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Metallic, metal oxide, and metalloid nanoparticles toxic effects on freshwater microcrustaceans: an update and basis for the use of new test species

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Abstract

In this article, we performed a literature review on the metallic, metal oxide, and metalloid nanoparticles (NP) effects on freshwater microcrustaceans, specifically focusing on (i) the main factors influencing the NP toxicity and (ii) their main ecotoxicological effects. Also, given that most studies are currently developed on the standard test species *Daphnia magna* Straus, we analyzed (iii) the potential differences in the biological responses between *D. magna* and other freshwater microcrustacean, and (iv) the ecological implications of considering only *D. magna* as surrogate of other microcrustaceans. We found that NP effects on microcrustaceans depended on their intrinsic properties as well as the exposure conditions. Among the general responses to different NP we identified body burial, feeding inhibition, biochemical effects, metabolic changes, and reproductive and behavioral alterations. The differences in the biological responses between *D. magna* and other

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freshwater microcrustacean rely on the morphology (size and shape), ecological traits (feeding mechanisms, life cycles) and intrinsic sensitivities. Thus, we strongly recommend the use of microcrustaceans species with different morphological, physiological, and ecological characteristics in future ecotoxicity tests with NP to provide relevant information with regulation purposes regarding the discharge of NP into aquatic environments.

Keywords: bibliometric analysis; Daphnia; functional groups; nanoparticles; toxicity

Introduction

Nanomaterials (NM) are characterized for having a size range between 1 and 100 nm, at least in one dimension (Batley et al. 2013; USEPA 2017). Among the most common NM, metallic, metal oxide, and metalloid (e.g. silica, calcium) nanoparticles (NP) are the most applied in nanoproducts worldwide (Handy et al. 2008; Soares and Soares 2021). NP are of great interest due to their unique properties including a large surface area in relation to volume, as well as their great reactivity and mobility (Jiang et al. 2009). For this reason, they have been applied in numerous fields such as medicine, electronics, cosmetics, food additives, water treatment, and soil decontamination (Singh 2015). The increasing use of NP inevitably leads to their discharge or release into the environment, particularly in continental aquatic systems via industrial and domestic effluents (Liu et al. 2013). This problem results in a need to deepen researches related to the area of nano-ecotoxicology in order to provide recommendations for environmental regulation and control that allow the protection of both aquatic biota and the ecosystem integrity.

In the European Union, data on ecotoxicological effects of NP have been improved under the application of REACH framework Directive (https://echa.europa.eu). However, up to date, most studies in the nano-ecotoxicology field have been developed specifically on standard species used for regulatory toxicology, being frequently focused on vertebrate aquatic organisms (Nelson et al. 2010; Fent et al. 2010; Priya et al. 2015; Lajmanovich et al. 2018; Cazenave et al. 2019). Among aquatic microorganism, most research has been mainly developed for some groups such as bacteria or algae

(Wang et al. 2019; Chen et al. 2019). However, less attention has been paid to the zooplankton organisms although they are highly important in the aquatic systems. In this sense, the zooplankton, particularly microcrustaceans (cladocerans and copepods), are an essential part of the trophic webs, as they are the link between primary producers and consumers, and gears in the processing of organic matter and the nutrients cycling (Mano and Tanaka 2016). Furthermore, due to their biological characteristics, they are excellent early indicators of environmental changes (Ferdous and Muktadir 2009). Cladocerans and copepods have short generation times, inhabit all aquatic systems, and have high sensitivity and frequency in aquatic systems that make them useful for monitoring purpose regarding the changes in water quality and environmental stress (Ferdous and Muktadir 2009).

Within freshwater microcrustaceans, *Daphnia magna* and some phylogenetically related species (e.g. *D. pulex*, *D.galeata*) seem to be the only ones used for the NP toxicological tests (Baun et al. 2008; Kahru et al. 2008; Izoton et al. 2019). These studies are insufficient if they are considered as surrogates of other microcrustacean species because their variations in morphological and physiological traits probably impose changes in the biological responses (Koivisto 1995). Apparently, there is a gap in the current knowledge of the effects of metallic, metal oxide, and metalloid NP on freshwater microcrustaceans other than *D. magna*, which must be addressed to reach a more representative and realistic scenario of the potential risk of zooplankton in freshwater systems (Baun et al. 2008).

In this article we performed a bibliometric analysis of the NP effects on freshwater microcrustaceans (cladocerans and copepods) to evaluate the paper production in the last 14 years (from 2007 to April 2021). Then, we performed a refined literature review of selected articles to analyse (i) the main factors influencing NP toxicity in microcrustaceans, (ii) their main ecotoxicological effects, (iii) the potential differences in the biological responses between *D. magna* and other freshwater microcrustacean and (iv) the ecological implications of considering only *D. magna* as surrogate of other microcrustaceans in NP toxicity studies. With this review, we expect to provide a holistic overview of the current advances on the effects of NP on freshwater microcrustaceans and encourage further studies including other species than the standard ones (e.g. *D. magna*).

Methodology

The present study was carried out through a bibliographical review of scientific research that were published between 2007 and April 2021 (see Baun et al. 2008 for more details on a previous period). Literature was compiled through online searches in Scopus dataset (https://www.scopus.com), Google Scholar (https://scholar.google.com), PubMed (https://pubmed.ncbi.nlm.nih.gov) and Scielo (https://scielo.org) using keywords and their combinations including "nanoparticles", "Cladocera", "Copepoda", "crustaceans", "microcrustaceans", and "freshwater". No limit was applied to language and subject area, and among the literature review, research and review articles were specifically chosen. A total of 155 articles were selected and included in this review based on author's criteria and the specific information provided on the NP effects on microcrustaceans. Our main criteria were to include (a) those articles that specifically analyzed individual effects in physiological, morphological, or ethological markers after metallic, metal oxide, and metalloid NP exposures; (b) articles regarding organic or plastic NP were excluded from this research because their properties, interaction with the water media, and toxicity mechanisms are different from those of metallic, metal oxide, and metalloid NP; (c) articles regarding the effects of NP on biological interactions or at the community level were also excluded. However, the latter were considered for the general analysis of ecological implications. For the bibliometric analysis, all articles were organized in a unique dataset and classified per year of publication, test species, and NP type.

In addition to the bibliometric analysis, we performed a refined literature review of selected articles to analyse the main factors influencing NP toxicity in microcrustaceans, identify their main ecotoxicological effects and to compare the biological responses of *D. magna* and other freshwater microcrustacean. Finally an additional literature review reporting ecological differences of microcrustacean species was included to identify the ecological implications of considering only *D. magna* as surrogate of other microcrustaceans in NP toxicity studies.

Results and discussion

The number of specific articles per year on the subject was variable. However we evidenced an exponential increase in the cumulative published articles from 2007 to 2021 (April), which demonstrate a rapid growth of this field (**Fig. 1a**). The main NP reported in microcrustaceans studies were silver (Ag), titanium dioxide (TiO_2), copper (Cu), zinc oxide (ZnO), gold (Au), cerium oxide (CeO_2), copper oxide (CuO), iron (Fe), and silica dioxide (SiO_2) (**Fig. 1b**).

Notably, over 80% of studies were focused on the Cladocera genus *Daphnia*, and specifically, *D. magna* species (**Fig. 1c**). From a taxonomical perspective, among cladocerans, all studies were focused on the Anomopoda order, being most of them representative of the filtering Anomopoda functional group (D-type), except for *Bosmina longirostris* (which is a selective filter feeders or B-type) and *Chydorus sphaericus* (which is filtering scrapers or C-type) (**Fig. 1 c**). No studies were performed on the Ctenopoda Order.

