The Toxicity of Nanoparticles to Organisms in Freshwater

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Contents

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1 Introduction

Nanoparticles (NPs) have existed for millions of years, formed from natural phenomena such as weathering, volcanic activities and formation of colloids in rivers (Sharma et al. 2015). The use of manufactured NPs started recently (Warheit 2018) and represents a human-made material which is being used increasingly. Currently, nanotechnology is a trillion dollar industry which is increasing exponentially. It can be assumed that naturally produced NPs have been in a form of equilibrium in nature, but engineered NPs are a growing concern among institutions and the public due to their possible negative consequences on living organisms (Moore 2006; Tiede et al. 2009). The knowledge that the scientific world has acquired to date is inadequate to draw conclusions on the actual release and fate of NPs in the natural environment, actual environmental exposure to NPs and the magnitude of harm they incur to living beings (Gottschalk and Nowack 2011; Bäuerlein et al. 2017). Establishing the safety of nanomaterials is important to protect the environment and health of organisms. The effects of NPs depend on many factors including their intrinsic properties, fate and bioavailability in the respective environment and response of the receptor organisms (Lapresta-Fernández et al. 2012). The toxicity of NPs to organisms has been the subject of study for the past decade (Klaine et al. 2008; Moore 2006; Levard et al. 2012); however, coherent, consistent and well-founded data are still lacking (Selck et al. 2016; Giese et al. 2018). Currently available data on exposure to NPs and effects on organisms are currently insufficient to conclude on the risks involved (Ma et al. 2013; Holden et al. 2014; Skjolding et al. 2016; Hjorth et al. 2017a, c), and the detection and quantification of NPs in the environment are challenging (Bundschuh et al. 2016).

The authors appreciate attempts made by several authors in reviewing published literature on freshwater nanoecotoxicology (Handy et al. 2008a, b; Baun et al. 2008; Fabrega et al. 2011; Ma et al. 2013; Vale et al. 2016; Rocha et al. 2017; Lei et al. 2018; Goswami et al. 2017). This review was written in view of giving the reader an exhaustive, holistic and comparable understanding on the effects of NPs on three major groups of freshwater organisms of various classes, in contrast to the majority of reviews which are devoted to specific classes of NPs. By doing this, authors address the issue of information and data heterogeneity. Also, care has been taken in the review to appreciate and stress the importance of the physicochemical characteristics and transformation of NPs in the environment with respect to their toxicity. Furthermore, the number of publications on nanoecotoxicity is increasing every year, and therefore, a concerted effort is needed to cohesively analyse them to better

understand potential risks of NPs in a background of ongoing, increasing research and development of nanotechnology applications. This review discusses our current understanding of environmental exposure to NPs, physicochemical characteristics of NPs and the aquatic environment that influence toxicity, their bioavailability, trophic transfer, toxicity, mechanisms of toxicity and behavioural toxicity in view of some key environmentally relevant freshwater organisms. The paper also identifies gaps in research and provides recommendations for future research needs to effectively develop our understanding on the risks of NPs to freshwater aquatic organisms and develop effective strategies to mitigate those risks. This review examined more than 350 articles including review papers with majority of those published in the last decade.

NPs are released into all ecosystems including freshwater, marine water, soil and air. However, the behaviour of NPs in the freshwater environment is likely to differ in each due to their unique environmental characteristics. For example, high salinity in the marine environment causes increased agglomeration, aggregation and precipitation of NPs which affects the bioavailability of NPs (Keller et al. 2010; Gambardella et al. 2015; Buffet et al. 2013). High surface area increases the potentiality of ion release from NPs (Mudunkotuwa and Grassian 2011), while higher aggregation reduces surface area for dissolution and any metal cations released from NPs are likely to be complexed by free chloride (Cl^-) ions present in saltwaters (Baker et al. 2014). Moreno-Garrido et al. (2015) report that the EC_{50} values of NPs for marine algae species are twofold higher than for freshwater species as per the published literature. Also, they claim that OECD documents on safety and toxicity tests for NPs do not have any specific references to marine water. The major source of NPs to soil is through the disposal of wastewater treatment plant (WWTP) sewage sludge, and NPs are unlikely to enter the soil in their original form due to organically rich reactive environments in the WWTP. The attachment of NPs to soil colloids is rapid, and therefore, the mobility of NPs away from the point of source could be limited in soils (El Hadri et al. 2018). Also, the assessment of the form of NPs in soil matrices is hampered by the relative lack of procedures for their characterization compared to aqueous media (Tourinho et al. 2012; Kraas et al. 2017). Due to the variations in the fate and behaviour of NPs and mode of organism exposure in different spheres, the authors have restricted this review to the freshwater environment only. However, this does not undermine the importance for more research related to the nanotoxicity to organisms in other environments which attract comparatively less nanoecotoxicology studies (Minetto et al. 2016).

Metal oxide NPs, metal NPs and carbon nanotubes (CNT) are the most relevant materials in terms of worldwide production volumes and exposure (Bundschuh et al. 2018; Tiede et al. 2016), while the OECD has highlighted silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO2) and cerium dioxide (CeO_2) NPs as high interest due to their widespread use, commercial importance and inherent properties (Baker et al. 2014). Rocha et al. (2015) reported that 85% of toxicological studies on marine bivalves are based on inorganic NPs and only 15% are on organic NPs. Also, more than 70% of inorganic NPs examined in saltwater are metal oxides and metals that mainly consist of TiO_2, Ag, Au, ZnO and CuO NPs (Minetto et al. 2016; Rocha et al. 2015). A large number of studies (~80%) on the effects of inorganic NPs on the

organisms considered in this review also reflect the production, release and exposure risk concerns of inorganic NPs in the freshwater environment (Fig. 2 and Table 2). However, a large proportion of those inorganic NPs are coated with organic capping agents. As an example, citrate and carboxylic acids are the most used reductant and capping agents in the synthesis of AgNPs (Sharma et al. 2014).

Low-throughput tests such as microcosms, mesocosms or field-scale studies are more representative of actual environmental conditions in comparison with high-throughput tests such as in vitro tests which lack environmental complexity. Therefore, using widely accepted key environmental organisms in ecotoxicology with corresponding in vivo tests is still highly regarded in environmental risk assessment of NPs. The European Chemical Agency mentions that in vitro data are relevant information for aquatic toxicity assessments, but also note that there are no EU/OECD guidelines for in vitro tests at the moment (Hjorth et al. 2017b). Therefore the authors have restricted this review to in vivo studies. In vitro assays have a role in hazard identification, but their usefulness in environmental risk assessment is limited (SCENIHR 2009; Mattsson and Simkó 2017). There is a limited correlation between in vitro and in vivo toxicity results (Sharifi et al. 2012). These reasons may have influenced the large number of in vivo studies published compared to in vitro studies as reflected in this review. However, this does not undermine the importance of alternative testing strategies in nanoecotoxicology risk assessment since there is an ongoing discussion and proposals to use those effectively (Hjorth et al. 2017b). Also, certain aspects of NP toxicity studies such as shape-dependent toxicity are based on in vitro experiments (Sharifi et al. 2012; Forest et al. 2017).

2 Nanoparticles (NPs) in the Environment

Several types of NPs are present in the environment and exposure to those particles is a reality. Therefore, it is important to understand the flow of NPs to the environment and exposure to assess the risks (Scown et al. 2010). Release of NPs to the environment may occur from the manufacturer through to the end user who consumes NP-enabled products (Sun et al. 2014). The majority of the products containing NPs belong to cosmetics and personal care products with sunscreen representing the dominant application (Boxall et al. 2007). A significant fraction of the NPs released to soil and air would end up in waterbodies as well while cosmetics, coatings, paints and pigments alone contribute 89–97% of total NP emissions to water (Keller et al. 2013). The data related to NP production and released volumes in the literature have large variations, and Giese et al. (2018) provide a comprehensive summary based on data from literature, their own surveys and modelling. About 250,000 metric tonnes are released to landfills, soil and air every year (Keller and Lazareva 2013), and it is predicted that about 69,000 metric tonnes of NPs are released globally to surface waters directly. This amount is increasing since the predicted NP production in 2019 is close to 600,000 metric

tonnes with an annual growth rate of 21.1% of NP production (Vale et al. 2016). It is estimated that around 10–30% of NPs released into the environment would end up in waterbodies in Asia, while it is 3–17% in Europe and 4–19% in North America (Keller and Lazareva 2013).

Usage of NPs are increasingly popular in several consumer and industrial sectors including health and fitness, home and garden, automotive, electronics, contaminant remediation and food and beverage (Vance et al. 2015; Cecchin et al. 2016). The number of inventories with catalogue products which contain NPs is rising globally (Hansen et al. 2016; Vance et al. 2015; Mcgillicuddy et al. 2017). Hansen et al. (2016) summarized the number of products listed yearly until 2015 in the Consumer Product Inventory (CPI 2018) and Nanodatabase (2018) which list products containing NPs or are based on nanotechnology available to the European market. The number of products listed in the Nanodatabase has increased from 2,231 to 3,038 from 2015 to date. There are several classes of engineered nanoparticles (NPs) based on chemical composition and morphology (Kümmerer et al. 2011). Though metal oxide NPs, SiO_2 NPs and CNTs are the most produced worldwide (Fig. 1), Silver (Ag) NPs are the most used in consumer products representing 25% of the products containing NPs (Bondarenko et al. 2013; Vance et al. 2015; Bundschuh et al. 2018). Due to their excellent antimicrobial action, Ag NPs are increasingly popular in medicines, cosmetics, personal care and certain clothing products (Boxall et al. 2007; Yameen et al. 2014). Manufacturers are not required to report the use of NPs in products except for a few NPs in some countries (e.g. carbon nanotubes in the USA). Also, manufacturers are not legally bound to label products that contain NPs (Kessler 2011) or may be ignorant with respect to specific information (Giese et al. 2018). A survey conducted by Piccinno et al. (2012) found that the manufacturers are reluctant to provide production amounts with respect to NPs. As per the listed NP-containing products in the Nanodatabase for 2018, the constituent NPs in 64% of the consumer products have not been disclosed (The

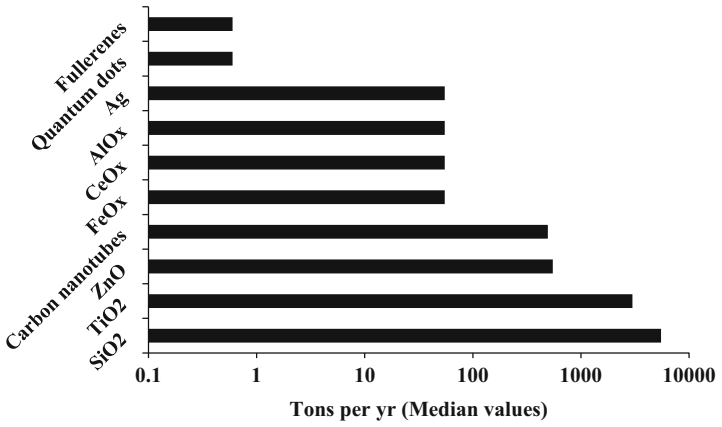

Fig. 1 Annual production volumes of nanoparticles (adapted from Piccinno et al. 2012)

Nanodatabase 2018), while metallic NPs (19.5%), metal oxide NPs (6.5%) and other types including organic NPs (9.5%) constitute the rest of the products. The majority of organic NPs are constituted of carbon (present in 2% of products), carbon nanotubes (2.1%), bamboo charcoal (1.4%), graphite (0.6%), carbon black (0.5%) and fullerene (0.3%).

Emission and environmental concentration levels are mainly estimated by using material flow models following the NP life cycle (Mueller and Nowack 2008; Boxall et al. 2007; Gottschalk et al. 2013; Sun et al. 2014, 2017; Piccinno et al. 2012; Markus et al. 2016, 2017; Jiménez et al. 2016) and analytical methods (Gottschalk et al. 2013; Chang et al. 2017; Aznar et al. 2017; Laborda et al. 2016a, b; Majedi and Lee 2016; Venkatesan et al. 2018; Vidmar et al. 2017; Yang et al. 2016; Gondikas et al. 2014, 2018; Folens et al. 2018; Markus et al. 2018; Hartmann et al. 2013; Chen and Ding 2012; Astefanei et al. 2014). However, there are number of known incorrect assumptions in all the models (Giese et al. 2018). Measured field data are essential to validate predicted environmental concentrations of NPs (Bäuerlein et al. 2017). Most NP analytical studies have so far concentrated on method development, but a rise of efforts to apply these methods to measure actual concentrations in the environment is observed (Aznar et al. 2017; Bäuerlein et al. 2017; Venkatesan et al. 2018; Peters et al. 2018). However, limitations in analytical methods in discriminating engineered NPs from naturally occurring NPs have caused results of models difficult to validate (Giese et al. 2018; Gondikas et al. 2014; Wagner et al. 2014). Also, factors such as transformation of NPs in the environment, aggregation and the copresence of dissolved ions may cause measurement of NP properties and concentrations less accurate (Majedi and Lee 2016). The physicochemical characteristics of the surrounding environment and NP properties such as coating agent and size have a huge impact on their fate and behaviour in the environment which demands careful attention in both modelling and analytical efforts (Ellis et al. 2016; Pu et al. 2016; Luo et al. 2018).Once released in to the environment, NPs undergo transformation and change their characteristics such as size compared to their pristine form (Nowack et al. 2012). For example, NPs may agglomerate in the environment, and the modelling considers agglomerates larger than 100 nm as well and thus targets the complete NP pool. In contrast, analytical methods may consider only the nanofraction, and therefore, the measured concentrations may indeed be smaller than the actual concentrations. However, this was true for certain types of NPs that were measured in a recent study conducted by Bäuerlein et al. (2017) in the Dutch environment, but the measured concentrations of AgNPs were higher than the predicted concentrations in sewage treatment plant effluent.

From various sources, Gottschalk et al. (2013) summarized predicted environmental concentrations of TiO_2 (10^{-3}–10^{1} μg L^{-1}), Ag (10^{-5}–10^{0} μg L^{-1}), ZnO (10^{-4}–10^{-3} μg L^{-1}), CNT (10^{-6}–10^{-3} μg L^{-1}), fullerenes (10^{-5}–10^{-4} μg L^{-1}) and CeO_2 NPs (10^{-3}–10^{-1} μg L^{-1}) in freshwater. In the year 2017, the global predicted environmental concentrations of SiO_2, CeO_2 and Ag NPs in the freshwater were 5,300, 7.0 and 0.3 ng L^{-1}, respectively, and predicted to increase up to 25,300, 46.7 and 2.1 ng L^{-1} in 2050, respectively. These increased concentrations correlate with the predicted increased release of NPs into the environment (Giese et al. 2018).

Based on the per capita contributions from the households, Markus et al. (2018) estimated the concentrations of ZnO, TiO_2 and AgNPs in the river Dommel in Netherlands to be 1.4 μg L^{-1}, 1.0 μg L^{-1} and 13.0 ng L^{-1}, respectively. Relatively few reports are available on actual application of analytical methods to determine the presence, properties and concentrations of NPs in freshwater samples collected from the environment. The concentration of nC_{60} was found up to 98 ng L^{-1} by using LC-MS in surface water samples collected from a creek in Hsinchu Science Park, Taiwan (Chen and Ding 2012). Comparatively, C_{60} and C_{70} concentrations were reported in using an UPLC-MS method with concentrations between 25 and 330 pg L^{-1} in freshwater samples collected from several ponds located around Barcelona's airport (Astefanei et al. 2014). Folens et al. (2018) reported Pt NP concentrations in the range of 0.05–0.9 ng L^{-1} by measuring with spICP-MS in the road dust leachate of Ghent, Belgium and Gothenburg, Sweden, which might be released into aquatic environment. Peters et al. (2018) reported actual environmental concentrations in the range of 0.3–2.5 ng L^{-1} for Ag NPs, 1.3–5.2 ng L^{-1} for CeO_2 NPs and 0.2–8.1 μg L^{-1} for TiO_2 NPs measured using spICP-MS in the samples collected from the rivers IJssel and Meuse in the Netherlands. Venkatesan et al. (2018) found TiO_2 particle concentration in the range of 260–659 ng L^{-1} in the Salt River, Arizona, USA, by measuring with spICP-MS. The morphological features of those particles were similar to the NPs present in sunscreens. In the last decade, predicting environmental concentrations of NPs by modelling has received considerable attention, but determining actual concentrations is critical for validating those estimates and reliable risk assessment and for regulating NP industry. However, the development of real-world parameters of NPs and concentrations remains scarce due to several limitations such as lack of appropriate analytical methods and complexity of the real sample matrices (Gondikas et al. 2018).

3 Why Be Concerned About NPs?

Engineered nanoparticles (ENPs) have been around for quite a while, but concerns about the risks associated with them rose a few years ago. Despite huge concerns, due to a lack of sample-related certified standards, analytical procedures and reliable units of measure (Mottier et al. 2017), the presence of NPs in the sources and receiving bodies like waste effluents, surface or groundwaters and sediments was not well documented (Mirzajani et al. 2013; Mitrano et al. 2012). However, the presence of NPs in the environment is a proven phenomenon as per recent studies, as discussed earlier. Bulk materials are usually defined in terms of properties like density, resistivity, magnetism and dielectric constant which are averaged for the whole unit. Compared to their bulk form, NPs possess unusual and different properties which cannot be explained with Newtonian mechanics, but only with quantum mechanics (Throbäck et al. 2007; Bhushan 2010; Poole and Owens 2003). Compared to naturally available NPs, engineered NPs may have different physical and chemical characteristics (Handy et al. 2008b). Unpredictable consequences due

to their colloidal nature and the dynamics of NPs in receiving environments represent a huge challenge in assessing their toxicity (Service 2004; Nowack and Bucheli 2007; Blaser et al. 2008; Diegoli et al. 2008; Hassellöv et al. 2008; Tiede et al. 2008). Chemical and physical properties like zeta potential and metal binding capacity are determined by the size of the particles (Madden et al. 2006) which varies significantly in NPs. Due to their small size, the behaviour of NPs in the environment and effects on organisms are different to those of conventional xenobiotics (Scown et al. 2010; Klaine et al. 2012). High surface to volume ratio and abundant reactive sites on the surface are some unique characteristics of NPs, and these along with their mobility could result in unexpected health hazards (Maynard et al. 2006; Wiesner et al. 2006). Also, physicochemical characteristics of both NPs and their surrounding environment and modalities of the suspension decide the attributes of the dispersed nanophase.

It was reported that creation of free radicals and oxidative damage is the main cause of adverse effects in cells (Auffan et al. 2009). Since NPs have a very large surface area in relation to volume, they may cause direct generation of oxyradicals which can attack DNA, proteins and membranes (Brown et al. 2001). Once in the cell, NPs may embed within the cell functional machinery resulting in different toxicological responses compared to conventional toxicants (Moore 2006). In addition to their own toxicity effects, NPs also influence the toxicity of other contaminants which are harmful to aquatic organisms (Tan and Wang 2014; Fan et al. 2016). NPs have the potential to bind toxicants and may carry them to sites in cells where these chemicals would not normally travel (Cheng et al. 2004; Pelkmans and Helenius 2002).

