to a chromatography system (LC, HPLC, GC, etc.) so that the compounds are not only identified by their mass fragmentation spectra but mainly by their chromatographic behaviour compared to standard samples (Chen et al. 2018).

2.4 Light Scattering

Particles scatter light of a wave length in the range of their size. This can be used to detect and to characterize NMs in suspensions. Light scattering techniques are comparably easy to handle instruments for the quick analysis of aqueous NM suspensions. In almost every laboratory dealing with NMs, some light scattering technique is available, predominantly dynamic light scattering (DLS). This method is useful to determine particle size distributions in terms of hydrodynamic diameters and to characterize the net surface charge of the NMs through measurement of the zeta potential using electrophoretic light scattering (ELS). The zeta potential is often of special interest since it drives suspension stability and interaction with environmental matrices. The working principle of DLS is the detection of the fluctuation of the scattered light intensity of a suspension. The stronger the signal fluctuates the faster the particles are moving, i.e. the smaller the particles are. By comparing the signal at a given time with the signal obtained at a previous time step an exponentially decaying autocorrelation function can be derived. From this decay a hydrodynamic diameter is calculated based on the assumption of the particles as hard spheres. In ELS the particles are subjected to an electrical field and move according to their zeta potential which is derived from the Doppler shift of the wavelength of the scattered light.

However, DLS has some major drawbacks. NM identification in terms of shape and chemical composition is impossible. Since for calculation of particle size distributions spherical, monodisperse particles are assumed light scattering techniques have limitations in analysing polydisperse samples (Tomaszewska et al., 2013). Larger particles or agglomerates/aggregates influence the measurements and can shift results towards elevated particle size distributions due to the high light scattering intensity of large particles. This may lead to masking of big volume fractions of smaller particles in the analysed sample. Typically, DLS is used for the characterisation of rather well defined monodisperse particle batches. For use in detection of NM in complex natural media a size separation before any DLS measurement is mandatory in order to obtain reasonable data.

Static or Multi Angle Light Scattering (SLS/MALS) is a light scattering technique that, as the name suggests, makes use of either multiple detectors at different angles to the sample or one detector that is moved to different angles towards the sample. This allows the measurement of absolute molar mass, size and most importantly also gives information on the shape and conformation of particles or polymeric materials (Lapresta-Fernandez et al., 2014). The multi-angle signal allows the measurement of the hydrodynamic diameter (similar to DLS) as well as the radius of gyration (the mean-square radius). For spherical particles these would be equal while for particles deviating from the spherical shape a form factor can be calculated in order to quantify this deviation and identify NMs with non-spherical shapes, such as rod and tubes as described e.g. for carbon nanotubes by Gogos et al. (2014). Just as with DLS no information on chemical composition of the NM is obtained and a separation of particles into different size classes should be performed in advance of MALS analysis.

Nanoparticle tracking analysis (NTA) is an inexpensive, quick to perform and reliable method to measure the particle size distribution and particle number concentration in liquids (Luo et al., 2017). NTA is a laser-scattering technique which tracks the Brownian motion of small particles and uses this information to calculate the size of particles. Luo et al. (2017) report on the different fields of application of NTA such as the assessment of the behaviour of NMs in aqueous environments for environmental, biological, food, and aquatic ecotoxicity studies. Nevertheless, due to the assumption of spherical particles during NTA measurement and calculations, electron microscopy techniques are recommended to receive more detailed information about the NM size and shape distribution in water samples (Luo et al., 2017).

Table 5: Analytical light scattering techniques

Analytical light scattering techniques with limits of detection (LOD) in complex systems and relevant analytical information, adapted from Labardo et al. (2016); DLS data from Malvern Zeta Sizer User Manual (2013), MALS data from Steppert et al. (2017).

Technique	Size LOD [nm]	Concentration LOD	Analytical Information	Advantages (+) Disadvantages (-)
NTA	20	10^9 L^{-1}	- size (average and distribution) - number concentration	+ liquid samples + low interference of magnetic fields from the instruments (also feasible for magnetic NMs) + inexpensive + quick to perform - highly sensitive to sample instability - interference of large particles - no information concerning elements or shape
DLS	<10 10-100 100-1000	0.5 mg mL^{-1} 0.1 mg mL^{-1} 0.01 mg mL^{-1}	- size (average and distribution) - number concentration - volume concentration - agglomeration behaviour - sedimentation - zeta potential	+ fast and inexpensive measurement of liquid samples - strong interference of bigger particles - not well-suited for polydisperse samples - assumption of spherical particles - no information concerning elements or shape
SLS/MALS	<10	10^8 L^{-1}	- size (hydrodynamic diameter) - shape	+ shape information (MALS) - highly sensitive to sample instability - not well-suited for polydisperse samples - no information concerning elements

2.5 Particle counters (air samples)

NMs from aerosols are conventionally trapped by filter material and further analysed as described later in chapter 4.3. Alternatively, particle counters are established techniques in research and work place monitoring (Pelzer et al., 2010). Conventionally, these are instruments for measuring the number concentration of NMs in air samples online with different time and size resolution capabilities. Pelzer et al. (2010) discuss the following instruments: scanning mobility particle sizer (SMPSTM), fast mobility particle sizer (FMPSTM), nanoparticle spectrometer model NPS500 (Nano-IDTM), electrical low pressure impactor (ELPI+TM), diffusion size classifier (DiSC), miniature diffusion size classifier (miniDiSC) and NanoTracer. Table 6 summarizes the particle counters described in this chapter. SPMSTM and FMPSTM are both particle sizer instruments with nearly identical operation principle and designed for stationary measurement e.g. in working place environments and not meant for mobile use. Both techniques are based on the highly resolved electrostatic classification of NMs followed by particle counting systems. Briefly, air is aspirated in the systems send through an electrostatic classification system. In a first step, larger particles are sorted out by an impactor, while nanosized materials proceed. The NMs are subsequently charged with a size-dependent electric charge. The subsequent compensation of this charge yields information about number concentration and size distributions in the range of 2.5-1000 nm (SPMSTM) and 5.6-560 nm (FMPSTM) by counting the particles per size fraction. Nano-IDTM can be used in two operation modes. In the first mode it is used like a SMPS and measures air samples with a constant sample flow of 0.2 L min-1 by electrostatic classification. In the second mode, the instrument works as a condensation particle counter, which means that the total number of NM is detected by an optical particle counter. The ELPITM is an analyser which can measure particle number concentrations and number-size distributions in a broad range of NM size from 6 nm to 10 µm online. The measurement principle is based on electrostatic particle loading followed by particle size classification in a multi-step low pressure cascade impactor resulting in electrical detection of the charged particles. Another class of instruments is dedicated for personal work place measurements and safety. The DiSC and miniDiSC are sensors for measurement of particle number and size in a time resolution of up to 1 second. This allows to gain information about the lung deposited surface area and also the mass of NM that might be ingested via inhalation. DiSC working principle is also based on electric loading of aerosols and later detection with electrometers (highly sensitive current amplifier). Data is stored on SD cards and data evaluation has to be performed on a computer. NanoTracer is a handset and also developed for personal work place measurements. The measurement is based on diffusion load of the particles. The charged particles pass two parallel electrodes and a very sensitive electrometer detects the transported surface charge of all particles. If a voltage is applied to the electrodes, small charged particles are deposited on the electrodes and a low electrical current can be measured. With that, particle number and mean particle sizes can be calculated.

However, these instruments are feasible for detection of NMs in air samples but they do not allow the identification of chemical composition and distinction from background particles. Further analysis of NMs collected on filter material is typically needed for that. Some instruments, such as ELPI+TM collect size fractions of particles during the measurement process. These fractions can then be further evaluated. Depending on the intended use, all instruments are quite promising for NM monitoring in air samples.

Table 6: Analytical particle counter techniques for air quality monitoring

Analytical particle counter techniques with limits of detection (LOD) in air and relevant analytical information, adapted from Pelzer et al. (2010)

Technique	Size LOD [nm]	Concen-tration LOD [particles cm^{-3}]	Analytical Information	Advantages (+) Disadvantages (-)
SMPS^{TM}	2.5	1	- size distribution - number concentration	+ very low detection limit + high number of size classification channels (up to 167 below 100 nm) + continuous measurement without data gaps + very precise measurements - stationary, not portable (~30 kg) - sensitive to changing NM sizes during measurements
FMPS^{TM}	5.6	300	- size distribution - number concentration	+ fast and continuous online measurement + precise measurement of quickly changing particle sizes - lower resolution compared to SMPS^{TM} - stationary, not portable (32 kg) - comparable high concentration LOD
Nano-ID^{TM}	5	1	- size distribution - number concentration	+ portable device + high-resolution of particle number and size distribution + easy to use - not feasible for areas with danger of explosion
ELPI+^{TM}	6	150	- size distribution - number concentration	+ online measurement of size distribution and number concentration + broad particle size range covered + sampling of size classified particles for further analysis stationary, not portable - frequent and thoroughly cleaning procedures necessary
DiSC	10	10^3	- mean particle size	+ portable, personal working place monitoring + high resolution measurement

Technique	Size LOD [nm]	Concen-tration LOD [particles cm^{-3}]	Analytical Information	Advantages (+) Disadvantages (-)
				- highly sensitive to particle morphology may result in deviations of results up to 30 % compared to SMPS - high detection limit, no use in clean rooms
miniDiSC	10	10^3	- mean particle size	analogue to DiSC
NanoTracer	10	10^3	- mean particle size	+ good time resolved measurements + measurement of personal exposure + small size - not feasible for areas with danger of explosion

2.6 Biological sensors

Some alternative approaches that may be promising for environmental monitoring are biological sensors as described by von der Kammer et al. (2012). These biosensors might be helpful tools for detecting simply the presence of NMs in aqueous samples when other instrumental methods fail or it would be too elaborate to run a whole detection program with a huge number of samples. These bioassays rely on specific responses of organisms to substances. Individual components of living systems also have been used as analytical tools for decades. Von der Kammer et al. (2012) report on specific association of e.g. Ag NMs with cell surface proteins and the differential expression of bacterial stress response genes with Ag NMs and TiO2 NMs. This indicates that biological reporter systems, that are highly specific to nanoparticle surfaces, could be developed. Based on these information it could be assumed that these sensors might be a helpful alternative for screening of samples but no detection method in terms of clear identification or quantification of NMs. Further techniques would be necessary to obtain reliable information about NMs in the samples.

3 Sample preparation procedures for NM detection

Working with pure NMs is a very exceptional case and mostly only done during quality control in NM production processes and lab studies. Especially environmental samples require some kind of preparation prior to the analysis of NMs by most analytical techniques. Sample preparation techniques are procedures to separate the NMs from or to remove the complex matrix. These methods are considered the most crucial step in NM detection in environmental compartments since highly sensitive detection techniques are available but almost all techniques require reasonably “clean” samples for accurate measurements. Laborda et al. (2016) reviewed a couple of sample preparation techniques such as digestion, separation and pre-concentration, centrifugation, filtration, liquid phase extraction, and solid phase extraction. In the following chapter, the most relevant sample preparation techniques will be briefly outlined.

3.1 Chromatography and related separation techniques

Chromatography and related separation techniques are predominantly used for sample pre-treatment and fractionation for further analysis with other techniques or detection devices. Size characterisation can be made by different separation techniques such as size exclusion chromatography (SEC), hydrodynamic chromatography (HDC), capillary electrophoresis (CE) and field-flow fractionation (FFF). Often these chromatography techniques are coupled with diode array, fluorescence, refractive index and MALS detectors.

SEC is a widely used technique for NM isolation. Briefly, a column is packed with porous packing materials which form the flow channels. Particles with a smaller diameter or equal to the pore size of the packing material are transported longer distances in the columns and have elevated retention times while larger particles can only pass bigger pores or are excluded to extra-particular region (Lapresta-Fernandez et al., 2014). By well defining the pore size of the stationary phase, NMs can be well separated from larger particles (Weinberg et al., 2011). Results related to the particle size can be confirmed by electron microscopy and chemical composition can be determined using mass spectrometry.

The separation of particles by HDC is based on their hydrodynamic radius. HDC is similar to SEC, except, due to the non-porous nature of the stationary phase; there are fewer possibilities of solid phase interactions.

CE allows species separation based on the charge and the size distribution (Lapresta-Fernandez et al., 2014). For selective separation of various NMs by size and surface charge density capillary-electromigration techniques are used. By modifying some of the most important electrophoretic separation variables such as pH, buffer concentration, and organic modifiers, the rate of migration can be varied. A review of the application of CE techniques has been presented by Surugau and Urban (2009). The authors conclude that further work is needed to enable analysis of complex samples containing NMs. Analysis of NMs in environmental samples will require development of complex sample preparation procedures, irrespective of the mode of electrophoresis implemented. Electrophoretic methods have shown potential for purification of carbon nanotubes by size (López-Lorente et al., 2011). This technique may have a potential for sample pre-treatment and separation of carbon-based NMs from e.g. aqueous samples.

FFF is a velocity based separation technique that does not require a stationary phase for separation. Separation occurs by differential retention of NPs in a stream of liquid flowing through a thin channel when an external field is applied perpendicular to the channel. One type of this technique is the asymmetric flow FFF (AF4). The field drives components unequally into different streams according to the physical properties of the NMs, causing the separation from 1 nm to 100 µm (Lapresta-Fernandez et al., 2014). FFF can be coupled off-line with several analytical detectors. Basically, to monitor the size distributions, the FFF needs to be coupled to a detector that responds to the nanoparticle number or mass concentration. Baalousha et al. (2011) conclude that FFF "allows continuous separation and sorting of complex samples as a function of diffusion coefficient/particle size, providing a continuous set (fractions) of monodisperse colloids/NPs, which simplifies further characterization of the particles.".

Table 7: Separation techniques with coupled NM analysis

Analytical separation techniques with LOD in complex systems and relevant analytical information adapted from Laborda et al. (2016)

Technique	Size LOD [nm]	Concen-tration LOD	Analytical information	Advantages (+) Disadvantages (-)
AF^4	1…5	1-10 µg L^{-1} (AF4-ICP-MS) 0.1 mg L^{-1} (AF4-UV-Vis) 1 mg L^{-1} (AF4-DLS)	- detection of complexed elements/NM - size (average and distribution) - mass concentration	+ NM information + elemental composition - elaborate sample pre-treatment (stabilisation, removal of matrix) for accurate size fractionation
CE	5	0.2 µg L^{-1} (CE-ICP-MS)	- detection of dissolved element/NM - size (average and distribution) - mass concentration	+ one of the most potent separation techniques - need of surfactants to increase surface charge of NMs - sensitive to clogging phenomena in the stationary phase
HDC	5	1 µg L^{-1} (HDC-ICP-MS)	- detection of dissolved element/NM - size (average and distribution) - mass concentration	+ separation of dissolved species from particles and particle size classification - sensitive to clogging phenomena in the stationary phase
SEC	< 3 (Au-NP)	-	- size classification according to retention times - connected to a MALS for molecular mass distributions	+ quick separation technique - effectiveness strongly depends on defining the pore size of the stationary phase - problems with clogging of the sample inside the column

3.2 Centrifugation

Centrifugation can be considered as the simplest approach to isolate particulates from an aqueous suspension or to separate NMs from dissolved species (Unrine et al., 2012). High centrifugal forces and long times are required and removal of NMs from supernatants might be incomplete, even at harsh ultracentrifugation conditions. However, solids like suspended sediment or organic matter cannot be separated from NMs by centrifugation. Nevertheless, centrifugation has been proven useful for isolation of dissolved species when ultrafiltration fails.

