# Chapter 16
# Ecotoxicological Effects of Nanomaterials on Growth, Metabolism, and Toxicity of Nonvascular Plants


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## 16.1 Introduction

Nanomaterials (NMs), defined as materials with one or more dimensions of the size of 1–100 nm (ASTM/E2456-06 2012; Reiners 2013), have gained increasing attention due to their unique properties, relative to their bulk counterparts, which impart them beneficial characteristics including a high specific surface area and reaction activity (Laurent et al. 2008) and the quantum confinement effects (Amelia et al. 2012). Nowadays with the increasing insertion of nanotechnology in our daily life, nanomaterials of different shapes and diameters have been developed and used in various consumer products, pharmaceuticals, cosmetics (Melo et al. 2015), and other commodities (Bradley et al. 2011; Ghasemzadeh et al. 2014; Singh 2017).

Nanoparticles (NPs), a subgroup of nanomaterials, are classified into various categories depending on their size, morphology, and chemical properties. The primary focus of this chapter is metal, metal oxide, carbon-based NPs, and quantum dots. Metal NPs, i.e., Cu, Ag, and Au, in nanometer range possess unique optical, electrical, and magnetic properties due to their localized surface plasmon resonance (LSPR) characteristics (Dreaden et al. 2012). They are considered as the potential candidates for catalysis due to their large surface area per volume or weight unit, compared to their bulk counterparts, typically functioning on metal surfaces (Roldan Cuenya 2010). Metal oxide nanoparticles represent a class of engineered nanomaterials that can be synthesized via several routes (Lang et al. 2011). The usual practices for manufacturing metal oxide nanoparticles are chemical vapor synthesis (Stankic et al. 2016) and the addition of oxidizing/precipitating agents during their synthesis (Sanchez-Dominguez et al. 2009). Metal oxide nanoparticles include both individual (e.g., $TiO_2$, $CeO_2$, $CrO_2$, ZnO, $Bi_2O_3$, and $MoO_3$) and binary oxides (e.g., $BaTiO_2$, InSnO, and $LiCoO_2$). They too have wide applications in industry.

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Carbon-based NPs comprise mainly of two major groups: fullerenes and carbon nanotubes (CNTs). Fullerenes are globular cage-like structures with various numbers of carbon atoms (e.g., $C_{60}$, $C_{70}$). They have created noteworthy commercial interest due to their remarkable electronic and structural properties, high strength, and versatility (Astefanei et al. 2015). The fullerene-$C_{60}$ is the most commercially attractive carbon-based NP due to its ability to increase the efficiency of drugs, cosmetics, and electronics (Bianco and Da Ros 2011). Carbon nanotubes (CNTs) have been listed at the third position of the most important engineered nanoparticles (ENPs) found in the consumer product inventories (Vance et al. 2015). They are elongated, tubular structure with a large length/diameter ratio (Ibrahim 2013). They can be metallic or semiconducting reliant on a chiral vector value (the way they are rolled up) (Aqel et al. 2012). Structurally, they resemble graphite sheet rolling upon itself. Depending on the number of carbon sheets, they can either be single-walled (SWNTs), double-walled (DWNTs), or multiwalled carbon nanotubes (MWNTs), respectively (Elliott et al. 2013). CNTs have many potential applications, e.g., in plastics, batteries, paints, composites, touch screens, and drug delivery (De Volder et al. 2013).

Semiconductor materials possess properties between metals and nonmetals and therefore have found various applications (Ali et al. 2017). Semiconductor NPs or quantum dots (QDs) possess wide bandgaps and therefore show significant alteration in their properties with bandgap tuning by exhibiting particle size-dependent tunable photoluminescence (Dybiec et al. 2007). These properties render them very useful materials in photocatalysis, photo optics, electronic devices (Chow and Jahnke 2013), biology, and medicine (Zhou et al. 2015). The most common commercially available QDs are CdSe/ZnS-QDs due to their bright and unique emission with wide excitation spectra and narrow emission bandwidths (Deerinck 2008).

The increased use of nanomaterials in several industrial applications and consumer products ranging from cosmetics to medicine (e.g., odor-resistant textiles, household appliances, including wound dressings) (Rai et al. 2009; Namasivayam et al. 2010; Chaudhari et al. 2012) during the past decade has led to a rise in concerns about the potential toxic effects of accidentally or incidentally released NPs into the environment and their likely access into ambient aquatic systems (Colvin 2003; Service 2008). Scientists have expressed concerns about the potential adverse effects of NPs to beneficial bacteria in the environment, especially in soil and water. Although toxic effects of NPs on bacterial, fungal, and mammalian cells have been well investigated (Shrivastava et al. 2007, 2009; Kim et al. 2009), their impact on the growth and biology of algae and nonvascular lower plants has not been sufficiently documented (Lee et al. 2005).

"Lower plants" represent a heterogeneous group of plants and plantlike organisms, including algae, bryophytes, and lichens, which are characterized primarily by their lack of vascular tissues (which circulate water and nutrients in higher plants) (Eddy et al. 1992). However, pteridophytes, which are also included in lower plants due to absence of seeds, have the vasculature (phloem and xylem tissues), like the seed-bearing "higher plants." This chapter is focused on algae and bryophytes, the nonvascular lower plants, because there is hardly any information on responses of sister groups (like lichens, pteridophytes) to NPs.

Algae represent a large and diverse group of photosynthetic eukaryotes. They comprise of many ancient and miscellaneous lineages, including various symbiotic relationships with animals and fungi. Moreover, they display many degrees of organismal complexity: they range from microscopic unicells to macroscopic bodies and also possess multicellular thalli more than a meter in length (De Clerck et al. 2012). Further, algal cell wall surface has an additional layer of rigid, porous cell wall for modulating the entry of foreign materials, ions, and particles (Chen et al. 2012). Algae are commercially important since they can be used as biofertilizers, pollution control agents (algae bioreactors) (Pimratcha et al. 2015), biofuels (Hannon et al. 2010), stabilizers of casein, and source of nutrition (B complex vitamins and minerals) and can be incorporated to cosmetics (Spolaore et al. 2006). They form a critical component of almost all aquatic and many terrestrial ecosystems. As primary producers in the aquatic ecosystem, they are important indicators for environmental pollution monitoring and therefore constitute widely used model organisms in ecotoxicity studies of nanomaterials (Ma and Lin 2013; Quigg et al. 2013). The observed toxicities of NPs to algae have been attributed mainly to three mechanisms (Schwab et al. 2011; Long et al. 2012): (1) reduction of photosynthetic rate due to inhibition of light transmittance (shading effect) (Miazek et al. 2015); (2) NP agglomeration and physical interaction with algal cells, leading to the internalization of NPs and the disruption of the cell membrane (García-Cambero et al. 2013); and (3) induction of intracellular reactive oxygen species (ROS) formation leading to membrane lipid peroxidation and changes in the concentration of nonenzymatic antioxidants and in the activity of antioxidant enzymes (Chen et al. 2012).

Like algae, bryophytes are also nonvascular plants that can grow on the surface of tree trunks or rocks and generally absorb water and nutrients direct through leaf surfaces from their immediate environment (Dymytrova 2009; Schröder et al. 2010; Harmens et al. 2011). Some species play an important role in the colonization of bare or degraded soil and facilitate the installation and maintenance of higher plants, a significant fact for a healthy plant-soil dynamics and sustainable ecosystem development. Moreover, these lower plants are recognized as good accumulators of pollutants, especially for metal trace elements (Garrec and Van Haluwyn 2002; Faburé et al. 2010). Furthermore, these organisms lack a root system ensuring their exposure to atmospheric pollutants and are excellent sensors of air quality for different contexts and pollutants (Faburé et al. 2010; Meyer et al. 2010; Lodenius 2013). Owing to these properties, bryophytes are considered excellent models for evaluating atmospheric pollutant impact on the environment in many parts of the world (Oishi 2013; Agnan et al. 2015; Vuković et al. 2015). The observed toxicities of NPs to bryophytes have been mainly attributed to (a) NP agglomeration that leads to the NP uptake by leaves (Canivet et al. 2014) and (b) overproduction of ROS/RNS and induction of glutathione status modulation (Canivet et al. 2015).

Since the last decade, the use of NPs in our daily life has considerably increased, and the ecotoxicity studies about NP effects on plants and animals come up rapidly. However, the published data on NP toxicity on algae and plants of sister groups are insufficient, and the experimental designs and testing conditions are inconsistent

across the studies. This chapter provides an outline of the NP ecotoxicological effects on these plants based on the data available in the published literature and focuses on the underlying mechanisms of NP toxicity.

## 16.2 Effects of Metal Nanoparticles

### *16.2.1 Gold Nanoparticles (Au-NPs)*

A recent study explored the impacts of amine-coated 10 nm gold NP contaminations on the green alga *Scenedesmus subspicatus* (Renault et al. 2008). The growth/mortality effects were determined by the algal cell numerations. The lethal dose for 50% of the population was reached within a 24 h exposure at $1.6 \times 10^5$ Au-NPs/cell, while mortality for the lowest contamination condition was 20%. TEM examination revealed that Au-NPs were strongly adsorbed by the cell wall of algae, leading to progressive intracellular and wall disturbances. However, bare and hyaluronic acid-capped Au-NPs (particle size 12.5 nm) were found to be harmless to *S. subspicatus* in contrast to bulk soluble gold that gave an $EC_{50}$ value 1.91 mg $L^{-1}$ (García-Cambero et al. 2013). The carbonate- and citrate-coated Au-NPs did not cause any significant toxicity to the green algae *Chlamydomonas reinhardtii* (Behra et al. 2015).

