Analysis of environmental nanoplastics: Progress and challenges

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ARTICLE INFO

ABSTRACT

Nanoplastics (<1000 nm) are gaining high attention worldwide as an emerging environmental contaminant because they are easier to be taken up by organisms and likely to pose higher ecological and health risks than microplastics (<5 mm). However, we are still lacking harmonized and reliable methodologies for analyzing nanoplastics in environments. Here, we reviewed 33 studies on state-of-art methodologies for pretreatment, separation, identification, and quantification of nanoplastics. Most of the studies successfully detected standard reference nanoplastics spiked in environmental samples but failed to separate and quantify nanoplastics from real field samples. Up to date, only five studies measured nanoplastics in real field samples, i.e., seawater, snow, air, sand, and agriculture soil samples, respectively. Raman spectroscopy and pyrolysis–gas chromatography/ mass spectrometry (py-GC–MS) are the most popular analytical methods. However, the current spectroscopic methods are time-consuming and cannot cover the whole nano-range due to the detection limit of particle size; determination of the mass concentration of nanoplastics by the mass spectrometry methods are destructive, thus requiring extra/subsamples to obtain physical information of nanoplastics. The major caveat is that the quantification is often conducted without chemical confirmation of polymer types, raising concerns about the reliability of current results. It is also worth noting that recovery tests and blank controls, both of which are general steps in the quantification of conventional chemical pollutants, are rarely reported in nanoplastic studies. More efforts should be made to enhance the reliability and accuracy of nanoplastic analysis in environmental samples, which can only be achieved with strict chemical confirmation and adequate quality assurance along with the whole analytical process.

1. Nanoplastics as an emerging environmental contaminant

Over the past decades, multifarious plastic productions have been used in a wide range of human needs for improvements in life quality [1]. The extensive consumption of plastics has resulted in severe environmental pollution globally, gaining increasing scrutiny from the scientific community [2]. Meanwhile, large plastic debris is likely to break down to microplastics (<5 mm) [3] and even nanoplastics (<1000 nm) [4–6]. Currently, the size definition of nanoplastic is still under debate. The definition of nanoplastic (<100 nm) follows the threshold commonly used in nanomaterial and nanotechnology [7,8], while we adopt 1 to 1000 nm as the size range of nanoplastic recommended more recently by Hartmann (2019) [9]. Due to the small size and higher potential for penetrating biological members, nanoplastic is expected to be more harmful than microplastic [10,11]. It is urgent to advance our understanding of the presence of nanoplastics in different environments and the potential toxicological effects on different organisms [12–16].

Microplastics have been detected and quantified in various environmental samples, but we still severely lack counterpart information for nanoplastics (<1000 nm). The analysis of nanoplastics in real field samples is at the infancy stage and protocols are still immature and under development [17]. Until now, 33 studies have focused on developing pretreatment (digestion and preconcentration), separation, identification, and quantification methods to detect nanoplastics, most of which detected standard model nanoplastics spiked in environmental samples but failed to extract and quantify environmental nanoplastics from real field samples [17–23]. Some available review papers and book chapters have discussed the methods for sampling, separation, quantification, and identification of microplastics and nanoplastics in complex

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Abbreviations

AF4	Asymmetrical flow field-flow fractionation
ATR-FTIR	Attenuated Total Reflection FTIR
Au–PS/PS-*b*-PAA	Gold–polystyrene-*block*-poly(acrylic acid)
CFC	Continuous flow centrifugation
CF3	Centrifugal field-flow fractionation
DLS	Dynamic light scattering
FE-SEM	Field emission scanning electron microscopy
HRTEM	High resolution transmission electron microscopy
ICP-MS	Inductively coupled plasma-mass spectrometry
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time of flight mass spectrometry
MALS	Multi-angle light scattering
NTA	Nanoparticle tracking analysis
PA-6	Polyamide-6
PC	Polycarbonate
PE	Polyethylene
PES	Polyethersulfone
PET	Polyethylene terephthalate
PHB	Polyhydroxybutyrate;
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PPC	Polypropylene carbonate
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
Py-GC–MS	Pyrolysis gas chromatography mass spectrometry
Py-GCToF	Pyrolysis-gas chromatography time of flight mass spectrometry
RISE	Raman imaging and scanning electron microscope
SEM	Scanning electron microscopy
SERS	Surface-enhanced Raman spectroscopy
SLS	Static light scattering
SP-ICP-MS	Inductively coupled plasma-mass spectrometry in single particle mode
TED-GC–MS	Thermal extraction desorption gas chromatography mass spectrometry
TEM	Transmission electron microscopy
TD-PTR-MS	Thermal desorption-proton transfer reaction-mass spectrometer
TOC	Total organic carbon
UC	Ultracentrifugation
UF	Ultrafiltration
UV–Vis	Ultraviolet–visible spectroscopy
XPS	X-ray photoelectron spectroscopy

environmental samples [1,24–29]. Up to date, only 5 studies reported the detection and analysis of nanoplastics in real field samples. The first study analyzed seawater samples collected from North Atlantic subtropical gyre and detected polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE), and polyvinyl chloride (PVC). Ultrafiltration, Dynamic Light Scattering (DLS), and pyrolysis, thermodesorption, and thermochemolysis coupled to GC–MS were applied to separate, preconcentrate, quantify, and identify the nanoplastics [12]. The second study detected PET in Alpine snow samples. Membrane filtration and thermal desorption-proton transfer reaction-mass

spectrometry (TD-PTR-MS) were used to separate, quantify, and identify nanoplastics [13]. Air samples were collected, digested, separated, and detected on Klarite substrates using surface-enhanced Raman spectroscopy (SERS) [30]. The other two studies analyzed sand and agricultural soil samples respectively, using pyrolysis–gas chromatography/mass spectrometry (py-GC–MS) [31,32].

