**NANO AND MICROPLASTIC ANALYSIS: FOCUS ON REMEDIATION
TECHNOLOGIES AND OCCURRENCE IN FRESHWATER ECOSYSTEMS**

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- Current sampling strategies for micro and nanoplastics (MPs and NPs) in the environment summarized
- Progress on isolation, characterization and determination reviewed.
- Applications focus on fresh water occurrence and remediation system presented
- Future trends within the field of MPs and NPs characterization and determination discussed.

Abstract

Plastic pollution is a global problem since its production reached 322 million tonnes in 2016, excluding fibers. Daily discharges of microplastics (MPs, defined as < 5 mm in size) are estimated in the range of 50,000 up to 15 million particles, whereas no information on nanoplastics (NPs, < 100 nm) release is available yet. Different processes further degraded these materials producing more MPs and NPs. This review attempts to fill a void of information on the state-of-art of the analysis of MP and NP (recently identified as emerging contaminants) offering a critical overview on modern instrumentation, newly developed workflows and promising techniques for their characterization (Raman and FT-IR spectroscopies and microscopies, pyrolysis and thermal desorption gas chromatography, imaging techniques, etc.). MP and NP sampling, identification and characterization are discussed. Finally, recent applications to establish their occurrence in fresh water ecosystem and the effectiveness of the proposed remediation technologies are considered.

Keywords: Sampling strategies; Analytical methods; Remediation; Microplastics; Nanoplastics; Future trends.

Abbreviations

AF⁴, Asymmetrical flow field-flow fractionation

ATR, attenuated total reflection

BAF, biological activated filter

BEPP, basic enzymatic purification protocol

CAS, conventional activated sludge

CLSM, confocal laser scanning microscopy

DAF, dissolved air flotation

EPS, expanded polyethylene

FPA-FT-IR, focal plane array Fourier transform infrared

FT-IR, Fourier transform infrared spectroscopy

HDPE, high density polyethylene

LCP, life cycle of plastics

LDPE, low density polyethylene

LS, light scattering

μFT-IR, Fourier transform infrared microscopy

μRaman, Raman microscopy

MALS, multi-angle light scattering

MBR, membrane bioreactor

MP, microplastics

NP, nanoplastics

PA, polyamide

PE, polyethylene

PET, polystyrene terephthalate

PLE, pressurized liquid extraction

POPs, persistent organic pollutants

PP, polypropylene

PS, polystyrene

PSNPs, polystyrene nanoplastics

PVC, polyvinyl chloride

PVC-P, plasticized polyvinyl chloride

PVC-U, unplasticized polyvinyl chloride

Py-GC-MS, pyrolysis gas chromatography-mass spectrometry

RSF: Rapid gravity sand filter

SEM: scanning electron microscopy

TEM. Transmission electron microscopy

TGA-DSC-GC-MS, thermogravimetry differential scanning calorimetry gas
chromatography-mass spectrometry

WWTP, wastewater treatment plant

1.-Introduction

Plastic pollution is a globally recognized problem [1]. Its production reached 322 million metric tonnes in 2016, excluding plastic fiber production. During the same period, approximately 90 million metric tonnes of textile fibers were produced. Over 60 % of this production corresponds to plastic fibers. The rest corresponds to cellulosic (6%) and natural fibers (27%) [2]. In addition to its growing production, plastics are long-lived and resistant to biodegradation, then, accumulative in the environment to a broad extent. Seas and oceans are the most affected and studied environmental compartments since it is estimated that annually, between 4 and 12 metric tonnes of plastic go into them—with a forecast for them to outweigh the amount of fish in 2050 [3-5]. However, no for a moment can we forget that all size of plastics —produced and mostly consumed and disposed of on land because of anthropogenic activity— have also been extensively reported in terrestrial and adjacent freshwater environments [6-9]. Main sources of microplastics (MPs, < 5 mm) and/or nanoplastics (NPs, <100 nm) are: (I) polymer nanoparticles manufactured for specific purposes, like cosmetic products, ink for 3D printers, and others [10]; (ii) fragmentation of plastics due to UV photodegradation, mechanical actions, hydrolysis or microbial degradation, as it is often the case in the marine environment, and (iii) wastewater treatment plants discharges including biosolids and effluents —daily discharges of MPs could range from 50,000 up to 15 million particles with no data on NPs [11, 12].

Although more research is needed to establish the impact of these contaminants in the environment and human health, there has been a considerable increase on the studies on the ecological consequences of MPs and NPs released into the environment [13, 14]. The reduced size of both types of plastics makes them susceptible of ingestion by organisms. Moreover, the higher binding affinity to cells and surface area-to-volume

ratio of NPs may add hazardous effects [13]. MPs and NPs exposure has already been associated with toxic effects, including enhanced immune response, decreased growth rate, fecundity, and negative impacts on subsequent generations [15]. In parallel, these particles can transport other harmful chemicals used as additives in their fabrication or adsorb to them due to MPs and NPs strong adsorption capacity facilitating the entry of these substances into organisms [16-18]. Thus, the need of robust analytical methods for detecting, characterizing and accurately measuring MPs and NPs both in the environment and in exposed organisms is imperative.

Searching of SCOPUS database (1 April 2018) using the keyword ‘microplastics’ or ‘nanoplastics’ combined with ‘environment’ resulted in 451 and 30 articles, respectively. This highlights how much the topic of MPs has been studied compared to that of NPs. **Fig. 1** shows the analysis of the number of papers per year pinpointing that the topic has experienced exponential growth in the number of articles published since 2013. Most of the scientific literature was on the characterization, analysis and fate of MPs in the marine environment. However, in the recent years research on freshwater systems was published. Two books related to characterization and analysis of MPs and NPs [15] and the impact into freshwater systems [6] were recently published. Several review papers discuss the increasing problems related with the presence of MPs in wastewaters and surface waters [8, 9, 19-22]. Some other papers were related to soil and sediment matrices [19, 23-26], food [27, 28], air [29, 30] and human health [31]. Even few of these review are particularly focused on NPs sources, fate and effects on the environment [16].

There are already two interesting reviews offering an overview on the analytical challenges but restricted to them [32, 33]. Furthermore, few reviews have already tackled some of the multiples steps of the analytical procedure, for example the standardization

of sampling [34], analytical techniques to quantify them in sediments [35], identification methods applied to fish extracts [36] or even the analysis of the organic compounds associated to them [37]. These reviews are small pieces that need to be incorporated in a whole.

This review integrates this previous information and describes current sampling strategies, extraction, characterization and quantification methodologies for MPs and NPs analysis with a focus on remediation technology and occurrence in freshwater ecosystems. It is based on literature data from the last four years (2015-2018) and refers to different types of freshwater ecosystem samples, many of them characterized by complex matrix composition. Instead of covering all the articles, selected publications highlighting the major trends were included. A structured overview of the fate and risk of MPs and NPs in freshwater and terrestrial environments and to human health is provided including a description of the analytical methodologies as well as their advantages and limitations. We also introduce technological perspectives related to remediation of MPs and NPs in the environment as an important tool to reduce the impact of these materials.

2. Occurrence, fate and risk in freshwater and terrestrial environments and to human health

The fate and behavior of MPs and NPs in freshwater ecosystems and terrestrial environments, and their transfer between organisms and from organisms to the environment, must be considered **Fig 2.** shows a conceptual map of MPs and NPs sources, transport and fate in freshwater ecosystems.

Several studies pointed out that MPs and NPs are widely distributed in water and sediments [8]. The presence in one or other compartment depends mostly on the plastic density that varies according to their chemical nature. The denser MPs settle out of the water column into the sediment. Several studies report how organic matter fouling and subsequent hetero-aggregation with suspended solids, algae or detritus will increase MPs settling. This could be even more important for NPs, because hetero-aggregation occurs frequently at the nanometre-scale. Then, river sediments could act a sink for MPs and NPs of higher density than water that act bridging terrestrial and freshwater systems contamination problems.