The effects of Au-NPs with amphiphilic polymer coating (AP) or amphiphilic coating to which 10 kDa polyethylene glycol chains were attached (AP-PEG) were also assessed on the green algae *Pseudokirchneriella subcapitata* (Van Hoecke et al. 2013). The AP Au-NPs were more toxic than the AP-PEG after a 72 h exposure with $EC_{50}$ values 7.5 and 39 mg Au-NPs $L^{-1}$, respectively. In another study Au-NP toxicity effects on *P. subcapitata* (formerly known as *Selenastrum capricornutum*) were evaluated by three biomass measuring techniques (coulter counting, cell counting in hemocytometer, and fluorescence of pigment extracts) (Hartmann et al. 2013). The coulter counting method gave unreliable results. Therefore, it was not considered suitable for biomass quantification. At 48 h, algal cultures showed follow the growth of the control sample – both with respect to cell number and pigment content – whereas at 72 h a leveling off was noticed in pigment content of all algal cultures exposed to Au-NP dispersions accompanied by a decrease in EC10. On the contrary, cell number growth rates followed continued exponential trends. The results also indicated that pigment (such as chlorophyll and carotenoid) synthesis was affected by the exposure to Au-NP dispersions despite the continued exponential cell growth. Although the $EC_{50}$ values were higher than the highest tested concentration, subsequent tests of the effects of the "Starch Control" (starch/glucose/MES solution in concentrations identical to that of the Au nanoparticle dispersions) revealed that the dispersion constituents, and not Au-NPs themselves, were largely responsible for inhibitory effects as well as the characteristic leveling off in pigment content after 48 h.

A recent study suggested that the naturally existing ions like zinc ions could modulate the toxicity of Au-NPs (Iswarya et al. 2017). The effects of Au-NPs with two surface cappings (citrate and PVP) and three different sizes (16, 27, and 37 nm) were explored on a predominant freshwater alga *Scenedesmus obliquus* in the sterile freshwater matrix. Among the different-sized Au-NPs, the highest toxicity (54%) was observed at 1 mg $L^{-1}$ of citrate-capped Au-NPs with particle size 37 nm, whereas PVP-capped Au-NPs showed 42% toxicity. A statistically significant reduction in the Au-NP (both citrate-capped/37 nm and PVP-capped/37 nm) toxicity was observed when $Zn^{2+}$ (5 mg $L^{-1}$) was added to the growth medium (12% for citrate-capped −37 and 11% for of PVP-capped −37). All the above information on the Au-NP toxicity on lower plants is summarized in Table 16.1, specifying the NP size, the algal species used, the half maximal effective concentration, the exposure time, and the effects observed.

**Table 16.1** Summarized results from Au-NP toxicity studies performed on the lower plants

| Species | Particle size (nm) | $EC_{50}$ (mg Au-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Scenedesmus subspicatus* (Chodat) | 10 | $1.6 \times 10^5$ Au-NPs per cell at 24 h | Agglutination, intracellular and wall disturbances | Renault et al. (2008) |
| *S. subspicatus* (Chodat) | 12.8 | 1.91 at 72 h | Agglomeration | García-Cambero et al. (2013) |
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 51 by dynamic light scattering (DLS) measurements 46 by NTA measurements | 36 at 48 h 38 at 72 h fluorescence of pigments/ 83 at 72 h hemocytometer counts | Formation of larger agglomerates/ aggregate during time, reduction of pigment (chlorophyll and carotenoid) synthesis | Hartmann et al. (2013) |
| *P. subcapitata* (Korshikov) F. Hindák | 4–5 | AP:7.5 at 72 h AP-PEG:39 at 72 h | Growth inhibition | Van Hoecke et al. (2013) |
| *Chlamydomonas reinhardtii* P.A. Dangeard (strain CC 125 and wall-free strain CC-400) | 5 | – | No effects observed | Behra et al. (2015) |
| *Scenedesmus obliquus* (Turpin) Kützing | 16 | PVP-capped: >1 | Aggregation, cytotoxicity | Iswarya et al. (2017) |
| | | Citrate-capped: >1 | | |
| | 27 | PVP-capped: >1 | | |
| | 37 | Citrate-capped: >0.1 and <1 | | |

### *16.2.2 Silver Nanoparticles (Ag-NPs)*

Ag-NPs have shown extensive adverse effects on growth and morphology of the green algae *Pithophora oedogonia* and *Chara vulgaris* in a dose-dependent manner (Dash et al. 2012). Exposure of algal thalli to increasing concentrations of Ag-NPs resulted in progressive chromosome instability, mitotic disturbance, depletion of chlorophyll content, and the associated morphological malformations in algal filaments. SEM micrographs revealed dramatic alterations in cell wall, characterized with cell wall rupture and degradation in the NP-treated *Pithophora*. Discoloration of filaments due to chloroplast contraction followed by disintegration, regional bulging of filaments, thinning and disruption of cell wall permitting exclusion of the chlorophyll pigments, adsorption of Ag-NPs on cell surface and organellar membranes, mitostatic effect, induction of chromosomal anomalies, and irreversible genetic damage were the significant detrimental effects of nanosilver recorded in the tested algae. Ag-NPs also caused growth inhibition on the green alga*Pseudokirchneriella subcapitata* with an $EC_{50}$ value 0.19 mg Ag-NPs $L^{-1}$ after 96 h incubation (Griffitt et al. 2008).

The short-term toxicity of citrate-stabilized Ag-NPs and ionic silver Ag(I) to the ichthyotoxic marine raphidophyte *Chattonella marina* has also been investigated (He et al. 2012). The addition of Ag-NPs to GSe medium caused aggregation and dissolution of Ag-NPs. Cellular uptake of dissolved Ag(I) was observed, and toxicity effects were much higher for Ag(I) than for Ag-NPs. However, these inhibitory effects of Ag(I) and Ag-NPs were completely removed by the addition of cysteine, a strong Ag(I) ligand, suggesting that the toxicity of Ag-NPs was due to the release of Ag(I). The growth inhibition effects of Ag-NPs have been studied also on the bloom-forming cyanobacterial *Microcystis aeruginosa* strain after a 10-day exposure (Duong et al. 2016). A dose-dependent reduction of the cell growth was observed by increasing Ag-NP concentrations. The $EC_{50}$ value based on the cell growth was 0.0075 mg $L^{-1}$, and the inhibition efficiency at the highest concentration of Ag-NPs (1 mg $L^{-1}$) was 98.7%. SEM and TEM images indicated shrunken and damaged cell wall attributed to toxicity of Ag-NPs.

The toxic effects of large-sized Ag-NPs (50 nm) were investigated on the freshwater microalga *Chlorella vulgaris* and the marine microalga *Dunaliella tertiolecta* after 24 h exposure (Oukarroum et al. 2012). Ag-NPs interacted directly with the *Chlorella vulgaris* cell surface forming large aggregates and caused significant decrease in chlorophyll contents and algal viability, while they induced ROS formation and lipid peroxidation in both algae, showing a variability in sensitivity (1 mg $L^{-1}$ Ag-NPs induced a 44% decrease of viable cells for *D. tertiolecta* and 33% for *C. vulgaris*). In another study, aggregation and dissolution behavior of gum arabic (GA)- and polyvinylpyrrolidone (PVP)-coated Ag-NPs were compared in a mixture of aquatic plants *Potamogeton diversifolius* and *Egeria densa* (Unrine et al. 2012). Plants released dissolved organic matter (DOM) into the water column either through active or passive processes in response to Ag exposure that bound Ag ions.

As a result, the plant-derived DOM stabilized PVP-Ag-NPs as the primary particles but removed GA-Ag-NPs from the water column, possibly by dissolution and binding of the released Ag ions on sediment and plant surfaces.

The extent and mechanisms of toxicity of two Ag-NPs with differing size distributions (AG1 and AG2) and capping agents were investigated on two model organisms, a green alga (*Chlamydomonas reinhardtii*) and a cyanobacterium (*Synechococcus leopoliensis*) (Taylor et al. 2016a). Their effects on the production of extracellular polymeric substances (EPS) were also assessed. Both silver forms had a significant effect on viability and membrane integrity in *C. reinhardtii* but hardly affected ROS production, whereas no toxicity effects were observed in *S. leopoliensis*. The levels of EPS produced by both the species were similar for all the treatments. The EPS composition was affected from AG1 in a concentration-dependent manner and conversely from AG2. Higher levels of lower molecular weight material were produced by *C. reinhardtii* in the presence of all silver forms. Reduction in growth rate was observed for *S. leopoliensis*, but the impact on viability and ROS was lower than for *C. reinhardtii* probably due to differences in relevant biological properties (e.g., algal cell size and cell wall composition).

The abovementioned toxicity data of Ag-NPs on lower plants are presented in Table 16.2, specifying the particle size of Ag-NPs, the algal species used, the half maximal effective concentration and exposure time, and the effects observed.

### *16.2.3 Platinum Nanoparticles (Pt-NPs)*

Pt-NP toxicity toward green microalgae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* was assessed (Sørensen et al. 2016), using the standard ISO tests for estimation of growth rate inhibition ($EC_{50}$ values of 15−200 mg Pt-NPs $L^{-1}$). By using a double-vial setup, cells were separated from Pt-NPs, which indicated that shading is an important artifact for Pt-NP toxicity. Membrane damage was not severe, but substantial oxidative stress was detected at 0.1–80 mg Pt-NPs $L^{-1}$ in both the algal species. Pt-NPs caused a growth rate inhibition and oxidative stress in *P. subcapitata*, in a low concentration of dissolved Pt, indicating the NP-specific toxicity of Pt. In addition, higher body burdens were measured in this species, possibly due to a favored binding of Pt to the polysaccharide-rich cell wall. However, in a previous study, the Pt-NP concentration causing total inhibition of algal growth was 22.2 mg $L^{-1}$ (Książyk et al. 2015). Similar results were obtained by analyzing the levels of photosynthetic pigments in *P. subcapitata* exposed to nanoparticles. In another study, where the acute toxicity of $PtCl_4$ and Pt-NPs was investigated, $EC_{50}$ values were 14 mg $L^{-1}$ after 48 h exposure and 28 mg $L^{-1}$ after 2 h, indicating that the toxicity was dependent on the exposure duration (Delgado et al. 2013).