Analytical ultracentrifugation (AUC) is performed using an ultracentrifuge combined with one or more optical detection systems, allowing the observation of the fractionation process of a species dispersed in solution with a known density. The sedimentation process is monitored by a scanning UV/Vis optical detection system that records the concentration profile with respect to radial distance from the rotor and time (the UV/Vis simply detecting an UV absorber, no NM specific information is gained). However, 2D mathematical and computational modelling advancements in sedimentation-velocity analytical ultracentrifugation (SV-AUC) allow for the mapping of sedimentation coefficient and diffusion coefficient distributions of species present in solution (Carney et al., 2011). Results obtained from Au NM standards by Carney et al. (2011) may open the possibility of applying the 2D SV-AUC method to naturally polydisperse samples, which exhibit dispersity in size, density and molecular weight. In this way, it would be possible to examine a particular population in a distribution, without requiring homogeneity in density. However, in samples with particles with widely varying aspect ratios, it would not be possible to apply the method without a priori knowledge on the aspect ratio of the specific (fractionated) populations. Due to the high sensitivity of this method towards e.g. surface coatings and corona built-up by organic substance, AUC may be probably less suited for characterisation of natural aqueous samples without extensive pre-treatment and purification.

3.3 Digestion

In order to analyse NM composition and concentration often digestion steps are performed. These typically serve the purpose of dissolving a solid sample for further analysis in, for example, ICP-AES or ICP-MS. In NM detection such steps can either serve the purpose of dissolving the NM, the matrix (e.g. tissue, microorganisms, sediment, sewage sludge, soil) or both (Laborda et al., 2016). Concentrated oxidizing acids like nitric acid, also in combination with hydrogen peroxide or hydrochloric acid, are commonly used to digest organic matrices at elevated temperatures or with microwave assisted techniques. In any case, these acid-based digestion methods are oriented to get information of the total element content from the NMs in the samples. Other digestion alternatives make use of alkaline reagents or enzymes. Those strategies may preserve the core of inorganic NMs and allow the direct detection, quantification and characterisation. Enzymatic digestion procedures by proteases or pectinases are in use to degrade biological material. However, the presence of organic residues can affect the quantitative recovery of the NMs when sp-ICP-MS or AF4-ICP-MS methods were validated for sizing and quantification of e.g. Ag NMs in spiked biological tissue (Gray et al., 2013). In any case, alteration of the NMs due to the digestion routines need to be characterised so that adapted digestion protocols can be developed with the properties/alteration of the NMs in mind (e.g. adapted digestion routines for easily dissolvable NMs).

3.4 Filtration, ultrafiltration and dialysis

Filtration with 0.45 µm filter material is no longer accepted to distinguish dissolved and particulate fractions as it was common practice for water samples for a long time period (Howard, 2010). In theory, a filter cutoff at 450 nm might at first sight seem to be useful for the isolation of NMs (having one dimension of 100 nm or less). However, this would only be suitable for quasi-spherical, non-agglomerated/aggregated particles; nanorods (or carbon nanotubes), having dimensions much greater than 450 nm or NM agglomerates/aggregates would, in theory, be removed during filtration (Howard, 2010). Serial filtration, usually in combination with ultrafiltration, has been used for size fractionation, quantification and characterization of each fraction by a number of techniques (Mitrano et al., 2014). Dialysis and ultrafiltration are based on the use of nanoporous membranes of different materials and nominal molecular weight cut-offs. Ultrafiltration is often used in studies concerning NM dissolution. The slow diffusive processes of dissolved species crossing the membranes are speeded up by using centrifugal forces.

In any case, there remain uncertainties whether there are interactions of NMs and dissolved species with the membranes and filter materials. This can significantly influence results and needs to be validated.

3.5 Extraction techniques

NMs can be extracted from solid or liquid samples using water or organic solvents. Clean-up procedures and stabilization are necessary to transfer the NMs in appropriate liquids for further analysis. The major challenge is to reach quantitative information about the extraction yield, especially in samples with an unknown NM load. Reported extraction media are for example aqueous surfactant solutions or toluene (for organic NMs such as fullerenes). In the case of extraction of NMs from solid samples such as soil or sediments ultrasound-assisted techniques are employed in order to detach soil/sediment-associated NMs and bring them into suspension for further analysis. These methods will for the most part not extract one type of NM specifically (although certain extraction media will extract a certain class/type of NM more or less effectively), but will yield mixtures of a multitude of engineered and natural NMs. An application note published by Dan et al. (2016) for sp-ICP-MS analysis of CeO2 NM proposed a sequential extraction of the NMs from spiked soil samples using tetrasodium pyrophosphate decahydrate solution with different concentrations (2.5 mM, 5 mM, and 10 mM). Results showed that a reasonable recovery rate could be reached for NMs spiked to soil systems. Further studies would be necessary to prove applicability for other NMs as well.

A very promising more sophisticated extraction technique for the extraction of NMs from environmental media is cloud point extraction (CPE). Here, just as described above, an aqueous surfactant/surfactant mixture is used to extract NMs from the sample. The micelles formed by the surfactants in the water phase can be adjusted in size by using specific surfactants/mixtures and consequently incorporate NMs matching that size (Liu et al., 2009; Majedi et al., 2014a; Majedi et al., 2014b; Majedi et al., 2016). In a second step the suspension is heated above the so-called cloud point where a phase separation into a surfactant rich phase composed of the NM containing micelles and a low-surfactant water phase is taking place, thereby effectively removing the suspended NMs from the suspension. By adding complexing agents such as EDTA, even selective extraction of Ag, Au, CuO and ZnO NMs may be achieved (Laborda et al., 2016). Using CPE for unknown samples, one has to consider the limitations in size of the micelles that also can change with the environmental conditions (pH, ionic strength, presence of natural organic matter) which may call for adjustments of the procedures. Further uncertainties remain concerning discrimination of larger NM aggregates or (hetero-) agglomerates and those with unknown coatings in environmental samples. If successful, CPE selectively removes NMs from suspensions while at the same time concentrating them for further analysis. In a setup where the surfactant solution is first used to extract/detach NMs from solids, e.g. a soil/sediment sample, and then used to perform CPE to extract the NM from the resulting suspension, extraction of NMs from soil/sediments becomes possible (El Hadri and Hackley, 2017).

Anionic exchange resins have been proposed for the selective extraction of noble metal NMs from environmental water samples (Li et al., 2012). The surface of the NMs has to be modified for adsorption onto the resin followed by an elution step to recover the NMs for later analysis.

Functionalized magnetic NMs have also been proposed for extraction of e.g. Au NMs from aqueous samples (Laborda et al., 2016).

The application of capture material for extraction of silver NMs from environmental water samples using activated charcoal was proposed by McGillicuddy et al. (2018). This method seems to be simple and facile for metal NMs in environmental samples with low natural background concentrations. A hydrochloric acid leaching procedure was also developed and allowed quantification of silver fraction captured by the charcoal. An average recovery of app. 95 % could be reached. This sampling technique might be quite interesting since samples can be measured in every analytical lab that has ICP-MS for chemical analysis, but nevertheless it is quite limited to a small number of (metallic) NMs. Furthermore, chemical digestion does not provide information about NM sizes and shape but only total (metal) elemental content.

In summary, a number of extraction techniques have been used to extract NMs from different samples. Most of these techniques have been established with and optimized for specific NMs. Nevertheless a specificity for only one type of NM cannot be assumed. For routine analysis in an environmental monitoring context extraction routines for different classes of NMs (e.g. inorganic, metallic, organic, etc.) should be developed with adapted routines considering the natural variability of the matrix in question.

4 NM detection in environmental compartments - Examples

In the previous chapter the most prominent and most promising methods for NM processing and detection in complex samples were described. As mentioned above, for detection and quantification of NM in environmental samples, a combination of methods is mandatory that involves sample preparation and pre-treatment steps in order to make samples feasible for measurement with the chosen techniques. These preparation steps serve the purpose of making the samples less complex by largely removing the (disturbing) matrix. So far, no standard methods exist for achieving this for the different environmental compartments. In this chapter, literature examples are given of how researchers have achieved the detection of NMs in the different environmental compartments. To date, many of the studies performed are not primarily concerned with monitoring manufactured NM in the environment and researchers have used plenty of methods that were suitable for specific study goals (i.e. the detection of very specific NMs at spiked high concentrations) but would not lend themselves to environmental detection applications of manufactured NMs. Nevertheless, these methods may be at least mentioned in the text. At the end of each subchapter, a table listing the most suitable detection and sample treatment techniques for each environmental compartment is presented based on the reviewed literature.

Delay and Frimmel (2012) have formulated the main analytical problems concerning environmental aqueous NM suspensions that hold true for any other compartment as for most techniques the samples must be transformed into a suspensions at some stage of the analysis:

(i) expected low concentrations, especially of engineered NMs,

(ii) high concentration of the organic or matrix components of the same elemental composition (interference during the ionisation within the measurements and overlay),

(iii) difficulties in sample preparation, artefacts and sample stability,

(iv) polydispersity,

(v) lack of reference and standard materials for calibration and validation.

To overcome these challenges, suitable sample pre-treatment steps have to be developed, validated and to be integrated in the laboratory routine. The procedures employed by researchers mentioned in this chapter may serve as a starting point for a more standardized approach in the future.

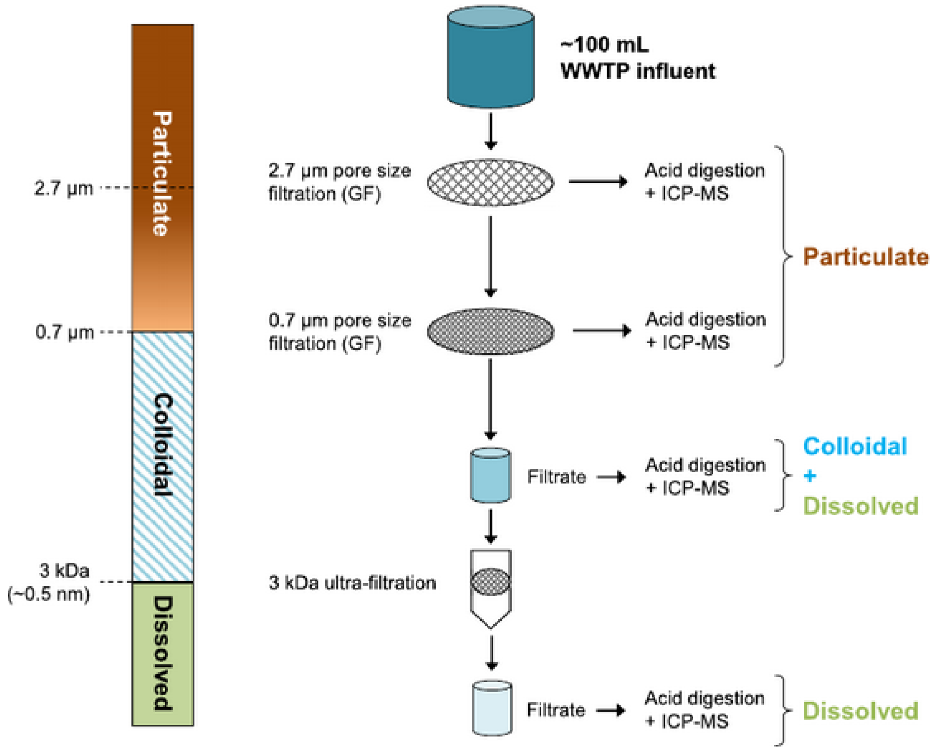
4.1 Water

Numerous approaches have been proposed for the detection and characterization of NMs in water samples (see also tables 2 to 5) including microscope-based techniques (TEM, SEM, autoSEM), light scattering methods and several based on chromatography (Tiede et al. 2009, Bäuerlein et al. 2017, Gondikas et al. 2018). Electron microscope-based techniques are in most cases relevant to distinguish manufactured NMs from natural occurring NMs of similar elemental composition, for example for TiO2 (von der Kammer et al. 2012). DLS and NTA (Gallego-Urrea et al. 2011) are mainly useful to show the presence of NMs in aqueous samples. However, these methods on their own are blind to the specific NM type concerning their chemical composition, shape and their accurate quantification. The most promising approaches involve the use of separation techniques such as filtration, FFF, liquid chromatography, SEC, gel electrophoresis or capillary electrophoresis (Tiede, Boxall et al. 2009) combined with element specific detectors.

Polesel et al. (2018) suggest a general scheme for sample pre-treatment involving sequential filtration in order to separate particulate, colloidal and dissolved fractions followed by acid digestion and ICP-MS analysis in order to enable Ti and Ag nanoparticles detection in waste water (figure 4). In this study, real waste water samples from two Norwegian waste water treatment plants were sampled and analysed concerning the occurrence, characterisation and fate of (nano)- particulate Ti and Ag.

Figure 4: NM separation from waste water

Fractionation procedure used to separate and characterize Ti and Ag as particulate, colloidal and dissolved (ionic) fractions. From Polesel et al. (2018)



The separation technique CPE followed by atom emission spectrometry analysis was proposed by Hartmann et al. (2013) for the analysis of Ag NMs in environmental aqueous samples. Samples of different complexity such as river water, treated and untreated municipal wastewater and synthetic solutions containing dissolved and particulate organic matter with a total organic carbon (TOC) content up to 10 mg L−1 were spiked with different concentrations of Ag-NMs. High recovery rates (>80%) were found in all cases. This could be interesting for routine analysis of Ag-NM in aqueous samples, but transferability to other kinds of NMs would have to be proven.

A field study by Bäuerlein et al. (2017) describes the detection of NMs in Danish surface waters including “dune and bank filtrates, surface waters and ground waters as well as influents, effluents and sludge of sewage treatment plants (STPs), and surface waters collected near airports and harbours”. Inorganic NMs (Ag, Au, TiO2) were analysed directly using sp-ICP-MS without excessive sample pre-treatment after an ultrasound treatment and filtering step (0.22

µm filters). Organic NMs such as fullerenes were first extracted from the water samples using solid phase extraction and the fullerenes were eluted from the extraction column using toluene. In a similar approach, Sanchís et al (2018) used liquid-liquid-extraction with toluene to detect fullerenes in Sava river water (Slovenia, Croatia and Serbia) using HPLC coupled to mass spectrometry.