The risk assessment methodologies of NP exposure are still at the research and development level. A number of authors have proposed approaches to NP risk assessment (Dekkers et al. 2016; Domercq et al. 2018; Garner et al. 2017; Hristozov et al. 2016), although a comprehensive risk assessment of NPs, data requirements, models and advancement related to NP production, release, exposure, fate and behaviour, risk characterization, etc. (Scott-Fordsmand et al. 2017) has not yet been developed. The models that are proposed each have their own limitations, and the tools used in characterization of NPs in exposure assessment and toxicology tests are not sufficient for risk assessment (Garner et al. 2017; Mattsson and Simkó 2017). Several parameters influence NP toxicity tests, but there exists a lack of scientific understanding of the importance of each parameter or the interactions between them for the toxicity endpoints in current test guideline (Hjorth et al. 2017c). The available test guidelines are not sufficient to analyse the behaviour of NPs in test media. For example, Wasmuth et al. (2016) concluded that the available OECD guidance document No. 29 which was designed to determine the rate and extent of ion release from metals does not cover analytical methods for NPs. Also, the development of toxicity test guidelines is still at early stages with only a few guidelines available, published recently (OECD 2017a, b, c). Accordingly, the lack of NP toxicity test data which are suited for regulatory decision-making is still a pressing issue (Hjorth et al. 2017c). Also, the currently available remediation or purification technologies may not reduce NPs to environmental permissible levels.

Furthermore, increasing NP release may cause further issues in wastewater and sewage sludge treatment plants which may pose a risk to microbes in the digestion systems (Wang and Chen 2016).

4 Physicochemical Characteristics of NPs on Ecotoxicity

NPs exhibit unique physicochemical properties compared to their bulk counterparts. Several researchers have tried to investigate the influence of physicochemical characteristics of NPs such as size, shape, surface properties and charge that could change their toxicity to organisms. Though the results seem to be inconsistent and conflicting, they suggest that the physicochemical properties of NPs could affect the toxicity to organisms. Therefore, these parameters need to be considered in environmental risk assessments and demand further research.

4.1 Size

The uptake and toxicity of NPs depend on the inherent properties of NPs and also the chemistry of the surrounding environment (Park et al. 2015). The behaviour of NPs depends on factors like size, shape, surface chemistry, surface area, functional groups, coatings, charge, aggregation, solubility, photochemistry, crystallinity and the presence of other compounds (Scown et al. 2010; Albanese et al. 2012; Shang et al. 2014; Fröhlich 2012; Clément et al. 2013; Hund-Rinke and Simon 2006; Barbero and Yslas 2016; Garner and Keller 2014). The size of NPs determines the physicochemical properties, adsorption, distribution, metabolism and excretion in the biological systems (Qu et al. 2017). The toxicity of AgNPs is size dependent with smaller particles which are more active (Lok et al. 2007). Choi and Hu (2008) observed that the inhibition of nitrifying organisms correlated with a fraction of AgNPs less than 5 nm in size. This was achieved through examining the correlation between nanoparticle size distribution, photocatalytic reactive oxygen species (ROS) generation, intracellular ROS accumulation and nitrification inhibition. They concluded that NPs of this size could be more toxic to bacteria than any other fractions of NPs.

Choi et al. (2008) saw no indication of internalization of AgNPs (14 nm in size) into the bacteria *Escherichia coli* since internalization of NPs into bacteria cells depends on the size of the NPs and only smaller NPs (<10 nm in size) could enter (Morones et al. 2005). Jiang et al. (2008) found that binding and activation of membrane receptors and subsequent protein expression in mammalian cells depend on nanoparticle size. Hoecke et al. (2009) reported that the toxicity of CeO_2 NPs to *Raphidocelis subcapitata* increased with decreasing particle size. Lei et al. (2016) observed increased toxicity of zero-valent iron (nZVI) NPs to *Chlorella pyrenoidosa* with decreasing particle size. Hartmann et al. (2010) studied the ecotoxicity of three

TiO_2 NPs with different sizes, to the alga *Pseudokirchneriella subcapitata*. They found that the smallest particle type (<10 nm) resulted in higher inhibition than the other two types (3–300 nm). Kim et al. (2010) investigated the effects of particle size of TiO_2 NPs on *Daphnia magna*. They showed that the particle fraction in between 400 and 800 nm increased antioxidant enzyme activities in comparison with the NPs which were less than 400 nm in size. Cui et al. (2017) reported that the longer Ag nanowires (NWs) (20 μm) were more toxic to *Daphnia magna* and *Daphnia galeata* than those that were shorter (10 μm). Similarly, Chae and An (2016) showed that larger Ag nanowires (AgNW) (20 μm) were more toxic to aquatic organisms than smaller ones (10 μm) by exposing *Chlamydomonas reinhardtii* and *Daphnia magna* and to Ag NWs.

However, Matzke et al. (2014) observed no clear-cut relationship between NP toxicity and size of NPs when the bacterium *Pseudomonas putida* was exposed to AgNPs. Li et al. (2010a) studied the effects of three different sized (36, 52 and 66 nm) Ag NPs but concluded that the toxicity was not a function of size possibly due to the large degree of aggregation of NPs in synthetic freshwater. Also, Lopes et al. (2014) studied the effects of ZnO NPs with two different particle sizes (30 and 80–100 nm) and ionic Zn. They found that the acute toxicity of ZnO NPs did not depend on particle size. Iswarya et al. (2017) exposed the alga to PVP-coated Au NPs in different sizes but observed no size-dependent toxicity. However, the toxicity of citrate-coated Au NPs depended on the size with the smaller particles being less toxic. The smaller-sized NPs reacted rapidly with the substances in the solution causing aggregation which may have caused less toxicity. Wiench et al. (2009) reported that the acute toxicity to *Daphnia magna* was independent of particle size, type of coating, aggregation of particles or the type of medium for TiO_2 and ZnO NPs.

4.2 Shape

Peng et al. (2011) observed that rod-shaped zinc oxide NPs (ZnO NPs) were more toxic to the alga *Phaeodactylum tricornutum* than sphere-shaped NPs. Bacchetta et al. (2018) observed higher internalization of spherical- and tube-shaped CNTs into *Daphnia magna* compared to the cubic NPs. They also reported that NP shape influenced the severity of pathogenesis with cubic NPs being more effective in terms of physical damage and cellular degeneration. Liu et al. (2018) observed that star-shaped Au NPs were more toxic to the fungus *Aspergillus niger*, *Mucor hiemalis* and *Penicillium chrysogenum* compared to the toxicity of spherical-shaped ones. Also, the toxicity of star-shaped NPs increased with smaller sizes. Nasser et al. (2016) suggested that shape and charge played an important role in the toxicity and uptake of Au NPs to *Daphnia magna*. Abramenko et al. (2018) observed higher toxicity of spherical-shaped Ag NPs to *Danio rerio* embryos compared with Ag nano-plates. In contrast Dai et al. (2015) saw no effect of NP form or shape on the toxicity of CuO NPs to *Capitella teleta*. Also, Silva et al. (2014) claimed that particle shape did not

contribute to the toxicity of organo-coated Ag NPs to *Escherichia coli* and *Daphnia magna*. Chauhan et al. (2011) claimed that the rod-shaped CdSe/CdS NPs penetrated tumour cells more rapidly than spherical NPs. Truong et al. (2015) suggested that nonspherically shaped, such as filamentous, discs or wormlike NPs were better as drug delivery carriers. However, Chithrani et al. (2006) observed higher uptake of spherical-shaped Au NPs into mammalian cells than the rod-shaped Au NPs.

4.3 Surface Properties

Though NP size still remains central in determining toxicity, studies suggest that other inherent factors like coating agents should be considered in toxicity studies (Silva et al. 2014). The role of the surface properties of NPs is poorly understood and cannot be generalized to determine the risks (Baumann et al. 2014; Saei et al. 2017). Surface properties of NPs are key factors in determining behaviour of NPs; multiple types of surface ligands pose new challenges in understanding the toxicity of NPs (Yu et al. 2013). NPs are highly reactive because of their large surface area. The surface chemistry and reactivity of NPs determine their interactions with the surface lining layers of biological tissues (Hoet et al. 2004) and transfer of NPs to higher levels through the food web (Geitner et al. 2016). Many NPs which are in development are complex and carry different coatings which can alter their surface properties (Nune et al. 2009; Daima et al. 2014). Currently many different types of compounds are being used as capping agents in commercial NP production (Table 1). The physicochemical characteristics of these different coatings lead NPs to behave differently in the environment. Different ligands impart different chemical properties and affect charge, particle size, surface area and aggregation of NPs (Elsaesser and Howard 2012; Lapresta-Fernández et al. 2012; Rana and Kalaichelvan 2013; Cupi et al. 2016b). NPs are stabilized against aggregation

Table 1 Different types of capping agents of NPs (Park et al. 2011; Sapsford et al. 2013; Singla and Kumar 2009; Shukla et al. 2008; Song et al. 2011; Levard et al. 2012; Sharma et al. 2014)

Category	Compound
Carboxylic acids	Citrate, oleic acid, mercaptosuccinic acid
Polymers	Polyvinylpyrrolidone, polyacrylate, polyvinylalcohol, polyacrylamide, polylactic acid, polyvinyl chloride, polystyrene, dodecanothiol
Polysaccharides	Gum Arabic, sophorolipids, chitosan, heparin, hyaluronic acid, cellulose, starch, alginic acid, dextran, maltose
Biological molecules	Bovine serum albumin, fatty acids, tyrosine
Inorganic coatings	Silver carbonate
Surfactants	Sodium dodecyl sulphate, cetyltrimethylammonium bromide, polyoxyethylene sorbitane monooleate
Organic coatings	Plant extracts, whole plant extracts, food sources from plant origin, triethanolamine, thioglycerol, hexamine, sodium dodecylbenzenesulfonates

and other chemical reactions like oxidation and sulfidation through adsorption or covalent attachment of organic compounds which provide electrostatic, steric or electrosteric repulsive forces between particles (Phenrat et al. 2008; Hotze et al. 2010). However, the impacts of different coatings on toxicity have been scarcely explored (Dominguez et al. 2015). It was shown that fullerene can cause oxidative damage in mammalian cells, and their toxicity is related to lipophilicity; reduction of lipophilicity with modification of the surface of fullerene by introducing aliphatic and hydroxyl groups resulted in reduced toxicity (Colvin 2003; Sayes et al. 2004). It has been reported that uncoated colloidal fullerenes may damage the brain of largemouth bass (Oberdörster 2004). Iron oxide NPs coated with ascorbate and dextran have been shown to be more toxic to the freshwater cladoceran *Daphnia magna* in comparison with the same NPs coated with citrate and polyvinylpyrrolidone (Baumann et al. 2014). Bozich et al. (2014) found that both the type of ligand and the charge of the NP surface affected the toxicity of Au NPs to *Daphnia magna* at acute and chronic level. Bone et al. (2012) found that the silver speciation from silver NPs (Ag NPs) varied significantly by coating type (gum Arabic and polyvinylpyrollidone) and the presence of plants (*Potamogeton diversifolius* and *Egeria densa*) in the medium, which reduced the toxicity of NPs to *Daphnia magna*. Interestingly, the fate and behaviour of NPs are changed by organisms as well. Adeleye and Keller (2016) observed charge reversal and change of surface properties of TiO_2 NPs by the extracellular polymeric substances (EPS) produced by *Chlamydomonas reinhardtii.* The presence of EPS may affect the bioavailability of NPs, their interactions with organisms and overall effects. Therefore, the authors suggested that the fate and effects of NPs cannot be simply predicted by the physicochemical characteristics of NPs.

4.4 Charge

The surface charge of NPs, measured as zeta potential, contributes to the adhesion of NPs on cell surfaces and hence is important in the toxicity of NPs. The NPs with the highest positive charge are the most toxic to the algae cells. Algal cells, having a negative charge on their surface, attract positive NPs to neutralize the charge, and this causes surface alterations resulting in cell death (Karunakaran et al. 2015). El Badawy et al. (2010) observed a surface charge-dependent toxicity of Ag NPs to bacteria (*Bacillus* sp.) when they were exposed to four different Ag NPs with different surface charges. Dominguez et al. (2015) showed that different types of coatings and the charge of NPs had an impact on ROS formation and gene expression in *Daphnia magna*. Nasser et al. (2016) suggested charge played an important role in toxicity and uptake of Au NPs to *Daphnia magna*.

5 Effects of the Surrounding Environment on NP Toxicity

OECD (2014) highlights the importance in identifying transformation, degradation and dissolution in the characterization of NPs in toxicity tests (Cupi et al. 2016a). Transformation of Ag NPs affects their surface properties, transportation, reactivity and toxicity in the environment (Xiu et al. 2011; Liu et al. 2010; Levard et al. 2011a, b). It is important to further assess the effects of the transformed NPs as well as fresh NPs to clearly understand how the transformation of NPs in the aquatic environment affects organisms (Levard et al. 2012). Biological systems have not evolved in the presence of ENPs which are produced today, and hence, the lack of knowledge about transport and fluxes of such particles present problems (Hoet et al. 2004; Dowling 2004). Generally, abiotic factors like pH, salinity, hardness of water and chemical oxygen demand (COD) influence the aquatic toxicity of chemicals (Li et al. 2013; Fabrega et al. 2011). The fate and toxicity of NPs in the aquatic environment are governed by physicochemical pathways which include aggregation and subsequent sedimentation, dissolution, adsorption to particulate and other solid surfaces, stabilization via surfactants and binding to natural organic matter (NOM) (Wang et al. 2016c; Boncel et al. 2015; Apul and Karanfil 2015; Köser et al. 2017; Ellis et al. 2018). Biological degradation, abiotic degradation, oxidation and reduction could also be of concern in some aquatic environments (Batley and Mclaughlin 2007). It was reported that the surface coatings change or are replaced with new coatings during their transit in water (Jarvie and King 2010). Less is known about the comparative toxicity of metallic NPs and their ionic forms (Xiu et al. 2012; Wang et al. 2016a). It has been found that NPs release ions into water over time and the rate and the degree of dissolution depend on their surface functionalization. Therefore, the biological toxicity of aged and freshly prepared NPs differ (Kittler et al. 2010). Strigul et al. (2009) studied the toxicity of boron NPs (B NPs) to *Daphnia magna*. Depending on the age of the test solution, the calculated 48 h LC_{50} values for B NPs ranged from 56 to 66 mg/L, and the difference in toxicity was attributed to dissolution of NPs releasing free ions. Once released into the environment, the toxicity also depends on the oxidation state of the NPs (Conway et al. 2015). Lei et al. (2016) found that the toxicity of nZVI to the alga *Chlorella pyrenoidosa* decreased after NPs was aged for 3 months in the medium, in comparison with the toxicity of fresh NPs. They attributed this to the surface oxidation of the NPs.

5.1 Media and Exposure System

Abiotic factors like pH, salinity, water hardness, temperature, different organic ligands and the components in the media affect the ecotoxicity of NPs (Handy et al. 2008b; Jin et al. 2010; Djurišić et al. 2015). The fate and transport of NPs in aquatic systems largely depend on the chemical characteristics of water (Garner and Keller 2014). Physicochemical factors in freshwater are different from brackish

or seawater. Therefore the behaviour and effects of NPs identified in one medium cannot readily be applied to other media.

Li et al. (2011) assessed the toxicity of ZnO NPs to *Escherichia coli* in five different media (ultrapure water, 0.85% NaCl, phosphate-buffered saline (PBS), minimal Davis (MD) and Luria-Bertani (LB)). They observed different toxicity levels in a range of media and recommended that attention be paid to the physico-chemical characteristics of NPs and media in bacterial toxicity tests. Li et al. (2013) found that the toxicity of ZnO NPs to *Escherichia coli* depended on the dissolution of NPs. Interestingly, toxicity was reduced by the presence of Ca^{2+} and Mg^{2+} in the medium which could compete with toxic Zn^{+} ions for binding sites on the organisms. Lopes et al. (2012) observed higher bacterial toxicity in Milli-Q water than in ASTM hard water which may be due to the interference of ions in ASTM hard water causing higher aggregation. von Moos et al. (2015) exposed *Chlamydomonas reinhardtii* to CuO NPs (10 mg/L) in five different exposure media. They observed that the media was decisive in determining toxicity regardless of the effects from NPs or ions. Similarly, Aravantinou et al. (2015) observed that the different sensitivity of the algae *Chlorococcum* sp. and *Scenedesmus rubescens* to ZnO NPs strongly depended on the algae medium. Zhang et al. (2016a) observed that media chemistry had profound effects on aggregation, dissolution and toxicity of TiO_2, ZnO and Ag NPs and CNTs to *Chlorella pyrenoidosa*. Seo et al. (2014) observed different toxicity levels in different media (ISO and moderately hard water (MHW)) when *Daphnia magna* was exposed to Ag, Cu and Zn NPs. Though the dissolution rate of NPs was higher in ISO medium, the toxicity was highest in MHW. Muna et al. (2017) found increased total Cu body burden from Cu NPs after exposing *Daphnia magna* in natural freshwater compared with OECD202 artificial medium. The Cu body burden in daphnids in natural freshwater bodies may be higher than laboratory predictions carried out using artificial media. Also, the total Cu body burden was higher in daphnids exposed to Cu NPs than Cu salt. Hu et al. (2017) reported higher toxicity of AgNPs and $AgNO_3$ to *Daphnia magna* in M4 medium in comparison with the surface water. For both forms of Ag, daphnids took up less and depurated more in the surface water. The authors suggested a reduced toxicity for the observation. However, Salieri et al. (2015) did not observe any significant influence of test media on toxicity of TiO_2 NPs to *Daphnia magna*. They believe this may be due to fast and strong agglomeration of NPs in all media, creating secondary particle size in the micrometre range. They did however report that the exposure volume of the medium had a significant influence on toxicity.

Nicolas et al. (2016) conducted standard algal growth inhibition tests (OECD 2011) with *Raphidocelis subcapitata* to test how the exposure system (24-well microplate, cylindrical vials and Erlenmeyer flasks) influenced the toxicity of TiO_2 NPs. They found that the exposure system significantly affected the results and recommended attention be paid during the algal growth inhibition test. Sørensen and Baun (2015) exposed the alga *Raphidocelis subcapitata* to AgNPs and $AgNO_3$ for 2 and 48 h in standard algal toxicity tests. Similar toxicity levels were observed for Ag^{+} in the two tests, whereas the toxicity of AgNPs was less toxic in 2 h test compared to 48 h test. Interestingly, ageing AgNPs in the medium for 48 h before

performing the 2 h test increased the toxicity, while ageing beyond 48 h prior to testing reduced the toxicity. Xiao et al. (2018) observed higher toxicity in a dynamic exposure system with a vibration speed of 140 rpm in comparison with static exposure when *Daphnia magna* was exposed to CuO NPs. The aggregation of NPs in the dynamic system was less, and therefore, they hypothesized that the reduced toxicity may be due to the lower hydrodynamic diameter (HDD) of NPs. Sørensen et al. (2016b) claimed that the acute toxicity of Ag NPs and CuO NPs to *Daphnia magna* after pulse exposure (1–2 h) was comparable to the effects levels of 24 h continuous exposure. They attributed this to rapid toxicokinetic and toxicodynamic features of NPs causing the same level of toxicity following a few hours of exposure, concluding that the dissolved fractions of NPs are responsible for the toxicity. With this, they suggested that the use of pulse exposure was more environmentally relevant for NP toxicity assessments than standard continuous exposure tests.

Media critically influence the toxicity of NPs; this is due to several reasons. The physicochemical characteristics of the medium affect the fate and behaviour of NPs. The constituents of the medium may react with dissolved ions from NPs causing complexation and aggregation or compete with them for binding sites on the organisms. In addition, an organism's sensitivity and response to NP exposure also depend on the medium. Due to these reasons, the toxicity of NPs in one medium cannot be readily applied to other media. Other than the media, the outcome of toxicity testing of NPs is highly influenced by the test duration, the time from the moment NPs are added to the test medium, dynamic vs static exposure and pulse vs continuous exposure.