There were no studies about the effects of NP on freshwater copepods, with the exception of some cyclopoid copepods (*Mesocyclops thermocyclopoides, M. longisetus, M. aspericornis*, *Eucyclops* sp., **Fig. 1c**), in comparison with those existing in marine or estuarine ones (e.g. Jarvis et al. 2013; Michalec et al. 2017; Wong et al. 2020). This may be due to their greater complexity for its culture in the laboratory, in the taxonomic identification, types of feeding, and fundamentally to the fact that there are not specific protocols for acute or chronic toxicity tests with freshwater copepods (Kulkarni et al. 2013).

Factors influencing NP toxicity in microcrustaceans

From our bibliographic analysis, we could identify that the toxic effects of NP on microcrustaceans strongly depend on intrinsic and extrinsic factors, such as their specific properties (e.g. coating, charge, size and shape), and on the environmental condition (e.g. physicochemical characteristics of the water) in which the studies were carried out, including the interaction with other pollutants. Such factors are individually described below and summarized in **Table 1**.

Size

Size has been considered as one of the most important factors in nanotoxicology since penetration is eased with decreasing size and bioavailability (Kim et al. 2010; Seitz et al. 2015; Hou et al. 2017). In general terms, it was assumed that small size increases the surface area-to-mass ratio, which increases the particle surface energy and thereby the reactivity (Jiang et al. 2009). Accordingly, the toxicity of NP is also inversely related to the aggregation of the particles because they can be more easily retained by external cell membranes. However, there are some controversies for microcrustaceans given that the main route for their exposure to NP is the filtering and digestion system rather than skin penetration (Roberts et al. 2007).

A study carried out with *D. magna* exposed to <50 or <100 nm sized Zn NP (0.1-10 mg L^{-1}) showed greater toxicity for the smaller ones after a 48 h-acute test. They were found inside the cells in a higher number than the bigger ones, as they gained a best access to most cell compartments. Then, protein and lipid metabolism disturbance were observed (Santo et al. 2014). Similar results were found in the same species exposed to 5-100 nm sized TiO_2 NP, being the smaller particles more toxic due to their potentiality to induce more reactive oxygen species (ROS). AgNP toxicity on *D. magna* also varies considerably between different particle sizes (Zhao & Wang 2012; Ivask et al. 2014; Seitz et al. 2015; Hou et al. 2017). It was suggested that smaller AgNP have higher dissolution rates and therefore higher release of silver ions in the gut or in body fluids, affecting different metabolic mechanisms (Hou et al. 2017).

On the other hand, conversely to the above mentioned studies, *D. magna* exposed to 5 mg L^{-1} of TiO_2 NP with a size fraction <200, <400, and <800 nm, showed higher antioxidant enzymes activities and accumulation with increasing size fraction (Kim et al. 2010). Therefore, it can be argued that microcrustaceans are susceptible to various NP sizes, not only smaller ones. However, this subject deserves more studies as the ingested NP can be more rapidly depurated, and not accumulated in the organisms, than those that, given their small size, pass directly through the biological membranes. Overall, as feeding mechanisms vary among microcrustacean species (see also section 2.2), other ones than *D. magna* should be included in further research to better understand the size influence on NP toxicity.

Shape

The shape constitutes another important factor in nano-ecotoxicity evaluation. This subject was reviewed by Kwak and An (2015) who also highlighted that most research have been made on three test crustacean species: the macrocrustacean *Hyalella azteca*, and the microcrustaceans *Daphnia similis*, and *D. magna*. Sohn et al. (2015) compared toxicity between Ag NP and Ag nanowires (NW) on different test organisms. *D. magna* showed to be the most sensitive after acute exposition to Ag NW, showing erratic swimming and large amounts of Ag NW in the gut tract. However, Ag NP were more toxic in freshwater media due to the higher amount of released Ag ions. This agrees with the results obtained by Artal et al. (2013) on *D. similis* and by Cui et al. (2017) on *D. magna* and *D. galeata* exposed for 48 h to multi-dimensional Ag NM. In this last case, the toxic effects of silver nanoparticles (AgNPs), silver nanowires (AgNWs), and silver nanoplates (AgPLs) were evaluated to *D. magna* and *D. galeata*, being the AgPLs the most toxic. In another study, the specific effects of various shaped (sphere, short rod, long rod) Au NP on *D. magna* was evaluated in terms of survival, uptake and production of ROS (Nasser 2018). The authors found an order of toxicity ranking with long rods, short rods and finally spheres due to a larger available surface area to interact with the organism along with a larger surface area to charge ratio. It is evident that the shape is intrinsically related to the size of the particles; however more studies are necessary to further elucidate the risk of multi-dimensional nanomaterials in the freshwater microcrustaceans.

Coating and charge

It has been widely reported that the type of coating employed in the NP synthesis influence their toxic effects on the organisms. This is because the NP stability in the media partially depends on the coating, and also because their composition could have toxic effects by their own. In this sense, Baumann et al. (2014) exposed *D. magna* neonates for 96 h to FeO NP (1-100 mg L^{-1}) with different types of coating. The immobilization rate was more drastic for FeO NP coated with ascorbate and dextran compared to the ones coated with citrate. However, citrate showed to be toxic for *D. magna* itself, leading to the formation of ROS.

Additionally, *D. magna* exposed for 48 h to CeO_2 NP (10 and 100 µg L^{-1}) coated with alginate or chitosan also showed different responses regarding the type of coating: the first one triggered oxidative stress, while the second one induced hyperactivity (Villa et al. 2020). An important factor affecting toxicity was the Z potential of NP. The alginate-coated CeO_2 NP showed a negative value while Z potential for the chitosan-coated ones was positive. Due to the negative charge of cells, the positively charged NP would penetrate faster than the negatively ones, then, cationic NP generally displayed higher toxicity (Abramenko et al. 2019). As the capping agent provides steric or electrostatic stabilization to NP, the chitosan coated CeO_2 NP were more stable as their dispersion was increased, while the alginate coated ones suffered from sedimentation and left small aggregates in the water column. The lack of stability of alginate coated NP could have led to Ce^{3+} release which have a relevant role in generating oxidative stress (Villa et al. 2020). Then, the coating characteristics will also determinate the amount of ions released from NP. In the case of Ag NP, that topic has been widely studied since their toxic effects are mainly due to the Ag ions released from them, and particularly, that ion is highly toxic for human health and environment (Sakamoto et al. 2015; de Souza et al. 2019). However, the Ag ion released into aquatic environments will also depend on other factors. For example, the presence of organic matter like humic acids decreases the amount of released ions from Ag NP (Ale et al., 2021), and high ionic strength increases particle agglomeration (and agglomerates still keep releasing ions) (de Souza et al. 2019).

Environmental conditions

The influence of environmental condition is a crucial point when the effects of different pollutant are evaluated through toxicity tests. In the case of NP, this aspect has been widely analyzed for many organisms, including microcrustaceans and other invertebrates (Stevenson et al. 2017; Renzi and Blašković 2019; Galhano et al. 2020; Ale et al. 2021). Among the different environmental factors, the influence of temperature, pH and organic matter are key factors when determining the NP toxicity. Changes in water temperature can affect the organism's responses to NP toxicity directly through alterations on the chemical conditions of the water media (e.g. decrease oxygen concentration) but also indirectly by modifying the organisms' biological functions such as metabolism, growth rate, development, reproduction, feeding behavior, etc. (Silva et al. 2018). All these mechanisms may modify the absorption, distribution, storage and elimination of NP in the animals. In this sense, Ag NP were shown to interact with temperature in freshwater invertebrate *Limnephilus* sp. performance, causing a stimulation of plant litter consumption, with potential consequences for the carbon fluxes in the water ecosystem (Batista et al. 2020).

The effects of NP on microcrustaceans can also be influenced by the pH condition of the water. The hydrogen ion concentration may exert its effect by affecting uptake sites, or by determining chemical changes in the NP in water. Gilbert et al. (2007) showed a pH-driven aggregation and disaggregation of FeO NP (6 nm sized), with larger aggregate radius at higher pH. These authors also pointed out that internal pH of the animals can also influence the NP toxicity. For example, the pH of the digestive tract in *D. magna* was found to vary from 6.8 to 7.2 and a change in this condition and the ionic strength in the animal stomach may lead to changes in particle aggregation, hereby also affecting particle uptake in the organism.