**Table 16.2** Summarized results from Ag-NP toxicity studies performed on lower plants

| Species | Particle size (nm) | $EC_{50}$ (mg Ag-NPs/$L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 20–30 | 0.19 at 96 h | Growth inhibition | Griffitt et al. (2008) |
| *Pithophora oedogonia* (Mont.) Wittrock *Chara vulgaris* Linn. | 10–15 | n.m.[a] | Depletion in algal chlorophyll content, chromosome instability, mitotic disturbance, morphological malformations in algal filaments | Dash et al. (2012) |
| *Chattonella marina* (Subrahmanyan) Hara et Chihara (strain CMPL01) | 56 | 10–20 uM at 1 h | Growth inhibition, aggregation, cellular uptake | He et al. (2012) |
| *Chlorella vulgaris* Beyerinck *Dunaliella tertiolecta* Butcher (CPCC-420) | 50 | n.m.[a] >1 at 24 h | Aggregation, decrease in chlorophyll content and algal viability, induction of ROS formation and lipid peroxidation | Oukarroum et al. (2012) |
| *Potamogeton diversifolius* Raf. | 49.3 (PVP) | Not assayed | Aggregation | Unrine et al. (2012) |
| *Egeria densa* Planch. | 12.0 (GA) | | | |
| *Microcystis aeruginosa* (Kützing) Kützing | 10–15 | 0.0075 | Growth inhibition, changes in cell structure and morphology | Duong et al. (2016) |
| *Chlamydomonas reinhardtii* P.A. Dangeard (CCAP strain 11/32c) *Synechococcus leopoliensis* (Raciborski) Komárek | 3–8 (AG1) 50 (AG2: PVP) | 0.038 (AG1) and 0.031 (AG2) at 72 h0.028 (AG1) and 0.01 (AG2) at 72 h | Growth inhibition only, variation on the composition of the produced EPS | Taylor et al. (2016a, b) |

[a]Not mentioned

## 16.3 Effects of Metal Oxide Nanoparticles

### *16.3.1 Alumina Nanoparticles*

The toxicological impact of $Al_2O_3$-NPs to lower plants was demonstrated on algal species *Scenedesmus* sp. and *Chlorella* sp. (Aruoja et al. 2015). The observed $EC_{50}$ value of $Al_2O_3$-NPs with particle size <50 nm after 72 h was 45.4 mg $L^{-1}$ for *Chlorella* sp. and 39.35 mg $L^{-1}$ for *Scenedesmus* sp. Bulk alumina (particle size <5 um) also

showed toxicity in a lower range ($EC_{50}$ = 110.2 mg $L^{-1}$ for *Chlorella* sp.; 100.4 mg $L^{-1}$ for *Scenedesmus* sp.). Additionally, chlorophyll content declined, and the cell surface was also affected (Sadiq et al. 2011b). Earlier, $Al_2O_3$-NPs with a particle size of 51 nm showed an $EC_{50}$ value 8.30 mg $L^{-1}$ at 96 h for the algal species *Pseudokirchneriella subcapitata* (Griffitt et al. 2008), whereas smaller $Al_2O_3$-NPs (particle size 8–21 nm) showed an $EC_{50}$ value >10–100 mg $Al_2O_3$ $L^{-1}$ at 72 h.

In addition, toxic effects of binary compounds of aluminum oxide alpha-forms (7 and 70 nm) and macro form (4um) on the growth of unicellular algae *Chlorella vulgaris* (Gosteva et al. 2015) were found to be concentration-dependent. A selective dependence of $Al_2O_3$-NPs toxicity on the size, concentration, and chemical nature of NPs was revealed. The $EC_{50}$ values for the small-sized $Al_2O_3$-NPs (7 and 70 nm) were around 1 mg $L^{-1}$, whereas no toxicity was observed for the macro form.

### *16.3.2 Cerium Oxide Nanoparticles ($CeO_2$-NPs)*

An assessment of the molecular and phenotypic effects of $CeO_2$-NPs was conducted with the unicellular green alga, *Chlamydomonas reinhardtii*, by using well-characterized monodispersed NPs (particle size 4– nm) based on the hypothesis that their toxicity is likely to be higher than the macro form (Taylor et al. 2016a, b). The potential toxicity of NPs was investigated by transcriptomics and metabolomics approaches in a wide range of exposure concentrations in order to provide insight into molecular toxicity pathways. Even though $CeO_2$-NPs inserted the intracellular vesicles within *C. reinhardtii*, they did not cause significant changes in the algal growth at any exposure concentration. At supra-environmental $CeO_2$-NPs concentrations, downregulation of photosynthesis and the carbon fixation perturbations were detected with further effects on energy metabolism.

In a previous study, on a short-term exposure, dissolved $Ce^{3+}$ decreased the photosynthetic yield in a concentration-dependent manner with $EC_{50}$ values of 7.5 uM for the wild type and 6.3 uM for a cell wall-free mutant strain of *C. reinhardtii*, whereas precipitated $CePO_4$(s) was not bioavailable and, hence, not toxic (Röhder et al. 2014). The intracellular ROS levels increased upon exposure to $Ce^{3+}$ with the effective concentrations being similar to those inhibiting photosynthesis. Moreover, $CeO_2$-NPs agglomerated in exposure media and caused a slight inhibition of photosynthesis and reduction of intracellular ATP content upon a short-term exposure at the highest (100 uM) concentrations possibly due to $Ce^{3+}$ ions co-occurring in the nanoparticle suspension, whereas no effect was observed for dispersed $CeO_2$-NPs as the dissolved $Ce^{3+}$ got precipitated with phosphate and, hence, was not bioavailable. Moreover, flocculation of algal cells upon exposure to agglomerated $CeO_2$-NPs and $Ce^{3+}$ was observed. The cell wall-free mutant and wild type of *C. reinhardtii* showed the same sensitivity to either $Ce^{3+}$ or $CeO_2$-NPs toxicity indicating that the cell wall does not have a protective effect against $CeO_2$-NPs or $Ce^{3+}$.

### *16.3.3 Titanium Dioxide Nanoparticles*

The studies on a potential impact of $TiO_2$-NPs on the environment have been conducted on green alga *Desmodesmus subspicatus* by determining its growth during a 72 h incubation period (Hund-Rinke and Simon 2006). Twenty-five nm-sized $TiO_2$-NPs ($EC_{50}$ = 44 mg $TiO_2$-NPs $L^{-1}$) were more toxic to *D. subspicatus* than 100 nm $TiO_2$-NPs (no toxicity observed). It was demonstrated that the smaller particles caused a clear dose-dependent reduction in the algal growth, whereas the larger ones showed less toxicity ($C < 50$ mg $TiO_2$-NPs $L^{-1}$). On similar lines, $TiO_2$-NPs (10, 30, 300 nm) were applied to the algal species *Pseudokirchneriella subcapitata*, and their effects assessed after 72 h incubation (Hartmann et al. 2010). Smaller $TiO_2$-NPs (<10 nm) showed inhibition (21% reduction) in growth rate at the concentration of 2 mg $L^{-1}$, whereas, 30 and 300 nm $TiO_2$-NPs showed a slight stimulation of algal growth. In earlier studies, $TiO_2$-NPs with particle size of 30 nm (Griffitt et al. 2008) and ~ 100 nm (Blaise et al. 2008) were shown not to be toxic to this alga.

In another study, *P. subcapitata* was used for toxicity assessment of not readily soluble NPs with standardized algal growth inhibition tests (Hartmann et al. 2013). $TiO_2$-NPs formed large (micron-sized) agglomerates/aggregates in a dose-dependent manner. Three biomass surrogate measuring techniques (coulter counting, cell counting in hemocytometer, and fluorescence of pigment extracts) were evaluated. The results showed a concentration-dependent reduction in algal growth by both the biomass quantification techniques, yielding an $EC_{50}$ value of 160 mg $TiO_2$-NPs $L^{-1}$ (by hemocytometer). The $EC_{50}$ value based on measurements of pigment fluorescence was found to be 200 mg $L^{-1}$ (the highest tested concentration was 560 $TiO_2$-NPs $L^{-1}$). *P. subcapitata* was also used for toxicity assessment of fine (140 nm) and ultrafine (~140 nm) $TiO_2$ (uf-$TiO_2$) particles after incubation for 72 h (Warheit et al. 2007). $EC_{50}$ values (95% fiducial limits) based on inhibition of growth and healthy average cell counts were 16 mg $L^{-1}$ for fine $TiO_2$-NPs and 21 mg $L^{-1}$ for uf-$TiO_2$-NPs. Aruoja et al. (2009) found that bulk $TiO_2$ ($EC_{50}$ = 35.9 mg Ti $L^{-1}$) were less toxic to this algal species than their nano formulations ($EC_{50}$ = 5.83 mg $TiO_2$-NPs $L^{-1}$). $TiO_2$-NPs formed characteristic aggregates entrapping the algal cells, thus contributing therefore to the toxic effect of $TiO_2$-NPs to algae. In a later study, it was indicated that the agglomerates entrapped nearly all algal cells so that the cells could mostly be seen inside the agglomerates and rarely in the surrounding medium (Aruoja et al. 2015). The high variability in the observed toxicity of $TiO_2$-NPs has been discussed by Menard et al. (2011), but no discernable correlation between primary particle size and toxic effect could be proved because the existing data were insufficient for confirmation (Menard et al. 2011).

Manier et al. (2016) indicated that the type of exposure system also affects the toxicity of $TiO_2$-NPs. Different exposure systems including the Erlenmeyer flasks and 24-well microplates (both using an orbital shake system) and an alternative system using cylindrical vials and magnetic stirring were used. After a 72 h exposure of *P. subcapitata* to two different types of $TiO_2$-NPs (particle size <10 and 20 nm), the authors found that the exposure systems applied to achieve the test can substantially

affect the ecotoxicological results and the subsequent calculated $EC_{50}$ values. The selected systems influenced both the interaction between algal cells and $TiO_2$-NPs as well as the growth inhibition level (Manier et al. 2016). The cytotoxicity potential of $TiO_2$-NPs was also assessed toward freshwater algal isolate *Scenedesmus obliquus* under dark and UV conditions at low exposure levels ($\leq$1 μg $mL^{-1}$) (Dalai et al. 2013). Statistically significant reduction in cell viability and photosynthetic pigment content and increase in ROS production and membrane permeability (light vs. dark) were observed. Cell viability at 1 μg $mL^{-1}$ concentration under UV illumination and dark conditions was 59.1% and 69.46%, respectively, for 72 h exposure period. Cellular uptake of NPs was indicated in electron micrographs, whereas fluorescence micrographs and images from confocal laser scanning microscopy (CLSM) brought out their probable genotoxic effects (Dalai et al. 2013).

