A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms

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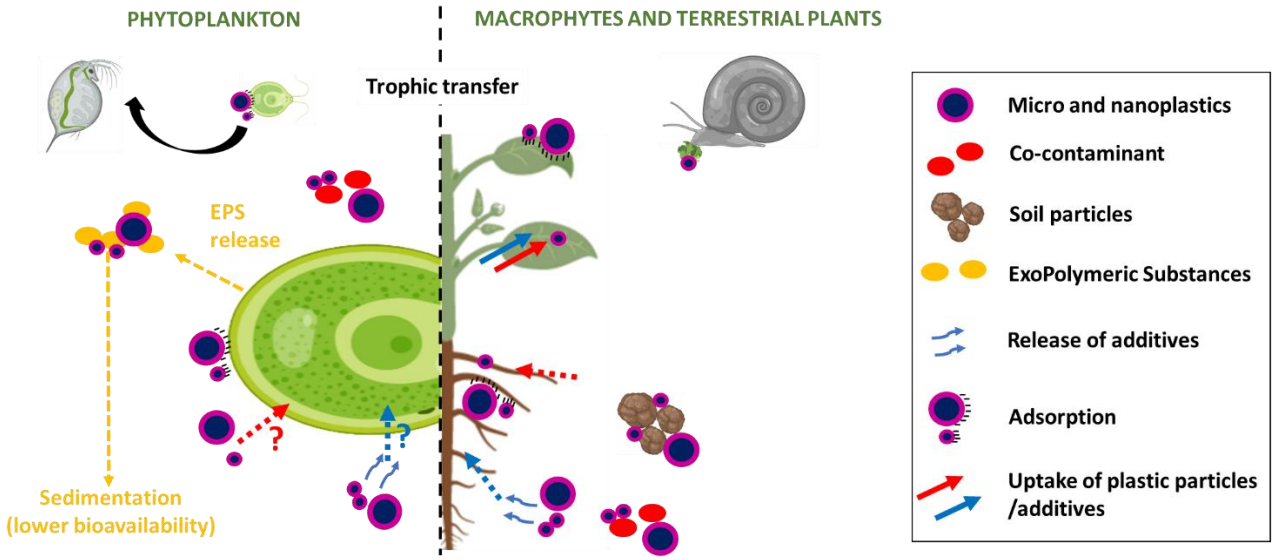
Abstract

Microplastic and nanoplastic contamination is widespread and affects aquatic and terrestrial ecosystems. Photosynthetic organisms are present in both media, they are primary producers, sink for CO_2, and they represent a major point of entry in the food chain. Here, the current knowledge on the fate and impacts of microplastics and nanoplastics in interaction with these organisms has been reviewed. As a general trend, plastic characteristics (smaller size and positive charge) play a crucial role in their toxicity towards photosynthetic organisms. Plastic leachates (containing additives) represent also a major source of toxicity, and some harmful compounds such as phthalate esters have been shown to accumulate in plants and generate a risk for the consumers. Adsorption of plastic particles has been evidenced for each type of photosynthetic organism, and uptake and translocation in terrestrial plants was evidenced for nanoplastics, leading to concerns for trophic chain contamination. The available techniques for the detection of microplastics and nanoplastics and their secondary products in biological samples and media are also listed. Finally, the current gaps of knowledge, specific challenges, and future research directions were also discussed.

Keywords: macrophyte, phytoplankton, plant, plastic

TOC. This critical review synthesizes the knowledge acquired on photosynthetic organisms both in aquatic and terrestrial compartments as well as the analytical techniques required for these studies.



1. Introduction

The word “plastics” gathers a wide range of synthetic polymer materials made of long organic chains deriving mainly from fossil fuel. Plastic production also implies the incorporation of additives for the processing or to improve plastic properties according to their future use; among them are catalysts, polymerization solvents, a wide range of organic and inorganic compounds, antioxidants or antimicrobials. Additives can represent up to 50% of the final material in some cases[1].

Since their discovery at the end of the 19th century, their use in our everyday life has rapidly and exponentially increased. Indeed, the annual production went from 1.5 million tons in the 50’s to close to 335 million tons in 2016[2]. They are used in a wide variety of sectors such as packaging, building, transportation, renewable energy, medical devices or sport equipment[2]. The most commonly encountered plastics in commercial products are thermoplastics and, among those, polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS) and polyethylene terephthalate (PET). These represent approximately 90% of world production[3].

Unfortunately, our waste management systems are not efficiently handling plastic waste; every year only 6% at the world level is recycled and about 28% is directly disseminated in the environment, the rest being incinerated or placed in landfills[2,4]. Since plastics are barely degradable, they are persistent[5] and are found in all environmental compartments, from mountains[6] to deep sea and even in remote locations far away from any anthropogenic activities such as in the Antarctic and Artic oceans (reviewed in [4,7]). Plastic waste has been first identified and studied in oceans (mainly in ocean gyres), however some analyses revealed a similar level of contamination in continental freshwaters[8] and in soils[9]. These plastics mainly originate from land sources (80%) which can be as diverse as mulching in agriculture, spreading of sewage sludge or leaks from discharge sites, and transit through rivers to finally reach oceans in which they represent from 60 to 80% of the marine debris and about 10 million tons[4,10]. Among the most widespread plastics found in the environment are PE (about 90%), PP and PS and mostly encountered in the form of fibers[11].

In the environment, plastics undergo modifications by chemical, physical and photo-degradations leading to changes in their physico-chemical properties (and possibly toxicity). For instance, one of the most common consequences of environmental weathering is the

appearance of carbonyl functional groups leading to an overall negative surface charge of the particles[12]. Weathering also has consequences on particle size distribution. Indeed, in the environment several classes of plastics have been defined: macroplastics (<1 m), mesoplastics (2.5 cm - 5 mm), microplastics (1 µm - 5 mm) and nanoplastics (<1 µm)[13]. The most commonly sampled size fraction in the environment is centered around 1.5 mm[11]. The persistence of plastics in the environment can also lead to the adsorption of diverse biological and chemical components such as heavy metals or organic compounds (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pharmaceuticals or pesticides among others)[14,15]. These adsorption processes have been shown to be enhanced on weathered plastics in comparison to pristine ones[16]. Finally, the weathering process can also lead to the release of additives incorporated in plastics during the processing since most of them are not covalently bound to the polymer matrix and can migrate towards the surface[17].

Quantifying plastics in the environment is challenging; adequate analytical techniques are being developed. Consequently, quantitative data is still scarce. Furthermore, the comparison between concentrations reported in different sites is most of the time difficult since they are presented in different units: items or mass per volume, per mass or per surface. Concentrations in soils were found ranging from 55.5 to 67500 mg/kg over different sites (reviewed in [18]). In freshwater aquatic systems, plastic concentrations also greatly vary between in average 4 items/L to as high as 25.8 items/L in Taihu lake in China[19] or 419 items/L in Saigon river (Vietnam)[20]. Few data expressed in mass/L are also available reporting concentrations from 0 to 700 µg/L in the Danube River[21] or up to 1.56 mg/L in an urban lake in Texas[22]. Overall, in seawater, plastic concentrations are about 3 orders of magnitude below those found in freshwaters with average concentrations below 0.01 item/L[20] but could reach 1770 items/L in most polluted area such as Southern North Sea[23].

The number of publications about the impact of micro and nanoplastics in the environment is rapidly increasing and good reviews have been published already on freshwater ecosystems[4,20,24,25], marine ecosystems[20,25–28], terrestrial ecosystems[18,29,30], agroecosystems[9] and on analytical methods[31].

In this review, we focus on photosynthetic organisms. These organisms are of tremendous importance for ecosystem functioning since they are at the base of the trophic chains as primary producers. Here, we summarize data obtained both on aquatic (freshwater and marine) and terrestrial photosynthetic organisms to try to identify general trends. Indeed, despite the fact that these organisms thrive in different environments, they share common characteristics which

makes the comparison relevant. For instance, they have the same autotrophic functioning unlike animals. They also possess thick cell walls which limit uptake processes. We thus detail results of micro and nanoplastic (including their leachates) uptake and toxicity to phytoplankton, macrophytes and terrestrial plants. Finally, we also develop on analytical techniques available to characterize micro and nanoplastics in such complex matrices.

2. Phytoplankton

If this group represents only about 1% of the photosynthetically active biomass of the world, it is responsible for about 45% of the world primary production, by fixing half of the atmospheric CO_2 and about a third of "anthropogenic" carbon over the last century[32].

Among the published papers on phytoplankton (Table 1), it is interesting to note that most of them focused on PS or PE (>75%) and green algae and diatoms (>75%). Microplastics are a bit more studied than nanoplastics (60% *vs.* 40%) and marine environments more than freshwater systems (60 *vs.* 40%). The parameters most commonly investigated were growth inhibition, photosynthetic activity and cell morphology.

2.1. Influence of plastic particle size

Overall, most of the studies investigated the impact of nanoplastics on phytoplankton and among them nearly 90% suggest that they have toxic effects[33–46]. For particles having size between 1 and 10 µm, 90% of the concerned studies show no toxicity[43,47–54]. Finally, for bigger particles mostly no impact was reported or even in some cases an enhanced algal growth[55,56].

According to these findings, some authors suggested to introduce a threshold size for plastic ecological risk assessment and to consider particles above 100 µm as low risk for microalgae[34].

Some studies evidenced a size dependent toxicity of plastic particles to phytoplankton[43,44,47,56,57]. For instance, Li *et al.*, exposed the microalga *Chlorella pyrenoidosa* (green alga) to PS particles and found EC_{50} of 6.9, 7.2 and > 64 mg/L for 0.1, 0.55 and 5 µm particles respectively[47]. The same result was evidenced with the green alga *Dunaliella tertiolecta* (0.05 µm PS particles were more toxic than 0.5 µm and 6 µm)[43]. This size dependent effect was also observed for PVC particles and diatoms[44,57]. However, few other studies show

similar EC_{50} values for three green algae (Chlorella, *Raphidocelis subcapitata* and *Scenedesmus obliquus)* exposed to PS particles ranging from 55 nm to 2 µm[35,40,58].

EC_{50} reported for phytoplankton vary between 0.5 to 13 mg/L for PS nanoparticles[33,40,47]. The highest reported plastic concentration in the aquatic environment so far is 1.56 mg/L[22] which suggests that plastic pollution represents a risk for phytoplankton populations in these highly contaminated area.

2.2. Influence of plastic particle surface charge

According to the literature, positively charged particles appear more toxic to phytoplankton than negatively charged particles[33,39,58–60]. Positively charged particles are usually more stable in the medium[33], and are attracted by phytoplankton cell walls through electrostatic interactions, since cell wall components such as cellulose bear negative charges[39,59]. Negatively charged particles tend to form large agglomerates and to interact less with phytoplankton. For instance, positively charged PS nanoparticles (50 nm) were characterized in natural seawater with a hydrodynamic diameter <200 nm and led to a decreased growth of *D. tertiolecta* with an EC_{50} of 13 mg/L. On the opposite, negatively charged PS nanoparticles (40 nm) agglomerated in seawater (> 1 µm) and no toxicity symptom was detected up to 50 mg/L[33]. Likewise, uncharged particles tended to be more toxic than negatively charged ones with a 45% decrease in growth of the same green alga at 250 mg/L while no toxic effect was detected with the negatively charged PS particles[43].

Over the different papers using functionalized particles, only one (over 8) reported toxicity of PS-COOH particles (70 nm) towards *S. obliquus*, however this toxicity was only visible at the highest exposure concentration: 1.1 g/L which is extremely high[38]. And the only study reporting no toxicity of PS-NH_2 particles to *Chaetoceros neogracile* (diatom) mentioned the fact that these particles once in the exposure medium had negative charges[53].

2.3. Influence of plastic particle chemical composition

Very few articles compared the impact of plastics of different chemical nature. Some of them showed no toxicity, possibly related with the fact that they used particles with size > 100 µm[34,61]. The most comprehensive study was performed by Zhu *et al*. and suggested that at equivalent diameter (74 µm) PVC could be more toxic than PS and PE with respective algal growth reduction of 29.0%, 24.7%, and 25.3% after 96h of exposure at 100 mg/L[57]. Indeed, in the literature, PVC has been shown to be toxic in all the studies in which it has been used[36,62] except when its size exceeded 100 µm[34]. In contrast, the few studies done on PE and PP microplastics showed no toxicity[34,49,50,54–56,61,63]. Following a species specific distribution approach, some authors suggested that polymethylmethacrylate (PMMA) was less harmful than PS nanoparticles for marine biota[45].