The presence of organic matter in the water media is another high important factor when evaluating NP toxicity. Specifically, organic matter has showed to mitigate the toxic effects of many NP on microcrustaceans. For example, Li et al. (2016 a) found that 5 mg L^{-1} of natural organic matter from a river reduced the impact of the phototoxicity of TiO_2 NP in *D. magna* and suppressed the generation of ROS. In addition, when *C. dubia* was exposed for 48 h to Cu NP or Ag NP, the mortality was reduced with increased concentrations of dissolved organic carbon (<10 mg L^{-1}) (Gao et al. 2009). Even more, *D. magna* exposed to Cu NP evidenced a great mitigation of toxicity by the addition of dissolved organic matter (Xiao et al. 2018). This result was due to complexation and surface adsorption to block the oxidation sites of NP by dissolved organic matter.

Daphnia magna exposed to Ag NP also increased their survival when humic acids were present in the media. That result was explained by the low bioavailability of NP because of their agglomeration and settling. Moreover, the decreased toxic effects of Ag NP may be due to the complexation of Ag ions with organic compounds serving as a radical scavenger (Gao et al. 2012). On the other hand, natural organic matter can also stabilize NP by the formation of a coating which provides electric and steric stabilization, decreasing agglomeration. Then, the NP behavior (and potential toxic effects on a test organism) will be highly determined by the exposure media (de Souza et al. 2019).

Overall, although NP stabilization and/or agglomeration will lead to a decrease in the dissolution rate, agglomerates still release Ag ions (de Souza et al. 2019). Thus, the ion release is an especially important factor highly recommended to be reported and it will partially depend on media characteristics.

Considering the large influence of the water quality and characteristics, the use of different environmental test conditions is also highly recommended when assessing the toxicological effects of NP on microcrustaceans (Seitz et al. 2013; Wang et al. 2015; Li et al. 2016; Stevenson et al. 2017; Renzi and Blašković 2019).

The presence of other stressors

NP may have unusual physicochemical properties or behavior in water in comparison with other pollutants (e.g. metals and pesticides), and a more complex scenario is evidenced when NP and other kind of stressors interact (Deng et al. 2017; da Silva et al. 2020). *D. magna* exposed to 0.1-0.2 mg ZnO NP L^{-1} and different proportions of *Microcystis* (10-30%) showed mutual attenuation of harmful effects (in terms of development delay, maturation, and number of neonates in the first brood). That attenuation was mainly attributed to the reduction of the toxicity of the cyanobacteria by the presence of NP, because NP can attach them to their surface, form aggregates in the medium that capture and encapsulate the cyanobacteria cells, or even release Zn^{2+} ions (which can interact with *Microcystis* surface or been internalized) (Wang et al. 2019).

On another hand, changes in physicochemical characteristics of the media due to the presence of NP can increase the potential toxicity of other stressors present in the environment. The bioconcentration of As augmented with increasing TiO_2 NP concentrations in water (2-20 mg L^{-1}). This effect was explained by the capacity of TiO_2 NP to increase arsenate absorption (Li et al. 2016 b). In another similar study, when *D. magna* was exposed for 48 h to TiO_2 NP (2 mg L^{-1}, size 100-5000 nm) and Ag ions (0.5-16 µg L^{-1}), the Ag toxicity was increased up to a 40% in terms of LC_{50} values. Again, the elevated toxicity of Ag was explained by the potential ingestion of the TiO_2 NP which acted as Ag ion carriers (Rosenfeldt et al. 2014).

Pacheco et al. (2018) evidenced synergism regarding the toxic effects in *D. magna* after a 21-days exposure to a mix of Au NP and microplastics. A recent study showed that a mixture of Ag NP and the herbicide glyphosate cause multigenerational toxic effects on *D. magna* (da Silva et al. 2020). In chronic expositions, both the parents and the F1 descendants exposed to that mixture suffered from a significant delay in the age at first brood and in the reproduction parameters. The authors suggest that such effect may be due to changes in the physicochemical characteristics of original compounds in the mixture, since glyphosate has the ability to complex metals at pH close to neutral, where the carboxyl and phosphonate are deprotonated. This recent field of pollutant interactions should be further investigated. Especially, employing another test species of microcrustacean than *D. magna* will appropriately assess the ecotoxicological effects of emerging pollution in complex environments.

Main ecotoxicological effects of NP on microcrustaceans

Several studies account for the ecotoxicological effects of NP on microcrustaceans at different scales of biological organization. Backgrounds of the main effects found in the selected literature review are shown in **Table 2**.

Body burial and formation of gas bubbles inside the body

Surface coating in microcrustaceans has been considered an important mode of NP toxic action, adversely affecting their moulting, mobility and survival. This phenomenon was observed after the exposure to different NP such as Ag, TiO_2, Co_3O_4, Mn_2O_3 and ZnO, among others (Dabrunz et al. 2011; Asghari et al 2012; Heinlaan et al. 2017; Renzi and Blašković 2019). It was also suggested that body burial condition increases with increasing NP concentration (Tao et al. 2011), and that surfactants mixtures can potentiate such effects (Renzi and Blašković 2019). However, it is possible that this effect quickly disappears in recovery phases, as it was demonstrated in *D. magna* (neonates ≤ 6 h) exposed to TiO_2 NP (Dabrunz et al. 2011). Also, the presence of dissolved organic materials in the water media can prevent the adhesion of particles onto the exterior surface of daphnids (Seitz et al. 2013) by stabilizing NP due to steric or electrostatic repulsion (Navarro et al. 2008; Hall et al. 2009).

Besides animals' surface coating, the formation of gas bubbles inside their valves is another physical effect of NP exposure in microcrustaceans. Renzi and Blašković (2019) registered this phenomenon at high TiO_2 NP concentrations, which caused drastic death of exposed daphnids after 24 h. This effect was caused as consequence of changes in the environmental conditions that generated gas oversaturation in the water media. Weitkamp and Katz (1980) previously reported that even if gas bubbles appear in certain conditions inside the animals, they contain mainly nitrogen but not oxygen. However, more research is necessary to fully understand such phenomenon in cladocerans.

Feeding inhibition and intestine obstruction

The most typical effects that microcrustaceans, especially daphnids, suffer from NP exposure are feeding inhibition and intestine obstruction. Kwon et al. (2015) analyzed the effects of TiO_2 NP and evidenced damage in the morphology of the microvilli membranes of *D. magna*, and proved that the uptake of such NP depended on their agglomeration. Similar results were found by other authors who considered other kinds of metallic NP such as Au, Ag, and CuO (Lovern et al. 2008; Asghari et al. 2012; Ribeiro et al. 2014; Seitz et al. 2015; Santos-Rasera et al. 2019) and other microcrustacean species like *C. silvestrii* (Mansano et al. 2018) and *Moina macrocopa* (Borase et al. 2018). Once the NP reach the filter membranes, the greatest retention is generally in the intestine, affecting growth and inducing individuals mortality (Zhu et al. 2010; Zhao and Wang 2012; Khan et al. 2014; Kwon et al. 2014).

NP can also penetrate the paratrophic membrane (PTM) of the midgut in daphnids, a structure which regulates the exchange of nutrients and enzymes, and prevents the colonization of microorganisms (Baumann et al. 2014). For example, CuO NP and Au NP were proved to pass the PTM and enter between the microvilli, meanwhile CuO NP also cause a destruction of the membrane (Lovern et al. 2008; Heinlaan et al. 2011).

Currently, numerous studies suggest that the acute tests underestimate the toxicity of NP, not only because of the short exposure period (generally 48 h), but also because of the lack of food provision, which is a *sine qua non* condition of acute toxicity tests (Baumann et al. 2014; Ribeiro et al. 2014). Therefore, we highlight these observations and highly encourage further studies considering long-term exposures and including other microcrustaceans species with different feeding mechanisms, such as selective scrapers or filters.

