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

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► To cite this version:

Camille Larue, Geraldine Sarret, Hiram Castillo-Michel, Ana Elena Pradas, Del Real. A critical review on the impacts of nanoplastics and microplastics on aquatic and terrestrial photosynthetic organisms. *Small*, 2021, 10.1002/sml.202005834 . hal-03373004

HAL Id: hal-03373004

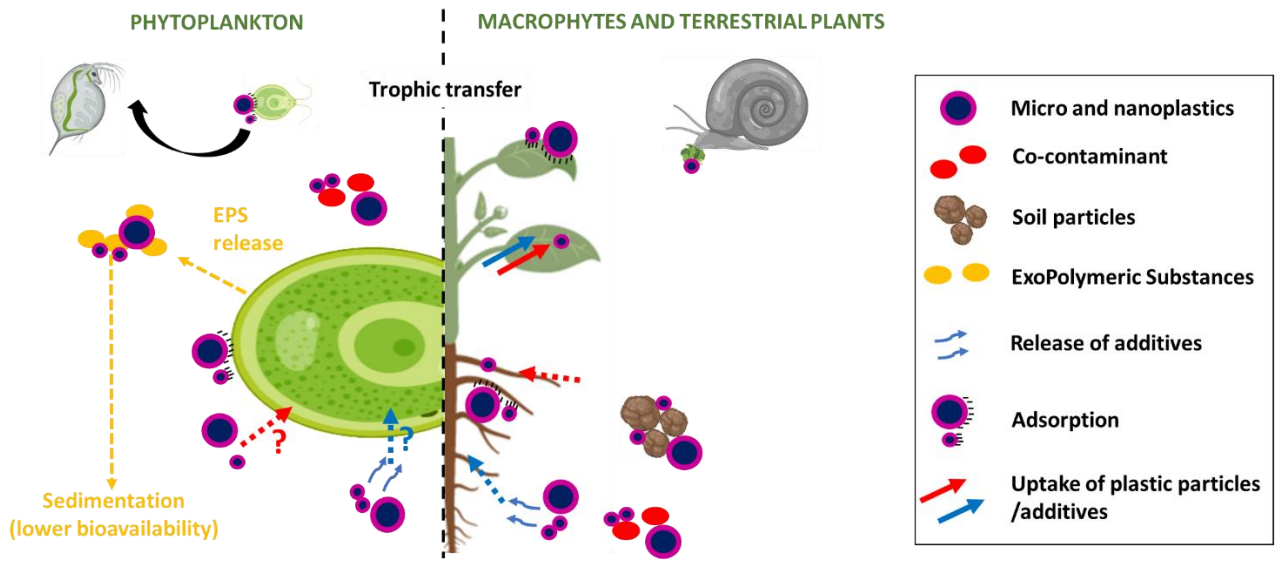
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Submitted on 11 Oct 2021

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35 **TOC.** This critical review synthesizes the knowledge acquired on photosynthetic organisms both in
36 aquatic and terrestrial compartments as well as the analytical techniques required for these studies.
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39

40 1. Introduction

41

42 The word “plastics” gathers a wide range of synthetic polymer materials made of long organic
43 chains deriving mainly from fossil fuel. Plastic production also implies the incorporation of
44 additives for the processing or to improve plastic properties according to their future use; among
45 them are catalysts, polymerization solvents, a wide range of organic and inorganic compounds,
46 antioxidants or antimicrobials. Additives can represent up to 50% of the final material in some
47 cases¹.

48 Since their discovery at the end of the 19th century, their use in our everyday life has rapidly
49 and exponentially increased. Indeed, the annual production went from 1.5 million tons in the
50 50’s to close to 335 million tons in 2016². They are used in a wide variety of sectors such as
51 packaging, building, transportation, renewable energy, medical devices or sport equipment².
52 The most commonly encountered plastics in commercial products are thermoplastics and,
53 among those, polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene
54 (PS) and polyethylene terephthalate (PET). These represent approximately 90% of world
55 production³.

56

57 Unfortunately, our waste management systems are not efficiently handling plastic waste; every
58 year only 6% at the world level is recycled and about 28% is directly disseminated in the
59 environment, the rest being incinerated or placed in landfills^{2,4}. Since plastics are barely
60 degradable, they are persistent⁵ and are found in all environmental compartments, from
61 mountains⁶ to deep sea and even in remote locations far away from any anthropogenic activities
62 such as in the Antarctic and Arctic oceans (reviewed in ^{4,7}). Plastic waste has been first identified
63 and studied in oceans (mainly in ocean gyres), however some analyses revealed a similar level
64 of contamination in continental freshwaters⁸ and in soils⁹. These plastics mainly originate from
65 land sources (80%) which can be as diverse as mulching in agriculture, spreading of sewage
66 sludge or leaks from discharge sites, and transit through rivers to finally reach oceans in which
67 they represent from 60 to 80% of the marine debris and about 10 million tons^{4,10}. Among the
68 most widespread plastics found in the environment are PE (about 90%), PP and PS and mostly
69 encountered in the form of fibers¹¹.

70

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

74 appearance of carbonyl functional groups leading to an overall negative surface charge of the
75 particles¹². Weathering also has consequences on particle size distribution. Indeed, in the
76 environment several classes of plastics have been defined: macroplastics (<1 m), mesoplastics
77 (2.5 cm - 5 mm), microplastics (1 μm - 5 mm) and nanoplastics (<1 μm)¹³. The most commonly
78 sampled size fraction in the environment is centered around 1.5 mm¹¹. The persistence of
79 plastics in the environment can also lead to the adsorption of diverse biological and chemical
80 components such as heavy metals or organic compounds (polycyclic aromatic hydrocarbons,
81 polychlorinated biphenyls, pharmaceuticals or pesticides among others)^{14,15}. These adsorption
82 processes have been shown to be enhanced on weathered plastics in comparison to pristine
83 ones¹⁶. Finally, the weathering process can also lead to the release of additives incorporated in
84 plastics during the processing since most of them are not covalently bound to the polymer
85 matrix and can migrate towards the surface¹⁷.

86

87 Quantifying plastics in the environment is challenging; adequate analytical techniques are being
88 developed. Consequently, quantitative data is still scarce. Furthermore, the comparison between
89 concentrations reported in different sites is most of the time difficult since they are presented
90 in different units: items or mass per volume, per mass or per surface. Concentrations in soils
91 were found ranging from 55.5 to 67500 mg/kg over different sites (reviewed in ¹⁸). In freshwater
92 aquatic systems, plastic concentrations also greatly vary between in average 4 items/L to as
93 high as 25.8 items/L in Taihu lake in China¹⁹ or 419 items/L in Saigon river (Vietnam)²⁰. Few
94 data expressed in mass/L are also available reporting concentrations from 0 to 700 $\mu\text{g/L}$ in the
95 Danube River²¹ or up to 1.56 mg/L in an urban lake in Texas²². Overall, in seawater, plastic
96 concentrations are about 3 orders of magnitude below those found in freshwaters with average
97 concentrations below 0.01 item/L²⁰ but could reach 1770 items/L in most polluted area such as
98 Southern North Sea²³.

99 The number of publications about the impact of micro and nanoplastics in the environment is
100 rapidly increasing and good reviews have been published already on freshwater
101 ecosystems^{4,20,24,25}, marine ecosystems^{20,25-28}, terrestrial ecosystems^{18,29,30}, agroecosystems⁹
102 and on analytical methods³¹.

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

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

113

114 2. Phytoplankton

115

116 If this group represents only about 1% of the photosynthetically active biomass of the world, it
117 is responsible for about 45% of the world primary production, by fixing half of the atmospheric
118 CO₂ and about a third of “anthropogenic” carbon over the last century³².

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

124

125

126 2.1. Influence of plastic particle size

127

128 Overall, most of the studies investigated the impact of nanoplastics on phytoplankton and
129 among them nearly 90% suggest that they have toxic effects³³⁻⁴⁶. For particles having size
130 between 1 and 10 μm, 90% of the concerned studies show no toxicity^{43,47-54}. Finally, for bigger
131 particles mostly no impact was reported or even in some cases an enhanced algal growth^{55,56}.

132 According to these findings, some authors suggested to introduce a threshold size for plastic
133 ecological risk assessment and to consider particles above 100 μm as low risk for microalgae³⁴.

134

135 Some studies evidenced a size dependent toxicity of plastic particles to
136 phytoplankton^{43,44,47,56,57}. For instance, Li *et al.*, exposed the microalga *Chlorella pyrenoidosa*
137 (green alga) to PS particles and found EC₅₀ of 6.9, 7.2 and > 64 mg/L for 0.1, 0.55 and 5 μm
138 particles respectively⁴⁷. The same result was evidenced with the green alga *Dunaliella*
139 *tertiolecta* (0.05 μm PS particles were more toxic than 0.5 μm and 6 μm)⁴³. This size dependent
140 effect was also observed for PVC particles and diatoms^{44,57}. However, few other studies show

141 similar EC₅₀ values for three green algae (*Chlorella*, *Raphidocelis subcapitata* and *Scenedesmus*
142 *obliquus*) exposed to PS particles ranging from 55 nm to 2 μm^{35,40,58}.