Gondikas et al. (2018) describe the application of sp-ICP-TOF-MS for the detection of TiO2 NMs in the case of surface waters and sediments. The authors state, that there are currently two promising approaches for the detection and quantification of NMs in environmental samples: The first approach is dedicated to bulk analysis and involves the measurement of specific parameters such as the elemental ratios, crystal phases, and isotope ratios of bulk materials (which might include NMs, colloids, larger minerals, organic matter, and living organisms) in order to quantify the total mass of engineered NMs under investigation. Since this approach is mass-based it may require large quantities of engineered NMs in the sample, depending on the natural background variation and on instrument precision. This might be difficult for most natural samples. The second approach is dedicated to specific analysis of the nanoscale fraction of the samples and involves the application of nanometrological techniques that are able to provide detailed characterization of individual NMs in terms of size and number distributions, such as sp-ICP-TOF-MS coupled with AF4. These proposed approaches reflect the complexity of the NM detection in environmental samples and can be seen as most promising approaches for aqueous samples with comparable low complexity.

Table 8 summarises the most suitable detection methods and sample preparation techniques for water samples. In general, the most promising combination of sample pre-treatment and measurement techniques might be FFF-ICP-MS since this provides concentration, size distribution and composition data and has already been successfully applied to aquatic environmental media. Often for aqueous samples sample pre-treatment for sp-ICP-MS measurement could be limited to filtering and ultrasonication even for such complex systems as fruit juices and waste water, making the detection of NMs in water samples the most technically mature monitoring application. However, standardisation and validation of protocols in light of the natural variability of the water contents still needs further work.

Table 8: **NM detection and separation methods and preparation techniques suitable for water samples**

Nanoparticle detection and separation techniques adapted from Gallego-Urrea et al., 2011; Gondikas et al. 2018; v.d.Kammer et al, 2012; Laborda et al. 2016

Detection/Separation Principle	Techniques
atomic spectroscopy	- ICP-MS - sp-ICP-MS - sp-ICP-TOFMS (single element and multi-element detection)
microscopy	- electron microscopy (TEM, SEM) - ESEM
light scattering	- nanoparticle tracking analysis (NTA) - dynamic light scattering (DLS)
chromatography/ separation	- field flow fractionation (FFF, AF4) - AF4 coupled with multiangle laser light scattering (MALS) - liquid chromatography - size exclusion chromatography (SEC)

Detection/Separation Principle	Techniques
filters	- trapping filters - ultrafiltration
capture materials	- activated charcoal as capture material
extraction	- CPE - liquid phase extraction - solid phase extraction

4.2 Soil and Sediment

Soil and sediments are the environmental compartments that are considered major sinks for manufactured NMs. NM released into water bodies will ultimately find their way into sediments due to agglomeration/aggregation and deposition processes (Luo et al., 2018) while NMs may be introduced into soil via sewage sludge, deposition from air, street run-off or NM applications. Soil and sediment samples are very challenging matrices for NM detection due to their very complex chemical composition and the polydispersity of grain sizes involved that include micro- and nanoscale materials (e.g. clay minerals).

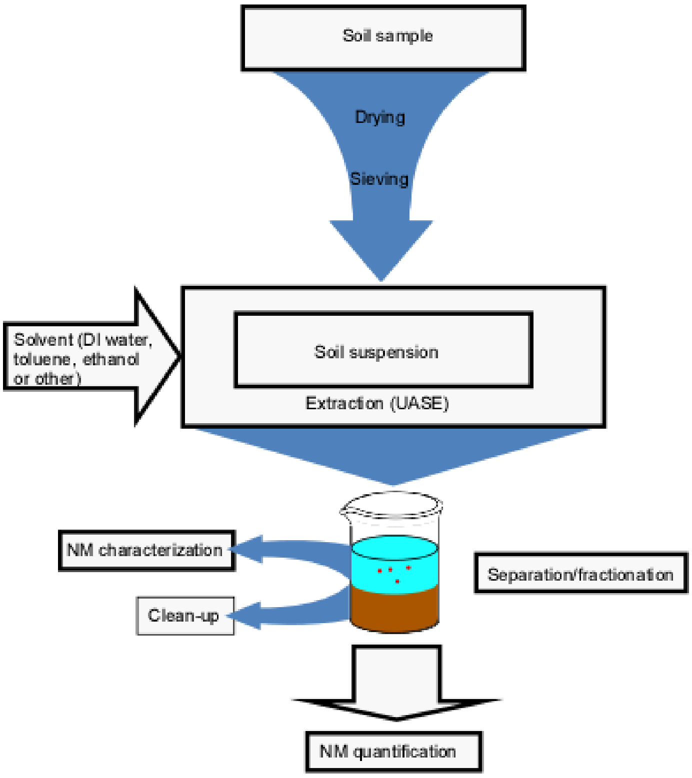
Most studies in the literature show results from soil samples that were spiked with NMs in quite high concentrations up to 1 g per kg soil, for example nano-TiSiO4 (Bouguerra et al., 2016). In this particular study the spiked soil samples were digested using aqua regia and analysed with ICP-MS to measure their Ti content. Procedures like that are limited to overall elemental information and will not generate NM specific data. Hence, total chemical digestion is generally not useful for environmental monitoring purposes due to the high natural elemental background and expected low NM concentrations. Only for NMs with very low elemental backgrounds, e.g. Au NMs, total chemical digestion of the sample may be an appropriate approach.

Fang et al. (2009) performed stability tests with TiO2 and soil suspensions using light scattering techniques to identify the fraction of NMs in suspension. Unfortunately, with this approach it is not feasible to detect and to identify NMs in natural undisturbed soil samples, but the general idea to remove NMs from soils or sediments for further analysis is a relevant approach as also suggested by Pachapur et al. (2016). A comprehensive study of Kuhlbusch et al. (2012) evaluated soil percolates concerning NM load in laboratory column studies. SEM-EDX was used to investigate the NMs in the eluate, so that size, shape and elemental information could be obtained in one measurement. However, this method is quite elaborate and less feasible for a high throughput of samples and only the mobile fraction of NMs that leaches from the sample is accessible for measurement.

In general, it is essential to quantitatively extract NMs from the soil/sediment sample for further analysis in order to gain knowledge of the total NM load of the sample. The further analysis should be preferably at the single-particle level, as described for CuO by Navratilova et al. (2015) and Ag NMs by Schwertfeger et al. (2017) and Mahdi et al. (2017) using sp-ICP-MS analysis. Sample pre-treatment for NM extraction is largely similar for soil and sediment samples. A general scheme proposed by Pachapur et al. (2016) is shown in figure 5. The procedure involves numerous pre-processing steps, such as sieving and drying and finally an ultrasound-assisted extraction step using a suitable solvent in order to effectively remove the NMs from the soil matrix. It becomes quickly obvious, that the many processing steps may have clear potential to significantly influence the results. Alternatively, CPE proved also to be useful to extract NMs from soil matrices (or suspensions) and was systematically studied by El Hadri and Hackley (2017) and El Hadri et al. (2018).

Figure 5: Characterization and quantification of NMs from soil

General scheme for the characterization and quantification of ENPs from soil. USAE = Ultrasound assisted extraction. Adapted from Pachapur et al. (2016).



AF4, ICP-MS and different microscopic techniques are mostly used for the analysis of the extracted samples. In addition to the time-consuming electron microscopic techniques Gogos et al. (2014) proposed a combination of AF4 and MALS for identification of MWCNTs by making use of their rod-like shape compared to other (native) soil particles and in particular soot, which is ubiquitously present in soils. This approach was successfully tested for soil extracts spiked with MWCNTs. However, at 1.6 to 4.0 mg g−1 soil the spiked concentrations of MWCNTs were far away from predicted environmental concentrations. Luo et al. (2018) studied the feasibility of NTA for characterizing the fate of Au and magnetite NMs in sediment-water systems. Results showed, that NTA is inexpensive, quick to perform and can correctly track the particle size distribution (PSD) and the particle number concentration (PNC) of the NMs, which cannot be obtained using other traditional methods, such as ICP–MS, ICP-AES, DLS or ELS. This method was successfully applied to a range of Au and magnetite NMs artificially spiked to sediment samples. However interference of larger particles may pose a problem for a wider application.

Table 9 shows the most promising detection and treatment techniques for soil and sediment samples. In general, a combination of CPE for extraction of NMs, followed by separation with AF4 and measurement using sp-ICP-MS presents itself as the most promising approach, yielding data on NM concentration, size distribution and composition. For routine analysis standardized sample handling protocols need to be developed in dependence of the soil properties and types/classes of NMs. For example, a protocol to extract and detect metal oxide NMs from soils depending on the natural organic matter content of the soil, etc..

Table 9: NM detection and separation methods in soil and sediment samples

Nanoparticle detection and separation techniques adapted from Gallego-Urrea et al., 2011; Gondikas et al. 2018; v.d.Kammer et al, 2012; Laborda et al. 2016; Makselon et al. 2018

Detection/Separation Principle	Techniques
atomic spectrometry	- ICP-MS - sp-ICP-MS - sp-ICP-TOFMS (single element and multi-element detection)
direct visualisation/ microscopy	- TEM, SEM - ESEM - automated SEM
light scattering (extracts)	- NTA - DLS
chromatography/ separation (extracts)	- FFF, AF^4 - AF^4 coupled with MALS - liquid chromatography
extraction	- ultrasound assisted solid-liquid extraction - sequential extraction - CPE

4.3 Air

Detection of particulate matter in air samples is a routine procedure in air quality checking. The detection of specific micropollutants in air monitoring was reviewed by e.g. Lay-Ekuakille et al. (2017). In air monitoring particulate matter smaller than 2.5 µm (PM2.5) is considered to be transported through diffusion. PM is collected by trapping filters and then further analysed. For example Bäuerlein et al. (2017) used Teflon filters that were digested together with the collected materials completely for further analysis using ICP-MS. Weighing using for example a piezo balance is performed to get information about mass concentrations. Electron microscopy (SEM-EDX) is used to gain information about the chemical composition and shape of the trapped particles. Advanced technology using helium microwave-induced plasma is also applied for NM detection in air samples by means of elemental composition analysis. Other methods are ICP-AES or ICP-MS. Unfortunately, in most cases, information about chemical composition or properties of single individual particles cannot be obtained with these techniques (Lay-Ekuakille et al., 2017).

Pelzer et al. (2010) give a very good overview about instruments for measuring the number concentration of nanoparticles in air samples for work place exposure monitoring or air quality monitoring. They discuss the following instruments: Scanning Mobility Particle Sizer (SMPSTM), Fast Mobility Particle Sizer (FMPSTM), Nanoparticle Spectrometer Model NPS500 (Nano-IDTM), Electrical Low Pressure Impactor (ELPI+TM), Diffusion Size Classifier (DiSC), miniature Diffusion Size Classifier (miniDiSC) and NanoTracer. However, these instruments do not allow a distinction from background particles.

Nanomechanical resonant filter-fibers have been proposed by Schmid et al. (2013) for Ag and silica NM detection in environmental or work place safety applications. With this sampling and detection method, real time monitoring of NM concentrations in air samples is possible. However, for accurate identification of unspecific NMs further analysis becomes necessary.

Table 10: NM detection and separation methods in air samples

Nanoparticle detection and separation techniques adapted from Gondikas et al. 2018; Lay-Ekuakille et al. (2017); Schmid et al. (2013)

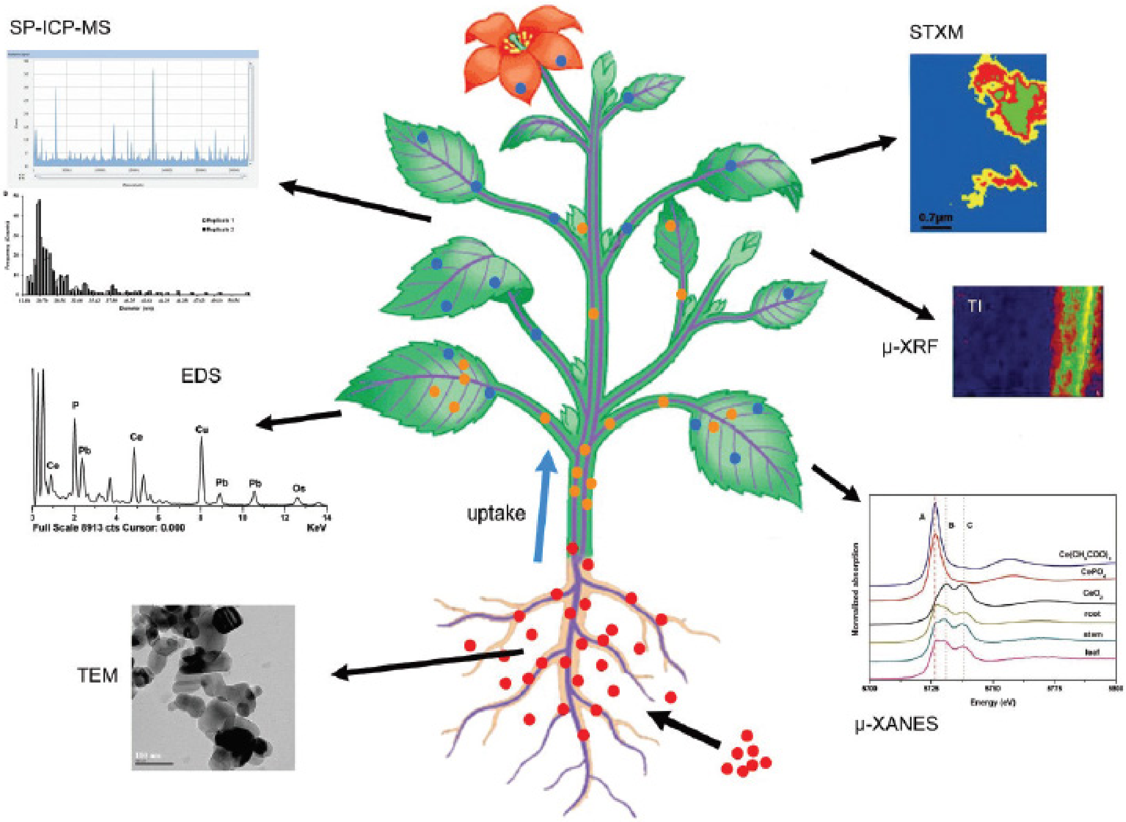
Detection/Separation Principle	Techniques
atomic spectrometry	- ICP-MS - sp-ICP-MS - sp-ICP-TOF-MS (single element and multi-element detection)
direct visualisation/ microscopy	- TEM, SEM - automated SEM and bulk elemental analysis
filters	- trapping filters - nanomechanical resonant filter-fibers
weighing	piezo balance
electroanalytics	- voltammetry of immobilizes NMs on an electrode surface - SMPS, FMPS^{TM}, Nano-ID^{TM}, ELPI+^{TM}, DiSC, miniDiSC, NanoTracer

4.4 Biota

The detection of manufactured NMs in biota, so far is mainly based around mechanistic (toxicity) studies. Consequently NM detection in biota has been done largely in rather high concentration scenarios and often with techniques unsuitable for environmental monitoring scenarios, due to their relatively high detection limits, mainly academic availability and low sample throughput. Figure 6 shows an overview of NM detection methods for application in plant samples suggested by Yan and Chen (2018). The detection techniques presented on the left side of the picture may available also in analytical labs (sp-ICP-MS, energy dispersive (X-ray) spectroscopy EDS, TEM). The methods presented on the right side of the figure are scanning transmission X-ray microscopy (STXM), micro X-ray fluorescence (µ-XRF) and X-ray absorption near edge structure (µ-XANES) which are methods that require X-ray sources or even synchrotron radiation and are less feasible for routine analysis and used more in an academic context.