Fresh aquatic biota can intake MPs and NPs from both, water and sediments. Impacts of MP and NPs in freshwater ecosystem biota have been extensively described. They are affecting microalgae, fish, bivalve, zebrafish, daphnia, nematodes, etc. [38, 39]. The toxicity of MP can be due to, (i) intrinsic toxicity from, physical damage and leaching of chemicals present in the plastic, (ii) as well as ability to sorb persistent organic pollutants (POPs) or pathogens [31]. A recent study demonstrated that MPs particles of polyamide (PA), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) caused intestinal damage in *Danio rerio* —selected as a model of vertebrate fish living in the water column—, including cracking of villi and splitting of enterocytes. This study also found that MP particle-induced lethality and reproductive dysfunction, reduction in intestinal calcium levels and increased expression of the oxidative stress gene *gst-4* were dependent on particle size in *Caenorhabditis elegans* —nematode living in the sediments [40]. These MPs effects are more related to their sizes than to their chemical composition. Interestingly, Net et al. [24] also pointed out as common deposit feeder as *Chironomus spp* larvae ingested MPs and could serve as indicator of MPs loads in freshwater systems. MPs and NPs can also absorb organic contaminants, mainly POPs

like perfluoralkyl substances [17, 34], polycyclic aromatic hydrocarbons, or organochlorine compounds [8, 41]. Not only POPs but also other emerging contaminants absorbed into their surface can be introduced into the food web through MPs and NPs ingestion [42]. Recently, interesting studies on the combined effects of MPs and emerging contaminants in freshwater species has been published. Guilhermino et al [38] performed exposure experiments with individual and mixtures of the antimicrobial florfenicol and MPs on the freshwater exotic bivalve *Corbicula fluminea*. The reported results indicated that *C. fluminea* was able to uptake MPs and florfenicol from water. Evidences of toxicological interactions were found being the mixture of MPs and florfenicol more toxic than their components separately. Furthermore, microbial colonization seems to be a common way, as reported in a recent paper of MPs in an urban river [31, 43]

Soils are major reservoirs of MPs and NPs, and it may represent 4-20 times higher than in marine environment [19, 23]. Agricultural common practices like plastic mulching or the application of treated sludge's to soil as amendments are important and offer opportunities from the economic point of view, —higher yields, better fruit quality and early marketing of seasonal crops. However, these practices are main sources of plastics to soil together with landfills. Several problems outlined including increases the degradation rate of soil organic matter and emission of greenhouse gases [25], inhibition of tetracycline dissipation as well as antibiotic resistance gens in soil [44].

Different studies already reported the presence of MPs in seafood products like bivalves and fish and more recently in commercial salts from different countries [27, 28]. Consequently, humans can be exposed with food to MPs. European shellfish consumers are expected to ingest up to 11,000 plastic particles per year [45].

There are very few studies on the presence of MPs in the air [29, 30] the fact that airborne fibrous MPs enter our respiratory system will be dependent upon size. In addition fibers can be associated with common air pollutants, like PAHs. The question remains because up until now, most of the studies reported detection of 50 μm fibers, but detection of smaller size, below 10 μm is needed. Only in that specific case we should be able to better assess the impact of air in human health. A very recent paper [46] reported the issue of MPs and human health and indicated that all different exposure pathways should be considered.

3. Analysis of MPs and NPs

Fig. 3 shows schematically the procedure for MPs and NPs characterization and quantification and highlights the principal methods for each step while **Tables 1-3** compile an overview on procedures applied in the latest studies characterizing and determining MPs and NPs in freshwater ecosystems. Some issues in the different steps of the workflow—sampling and processing; extraction, clean-up and separation; detection and quantification— need some consideration to ensure the reliability of analytical results and to:

- (i) avoid sample contamination during sampling, transport and laboratory manipulation.
- (ii) develop appropriate reference materials for quality control and proceed with a thorough validation of analytical methods to decrease inter-assay and -laboratory variability to ensure harmonization and data comparability.

<Table 1-3 near here>

Precautions taken during sampling to avoid sample contamination in the field include limiting sample material exposure to MPs and NPs sources, such as air, synthetic textiles and plastic materials. If some plastic materials are used for sampling and transportation, blank samples are also analyzed to quantify their contribution to the MPs and NPs load of the sample. The topic of the blank samples is of special interest in studies dealing with fibers since operator clothes or any textile material can be a source of contamination. Then, if blank samples are not analyzed the results obtained might be of limited validity. To prevent airborne or other contamination in the laboratory or during sample analysis, nitrile gloves, cotton laboratory coat, and shower cap are worn during the whole process. All containers are covered with aluminum foil if not in use. Sample pretreatment was performed in a fume hood, and a sticky dust drum is used to clean the desktop, hands, and clothes. Blank controls are also carried out following the same analytical procedures as for the samples to assess the contamination.

Moreover, the entire method starting from the sample preparation to the analytical detection need to be critically evaluated. Therefore, a proper validation must be implemented, which also favor comparability between different studies. This includes, for example, the determination of within-site variability for the sampling process or the determination of recovery rates for the separation methods used during the sample preparation. The reported analytical methods only in some cases estimate recoveries using spiked samples, and in very few cases evaluate sensitivity through the limit of detection or quantification. Another problem is the variety of MPs or NPs that exists and their different physic-chemical properties. Roch & Brinker [82] tested the effects of digestion with sodium hydroxide and nitric acid on a range of polymers: high density polyethylene (HDPE), low density polyethylene (LDPE), PP, PS, expanded polystyrene (EPS), polyethylene terephthalate (PET), PA, plasticized PVC (PVC-P) and unplasticized PVC (PVC-U). After digestion, PA

was completely dissolved and statistically significant changes were observed in the surface area of EPS, LDPE, PVC-P and PVC-U. Thus, establishing the legitimacy of the research findings requires an increasing emphasis on quality control, which the weakest point of the methods developed up to now. Validation and standardization of analytical methods is still limited partly due to the lack of reference methods, reference materials and inter-calibration exercises.

3.1. Sampling of NPs and MPs

3.1.1. Sampling in wastewaters and surface waters

There is not a general agreement on the sampling. An excellent review paper on sampling and analysis of MPs in the environment was recently published [33] describes sampling devices like manta trawl, Neuston catamaran and bong nets, most of them designed for marine water samples. Such devices can be easily adapted for surface and river waters sampling. Bulk sampling and the use of Neuston and conical plankton nets seems to be the most common approach to sample surface water. Net-based devices also incorporate a flowmeter to determine the water volume passed through. Mesh size is a critical point since it determines the minimum size of MPs detected. The most common was 300 μm but recently lower mesh sizes of 150 and 80 μm were used too [91]. The use of lower mesh size enlarges the MPs detected. However, the smaller the net mesh size, greater likelihood of clogging due to suspended organic matter. In this sense, Dris et al. [91] addressed the representativeness of sampling MPs in the Seine river as it passes through Paris and pointed out most of these problems. The use of longer time sampling periods (3 min versus 1 min) reduced variability between consecutive samples and the results obtained with 80 μm mesh size were 100,000 times higher than using 450 μm mesh. However, tests to prevent net clogging show that the 80 μm net stopped from sampling

when the volume surpassed 8 m³ in conditions of total suspended solid levels over 10 mg·L⁻¹, which is already reached with 1 min sampling when the river flow is high. To overcome this problem, these authors proposed multiplying the number of samples instead of increasing the volume to get more representativeness. Other solution in progress is the use of filter cascades that result in a size fractionation during the sampling and the reduction of the matrix burden of the small mesh sizes [11, 48]. Samples taken in the water column have only been reported occasionally. Sampling is carried out by direct filtration of the water with submersible teflon pumps or is reported by the acquisition of batch samples [62, 64]. Sampling at the wastewater treatment plants is carried out also using these pump filtration devices [11, 48, 51, 52, 55, 56], grab samples [57] or 24 h composite samples [58]. Ziajahromi et al. [68] applied a high-volume home made sampling device that fractionated MPs in situ. Different volumes in the wastewater treatment plant were sampled, varying from 40 up to 200 L depending on the mesh size and the type of samples. In this respect, i.e. for a mesh size of 25 µm 100 L were collected whereas for a mesh size of 200 µm up to 200 L were samples were collected. The volume was dependent on the type of water too, for reversed osmosis samples up to 200 L were samples whereas the volume for primary effluent between 16-100 L.