Studies on the measurement of nanoplastics in real field samples are scarce. There is still a long journey ahead from method development in the laboratory to the identification and quantification of real nanoplastics in the field. The present review aimed to provide an overview of
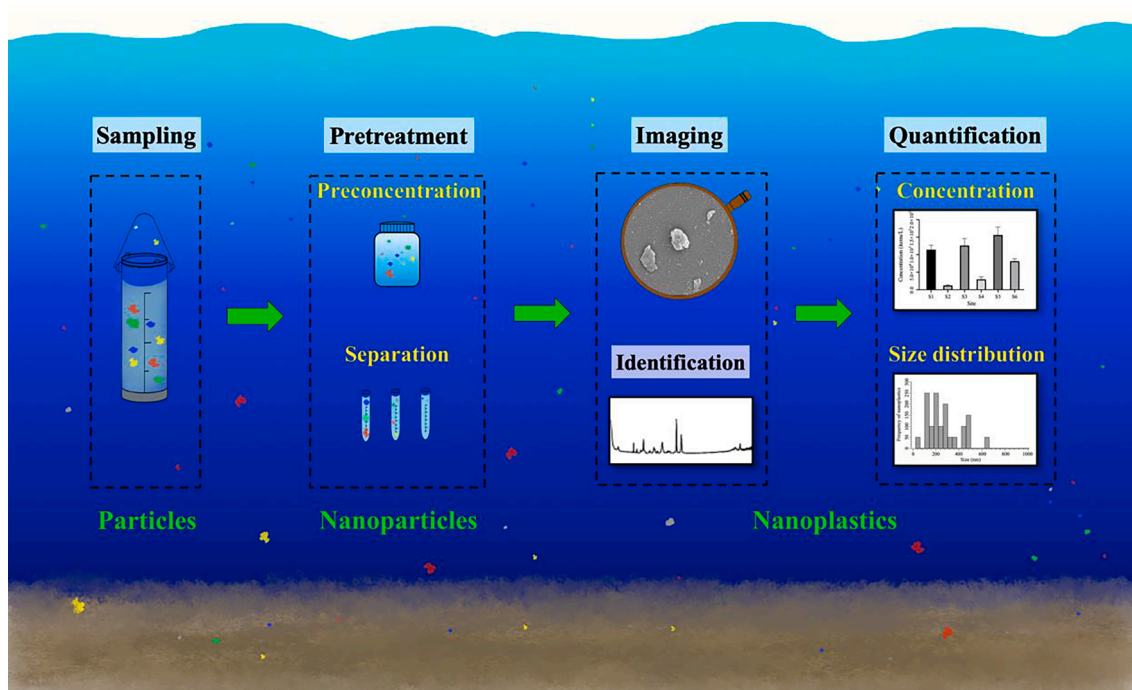

Fig. 1. A general process of analyzing nanoplastics in environmental samples.

the state of art methodologies for pretreatment, identification, and quantification of nanoplastics, i.e., a general process of analyzing nanoplastics in environmental samples (Fig. 1). The feasibility and reliability of these methodologies were also discussed when generalizing to real field samples. Data from peer-reviewed manuscripts, books, and reports on nanoplastic measurements published by the beginning of December 2020 were collected. The keywords used in the search were 'nanoplastic', 'separation', 'extraction', 'characterization', 'identification', and 'quantification'. The data resources included Science Direct, Web of Science, Wiley Online Library, Directory of Open Access Journals, Spring Link, EBSCOhost, BioMed Central, and PubMed Central. After careful screening, a total of 33 publications were analyzed. The results elaborated on the current progress and challenges in the detection of environmental nanoplastics.

2. Pretreatment of samples

Nanoplastics present in various environmental matrices such as freshwater and seawater, wastewater, sediments, soils, foods, and biota tissues [11,33,34]. These environmental or biological samples contain complex organic and inorganic substances [35–37]. Nanoplastic and natural particles in the environment are highly heterogeneous [7].

Natural organic matters and non-plastic particles may form homo- or hetero-aggregate with nanoplastic in complex environmental samples [8,38]. Thus, before identification and quantification, pretreatment is an essential step to remove impurities but retain nanoplastics in their original properties and amounts [2]. Special attention should be given to avoid contamination in this pretreatment step. All tools and setups should be made from non-polymer materials, and potential contamination due to airborne particles and synthetic fibers needs to be minimized, for instance, by using laminar flow benches or an air filtration system. As contamination is difficult to be fully avoided, procedural blank samples and recovery tests should be always included.

2.1. Digestion of matrix

Field samples such as wastewater, organisms, soil, and sediments usually contain high organic matrices. Nanoplastics should be separated/extracted from these organic matrices for further analysis. Various approaches for the digestion of organic matrices have been applied in microplastic analysis [39]. Digestion agents for particle separation comprise acid treatment, e.g., 65% nitric acid [40,41], 30% hydrogen peroxide [42,43], and alkaline treatment with sodium hydroxide/potassium hydroxide [44,45]. Additionally, enzymatic protocol that is

Table 1
Methods for digestion, preconcentration, and separation of nanoplastics.

Type of sample	Nanoplastic spikes[a]	Pretreatment			Recovery rate (%)	Refs.
		Digestion	Preconcentration	Separation		
Commercial products						
Standard nanobeads	PS	–	–	AF4	–	[54]
	PS, PMMA	–	–	AF4, CF3, optical tweezer	–	[55]
	PS, PE, PA, PVC	–	–	PC membrane filter coated with Al	–	[56]
	PE	–	$[C_8C_1Im][NTf_2]$ extraction	–	–	[57]
	Au–PS/PS-*b*-PAA	–	–	AF4	–	[58]
	PS	–	–	PC membrane filtration	–	[59]
Commercial facial scrubs	–	–	–	Five-step membrane filtration	–	[18]
Fragmented nanoplastics made in lab						
Degraded PS, PE, PE/PP	–	–	–	AF4	–	[59]
Degraded PHB	–	–	UF, centrifugation	–	–	[5]
Degraded PVC powders	–	–	–	Two-step membrane filtration	–	[60]
Plastic teabag leachate	–	–	–	Cellulose membrane filtration	–	[61]
Environmental samples						
Air	PS, PMMA	30% H_2O_2	–	Glass fiber membrane filtration	–	[30]
Drinking water	PS	–	Crossflow UF	AF4	12.7–54.0	[17]
Seawater	PS, PE particles, fragmented PMMA, PA6	–	–	Optical tweezer	–	[23]
	–	–	UF	PES membrane filtration	–	[12]
Alpine Snow	PS	–	–	PTFE membrane filtration	15%	[13]
River water	PS	–	–	Nylon membrane filtration	–	[62]
	PP, PS, PVC	–	–	PTFE membrane filtration	–	[21]
	Pd-doped PS	Microwave-assisted acid	CFC	SCFA filtration	88.4	[52]
	Carboxylated PS with Au nanoparticles	–	–	Syringe filtration	–	[53]
	PS	KOH	–	Membrane filtration	80.9–109.2	[45]
River, sea, WWTP influent and effluent	PS, PMMA	–	Cloud point extraction	Glass-microfiber membrane filtration	PS: 84.6–96.6, PMMS: 76.5–96.6	[19]
Sand	–	–	–	Sartorius filtration	–	[31]
Agricultural soils	–	–	–	Sartorius filtration, AF4	–	[32]
Sediment	–	30% H_2O_2	Freeze-drying	Glass-microfiber membrane filtration	–	[63]
Fish meat	PS	HNO_3 and Proteinase K	–	AF4	60–70	[51]
Fish	PS	10% KOH	–	Membrane filtration	88.7–106.8	[45]
Egg shells	PS	HCl and TMAH	UC	AF4	54–63	[20]

NOTE: [a] Spiking nanoplastics are spherical if not specified.

mild and non-destructive can be used for tissue digestion [46,47], which has also been used for nanoplastic analysis [48].