Figure 6: NM characterization in plants

Schematic diagram representing a regular work flow of NMs characterization in plants. A selection of analytical techniques is shown. Red dots indicate NMs at the moment of application. Yellow and blue dots indicate different elemental species of NMs after biotransformation in plants. From Yan and Chen (2018).



TEM, SEM and element-specific detection techniques (ICP-MS, ICP-AES) are presented by e.g. Tan et al. (2018) or von der Kammer et al. (2012) for the analysis of NM in biota. The critical review of Tan et al. (2018) is focussed on TiO2 NMs interaction with soil components and plants. Von der Kammer et al. (2012) report on conceptual case studies for analysing different NM in biota in general. For tissue material, e.g. cryogenic SEM is proposed to image surface associations of NMs with cells. A recent publication of Naghdi et al. (2017) presents experimental approaches to investigate nano-pollutants in environmental samples and organisms such as mice, rats, zebrafish and mussel, and also E. coli bacteria, human cell lines and fibroblasts. In this study, techniques such as SEM, TEM, DLS, atomic force microscopy (AFM) or X-ray diffractometry were applied to identify the NMs and to study their characteristics.

Detection procedures of NMs in biological samples that are suitable for environmental monitoring scenarios, typically involve digestion of the complex biomatrix as a sample pre-treatment to separate NMs from the matrix for further analysis after purification and maybe also pre-concentration of the extracts (Knopf et al., 2017). Dan et al. (2015) detected Au NM in plants using sp-ICP-MS after enzymatic digestion of the plant material. Gray et al. (2013) describe an alkaline digestion using tetramethylammonium hydroxide in order to analyse Ag and Au NMs in Daphnia magna and Lumbriculus variegatus. As a media somewhat similar to biota, Dudkiewicz et al. (2011) describe the application of electron microscopy to investigate NMs in food. In most cases, the complex matrix is removed and pre-treatment techniques such as AF4 are applied for conditioning and analysis of the NM dispersions. Wagner et al. (2015) suggest a general procedure for the extraction of NMs from complex matrices for measurement using AF4-ICP-MS (figure 7). For the exemplary case of SiO2 NMs in tomato soup this involves homogenization, extraction, enrichment and stabilization steps that need to be undertaken in order to facilitate a subsequent AF4-ICP-MS measurement yielding size distribution and concentration data of the NMs.

Figure 7: Generic procedure for NM extraction from complex matrices

Generic multi-step procedure for development of a sample preparation method to extract NMs from a complex matrix. Specific details for the example separation of SiO_2-NMs from tomato soup are given on the right side of the scheme (numbered sub-steps can be performed as stand-alone or in combination with other listed sub-steps), from Wagner et al. (2015).

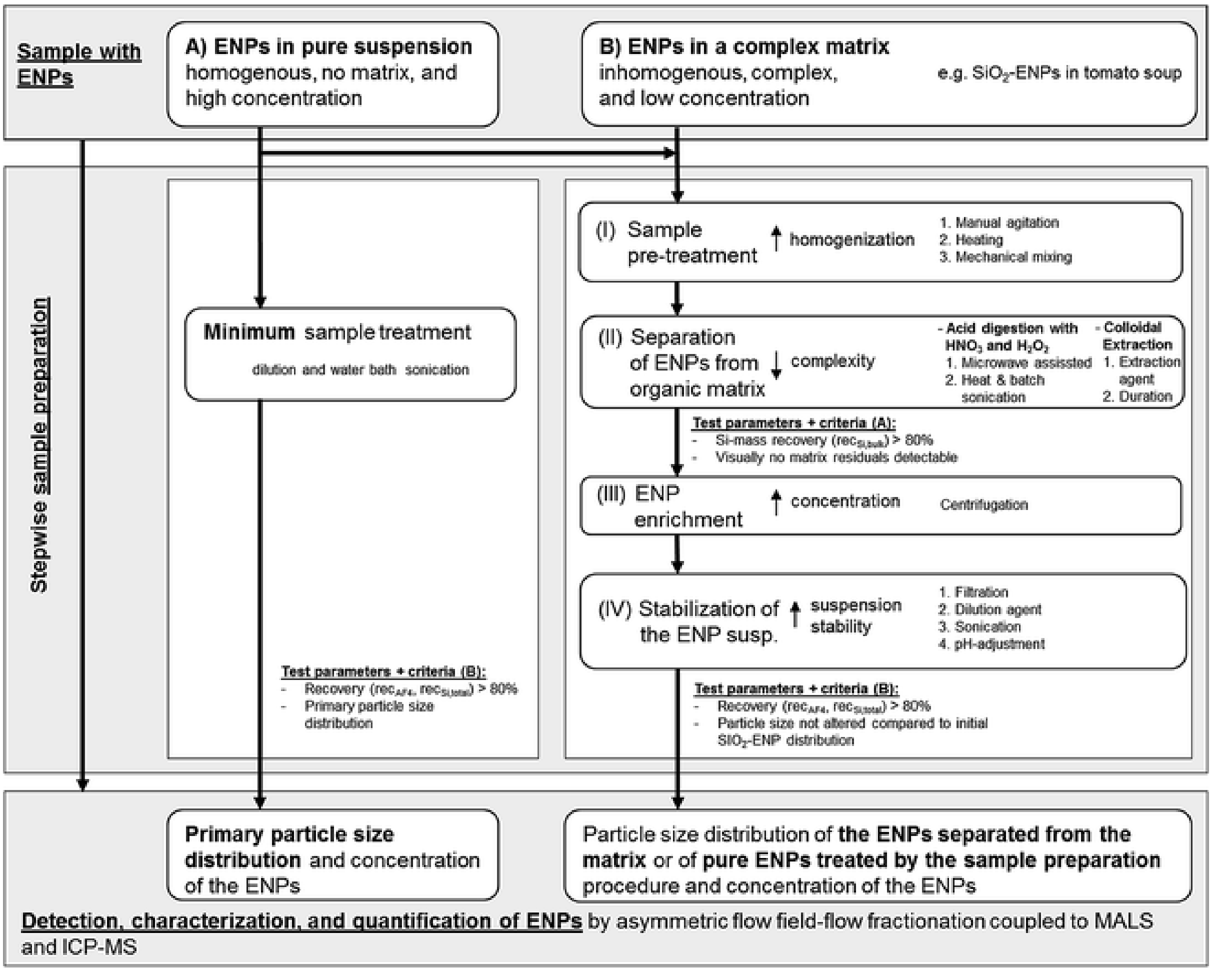


Table 11 shows the most promising detection and treatment techniques for biological samples. In general, a combination of a suitable digestion or extraction routines of NMs, followed by separation with AF4 and measurement using sp-ICP-MS presents itself as the most promising approach, yielding data on NM number concentration, size distribution and composition. For routine analysis standardized sample handling protocols need to be developed with a specific focus on the digestion step that needs to be validated for its impact on the NMs.

Table 11: NM detection and separation methods in biological samples

Nanoparticle detection and separation techniques adapted from Tan et al. (2018); von der Kammer et al. (2012); Zamora-Perez et al., 2018; Naghdi et al. (2017)

Detection/Separation Principle	Techniques
atomic spectrometry	- ICP-MS - sp-ICP-MS - sp-ICP-TOFMS (single element and multi-element detection)
direct visualisation/ microscopy	- TEM, SEM - TEM-EDX - HEDFM
chromatography/ separation (extracts)	- FFF, AF^4 - AF^4 coupled with MALS
light scattering (extracts)	- NTA - DLS
digestion	- alkaline digestion of biomatrix - enzymatic digestion of biomatrix - total digestion using concentrated acids (for overall content measurements)

4.5 Sewage sludge

Waste water treatment plants (WWTP) are considered major sinks for manufactured NMs, as well as release paths for their introduction into the environment via the WWTP effluents, sludge and cleared water (Brar et al., 2009, Kim et al., 2012, Kiser et al., 2009). To analyse these media for NMs, the highly complex matrix first has to be removed since wastewater and sewage sludge have a high load of disturbing dissolved species and particulate matter. This is e.g. performed by chemical digestion followed by ICP-MS measurement of the dissolved species (Bitragunta et al., 2017), which could be feasible for metal and metal oxide NMs with a low background concentrations.

Bäuerlein et al. (2018) describe the analysis of TiO2, Ag and Au NM in sludge samples from Dutch waste water treatment plants by SEM-EDX. Samples were placed on aluminium sample holders and the organic material was removed using plasma discharge leaving the inorganic NMs to be analysed. However, the authors note “the time-consuming nature of this method and the slim chance of success” due to the low NM load and low through-put of the SEM analysis. Nevertheless, this study is one of the few with analysis of real environmental samples without spiking of NMs.

Kiser et al. (2009) achieved the analysis of Ti –particles in the biosolids of WWTPs using a combination of total digestion using HNO3/H2SO4, partial digestion of only the organic matrix using H2O2, filtering and ICP-AES and SEM-EDX analysis. The ICP-AES measurements of filtered and unfiltered samples provided tenuous information of nanoscale Ti-particles. The detailed SEM-EDX analysis of the composition of the particles that varied from pure Ti over titanium oxides TiOx to Ti in co-occurrence with other elements such as iron, calcium, silica, and oxygen atoms lead the authors to conclude that the Ti-solids stem partly from food additives and partly from natural sources. A detailed analysis of the proportions was not possible due to the qualitative nature of the SEM analysis.

Another procedure described by Kim et al. (2012) involved freeze dried sewage sludge samples that were gently ground with a mortar and pestle, re-suspended in de-ionized water and sonicated in an ultrasonic bath. Afterwards, the slurry solution was diluted 200-fold with methanol and e.g. Ti concentration was measured by ICP-AES. A drop of the methanol suspension was then placed onto a TEM grid and allowed to evaporate for further microscopic analysis using TEM-EDX.

Light scattering techniques were used for supernatants analysis of suspended and filtered sludge samples to determine NM size distributions by Hennebert et al. (2017) and combined with TEM-EDX measurements for information of the chemical composition.

Table 12 summarises the most promising detection and treatment techniques for sewage sludge samples. Sewage sludge with its high content of organic and inorganic substances is one of the most challenging matrices considering the reliable detection of NMs. The literature describes different approaches based on digestion of the sludge-matrix or extraction of the NMs. If these crucial steps can be established in a standardized fashion separation with AF4 and measurement using sp-ICP-MS may be the most promising approach, yielding data on NM number concentration, size distribution and composition.

Table 12: NM detection and separation methods for sewage sludge samples

Nanoparticle detection and separation techniques adapted from Polesel et al., 2018, Bitragunta et al., 2017

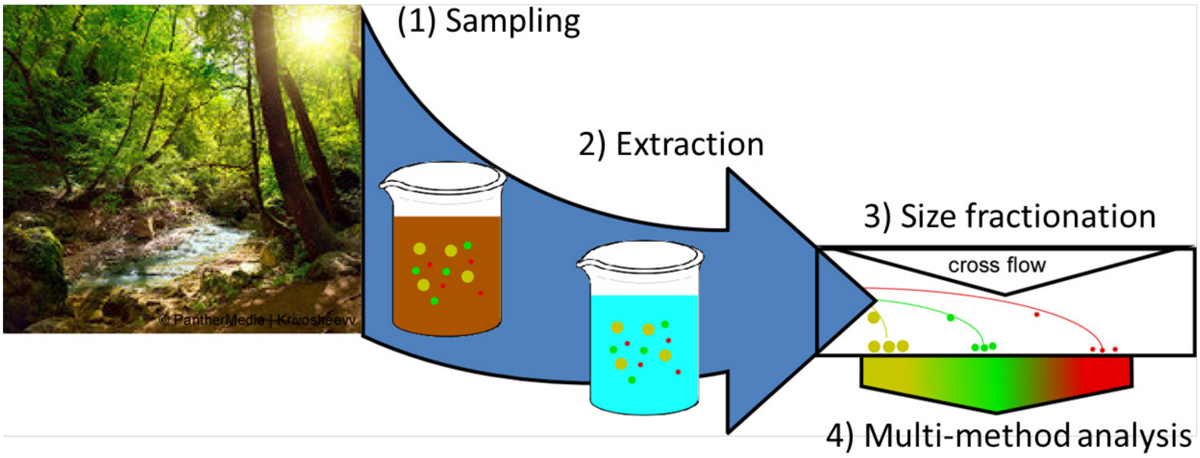
Detection/Separation Principle	Techniques
atomic spectrometry	- ICP-MS - sp-ICP-MS - sp-ICP-TOF-MS (single element and multi-element detection)
direct visualisation/ microscopy	- TEM, SEM - automated SEM and bulk elemental analysis
light scattering (extracts)	- NTA - DLS - MALS
chromatography/ separation (extracts)	- FFF, AF^4 - liquid chromatography
extraction	- sequential extraction
digestion	- total digestion - partial digestion

5 Conclusions /Need for action/Recommendations

From the current literature survey, it can be concluded that sophisticated analytical methods and techniques are available to analyse NMs in general and also in environmental samples. To get sufficient information about NMs in environmental compartments it is always essential to use a combination of methods for detection and analysis since some methods are dedicated to visualisation and thereby evaluation of primary particle size and shape (electron microscopy), others to gain information about hydrodynamic particle size (e.g. HDC, FFF, light scattering techniques) or elemental composition (atomic spectroscopy methods) and yield different concentration data (overall elemental concentration, NM mass concentration, number concentration, etc.).

Figure 8: Scheme of general workflow for NM detection in environmental samples

Relevant steps from sampling to extraction, size fractionation and analysis that have to be validated to ensure robustness of NM detection in environmental samples.



