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REVIEW ARTICLE

The genus *Artemia*, the nanoplastics, the microplastics, and their toxic effects: a review

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Abstract

Plastic pollution is a threat to the marine environment, the destination of mismanaged plastic. Due to reduced size, microplastics and nanoplastics (MNPs) can interact with a wide range of organisms. Non-selective filter feeder zooplanktonic microcrustaceans are potential targets for MNP accumulation. Zooplankton is a key group for the food web, linking primary producers to secondary consumers. The genus *Artemia* has been widely used to investigate the effects of plastic particles on the biota. The present work critically reviewed the ecotoxicological studies about plastic particles and *Artemia*, pointing out methodological aspects and effects caused by MNPs, highlighting their importance and limitations, and suggesting directions for future research. We analyzed twenty-one parameters into four categories: characteristics of plastic particles, general particularities of brine shrimp, methodologies of the cultures, and toxicological parameters. The principal gaps in the area are the lack of methodological standardization regarding the physicochemical parameters of the particles, the biology of the animals, and culture conditions. Even though few studies performed realistic exposure scenarios, results indicate MNPs as potential harmful contaminants to microcrustaceans. The main effects reported were particle ingestion and accumulation followed by reduced brine shrimp survival/mobility. The present review poses *Artemia* as suitable animals for investigations concerning the risks of MNP exposure at the individual level and to the ecosystems, although protocol standardization is still needed.

Keywords Brine shrimp · Ecotoxicology · Microcrustacean · Plastic · Microplastic · Realistic exposure scenarios · Polystyrene · Zooplankton

Introduction

Plastics are organic synthetic polymers, malleable and moldable in different shapes (da Costa et al. 2016). Due to their versatility, durability, and large global production, these materials are widely used by human society, leading some authors to coin the term the "Age of Plastics" (Avio et al. 2017). Plastic production in 2019 was 368 Mt, with disposable packing as the main product (Plastics The Facts 2020). Plastics used to be considered inert materials due to their chemical properties and apparent lack of toxicity, resulting in disordered use and discard (Worm et al. 2017). About 60% of all plastics ever produced are accumulating in the environment (Geyer et al. 2017). Thus, the same characteristics that bring plastics' benefits are the ones that make them a persistent contaminant, accumulating in the environment for long periods (da Costa et al. 2016; Geyer et al. 2017; Worm et al. 2017; Rhodes 2018; Ganesh Kumar et al. 2020; Li et al. 2020). In this context, estuarine and marine environments are a huge concern for environmental agencies and the scientific community because these are the destination of plastic polymers (Ferreira et al. 2019; Lebreton & Andrady 2019). The presence of plastic in the marine ecosystem has been reported in different areas, from coastal zones highly polluted to remote regions of the planet such as the deep sea (Thompson et al. 2009) and even in the Antarctic continent (Bergami et al. 2020).

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Plastics are classified regarding their size as macroplastic (> 200 mm), mesoplastic (5–200 mm), large microplastic (1–5 mm), small microplastic (25 µm to 1 mm), and nanoplastic (1–100 nm or 1–1.000 nm) (Eriksen et al. 2014; Koelmans et al. 2015; da Costa et al. 2016; Worm et al. 2017; Gigault et al. 2018; Montagner et al. 2021). Microplastics and nanoplastics (MNPs) are also defined as primary or secondary according to their origins. The primary is those manufactured in micrometric and nanometric scales (Cole et al. 2011; Piccardo et al. 2020). These materials are used in the composition of several products, including cosmetics, biomedical devices, textiles, medical diagnostics, and electronics (Fendall & Sewell 2009; Koelmans et al. 2015; Guerranti et al. 2019). Primary MNPs may be released by the original products and reach aquatic ecosystems through runoff (Andrady 2011; Koelmans et al. 2015). On the other hand, secondary MNPs are produced as a result of the breakdown of larger plastic debris, such as macroplastics and mesoplastics, into smaller fragments due to environmental conditions (Andrady 2011; Piccardo et al. 2020). Photo-oxidative degradation (light action) can cleave the chemical bonds of polymers' chains and in combination with physical forces (friction, wind, and waves) generate smaller particles of plastics (Koelmans et al. 2015; Avio et al. 2017).