5.2 Natural Organic Matter

Studies suggest that the presence of some organic and inorganic substances in the medium could change the properties of NPs which contribute to determining the fate and toxicity of NPs (Metreveli et al. 2016; Gunsolus et al. 2015; Wang et al. 2018; Luoma et al. 2016). NPs may be more stable in natural waters than in synthetic waters where no NOM is present (Batley and Mclaughlin 2007). NOM present in media could form a layer on NPs and increase the stability of NPs (Baalousha and Lead 2013; Omar et al. 2014). Once released to the aquatic environment, NOM coated on NPs changes the reactivity and bioavailability of NPs to organisms (Aiken et al. 2011). Also, DOM may promote the mobility of the NPs in the aquatic environment (Ren et al. 2017). Liberation of ions from NPs is influenced by the presence of NOM in water (Wang et al. 2015). Jiang et al. (2015) observed that NOM affected the dissolution kinetics of ZnO NPs and found that the dissolution rate constants and dissolved Zn concentrations increased with increased NOM concentration. In addition, they found that the aromatic carbon content of NOM played a key role in promoting dissolution. Li et al. (2016b) studied the effects of DOM in the medium on the generation of ROS and the acute toxicity of metal

oxide NPs to *Escherichia coli*. They observed that different photo-reactivity of humic and fulvic acids resulted in different effects on ROS generation and acute toxicity of NPs. Seitz et al. (2015) found that the pH and dissolved organic matter (DOM) in water considerably influenced the acute and chronic toxicity of Ag NPs to *Daphnia magna*. Xiao et al. (2018) observed that the toxicity of CuO NPs to *Daphnia magna* was mitigated in the presence of DOM. There are different views about the effects of NOM on the toxicity of NPs. However, NOM have been shown to influence the stability, dissolution, reactivity, bioavailability and mobility of NPs which directly or indirectly affected the toxicity.

5.3 Sulfidation

In natural waters, Ag NPs will preferentially transform to Ag_2S or AgCl as per the thermodynamic constraints. Also, the transformation will depend on pH and redox potential (Eh) and the composition of natural waters; by knowing those values, it is possible to predict the speciation of silver in simple systems. Under aerobic conditions, formation of silver chloride species is predicted, but under anaerobic conditions, sulfidation is predicted (Levard et al. 2012). Bioavailability and toxicity of ions change with sulfidation, and it was found that the toxicity of Ag^+ to *Daphnia magna* decreased by about fivefold in the presence of environmentally relevant levels of sulphide (Bianchini and Wood 2008; Bianchini et al. 2002). Guo et al. (2017) observed that the toxicity depended on sulfidation rate when the bacteria *Escherichia coli* was exposed to Ag NPs. Reinsch et al. (2012) observed decreasing toxicity of Ag NPs to *Escherichia coli* with increasing sulfidation (Ag_2S:Ag^0 ratio).

5.4 Other Factors

The interactions between NPs and bacteria can be affected by several other factors such as the pH and ionic strength of the medium and the photocatalytic activity of NPs under different irradiation conditions (Djurišić et al. 2015). Pagnout et al. (2012) observed toxicity changed with different electrolytes (NaCl, $CaCl_2$, Na_2SO_4) in the medium when *Escherichia coli* was exposed to TiO_2 NPs. Also, they observed that the toxicity changed with pH which may cause changes in the surface charge of NPs resulting in different interactions between bacteria and NPs. Bhuvaneshwari et al. (2015) observed increased toxicity of ZnO NPs to *Scenedesmus obliquus* under UV-C irradiation compared with that under visible light. They ascribed this to increased ROS production in UV-C irradiated algal cells compared with cells under other irradiation conditions. Sendra et al. (2017) reported a significant increase in the toxicity of TiO_2 NPs and bulk TiO_2 under UVA irradiation in comparison with that observed invisible light. Ratti et al. (2016) observed light-enhanced antimicrobial activity of NPs when *Escherichia coli* and

Bacillus subtilis were exposed to AgNPs. Lee and An (2013) exposed *Raphidocelis subcapitata* to ZnO and TiO_2 NPs under visible, UVA and UVB irradiation conditions. Though the growth of algae was inhibited under all conditions, there was no significant toxicity difference among the light conditions.

Physicochemical characteristics of NPs alter upon environmental release with time under the influence of the surrounding environment, thereby affecting their impact on organisms. Several environmental factors such as media composition, exposure scenario, sulfidation, irradiation, pH and ionic strength of media influence the toxicity of NPs. Most ecotoxicological studies (Table 2) to date have focused on the effects of as-prepared NPs on organisms; few studies have evaluated the effects of the transformation of NPs on toxicity. More studies focusing on this aspect which are biologically and environmentally relevant are warranted.

5.5 NP Stability and Aggregation

Aggregation, sulfidation and oxidation are examples of changes that could happen to varying degrees (Fortner et al. 2005; Brant et al. 2005; Teeguarden et al. 2007; Garner and Keller 2014; Conway et al. 2015). Size and aggregation are the crucial factors in determining the ecotoxicity of carbon NPs, while solubility and speciation determine the toxicity of metal oxide NPs (Blinova et al. 2010). The degree, kinetics and size range of aggregates depend on the characteristics of the particles, the characteristics of the environment and the concentrations of the particles (Phenrat et al. 2007; Hyung et al. 2007). Negatively charged NPs are electrostatically stabilized when the negative charge is strong enough to repel NPs from each other to overcome attractive forces. However, the presence of counterions in the solution will reduce the repulsive forces resulting in decreased stability. Several researchers provided supportive evidence for this phenomenon claiming that the different ionic strengths of the environment affect the aggregation and stability of NPs (El Badawy et al. 2010; Li et al. 2010c; Liu et al. 2011; Delay et al. 2011). Even a slight increase in salinity decreases colloids by particle aggregation and precipitation (Stolpe and Hassellöv 2010). The ionic strength of freshwater systems ranges from 1 to 10 mM and that of seawater is about 700 mM (Levard et al. 2012). However, there is a tendency for less aggregation when NPs are stabilized sterically other than solely by surface charge. Attachment of certain polymers causes steric stabilization, and several researchers demonstrated that adsorption of compounds in natural waters induced steric forces that resist aggregation (Fabrega et al. 2009; Delay et al. 2011; Chinnapongse et al. 2011; Cumberland and Lead 2009). Polyelectrolytes exhibit additional electrosteric forces in addition to steric stabilization which makes them excellent in stabilizing NPs (Badawy et al. 2010).

Aggregation is a crucial factor in determining NP toxicity. In general, the majority of studies support the idea that the aggregation of NPs reduces the toxicity to organisms though some researchers have claimed otherwise. Several researchers reported a correlation between aggregation of NPs and their toxicity to isolated

Table 2 Summary list of NP effect studies involving freshwater species of bacteria, algae, *Daphnia* and bivalves

Reference	Materials tested	Organisms	Effects studied	Endpoints
Bacteria				
Hwang et al. (2008)	Ag and Au NPs, $AgNO_3$	*Escherichia coli*	Toxic mode of action	Oxidative stress, membrane/protein damage, DNA damage
Strigul et al. (2009)	B, TiO_2 and Al NPs	*Vibrio fischeri*	Toxicity of NPs	60 days EC_{50} (mg/L) – B NPs, 56–66; TiO_2 NPs, ND; Al NPs, ND
Jiang et al. (2009)	Al_2O_3, SiO_2 and ZnO NPs	*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*	Toxicity of NPs and mechanisms of toxicity	Mortality% *B. subtilis*: Al_2O_3, 57%; SiO_2, 40%; ZnO, 100% *E. coli*: Al_2O_3, 36%; SiO_2, 58%; ZnO, 100% *P. fluorescence*: Al_2O_3, 70%; SiO_2, 70%; ZnO, 100% Membrane damage (cell pitting) by NP attachment
Baek and An (2011)	CuO, ZnO, NiO and Sb_2O_3 NPs	*Escherichia coli*, *Bacillus subtilis*, *Streptococcus aureus*	Toxicity of NPs	24 h EC_{50} (mg/L) – *E. coli*: CuO, 28.6; ZnO, 15.7; NiO, 160.2; Sb_2O_3, 265.5 *B. subtilis*: CuO, 61.1; ZnO, 85.8; NiO, 121.9; Sb_2O_3, 144.7 *S. aureus*: CuO, 65.9; ZnO, >125; NiO, 121.1; Sb_2O_3, 324.0
Choi et al. (2008)	Ag NPs, $AgNO_3$, AgCl colloides	Nitrifying bacteria, *Escherichia coli*	Size, bioavailability and ROS-dependent toxicity of NPs	Nitrifying bacteria (% inhibition): AgNPs, 86; Ag^+, 42; AgCl, 46 *E. coli* (% inhibition): AgNPs, 55; Ag^+, 100; AgCl, 66 Membrane damage (cell pitting) by NP attachment
Lopes et al. (2012)	Sodium dodecyl sulphate/didodecyl dimethylammonium	*Vibrio fischeri* and *Salmonella typhimurium*	Toxicity of NPs and genotoxicity of NPs	30 min EC_{50} (mg/L) (*V. fischeri*) ASTM medium – SDS/DDAB, 24.5; Mo/NaO, 36.5; TiO_2, NT; $TiSiO_4$, NT;

	bromide (SDS/DDAB), monoolein and sodium oelate (Mo/NaO), TiO_2, $TiSiO_4$, CdSe/ZnS, Au nanorods			CdSe/ZnS, 0.15; Au nanorods, 0.22 Milli-Q – SDS/DDAB, 13.6; Mo/NaO, 68; TiO_2, NT; $TiSiO_4$, 51; CdSe/ZnS, 0.08; Au nanorods, 0.22
Heinlaan et al. (2008)	ZnO, CuO and TiO_2 NPs, ZnO, $ZnSO_4$, $CuSO_4$, TiO_2, CuO	*Vibrio fischeri*	Toxicity of NPs	72 h EC_{50} (mg/L) – ZnO, 1.8; ZnO NPs, 1.9; $ZnSO_4$, 1.1; CuO, 3,811.0; CuO NPs, 79; $CuSO_4$, 1.6; TiO_2, ND
Fabrega et al. (2009)	Ag NPs	*Pseudomonas fluorescens*	Toxicity of NPs	Growth inhibition
Jin et al. (2010)	Ag NPs	*Bacillus subtilis*, *Pseudomonas putida*	Toxicity of NPs, effect of water chemistry on toxicity	Membrane damage, survival
Li et al. (2013)	ZnO NPs	*Escherichia coli*	Effect of water chemistry on dissolution and toxicity of NPs	Percentage mortality
Choi et al. (2010)	Ag NPs, $AgNO_3$	*Escherichia coli*	Toxicity of NPs in planktonic and biofilm cultures	Minimum bactericidal concentration (MBC) (mg/L) AgNPs, planktonic, 38 biofilms, 10 Ag^+, planktonic, 2.4 biofilms, 1.2
Lok et al. (2006)	Ag NPs, $AgNO_3$	*Escherichia coli*	Mode of antibacterial action	Membrane damage
Binaeian et al. (2012)	Ag NPs, $AgNO_3$	*Vibrio fischeri*	Toxicity of NPs	30 min EC_{50} (mg/L) – AgNPs (biological method), 34.5; AgNPs (chemical reduction method), 29.3; Ag^+, 1.8
Greulich et al. (2012)	Ag NPs, $AgNO_3$	*Escherichia coli*, *Staphylococcus aureus*	Toxicity of NPs	MBC (mg/L) (for both types of bacteria) AgNPs, 12.5–50; Ag^+, 0.5–5.0
Li et al. (2010b)	Ag NPs	*Escherichia coli*	Growth, permeability and morphology	Leakage of reducing sugars and proteins, change of respiratory chain dehydrogenases, membrane damage (cell pitting)
Sondi and Salopek-Sondi (2004)	Ag NPs	*Escherichia coli*	Mechanism of antimicrobial toxicity of NPs	Growth inhibition, membrane damage (cell pitting), NP accumulation in the membrane

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Su et al. (2009)	Ag/silicate nanohybrids	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyrogens*	Mode of antibacterial action Toxicity of NPs	Growth inhibition, cell death, membrane damage due to ROS production by NPs
Brayner et al. (2006)	ZnO NPs	*Escherichia coli*	Toxicity of NPs	Membrane damage, NP accumulation in the membrane, cellular internalization of NPs
Feng et al. (2015)	Au NPs	*Bacillus subtilis*, *Shewanella oneidensis*	Charge and ligand type on surface binding and toxicity	Viability, membrane damage by associated NPs on bacterial cells, NP localization in cells
Hossain and Mukherjee (2012)	CdO NPs	*Escherichia coli*	Toxicity of NPs on growth, morphology and cell division	Growth inhibition, morphological changes, membrane damage by ROS generation, effects on cell division
Thill et al. (2006)	CeO_2 NPs	*Escherichia coli*	Toxicity of NPs	Growth inhibition, membrane damage by adsorption of NPs on bacteria cells
Kumar et al. (2011)	TiO_2 and ZnO NPs	*Escherichia coli*	Toxicity of NPs by oxidative stress and DNA damage	Cell viability, membrane damage, cellular intake, enzyme activity, DNA damage
Li et al. (2011)	ZnO NPs	*Escherichia coli*	Toxicity of NPs in different media	Mortality, growth inhibition
Reinsch et al. (2012)	Ag NPs	*Escherichia coli*	Effect of sulfidation on acute toxicity	Growth inhibition
Pagnout et al. (2012)	TiO_2 NPs	*Escherichia coli*	Role of electrostatic interactions on toxicity	Viability, growth inhibition
El Badawy et al. (2010)	Ag NPs	*Bacillus* sp.	Surface charge-dependent acute toxicity	Viability, oxygen consumption
Guo et al. (2017)	Ag NPs	*Escherichia coli*	Acute toxicity in the presence of anions	Viability

Choi and Hu (2008)	Ag NPs	Nitrifying bacteria	Size- and ROS-dependent toxicity	30 min EC_{50} (mg/L) – AgNPs, 0.14; AgCl, 0.25; Ag^+, 0.27; membrane damage (cell pitting)
Li et al. (2016b)	Metal oxide NPs	*Escherichia coli*	Influence of DOM on photogenerated ROS formation and acute toxicity of NPs	Survival rate, ROS generation
Xiu et al. (2012)	Ag NPs, $AgNO_3$	*Escherichia coli*	Particle-specific toxicity, effect of size and coating	Survival rate, effects of NP morphology
Kvitek et al. (2008)	Ag NPs	*Escherichia coli*	Effect of surfactants and polymers on stability and antibacterial activity of NPs	Growth inhibition
Lok et al. (2007)	Ag NPs, $AgNO_3$	*Escherichia coli*	Oxidation and antibacterial activity of NPs	Growth inhibition
Werlin et al. (2011)	CdSe Qds	*Pseudomonas aeruginosa*	Trophic transfer of NPs from bacteria to protozoa and biomagnification	Bioaccumulation of QDs in *P. aeruginosa* and trophic transfer to *Tetrahymena thermophila*
Jaiswal and Mishra (2018)	Ag NPs, $AgNO_3$	*Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*	Toxicity of NPs with improved stability	MIC and minimum biocidal concentration (MBC), Growth inhibition, cytotoxicity MIC (mg/L) – Cur-AgNPs: *P. aeruginosa*, 10; *E. coli*, 5; *B. subtilis*, 2.5; *S. aureus*, 5 MIC (mg/L) – AgNPs: *P. aeruginosa*, 10; *E. coli*, 15; *B. subtilis*, 10; *S. aureus*, 10 MIC (mg/L) – Ag^+: *P. aeruginosa*, 1.25; *E. coli*, 0.6; *B. subtilis*, 2.5; *S. aureus*, 1.25
Matzke et al. (2014)	Ag NPs, $AgNO_3$	*Pseudomonas putida*	Toxicity of differently sized and coated NPs	72 h EC_{50} (μg/L) – AgNPs, 0.25–13.4; Ag^+, 0.16
Akhil and Sudheer Khan (2017)	ZnO NPs	*Pseudomonas aeruginosa*, *Staphylococcus aureus*	Effects of humic acid on toxicity of NPs	Percentage toxicity, ROS, lipid peroxidation

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Miao et al. (2018)	TiO_2 NPs, CeO_2 NPs	*Cytophagaceae*, *Methylotenera* genera	Effect on metabolic activities of microbial communities	Enzyme activities, quantitative PCR, and high-throughput sequencing
Miao et al. (2018)	Ag NPs	Benthic microbial communities	Influence of NPs on benthic oxygen consumption	Oxygen concentration distributions
Algae				
Taylor et al. (2016b)	CeO_2 NPs	*Chlamydomonas reinhardtii*	Molecular toxicity of NPs with exposure concentration	Transcriptomics and metabolic profiling, NP internalization
Hoecke et al. (2009)	CeO_2 NPs	*Raphidocelis subcapitata*	Acute toxicity of NPs	72 h IC_{50} (mg/L) – 10.2–19.1
Rogers et al. (2010)	CeO_2 NPs	*Raphidocelis subcapitata*	Physicochemical behaviour and toxicity of NPs	72 h IC_{50} (mg/L) – 10.3 Membrane permeability, ROS production
Oukarroum et al. (2012)	Ag NPs	*Chlorella vulgaris*	Toxicity of NPs	Cell viability, ROS production, enzyme activity, chlorophyll content
Navarro et al. (2008)	Ag NPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Acute toxicity	1–5 h EC_{50} (nM) – AgNPs, 829.0–3,300.0 1–2 h EC_{50} (nM) – Ag^+ ($AgNO_3$), 184–188 1–5 h EC_{50} (nM) – Ag^+ (AgNPs), 8–33
Miao et al. (2010)	Ag NPs	*Ochromonas danica*	Toxicity and intracellular uptake of NPs	7 days EC_{50} (pM) – dissolved Ag^+ (from AgNPs), 1.27 7 dEC_{50} (nM) – Ag^+, 49.1
Angel et al. (2015)	CeO_2 NPs	*Raphidocelis subcapitata*	Acute toxicity, mechanism of toxicity	72 h IC_{50} (mg/L) – 7.6–28 ROS production, hyperspectral imaging
Adeleye and Keller (2016)	TiO_2 NPs	*Chlamydomonas reinhardtii*	Influence of algal EPS on TiO_2 Nanoparticles	Interaction, surface charge and aggregation of NPs
Zhou et al. (2016)	Ag NPs, $AgNO_3$	*Chlorella pyrenoidosa*	Role of EPS on bioaccumulation and toxicity of Ag nanoparticles	96 h IC_{50} (mg/L) – AgNPs, 39.2–140.0; Ag^+, 12.0 Interactions, accumulation of Ag
Ribeiro et al. (2014)	Ag NPs, $AgNO_3$	*Raphidocelis subcapitata*	Toxicity of NPs	96 h EC_{50} (μg/L) – AgNPs, 32.40; Ag^+, 33.79

