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Abstract

Widespread use of nanoparticles for different applications has diffused their presence in the environment, particularly in water. Many studies have been conducted to evaluate their effects on aquatic organisms. Microalgae are at the base of aquatic trophic chains. These organisms which can be benthic or pelagic, meaning that they can enter into interaction with all kinds of

particulate materials whatever their density, and constitute an interesting model study. The purpose of this review was to gather more than sixty studies on microalgae exposure to the different nanoparticles that may be present in the aquatic environment. After a brief description of each type of nanoparticle (metals, silica and plastic) commonly used in ecotoxicological studies, techniques to monitor their properties are presented. Then, different effects on microalgae resulting from interaction with nanoparticles are described as well as the parameters and techniques for monitoring them. The impacts described in the literature are primarily shading, ions release, oxidative stress, adsorption, absorption and disruption of microalgae barriers. Several parameters are proposed to monitor effects such as growth, photosynthesis, membrane integrity, biochemical composition variations and gene expression changes. Finally, in the literature, while different impacts of nanoparticles on microalgae have been described, there is no consensus on evidence of nanomaterial toxicity with regard to microalgae. A parallel comparison of different nanoparticle types appears essential in order to prioritize which factors exert the most influence on toxicity in microalgae cultures: size, nature, surface chemistry, concentration or interaction time.

Keywords: microalgae; toxicity; nanoparticles; characterization; interaction

1. Introduction

At the nanoscale, materials have properties such as electrical and thermal specific conductivity, which make them interesting to develop novel applications. Nanomaterials are defined by European Union commission (2011) as natural or manufactured materials formed by at least 50% of particles with dimensions comprised between 1 and 100 nm. But this definition is not admitted by all organizations (ISO, USA legislation…) (Jeevanandam et al. 2018). Nanoparticles (NPs) are part of the nanomaterial family and the British Standard institution (2011) define them as materials possessing three external nanoscale dimensions.

For the last two decades, they have been used in a large panel of industrial applications leading to their presence in the aquatic environment, which raises concern about their environmental impact (Moore 2006). Their marked presence in the environment has led to awareness and significant research efforts have been devoted to understanding their potential toxicity on aquatic organisms at the species and cellular levels, as well as the underlying mechanisms. Microalgae are very common organisms present in all aquatic systems and at the lowest level of the trophic chain. Knowledge of their potential interactions with NPs is very sparse and difficult to compare, mostly due to the fact that tracking NPs in a medium as complex as the aquatic environment is still very challenging (Selck et al. 2016). Recent reviews on nanomaterials and the methods to assess their ecotoxicity to marine life, are already present in literature (Handy et al. 2012; Selck et al. 2016)highlighting the challenges and research priorities in this field. To be more specific, the present review synthesizes literature focusing on microalgae and NP interactions in view of analyzing the potential toxicity and impacts of NPs on microalgae. The study of such interaction relies on proper characterization of both NPs and microalgae in order to evaluate their changes during interaction and to improve our understanding of the interaction pathways. The usual strategies

allowing access to such information are presented here together with novel techniques providing new insights into nanomaterial-organism interactions.

In the first part, nanoparticle types, applications and properties are presented to highlight the diversity of these materials. Secondly, different effects arising from interaction with microalgae are described as well as parameters and techniques for monitoring them. Finally, the chemistry of nanoparticles is studied so as to determine whether this property has an impact on interactions.

2. NPs types, applications and properties

The generic term of NP encompasses a wide variety of particles with different natures, origins (natural *vs* engineered) and applications. Man-made NPs are most often referred to as metallic nanoparticles, and mineral and organic NP are also used for a wide range of applications such as electronics, medicine, cosmetics, packaging, remediation (López-Serrano et al. 2014). As the use of engineered nanomaterials has globally increased in recent years (Lapresta-Fernández et al. 2012), they may leach from engineered products because of their small size, pass through most depuration systems and unintentionally enter the aquatic environment.

2.1. Types and applications

2.1.1. Metals and oxides

Among the first NPs to be synthesized, NPs in noble metals as silver and gold, are now widely commercialized. Table 1 presents the principal characteristics of the kind of nanoparticles used in ecotoxicological studies. Gold NPs are used not only in the medical field and in molecular biology (Lapresta-Fernández et al. 2012) but also in paints and electronic components (Renault et al. 2008). Silver nanoparticles are employed primarily as

antimicrobial agents in the medical field, in textiles and in cosmetic products (Zhang et al. 2015). Platinum NPs are also used in biomedicine applications, automotive exhaust converters and as electrochemical sensors (Ksiazyk et al. 2015). With their catalytic, magnetic and electrical properties, cobalt NPs are used in magnetic fluids and in the biomedical field (Ansari et al. 2017).

Metallic oxide NPs represent a second kind of metallic nanomaterial (Table 2). Copper oxide (CuO) is exploited in antifouling paints for its antimicrobial properties (Almeida et al. 2007; Sandberg et al. 2007). Likewise, studies have focused on cerium oxide NPs as antibacterial products, catalysts and for their use in pharmaceutical drugs (Trovarelli et al. 1999; Celardo et al. 2011). Nickel oxide NPs are frequently used in industry as catalysts for alkaline battery cathodes, electrochromic and magnetic materials or as pigments for ceramics and glass (Gong et al. 2011; Oukarroum et al. 2017). Zinc and titanium oxide NPs are photoreactive. Their utilization as bleaching agents, photocatalysts and transparent agents renders titanium oxide (TiO_2) NPs one of the most produced in the world (Wang et al. 2008; Robichaud et al. 2009). TiO_2 NPs are also widely used in drug delivery, health and in plastic products (Vance et al. 2015). Zinc oxide (ZnO) is a molecule with a high capacity to absorb UV radiations, a property that is used in solar creams, cosmetics and paints (Osmond and McCall 2010; Suman et al. 2015). These ZnO NPs have additional antibacterial properties that explain their presence in toothpaste and textiles (Lee and An 2013; Yung et al. 2017).

Some other metal oxides under nanoparticle forms a such as lanthane, aluminium and iron have been less frequently studied. Fuel cell construction, magnetic data storage, biomedicine and sewage treatment are applications of lanthane oxide (La_2O_3) NPs (Balusamy et al. 2015). Aluminium oxide nanoparticle applications (Al_2O_3) are linked to polymer modification and hot fluid transfer (Pakrashi et al. 2013). Iron NPs with zero valence (ZVI) are used mostly in

112 environmental restoration, the food industry and medical diagnosis (Kadar et al. 2012;
113 Adeleye and Keller 2016).

114 ### 2.1.2. Silica (SiO_2)

115 Silica, the main component in the mass of continental crust, can naturally be found as NPs
116 in oceans (Fujiwara et al. 2008). Indeed, it can possess a crystalline or an amorphous form.
117 Crystalline silica is found in the manufacturing industry, mines and is known to be highly
118 toxic by inhalation (silicose, INRS data). Silica NPs (< 100nm) are used in various domains
119 such as cosmetics, anti-caking agents (E551) in food products (Athinarayanan et al. 2014).
120 The OECD has requested amorphous silica NPs as a NP reference in risk and toxicity
121 measurement for nanomaterial evaluation (Aruoja et al. 2015).

122 ### 2.1.3. Plastics

123 Particles of plastic are present in the environment and were identified in the 1970s; they
124 form an emerging issue. More specifically, the smallest ones called nanoplastics (whose
125 presence was recently described) (Ter Halle et al. 2017) were considered as being very toxic
126 to *Scenedesmus obliquus* and *Daphnia magna* (Besseling et al. 2014). Tested concentrations
127 in this study are nevertheless higher than environmental concentrations. Nanoplastics have
128 been defined as particles originating from the degradation of larger plastic debris having
129 accumulated for several decades in the aquatic environment. Besides these fragmented
130 particles, polymeric nanobeads (used in several industries) may also enter the aquatic habitat.
131 Such nanobeads, also named polymer colloids, are widely used as material strengtheners, in
132 medical activities (antimicrobial properties, drug delivery…) and in cosmetics products
133 (Daniel and Astruc 2004; Salata 2004; Aitken et al. 2006; Besseling et al. 2014; Nolte et al.
134 2016). Lastly, polymeric fibers originating from clothes washing have also been detected as
135 they entered the aquatic environment (Falco et al. 2017) through wastewater treatment plants.

Due to the high quantities of plastics entering the aquatic environment each year, around 1.7 kg/day/inhabitant (Jambeck et al. 2015), the potential accumulation of these newly identified nanomaterials is of concern, but their concentration in the environment has yet to be reliably measured. Seven major types of polymers have been identified in the aquatic environment. Nonetheless, in interaction and impact studies, polystyrene is overrepresented (Phuong et al. 2016) due to its commercial availability at diverse sizes (from nano to micro size beads) with different surface charges: neutral, anionic and cationic (Long et al. 2015; Libralato et al. 2017). Some studies have focused on other polymers as PVC, PE and PP but only on a micro scale (Lagarde et al., 2016; Davarpanah et al., 2015; Zhang et al., 2016). Concerning nanoplastics, only the impacts of polystyrene NPs on microalgae have been studied so far (Table 3).

2.2. NPs properties

Some properties such as their size are common to all NPs but many other properties differ, so it is important to characterize them (Handy et al. 2012) using as these properties may strongly modify NPs behavior in contact with aquatic organisms. Among a variety of emerging approaches to characterize nanomaterials in ecotoxicological tests (Selck et al., 2016), the techniques described below help to assess changes in the structure and morphology of NPs before or after interactions with microalgae.