143
144 EC₅₀ reported for phytoplankton vary between 0.5 to 13 mg/L for PS nanoparticles^{33,40,47}. The
145 highest reported plastic concentration in the aquatic environment so far is 1.56 mg/L²² which
146 suggests that plastic pollution represents a risk for phytoplankton populations in these highly
147 contaminated area.

148
149

150 2.2. Influence of plastic particle surface charge

151
152 According to the literature, positively charged particles appear more toxic to phytoplankton
153 than negatively charged particles^{33,39,58–60}. Positively charged particles are usually more stable
154 in the medium³³, and are attracted by phytoplankton cell walls through electrostatic interactions,
155 since cell wall components such as cellulose bear negative charges^{39,59}. Negatively charged
156 particles tend to form large agglomerates and to interact less with phytoplankton. For instance,
157 positively charged PS nanoparticles (50 nm) were characterized in natural seawater with a
158 hydrodynamic diameter <200 nm and led to a decreased growth of *D. tertiolecta* with an EC₅₀
159 of 13 mg/L. On the opposite, negatively charged PS nanoparticles (40 nm) agglomerated in
160 seawater (> 1 μm) and no toxicity symptom was detected up to 50 mg/L³³. Likewise, uncharged
161 particles tended to be more toxic than negatively charged ones with a 45% decrease in growth
162 of the same green alga at 250 mg/L while no toxic effect was detected with the negatively
163 charged PS particles⁴³.

164 Over the different papers using functionalized particles, only one (over 8) reported toxicity of
165 PS-COOH particles (70 nm) towards *S. obliquus*, however this toxicity was only visible at the
166 highest exposure concentration: 1.1 g/L which is extremely high³⁸. And the only study reporting
167 no toxicity of PS-NH₂ particles to *Chaetoceros neogracile* (diatom) mentioned the fact that
168 these particles once in the exposure medium had negative charges⁵³.

169
170

171 2.3. Influence of plastic particle chemical composition

172

173 Very few articles compared the impact of plastics of different chemical nature. Some of them
174 showed no toxicity, possibly related with the fact that they used particles with size
175 $> 100 \mu\text{m}^{34,61}$. The most comprehensive study was performed by Zhu *et al.* and suggested that
176 at equivalent diameter (74 μm) PVC could be more toxic than PS and PE with respective algal
177 growth reduction of 29.0%, 24.7%, and 25.3% after 96h of exposure at 100 mg/L⁵⁷. Indeed, in
178 the literature, PVC has been shown to be toxic in all the studies in which it has been used^{36,62}
179 except when its size exceeded 100 μm^{34} . In contrast, the few studies done on PE and PP
180 microplastics showed no toxicity^{34,49,50,54–56,61,63}. Following a species specific distribution
181 approach, some authors suggested that polymethylmethacrylate (PMMA) was less harmful than
182 PS nanoparticles for marine biota⁴⁵.
183 It seems that this difference in toxicity could be related to plastic leachate composition^{64,65}. For
184 instance, Capolupo *et al.* generated plastic leachates (PP, PET, PS, PVC, 1000 μm)⁶⁴ and
185 detected plasticizers, antioxidants, antimicrobials, lubricants, vulcanizers (between ng to
186 hundreds of $\mu\text{g/L}$) with highest amount of additives in the PVC leachates and the least in PE
187 leachates. Leachate composition varied according to the solution used (freshwater *vs.* seawater).
188 This was also confirmed in another study showing the importance for leachate composition of
189 solution pH and leaching time^{66,67}. All plastic leachates, except the one derived from PET,
190 inhibited algal growth with EC₅₀ of 1.62%, 23.5% and 64% (of the initial leachate) for PVC,
191 PS and PP, respectively.

192

193

194 2.4. Influence of phytoplankton type and exposure conditions

195

196 Several studies suggested a different sensitivity to plastics according to phytoplankton
197 species^{43,63,64,68}. For instance, EC₅₀ have been calculated for 3 marine microalgae exposed to
198 PMMA nanoparticles showing that the diatom species was the most sensitive (83.4 mg/L) and
199 green alga and haptophyte had similar sensitivity (132.5 and 123.8 mg/L, respectively).
200 Sometimes this difference was related to exopolymeric substance (EPS) production^{51,68–70}.
201 In addition, the phytoplankton growth phase (lag, logarithmic, stationary or death phase) used
202 for the exposure experiments can also affect the results. Some studies (less than 15%) compared
203 the sensitivity of phytoplankton populations when in stationary phase rather than in logarithmic
204 growth showing a lower sensitivity^{34,35,41,51,70}. For instance, PS nanoparticles (50 nm) decreased
205 growth, chlorophyll concentration and increased reactive oxygen species (ROS) production of
206 the diatom *C. neogracile* in logarithmic growth while the effect were mostly non detectable in

207 stationary phase⁴¹. Authors suggested that this difference can be related with the higher EPS
208 production by diatoms when in stationary phase that would lead to nanoplastic hetero-
209 aggregation and sedimentation decreasing plastic bioavailability in the water column. Indeed,
210 EPS secretion has been highlighted in several studies as a way to mitigate plastic
211 toxicity^{35,37,61,70} and can vary according to growth phase, algal species and the type of plastic
212 considered^{61,69,71}.

213 During exposure, the medium composition (ions, salinity, hardness, pH, organic matter) will
214 also influence plastic behavior through the formation of a corona around the particles^{37,59} and
215 thus influence plastic interactions with other components of the system⁵³. For instance, the
216 addition of humic acid (5 mg/L) in the exposure medium alleviated the toxicity of PS
217 nanoplastics (75 mg/L) to *S. obliquus* by decreasing the adsorption of PS particles to alga
218 surface⁵⁸.

219

220

221 2.5. Cocktail effects in presence of other contaminants

222

223 Some authors have investigated the toxicity of plastics together with other contaminants such
224 as heavy metals (Cu^{37,54,62}, Ti⁶⁰, Au⁴⁸, Pb⁷²) or organic compounds (dibutyl phthalate⁴⁷,
225 chlorpyrifos⁵⁰, glyphosate⁷³, phenanthrene³⁴, triclosan⁵⁷, procainamide and doxycycline⁵²).
226 Most of the time antagonistic effects were identified. Indeed, the other contaminant studied was
227 found adsorbed at the plastic surface and thus became less bioavailable to the exposed
228 organisms^{34,47,50,57,72,73}. Those interactions are controlled by plastic surface charge. For instance,
229 adding positively charged PS particles (6 µm) to TiO₂ nanoparticles enhanced their toxicity
230 (+20%) to *Chlorella vulgaris* (green alga) while adding negatively charged PS particles led to
231 a decrease of about 20% in toxicity⁶⁰. However, some studies reported no influence of the
232 addition of plastics on the toxicity of co-contaminants (Cu pollution)^{37,54}. Finally, in few cases,
233 the mixture appeared to be more toxic than the contaminants considered alone (mixture with
234 Au nanoparticles⁴⁸ or with pharmaceuticals⁵²) but the lack of experimental details (plastic size
235 or type) does not allow to go further in the explanation.

236

237

238 2.6. Pristine model versus environmentally aged plastic particles

239

240 So far studies investigating plastic toxicity to phytoplankton have mostly focused on pristine
241 particles, predominantly PS spheres. Actual plastic debris found in the environment have a large
242 variety of shapes (from round particles to fibers, mainly), size distribution and surface
243 properties. In addition, these properties are supposed to evolve with the aging, which will likely
244 result in different toxicities. So far, only one study comparing the impact of pristine plastic
245 particles and “environmental” plastics has been published. It demonstrated that the microalga
246 *S. subspicatus* was more sensitive to PE plastic coming from the North Atlantic gyre than to PE
247 plastic made from pristine reference material⁶³. The aged plastic contained higher
248 concentrations of metals (Ti, Al, Fe and Zn). Likewise, *C. vulgaris* growth was less impacted
249 by pristine PVC particles than by aged (2 months) PVC ones (35% growth inhibition vs. 28%
250 for pristine particles)⁶². FTIR showed that aged material suffered modifications of functional
251 groups: increased in hydroxyl groups and aromatic carbon-carbon double bond but decreased
252 carbon hydrogen bond. In another study, authors performed an accelerated aging step on PE
253 particles by exposing them in a xenon chamber to high temperature, humidity and light
254 irradiance conditions for up to 4 weeks⁶⁷. The aging step caused surface cracks and
255 fragmentation of the particles, increased their surface area and carbonyl contents, and promoted
256 the release of pigments. The 4-week aged particle leachates were more toxic to cyanobacteria
257 than the 2-week aged particle leachate and the non-aged particle leachate.