Digestion is not included for relatively clean water samples like seawater and drinking water [12,49,50] but has been applied in studies that analyzed complex samples, such as fish, and eggshells [20,45,51] (Table 1). Two studies investigated nanoplastics in fish samples. In the first study, nitric acid (67–69%) and proteinase K (3 mg/mL) were used for digesting 0.1 g frozen fish homogenate [51]. In the second study, the authors fed PS nanoplastics to fish and then used 10% KOH solution to digest fish tissue at 50 °C for 36 h [45]. In a third study, eggshells were utilized as a model environmental matrix for nanoplastic method development [20]. One gram of eggshells and PS nanoplastics were digested using a two-step digestion method. First, HCl (0.01 M) was added into samples (50 °C, 2 h). Then the residuals were kept at room temperature (at pH = 4.5) for 5 days to further dissolve the $CaCO_3$ layer of the eggshells. After, 5% TMAH solution (60 °C, 1 h) was used to further dissolve organic matters that were not digested during the acid process. In an aerosol study, the collected airborne particles were rinsed in a glass container with 30% hydrogen peroxide solution (H_2O_2) for 24 h at room temperature [30]. Microwave-assisted acid digestion was used for Pd-doped PS nanoplastics and suspended particulate matter [52]. BCR-414 (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Se, V, and Zn), ERMR-EC680m (As, Cd, Cr, Hg, Pb, Sb, Sn, and Zn), and the Pd concentration for all fractions that could not be introduced directly into the inductively coupled plasma-mass spectrometry (ICP-MS) identification were digested at 230℃ (20 min ramp and 5 min hold time). Different from traditional ICP-MS, ICP-MS operated in single particle (SP) mode can directly detect metal-containing nanoparticles without digestion, like Au-labeled PS nanoplastics with high sensitivity [53].

2.2. Preconcentration of nanoplastics

Preconcentration of nanoplastics, such as ultrafiltration, ultracentrifugation, continuous flow centrifugation, and cloud point extraction [52,64–66] is a solution to improve the limit of detection (LOD) and limit of quantification (LOQ) of existing methods [12,19]. Ultrafiltration equipped with polyethersulfone (PES)-based cell has been used to concentrate and separate nanoplastics in relative clean seawater and drinking water [12,17]. After ultrafiltration, a small volume of retentate was kept for subsequent characterization, with an enrichment factor up to 500 [19]. Ultracentrifugation has been used to enrich low concentrations of spiking PS nanoplastics in digested eggshell samples (at 40000 rpm, 20 min), increasing number concentrations to reach the LOD of the instrument for following separation and quantification [20]. Continuous flow centrifugation (CFC) has been reported as an efficient sampling method for fine particulates (1–25 μm) in natural water at a high flow rate of several hundred liters per hour [67,68]. Also, CFC was successfully used to enrich small microplastics (model microplastics ranged from 1 μm to 1 mm) of a variety of polymer types [69]. A recent study showed that operating two continuous flow centrifuges sequentially at different rotational speeds (2000–4000 rpm followed by 17000 rpm) enabled size- and density-selective preconcentration and separation of commercial Pd-doped PS nanoplastics in river water samples [52]. Cloud point extraction (CPE) is based on the solubilization of surfactant solution and phase separation to separate and preconcentrate analytes [66,70], which is a simple, safe, inexpensive, and nonpolluting approach. This cost-effective technique can be used to concentrate inorganic nanoparticles, particularly for the nanoparticles with hydrophobic coatings, in a complex matrix without changing the original size and morphology of nanoparticles. The CPE method based on surfactant (Triton X-45) was used to enrich the spiked PS and polymethyl methacrylate (PMMA) nanoplastics from wastewater samples. The targeted nanoplastics were captured in the surfactant micelle and well separated from the solvent after ultracentrifugation [19]. Except CPE, ionic liquids were proposed as extractants to separate nanoplastics from aqueous solutions. Some ionic liquids are sufficiently hydrophobic for

applications in extracting non-polar compounds from an aqueous phase, such as $[C_8C_1Im][NTf_2]$ [57]. It has been used to simulate the extraction of PE nanoplastics from water solution. Oxidized moieties of plastics can also be efficiently solvated, given the amphiphilic nature of the $[C_8C_1Im][NTf_2]$, allowing also realistic particles to be extracted into solvent. Different from above-mentioned preconcentration methods, freeze-drying can remove all the solvent in samples and make the liquid samples more convenient for following analysis [63,71].

2.3. Separation of nanoplastics

Size-based separation is a common approach to separate particles in samples, such as filtration, field flow fractionation (FFF), and size-exclusion chromatography. Among these, membrane filtration is the most used method in nanoplastic separation (Table 1). Membrane filters can be used to exclude macro- and large micro- particles. Different studies used membrane filters of different pore sizes and materials [12,13,30,59,62]. In the study of nanoplastics in North Atlantic Subtropical Gyre, 1.2 μm pore sized PES membrane was used to filter out large particles before the preconcentration step [12]. A similar pretreatment step was also reported but with a 1 μm pore sized glass fiber membrane in the study on nanoplastics in wastewater [19]. The 2.5 μm pore sized cellulose filters were used to separate microplastics and nanoplastics from teabag leachate [61]. Polytetrafluoroethylene (PTFE) filters with a pore size of 0.1–0.45 μm were utilized to sort nanoplastics from microplastics in aqueous water and melted Alpine snow samples [11,19]. Sometimes, multi-step filtration is necessary to reduce particle clogging. A five-step filtration by using 20–25 μm, 2.5 μm, 0.45 μm, and two times of 0.1 μm syringe filters was developed for the size selection of PE nanoplastics in facial scrubs [18]. Zhang et al. conducted a two-step filtration by using 10 μm and two times of 1 μm PES membranes to ensure that the filtered particles in the filtrate were in the nanometer scale [60]. Metal coated membrane filter, such as aluminum coated polycarbonate (PC) filter, was also used to avoid substrate interference in the following identification steps [56].