It seems that this difference in toxicity could be related to plastic leachate composition[64,65]. For instance, Capolupo *et al*. generated plastic leachates (PP, PET, PS, PVC, 1000 µm)[64] and detected plasticizers, antioxidants, antimicrobials, lubricants, vulcanizers (between ng to hundreds of µg/L) with highest amount of additives in the PVC leachates and the least in PE leachates. Leachate composition varied according to the solution used (freshwater *vs*. seawater). This was also confirmed in another study showing the importance for leachate composition of solution pH and leaching time[66,67]. All plastic leachates, except the one derived from PET, inhibited algal growth with EC_{50} of 1.62%, 23.5% and 64% (of the initial leachate) for PVC, PS and PP, respectively.

2.4. Influence of phytoplankton type and exposure conditions

Several studies suggested a different sensitivity to plastics according to phytoplankton species[43,63,64,68]. For instance, EC_{50} have been calculated for 3 marine microalgae exposed to PMMA nanoparticles showing that the diatom species was the most sensitive (83.4 mg/L) and green alga and haptophyte had similar sensitivity (132.5 and 123.8 mg/L, respectively). Sometimes this difference was related to exopolymeric substance (EPS) production[51,68–70].

In addition, the phytoplankton growth phase (lag, logarithmic, stationary or death phase) used for the exposure experiments can also affect the results. Some studies (less than 15%) compared the sensitivity of phytoplankton populations when in stationary phase rather than in logarithmic growth showing a lower sensitivity[34,35,41,51,70]. For instance, PS nanoparticles (50 nm) decreased growth, chlorophyll concentration and increased reactive oxygen species (ROS) production of the diatom *C. neogracile* in logarithmic growth while the effect were mostly non detectable in

stationary phase[41]. Authors suggested that this difference can be related with the higher EPS production by diatoms when in stationary phase that would lead to nanoplastic hetero-aggregation and sedimentation decreasing plastic bioavailability in the water column. Indeed, EPS secretion has been highlighted in several studies as a way to mitigate plastic toxicity[35,37,61,70] and can vary according to growth phase, algal species and the type of plastic considered[61,69,71].

During exposure, the medium composition (ions, salinity, hardness, pH, organic matter) will also influence plastic behavior through the formation of a corona around the particles[37,59] and thus influence plastic interactions with other components of the system[53]. For instance, the addition of humic acid (5 mg/L) in the exposure medium alleviated the toxicity of PS nanoplastics (75 mg/L) to *S. obliquus* by decreasing the adsorption of PS particles to alga surface[58].

2.5. Cocktail effects in presence of other contaminants

Some authors have investigated the toxicity of plastics together with other contaminants such as heavy metals (Cu[37,54,62], Ti[60], Au[48], Pb[72]) or organic compounds (dibutyl phtalate[47], chlorpyrifos[50], glyphosate[73], phenanthrene[34], triclosan[57], procainamide and doxycycline[52]). Most of the time antagonistic effects were identified. Indeed, the other contaminant studied was found adsorbed at the plastic surface and thus became less bioavailable to the exposed organisms[34,47,50,57,72,73]. Those interactions are controlled by plastic surface charge. For instance, adding positively charged PS particles (6 µm) to TiO_2 nanoparticles enhanced their toxicity (+20%) to *Chlorella vulgaris* (green alga) while adding negatively charged PS particles led to a decrease of about 20% in toxicity[60]. However, some studies reported no influence of the addition of plastics on the toxicity of co-contaminants (Cu pollution)[37,54]. Finally, in few cases, the mixture appeared to be more toxic than the contaminants considered alone (mixture with Au nanoparticles[48] or with pharmaceuticals[52]) but the lack of experimental details (plastic size or type) does not allow to go further in the explanation.

2.6. Pristine model versus environmentally aged plastic particles

So far studies investigating plastic toxicity to phytoplankton have mostly focused on pristine particles, predominantly PS spheres. Actual plastic debris found in the environment have a large variety of shapes (from round particles to fibers, mainly), size distribution and surface properties. In addition, these properties are supposed to evolve with the aging, which will likely result in different toxicities. So far, only one study comparing the impact of pristine plastic particles and "environmental" plastics has been published. It demonstrated that the microalga *S. subspicatus* was more sensitive to PE plastic coming from the North Atlantic gyre than to PE plastic made from pristine reference material[63]. The aged plastic contained higher concentrations of metals (Ti, Al, Fe and Zn). Likewise, *C. vulgaris* growth was less impacted by pristine PVC particles than by aged (2 months) PVC ones (35% growth inhibition *vs*. 28% for pristine particles)[62]. FTIR showed that aged material suffered modifications of functional groups: increased in hydroxyl groups and aromatic carbon-carbon double bond but decreased carbon hydrogen bond. In another study, authors performed an accelerated aging step on PE particles by exposing them in a xenon chamber to high temperature, humidity and light irradiance conditions for up to 4 weeks[67]. The aging step caused surface cracks and fragmentation of the particles, increased their surface area and carbonyl contents, and promoted the release of pigments. The 4-week aged particle leachates were more toxic to cyanobacteria than the 2-week aged particle leachate and the non-aged particle leachate.

2.7. Toxicity mechanisms

Among the most discussed toxicity mechanisms in the literature is the particle adsorption on the surface of the organisms leading to a physical blockage of light, gases and nutrients decreasing growth and photosynthetic activity[33,35,36,39,44,60,74]. As for other carbon based nanomaterials, the involvement of shading effect has also been reported[44]. Beside these physical effects, some studies evidenced effects related to the chemistry of particles, inducing oxidative stress[42,60,62,75] and structural damages[35,37,42,46,47,75]. A recent study suggests that for the smallest particles (nanoplastics) the "chemical" effect predominates (increased ROS production, decreased photosynthesis) while for bigger particles (microplastics) toxicity is mainly driven by "physical" effects (limitation of exchanges with the medium)[58]. Additionally, the additives present in plastics, which can be released during aging, as well as the secondary metabolites after aging of these additives, can be responsible for the toxicity[64,76].

Very recently, few articles investigating molecular processes modified after plastic exposure were released[75,77,78]. Overall they confirmed the impact of plastic particles (in these cases PS) on genes involved in photosynthesis (downregulation)[78] and in oxidative stress regulation (upregulation)[75]. One of the known consequences of oxidative stress is the impairment of microtubule polymerization. Ripken *et al*., observed a massive upregulation in genes involved dynein-related proteins, dynein being a microtubule-associated motor protein implied in cytoskeletal dynamics suggesting that the organism was facing oxidative stress[78]. New findings were also suggested such as the impact on fatty acid synthesis. For instance, KCS gene expression was upregulated in *Euglena gracilis* possibly leading to higher extracellular secretion and hetero-aggregation phenomenon between algal cells and plastic particles[75]. Likewise, in *Cladocopium*, after PS exposure 4 genes encoding proteins found in cell surface receptors were up-regulated. These genes control cell dissociation and thus possibly influence adhesion between neighboring cells and extracellular matrix composition and could explain changes in cell aggregation[78]. Cell division was also impacted with a decrease in the expression of CTR1 gene related to the mitogen-activated protein kinases (MAPK) signaling pathway in *E. gracilis*[75] and a decrease of 6 genes with RCC1 (regulator of chromosome condensation) and 3 genes with CDC domains in *Cladocopium*[78]. Finally, gene expression was altered to different extent between 2 microalgae confirming different sensitivities according to species[78].

2.8. Accumulation and trophic transfer

No investigation has been done so far on the potential internalization of plastic particles in phytoplankton because of the lack of adapted techniques.

However, positively charged particles tend to adsorb on phytoplankton surface. As these organisms represent the first level of trophic chains, it suggests that adsorbed plastic can be transferred to the next trophic level. This has been confirmed by few studies showing the transfer of particles from *Chlamydomonas reinhardtii (green alga)* to the crustacean *Daphnia magna* and to the fish *Orizias sinensis* and *Zacco temminckii*[74]. A higher toxicity of nanoPS to *D. magna* (6 times higher mortality) has also been evidenced when these organisms were exposed through their diet (*S. obliquus* exposed to nanoPS) than directly in the water column[38].

2.9. Plastic particle degradation by phytoplankton

If plastic can be toxic to phytoplankton, on the opposite some articles report plastics as a new opportunity for organisms as vectors for long scale traveling or as a new carbon source available for development. Analyses performed on plastic debris from the open sea have highlighted the preferential development of microbial communities on their surface, called the plastisphere[79–83].

Since the discovery in 2016, that some bacteria (in particular *Ideonella sakaiensis*) are able to hydrolyze PET thanks to a specific enzyme called PETase, a lot of efforts has been put to take advantage of micro-organisms for plastic biodegradation. Microalgae seem to be a good candidate for this task through two main pathways: (i) the natural release of toxins and ligninolytic and exopolysaccharide enzymes and (ii) the synthetic production of PETase[84]. Indeed, cyanobacteria such as Phormidium, when growing on a low density PE film, were able to use about 4% of its carbon for their growth leading to the biodegradation of the film[77]. Likewise, *Anabaena spiroides* (cyanobacteria), *Navicula pupula* (diatoms) and *Scenedesmus dimorphus* (green algae) were able to biodegrade LDPE in their respective growth media to different extent: 8.18%, 4.44% and 3.74%, respectively[85]. However, as highlighted by recent reviews[82,83], biodegradation rates by microorganisms in real conditions are probably much lower than in laboratory conditions, and the nature and potential toxicity of the degradation products are still unknown.

On the other hand, some author used genetically modified microalgae to produce PETase. For instance, a lysate of modified *C. reinhardtii* incubating at 30°C for 4 weeks led to the apparition of dents and holes on a PET film and the detection of terephtalic acid (TPA) which is the fully degraded form of PET[86]. The same was also achieved by using *Phaeodactylum tricornutum* (diatoms) cells in growing medium under more environmentally relevant conditions[87]. Moreover, it has been shown thanks to a trophic chain study (bacteria, microalga, zooplankton) combined with the use of ^{13}C labeled PE microparticles that the plastic could be partly mineralized, used for cell growth and ^{13}C was found in cell membranes of upper trophic organisms[88].

3. Macrophytes

Macrophytes may be exposed to micro and nanoplastics *via* the aquatic medium for the shoots, and *via* the sediment for their root system. In this review, we have also included dead biomass of macrophytes, which plays an important role in the aquatic communities and aquatic food webs (Table 1). Only global endpoints such as biomass and pigments have been investigated so far, on a limited number of organisms, and for a limited type of plastic particles.

3.1. Plastic particle toxicity

Kalcikova *et al*. evaluated the effect of PE microplastics from cosmetic products on duckweed (*Lemna minor*)[89]. Specific leaf growth rate and concentration of photosynthetic pigments in duckweed leaves were not affected, whereas root growth was significantly decreased with 50 mg/L PE microbeads; the authors suggested that this could be due to mechanical blocking. Surface properties seemed to influence microplastic toxicity. Indeed, sharp particles reduced the viability of root cells, while the impact of microplastics with a smooth surface was negligeable. The same group showed that microplastics incubated in wastewater at different organic loads had different toxicity to *L. minor* [90]. Van Weert *et al*. evaluated the impacts of PS nanoplastics (50-190 nm) and microplastics (20-500 µm) on the macrophytes *Myriophyllum spicatum* and *Elodea* sp., after exposure to plastic amended sediment[91]. Microplastics did not produce consistent dose-effect relationships, and nanoplastics significantly induced an increased root biomass compared to shoot biomass of *M. spicatum*. For *Elodea* sp., the biomass and growth parameters tested were positively correlated to nanoplastic concentration.

3.2. Accumulation and trophic transfer

Published data concerns microplastics only. Some lab-based experiments investigated the sorption of microplastics on macrophytes. Sundbaek *et al*. evaluated the adsorption of fluorescent PS microplastics of 20 µm diameter on the surface of the macroalga *Fucus vesiculosus*[92]. After 2h in filtrated seawater containing 2.65 mg/L microplastics (corresponding to 597 items/mL), the algal surface contained on average 3.99 items/mm^2 (corresponding to 2787 items/g dry weight DW), but only 0.22 item/mm^2 after washing in filtrated seawater. Gutow *et al*. did the same kind of experiment at lower concentrations of PS (1.4 to 55.7 items of 10 µm diameter per mL), and also observed a sorption to the surface of *F. vesiculosus*, with

an average of 0.04 item/mm^2 after washing for the highest concentration[93]. Similarly, Mateos-Cardenas *et al*. showed that 10–45 µm PE microplastics can strongly adsorb to all surfaces of *L. minor*, although photosynthetic efficiency and plant growth were not affected by microplastics[94]. To our knowledge, there is no data on the transfer of micro or nanoplastics from the surface of macrophytes to the inside of plant tissues. Likewise, the possible uptake of plastic particles from the sediment to the root system of macrophytes is not yet documented.