Biochemical, metabolic, and genetic effects

Once NP enter the cells, they can cause different types of damages, among which oxidative stress is one of the most studied in several species (Li et al. 2015; Dominguez et al. 2015). Short exposures to CuO and ZnO NP between 0.3 and 1.1 mg L^{-1} caused inactivation of glutathione-*S*-transferase (GST) enzyme, increased the production of oxidized glutathione, generated lipid peroxidation, and increased the induction of metallothionein in *D. magna* neonates (Mwaanga et al. 2014). In the same species, TiO_2 NP at concentrations between 1-10 mg L^{-1} caused an increase in the activity of antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and GST (Kim et al. 2010). These effects were highly dependent on the size of the NP analyzed (between ~200 and 800 nm) and the suggested toxicity mechanism was the generation of ROS (Kim et al. *op cit*).

Mansano et al. (2018) also documented ROS generation by rod-shaped CuO NP at concentrations between 0.5 and 10 µg L^{-1} in the Neotropical species *Ceridaphnia silvestrii* after a 48 h-exposure, causing high mortality in the exposed individuals. The energetic costs of antioxidant defenses activation and repair mechanisms -induced by oxidative stress- could probably lead to a reduction of energy reserves. Accordingly, daphnids exposed to CuO NP suffered from a reduction of glycogen, lipid and protein concentrations (Adam et al. 2015). This demonstrates that when the organism is exposed to a stressor, additional energy may be required. As a result, it will need to rely more on its energy reserves, especially when the energy supplied by food intake is not sufficient for compensation. It is also possible that, when exposed to a stressor, energy reserves are even more reduced due to lower food intake because of decreased filtration and ingestion (Ferrando and Andreu 1993).

As regards of genetic damages, information about microcrustaceans such as *D. magna* and the genotoxicity mechanisms are clearly described by Mahaye et al. (2017). All works in this line were performed in *D. magna*, and among genotoxic responses, different gene expressions and DNA strand breaks were specifically documented at different NP exposures such as Ag (Park et al. 2010; Poynton et al. 2012), CeO_2 (Lee et al. 2009), TiO_2 (Lee et al. 2009), and Au (Dominguez et al. 2015). It was also noticed that the genotoxicity of NP was highly dependent on the physicochemical properties (e.g. size, coating, surface chemistry, etc.), the presence of co-pollutants, and the physiological attributes of the organisms cells, such as the thickness of the cellular barrier (Sood et al. 2011).

Although there are few studies in this line, with some contradictory results (Adam et al. 2015), these aforementioned effects (together with feeding inhibition and intestine obstruction) are thought to be responsible for the general mortality observed in microcrustaceans after NP exposures.

Reproductive impairments

Alterations in reproductive parameters due to NP exposure are usually related to a decrease in food consumption and, therefore, to lower energy availability (e.g. lipids) for offspring production (Völker et al. 2013; Saebelfeld et al. 2017; Gebara et al. 2019; Park et al. 2021). Several types of NP can generate reproductive disturbances in different microcrustaceans species. Ag NP altered daphnids (*D. magna*, *D. pulex,* and *D. galeata*) reproduction by inducing low offspring number (Zhao and Wang 2012; Zhao et al. 2012; Völker et al. 2013, Rossetto et al. 2014; Adam et al. 2015; Hartmann et al. 2019). Moreover, a recent study showed that such offspring reduction due to Ag NP could be exacerbated in successive generations, causing embryonic developmental arrests and abnormalities (Park et al 2021). Within the Daphnidae group, *C. silvestrii* also documented a reduction in the number of offspring when exposed to different concentrations (0.5-10 μg L^{-1}) of CuO NP (Mansano et al. 2018), which was coupled with oxidative stress and altered lipid levels. The cladoceran *B. longirostris* decreased the net reproductive rate, the probability of survival at maturation, and the intrinsic population growth rate after the exposure to Ag NP (1-3 μg L^{-1}). The inhibition of algal food ingestion caused a decrease in the energy reserves as well as the high mortality of the individuals, especially during juvenile stages (Sakamoto et al. 2015).

Exposure to Fe_3O_4 NP (0.25-44.23 mg L^{-1}) affected body length and the number of eggs and neonates in *C. silvestrii* (Mansano et al. 2018; Gebara et al. 2019). Concentrations between 0.5 and 8 mg L^{-1} of TiO_2 NP caused reproductive toxicity in *D. magna* by molting disruption. In that study, after reproduction, neonates exposed to TiO_2 NP suffered from biological surface coating that impaired molting, growth, and survival (Dabrunz et al. 2011). Such effects could be exacerbated over generations causing a population collapse as found by Jacobasch et al. (2014) in *Daphnia* exposed to the same NP.

$ZnSO_4$ and ZnO NP at concentrations of 0.1 and 0.3 mg L^{-1} also caused a significant inhibition in *D. magna* reproduction. However, animals exposed to ZnO NP showed a complete recovery in the full reproduction potential while those exposed to $ZnSO_4$ NP did not, and presented a dose-dependent reduction in their lifespan (Bacchetta et al. 2017).

Behavioral alterations

Studies about the effects of NP on the microcrustaceans behavior are very few, and non-existent to our knowledge in freshwater copepods, whereas the available ones are focused on cladocerans of the *Daphnia* genus (e.g. *D. magna, D. similis, D. pulex, D. carinata*). Overall, like for other xenobiotics, the effects of NP on microcrustaceans behavior is closely related to their energy metabolism and to ecological parameters such as food intake (Artells et al. 2013; Galhano et al. 2020). Thus, together with an increased risk of predation, behavioral effects could cause growth inhibition, reproductive decline, as well as disruptions in the food web interactions.

Among the all behavioral patterns, Lovern et al. (2007) examined the heart rate, hopping frequency, and appendage movement in *D. magna* exposed to 1 mg L^{-1} of TiO_2 NP. The authors found that such NP caused an increase in all these traits. Noss et al. (2013) also showed that 5 and 20 mg L^{-1} of TiO_2 NP caused a concentration-dependent aggregation of *D. magna* in the central region of a vessel and a reduction in their swimming activity. This aggregation phenomenon was interpreted as a kind of swarming behavior, a well-known response of different cladocerans towards other compounds (Gutierrez et al. 2013; Čolović et al. 2013; Noss et al. 2013; Szulkin et al. 2006). However, Noss et al. (*op cit.*) also noticed that the swarming behavior disappeared after 24 h at all tested concentrations. In this way, Galhano et al. (2020) showed that *D. magna* exposed to 12.5 µg L^{-1} of TiO_2 NP increased the swimming height, and suggested that the observed irregular swimming behavior could be indicative of the impairment of the nervous system with a consequent loss of orientation. The authors also reported that this response was overcome after 96 h, probably due to NP sedimentation. The swimming velocity of *D. similis* and *D. pulex* was another analyzed behavioral parameter under NP toxicity. A 48-h exposure to 1 mg L^{-1} of CeO_2 NP caused a decrease of 30% and 40% of this behaviour, respectively (Artells et al. 2013).

On the other hand, swimming alteration was also evidenced in *D. magna* neonates exposed to FeO NP (250 mg iron L^{-1}) which was linked to turbulences induced by the natation of the organisms. Then, that movement would induce the release of the coating agent, and the high loads of the NP would induce the complete immobilization of the neonates without killing them (Baumann et al. 2014).

Contrary to the above mentioned studies, it was also reported that some NP do not affect the cladocerans behavior, as it was found in *D. magna* exposed to wastewater-borne Ag NP and a standardized culture media-dispersed Ag NP (Galhano et al. 2020). Nevertheless, further studies including other kind of NP and microcrustaceans species are necessary to find out whether the behavioral responses are dependent on the type of NP, size, and matrices.