2.2.1. Nature, shape and size

The chemical nature of NPs can be determined by Energy Dispersive X-ray spectroscopy (EDX) SEM or by induced coupled plasma-mass spectrometry (ICP-MS) in most cases (excluding plastics), this latter being an emerging tool to also provide size distribution (Selck et al., 2016).

X-ray techniques can offer information on crystalline phase and crystallization type. Sadiq et al. (2011) used X-ray diffraction (XRD) on TiO_2 NPs to identify an anatase phase size of 17 nm. Surface chemistry characterization of cerium oxides was also performed by X-rays to determine Ce^{3+}/Ce^{4+} ratio (Pulido-Reyes et al. 2015). XRD was used to follow the valence state of nickel NPs (Gong et al. 2011). In the same study, X-ray photoelectronic spectroscopy (XPS) was used to assess nickel bioreduction and Ni^{2+}/Ni^{0} ratio. Analysis of EDX, often associated with SEM or TEM, may identify elements in nanoparticle agglomerates. By this technique, the presence of metal oxide NPs translocated into cells can be confirmed (Oukarroum et al. 2017 for nickel oxide and copper oxide nature in Perreault et al. 2012). Moreover, TEM is also an interesting technique in so far as it can also be used for morphologic and crystallographic characterization of NPs (Barwal et al. 2011).

Most studies check to see if the size of NPs after interaction remains the same using different methods among which DLS (Dynamic Light Scattering) predominates (Van Hoecke et al. 2008; Wang et al. 2008; Hartmann et al. 2010; Casado et al. 2013; Pakrashi et al. 2013; Nolte et al. 2016; Adeleye et al. 2016; Iswarya et al. 2016; Bergami et al. 2017; Yue et al. 2017; Chen et al. 2018). This non-invasive technique allows characterization of particles in solution by measuring the distribution of their hydrodynamic radius. After interaction of a light beam with particles, Brownian particle motion generates light scattering at different intensities. Light intensity variation is measured and NP diameter is obtained using the Stokes-Einstein relation. DLS is appropriate if the NP size is comprised between 0.5 nm and 10.0 µm. This method also evaluates NP aggregation. In their study, Wang et al. (2008) monitored the impact of interaction on TiO_2 NP size (21 nm). Their observations demonstrate particle agglomeration forming objects of around 900 nm in the culture medium of *C. reinhardtii,* thereby modifying NP properties (i.e. specific surface reduction).

184 Image analysis in microscopy is also used to assess the size distribution of NPs: Leclerc
185 and Wilkinson used transmission electron microscopy (TEM) image analysis on silver NPs,
186 Pulido-Reyes on cerium oxide NP and Aruoja in a comparative study of nanometals (Leclerc
187 and Wilkinson 2014; Aruoja et al. 2015; Pulido-Reyes et al. 2015) to determine the size
188 distribution of particles after interaction. In addition, size measurement comparison by TEM
189 and DLS was performed for silica NPs in interaction with *P. subcapitata*, by Van Hoecke et
190 al. (2008). Their results indicated a difference in measured sizes between the two techniques.
191 They considered DLS more reliable due to a higher analysed volume and to the fact that NPs
192 can be analyzed in their solution, which is a major advantage of this technique.

193 UV-Visible spectrometry is another technique used to study particle stability in the
194 medium. Peak absorption measurement can be characteristic and give access to information
195 such as the concentration or size of the NPs (Haiss et al. 2007). For example, Renault et al.
196 (2008) monitored the concentration of 10 nm NPs absorbing at a 520 nm wavelength in the
197 culture medium and observed a decrease of gold NP concentration in Arcachon's water
198 compared to ultrapure water. Bhattacharya et al., (2010) also suggested that NP adsorbed on
199 microalgae cell walls could be quantified by UV-visible spectrometry. Light absorption
200 measurements directly performed in the growth medium at 260 nm corresponding to a higher
201 absorbance band of polystyrene facilitated quantification of the concentration of polystyrene
202 NP in the medium.

203 **2.2.2. Surface chemistry and charge density**

204 Zeta potential measurements provide information, by electrophoretic mobility
205 measurements, on the charge density of particles and medium viscosity. . Many of surveys
206 monitoring charge have provided elements on the nature of interaction and ionic forces
207 (Bhattacharya et al. 2010; Hartmann et al. 2010; Saison et al. 2010; Perreault et al. 2012;

Casado et al. 2013; Lee and An 2013; Manier et al. 2013; Pakrashi et al. 2013; von Moos and Slaveykova 2014; Cheloni et al. 2016; Nolte et al. 2016; Adeleye et al. 2016; Bergami et al. 2017; Sendra et al. 2017a). These works have strongly contributed to the understanding of particle stability as recommended by Handy et al. (2012). For example, when studying their stability within a complex medium, Bhattacharya et al., (2010) showed a difference of aggregation behavior in NPs of the same chemical nature but with two different surfactants. This point is very important since many NPs are synthesized with different molecules coated on their surface through which they are stabilized in suspension or given specific properties (cf. Tables 1, 2 and 3).

2.2.3. Dissolved fraction

Eventually, some NPs in solution can undergo ageing in time and may release ions or molecules. Then, it becomes necessary to quantify the dissolved fraction in the medium. In the case of metallic particles, ICP-MS has been extensively used to this purpose: for dissolved nanocopper (Cheloni et al. 2016) or for platinum and silver NPs (Ksiazyk et al. 2015; Navarro et al. 2015; Yue et al. 2017). This technique has also been used to track nickel oxide nanoparticle solubility and to quantify bioabsorption rates in cells (Gong et al. 2011).

3. Microalgae's role and defence strategies in aquatic systems

Microalgae are microscopic autotrophic organisms, living in unicellular form or forming colonies, at the base of all aquatic trophic webs. The share of primary production due to oceanic phytoplankton has been estimated at between 40% and 50% of net primary production (NPP) (MacIntyre et al. 2000; Geider et al. 2001). In settling, they confine the carbon of carbon dioxide (Bowler et al. 2009) at the bottom of oceans. Microalgae, which are

231 present in fresh and sea-water ecosystems, can be divided into two groups. Benthic
232 microalgae are based on aquatic bottoms, while pelagic microalgae are found in the water
233 column and form phytoplankton with cyanophytes, meaning that they can interact with all
234 kinds of particulate materials whatever their density.

235 Many ecotoxicology studies at the physico-chemistry, molecular biology or biochemistry
236 levels, use microalgae - including green and brown - as models for experimentation. These
237 unicellular organisms can be strongly impacted by various pollutions. The position of primary
238 producers implies that there will be transfers along the trophic chain affecting the organisms
239 consuming them. Consumers can be directly affected by a lack of food - microalgae do not
240 support pollution - or indirectly, by accumulating more and more pollutants via their food
241 (Cedervall et al. 2012; Besseling et al. 2014). However, it has also been shown that
242 microalgae can set up systems of responses to these pollutants allowing them to defend
243 themselves or adapt. These responses can be observed at different levels:

244 *morphological responses, with modifications in size and shape as a result of contact
245 with metals (Jamers et al. 2009).

246 *biochemical responses, such as phytochelatins, polypeptides which are able to
247 confine metals, are synthesized in *C. reinhardtii* with apost-translational enzymatic pathway
248 to evacuate TME from the cells (Howe and Merchant 1992).

249 *molecular responses, such as induction of gene over-expression encoding thioredoxin
250 (cadmium chelation) and glutathione peroxidase (H_2O_2 transformation in H_2O) in *C.
251 reinhardtii* in the presence of cadmium (115µM) to limit damages due to oxidative stress
252 (Jamers et al. 2013).

253 The defence systems of microalgae against metals have been described in the literature.
254 Are they adapted as protection mechanisms against NPs? Methods of interactions are

255 presented in the following section to characterize the response of microalgae in the presence
256 of NPs.

257

258 4. How to highlight microalgae/NP interactions and associated impacts?

259 4.1. Direct interactions visualization: microscopy at different scales

260 Several techniques of microscopy visualize of interactions between NPs and microalgae
261 (Figure 1) at different scales down to the nanometer range. First, scanning electronic
262 microscopy (SEM) screens elements up to a hundred nanometers by collecting reflection
263 secondary electrons of a sample after excitation by a laser beam. Coupling a X-ray detector
264 with SEM gives the advantage of providing information on the nature of the detected element.
265 For example, Barwal used this type of detector to prove the presence of silver atoms inside *C.*
266 *reinhardtii* (Barwal et al. 2011).

267 Second, transmission electron microscopy (TEM) makes it possible to visualize very
268 small particles (until nanometer size) by transmitting electron detection. Despite its time-
269 consuming nature, this technique is regularly used for analysis of metallic NPs such analysis
270 as gold NPs (Schrand et al. 2010). Renault et al. (2008) have notably monitored the impact of
271 the distribution of nanomaterials in *Scenedesmus subspicatus*.

272 Following interaction with TiO_2 used confocal microscopy was used to visualize NPs
273 (primary size: 35.1 nm, secondary size: 3580 nm), after aggregation at the surface of *P.*
274 *subcapitata* (Metzler et al. 2011). Drawbacks of this technique compared to the others is that
275 only a visualization of nanomaterials agglomerates is possible due to a detection limit of a few
276 hundred nanometers (Röhder et al. 2014). Moreover, contrary to SEM and TEM, confocal
277 microscopy necessitates fluorophore probe with the risk of fluorophore release (Salvati et al.