Beside these laboratory studies, the presence of microplastics on macrophytes collected in natural environment was investigated. Goss *et al*. showed that 75% of marine turtle seegrass (*Thalassia testudinum*) collected from an area impacted by urban discharges had encrusted microplastics[95]. Microfibers, beads, and other microplastic fragments were found adhered to seagrass blade surfaces, often overgrown by periphyton and various epibiont organisms. This suggests that these macrophytes may represent a pathway for plastics into marine food web. Saley *et al*. found 8.65 ± 6.44 and 2.34 ± 2.19 items/g on the surface of the marine macrophytes *Endocladia muricata* and *Pelvetiopsis limitata* growing in a remote marine reserve on the open coast of California[96]. Densities were higher in the herbivorous snail, *Tegula funebralis*, at 9.91 ± 6.31 items/g, potentially due to bioaccumulation[96].

Posidonia oceanica is a macrophyte endemic of the Mediterranean sea, considered a key species because it serves as habitat and food source for many marine organisms. Egagropiles, which are spheroids made of dead biomass of *P. oceanica*, ubiquitous in the Mediterranean coastal zone, also constitute a habitat and food source for the fauna and microfauna of sandy beaches. Pietrelli *et al*. showed that 53% of these spheroids contained plastics[97]. These plastic particles may have sorbed to the leaf blades during plant growth and also after their death. Remy *et al*. found that 27.6% of the digestive tracts of detritivorous invertebrates feeding on dead biomass of *P. oceanica*, collected in a Mediterranean coastal zone, contained plastic fibers of various sizes[98].

To our knowledge, there is no study on the biodegradation of micro or nanoplastics by macrophytes.

4. Terrestrial plants

One of the main identified routes for the entry of plastics into soils is the use of plastic mulch in agriculture[9]. Hegan *et al*. simulated the accumulation of plastic residues after repeated field mulching and they found that, after 121 years, the cumulative reduction yield per surface area would be greater than the benefit of using the mulching[99]. Traditional polymers used for this purpose were low and high density PE (LDPE, HDPE) but in recent years, different biodegradable polymers such as polylactic acid (PLA), poly-hydroxyalkanoate (PHA) or cellulosic and starch based polymers are being tested. Besides, plastic residues from consumer products arrive to soils through the application of sewage sludge as fertilizer and, to a lesser extent, from atmospheric deposition, runoff from aquatic bodies and polymer seed coating[100]. Urban soils can also present plastic pollution due to the release of fragments or leachates from plastics used in outdoor applications[101].

There is still a limited number of studies addressing the effects of micro and nanoplastics in terrestrial plants (17 studies, Table 1), most of them focused on crops (13 studies) and PS particles (11 studies). Overall, studies regarding the impacts of microplastics (10 studies) are performed in quite similar experimental conditions: pot experiments, long exposures (1 to 5 months), and similar plastic concentrations (0.001-2% w/w). The main parameters studied are germination, plant growth and photosynthesis. In the case of nanoplastics (10 studies), all available studies were performed in soil free media. Besides parameters considered for microplastics, these studies also included cyto- and genotoxicity, oxidative stress, genomics and metabolomics studies.

4.1. Influence of plastic particle size, shape and surface charge

Only three studies have addressed the size dependent toxicity of plastic particles in plants. In a pot experiment, Qi *et al*.[102] found that microparticles (50 µm to 1 mm) of LDPE and starch based biodegradable plastic produced slightly more negative effects on root and total biomass of wheat plants than macroplastics (7 x 1.5 mm average) of the same polymers. A size and dose dependent delay in germination was observed in *Lepidium sativum* exposed to 50, 500 and 4800 nm fluorescent PS particles[103]. It was stronger for the biggest particles, which accumulated in seed testa probably blocking water uptake. Seedlings 72h after germination showed decreased root growth for 50 nm particles treatment but the opposite in 500 nm treatment. In another study, seedlings of *Vicia faba* (36-48h after germination) were exposed to 5 µm and 100 nm PS fluorescent particles at 0, 10, 50, and 100 mg/L for 48h[104]. In this study, root biomass and length were more decreased by microparticles while oxidative stress measured as malondialdehyde

(MDA) and micronucleus frequency were mostly affected in nanoparticle treatments. Laser confocal microscopy showed larger number of particles accumulated in the root tips of plants exposed to 100 mg/L nanoparticles than in the same dose of microparticles.

Some studies also pointed to the shape, as one of the variables controlling plastic toxicity. Synthetic microsized fibres (mixture of nylon and acrylic) induced similar effects on the germination rate, shoot length and chloropyll a/chloropyll b ratios of *Lolium perenne* than HDPE and PLA microparticles, even when applied at hundred times lower concentration (0.001% *vs.* 0.1%)[105].

In another study, *Allium fistulosum* was exposed to polyester (PES) fibres (0.2% w/w), PA beads and microparticles of HDPE, PP, PS and PET (2% w/w) for two months[106]. PES fibres induced the strongest effects on studied plants traits.

To date, only one study has addressed the influence of particle charge on plastic toxicity to terrestrial plants. Positively charged PS-NH_2 nanoplastic were more toxic to *Arabidopsis thaliana* than negatively charged PS-SO_3H nanoplastics, although the uptake was higher in the latter[107].

4.2. Influence of plastic particle chemical composition

Qi *et al*. found that the biodegradable starch-based plastic induced stronger negative effects on wheat than LDPE[102]. This was attributed to the components (37.1% pullulan, 44.6% PET and 18.3% polybutylene terephthalate (PBT)) of the biodegradable plastic that may be more toxic than LDPE. The authors observed important changes in chemical and bacterial composition of the rhizosphere in soils spiked with starch based plastic that may explain, at least in part, observed effect on wheat plants [108].

Microparticles of PLA, were shown to be more toxic to *L. perenne* than microparticles of HDPE with similar size at the same concentration[105]. PLA decreased germination rate by 7% and decreased shoot length after 30 days whereas the exposure to HDPE increased root biomass.

In another study, PA beads induced higher effects than the microparticles of PEHD, PP, PS and PET[106]. According to the authors, the lower toxicity induced by PEHD, PET, PP and PS microparticles could be explained because they were mainly composed by H and C, whereas PA beads contained N and additives that can alter soil chemistry.

Other studies have shown that leachates derived from plastic degradation may contain polymer monomers and/or additives that can cause toxicity to plants. For instance, genotoxicity and cytotoxicity were induced in *A. cepa* exposed to leachates from the composting of a new biodegradable nanocomposite of PLA and nanoclays[109]. Leachates from HDPE and compostable plastic bags delayed and advanced the germination of the coastal dune species *Thinopyrum junceum* and *Glaucium flavum*, respectively[110] . However, no negative effect has been found in *Brassica rapa* var. chinensis exposed to the biodegradation products of PBAT (butylene adipate-coterephthalate)[111].

4.3. Cocktail effects in presence of other contaminants

Only two studies have explored the combined toxicity of plastics with other pollutants in terrestrial plants, both in hydroponics conditions. Dong *et al*. investigated the phytotoxicity of PS and PTFE microparticles alone or in combination with As(III) over a range of concentrations (0.004-0.2 mg/L plastics, 0.04-4 mg/L As(III)) for rice plants[112]. Lian *et al*. investigated the impact of PS nanoparticles (0, 10 mg/L) on the toxicity of Cd (0, 20 µM) in wheat[113]. In both cases, although the exposure to plastic particles alone caused negative effects on plants, their combined exposure with As and Cd were less toxic than the exposure to As and Cd alone. The alleviation of toxicity by plastic particles was mainly explained by the adsorption of the pollutant to plastic surface that reduced total concentrations in plants. The reduction of root activity caused by plastics may also decrease the uptake of As and Cd.

Besides this, PS nanoparticles increased the concentration of carbohydrates which may trap free radicals reducing Cd toxicity to wheat[113].

4.4. Toxicity mechanisms

Phytotoxicity caused by plastic seems to depend on a wide range of variables including polymer type, presence of additives, size, shape, surface charge of plastics and dose.

Mechanisms underlying observed effects on plants can be indirect through changes in the physical environment of soils which include alterations in the profile of water-stable aggregates[105], soil bulk density, soil aggregation and water cycling[106]. Atuanya *et al*. already

503 suggested that the reduction of growth found in *Zea mays* plants grown in soils containing 2.5%
504 w/w of PE granules was related to an increase in the bulk density of the soil[114]. However, only
505 van Kleunen *et al*. have used cork microparticles as additional control to test the hypothesis of
506 changes in soil structure as the cause of microparticle toxicity[101], and suggested, contrary to
507 Atuanya *et al*., that particles improved the drainage or aeration of the soil. Cork granules
508 showed less negative effects than ethylene propylene diene monomer (EPDM) particle on
509 *Plantago lanceolate*[101], which makes it necessary to reconsider the results from the previous
510 studies that did not use this kind of control. The phytotoxicity of plastic residues have also been
511 attributed to a direct chemical toxicity due to the leaching of polymers or additives[105,108–110].
512 But none of these studies have quantified the release of additives. Qi *et al*.[108] and De Souza
513 Machado *et al*.[106] also suggested that the presence of plastics in soil can alter C:N ratios, thus
514 affecting nutrient balance for plants. These physical or chemical changes in the soil can also
515 affect soil biota (earthworms[102,105], soil microbial community[106,108]) indirectly affecting plant
516 development.
517
518 Microscopic observations of particle accumulation in seed and root surfaces suggested that the
519 inhibition of water and nutrient uptake was one of the main mechanism altering germination
520 and plant development[104,115,116,103]. However, this hypothesis was not verified by nutrient or
521 water content measurements. On the opposite, Lian *et al*. found an increase of seed vigour and
522 seedling length in wheat seeds exposed to PS nanoparticles that was accompanied by an
523 increase in water uptake[117]. And, Taylor *et al*. found the accumulation of micro and nano
524 spheres of PS in the root cap of *Arabidopsis thaliana* and *T. aestivum*, but no evidence of uptake
525 or negative effects[118].
526
527 Dose-dependent decreases in mitotic index have been reported in *A. cepa*[115,119] and in *V. faba*[104]
528 seedlings exposed to PS nanoparticles. In the case of *A. cepa*, the presence of nanoplastics inside
529 cells interfering with some organelles was reported for the first time. The presence of lipid
530 bodies in the cytoplasm of exposed plants revealed some alterations in lipid mobilization. Non
531 monotonic oxidative stress was also reported for these two species suggesting that antioxidant
532 systems in plants may prevent lipid peroxidation to a certain dose of nanoplastics and that other
533 toxicity mechanisms may trigger observed cyto and genotoxicity. Wheat plants exposed to PS
534 nanoparticles for three weeks showed lower contents of the non-enzymatic antioxidants proline,
535 glycine and glutamine than the controls[117]. Lian *et al*. have suggested that PS nanoparticles can
536 reduce the concentration of free radicals reducing oxidative stress in wheat[113]. In both studies,

metabolomics analysis revealed an acceleration of carbohydrate and aminoacid metabolism which can be a mechanism to cope with PS toxicity. However, Sun *et al.* found ROS production and inferences in the regulation of genes related with oxidative stress in *A. thaliana* exposed to PS nanoparticles[107].

Finally, nutrient imbalances, changes in root morphology and alterations in shoot to root ratio have been reported[106,113,117].

4.5. Accumulation and trophic transfer

To date there is no information about the uptake of microparticles by plants and their potential trophic transfer. Due to their small size, nanoplastics are more likely to be taken up by plants and transported to the edible parts. The use of fluorescent PS nanoparticles have allowed to show the absorption of nanoplastics by the roots of *L. sativum*[103], *V. faba*[104] and *A. cepa*[115] and the translocation to the shoots of *T. aestivum*[117]. By combining two photon excitation and time resolved (TPE-TR) optical imaging, fluorescence lifetime imaging (FLIM) microscopy and transmission electron microscopy (TEM), Zhang *et al*. showed the uptake and translocation of styrene maleic anhydride nanoplastics (SMA-PNPs) in *Murraya exotica*[120]. A recent article just reported the trophic transfer of PS fluorescent nanoplastics from soils (100 mg/kg) to mung beans and then to African giant snails[121]. These studies provide significant evidence of nanoplastic uptake by plants. However, they did not provide quantitative information and they used only model nanoplastics in quite unrealistic exposure conditions. This information does not allow yet to assess the risk of transfer of plastic particles from terrestrial plants to the food chain.

Still, pot studies[122] and survey of commercialized vegetables from greenhouses[123,124] showed that vegetables and cereals accumulate high amounts of phthalates, leading to a high risk of trophic transfer of these additives through the diet[123].