Rodea-Palomares et al. (2011)	CeO_2 NPs	*Raphidocelis subcapitata*	Toxicity (growth rate measured by different methods) of NPs	24 h EC_{50} (mg/L) – OD, 0.88–16.5; cell counting, 4.25–56.7; ATP, 2.4–20.3
Hartmann et al. (2010)	TiO_2 NPs	*Raphidocelis subcapitata*	Algal toxicity of different types of NPs and influence of NPs on Cd toxicity	72 h EC_{50} (mg/L) – Degussa P25, 71.1; LW-S, 145.0; UV 100, 241.0 Cd toxicity
Lei et al. (2016)	Nano zero-valent iron, Fe_2O_3 and Fe_3O_4 NPs, $FeCl_3$	*Chlorella pyrenoidosa*	Effects of particle size, crystal phase, oxidation state and ageing of NPs on toxicity	96 h IC_{50} (mg/L) – ZVI NPs, 3.2–91.3; Fe_2O_3 NPs, 71.0–132.0; Fe_3O_4 NPs, ~32.0; Fe^{2+}, 1.0
Nicolas et al. (2016)	TiO_2 NPs	*Raphidocelis subcapitata*	Influence of exposure system on NP toxicity	72 h EC_{50} (mg/L) – 24 well plates, 8.5–>50; cylindrical vials, 2.7–39; Erlenmeyer flasks, >50
von Moos et al. (2015)	CuO NPs	*Chlamydomonas reinhardtii*	Sublethal effects in different media	Cellular growth, chlorophyll autofluorescence, intracellular oxidative stress and membrane integrity
Lee et al. (2015)	Au NPs	*Euglena gracilis* and *Chlamydomonas reinhardtii*	Toxicity and trophic transfer of NPs from algae to *Daphnia*	48 h EC_{50} (mg/L) *E. gracilis* – 9.18; *C. reinhardtii*, ND Trophic transfer from algae to *D. magna*
Bouldin et al. (2008)	Carboxyl NPs	*Raphidocelis subcapitata*	Toxicity and trophic transfer of NPs from algae to *Daphnia magna*	96 h EC_{50} (μg/L) – 37.1 Uptake of NPs
Zhao et al. (2016)	CuO NPs	*Chlorella pyrenoidosa*	Adhesion, uptake and toxicity of NPs	72 h EC_{50} (mg/L) – 45.7 NP internalization, membrane damage, ROS production, mitochondrial dysfunction
Aruoja et al. (2009)	CuO, ZnO and TiO_2 NPs, CuO, TiO_2, ZnO, $ZnSO_4$	*Raphidocelis subcapitata*	Toxicity of NPs	72 h EC_{50} (mg/L) – ZnO NPs, 0.042; TiO_2 NPs, 5.83; CuO NPs, 0.71; Zn^{2+}, 0.037–0.042; Ti^{2+}, 35.9; Cu^{2+}, 0.02–11.5
Ji et al. (2011)	Al_2O_3, SiO_2, ZnO, TiO_2	*Chlorella* sp.	Toxicity of NPs	6 days EC_{30} (mg/L) – ZnO NPs, 20.0; TiO_2 NPs, 30.0; Al_2O_3 and SiO_2 NPs, ND

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Sørensen et al. (2016a)	Pt NPs	*Raphidocelis subcapitata*, *Chlamydomonas reinhardtii*	Toxicity of NPs	48 h EC_{50} (mg/L) – *P. subcapitata*, 15; *C. reinhardtii*, 201
Dorobantu et al. (2015)	AgNPs, $AgNO_3$	*Chlorella protothecoides*, *Euglena gracilis*	Toxicity of NPs and mechanisms of toxicity	Growth inhibition, membrane damage
Aravantinou et al. (2015)	ZnONPs	*Chlorococcum* sp., *Scenedesmus rubescens*	Effect of media on toxicity of NPs	Growth inhibition 48 h IC_{50} (mg/L) – *Chlorococcum* sp., 0.77–7.99; *S. rubescens*, 42.68–300.59; 72 h IC_{50} (mg/L) – *Chlorococcum* sp., 1.78–2.03; *S. rubescens*, 22.56–>810.0; 96 h IC_{50} (mg/L) – *Chlorococcum* sp., 0.75–893.55; *S. rubescens*, 14.27–>810.0
Aruoja et al. (2015)	Al_2O_3, Co_3O_4, CuO, Fe_3O_4, MgO, Mn_3O_4, Sb_2O_3, SiO_2, ZnO, TiO_2, WO_3 and Pd NPs	*Raphidocelis subcapitata*	Toxicity of different NPs	Growth inhibition 72 h EC_{50} (mg/L) – CuO, ZnO, Pd NPs; 0.1–1.0; Co_3O_4, Fe_3O_4, Mn_3O_4, TiO_2 NPs; 1.0–10.0, Al_2O_3, SiO_2, WO_3 NPs; 10.0–100.0, MgO, Sb_2O_3 NPs; >100.0
Becaro et al. (2015)	AgNPs	*Raphidocelis subcapitata*	Toxicity of NPs	Growth inhibition 7 days EC_{50} (mg/L) – 1.09
Iswarya et al. (2017)	Au NPs	*Scenedesmus obliquus*	Modulatory effects of Zn^{2+} on the toxicity of NPs	Acute toxicity
Karunakaran et al. (2015)	Al_2O_3, SiO_2	*Porphyridium aerugineum*, *Geitler*	Toxicity of NPs based on size, contact angle and zeta potential	Growth inhibition, chlorophyll content and protein content of algae
Lee and An (2013)	ZnO, TiO_2	*Raphidocelis subcapitata*	Effect of NPs under different irradiation conditions	72 h EC_{50} (mg/L) – ZnO NPs – visible, UVA, UVB: <0.05; 72 h EC_{50} (mg/L) – ZnO NPs – visible, 2.53; UVA, 3.0; UVB, 2.95
Li et al. (2015b)	AgNPs, $AgNO_3$	*Euglena gracilis*	Toxicity and association of NPs	1 h EC_{50} (nM) – Ag NPs, 1,858.0; Ag^+, 85 2 h EC_{50} (nM) – Ag NPs, 1,487.0; Ag^+, 89 Cell morphology, uptake

Müller et al. (2016)	Cu NPs, Cu^{2+}	*Chlamydomonas reinhardtii*	Toxicity of NPs	Photosynthetic yield 1 h EC_{50} (μM) – Cu NPs, 101.0–219.0; Cu^{2+} (dissolved), 1.23–1.29; Cu^{2+}, 1.12; 2 h EC_{50} (μM) – Cu NPs, 29.0–30.0; Cu^{2+} (dissolved), 0.23–0.4; Cu^{2+}, 0.39
Nogueira et al. (2015)	Graphene oxide NPs	*Raphidocelis subcapitata*	Toxicity of NPs	Growth inhibition, ROS production, membrane damage
Oukarroum et al. (2017)	NiO NPs	*Chlorella vulgaris*	Toxicity of NPs	Cell viability, chlorophyll content, ROS production
Qian et al. (2016)	AgNPs, $AgNO_3$	*Microcystis aeruginosa*, *Chlorella vulgaris*	Toxicity of NPs and detoxification strategies	Growth inhibition, ROS production, gene transcription and protein expression analysis, chlorophyll content 48 h EC_{50} (nM) – *M. aeruginosa*: AgNPs, 731.7; Ag^{+}, 146.0; 96 h EC_{50} (nM) – *M. aeruginosa*: AgNPs, 351.4; Ag^{+}, 44.6; EC_{50} – *Chlorella vulgaris*: ND
Sendra et al. (2017)	TiO_2 NPs, Bulk TiO_2	*Chlamydomonas reinhardtii*	Toxicity of NPs under different irradiation conditions	Growth inhibition, yield reduction, ROS production, membrane damage, production of exopolymeric substances (EPS) 72 h EC_{50} (mg/L) – UVA + visible light – TiO_2 NPs, 2.3; bulk TiO_2, 1.35; 72 h EC_{50} (mg/L) – UVA – TiO_2 NPs, 551.7; bulk TiO_2, 423.7
Sørensen and Baun (2015)	AgNPs, $AgNO_3$	*Raphidocelis subcapitata*	Effect of timing in algal toxicity testing of NPs	Growth inhibition 2 h EC_{50} (μg/L) – AgNPs, 50–710; Ag^{+}, 6.0; 48 h EC_{50} (μg/L) – AgNPs, 75–310; Ag^{+}, 4.9; 2 h EC_{50} (μg/L) – AgNPs + cysteine, 1,000.0–1,800.0; Ag^{+} + cysteine, >240.0; 48 h EC_{50} (μg/L) – AgNPs + cysteine, 670.0–3,700.0; Ag^{+} + cysteine, 270.0
Stevenson et al. (2013)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Mitigation of AgNP toxicity by EPS	Chlorophyll concentration

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Taylor et al. (2016a)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Toxicity of NPs and effects on EPS	Growth inhibition, cell viability, ROS production
Wang et al. (2016b)	AgNPs, $AgNO_3$	*Chlamydomonas reinhardtii*	Cellular internalization and biotransformation of NPs	Uptake, distribution and speciation analysis of NPs
Yue et al. (2017)	AgNPs, $AgNO_3$	*Euglena gracilis*	Interaction of NPs with algal cells	Uptake, enzyme activity, interaction with proteins
Zhang et al. (2017)	TiO_2 NPs, organochlorine	*Chlorella pyrenoidosa*	Toxic interactions between NPs and OC contaminants	Oxidative stress, bioaccumulation
Zhang et al. (2016a)	TiO_2, ZnO, Ag NPs, CNTs	*Chlorella pyrenoidosa*	Physicochemical transformation and toxicity of NPS	Cell viability
Dauda et al. (2017)	TiO_2 NPs	*Chlorella vulgaris*	Toxicity of NPs	Oxidative stress
Zhang et al. (2016b)	Fe_xO_y NPs	*Raphidocelis subcapitata*	Toxicity of NPs	72 h EC_{50} (mg/L) – 0.05–0.09
Metzler et al. (2018)	TiO_2 NPs	*Raphidocelis subcapitata*	Influence of algae age and population on toxicity of NPs	Growth inhibition, chlorophyll content, oxidative stress
Yu et al. (2018)	TiO_2, SiO_2 and Ag NPs, CdTe/CdS QDs	*Chlamydomonas reinhardtii*	Effects of NPs on Cd toxicity	Growth inhibition, photosynthesis, bioaccumulation, oxidative stress
Ozkaleli and Erdem (2018)	TiO_2 NPs	*Raphidocelis subcapitata*	Mechanisms of NP toxicity	Growth inhibition, lipid peroxidation 24 h EC_{50} (mg/L) – 18.12–98.3, 72 h EC_{50} (mg/L) – 3.58–12.14
Bhuvaneshwari et al. (2017)	nZVI	*Scenedesmus* sp.	Trophic transfer of NPs to *Ceriodaphnia dubia*	Acute toxicity, uptake, enzyme activity 72 h EC_{50} (mM) – 7.12–24.29
Bhuvaneshwari et al. (2018b)	TiO_2 NPs	*Dunaliella salina*	Trophic transfer of NPs to *Artemia salina*	Acute toxicity, uptake, enzyme activity 72 h EC_{50} (mg/L) – 11.35
Akhil and Sudheer Khan (2017)	ZnO NPs	*Chlorella pyrenoidosa*	Effects of humic acid on toxicity of NPs	Chlorophyll content, ROS, lipid peroxidation

Cladocerans				
Allen et al. (2010)	Ag NPs, $AgNO_3$	*Daphnia magna*	Effects of filtration, coating and presence of food on toxicity of NPs	48 h LC_{50} (μg/L) – Ag NPs, 0.7–16.7; Ag^+, 1.1–1.4
Zhu et al. (2010b)	TiO_2	*Daphnia magna*	Trophic transfer of TiO_2 NPs from *D. magna* to *D. rerio*	Accumulation of NPs in *D. magna* and *D. rerio*
McTeer et al. (2014)	Ag NPs, $AgNO_3$	*Daphnia magna*	Trophic transfer of Ag from algae to *Daphnia*, feeding behaviour	Feeding behaviour, accumulation of Ag NPs in *D. magna*
Skjolding et al. (2014a)	Au NPs	*Daphnia magna*	Uptake and depuration of NPs	Uptake, depuration
Skjolding et al. (2014b)	ZnO NPs, $ZnCl_2$	*Daphnia magna*	Influence of surface functionalization on acute toxicity, uptake and trophic transfer of NPs	Acute toxicity – 48 h LC_{50} (mg/L) – Zn^{2+}, 0.41; ZnO NPs, 1.9–15.5 Uptake, trophic transfer from *D. magna* to *D. rerio*
Rosenkranz et al. (2009)	Carboxylated polystyrene NPs	*Daphnia magna*	Influence of size of NPs on uptake, accumulation and depuration of NPs	Uptake, accumulation, depuration
Heinlaan et al. (2008)	ZnO, CuO and TiO_2 NPs, $ZnSO_4$, $CuSO_4$	*Daphnia magna*	Acute toxicity and bioavailability of NPs	48 h EC_{50} (mg/L) – ZnO, 8.8; ZnO NPs, 3.2; $ZnSO_4$, 6.1; CuO, 165; CuO NPs, 3.2; $CuSO_4$, 0.17; TiO_2, ND
Dabrunz et al. (2011)	TiO_2 NPs	*Daphnia magna*	Acute toxicity, biological surface coating and moulting inhibition	72 h EC_{50} (mg/L) – 3.8, 96 h EC_{50} (mg/L) – 0.73 Moulting disruption
Lu et al. (2017)	Cu and Cr NPs	*Daphnia magna*	Acute and chronic toxicity	48 h EC_{50} (mg/L) – Cu NPs, 0.63; Cr NPs, 1.57 Feeding behaviour, enzyme activity (AChE, SOD,CAT and GST)
Zhao and Wang (2011)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute and chronic toxicity of NPs and Ag^+, effects of diet-borne and waterborne NPs and Ag^+	48 h EC_{50} (μg/L) – Ag^+, 2.51; Ag NPs, ND Acute toxicity, growth, reproduction, uptake

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Rainville et al. (2014)	AgNPs, $AgNO_3$	*Daphnia magna*	Acute toxicity (lethal and sublethal)	48 h LC_{50} (μg/L) – Ag^+, 4.5; Ag NPs, 47.2–429.9 Enzyme activity (CAT and GAPDH), protein identification
Li et al. (2010a)	Ag, Au and Ag-Au NPs	*Daphnia magna*	Comparative toxicity of NPs, effects of size of NPs	48 h LC_{50} (μg/L) – Au NPs, 65–75; Ag NPs, 3–4; Ag-Au NPs, 12–59
Shen et al. (2015)	Ag NPs and $AgNO_3$	*Daphnia magna*	Acute toxicity of NPs and Ag^+	8 h LC_{50} (μg/L) – Ag^+, 0.4; Ag NPs, 5.0–144.2
Adam et al. (2015a)	ZnO and CuO NPs, $ZnCl_2$, $CuCl_2{\cdot}2H_2O$	*Daphnia magna*	Uptake and elimination of NPs under chronic exposure	Uptake and elimination of NPs
Wiench et al. (2009)	TiO_2 and ZnO NPs	*Daphnia magna*	Acute and chronic effects on mobility and reproduction	Mortality, reproduction
Kim et al. (2010)	TiO_2 NPs	*Daphnia magna*	Oxidative stress based on size of NPs	Enzyme activity (CAT, GST, GSH and GPx)
Dominguez et al. (2015)	Au NPs	*Daphnia magna*	Effects of charge and surface ligand properties of NPs on oxidative stress and gene expression	ROS production, gene expression
Strigul et al. (2009)	TiO_2, B and Al NPs	*Daphnia magna*	Acute toxicity based on ageing of NPs	48 h LC_{50} (mg/L) – B NPs, 6.7; TiO_2 NPs, ND; Al NPs (ALEX), 7.4; Al NPs (L-ALEX), 107.5
Adam et al. (2014)	ZnO NPs, $ZnCl_2$	*Daphnia magna*	Chronic toxicity, NP aggregation and dissolution	21 days EC_{50} (reproduction) (mg/L) – ZnO NPs, 0.112; Zn^{2+}, 0.082 Growth, reproduction, accumulation
Adam et al. (2015b)	CuO NPs and $CuCl_2{\cdot}2H_2O$	*Daphnia magna*	Chronic toxicity of NPs and Cu^{2+}	21 days EC_{50} (reproduction) (mg/L) – CuO NPs, 1.041; Cu^{2+}, 0.022 Reproduction, length

Conine and Frost (2017)	Ag NPs	*Daphnia magna*	Effects of food and nutrition in responding to NP exposure	48 h LC_{50} (μg/L) – AgNPs (without food), 5.2; AgNPs (with food), 17.0
Tan et al. (2016b)	TiO_2 NPs, Cd	*Daphnia magna*	Toxicity of Cd in the presence and absence of well-dispersed NPs	Toxicity, uptake and accumulation of Cd
Lu et al. (2017)	Cu and Cr NPs	*Daphnia magna*	Toxicity of NPs	48 h LC_{50} (μg/L) – Cu NPs, 0.63; Cr NPs, 1.57 Chronic toxicity of NPs (AChE, CAT, SOD, GST), feeding rate
Nasser et al. (2016)	Au NPs	*Daphnia magna*	Effect of shape and charge of NPs on acute toxicity, oxidative stress and moulting	24 h LC_{50} – Au NPs (positively charged), 6.1–50.0 μg/L; Au NPs (negatively charged), 10–50 mg/L ROS production; moulting inhibition
Fan et al. (2016)	TiO_2 NPs, Cu	*Daphnia magna*	Influence of NPs on Cu toxicity	Uptake and bioaccumulation of Cu, enzyme activity
Sørensen et al. (2016c)	Ag and CuO NPs, $AgNO_3$, $CuCl_2$	*Daphnia magna*	Acute and chronic effects of NPs and ions after standard and pulse exposure	48 h LC_{50} (μg/L) – CuONP, 5.2; AgNP, ND; Ag^+, 4.0; Cu^{2+}, 0.05, 24 h LC_{50} (μg/L) – CuONP, 6.5; AgNP, 130; Ag^+, 4.5; Cu^{2+}, 0.1 EC_{50} values after 1, 2 and 3 h pulse exposure, moulting inhibition, body length, offspring production
Georgantzopoulou et al. (2016)	Ag NPs, $AgNO_3$	*Daphnia magna*	Influence of NPs on multixenobiotic resistance	Inhibition of multixenobiotic resistance transporters
Iswarya et al. (2016)	TiO_2 NPs (anatase and rutile)	*Ceriodaphnia dubia*	Individual and binary mixture toxicity of anatase and rutile NPs	48 h LC_{50} (visible irradiation) (mg/L) – anatase NPs, 37.0; rutile NPs, 48.0 48 h LC_{50} (UV irradiation) (mg/L) – anatase NPs, 22.5; rutile NPs, 23.7 LC_{50} values for binary mixtures

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Bhuvaneshwari et al. (2016)	ZnO NPs, Zn^{2+} ions	*Ceriodaphnia dubia*	Relative contribution of dissolved ions and NPs to acute toxicity and accumulation	48 h LC_{50} (mg/L) – ZnO NPs, 0.431–0.701; Zn^{2+}, 1.048–2.046 Bioaccumulation
Sakka et al. (2016)	AgNPs	*Daphnia magna*	Behaviour and chronic effects of differently stabilized NPs	Reproduction, mass balance, uptake, depuration
Tan et al. (2016a)	TiO_2 NPs	*Daphnia magna*	Influence of Ca in the medium on NP uptake and uptake routes	Accumulation, depuration
Chen et al. (2016)	Fullerene (nC_{60}) NPs	*Daphnia magna*	Effect of subcellular distribution on NP uptake and transfer efficiency from algae to *Daphnia*	Bioaccumulation in *Scenedesmus obliquus* and *D. magna*
Botha et al. (2016)	Au NPs	*Daphnia magna*	Adsorption, uptake and distribution of NPs	Reproduction, imaging
Zhao and Wang (2012a)	Ag NPs, Ag^{+}	*Daphnia magna*	Influence of surface coating on acute toxicity	48 h LC_{50} (μg/L) – AgNPs, 1.1–28.7; Ag^{+}, 0.57–1.1 Bioaccumulation
Zhu et al. (2010a)	TiO_2 NPs	*Daphnia magna*	Toxicity and bioaccumulation	72 h EC_{50} (mg/L) – 1.62, 72 h LC_{50} (mg/L) – 2.02 Bioaccumulation, offspring production, reproductive defects, growth retardation
Blinova et al. (2010)	CuO and ZnO NPs, $CuSO_4$ and $ZnSO_4.7H_2O$	*Daphnia magna*	Toxicity of NPs in natural waters	48 h EC_{50} (mg/L) – CuO NPs, 2.6–24; ZnO NPs, 2.6–9.0; Cu^{2+}, 0.07–0.92; Zn^{2+}, 1.4–2.0
Baumann et al. (2014)	Iron Oxide NPs	*Daphnia magna*	Effects of coating on acute toxicity	96 h EC_{50} (mg/L) – 27.9–>100