Field flow fractionation (FFF) is a separation technique typically for size and molar mass fractionation of biopolymers, proteins, polymers, and nanoparticles [72]. The main separation fields include thermal, electric, magnetic, centrifugal, and cross-flow, covering the whole nanometer range to < 100 μm [26]. The most used FFF for nanoplastic separation in water samples is the asymmetrical flow field-flow fractionation (AF4) [17,54,58]. AF4 has also been used for separating nanoplastics from fish tissue and soil samples [32,51]. Surfactants like SDS could be the mobile phase of AF4, which was used to disperse PS nanoplastics in digested eggshell residue [20]. In addition to spherical PS nanoplastics, some manufactured nanoplastics of irregular shapes can also be separated by AF4. El Hadri et al. proposed a top-down process from manufacture to characterization of nanoplastics (PS, PE, and environmentally pre-degraded PE/PP) using AF4 and coupled multi-angle laser light scattering (MALS) [59]. Besides AF4, centrifugal field-flow fractionation (CF3) hyphenated to Raman technology was developed to separate fractionation of PS and PMMA nanoplastics with higher resolution than AF4 [55]. FFF techniques can be coupled with mass spectrometric detectors or UV, refractive index, or fluorescence detectors that also provide quantitative information of particles [54,73].

Optical tweezers can trap and manipulate micro- and nanoparticles dispersed in liquid by exploiting the tiny forces that light exerts on the matter [74,75]. Optical forces are generated by momentum exchange between the photons and particles during the light-matter interaction. The laser beam-generated forces can confine a particle in an optical potential well, which is called optical trapping. When coupled with Raman spectroscopy, optical tweezer enables chemical analysis of the trapped particles. Gillibert et al. used optical tweezers with Raman spectroscopy (Raman Tweezers) to trap PS, PE, and PMMA particles in size of tens of μm to 90 nm [23]. Hence, optical tweezer is a promising separation method to trap nanoplastics for subsequent identification

with a Raman microscope [55].

3. Identification of nanoplastics

After the separation of nanosized particles from the environmental matrix, chemical identification of these nanosized particles is critical to ensure the nanosized particles to count are plastic (Fig. 1). In microplastic analysis, vibrational spectroscopy is usually hyphenated with optical microscopy to identify polymer types as well as the particle size and morphology [76,77]. This methodology, however, becomes increasingly difficult with decreasing particle size (below 10 μm) [78,79]. In Table 2, 25 out of 33 studies used different methods for chemical identification. The methods can be categorized into two groups, spectroscopy, and mass spectrometry.

3.1. Spectroscopy

Spectroscopic technique is the most used chemical identification method for nanoplastic (Table 2). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy has been used to identify microplastics in facial scrubs, plastic teabag leachate, and biodegradable

plastic leachate [5,18,61]. Compared with FTIR (LOD of ~ 20 μm), Raman spectroscopy can measure smaller microparticles in the size of ~ 1 μm [78]. Raman is a non-contact technique requiring minimal sample preparation, with a highly specific fingerprint spectrum and with negligible interference from water [78]. By collecting the unique spectra at each pixel, a Raman image can be generated to directly visualize the particles [82]. Raman imaging was used to visualize and identify paint-polishing dust samples collected from a driveway and estimated billions-trillions of microplastics and nanoplastics down to ~ 200 nm [22]. However, the spatial resolution of Raman imaging depends on the wavelength of the laser and the numerical aperture of the spectroscope [87]. Hence, Raman imaging cannot detect nanoplastics smaller than the spatial resolution of the spectroscope, implying that Raman imaging cannot cover the whole nano-range [22]. When integrated with scanning electron microscopy, Raman can be used to identify individual nanoplastics. This technique allowed the acquisition of morphological information and the chemical composition of PVC particles as small as 360 nm [60]. A similar method named SEM-Raman was successfully applied to identify PS nanoplastics in the size of ~ 100 nm [56]. Optical tweezer can overcome the problem of low Raman scattering, thus Raman tweezer has realized the analysis of nanoplastics (including PP,

Table 2
Methods for identification and quantification of nanoplastics.

Type of sample	Nanoplastic spikes[a]	Identification	Quantification	Quality control	Chemical composition	Refs.
Spectroscopy						
Standard nanobeads	PS, PMMA	On-line Raman microscope	AF4 and CF3-UV-MALS	Procedure blank	Spiked PS and PMMA	[55]
	PS, PE, PA, PVC	RISE microscopy, SEM	SEM	–	Spiked PE	[56]
	PS	SERS	FE-TEM	–	Spiked PS	[80]
Air	PS, PMMA	SERS	Raman images	Lab and field blanks	Spiked plastics, PS and PET in air	[30]
River water	PS	SERS, FE-SEM, UV–Vis	HRTEM	–	Spiked PS	[62]
Seawater	PS	SERS, TEM	TEM, DLS	–	Spiked PS	[81]
	PS, PE, PMMA and PA6	Raman Tweezer	Raman images	–	Spiked PS, PE, PMMA, PA6	[23]
Degraded PVC powders	–	RISE microscopy, SEM	Raman images	–	Degraded PVC	[60]
Vehicle's clear coating	PS	Confocal Raman imaging, SEM	Raman images	–	Spiked PS, poly-acrylic	[82]
	PS	Confocal Raman imaging, SEM	Raman images	–	Spiked PS	[83]
Commercial facial scrubs	–	ATR-FTIR, XPS, SEM, TEM,	SEM, DLS	Procedure blank	PE	[18]
Plastic teabag leachate	–	ATR-FTIR, XPS, SEM	SEM, NTA	Four kinds of control experiments	Nylon and PET	[61]
Biodegradable PHB	–	ATR-FTIR, SEM	DLS, NTA, TOC	Procedure blank	Degraded PHB	[5]
Degraded PS, PE, PE/PP	–	ATR-FTIR, SEM, TEM	AF4-UV-MALS, DLS	–	Degraded PS, PE	[59]
Mass spectrometry						
Standard nanobeads	Au–PS/PS-*b*-PAA	SP-ICP-MS, TEM	AF4-UV	–	Spiked Au–PS/PS-*b*-PAA	[58]
Grinded PS and PET powders	–	MALDI − TOF MS	MALDI − TOF MS	Procedure blank	Grinded PS	[63]
Drinking water	PS	Py-GC–MS	AF4-UV-MALS	–	Spiked PS	[17]
Seawater	–	Py-GC–MS	DLS	Three kinds of control experiments	PVC, PET, PS	[12]
River water	PP, PS, PVC	Py-GCToF	Py-GCToF	–	Spiked PS	[21]
River, sea, WWTP influent and effluent	PS, PMMA	Py-GC–MS, TEM	Py-GC–MS	–	Spiked PS and PMMA	[19]
Sand	–	Py-GC–MS, TEM/EDS	Py-GC–MS, DLS	–	PVC, PS	[31]
Agricultural soils	–	Py-GC–MS	AF4-UV-SLS	Procedure blank	PE, PS, PVC	[32]
Drinking, tap, and river water	Carboxylated PS with Au nanoparticles	SP-ICP-MS	SP- ICP-MS	–	Spiked PS	[53]
Snow	PS	TD-PTR-MS	TD-PTR-MS	Procedure blank	Spiked PS, PET in snow	[13]
River water and fish	PS, PET	MALDI-TOF MS	MALDI-TOF MS	–	Spiked PS and PET	[45]
No polymer type confirmation						
Standard nanobeads	PS	–	AF4-UV-MALS	–	–	[54]
	PS	Fluorescence microscope	DLS, NTA	–	–	[84]
Fragmented PE	–	SEM	DLS, NTA	Milli-Q water blank	–	[85]
Degraded PS	–	FE-SEM	NTA	–	–	[86]
Egg shells	PS	TEM	AF4-UV-MALS	Control sample, procedure blank	–	[20]
Fish meat	PS, PE	SEM	AF4-MALS	–	–	[51]