4.6. Plastic particle degradation in the rhizosphere

The rhizosphere being a zone of intense bacterial and fungal activity, and some strains having been shown to degrade plastics, a few studies examined the fate of plastics in the rhizosphere. To our knowledge, the biodegradation of nano and micro-plastics in the rhizosphere has not been studied so far. Two studies investigated the degradation of macroplastics in the rhizosphere of plants, or in contact with rhizosphere fungi or bacteria. One study showed a loss

of weight for biodegradable PLA, not for PET[125], and the second one showed changes in physical and chemical properties of PE strips, but non reproducible losses of weight[126].

5. Methods for detection and characterization of micro and nanoplastics in photosynthetic organisms

The widespread environmental presence of micro and nanoplastics and the potential adverse effects to photosynthetic organisms demand the development of adapted detection and characterization techniques that provide access to their chemical nature, shape and size, surface chemistry, dissolved fraction and leachate composition. In particular, for nanoplastics methodological challenges are associated with detection and recovery of these carbon-based materials in complex natural matrices (such as photosynthetic organisms). Among a variety of analytical methods to characterize plastic contaminants in ecotoxicology, the techniques described below are the most widely used to detect and characterize their changes in chemistry and morphology before or after interactions with photosynthetic organisms. Figure 1 presents a summary of the main sample preparation steps and information obtained from these techniques techniques and more details are provided in Table 2.

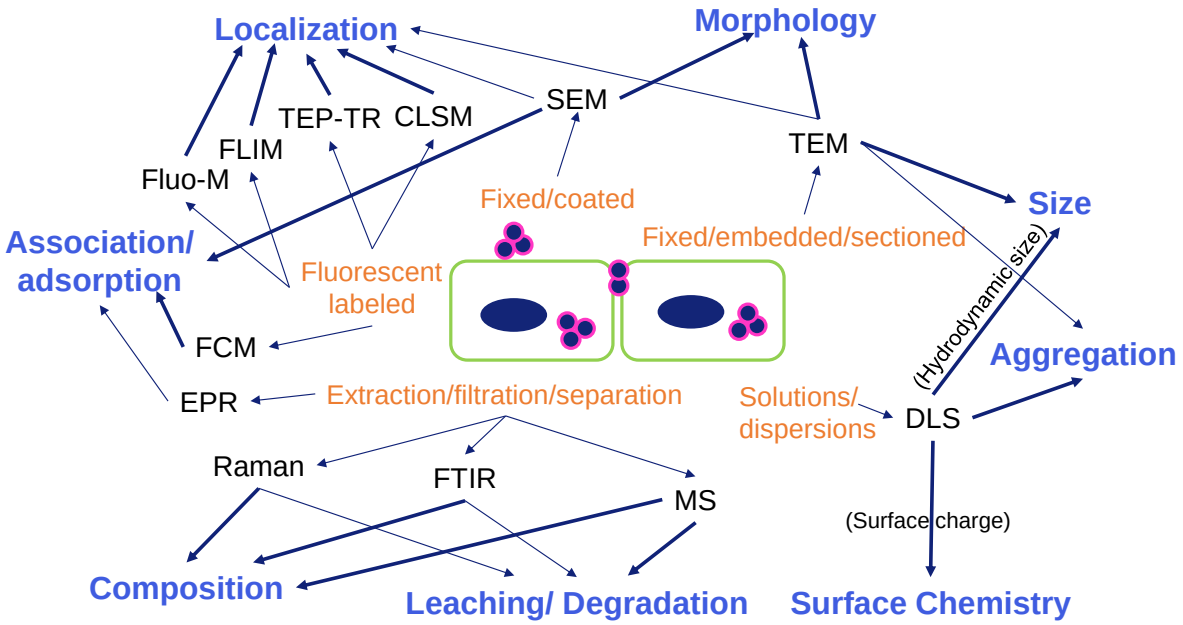


Figure 1. Analytical techniques used for detection and characterization of micro and nanoplastics in photosynthetic organisms. In green (general) sample preparation requirements, in blue the characterization properties obtained (darker arrows are indicative of the main use of the technique).

Acronyms: confocal laser scanning microscopy (CLSM), electron paramagnetic resonance (EPR), flow cytometry (FCM), fluorescence lifetime imaging (FLIM), fluorescence microscopy (Fluo-M), mass spectrometry based techniques (MS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), two-photon excitation and time resolved detection imaging (TPE-TR), dynamic and electrophoretic light scattering (DLS), Raman (micro)spectroscopy (Raman), Fourier transform infrared (micro)spectroscopy (FTIR).

5.1. Microscopy

The use of microscopy techniques in different modalities is one of the recurrent methods for plastic identification from environmental samples and is often followed by chemical identification by means of vibrational spectroscopy or mass spectrometry techniques. In ecotoxicological studies of micro and nano plastics in photosynthetic organisms, a variety of microscopic methods have been used and are below divided in electron, fluorescence, and laser based methods.

5.1.1. Electron based microscopy

High resolution images can be obtained using electron based methods in both scanning (SEM) and transmission (TEM) modes. These methods are often used before interaction with organisms in order to characterize size and morphology of micron and nano size plastics. Electron microscopy applications in the characterization of plastic interactions with organisms demands careful sample preparation protocols (dehydration, fixation, staining, coating) and the contrast offered by plastics does not allow unambiguous identification. In order to gain additional information, for example to discriminate plastic from inorganic particles, the use of energy dispersive X-ray fluorescence detection is advised. Despite of this fact, electron based microscopy has been widely used to characterize associations of micro and nanoplastics with phytoplankton[33,35,36,38,39,41,42,44,53,57,60,62,69,70,73,74,81] with a predominant use of SEM. In terrestrial plants, evidence of cell internalization in *A. cepa* exposed to nanoPS has been obtained from transmission electron microscopy images[115].

5.1.2. Fluorescence microscopy

This microscopy method has been used to determine the interaction of micro and nanoplastics with photosynthetic organisms and in particular has provided evidence of aggregation with phytoplankton [33,34,37,56,69,70,74] and internalization of micro[103] and nano[107,115] plastics in terrestrial plants. This is of outmost relevance to evaluate uptake mechanisms and to perform risk analysis of food chain transfer in the environment through primary producers. However, this method can only be applied on model fluorescent plastic materials and do not fully represent the myriad of shapes, surface properties and sizes found in the environment. For this reason and, due to the importance of environmental weathering in determining the fate of plastic in the environment[4,100], results from this technique require careful interpretation when extrapolated to real environmental plastic pollution scenarios.

Staining dyes (Oil red EGN, Eosin B, Hostasol Yellow 3G, Nile red and Rose Bengal) could be used in staining protocols after exposure, the issue is that the affinity of these dyes for plastics *vs*. biogenic material would complicate interpretation. Hence, existing protocols require digestion, extraction, or filtration steps to remove biogenic material. These protocols may be used for testing plastic presence in photosynthetic organisms from environmental samples[127]. However, this approach does not allow for precise cellular and tissue localization.

5.1.3. Laser scanning microscopy

Confocal laser scanning microscopy (CLSM) allows direct and non-invasive serial optical sectioning of intact living photosynthetic organisms. This method requires minimum sample preparation. However, as in the case of fluorescence microscopy, specimens are treated with fluorescent dyes to make selected objects, in this case plastics, visible. By performing successive slices (z-stack) images can be arranged to create a 3D image. Their application in studying photosynthetic organisms is advantageous but careful selection of the excitation/emission wavelength of the labelled plastic material should be done in order to prevent autofluorescence from the specimen to mask its signal. In plant leaves, the primary contributor of autofluorescence is chlorophyll, but lignins, carotenes, and xanthophylls also produce a significant level of fluorescence emission. This technique has been used to investigate nanoplastic association with microalgae[42,53,60,62,69,70,74]. In terrestrial plants, with aid of CLSM the presence of microplastics in the seed capsules, and at later stage in the root hairs of *L.*

sativum was identified[103]. In *V. faba*, 100 nm PS particles were observed entering the roots[104] and in *T. aestivum* CLSM demonstrated uptake of PS nanoparticles in roots[117].

Other techniques based on laser scanning are two-photon excitation and time-resolved detection (TPE-TR) and fluorescence lifetime imaging (FLIM). Zhang *et al*. used TPE-TR as a non-invasive approach to investigate kinetics and uptake mechanisms of Eu luminescent styrene-co-maleic anhydride SMA-NPs in *M. exotica*[120]. This novel approach exploits the differential fluorescence persistence of the Eu luminescence and the autofluorescence of plant tissues (~1 ms *vs*. <100 ns) to set an appropriate time delay prior to Eu luminescence detection to avoid interference from autofluorescence. It offers the advantage of wide field of view (32x32 mm^2) and large imaging depth that enables *in vivo* analysis in plants and other organisms. However, the resolution obtained is 60 µm, for high resolution studies and for a more detailed localization FLIM can be used in combination. Similarly, FLIM takes advantage of the longer fluorescence lifetime of the Eu-luminescent nanoplastics to eliminate autofluorescence interference; the resolution is sub-micrometric[128]. Results from this study revealed inter cell wall deposition of SMA-NPs in the lignified epidermis of *M. exotica* plants that represents a path of uptake and translocation, and revealed similar accumulation kinetics at different positions along the plant stems[120]. Efforts should be made to produce luminescent plastic particles with characteristics similar to those found in the environment. It is worth noting that the use of lanthanide doped or metal doped particles would enable detection of these materials in complex biological systems through the use of element sensitive techniques (bulk and microscopy) typically used for metal engineered nanoparticles[129,130]. For instance, PS-Pd nanoplastic have been recently used to confirm the internalization of PS nanoplastics by *A. thaliana*[107].

5.2. Flow cytometry

An additional method that can be successfully coupled to laser induced fluorescence (natural or following staining) is flow cytometry (FCM). However, this method is a bulk technique and no spatial information is obtained. This technique screens a sample containing cells or particles suspended in a fluid and flows one cell at a time through a laser beam. FCM is able to analyse quickly and simultaneously large quantities of cells in suspension and several parameters (cell characteristics and function, plastic association with cells). MicroPS were quantified using FCM in microalgal cultures and allowed the distinction between free suspended micro-PS,

hetero-aggregates, and microalgae[51]. These results underline the potential interactions of phytoplankton cell and residual organic matter with microplastics and their influence on bioavailability in aquatic systems.

5.3. Vibrational (micro)spectroscopy

Detailed chemical information about plastic materials can be obtained from vibrational spectroscopy methods. Fourier transformed infrared (FTIR) and Raman spectroscopy being the most popular methods and offering complementary information. Moreover, both methods can be coupled to a microscope offering spatially resolved chemical composition. These techniques can provide insight about the chemical changes in the plastic material induced by aging in the environment[62,67,81]. Though direct analysis (*in situ*) in photosynthetic organisms by these techniques, in micro-spectroscopy mode, is in principle feasible, this has not been attempted yet (to the best of our knowledge) and limitations exist due to spatial resolution or sample preparation.

FTIR produces a molecular fingerprint resulting from the change in dipole moment of chemical bonds, and allows the detection of various types of plastics such as PS or PE due to characteristic bands[116]. The commonly used analytical modes are transmission, attenuated total reflection and reflectance. The three modes may be used in combination with a microscope and the spatial resolution is around 10 µm for bench-top instrument with a globar source (but it may reach the diffraction limit if a synchrotron source is used)[132]. Though the direct detection of plastic materials may be challenging, FTIR micro-spectroscopy may be used to assess biomolecular changes in aquatic and terrestrial plants induced by exposure to these materials. Resolution of FTIR micro-spectroscopy can reach nanoscale when coupled to an atomic force microscopy tip (in thermal expansion or near-field mode) but this significantly complicates sample preparation protocols[132].

Raman provides a molecular fingerprint spectrum based on the polarizability of chemical bonds[131]. Raman microscopy allows the characterization of microplastics down to 300 nm, in some cases it can provide additional information about contained fillers and pigments, and it does not suffer from water interference. However, some drawbacks are the weak nature of the signals (approx. 10^{-8} photons reaching the sample produce Raman signal), increasing

measurement duration, and interference from sample autofluorescence, that depend on the organism and the plastic material characteristics such as color, biofouling and degradation[133].