258

259

260 2.7. Toxicity mechanisms

261

262 Among the most discussed toxicity mechanisms in the literature is the particle adsorption on
263 the surface of the organisms leading to a physical blockage of light, gases and nutrients
264 decreasing growth and photosynthetic activity^{33,35,36,39,44,60,74}. As for other carbon based
265 nanomaterials, the involvement of shading effect has also been reported⁴⁴. Beside these physical
266 effects, some studies evidenced effects related to the chemistry of particles, inducing oxidative
267 stress^{42,60,62,75} and structural damages^{35,37,42,46,47,75}. A recent study suggests that for the smallest
268 particles (nanoplastics) the “chemical” effect predominates (increased ROS production,
269 decreased photosynthesis) while for bigger particles (microplastics) toxicity is mainly driven
270 by “physical” effects (limitation of exchanges with the medium)⁵⁸. Additionally, the additives
271 present in plastics, which can be released during aging, as well as the secondary metabolites
272 after aging of these additives, can be responsible for the toxicity^{64,76}.

273 Very recently, few articles investigating molecular processes modified after plastic exposure
274 were released^{75,77,78}. Overall they confirmed the impact of plastic particles (in these cases PS)
275 on genes involved in photosynthesis (downregulation)⁷⁸ and in oxidative stress regulation
276 (upregulation)⁷⁵. One of the known consequences of oxidative stress is the impairment of
277 microtubule polymerization. Ripken *et al.*, observed a massive upregulation in genes involved
278 dynein-related proteins, dynein being a microtubule-associated motor protein implied in
279 cytoskeletal dynamics suggesting that the organism was facing oxidative stress⁷⁸. New findings
280 were also suggested such as the impact on fatty acid synthesis. For instance, KCS gene
281 expression was upregulated in *Euglena gracilis* possibly leading to higher extracellular
282 secretion and hetero-aggregation phenomenon between algal cells and plastic particles⁷⁵.
283 Likewise, in *Cladocodium*, after PS exposure 4 genes encoding proteins found in cell surface
284 receptors were up-regulated. These genes control cell dissociation and thus possibly influence
285 adhesion between neighboring cells and extracellular matrix composition and could explain
286 changes in cell aggregation⁷⁸. Cell division was also impacted with a decrease in the expression
287 of CTR1 gene related to the mitogen-activated protein kinases (MAPK) signaling pathway in
288 *E. gracilis*⁷⁵ and a decrease of 6 genes with RCC1 (regulator of chromosome condensation) and
289 3 genes with CDC domains in *Cladocodium*⁷⁸. Finally, gene expression was altered to different
290 extent between 2 microalgae confirming different sensitivities according to species⁷⁸.

291

292

293 2.8. Accumulation and trophic transfer

294

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

297 However, positively charged particles tend to adsorb on phytoplankton surface. As these
298 organisms represent the first level of trophic chains, it suggests that adsorbed plastic can be
299 transferred to the next trophic level. This has been confirmed by few studies showing the
300 transfer of particles from *Chlamydomonas reinhardtii* (green alga) to the crustacean *Daphnia*
301 *magna* and to the fish *Orizias sinensis* and *Zacco temminckii*⁷⁴. A higher toxicity of nanoPS to
302 *D. magna* (6 times higher mortality) has also been evidenced when these organisms were
303 exposed through their diet (*S. obliquus* exposed to nanoPS) than directly in the water column³⁸.

304

305

306 2.9. Plastic particle degradation by phytoplankton

307

308 If plastic can be toxic to phytoplankton, on the opposite some articles report plastics as a new
309 opportunity for organisms as vectors for long scale traveling or as a new carbon source available
310 for development. Analyses performed on plastic debris from the open sea have highlighted the
311 preferential development of microbial communities on their surface, called the plastisphere^{79–}
312 ⁸³.

313 Since the discovery in 2016, that some bacteria (in particular *Ideonella sakaiensis*) are able to
314 hydrolyze PET thanks to a specific enzyme called PETase, a lot of efforts has been put to take
315 advantage of micro-organisms for plastic biodegradation. Microalgae seem to be a good
316 candidate for this task through two main pathways: (i) the natural release of toxins and
317 ligninolytic and exopolysaccharide enzymes and (ii) the synthetic production of PETase⁸⁴.
318 Indeed, cyanobacteria such as *Phormidium*, when growing on a low density PE film, were able
319 to use about 4% of its carbon for their growth leading to the biodegradation of the film⁷⁷.
320 Likewise, *Anabaena spiroides* (cyanobacteria), *Navicula pupula* (diatoms) and *Scenedesmus*
321 *dimorphus* (green algae) were able to biodegrade LDPE in their respective growth media to
322 different extent: 8.18%, 4.44% and 3.74%, respectively⁸⁵. However, as highlighted by recent
323 reviews^{82,83}, biodegradation rates by microorganisms in real conditions are probably much
324 lower than in laboratory conditions, and the nature and potential toxicity of the degradation
325 products are still unknown.

326 On the other hand, some author used genetically modified microalgae to produce PETase. For
327 instance, a lysate of modified *C. reinhardtii* incubating at 30°C for 4 weeks led to the apparition
328 of dents and holes on a PET film and the detection of terephthalic acid (TPA) which is the fully
329 degraded form of PET⁸⁶. The same was also achieved by using *Phaeodactylum tricorutum*
330 (diatoms) cells in growing medium under more environmentally relevant conditions⁸⁷.
331 Moreover, it has been shown thanks to a trophic chain study (bacteria, microalga, zooplankton)
332 combined with the use of ¹³C labeled PE microparticles that the plastic could be partly
333 mineralized, used for cell growth and ¹³C was found in cell membranes of upper trophic
334 organisms⁸⁸.

335

336

337 3. Macrophytes

338

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

344

345 3.1. Plastic particle toxicity

346

347 Kalcikova *et al.* evaluated the effect of PE microplastics from cosmetic products on duckweed
348 (*Lemna minor*)⁸⁹. Specific leaf growth rate and concentration of photosynthetic pigments in
349 duckweed leaves were not affected, whereas root growth was significantly decreased with 50
350 mg/L PE microbeads; the authors suggested that this could be due to mechanical blocking.
351 Surface properties seemed to influence microplastic toxicity. Indeed, sharp particles reduced
352 the viability of root cells, while the impact of microplastics with a smooth surface was
353 negligible. The same group showed that microplastics incubated in wastewater at different
354 organic loads had different toxicity to *L. minor*⁹⁰. Van Weert *et al.* evaluated the impacts of PS
355 nanoplastics (50-190 nm) and microplastics (20-500 µm) on the macrophytes *Myriophyllum*
356 *spicatum* and *Elodea* sp., after exposure to plastic amended sediment⁹¹. Microplastics did not
357 produce consistent dose-effect relationships, and nanoplastics significantly induced an
358 increased root biomass compared to shoot biomass of *M. spicatum*. For *Elodea* sp., the biomass
359 and growth parameters tested were positively correlated to nanoplastic concentration.

360

361

362 3.2. Accumulation and trophic transfer

363

364 Published data concerns microplastics only. Some lab-based experiments investigated the
365 sorption of microplastics on macrophytes. Sundbaek *et al.* evaluated the adsorption of
366 fluorescent PS microplastics of 20 µm diameter on the surface of the macroalga *Fucus*
367 *vesiculosus*⁹². After 2h in filtrated seawater containing 2.65 mg/L microplastics (corresponding
368 to 597 items/mL), the algal surface contained on average 3.99 items/mm² (corresponding to
369 2787 items/g dry weight DW), but only 0.22 item/mm² after washing in filtrated seawater.
370 Gutow *et al.* did the same kind of experiment at lower concentrations of PS (1.4 to 55.7 items
371 of 10 µm diameter per mL), and also observed a sorption to the surface of *F. vesiculosus*, with

372 an average of 0.04 item/mm² after washing for the highest concentration⁹³. Similarly, Mateos-
373 Cardenas *et al.* showed that 10–45 µm PE microplastics can strongly adsorb to all surfaces of
374 *L. minor*, although photosynthetic efficiency and plant growth were not affected by
375 microplastics⁹⁴. To our knowledge, there is no data on the transfer of micro or nanoplastics
376 from the surface of macrophytes to the inside of plant tissues. Likewise, the possible uptake of
377 plastic particles from the sediment to the root system of macrophytes is not yet documented.
378 Beside these laboratory studies, the presence of microplastics on macrophytes collected in
379 natural environment was investigated. Goss *et al.* showed that 75% of marine turtle seagrass
380 (*Thalassia testudinum*) collected from an area impacted by urban discharges had encrusted
381 microplastics⁹⁵. Microfibers, beads, and other microplastic fragments were found adhered to
382 seagrass blade surfaces, often overgrown by periphyton and various epibiont organisms. This
383 suggests that these macrophytes may represent a pathway for plastics into marine food web.
384 Saley *et al.* found 8.65 ± 6.44 and 2.34 ± 2.19 items/g on the surface of the marine macrophytes
385 *Endocladia muricata* and *Pelvetiopsis limitata* growing in a remote marine reserve on the open
386 coast of California⁹⁶. Densities were higher in the herbivorous snail, *Tegula funebris*, at 9.91
387 ± 6.31 items/g, potentially due to bioaccumulation⁹⁶.