In addition, a comparative study was conducted by Sadiq et al. (2011a, b) to demonstrate the toxic effects caused by $TiO_2$-NPs toward the freshwater algae (*Scenedesmus* sp. and *Chlorella* sp.) isolated from freshwater environment after 72 h incubation. The particles had a growth-inhibiting effect for both species ($EC_{50}$ = 16.12 mg $TiO_2$-NPs $L^{-1}$ for *Chlorella* sp.; $EC_{50}$ = 21.2 mg $TiO_2$-NPs $L^{-1}$ for *Scenedesmus* sp.). Bulk (micron-sized) $TiO_2$ also showed toxicity though to a lesser extent ($EC_{50}$ = 35.50 mg $TiO_2$-NPs $L^{-1}$ for *Chlorella* sp.; $EC_{50}$ = 44.40 mg $TiO_2$-NPs $L^{-1}$ for *Scenedesmus* sp.). A concentration-dependent reduction in the fluorescence of chlorophyll content was also observed (Sadiq et al. 2011a, b). These species were also used in a comparative study of the photocatalytic activity of P25 $TiO_2$-NPs under dark, visible light and UVA conditions (Roy et al. 2016). *Chlorella* was more sensitive toward the toxicity effects than *Scenedesmus*. Furthermore, at the highest exposure concentration, ROS generation was found correlated with inactivation of the antioxidant enzymes (SOD and GSH) for both the algae under visible light and UVA conditions. Additionally, $TiO_2$-NPs increased catalase activity and LPO release, indicating the membrane damage, particularly high in *Chlorella*, which is a single-cell algae and therefore is more susceptible to $TiO_2$-NP uptake in comparison to *Scenedesmus*, which shows a high colonization tendency.

Moreover, toxicity of NPs of binary compounds of titanium dioxides (with particle size 5, 50, 90, and 350 nm) was studied again on the unicellular alga *Chlorella vulgaris* (Gosteva et al. 2015). Substantiating the findings of Hartmann et al. (2010), this study revealed a selective dependence of $TiO_2$-NP toxicity on size and concentration of NPs. $TiO_2$-NPs with particle size 5 and 90 nm were classified to the category "acute toxicity 1," whereas no acute toxicity was registered for particle size 50 nm. Physiological, biochemical, and molecular genetic levels were assessed on the unicellular green alga*Chlamydomonas reinhardtii* after application of $TiO_2$-NPs (Wang et al. 2008). Growth inhibition was observed during the first 2–3 days of incubation with $TiO_2$-NPs, but later a dose-dependent recovery was observed. Oxidative stress occurred within the cell after exposure to $TiO_2$-NPs, which caused an increase in malondialdehyde levels, while four stress response genes (sod1, gpx, cat, and ptox2) were upregulated in cultures containing even 1 mg $L^{-1}$ of $TiO_2$-NPs. The maximum transcripts of cat, sod1, gpx, and ptox2 occurred at 1.5, 3, 3, and 6 h, respectively, proportional to the initial concentration of the NPs.

Kulacki and Cardinale (2012) examined how $TiO_2$-NPs (ranging from 0 to 300 mg $TiO_2$-NPs $L^{-1}$) affect the population dynamics and production of biomass across a range of the North American freshwater algae (*Anabaena* spp., *Navicula subminuscula*, *Nitzschia pusilla*, *Oscillatoria* spp., *Planothidium lanceolatum*, *Scenedesmus quadricauda*, *Selenastrum minutum*, *Spirogyra communis*, *Stigeoclonium tenue*, *Tabularia fasciculata*). The effects of $TiO_2$-NPs on the population growth rate of each algal species over a period of 25 days were not significant ($p = 0.376$), even though there was a considerable species-specific differentiation in responses (strong inhibition of maximum growth rate for *Spirogyra communis*, whereas strong stimulation of maximum growth rate for *Stigeoclonium tenue*). On the contrary, exposure to $TiO_2$-NPs tended to increase the maximum biomass achieved by species in culture ($p = 0.06$).

Finally, the effects of $TiO_2$-NPs and bulk particles on the marine microalga *Nitzschia closterium* were evaluated with reference to growth, oxidative stress, and cellular uptake after 96 h incubation (Xia et al. 2015). Toxicity of $TiO_2$-NPs to algal cells significantly increased with decreasing nominal particle size, and the $EC_{50}$ values were 88.78, 118.80, and 179.05 mg $L^{-1}$ for 21, 60, and 400 nm NPs, respectively. The growth was significantly inhibited on exposure to 5 mg $L^{-1}$ of 21 nm $TiO_2$ NPs. Activities of antioxidant enzymes, viz., peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT), were induced at the beginning and thereupon were inhibited, whereas malondialdehyde (MDA) levels and reactive oxygen species (ROS) increased following the exposure to 5 mg $L^{-1}$ $TiO_2$ NPs, indicating damages on the cell membrane. Flow cytometry and TEM studies and Ti content measurements indicated that $TiO_2$-NPs were internalized in *N. closterium* cells. The level of extracellular ROS was negligible, as compared to the intracellular ROS level, suggesting that the elevated $TiO_2$ toxicity in marine environments is related to increased ROS levels caused by internalization of $TiO_2$-NPs.

The abovementioned toxicity data of $TiO_2$-NPs are presented in Table 16.3, specifying the particle size, the algal species used, the half maximal effective concentration, and the exposure time used.

**Table 16.3** Summarized results from $TiO_2$-NP toxicity studies performed on algae

| Species | Particle size (nm) | $EC_{50}$ (mg $TiO_2$-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Desmodesmus subspicatus* Chodat | 25<br>100 | 44 at 72 h | Reduction of fluorescence intensity | Hund-Rinke and Simon (2006) |
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 140 | 16 (fine $TiO_2$-NPs) at 72 h | Growth inhibition | Warheit et al. (2007) |
| | Mean~ 140 | 21 (uf-C $TiO_2$-NPs) at 72 h | | |

(continued)

**Table 16.3** (continued)

| Species | Particle size (nm) | $EC_{50}$ (mg $TiO_2$-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *P. subcapitata* (Korshikov) F. Hindák | 30 | n.m.[a] | Growth inhibition | Griffitt et al. (2008) |
| *Chlamydomonas reinhardtii* P.A. Dangeard | 21 | n.m.[a] >10 at 72 h | Aggregate during time, growth inhibition, lipid peroxidation, increase of malondialdehyde level, upregulation of sod1, gpx, cat, and ptox2 gene transcripts | Wang et al. (2008) |
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | <100 | n.m.[a] at 72 h | No toxicity effects mentioned | Blaise et al. (2008) |
| *P. subcapitata* (Korshikov) F. Hindák | 25<br>70 | 5.83 at 72 h<br>35.9 at 72 h | Aggregate during time, growth inhibition | Aruoja et al. (2009) |
| *P. subcapitata* (Korshikov) F. Hindák | 10<br>30<br>300 | 241 at 72 h,<br>71.1 at 72 h,<br>145 at 72 h | Concentration-dependent aggregation, reduction of fluorescence intensity | Hartmann et al. (2010) |
| *Scenedesmus* sp. | 25 | 21.2 ($TiO_2$-NPs) at 72 h, | Reduction of fluorescence intensity | Sadiq et al. (2011a, b) |
| | | 35.5 (bulk $TiO_2$-NPs) at 72 h | | |
| | | 16.12 ($TiO_2$-NPs) at 72 h, | | |
| *Chlorella* sp. | | 44.4 (bulk $TiO_2$-mPs) at 72 h | | |
| *Anabaena* spp., *Navicula subminuscula* Manguin, *Nitzschia pusilla* Grunow, *Oscillatoria* spp., *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, *Scenedesmus quadricauda* (Turpin) Brébisson, *Selenastrum minutum* (Nägeli) Collins, *Spirogyra communis* (Hassall) Kützing, *Stigeoclonium tenue* (C.Agardh) Kützing, *Tabularia fasciculata* (C. Agardh) D.M.Williams and Round | 27 | n.m.[a] | Moderate growth inhibition across all species, increase of maximum biomass | Kulacki and Cardinale (2012) |

(continued)

**Table 16.3** (continued)

| Species | Particle size (nm) | $EC_{50}$ (mg $TiO_2$-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 21 | 200 at 72 h (fluorescence of pigments), 160 at 72 h (hemocytometer counts) | Formation of larger agglomerates/aggregate during time, reduction of pigment (chlorophyll and carotenoid) synthesis | Hartmann et al. (2013) |
| *Scenedesmus obliquus* (Turpin) Kützing | 25 | n.m.[a] >1 at 72 h | Agglomeration, ROS generation, genotoxicity, increased membrane permeability and $TiO_2$-NP uptake | Dalai et al. (2013) |
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 8–21 | >1–10 at 72 h | Growth inhibition, agglomeration | Aruoja et al. (2015) |
| *Chlorella vulgaris* Beyerinck | 5<br>50<br>90<br>350 | 1.153 at 72 h,<br>>100 at 72 h,<br>7.7 at 72 h,<br>>100 at 72 h | Growth inhibition | Gosteva et al. (2015) |
| *Nitzschia closterium* (Ehrenberg) W.Smith | 21<br>60<br>400 | 88.78<br>118.80<br>179.05 | Aggregation, growth inhibition, decrease in the activities of SOD, CAT, and POD, increase in MDA and ROS levels, damages in cell membrane, NP internalization | Xia et al. (2015) |
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | <10 | 8.5 at 72 h (24-well microplates), 2.7 at 72 h (in cylindrical vial systems), >50 at 72 h (in Erlenmeyer flasks) | Growth inhibition | Manier et al. (2016) |
| | 20 | >50 at 72 h (24-well microplates), 39 at 72 h (in cylindrical vial systems), >50 at 72 h (in Erlenmeyer flasks) | | |
| *Scenedesmus* sp. | 21 | 7.6 at 72 h under dark conditions | Growth inhibition under UVA, ROS generation, decreased SOD activity and GSH levels especially under UVA, increased catalase levels, LPO release and $TiO_2$-NP uptake | Roy et al. (2016) |
| | | 4.1 at 72 h under visible light | | |
| | | 2.7 at 72 h under UVA | | |
| *Chlorella* sp. | | 5.9 at 72 h under dark conditions | | |
| | | 2.16 at 72 h under visible light | | |
| | | 1.5 at 72 h under UVA | | |