Although filtration by any of the previously described devices is the preferred method for sampling water, is not trouble-free. Synthetic fibers and particles having dimensions < 25 µm easily bypass the filtration devices. This is of particular interest for fibers and NPs because they are not always retained in nets or mesh. There is an absolute lack of standardized method to sample fibers and NPs, In the case of fibers, some studies used bulk samples but this requires a high fiber-concentration to identify them [59]. Up to our knowledge NPs has not been reported in any type of water yet. This will be the strategic

gap that probably will remain in a near future between the goals within this field and the expected reality.

3.1.2. Sampling solid samples to determine NPs and MPs

Sediment samples are mostly surface sediments sampled using sediment grabs (e.i. van Veen drag, stain steel spoons, etc.), or by the collection of grid samples (commonly samples are from the top 2 up to 5 cm) [73]. The sampling procedure used will affect how the corresponding results are reported. For example, studies that use grid samples usually report the results per surface sampled (e.g., m^2), whereas studies based on aerial bulk samples give the results referred to the volume or mass of the collected sample (e.g., m^3 or kg). Sludges are commonly collected with stainless steel bucket or cup [48] or as a pellets [72]. Common pretreatment operations carried out at the laboratory are air or oven dried at 45 °C and sieved. Suspended particulate matter is sampling using continuous centrifugation systems [52].

The applications of corers allows the determination of MP depth profiles but results in small sample volumes (25 cm^3). The use of the HTH sediment corer, which is a rope-deployed gravity corer for collecting soft surface sediments without disturbance, were only reported to get sediment samples taken at each 0.5 m depth to 1.5 m in the Edgbaston Pool, a shallow eutrophic lake in central Birmingham, UK [74].

Commonly benthic macroinvertebrates are sampled with a nylon nets. Clams and other bivalves are taken with a bottom fauna trawl. Fish samples are commonly taken by electrofishing, with nets or hook and line. The electrofishing is the best way to ensure an exhaustive and representative sampling. However, electrofishing is not possible in all the river parts because it needs of affordable parts with not too high steam. Fishing using nets or hook and line is not easy and requires training, especially in highly contaminated river

where the fish population is not too abundant. An alternative widely employed is to buy fish to the local fishermen. Commonly, in the case of fish the digestive tract is the only part analyzed. Several techniques preserve this part using 70 % of ethanol, 4 % formalin [39, 79, 84].

3.2. Extraction and isolation of microplastics

3.2.1. Water samples

The MPs isolated by the nets and sieves are commonly transferred to glass bottles using water. Once in the laboratory, they are filter again using stain steel sieves or glass fiber filters. The identification of MP particles is often difficult due to the organic matter that could be easily confuse with MPs. Thus, the destruction of natural organic matter is unavoidable to minimize the possibility of misidentification or underestimation of small plastic particles. The destruction of natural material can be carried out by chemical or enzymatically catalyzed reactions. Chemical destruction of natural organic matter is achieved through the treatment of the sample with hydrogen peroxide, mixtures of hydrogen peroxide and sulfuric acid, or hydrogen peroxide in presence of iron II as catalyst (Fenton's reagent) reactions. A basic enzymatic purification protocol (BEPP) to eliminate the organic matter always consist of an initial treatment with sodium dodecyl sulfate (SDS) (pH 8–8.2) and then, a treatment with several enzymes alone or in combination that vary depending on the sample characteristic and could include cellulases, proteases, lipases and amylases of different origins [47-49, 53].

Density separation is also frequently reported in water prior or after the organic matter elimination but commonly after. The solutions used to separate MPs are NaCl, ZnCl₂ (1.64 g/mL), KHCO₂ and NaI. There is no consensus on the best method to analyze the

sample or the water characteristics that make more appropriate one or other. As **Table 1** pointed out, the majority of isolation techniques are based on the same principle however, specific procedures vary from author to author.

Interestingly, Rodrigues et al [47] compared six distinct MPs extraction methods, selected according to their common application and efficiency: density separation methods using sugar, olive oil and zinc chloride, as well as organic matter degradation methods with hydrogen peroxide (wet peroxide oxidation) and BEPP (enzymatic digestion). To assess the effectiveness of distinct methods of MPs isolation the efficiency of density separation and organic matter degradation, economical cost and time spent, simplicity as well as the quality and the total mass of recovered polymers (from the top and the net of the density separator) were considered. After the application of distinct methods on artificial samples, the ZnCl_2 density separation, wet peroxide oxidation, BEPP + wet peroxide oxidation and BEPP formed the group with the highest percentages of efficiency (all above 90%), followed by the olive oil density separation, and finally by non-treated samples and density separation with sugar (with the lowest percentages). Regarding the methods with organic matter degradation, wet peroxide oxidation and its combination with a BEPP were more efficient than the BEPP alone. So it became evident the need to develop additional comparison studies. Further studies are needed to establish the best assessment procedure among the different methods.

3.2.2. Soil, sediments and sludges

To our knowledge, the analysis of NPs have not been reported in any of these matrices. The most used technique for the separation of MPs of the sediment particles is the separation by density. In a high density solution, particles of MPs float, while very dense sediment particles settle at the bottom. Several methods have been described; all of them based on the separation of the MPs because of their lower density. Many of those methods

are based on the separation proposed by Thompson et. [1] in 2004 who separate MPs from sediment in a saturated solution of NaCl (density 1.2 g/mL). Modifications to this method include the use of various salts (water alone, NaI ZnCl₂ or potassium formate) to adjust the density of the liquid and ensure floating MPs present in the sediment (which are those of higher density). Despite that sediments are quite rich in organic matter that can float along with the MPs in density-based separation, the chemical digestion of the organic matter (using H₂O₂) so used in the case of water samples, are used only in few studies [66, 77]. Up to our knowledge, the enzymatic digestion was not reported yet for this type of samples.

An additional technique that could help on density separation is elutriation that separates lighter particles from heavier ones through an upward flow of liquid and/or gas. Mahon et al. [72] constructed an elutriation column, based on a previous design to extract MPs from sludges. The average extraction efficiency rate of the elutriation column for the spiked sediment samples was 90-94 % for the HDPE particles. The elutriation process was less efficient for the PVC particles, which resulted in an average extraction efficiency of 80%. After the elutriation a density separation clean-up with ZnCl₂ is still required.

There are few examples of other alternative methods, but one of them based on pressurized fluid extraction (PFE) deserves mention because due to its simplicity, it can address some limitations of the previous MP methods. The method applied to a composted municipal waste sample with spike recoveries ranging from 85% to 94%. Although the results from municipal waste samples and soil samples collected from an industrial area indicated that this method is a promising alternative for determining the concentration and identity of MPs in environmental samples, it must also be taken into account that so far only an application of the method has been published.

3.2.3. Biota samples

Unlike what happens in sediment extraction based almost exclusively in density separation, in the case of biota, extraction methods are mostly based on wet digestion with acids, bases or even hydrogen peroxide. In the case of fish, all methods described in Table 3 are only applied to the digestive tract and not the full specimen. The reasons are, first that MPs accumulate mainly in the fish gastrointestinal tract and second, that the digestion process have to be soft since the MPs are organic and also degrade if the treatment is too drastic. The organic matter oxidation has been achieved using HNO_3 , H_2O_2 , NaOH , KOH or their mixtures. Only in one study, after digestion, density separation was reported [82] in order to better separate MPs. These digestion or wet oxidation methods contrast with those that propose just to analyze the stomach content that is isolated over a glass fiber filter. Only simple operations as washing with distilled water, dry in an oven at 70 °C overnight are included. MPs are separated from the organic matter using forces.