278 2011). Other techniques are marginally used, such as dark field microscopy, which diffuses of
279 structures coupled with hyperspectral imaging. This was proposed by Leclerc and Wilkinson
280 (2014) in order to visualize internalization of silver particles. Atomic force microscopy
281 (AFM) is used to study the topographic surface of microalgae (Nolte et al. 2016). Recently,
282 Raman spectroscopy at 532 nm was proposed as a tool to study the localization of polystyrene
283 nanoparticles in human cells (Dorney et al. 2012). It is described as optimal in terms of signal
284 intensity and permits live cell analysis; Ivask et al. (2017) have suggested that it could also be
285 used in microalgae-nanoparticle interaction studies. Surface-Enhanced Raman Spectroscopy
286 or SERS, has been used on *Pseudokirchneriella subcapitata* to detect gold NP biosynthesis
287 (Lahr and Vikesland 2014). The resonance of gold NPs in the SERS technique enhances the
288 Raman signal of living cells, with high resolution and decreased signal collection times,
289 permitting non-destructive measurements (Kneipp et al. 2002).

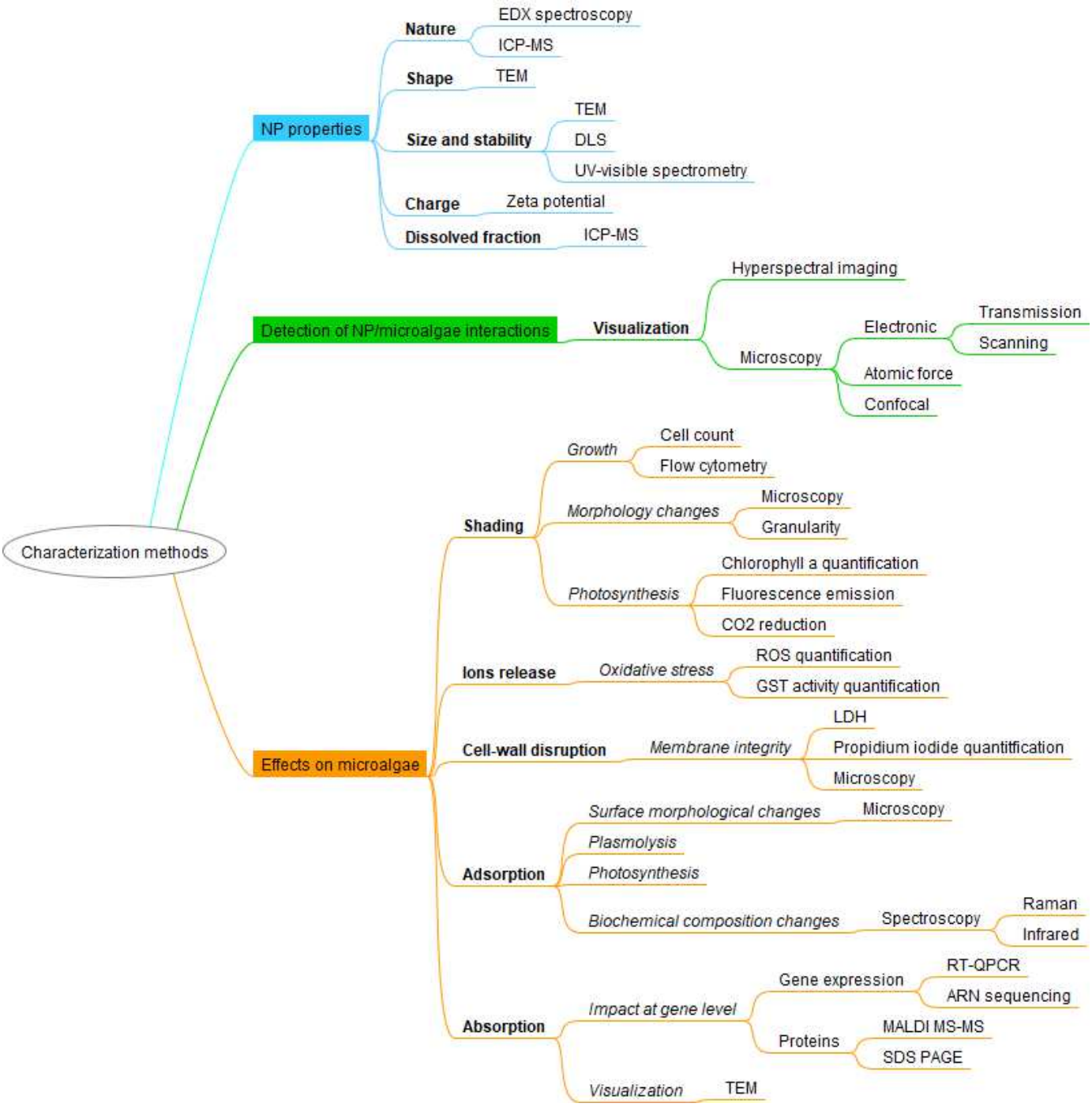
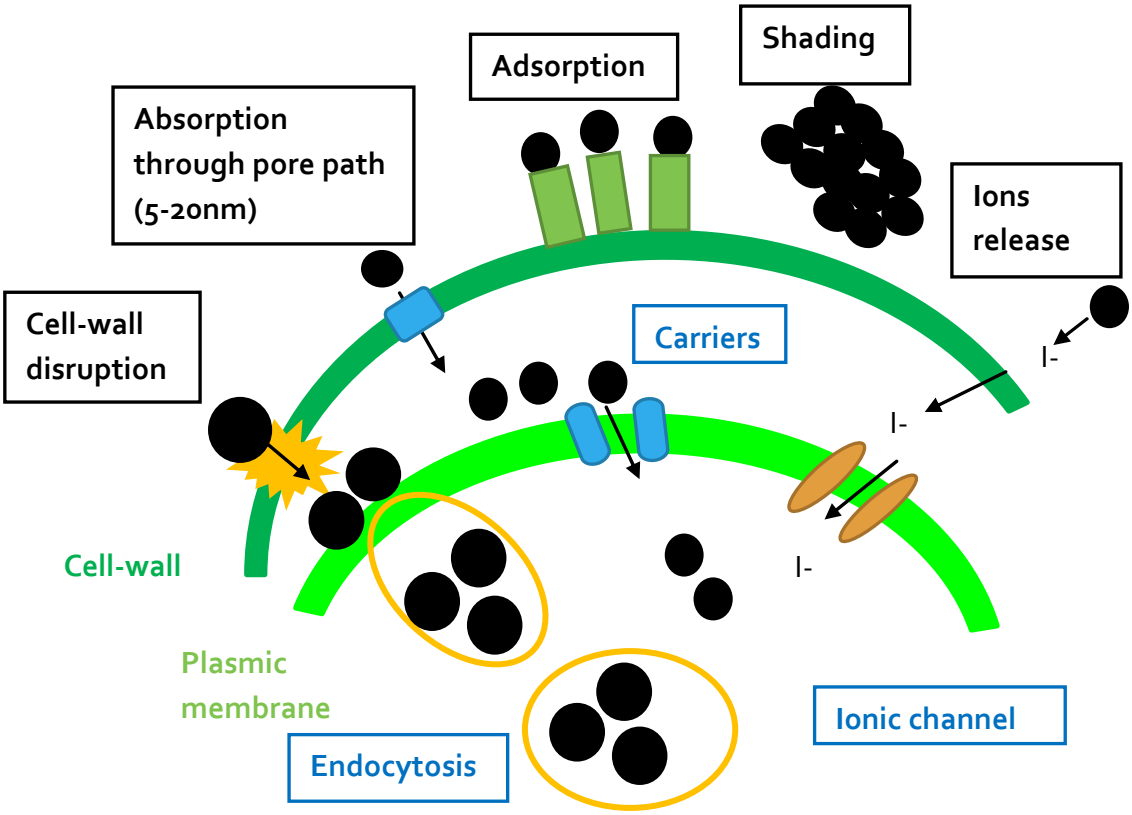


Figure 1: *Characterization methods of microalgae-NP interactions: NP properties, detection of NP/microalgae interactions and effects on microalgae.*

290

291 **4.2. Monitoring effects of interactions**

292 Several kinds of interaction between microalgae and NPs are possible. They are
293 summarized in Figure 2: shading, ion release, adsorption, absorption and cell-wall disruption.
294 NP-microalgae interactions may involve changes in microalgae state (physiological,
295 biochemical and molecular). To characterize these impacts, several approaches are necessary
296 and have been presented in the literature. They have been partially collected and are
297 commented on here (Tables S1, S2 and S3 given in supplementary data summarize all the
298 cited studies).



299

Figure 2: NPs potential interactions with barriers of microalgae (cell-wall and plasmic membrane): ion release (absorption through ionic channel), shading effect, adsorption (block ionic exchange) and absorption by pore pathway, cell-wall disruption or endocytosis.

300 **4.2.1. Growth of microalgae**

All type of interactions can impact microalgae growth, which is the most commonly monitored parameter. Measurement of microalgae growth is done mainly by cell count (Renault et al. 2008; Wang et al. 2008; Hartmann et al. 2010; Lee and An 2013; Aruoja et al. 2015; Ksiazyk et al. 2015; Sjollema et al. 2016; Zhang et al. 2016; Bergami et al. 2017; Dauda et al. 2017; Oukarroum et al. 2017). Other studies evaluate the health of microalgae by measuring cellular density and chlorophyll fluorescence by flow cytometry (Manier et al. 2013; Melegari et al. 2013; von Moos et al. 2015; Cheloni et al. 2016; M. Sendra et al. 2017a). Morphometry, which is the study and analysis of geometry (3D), is also used to follow microalgae morphological variations after interaction with copper NPs (von Moos et al. 2015).