[a]Not mentioned

### 16.3.4 Zinc Oxide Nanoparticles

Toxicity of ZnO-NPs to the algae *Pseudokirchneriella subcapitata* was determined by the OECD 201 algal growth inhibition test (Aruoja et al. 2009). $EC_{50}$ values of bulk and nano ZnO particles were both similar to that of $ZnSO_4$ (at 72 h $EC_{50}$ ~ 0.04 mg ZnO-NPs $L^{-1}$), and no aggregation formation was observed. This was attributed to the dissolved Zn possibly because most of the ZnO is dissolved at these low concentrations (Franklin et al. 2007). The toxicity data were close to those obtained by Franklin et al. (2007) who showed $EC_{50}$ values for the same algal species to be 0.063 mg Zn $L^{-1}$ for bulk ZnO and 0.068 mg Zn $L^{-1}$ for nano ZnO after 72 h exposure. In another study performed on *P. subcapitata*, $EC_{50}$ value was between 0.1 and 1 mg ZnO-NPs $L^{-1}$ with particle size 8–21 nm (Aruoja et al. 2015).

Toxicity of ZnO-NPs with particle size 20 nm was also evaluated on the unicellular alga *Chlorella vulgaris* (Morgalev et al. 2015). A concentration-dependent reduction in the fluorescence of growth rate was observed after application of ZnO-NPs. The detected value of $EC_{50}$ was 0.17 mg ZnO-NPs $L^{-1}$. In assessing the maximum effect of ZnO-NPs on *Chlorella* and other organisms according to GHS and EU Directive 93/67/ EEC, they were assigned to dangerous substances with a high-degree toxicity "acute toxicity 1."

### 16.3.5 Iron Oxide and Zerovalent Iron Nanoparticles

Engineered zerovalent nano-iron particles (Fe-NPs) hold promise for remediation of several pollutants, but their impact on the environment is not completely clear. The effects of three types of nZVI, (a) Nanofer 25 (uncoated), (b) Nanofer 25S (surface coated with a Na-acrylic copolymer), and (c) Nanofer STAR (Surface stabilized Transportable Air-stable Reactive) powder with an inorganic coating, were assessed on the growth, cell morphology, and metabolic status of marine microalgae *Pavlova lutheri*, *Isochrysis galbana*, and *Tetraselmis suecica* after 23-day exposure (Kadar et al. 2012). The algal growth rate, size distribution, and cellular structure were not altered significantly in any of the three species. The total cellular lipid content increased in *T. suecica* grown on media enriched with uncoated Nanofer 25 and in *P. lutheri* with Nanofer STAR, when compared at equimolar exposures. Furthermore, there occurred a significant change in fatty acid composition complementing the Nanofer STAR-mediated increase in lipid content of *P. lutheri*. Likewise, Zehnder medium fortified with zerovalent Fe-NPs (Nanofer 25 and Nanofer 25S) boosted the growth of four green algae (*Desmodesmus subspicatus*, *Dunaliella salina*, *Parachlorella kessleri*, and *Raphidocelis subcapitata*), two eustigmatophycean algae (*Nannochloropsis limnetica* and *Trachydiscus minutus*), and the cyanobacterium *Arthrospira maxima* (Pádrová et al. 2015). In all the species studied, zerovalent Fe-NPs induced lipid accumulation, the saturated and monounsaturated fatty acid (except palmitoleic acid) and polyunsaturated fatty acid contents in cells. The authors suggested that these particles may provide a source of

iron that increases cell growth and enhances metabolic changes leading to higher lipid production and changes in the composition of fatty acids.

In another study, toxicities of four zerovalent Fe-NPs of different sizes (20, 50, 80, and 100 nm), $Fe_2O_3$-NPs of two sizes (30 and 20 nm) of different crystal phases (α, γ), and $Fe_3O_4$-NPs of one size (20 nm) were assessed with green alga *Chlorella pyrenoidosa*, focusing on the effects of particle size, crystal phase, oxidation state, and environmental aging (Lei et al. 2016). The results indicated a significant increase in toxicity as particle size decreased. The algal growth inhibition decreased with oxidation of the NPs with an order of zerovalent Fe-NPs > $Fe_3O_4$ NPs > $Fe_2O_3$ NPs, while α-$Fe_2O_3$ NPs ($EC_{50}$ = 71 mg $L^{-1}$) presented significantly higher toxicity than γ-$Fe_2O_3$ NPs ($EC_{50}$ = 132 mg $L^{-1}$). The $EC_{50}$ values after a 96 h exposure to zerovalent Fe-NPs were for 100 nm (91.3 mg $L^{-1}$) > 80 nm (81.2 mg $L^{-1}$) > 50 nm (74.1 mg $L^{-1}$) > 20 nm (19.8 mg $L^{-1}$). The NP-induced oxidative stress was the main toxic mechanism, which could give a possible explanation in the difference in algal toxicity caused by NPs with the contribution of agglomeration and physical interactions.

The effects of Fe-NPs have also been assessed on the bryophyte *Physcomitrella patens* subsp. *patens* after foliar exposure (Canivet et al. 2015). The effects (cytotoxicity, oxidative stress, lipid peroxidation of membrane) of Fe-NPs from industrial emissions of metallurgical industries were determined through the axenic culture of *P. patens* exposed at five different concentrations (5 ng, 50 ng, 500 ng, 5 mg, and 50 mg per plant). At concentrations tested over a short period (24 h, 72 h), the levels of ROS, MDA, and glutathione were not significantly disturbed, but after internalization (168 h) the Fe-NPs could interact with the intracellular medium and cause cytotoxic effects and/or oxidative stress. Additionally, confocal microscopy experiments revealed that Fe-NPs (particle size 20–80 nm) penetrated the leaves of *Aphanorrhegma patens* when applied as mineral water suspensions (Canivet et al. 2014). By the way, this was the first demonstration of NP uptake by a bryophyte, and the actual penetration mechanism remains mysterious as also in higher plants.

### *16.3.6 Copper Oxide Nanoparticles*

In a recent study, toxicity of CuO-NPs toward the algal species *Pseudokirchneriella subcapitata* was estimated by the OECD 201 algal growth inhibition test (Aruoja et al. 2009). CuO-NPs with mean particle size 30 nm were found to cause higher toxicity (72 h $EC_{50}$ = 0.71 mg CuO-NPs $L^{-1}$) than one caused by bulk CuO (72 h $EC_{50}$ = 11.55 mg CuO-NPs $L^{-1}$). No aggregates were observed in the growth medium. These findings are in agreement with those of a previous study where 15–45 nm CuO-NPs gave 0.54 mg CuO-NPs $L^{-1}$ $EC_{50}$ value at 96 h (Griffitt et al. 2008). The toxicity of CuO-NPs to algae has also been assessed in the presence of dissolved organic matter (DOM). One of the main fractions of DOM (Suwannee river fulvic acid) (20 mg $L^{-1}$) was added alone or in the presence of CuO-NPs in the culture medium of the prokaryotic alga *Microcystis aeruginosa* (Wang et al. 2011). Internalization of CuO-NPs was observed in the intact algal cells at certain locations

(e.g., thylakoids and granules), and the cell uptake was enhanced by Suwannee river fulvic acid (SRFA). The main form of intracellular NPs observed was $Cu_2O$, indicating that intracellular environment may reduce CuO into $Cu_2O$. The increased CuO nanotoxicity observed in the presence of SRFA was related to the decreased rate of aggregation formation, the higher $Cu^{2+}$ release, and the induction in the internalization of CuO-NPs.

In another study, the short-term effects of core-shell copper oxide NPs (CS-CuO-NPs) in two different agglomeration states on the green alga *Chlamydomonas reinhardtii* were examined, and toxicity was investigated with regard to change in cellular population structure, primary photochemistry of photosystem II, and the ROS formation (Saison et al. 2010). CS-CuO-NPs induced cellular aggregation processes and reduced chlorophyll levels by inhibiting photosystem II. This process (inhibition of photosynthetic electron transport) induced a strong energy dissipation process via non-photochemical pathways indicating the formation of reactive oxygen ROS. However, no ROS formation was observed when *C. reinhardtii* was exposed to the core without the shell or to the shell only. The toxicity of carbon-coated copper nanoparticles (Cu-NPs) was also investigated using the alga *C. reinhardtii* and compared with effects of dissolved $Cu^{2+}$ (provided as $CuSO_4$) (Müller et al. 2015). The Cu-NPs agglomerated in the medium from original size of 6–7 nm to average particle sizes of 140–200 nm possibly due to the hydrophobic properties of the carbon coating. Cu-NPs strongly decreased the photosynthetic yield of *C. reinhardtii* after 1–2 h exposure to dissolved $Cu^{II}$ in a concentration range 1–100uM, whereas this decrease occurred in a concentration range of 0.1–10 uM for $CuSO_4$. Cu-NP effects on photosynthetic yield were similar for the same concentration of dissolved $Cu^{2+}$ for 1 h exposure and slightly stronger after longer exposure times. After the addition of EDTA as a strong ligand for $Cu^{II}$, toxicity of both dissolved $Cu^{II}$ and of Cu-NPs was completely suppressed.