388

389

390 *Posidonia oceanica* is a macrophyte endemic of the Mediterranean sea, considered a key
391 species because it serves as habitat and food source for many marine organisms. Egagropiles,
392 which are spheroids made of dead biomass of *P. oceanica*, ubiquitous in the Mediterranean
393 coastal zone, also constitute a habitat and food source for the fauna and microfauna of sandy
394 beaches. Pietrelli *et al.* showed that 53% of these spheroids contained plastics⁹⁷. These plastic
395 particles may have sorbed to the leaf blades during plant growth and also after their death. Remy
396 *et al.* found that 27.6% of the digestive tracts of detritivorous invertebrates feeding on dead
397 biomass of *P. oceanica*, collected in a Mediterranean coastal zone, contained plastic fibers of
398 various sizes⁹⁸.

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

401

402 4. Terrestrial plants

403

404 One of the main identified routes for the entry of plastics into soils is the use of plastic mulch
405 in agriculture⁹. Hegan *et al.* simulated the accumulation of plastic residues after repeated field
406 mulching and they found that, after 121 years, the cumulative reduction yield per surface area
407 would be greater than the benefit of using the mulching⁹⁹. Traditional polymers used for this
408 purpose were low and high density PE (LDPE, HDPE) but in recent years, different
409 biodegradable polymers such as polylactic acid (PLA), poly-hydroxyalkanoate (PHA) or
410 cellulosic and starch based polymers are being tested. Besides, plastic residues from consumer
411 products arrive to soils through the application of sewage sludge as fertilizer and, to a lesser
412 extent, from atmospheric deposition, runoff from aquatic bodies and polymer seed coating¹⁰⁰.
413 Urban soils can also present plastic pollution due to the release of fragments or leachates from
414 plastics used in outdoor applications¹⁰¹.

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

424

425 4.1. Influence of plastic particle size, shape and surface charge

426

427 Only three studies have addressed the size dependent toxicity of plastic particles in plants. In a
428 pot experiment, Qi *et al.*¹⁰² found that microparticles (50 µm to 1 mm) of LDPE and starch
429 based biodegradable plastic produced slightly more negative effects on root and total biomass
430 of wheat plants than macroplastics (7 x 1.5 mm average) of the same polymers. A size and dose
431 dependent delay in germination was observed in *Lepidium sativum* exposed to 50, 500 and 4800
432 nm fluorescent PS particles¹⁰³. It was stronger for the biggest particles, which accumulated in
433 seed testa probably blocking water uptake. Seedlings 72h after germination showed decreased
434 root growth for 50 nm particles treatment but the opposite in 500 nm treatment. In another
435 study, seedlings of *Vicia faba* (36-48h after germination) were exposed to 5 µm and 100 nm PS
436 fluorescent particles at 0, 10, 50, and 100 mg/L for 48h¹⁰⁴. In this study, root biomass and length
437 were more decreased by microparticles while oxidative stress measured as malondialdehyde

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

441 Some studies also pointed to the shape, as one of the variables controlling plastic toxicity.
442 Synthetic microsized fibres (mixture of nylon and acrylic) induced similar effects on the
443 germination rate, shoot length and chlorophyll a/chlorophyll b ratios of *Lolium perenne* than
444 HDPE and PLA microparticles, even when applied at hundred times lower concentration
445 (0.001% vs. 0.1%)¹⁰⁵.

446 In another study, *Allium fistulosum* was exposed to polyester (PES) fibres (0.2% w/w), PA
447 beads and microparticles of HDPE, PP, PS and PET (2% w/w) for two months¹⁰⁶. PES fibres
448 induced the strongest effects on studied plants traits.

449 To date, only one study has addressed the influence of particle charge on plastic toxicity to
450 terrestrial plants. Positively charged PS-NH₂ nanoplastic were more toxic to *Arabidopsis*
451 *thaliana* than negatively charged PS-SO₃H nanoplastics, although the uptake was higher in the
452 latter¹⁰⁷.

453

454 4.2. Influence of plastic particle chemical composition

455

456 Qi *et al.* found that the biodegradable starch-based plastic induced stronger negative effects on
457 wheat than LDPE¹⁰². This was attributed to the components (37.1% pullulan, 44.6% PET and
458 18.3% polybutylene terephthalate (PBT)) of the biodegradable plastic that may be more toxic
459 than LDPE. The authors observed important changes in chemical and bacterial composition of
460 the rhizosphere in soils spiked with starch based plastic that may explain, at least in part,
461 observed effect on wheat plants¹⁰⁸.

462 Microparticles of PLA, were shown to be more toxic to *L. perenne* than microparticles of HDPE
463 with similar size at the same concentration¹⁰⁵. PLA decreased germination rate by 7% and
464 decreased shoot length after 30 days whereas the exposure to HDPE increased root biomass.

465 In another study, PA beads induced higher effects than the microparticles of PEHD, PP, PS and
466 PET¹⁰⁶. According to the authors, the lower toxicity induced by PEHD, PET, PP and PS
467 microparticles could be explained because they were mainly composed by H and C, whereas
468 PA beads contained N and additives that can alter soil chemistry.

469

470 Other studies have shown that leachates derived from plastic degradation may contain polymer
471 monomers and/or additives that can cause toxicity to plants. For instance, genotoxicity and
472 cytotoxicity were induced in *A. cepa* exposed to leachates from the composting of a new
473 biodegradable nanocomposite of PLA and nanoclays¹⁰⁹. Leachates from HDPE and
474 compostable plastic bags delayed and advanced the germination of the coastal dune species
475 *Thinopyrum junceum* and *Glaucium flavum*, respectively¹¹⁰. However, no negative effect has
476 been found in *Brassica rapa* var. *chinensis* exposed to the biodegradation products of PBAT
477 (butylene adipate-coterephthalate)¹¹¹.

478

479

480 4.3. Cocktail effects in presence of other contaminants

481

482 Only two studies have explored the combined toxicity of plastics with other pollutants in
483 terrestrial plants, both in hydroponics conditions. Dong *et al.* investigated the phytotoxicity of
484 PS and PTFE microparticles alone or in combination with As(III) over a range of concentrations
485 (0.004-0.2 mg/L plastics, 0.04-4 mg/L As(III)) for rice plants¹¹². Lian *et al.* investigated the
486 impact of PS nanoparticles (0, 10 mg/L) on the toxicity of Cd (0, 20 μ M) in wheat¹¹³. In both
487 cases, although the exposure to plastic particles alone caused negative effects on plants, their
488 combined exposure with As and Cd were less toxic than the exposure to As and Cd alone. The
489 alleviation of toxicity by plastic particles was mainly explained by the adsorption of the
490 pollutant to plastic surface that reduced total concentrations in plants. The reduction of root
491 activity caused by plastics may also decrease the uptake of As and Cd.

492 Besides this, PS nanoparticles increased the concentration of carbohydrates which may trap free
493 radicals reducing Cd toxicity to wheat¹¹³.

494

495

496 4.4. Toxicity mechanisms

497

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

500 Mechanisms underlying observed effects on plants can be indirect through changes in the
501 physical environment of soils which include alterations in the profile of water-stable
502 aggregates¹⁰⁵, soil bulk density, soil aggregation and water cycling¹⁰⁶. 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 soil¹¹⁴. 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 toxicity¹⁰¹, 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 uptake¹¹⁷. 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 effects¹¹⁸.

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 controls¹¹⁷. Lian *et al.* have suggested that PS nanoparticles can
536 reduce the concentration of free radicals reducing oxidative stress in wheat¹¹³. In both studies,

537 metabolomics analysis revealed an acceleration of carbohydrate and aminoacid metabolism
538 which can be a mechanism to cope with PS toxicity. However, Sun *et al.* found ROS production
539 and inferences in the regulation of genes related with oxidative stress in *A. thaliana* exposed to
540 PS nanoparticles¹⁰⁷.