Bozich et al. (2014)	Au NPs	*Daphnia magna*	Effects of surface chemistry, charge and ligand type on acute and chronic toxicity	Mortality, reproduction, body size
Bone et al. (2012)	Ag NPs	*Daphnia magna*	Effects of biotic and abiotic interactions on fate and toxicity of NPs	Mortality (percentage)
Seo et al. (2014)	Ag, CuO and ZnO NPs	*Daphnia magna*	Effects of physiochemical properties of media on toxicity	48 h EC_{50} (μg /L) – AgNPs, 4.94; CuO NPs, 980.0; ZnO NPs, 1,950.0
Salieri et al. (2015)	TiO_2 NPs	*Daphnia magna*	Influence of exposure mode on toxicity	96 h EC_{50} (mg/L) – 32–82
Seitz et al. (2015)	Ag NPs, $AgNO_3$	*Daphnia magna*	Effects of NP properties, media pH and dissolved organic matter on toxicity	48 h EC_{50} (μg /L) – AgNPs, 3.9–33.4, Ag^+: 1.7–3.0
Rosenfeldt et al. (2015)	TiO_2 NPs	*Daphnia magna*	Role of crystalline phase of NPs and OM on Cu toxicity	Acute toxicity of Cu
Kim et al. (2016)	Ag NPs	*Daphnia magna*	Influence of NP on As, Cu and Cd toxicity and bioaccumulation	Acute toxicity of Cu, Ad and Cd
Liu et al. (2015)	TiO_2 NPs and nanosheets	*Daphnia magna*	Influence of different facets of NPs on NP and cu toxicity	Enzyme activity, bioaccumulation of NPs and Cu
Simon et al. (2015)	MWCNT	*Daphnia magna*	Population level effects of MWCNTs in *D. magna* exposed to pulses of triclocarban	96 h EC_{50} (μg /L) – 12.65–33.16 Size, reproduction
Wang et al. (2014)	MWCNT, Ni	*Daphnia magna*	Effects of MWCNTs on the toxicity of Ni under different pH levels	Acute toxicity and bioaccumulation of Ni
Li et al. (2015a)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity at sublethal levels by NMR-based metabolomics	Metabolic profile

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Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Sakamoto et al. (2015)	Ag NPs, Ag^+	*Daphnia magna*, *Daphnia galeata*, *Bosmina longirostris*	Role of free silver in acute and chronic toxicity of NPs	48 h EC_{50} (μg/L) – AgNPs: *D. magna*, 2.43; *D. galeata*, 2.16; *B. longirostris*, 2.90 Ag^+: *D. magna*, 0.25; *D. galeata*, 0.16; *B. longirostris*, 0.22 Swimming behaviour, offspring production
Strigul et al. (2009)	B NPs, TiO_2 NPs, Al NPs	*Daphnia magna*	Toxicity of NPs	48 h LD_{50} (mg/L) – B NPs, 6.7; Al NPs, 7.48–107.58; TiO_2 NPs, ND
Hoecke et al. (2009)	CeO_2 NPs	*Daphnia magna*	Acute and chronic toxicity of NPs	21 days EC_{50} (mg/L) – survival, 36.9–71.1 Reproduction, 20.5–42.7
Ribeiro et al. (2014)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute toxicity	48 h LC_{50} (μg/L) – without food – AgNPs, 11.02; Ag^+, 1.05; with food AgNPs, 72.0; Ag^+, 3.38 24 h EC_{50} (feeding inhibition) (μg/L) – AgNPs, 13.64; Ag^+, 2.03 21 days EC_{50} (reproduction) (μg/L) – AgNPs, 1.0; Ag^+, 0.385
Lee et al. (2015)	Au NPs	*Daphnia magna*	Toxicity and trophic transfer of NPs from algae from *E. gracilis* and *C. reinhardtii*	48 h LC_{50} (mg/L) *Daphnia magna* – 0.02 Trophic transfer from *E. gracilis* and *C. reinhardtii* to *D. magna*
Bouldin et al. (2008)	Carboxyl NPs	*Ceriodaphnia dubia*	Toxicity and trophic transfer of NPs from algae *Raphidocelis subcapitata* to *Daphnia*	48 h LC_{50} (μg/L) – >110.0 Localization of NPs in *Daphnia*
Lovern et al. (2008)	Au NPs	*Daphnia magna*	Intake of NPs	Localization of NPs, intake and retention of NPs
Wray and Klaine (2015)	Au NPs	*Daphnia magna*	Influence of physicochemical properties on NP uptake and elimination	Uptake, elimination, bioaccumulation

Scanlan et al. (2013)	Ag NPs, $AgNO_3$	*Daphnia magna*	Acute toxicity and internalization of NPs	Gene expression, NP localization
Ribeiro et al. (2017)	Ag NPs, $AgNO_3$	*Daphnia magna*	Bioaccumulation from waterborne and dietary NPs	Uptake, elimination
Stanley et al. (2016)	MWCNTs	*Daphnia magna*	Acute toxicity and sublethal effects	48 h LC_{50} (mg/L) – 29.3, 48 h EC_{50} (swimming velocity) (mg/L), 6.7; 14 days LC_{50} (mg/L), 4.3; 14 days EC_{50} (reproduction) (mg/L), 5.0 Feeding rate
Lovern and Klaper (2006)	C_{60} and TiO_2 NPs	*Daphnia magna*	Acute toxicity, swimming behaviour	48 h LC_{50} (mg/L) – filtered TiO_2, 5.5; unfiltered TiO_2, ND 48 h LC_{50} (μg/L) – filtered C_{60}, 460; 48 h LC_{50} (mg/L) – unfiltered C_{60}, 7.9 Swimming behaviour
Pokhrel and Dubey (2012)	Ag NPs	*Daphnia magna*	Swimming behaviour in the presence of *Anax junius*	Survival, reproduction, swimming behaviour
Artells et al. (2013)	CeO_2 NPs	*Daphnia similis*, *Daphnia pulex*	Effects of NPs on acute toxicity and swimming behaviour	48 h LC_{50} (mg/L) – *D. similis*, 0.26; *D. pulex*, 91.79 Swimming velocity, localization of NPs
Lovern et al. (2007b)	TiO_2 and C_{60} NPs, $C_{60}H_xC_{70}H_x$	*Daphnia magna*	Behavioural and physiological changes	Hopping frequency, feeding appendage and postabdominal curling movement, heart rate
Noss et al. (2013)	TiO_2 NPs	*Daphnia magna*	Effects of NPs on swimming behaviour	Swimming behaviour and velocity
Qin et al. (2015)	AgNPs	*Daphnia carinata*	Impact of predator cues on responses to NPs	48 h LC_{50} (μg/L) – predator absent, 1.75; predator present, 4.61 Enzyme activity (SOD, CAT), offspring production
Bacchetta et al. (2018)	CNPs and CNCs	*Daphnia magna*	Influence of shape on acute toxicity, uptake and morphological alterations	48 h LC_{35} (mg/L) – CNP, 50; 48 h LC_{20} (mg/L) – CNC, 50 Uptake, microscopic and histological analysis

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Cui et al. (2017)	Ag NPs, Ag NWs, Ag PLs, $AgNO_3$	*Daphnia magna*, *Daphnia galeata*	Dimension-dependent acute toxicity	48 h LC_{50} (μg/L) – AgPLs: *D. magna*, 39.42; *D. galeata*, 34.90; AgNPs, *D. galeata*, 36.51; AgNWs (10 nm), *D. galeata*, 214.19; AgNWs (20 nm), *D. galeata*, 149.26; Ag^+, *D. magna*, 3.09; *D. galeata*, 1.94
Lv et al. (2017)	C_{60}	*Daphnia magna*	Mechanisms underlying the acute toxicity of NPs	72 h EC_{50} (mg/L) – 14.9, 72 h LC_{50} (mg/L) – 16.3 Feeding rate, digestive enzyme activities, morphological changes, oxidative stress
Das et al. (2013)	AgNPs, capped and uncapped TiO_2 NPs	*Daphnia magna*	Acute and chronic toxicity	48 h LC_{50} (μg/L) – AgNPs, 2.75 48 h LC_{50} (mg/L) – capped TiO_2 NPs, ND, uncapped TiO_2 NPs, 7.75
Becaro et al. (2015)	AgNPs	*Daphnia similis*	Toxicity of NPs	Acute toxicity 48 h EC_{50} (μg/L) – 0.26
Bacchetta et al. (2016)	ZnO NPs, $ZnSO_4$	*Daphnia magna*	Role of Zn^{2+} in Zn NP cytotoxicity	Acute toxicity, cytotoxicity 48 h EC_{50} (mg/L) – Zn^{2+}, 0.99; ZnO NPs, ND 48 h LC_{50} (mg/L) – Zn^{2+}, 1.37; ZnO NPs, ND
Conine and Frost (2017)	AgNPs	*Daphnia magna*	Effects of algal particles and animal nutrition on toxicity	Acute toxicity, growth 48 h LC_{50} (μg/L) – algae fed (continuous), 17.0; nonfed, 5.2 48 h LC_{50} (μg/L) – algae fed (2 h intervals), 3.9; nonfed, 4.5
Cupi et al. (2016a)	Zn, TiO_2, AgNPs	*Daphnia magna*	Influence of pH and media composition on toxicity of NPs	48 h EC_{50} (μg/L) – AgNPs: US-EPA medium, 51.0; M7 medium, 66.0 48 h EC_{50} (mg/L) – TiO_2NPs: US-EPA medium, 14.0; M7 medium, >100.0 48 h EC_{50} (mg/L) – ZnONPs: US-EPA medium, 0.047; M7 medium, 4.9

Lu et al. (2017)	Cu NPs, Cr NPs	*Daphnia magna*	Acute and chronic toxicity of NPs	Acute toxicity, enzyme activity, food intake
Lv et al. (2017)	C_{60} NPs, Si NPs	*Daphnia magna*	Mechanisms of NP toxicity	Acute toxicity, accumulation, feeding rate, enzyme activity, oxidative stress 48 h EC_{50} (mg/L) – 28.5, 72 h EC_{50} (mg/L) – 16.3
Vijayakumar et al. (2016)	ZnO NPs	*Ceriodaphnia cornuta* *Moina micrura*	Protective effects of chitosan against ZnO NP toxicity	Bioaccumulation, behavioural effects, mechanical damage
Wu et al. (2017a)	CuO NPs	*Daphnia magna*	Effects of waterborne and dietary exposure of NP	Chronic toxicity, bioaccumulation
Xiao et al. (2018)	Cu NPs	*Daphnia magna*	Impact of water chemistry on particle-specific toxicity of NPs	Acute toxicity 48 h LC_{50} (mg/L) – static exposure and no DOC, 0.024–0.076 48 h LC_{50} (mg/L) – static exposure and DOC, 0.5–3.5 48 h LC_{50} (mg/L) – dynamic exposure and no DOC, 0.02–0.15 48 h LC_{50} (mg/L) – dynamic exposure and no DOC, 0.3–2.1
Zhang et al. (2016b)	Fe_xO_y NPs	*Daphnia magna*	Toxicity of NPs	72 h EC_{50} (mg/L): 20.8–65.9
Sá-Pereira et al. (2018)	TiO_2 NPs	*Daphnia magna*	Protein profiling as a bio-marker for NP toxicity	Acute toxicity, uptake, distribution, protein profiling 48 h EC_{50} (mg/L) – 30.0
Brun et al. (2017)	Polystyrene NPs	*Daphnia magna*	Brood pouch-mediated NP uptake	Uptake
Gökçe et al. (2018)	TiO_2, ZnO NPs	*Daphnia magna*	Effects of NPs on population dynamics	Acute toxicity, chronic toxicity 96 h LC_{50} (mg/L) – TiO_2NPs, 1.8; ZnONPs, 0.7 21 days LC_{50} (mg/L) – TiO_2NPs, 1.0; ZnONPs, ND

(continued)

Table 2 (continued)

Reference	Materials tested	Organisms	Effects studied	Endpoints
Gonçalves et al. (2018)	ZnO NPs	*Daphnia magna*	Comparative assessment of toxicity	Acute toxicity, chronic toxicity 48 h EC_{50} (μM) – 27.1–34.5
Heinlaan et al. (2017)	Co_3O_4 NPs Mn_2O_3 NPs	*Daphnia magna*	Body burden of NPs	Acute toxicity, ROS generation, feeding behaviour, bioaccumulation 48 h LC_{50} (mg/L) – Co_3O_4 NPs, >100.0; Mn_2O_3 NPs, >100.0
Hu et al. (2017)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity of NPs and Ag^+	Acute toxicity, chronic toxicity, bioaccumulation, biomagnification
Jensen et al. (2017)	Au NPs	*Daphnia magna*	Uptake of NPs and related artefacts	Microscopic analysis
Kim et al. (2017)	CuO and ZnO NPs $CuSO_4$, $ZnSO_4$	*Daphnia magna*	Time-dependent toxicity of NPs	48 h EC_{50} (μg/L) – CuO NPs, 1,090.0; Cu^{2+}, 56.0 ZnO NPs, 1,400.0; Zn^{2+}, 800.0
Liu et al. (2017)	Graphene oxide NPs	*Daphnia magna*	Transgenerational effects of NPs	Mortality, body size, time to first brood
Pacheco et al. (2018)	Au NPs	*Daphnia magna*	Toxicological interactions by chronic exposure to NPs and microplastics	Mortality, growth inhibition, number of broods, total offspring
Tan and Wang (2017)	TiO_2	*Daphnia magna*	Influence of NPs on dietary metal uptake	Metal assimilation
Bhuvaneshwari et al. (2018a)	ZnO NPs, ZnO	*Ceriodaphnia dubia*	Trophic transfer of NPs from alga *Scenedesmus obliquus*	Toxicity, uptake, algae cell viability
Bhuvaneshwari et al. (2017)	nZVI	*Ceriodaphnia dubia*	Trophic transfer of NPs from alga *Scenedesmus* sp.	Acute toxicity, uptake, enzyme activity 48 h EC_{50} (mM) – 14.22–15.64
Gaiser et al. (2011)	AgNPs, CeO_2 NPs	*Daphnia magna*	Acute and chronic toxicity	Acute toxicity (percentage), moulting and growth inhibition, mortality, feeding rate, swimming velocity

Blinova et al. (2017)	Fe_3O_4 NPs, Fe_3O_4	*Daphnia magna*	Toxicity of NPs	Acute toxicity, reproduction 48 h EC_{50} (μg/L) – Fe_3O_4 NPs, > 100, Fe_3O_4, > 100
Blinova et al. (2013)	Ag NPs, $AgNO_3$	*Daphnia magna*	Toxicity of NPs in different media	Acute toxicity, reproduction 48 h EC_{50} (μg/L) – AgNPs, 40.2–236.3; Ag^+, 2.2–12.9
Khan et al. (2014)	Au NPs	*Daphnia magna*	Bioaccumulation and epithelial uptake	Uptake, elimination, internalization
Muna et al. (2017)	CuO NPs, $CuSO_4$	*Daphnia magna*	Acute toxicity and effect of medium on Cu body burden	Percentage mortality, body burden, body length, dry weight
Xiao et al. (2015)	Cu NPs and ZnO NPs	*Daphnia magna*	Toxicity and accumulation of NPs and dissolved ions	Acute toxicity, accumulation, 48 h LC_{50} (mg/L) – CuNPs, 0.093; Cu^{2+}, 0.028 48 h LC_{50} (mg/L) – ZnONPs, 0.99; Zn^{2+}, 1.01
Akhil and Sudheer Khan (2017)	ZnO NPs	*Daphnia magna*	Effects of humic acid on toxicity of NPs	Percentage mortality, accumulation, morphology changes

ACP acid phosphatase, *CAT* catalase, *CNC* cubic-shaped carbon nanoparticle, *CNP* carbon nanopowder, *CSP* caspase, *DOM* dissolved organic matter, *EC* effective concentration, *EPS* extra polymeric substances, *GPx* glutathione peroxidase, *GST* glutathione S-transferase, *HSP* heat shock protein, *IC* inhibitory concentration, *LD* lethal dose, *LDH* lactate dehydrogenase, *LPO* lipid peroxidation, *MBC* minimum bactericidal concentration, *MIC* minimum inhibitory concentration, *MT* metallothionein, *MWCNT* multi-walled carbon nanotube, *ND* not defined, *NT* not tested, *nZVI* nano zero-valent iron, *OM* organic matter, *QD* quantum dots, *ROS* reactive oxygen species, *SOD* superoxide dismutase

strains of bacteria (Kvitek et al. 2008; Lok et al. 2007; Bradford et al. 2009). They demonstrated that aggregation mitigates the potential toxicity of NPs. It is generally accepted that aggregation reduces toxicity to aquatic organisms. Low environmental concentrations lead to less aggregation, and hence, unlike traditional toxicants, it is possible that low concentrations are more toxic than higher concentrations with time (Tiede et al. 2009). Lok et al. (2007) observed higher aggregation in high salt media resulting in loss of antibacterial activities of AgNPs to *Escherichia coli*. Fernandes et al. (2006) suggested that NPs would disaggregate in the presence of household or industrial detergents. Limbach et al. (2008) found that protein breakdown products and surfactants in wastewater change the zeta potential of NPs causing stabilization. Oleszczuk et al. (2015) found that certain surfactants decrease the toxicity of TiO_2 NPs to *Daphnia magna*, and they hypothesized that the surfactants increase the aggregation of NP, reducing the bioavailability to daphnids. In contrast, there are instances where higher toxicity was observed with the aggregation of NPs. In one such study, Kashiwada (2006) found increased salinity caused higher toxicity by NPs to medaka eggs though NPs showed higher aggregation in saline media.

5.6 *Influence of NPs on Other Contaminant Effects*

There is also evidence that NPs influence the toxicity of other contaminants (Deng et al. 2017) and that influence is mitigated by the characteristics of the aquatic environment, such as the presence of organic matter. Fan et al. (2016) observed a reduction in Cu accumulation in *Daphnia magna* in the presence of TiO_2 NPs, but humic acids decreased that reducing effect. In a similar study, Rosenfeldt et al. (2015) observed a twofold decrease in Cu toxicity to *Daphnia magna* in the presence of TiO_2 NPs in the medium. They attributed this to the adsorption of Cu to NPs leading to a reduction in the bioavailability of Cu as the cause of toxicity reduction. In another study, Liu et al. (2015) found that TiO_2 NPs increased Cu accumulation in *Daphnia magna*, while TiO_2 nanosheets decreased Cu accumulation. Interestingly, the presence of Cu^{2+} in the medium caused agglomeration and sedimentation of TiO_2 NPs causing decreased NP bioaccumulation. Hartmann et al. (2010) investigated the toxicity of cadmium (Cd^{2+}) ions in the presence of TiO_2 NPs. Toxicity from Cd was reduced in the presence of TiO_2 NPs compared to Cd alone due to the decreased bioavailability of Cd resulting from the sorption of Cd to NPs. Kim et al. (2016) observed decreased bioaccumulation of Cu, while both acute toxicity and bioaccumulation of Cd increased in the presence of citrate-coated Ag NPs after 24 h exposure of *Daphnia magna*. Simon et al. (2015) observed a reduction in the toxicity of triclocarban to *Daphnia magna* in the presence of multiwalled carbon nanotubes (MWCNT). In contrast, Wang et al. (2014) observed increased toxicity of Nickel (Ni) to *Daphnia magna* in the presence of hydroxylated MWCNTs. They found that this was due to the uptake of Ni-adsorbed NPs. NPs could also influence the multixenobiotic resistance (MXR) of aquatic organisms. Georgantzopoulou et al. (2016) reported similar findings when they exposed *Daphnia magna* to Ag NPs.