The toxicity effects of sonicated and non-sonicated CuO suspensions (<50 nm) were elucidated on macrophytic (*Nitellopsis obtusa*) and microphytic (*Chlorella* spp.) algae cells (Manusadžianas et al. 2012). Cell lethality and resting potential depolarization were used to measure the NP effects on *N. obtusa*, whereas photosynthetic efficiency was assessed on *Chlorella* spp. There were no substantial differences between the effects of non-sonicated and sonicated CuO-NP suspensions. The particles rapidly reagglomerated within 5 min after sonication. The lethal concentrations of CuO-NPs did not evoke a rapid cell membrane depolarization in *N. obtusa* within the initial 90 min period, indicating that charophyte cell wall might have delayed the NP toxic effects. Significant cell membrane depolarization could be observed only after a 6 h exposure. In addition, rewash lethality tests revealed that 5 min exposure in 100 mg CuO-NPs $L^{-1}$ concentration induced algal cells mortality by 70% after 8 days, whereas 6 h exposure at 0.64 mg $L^{-1}$ of $Cu^{2+}$ evoked less than 40% cell mortality. The observed lethal effects of algae cells as well as delayed cell membrane depolarization were evoked by nanoparticles or their agglomerates per se, but not by dissolved Cu, as neither chemical analysis nor biological testing confirmed the presence of $Cu^{2+}$ in toxic amounts.

The macrophyte *Lemna gibba* was also used to evaluate CuO-NP toxicity. On exposure to CuO-NPs or soluble copper for 48 h, photosynthetic activity was inhibited due to inactivation of photosystem II reaction centers, causing a decrease in electron transport rate and an increase of thermal energy dissipation (Perreault et al. 2014). Toxicity of CuO-NPs was mainly driven by copper ions released from particles due to the NPs' tendency to agglomerate in the culture medium.

The data on CuO-NPs versus algae are presented in Table 16.4.

**Table 16.4** Summarized results from CuO-NP toxicity studies performed on lower plants

| Species | Particle size (nm) | $EC_{50}$ (mg CuO-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | 15–45 | 0.54 at 96 h | Growth inhibition | Griffitt et al. (2008) |
| *P. subcapitata* (Korshikov) F. Hindák | 30 | 0.71at 72 h | Growth inhibition | Aruoja et al. (2009) |
| *Chlamydomonas reinhardtii* P.A. Dangeard (wall-free strain CC-400) | 81 | n.m.[a] | Formation of aggregates, inhibition of photosynthesis, ROS formation | Saison et al. (2010) |
| *Microcystis aeruginosa* (Kützing) Kützing | <10 | 0.47 at 72 h | Growth inhibition, ROS formation, DNA damage, membrane integrity damage | Wang et al. (2011) |
| *Nitellopsis obtusa* (N.A. Desvaux) J. Groves | ~30 | 4.3 for non-sonicated NPs at 96 h | Growth inhibition, agglomeration, inhibition of photosynthesis, cell membrane depolarization | Manusadžianas et al. (2012) |
| | | 2.8 for sonicated NPs at 96 h | | |
| *Chlorella* sp. | | 57 for non-sonicated NPs at 30 min | | |
| | | 47 for sonicated NPs at 30 min | | |
| *Lemna gibba* L. | 30–40 | n.m.[a] | Growth inhibition, inactivation of photosystem II reaction centers, decrease in electron transport rate, increase of thermal energy dissipation | Perreault et al. (2014) |
| *C. reinhardtii* P.A. Dangeard | 6–7 | 1.87 ± 3 at 2 h | Agglomeration, inhibition of photosynthesis | Müller et al. (2015) |

[a]Not mentioned

### 16.3.7 Nickel Oxide Nanoparticles

NiO-NPs (20 nm average size) were found to provoke a severe growth inhibition on a marine microalga strain of *Chlorella vulgaris* in a sterilized enriched seawater medium (f/2 medium) when treated with 40–50 mg $L^{-1}$ during 72–120 h of exposure, with $EC_{50}$ being 32.28 mg NiO $L^{-1}$ at 72 h and 44.33 mg NiO $L^{-1}$ at 120 h (Gong et al. 2011). The observed inhibitory effect was accompanied by cellular structural alterations such as cytomembrane breakage (detached or degraded plasma membrane), plasmolysis (leak of cytosol), and disorder of thylakoids. At the same time, living algae showed a tendency to increase the agglomeration-deposition capacity of NiO-NPs as well as to reduce them for zero valence nickel.

In another study, the aquatic plant *Lemna gibba* was used to investigate and compare the toxicity induced by 30 nm NiO-NPs and nickel(II) oxide as bulk (NiO Bulk) (Oukarroum et al. 2015). Plants were exposed for 24 h to NiO-NPs, or NiO Bulk caused agglomerations of NiO-NPs in culture medium, due to ionic strength. Both NPs and bulk enhanced ROS formation, especially at 1000 mg $L^{-1}$ (five times compared to control), indicating the cellular oxidative stress. Both types of NiO induced a strong inhibitory effect on the PSII quantum yield, indicating a reduction of the photosynthetic electron transport performance due to damage to the structural and functional properties of PSII (Oukarroum et al. 2015).

Likewise, *Chlorella vulgaris* exposed to NiO-NPs for 96 h showed cellular alterations, which were related to NiO-NP concentration ($EC_{50}$ of 13.7 mg $L^{-1}$). They particularly inhibited cell division (relative cell size and granularity), deteriorated the photosynthetic apparatus (chlorophyll synthesis and photochemical reactions of photosynthesis), and induced oxidative stress (ROS formation). The TEM and X-ray analysis indicated that NiO-NPs were able to cross biological membranes and accumulate inside the algal cells (Oukarroum et al. 2017). In addition, 20 nm NiO-NPs displayed severe inhibitory effect on the growth of *C. vulgaris* after 96 h exposure with $EC_{50}$ value of 31.4 mg $L^{-1}$ (Li et al. 2017). The changes observed were plasmolysis with a shriveled cell shape, disruption of plasma membrane, cytosol leakage, and disorders in thylakoid grana lamella. Moreover, NP aggregation as well as partial reduction to $Ni^0$ could be observed, suggesting a possible remediation strategy of aquatic pollution (Li et al. 2017). These results for 20 nm NiO-NPs are comparable to the 0.35 mg NiO $L^{-1}$ $EC_{50}$ value obtained in a previous study for *Pseudokirchneriella subcapitata* (Griffitt et al. 2008). Data on toxicity effects of NiO-NPs are presented in Table 16.5.

### 16.3.8 Silica Oxide Nanoparticles

In order to test the hypothesis that the ecotoxicity of nanoparticles is related to their surface area and not to their mass, toxicity of silica ($SiO_2$) nanoparticles was monitored on the growth of *Pseudokirchneriella subcapitata* exposed to stable silica suspensions (Van Hoecke et al. 2008). Commercial Ludox suspensions of NPs with diameters 12.5 and 27 nm were toxic, with 20% effect concentration ($EC_{20}$) values on growth rate of 20.0 and 28.8 mg $L^{-1}$, respectively, at 72 h. Because no aggregation was

**Table 16.5** Summarized results from NiO-NP toxicity studies performed on lower plants

| Species | Particle size (nm) | $EC_{50}$ (mg NiO-NPs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* (Korshikov) F.Hindák | 5–20 | 0.35 at 96 h | Growth inhibition | Griffitt et al. (2008) |
| *Chlorella vulgaris* Beyerinck | 20 | 32.28 at 72 h<br>44.33 at 120 h | Plasmolysis, cytomembrane breakage, and thylakoid disorder | Gong et al. (2011) |
| *Lemna gibba* L. | 30 | n.m.[a]/at 24 h | Increase in ROS formation, inhibition of the photochemical activity of the photosystem II (PSII), reduction in the quantum yield of PSII electron transport | Oukarroum et al. (2015) |
| *Chlorella vulgaris* Beyerinck | 20 | 31.4 at 96 h | Growth inhibition, plasmolysis | Li et al. (2017) |
| *C. vulgaris* Beyerinck | 30 | 13.7 at 96 h | Agglomeration formation, inhibition in cell division, deterioration of photosynthetic apparatus, ROS formation | Oukarroum et al. (2017) |

[a]Not mentioned

observed and the dissolution of NPs was negligible, the toxicity was attributable to the solid nanospheres. There was no significant difference in toxicity on expressing the concentration as a surface area. The 72 h $EC_{20}$ values were 4.7 and 3.9 mg $L^{-1}$. Silica bulk material was found to be nontoxic up to 1 g $L^{-1}$. TEM studies with 100 mg $SiO_2$-NPs $L^{-1}$ (particle size 12.5 and 27 nm) elucidated no evidence of particle uptake even though the particles clearly adhered to the cell wall surface (Van Hoecke et al. 2008).

In a different study, 96 h exposure of *Scenedesmus obliquus* to $SiO_2$-NPs (10–20 nm, 25–200 mg $L^{-1}$) resulted in a significant concentration-dependent decrease in chlorophyll content, whereas the carotenoid content was unaffected. $EC_{50}$ value could not be determined since $SiO_2$-NPs were not toxic probably due to shading on the cell surface (Wei et al. 2010a).

## 16.4 Carbon-Based Nanoparticles

### *16.4.1 Fullerene*

The effects of carbon fullerene $C_{60}$ were investigated on *Chlamydomonas reinhardtii*. The assays included also a bioaccumulation test to observe whether the algae accumulate nanomaterials and whether they have negative effects on *Daphnia*

*magna* (water flea), a small planktonic crustacean belonging to the subclass Phyllopoda and consecutively on the whole trophic chain (Luo 2007). Population changes were measured over an initial period of 48 h, which was then extended to 480 h to estimate the long-term effects. The effects were long lasting, as the algal population treated with 10 mg $L^{-1}$ of $C_{60}$-NPs was unable to recover within a 20-day period. $C_{60}$ treatment caused color changes, cell lysis, and difficulties in reproduction. More algae died on 1 mg $L^{-1}$ of $C_{60}$, compared to 10 mg $L^{-1}$ of $C_{12}$. $C_{60}$ NPs were also more toxic to *D. magna* than $C_{12}$. Bioaccumulation studies indicate the relocation of nanomaterials from the alga to *Daphnia*, primarily through water but also through the alga, but the trend is not conclusive. Dynamic light scattering studies indicated aggregate formation, when the particles were introduced in an aquatic environment that led to the induction of oxidative stress.