541 Finally, nutrient imbalances, changes in root morphology and alterations in shoot to root ratio
542 have been reported^{106,113,117}.

543

544 4.5. Accumulation and trophic transfer

545

546 To date there is no information about the uptake of microparticles by plants and their potential
547 trophic transfer. Due to their small size, nanoplastics are more likely to be taken up by plants
548 and transported to the edible parts. The use of fluorescent PS nanoparticles have allowed to
549 show the absorption of nanoplastics by the roots of *L. sativum*¹⁰³, *V. faba*¹⁰⁴ and *A. cepa*¹¹⁵ and
550 the translocation to the shoots of *T. aestivum*¹¹⁷. By combining two photon excitation and time
551 resolved (TPE-TR) optical imaging, fluorescence lifetime imaging (FLIM) microscopy and
552 transmission electron microscopy (TEM), Zhang *et al.* showed the uptake and translocation of
553 styrene maleic anhydride nanoplastics (SMA-PNPs) in *Murraya exotica*¹²⁰. A recent article
554 just reported the trophic transfer of PS fluorescent nanoplastics from soils (100 mg/kg) to mung
555 beans and then to African giant snails¹²¹. These studies provide significant evidence of
556 nanoplastic uptake by plants. However, they did not provide quantitative information and they
557 used only model nanoplastics in quite unrealistic exposure conditions. This information does
558 not allow yet to assess the risk of transfer of plastic particles from terrestrial plants to the food
559 chain.

560 Still, pot studies¹²² and survey of commercialized vegetables from greenhouses^{123,124} showed
561 that vegetables and cereals accumulate high amounts of phthalates, leading to a high risk of
562 trophic transfer of these additives through the diet¹²³.

563

564 4.6. Plastic particle degradation in the rhizosphere

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

570 of weight for biodegradable PLA, not for PET¹²⁵, and the second one showed changes in
 571 physical and chemical properties of PE strips, but non reproducible losses of weight¹²⁶.

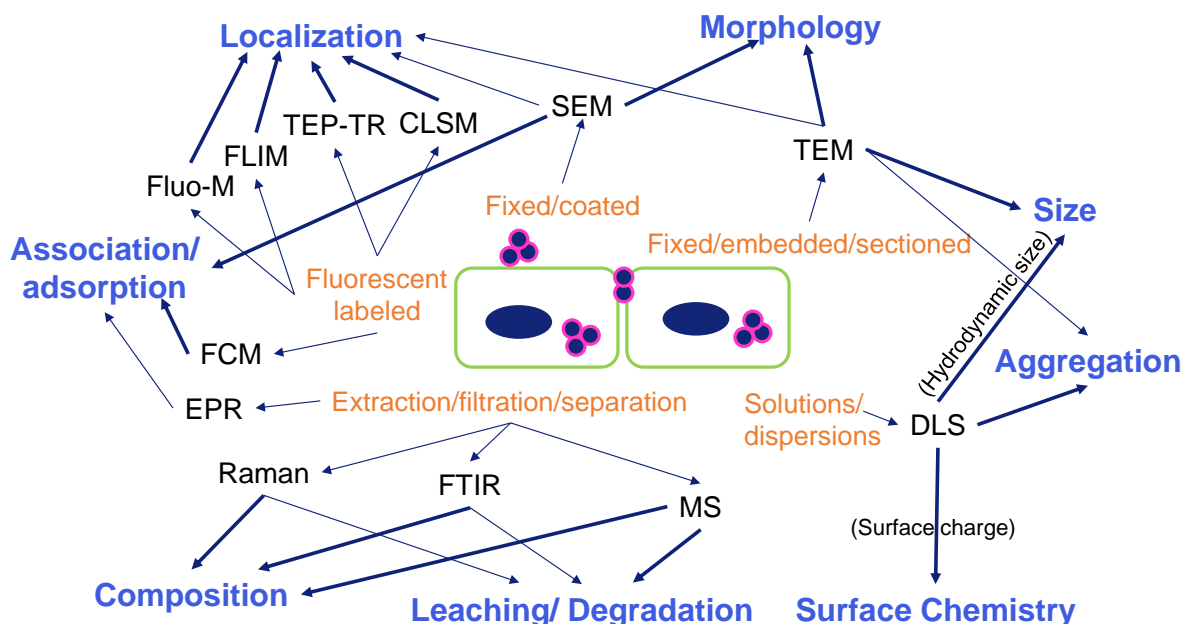
572

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

575

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

587



588

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

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

598

599 5.1. Microscopy

600

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

607

608

609 5.1.1. Electron based microscopy

610

611 High resolution images can be obtained using electron based methods in both scanning (SEM)
612 and transmission (TEM) modes. These methods are often used before interaction with
613 organisms in order to characterize size and morphology of micron and nano size plastics.
614 Electron microscopy applications in the characterization of plastic interactions with organisms
615 demands careful sample preparation protocols (dehydration, fixation, staining, coating) and the
616 contrast offered by plastics does not allow unambiguous identification. In order to gain
617 additional information, for example to discriminate plastic from inorganic particles, the use of
618 energy dispersive X-ray fluorescence detection is advised. Despite of this fact, electron based
619 microscopy has been widely used to characterize associations of micro and nanoplastics with
620 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
621 plants, evidence of cell internalization in *A. cepa* exposed to nanoPS has been obtained from
622 transmission electron microscopy images¹¹⁵.

623

624

625 5.1.2. Fluorescence microscopy

626

627 This microscopy method has been used to determine the interaction of micro and nanoplastics
628 with photosynthetic organisms and in particular has provided evidence of aggregation with
629 phytoplankton^{33,34,37,56,69,70,74} and internalization of micro¹⁰³ and nano^{107,115} plastics in
630 terrestrial plants. This is of utmost relevance to evaluate uptake mechanisms and to perform
631 risk analysis of food chain transfer in the environment through primary producers. However,
632 this method can only be applied on model fluorescent plastic materials and do not fully
633 represent the myriad of shapes, surface properties and sizes found in the environment. For this
634 reason and, due to the importance of environmental weathering in determining the fate of plastic
635 in the environment^{4,100}, results from this technique require careful interpretation when
636 extrapolated to real environmental plastic pollution scenarios.

637 Staining dyes (Oil red EGN, Eosin B, Hostasol Yellow 3G, Nile red and Rose Bengal) could
638 be used in staining protocols after exposure, the issue is that the affinity of these dyes for plastics
639 vs. biogenic material would complicate interpretation. Hence, existing protocols require
640 digestion, extraction, or filtration steps to remove biogenic material. These protocols may be
641 used for testing plastic presence in photosynthetic organisms from environmental samples¹²⁷.
642 However, this approach does not allow for precise cellular and tissue localization.

643

644

645 5.1.3. Laser scanning microscopy

646

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

659 *sativum* was identified¹⁰³. In *V. faba*, 100 nm PS particles were observed entering the roots¹⁰⁴
660 and in *T. aestivum* CLSM demonstrated uptake of PS nanoparticles in roots¹¹⁷.
661 Other techniques based on laser scanning are two-photon excitation and time-resolved detection
662 (TPE-TR) and fluorescence lifetime imaging (FLIM). Zhang *et al.* used TPE-TR as a non-
663 invasive approach to investigate kinetics and uptake mechanisms of Eu luminescent styrene-
664 co-maleic anhydride SMA-NPs in *M. exotica*¹²⁰. This novel approach exploits the differential
665 fluorescence persistence of the Eu luminescence and the autofluorescence of plant tissues (~1
666 ms *vs.* <100 ns) to set an appropriate time delay prior to Eu luminescence detection to avoid
667 interference from autofluorescence. It offers the advantage of wide field of view (32x32 mm²)
668 and large imaging depth that enables *in vivo* analysis in plants and other organisms. However,
669 the resolution obtained is 60 μm, for high resolution studies and for a more detailed localization
670 FLIM can be used in combination. Similarly, FLIM takes advantage of the longer fluorescence
671 lifetime of the Eu-luminescent nanoplastics to eliminate autofluorescence interference; the
672 resolution is sub-micrometric¹²⁸. Results from this study revealed inter cell wall deposition of
673 SMA-NPs in the lignified epidermis of *M. exotica* plants that represents a path of uptake and
674 translocation, and revealed similar accumulation kinetics at different positions along the plant
675 stems¹²⁰. Efforts should be made to produce luminescent plastic particles with characteristics
676 similar to those found in the environment. It is worth noting that the use of lanthanide doped or
677 metal doped particles would enable detection of these materials in complex biological systems
678 through the use of element sensitive techniques (bulk and microscopy) typically used for metal
679 engineered nanoparticles^{129,130}. For instance, PS-Pd nanoplastic have been recently used to
680 confirm the internalization of PS nanoplastics by *A. thaliana*¹⁰⁷.