A generalized method for NM detection in environmental samples would consist of standardized sampling procedures followed by an extraction step that serves to largely remove the complex matrix followed by a size fractionation step which would then lead into a multi-method analysis depending on the desired information depth as illustrated in figure 8. An all-encompassing method that allows for the detection and identification as well as measurement of the concentration of NMs without prior knowledge/specification on what to expect, i.e. the detection of unspecified NMs in an environmental monitoring scenario, would need to provide information about NM size, concentration, composition, and possibly shape. An overview of the different techniques that can be used to detect these different properties of NMs is given in table 13. These techniques need to be combined with a suitable size separation technique such as AF4, SEC, HDC, CE, filtration or centrifugation, with AF4 being used the most in the current literature.

Table 13: NM detection techniques according to relevant properties

Overview of the different techniques to be used to detect the different properties of NMs. The most promising methods for a combinatory approach are in **bold**.

NM property	Techniques/Methods
size	DLS, MALS, NTA, electron microscopy, **sp-ICP-MS**

NM property	Techniques/Methods
concentration	ICP-AES/MS (overall conc.), **sp-ICP-MS (sp level)**
composition	EM-EDX, ICP-AES/MS (overall comp.), **sp-ICP-TOF-MS**
shape	electron microscopy, **MALS**

Naturally, all techniques have advantages and disadvantages and not least, the purchase prices of the instruments will also determine the techniques which are the most promising for routine analytics. From all the techniques evaluated for routine NM detection and quantification, sp-ICP-MS seems to be the fastest developing method for the detection of metal and metal-oxide NMs and many laboratories try to make use of this technique since it is possible with almost any ICP-MS machine without enormous technical or financial effort. Manufacturers provide technical notes for their machines to support the customers in using these techniques (Liu et al., 2018). In combination with appropriate sample pre-treatment and preparation, fractionation, purification and stabilisation for measurement, this is a quite robust approach for NM detection in environmental samples.

The most promising method in terms of information depth provided about NMs seems to be sp-ICP-TOF-MS, which can potentially yield concentration, size and composition data. With very low detection limits for NMs and quasi simultaneous multi-element analysis it is even potentially possible to differentiate between natural and engineered NMs. However, this could not be used as a stand-alone technique since information about e.g. particle shape if needed cannot be provided. Due to the high price for such a high-end machine, it might hardly find its way in analytical labs for routine analysis, unless there is a need for routine analysis in environmental samples e.g. sewage or effluent defined by regulatory obligations.

To date, it is hardly possible to analyse environmental samples without having any idea concerning NM load. Most or almost all studies use model NMs that were spiked to environmental samples to prove feasibility of the individual methods or techniques, extraction procedures etc.. In general, sampling, sample handling and preparation for measurements are leading to the biggest uncertainties in NM detection in environmental compartments. Validation of the detection methods seems to be crucial since recovery rates and accuracy of the methods are often unknown or very broad.

A general workflow for NM monitoring shall be exemplified for the case of a soil sample (for other compartments the workflow would be similar with appropriate choice of extraction methods). The sample to be analysed for NMs would need to undergo an extraction procedure to separate the NMs from the soil matrix. While such an extraction procedure can be optimized for a specific NM, in general, the extraction will be blind to the specific type of NM and the resultant extract would consist of a mixture of a multitude of manufactured and natural nano- and non-nano materials when it comes to size, shape and composition that will be extracted with different efficiency. In terms of specialized extraction procedures, different procedures for different classes of NMs, such as metal oxides, metals and organic/carbon nanoparticles, seem appropriate with adaptations of the procedures concerning dissolvable NMs. Additionally, the extraction procedures need to be adapted according to different soil properties, such as natural organic matter content or ionic strength. Based on known recovery rates the actual content of NMs in the soil sample can be calculated from the results of the subsequent analysis. The extraction step needs to be followed by a size fractionation step as most NM detection and identification methods have problems with polydisperse samples. The methodologically simplest approach would be a two-step filtering procedure to remove larger particles as well as soluble backgrounds. An elemental analysis of the different filtrates by, for example, ICP-MS would yield a general idea about what elements are present in nano-particulate form and in what concentration (although potentially missing NMs that could not be detached from larger background particles). However, no information about the specific type of NM (e.g. manufactured TiO2 vs. natural TiO2 vs. natural NM with Ti-impurities, etc.) or the detailed size distribution is gained. Furthermore the recovery rates for filtration steps are poor and subject to great uncertainty. In this sense, Fabricius et al. (2014) indicated that aiming to establish a standard protocol for quantification of metallic NMs with ICP-MS, ultrafiltration combined with microwave digestion would be the best practice to maximize the measurements. The lack of detailed information of such simple procedures could be attenuated to some degree by additional electron microscopic investigations of the filtered/sedimented materials. A more sophisticated size fractionation procedure would make use of e.g. AF4 to fractionate the extracted materials according to their hydrodynamic diameter. Once again an elemental analysis would provide information on composition, now for a more detailed size range. However, for a detailed identification of the NMs contained in the fractionized samples further information is needed, as still, the interpretation of the elemental compositions in terms of NM content is solely dependent on the assumptions made during the analysis (e.g. assuming that all the measured Ti is present as TiO2 of a specific size and not as component in natural NMs). The further information needed can be gained from single particle analysis. Using an ICP-MS in single-particle mode can give detailed information on size distribution and NM concentration. The use of sp-ICP-TOF-MS further increases the information depth to yield detailed compositional information potentially allowing to identify mixed composition particles and even distinguish engineered from natural nanomaterials by their elemental composition profile.

5.1 Need for action

As stated above, no singular technique provides a straight forward way of detecting arbitrary NMs in environmental samples and a combinatory approach must be implemented. To reiterate, an all-encompassing method for NM detection in environmental samples would need to provide information on NM size, concentration, composition and shape. In order to perform such measurements suitable extraction, pre-sorting and if necessary pre-concentration procedures need to be developed, validated and coupled. A general scheme, in line with the conclusions of European projects such as nanoMONITOR (nanoMONITOR Guidance, 2018), consists of:

(i) validated sampling procedures for the different environmental compartments and NMs

(ii) validated extraction techniques to separate NMs or specific NMs (e.g. organic vs. inorganic) from the matrix

(iii) validated size separation technique

(iv) a multi-method analysis which provides information about NM size, concentration, composition and shape

For every step along the path, from sampling, over extraction and size separation to analysis there exists a body of work in the literature, not least summarized in this report. Based on this knowledge a standardization and validation of every single step should be pursued. Following that/in parallel to that a standardization and validation of the coupling of the different steps to a full-fledged combinatory approach needs to be implemented.

This is easier said than done since one procedure that may be suitable for one NM under certain conditions at a certain concentration in one environmental compartment but may not be generalizable for the multitude of other possible manufactured NMs as well as within the natural variability of the environmental compartments. Furthermore, the lack of reference and standard materials for calibration and validation greatly hinders the efforts in this direction. Radiolabelling techniques may help to proof the concepts and robustness of the whole separation and detection processes through sampling, sample pre-treatment and analysis. A review of Yin et al. (2017) summarizes the current status of application of isotope tracers to study the environmental fate and bioaccumulation of metal-containing engineered NMs. While these studies can only be performed in controlled lab areas the extremely high sensitivity of radiotracer detection allows the detection of suitably labelled NMs at environmentally relevant concentrations in any environmental compartment irrespective of the complexity of the matrix (Hildebrand and Franke 2012, Hildebrand et al. 2015, Schymura et al. 2017). Therefore the radiolabelling of NMs may provide a possibility of independent validation; independent in the sense of not depending on the methods that are to be validated.

In consequence of the preceding the need for action presents itself in the following categories.

5.1.1 Identification of most relevant NMs/compartments

Many of the existing procedures for NM sampling, extraction and size separation in the literature have been developed/optimized for specific NMs, often for Au NMs which are of little environmental significance. This is reasonable in the first development stages of techniques but for a future targeted development of environmental monitoring procedures the most relevant NMs in terms of expected environmental relevance (e.g. exposure, hazard, etc.) and the key compartments should be identified in order to steer the development of techniques and consequent validation of NM mass flow models towards the most crucial issues. With time this relevancy evaluation should be updated as more data accumulates or NM application/production patterns change.

Short-term:

- Identification of most pressing NMs

Medium-term:

- Constant update/re-evaluation of the assessment

5.1.2 Development of sampling and extraction procedures

The most pressing issue for environmental NM detection is the development of standardized sampling and extraction techniques for the different environmental compartments. In consequence of the large variety of NMs and the variability of environmental conditions within the different compartments the starting point for development should in the short term be guided by the identified relevancy of NMs mentioned above. Detailed handling procedures for the identified most relevant NM should be developed that take the natural variability into account. For example, an extraction procedure for TiO2 NMs from soil needs to come with instructions for adaptations based on the soil properties (e.g. pH, ionic strength, natural organic matter content, etc.). While this would potentially produce a solution for the most pressing issues a wider approach is mandatory in the medium-term. The developed extraction techniques for specific NMs should be tested for their applicability for wider classes of NMs that show similar properties and characteristics. For example, extraction techniques that are applicable for NM classes, such as metal oxide NMs, metallic NMs and organic/carbon NMs with adaptations for dissolvable NMs. In all cases, validation and standardisation efforts should be pursued via international, possibly blinded, round robin tests in order to improve repeatability and comparability of the developed procedures. Blind tests in which the analysing laboratory does not know how much NM of which size is contained in the sample serve as a "final" validation in procedure development. The feasibility of these goals is dependent on the identification and provision of standard reference materials that can serve to ensure repeatability and comparability in between laboratories. Techniques such as (radio-)isotopic labelling may provide valuable independent reference measurements for such procedure development.

Short-term:

- Development of extraction techniques for specific relevant NMs with adaptations and SOPs according to the natural variability in the sampled compartment.
- Identification and provision of standard reference materials
- Testing and validation of the developed procedure in international round robin test.

Medium-term:

- Adaptation of the developed procedure for the wider classes of NMs
- Testing and validation of the developed procedures in international round robin test

Long term:

- Establishment of a library of extraction procedures with SOPs according to the NMs/classes of NMs, the compartment and the detailed environmental conditions within the compartment.

5.1.3 Implementation of a combinatory approach for NM detection/identification

As concluded from the literature survey a detection and identification of NMs in environmental samples without prior knowledge of the NM to detect must rely on a combination of separation and detection techniques. For a reliable identification and quantification of manufactured NMs in environmental samples information about the material's size, concentration and chemical composition is needed. Based on the existing body of work, not least summarized in this report, the most suitable methods for a combinatory approach should be identified (suggestions based on the state of the art can be found in this report) and validated SOPs for the different methods should be developed/extracted from the existing literature. The feasibility and reliability of the combinatory approach should be tested and validated in the short term for the identified most relevant NMs known. This should be done in combination with the development of the extraction procedure mentioned above for samples of NMs spiked to a complex matrix. The use of a specific NM at this stage allows the implementation of the approach with relative "ease" as the data evaluation can be cut short considerably, if it is known what to look for. Furthermore this "single NM" approach should be tested with real environmental samples. In a next step, the approach should be implemented and validated for the analysis of NM mixtures (mixtures of different sizes as well as mixtures of different chemical composition). Validation efforts for the single methods and steps should be pursued with inter-laboratory round robin tests of the developed SOPs and possibly blind testing for specific NMs/NM mixtures. Standardisation of the single steps should be aimed for in order to improve repeatability and comparability of measured environmental data. The feasibility of these goals is dependent on the identification and provision of standard reference materials that can serve to ensure repeatability and comparability in between laboratories. Techniques such as (radio-)isotopic labelling may provide valuable independent reference measurements for such procedure development.

Short-term:

- Choice of most promising combinatory approach for NM separation/detection (for example: CPE, AF^4, MALS, sp-ICP-TOF-MS)
- Development of SOPs for a specific relevant NM for the different combined techniques
- Testing and validation of the methods in combination

Medium-term:

- Establishment of a SOP for environmental monitoring of the relevant NM
- Adaptation of the developed procedure for the wider classes of NMs
- Testing and validation of the developed combined procedures in international round robin test

Long-term:

- Establishment of the combinatory approach for environmental samples without prior limitation of NMs to be detected
- Testing and validation of the developed combined procedures in international round robin test

5.1.4 Instrument development/data handling

There are sophisticated analytical methods and techniques available to analyse NMs in general and also in environmental samples. For environmental monitoring purposes the different types of detection should be integrated in a way suitable for routine analysis. Furthermore instrumental advancements towards a wider spectrum of detectable NMs (e.g. compatibility for metal, metal oxide and organic NMs), lower detection limits and especially to align the detection limits of the different methods, so that potential pre-concentration steps could be avoided should be explored. Additionally, a combinatory approach of different methods is set to produce a lot of data to be evaluated. In the case of an arbitrary environmental sample, NM size, shape, composition and concentration data need to be brought together on a single particle basis (for example, chemical composition data for a single particle from sp-ICP-MS with shape information from MALS, etc.). Data handling and evaluation routines need to be developed that allow for an automated processing and evaluation of such data packages. A database of manufactured and natural NMs should be developed for the data to be matched against in order to identify the detected NMs.

Shot-term:

- Continuous improvement towards lower detection limits
- Increase availability/applicability of promising techniques (e.g.: sp-ICP-TOF-MS)
- Widening of the spectrum of detectable NMs (i.e. sp-ICP-MS with carbon NMs)

Medium-term:

- Automation of experimental steps
- Automation of data handling
- Combination of techniques in single technical solution

Long-term:

- Establishment of a data library of different natural and manufactured NMs against which data from environmental monitoring application can be checked for routine NM identification in environmental samples

6 List of references

This lists the evaluated literature in its entirety, even if not directly cited in the text above.

Allabashi, R., Stach, W., De La Escosura-Muñiz, A., Liste-Calleja, L. and Merkoçi, A. (2009): ICP-MS: a powerful technique for quantitative determination of gold nanoparticles without previous dissolving. J. Nanopart. Res. 11(8): 2003-2011.

Alvarez, P.J., Colvin, V., Lead, J. and Stone, V. (2009): Research priorities to advance eco-responsible nanotechnology. ACS Nano 3(7): 1616-1619.

Baalousha, M., Stolpe, B. and Lead, J.R. (2011): Flow field-flow fractionation for the analysis and characterization of natural colloids and manufactured nanoparticles in environmental systems: a critical review. J. Chromatogr. A. 1218(27): 4078-4103.

Bäuerlein, P.S., Emke, E., Tromp, P., Hofman, J.A.M.H., Carboni, A., Schooneman, F., De Voogt, P. and Van Wezel, A.P. (2017): Is there evidence for man-made nanoparticles in the Dutch environment. Sci. Total Environ. 576: 273-283.

Bitragunta, S.P., Palani, S.G., Gopala, A., Sarkar, S.K. and Kandukuri, V.R. (2017): Detection of TiO2 Nanoparticles in Municipal Sewage Treatment Plant and Their Characterization Using Single Particle ICP-MS. Bull. Environ. Contam. Toxicol. 98(5): 595-600.