The field of the biota is the only one in which NPs have been really studied. Acid or enzymatic digestion has been widely applied to the determination of nanomaterials in complex matrices [92, 93]. Correia & Loeschner [85] compared acid and enzymatic digestion with the protease proteinase K. Although the acid treatment was successful for complete digestion of the fish, it led to agglomeration of the PSNPs. Instead, using enzymatic digestion particles larger than 1 μm were not detected in the investigated samples. This pointed out the additional problems in the analysis of nanoparticles.

3.3. Detection

The characterization, identification and quantification of MPs is a process commonly carried out in 2 steps.

(i) detection, quantification and sizing (by naked eye or using a microscope). At this point, the review Hidalgo et. [94] on MPs in the marine environment deserves mention, since it established the rules for the MPs visual classification and it has become a guideline followed in many studies. Microplastic particles are identified because they unnatural coloration (e.g. bright blue and multi-colored) and/or unnatural shape (e.g. fragments with sharp edges, perfectly spherical). Some simple test can help in the visual inspection (i.e. staining of natural and non-plastics particles [68], heating MPs at $> 100^{\circ}\text{C}$ [26], hot needle point for fibers [82]). **Fig. 4** shows how morphology of the plastic particles collected in the field was compared to plastic particles extracted from a variety of personal care products containing polyethylene [54]. Microplastics quantification is often performed by visual methods only.

2—characterization/identification of the plastics are well establish by Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy or their microscopy versions ($\mu\text{FT-IR}$ or μRaman). The spectroscopic investigation is performed on all the isolated particles without any visual selection before analysis. With respect to FT-IR spectroscopy, mainly the attenuated total reflection (ATR) technique. The inconveniences of this technique is the lack of sensitivity and that is not so efficiency to detect low size MPs. The use of the μ -version involves the examination of those particles that are visually identified as MPs. This version is time-consuming, not automatable and prone for underestimation because involves a subjective visual sorting that depends on the analyst experience and only examines a small percentage of the filter. Latest advances in the imaging version of these techniques as focal plane array (FPA)-FT-IR detectors that simultaneously acquiring thousands of spectra within minutes and imaging the entire membrane filters leads to more robust results that are subject to minimal analytical bias [57]. Apart from different IR absorption or Raman scattering properties,

scanning electron microscopy (SEM) [62, 65, 66, 72], pyrolysis-gas chromatography/mass spectrometry (Pyr-GC-MS) [43, 63] or thermogravimetric coupled to differential scanning calorimetry (TGA-DSC) with GC-MS [70], are qualitative techniques also applied for identifying and characterizing MPs.

Interesting also to cite the work of G. Renner et al. [95] who after reviewing 170 papers on MPs and NPs detection from 2015-2017 find out that still visual examination is the most used technique, up to 79% of the studies whereas techniques like FTIR and Raman are only used 28 and 14 % respectively. Finally, GC-MS wither using pyrolysis or thermal extraction is only used 7 %. Indeed, py-GC-MS is able to identify the polymer type and contained additives simultaneously. This last technique is very promising because there are already existing GC-MS libraries on MPs (polymers) available.

In contrast to MPs, there are currently no robust analytical methods for detection and quantification of NPs in environmental matrices. Theoretically, some of the analytical methods used for detecting and characterizing inorganic NPs, like metal and metal oxide NPs, should be applicable for the detection of MPs [92, 93]. Mostly SEM, transmission electron microscopy (TEM) and other electron microscopy techniques but also optical microscopy as confocal laser scanning microscopy (CLSM) have been widely used in polymer science (including the SEM application to characterize MPs in environmental samples) and are considered as the reference technique to characterize several types of nano-sized plastic structures and particles. These techniques were found to be suitable to detect and characterize the intake of NPs in simple matrices (e.g., *Daphnia magna*, *Oryzias sinensis*, etc). Fig. 5 shows the analysis of *D. magna* via CLSM and used Z-stack imaging to confirm the presence of nPS in the inner guts.

However, detecting NPs in a fish matrix is more challenging as it is difficult to distinguish the NPs from the organic fish matrix due to the similar density (typically between 0.9 and 1.4 g cm⁻³) and chemical composition (mainly carbon, hydrogen). Correia & Loeschner [85] used AF⁴ for separating the PSNPs from the digested fish and to determine their size by multi-angle light scattering (MALS). The PSNPs could be easily detected by following their light scattering (LS) signal with a limit of detection of 52 µg/g fish. However, the asymmetric field flow fractionation (AF⁴)-MALS method failed to detect the PE particles in fish, due to the presence of an elevated LS background, indicating that an analytical method developed for a certain type of NPs may not be directly applicable to other types of NPs and may require further adjustment. Despite the current limitations, this is a promising methodology for detecting NPs in complex environmental matrices.

4. Control and remediation techniques

There are different possibilities to control and reduce any type of plastic contamination. Summarized in **Table 4**. The consumption of plastics has promoted the creation of a legislative body in many countries, which regulates the use and disposal of plastic in order to reduce their presence in the environment. This issue is collateral to the topic of this review. However, there are two excellent reviews very recommendable for the interested reader that covered the situation in the United States of America [3] and Europe [96] from the perspective of the life cycle of plastics (LCP). Both reviews, identified regulatory gaps and limitations and how they can be addressed in order to limit and prevent environmental exposure and hazards of MPs and NPs.

Both reviews also identified the pressing need for implementing and enforcing the existing legislation, as well as to improve measures of source control, waste management and clean-up. Strategies based on citizens enrolment and behavior changes, involving

recycling and consumption/demand of disposal plastics should also be prioritized in order more ambitious recycle and recovery targets.

In addition to these regulatory and social measures, several remediation technologies are being used to overcome with the plastic pollution [97, 99-105]. These remediation measures could be divided into technological and biotechnological techniques. The former involved advanced wastewater treatment technologies since waste water treatment plants (WWTPs) can offer a solution to reduce the point source input of microliter and MPs into the environment... There are several studies that evaluate the capacity of these technologies to remove plastics [48, 97, 98]. The membrane bioreactor (MBR) process had a slightly better removal efficiency of MPs (99.4%) compared to the overall conventional activated sludge (CAS)-based process (98.3%). Rapid gravity sand filters (RSF), Dissolved Air Flotation (DAF) and Membrane Bioreactor (MBR) were used as full tertiary treatment in Finland. Wastewater containing MPs was treated and all three technologies offered removal rates of MPs higher than 95% from primary and secondary effluents [98]. The biologically active filter (BAF) is also an important process to remove these compound [97]

The second strategy to solve the problem of MPs is to use more biodegradable materials. Bio-based polymers are obtained from renewable starting materials, such as starch, cellulose, lignin and bioethanol and most of them are biodegradable (i.e. polylactitol and biodegradable polyethylene). A major problem with biodegradable plastics is that they have the potential to be biodegraded, but this process requires suitable conditions and microorganisms. Some of their degradation products were already identified in water [106].

The last one is also based on bioengineering-based solution but in this case is using microorganisms capable of degrading plastics, like different types of bacterium, fungus and or enzymatic hydrolysis. Multiple efforts have been made to identify and isolate microorganisms capable of utilizing synthetic polymers and recent results point towards the viability of a solution for this problem based on the biodegradation of plastics resorting to selected microbial strains [100-103]. Yoshida et al.[102] showed how the biodegradation of plastics by specialized bacteria could be a viable bioremediation strategy. The new species, *Ideonella sakaiensis*, breaks down the plastic by using two enzymes to hydrolyze PET and a primary reaction intermediate, eventually yielding basic building blocks for growth. The enzymatic hydrolysis of PE have also been reported [100, 101]. The fungus *Zalerion maritimum* was tested also to degrade polyethylene indicate that this naturally occurring fungus may actively contribute to the biodegradation of MPs, requiring minimum nutrients [103]. Despite the alternatives that seem promising, the remediation of macro and MPs is in their infancy. Strategies for the future are defined, and is expected that the number of applications to grow exponentially in the coming years.