Differences in responses between *D. magna* and other freshwater microcrustaceans

Daphnia magna Straus is a Holarctic species, typical of shallow and ephemeral water bodies (Brooks 1965). Although its use in ecotoxicological studies was criticized due to its low representativeness (Buikema et al. 1982; Baird et al. 1989; Koivisto et al. 1992), it is still employed as a test organism in numerous environmental studies because of its sensitivity to environmental changes. *D. magna* accounts for numerous advantages for laboratory assays such as its genetically identical offspring, its short life cycle and reproduction, and its well-known functional biology (De Bernardis and Peters 1987; Lampert 2006).

Among cladocerans of *Daphnia* genus, the toxic effects of different NP have been carried out employing mainly *D. magna* as test species (Izoton et al. 2017). Other species of the same genus have also been used in toxicological tests because they share the aforementioned advantages. However, the differences found in sensitivity within the same genera (Völker et al. 2013; Artells et al. 2013; Song et al. 2015; Sakamoto et al. 2015) suggests that other freshwater microcrustaceans, phylogenetically and/or functionally more distant, could respond differently to the presence of NP. The analyzed bibliography indicated that these differences come from individual (intrinsic) and population (ecological) sensitivities, in which morphological, physiological, genetic, and ethological aspects interact with the environment. Nonetheless, some general characteristics described below could be the driving factors of the differences in the NP toxicity between *D. magna* and other freshwater microcrustaceans. The main morphological and ecological traits of each specific group can be found in **Table 3**.

Differences in the size and shape of organisms

In general terms, the body size is one of the most analyzed traits when comparing species sensitivities to the same xenobiotic, particularly in the case of NP. The main reason is because the body size is related to metabolism. Overall, it has been found that larger organisms are less sensitive than smaller ones given that the latter have faster metabolic rates, which favors the entry of contaminants into tissues and cells (Vesela and Vijverberg 2007; Lopes et al. 2014). As regard NP, the high sensitivity of smaller cladocerans is explained by the fact that the exposure area is greater in the smaller species because of the high surface area in relation to body mass. Moreover, particles and ions can be collected faster through water filtration, quickly transferred into the gut, and potentially be taken up rapidly because of their higher respiration and circulation rate in comparison with larger organisms. Evidence was found in 5 Cladocera species (*D. magna*, *D. pulex*, *D. galeata*, *C. dubia*, and *C. sphaericus*) exposed to Cu NP. The toxicity caused by such particles in 5 different Cu suspensions (with nominal sizes of 25, 50, 78, 100 and 500 nm) increased with decreasing body length and surface area of the organisms (Song et al. 2015). The same pattern was found in a comparative study which analyzed the acute effects of Ag NP at concentrations between 1 and 3.73 μg L^{-1}, on *D. galeata*, *B. longirostris* and *D. magna* (Sakamoto et al. 2015).

In addition to body size, some other morphological aspects seem to determine the sensitivity of NP among microcrustacean species. For example, differences in response to CeO_2 NP between *D. pulex* (48 h EC50=0.26 mg L^{-1}) and *D. similis* (48 h EC50=91.7 mg L^{-1}) were explained by the presence of reliefs on the cuticle and a longer distal spine in *D. similis* which act as traps for the CeO_2 aggregates (Artells et al. 2013). These same, or even more marked differences, could be found in other microcrustacean organisms with more conspicuous structures or appendages in their body such as *Ilyocryptus* spp..

Differences in feeding mechanisms

It was mentioned before that feeding inhibition is one of the most important mechanisms of NP acute toxicity in microcrustaceans. The studies analyzed in this review suggest two main ways by which NP can generate it. One of them is through the obstruction of the filtering apparatus (Asghari et al. 2012; Ribeiro et al. 2014; Mansano et al. 2018), and the other through the decrease of food availability due to sedimentation of the microalgae in the water-column (Campos et al. 2013; Bundschuh et al. 2016; de Luca et al. 2018).

However, the relative importance of these mechanisms in the toxicity of NP on microcrustaceans is still difficult to estimate, since the experiments in this line were focused on daphnids, such as *Daphnia* and *Ceriodaphnia* spp. These daphnids belong to the same class within the functional classifications based on the type of feeding of microcrustaceans (Barnett et al. 2007; Rizo et al. 2017), which differs on several aspects from the other classes (**Table 3**). Alterations in species belonging to other feeding classes have been neglected, with some exceptions such as *C. sphaericus* and *B. longirostris* (e.g. Song et al., 2015; Sakamoto et al. 2015).

Sidids (S-type) and bosminids (B-type) share a feeding mode similar to daphnids. However, the formers use their first five legs in filtering instead of their third and fourth legs as daphnids do. Bosminids use active swimming while filtering, with less developed thoracic limbs. These particularities suggest that the obstruction of filter bristles by NP could have greater consequences in daphnids and sidids than in bosminids. Chydorids, macrothricids, and ilyocryptids inhabit the littoral zone and use their second limb to scrape food from the substrates. Probably, the sedimentation of NP constitutes a critical pathway to them as occurs in other benthic invertebrates (Baun et al. 2008).

On the other hand, copepods also differ in their dietary and feeding strategies (food acquisition) from cladocerans. Copepods have mixed food preferences depending on the life stage, and these in turn may influence the amount of NP ingested. More specifically, cyclopoid copepods, are predominately microphagous carnivores (i.e. predators), harpacticoids are predominately omnivorous, while many calanoid species are selective filtering feeders (Vijverberg 1989). This suggests that at more studies on the latter are necessary, given that they could represent the worst-case scenario among copepods because its filtering apparatus may be more susceptible to mechanical damages.

Differences in life cycles and reproductive aspects

The relationship between the organisms' life-history strategies and the responses to stress situations generated by toxicants has not been deeply analyzed. However, microcrustacean species with distinct life histories could react differently to stress because, under such conditions, organisms preserve the most important life-history traits for their own fitness, at the cost of the less critical ones (Sibly and Calow 1989). *Daphnia* genus differs in these aspects from other cladeceran and copepod species. For example, large cladocerans such as *D. magna* increase their fecundity by postponing reproductive effort to a later part of their life cycle, while small cladocerans tend to maximize reproductive effort at the cost of future survival (Lynch 1980). Daphnids produce large clutches of small neonates, whereas the opposite occurs for smaller cladocerans like *Bosmina* sp. and *Chydorus* sp. (Lynch 1980; Koivisto et al. 1992). Copepods usually have a longer life cycle than *Daphnia* species, and it is quite more complex because it includes six naupliar stages, five copepodid stages, and finally adults (Santer 1998). Moreover, copepods have sexual reproduction, an extremely sensitive process that is almost absent in cladocerans that have parthenogenetic reproduction. All these characteristics may increase the sensitivity of copepod populations to NP exposures. Although this remains to be assessed for metallic NP, there are already studies with organic nanoparticles showing reduced copepepods fertility (Templeton et al. 2006).

Differences in the intrinsic sensitivity of each species

Despite some morphological and physiological characteristics can provide general patterns of sensitivity to NP, it is also documented that different groups of species present particular intrinsic sensitivities to different kind of contaminants. In this way, several authors agree that *D. magna* has a remarkable tolerance to different xenobiotics, although this generalization has not been demonstrated for NP yet. For example, Vesela and Vijverberg (2007) found EC_{50} values in *D. magna* exposed to Zn much higher than it would be expected based on its body size. Some authors suggest that this particular tolerance could be related to evolutionary adaptations since *D. magna* is an ecologically adapted species to small ponds and rock pools with highly variable temporal changes in water chemistry, which may be transferred to xenobiotics (Koivisto 1995). Moreover, such tolerance has been related to an efficient detoxification system like the large amount of metallothioneins and protein ligands per unit of body weight (Vesela and Vijverbg 2007).