Several studies have shown an inhibition of growth in *P. subcapitata* in contact with ZnO and TiO_2 NPs (Lee et al. 2013) as well as for silver and platinum NPs (Ksiazyk et al. 2015).

At a morphological level, cell dimension alterations have been examined after $AgNO_3$ NP (*Chlorella autotrophica*) and gold NP (*S. subspicatus*) exposure for concentrations of 5 mg/L and $1{,}6x10^{-6}$ mg/L (Renault et al. 2008; Sendra et al. 2017b). Moreover, modification of cellular complexity (granularity) obtained through a measure of scattered light (Side Scatter, SSC) by flux cytometry, is also observed for Ag and Ce NP at 24 h (Sendra et al. 2017b). According to Saison et al. (2010), cerium oxide NPs increase microalgae granularity and cause aggregation phenomena, which may be accompanied by cellular coloration loss, as in *Chlorella kessleri* in the presence of silica NPs (5 nm) (Fujiwara et al. 2008).

4.2.2. Shading

The reduction of captured light, called shading phenomena, was described by Schwab et al. (2011) and Cheloni et al. (2016) who showed a correlation between photosynthesis inhibition and shading linked with carbon and oxide copper NPs. Chen et al. (2018)

demonstrated a correlation between growth inhibition and shading linked with carbon, oxide copper and cobalt NPs) on three microalgae species *Platymonas subcordiforus, Chaetoceros curvisetus* and *Skeletonema costatum* (Chen et al. 2018). Nevertheless, few studies have taken into account, much less underlined this effect.

Moreover, photosynthesis, a key mechanism of algae functioning, has frequently been explored in interactions (Navarro et al. 2008a; Bhattacharya et al. 2010; Zhang et al. 2016) such as the analysis of photosystem II photoinhibition (Saison et al. 2010). Photocatalytic capacity has also been monitored through the quantification of chlorophyll a (Besseling et al. 2014), a pigment found in all microalgae. Extraction of chlorophyll a and quantification by spectrophotometry technic are easy to set up and not expensive but can overestimate chlorophyll content (Sartory 1985; Hosikian et al. 2010). During photosynthesis, CO_2 and water are captured and transformed into sugar and O_2 under light effect. Monitoring CO_2 reduction elucidate the alteration of algal photosynthesis functioning and inform on a possible cellular respiration phenomenon (Bhattacharya et al. 2010). It should be noted that the photosynthetic system is sensitive to the presence of NPs as some researchers have observed a drop in photosynthetic activity (von Moos et al. 2015). Photosynthesis can be impacted in two different manners: (1) either indirectly by the reduction of captured light (2) or directly by photoinhibition of photosystem II and photocatalysis (Navarro et al. 2008a). In *C. reinhardtii* a photosystem II (PS II) photoinhibition mechanism has also been revealed in the presence of cerium oxide NP covered by polystyrene (80 nm; 0,01 g/L), which led to chlorophyll deterioration (Saison et al. 2010).

4.2.3. Ion release

NPs in solution can release ions and this is particularly frequent in the case of metallic oxides NPs. Concerning silver NP studies (Table 5), most have reported internalisation in

different types of microalgae (*C. reinhardtii, C. vulgaris, S. obliquus and E. gracilis*) due to the release of Ag^+ ions and an agglomeration of ions inside the cell (Navarro et al. 2008b; Barwal et al. 2011; Leclerc 2013; Ksiazyk et al. 2015; Wang et al. 2016; Yue et al. 2017; Zhang et al. 2017). Techniques for quantifying the dissolved fraction have previously been described (§ 2.2.3).

Moreover, ion release can induce oxidative stress and photosynthesis effects on microalgae as has been reported for copper oxide NPs (Saison et al. 2010; Perreault et al. 2012; von Moos et al. 2015). Some NPs such as ZnO and TiO_2 induce a photocatalysis phenomenon in *P. subcapitata* (Lee et al. 2013) leading to reactive oxygen species (ROS) formation taking part in secondary oxidative stress due to ion release.

Oxidative stress occurs more widely after every environmental modification (Pinto et al. 2003). Oxidation phenomenon is a frequently reported mechanism after contact with reactive NPs such as oxides or dissolved particles in ions. Glutathione S transferase (GST) activity, an enzyme implicated in xenobiotic detoxification and in reparation of oxidized macromolecules, was studied by Dauda et al. (2017) in *C. vulgaris* after incubation with TiO_2 NP. Increased enzymatic activity was observed indicating that a cellular defence against ROS had been established (Dauda et al. 2017). In parallel, it was shown by (Leclerc and Wilkinson 2014) that Ag^+ ions salting-out by Ag NP induced ROS production responsible for Ag NP toxicity on *C. reinhardtii.* In this species as well, increased ROS concentration coupled with lipidic membrane peroxidation was brought to light in the presence of copper oxide at 1000 mg/L (Melegari et al. 2013). A majority of studies use ROS quantification to monitor oxidative stress in comparison with GST activity monitoring (Bhattacharya et al. 2010; Lee et al. 2013; Leclerc and Wilkinson 2014, Tables 4, 5 and 6).

4.2.4. Cell-wall and membrane effect

373 ROS production can cause important cellular damages such as loss of membrane fluidity
374 and oxidation of unsaturated lipids…(Aruoja et al. 2015; Bhattacharya et al., 2010; Hartmann
375 et al. 2010; Lapresta-Fernández et al. 2012; Melegari et al. 2013 (by flow cytometry);
376 Pakrashi et al. 2013; Perreault et al. 2012; Pulido-Reyes et al. 2015; Röhder et al. 2014;
377 Saison et al. 2010; Sendra et al. 2017a). Several techniques are focused on the ROS
378 measurement: electron spin resonance (ESR) which is sparsely used but referenced in the
379 literature (Lapresta-Fernández et al. 2012), fluorescence detection of DCFH-DA (2,7-
380 dichlorodihydrofluorescein diacetate) (Saison et al. 2010; von Moos et al. 2015) and the
381 lipidic peroxidation test with malondialdehyde (MDA) (Wang et al. 2008).

382 Furthermore, changes can appear at a cellular membrane integrity level, which is followed
383 by the lactate dehydrogenase test, where an increase of cytolytic enzyme in the surrounding
384 medium reveals membrane damage (Pakrashi et al. 2013). Membrane integrity can also be
385 monitored by propidium iodide fluorophore, which intercalates in nucleic acid strands of cells
386 with damaged membranes (Prado et al. 2011; Xia et al. 2015). These alterations can also be
387 visualized by microscopy. Microalgae structural alterations, for example, can be observed
388 using TEM and have been viewed as a distortion of cell walls, resulting from the interaction
389 of *P. subcapitata* with zinc NPs (Lee and An 2013). Microscopy techniques such as AFM can
390 be used to measure the energy necessary to cell-wall disruption in *Tetraselmis suecica* (Lee et
391 al. 2013).

392 Cellular metabolism may be impacted as well. Melegari et al. (2013) demonstrated -for
393 example- decreased esterase activity in the presence of copper oxide. This marker is crucial
394 for phospholipid membrane metabolism in *C. reinhardtii* (Li et al. 2011).

395 The cell wall and the plasma membrane are cellular compartments that can be particularly
396 affected by the physical or biochemical impact of NPs, respectively. Studies on *C. vulgaris*

have shown membrane destructuration in the presence of nickel oxide (Gong et al. 2011) and zinc oxide (Suman et al. 2015) NPs. Aluminium oxide NPs at 1 µg/mL leading to cell-wall and membrane damage in *C. ellipsoidea* (Pakrashi et al. 2013). This study was performed by microscopy and a lactacte deshydrogenase (LDH) test providing information on membrane integrity.

Indirect effects are also observed on physical barriers of microalgae. In this way, photocatalysis induced by the presence of TiO_2 NPs generates peroxidation of functional groups at the cell wall and membrane levels. Damage at the level of phospholipidic chains in *C. reinhardtii* is revealed by infrared analysis and SEM (Chen et al. 2017). For the same NPs, disruption of the cell wall was observed in *Nitzschia closterium* (Xia et al. 2015).

4.2.5. Adsorption

A key question raised by many researchers is whether or not these NPs should be adsorbed or if an absorption phenomenon could happen that could lead to particle internalization. NP localisation at the surface (adsorption) or inside cells (absorption) is partially possible through microscopy techniques.

With SEM, Balusamy's study demonstrated the attachment of lanthane oxide (La_2O_3) NPs on microalgae without producing morphological changes in *Chlorella sp*. (Balusamy et al. 2015). Contrarily, in the interaction of titane oxide NPs with *C. reinhardtii*, surface morphological modifications due to the presence of NPs have been observed (Chen et al. 2017). Overlapping aspects could be revealed by SEM, as titane oxide NPs (TiO_2) appears, covering *Pseudokirchneriella subcapitata* in several non-uniform layers (Metzler et al. 2011).

By TEM, cerium oxide NPs are detected at the surface but not inside *P. subcapitata* (Pulido-Reyes et al. 2015). Furthermore, a method to observe NP adsorption could be confocal microscopy. With AFM (topographic surface), Nolte et al. (2016) observed a cell

421 wall with fibrillar structure offering numerous attachment points for polystyrene nanobeads,
422 with preferential adsorption of PS-NH_2.

423 Propidium iodide used after interaction of zinc oxide NPs with *P. subcapitata* highlights
424 interaction of NPs with surface sites of the cell wall leads to formation of distorted
425 morphological features and the loss of membrane integrity (Suman et al. 2015).