5. Conclusions and future trends

NPs and MPs analysis is still challenge and under development. Although additional time and resources are needed to refine analytical characterization, the overview presented here reveal analytical developments of major importance for NPs and MPs detection, characterization, and quantification. An assessment of the proposed analytical methods so far, makes clear that the procedures for sampling and extraction of MPs are more or less defined. From water, MPs are sampled and extracted simultaneously mainly with nets, sieves or filters. From soil, sediment or sludge, MPs are separated by density using

saline solutions while from biota, acid digestion is the preferred method. Most of the studies still identify and quantify the MPs by visual inspection, using in the best cases, dissection or optical microscopes. However, the recommendation is to ensure the nature and type of MPs using a technique able to establish their chemical composition. Important advances are expected in this field in the near future. The traditional use of Raman and FT-IR, is nowadays already reinforced by the emergence of a number of promising techniques, such as GC-MS preceded of a pyrolysis or TDS and especially new hyperspectral imaging techniques. The latter in addition to information about the location of the MPs provide information on chemical composition. On the contrary, in the case of the NPs sampling and pre-treatment of the sample prior to the determination are still in a very preliminary step while the techniques proposed to characterize distribution size and chemical composition of nanomaterials in complex matrices are applied.

Another important analytical aspect which should be noted in the determination of NPs and MPs is the lack of standardization and homogenization of the analytes methods that often prevent compare the results between the different studies. An assessment of the analytical methods pinpointed that several technological and methodological challenges remain ahead for the implementation of fully validated solutions for MPs and NPs. So far, the only effort made has been the establishment of guidelines to visually identify MPs. This field not yet addressed, is now highlighted not only here but also in many other reports and hopeful in the near future, the first reference materials and inter-laboratory exercises to help to standardize analytical methods and establish workflows for the validation of analytical methods applied will start to appear.

Last, but not least, although a large number of further studies are still needed to establish sources, presence, transport and fate of MPs and NPs on the environment, the existing ones already highlight their ubiquity not only in the marine environment but in all

terrestrial environments including freshwater ecosystems around the world. So much so, that the various Governments are beginning, although in an early way, to deal with the problem of MPs and NPs in the environment. Some regulations to reduce the plastic disposal of and to recycle and reuse them, or to prohibit their use in cosmetic products already are a reality. Biodegradable plastics are also an alternative with good prospects. However, these corrective measures must be accompanied by remediation techniques that allow to eliminate NPs and MPs that are coming or are already in the environment. In this sense progress made can be optimistic.

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Table 1

Sampling, extraction and analytical methods for characterization and/or determination of NPs and MPs in water from 2015 to 2018