In addition to the high intrinsic tolerance of *D. magna*, studies with other xenobiotics have also demonstrated its high recovery capacity in comparison with other species (López-Mancisidor et al. 2008; Duquesne and Küster 2010). Given the variety of life cycles, it is expected that different microcrustacean species differ from *D. magna* in their intrinsic ability to recover from exposure to NP. The resilience of daphnids and other species of microcrustaceans to NP exposure has not been analyzed yet. The importance of deepen into this aspect lies in the fact that the variable recovery times to NP exposure of the different taxonomic and functional groups in a community could generate ecological imbalances in the system.

Up to date, the Neotropical cladoceran *C. silvestrii* is considered the most sensitive species to NP in general terms (de Lucca et al. 2018; Mansano et al. 2018; Gebara et al. 2019). However, *C. dubia* and *M. macrocopa* also have been proposed as experimental models due to their particular sensitivity to Au and Al_2O_3 NP, respectively (Pakrashi et al. 2013; Borase et al. 2019). Further studies performing sensitivity curves with a great number of species are encouraged to provide a general overview of the real sensitivity that characterizes each one in case of NP exposure.

The ecological implications of considering only *D. magna* as surrogate of other microcrustaceans in NP toxicity studies

Because of the large ecological and functional differences between *D. magna* and other microcrustaceans, it is expected that the range of sensitivity to NP in this zoological group would be remarkably high. Depending on their feeding habits and its relative importance as food for higher trophic groups, microcrustaceans occupy different places in the food chain. Thus, considering the sole sensitivity of *D. magna* -or functionally similar species- as surrogates of other microcrustaceans leads to uncertainties about the consequences of NP in the ecosystem.

Furthermore, it is well known that industrial and domestic wastewater treatment plants, representing the most important sources of NP to aquatic ecosystems (Liu et al. 2013), usually release their effluents in aquatic systems where *D. magna* hardly represents the microcrustacean community (e.g.

rivers). The discharge areas are usually represented by small sized species, mainly chydorids cladocerans, and cyclopoid copepods, because they are usually more tolerant to pollutants and eutrophic conditions. These groups are the least considered in ecotoxicological studies, despite they play a particularly important ecological role, like the nutrient cycling.

Finally, given the limited representation of *D. magna*, future research on NP should include the development of toxicity tests with species of different regions and functional groups as it has been done for other xenobiotics such as pesticides (Arias-Andrés et al. 2014; Garza-León et al. 2017) and heavy metals (Bossuyt et al. 2005; Rodgher et al. 2010).

Final remarks

The increasing production, use and release of NP to the environment constitute a risk to aquatic systems and their biota. Thus, it is necessary to deepen researches related to the area of nano-ecotoxicology in order to provide recommendations for environmental regulation and control that allow the protection of both aquatic biota and the ecosystem integrity.

In this article, we reviewed the state of the art of the main factors influencing NP toxicity in aquatic microcrustaceans, and their main ecotoxicological effects. We found that NP effects on microcrustaceans depend on their intrinsic properties (e.g. coating, charge, size, shape and the physicochemical characteristics of the water). Among the general responses of the organisms exposed to NP, the most typical ones are body burial, feeding inhibition, biochemical effects, metabolic changes, and reproductive and behavioral alterations. However, we observed that most studies were performed on *Daphnia magna* and there is a notable gap on morphological, physiological, reproductive and behavioural studies on other different species within the microcrustacean group. Thus, to achieve a more complete understanding of the deleterious effect exerted by NP on microcrustaceans and its realistic consequences for the ecosystems, we recommend the inclusion of a greater number of species in toxicological tests, including different functional groups apart from that of *D. magna*. This would provide a more complete database to generate more accurate regulation measures.

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Competing Interests

The authors declare that they have no competing interests

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Table 1: Summary of the main factors influencing NP toxicity in microcrustaceans described in the text. The table provides a general expected effect of such factors on the NP toxicity although exceptions can be well documented, and a brief detail for each expected effect. The provided references are those considered in the selected literature review.

Factors influencing NP toxicity in microcrustaceans			
	General expected effects	**Details**	**Reference**
Size	Smaller nanoparticles (NP) are more toxic than larger ones	Smaller sizes increase the surface area-to-mass ratio, which increases the particle surface energy and thereby the reactivity of NPs	Hou et al. 2017; Ivask et al. 2014; Jiang et al. 2009; Kim et al. 2010; Seitz et al. 2015; Zhao & Wang 2012
Shape	NP are more toxic than nanowires (NW)	Higher potential to release ions from the NP	Cui et al. 2017; Kwak and An 2015; Sohn et al. 2015
Coating and charge	Different toxicity according to the type of coating employed in the NP synthesis	The coating composition influences on the NP stability in the media and has toxic effects itself	Baumann et al. 2014 ; Villa et al. 2020
	Different toxicity according to the water temperature	Alterations on the chemical conditions of the water media (e.g. decrease oxygen concentration) and influence on the organisms' biological functions	Silva et al. 2018; Batista et al. 2020
Environmental conditions	Different toxicity according to the water pH	The hydrogen ion concentration may affect uptake sites determinate chemical changes of the NP in water	Gilbert et al. 2007
	Different toxicity at different organic matter concentration and nature	Natural organic matter can stabilize NP by the formation of a coating which provides electric and steric stabilization and decreasing agglomeration	de Souza et al. 2019; Gao et al. 2009; Li et al. 2016a; Xiao et al. 2018
The presence of other stressors	Interactions can enhance (synergism) or diminish (antagonism) the toxic effects of NP	Changes in the physicochemical characteristics of original compounds in the mixture	da Silva et al. 2020; Deng et al. 2017; Li et al. 2016b; Pacheco et al. 2018; Rosenfeldt et al. 2014; Wang et al. 2019

Table 2: Summary of selected case studies documenting the general toxic effects of metallic, metal oxide and metalloid nanoparticles on microcrustaceans, mainly represented by *Daphnia magna*. Note that the case of studies provided in the table are not the unique in the current bibliography but they were selected as being well representative of the toxicity mechanisms described in the main text of this review.

Test species	NP type and concentrations	Exposure time	Environmental characteristic	Effective concentration*	Reference
Body burial and formation of gas bubbles inside the body					
Daphnia magna	TiO_2; particle size: 94-205 nm; concentrations exposed: 0.5 to 8 mg L^{-1}	72-96 h	Elendt M4 medium	EC_{50} 72h: 3.8 mg L^{-1}; EC_{50} 96h: 0.73 mg L^{-1}	Dabrunz et al. 2011
Daphnia magna	CuO; particle size of ~30 nm; concentrations exposed: 0.5 and 4 mg L^{-1}	48 h	STM medium	EC_{50} 48h: 4 mg L^{-1}	Heinlaan et al. 2017
Daphnia magna	ZnO, TiO_2; particle size: <21 nm and <100 nm for ZnO and TiO_2 respectively; concentrations exposed: 1.12 and 113.18 mg L^{-1} for ZnO and TiO_2 respectively	24-96 h	Standadized medium (OECD)	EC_{10} 24h: 1.12 mg ZnO L^{-1}; EC_{10} 24h: 113.18 mg TiO 2 L^{-1}	Renzi and Blašković 2019
Daphnia magna	TiO_2; particle size: 6-21 nm; concentrations exposed: 0.02 to 2.00 mg L^{-1}	up to 21 days	ASTM enriched with selenium, vitamins and seaweed extract (Marinure®)	EC_{50} 72h: 3.8 mg L^{-1}; EC_{50} 96h: 0.73 mg L^{-1}	Seitz et al. 2013
Feeding inhibition and intestine obstruction					
Daphnia magna	Ag (two types and a suspension of silver nanoparticles); particle size: 5-25 nm; concentrations exposed: 0.001 to 0.32 mg L^{-1}	48 h	Elendt M4 medium	EC_{50} 48h: 0.004; 0.002 and 0.187 mg L^{-1}	Asghari et al. 2012
Dpahnia magna	Fe (functionalized with four different coatings: ascorbate, citrate, dextran, and polyvinylpyrrolidone); particle size: 5.2 ± 0.4 nm and 6.1 ± 0.6 nm (depending on the coating agent); concentrations tested: 1 to 100 mg L^{-1}	96 h	Elendt M7 medium	EC_{50} 96h: 50, 68.4, 27.9 and >100 mg L^{-1} for each coating agent	Baumann et al. 2014
Dpahnia magna	CuO; particle size: ~30 nm; concentrations exposed: 0.5 and 4 mg L^{-1}	48 h	STM medium	EC_{50} 48h: 4 mg L^{-1}	Heinlaan et al. 2011