Blaser, S.A., Scheringer, M., Macleod, M. and Hungerbuhler, K. (2008): Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci. Total. Environ. 390(2-3): 396-409.

Boschi, F. and De Sanctis, F. (2017): Overview of the optical properties of fluorescent nanoparticles for optical imaging. Eur. J. Histochem. 61(3):

Bouguerra, S., Gavina, A., Ksibi, M., Rasteiro Mda, G., Rocha-Santos, T. and Pereira, R. (2016): Ecotoxicity of titanium silicon oxide (TiSiO4) nanomaterial for terrestrial plants and soil invertebrate species. Ecotoxicol. Environ. Saf. 129: 291-301.

Bradford, A., Handy, R.D., Readman, J.W., Atfield, A. and Mühling, M. (2009): Impact of Silver Nanoparticle Contamination on the Genetic Diversity of Natural Bacterial Assemblages in Estuarine Sediments. Environ. Sci. Technol. 43(12): 4530-4536.

Brar, S.K., Verma, M., Tyagi, R.D. and Surampalli, R.Y. (2010): Engineered nanoparticles in wastewater and wastewater sludge--evidence and impacts. Waste Manag. 30(3): 504-520.

Brown, K., Thurn, T., Xin, L., Liu, W., Bazak, R., Chen, S., Lai, B., Vogt, S., Jacobsen, C., Paunesku, T. and Woloschak, G.E. (2018): Intracellular in situ labeling of TiO2 nanoparticles for fluorescence microscopy detection. Nano Res. 11(1): 464-476.

Buffet, P.E., Amiard-Triquet, C., Dybowska, A., Risso-De Faverney, C., Guibbolini, M., Valsami-Jones, E. and Mouneyrac, C. (2012): Fate of isotopically labeled zinc oxide nanoparticles in sediment and effects on two endobenthic species, the clam Scrobicularia plana and the ragworm Hediste diversicolor. Ecotoxicol. Environ. Saf. 84: 191-198.

Bundschuh, M., Filser, J., Lüderwald, S., Mckee, M.S., Metreveli, G., Schaumann, G.E., Schulz, R. and Wagner, S. (2018): Nanoparticles in the environment: where do we come from, where do we go to. Environ. Sci. Eur. 30(1): 6.

Bustos, A.R.M. and Winchester, M.R. (2016): Single-particle-ICP-MS advances. Anal. Bioanal. Chem. 408(19): 5051-5052.

Chen, Z., Westerhoff, P. and Herckes, P. (2008): Quantification of C60 fullerene concentrations in water. Environ. Toxicol. Chem. 27(9): 1852-1859.

Contado, C. (2017): Field flow fractionation techniques to explore the “nano-world”. Anal. Bioanal. Chem. 409(10): 2501-2518.

Da Sliva, B.F., Perez, S., Gardinalli, P., Singhal, R.K., Mozeto, A.A. and Barcelo, D. (2011): Analytical Chemistry of metallic nanoparticles in natural environments. Trends Anal. Chem .30(3): 528-540.

Dan, Y., Ma, X., Zhang, W., Liu, K., Stephan, C. and Shi, H. (2016): Single particle ICP-MS method development for the determination of plant uptake and accumulation of CeO2 nanoparticles. Anal. Bioanal. Chem. 408(19): 5157-5167.

Dan, Y., Zhang, W., Xue, R., Ma, X., Stephan, C. and Shi, H. (2015): Characterization of Gold Nanoparticle Uptake by Tomato Plants Using Enzymatic Extraction Followed by Single-Particle Inductively Coupled Plasma–Mass Spectrometry Analysis. Environ. Sci. Technol. 49(5): 3007-3014.

Delay, M. and Frimmel, F.H. (2012): Nanoparticles in aquatic systems. Anal. Bioanal. Chem. 402(2): 583-592.

Deline, A.R. and Nason, J.A. (2019): Evaluation of labeling methods used for investigating the environmental behavior and toxicity of metal oxide nanoparticles. Environ. Sci.: Nano 6(4): 1043-1066.

Domingos, R.F., Baalousha, M.A., Ju-Nam, Y., Reid, M.M., Tufenkji, N., Lead, J.R., Leppard, G.G. and Wilkinson, K.J. (2009): Characterizing Manufactured Nanoparticles in the Environment: Multimethod Determination of Particle Sizes. Environ. Sci. Technol. 43(19): 7277-7284.

Dudkiewicz, A., Tiede, K., Loeschner, K., Helene, L., Jensen, S., Jensen, E., Wierzbicki, R., Aba, B. and Molhave, K. (2011): Chracterization of nanomaterials in food by electron microscopy. Trends Anal. Chem. 30(1): 28-43.

Dudkiewicz, A., Lehner, A., Chaudhry, Q., Molhave, K., Allmaier, G., Tiede, K., Boxall, A.B.A., Hofmann, P. and Lewis, J. (2019): Development of a sample preparation approach to measure the size of nanoparticle aggregates by electron microscopy. Particuology 45: 49-57.

Eastern Research Group, Inc. 2008. Sampling and Analysis of Nanomaterials in the Environment: A State-of-the-Science Review - Final report. United States Environmental Protection Agency.

Eduok, S., Ferguson, R., Jefferson, B., Villa, R. and Coulon, F. (2017): Aged-engineered nanoparticles effect on sludge anaerobic digestion performance and associated microbial communities. Sci. Total. Environ. 609: 232-241.

El Hadri, H. and Hackley, V.A. (2017): Investigation of cloud point extraction for the analysis of metallic nanoparticles in a soil matrix. Environ. Sci.: Nano 4(1): 105-116.

El Hadri, H., Louie, S.M. and Hackley, V.A. (2018): Assessing the interactions of metal nanoparticles in soil and sediment matrices – a quantitative analytical multi-technique approach. Environ. Sci.: Nano 5(1): 203-214.

European Commission Directorate-General, Health and Consumer Protection. 2006. The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies.

Fabricius, A.L., Duester, L., Meermann, B. and Ternes, T.A. (2014): ICP-MS-based characterization of inorganic nanoparticles--sample preparation and off-line fractionation strategies. Anal. Bioanal. Chem. 406(2): 467-479.

Fang, J., Shan, X.Q., Wen, B., Lin, J.M. and Owens, G. (2009): Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. Environ. Pollut. 157(4): 1101-1109.

Gallego-Urrea, J.A., Tuoriniemi, J. and Hassellöv, M. (2011): Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples. Trends Anal. Chem. 30(3): 473-483.

Gogos, A., Kaegi, R., Zenobi, R. and Bucheli, T.D. (2014): Capabilities of asymmetric flow field-flow fractionation coupled to multi-angle light scattering to detect carbon nanotubes in soot and soil. Environ. Sci.: Nano 1(6): 584-594.

Gogos, A., Wielinski, J., Voegelin, A., Emerich, H. and Kaegi, R. (2019): Transformation of cerium dioxide nanoparticles during sewage sludge incineration. Environ. Sci.: Nano 6: 1765-1776.

Gondikas, A., Von Der Kammer, F., Kaegi, R., Borovinskaya, O., Neubauer, E., Navratilova, J., Praetorius, A., Cornelis, G. and Hofmann, T. (2018): Where is the nano? Analytical approaches for the detection and quantification of TiO engineered nanoparticles in surface waters. Environ. Sci.: Nano 5(2): 313-326.

Gondikas, A.P., Von der Kammer, F., Reed, R.B., Wagner, S., Ranville, J.F. and Hofmann, T. (2014): Release of TiO Nanoparticles from Sunscreens into Surface Waters: A One-Year Survey at the Old Danube Recreational Lake. Environ. Sci. Technol. 48(10): 5415-5422.

Gottschalk, F., Sonderer, T., Scholz, R.W. and Nowack, B. (2009): Modeled Environmental Concentrations of Engineered Nanomaterials (TiO, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environ. Sci. Technol. 43(24): 9216-9222.

Gottschalk, F., Sun, T. and Nowack, B. (2013): Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. Environ. Pollution. 181: 287-300.

Gray, E.P., Coleman, J.G., Bednar, A.J., Kennedy, A.J., Ranville, J.F. and Higgins, C.P. (2013): Extraction and analysis of silver and gold nanoparticles from biological tissues using single particle inductively coupled plasma mass spectrometry. Environ. Sci. Technol. 47(24): 14315-14323.

Greswell, R.B., Rahman, S.H., Cuthbert, M.O. and Tellam, J.H. (2010): An inexpensive flow-through laser nephelometer for the detection of natural colloids and manufactured nanoparticles. J. Hydrol. 388(1-2): 112-120.

Gubala, V., Johnston, L.J., Liu, Z., Krug, H., Moore, C.J., Ober, C.K., Schwenk, M. and Vert, M. (2018): Engineered nanomaterials and human health: Part 1. Preparation, functionalization and characterization (IUPAC Technical Report). Pure Appl. Chem. 90(8): 1283-1324.

Hartmann, G., Hutterer, C. and Schuster, M. (2013): Ultra-trace determination of silver nanoparticles in water samples using cloud point extraction and ETAAS. J. Anal. At. Spectrom. 28(4): 567.

Hassellöv, M., Readman, J.W., Ranville, J.F. and Tiede, K. (2008): Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Ecotoxicology 17(5): 344-361.

Hennebert, P., Anderson, A. and Nerdy, P. (2017): Mineral Nanoparticles in Waste: Potential Sources, Occurrence in Some Engineered Nanomaterials Leachates, Municipal Sewage Sludges and Municipal Landfill Sludges. J. Biotechnol. Biomater. 7(2): 1000261.

Hildebrand, H. and Franke, K. (2012): A new radiolabeling method for commercial Ag0 nanopowder with 110mAg for sensitive nanoparticle detection in complex media. J. Nanopart. Res. 14(10): 2435.

Hildebrand, H., Schymura, S., Holzwarth, U., Gibson, N., Dalmiglio, M. and Franke, K. (2015): Strategies for radiolabeling of commercial TiO2 nanopowder as a tool for sensitive nanoparticle detection in complex matrices. J. Nanopart. Res. 17(6): 2435.

Howard, A.G. (2010): On the challenge of quantifying man-made nanoparticles in the aquatic environment. J. Environ. Monit. 12(1): 135-142.

Isaacson, C.W., Kleber, M. and Field, J.A. (2009): Quantitative analysis of fullerene nanomaterials in environmental systems: a critical review. Environ. Sci. Technol. 43(17): 6463-6474.

Jośko, I., Oleszczuk, P. and Skwarek, E. (2016): The bioavailability and toxicity of ZnO and Ni nanoparticles and their bulk counterparts in different sediments. J. Soils. Sediments. 16(6): 1798-1808.

Kazemi-Beydokhti, A. and Hajiabadi, S.H. (2018): Rheological investigation of smart polymer/carbon nanotube complex on properties of water-based drilling fluids. Colloids Surf. A. 556: 2329.

Kennedy, A.J., Hull, M.S., Bednar, A.J., Goss, J.D., Gunter, J.C., Bouldin, J.L., Vikesland, P.J. and Steevens, J.A. (2010): Fractionating Nanosilver: Importance for Determining Toxicity to Aquatic Test Organisms. Environ. Sci. Technol. 44(24): 9571-9577.

Kim, B., Murayama, M., Colman, B.P. and Hochella, M.F. (2012): Characterization and environmental implications of nano- and larger TiO2 particles in sewage sludge, and soils amended with sewage sludge. J. Environ. Monit. 14: 1129-1137.

Kiser, M.A., Westerhoff, P., Benn, T., Wang, Y., Pérez-Rivera, J. and Hristovski, K. (2009): Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants. Environ. Sci. Technol. 43(17): 6757-6763.

Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., Mclaughlin, M.J. and Lead, J.R. (2008): Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27(9): 1825-1851.

Knopf, B., Meisterjahn, B., Schröder, N., Rüdel, H. and Koschorreck, J. (2017): Development of a method for the analysis of nanoparticles in homogenized biota tissue from the German Environmental Specimen Bank. Scientific Stakeholder Meeting on Nanomaterials in the Environment, UBA.

Koelmans, A.A., Nowack, B. and Wiesner, M.R. (2009): Comparison of manufactured and black carbon nanoparticle concentrations in aquatic sediments. Environ. Poll. 157(4): 1110-1116.

Kuhlbusch, T.A., Nickel, C., Hellack, B., Gartiser, S., Flach, F., Schiwy, A., Maes, H., Schäffer, A., Erdinger, L., Gabsch, S. and Stintz, M. (2012): Fate and behaviour of TiO2 nanomaterials in the environment, influenced by their shape, size and surface area. UBA Texte 25.

Laborda, F., Bolea, E., Cepriá, G., Gómez, M.T., Jiménez, M.S., Pérez-Arantegui, J. and Castillo, J.R. (2016): Detection, characterization and quantification of inorganic engineered nanomaterials: A review of techniques and methodological approaches for the analysis of complex samples. Anal. Chim. Acta 904 10-32.

Laborda, F., Bolea, E. and Jiménez-Lamana, J. (2014): Single Particle Inductively Coupled Plasma Mass Spectrometry: A Powerful Tool for Nanoanalysis. Anal. Chem. 86(5): 2270-2278.

Lapresta-Fernandez, A., Salinas-Castillo, A., Anderson De La Llana, S., Costa-Fernandez, J.M., Dominguez-Meister, S., Cecchini, R., Capitan-Vallvey, L.F., Moreno-Bondi, M.C., Pilar Marco, M., Sanchez-Lopez, J.C. and Anderson, I.S. (2014): A General Perspective of the Characterization and Quantification of Nanoparticles. Imaging, Spectroscopic and Separation Techniques. Crit. Rev. Solid State 39(6): 423-458.

Larner, F. and Rehkämper, M. (2012): Evaluation of Stable Isotope Tracing for ZnO Nanomaterials—New Constraints from High Precision Isotope Analyses and Modeling. Environ. Sci. Tech. 46(7): 4149-4158.

Lay-Ekuakille, A., Ikezawa, S., Mugnaini, M., Morello, R. and De Capua, C. (2017): Detection of specific macro and micropollutants in air monitoring: Review of methods and techniques. Measurement 98: 49-59.

Lee, S., Bi, X., Reed, R.B., Ranville, J.F., Herckes, P. and Westerhoff, P. (2014): Nanoparticle Size Detection Limits by Single Particle ICP-MS for 40 Elements. Environ. Sci. Technol. 48(17): 10291-10300.

Leopold, K., Philippe, A., Wörle, K. and Schaumann, G.E. (2016): Analytical strategies to the determination of metal.containing nanoparticles in environmental waters. Trends Anal. Chem. 84: 107-120.

Li, L., Leopold, K. and Schuster, M. (2012): Effective and selective extraction of noble metal nanoparticles from environmental water through a noncovalent reversible reaction on an ionic exchange resin. Chem. Commun. 48(73): 9165.