681

682

683 5.2. Flow cytometry

684

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

692 hetero-aggregates, and microalgae⁵¹. These results underline the potential interactions of
693 phytoplankton cell and residual organic matter with microplastics and their influence on
694 bioavailability in aquatic systems.

695
696

697 5.3. Vibrational (micro)spectroscopy

698

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

708 FTIR produces a molecular fingerprint resulting from the change in dipole moment of chemical
709 bonds, and allows the detection of various types of plastics such as PS or PE due to
710 characteristic bands¹¹⁶. The commonly used analytical modes are transmission, attenuated total
711 reflection and reflectance. The three modes may be used in combination with a microscope and
712 the spatial resolution is around 10 μm for bench-top instrument with a global source (but it may
713 reach the diffraction limit if a synchrotron source is used)¹³². Though the direct detection of
714 plastic materials may be challenging, FTIR micro-spectroscopy may be used to assess
715 biomolecular changes in aquatic and terrestrial plants induced by exposure to these materials.
716 Resolution of FTIR micro-spectroscopy can reach nanoscale when coupled to an atomic force
717 microscopy tip (in thermal expansion or near-field mode) but this significantly complicates
718 sample preparation protocols¹³².

719

720 Raman provides a molecular fingerprint spectrum based on the polarizability of chemical
721 bonds¹³¹. Raman microscopy allows the characterization of microplastics down to 300 nm, in
722 some cases it can provide additional information about contained fillers and pigments, and it
723 does not suffer from water interference. However, some drawbacks are the weak nature of the
724 signals (approx. 10^{-8} photons reaching the sample produce Raman signal), increasing

725 measurement duration, and interference from sample autofluorescence, that depend on the
726 organism and the plastic material characteristics such as color, biofouling and degradation¹³³.

727

728

729 5.4. Dynamic light scattering (DLS)

730

731 DLS is currently the most widely used technique for the characterization of micro and
732 nanoplastic (size range from 0.7 nm - 10 μm) hydrodynamic diameter¹³⁴. This technique offers
733 affordability and easy usage with built-in automatized data analysis, but it has some limitations:
734 (i) it is a blind technique, so it can be used only on nanoplastic suspensions in water or in
735 nutrient solutions devoid of other types of particles, and (ii) it does not provide reliable results
736 for polydispersed suspensions (containing various particle size categories). The hydrodynamic
737 diameter is hence the most commonly reported parameter since it reflects the size of the particle
738 in suspension and includes coatings or surface modifications. The available instruments for
739 DLS offer also the capability to determine the electrophoretic movement of the particles when
740 applying an electric field, this allows to calculate the zeta potential value of the particles, *i.e.*
741 the surface charge of particles. This is a key indicator of the stability of the colloidal dispersion;
742 its magnitude denotes the degree of electrostatic repulsion between adjacent similarly charged
743 particles in the dispersion. This technique has been applied as routine in most of the research
744 work included in this review (60% of the cited studies), in most cases for characterization of
745 the hydrodynamic size of the particles in different growth and exposure media.

746

747

748 5.5. Magnetic based

749

750 Electron paramagnetic resonance (EPR) spectroscopy is a technique used for the
751 characterization of paramagnetic compounds. Paramagnetic compounds and atoms are
752 characterized by the presence of unpaired electrons, EPR spectroscopy measures electron spin
753 transitions in molecules in the presence of an external magnetic field¹³⁵. This technique allows
754 to study for example the formation of environmentally persistent free radicals and oxygen free
755 radicals formed on the surface of microplastics when exposed to sunlight¹³⁶. In the study of
756 Casabianca *et al.*, EPR was used to investigate the associations of marine phytoplankton and
757 PET plastics in seawater⁶⁸. In this study a paramagnetic surfactant dodecyldimethylammonium-

758 2,2,6,6-tetramethyl-piperidine-N-oxide bromide (CAT12) with good affinity for insertion into
759 the cell membrane was used to probe cell-plastic affinity⁶⁸. The diatom *S. marinoi* presented
760 an exponentially increasing adsorption to PET as a function of incubation time. On the other
761 hand, *L. polyedrum* showed lower adsorption and a different mechanism of interaction with the
762 plastic surface. This demonstrates the applicability of this technique to obtain useful
763 information about the mechanisms of adhesion of microalgae in plastic surfaces.

764

765 5.6. Mass spectrometry based techniques

766

767 Mass spectrometry (MS) based techniques measure the mass-to-charge ratio of ions. The most
768 commonly used separation methods are gas chromatography (GC) and (high performance)
769 liquid chromatography (HPLC/LC). These methods have the advantage of being able to provide
770 chemical composition of plastic materials from relatively high mass and representative samples,
771 but the down side is that they are destructive²⁵.

772 In Chae *et al.* the leachates from micro-sized PE spheres in algal growth medium were analyzed
773 qualitatively by HPLC-MS⁵⁶. The results reported the presence of bisphenol A, phthalates,
774 dibutyl phthalate, and UV stabilizers (such as octyl methoxycinnamate) in the medium. Another
775 study by Tetu *et al.* in *Prochlorococcus* photosynthetic bacteria⁶⁵ found that leachates from
776 HDPE and PVC contained 5877 and 10658 components, respectively. This was a broad
777 chemical screening that did not allow identification of individual chemical species. This study
778 also demonstrated the enrichment of elements such as Zn, Sr and Cu through the use of
779 inductively coupled plasma (ICP-MS).

780 Similarly, Capolupo *et al.* used a combination of GC-MS and ICP-MS to screen the organic
781 and inorganic composition of fresh and marine water leachates produced by car rubber tire, PP,
782 PET, PS and PVC microplastics⁶⁴. This analysis revealed the presence of additives
783 (benzothiazole, phthalide, acetophenone) and metals (Co, Zn and Pb) with concentrations
784 varying depending on the plastic type. Thermal decomposition and extraction variants of GC-
785 MS exist such as pyrolysis-GC-MS and thermo extraction and desorption (TED-GC-MS). In
786 pyro-GC-MS microplastics are thermally decomposed (pyrolyzed) and the gas formed is cryo
787 trapped and separated on a chromatographic column, this method has been successfully used to
788 identify polymer types and additives in marine microplastics¹³⁷. However, it was unsuccessful

789 to detect PS in the roots of *A. thaliana* and *T. aestivum* because of the interference with styrene
790 peak, which can be a degradation product of the pyrolysis of plant tissues¹¹⁸. TED-GC-MS
791 combines thermo gravimetric analysis for thermal degradation and solid phase extraction for
792 identification of plastics in environmental samples¹³⁸.

793 Recently, Bolea-Fernandez *et al.* presented a new approach based on ICP-MS detection
794 operated in single-event mode or single particle (SP) ICP-MS mode¹³⁹. This method relies on
795 ultra-fast monitoring of the signal intensity spikes for the mass-to-charge ratio of C¹³⁺ produced
796 by individual particles. The method was demonstrated with PS microspheres (1-2.5 µm) and
797 allows for quantitative determination of mass concentration and size distribution. This is a very
798 promising method that requires further optimization to be routinely used in environmental
799 samples such as photosynthetic organisms. In fact, for all mass spectrometry based techniques
800 the feasibility to apply them to plastic materials interacting or internalized in photosynthetic
801 organisms requires more study to develop adequate sample preparation protocols.

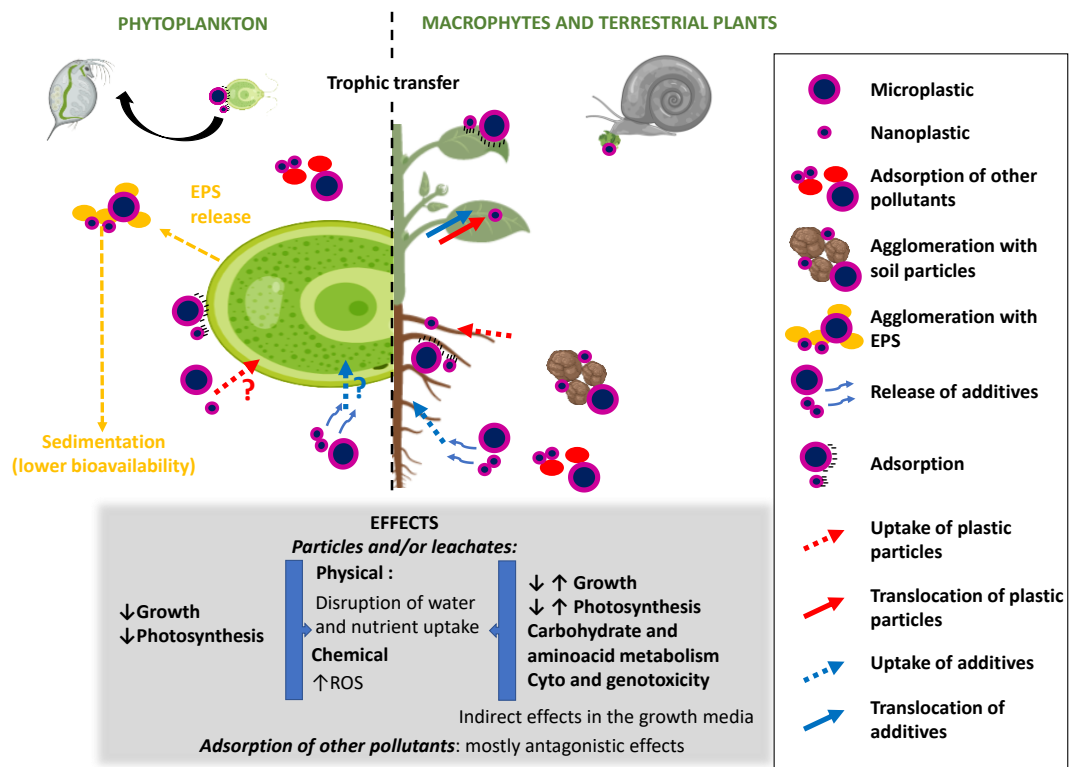
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803 6. Conclusions, knowledge gaps and future directions