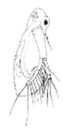
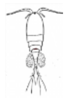
Daphnia magna	Au; particle size: 300–700 nm; concentration exposed: 600 µg L^{-1}	24 h	moderately hard freshwater (MHW)	not reported	Khan et al. 2014
Daphnia magna	TiO_2; particle size: 5 ± 2 nm and 23 ± 7 nm; concentration exposed: 25 mg L^{-1}	48 h	moderately hard freshwater (MHW)	not reported	Kwon et al. 2015
Daphnia magna	FeO (two different types); particle size: 20–30 nm and 20–60 nm; concentration exposed: 50 mg L^{-1}	48 h	moderately hard freshwater (MHW)	not reported	Kwon et al. 2014
Daphnia magna	Au; particle size: 17-23 nm; concentration exposed: 5 mg L^{-1}	24 h	moderately hard reconstituted water (MHRW)	not reported	Lovern et al. 2008;
Ceriodaphnia silvestrii	CuO; particle size: 8-28 nm ; concentrations exposed: 7 to 19 µg L^{-1} (acute test) and 0.5 to 10 µg L^{-1} (chronic test)	8 days	reconstituted water, Brazilian Association of Technical Standards (ABNT)	EC_{50} 48h: 12.6 µg L^{-1}	Mansano et al. 2018
Daphnia magna	Ag; particle size: 127–132 nm; concentrations exposed: from 5 µg L^{-1} to 25 µg L^{-1}	48 h	ASTM medium	EC_{50} 48h: 11.02 µg L^{-1} and 3.38 µg L^{-1} in presence and absence of food respectively	Ribeiro et al. 2014
Daphnia magna	CuO; particle size: 25 -80 nm; concentrations exposed: 0.015 to 16 mg L^{-1}	21 days	reconstituted water, Brazilian Association of Technical Standards (ABNT)	EC_{50} 48h: 0.05 mg L^{-1} for size of 25 nm, 2.34 mg L^{-1} for size of 40 nm and 2.36 mg L^{-1} for size of 80 nm	Santos-Rasera et al. 2019
Daphnia magna	Ag; particle size: 3 nm; concentrations exposed: 1 to 78 µg L^{-1}	21 days	ASTM medium	EC_{50} 48h: ~1.7 µg L^{-1} at lower pH and DOM levels; ~3 µg L^{-1} at high pH and DOM levels	Seitz et al. 2015
Daphnia magna	TiO_2; particle size: 225.5–1164.7 nm (after 1 h of its addition to the culture medium); concentrations exposed: 0.1 to 100 mg L^{-1} (acute test) and 0.1 to 5 mg L^{-1} (chronic test)	21 days	standard culture medium (OECD 202)	EC_{50} 72h: 1.62 mg L^{-1}	Zhu et al. 2010
Biochemical, metabolic and genetic effects					
Daphnia magna	ZnO and CuO; particle size: 30 nm; concentration tested: 0.04 to 2.57 and 0.18 to 19.97 mg L^{-1} respectively in acute tests.	96 h	OECD recommended ISO test medium	EC_{10} 96h: 0.52 mg L^{-1} for Zn; EC_{10} 96h: 1.97 mg L^{-1} for CuO	Adam et al. 2015

Daphnia magna	Au (different surfaces groups); particle size: ~ 4 nm; concentrations tested: 1-50 μg L^{-1}	24 h	moderately hard reconstituted water (MHRW)	not reported	Dominguez et al. 2015
Daphnia magna	TiO_2; particle sizes: from < 200 to < 800 nm; concentrations exposed: 1 -10 mg L^{-1}	21 days	synthetic moderately hard water (MHW)	EC_{50} 48h: >10 mg L^{-1}	Kim et al., 2010
Daphnia magna	CeO_2, SiO_2 and TiO_2; particle sizes: 7 and 10 nm for SiO2, 7 and 20 nm for TiO2 and 15 and 30 nm for CeO2; concentrations exposed: 1 mg L^{-1}	24 and 96 h	standard medium (OECD)	not reported	Lee et al. 2009
Ceriodaphnia silvestrii	CuO; particle size: between 8 and 28 nm ; concentrations exposed: 7 to 19 μg L^{-1} (acute test) and 0.5 to 10 μg L^{-1} (chronic test)	8 days	reconstituted water, Brazilian Association of Technical Standards (ABNT)	EC_{50} 48h: 12.6 μg L^{-1}	Mansano et al. 2018
Daphnia magna	CuO and ZnO; particle size: <50 nm for CuO and <100 nm for ZnO; concentrations exposed: 0.3 to 1.1 mg L^{-1}	72 h	synthetic moderately hard water (MHW)	not reported	Mwaanga et al. 2014
Daphnia magna	Ag; particle size: <50nm; concentrations exposed: 0.5 to 1.5 μg L^{-1}	21 days	Elendt M4 medium	EC_{50} 24h: >1 and <2 μg L^{-1}	Park et al. 2010;
Daphnia magna	Ag; particle size: 35 nm; concentrations exposed: based on the LC_{50} (1 to 31 μg L^{-1})	10 days	moderately hard reconstituted water (MHRW)	EC_{50} 24h: 1.8 μg L^{-1} and 10.6 μg L^{-1} for citrate-coated and PVP-coated Ag	Poynton et al. 2012
Reproductive impairments					
Daphnia magna	ZnO and CuO; particle size: 30 nm; concentration tested: 0.04 to 2.57 and 0.18 to 19.97 mg L^{-1} respectively	96 h	OECD recommended ISO test medium	EC_{10} 96h: 0.52 mg L^{-1} for Zn; EC_{10} 96h: 1.97 mg L^{-1} for CuO	Adam et al. 2015
Daphnia magna	ZnO; particle size: <50 nm; concentrations tested: 0.1 -0.3 mg L^{-1}	21 days	comercial mineral water	not reported	Bacchetta et al. 2017
Ceriodaphnia silvestrii	FeO; particle size: < 50 nm; concentrations exposed: 0.25-44.23 mg L^{-1}	7 days	soft synthetic water (ASTM)	EC_{50} 48h: >100 mg L^{-1}	Gebara et al. 2019
Daphnia magna	TiO2; particle size: between 375 nm and 694 nm; concentrations tested: 1.19 -6 mg L^{-1}	21 days	Elendt M4 medium	EC_{50} 21 days: 1.19 mg L^{-1} for the fifth generation	Jacobasch et al. 2014
Ceriodaphnia silvestrii	CuO; particle size: between 8 and 28 nm; concentrations exposed: 7 to 19 μg L^{-1} (acute test) and 0.5 to 10 μg L^{-1} (chronic test)	8 days	reconstituted water, Brazilian Association of Technical Standards (ABNT)	EC_{50} 48h: 12.6 μg L^{-1}	Mansano et al. 2018