Liang, H.W., Wang, L., Chen, P.Y., Lin, H.T., Chen, L.F., He, D. and Yu, S.H. (2010): Carbonaceous nanofiber membranes for selective filtration and separation of nanoparticles. Adv. Mater. 22(42): 4691-4695.

Linsinger, T.P.J., Chaudhry, Q., Dehalu, V., Delahaut, P., Dudkiewicz, A., Grombe, R., Von Der Kammer, F., Larsen, E.H., Legros, S., Loeschner, K., Peters, R., Ramsch, R., Roebben, G., Tiede, K. and Weigel, S. (2013): Validation of methods for the detection and quantification of engineered nanoparticles in food. Food Chem. 138(2-3): 1959-1966.

Liu, J. (2005): Scanning transmission electron microscopy and its application to the study of nanoparticles and nanoparticle systems. J. Electron. Microsc. (Tokyo) 54(3): 251-278.

Liu, J.-F., Chao, J.-B., Liu, R., Tan, Z.-Q., Yin, Y.-G., Wu, Y. and Jiang, G.-B. (2009): Cloud Point Extraction as an Advantageous Preconcentration Approach for Analysis of Trace Silver Nanoparticles in Environmental Waters. Anal. Chem. 81(15): 6496-6502.

Liu, W., Shi, H. and Stephan, C. (2018): Analysis of Cerium Dioxide Nanoparticles in Soil Using Single Particle ICP-MS. Perkin Elmer Application Note.

Lu, P.J., Fu, W.E., Huang, S.C., Lin, C.Y., Ho, M.L., Chen, Y.P. and Cheng, H.F. (2018): Methodology for sample preparation and size measurement of commercial ZnO nanoparticles. J. Food Drug Anal. 26(2): 628-636.

Luo, P., Morrison, I., Dudkiewicz, A., Tiede, K., Boyes, E., O'toole, P., Park, S. and Boxall, A.B. (2013): Visualization and characterization of engineered nanoparticles in complex environmental and food matrices using atmospheric scanning electron microscopy. J. Microsc. 250(1): 32-41.

Luo, P., Roca, A., Tiede, K., Privett, K., Jiang, J., Pinkstone, J., Ma, G., Veinot, J. and Boxall, A. (2018): Application of nanoparticle tracking analysis for characterising the fate of engineered nanoparticles in sediment-water systems. J. Environ. Sci. (China) 64, 62-71.

Luo, Z.-X., Wang, Z.-H., Xu, B., Sarakiotis, I.L., Laing, G.D. and Yan, C.-Z. (2014): Measurement and characterization of engineered titanium dioxide nanoparticles in the environment. J. Zhejiang Univ. Sci. A 15(8): 593-605.

Ma, X., Geisler-Lee, J., Geiser-Lee, J., Deng, Y. and Kolmakov, A. (2010): Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci. Total. Environ. 408(16): 3053-3061.

Mahdi, K.N.M., Peters, R.J.B., Klumpp, E., Bohme, S., Ploeg, M.V.D., Ritsema, C. and Geissen, V. (2017): Silver nanoparticles in soil: Aqueous extraction combined with single-particle ICP-MS for detection and characterization. Environ. Nanotechnol. Monit. Manage. 7: 24-33.

Maier, M. and Wegenke, M. (2017): Detection of nanoparticles in rivers and industrial wastewaters. Scientific Stakeholder Meeting on Nanomaterials in the Environment, UBA.

Majedi, S.M., Kelly, B.C. and Lee, H.K. (2014a): Evaluation of a cloud point extraction approach for the preconcentration and quantification of trace CuO nanoparticles in environmental waters. Anal. Chim. Acta 814: 39-48.

Majedi, S.M., Kelly, B.C. and Lee, H.K. (2014b): Toward a robust analytical method for separating trace levels of nano-materials in natural waters: cloud point extraction of nano-copper(II) oxide. Environ. Sci. Pollut. Res. 21(20): 11811-11822.

Majedi, S.M. and Lee, H.K. (2016): Recent advances in the separation and quantification of metallic nanoparticles and ions in the environment. Trend Anal. Chem. 75: 183-196.

Makselon, J., Siebers, N., Meier, F., Vereecken, H. and Klumpp, E. (2018): Role of rain intensity and soil colloids in the retention of surfactant-stabilized silver nanoparticles in soil. Environ. Poll. 238: 1027-1034.

Malvern Malvern Zeta Sizer ZS User Manual.

Mattarozzi, M., Suman, M., Cascio, C., Calestani, D., Weigel, S., Undas, A. and Peters, R. (2017): Analytical approaches for the characterization and quantification of nanoparticles in food and beverages. Anal. Bioanal. Chem. 409(1): 63-80.

Mcgillicuddy, E., Murray, I., Kavanagh, S., Morrison, L., Fogarty, A., Cormican, M., Dockery, P., Prendergast, M., Rowan, N. and Morris, D. (2017): Silver nanoparticles in the environment: Sources, detection and ecotoxicology. Sci. Total Environ. 575: 231-246.

Mcgillicuddy, E., Morrison, L., Cormican, M., Dockery, P. and Morris, D. (2018): Activated charcoal as a capture material for silver nanoparticles in environmental water samples. Sci. Total Environ. 645: 356-362.

Meermann, B. and Nischwitz, V. (2018): ICP-MS for the analysis at the nanoscale – a tutorial review. J. Anal. At. Spectrom. 33: 1432-1468.

Mitrano, D.M., Rimmele, E., Wichser, A., Erni, R., Height, M. and Nowack, B. (2014): Presence of nanoparticles in wash water from conventional silver and nano-silver textiles. ACS Nano 8(7): 7208-7219.

Montaño, M.D., Badiei, H.R., Bazargan, S. and Ranville, J.F. (2014a): Improvements in the detection and characterization of engineered nanoparticles using spICP-MS with microsecond dwell times. Environ. Sci.: Nano 1(4): 338-346.

Montaño, M.D., Lowry, G.V., Von Der Kammer, F., Blue, J. and Ranville, J.F. (2014b): Current status and future direction for examining engineered nanoparticles in natural systems. Environ. Chem. 11(4): 351.

Montaño, M.D., Olesik, J.W., Barber, A.G., Challis, K. and Ranville, J.F. (2016): Single Particle ICP-MS: Advances toward routine analysis of nanomaterials. Anal. Bioanal. Chem. 408(19): 5053-5074.

Mourdikoudis, S., Pallares, R.M. and Thanh, N.T.K. (2018): Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale 10(27): 12871-12934.

Mudalige, T.K., Qu, H., Van Haute, D., Ansar, S.M. and Linder, S.W. (2018): Capillary electrophoresis and asymmetric flow field-flow fractionation for size-based separation of engineered metallic nanoparticles: A critical comparative review. Trends Anal. Chem. 106: 202-212.

Mukherjee, K. and Acharya, K. (2018): Toxicological Effect of Metal Oxide Nanoparticles on Soil and Aquatic Habitats. Arch. Environ. Contam. Toxicol. 75(2): 175-186.

Murashov, V. and Howard, J. 2011. Nanotechnology Standards. Springer-Verlag New York Inc.

Mwilu, S.K., Siska, E., Baig, R.B.N., Varma, R.S., Heithmar, E. and Rogers, K.R. (2014): Separation and measurement of silver nanoparticles and silver ions using magnetic particles. Sci. Total Environ. 472: 316-323.

Naasz, S., Weigel, S., Borovinskaya, O., Serva, A., Cascio, C., Undas, A.K., Simeone, F.C., Marvin, H.J.P. and Peters, R.J.B. (2018): Multi-element analysis of single nanoparticles by ICP-MS using quadrupole and time-of-flight technologies. J. Anal. At. Spectrom. 33(5): 835-845.

Naghdi, M., Metahni, S., Ouarda, Y., Brar, S.K., Das, R.K. and Cledon, M. (2017): Instrumental approach toward understanding nano-pollutants. Nanotechnol. Environ. Eng. 2(1): 1723.

Nanomonitor 2018. Guidance on the sampling methods and analytical techniques for the measurement and monitoring of engineered nanomaterials in the environment.

Navarro, E., Baun, A., Behra, R., Hartmann, N.B., Filser, J., Miao, A.-J., Quigg, A., Santschi, P.H. and Sigg, L. (2008): Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology 17(5): 372-386.

Navratilova, J., Praetorius, A., Gondikas, A., Fabienke, W., Von Der Kammer, F. and Hofmann, T. (2015): Detection of Engineered Copper Nanoparticles in Soil Using Single Particle ICP-MS. IJERPH 12: 15756-15768.

Neal, C., Jarvie, H., Rowland, P., Lawler, A., Sleep, D. and Scholefield, P. (2011): Titanium in UK rural, agricultural and urban/industrial rivers: Geogenic and anthropogenic colloidal/sub-colloidal sources and the significance of within-river retention. Sci. Total Environ. 409(10): 1843-1853.

Nilsson, P.T., Isaxon, C., Eriksson, A.C., Messing, M.E., Ludvigsson, L., Rissler, J., Hedmer, M., Tinnerberg, H., Gudmundsson, A., Deppert, K., Bohgard, M. and Pagels, J.H. (2013): Nano-objects emitted during maintenance of common particle generators: direct chemical characterization with aerosol mass spectrometry and implications for risk assessments. J. Nanopart. Res. 15(11): 1634.

Nischwitz, V., Gottselig, N., Missong, A., Klumpp, E. and Braun, M. (2018): Extending the capabilities of field flow fractionation online with ICP-MS for the determination of particulate carbon in latex and charcoal. J. Anal. At. Spectrom. 33(8): 1363-1371.

Nowack, B., Baalousha, M., Bornhöft, N., Chaudhry, Q., Cornelis, G., Cotterill, J., Gondikas, A., Hassellöv, M., Lead, J., Mitrano, D.M., Von Der Kammer, F. and Wontner-Smith, T. (2015): Progress towards the validation of modeled environmental concentrations of engineered nanomaterials by analytical measurements. Environ. Sci.: Nano 2(5): 421-428.

Okello, V.A., Du, N., Deng, B. and Sadik, O.A. (2011): Environmental applications of poly(amic acid)-based nanomaterials. J. Environ. Monit. 13(5): 1236-1245.

Pachapur, V.L., Dalila Larios, A., Cledon, M., Brar, S.K., Verma, M. and Surampalli, R.Y. (2016): Behavior and characterization of titanium dioxide and silver nanoparticles in soil. Sci. Total. Environ. 563-564: 933-943.

Páscoa, R.N.M.J., Tóth, I.V., Almeida, A.A. and Rangel, A. (2011): Spectrophotometric sensor system based on a liquid waveguide capillary cell for the determination of titanium: Application to natural waters, sunscreens and a lake sediment. Sens. Actuators. B Chem. 157(1): 51-56.

Pelzer, J., Bischof, O., Van Den Brink, W., Fierz, M., Isherwood, H., Gnewuch, H., Knecht, A., Krinke, T. and Zerrath, A. (2010): Geräte zur Messung der Anzahlkonzentration von Nanopartikeln. Gefahrstoffe-Reinhaltung der Luft 11-12: 469-477.

Peters, R., Herrera-Rivera, Z., Undas, A., Van Der Lee, M., Marvin, H., Bouwmeester, H. and Weigel, S. (2015): Single particle ICP-MS combined with a data evaluation tool as a routine technique for the analysis of nanoparticles in complex matrices. J. Anal. At. Spectrom. 30(6): 1274-1285.

Peters, R.J.B., Van Bemmel, G., Milani, N.B.L., Den Hertog, G.C.T., Undas, A.K., Van Der Lee, M. and Bouwmeester, H. (2018): Detection of nanoparticles in Dutch surface waters. Sci. Total Environ. 621: 210-218.

Petersen, E.J., Flores-Cervantes, D.X., Bucheli, T.D., Elliott, L.C., Fagan, J.A., Gogos, A., Hanna, S., Kägi, R., Mansfield, E., Bustos, A.R., Plata, D.L., Reipa, V., Westerhoff, P. and Winchester, M.R. (2016): Quantification of Carbon Nanotubes in Environmental Matrices: Current Capabilities, Case Studies, and Future Prospects. Environ. Sci. Technol. 50(9): 4587-4605.

Petersen, E.J., Mortimer, M., Burgess, R.M., Handy, R., Hanna, S., Ho, K.T., Johnson, M., Loureiro, S., Selck, H., Scott-Fordsmand, J.J., Spurgeon, D., Unrine, J., Van Den Brink, N.W., Wang, Y., White, J. and Holden, P. (2019): Strategies for robust and accurate experimental approaches to quantify nanomaterial bioaccumulation across a broad range of organisms. Environ. Sci.: Nano 61: 619-1656.

Pico, Y., Alfarham, A. and Barcelo, D. (2017): Analysis of emerging contaminants and nanomaterials in plant materials following uptake from soils. Trends Anal. Chem. 94: 173-189.

Plata, D.L., Reddy, C.M. and Gschwend, P.M. (2012): Thermogravimetry–Mass Spectrometry for Carbon Nanotube Detection in Complex Mixtures. Environ. Sci. Technol. 46(22): 12254-12261.

Plathe, K.L., Von Der Kammer, F., Hassellöv, M., Moore, J., Murayama, M., Hofmann, T. and Hochella, M.F. (2010): Using FlFFF and aTEM to determine trace metal–nanoparticle associations in riverbed sediment. Environ. Chem. 7(1): 82.

Polesel, F., Farkas, J., Kjos, M., Almeida Carvalho, P., Flores-Alsina, X., Gernaey, K.V., Hansen, S.F., Plosz, B.G. and Booth, A.M. (2018): Occurrence, characterisation and fate of (nano)particulate Ti and Ag in two Norwegian wastewater treatment plants. Water. Res. 141: 19-31.

Praetorius, A., Scheringer, M. and Hungerbühler, K. (2012): Development of environmental fate models for engineered nanoparticles--a case study of TiO2 nanoparticles in the Rhine River. Environ. Sci. Technol. 46(12): 6705-6713.

Praetorius, A., Gundlach-Graham, A., Goldberg, E., Fabienke, W., Navratilova, J., Gondikas, A., Kaegi, R., Günther, D., Hofmann, T. and Von Der Kammer, F. (2017): Single-particle multi-element fingerprinting (spMEF) using inductively-coupled plasma time-of-flight mass spectrometry (ICP-TOFMS) to identify engineered nanoparticles against the elevated natural background in soils. Environ. Sci.: Nano 4(2): 307-314.

Proulx, K., Hadioui, M. and Wilkinson, K.J. (2016): Separation, detection and characterization of nanomaterials in municipal wastewaters using hydrodynamic chromatography coupled to ICPMS and single particle ICPMS. Anal. Bioanal. Chem. 408(19): 5147-5155.

Proulx, K. and Wilkinson, K.J. (2014): Separation, detection and characterisation of engineered nanoparticles in natural waters using hydrodynamic chromatography and multi-method detection (light scattering, analytical ultracentrifugation and single particle ICP-MS). Environ. Chem. 11(4): 392.