Zhang et al. (2017) evaluated the joint toxicities of TiO_2 NPs with four different organochlorine contaminants (OC) towards the alga *Chlorella pyrenoidosa*. The results indicated that there were synergistic, antagonistic and additive effects between TiO_2 NPs and OCs on the alga. Similarly, Yu et al. (2018) reported synergistic and antagonistic effects of TiO_2, SiO_2 and Ag NPs and CdTe/CdS QDs on Cd^{2+} toxicity towards alga *Chlamydomonas reinhardtii*. Li et al. (2017a) also observed increased toxicity of Cd^{2+} ions to *Daphnia magna* in the presence of TiO_2 NPs. These studies show that the NPs influence the effects of existing environmental contaminants on organisms and therefore highlight the importance of systematic studies of toxicological effects of NPs due to their own effects plus their influence on other contaminant effects in environmental risk assessment.

6 The Toxicity of NPs to Freshwater Organisms

Human and industrial wastes enter waterways, and hence, it is inevitable that NPs also end up in waterbodies due to the mass use of products containing NPs (Daughton 2004; Moore 2002). Ingestion and inhalation are considered as the major routes of NP uptake by terrestrial organisms (Dowling 2004; Warheit 2004; Moore and Allen 2002). In addition, there might be other routes of exposure in aquatic organisms, such as uptake through gills and surface epithelia (Moore 2006; Oberdörster 2004). Once internalized into invertebrates, the gut epithelium, the cellular immune system and the hepatopancreas are likely targets for reactive mechanisms, while the liver is a probable target in fish (Moore 1990; Moore et al. 2004). Eukaryotes have developed advanced mechanisms, endocytosis (100 nm or less) and phagocytosis (100–10,000 nm) for cellular internalization of particles (Na et al. 2003; Pelkmans and Helenius 2002; Moore 2006). Contamination of waterways is not only harmful for aquatic biota but also to terrestrial organisms including humans by direct or indirect exposure to NPs via direct ingestion, inhalation of water aerosols, skin contact or food (Daughton 2004). The ability of water treatment plants to treat NPs is still doubtful, and in particular, uncharged or anionic NPs could pass through into the sewage effluent. Also, some studies showed that there is a potential for NPs to harm important bacteria in sewage treatment plants (Choi et al. 2008; Kwak et al. 2001; Ghafari et al. 2008; Nyberg et al. 2008) which may put freshwater aqueous ecosystems under threat from other contaminants.

Metallic NPs have the potential to dissolve and release ions into the aquatic media. Some researchers claim that these liberated ions are the only cause of toxicity to aquatic organisms, while other studies indicate that NPs are the major cause of toxicity (Li et al. 2017b; Abramenko et al. 2018). This debate is still prevalent though the effects of metallic NPs have been intensively studied in the past decades (Wang et al. 2016a). In general, the toxicity of NPs is compared to the toxicity of the counterpart bulk material, usually metal salts, to test this hypothesis (Djurišić et al. 2015). Evaluation of acute and chronic toxicity and mechanism of toxicity is crucial in environmental risk assessment of NPs in protecting the organisms and

setting up guidelines. A considerable number of studies have been undertaken to date in assessing the toxicity of different NPs and the sensitivity of organisms to NPs and on the mechanisms of toxicity.

6.1 The Toxicity of NPs to Bacteria

Toxicity to bacteria is an area of concern due to the possibility that important biogeochemical processes and other organisms in the aquatic environment may be affected detrimentally by the release of NPs to aquatic systems (Neal 2008). Several studies have evaluated the effects on non-pathogenic or environmentally relevant bacteria (Bondarenko et al. 2013; Hwang et al. 2008; Strigul et al. 2009; Jiang et al. 2009; Bradford et al. 2009; Baek and An 2011; Choi et al. 2008; Lopes et al. 2012; Heinlaan et al. 2008; von Moos and Slaveykova 2014). Also, due to the antimicrobial effects of certain NPs, the effects on pathogenic bacteria have been heavily studied due to the potential applications of NPs in medical and healthcare products. Studies on silver dominate these as a result of the excellent antimicrobial properties of Ag NPs (Marambio-Jones and Hoek 2010; Rai et al. 2009; Atiyeh et al. 2007; Durán et al. 2010; Fabrega et al. 2009).

6.1.1 Acute and Chronic Toxicity

More than 60% of the studies have looked into the acute toxicity of NPs to bacteria, while approximately 40% of studies have assessed other cellular effects such as membrane damage, morphological changes, oxidative stress, uptake, internalizations, enzyme activity, protein damage and DNA damage (Table 2). Currently, there are no standard bacterial species recommended for toxicity assessments. However, *Escherichia coli* has been the most preferred organism since 45% of studies have used it to assess the effects of NPs on bacteria (Fig. 2a). More than 40% of studies have assessed the effects of Ag NPs on bacteria (Fig. 2d) possibly due to the increased antimicrobial properties of Ag compared to other metals which is a huge concern in environmental toxicity and also due to the importance of Ag NPs in the medical field. Additionally, ZnO and TiO_2 NPs which have antimicrobial properties have been used in 15% and 11% of studies, respectively. Most studies have focused on single species, while there are a few studies at the community level (Miao et al. 2018; Colman et al. 2012; Kumar et al. 2012; Pradhan et al. 2011; Ge et al. 2013; Frenk et al. 2013; Li et al. 2016a; Asadishad et al. 2018; Ma et al. 2015) which report on changes in microbial biomass, community activity, community composition, microbial diversity, community richness, genome and structural diversity. Risk assessment of the effects of NPs on bacterial communities is particularly important due to the detrimental effects on treatment processes.

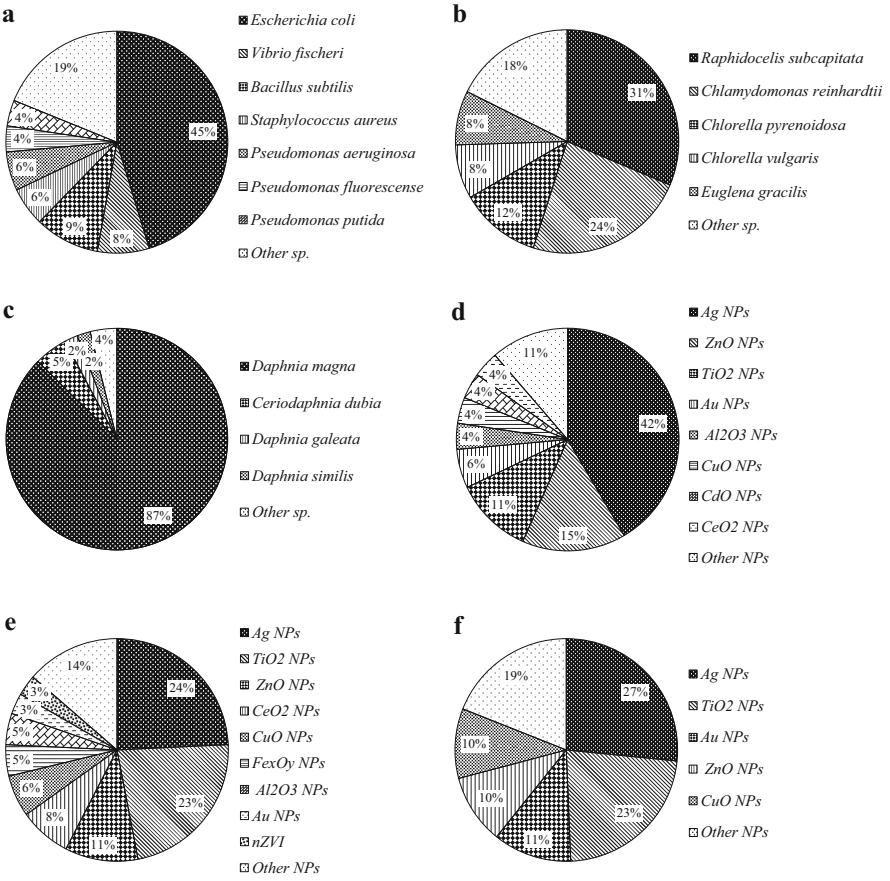

Fig. 2 (**a**) Percentage use of different (**a**) bacteria (**b**) algae and (**c**) *Daphnia* species in NP studies; percentage use of different types of NPs to study effects on (**d**) bacteria, (**e**) algae and (**f**) daphnia in NP studies (the data are based on the previous publications referred in this review)

6.1.2 Toxicity from NPs or Ions

The toxicity of NPs is widely attributed to the dissolved ions rather than NPs themselves (Jiang et al. 2009). Fabrega et al. (2009) investigated the impact of Ag NPs on *Pseudomonas fluorescens* in the presence of river humic acids (RHA) at different pH values. They found that the bacterial growth was entirely inhibited at 2 mg/L under all conditions and adversely affected at 0.2 mg/L concentration under some conditions. A similar toxicity was observed in the absence of RHA at pH 9 only. With these results, the authors concluded that there was a specific nanoparticle effect which could not be explained by just dissolved Ag^{+}. Jin et al. (2010) studied the influence of inorganic aquatic chemistry on Ag NP stability

and bacterial viability. They found that the antibacterial activity of Ag NPs was much lower than Ag^+, when compared on the basis of mass added. Choi et al. (2010) found Ag^+ was more toxic to *Escherichia coli* biofilms than Ag NPs with minimum bactericidal concentrations of 1.2 mg/L and 10 mg/L, respectively. Ag NPs were highly aggregated in the presence of biofilms causing increased size of NPs by a factor of 40 causing reduced toxicity. The aggregation may be due to a change of ionic strength in the medium caused by biofilms and interactions with various complexing agents produced by biofilms. Xiu et al. (2012) suggested that the antimicrobial effects of AgNPs are primarily from Ag^+ released from NPs rather than the morphological properties of the particles after exposing *Escherichia coli* to AgNPs with different coatings and sizes. Dorobantu et al. (2015) observed no damage to membranes of the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* when exposed to Ag NPs. However, though AgNPs were toxic to bacteria, only $AgNO_3$ caused membrane damage, and therefore, they decided that only Ag^+ ions were responsible for biological action against microorganisms. However, Lok et al. (2006) claimed that Ag NPs were more toxic to *Escherichia coli* than Ag^+ though they found the mode of action of NPs was similar to Ag^+. The effective concentrations for toxicity of AgNPs and Ag^+ were at nanomolar and micromolar level, respectively. Similarly, Baek and An (2011) attributed the toxicities to particle-specific toxicity rather than ionic effects when *Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus* were exposed to metal oxide NPs. Most studies on metallic NP toxicity support the hypothesis that the toxicity of NPs to bacteria is primarily caused by the ions released from NPs and the toxicity of NPs is less than their bulk form. However, as above, there are instances where authors conclude that the NPs are more toxic than the ions.

6.1.3 Mechanisms of Toxicity

Sondi and Salopek-Sondi (2004) found that Ag NP-treated *Escherichia coli* cells were damaged, while Ag NPs were found accumulated in the bacterial membrane. They concluded that such changes in morphology would significantly increase the permeability of the membrane resulting in death of the cell. Jiang et al. (2009) suggested that the toxicity to *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* was affected by the attachment of NPs to the surface of bacteria upon exposure to Al_2O_3, SiO_2 and ZnO NPs. Thill et al. (2006) found that CeO_2 NPs come into contact with *Escherichia coli* cells to cause toxicity. They observed a strong absorption of NPs to the surface of bacterial cells and reduction of NPs which was correlated with the cytotoxicity. Su et al. (2009) found that the nanohybrids made up of Ag and silica were adhered to the surface of the bacteria cells. Subsequent toxicity studies revealed that the toxicity was related to the cell death caused by loss of membrane integrity due to the formation of ROS. Lok et al. (2006) observed that AgNPs destabilized the outer membrane, collapsed the plasma membrane potential and depleted the levels of intracellular ATP when *Escherichia coli* was exposed to AgNPs and Ag^+. Li et al. (2010b) observed that NPs destroyed the permeability

of the membrane of *Escherichia coli* and some membrane enzymes were depressed upon exposure to AgNPs. Brayner et al. (2006) observed the membrane disorganization of *Escherichia coli* cells as a result of exposure to ZnO NPs which led to NP accumulation in the bacterial membrane and internalization as verified by TEM images. Feng et al. (2015) studied the toxicity of cationic and anionic Au NPs to the Gram-negative bacteria *Shewanella oneidensis* and the Gram-positive bacteria *Bacillus subtilis*. Au NPs coated with cationic polyelectrolyte PAH were associated most with the bacterial surfaces and caused greatest membrane damage causing highest toxicity. Hossain and Mukherjee (2012) observed morphological changes of *Escherichia coli* cells to the filamentous form followed by filamentation-associated clumping with increased CdO NP concentrations. Also, the cell surface was severely damaged, and cell division proteins were affected upon exposure to NPs. The researchers attributed intracellular oxidative stress as a cause of these changes. Kumar et al. (2011) assessed the toxicity of TiO_2 and ZnO NPs to *Escherichia coli* and observed that the exposure caused oxidative stress leading to genotoxicity and cytotoxicity. Both NPs caused induction of reactive oxygen species (ROS) and DNA damage. Genotoxic effects were also reported by Lopes et al. (2012) when bacterium *Salmonella typhimurium* was exposed to sodium dodecyl sulphate and didodecyl dimethylammonium bromide NPs. The mechanisms of toxicity of NPs to bacteria are complex although membrane damage by the production of ROS and physical damage by NPs have attracted most attention (Hwang et al. 2008). Membrane damage causes severe effects including the inability to properly regulate transportation through the plasma membrane. Attachment of NPs onto the surface of the bacteria is emphasized, while accumulation of NPs in the membrane has also been observed. In addition, some researchers have reported additional adverse effects such as DNA and protein damage and enzyme inactivation (Kumar et al. 2011).

6.2 The Toxicity of NPs to Freshwater Algae

Freshwater microalgae are primary producers in the environment and hence carry out a pivotal role in the food chain. Therefore, any abnormal structural or population changes of the organism will affect higher organisms which directly or indirectly feed on them (Nyholm and Peterson 1997). This highlights the importance of assessing any causes for such changes and the effects due to such causes. Their main habitats, freshwater bodies, are always under threat of chemicals released by households and industries. Also, toxicity tests with algae are recommended internationally by organizations as a source of basic information to understand environmental hazards (OECD 2011; ASTM 2012).

6.2.1 Acute and Chronic Toxicity

Most acute toxicity tests have been performed over 72 h (>40%), a time recommended for algae by the OECD; 18 and 15% of studies report 96 and 48 h toxicity tests, respectively. Several methodologies have been used to measure the acute toxicity of NPs to algae, although growth inhibition has been predominantly used. Growth inhibition can be evaluated using several techniques and methodologies including cell counting, ATP measurement, optical density measurement and chlorophyll content. Other endpoints assessed include membrane damage, oxidative stress, uptake, accumulation, cell morphology, mitochondrial dysfunction, cellular growth and metabolic profiling (Table 2). *Raphidocelis subcapitata* (31%), *Chlamydomonas reinhardtii* (24%), *Chlorella pyrenoidosa* (12%), *Chlorella vulgaris* (8%) and *Euglena gracilis* (8%) have been the most preferred species in NP studies (Fig. 2b). Most studies on algae have assessed the effects of Ag NPs (24%) followed by TiO_2 (23%), ZnO (11%) and CeO_2 (8%) (Fig. 2e).

6.2.2 Toxicity from NPs or Ions

Lee and An (2013) exposed the alga *Raphidocelis subcapitata* to ZnO NPs and concluded that the observed toxicity was almost entirely caused by the dissolved free Zn^{2+} ions. Li et al. (2015b) exposed the alga *Euglena gracilis* to AgNPs in the presence and absence of cysteine which is a strong silver ligand. The effects of NPs on photosynthesis decreased in the presence of cysteine suggesting that the effects of AgNPs were mediated by the dissolved Ag^{+}. Müller et al. (2016) exposed the alga *Chlamydomonas reinhardtii* to Cu NPs and corresponding dissolved fraction of Cu^{2+} ions and observed that the toxicity was similar. Also, when the same experiments were performed in the presence of EDTA which is a strong metal ion chelator, the toxicity of both NPs and Cu^{2+} decreased. These results indicated that the toxicity of Cu NPs arises mostly from the dissolved fraction of Cu^{2+} ions. Despite Zn^{2+} being toxic, Iswarya et al. (2017) saw a reduction in toxicity of Au NPs to the alga *Scenedesmus obliquus* with the addition of Zn^{2+} ions to the medium. Navarro et al. (2008) examined the short-term toxicity of Ag^{+} and Ag NPs to photosynthesis in *Chlamydomonas reinhardtii*. They found that the toxicity of Ag^{+} in terms of EC_{50} was about 18 times higher compared to Ag NPs. However, the observed toxicity by Ag NPs could not be fully explained relative to the Ag^{+} measured in the Ag NP suspension, and the toxicity of Ag NPs appeared to be much higher when compared as a function of Ag^{+} concentration. When the alga *Raphidocelis subcapitata* was exposed to Ag NPs, the toxicity from 2 to 48 h did not increase at the corresponding ionic release rate. Also, the addition of cysteine in equimolar concentrations to silver did not eliminate toxicity. Therefore, Sørensen and Baun (2015) suggested that the dissolution cannot be the only process which contributes to the algal toxicity.

6.2.3 Mechanisms of Toxicity

Angel et al. (2015) found that the presence of dissolved organic carbon (DOC) reduced the toxicity of NPs to *Raphidocelis subcapitata*. The presence of DOC substantially reduced the sorption of NPs to the algal cells, and therefore, they concluded that sorption was the cause of the toxic mechanism. However, though they stopped ROS generation by using UV filters, the toxicity observed was still similar to the levels when ROS was present. They concluded, in contrast to many other findings, that the toxicity was not caused by localized exposure to ROS. Rogers et al. (2010) assessed the effects of CeO_2 NPs and CeO_2 macro-particles (<5 μm) to *Raphidocelis subcapitata*. They concluded that the effects were due to membrane damage of cells by lipid peroxidation caused by the production of hydroxyl radicals. Sørensen et al. (2016a) observed growth inhibition in *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii* following exposure to Pt NPs and attributed toxicity to oxidative stress caused by ROS production. Higher body burden of NPs was found in *Raphidocelis subcapitata*, possibly due to favoured binding of NPs to the polysaccharide-rich cell wall. Interestingly, the accumulation of intracellular ROS levels was comparatively less in *Raphidocelis subcapitata* though it was the most sensitive species. Membrane damage was not observed in both algae species. Bhuvaneshwari et al. (2015) noted significant toxicity correlated with intracellular ROS generation in the alga *Scenedesmus obliquus* when exposed to ZnO NPs. Substantial membrane damage and a significantly enhanced lactate dehydrogenase (LDH) enzyme release into the medium were also observed. Nogueira et al. (2015) exposed the alga *Raphidocelis subcapitata* to grapheme oxide NPs and observed increased ROS production and membrane damage in algal cells which was suggested as the cause of observed growth inhibition. Oukarroum et al. (2017) suggested that several cellular alterations, such as the inhibition in cellular division processes, the deterioration of photosynthetic apparatus and the generation of ROS, caused the cell viability in alga *Chlorella vulgaris* to decrease when exposed to NiO NPs. Qian et al. (2016) saw increased ROS production and lipid peroxidation in the cyanobacterium *Microcystis aeruginosa* when exposed to AgNPs. They also showed that ROS inhibited SOD and POD transcription and expression. In contrast, ROS production was mediated by the induction of SOD and POD activity and the expression of the antioxidant enzyme glutamine synthetase in *Chlorella vulgaris* at same exposure scenario. Dauda et al. (2017) reported a significant increase in GST and peroxidase (POD) enzymes in *Chlorella vulgaris* upon exposure to TiO_2 NPs.