804

805 Figure 2 provides a synthesis of the current knowledge on the fate and effects of micro and
806 nanoplastics on photosynthetic organisms. The overview of the literature shows that plastics
807 have significant impacts on photosynthetic organisms. There are comparatively much less
808 studies on terrestrial crop plants than on aquatic organisms, although micro and nanoplastics
809 are ubiquitous in agricultural soils. The observed effects seem to be modulated by particle size,
810 surface charge, composition and shape of the particles, as well as their agglomeration and
811 weathering state. The impact of these parameters have been investigated more in depth in
812 phytoplankton and results show that overall nanoplastics (<1 µm) are more toxic, especially
813 those bearing positive surface charges, with EC₅₀ between 0.5 to 13 mg/L for PS nanoparticles.
814 PVC and PS particles tend to be the most toxic while PE and PMMA have less effects on
815 phytoplankton. The effects of biodegradable plastics have been studied in soils and they can
816 induce similar or even more phytotoxicity than classical polymers. Bigger plastic particles (>
817 100 µm) showed little effect.

818



819

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

823

824

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

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

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

841

842

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

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

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

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

872

873

874 **Funding:**

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

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1248 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
 1249 photosynthetic organisms. catalase (CAT), database of essential genes (DEG), diethylhexyl phthalate (DEHP), half maximal effective concentration
 1250 (EC₅₀), ethylene propylene diene monomer (EPDM), exopolymeric substances (EPS), humic acid (HA), high density (HD), half maximal inhibitory
 1251 concentration (IC₅₀), low density (LD), malondialdehyde (MDA), polyethylene (PE), polyethersulfone (PES), polyethylene terephthalate (PET),
 1252 polylactic acid (PLA), poly(methyl methacrylate) (PMMA), peroxidase (POD), polypropylene (PP), polystyrene (PS), polytetrafluoroethylene
 1253 (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									
<i>Scenedesmus subspicatus</i> <i>Thalassiosira weissiflogii</i>	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 <i>T. weissiflogii</i> - PEN exposure caused growth inhibition of <i>S. subspicatus</i> for all exposure concentrations tested - recovery after some time - PEN seemed more toxic (metal release?)	63
<i>Raphidocelis subcapitata</i> <i>Skeletonema costatum</i>	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
<i>Skeletonema marinoi</i> <i>Lingulodinium polyedrum</i>	seawater (artificial)	PET		sheets 4 x 20 mm		up to 16 d, log phase		- interactions between <i>S. marinoi</i> cells and plastic surface, exponentially increasing as a function of incubation time - <i>L. polyedrum</i> showed lower adsorption. Siloxane groups of the silica frustule involved in the binding with the hydrophobic plastic surface	68

<i>Dunaliella salina</i> <i>Scenedesmus rubescens</i> <i>Chlorella saccharophila</i> <i>Stichococcus bacillaris</i>	seawater (f/2 medium)	expanded PS		fragment: 2 mm to 10 cm ² sphere: 2 to 10 mm	leachate: 0.1 g / 50 mL or 10 and 30 items/50 mL, checked for 28 d	7 d		<ul style="list-style-type: none"> - 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
<i>Phaeodactylum tricorutum</i>	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)	<ul style="list-style-type: none"> - 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
<i>Chlamydomonas reinhardtii</i>	fresh water (SE medium)	PS		300 - 600 nm	5 to 100 mg/L	10 d, log phase		<ul style="list-style-type: none"> - 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
<i>Chlorella pyrenoidosa</i>	seawater (artificial)	PS		0.1, 0.55, 5 µm	up to 64 mg/L	96 h, log phase	DBP : dibutyl phtalate	<ul style="list-style-type: none"> - IC₅₀ ≈ 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
<i>Euglena gracilis</i>	fresh water (sterilized growth medium)	PS		100 nm, 5 µm	1 mg/L	96 h	Cd (0.5 mg/L)	<ul style="list-style-type: none"> - 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

<i>Scenedesmus obliquus</i>	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	<ul style="list-style-type: none"> - no significant impact on the EC₅₀ - 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
<i>Microcystis aeruginosa</i>	fresh water (BG-11 medium)	PE		<500 µm	leachate: 0.5 g / 300 mL, for up to 96 h	12 h		<ul style="list-style-type: none"> - 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
<i>Symbiodinium tridacnidorum, Cladocopium sp.</i>	seawater (artificial + f/2 medium)	PS		42 nm	0.01, 0.1, 10 mg/L	10 d		<ul style="list-style-type: none"> - 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
<i>Euglena gracilis</i>	fresh water (sterilized growth medium)	PS		100 nm, 5 µm	1 mg/L	96 h		<ul style="list-style-type: none"> - 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
<i>Raphidocelis subcapitata</i>	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)	<ul style="list-style-type: none"> - 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
<i>Dunaliella salina</i>	seawater (f/2 medium)	PE		200 µm	up to 350 mg/L	6 d		<ul style="list-style-type: none"> - 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

<i>Microcystis panniformis</i> <i>Scenedesmus sp.</i> <i>Tetraselmis sp.</i> <i>Gloeocapsa sp.</i>	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
<i>Tetraselmis chuii</i>	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
<i>Chlorella vulgaris</i>	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
<i>Phaeodactylum tricorutum</i>	seawater (natural + f/2 medium)	PE	virgin or oxidized	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
<i>Isochrysis galbana</i>	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
<i>Chaetoceros neogracile</i>	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?	
<i>Chlorella vulgaris</i>	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			<ul style="list-style-type: none"> - 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
<i>Prochlorococcus</i>	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		<ul style="list-style-type: none"> - 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
<i>Chlorella sp.</i>	seawater (artificial)	PS	-NH ₂ -COOH - non functionalized	6 μm	1000 mg/L	72 h, log phase	TiO ₂ nanoparticles	<ul style="list-style-type: none"> - EC₅₀ TiO₂ = 81 μM - only 15% toxicity of MP at 1000 mg/L - NH₂ and non-functionalized MPs enhanced TiO₂ toxicity (oxidative stress) -COOH MPs decreased TiO₂ toxicity (hetero-aggregation) 	60
<i>Phaeodactylum tricorutum</i>	seawater (artificial + f/2 medium)	PS		50, 100 nm	0.1 to 50 mg/L	72 h		<ul style="list-style-type: none"> - 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

<i>Chaetoceros neogracile</i>	seawater	PS	-NH ₂	0.5, 2 µm (50 nm)	2.5 mg/L	72 h, log phase		<ul style="list-style-type: none"> - 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
<i>Tetraselmis chuii</i> <i>Nannochloropsis gaditana</i> <i>Isochrysis galbana</i> <i>Thalassiosira weissflogii</i>	seawater (natural + f/2 medium)	PMMA		40 nm, 50 nm	0 to 300 mg/L	96 h		<ul style="list-style-type: none"> - <i>T. weissflogii</i> and <i>T. chuii</i> were respectively the most sensitive (EC₅₀ = 83.75 mg/L) and least sensitive species (EC₅₀ = 132.52 mg/L) 	45
<i>Karenia mikimitoi</i>	seawater (natural)	PVC		1 µm	100 mg/L	24, 48, 72, 96 h, log phase		<ul style="list-style-type: none"> - 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
<i>Skeletonema costatum</i>	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)	<ul style="list-style-type: none"> - 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 < PVC 1 µm) because of the minimum particle size - antagonist interactions 	57
<i>Raphidocelis subcapitata</i>	fresh water	PE		63 - 75 µm	130 mg/L	5 d		<ul style="list-style-type: none"> - higher growth with microplastics - hypothesis: microplastics could serve as substrates for growth 	55
<i>Chlamydomonas reinhardtii</i>	fresh water	PS		<100 nm	50 mg/L (up to 100 mg/L for toxicity)	72 h		<ul style="list-style-type: none"> - 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)				
<i>Chlorella pyrenoidosa</i>	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
<i>Tetraselmis chuii</i>	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
<i>Microcystis aeruginosa</i>	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
<i>Dunaliella tertiolecta</i>	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>i.e.</i> possible trophic transfer) - positively charged particles showed no agglomeration, inhibition of algal growth (EC ₅₀ = 13 mg/L)	33
<i>Tisochrysis lutea</i> <i>Heterocapsa triquetra</i> <i>Chaetoceros neogracile</i>	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 <i>C. neogracile</i> during stationary growth phase - high adsorption with <i>T. lutea</i> and <i>H. triquetra</i> , increasing with the age of the culture for both species - no effects of PS on microalgal growth and chlorophyll fluorescence	51