426 Van Hoecke et al. (2008) investigated the adhesion of silica NPs at the surface of
427 *Pseudokirchneriella subcapitata.* This adsorption phenomenon was reported for cerium oxide
428 particles in *P. subcapitata* and *C. reinhardtii* (Röhder 2014; Pulido-Reyes et al. 2015), as
429 well. A cell wall attachment could involve a physiologic disorder of microalgae, as a
430 photosynthesis impediment in *Chlorella* with nanopolystyrene (Bhattacharya et al. 2010) or
431 thylakoid disorganization. It has been demonstrated that, after adhesion, TiO_2 particles could
432 also disrupt the cell wall, leading to plasmolysis (Xia et al. 2015). Moreover, plasmolysis, a
433 water loss phenomenon by cell, is also induced after nickel oxide NPs adhesion in *C. vulgaris*
434 (Gong et al. 2011).

435 Spectroscopic tools (Infrared and Raman) also detect chemical modifications of
436 microalgae (Meng et al. 2014) during interaction with NPs. Chen et al. (2017) monitored TiO_2
437 NP effects by Fourier transform infrared spectroscopy in attenuated total reflection (FTIR-
438 ATR). Infrared spectra monitored a peak decline of functional groups such as C-N, C=O, C-
439 O-C and P=O, which are characteristic of cell-wall and membrane components during
440 photocatalysis of TiO_2 NP at the surface of *C. reinhardtii* (Chen et al. 2017). Infrared
441 spectroscopy was also used to monitor cerium oxide and aluminium oxide nanoparticle effects
442 on microalgae (Pakrashi et al. 2013; Pulido-Reyes et al. 2015). One of the major interests of
443 these techniques, in the case of nanoparticle adsorption on microalgae, is to inform on which
444 chemical groups can be impacted.

445 In the case of plastics, adsorption mechanisms have been investigated mainly with
446 microplastics (Lagarde et al. 2016). At this size, it has been shown that microalgae formed
447 cohesive hetero-aggregates with plastics by excretion of exopolymeric substances (EPS)
448 leading to their sedimentation (Long et al. 2015). At the nanoscale, adsorption of nanoplastics
449 was observed on *Scenesdesmus* and *Chlorella* (Bhattacharya et al. 2010) but in this case, there
450 was no indication of an impact on the sinking rate of microalgae.

451 **4.2.6. Absorption**

452 For very small particles (<20 nm), internalization can be visualised only by TEM
453 technique (Aruoja et al. 2015). NP uptake seems to adopt two ways, one natural via cell wall
454 pores and another unnatural via mechanical destructuration. The cell wall is semi-permeable
455 and can allow the crossing of very small particles (Navarro et al. 2008a). Once the uptake had
456 been completed, in which cellular compartments were NPs found?

457 Regarding the uptake of NPs, nickel oxide NP agglomerates were observed by TEM in the
458 cytoplasm of *C. vulgaris* (Oukarroum et al. 2017). In the same manner, copper oxide
459 aggregates were found in *C. reinhardtii* in the same compartment (Perreault et al. 2012). In
460 addition, cerium oxide NPs were found in *C. reinhardtii* around cells and intracellular vesicles
461 (Taylor et al. 2016).

462 TEM verified the internalization of silver, cerium, silica particles (Fujiwara et al. 2008;
463 Taylor et al. 2016; Wang et al. 2016). In some studies, aggregation was observed inside the
464 cell. Nickel oxide nanometric agglomerates are detected in *C. vulgaris* cytoplasm (Gong et al.
465 2011; Oukarroum et al. 2017), supposing NP uptake after plasma membrane disruption.
466 Moreover, in *C. reinhardtii*, Perreault et al., (2012) showed copper oxide aggregates of one
467 hundred nanometers potentially stocked in vacuoles.

468 **4.2.7. Impact at gene level**

469 Various monitoring techniques of molecular response have been described in the
470 literature. Techniques such as SDS-PAGE electrophoresis and MALDI MS-MS mass
471 spectrometry are utilized to identify proteins associated with silver NPs (Barwal et al. 2011).

472 After stress or direct environmental disruption, genetic expression offers a means of
473 following over/under-expression of genes. A RT-PCR (Reverse Transcriptase-Polymerase
474 Chain Reaction) study has been performed on genes of oxidative stress responses (superoxide
475 dismutase, glutathione peroxidase, catalase, and phototaxis). In this study, gene transcripts
476 linked with photosynthesis (psbA, nucleic gene encoding protein of liaison D1, part of
477 photosystem II reactive body; rbcS, small subunit gene of ribulose 1-5 bis phosphate
478 carboxylase) and with carotenoid biosynthesis pathway, (pds, gene encoding phytoene
479 desaturase) are also tracked (Wang et al. 2008). An exopolysaccharide biosynthesis pathway
480 may also be studied by quantitative PCR. Gene expression of UDP glucose 4-6 dehydratase
481 (UGLD), UDP-glucuronate decarboxylase (UGD), UDP glucose 4-epimerase (UGE) and
482 phosphoglucomutase (PG), implicated in the biosynthesis pathway of polysaccharides have
483 been monitored after interaction of microalgae *Chlamydomonas reinhardtii* with microplastics
484 (Lagarde et al. 2016). In addition, a transcriptomic approach was proposed by Taylor et al.
485 (2016), consisting of a gene survey encoding photosynthesis- implicated proteins. An ARN
486 study by sequencing (Simon et al. 2013) and an ARN messenger expression of CTR_2 protein
487 (copper carrier) (Leclerc and Wilkinson 2014) have also been reported for NPs.

488 The presence of particles inside the cell may modify the gene expression of microalgae. A
489 long-term study (70 days) with polypropylene and high-density polyethylene microplastics
490 has shown over-expression of the genes associated with the xylose biosynthesis pathway
491 (UGD: UDP-glucuronate decarboxylase) in *C. reinhardtii* (Lagarde et al. 2016). To our
492 knowledge, the same mechanisms could be suggested in the presence of nanoplastics but no

493 study on sugar biosynthesis pathway gene expression has been conducted so far in
494 microalgae.

495 Finally, Simon et al. (2013) reported for *C. reinhardtii* a decrease at the transcript level
496 linked with photosynthesis (growth reduction) in the presence of titane oxide or zinc NPs, and
497 a rise of gene transcripts linked to proteasome (increase of detoxification system, proteins
498 bend incorrectly). This study also underlines an enhancement of transcript levels encoding
499 cell wall components in the presence of silver NPs (Simon et al. 2013). Schiavo et al. (2016)
500 followed the genotoxicity of ZnO NPs with a COMET assay and reported effects from 5 mg
501 Zn/L.

502

503 5. Conclusion: Which NPs for which measured impact?

504 According to NP properties, measured impact on microalgae can widely differ. More than
505 sixty studies investigating the exposure of different microalgae to NPs with their reported
506 effects (see supplementary data) were summarized in Table 4 to investigate which NPs lead to
507 what impacts?

508 Silver is one of the most widely studied NPs (17/60 studies) as it is suspected of toxicity
509 contrarily to lanthanum oxide (La_2O_3), aluminium oxide (Al_2O_3), nickel oxide (NiO) and
510 manganese oxide (MgO) NP (Table 4). Most studies have reported internalization of Ag in
511 different types of microalgae (*C. reinhardtii, C. vulgaris, S. obliquus and E. gracilis*) due to
512 the release of Ag^+ ions and an agglomeration of ions inside the cell (Navarro et al. 2008b;
513 Barwal et al. 2011; Leclerc 2013; Ksiazyk et al. 2015; Wang et al. 2016; Yue et al. 2017;
514 Zhang et al. 2017). In most of these studies, the toxicity of Ag NPs appeared at very low
515 concentrations (0.01 mg/L (Leclerc and Wilkinson 2014), Table S1). Microalgae

photosynthesis was impacted by Ag NPs between 0.03 and 5 mg/L (Leclerc 2013; Navarro et al. 2015; Lodeiro et al. 2017; Sendra et al. 2017b; Yue et al. 2017; L. Zhang et al. 2017). Moreover, toxic concentrations varied from 0.26 to 4.91 mg/L (Table S1), depending on the surfactant, for the same Ag NPs (Navarro et al. 2015). However, in two of these studies no adverse effects were reported for a concentration of 4.63×10^{-4} M Ag NP on *Thalassiosira weissflogi* and *Chlorella vulgaris* (Miao et al. 2009; Eroglu et al. 2013). While other pure metallic NPs such as Au, Co or Pt NPs have been less widely studied, internalization was also observed on *Scenedesmus suspicatus* and *C. reinhardtii*, for small Au NPs (10 nm; Renault et al., 2008) whereas it did not appear for Co NPs. All metallic NPs presented an effect on growth and photosynthesis activity when investigated. As for Ag, the toxicity of Co NPs was supposedly induced by release of Co^{2+} ions, showing that the quantification of ion release is of major importance when studying the interaction of microorganisms with metallic NPs. In addition, the aggregation of Co NPs may lead to a shading effect on three microalgae species: *Platymonas subcordiforus, Chaetoceros curvisetus* and *Skeletonema costatum* (Chen et al. 2018). Overall, decrease of microalgae growth and photosynthesis activity are the most widely studied criteria, and they appear to be impacted in the majority of studies involving metallic and metallic oxide NPs, findings supporting the idea that metal containing NPs are toxic to microalgae. However, in all cases except TiO_2 NPs, the growth inhibition was monitored together with metallic ion release, raising questions on the actual toxicity of particles compared to ions (Lee and An 2013; Suman et al. 2015; Schiavo et al. 2016; Yung et al. 2017). Meanwhile, some studies still reported no effect at all of NPs on microalgae, for example Miller et al. (2010), who did not note any effect of TiO_2 NPs. Balusamy et al. (2015) reported no effect at all for lanthane oxide (La_2O_3) NPs at a high concentration (1000 mg/L), on *Chorella sp.* growth but for the same concentration, lethal effects were observed in *Daphnia magna,* showing that microalgae response is species-specific. On the contrary,

541 amorphous silica NPs, considered as biosafe, affected the growth of freshwater microalgae
542 according to Fujiwara et al. (2008) and Van Hoecke et al. (2008).