5.4. Dynamic light scattering (DLS)

DLS is currently the most widely used technique for the characterization of micro and nanoplastic (size range from 0.7 nm - 10 µm) hydrodynamic diameter[134]. This technique offers affordability and easy usage with built-in automatized data analysis, but it has some limitations: (i) it is a blind technique, so it can be used only on nanoplastic suspensions in water or in nutrient solutions devoid of other types of particles, and (ii) it does not provide reliable results for polydispersed suspensions (containing various particle size categories). The hydrodynamic diameter is hence the most commonly reported parameter since it reflects the size of the particle in suspension and includes coatings or surface modifications. The available instruments for DLS offer also the capability to determine the electrophoretic movement of the particles when applying an electric field, this allows to calculate the zeta potential value of the particles, *i.e.* the surface charge of particles. This is a key indicator of the stability of the colloidal dispersion; its magnitude denotes the degree of electrostatic repulsion between adjacent similarly charged particles in the dispersion. This technique has been applied as routine in most of the research work included in this review (60% of the cited studies), in most cases for characterization of the hydrodynamic size of the particles in different growth and exposure media.

5.5. Magnetic based

Electron paramagnetic resonance (EPR) spectroscopy is a technique used for the characterization of paramagnetic compounds. Paramagnetic compounds and atoms are characterized by the presence of unpaired electrons, EPR spectroscopy measures electron spin transitions in molecules in the presence of an external magnetic field[135]. This technique allows to study for example the formation of environmentally persistent free radicals and oxygen free radicals formed on the surface of microplastics when exposed to sunlight[136]. In the study of Casabianca *et al.*, EPR was used to investigate the associations of marine phytoplankton and PET plastics in seawater[68]. In this study a paramagnetic surfactant dodecyldimethylammonium-

2,2,6,6-tetramethyl-piperidine-N-oxide bromide (CAT12) with good affinity for insertion into the cell membrane was used to probe cell-plastic affinity[68]. The diatom *S. marinoi* presented an exponentially increasing adsorption to PET as a function of incubation time. On the other hand, *L. polyedrum* showed lower adsorption and a different mechanism of interaction with the plastic surface. This demonstrates the applicability of this technique to obtain useful information about the mechanisms of adhesion of microalgae in plastic surfaces.

5.6. Mass spectrometry based techniques

Mass spectrometry (MS) based techniques measure the mass-to-charge ratio of ions. The most commonly used separation methods are gas chromatography (GC) and (high performance) liquid chromatography (HPLC/LC). These methods have the advantage of being able to provide chemical composition of plastic materials from relatively high mass and representative samples, but the down side is that they are destructive[25].

In Chae *et al*. the leachates from micro-sized PE spheres in algal growth medium were analyzed qualitatively by HPLC-MS[56]. The results reported the presence of bisphenol A, phthalates, dibutyl phthalate, and UV stabilizers (such as octyl methoxycinnamate) in the medium. Another study by Tetu *et al*. in *Prochlorococcus* photosynthetic bacteria[65] found that leachates from HDPE and PVC contained 5877 and 10658 components, respectively. This was a broad chemical screening that did not allow identification of individual chemical species. This study also demonstrated the enrichment of elements such as Zn, Sr and Cu through the use of inductively coupled plasma (ICP-MS).

Similarly, Capolupo *et al*. used a combination of GC-MS and ICP-MS to screen the organic and inorganic composition of fresh and marine water leachates produced by car rubber tire, PP, PET, PS and PVC microplastics[64]. This analysis revealed the presence of additives (benzothiazole, phtalide, acetophenone) and metals (Co, Zn and Pb) with concentrations varying depending on the plastic type. Thermal decomposition and extraction variants of GC-MS exist such as pyrolysis-GC-MS and thermo extraction and desorption (TED-GC-MS). In pyro-GC-MS microplastics are thermally decomposed (pyrolyzed) and the gas formed is cryo trapped and separated on a chromatographic column, this method has been successfully used to identify polymer types and additives in marine microplastics[137]. However, it was unsuccessful

to detect PS in the roots of *A. thaliana* and *T. aestivum* because of the interference with styrene peak, which can be a degradation product of the pyrolysis of plant tissues[118]. TED-GC-MS combines thermo gravimetric analysis for thermal degradation and solid phase extraction for identification of plastics in environmental samples[138].

Recently, Bolea-Fernandez *et al*. presented a new approach based on ICP-MS detection operated in single-event mode or single particle (SP) ICP-MS mode[139]. This method relies on ultra-fast monitoring of the signal intensity spikes for the mass-to-charge ratio of C^{13+} produced by individual particles. The method was demonstrated with PS microspheres (1-2.5 µm) and allows for quantitative determination of mass concentration and size distribution. This is a very promising method that requires further optimization to be routinely used in environmental samples such as photosynthetic organisms. In fact, for all mass spectrometry based techniques the feasibility to apply them to plastic materials interacting or internalized in photosynthetic organisms requires more study to develop adequate sample preparation protocols.

6. Conclusions, knowledge gaps and future directions

Figure 2 provides a synthesis of the current knowledge on the fate and effects of micro and nanoplastics on photosynthetic organisms. The overview of the literature shows that plastics have significant impacts on photosynthetic organisms. There are comparatively much less studies on terrestrial crop plants than on aquatic organisms, although micro and nanoplastics are ubiquitous in agricultural soils. The observed effects seem to be modulated by particle size, surface charge, composition and shape of the particles, as well as their agglomeration and weathering state. The impact of these parameters have been investigated more in depth in phytoplankton and results show that overall nanoplastics (<1 µm) are more toxic, especially those bearing positive surface charges, with EC_{50} between 0.5 to 13 mg/L for PS nanoparticles. PVC and PS particles tend to be the most toxic while PE and PMMA have less effects on phytoplankton. The effects of biodegradable plastics have been studied in soils and they can induce similar or even more phytotoxicity than classical polymers. Bigger plastic particles (> 100 µm) showed little effect.

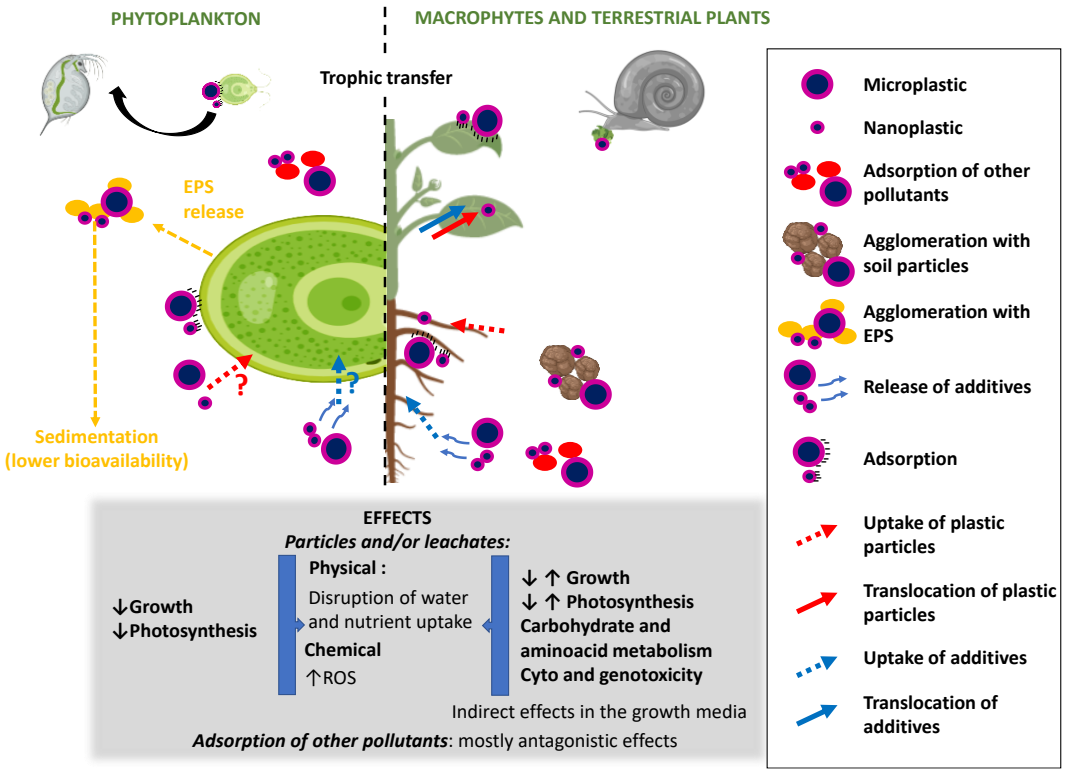


Figure 2. Synthesis of the interactions observed between micro and nanoplastics and photosynthetic organisms. Processes observed in both phytoplankton and macrophytes and terrestrial plants are placed in the middle of the scheme. (EPS: exopolymeric substances)

Phytotoxicity was visible mainly through decreased growth and impaired photosynthesis, the suggested mechanisms are either physical by disruption of the fluxes of water and nutrients or chemical by ROS induction. Other parameters can possibly influence the output of toxicity studies and should also be further investigated such as the influence of the species, developmental stages, experimental systems or exposure duration. It has also been shown that part of the toxicity is directly related with the leaching of additives and weathering products. Toxicity studies should more systematically characterize the leachates, in order to clearly identify them as sources of phytotoxicity. More studies using genomic, proteomic and metabolomic techniques are needed to understand the mechanisms causing phytotoxicity in photosynthetic organisms exposed to plastics.

Trophic transfer is also of concern since it has been proven (both in the lab and in the field) that plastic particles can adhere to aquatic organism surface and thus be transferred to the upper trophic level. However, there is a lack of quantification of plastic inside the organisms (both aquatic and terrestrial) so the question of plastic internalization inside plant tissues, and in

particular in edible parts in crop plants, is still a matter of debate. Uptake of additives such as phthalate esters in vegetables has been evidenced, leading to human exposure through the diet.

In addition, and following the trend of the research on engineered nanoparticles, studies should include aged plastics, which are more representative and environmentally relevant. So far, pristine spherical PS particles have been the most studied, although they are not representative of plastic particles spread in the environment, and even less representative of real aged particles. Working with these "real" particles is not ideal because experiment cannot be reproduced and results obtained are not comparable. Another strategy is to work with particles (and their leachates) after aging in controlled conditions. Also, in a more complex and realistic environment, plastic particles most probably interact with other contaminants and affect their toxicity, this should also be investigated further especially since aged plastic particles have a high sorption capacity. In addition, the response of organisms to combined stress (for example drought and micro nanoplastics) has been very little studied so far.

Concerning biodegradation, although some laboratory studies evidenced some loss of weight and some chemical degradation by microorganisms including photosynthetic ones, processes in environmental conditions and secondary products remains to be determined, and the kinetics is probably much slower than in laboratory experiments. The increasing concentrations of nano and micro-plastics in environmental compartments is the best evidence of the slow kinetics of both abiotic and biotic degradations.

More in depth research about nanoplastics will require the development of advanced detection techniques and sample preparation protocols that allow quantification of plastics in photosynthetic organisms. Main challenges include the detection limit of the techniques compared to environmental concentrations, and the similarity between micro and nanoplastics and natural organic compounds. In order to elucidate internalization in tissues and intra-cellular localization, microscopy methods capable of detecting non-labeled plastic particles unambiguously would be a breakthrough.

Other recommendation that can be formulated is the need for standardization of the methods for better intercomparison of the studies (using standard exposure conditions, standard methods for aging, …) and the need for categorization. Indeed, micro and nanoplastics include a vast variety of compounds with different properties and toxicities, and there is a need to categorize them to speed the risk assessment process[34,140].

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Ana E. Pradas del Real thanks "Atración de Talento" program from Comunidad de Madrid for her fellowship. G. Sarret (ISTerre) is part of Labex OSUG (ANR10 LABX56).