<i>Raphidocelis subcapitata</i>	fresh water (ISO medium)	PS	-COOH -NH ₂ - non functionalized	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
<i>Skeletonema costatum</i>	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
<i>Chlamydomonas reinhardtii</i>	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
<i>Thalassiosira pseudonana</i> <i>Dunaliella tertiolecta</i> <i>Chorella vulgaris</i>	seawater (f/2 medium) fresh water (BG-11 medium)	PS	-COOH and uncharged	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
<i>Tetraselmis chuii</i>	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
<i>Scenedesmus obliquus</i>	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 <i>Daphnia magna</i>	38

<i>Raphidocelis subcapitata</i>	algal growth medium	PS-PEI (polyethyleneimine polystyrene)		55 nm, 110 nm	0.1 to 1 mg/L	72 h		<ul style="list-style-type: none"> - trophic chain experiment - toxicity from 0.40 mg/L to 416.5 mg/L according to considered species: <i>R. subcapitata</i> ≥ <i>D. magna</i> > <i>T. platyurus</i> > <i>V. fischeri</i>. - EC₅₀ = 0.5 mg/L 	40
<i>Amphora sp</i> <i>Ankistrodesmus angustus</i> <i>Phaeodactylum tricorutum</i>	seawater (artificial)	PS		23 nm	10, 100 µg/L	13 d, stationary phase		<ul style="list-style-type: none"> - PS induced acceleration in <i>Amphora sp.</i> 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 <i>A. angustus</i> and <i>P. tricorutum</i> EPS samples - effects of PS on EPS assembly kinetics mainly depended on the hydrophobic interactions of PS with EPS polymers 	70
<i>Scenedesmus Chlorella</i>	fresh water (Alga-growth medium)	PS	-NH ₂ -COOH	20 nm	80 to 800 mg/L	2 h		<ul style="list-style-type: none"> - 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									
<i>Lemna minor</i>	hydroponics, Steinberg medium	PE, pristine and aged in wastewater		140 ± 80 µm, max 1000 µm	100 mg/L	7 d		<ul style="list-style-type: none"> - 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
<i>Myriophyllum spicatum</i> , <i>Elodea sp.</i>	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		<ul style="list-style-type: none"> - increased root biomass for both macrophytes for NPs - decreased shoot length for <i>M. spicatum</i> for NPs - no effect on biomass, relative growth rate, shoot length for both macrophytes, except a decreased shoot length for <i>M. spicatum</i> for MPs 	91

<i>Lemna minor</i>	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									
<i>Vigna radiata</i>	soil	PS		20 nm	0, 10, 100 mg/kg	10 d		- decreased root growth - particle accumulation in leaves - trophic transfer to snails	121
<i>Oryza sativa</i>	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
<i>Allium cepa</i>	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
<i>Triticum aestivum</i>	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
<i>Arabidopsis thaliana</i>	agar, hydroponics, soil	PS	- SO_3H - COOH - NH_2	55, 71, 120, 220 nm	agar : 10, 50, 100 $\mu\text{g/L}$	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 ₂ O ₂ accumulation in the root tips and root maturation zone + accumulation and toxicity of PS-NH ₂ >>PS-SO ₃ H + 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 ₃ H + 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	
<i>Arabidopsis thaliana</i> , <i>Triticum aestivum</i>	Germination test and early development	PS		40 nm, 1 µm	0.029 g/L	<i>T. aestivum</i> : 5 d, <i>A. thaliana</i> : 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
<i>Lolium perenne</i>	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
<i>Lepidium sativum</i>	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
<i>Allium fistulosum</i>	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				<ul style="list-style-type: none"> - 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) 	
<i>Allium cepa</i>	hydroponics	PS		100 nm	5, 10, 15, 20, 25 µg/mL	3, 6, 12, 24 h		<ul style="list-style-type: none"> - dose response decrease of mitotic index and increase in chromosome aberrations - decrease of root growth 	119
<i>Vicia faba</i>	germination test	PS		100 nm, 5 µm	10, 50, 100 mg/L	48 h		<ul style="list-style-type: none"> - 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 weight - 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
<i>Plantago lanceolata</i>	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		<ul style="list-style-type: none"> - reduced plant survival - non monotonic response in total biomass - decrease in root weight 	101
<i>Alopecurus pratensis</i> , <i>Festuca guestfalica</i> , <i>Lolium perenne</i> , <i>Galium album</i> , <i>Leucanthemum ircutianum</i> , <i>Leucanthemum vulgare</i> ,	artificial soil (mixture sand and vermiculite)	EPDM		0.5–2.5 mm	5 % v/v	1 week		<ul style="list-style-type: none"> - reduction of aboveground biomass and root weight and increase in root length but reduced weight - reduced competitive interactions 	101

<i>Prunella vulgaris</i> , <i>Daucus carota</i>									
<i>Triticum aestivum</i>	Germination test + hydroponics	PS		100 nm	0.01, 0.1, 1, 10 mg/L	5 d, 21 d		<ul style="list-style-type: none"> - 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
<i>Thinopyrum junceum</i> , <i>Glaucium flavum</i>	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		<ul style="list-style-type: none"> - 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
<i>Triticum aestivum</i>	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		<ul style="list-style-type: none"> - 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)					
<i>Murraya exotica</i>	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
<i>Triticum aestivum</i>	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
<i>Brassica rapa</i> subsp. <i>Chinensis</i> , <i>Cucumis sativus</i> , <i>Solanum melongena</i> , <i>Phaseolus vulgaris</i> , <i>Capsicum annuum</i> , <i>Raphanus sativus</i> , <i>Solanum lycopersicum</i>	survey greenhouses, open fields and markets	phthalate esters			0.26-2.53 mg/kg			- concentration of phtalates in vegetable leaves between 0.95 and 8.09 mg/kg	124

<i>Brassica rapa Chinensis</i>	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
<i>Gossypium hirsutum</i>	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
<i>Capsicum annuum, Cucumis sativus, Brassica oleracea, Raphanus sativus, Brassica chinensis, Lactuca sativa, Chrysanthemum coronarium, Apium graveolens, Spinacia oleracea, Brassica juncea</i>	survey greenhouses	phthalate esters			2.56 - 2.23 mg/kg			- concentration of phthalates in vegetables between 0.51 and 7.16 mg/kg - accumulation in leaves and stems	123
<i>Allium cepa</i>	germination test	nanocomposites of PLA and nanoclays		leachates from composting	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
<i>Zea mays</i>	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

<i>Benincasa hispida</i> , <i>Cucumis sativus</i> , <i>Cucurbita moschata</i> , <i>Brassica parachinensis</i> , <i>Momordica charantia</i> , <i>Lactuca sativa</i> , <i>Capsicum frutescens</i> , <i>Luffa cylindrical</i> , <i>Ipomoea aquatica</i> , <i>Lycopersicon esculentum</i>	soil	film mulch DEHP		pieces of 12 cm diameter and 0.012 mm thickness	0,6 % (w/w)	6 weeks		<ul style="list-style-type: none"> - significant concentrations of DEHP accumulated by <i>Benincasa hispida</i>, <i>Cucumis sativus</i>, <i>Cucurbita moschata</i> and <i>Brassica parachinensis</i> - concentrations raised in this later are close to the dietary daily intake limit - no phytotoxicity 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 <i>A. cepa</i> 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	fluorescence 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 (<i>L. sativum</i>) - uptake of plastic nanoparticles in roots (<i>V. faba</i> , <i>T. aestivum</i>)	
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 <i>in vivo</i> - used for kinetic studies of Eu marked nanoplastics in <i>M. exotica</i>	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 <i>M. exotica</i>	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 (<i>S. marinoi</i> , <i>L. polyedrum</i>)	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

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