Note: [a] Spiking nanoplastics are spherical if not specified.

PE, PS, and nylon) down to ~ 100 nm [23,55]. Surface-enhanced Raman spectroscopy (SERS) is an approach to increase the electric field intensity of nanostructure. Commercial SERS substrate, such as Klarite, can be used as a substrate for PS and PMMA nanoplastics down to 450 nm extracted from ambient airborne particles [30]. A surface substrate of silver-coated gold nanostars (AuNSs@Ag) inserted into anodized aluminum oxide (AAO) nanopores (AuNSs@Ag@AAO) detected 400 nm PS particles in water samples [80]. Silver colloid can also be used as an active substrate for SERS [62,81]. Raman has the potential for direct analysis of aqueous samples because of its spectroscopic insensitivity to water. A hyphenation of particle separation and characterization (FFF-MALS) with subsequent chemical identification by Raman enabled the identification of different particles in the size of 200 nm to 5 μm [55].

Besides FTIR and Raman spectroscopes, X-ray photoelectron spectroscopy (XPS) was also employed to confirm the chemical composition of PS nanobeads [88], primary PE nanoplastics in cosmetics [18], and plastic teabag leachate [61]. XPS can also observe changes in the surface oxidation of nanoplastics [88,89]. Overall, most spectroscopic methods for nanoplastic identification are coupled with microscopes, allowing simultaneously obtain optical images and chemical compositions.

3.2. Mass spectrometry

Mass spectrometry such as pyrolysis–gas chromatography-mass spectrometry (Py-GC–MS) has been increasingly used for microplastic and nanoplastic identification, whereby samples treated by thermal methods release gaseous compounds that are further transferred to gas chromatography-mass (GC) for the identification of chemical compositions [90–92]. This technique requires little sample pretreatment because it directly examines the solid polymer sample [92]. For example, water samples contain nanoplastics can be placed on a filament or in a furnace of py-GC–MS for polymer identification. Py-GC–MS has been successfully used to identify standard nanoplastics spiked in drinking water, river water, seawater, wastewater, and sand/soil extraction water [17,19,31,32]. Py-GC–MS has also been used for the identification of nanoplastics (<1.2 μm) in the colloidal fraction of seawater samples collected from the North Atlantic Subtropical Gyre [12]. Sullivan et al. used pyrolysis–gas time-of-flight mass spectrometry (py-GC-ToF) to identify standard PS nanoplastics (>0.45 μm) spiked in river water samples [21]. Based on thermal desorption–proton transfer reaction–mass spectrometry (TD-PTR-MS), Materic et al. successfully identified PET nanoplastics in snow samples without a preconcentration step, even when the snow samples contained mixtures of organic compounds [13]. Recently, a new technique–matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) was employed to detect spiked PS and PET nanoplastics in water and fish samples [45]. In another study, MALDI-TOF MS was used to identify nano-sized powders grinded from aviation plastic cups [63]. Although they included PS and PET nanoplastic analysis in sediment samples, they mixed micron-sized and nano-sized particles together for mass spectrometry identification. The analysis results of the particle mixture are difficult to tell the identified plastics are from micro- or nano- size fraction, and this is the reason we excluded this study from our count of real field sample reports.

ICP-MS can detect metal-doped plastic particles. After removing polymer shells, the metal cores can be identified and quantified. This new approach shows high sensitivity [93–95] but the morphological features of the identified particles cannot be obtained due to the destructive process. In comparison, a novel SP-ICP-MS that works on a particle-by-particle basis is a non-destructive technique for nanoplastic samples. Jiménez-Lamana et al. conjugated nanoplastics with functionalized gold-containing nanoparticles (NPs), thus making them detectable by highly sensitive SP-ICP-MS [53]. Barber et al. used SP-ICP-MS to develop an analysis method on colloidal-sized nanoplastics consisting of a gold–polymer nanocomposite (polystyrene-*block*-poly(acrylic acid), Au–PS/PS-*b*-PAA) [58]. A significant difference from

spectroscopic methods is that mass spectrometry methods require sub/extra samples to obtain information on the morphology and chemical composition of nanoplastics separately. For example, SEM and transmission electron microscopy (TEM) are widely used for characterizing the morphology of nanoplastics in different matrices [18,59,61,86,96] and also the changing morphology of nanoplastics during separation procedure [19,20,51].