543 Another issue which may modify the effects of NPs on microalgae concerns particle
544 surface properties, and this parameter calls for in-depth characterization in exposure tests. The
545 coating effect of ZnO was studied by Yung et al. (2017) who showed that hydrophilic NPs
546 inhibited microalgae growth more than hydrophobic NPs. The surface properties of NPs were
547 likewise highlighted in impact measurements with the recent upsurge of research on
548 nanoplastics. Several studies reporting the effect of polystyrene (PS) NPs have been carried
549 out in various environments: fresh, brackish and sea-water (Besseling et al. 2014; Sjollema et
550 al. 2016; Bergami et al. 2017). Growth inhibition of *P. subcapitata* and *Scenesdesmus*
551 *obliquus* has been described with amide-polystyrene NPs (Besseling et al. 2014; Nolte et al.
552 2016; Bergami et al. 2017) whereas this phenomenon has not been observed with polystyrene
553 nanoparticles coated with carboxylic chemical groups, suggesting the importance of
554 nanoparticle surfactants (Table 6, Bhattacharya et al. 2010; Nolte et al. 2016; Bergami et al.
555 2017). PS NPs with NH_2 groups at their surface induced higher oxidative stress compared to
556 COOH coating, and toxicity was observed at lower concentrations (12.97 mg/L vs. more than
557 100 mg/L for COOH, Bhattacharya et al. 2010; Bergami et al. 2017). These results show that
558 monitoring the surface properties of NPs is of major importance in understanding interaction
559 pathways.

560 Different sizes of PS NP, ranging from 20 to 500 nm, have also been tested, but no
561 internalization of polymeric NPs has been mentioned (Table 4, S3). The minimal observed
562 toxicity concentration of PS NPs was found to be 6.5 mg/L (Bhattacharya et al. 2010).
563 However, the effects of nanoplastics on microalgae have been only sparsely described due to
564 the difficulty of their detection compared to metallic NPs and the low number of kinds of
565 nanoplastics that are currently commercially available. Lastly, even if the effects all of all

566 kinds of studied particles are often described as size-dependent, up until now no direct
567 correlation between the size of NPs and the described effects has been found. For example,
568 Fujiwara et al. (2008) suggested that the toxicity of silica NP was size-dependent, while Van
569 Hoecke et al. (2008) observed similar effects for two silica NP with different sizes and
570 different surface areas.

571 Overall, characterizing the properties of NPs in exposure experiments appears decidedly
572 important as a means of understanding the interaction pathways and potential impacts of these
573 particles on microalgae. Among several parameters currently used to monitor impacts
574 (photosynthesis, membrane integrity, biochemical composition changes, gene expression
575 changes...) growth is one the simpler parameters to record, and remains the most widely
576 mentioned as an indicator of nanoparticle toxicity on microalgae (Table 4). However, the
577 other parameters may be more informative as regards the mechanisms involved during
578 interaction and should also be accurately monitored. The most frequently used techniques in
579 nanoparticle-microalgae interaction monitoring are microscopy, biochemical assay (ROS,
580 propidium iodide quantification), genetic tools and spectroscopy. All of them provide data
581 that are complementary and most of the time, several are necessary to understand the impacts
582 of interaction on microalgae while providing sensitive information on NP properties and
583 modifications.

584 To conclude, among all the studies reported here, size, NP concentration and time of
585 interaction have greatly varied a fact rendering comparison difficult. What is more, methods
586 of characterization have differed from one study to another, with each of them providing only
587 some pieces of evidence. NPs is a generic name encompassing a wide-ranging variety of
588 materials that can pronouncedly differ in chemical nature, shape, surface properties and size.
589 It consequently appears very difficult to identify common pathways in their interactions with
590 microalgae. And lastly, primary producers also present diversified shapes and barriers

591 (frustule, cell-wall, plasmic membrane) which can be an obstacle to interpretation of the key
592 NP factors resulting in the impacts.

593 *Table 4: Effect synthesis of NPs interactions with microalgae. Red: effect reported in a majority of*
594 *studies (more than half), orange: effect reported in some studies but no frequently mentioned (less*
595 *than half), yellow: effect no clearly demonstrates (studies VS studies), green: no effect, white: not*
596 *investigated*

NP type	Study number	Recorded effect	Growth	Oxidative stress	Photosynthesis	Internalisation	Ions	Others
Ag	17	15/17						Gene overexpressed
CeO_2	15	12/15						Adsorption Cell membrane damage
ZnO	14	13/14						
PS	13	9/13						
TiO_2	11	9/11						Modification gene expression Shading effect
CuO	5	5/5						
Au	4	3/4						Adsorption
Co	3	3/3						Adsorption
NiO	2	2						
Si	2	2						
Pt	1	1						
La_2O_3	1	0						

Fe_2O_3	1	1						
MgO	1	1						
Al_2O_3	1	1						
QD	1	1						

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Table 1: Synthesis of metallic NP characteristics (type, surfactant, charge, primary size (diameter, aggregation (size instability of NPs) and microalgae exposed to NPs (environment, species and cell-wall presence/absence): n.a.: not indicated

<table>
<tr><th></th><th colspan="5">Nanoparticles</th><th colspan="3">Microalgae</th></tr>
<tr><th>Authors</th><th>Type</th><th>Surfactant</th><th>Charge (mV)</th><th>Primary size (nm)</th><th>Aggregation</th><th>Environment</th><th>Species</th><th>Cell-wall</th></tr>
<tr><td>Renault et al. 2008</td><td rowspan="5">Au</td><td>amine</td><td rowspan="14">n.a.</td><td>10</td><td>yes</td><td rowspan="15">Freshwater</td><td>Scenedesmus suspicatus</td><td rowspan="13">yes</td></tr>
<tr><td rowspan="4">Iswarya et al. 2016</td><td rowspan="2">citrate</td><td>16</td><td rowspan="2">no</td><td rowspan="4">Chlamydomonas reinhardtii</td></tr>
<tr><td>27</td></tr>
<tr><td rowspan="2">PVP</td><td>37</td><td rowspan="4">yes</td></tr>
<tr><td>16</td></tr>
<tr><td>27</td><td></td></tr>
<tr><td>37</td><td></td></tr>
<tr><td>Eroglu et al. 2013</td><td rowspan="3">no</td><td>30 by 14</td><td>n.a.</td><td>Chlorella vulgaris</td></tr>
<tr><td rowspan="2">Ksiazyk et al. 2015</td><td>Pt</td><td>51</td><td rowspan="2">yes</td><td rowspan="2">Pseudokirchneriella subcapitata</td></tr>
<tr><td rowspan="8">Ag</td><td>34</td></tr>
<tr><td>Simon et al. 2013</td><td>polyacrylate</td><td>-25.5</td><td>20</td><td>no</td><td>Chlamydomonas reinhardtii</td></tr>
<tr><td>Eroglu et al. 2013</td><td>n.a.</td><td rowspan="4">n.a.</td><td>10</td><td>n.a.</td><td>Chlorella vulgaris</td></tr>
<tr><td>Kustov and Abramenko 2016</td><td>SDS</td><td>10 to 12</td><td>n.a.</td><td>Chlorella vulgaris</td></tr>
<tr><td>Leclerc and Wilkinson 2014</td><td>polyacrylate</td><td>5</td><td rowspan="3">no</td><td rowspan="3">Chlamydomonas reinhardtii</td></tr>
<tr><td rowspan="2">Barwal et al. 2011</td><td rowspan="2">no</td><td>5;15</td><td>no</td></tr>
<tr><td>5;35</td><td>yes</td></tr>
</table>

Navarro et al. 2008b	carbonate	-36	25	yes			
		-36	40				
Navarro et al. 2015	chitosan	-5.1	25	no			
	citrate	-29	17				
	dexpanthenol	-3.8	456	yes			
	gelatine	-6.8	52	no			
	lactate	-3.5	350	yes			
	sodium dodecyl benzenesulfonate	-29	45	no			
	polyethylene glycol (PEG)	-5.5	70				
	polvinylpolypyrrolidone (PVP)	-6.3	84	yes			
Wang et al. 2016	polvinylpolypyrrolidone (PVP)	-21	7.5-16.5	n.a.			
Miao et al. 2010	carbonate	n.a.	<10	yes		*Ochromonas danica*	
Zhang L. et al. 2017	fluorescent	-15.5	2	no		*Scenedesmus obliquus*	
Yue et al. 2017	citrate	-25	19.4	yes		*Euglena gracilis*	
Sendra et al. 2017a	no	-5.6	16.7		Brackish water	*Dunaliella salina*	no
Burchardt et al. 2012		-38	20		Sea water	*Thalassiosira pseudonana*	frustule
		-49	40				
		-48	100				
Miao et al. 2009	carbonate	n.a.	60-70			*Thalassiosira weissflogii*	

Lodeiro et al. 2017		Tween 20 and Polyethylene Glycerol Trioleate	-4	48			*Chaetoceros curvisetus*	
Zhang X. et al. 2017		n.a.	n.a.	45			*Chlorella vulgaris*	yes
Chen et al. 2018	Co	no	-13.8	30			*Platymonas subcordiforus*	yes
							Chaetoceros curvisetus	frustule
							Skeletonema costatum	