Several ecotoxicological studies have shown the negative impacts of MNPs (reviewed by Botterell et al. 2019). Studies with MPs in the marine environment became more frequent in the 2000s (Auta et al. 2017), when they were no longer considered emerging contaminants but instead were recognized as an emergent threat to ecotoxicology. Nevertheless, NPs are potentially more harmful to biological systems (Koelmans et al. 2015; da Costa et al. 2016); due to their reduced size (Piccardo et al. 2020), they are in contact with marine biota and may cross the biological barrier (Loos et al. 2014; Rossi et al. 2014). Their reduced size favors ingestion (or internalization) and accumulation of the plastic particles (Setälä et al. 2014; Foley et al. 2018; Wan et al. 2019), mainly affecting the development, survival, and reproduction of several organisms (Lee et al. 2013; Della Torre et al. 2014; Pinsino et al. 2017; Wan et al. 2019; Trestrail et al. 2020). Among the taxa studied are cnidarians (Morais et al. 2020), crustaceans (Cole et al. 2013; Sun et al. 2017), rotifers (Manfra et al. 2017), annelids (Van Cauwenberghe et al. 2015), mollusks (Browne et al. 2008; Setälä et al. 2016), echinoderms (Murano et al. 2020), and marine mammals (Besseling et al. 2015). Besides plastic accumulation in animals, another concern is the possible propagation to other taxa through trophic transfer (Cedervall et al. 2012; Setälä et al. 2014; Mattsson et al. 2015; Batel et al. 2016). Furthermore, due to the chemical properties of these particles, such as a higher surface area to volume ratio and hydrophobicity, they have a high capacity to adsorb other compounds like persistent organic pollutants (POPs) and heavy metals (Mato et al. 2001; Holmes et al. 2012; Wan et al. 2019). Thus, MNPs may impact the ecosystems as isolated particles and vectors to several notoriously toxic compounds (Mato et al. 2001). Additionally, plastic additives and polymer monomers can leach from particles, posing a potential harm to biota.

The zooplankton is a group of tiny aquatic organisms that inhabit marine and/or freshwater environments (Ferdous & Muktadir 2009) and exhibit a large morphological and taxonomic diversity (Kiørboe 2011). Their principal food source is phytoplankton and has an essential ecological role in aquatic ecosystems representing the main connection between primary producers and more elevated trophic levels of the food web (Sommer et al. 2002; Ferdous & Muktadir 2009; Sun et al. 2017). Although filter feeding is observed in several zooplankton taxa (Kiørboe 2011; Wirtz 2012), the feeding zone is characterized by a relatively low concentration of nutrients. So a volume filtration of about 10^6 times their own body's volume per day is necessary for nutritional maintenance (Kiørboe 2011). Thus, these animals are in continuous contact with suspended particles in the medium, such as plastic particles. Consequently, the ingestion may be the main entrance of plastics in zooplankton organisms (Wan et al. 2019). Studies have reported plastic debris ingestion by zooplankton in both laboratory experiments (Cole et al. 2013; Lee et al. 2013; Setälä et al. 2014) and the natural environment (Sun et al. 2017). So zooplankton have been considered the most susceptible biota group to the toxic effects of plastics (Foley et al. 2018). The interactions with plastic particles may cause harmful effects like changes in feeding behavior, development impairment, growth damage, and reduction of reproduction capacity and survival time (Lee et al. 2013; Foley et al. 2018; Botterell et al. 2019). Furthermore, the ingestion of plastic debris by zooplankton may also impact the ecosystems. Due to their relevant role in the energy flow in the aquatic food chain, these animals can become vectors of these contaminants and lead to plastic transfer to other trophic levels (Cole et al. 2013; Setälä et al. 2014; Sun et al. 2017; Foley et al. 2018). For these reasons, it is crucial to understand the impact of plastic debris in different zooplankton groups to establish a proper ecological risk assessment of plastic contaminants (Sun et al. 2017).