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Table 1. Summary of the main technical details of studies published in the literature so far dealing with the impacts of nano and microplastics on photosynthetic organisms. catalase (CAT), database of essential genes (DEG), diethylhexyl phthalate (DEHP), half maximal effective concentration (EC_{50}), ethylene propylene diene monomer (EPDM), exopolymeric substances (EPS), humic acid (HA), high density (HD), half maximal inhibitory concentration (IC_{50}), low density (LD), malondialdehyde (MDA), polyethylene (PE), polyethersulfone (PES), polyethylene terephthalate (PET), polylactic acid (PLA), poly(methyl methacrylate) (PMMA), peroxidase (POD), polypropylene (PP), polystyrene (PS), polytetrafluoroethylene (PTFE), polyvinyl chloride (PVC), reactive oxygen species (ROS), superoxide dismutase (SOD)

Species	Growth or living medium	Plastic type	Coating	Size	Concentration	Exposure duration	Co exposure	Effects	Ref
Microalgae									
Scenedesmus subspicatus *Thalassiosira weissiflogii*	fresh water seawater	pristine PE (PER) and PE from ocean (PEN)		350 µm	0 to 1 mg/L	48 h		- no influence on cell growth of *T. weissiflogii* - PEN exposure caused growth inhibition of *S. subspicatus* for all exposure concentrations tested - recovery after some time - PEN seemed more toxic (metal release?)	63
Raphidocelis subcapitata *Skeletonema costatum*	fresh water seawater, (DM medium, TG201 medium)	CTR (car tire rubber), PP, PET, PS, PVC		leachates of 1000 µm particles	80 g/L for leachate preparation	72 h		- in the leachates: plasticizers, antioxidants, antimicrobials, lubricants, vulcanizers (between ng to hundreds of µg/L) - CTR and PVC contained the most additives *vs.* PET - different leachate composition with fresh or sea waters for inorganic contaminants - all leachates (except PET) inhibited algal growth - sorption of some inorganic nutrients at the plastic surface (especially PS particle and Mg)	64
Skeletonema marinoi *Lingulodinium polyedrum*	seawater (artificial)	PET		sheets 4 x 20 mm		up to 16 d, log phase		- interactions between *S. marinoi* cells and plastic surface, exponentially increasing as a function of incubation time - *L. polyedrum* showed lower adsorption. Siloxane groups of the silica frustule involved in the binding with the hydrophobic plastic surface	68

Dunaliella salina *Scenedesmus rubescens* *Chlorella saccharophila* *Stichococcus bacillaris*	seawater (f/2 medium)	expanded PS		fragment: 2 mm to 10 cm² sphere: 2 to 10 mm	leachate: 0.1 g / 50 mL or 10 and 30 items/50 mL, checked for 28 d	7 d		- leachate exposure (especially from smaller fragments) increased photosynthetic activity in all 4 species - in the leachates: hexabromocyclododecane, UV326, bisphenol-A - different leachate composition according to particle size	76
Phaeodactylum tricornutum	seawater (artificial)	PE PVC		150 µm 250 µm	50, 1000, 50000 mg/L	4 d - 9 d, log and stationary phases	phenanthrene (0.8 mg/L)	- both plastic types did not influence algal growth and lipid accumulation after 4d but minimized algal inhibition by the action of Phe (more effects at 9 d) - polymer-size was the key factor influencing plastic toxicity	34
Chlamydomonas reinhardtii	fresh water (SE medium)	PS		300 - 600 nm	5 to 100 mg/L	10 d, log phase		- microalgae density decreased as plastic concentration increase: highest inhibitory rate was 45.8% - inhibition of EPS release - decrease in chloro fluorescence yield/photosynthetic activities - increase of soluble proteins and MDA content - plastic wrapped on algae surface inducing membrane damages	46
Chlorella pyrenoidosa	seawater (artificial)	PS		0.1, 0.55, 5 µm	up to 64 mg/L	96 h, log phase	DBP : dibutyl phtalate	- $IC_{50} \approx 7$ mg/L for PS alone - size dependent inhibitory effect - modeling showed antagonist interactions between the 2 contaminants (20 mg/L PS lead to a 20% decrease in the toxicity of DBP) - biomarkers: cell volume, morphological complexity and chlorophyll fluorescence	47
Euglena gracilis	fresh water (sterilized growth medium)	PS		100 nm, 5 µm	1 mg/L	96 h	Cd (0.5 mg/L)	- toxicity of microPS > nanoPS (growth inhibition, oxidative damage, decreased photosynthesis pigment) - microPS alone or with Cd caused cavitation and increased the number and volume of vacuoles - combined exposure toxicity Cd + nanoPS > Cd+microPS - DEGs in Cd + nanoPS enriched in metabolism-related pathways - no adsorption of 0.5 mg/L Cd to 1 mg/L PS microbeads	77

Scenedesmus obliquus	fresh water (BG-11 medium)	PS	-COOH -NH_2	0.1, 0.5, 1, 2 µm	75 mg/L and up to 250 mg/L	150 h, log phase	humic acid	- no significant impact on the EC_{50} - larger size particles blocked light transport affecting photosynthesis - smaller particles destroyed cell walls after adsorption - HA alleviated toxicity of small particles by forming a corona around, decreasing plastic affinity for algae	58
Microcystis aeruginosa	fresh water (BG-11 medium)	PE		<500 µm	leachate: 0.5 g / 300 mL, for up to 96 h	12 h		- aging led to surface cracks and fragmentation, increased surface area and carbonyl contents, and promoted the release of Pb chromate pigment - Cr and Pb leached more under acidic condition, rather than neutral and alkali environment - high concentration of NaCl favored leaching - concentration of leachate >10 µg/L decreased photosynthesis - growth inhibition increased with leachate concentration	67
Symbiodinium tridacnidorum, Cladocopium sp.	seawater (articifial + f/2 medium)	PS		42 nm	0.01, 0.1, 10 mg/L	10 d		- cell number and aggregation reduced - no clear correlation with plastic concentration - genes involved in dynein motor function upregulated *vs.* genes related to photosynthesis, mitosis, and intracellular degradation downregulated - different sensitivity to nanoplastics between species	78
Euglena gracilis	fresh water (sterilized growth medium)	PS		100 nm, 5 µm	1 mg/L	96 h		- induction of vacuoles and superoxide dismutase and peroxidase activities, reduction of pigment contents - dysregulation in gene expression in cellular processes, genetic information processing, organismal systems, and metabolism	75
Raphidocelis subcapitata	fresh water (WC medium)	PS	-COOH	< 1µm	0.5 to 50 mg/L	72 h, 7 d, log phase	Cu (1 to 200 µg/L)	- algae secreted EPS, creating a protein corona around nanoplastics - no adsorption of Cu on plastic surface - PS had no impact on Cu toxicity (no toxicity) - plastic led to structural damages after 72 h	37
Dunaliella salina	seawater (f/2 medium)	PE		200 µm	up to 350 mg/L	6 d		- growth and photosynthetic activity enhanced with exposure to plastic - cell morphology (size and granularity) not impacted - implication of trace concentrations of additive chemicals (endocrine disruptors, phthalates, stabilizers) ?	56

Microcystis panniformis Scenedesmus sp. Tetraselmis sp. Gloeocapsa sp.	fresh water (BG-11 medium) seawater (f/2 medium)	PMMA PS		<106 µm, 106 to 250 µm	12.5 and 125 mg/L	21 d		- cell abundance decreased (up to 42%) - formation of homo-aggregates (microalgae and EPS) and hetero-aggregates (microalgae, EPS and microparticles) - hetero-aggregation was dependent on particle size and yield production of EPS, which was species specific	69
Tetraselmis chuii	seawater (f/2 medium)	unknown composition		1 to 5 µm	0.3, 0.9, 4 mg/L	96 h log phase	Au nanoparticle (5 nm), 0.1, 0.3, 3 mg/L	- Au nanoparticle alone and microplastics alone did not cause significant decrease of average specific growth rate, - the mixture at the highest concentration was toxic	48
Chlorella vulgaris	seawater (artificial)	PVC		??	10, 100, 1000 mg/L	aging: 2 months exposure : 10 d	Cu (0.2, 0.5, 1 mg/L)	- aging led to surface structural changes and increased hydroxyl groups and aromatic carbon-carbon double bond but decreased carbon hydrogen bond - toxicity at 10 mg/L but not above - aged particles were more toxic (35 *vs.* 28% growth inhibition) - oxidative stress (increased of SOD and MDA) - algal growth enhanced by Cu (0.5 mg/L) + aged PVC (10 mg/L) after 10 days (but decreased at earlier stages)	62
Phaeodactylum tricornutum	seawater (natural + f/2 medium)	PE	virgin or oxydized	from 1 to 500 µm	0.01 to 25 mg/L	72 h, log phase		- no acute toxicity - standard ecotoxicological endpoints not sufficiently sensitive to assess the potential effects of microplastics (*vs.* for nanoplastics) - EC_{50} > 25 mg/L	49
Isochrysis galbana	seawater (Walne medium + surfactant)	PE		from 2 to 6 µm	up to 25 mg/L	72 h	CPF: chlorpyrifos (up to 4 mg/L)	- no impact of plastics on growth - growth decreased by exposure to CPF from 2 mg/L - 80% of CPF was sorbed onto plastic surfaces (5 mg/L) - lower percentages of inhibition when CPF in association with plastics - microplastics less bioavailable than CPF	50
Chaetoceros neogracile	seawater	PS	$-NH_2$	50 nm	0.05, 5 mg/L	4 d, log and stationary phases		- more toxicity during exponential than stationary phase (ROS production) - relation with higher concentration of transparent exopolymer particles at stationary phase leading to NP aggregation and/or with the fact that dividing cells during	41

								exponential phase may be intrinsically more sensitive to stress?	
Chlorella vulgaris	simulated acidic, saline, basic water and natural waters river, lake, wetland, seawater	polyurethane foam (PUF)		leachates: 3 mm side, 12 months aging	350 mg/L			- release amount of additives increased with increasing solution pH and leaching time - maximum amount after 12–24 h - 3,3′-diaminobenzidine-like substances identified in the leachate - leaching basic water > saline water > seawater > lake > river > wetland - Fv/Fm decreased with increasing leachate concentrations - only high content of plastics (1.6 g/L) decreased photosynthesis	66
Prochlorococcus	seawater (artificial)	HDPE from bags PVC from matting		1-2 cm^2	leachates: 50 g/L plastic pieces for 5 d	72 h, log phase		- leachate exposure strongly impaired growth and photosynthetic capacity and resulted in genome-wide transcriptional changes - PVC leachate had a considerably greater effect than HDPE	65
Chlorella sp.	seawater (artificial)	PS	$-NH_2$ -COOH - non functionalized	6 µm	1000 mg/L	72 h, log phase	TiO_2 nanoparticles	- EC_{50} TiO_2 = 81 µM - only 15% toxicity of MP at 1000 mg/L - NH_2 and non-functionalized MPs enhanced TiO_2 toxicity (oxidative stress) -COOH MPs decreased TiO_2 toxicity (hetero-aggregation)	60
Phaeodactylum tricornutum	seawater (artificial + f/2 medium)	PS		50, 100 nm	0.1 to 50 mg/L	72 h		- after 24 h, oxidative stress, damage to the photosynthetic apparatus, DNA damage and depolarization of mitochondrial and cell membrane from 5 mg/L - after 72 h, inhibition of growth and chlorophyll - smallest NPs (50 nm) induced greater effects at 24 h while bigger nanoparticles (100 nm) did at 72 h - strong adsorption	42

Chaetoceros neogracile	seawater	PS	$-NH_2$	0.5, 2 µm (50 nm)	2.5 mg/L	72 h, log phase		- strong aggregation - negative charges in the culture medium decreasing interactions with microalgae : no adsorption - cell growth, morphology, photosynthesis, reactive oxygen species levels and membrane potential unaltered - significantly decreased cellular esterase activity and neutral lipid content - microplastic-exposed cells modulate their energy metabolism to properly acclimate to stress conditions	53
Tetraselmis chuii *Nannochloropsis gaditana* *Isochrysis galbana* *Thalassiosira weissflogii*	seawater (natural + f/2 medium)	PMMA		40 nm, 50 nm	0 to 300 mg/L	96 h		- *T. weissflogii* and *T. chuii* were respectively the most sensitive (EC_{50} = 83.75 mg/L) and least sensitive species (EC_{50} = 132.52 mg/L)	45
Karenia mikimitoi	seawater (natural)	PVC		1 µm	100 mg/L	24, 48, 72, 96 h, log phase		- dose dependent adverse effect on growth - decreased in chlorophyll content and photosynthetic efficiency - related to physical blockage (algae wrapped by plastic) and aggregation - after 96 h decrease of the toxicity	36
Skeletonema costatum	seawater (artificial seawater + f/2 medium)	PE PS PVC		74 µm (+ 1 µm PVC)	up to 100 mg/L	96 h, log phase	TCS: triclosan (up to 0.4 mg/L)	- TCS had obvious inhibition effect - single particles also had significant inhibition effect which followed the order of PVC 1 µm > PVC > PS > PE - joint toxicity of PVC and PVC 1 µm with TCS, maybe related to higher adsorption capacity of PVC for TCS - joint toxicity of PVC 1 µm most significant (PE < PVC < PS < PVC1 µm) because of the minimum particle size - antagonist interactions	57
Raphidocelis subcapitata	fresh water	PE		63 - 75 µm	130 mg/L	5 d		- higher growth with microplastics - hypothesis: microplastics could serve as substrates for growth	55
Chlamydomonas reinhardtii	fresh water	PS		<100 nm	50 mg/L (up to 100 mg/L for toxicity	72 h		- trophic transfer study with 4 levels - adsorption of nanoplastics on algae surface and transfer to higher trophic level through diet with toxicity - no toxicity to microalgae (through chlorophyll fluorescence evaluation)	74