The nanocrystalline fullerene ($nC_{60}$) uptake amounts and trophic transfer efficiency to the predator (*Daphnia magna*) through dietary exposure to algae or algal subcellular fractions (*Scenedesmus obliquus*) have also been investigated (Chen et al. 2016). The $nC_{60}$-contaminated algae were separated into the cell wall (CW), cell organelle (CO), and cell membrane (CM) fractions. The highest $nC_{60}$ distribution was in CW, followed by CO and CM subparts. Further, the sublethal concentration for *S. obliquus* has been determined as 0.09 mg $L^{-1}$ after 72 h exposure (Tao et al. 2015). During a sublethal experiment of $C_{60}$ that was carried out for 60 days, the photosynthesis processes, the photosynthetic polysaccharide products, soluble protein, and total lipids in *S. obliquus* were decreased. Additionally, chlorophyll *a* and chlorophyll *b* were negatively impacted possibly due to the 40% algal $Mg^{2+}$ decline at the sublethal concentration (0.09 mg $L^{-1}$) of $C_{60}$. The decline was due to inhibition of $Mg^{2+}$-ATPase activity caused by $nC_{60}$ aggregates. On the other hand, on comparing the highest and lowest sensitivity responses of a bioassay on *Pseudokirchneriella subcapitata*, Blaise et al. (2008) found fullerene $C_{60}$ to be less toxic than other NPs and placed it in the "not toxic" category (>100 mg $L^{-1}$) (Blaise et al. 2008).

*Pseudokirchneriella subcapitata* as well as the crustacean *D. magna* were used in a series of toxicity tests for studying the influence of $C_{60}$ aggregates on toxicity and bioaccumulation (Baun et al. 2008). $C_{60}$ powder was stirred in water over 2 months, and the aggregates formed were mixed with four environmental contaminants (atrazine, methyl parathion, pentachlorophenol-PCP, and phenanthrene) with different physicochemical properties and toxic modes of action, 5 days prior to testing. The sorption to $C_{60}$ aggregates was 85% for phenanthrene and 10% for the rest of the compounds. In the presence of $C_{60}$ suspensions, the toxicity of phenanthrene increased (from 720 μg $L^{-1}$ to 430 μg $L^{-1}$), and the toxicity of PCP decreased (from 36 μg $L^{-1}$ to 70 μg $L^{-1}$), and a consequent increase in toxicity was found for phenanthrene after addition of $C_{60}$ to the aqueous solution. Addition of $C_{60}$ suspensions reduced the toxicity of PCP. Finally, no enhanced bioaccumulation of phenanthrene was observed in the presence of $C_{60}$ (Baun et al. 2008).

### *16.4.2 Carbon Nanotubes (CNTs)*

In a recent study, multiwalled CNT (MWNT) material was carboxylated by microwave-assisted acid oxidations (f-MWNTs) and was examined for potential toxicity effects, using the unicellular marine chlorophyte alga*Dunaliella tertiolecta* (Wei et al. 2010a, b). Concentrations 5 and 10 mg f-MWNTs $L^{-1}$ caused substantial growth lag phase, and the $EC_{50}$ value at 96 h was 0.82 mg $L^{-1}$. This impact is in line with the 72 h $IC_{25}$ value (1.04 mg $L^{-1}$) of single-walled carbon nanotubes (SWCNTs) on the growth of green alga *Pseudokirchneriella subcapitata* (Blaise et al. 2008). Especially, at 10 mg $L^{-1}$ f-MWNTs, 36% reduction in exponential growth rate was observed indicating the presence of oxidative stress and 22% reduction in photosystem II (PSII) quantum yield (Wei et al. 2010a, b). The results differed in a later study, where oxidized SWCNTs (f-SWCNTs) caused 30% growth inhibition, 18% decrease in the photosynthetic yield, and 95% reduction in the intracellular glutathione levels of *Dunaliella tertiolecta* (Thakkar et al. 2016).

In a study of growth inhibition and photosynthetic activity in *Chlorella vulgaris* and *P. subcapitata*, $EC_{50}$ values were 1.8 mg CNTs $L^{-1}$ and 20 mg CNTs $L^{-1}$, respectively, in well-dispersed suspension whereas 24 mg CNTs $L^{-1}$ and 36 mg CNTs $L^{-1}$, respectively, in agglomerated suspension (Schwab et al. 2011). The photosynthetic activity was not affected, whereas growth inhibition was correlated to the shading of CNTs and the agglomeration of algal cells, suggesting that the growth might be affected by the shading caused by the CNTs and by alga-CNT agglomerates. However, in another study the toxicological effects of MWCNTs were not dose-dependent (Pereira et al. 2014). Exposure of *C. vulgaris* to MWCNTs induced SOD activity, decreased intracellular ATP levels, and further induced ultrastructural cell damage. Uptake of MWCNTs was observed when cells were cultured in BB medium, but this internalization was not repeated when cells were cultured in Seine river water. The toxicity of MWCNTs was also investigated in *Chlorella* sp. focusing on the four possible mechanisms for the algal growth inhibition (i.e., oxidative stress, agglomeration, physical interactions, and shading effects) and their correlation to the MWCNT size and concentration. At MWCNT concentrations near $EC_{50}$ at 96 h, the oxidative stress accounted for approximately 50% of the algal growth inhibition, whereas 25% of it owes to agglomeration-physical interactions and 25% to the shading effects (Long et al. 2012). Moreover, toxicity of MWCNTs toward *Chlorella pyrenoidosa* was investigated in the presence of different dissolved organic matters, i.e., a natural originated humic acid (HA) and two synthetic surfactants [sodium dodecylbenzenesulfonate (SDBS) and octyl phenoxy polyethoxyethanol (TX100)] (Zhang et al. 2015). Cell internalization of MWCNTs and induction of oxidative stress were promoted by SDBS and TX100, while HA alleviated the MWCNT toxicity by limiting the cell internalization of MWCNTs and reducing the oxidative stress.

The acute aquatic toxicity of SWCNTs (~20 um in length and 1 ~ 1.2 nm in diameter) has been evaluated toward two freshwater microalgae (*Raphidocelis sub-*

*capitata* and *Chlorella vulgaris*) after a 72 h incubation period (Sohn et al. 2015). The SWCNTs inhibited the growth of *R. subcapitata* and *C. vulgaris* with $EC_{50}$ values of 29.99 and 30.96 mg CNTs $L^{-1}$, respectively, and were classified as "acute category 3" in the Globally Harmonized System (GHS) of classification and labeling of chemicals. A study of the effects of SWCNTs was undertaken on a population of microalga *Chromochloris zofingiensis* with a special focus on the profile and production of pigments and fatty acids (Wang and Yang 2013). The alga after a 6-day incubation with SWCNTs showed biomass enhancement at low concentrations (40–160 mg $L^{-1}$) and inhibition at high concentrations (320 mg $L^{-1}$). By contrast, fatty acids and pigments accumulation decreased over the range of the tested concentrations indicating an increasing sensitivity of the inhibitive toxicity markers as follows: biomass and fatty acids < primary carotenoids < chlorophylls < secondary carotenoids. The data recorded for toxicity of CNTs are shown in Table 16.6.

## 16.5 Quantum Dots

Potential toxicity of quantum dots (QDs) was assessed by using *Chlamydomonas reinhardtii* as a model system (Wang et al. 2008). The response of the organism to QDs was initially assessed by growth kinetics that showed growth inhibition and formation of aggregates during the first 2–3 days of cultivation ($EC_{50}$ value = 5 mg QDs $L^{-1}$), followed by a rapid recovery and reduction of cell aggregation as the culture proceeded. Cellular oxidative stress occurred 6 h after exposure to QDs, as confirmed by the transcriptional expression profiling of three stress response genes (*SOD1*, *GPX*, and *CAT*). The expression of these genes was temporarily enhanced in cultures containing 0.1 mg QDs $L^{-1}$, with the maximum transcripts of *SOD1*, *GPX*, and *CAT* occurring after 3 h of treatment, proportionally to the initial concentration of QDs. As the cultures continued, recovery in growth was observed, and the extent of recovery, as indicated by the final cell concentration, was dosage-dependent. In another study, the adsorption of carboxyl-functionalized polymer-coated QDs (CdSe/ZnS-QDs) and their effects on *C. reinhardtii* photosynthesis were examined (Lin et al. 2009). The amount of QDs adsorbed onto algae logarithmically depends upon the equilibrium concentration of the QDs with Freundlich constants determined as $k = 0.588$ ppm$^{1-n}$ and $n = 0.629$. Furthermore, $CO_2$ depletion and $O_2$ production assays showed a significantly inhibited photosynthetic activity of the alga exposed to QDs in concentrations above 100 ppm and 5 ppm, respectively, suggesting the potential impact of NP adsorption on the obstruction of gas flow and nutrients uptake for the algae.