4. Quantification of nanoplastics

Quantification should be the final step of the general process of analyzing nanoplastics in environmental samples (Fig. 1). Before quantifying nanoparticles, we should confirm the polymer types of particles. Quantitative data on particle size distribution, particle concentration, and mass concentration are of utmost importance in further assessing the potential impact and risk of nanoplastics, as we still do not know how many nanoplastics are in the environment. Although the current quantification methods are not yet mature, microscopic techniques and light scattering techniques, e.g., SEM, TEM, dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), multi-angle light scattering (MALS), and SP-ICP-MS, have been applied to quantify particle size distribution and particle concentration of nanoplastics, and mass spectrometry methods have been used to determine mass concentration of nanoplastics.

4.1. Particle size distribution and concentration

Microscopic techniques are convenient ways to determine particle size distribution and concentration when analyzing nanoplastics. Microscopes coupled on FTIR and Raman spectroscopes, SEM, and TEM are widely used to measure the sizes or concentrations in neoplastic studies [56,60,62,80]. DLS can also characterize the size of nanoparticles by measuring fluctuations in scattered light intensity due to the Brownian movement of the nanoparticles [97]. A major limitation of DLS is that the intensity of the scattered light is proportional to the sixth power of the particle diameter so it is highly sensitive to the presence of large particles [98]. NTA can generate videos of a population of nanoparticles moving under Brownian motion in liquid suspension when illuminated by laser light. A laser illumination device in conjunction with an ultramicroscope can visualize nanoparticles in the liquid sample that pass through the laser beam, by capturing the scattered light of particles using the instrument charged-coupled device camera [99,100]. Hernandez et al. applied DLS to quantify the size distribution of fragmented PE nanoplastics in facial scrubs and further confirmed it by SEM [18]. In another study, the same group used NTA and SEM to successfully quantify the number concentration and size distribution of nanoplastics in teabag leachate [61]. However, DLS and NTA have considerable limitations when analyzing the polydisperse and non-spherical nanoplastics in field samples [12,54]. So far, DLS and NTA have been most used for high-concentration and monodisperse nanoplastics, such as the standard plastic nanospheres spiked for laboratory tests [59,84,86,96]. The nanoplastics in real water samples are probably too dilute for DLS detection even after UF concentration [12].

Static light scattering (SLS) or multi-angle laser light scattering (MALS) coupled with AF4 or CF3 can separate nanoplastics in different size fractions and obtain the size distribution of the separated particles [20,32,51]. UV detector can also be used in AF4-MALS to acquire particle sizes by absorbance indication [17,55]. However, the light scattering methods do not provide chemical information and cannot distinguish particles of similar molecular weight but different chemical compositions. Thus, the quantification results are not specific to nanoplastics but also non-plastic particles in environmental samples. SP-ICP-MS has also be used to analyze the particle size and particle concentration of nanoplastics by measuring the conjugated metal-containing nanoparticles with a quantification limit of 8.4×10^5 items/L [53].

4.2. Mass concentration

Mass spectrometry used in nanoplastic analysis provides information on the mass fraction of a polymer [101,102]. In general, a single hand-picked plastic particle is thermally degraded in an inert atmosphere and then the pyrolysis fragments (ions) of the polymer structure of the particle are separated by gas chromatography and characterized by mass spectrometry [103–105]. A challenge of applying the mass spectrometry method is that a polymer can produce a large number of volatile substances and isomers upon thermal decomposition, which results in a complex chromatogram with overlapping peaks of similar substances and isomers. Moreover, the decomposition of various natural substances that coexist with nanoplastics in a field sample could also produce similar pyrolysis breakdown fragments (ions). Thus, both selection of main indicator compounds and sample purification must be carefully considered. Particularly, when the analytical method is based on unspecific indicators, sample purification is critical to remove interfering constituents from the environmental sample. Another drawback of the thermal analysis methods is the limited amount of sample introduction (~0.5 mg) to the instrument, especially for aqueous samples. To enhance the sample introduction and representative coverage, thermal extraction desorption gas chromatography mass spectrometry (TED-GC–MS) allows the introduction of a relatively large sample, e.g., the whole filter, and avoids the issues of subsampling and homogeneity of the filter.

Four studies applied mass spectrometry methods to analyze nanoplastics in real field samples [12,13,31,32]. Using py-GC–MS, Ter Halle et al. estimated that the spectrometry signal of colloidal aromatic fraction of seawater was attributed to a mixture of 73% PVC (±18%), 18% PET (±16%), and 9% PS (±10%) in the North Atlantic subtropical gyre seawater samples [12]. Using py-GC–MS, Wahl et al. found PE, PS, and PVC nanoplastics in water extract from agricultural soil samples [32]. Similarly, Davranche et al. found PS and PVC in water extracts from beach sand samples [31]. Materić et al. showed low concentrations of PET nanoplastics (5.4–27.4 ng/mL) in surface snow samples from Austrian Alps using TD-PTR-MS [13]. Recently, some other mass spectrometry methods have been validated for the quantification of spiked standard nanoplastics. Sullivan et al. used py-GCToF to identify the specific markers ions of PS, PP, and PVC. With the combination of known nanoplastics as internal standards, this method was applied for the semi-quantification of PS in river water samples [21]. TED-GC–MS allows the analysis of plastic in environmental samples without pretreatment to remove (in)organic matrixes and also enables relatively large samples (up to 100 mg) [95]. TED-GC–MS combines thermal extraction of thermogravimetric analysis products onto a solid-phase adsorber, which comprises the main difference to py-GC–MS [26]. The pyrolysis fragments are subsequently thermally desorbed into gas chromatography mass spectrometry to enable the identification and quantification of the polymer [95,103]. MALDI-TOF MS is a powerful technique for (bio)polymer analysis due to its soft ionization and wide mass detection ranges [45]. Using this new technique, Lin et al. developed a simple thermal pretreatment at 380 °C that can facilitate the fragmentation of PS and significantly enhances the intensities of fingerprint peaks in low-mass regions, with a LOD of 25 ng for PS nanoplastics [45]. By using the same technique, another study reached lower LOD of 9.8 ng for PS nanoplastics mixed with the dithranol and silver trifluoroacetate (matrix and cationization agents respectively) at a certain volume ratio [63]. New strategies for nanoplastic quantification include doping or labeling nanoplastics with metals before using ICP-MS [52,53,58]. However, these methods have been only tested at lab scale and are difficult to be applied in more complex field samples that contain other particles that may compete for doping or labeling. Thus, selective separation steps must be developed for environmental matrixes reduction.