963

Table 2: Synthesis of oxide metallic nanoparticle characteristics (type, surfactant, charge, primary size (diameter, aggregation (size instability of nanoparticles) and microalgae exposed to nanoparticles (environment, species): n.a.: not indicated

	Nanoparticles					Microalgae		
Authors	Type	Surfactant	Charge (mV)	Primary size (nm)	Aggregation	Environment	Specie	Cell-wall
Cheloni et al. 2016	CuO	no	-25	30-50	yes	Freshwater	*Chlamydomonas reinhardtii*	yes
von Moos et al. 2015		poly(styrene-co-butyl acrylate)	-20	30-50				
Perreault et al. 2012			-51	30-40				
Saison et al. 2010		PS	n.a.	80				no
Melegari et al. 2013		no	-35	30-40				yes
Kulacki and Cardinale 2012	TiO_2		n.a.	27			*Scenedesmus quadricauda*	
Chen et al. 2017				20-30			*Chlamydomonas reinhardtii*	
Wang et al. 2008				21				
Hartmann et al. 2010			-23	10			*Pseudokirchneriella subcapitata*	
			-21	30				
			-25	300				
Dauda et al. 2017			n.a.	<25	n.a.		*Chlorella vulgaris*	
Simon et al. 2013			-24	5	no		*Chlamydomonas reinhardtii*	
Lee and An 2013			-13.3	21	yes		*Pseudokirchneriella subcapitata*	
Miller et al. 2010			n.a.	15-20		Sea water	*Thalassiosira pseudonana*	frustule
			n.a.	15-20			*Dunaliella tertiolecta*	yes
Xia et al. 2015			-13.2	21			*Nitzschia closterium*	frustule
			-8.1	60				
			-6.4	400				
Hu et al. 2017			-19	5			*Isochrysis galbana*	yes
Taylor et al. 2016	CeO_2	PVP	n.a.	4-5	no	Freshwater	*Chlamydomonas reinhardtii*	
Pulido-Reyes et al. 2015		58% Ce^{3+}	-12.2	5	yes		*Pseudokirchneriella subcapitata*	
		28% Ce^{3+}	-25.6	7				

		40% Ce^{3+}	-21.2	18				
		26% Ce^{3+}	-24.4	50				
		36% Ce^{3+}	-21.1	350 on 20				
Manier et al. 2011		no	20	25				
Manier et al. 2013		citrate triammonium, non- aged	-21	10				
		citrate triammonium, aged 3days	n.a.					
		citrate triammonium, aged 30 days	n.a.					
Sendra et al. 2017b		no	23.6	26			*Chlorella autotrophica*	
Röhder et al. 2014		no	0	140			*Chlamydomonas reinhardtii*	no
Sendra et al. 2017a			0	140				yes
			4	26				
			-22	9				
			3.2	26		Sea water	*Phaeodactylum tricornutum*	yes
			3.2	26			*Namnochloris atomus*	
			-10	9			*Phaeodactylum tricornutum*	
			-10	9			*Namnochloris atomus*	
Simon et al. 2013	ZnO	no	2.7	20	no	Freshwater	*Chlamydomonas reinhardtii*	
Lee and An 2013			-23.3	<100	yes		*Pseudokirchneriella subcapitata*	
Yung et al. 2017			n.a.	23			*Chlamydomonas reinhardtii*	
		3-aminopropyltrimethoxysilane	-	24				
		dodecyltrichlorosilane		23				
		no	n.a.	23			*Chlorella pyrenoidosa*	
		3-aminopropyltrimethoxysilane	-	24				
		dodecyltrichlorosi		23				

		lane						
		no	n.a.	23			*Pseudokirchneriella subcapitata*	
		3-aminopropyltrimethoxysilane	-	24				
		dodecyltrichlorosilane		23				
		no	n.a.	23		Sea water	*Thalassiosira pseudonana*	frustule
		3-aminopropyltrimethoxysilane	-	24				
		dodecyltrichlorosilane		23				
Schiavo et al. 2016			-10	100			*Dunaliella tertiolecta*	yes
Suman et al. 2015			n.a.	40-48	n.a.		*Chlorella vulgaris*	
Miller et al. 2010				20-30	yes		*Thalassiosira pseudonana*	frustule
Schiavo et al. 2017				20-30			*Dunaliella tertiolecta*	yes
				<100				
Peng et al. 2011				6.3-15.7			*Thalassiosira pseudonana*	frustule
				242-862				
				6.3-15.7			*Chaetoceros gracilis*	
				242-862				
				6.3-15.7			*Phaeodactylum tricornutum*	
				242-862				
Balusamy et al. 2015	La_2O_3	no	14	60	no	Freshwater	Chlorella sp.	yes
Oukarroum et al. 2017	NiO		n.a.	30	yes		*Chlorella vulgaris*	
Gong et al. 2011				20				
He et al. 2017	Fe_2O_3	n.a.		<30	n.a.		*Scenedesmus obliquus*	
	MgO			<50				
Pakrashi et al. 2013	Al_2O_3	no	12	82	yes		*Chlorella ellipsoidea*	
			17	247				

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966 *Table 3: Synthesis of plastic, silica and Quantum Dot (QD)nanoparticles characteristic (type, surfactant, charge, primary size (diameter, aggregation*
967 *(size instability of nanoparticles) and microalgae exposed to nanoparticles (environment, species and cell-wall presence/absence): n.a: not indicated*

	Nanoparticles					Microalgae		
Authors	Type	Surfactant	Charge (mV)	Primary size (nm)	Aggregation	Environment	Specie	Cell-wall
Bhattacharya et al. 2010	PS	COOH	-40	20	yes	Freshwater	*Scenesdesmus*	yes
		NH_2	106					
		COOH	-40				*Chlorella*	
		NH_2	106					
Besseling et al. 2014		SDS	n.a.	70	n.a.		*Scenesdesmus obliquus*	
Nolte et al. 2016		COOH	-54	110	yes		*Pseudokirchneriella subcapitata*	
		NH_2	106	20				
Sjollema et al. 2016		COOH	n.a.	500	n.a.		*Chlorella vulgaris*	
						Sea water	*Thalassiosira pseudonana*	
		no		50		Brackish water	*Dunaliella tertiolecta*	
				500				
Bergami et al. 2017		COOH	-66	40	yes			
		NH_2	53	50				
Li 2015		Fluorophore	-24	50	no	Freshwater	*Euglena gracilis*	no
							Haematococcus pluvialis	yes

							Chlamydomonas reinhardtii	yes
								no
			-44	500				yes
								no
Casado et al. 2013		Polyethyleneimine	40	55	yes		*Pseudokirchneriella subcapitata*	yes
				110				
Fujiwara et al. 2008	Si	no	n.a.	5	n.a.	Freshwater	*Chlorella kessleri*	yes
				26				
				78				
Van Hoecke et al. 2008			-	12.5	no		*Pseudokirchneriella subcapitata*	
				27				
Wang et al. 2008	QD		n.a.	3.5-4.5	yes		*Chlamydomonas reinhardtii*	

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Supplementary data

Table S1: Synthesis of metallic nanoparticles effect on microalgae (concentration of toxicity effect, endpoints tested: growth, shading, photosynthesis, oxidative stress, ion release, internalization); n.a.: not indicated

	Nanoparticles		Microalgae	Toxicity effect			Endpoints: effects						
Authors	Type	Surfactant	Specie	Yes/No	Effect concentration (mg/L)	Time (h)	Growth	Shading	Photosynthesis	Oxidative stress	Ions	Internalization	Others
Renault et al. 2008	Au	amine	*Scenedesmus suspicatus*	yes	$1{,}59x10^{-6}$	24	yes	n.a.	n.a.	n.a.	n.a.	yes	adsorption
Iswarya et al. 2016		citrate	*Chlamydomonas reinhardtii*		0.01	72						n.a.	
		PVP											n.a.
Eroglu et al. 2013		no	*Chlorella vulgaris*	no	n.a.	n.a.	n.a.		yes		n.a.		carotenoïd production
Ksiazyk et al. 2015	Pt		*Pseudokirchneriella subcapitata*	yes	22.2	72	yes		n.a.		no		drop of chlorophyll
	Ag				5						yes		drop of chlorophyll
Simon et al. 2013		polyacrylate	*Chlamydomonas reinhardtii*		1	2	n.a.	yes	yes	no		yes	gene overexpressed
Eroglu et al. 2013		n.a.	*Chlorella vulgaris*	no	n.a.	n.a.		n.a.	yes	n.a.	n.a.	n.a.	carotenoïd production
Kustov and Abramenko 2016		SDS	*Chlorella vulgaris*	yes	0.067	22	yes		n.a.		yes		n.a.
Leclerc and Wilkinson 2014		polyacrylate	*Chlamydomonas reinhardtii*		0.01	2	n.a.					yes	gene overexpressed
Barwal et al. 2011		No			n.a.	n.a.							n.a.
Navarro et al. 2008b		carbonate			7.75	5			yes				
Navarro et					2.72	1						n.a.	