In addition, when *C. reinhardtii* was exposed to increasing QD concentrations, dissolution increased with decreasing pH (Domingos et al. 2011). QDs were accumulated by the algal cells (in a size-dependent manner), though the particles may have been dissolved upon entry into the cells. Whole transcriptome screening using

**Table 16.6** Summarized results from CNT toxicity studies performed on algae

| Species | Type | Length (μm) and outer diameter (nm) | $EC_{50}$ (mg CNTs $L^{-1}$) – incubation time | Effects observed | Reference |
|---|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | SWCNTs | 2–5 and 1.2–1.5 | n.m.[a] >1.04 at 72 h | Growth inhibition | Blaise et al. (2008) |
| *Dunaliella tertiolecta* Butcher | Oxidized MWCNTs | 50 and 20–30 | 0.82 at 96 h | Aggregation, growth inhibition, reduction of photosynthesis, oxidative stress indication | Wei et al. (2010a, b) |
| *Chlorella vulgaris Beyerinck Pseudokirchneriella subcapitata* (Korshikov) F. Hindák | CNTs | 2–5 and 5–15 | 1.8 mg CNTs $L^{-1}$ and 20 mg CNTs $L^{-1}$ in well-dispersed suspensions at 96 h/24 mg $CNTL^{-1}$ and 36 mg CNT $L^{-1}$ in agglomerated suspensions at 96 h | Aggregation, growth inhibition | Schwab et al. (2011) |
| *Chlorella* sp. | MWCNT10 | 0.8 and <10 | 38.7 (light) and 70.8 (dark) at 96 h | Agglomeration, growth inhibition, increase in MDA and ROS levels | Long et al. (2012) |
| | MWCNT40 | 1.7 and 20–40 | 12.6 (light) and 40.7(dark) at 96 h | | |
| | MWCNT100 | 3.2 and 60–100 | 10.8 (light) and 45 (dark) at 96 h | | |
| *Chromochloris zofingiensis* Dönz | SWCNTs | n.m.[a] | Not assayed | Biomass enhancement at concentrations 40–160 mg $L^{-1}$ and inhibition at 320 mg $L^{-1}$, decrease in fatty acids and pigment accumulation | Wang and Yang (2013) |
| *C. vulgaris* Beyerinck | MWCNTs | 40–60 and 20–40 | Nonconclusive | Growth inhibition, increase in SOD activity, decrease of intracellular ATP levels, internalization of MWCNTs, inhibition of photosynthesis at 96 h | Pereira et al. (2014) |

| | | | | | |
|---|---|---|---|---|---|
| *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen and O.M.Skulberg<br>*Chlorella vulgaris* Beyerinck | SWCNTs | 20 and 1–1.2 | 29.99 at 72 h<br>30.96 at 72 h | Growth inhibition | Sohn et al. (2015) |
| *Chlorella pyrenoidosa* H. Chick | MWCNTs | 3.2 and 70 | 14.5 at 96 h<br>10.0 (with SDBS) at 96 h<br>12.6 (with TX100) at 96 h<br>23.1 (with HA) at 96 h | Growth inhibition, oxidative stress induction, agglomeration, internalization of MWCNTs | Zhang et al. (2015) |
| *Dunaliella tertiolecta* Butcher | Oxidized SWCNTs | 5–30 and 1.1 | n.m.[a] >20 at 120 h | Growth inhibition, decrease in photosynthesis activity, reduction of the GSH levels | Thakkar et al. (2016) |

[a]Not mentioned

RNA-Seq analysis identified 174 transcripts that were specifically upregulated by QDs. Moreover, pathways linked to transmembrane activity, proteolysis involving proteasome activation, and ubiquitin-mediated processes were observed. In a different study, effects of CdSe/ZnS-QDs were studied on the availability of Cu and Pb on two strains of *C. reinhardtii* (a wall-less and a walled strain containing glycoproteins) and a microalga (*Chlorella kesslerii*) that possesses a cellulosic cell wall (Worms et al. 2012). The results indicated that QDs decreased the intracellular Cu and Pb contents (non-extractable by EDTA) to almost half in *C. kesslerii* and in the walled strain of *C. reinhardtii* but increased them about 3.5–4 times in the wall-less strain, suggesting that CdSe/ZnS-QDs could influence metal bioavailability due to the interactions of QDs with the cell wall.

As the stability of NPs in seawater is an important requisite for efficient interactions with living organisms, the effects of water-soluble CdSe-QDs were assessed on the marine microalga *Phaeodactylum tricornutum* (Morelli et al. 2012). High QD concentrations (>0.5 nM) caused a dose-dependent inhibition of growth rate and induced ROS formation as well as modulation of SOD and CAT activities. Similarly, functionalized CdSe/ZnS-QDs (amine- and carboxyl-) showed limited toxicity to the marine diatom *Thalassiosira pseudonana* after a 5-day exposure under varied nutrient conditions (enriched versus nitrogen-limited media) (Zhang et al. 2013). Production of proteins in *T. pseudonana* was induced suggesting that these extracellular proteins might be involved in the detoxification of QDs by this alga via the Cd release of QDs.

The bioaccumulation kinetics of thioglycolic acid-stabilized CdTe quantum dots (TGA-CdTe-QDs) was investigated in a freshwater alga *Ochromonas danica* (Wang et al. 2013). Flow cytometry measurements showed high photoluminescent intensity in cells during the exposure time (1, 5, 10, 15, 20, 30, 40, 50, 60 min) suggesting internalization of TGA-CdTe-QDs, while a significant NP uptake was observed through the mechanism of micropinocytosis. The intracellular TGA-CdTe-QDs had negligibly direct acute effects on the algae, and their toxicity was mainly caused by Cd ion liberation into the bulk medium. Quick elimination in the photoluminescent intensity of cellular TGA-CdTe-QDs was also observed, and it was correlated to QD dissolution, surface modification, or expulsion out of the cells. In another report, cytotoxicity of two types of QDs, i.e., carbon QDs (N,S-doped CQDs, N-doped CQDs, no-doped CQDs) and metal QDs (CdTe-QDs, CdS-QDs, $CuInS_2$/ZnS-QDs), was investigated on *Chlorella pyrenoidosa* (Xiao et al. 2016). On treating *C. Pyrenoidosa* with various concentrations of QDs, the total protein and chlorophyll *a* contents were reduced in a dose-response manner. The $EC_{50}$ values (mg $L^{-1}$) of CQDs and MQDs (shown in Table 16.7) were determined by a growth inhibition biotest (algal cells counting) for 96 h, and their toxicity order was $CuInS_2$/ZnS-QDs < no-doped CQDs < N-doped CQDs < N,S-doped CQDs < CdS-QDs < CdTe-QDs. QDs enhanced the activity of antioxidant enzyme superoxide dismutase (SOD) and decreased the reduced glutathione (GSH) level in a dose-dependent manner. Additionally, QDs enhanced the accumulation of malondialdehyde (MDA). Finally, the toxicity of CQDs was smaller than MQDs, with the toxicity of $CuInS_2$/ZnS-QDs being the smallest one (Xiao et al. 2016).

**Table 16.7** Summarized results from QD toxicity studies performed on algae

| Species | Type of QDs | $EC_{50}$ (mg QDs $L^{-1}$) – incubation time | Effects | Reference |
|---|---|---|---|---|
| *Chlamydomonas reinhardtii* P.A. Dangeard | CdTe-QDs | 5 at 72 h | Cell aggregation, growth inhibition, lipid peroxidation gene expression, temporary enhancement of sod1, gpx, and cat genes | Wang et al. (2008) |
| *C. reinhardtii* P.A. Dangeard | CdSe/ZnS-QDs | n.m.[a] | Inhibition of photosynthesis, reduction of $CO_2$ depletions, reduction of oxygen production | Lin et al. (2009) |
| *C. reinhardtii* P.A. Dangeard | CdTe/CdS-QDs | n.m.[a] | Increase in dissolution, QD uptake, upregulation of transmembrane activity pathway proteolysis involving proteasome activation and ubiquitin-mediated processes | Domingos et al. (2011) |
| *Phaeodactylum tricornutum* Bohlin | CdSe-QDs<br>CdSe/ZnS-QDs | n.m.[a] >0.5 nM | ROS formation, increase in SOD and CAT activities | Morelli et al. (2012) |
| *Chlamydomonas reinhardtii* P.A. Dangeard (wall-less and walled strain containing glycoproteins)<br>*Chlorella kessleri* Fott and Nováková | CdSe/ZnS-QDs | n.m.[a] | Twofold decrease in the intracellular contents of Cu and Pb in *C. kesslerii* and in the walled strain of *C. reinhardtii*, 3.5–4 times increase in the intracellular contents of Cu and Pb in the wall-less strain of *C. reinhardtii* | Worms et al. (2012) |
| *Ochromonas Danica* E.G. Pringsheim | TGA-CdTe-QDs | n.m.[a] | QD uptake through micropinocytosis, limited exocytosis/expulsion and dissolution. Quick photoluminescence elimination | Wang et al. (2013) |
| *Thalassiosira pseudonana* Hasle and Heimdal | CdSe/ZnS-QDs (amine- and carboxyl-) | n.m.[a] | Aggregation of amine-functionalized QDs, increase in the production of extracellular proteins | Zhang et al. (2013) |
| *Chlorella pyrenoidosa* H. Chick | N, S-doped CQDs | 38.56 at 96 h | Reduction of total proteins and chlorophyll *a* contents, induction of SOD activity and MDA accumulation, decrease in GSH levels | Xiao et al. (2016) |
| | N-doped CQDs | 185.83 at 96 h | | |
| | No-doped CQDs | 232.47 at 96 h | | |
| | CdTe-QDs | 0.015 at 96 h | | |
| | CdS-QDs | 4.88 at 96 h | | |
| | $CuInS_2$/ZnS-QDs | 459.5 at 96 h | | |

[a]Not mentioned

## 16.6 Conclusions

The quick growth of nanotechnology over the years has led to rapid development of its commercial applications, which involves the use of a great variety of manufactured NPs. The use of these organic and inorganic nanomaterials may result in the surreptitious discharge of these materials into the environment through soil, sediment, and biosolids from wastewater treatment. Algae and other nonvascular plants constitute an important component of our ecosystem, and toxic effects of nanoparticles on their growth attract serious concerns. Most of the studies conducted on NP toxicity to nonvascular plants have been focused on algae; only a few could encompass bryophytes. The toxic action of NPs can involve some distinct mechanisms, but drawing a general conclusion regarding factors that determine the toxicological effects of NPs is not possible, because toxicity data generated thus far are conflicting and inconsistent. There are indications that NPs might interact directly with algae due to secondary particle size and/or specific surface area or indirectly through release of toxic substances into the exposure media. Further, the duration of exposure to NPs may be an important parameter for the assessment of their toxicity potential (even at low concentrations), which may be more representative of real environmental conditions. Additional comprehensive investigations are urgently and immensely required to examine the impact of NPs on the food chain and the environment and also to be able to reach at logical conclusions for establishing regulations over the use, confinement, and disposal of NPs for the protection of the ecosystem and humankind.

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