Miao et al. (2010) studied the behaviour and toxicity of Ag NPs to the freshwater alga *Ochromonas danica* to determine whether there were any other mechanisms in algal toxicity other than due to the Ag^+ liberated outside the cells. They demonstrated that the Ag NPs were taken inside the cells where they exerted their toxic effects. However, they did not discuss how the NPs exerted toxic effects inside the cells. Dorobantu et al. (2015) observed that Ag NPs caused membrane damage in the alga *Euglena gracilis*, but not in *Chlorella protothecoides*. In addition Ag NPs caused morphological changes in *Euglena gracilis* altering the shapes from spindle

to round with the cells showing increased diameter. Ag^+ ions from $AgNO_3$ caused membrane damage in both algae causing intracellular material leaking out of the cells resulting in a depressed volume of cells. Hartmann et al. (2010) evaluated the toxicity of TiO_2 NPs to the alga *Pseudokirchneriella subcapitata* and suggested that the observed decreased growth rate could be caused by the adhesion of NPs onto the algal cell surfaces. Ozkaleli and Erdem (2018) observed lipid peroxidation of the alga *Raphidocelis subcapitata* cell membrane upon exposure to TiO_2 NPs, resulting in the deformation of the membrane structure. Li et al. (2015b) reported a doubling of cell volume when the alga *Euglena gracilis* was exposed to AgNPs. They suggested that the enlargement was a result of unspecific interactions of Ag^+ ions released from AgNPs with the thiol groups of glycoproteins in the pellicle. However, they did not observe any internalization of NPs into the algal cells. Ji et al. (2011) excluded the effects of ions or shading for the observed toxicity of ZnO NPs to the alga *Chlorella* sp. but concluded that the toxicity was caused by entrapping and wrapping by the NPs.

Zhou et al. (2016) observed increased toxicity and cell internalization of Ag NPs in the absence of EPS compared to the presence of EPS. EPS could bind both NPs and Ag^+ reducing the internalization and toxicity. Stevenson et al. (2013) investigated the toxicity of Ag NPs to the populations of the alga *Chlamydomonas reinhardtii* at different phases of batch culture and found that the toxicity was highest for the cultures at early phases in growth. Dynamic process modelling, incorporating algal growth rate, dissolution, bioaccumulation and extracellular DOC production, revealed that the DOC was a strong factor mitigating the toxicity of NPs. Kadukova (2016) exposed the alga *Parachlorella kessleri* to $AgNO_3$ and noted that the Ag^+ was removed from the medium by biosorption by algae. Interestingly, the majority of Ag was released back into the medium in the next 14 days, while the algal cells had formed Ag NPs inside, within that period. Those NPs were comparatively less toxic against algal cells than Ag^+ ions at the same Ag concentrations. The surface charge of the algae cells and the NPs is a major determinant in causing toxicity. There is a high tendency for negatively charged NPs to bind on the positively charged algal surfaces. Also, once bound to the cells, the charge density of the NPs decreases which favours the adsorption of more NPs resulting in large clusters. It is widely accepted that the sorption of NPs on the algal surfaces facilitates the localized exposure to ROS resulting in oxidative damage to the cell membranes. However, sorption of NPs might cause toxicity even without the production of ROS. Certain other effects were also reported including adverse effects to morphology, cell division, gene expression and even physical effects. Also, algae have their own mechanisms to mitigate the NP toxicity into certain extent.

6.3 *The Toxicity of NPs to* Daphnia

Invertebrates are the most widely distributed living macroorganisms on earth. Their presence in almost all ecological niches, fast and high rate of reproduction, short life

span and relatively high sensitivity to pollutants make them excellent candidates for ecotoxicological studies. Among invertebrates, *Daphnia* sp. is the first choice for standard toxicity tests among control agencies (Jonczyk and Gilron 2005). Except in extreme environments, this organism is present in all aquatic habitats and possesses all the above-mentioned positive characteristics for standard tests (Cattaneo et al. 2009). Daphnids exert strong grazing effects and support the aquatic food web. They feed on several sources like bacteria, algae, other invertebrates and plants and enter the trophic chain at intermediary level by being a preferential prey for larger organisms like fish, birds and humans. This also makes them a possible important linkage for passing contaminants through the food chain which should be studied for any such contaminants which are suspected of being capable of bioconcentration and bioaccumulation (Zhu et al. 2010b).

6.3.1 Acute and Chronic Toxicity

The *Daphnia* spp. 48 h acute test is one of the most widely used aquatic standardized tests, and this is reflected in NP toxicity studies. However, there are some suggestions to improve the sensitivity by prolonged exposure up to 72 h or the 48 h test duration followed by a 24 h recovery period (Novak et al. 2018). LC_{50} and EC_{50} are the most common endpoints used, while other effects such as uptake, accumulation, feeding rate, reproduction, enzyme activity, oxidative stress and morphological changes are reported (Table 2). More than 87% of studies have used *Daphnia magna* as the test species (Fig. 2c) possibly as a result of its inclusion in regulatory chemical testing, guidelines and international standards (Baun et al. 2008). The majority of studies have tested against Ag NPs (27%) followed by TiO_2 (23%), Au NPs (11%) ZnO NPs (10%) and CuO NPs (10%) (Fig. 2f).

6.3.2 Toxicity from NPs or Ions

There are different views on whether NPs or liberated ions from NPs cause the toxicity to *Daphnia* sp. Some evidence suggests that the ions are the cause and the NPs merely represent a source of ions, while several other studies suggest cumulative effects or more adverse effects from NPs. Li et al. (2015a) observed significant changes in the metabolomic profile of *Daphnia magna* after exposure to Ag NPs and Ag^+ for 48 h. The changes in metabolites of daphnids exposed to Ag NPs were identical to those exposed to Ag^+, and therefore, they concluded that Ag^+ is the dominant cause of toxicity. Sakamoto et al. (2015) observed higher toxicity for *Daphnia magna*, *Daphnia galeata* and *Bosmina longirostris* after exposure to Ag NPs compared to Ag^+. However, the 48 h EC_{50} values of Ag NPs based on Ag^+ concentrations were comparable with those of Ag^+, and therefore, they concluded that the effects of NPs were due to liberated Ag^+ from AgNPs. Zhao and Wang (2011) observed no toxicity from AgNPs to *Daphnia magna* when the

liberated Ag^+ ions were complexed by cysteine, suggesting that the toxicity was primarily caused by Ag^+. Shen et al. (2015) exposed *Daphnia magna* to seven types of Ag NPs with different sizes and coatings in $NaNO_3$ medium for 8 h to identify Ag species responsible for acute toxicity. The LC_{50} values of the seven Ag NPs as free Ag^+ agreed well with that of $AgNO_3$, and therefore, they concluded that the Ag^+ is exclusively responsible for acute toxicity. Bacchetta et al. (2016) noted the toxicity of ZnO NPs to *Daphnia magna* was similar to the toxicity from Zn^{2+} and therefore concluded that the toxicity was caused by released ions from NPs. Adam et al. (2014) found chronic effects of ZnO NPs (EC_{50}, 0.112 mg/L) and ionic Zn (EC_{50}: 0.082 mg/L) in *Daphnia magna* following exposure for 21 days. They studied the influence of free, dissolved and aggregated Zn fractions in the medium and concluded that the dissolved fraction was largely responsible for the chronic toxicity. Adam et al. (2015b) concluded that the ions from the dissolution of Cu NPs caused toxicity to *Daphnia magna* by exposing them to CuO and ZnO NPs and Cu and Zn salts for 21 days.

In contrast, there are reports by some researchers regarding the toxicity of NPs which cannot be explained by ionic effects (Navarro et al. 2008; Fabrega et al. 2009; Yin et al. 2011). Allen et al. (2010) observed that coffee-coated AgNPs were more toxic to *Daphnia magna* than Ag^+. Pakrashi et al. (2017) observed that AgNPs significantly affected the reproduction process of the first two broods in comparison with $AgNO_3$ which affected only the first brood. Based on this, they suggested that AgNPs may have longer adverse effects than Ag^+ ions. Bhuvaneshwari et al. (2016) claimed that the relative contribution of dissolved ions from NPs towards acute toxicity to *Ceriodaphnia dubia* was less than that of ZnO NPs. When *Daphnia magna* was exposed to CuO NPs and $CuSO_4$, Kim et al. (2017) observed that the dissolved Cu^{2+} ion concentration from CuO NPs after 72 h was much less than the 72 h median effective concentration of $CuSO_4$. These authors therefore suggested that the observed median toxicity of CuO NPs at 72 h was caused by the particles rather than by the dissolved ions. Xiao et al. (2015) reported that the relative percentage contributions of dissolved ions from CuO and ZnO NPs were 26% and 31%, respectively, when *Daphnia magna* was exposed to NPs. Therefore, they concluded that the particles rather than the dissolved ions were the main source of toxicity.

6.3.3 Ingestion into *Daphnia*

Ingestion via active and passive diffusion is the most common way of NP uptake by daphnids. Many NPs are lipophilic, and the ingested NPs are highly likely to be found in storage cells which contain lipids such as triacylglycerol and glycogen (Goulden and Hornig 1980; Moore 2006). The size of the particles daphnids can uptake depends on their body size. *Daphnia magna* can ingest particles up to about 70 μm, and the minimum size depends on the distances between setulae on thoracic limbs, which do not depend on the age or size since the gap is constant throughout

(Burns 1968; Geller and Müller 1981). Zhao and Wang (2011) observed a linear and positive correlation between Ag concentration in the daphnids and the concentration in the medium after exposing *Daphnia magna* to Ag NPs. Also, at same Ag exposure concentration levels, the Ag body burden from Ag NPs was two to three orders of magnitude higher than that from $AgNO_3$, showing the potential of daphnids to accumulate Ag NPs due to ingestion of NPs into their gut environment. Zhao and Wang (2012b) demonstrated that the Ag NP influx rate of *Daphnia magna* decreased with increased NP size. Also, they found 60% of Ag distributed in the gut of daphnids and concluded that ingestion was the dominant uptake pathway. Similarly, Skjolding et al. (2014a) observed a higher uptake of smaller mercaptoundecanoic acid-coated Au NPs than bigger particles. However, no such correlation was observed for citrate-coated Au NPs. In contrast, Rosenkranz et al. (2009) reported a lower uptake of smaller carboxylated polystyrene NPs (20 nm) in terms of mass compared to larger particles (1,000 nm). Tan et al. (2016b) observed that the uptake of polyacrylate-coated TiO_2 NPs by *Daphnia magna* depended on the calcium concentration in the medium. At low Ca concentrations, NPs were ingested via endocytosis and passive drinking and distributed throughout the body, with the highest NP concentration at the abdominal zone and gut. In contrast, NPs were actively ingested and concentrated only in the gut at high Ca concentration levels in the medium. Conine and Frost (2017) found that the presence of food reduced the toxicity of AgNPs in terms of the growth and survival of *Daphnia magna*. They also found that toxicity was greater for animals fed with P-rich algae compared to P-poor algae. The algal-bound AgNPs were not toxic at any tested concentrations, and they suggested that the reduced toxicity in daphnids fed with P-rich algae was due to higher removal efficiency of Ag NPs by P-rich algae from the medium leaving less for uptake by daphnids. They also suggested that the algae may convert NPs to non-toxic form to daphnids, while the nutrition and overall health of daphnids also play a role in responding to NPs. Skjolding et al. (2014b) studied the influence of surface functionalization of ZnO NPs and observed fast uptake of ZnO NPs and ZnO-octyl NPs compared to ZnO-OH NPs. Daphnids ingest NPs via active and passive diffusion, while the body size of the daphnids and the concentration of NPs in the medium positively correlate with ingestion. The body burden of NPs may be higher than their bulk counterparts due to the higher NP accumulation in the guts. The size of the NPs influences the ingestion though there are conflicting views on the correlation of size and ingestion rate. Also, several other factors such as media composition, the presence of food and the surface functionalization of NPs influence the ingestion.

6.3.4 Mechanisms of Toxicity

The widely accepted key toxic mechanism for acute toxicity from metals and metal NPs to invertebrates such as daphnids is the inhibition of Na^+/K^+-ATPase activity and the prevention of the absorption of Na^+ ions which could induce ionoregulatory

failure and finally cause the death of the organism (Bianchini and Wood 2003; Kennedy et al. 2012; Rüdel et al. 2015). In addition to this, several other effects are reported at acute and chronic level. Bacchetta et al. (2016) exposed *Daphnia magna* to ZnO NPs and noted morphological changes in the digestive epithelium. They attributed these effects to the dissolved Zn^{2+} from NPs. Zn^{2+} ions enter into the gut enterocyte cytoplasm and resulted in altered mitochondria membrane permeability causing ROS production, which stimulates the extensive autophagy process eventually causing cell and animal death. Chae and An (2016) reported structural damage to the digestive organs of *Daphnia magna* along with the production of lipid droplets and concluded that AgNPs adversely affected nutrient uptake leading to immobility and death. Das et al. (2013) suggested that the observed decreased reproduction, growth inhibition and erratic behaviour of *Daphnia magna* from chronic exposure to TiO_2 and Ag NPs could be due to the uptake of NPs in their gut plus decreased enzyme activity. Zhu et al. (2010a) observed growth retardation and reproductive defects in *Daphnia magna* upon exposure to TiO_2 NPs. A significant amount of NPs accumulated in the body interfered with food intake which could conceivably be the cause. Blinova et al. (2017) saw long-term effects on reproductive potential with decreased number of neonates hatched from ephippia when *Daphnia magna* was exposed to Fe_3O_4 NPs. Lv et al. (2017) observed reduced digestive enzyme activities in *Daphnia magna* upon exposure to C_{60} and Si NPs. They also reported a concentration-dependent increase in SOD and LPO levels. However, the SOD activity decreased at a higher dose of C_{60} exposure after 72 h along with increased levels of MDA. They suggested this may be due to the breakdown of the antioxidant system at high concentrations over lengthy exposures. Ulm et al. (2015) found increased GSH, CAT and AChE activity levels in *Daphnia magna* upon exposure to TiO_2 NPs. When Dabrunz et al. (2011) exposed *Daphnia magna* to TiO_2 NPs for 96 h, they observed that the second moulting was disrupted due to the biological surface coating of NPs on the daphnids. Disruption to moulting directly results in reduced reproduction rates. Vijayakumar et al. (2016) noted ingestion of ZnO NPs in *Ceriodaphnia cornuta* and *Moina micrura* which caused blackening of the intestine, rupture of intestinal wall, shrinkage of the abdomen and loss of carapace and antennae leading to structural deformities. Rainville et al. (2014) reported increased protein carbonylation indicating ROS, changed vitellogenin levels and higher haemoglobin levels indicating cellular respiration from Ag NP exposure in *Daphnia magna*. NPs would have adverse effects on ionoregulatory processes, digestive system, growth, reproduction, behaviour, oxidative stress and moulting. *Daphnia* acute and chronic tests are widely used by regulatory regimes. However, the toxicity of NPs might not be reflected within the scope of the tests, and careful consideration of the mechanisms of toxicity is important to analyse effects. It is also reported that daphnids release certain proteins creating eco-corona around NPs (Nasser et al. 2016) resulting in heightened uptake and toxicity which warrants careful investigation of NP risks under environmentally relevant scenarios.

6.4 Bioaccumulation and Trophic Transfer of NPs

NPs in current use are expected to persist in the aquatic environment in different forms. Bioaccumulation of NPs is significant and calls for more research even though the emission of NPs to the aquatic environment is low, because of their limited degradability combined with the probability that they will be fed on by many invertebrates (Baun et al. 2008). To understand the trophic transfer of NPs through the food web, it is important to understand the mode of action at cellular and higher levels within individual organisms (Aschberger et al. 2011). Cells use different routes to internalize NPs, and a particular preferred route is chosen based on NP properties like size, shape and surface characteristics (Yameen et al. 2014). Any foreign materials which the cell finds harmful are transported to the lysosomes where they are digested. Therefore, in medical nanotechnology, many NPs are designed to enter the target cell through the caveolae to avoid degradation (Na et al. 2003; Panyam and Labhasetwar 2003). Once they are released into the environment after use, their non-degradative nature might negatively affect aquatic organisms. Bioaccumulation and biomagnification of NPs through the food webs are yet to be properly understood, with more research required on the influential physicochemical characteristics of NPs and trophic transfers (Zhu et al. 2009). The potential of accumulation and biomagnification of NPs may be higher in comparison with conventional contaminants, but the current testing paradigms do not emphasize the importance of evaluating the ecological impacts in this context (Wu et al. 2017a).

6.4.1 Bioaccumulation of NPs

Cellular uptake and accumulation of NPs may determine the toxicity (Taylor et al. 2016a). However, studies show contradictory results regarding the internalization mechanisms and where NPs accumulate inside the algae cells. Some reports claim that NPs enter into the cells, while others claim that they are just absorbed onto the cellular surface of algal cells or restricted to the outer region including the cell wall or periplasmic space. Taylor et al. (2016a) noted NPs in the periplasmic space of *Chlamydomonas reinhardtii* algal cells when they were exposed to Ag NPs. However, there were no Ag NPs accumulated inside the vesicle or the endosome around the cell, excluding the possibility of endocytosis or passive diffusion which is proposed to be the most feasible route (von Moos et al. 2014; Behra et al. 2013) for cellular internalization. In contrast, they observed Ag_2S particles in the cytoplasm which they suggested were present as a result of sulfidation of Ag^+ ions from Ag NPs. Sulfidation is widely accepted as a mechanism for the complexation and sequestration of heavy metals in plants to mitigate the toxicity (Chen et al. 2013). Lee et al. (2015) found that the bioaccumulation of Au NPs in *Euglena gracilis* was higher than in *Chlamydomonas reinhardtii* and noted that the reason might be the difference in the physical structure of organisms and the surface area available for interaction with NPs. They also observed the transfer of NPs to *Daphnia magna*

after feeding them with Au NP-treated algae. Zhao et al. (2016) observed internalization of CuO NPs into *Chlorella pyrenoidosa* cells by endocytosis followed by storage in the vacuole. Yue et al. (2017) observed cell-associated Ag in the alga *Euglena gracilis* when exposed to Ag NPs. However, Ag NPs did not enter into the algal cells, only absorbed onto the algal surface.