5. Reliability and accuracy of nanoplastic analysis

Analyzing nanoplastics in field samples remains a big methodological challenge. Available studies failed to provide complete chemical information of all quantified nanoplastics. In other words, it is uncertain whether the quantified nanoparticles are nanoplastics, which questions the reliability of the current data [26]. The advantages and disadvantages of current techniques for digestion, preconcentration, separation, identification, and quantification are summarized in Table 3. Practical consideration associated with the reliability and accuracy of nanoplastic analysis is discussed below.

Nanoplastics co-occur with other natural organic or inorganic substances in the natural environment as heteroaggregates and are expected at much lower concentrations than natural particles [108,109]. Therefore, efficient preconcentration and separation are essential for detecting nanoplastics from complex environmental matrices. Acid digestion can effectively remove organic substances but may lead to destabilization and agglomeration of nanoplastics [51]. In comparison, enzymatic digestion has the advantage to avoid the problem of forming nanoplastic aggregates but requires elaborate optimization, more treatment steps, and longer processing time than acid digestion. To increase the efficiency of enzymatic digestion, a series of enzymes have been compared for purifying microplastics in environmental samples, such as amylase, protease, and lipase [47]. As enzymatic or H_2O_2 digestion usually take hours to days, contamination from ambient air or polymer container contamination could be introduced into solutions [47,110]. Also, the cost of enzymic digestion can be a lot higher than acid digestion when dealing with a large number of samples [111]. KOH solution is a widely-used and cheap digestion method for microplastic extraction with high recovery efficiency [112]. It can take several days to digest mussel or fish tissues by 10% KOH solution [112]. Preconcentration is necessary when the original concentration of nanoplastics in collected samples is lower than the LOD and LOQ of the quantification instrument [12,19]. For relatively clean samples like seawater, ultrafiltration could efficiently concentrate a large volume of water samples over ultracentrifugation, cloud point extraction, and ionic liquids extraction. To treat complex field samples, prefiltration with regular filter renewal is recommended to avoid membrane blockage in ultrafiltration.

Filtration is the most popular nanoplastic separation method (Table 1). Choosing membrane filters with proper pore sizes is a trade-off task for balancing between the capture of small particles, filter clogging, and volume of sample throughput. Sequential filtration using increasingly smaller pore sizes can minimize filter clogging meanwhile capture particles in different size ranges [18,60]. Alternatively, AF4/CF3 can be coupled with a UV detector and MALS to realize separation and size quantification simultaneously [15,49]. AF4/CF3 technique has been shown a powerful tool for sorting PS nanoplastics with a wide colloidal size range from 1 to 800 nm [54]. This size-based method, however, cannot exclude non-polymer particles in a similar size range out of the quantification results. Thus, AF4-MALS method has been only used for standard nanoplastics with known chemical compositions and sizes [20,51]. The loss of nanoplastics during the separation step should be quantified and the recovery test should be conducted. Alarmingly, among the 33 nanoplastic methodological studies, only 8 studies reported the recovery rate of their methods (Table 1).

The development of identification and quantification methods often use PS nanobeads as standard model nanoplastics (20 out of 33 detection studies; Table 2). MALS, DLS, and NTA have been successfully used for quantification of size distribution and number concentration of the PS nanobeads [5,17,51,61]. However, these methods cannot be directly applied for field samples because nanoplastics and non-plastic nanoparticles in field samples cannot be completely separated by the current separation methods. Thus, distinguishing plastic and non-plastic nanoparticles before quantification is essential to assess the reliability and accuracy of data. We emphasize that quantification should be the final step of analyzing nanoplastics in environmental samples. Quantitative

Table 3

The advantages and disadvantages of current techniques for nanoplastic analysis.

Techniques	Advantages	Disadvantages	Ref.
Digestion			
Proteinase K	↗ Avoid forming nanoplastic aggregates ↗ Fast	↘ Relatively expensive ↘ Need multiple digestion steps ↘ Air pollution risk	[51]
HCl and TMAH	↗ Relatively high recovery efficiency	↘ Cause destabilization and agglomeration ↘ Need surfactant to disperse particles	[20]
KOH	↗ Inexpensive ↗ Relatively fast ↗ High recovery efficiency	↘ Risk for changing properties of nanoplastic	[106,107]
Preconcentration			
Ultrafiltration	↗ Large sample volume ↗ Simple operation ↗ High concentration factor	↘ Interaction with membrane cube ↘ Clogging for real field waters ↘ Setup not plastic free	[5,12,17,104]
Centrifugation	↗ Simple ↗ Mild to particles	↘ Small sample volume ↘ No separation from particulate matrix	[20,105]
Cloud point extraction	↗ Inexpensive ↗ High concentration factor	↘ Small sample volume	[19]
$[C_8C_1Im][NTf_2]$ extraction	↗ Effective to oxidized nanoplastics	↘ Little-used ↘ Expensive	[57]
Separation			
Membrane filtration	↗ Relatively cheap ↗ Diverse in kind	↘ Small sample volume ↘ Low flow rates with small pores ↘ Multiple filtration steps for high turbidity water ↘ Some membranes can adsorb nanoplastics	[12,13,18,19,21,56,60,61]
AF4/CF3	↗ Easy for online coupling ↗ No need of stationary phase	↘ Difficult to obtain complete separation ↘ Particles need polymer identification ↘ Difficult operation	[17,20,51,54,59]
Optical tweezer	↗ High separation resolution ↗ Non-contact with particles ↗ Coupling with Raman spectroscope	↘ Only for transparent particles ↘ Heat effect of laser ↘ Little-used	[23,55]
Identification and quantification			
FTIR	↗ Simple and fast ↗ Effective for thin film of nanoplastics ↗ Particle quantification with polymer identification	↘ High concentration for nanoplastics ↘ Water interference	[5,18,59,61]
Raman	↗ Relatively high resolution ↗ No water interference ↗ Coupling with different microscopes	↘ Fluorescent interference ↘ Low intensity of signal ↘ Trade-off between measurement time and representativeness	[22,23,55,56,60,83]
MS	↗ Little sample mass ↗ Several polymer types for a single run ↗ Mass/number concentration	↘ Need preconcentration ↘ Some polymer types are not included ↘ LOD depends on polymer type ↘ No morphological information ↘ Metal label is needed for ICP-MS	[12,13,17,19,21,52]
XPS	↗ Surface characterization	↘ No polymer type information ↘ Expensive	[18,61]
SEM/TEM	↗ High resolution ↗ Size and number of particles	↘ Need sample preparation for nanoplastics ↘ Particles need polymer identification ↘ Representativeness problem ↘ Expensive	[5,18,20,22,51,56,59–61,83,86]
MALS	↗ Online coupling with AF4/CF3 ↗ Size distribution of particles	↘ Need perfect separation of nanoplastics ↘ Need polymer type identification	[17,20,51,54,55,59]
DLS	↗ Simple and fast ↗ Suitable for nano-sized particles ↗ Size distribution of particles	↘ Not suitable for polydisperse particles ↘ Only for spherical particles ↘ Need polymer identification for environmental samples	[5,12,18,59]
NTA	↗ Simple and fast ↗ Suitable for nano-sized particles ↗ Size distribution and number concentration of particles	↘ Complex in operation ↘ Only for spherical particles	[5,61,86]
TOC	↗ Carbon concentration	↘ Destructive ↘ Little-used	[5]