Invertebrate zooplankton, especially microcrustaceans, are remarkable for ecotoxicological investigations. The widespread use of aquatic invertebrates in this field is due to their ease of laboratory maintenance, sensitivity to substances, and ethical and economic considerations (Piazza et al. 2012). Several toxicological/ecotoxicological studies have been reported for marine and freshwater species, mostly the first one (Pisani et al. 2022; Li et al. 2023). Nevertheless, due to a great biological and environmental diversity, it is essential to amplify the studies to marine environments. Thus, the development of assays using microcrustaceans from saltwater is in progress, notably with the genus *Artemia* (Manfra et al. 2016).

The genus *Artemia* (subphylum Crustacea; class Branchiopoda; order Anostraca)—also known as brine shrimp—is a non-selective filter feeder microcrustacean (Nunes et al. 2006) that can potentially accumulate MNPs. These arthropods have a worldwide distribution and inhabit saline lakes and coastal regions on all five continents (Vanhaecke et al. 1987). The main characteristic of this group is tolerance to a wide range of salinity (5 to 250)—known as euryhaline—and temperature (6 to 35 °C) (Oliveira & Vaz 2018). Brine shrimp is a model organism widely used in ecotoxicological research, and it is one of the most studied animals in saline environments (Libralato 2014). Besides being part of zooplankton, the genus *Artemia* shows other beneficial features to ecotoxicological studies, such as high-quality commercial cysts storable for prolonged periods, easy culture and maintenance in the laboratory, low cost, short life cycle (2 to 4 months), small size (8 to 12 mm for adults), and a broad ecology and biology knowledge of the genus (Sorgeloos et al. 1978; Vanhaecke et al. 1980; Nunes et al. 2006; Dvorak et al. 2012; Manfra et al. 2012; Libralato et al. 2016). Environmental agencies are encouraging scientists to follow standard protocols for toxicological assays using brine shrimp, particularly the protocols designed by agencies from the USA (ISO/TS 20787) and Italy (APAT CNR-IRSA 8060). Moreover, different authors have emphasized the importance of experimental design harmonization for toxicological investigations with *Artemia* (Libralato 2014; Kos et al. 2016; Libralato et al. 2016; Johari et al. 2019; Lish et al. 2019). Standardized laboratory procedures may enable the genus *Artemia* to effectively address ecotoxicological issues (Nunes et al. 2006) and contribute to the development of the ecotoxicology field.

The present work aimed to critically review the ecotoxicological studies about MNPs in the genus *Artemia*, showing the main methodological aspects used and the toxic effects observed, followed by a discussion of research gaps and further directions to future studies in the field.

Data colleting

We carried out literature research to a broad analysis of published data about plastic particles in the microcrustacean of the genus *Artemia*, in the databases of the platforms PubMed (https://pubmed.ncbi.nlm.nih.gov/), SciELO (https://www.scielo.org/), ScienceDirect (https://www.sciencedirect.com/), Portal de Periódicos CAPES (https://periodicos.capes.gov.br/), and Google Scholar (https://scholar.google.com.br/). The following combinations of keywords were used: "*Artemia* + *plastic*"; "*Artemia* + *microplastic*"; "*Artemia* + *nanoplastic*"; "*brine shrimp* + *plastic*"; "*brine shrimp* + *microplastic*"; and "*brine shrimp* + *nanoplastic*." The terms were searched in titles, keywords, and abstracts of the manuscripts. Our selection process focused on studies that evaluated ecotoxicological endpoints related to the microcrustacean.