Several studies have looked into the bioaccumulation of NPs in daphnids. Waterborne exposure and diet are the major routes for uptake of NPs in daphnids. NPs may enter the body or be retained by attaching to the body surface including the carapace. The concentration of NPs, media composition and physicochemical characteristics of NPs such as size and charge influence the uptake and retention of NPs in daphnids. Ribeiro et al. (2017) concluded that waterborne exposure to Ag NPs causes more accumulation of Ag than dietary exposure in *Daphnia magna*. However, more Ag from $AgNO_3$ was accumulated through the diet. Similarly, Wu et al. (2017a) observed a higher uptake, retention of NPs and attachment to the carapace surface of *Daphnia magna* upon waterborne dermal exposure to CuO NPs when compared to feeding exposure. Oral exposure was predominant in feeding exposure through NP-treated algae, and the ingested Cu was regulated within the body and transferred to other biological compartments such as neonates and carapaces which may have caused less toxicity. Botha et al. (2016) observed that the uptake of Au NPs into *Daphnia magna* was related to NP concentration in the medium and the charge of NPs. NPs were seen adsorbed to the surface of daphnids and in the gut, but there were no evidence of NP internalization into the body cavity. No effects on reproduction or moulting patterns were observed. Wray and Klaine (2015) observed that the uptake and elimination of Au NPs by *Daphnia magna* were influenced by the size and surface charge of NPs, whereas shape of NPs was non-influential. However, they also found no evidence for NP internalization into the body with NPs restricted to the gut lumen and the carapace. Adam et al. (2014) found increased concentrations of Zn in *Daphnia magna* with increased ZnO NPs and Zn^{2+} concentrations in the media after exposing them for 21 days. In a similar study, Adam et al. (2015a) observed localization of CuO NPs in the gut of *Daphnia magna* when they were exposed to CuO NPs for 10 days. However, CuO were not internalized in the cells and were easily eliminated. Khan et al. (2014) observed accumulation of Au NPs in the gut lumen of *Daphnia magna*, but there was no internalization into the gut epithelial cells. Zhu et al. (2010a) found that significant amount of TiO_2 NPs accumulated in the body and *Daphnia magna* and had difficulty in eliminating these NPs. Tan et al. (2016a) reported that Ca concentration in the medium influenced NP uptake into *Daphnia magna*. They observed TiO_2 NPs distributed throughout the daphnid while NPs were concentrated in the gut at high Ca concentrations. Vijayakumar et al. (2016) observed the bioaccumulation of ZnO NPs in the gut region of *Ceriodaphnia cornuta* and *Moina micrura*. Pakrashi et al. (2017) exposed *Daphnia magna* to AgNPs and saw the NPs accumulated in the gut and non-gut tissues. Interestingly, a higher degree of positive correlation between the concentration of Ag in the non-gut tissue was found. Xiao et al. (2015) reported that the bioaccumulation of NPs or dissolved ions from NPs were concentration dependent. At low concentrations, *Daphnia magna* accumulated more dissolved ions from

Cu and ZnO NPs (0.05 and 0.5 mg/L, respectively), while the particles were accumulated more at high concentrations (0.1 and 1 mg/L). Scanlan et al. (2013) observed similar or higher concentrations of Ag levels in the haemolymph of *Daphnia magna* in comparison with the initial concentration of Ag NWs in the medium indicating effective bioaccumulation during filter feeding. Lovern et al. (2008) used electron microscopy to observe accumulation and to investigate the presence and distribution of Au NPs in gut tissues of *Daphnia magna* exposed for 24 h. They observed movement of NPs to the posterior region of the gut, and there were no large blockages, and minimal deaths were observed. Therefore, they concluded that the particles are cleared with time in waste pellets.

Correlation between accumulation of NPs in daphnids and their eggs is also reported. Sá-Pereira et al. (2018) found NPs in the digestive tract, mainly in the gut and in the eggs of the brood pouch of *Daphnia magna* when exposed to TiO_2 NPs. Also, the penetration of Ti into epithelial region was higher at higher concentration levels. When *Daphnia magna* was exposed to polystyrene NPs, Brun et al. (2017) noted accumulation of NPs in or on the lipophilic cells in the early stages of embryonic development, while the embryo is still surrounded by a chorion. However, they did not observe any NPs accumulated neither in the gut epithelium nor in lipid droplets in the adults. Sakka et al. (2016) observed higher mortality and reproductive effects in *Daphnia magna* correlated with the uptake of Ag NPs.

6.4.2 Trophic Transfer of NPs

Studies show that NPs are taken up and accumulated inside organisms which are transferred to higher trophic levels. Transfer of nanoparticles up through the food chain is a primary concern since it affects the balance of the ecosystem putting ecosystem health at risk (Bhuvaneshwari et al. 2018a; Wu et al. 2017b). Since organisms may feed on NP-contaminated food, it is important to understand the role of the trophic route (Bour et al. 2015). Dietary intake of NPs may cause significant effects on growth, survival and reproduction (Bhuvaneshwari et al. 2018a). Certain metals and NPs are accumulated more through dietary intake than waterborne exposure (Ribeiro et al. 2017), while certain other NPs are accumulated more through the waterborne exposure (Bhuvaneshwari et al. 2018b). The effects from NPs ingested via dietary intake may have different mechanism of toxicity compared to direct exposure (Bour et al. 2015). Werlin et al. (2011) showed that the CdSe quantum dots can be transferred from the bacteria *Pseudomonas aeruginosa* to the protozoa *Tetrahymena thermophile* with the Cd concentration in the protozoa five times higher than that found in the bacteria. Chae and An (2016) observed that the Ag NWs were transferred from the alga *Chlamydomonas reinhardtii* to *Danio rerio* through *Daphnia magna*. Renault et al. (2008) showed that Au NPs were transferred from the freshwater alga *Scenedesmus subspicatus* to *Corbicula fluminea*. Bouldin et al. (2008) observed the transfer of carboxyl quantum dots from *Raphidocelis subcapitata* to *Ceriodaphnia dubia* through dietary intake. Chen et al. (2016) found that the trophic transfer of fullerene NPs from *Scenedesmus obliquus* to

Daphnia magna was dependent on subcellular distribution of NPs in alga cell. They observed that the highest NP transfer occurs via the cell wall followed by cell organelle and cell membrane. McTeer et al. (2014) reported the transfer of Ag to *Daphnia magna* from AgNP- and $AgNO_3$-treated alga, *Chlamydomonas reinhardtii*. Bhuvaneshwari et al. (2018a) observed the transfer of ZnO NPs from the alga *Scenedesmus obliquus* to *Ceriodaphnia dubia* with the BMF found to be nearly one causing ultrastructural damage and degradation of internal organs in *Daphnia*. Larguinho et al. (2014) reported that Au NPs transferred from the alga *Dunaliella salina* to the bivalve *Mytilus galloprovincialis*. However, they did not observe any significant morphological alterations in mussel digestive glands or activation of any antioxidant enzymes tested. Zhu et al. (2010b) observed trophic transfer of TiO_2 NPs from *Daphnia magna* to *Danio rerio* by dietary exposure. Although they observed lower biomagnification from dietary intake than from aqueous exposure, the higher body burden in the dietary exposure group led them to conclude that trophic transfer is a major route of potential NP exposure. Skjolding et al. (2014b) observed trophic transfer of ZnO NPs and ZnO-octyle NPs from *Daphnia magna* to *Danio rerio*. However, daphnids did not uptake ZnO-OH NPs, and therefore, these NPs were not available for trophic transfer. This demonstrates that surface functionalization influences the trophic transfer of NPs. Cano et al. (2018) observed the trophic transfer of MWCNTs from *Daphnia magna* to *Pimephales promelas* which was found to be dependent on the size of the particles. However, Bhuvaneshwari et al. (2017) did not observe any transfer of nZVIs from the treated alga *Scenedesmus* sp. to *Ceriodaphnia dubia* though the algae had taken up NPs. Similarly, Bhuvaneshwari et al. (2018b) did not observe any trophic transfer of TiO_2 NPs from the treated alga *Dunaliella salina* to *Artemia salina*. However, Hu et al. (2017) observed the transfer of Ag from AgNP-treated *Chlorella pyrenoidosa* to *Daphnia magna*. In this case the biomagnification factor (BMF) was 0.5, and therefore, they concluded that there was no biological magnification of NPs from algae to daphnids.

6.5 *Effects of NPs on Behaviour of Aquatic Organisms*

In addition to the direct effects of the contaminants to organisms, behavioural effects are also critically important. Behaviour is a sensitive measure of an organism's response to stress, and noticeable changes can be observed at concentrations of contaminants which are orders of magnitude less than that which cause mortality (Weis and Candelmo 2012). Behavioural ecotoxicity tests are becoming increasingly popular because of their high sensitivity at low concentrations and early response (Yeardley et al. 1996). Though the importance of behavioural tests is appreciated in ecotoxicology tests, far less attention has been received (Melvin and Wilson 2013). Most currently available standard tests mention the obligation to document abnormal behaviour, but this is not quantitatively sufficient for any risk assessments (Postma and Keijzers 2014). Most of the behavioural activities which are used in experiments

are related to feeding (feeding rate, filtration rate, predator response) or movement (swimming, avoidance, burrowing).

There are a few studies on the effects of the NPs on the behaviour of aquatic species with most relating to daphnids. The adhesion of aggregates of NPs to the exoskeleton of *Daphnia* sp. may lead to different probability of survival, loss of mobility and physical damage (Baun et al. 2008). Stanley et al. (2016) exposed *Daphnia magna* to multiwalled carbon nanotubes (MWCNTs) for 48 h and found LC_{50} as 29.3 mg/L and EC_{50} (swimming velocity) as 6.7 mg/L. They concluded that behavioural tests are more sensitive than traditional acute toxicity tests for materials which are toxic physically rather than chemically. Also, they suggested that use of only survival endpoints to set environmental guidelines could underestimate potential hazards and risks of NPs to the environment. Lovern and Klaper (2006) observed *Daphnia magna* showing abnormal behaviour such as sporadic swimming in small circles, persistent ramming to vessel walls and inability to swim down from the surface when exposed to fullerene NPs. Artells et al. (2013) studied the effects of CeO_2 NPs on the survival and swimming behaviour of *Daphnia similis* and *Daphnia pulex*. Swimming velocities decreased in the range of 30–40% in both species when treated with 1 mg/L NPs. At higher concentrations (10 and 100 mg/L), the swimming velocity of *Daphnia similis* was more impacted than *Daphnia pulex*. Noss et al. (2013) studied the swimming behaviour of *Daphnia magna* after treating with TiO_2 NPs. They observed a treatment-dependent swarming in the centre of the test vessels during the initial period. The swimming velocities increased with increased body length but significantly reduced after 96 h of exposure. Vijayakumar et al. (2016) observed abnormal behaviour in *Ceriodaphnia cornuta* and *Moina micrura* upon exposure to ZnO NPs. The restricted and reduced movements were attributed to the adhesion and agglomeration of NPs on the carapace and the filter apparatus. When Strigul et al. (2009) exposed *Daphnia magna* to 2.5 mg L^{-1} B NPs, they were actively swimming compared with the control group. However, when the concentration increased to 8 mg L^{-1}, they were less active, while they were very slow at 25 mg L^{-1}.

O'Keefe et al. (1998) suggested that the predation risk of daphnids depends on their swimming behaviour. Pokhrel and Dubey (2012) investigated the potential impacts of citrate-coated Ag NPs on the behaviour of *Daphnia magna* in the presence of the predatory dragonfly *Anax junius*. In the absence of Ag NPs, daphnids avoided predators with both horizontal and vertical movements which are different to the control. However, they did not show any difference in vertical movement when treated with Ag NPs suggesting that Ag NPs may have potential implications on daphnid populations with increased vulnerability to predation. Lovern et al. (2007a) quantified the behavioural responses of *Daphnia magna* at sublethal concentrations of TiO_2 and fullerene NPs. Both treatments caused significant increase in hopping frequency and appendage movement suggesting increased risk of predation and reproductive decline. Lu et al. (2017) observed a decrease in the ingestion and filtration rate of algae by *Daphnia magna* upon exposure to increased concentrations of TiO_2 NPs, and the researchers attributed this to the observed chronic toxicity. Similarly, Lv et al. (2017) saw a reduction in ingestion and filtration rate of *Daphnia*

magna upon exposure to C_{60} and Si NPs. Heinlaan et al. (2017) suggested that the reduced algal feeding rate of Co_3O_4 and Mn_2O_3 NPs-exposed *Daphnia magna* was not particle specific since similar results were obtained for daphnids exposed to relevant metals. Gaiser et al. (2011) observed reduced feeding in *Daphnia magna* when they were exposed to CeO_2 NPs which was ascribed to potential replacement or coating of algae by NPs and filling the intestine with particles. Zhu et al. (2010a) observed drastic reductions in food intake when *Daphnia magna* was exposed to TiO_2 NPs. The chronic toxicity of NPs was ascribed to poor food intake and malnutrition. McTeer et al. (2014) observed a significant reduction of feeding when *Daphnia magna* were fed with Ag NP- and $AgNO_3$-contaminated algae compared to the control. They concluded that this reduction was due to the presence of Ag in algae.

In general, behavioural tests are fast and more sensitive than conventional acute and chronic ecotoxicity tests. These characteristics are particularly useful in assessing NP toxicity. NPs tend to transform and aggregate in the medium exerting huge challenges in assessing toxicity by conventional tests. Also, due to numerous types of NPs entering into the market, it is a huge challenge to assess the toxicity due to time consuming nature and the cost of conventional tests. These issues can be overcome by choosing comparatively faster and cheaper behavioural tests. There is a growing interest to develop lab-on-a-chip behavioural tests (Wang et al. 2017; Cartlidge et al. 2017) which are fast and sensitive with added advantages.

7 Conclusions and Recommendations

1. Nanotechnology is a booming industry and more applications are continuously being found. Therefore, release of NPs to the aquatic environment during their manufacture or use is unavoidable. The exact concentrations of NPs in waterbodies are yet to be assessed, and only limited predicted data are available with huge assumptions since there is also a lack of published data on NP-containing products. There has been increasing interest in research on the fate and effects of NPs in the environment, but the scientific community has not been able to come to a general consensus to accurately design regulatory requirements or guidelines.
2. Efforts have been taken to assess the flow of NPs into the environment and the exposure levels. Recent developments in material flow modelling are noteworthy. Also, recent efforts to accurately measure the environmental concentrations of NPs by analytical methods are a positive sign which also support in verifying the values predicted by models. However, factors such as the complexity of real sample matrices, transformation and aggregation of NPs once released into the environment and limitations in the analytical methods are causing a huge challenge in accurately measuring the environmental concentrations, while there is considerable uncertainty in models resulting in lack of reliable data. Therefore, estimates of more refined levels are needed, and further

research is needed for determination of actual environmental concentrations of NPs for reliable risk assessment and for regulating NP industry.

3. NPs possess special physicochemical characteristics due to their smaller size which may have different effects on organisms compared to their bulk counterparts. However, the presence of NPs in the environment is still not well documented due to a lack of sample-related certified standards, analytical procedures and reliable units of measurement. Also, toxicity tests and risk assessment methodologies specific to NPs are still at the research and development stage. Further, the available technologies are not sufficient to remediate them to environmental permissible levels.
4. The effect of the particle properties of NPs on toxic responses has been heavily studied in the last decade, and it has been found that certain physicochemical properties such as size, shape and surface functionality of NPs influence toxicity. However, conflicting and inconsistent results demand further research to make sound conclusions to protect the organisms from adverse effects of NPs. It is recommended to focus on the NP properties that are already known to influence toxicity. In the meantime, it is required to put more attention into systematic approaches to design NP structures with minimal adverse effects to the environment.
5. The surrounding environment largely influences the transformation of NPs once they are released into the aquatic environment. The presence of NOM, media constituents and kinetics of transformations make it significantly difficult to predict transformations in complex natural environments. Further research is required to develop methodologies and generate data on the fate and transport of NPs in the environment and how these affect organisms. Site-specific studies are recommended since mechanisms of transformation depend on the characteristics of any particular environment. Also, knowledge gaps exist, and further studies are required regarding effects of ageing of NPs since kinetics of particle degradation and kinetics of biological impact are extremely important to tease out mechanisms of interaction and mode of action. NPs interact with other chemicals in the environment. These interactions influence their very own toxicity and also the effects of those chemicals to aquatic organisms. Therefore, it is required to take multiple chemical interactions into account in environmental risk assessment of NPs.
6. Opinions still differ in what causes the effects of metallic NPs on living organisms. Contradictory views on whether NPs, ions released from NPs or a combination of both cause toxicity is still an issue to understand the nature of the metallic NPs' toxicity as well as their toxicity mechanisms. In general, the effects seem to depend on several factors such as the type of NPs, the surrounding environment and the organism. Therefore, more focused research is required to address this to better understand the toxic potential of the metallic NPs and make accurate risk assessments on them.
7. The toxicity data generated even with standard toxicity tests are not consistent. Compared with the traditional contaminants, there are several variables in assessing the effects of NPs to organisms. The influence of particle properties

on the toxicity of NPs is a major challenge to compare and make conclusive decisions based on obtained acute and chronic toxicity values. Therefore, it is necessary to develop robust procedures to generate data with a high degree of credibility. With such variable results, it is difficult to extrapolate the sensitivity of different species, and therefore, it is recommended that toxicity tests be conducted with a broad range of taxa to protect different organisms in aquatic environments.

8. Mechanisms of NP toxicity are also still not well understood. The underlying mechanisms for the toxic interactions of NPs are complex, possibly involving various processes mediated through reactive oxygen species (ROS) generation and oxidative stress. These mechanisms are currently regarded as the best-developed paradigm for NP toxicity. Other toxicity mechanisms include membrane damage, protein denaturation, DNA damage, behavioural effects, physical damage, etc. Improved understanding on the mechanisms of NP toxicity is crucial in risk assessment of NPs since conventional toxicity tests may not reflect the risks associated with NPs.
9. NPs can be ingested and accumulated inside the organism or absorbed onto the surface which may lead to trophic transfer of NPs through the food web. Studies on the bioaccumulation and trophic transfer of NPs are very limited, and therefore, more research is recommended to understand the effects on organisms at higher levels.
10. Due to the low NP concentrations in field conditions, the toxicity or any other physiological effects in organisms are unlikely to be prominent enough for detection. Behavioural effects may be more sensitive and would be efficient in certain situations to evaluate effects. Also, behavioural toxicity tests are fast and cheaper which could be helpful in assessing the toxicity of ever-increasing varieties of NPs. Further, behavioural tests may be more relevant in addressing challenges posed by NPs such as transformation and aggregation. However, attention to such tests is still lacking, and further research is recommended.

8 Summary

Both the use and the number of applications of nanoparticles have expanded rapidly in recent years. This has led to increasing concern regarding the impact of nanoparticles on ecosystem health. Toxicological research in this area is therefore of utmost importance in order to determine the risks of nanoparticles to organisms in the environment. The goal of this review is to analyse recent literature in this interdisciplinary research field, with special focus on the freshwater environment. The paper begins with summarizing knowledge of current production and use of nanoparticle production and exposure concerns in the environment. The major physicochemical characteristics of NPs are examined and their subsequent fate, behaviour and toxicity to aquatic organisms. We review literature regarding the toxicity of nanoparticles to freshwater organisms at different trophic levels involving

studies on bacteria, algae and *Daphnia*. Finally this review examines the less understood behavioural effects of nanoparticles on freshwater organisms. This aspect necessarily focuses on inorganic nanoparticles due to their industrial use and production although the effects of organic nanoparticle should not be overlooked. It is a huge challenge to accurately predict the environmental concentrations of nanoparticles, their fate and behaviour in the environment and to assess the risks posed to aquatic organisms. However, the work carried out by the nanotoxicology community over recent years is commendable. Through analysis, this review contributes to improved understanding on the effects of NPs while also identifying current research gaps and suggesting future research areas in nanotoxicology.

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