Rearick, D.C., Telgmann, L., Hintelmann, H., Frost, P.C. and Xenopoulos, M.A. (2018): Spatial and temporal trends in the fate of silver nanoparticles in a whole-lake addition study. PLoS One 13(8): 1-18.

Reddy Pullagurala, V.L., Adisa, I.O., Rawat, S., Kim, B., Barrios, A.C., Medina-Velo, I.A., Hernandez-Viezcas, J.A., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2018): Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environ. Pollut. 241: 1175-1181.

Richman, E.K. and Hutchison, J.E. (2009): The nanomaterial characterization bottleneck. ACS Nano 3(9): 2441-2446.

Roebben, G., Linsinger, T., Lamberty, A. and Emons, H. (2010): Metrological traceability of the measured values of properties of engineering materials. Metrologia 47(2): S23-S31.

Romero-Franco, M., Bilal, M., Godwin, H.A. and Cohen, Y. (2018): Assessment of information availability for environmental impact assessment of engineered nanomaterials. J. Nanopart. Res. 20(12): 1634.

Sanchís, J., Milačič, R., Zuliani, T., Vidmar, J., Abad, E., Farré, M. and Barceló, D. (2018): Occurrence of C60 and related fullerenes in the Sava River under different hydrologic conditions. Sci. Total. Environ. 643: 1108-1116.

Sanchís, J., Bosch-Orea, C., Farré, M. and Barceló, D. (2015): Nanoparticle tracking analysis characterisation and parts-per-quadrillion determination of fullerenes in river samples from Barcelona catchment area. Anal. Bioanal. Chem. 407(15): 4261-4275.

Schierz, A., Parks, A.N., Washburn, K.M., Chandler, G.T. and Ferguson, P.L. (2012): Characterization and quantitative analysis of single-walled carbon nanotubes in the aquatic environment using near-infrared fluorescence spectroscopy. Environ. Sci. Technol. 46(22): 12262-12271.

Schmid, S., Kurek, M., Adolphsen, J.Q. and Boisen, A. (2013): Real-time single airborne nanoparticle detection with nanomechanical resonant filter-fiber. Sci. Rep. 3(1): 109.

Schwertfeger, D.M., Velicogna, J.R., Jesmer, A.H., Saatcioglu, S., Mcshane, H., Scroggins, R.P. and Princz, J.I. (2017): Extracting Metallic Nanoparticles from Soils for Quantitative Analysis: Method Development Using Engineered Silver Nanoparticles and SP-ICP-MS. Anal. Chem. 89(4): 2505-2513.

Schymura, S., Fricke, T., Hildebrand, H. and Franke, K. (2017): Elucidating the Role of Dissolution in CeO2 Nanoparticle Plant Uptake. Angew. Chem. Int. Ed. Engl. 56(26): 7411-7414.

Simonet, B.M. and Valcárcel, M. (2009): Monitoring nanoparticles in the environment. Anal. Bioanal. Chem. 393(1): 17-21.

Stasinakis, A.S. (2012): Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour. Technol. 121: 432-440.

Stephan, C. and Thomas, R. (2017): Single-Particle ICP-MS: A Key Analytical Technique for Characterizing Nanoparticles. Spectroscopy 32(3): 12-25.

Steppert, P., Burgstaller, D., Klausberger, M., Tover, A., Berger, E., Jungbauer, A. (2017): Quantification and characterization of virus-like particles by size-exclusion chromatography and nanoparticle tracking analysis. J. Chromatogr. A. 1287: 89-99.

Stolpe, B., Hassellöv, M., Andersson, K. and Turner, D.R. (2005): High resolution ICPMS as an on-line detector for flow field-flow fractionation; multi-element determination of colloidal size distributions in a natural water sample. Anal. Chim. Acta 535(1-2): 109-121.

Suga, M., Asahina, S., Sakuda, Y., Kazumori, H., Nishiyama, H., Nokuo, T., Alfredsson, V., Kjellman, T., Stevens, S.M., Cho, H.S., Cho, M., Han, L., Che, S., Anderson, M.W., Schüth, F., Deng, H., Yaghi, O.M., Liu, Z., Jeong, H.Y., Stein, A., Sakamoto, K., Ryoo, R. and Terasaki, O. (2014): Recent progress in scanning electron microscopy for the characterization of fine structural details of nano materials. Prog. Solid State Chem. 42(1-2): 1-21.

Sung, H.K., Jo, E., Kim, E., Yoo, S.-K., Lee, J.-W., Kim, P.-J., Kim, Y. and Eom, I.-C. (2018): Analysis of gold and silver nanoparticles internalized by zebrafish (Danio rerio) using single particle-inductively coupled plasma-mass spectrometry. Chemosphere 209: 815-822.

Surugau, N. and Urban, P.L. (2009): Electrophoretic methods for separation of nanoparticles. J. Sep. Sci. 32(11): 1889-1906.

Tan, W., Peralta-Videa, J.R. and Gardea-Torresdey, J.L. (2018): Interaction of titanium dioxide nanoparticles with soil components and plants: current knowledge and future research needs – a critical review. Environ. Sci.: Nano 5(2): 257-278.

T.E. Commision. Commission Recommendation of 18 October 2011 on the definition of nanomaterial. (2011),

Tiede, K., Boxall, A.B., Tear, S.P., Lewis, J., David, H. and Hassellov, M. (2008): Detection and characterization of engineered nanoparticles in food and the environment. Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk. Assess. 25(7): 795-821.

Tiede, K., Boxall, A.B.A., Tiede, D., Tear, S.P., David, H. and Lewis, J. (2009): A robust size-characterisation methodology for studying nanoparticle behaviour in 'real' environmental samples, using hydrodynamic chromatography coupled to ICP-MS. J. Anal. At. Spectrom. 24(7): 964.

Tomaszewska, E., Soliwoda, K., Kadziola, K., Tkacz-Szczesna, B., Celichowski, G., Cichomski, M., Szmaja, W. and Grobelny, J. (2013): Detection Limits of DLS and UV-Vis Spectroscopy in Characterization of Polydisperse Nanoparticles Colloids. J. Nanomat. 2013: 1-10.

Trefry, J.C., Monahan, J.L., Weaver, K.M., Meyerhoefer, A.J., Markopolous, M.M., Arnold, Z.S., Wooley, D.P. and Pavel, I.E. (2010): Size Selection and Concentration of Silver Nanoparticles by Tangential Flow Ultrafiltration for SERS-Based Biosensors. J. Am. Chem. Soc. 132(32): 10970-10972.

Tuoriniemi, J., Cornelis, G. and Hassellöv, M. (2012): Size Discrimination and Detection Capabilities of Single-Particle ICPMS for Environmental Analysis of Silver Nanoparticles. Anal. Chem. 84(9): 3965-3972.

Unrine, J.M., Colman, B.P., Bone, A.J., Gondikas, A.P. and Matson, C.W. (2012): Biotic and abiotic interactions in aquatic microcosms determine fate and toxicity of Ag nanoparticles. Part 1. Aggregation and dissolution. Environ. Sci. Technol. 46(13): 6915-6924.

Van Koetsem, F., Geremew, T.T., Wallaert, E., Verbeken, K., Van Der Meeren, P. and Du Laing, G. (2015): Fate of engineered nanomaterials in surface water: Factors affecting interactions of Ag and CeO2 nanoparticles with (re)suspended sediments. Ecolog. Eng. 80: 140-150.

Vance, M.E., Kuiken, T., Vejerano, E.P., Mcginnis, S.P., Hochella, M.F., Rejeski, D. and Hull, M.S. (2015): Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J. Nanotechnol. 6: 1769-1780.

Von Der Kammer, F., Ferguson, P.L., Holden, P.A., Masion, A., Rogers, K.R., Klaine, S.J., Koelmans, A.A., Horne, N. and Unrine, J.M. (2012): Analysis of engineered nanomaterials in complex matrices (environment and biota): General considerations and conceptual case studies: Analysis of engineered nanomaterials in complex samples. Environ. Tox. Chem. 31(1): 32-49.

Wagner, S., Legros, S., Loeschner, K., Liu, J., Navratilova, J., Grombe, R., Linsinger, T.P.J., Larsen, E.H., Von Der Kammer, F. and Hofmann, T. (2015): First steps towards a generic sample preparation scheme for inorganic engineered nanoparticles in a complex matrix for detection, characterization, and quantification by asymmetric flow-field flow fractionation coupled to multi-angle light scattering and ICP-MS. J. Anal. At. Spectrom. 30(6): 1286-1296.

Wagner, S., Gondikas, A., Neubauer, E., Hofmann, T. and Von Der Kammer, F. (2014): Spot the Difference: Engineered and Natural Nanoparticles in the Environment-Release, Behavior, and Fate. Angew. Chem. Int. Ed. 53: 12398 – 12419.

Wahlund, K.-G. (2013): Flow field-flow fractionation: Critical overview. J. Chromatog. A 1287: 97-112.

Wei, L., Ding, J., Xue, M., Qin, K., Wang, S., Xin, M., Jiang, J. and Zhao, Q. (2019): Adsorption mechanism of ZnO and CuO nanoparticles on two typical sludge EPS: Effect of nanoparticle diameter and fractional EPS polarity on binding. Chemosphere 214: 210-219.

Weigel, S., Peters, R., Loeschner, K., Grombe, R. and Linsinger, T.P.J. (2017): Results of an interlaboratory method performance study for the size determination and quantification of silver nanoparticles in chicken meat by single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS). Anal. Bioanal. Chem. 409(20): 4839-4848.

Weinberg, H., Galyean, A. and Leoopold, M. (2011): Evaluating engineered nanoparticles in natural waters. Trends Anal. Chem. 30(1): 72-83.

Wills, J.W., Summers, H.D., Hondow, N., Sooresh, A., Meissner, K.E., White, P.A., Rees, P., Brown, A. and Doak, S.H. (2016): Characterizing Nanoparticles in Biological Matrices: Tipping Points in Agglomeration State and Cellular Delivery. ACS Nano 11(12): 11986-12000.

Wiltsche, H., Winkler, M. and Tirk, P. (2015): Matrix effects of carbon and bromine in inductively coupled plasma optical emission spectrometry. J. Anal. At. Spectrom. 30(10): 2223-2234.

Witzler, M., Küllmer, F., Hirtz, A. and Günther, K. (2016): Validation of Gold and Silver Nanoparticle Analysis in Fruit Juices by Single-Particle ICP-MS without Sample Pretreatment. J. Agric. Food Chem. 64(20): 4165-4170.

A. Yan and Z. Chen. Detection Methods of Nanoparticles in Plant Tissues, in Özge, C. (ed.) New Visions in Plant Science. Intechopen, (2018).

Yin, Y., Tan, Z., Hu, L., Yu, S., Liu, J. and Jiang, G. (2017): Isotope Tracers To Study the Environmental Fate and Bioaccumulation of Metal-Containing Engineered Nanoparticles: Techniques and Applications. Chem. Rev. 117(5): 4462-4487.

Zamora-Perez, P., Tsoutsi, D., Xu, R. and Rivera Gil, P. (2018): Hyperspectral-Enhanced Dark Field Microscopy for Single and Collective Nanoparticle Characterization in Biological Environments. Materials 11(2): 243.

Zänker, H. and Schierz, A. (2012): Engineered nanoparticles and their identification among natural nanoparticles. Annu. Rev. Anal. Chem. 5: 107-132.

Zhang, M., Yang, J., Cai, Z., Feng, Y., Wang, Y., Zhang, D. and Pan, X. (2019): Detection of engineered nanoparticles in aquatic environments: current status and challenges in enrichment, separation, and analysis. Environ. Sci.: Nano 6(3): 709-735.

Appendix

Table 14: List of identified NM detection and separation methods

Principle	Techniques	Relevant NM samples and environmental compartments
element-specific	ICP-MS, single particle ICP-MS, spICP-TOFMS (single element and multi-element detection)	water, soil, sediments, air, biota (depending on sample preparation), bulk, single particles, carbon NMs
	Laser ablation ICP-MS	bulk samples
	GC-MS	organic CNTs versus PAHs
	HPLC-atmospheric pressure ionization mass spectrometry	C60 fullerenes
	Argon induced inductively coupled plasma atomic emission spectrometry (Ar-ICP-AES)	chemical composition of NMs from air samples
	Argon induced inductively coupled plasma mass spectrometry (Ar-ICP-MS)	chemical composition of NMs from air samples
direct visualisation/ microscopy	Electron microscopy (TEM, SEM)	e.g. sediments, tissue, biota
	Atomic force microscopy (AFM)	CNTs
	Laser confocal microscopy	NMs and clusters in e.g. biological material
	Hyperspectral reflectanceimaging	tissue
	NIRF microscopy (near infrared fluorescence)	
	automated SEM (autoSEM) with bulk elemental analysis	sediment samples, samples from air-water-interface (e.g. TiO_2 from sunscreen)
	ESEM	wet, uncoated environmental specimens
	cryogenic SEM	tissue, plants, biota
	confocal scanning laser microscopy	biota (cells, tissue) → accessible to many laboratories
light scattering	nanoparticle tracking analysis (NTA)	water/extracts

Principle	Techniques	Relevant NM samples and environmental compartments
	dynamic light scattering (DLS)	water/extracts, pure samples
biological sensors	e.g. aptamer modified samplers	*in situ* sampling in aquatic environments
chromatography/ separation	field flow fractionation (FFF, AF4)	water/extracts preparation/fractionation for later measurements using e.g. ICP-MS
	AF4 coupled with multiangle laser light scattering (MALLS)	direct NM size analysis
	liquid chromatography	water/extracts
	size exclusion chromatography (SEC)	water/extracts
	gel electrophoresis	water/extracts
	capillary electrophoresis	water/extracts
X-ray-based	X-ray adsorption	elemental analysis for determination of NM composition (and detection)
	X-ray fluorescence	
	X-ray photoelectron spectroscopy	
helium microwave-induced plasma systems	He-MIP-AES	commercial particle analyser system for NMs in air samples
filters	trapping filters	NMs in air samples
	size exclusion filters	water
	nanomechanical resonant filter-fibers	air
	ultrafiltration	water
weighing	piezo balance	NMs from air samples on trapping filters
beta ray attenuation		NMs in air samples
electroanalytics	particle collision coulometry (PCC)	metallic NMs, metal oxide NMs
	voltammetry of immobilized particles (VIP)	NMs are immobilized on an electrode surface and thereby separated from the media where they were suspended in
chemical sensors		far from being successful applied to real samples

Principle	Techniques	Relevant NM samples and environmental compartments
capture materials	activated charcoal as capture material	e.g. silver NMs in water samples
extraction	cloud point extraction (CPE) and electrothermal atomic absorption spectrometry (ETAAS)	water
	sequential extraction	soil samples