al. 2015		chitosan			3.03								
		citrate			4.91								
		dexpanthenol			0.26								
		gelatine			4.41								
		lactate			1.89								
		sodium dodecyl benzenesulfonate			3.51								
		polyethylene glycol (PEG)			1.19								
		polvinylpolypyrrolidone (PVP)			0.74								
Wang et al. 2016		polvinylpolypyrrolidone (PVP)			2	48			n.a.			yes	
Miao et al. 2010		carbonate	*Ochromonas danica*		0.14		yes					n.a.	
Zhang L. et al. 2017		fluorescent	*Scenedesmus obliquus*		0.03	96	n.a.		yes	yes		yes	transcriptomic study
Yue et al. 2017		citrate	*Euglena gracilis*		0.16	1	n.a.						enzymatic activity inhibition
Sendra et al. 2017b		no	*Dunaliella salina*		5	72	yes			n.a.		n.a.	n.a.
Burchardt et al. 2012			*Thalassiosira pseudonana*		0.01				n.a.				
					0.02								
Miao et al. 2009		carbonate	*Thalassiosira weissflogii*	no	0.4	48	no		no				EPS production
Lodeiro et al. 2017		Tween20 et Polyethylene Glycerol Trioleate	*Chaetoceros curvisetus*	yes	0.041		yes		yes	no	no		n.a.
Zhang X. et al. 2017		n.a.	*Chlorella vulgaris*		1	96			no	yes	yes	yes	
Chen et al. 2018	Co	no	*Platymonas subcordiforus*		67.2		yes		yes	n.a.		no	Adsorption
			Chaetoceros curvisetus		38.6								
			Skeletonema costatum		21.5			yes					

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Table S2: Synthesis of oxide metallic nanoparticle effect on microalgae (concentration of toxicity effect, endpoints tested: growth, shading, photosynthesis, oxidative stress, ions releasing, internalization); n.a.: not indicated

	Nanoparticles		Microalgae	Toxicity effect			Endpoints: effects						
Authors	Type	Surfactant	Specie	Yes/No	Effect concentration (mg/L)	Time (h)	Growth	Shading	Photosynthesis	Oxidative stress	Ions	Internalization	Others
Cheloni et al. 2016	CuO	no	*Chlamydomonas reinhardtii*	yes	0.8	4	no	yes	n.a.	no	yes	n.a.	n.a.
von Moos et al. 2015					10	5		n.a.	yes	yes		no	membrane permeability
Perreault et al. 2012		poly(styrene-co-butyl acrylate)			4	6	n.a.				n.a.	yes	n.a.
Saison et al. 2010		PS			20	6						n.a.	
Melegari et al. 2013		no			150	72	yes		n.a.	n.a.	yes	yes	carotenoid levels decrease
Kulacki and Cardinale 2012	TiO_2		*Scenedesmus quadricauda*		n.a.	n.a.					no	no	n.a.
Chen et al. 2017			*Chlamydomonas reinhardtii*		130	3	n.a.	yes		yes		n.a.	
Wang et al. 2008					10	72	yes	n.a.	no				gene overexpressed
Hartmann et al. 2010			*Pseudokirchneriella subcapitata*		241				n.a.				n.a.
					71.1								
					145								
Dauda et al. 2017			*Chlorella vulgaris*		0.1	96					n.a.		
Simon et al. 2013			*Chlamydomonas reinhardtii*		1	2	n.a.	yes	yes	no	no	yes	gene underexpressed
Lee and An 2013			*Pseudokirchneriella subcapitata*		2.5	72	yes	no	n.a.	yes	n.a.	n.a.	n.a.
Miller et al. 2010			*Thalassiosira pseudonana*	no	> 1	96	no	n.a.		no			
			Dunaliella tertiolecta										
Xia et al. 2015			*Nitzschia closterium*	yes	89		yes			yes		yes	membrane damage
					119								
					179								
Hu et al.			*Isochrysis*		20			no	no	n.a.		n.a.	n.a.

2017			*galbana*										
Taylor et al. 2016	CeO_2	PVP	*Chlamydomonas reinhardtii*		80	72	no	n.a.			yes	yes	gene overexpressed
Pulido-Reyes et al. 2015		58% Ce^{3+}	*Pseudokirchneriella subcapitata*		1		yes		n.a.	yes	n.a.	n.a.	adsorption
		28% Ce^{3+}		no	50		no			no			
		40% Ce^{3+}								n.a.			
		26% Ce^{3+}											
		36% Ce^{3+}		yes	5		yes						
Manier et al. 2011		no			11.5				n.a.				n.a.
Manier et al. 2013		citrate triammonium, non aged			5.6					n.a.			adsorption
		citrate triammonium, aged 3days			4.1								
		citrate triammonium, aged 30 days			6.2								
Sendra et al. 2017b		no	*Chlorella autotrophica*		1				no	no	yes		change in cell complexity
Röhder et al. 2014			*Chlamydomonas reinhardtii*		4.36	2	n.a.		yes	yes			adsorption
					3.66								
Sendra et al. 2017a					100	72	yes		n.a.		n.a.	yes	cell membrane damage
					200		no					no	
			Phaeodactylum tricornutum		10		yes					yes	cell membrane damage
			Namnochloris atomus		200		no					no	n.a.
			Phaeodactylum tricornutum										
			Namnochloris atomus	no						no			
Simon et al. 2013	ZnO		*Chlamydomonas reinhardtii*	yes	1	2	n.a.	yes	yes	yes	no	yes	gene overexpressed
Lee and An 2013			*Pseudokirchneriella subcapitata*		<0.05	72	yes	n.a.	n.a.		yes		membrane destabilization
Yung et al. 2017			*Chlamydomonas reinhardtii*		<10	96			yes	n.a.		n.a.	n.a.
		3-			10								

		aminopropyltrimethoxysilane											
		dodecyltrichlorosilane			15								
		no	*Chlorella pyrenoidosa*		20								
		3-aminopropyltrimethoxysilane			30								
		dodecyltrichlorosilane			40								
		no	*Pseudokirchneriella subcapitata*		20								
		3-aminopropyltrimethoxysilane			25								
		dodecyltrichlorosilane			35								
		no	*Thalassiosira pseudonana*		5								
		3-aminopropyltrimethoxysilane			5								frustule formation decrease
		dodecyltrichlorosilane			10								n.a.
Schiavo et al. 2016			*Dunaliella tertiolecta*		5	72			n.a.	yes	n.a.	yes	cellular division inhibition
Suman et al. 2015			*Chlorella vulgaris*		100						yes		membranar damage,lipidic peroxidation
Miller et al. 2010			*Thalassiosira pseudonana*		0.5	96				n.a.		n.a.	n.a.
			Dunaliella tertiolecta		1								
Schiavo et al. 2017					2.2	72							
Peng et al. 2011			*Thalassiosira pseudonana*		10							yes	adsorption
			Chaetoceros gracilis									n.a.	
			Phaeodactylum tricornutum	no			no					yes	
												n.a.	
Balusamy et al. 2015	La_2O_3	no	Chlorella sp.	no	1000						n.a.		chlorophyll level increase
Oukarroum	NiO		*Chlorella*	yes	13.7	96	yes		yes	yes	yes	yes	n.a.

et al. 2017			*vulgaris*										
Gong et al. 2011					32.28	72		yes	n.a.	n.a.		n.a.	
He et al. 2017	Fe_2O_3	n.a.	*Scenedesmus obliquus*		40	96		n.a.	yes	yes	n.a.		
	MgO				0.8	72							lipidic content and peroxydase activity increase
Pakrashi et al. 2013	Al_2O_3	no	*Chlorella ellipsoidea*		0.001				n.a.	no	yes		

Table S3: Synthesis of plastic, silica and Quantum Dot (QD) nanoparticle effect on microalgae (concentration of toxicity effect, endpoints tested: growth, shading, photosynthesis, oxidative stress, internalization); n.a.: not indicated

	Nanoparticles		Microalgae	Toxicity effect			Endpoints: effects					
Authors	Type	Surfactant	Specie	Yes/No	Effect concentration (mg/L)	Time (h)	Growth	Shading	Photosynthesis	Oxidative stress	Internalization	Others
Bhattacharya et al. 2010	PS	COOH	*Scenesdesmus*	yes	6.5	2	n.a.	maybe	yes	no	no	n.a.
		NH_2								yes		
		COOH	*Chlorella*							no		
		NH_2								yes		
Besseling et al. 2014		SDS	*Scenesdesmus obliquus*	yes	1000	72	yes	n.a.	yes	n.a.		
Nolte et al. 2016		COOH	*Pseudokirchneriella subcapitata*	no			no		n.a.			
		NH_2		yes	40		yes				n.a.	
Sjollema et al. 2016		COOH	*Chlorella vulgaris*	yes	250		no	yes	no		no	
			Thalassiosira pseudonana									
			Dunaliella tertiolecta									
		no					yes					

					251							
Bergami et al. 2017		COOH		no	50		no	n.a.	n.a.	no	n.a.	
		NH_2		yes	12.97		yes			yes		
Li 2015		fluorophore	*Euglena gracilis*	yes	1000	2	n.a.	n.a.	n.a.	n.a.	no	Morphologic changes
			Haematococcus pluvialis	no								Flagella covered by particles
			Chlamydomonas reinhardtii wild type	no								EPS excretion
			Chlamydomonas reinhardtii mutant									EPS excretion
Casado et al. 2013		Polyethyleneimine	*Pseudokirchneriella subcapitata*	yes	0.58 (55nm) 0.54 (110nm)	72	yes				n.a.	
Fujiwara et al. 2008	Si	no	*Chlorella kessleri*	yes	800(0.8%m/v)	96	yes	n.a.	yes	n.a.	yes	n.a.
					7100(7.1%m/v)						no	
					9100 (9.1%m/v)							
Van Hoecke et al. 2008			*Pseudokirchneriella subcapitata*		20	72			n.a.			
					28.8							
Wang et al. 2008	QD		*Chlamydomonas reinhardtii*		5				no	yes	n.a.	Gene overexpressed