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
MPs						
Synthetic water	-----	-----	11 plastics LD/HDPE, PP, PS, PVC, PET	Six distinct methods: -Density separation using sugar, olive oil and Cl_2Zn -Organic matter degradation with H_2O_2 and <i>BEPP</i>	Stereomicroscope FTIR spectroscopy	[47]
Wastewater Lake water	Samples collected with a 10-L stainless steel bucket attached to a wire and poured to a cascade of 2 test sieves with mesh sizes of 0.25 and 5.0 mm.	Materials retained on the 0.25mm sieve rinsed with H_2O_2 to weighed glass beakers	All types Method tested with PET and PS	Sieved samples were placed in 75 °C Drying oven for > 40 h <u>Organic matter degradation</u> H_2O_2 with Fe^{2+} Filtrated through gridded membrane filters (0.8 mm) with glass fiber filters (1.5 mm) Dry for > 24 h at room temperature. For influent samples, <i>BEPP</i> with cellulases	Optical microscope μFTIR μRaman	[48]
Surface water	Not specified	Frozen	All types	Two size fractions using a 500 μm stainless steel sieve. Fraction < 500 μm , <i>BEPP</i> digestion with SDS, protease and cellulose Chemical treatment with H_2O_2	μFTIR Raman spectroscopy	[49]
Surface water	3 L from the top 8 to 18 cm	None	All types	Vacuum filtered through a Whatman 47 mm diameter, 0.45 μm gridded filter paper	Stereo-microscope (45 ×) μFTIR	[50]
Wastewater	Effluent flow directed across a stack of 0.355 mm and 0.125 mm sieves at peak flow for 2h	Content of the sieves transferred to a jar with distilled water	All types	Organic matter degradation using H_2O_2 with Fe^{2+} at 70°C Filtered through 0.8 mm membrane	Dissecting stereomicroscope (45x) combined with a digital camera	[51]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
Influent and effluent wastewater	2 L of water sample in glass jars	NaCl added to the saturation point (1.2 kg NaCl L ⁻¹)	All types	Filtration over 0.7 µm glass filters	Light microscopy to count particles and record shape After filtration through Al ₂ O ₃ µFTIR	[52]
Effluent of WWTP	Custom made mobile pump device	----	All types	Filter the purified water samples onto 0.2 µm aluminum oxide filters in an in-house fabricated filter funnel. <i>BEPP</i> cleaned up with SDS, protease, lipase and cellulasa	Visual inspection FTIR ATR-FTIR µ-FTIR	[53]
Surface water	Neuston nets (0.52 × 0.36 m) of 333- µm mesh 15-20 min + River flow	Rinse material of the net with distilled water and place in 1 L bottles	All types	Samples were first run through 4.75-mm and 330- µm stacked sieves. The 0.330–4.75 mm fraction was stored in glass beakers in a drying oven at 75°C. Organic material was degraded through wet peroxide oxidation (0.05 mol/L Fe(II) and 30% H ₂ O ₂) at ~75°C NaCl (= 6 mol/L) for a salinity- based density separation. Filtration over 0.7 µm glass filters	Count by dissection microscopy Py-GC-MS	[43]
Surface waters	Plankton nets (0.2 m diameter, 0.51 m long) with 153 µm mesh size. Samples collected for 1 h	Net rinsed with distilled water (0.25 m ³) into a series of sieves (4000, 2000, 500, 250, 125, and 63 µm aperture size).	Any type. Method validate for PE	Material of two bigger sieves discarded Other dry at 200 mL beaker, and dried overnight at 90 °C After drying, wet digestion with 30% H ₂ O ₂ treatment catalyzed by Fe ²⁺ at 75 °C. Addition of NaCl to perform density separation and floating particles rinsed with distilled water and transferred to a glass petri dish.	Visualized under a reflected microscope	[54]
Wastewater effluents	Filtered through a set of Tyler	Sieve contents transferred	Any type	After drying, wet digestion with 30% H ₂ O ₂ treatment catalyzed by Fe ²⁺	Visual analysis using dissection microscope	[55]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
	sieves at a flow rate of 12–18 L per minute for a period of 2–24 h using a pump	into sample containers and preserved in 70% isopropyl alcohol		After processing, samples filtered using the 0.125 mm sieve and particulates transferred to glass Petri dishes using distilled water		
Influent after 19 mm coarse screening, grit and grease effluent, primary effluent, and the final effluent	30 L (3 x 10 L) passed through steel sieves (65 µm)	This debris washed into clean glass bottles using distilled water	Any type	The samples vacuum filtered through Whatman No. 1 qualitative circles, 90 mm filter paper, with a pore size of 11 µm.	The filter was observed under a dissection microscope. Confirmation by FT-IR	[56]
Influent and effluent of wastewaters	Assembled stack of sieves with mesh sizes 400 - 45 µm	---	Any type	Water samples were centrifuged, filtered and treated with hypochlorite	Visually examined using a microscope	[11]
wastewater	10 L of grab sample	----	Any type. Validated with PE, PP, PS or PVC	Filtration of the water sample using glass fiber filter. Wet digestion with 30% H ₂ O ₂	PA-FT-IR imaging	[57]
Wastewater and surface water	Treated water collected using an automatic sampler and 24-h averaged samples Surface waters (i) Plankton net (mesh size of 80mm), and (ii)	-----	Any type	Filtered on glass fibre filters (1.6 µm).	Visually examined using a stereomicroscope (16x)	[58]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
	Manta trawl (mesh size of 330mm)					
River water	Fibers: 300 mL of grab water sample MPs collected by 300 µm mesh net in water for 60 s	The net rinsed from the outside and collected in a glass container	Macroplastics All type of MPS and anthropogenic fibers	Enzymatic digestion: + SDS in an oven at 70 °C for 24 h + Protease, amylase, lipase at 40 °C for 48 h Chemical oxidation: + H ₂ O ₂ , at 40 °C for 48 h Density separation with saturated ZnCl ₂ (1.64 g mL ⁻¹) Filtered through glass fiber filters (2.7 µm porosity)	Macroplastics: FT-IR MPs and fibers: Visual examination using a stereomicroscope at 16–160X µFT-IR	[59]
River water	Manta trawl equipped with 300 µm mesh size, 60 × 20 cm opening	Samples sifted in three size (<1 mm; 1 - 5 mm; > 5 mm) and stored in glass vials with 30% H ₂ O ₂ at 4 °C	Any type of MPs	Preliminary washed and MPs manually separated from the organic matter using a stereomicroscope. Drying at 50 °C	Visual examination using a stereomicroscopy FT-IR	[60]
Lake water	Trawl net (50cmX 100 cmX 150 cm, 112 mm mesh size) towed on the surface of the lake by the side of a vessel at a speed of 5 kmh ⁻¹	The floating debris retained in the net transferred into a 1 L glass bottle, and preserved with 5% methyl aldehyde	Any type	Sample passed through a 1mm mesh size stainless steel sieve Suspected MPs were transferred to petri dishes Water passed through the sieve was added of KHCO ₂ (1.54 g mL ⁻¹) for density separation Floating particles digested using 30% H ₂ O ₂ at 60 °C overnight, then filtrated onto glass fiber paper filters (1.2 mm pore size), and dried in desiccators.	Materials retained on the sieve were visually examined. Further examination	[61]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
Surface water	25 L collected n using a pump at a depth of 1 m, and then filtered with a 48 μ m stainless steel sieve	All types	Washed into a 50 mL glass jar using pure water. Fixed with formalin at 5 %	Treated with 30% H ₂ O ₂ for 12 h Filtered through a gridded 0.45 μ m glass microfiber filter paper. Filter paper was placed in a covered glass dish and dried in the oven at 50 °C	Microscope with a digital camera μ -Raman SEM	[62]
Lake water	-45–60 manta tow net (333 μ m mesh size, 3 m length, frame opening 14 cm deep by 85 cm wide)	Sieved samples quantitatively transferred to a glass breaker, and dried at 90 °C	Any type	After drying, wet digestion with 30% H ₂ O ₂ treatment catalyzed by Fe ²⁺ at 75 °C. Then, heated for 30 min at 75 °C Density separation with 5 M NaCl (~1.15 g/mL) The remaining supernatant solution was filtered through 180 μ m Nylon filter	Microscopy, Pyr-GC-MS, FTIR	[63]
Lake and River water	20 L of water (0-20 cm depth) by a Teflon pump and passed through a 50 μ m stainless steel sieve	Residue on the sieve rinsed with water into a 50 mL glass jar and added with 5% formalin	Any type	Treated with 30% H ₂ O ₂ at room temperature under dark for 24 h Filtered through a glass microfiber filter paper with a pore size of 0.45 μ m Filter paper was placed in a clean petri dish	Inspected visually using a stereoscopic microscope 160X SEM	[64]
Lake water	Manta trawl (61 cm wide by 18 cm high) with a 3 m long, 333 μ m mesh bag and 333 μ m removable cod-end.	The collected material was then preserved in 70% ethanol until processing in the laboratory	Any type	Samples were filtered through a 250 μ m mesh brass sieve Remaining material on the sieve reconstituted to a known volume (typically 1250 mL). Each 250 mL sub-sample + 20 mL of a 0.05 M Fe ²⁺ + 20 mL of 30% H ₂ O ₂ while on a 75 °C stirring for 30 min Digested samples were once again	Inspected visually dissecting microscope0 (6.3 - 50.0x) Confirmation by SEM	[65]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
				filtered through a 250 µm brass sieve		
Lake water	Nylon plankton net (0.65 m in diameter, 1.55 m in length and 333 µm in mesh size) and grab samples	---	Any type	Samples from the plankton net and surface water filtered through a 47-mm-diameter filter of 100 µm Filters treated with 30% H ₂ O ₂ at 70 °C for 72 h The liquid in the bottles was filtered again	Microscopic observation Confirmation by µ-FT-IR and SEM/EDS	[66]
River water	Manta net (rectangular opening of 60 cm x 18 cm, 300 µm mesh)	Sample transferred to a glass breaker and stored in 10% NaCl	Any type	Samples filtered through a stacked series of metal sieves (5 mm, 1 mm and 300 µm) > 300 µm fractions enzymatically treated (SDS, lipase, protease, amylase, chitinase, cellulase) Density separation (23% NaCl; 1.16 g cm ⁻³) Samples rinsed on a 300 µm mesh	Inspected visually using a stereoscopic microscope Confirmed by FT-IR	[67]
Wastewater	Device consisted of four removable stainless steel mesh screens stacked on top of each other, with the largest mesh screen at the top cover by a PVC holder	Rinsed into a glass beaker with 100–500 mL of distilled water	Any type Validate with PS particles 250–500 µm	The water content to 100 mL by drying in an oven at 90 °C Add 30% H ₂ O ₂ solution at 60 °C 24 h Density separation NaI (1.49 g/mL) Centrifuged 15 min at 3500 rpm Buoyant particles filtered by stainless steel mesh screen with a 25 µm	A staining method was applied using Rose-Bengal solution, which stains natural and non-plastics particles Visual inspection FT-IR	[68]
Wastewater	-----	Centrifugation and analysis of the obtained pellets	Any type. Method validated with	Pellets treated with H ₂ O ₂ (30 %) with Fe ²⁺	FP µFT-IR	[69]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
			PE, PP, PVC, nylon			
Wastewater samples	25 L and 57 L and filtered using 1 mm and 12 μ m sieves	----	Any type 12 μ m and 1 mm. Method validated with PE and PP	Density separation: The filter residues transferred into an Erlenmeyer flask and filled with $ZnCl_2$ solution ($\rho = 1.85$ g/ml) The particles floating separated by a fractionating column and filtration through 12 μ m filter. Subsequently, biomass residues were oxidized using H_2O_2 (30%) overnight Filtered on cellulose-acetate filters (0.1 μ m)	TGA-DSC	[70]
Lake water	Manta Trawl (mesh size 0.3 mm, 60 $\times$ 18.5 cm) and	Sieving cascade with 1, 0.5 and 0.3 mm mesh sizes	Any type	Density separation using NaCl (1.2 g/cm ³) Supernatant pumped down through a vacuum exhaustor. Treated with HCl for 48 h at room temperature and then, at 70 °C for 1 h Filtered through a glass fiber filter	>5 mm were identified via naked eye Labeled counting grid and examined visually under UV-light Using a UV-microscope	[71]
Effluent wastewater	Two wastewater samples (25 L and 57 L)	Filtered using 1 mm and 12 μ m sieves. Residues transferred to an Erlenmeyer	Any type. Validated with PE and PP	Density separation using with $ZnCl_2$ solution ($\rho=1.85$ g/ml) Chemical oxidation with H_2O_2 extract was filtered using weighed in and rinsed cellulose-acetate filters (0.1 μ m)	TGA-DSC	[70]