Daphnia magna	CuO; particle size: 30–40 nm; concentrations exposed: 0.79-7.80 mg L^{-1}	21 days	Elendt M4 medium	EC_{50} 48h: 22 mg L^{-1}	Rossetto et al. 2014;
Daphnia magna, *D. galeata*, and *Bosmina longirostris*	Ag; particle size: 79.9 nm; concentrations exposed: 1.55-17.44 µg L^{-1}	~60 days	COMBO medium	EC_{50} 48h: 2.16 µg L^{-1} for *D. galeata*; EC_{50} 48h: 2.43 µg L^{-1} for *D. magna*	Sakamoto et al. 2015
D. magna, *D. pulex*, and *D. galeata*	Ag; particle size: 57.6±1.20 nm; concentrations exposed: 1.25-10 µg L^{-1}	21 days	Elendt M4 medium	EC_{50} 48h: 121, 8.95 and 13.9 µg L^{-1} for *D. magna*; *D. pulex* and *D. galeata*	Völker et al. 2013,
D. magna	Ag; particle size: 57.01 ±1.2 nm; concentrations exposed: 0.4-50 µg L^{-1}	21 days	Elendt M4 medium	EC_{50} 48h: 0.063 mg L^{-1}	Park et al. 2021
Behavioral alterations					
Daphnia similis and *Daphnia pulex*	CeO_2; particle size: 3 to 8 nm; concentrations exposed: 1 - 100 mg L^{-1}	48 h	commercialized natural water (Cristaline®)	EC_{50} 48 h: 0.26 and 91.79 mg L^{-1}	Artells et al. 2013
Dpahnia magna	Fe; particle size: 5.2 ± 0.4 nm and 6.1 ± 0.6 nm (depending on the coating agent); concentrations tested: 1 -100 mg L^{-1}	96 h	Elendt M7 medium	EC_{50} 96h: 50, 68.4, 27.9 and >100 mg L^{-1} for each coating agent	Baumann et al. 2014
Daphnia magna	Ag and TiO_2; particle size: 15.6 ± 2.2 nm; concentrations tested: 25 -125 µg L^{-1} for Ag and 12.5 - 100 µg L^{-1} for TiO_2	96 h	ASTM reconstituted hard water, and supplemented with vitamins and selenium.	EC_{50} 96h: 113.8 µg Ag L^{-1}; EC_{50} 96h for TiO_2 not reported	Galhano et al. 2020
Daphnia magna	TiO_2; particle size: 30 nm; concentration tested: 2 mg L^{-1}	1 h	moderately hard reconstituted water (MHRW)	not reported	Lovern et al. 2007
Daphnia magna	TiO_2; particle size 21 nm; concentration tested: 5 and 20 mg L^{-1}	96 h	ASTM medium	not reported	Noss et al. 2013

***** Effective concentration (EC) documented in the table refers to the lethal (mortality or immobilization) concentration/s reported in the article obtained through acute toxicity test. Note that the exposition time could be different as well as the registered endpoint (e.g.: EC_{50}; EC_{10}).

Table 3. Main morphological and ecological differences among the microcrustacean groups (Cladocera and Copepoda) analysed in this review. The feeding mechanisms were characterized according to Vijverberg (1989) and Barnett et al. (2007). *The organism's averaged size is based on the bibliography including a wide variety of species within each group.

	Body shape	Average size	Feeding mechanisms	Life cycle and reproductive aspects
Cladocera				
Daphnidae		<1 to 5 mm	D-type: use their third and fourth legs in filtering	Short life cycle, habitat typically limnetic, asexual reproduction. Production of large clutches of small neonates.
Sididae		<2 mm	S-type: use their first five legs in filtering	Short life cycle, habitat littoral-limnetic, asexual reproduction. Production of large clutches of small neonates.
Bosminidae		0.4 to 0.6 mm	B-type: use their first five legs in filtering	Short life cycle, habitat typically limnetic, asexual reproduction. Production of small clutches of large neonates.
Chydoridae		<0.5 mm	C-type:use their second limb to scrape food from the substrate	Short life cycle, habitat littoral-benthic, asexual reproduction. Production of small clutches of large neonates.
Macrothricidae		<1mm	Similar to Chydoridae: use their second limb to scrape food from the substrate	Short life cycle, habitat littoral-benthic, asexual reproduction. Production of small clutches of large neonates.
Ilyocryptidae		<0.5 mm	Similar to Chydoridae:use their second limb to scrape food from the substrate	Short life cycle, habitat littoral-benthic, asexual reproduction. Production of small clutches of large neonates.
Copepoda				
Calanoida		0.5 to 3 mm	selective filtering feeders, mainly phytoplankton	Long life cycle, habitat typically limnetic, sexual reproduction
Cyclopoida		<2 mm	commonly carnivorous, mainly feeding on other zooplankton organisms, but also on algae, bacteria and detritus	Long life cycle, habitat littoral-limentic, sexual reproduction
Harpacticoida		<2 mm	generally omnivorous (feed on ciliates, rotifers, algae, bacteria and detritus)	Long life cycle, habitat primarily benthic, sexual reproduction

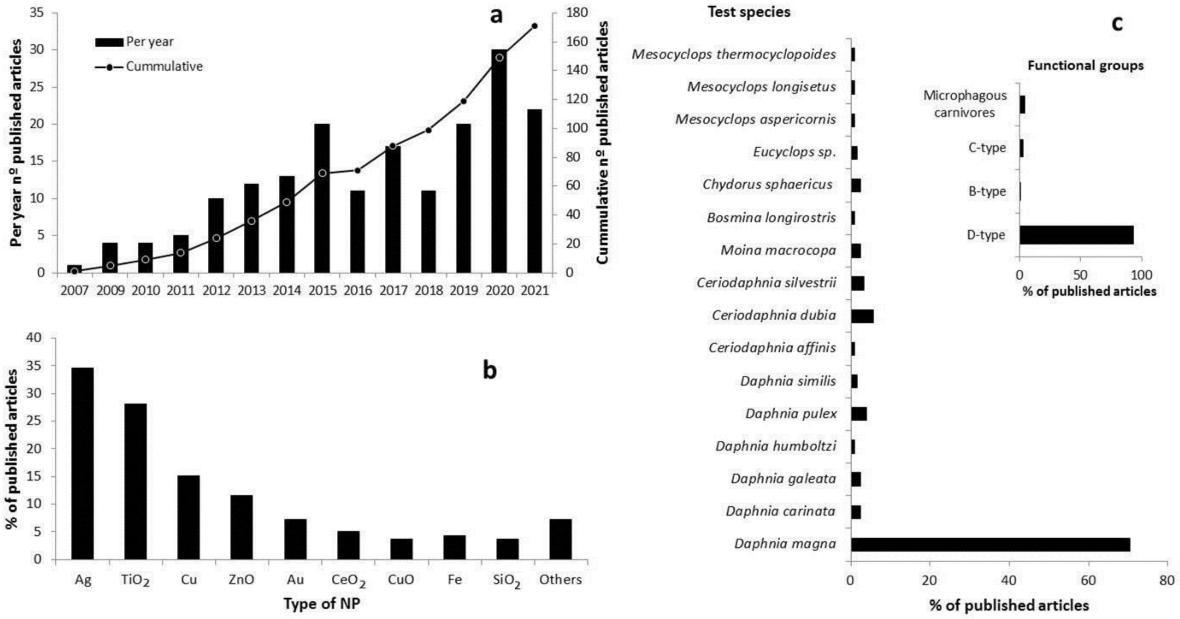


Figure 1: Bibliometric analysis on the effects of metallic, metal oxide, and metalloid NP to freshwater microcrustaceans (including cladocerans and copepods). (a) Number of per year and cumulative specific articles on the subject from 2007 to April 2021, (b) main inorganic NP analyzed during the mentioned period, and (c) species (scientific names) and the functional groups on which the studies on metallic, metal oxide, and metalloid NP were carried out. D-type: filtering Anomopoda (Daphnia, Ceriodaphnia and Moina), B-type: selective filter feeders (Bosmina), C-type: filtering scrapers (Chydorus), Microphagous carnivorous (Mesocyclops and Eucyclops).

Highlights

Nanparticles effects depend on intrinsic and external factors.

Nanoparticles affect the morphology, physiology and behavior.

Effects on Daphnia differ from other microcrustaceans.

The use of more diverse test species is suggested.

Graphical Abstract

Metallic, metal oxide, and metalloid nanoparticles toxic effects on freshwater microcrustaceans: an update and basis for the use of new test species

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