data including particle size distribution, particle concentration, and mass concentration, are critical and necessary to assess the potential impact and risk of nanoplastics. The available quantitative data of nanoplastic are often obtained from extra/subsamples without chemical confirmation, which limits the accuracy and feasibility of nanoplastic risk assessment [51,54,59,86].

Mass-spectrometry (MS)-based methods allow simultaneous identification of polymer composition, quantitation, or semi-quantitation of nanoplastic mass. By using a Curie-Point py-GC–MS, eight common polymers including PE, PP, PS, PET, PVC, PMMA, PC, and PA were detected in a single run [113–115]. The only two reports of nanoplastics in real field samples both applied MS-based methods [10,11]. Nevertheless, the sole use of py-GC–MS can only determine the mass of polymers per sample but not the particle number or morphology of nanoplastics [116]. Moreover, lower LODs of py-GC–MS are needed for the routine determination of low-concentration nanoplastics in the field [24]. In recent studies, the lowest LOD of PMMA nanoparticles in water samples was calculated to be 0.6 µg/L [19], and that of PS nanoparticles in fish samples was 52 µg/g [51]. Py-GC–MS is highly sensitive to organic impurities, particularly necessitating pretreatment for samples

with a high content of organic matters like soil, sediment, and biological samples [103].

Spectroscopy (e.g., Raman) coupled with microscopy (e.g., confocal, SEM) is considered a more suitable quantification method to simultaneously quantify the size, shape, and concentration, and chemical composition of nanoplastics [22,23,55,60]. The smallest detectable nanoplastics by confocal Raman imaging is 200 nm poly-acrylic nanoplastics [22]. Although the diffraction limitation of Raman is approximately 300 nm to 500 nm, depending on the numerical aperture and the criterion for single point differentiation [117], the spatial resolution of Raman can be down to 100 nm when coupled with SEM for standard PS nanobeads [22]. In addition to the diffraction limitation, how to improve the sensitivity and reduce the fluorescence interference upon laser excitation is another question. Although surface-enhanced Raman scattering substrates can help increase the electromagnetic field around the surface of the material, incorporating plasmonic nanoparticles with these metal substrates (e.g., silver, gold, or copper) are expensive, time-consuming, and needs highly specialized techniques such as electron-beam lithography, colloid immobilization, and soft lithography [80,118]. In practice, Raman sequentially scans and analyzes individual single particles on a filter. Therefore, there is another question of how many particles need to be analyzed to obtain statistically meaningful results [119]. It is feasible to analyze all separated particles when the total number of particles in a sample is low, e.g., bottled water and tap water samples [49,50] but a trade-off between measurement time and representativeness of analyzed particles is inevitable for most field samples. Regardless of different types of samples and different analytical methodologies, proper control experiments and procedural blanks allow for high reliability and accuracy of the quantitative results, however, only 11 out of 33 studies performed control experiments or included procedural blanks (Table 2).

6. Challenges and perspectives

Quantitative data on the occurrence and distribution of environmental microplastics are available but we are lacking harmonized and reliable methodologies for analyzing nanoplastics in the environment. Although 33 studies developed pretreatment, detection, and quantification methods for different types of samples, only 5 analyzed real field samples (Table 2).

Due to the immaturity of the current detection methods, our knowledge of the environmental status of nanoplastics is severely lacking. Several major methodological challenges remain unsolved and the first key question to be answered is what the forms of nanoplastics existing in real environments are. While the presence of nanoplastics has been confirmed, the actual forms of nanoplastics are still under-investigated. A majority of nanoplastics result from the erosion and fragmentation of microplastics as well as larger plastic debris from various sources and consequently become highly polydisperse and heterogeneous [120]. Heteroaggregates are likely formed from nanoplastics of diverse shapes, colors, polymer types, sizes, as well as other natural and anthropogenic materials [108,109]. Learning from colloid and particle science, we hypothesize that environmental nanoplastics do not present in single-particle form but aggregate with clay and organic matters [121,122]. Further studies are required to test whether this happens in the natural field.

The complicated forms of nanoplastics challenge both sampling and analyzing nanoplastics in the environment [123]. Despite the only two studies quantified the mass concentrations of nanoplastics in seawater and snow samples, the form and morphology of nanoplastics were not studied because of both using destructive MS-based methods. The complex form of nanoplastics existing in the environment makes pretreatments to extract nanoplastics from aggregations and matrices difficult. Currently, the most powerful pretreatment methods such as AF4/CF3 are developed on standard plastic nanobeads or degraded nanoplastics from commercial microplastics, which are inapplicable in

detecting nanoplastics in complex field samples. These treatment methods cannot completely separate plastic and non-plastic nanoparticles, thus chemical identification is a top priority to ensure reliable and accurate quantification. We should only obtain quantitative data after the confirmation on polymer types of nanoplastics. One future direction is to develop a high-throughput nano-analytical system, which enables simultaneously determined the chemical identity and morphology of nanoparticles, with an automatic quantification algorithm. While enormous efforts are needed to develop and realize the ideal instrument for analyzing nanoplastics, focused studies on the separation method towards a full separation of plastic and non-plastic nanoparticles will significantly simplify the subsequent steps of identification and quantification.

Nanoplastics as a new emerging environmental contaminant is one of the most challenging eco-environment issues of our time. The question of how many nanoplastics are actually in the environment remains unknown due to lack of efficient and accurate methodologies. The highlighted advantages and disadvantages of the existing methodologies imply the uncertainty of the current data. We are still far away from understanding the fates and impacts of nanoplastics, calling for harmonized and reliable methodologies of analyzing nanoplastics in real environmental samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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