Table 2

Sampling, extraction and analytical methods for characterization and/or determination of NP and MPs in sediment from 2015 to 2018

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
MPs						
River sediment (Australian temperate urban river)	Surface sediment 2 kg	Dried in an oven at 50 °C for 48–72 h	Any type, >63 µm -5000 µm	Mesh 2 mm to remove large rocks and debris Remaining sediment treated with hyper-saturated saline solution (100 g L ⁻¹), settled 24 h and filtered through 63 µm mesh	Visual enumeration using dissecting microscope at 50 X	[24]
Clay, silt and sandy oils	Not described	Air-dried and sieved at 2 mm Spiked at 0.05, 0.1, 0.2, 0.5, 1 % of MPs	LDPE < 150 µm and PP <400 µm	10 g spiked soil + distilled water after stirring left settled overnight. Floating SOM, MPs and other impurities (on the surface water) were poured off and filtered using filter paper (pore diameter < 3 µm). Repeat 4 times including ultrasound	Visual enumeration after putting the material on a glass slide using a microscope before and after heating at 130 °C for 1 min	[26]
Sludge	Sludge samples (150–200 mL) collected with a 10-L stainless steel bucket or a 0.25-L stainless steel cup from activated sludge after the aeration basin, MBR sludge and digested sludge	Poured to glass flasks with the help of a metal funnel Sludge (0.1 g dw) were poured to glass petri dishes and dried at 45 °C for 18 h	Any type	Dissolved in water Filtration over 0.7 µm glass fiber filters	Optical microscope, µFTIR µRaman	[48]
Sludge, sediment and SPM	SPM: continuous centrifugation system	Not reported	Any type	20 g sediment, sludge or SPM + distilled water+ NaCl to saturation (1.2 g/mL) Filtration over 0.7 µm glass fiber paper filters	Light microscopy to count particles and record shape After filtration through Al ₂ O ₃ FTIR	[52]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
	Sediment: Van Veen grab sample					
Sewage sludge	Pellets obtained from WWTP	Pellets of TD sludge placed in water for 1 week transferred to a water bath (30 °C) for 24 h, and placed in an “end-over-end” shaker	Any type Validated for HDPE, PVC	Elutriation using a solution of ZnCl ₂ Filtered onto glass fiber filter (1.2 µm)	Examined using stereomicroscopy Confirmed by FT-IR SEM	[72]
Sediment	1 L of sediment collected with a Van Veen grab (0.25 m ² sampling surface),	Placed in a glass jar	Any type	Two-step density separation method: 500 g sediment + 1 L of saturated NaCl Pass through 48 µm stain steel sieve and MPs intercepted washed into a beaker Then, used 60% NaI solution to extract high density MPs Finally treated with H ₂ O ₂	Microscope with a digital camera µ-Raman spectroscopy SEM	[62]
River sediments	0.5 x 0.5 m quadrant of the first 5 cm	Moved to clean tin cups and dry in an oven at the laboratory	Any type, 7 identified PP, PS, Rayon, PVS	Concentrated NaCl (1.2 g mL ⁻¹) solution added and, after it settled for 24 h, the supernatant was vacuum-filtered. Filtrate through glass fiber filter paper Filter paper with MPs dried to constant weight.	Microscopic analysis µ-FT-IR	[73]
River sediments	Samples taken from 0.5 to 1.5 m using transect with a gravity corer	-----	Any type, 500 µm ⁻¹ mm size PS, PE, PP	Size- and density separation using distilled water and a sieve	Binocular microscope (x40) FT-IR Raman	[74]
River sediments	Depth of 2 cm using a stainless-steel shovel and a 20 × 20	Transferred into an aluminum and dried at 50	Any type	30 g dried sediments + 200 mL of saturated NaCl Cleaned in an ultrasonic bath for 5 min	Microscopic imaging µ-FTIR microscopic imaging Study on metal accumulation:	[75]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
	cm wooden frame with a 2-cm height	°C for at least 48 h		Filtrated through a glass microfiber filter with 1 µm pore size Dried at 50 °C for 48 h and stored in Petri dishes	SEM/EDS ICP-MS	
Lake sediments	Six 20 cm × 20 cm quadrats sediments collected from the top 2 cm	Preserved in plastic bags and dried	Any type	Sieved with 1 mm mesh size Sieved material separated by density with KCO ₂ H (1.5 g/cm ³) Filtered through glass fiber filter 1.2 µm Filters transferred to petri dishes, oven-dried at 60 °C for 30 min to 1 h	>1 mm examined with naked eyes <1 mm examined under a stereomicroscope. Confirmed by Raman spectroscopy	[76]
Lake sediment	Collected 3 times using a Peterson sampler and pooled	Kept in an aluminum foil bag	Any type	Density separation using saturated NaCl Filtration through 5 µm pore filter Filters treated with 30% H ₂ O ₂ at 70 °C for 72 h The liquid in the bottles was filtered again	Microscopic observation Confirmation by µ-FT-IR and SEM/EDS	[66]
Lake sediments	An area of 0.25 m ² delimited using a stainless steel frame and the top 3 cm collected.	After sieved preserved in glass jars	Any type	Sieved with 5 mm mesh sized Weighed, dried at 40 °C and reweighed to determine the water content and dry weight. Density separation using NaCl (1.2 g/cm ³) Supernatant pumped down through a vacuum exhauster Treated with HCl for 48 h at room temperature and then, at 70 °C for 1 h Filtered through a glass fiber filter	>5 mm were identified via naked eye Labeled counting grid and examined visually under UV-light Using a UV-microscope	[71]
River and river shore sediments	Stainless steel spoon in an area of 30 cm ² to a depth of 2–3 cm, resulting in one bulk sample of 3–4 kg.	Stones and objects >10 mm removed before each sample was taken. Sediment dried	All types > 63 µm	Density separation using NaCl (1.2 g/cm ³) Floating particles were transferred through the glass hose of the filter flasks to glass fiber filter Treated with a mixture of H ₂ O ₂ (30%) and H ₂ SO ₄ . Filter through a glass filter fiber	>630 µm particles were counted with the naked eye 63–200 µm particles and 200–630 µm particles were visually identified and counted using binocular microscope 40–100× Confirmed by FT-IR	[77]

Type of sample	Sampling procedure	Pre-treatment	Plastic	Extraction	Characterization	Ref.
		at the laboratory				
Soil and compose wastes	-----	----	HDPE, PS, PVC, PE, PET	PLE –pre-extraction at 100 °C for 5 min, 3 cycles, 45 mL, 1500 psi Extraction with DCM at 180 °C for 9 min, 2 cycles 80 mL, 1500 psi	FT-IR	[78]
Soil	-----	-----	LDPE, PP	Soil (10 g) + distilled water Floating SOM, MPs and other impurities were poured off and filtered using filter paper (pore diameter 3 µm)	Observation under the microscope (6.4 X) Glass slide was then heated in an oven for 3–5 s at 130 °C Plastics were transformed	[26]

Table 3

Selected sampling, extraction and analytical methods for characterization and/or determination of NPs and MPs in biota from 2015 to 2018