					assessment)				
Chlorella pyrenoidosa	fresh water (BG-11 medium)	PS		0.1, 1 µm	10, 50, 100 mg/L	30 d, log and stationary phases		- dose dependent growth inhibition - decrease of photosynthetic activity, structural damages - defense mechanisms: cell wall thickening, homo and hetero aggregation when in stationary growth phase	35
Tetraselmis chuii	seawater (f/2 medium)	??		1-5 µm	0.75 to 48 mg/L	96 h, log phase	procainamide, doxycycline (pharmaceuticals): 4 to 256 mg/L	- plastics alone no effects on growth rate up to 41.5 mg/L - chlorophyll significantly reduced at 0.9 and 2.1 mg/L plastics, but not at higher concentrations - EC_{50} (growth rate and chlorophyll, respectively): 104 and 143 mg/L for procainamide alone; 125 and 31 mg/L for procainamide + plastics; 22 and 14 mg/L for doxycycline alone; 11 and 7 mg/L for + plastics - plastics-pharmaceutical mixtures more toxic than the pharmaceuticals alone	52
Microcystis aeruginosa	fresh water (BG-11 medium)	PS	$-NH_2$	200 nm	up to 20 mg/L	96 h, log phase	glyphosate (up to 7 mg/L)	- 5 mg/L glyphosate --> strong inhibitory effect while 5 mg/L nanoPS-NH_2 had no apparent effect on the growth - chlorophyll decreased at 10 mg/L after 96 h - nanoPS-NH_2 combined with glyphosate showed antagonistic effects (adsorption) - glyphosate presence enhanced dispersion stability	73
Dunaliella tertiolecta	seawater (natural)	PS	-COOH $-NH_2$	40 nm, 50 nm	up to 50 mg/L	72 h		- negatively charged particles lead to agglomeration >1 µm with no toxicity & adsorption on algae surface (*i.e.* possible trophic transfer) - positively charged particles showed no agglomeration, inhibition of algal growth (EC_{50} = 13 mg/L)	33
Tisochrysis lutea *Heterocapsa triquetra* *Chaetoceros neogracile*	seawater (f/2 medium)	PS		2 µm	4 µg/L (and 40 µg/L)	over one culture cycle, log and stationary phases		- hetero-aggregation for *C. neogracile* during stationary growth phase - high adsorption with *T. lutea* and *H. triquetra*, increasing with the age of the culture for both species - no effects of PS on microalgal growth and chlorophyll fluorescence	51

Raphidocelis subcapitata	fresh water (ISO medium)	PS	-COOH -NH_2 - non function alized	0.11 µm, 0.02 µm, 0.05 to 0.5 µm	10 mg/L	2 h		- influence of surface functionalization and water hardness (Ca^{2+}) on nanoparticle behavior - positively and neutral nanoparticles strongly attached to algae surface (*vs*. negatively charged) - medium hardness and particle concentration influenced interactions between nanoparticles and algae	59
Skeletonema costatum	seawater	PVC		1 µm, 1 mm	up to 50 mg/L, up to 2000 mg/L for mesoPVC	96 h, log phase		- growth inhibition up to 39.7% after 96 h and inhibition of photosynthesis for microplastics - no toxicity for mesoPVC - shading effect was not a reason for toxicity - adsorption	44
Chlamydomas reinhardtii	fresh water (TAP medium)	PP HDPE		400 µm - 1000 µm	1000 mg/L	78 d, log phase		- no impact on algae growth in the first days - no change in stress response gene expression after 78 d - after 20 d with PP higher production of EPS leading to hetero-aggregation - increased gene expression for sugar biosynthesis (mostly for HDPE) - both plastics were colonized	61
Thalassiosira pseudonana Dunaliella tertiolecta Chorella vulgaris	seawater (f/2 medium) fresh water (BG-11 medium)	PS	-COOH and uncharg ed	0.05, 0.5, 6 µm	25, 250 mg/L	72 h, log phase		- no effect on photosynthesis - growth decreased by up to 45% by uncharged particles only at 250 mg/L - size dependent effect (the smaller the more toxic)	43
Tetraselmis chuii	seawater	PE		1 - 5 µm	0.046 to 1.472 mg/L	96 h, log phase	Cu (0.02 to 0.64 mg/L)	- no significant effects of plastics on algal growth - Cu alone significantly decreased the growth (EC_{50} = 0.139 mg/L) - EC_{50} Cu + PE = 0.145 mg/L - no significant differences between the toxicity curves of Cu in the presence and absence of PE	54
Scenedesmus obliquus	fresh water (WC medium)	PS	-COOH	70 nm	up to 1100 mg/L	72 h, log phase		- nanoPS reduced the growth (-2.5%) and chlorophyll concentration in algae at the highest concentration - trophic chain experiment with *Daphnia magna*	38

Raphidocelis subcapitata	algal growth medium	PS-PEI (polyethyleneimine polystyrene)		55 nm, 110 nm	0.1 to 1 mg/L	72 h		- trophic chain experiment - toxicity from 0.40 mg/L to 416.5 mg/L according to considered species: *R. subcapitata* ≥ *D. magna* > *T. platyurus* > *V. fischeri*. - EC_{50} = 0.5 mg/L	40
Amphora sp *Ankistrodesmus angustus* *Phaeodactylum tricornutum*	seawater (artificial)	PS		23 nm	10, 100 µg/L	13 d, stationary phase		- PS induced acceleration in *Amphora sp*. EPS assembly - after 72 h PS-EPS aggregation reached equilibrium, forming microscopic gels of 4–6 µm in size - PS only cause moderate assembly kinetic acceleration for *A. angustus* and *P. tricornutum* EPS samples - effects of PS on EPS assembly kinetics mainly depended on the hydrophobic interactions of PS with EPS polymers	70
Scenedesmus *Chlorella*	fresh water (Alga-gro medium)	PS	$-NH_2$ -COOH	20 nm	80 to 800 mg/L	2 h		- preferential adsorption of positively charge PS (vs. negatively charged because of the interactions between plastic beads and cellulose components) - adsorption led to decreased photosynthesis (physical blockage of light and air?) - adsorption led also to increased ROS production	39
Macrophytes									
Lemna minor	hydroponics, Steinberg medium	PE, pristine and aged in wastewater		140 ± 80 µm, max 1000 µm	100 mg/L	7 d		- pristine particles: decreased root growth *vs.* aged particles: root growth decreased or no effect, depending on the aging medium - visible coating for aged beads, not characterized - adsorption of co-contaminant: possible in case of aged particles, not characterized	90
Myriophyllum spicatum, *Elodea sp.*	sediment amended with plastics + Smart and Barko medium	PS		50 to 190 nm, 20 to 500 µm	0.1, 0.3, 1, 3 and 10% sediment DW	21 d		- increased root biomass for both macrophytes for NPs - decreased shoot length for *M. spicatum* for NPs - no effect on biomass, relative growth rate, shoot length for both macrophytes, except a decreased shoot length for *M. spicatum* for MPs	91

Lemna minor	hydroponics, Steinberg medium	PE, sharp and smooth surface		30 - 600 µm	10 to 100 mg/L	7 d		- no effect on specific leaf growth rate and on photosynthetic pigments - decrease in root growth due to mechanical blocking - reduced root cell viability for sharp particles	89
Terrestrial plants									
Vigna radiata	soil	PS		20 nm	0, 10, 100 mg/kg	10 d		- decreased root growth - particle accumulation in leaves - trophic transfer to snails	121
Oryza sativa	hydroponics	PS, PTFE		10 µm	0.04, 0.1, 0.2 g/L	10 d	As 1.6, 3.2, 4 mg/L	- PS and PTFE affected transpiration and stomata inhibiting root vigor, lowering photosynthesis and decreasing biomass - As relatively more toxic than microplastics - low doses of plastic reduced the toxicity induced by As but higher doses increased the negative effects	112
Allium cepa	germination test and early development	PS		83 nm	0.01, 0.1, 1 g/L	72 h		- dose dependent decrease of root length - decrease of mitotic index at 0, and 1 g/L - frequency of abnormal metaphases and ana/telophases increased with the dose - cytological abnormalities at all concentrations - non monotonic oxidative stress - dose-dependent H_2O_2 production in the root epidermis, cortical cylinder and vascular tissues - internalization of PS by cells and accumulation in vacuoles, cytoplasm and less often in the nucleus - accumulation of high density bodies in the cytoplasm	115
Triticum aestivum	hydroponics	PS, PTFE		87 nm	0, 10 mg/L	3 weeks	Cd 0, 20 µM	- presence of PS reduced Cd contents in leaves and alleviated Cd toxicity - alteration in the morphology of root tips - increase in the activity of CAT and POD vs decrease in SOD - reduction in Fe, Mn and Cu concentrations - PS increase MDA in leaves but PS-Cd showed less MDA than Cd alone - PS increased carbohydrate and aminoacid metabolisms and promoted the formation of long lived radicals	117
Arabidopsis thaliana	agar, hydroponics, soil	PS	- SO_3H - COOH - NH_2	55, 71, 120, 220 nm	agar : 10, 50, 100 µg/L	agar : 10 d hydroponics :		- agar: decrease of root growth (dose and charge dependent + overregulation of peroxidases and plant pathogen genes +	107

					hydroponics: 50 µg/L soil : 0.3, 1.0 g/kg	7 d soil : 7 weeks		H_2O_2 accumulation in the root tips and root maturation zone + accumulation and toxicity of PS-NH_2>>PS-SO_3H + alterations in the morphology of root maturation zone - hydroponics: positively charged mainly accumulated in stele and maturation zone of roots + negatively charged mainly accumulated in root epidermis and root hairs + increase in exudation of oxalate PS-NH_2>>PS-SO_3H + accumulation in the epidermal cells and catheter of the xylem of roots for 120 nm negatively charged - soil: decrease of biomass and chlorophyll contents at high dose	
Arabidopsis thaliana, Triticum aestivum	Germination test and early development	PS		40 nm, 1 µm	0.029 g/ L	*T. aestivum*: 5 d, *A. thaliana*: 12 d		- accumulation in the root surface, especially root tip but no evidence of internal uptake - no effects on seed germination, root length or plant height	118
Lolium perenne	soil (+earthworms)	HDPE, PLA, acrylic and nylon fibers		HDPE: 102.6 µm, PLA fibers: 65.6 µm, >2 mm-16 mm	0.1 % particles w/w, 0.001% fibers w/w	30 d		- reduced germination - PLA decreased shoot length - HDPE increased root biomass	141
Lepidium sativum	germination test	not specified		50, 500 and 4800 nm	10^3, 10^4, 10^5, 10^6 ,10^7 particles/ mL	72 h		- dose and size dependent delay on germination - reduced root growth after 24 h in 50 nm treatment and increase in 500 nm but no differences at 48 and 72h - accumulation of particles in the testa of seeds and in the radicles, shoots, leaves and specially root hairs of seedlings.	103
Allium fistulosum	soil	PA, PES fibers, PEHD, PP, PS, PET		PA: 15-20 µm, PES fibers: length 5000 µm/ diameter 8 µm, PEHD: 643 µm,	2% w/w particle, 0.2 % w/w fibers	1.5 months (after 1.5 month soil incubation)		- PES and PS increased root biomass - PA decreased root/leaf dry mass, PES, PET, PP, PEHD and PS increased it - increase in total root length, decrease in root diameter and decrease in total root area - PA decreased root tissue density, PS and PES increased it - PES and PP increased mycorrhiza root colonization, PET decreased it	142

				PET: 376 µm, PP: 816 µm PS: 754 µm				- alterations in dry weight related to water contents (PA decrease in dry biomass with increase in water content, the opposite for PES) - PA decreased C:N ratios and PES increased it - PA increased aboveground leaf and PES increased belowground bulb - toxicity(fibers>>microplastics) (PA>PEHD, PP, PS and PET)	
Allium cepa	hydroponics	PS		100 nm	5, 10, 15, 20, 25 µg/mL	3, 6, 12, 24 h		- dose response decrease of mitotic index and increase in chromosome aberrations - decrease of root growth	[119]
Vicia faba	germination test	PS		100 nm, 5 µm	10, 50, 100 mg/L	48 h		- exposure to 5 µm particles decreased root length and relative root elongation at 50 and 100 mg/L - 100 nm decreased relative root elongation at 100 mg/L - 5 µm treatment at 100 mg/L and all 100 nm treatments decreased root weigh - higher uptake of 100 nm particles than 5 µm ones - SOD increased in all treatments. CAT activity showed significant change in nearly all treatments - no effect on MDA for the 5 µm treatments, decrease in the low dose of 100 nm and increase at high dose. - for 100 nm, dose dependent increase in micronucleus frequency - genotoxic and oxidative damage (nano>>micro)	[104]
Plantago lanceolata	artificial soil (quartz, sand,vermiculite)	EPDM		0.5–2.5 mm	0, 0.25, 0.5, 1,2, 4, 8, 16, 32, 64 % v/v	7 weeks		- reduced plant survival - non monotonic response in total biomass - decrease in root weight	[101]
Alopecurus pratensis, Festuca guestfalica, Lolium perenne, Galium album, Leucanthemum ircutianum, Leucanthemum vulgare,	artificial soil (mixture sand and vermiculite)	EPDM		0.5–2.5 mm	5 % v/v	1 week		- reduction of aboveground biomass and root weight and increase in root length but reduced weight - reduced competitive interactions	[101]