Type of Sample	Sampling	Pre-treatment	Plastic	Extraction	Characterization	Ref.
MPs						
Gudeon (<i>Gobio gobio</i>)	Electrofishing	Digestive tract was removed and stored in 70% ethanol and dissected.	All types	Isolation of the digestive tract content	Direct visual inspection of the digestive content under a dissecting microscope	[79]
Chironomid larvae	Hand-held nylon net (mesh size 500 µm, dimension 30 × 30 cm)	fixed using 70% ethanol	All types	HNO ₃ 55 % (3 mL) × 6 h, then, 1 h at 100 °C and filtration through 2 µm Millipore membrane filter	Light microscope at × 100 magnification	[24]
Clams	A bottom fauna trawl was used to collect clams	Grind at the laboratory	Any type	Clams treated with 30% H ₂ O ₂ at 70 °C for 72 h The liquid in the bottles was filtered again	Microscopic observation Confirmation by µ-FT-IR and SEM/EDS	[66]
European flounder (<i>Platichthys flesus</i>) European smelt (<i>Osmerus eperlanus</i>)	Fyke nets	Dissection	All types, specially fibers	Guts section was cut longitudinally and opened Digestive tract was opened in small sections. Using pins, a thorough search was then undertaken of the opened gut section.	Visual inspection Dissection microscope FT-IR	[80]
<i>Hoplosternum littorale</i>	From the fisherman of the area	Injected the abdomen with 4 % formalin. The whole fish immersed in formalin for fixation	All types	The digestive tract of each animal was opened with a scalpel, its content washed with distilled water through a 63 µm mesh sieve, and the gut weight measured for each fish. Transferred all the gut content to Petri Dishes.	Visual identification carried out in a petri dish covered with watch glass to avoid airborne contamination After drying, MP counting and identification in a dissecting microscope (45X).	[39]

Type of Sample	Sampling	Pre-treatment	Plastic	Extraction	Characterization	Ref.
				Plastic fragments were dried in oven at 70 °C for 24h		
bluegill (<i>Lepomis macrochirus</i>), longear (<i>Lepomis megalotis</i>), sunfish (Centrarchidae)	Hook-and-line and cast nets	Isolation of the stomach content	All types ranging from 50 to 5000 µm in size	Stomach contents washed with distilled deionized water through four filters, 1000 µm, 243 µm, 118 µm, and 53 µm	Resultant stomach contents examined using a stereomicroscope with 10× oculars	[81]
Goby (Neogobius melanostomus), common barbel (Barbus barbus)	Routine survey	Dissection of the gut	All types. Tested with: HDPE, LDPE, PP, PS, EPS, PET, PVC	NaOH at 50 °C for 15 min H ₂ O + HNO ₃ at 50 °C for 15 min plus at 80 ° for 15 min Filtration 0.45 µm filter Clean-up for saponins: digestion with NaOH at 50 °C and filtration. Clean-up for mineral loads: density separation with NaI and filtration	Dissecting microscope Suspicious particles and fibers verified using a hot needle point Number of MP particles per fish (intensity) Maximum length of each particle or fiber measured.	[82]
Rutilus rutilus (roach)	Electrofishing	Dissection of the entire digestive tract		Contents were spread on a glass microfibre filter paper (47 mm diameter, 1.2 µm mesh). MPs isolated with a forceps for characterization	Binocular microscope 6x Raman spectroscopy	[83]
Cyprinid (<i>Gymnocypris przewalskii</i>)	Fishermen	Dissection of the digestive tract	All types	Digested using a 10% potassium hydroxide solution. The digested solutions were filtrated onto glass filters and stored in Petri Dishes	Stereomicroscope up to 40 × Raman spectroscopy (of the MP separated with a tweezer)	[61]
Frehwater fish and birds	Not specified	Dissection of the digestive tract and storage in ethanol 70 %	All types	Contents were spread on a glass microfibre filter paper (47 mm diameter, 1.2 µm mesh).	Stereomicroscope up to 40 x Chemical analyses of potential contaminants binding to MPs	[84]
NPs						
Not specified	Not specified	Frozen fish homogenate	100 nm PSNPs PE	Enzymatic digestion with protein kinase K	Optical microscopy examination DLS, SEM	[85]

Type of Sample	Sampling	Pre-treatment	Plastic	Extraction	Characterization	Ref.
		mixed with water		Acid digestion HNO ₃	AF4-MALS (LODs 52 µg/g fish)	
<i>Danio rerio</i>	-----	-----	Fluorescent PSNPs	-----	Zebrafish were imaged using an Axioskop and fluorescence was captured with a camera	[86]
						[87]
<i>Chlamydomonas reinhardtii</i> , water flea <i>Daphnia magna</i> , secondary-consumer fish <i>Oryzias latipes</i> , and end-consumer fish <i>Zacco temminckii</i>	Laboratory study	-----	Fluorescent PSNPs	-----	Optical microscopy with fluorescence filter CLSM Bio-TEM	[88]
Crustacean <i>Daphnia galeata</i>	Laboratory test	-----	Fluorescent PSNPs 20 to 100 nm	-----	Optical microscopy with fluorescence filter	[89]
<i>Daphnia magna</i>	Laboratory test	-----	Fluorescent PSNPs	-----	Optical microscopy with fluorescence filter Confocal microscopy TEM	[90]

Table 4. Different strategies develop to control any type of plastic contamination

Strategy	Action	Ref.
Legislation	United States Microbead-Free Waters Act was signed into law in December 2015.	[3]
	EU chemical Regulation, the Packaging and Packaging Waste Directive including the amending Directive regarding regulation of the consumption of lightweight plastic carrier bags	[96]
	Directive on the Landfill of Waste	[96]
	Ban or imposed fees on single-use plastics items	[96]
Public enrolment	Educate the public about plastic microbeads	[3, 96]
Engineering tools (Advances treatments)	MBR treating primary effluent and different tertiary treatment technologies (discfilter, rapid sand filtration and dissolved air flotation) treating secondary effluent.	[97]
	Mechanical, chemical and biological treatment (activated sludge) and biologically active filter (BAF)	[98]
	Comparison of MBR and CAS	[48]
Biotechnological tools	Production of biodegradable plastics biopolymer polylactic,acid (PLA), polyhydroxyalkanoates and polylactide	[99]
	Use of organisms capable of degrading plastics. Enzymes degrading plastics (polyethylene)	[100, 101]
	Use of organisms capable of degrading plastics. Bacterium that degrades and assimilates poly(ethylene terephthalate)	[102]
	Use of organisms capable of degrading plastics. Marine fungus Zalerion maritimum degrades polyethylene	[103]

FIGURE CAPTIONS

Fig. 1. Literature on MPs and NPs in the environment (source SCOPUS) since year 2006, analyzed by year.

Fig. 2. Conceptual map of the source, transport and fate of MPs and NPs

Fig 3. Possible strategies described in literature for the analysis of MPs in sediment and water samples starting with the sampling to the report of the results. The sample preparation is split in pretreatment, the density separation, and the posttreatment of the separated MPs. Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy energy-dispersive X-ray spectroscopy (SEM-EDS), pyrolysis- or thermal desorption-gas chromatography/mass spectrometry (Pyr-GC-MS, TDS-GC-MS) are deployed for the analysis.

Fig. 4. (A) MPs recovered in samples in the 1) 63e125 mm size category, 2) 125e250 mm size category, 3) 250e500 mm size category, and 4) 500e2000 mm size category. (B) MPs recovered from personal care products in the 1) 63e125 mm size category, 2) 125e250 mm size category, 3) 250e500 mm size category, and 4) 500e2000 mm size category. Examples of different particles classifications are labeled i) primary microplastic, ii) secondary microplastic, and iii) non-microplastic particles excluded during the counting step [54].

Fig. 5. Confocal laser scanning microscope (CLSM) images of *Daphnia magna* that were not exposed to nano-sized polystyrene (nPS; green emissions) (a) and that were exposed through the diet (b,c). Z-stack image of individual exposed through the diet (b), observed using CLSM (d). Z-stack image (d) provides evidence of nPS uptake through dietary exposure of *D. magna*. Green fluorescent nPS was observed in the gut of exposed *D. magna*. Scale bar = 200 μm . [88].

Fig. 1.

Figure 2.

Fig. 3

Fig. 4

Fig. 5