Prunella vulgaris, Daucus carota									
Triticum aestivum	Germination test + hydroponics	PS		100 nm	0.01, 0.1, 1, 10 mg/L	5 d, 21 d		- germination rate not affected - improved seed vigor at 0.01 and 10 mg/L - increase in root elongation and water imbibition - increase in chlorophyll content at 0.1 and 1 mg/L and of shoot biomass at 0.01 and 0.1 mg/L - root biomass increased at 0.01, 0.1 and 1 mg/L but reduced shoot to root ratio - root length, average diameter, surface area and root volume increased at all doses except at 10 mg/L - dose dependent increase in C and N contents - reduction in micronutrient concentration (Fe, Mn, Cu and Zn) - leaf metabolomic alterations (energy and aminoacid metabolims) - uptake and transport to the shoots	[117]
Thinopyrum junceum, Glaucium flavum	germination test	non biodegradable bags (HDPE), compostable bags: non exposed, beach exposed, marine exposed		bag leachates	Water-to-plastic ratios of 100, 10 and 5	until germination		- alteration of germination time, radicle and hypocotyl length and in bellow to aboveground length ratio depending on the treatment and the plant species - low concentration of leachates from marine-exposed bags increased seed germination - seedling anomalies in high concentration treatments	[110]
Triticum aestivum	soil (+earthworms)	LDPE, starch-based biodegradable		6.92 x 6.10 mm for LDPE, 6.98 x 6.01 mm for Bio and micro (12.5% of 1 mm to 500 µm,	1% w/w	2 and 4 months		- changes in bacterial composition of the rhizosphere and increase in emitted volatiles for the Bio treatment - increase in pH - increase in C:N ratios (micro>>macro, LDPE>>Bio)	[108]

				62.5% of 500 µm to 250 µm and 25% of 250 µm to 50µm)					
Murraya exotica	hydroponics	Styrene maleic anhydride		12 nm	1.8, 5.5, 18, 55, µg/mL	0 - 8 h		- lignified epidermis is the the active tissue for the uptake (apoplastic transport) - translocation to the shoots through the sap	[120]
Triticum aestivum	soil (+earthworms)	LDPE, starch-based biodegradable		6.92 x 6.10 mm for LDPE, 6.98 x 6.01 mm for Bio and micro (12.5% of 1 mm to 500 µm, 62.5% of 500 µm to 250 µm and 25% of 250 µm to 50µm)	1% w/w	2 and 4 months		- decrease in root and total biomass, including fruit biomass - increase in shoot to root ratio. In the Bio treatment, decrease in number of leaves and leaf area - thinner stems (negative effect micro>>macro plastics, Bio>>LDPE) - presence of earthworm alleviated toxicity	[143]
Brassica rapa subsp. Chinensis, Cucumis sativus, Solanum melongena, Phaseolus vulgaris, Capsicum annuum, Raphanus sativus, Solanum lycopersicum	survey greenhouses, open fields and markets	phatalate esters			0.26-2.53 mg/kg			- concentration of phtalates in vegetable leaves between 0.95 and 8.09 mg/kg	[124]

Brassica rapa Chinensis	soil	film mulch PBAT		2 x 2 x 0.1 cm^3	0,6 % (w/w)	1 month		- non significant effects on plant growth	111
Gossypium hirsutum	field study	film mulch (polymer not specified)		0-25, 25-100, 100-200 cm^2	250, 500, 1000, 1500, 2000 kg/hm^2	5 months		- reduction in the yield	99
Capsicum annuum, Cucumis sativus, Brassica oleracea, Raphanus sativus, Brassica chinensis, Lactuca sativa, Chrysanthemum coronarium, Apium graveolens, Spinacia oleracea, Brassica juncea	survey greenhouses	phatalate esters			2.56 - 2. 23 mg/kg			- concentration of phatalates in vegetables between 0.51 and 7.16 mg/kg - accumulation in leaves and stems	123
Allium cepa	germination test	nanocomposites of PLA and nanoclays		leachates from compostin g	50 g nanocomposite/ 300 g compost, 100 g of this mixture in 400 mL water	until 2cm root		- inhibition of cell division and mitosis - higher frequency of chromosomal breaks and micronucleus	109
Zea mays	soil	PE		granules, not specified	0.83, 1.67, 2.50, 3.33 %	8 weeks		- increase in height for low dose treatment decrease for the others - dose-dependent decrease of stem girth	114

Benincasa hispida, *Cucumis sativus*, *Cucurbita moschata*, *Brassica parachinensis*, *Momordica charantia*, *Lactuca sativa*, *Capsicum frutescens*, *Luffa cylindrical*, *Ipomoea aquatica*, *Lycopersicon esculentum*	soil	film mulch DEHP		pieces of 12 cm diameter and 0.012 mm thickness	0,6 % (w/w)	6 weeks		- significant concentrations of DEHP accumulated by *Benincasa hispida*, *Cucumis sativus*, *Cucurbita moschata* and *Brassica parachinensis* - concentrations raised in this later are close to the dietary daily intake limit - no phytotoxicty evaluated	122

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1256 Table 2. Summary table of the main techniques used for plastic characterization

Technique	Physical principle	Type	Chemical specificity	Resolution	Detection limit	Sample preparation	Limitations	Obtained information	Ref.
scanning electron microscopy (SEM)	electron scattering	2D microscopy	elemental only if coupled to X-ray emission detection (EDS)	few nm	particle/ particle aggregate	chemically fixed, dehydrated, resin embedded sample and coated to minimize surface charging and improve resolution	- no chemical specificity - elemental specificity if coupled to EDS	- particle localization → mainly surface association on phytoplankton based on shape but not specific	33,35,36,38,39,41,42,44,53,57,60,62,69,70,73,74,81
transmission electron microscopy (TEM)	electron transmission	2D microscopy	- elemental only if coupled to EDS - crystal line phase if coupled to electron diffraction	nm	particle/ particle aggregate	chemically fixed, dehydrated, resin embedded sample and sectioned to 50-100 nm thickness to allow electron transmission	- no chemical specificity - elemental specificity if coupled to EDS - crystalline phase if coupled to electron diffraction	- particle localization and cell internalization in *A. cepa* based on shape but not specific	115
fluorescence microscopy	UV-Vis light absorption and emission	2D microscopy	fluorescence specific to the labelled particle or staining dye molecule	100s of nm	particle aggregate	- sectioning for plant tissues - applied on model fluorescent plastics or stained with specific dyes (e.g. oil red EGN, Eosin B, Hostasol Yellow 3G, Nile red and Rose Bengal)	- limited to fluorescent labelled plastic materials or based on staining protocols affected by affinity to the plastic of interest *vs.* sample organic component (e.g. lignins, carotens in plants)	- interaction of micro and nanoplastics with photosynthetic organisms - evidence of aggregation with phytoplankton - internalization of micro and nanoplastics in terrestrial plants	33,34,37,56,69,70,74,107,115,103
confocal laser scanning microscopy (CLSM)	UV-Vis light absorption and emission	3D microscopy	fuorescence specific to the labelled particle or	100s of nm	particle aggregate	applied on model fluorescent plastics or stained with specific dyes	limited to fluorescent labelled plastic materials or based on staining protocols affected	- 3D information is obtained - nanoplastic association with microalgae	42,53,60,62,69,70,74,104,103,117

			staining dye molecule				by affinity to the plastic of interest *vs*. sample organic component (e.g. lignins, carotens in plants)	- presence of microplastics in the seed capsules of terrestrial plants, and at later stage in the root hairs (*L. sativum)* - uptake of plastic nanoparticles in roots (*V. faba*, *T. aestivum*)	
two-photon excitation (time resolved) (TPE)	UV-Vis light absorption and emission	2D microscopy	fluorescence specific to the labelled particle	60 µm	large particle aggregates	no sample preparation	low resolution and applied on long fluorescence life time labelled plastics (e.g. Eu doped)	- non-invasive technique applied *in vivo* - used for kinetic studies of Eu marked nanoplastics in *M. exotica*	[120]
fluorescent lifetime imaging	UV-Vis light absorption and emission	2D microscopy	fluorescence specific to the labelled particle	100s of nm	particle aggregate	no sample preparation	applied on long fluorescence life time labelled plastics (e.g. Eu doped)	- cell wall deposition of nanoplastics in the lignified epidermis of *M. exotica*	[120]
Fourier transformed infrared spectroscopy (FTIR)	infrared light absorption of molecular bond dipoles	2D microscopy/ bulk solids	chemical functional groups (polar bonds)	10 µm	particle aggregate	- bulk solids require extraction, filtration or separation protocols - microscopy requires resin embedding and microtome sectioning	- water interference, samples must be IR active and the acquisition is slow if the objective is to identify individual micro-particles and obtain FTIR spectra.	- chemical changes in the plastic material induced by aging in the environment - carbonyl oxidation in lead chromated microplastics - photo-oxidative degradation in some microparticles	[62,67,81]
Raman spectroscopy	inelastic light scattering from chemical bonds	2D microscopy/ bulk solids	chemical functional groups	300 nm	particle aggregate	- bulk solids require extraction, filtration or separation protocols - microscopy requires resin embedding and microtome sectioning.	- low scattering yield affects detection limit in bulk solid analysis - sample autofluorescence complicates detection in	- microplastic structure remain stable during aging (4 weeks at 50% humidity, 60°C, and 1200 W m^{-2} irradiance)	[67]

						- confocal Raman microscopy can be applied in non-sectioned samples	microscopy applications		
flow cytometry	light absorption and emission	bulk suspension	fluorescence specific to the labelled particle or staining dye molecule	individual cells	aggregates on cells	suspension dilutions	- limited to fluorescent labelled plastic materials or based on staining protocols affected by affinity to the plastic of interest *vs.* sample organic component (e.g. lignins, carotens in plants)	- microplastic quantification in microalgal cultures - distinction between free suspended microplastics, hetero-aggregates, and microalgae	[51]
dynamic light scattering (DLS)	UV-Vis light scattering	bulk suspension	no chemical information	hydrodynamic diameter 0.7 - 10 µm	dispersed particles at ppm level	suspension dilutions	- average hydrodynamic size and surface charge - does not provide reliable results on polydispersed suspensions	- mainly used for pre-characterization of particles in exposure media	used in 60% of the studies cited in this review
electron paramagnetic resonance (EPR)	magnetic electron spin transitions	bulk suspension	electronic structure in paramagnetic compounds	n/a	ppm	suspension dilutions	- average paramagnetic electronic transitions - use of paramagnetic compounds	- investigate the associations of marine phytoplankton and plastics in seawater (*S. marinoi*, *L. polyedrum*)	[68]
high performance liquid chromatography (HPLC)	ionization and mass spectrometry	bulk solution	mass to charge ratio of ionized fragmented compound	n/a	sub-ppm	digestion/extraction, filtration and/or separation protocols	- average chemical composition - destructive technique	- identification of plasticizer and stabilizer molecules in leachates of microplastics - high-throughput screening of leachate molecules from plastics	[56,65]

gas chromatography (GC)	ionization and mass spectrometry	bulk solution	mass to charge ratio of ionized fragmented compound	n/a	sub-ppm	digestion/extraction, filtration and/or separation protocols	- average chemical composition - destructive technique	- identification of marine water leachates produced by different types of microplastics	64
inductively coupled plasma (ICP-MS)	ionization and mass spectrometry	bulk solution	elemental composition / element isotopes	n/a	sub-ppm	digestion/extraction, filtration and/or separation protocols	- average chemical composition - destructive technique	- elemental associations with plastics (Cu, Zn and Sr) - potential use for indirect detection of plastics (doped with metals)	64,65,129,130
pyrolysis gas chromatography (py-GC-MS)	ionization and mass spectrometry	bulk solid	mass to charge ratio of ionized fragmented compound	n/a	sub-ppm	isolation of plastic particles/ bulk solid	- average chemical composition - destructive technique	- identification of polymer types and additives in marine microplastics	137

1257