Four tables were generated with 21 parameters from the bibliographic survey. We grouped the parameters in tables according to the following topics: (i) characteristics of plastic particles, (ii) general particularities of brine shrimp, (iii) methodologies of cultures, and (iv) toxicological parameters analyzed. All terms and concepts used to describe the parameters agree with those used in the original studies.

Characteristics of plastic particles

We found 25 full papers that investigated the effects of plastic particles in microcrustacean *Artemia*. No review article was found. Studies include three types of plastic particles: nanoplastics, microplastics, and microfibers (MFs) (Table 1). The most studied ones were MPs (15 studies), followed by NPs (7 studies), MFs (2 studies), and MPs plus MFs (1 study).

The prevalence of MPs studies can be explained by the fact that these particles have gained more prominence since the 2000s (Auta et al. 2017) when investigations into the deleterious effects of MPs on the biota exponentially increased (da Costa et al. 2016; Avio et al. 2017). On the other hand, studies of NPs are more recent, reporting evidence of the potential and deleterious effects of NP on biological systems (Koelmans et al. 2015; da Costa et al. 2016). The current trend of investigations nevertheless is the extension of studies about NPs. The currently available research on the effects of MNPs on the genus *Artemia* still shows knowledge gaps, which highlights the need for further investigation.

The MFs are one of the most common plastic particles found in the environment (Cole 2016), and there is still a debate about their classification. The present study followed the recommendation of Schmid et al. (Schmid et al. 2021) that consider MFs as part of microplastic, being differentiated by their shape with a longer length and reduced diameter (Schmid et al. 2021). The more prevalent plastic particles in studies with brine shrimp are the spherical ones (MPs but also NPs). This fact also occurs in works with other aquatic organisms (Cole 2016). Although microfibers are usually found in the oceans (Geyer et al. 2017), spherical plastic microparticles and nanoparticles are widely manufactured and commercialized by the biotechnology industry, including companies such as Merck and Thermo Fisher, which makes them more readily available for researchers to use in ecotoxicological assays. In this sense, the use of spherical plastic particles mimics the impact of primary MNPs on the biota. However, more investigations are needed to

Table 1 Characteristics of plastic particles evaluated in publications using the genus *Artemia*

Reference	Particle	Type/size	Preparation of suspensions	Particle characterization	Concentration units
Bergami et al. 2016	NP	PS-COOH (40 nm); PS-NH$_2$ (50 nm)	NSW; vortex	TEM; DLS	5.0, 10, 25, 50, 100 mg/L
Batel et al. 2016	MP	Unknown (1–5 µm); PE (10–20 µm)	–	–	0.5 mg; 2.5 mg; ($\sim 1.2\times 10^6$ particles/20,000 nauplii)
Cole 2016	MF	Nylon (polyamide) (10 µm × 40 µm)	–	Optical microscopy	100 particles/mL
Bergami et al. 2017	NP	PS-COOH (40 nm); PS-NH$_2$ (50 nm)	NSW; vortex	TEM; DLS	0.1 and 1.0 mg/L; 0.5, 1.0, 1.5, 2.5, 5.0 mg/L
Gambardella et al. 2017	MP	PS (0.1 µm)	NSW; sonication (1 min)	DLS	0.001, 0.01, 0.1, 1.0, 10 mg/L
Kokalj et al. 2018	MP	PE; PET; (0.02–250 µm)	–	SEM; DLS	100 mg/L
Wang et al. 2019b	MP	PS (10 µm)	ASW; sonication (5 min)	FTIR; particle counter	1.0, 10, 100, 1000, 10,000 particles/mL; 0.1, 1.0, 10, 100, 1000 particles/mL
Mishra et al. 2019	NP	PS (50–70 nm and 100–120 nm)	–	DLS; SEM	0.5, 1.0, 10, 50, 100, 150, 200 mg/L
Peixoto et al. 2019	MP	FRM (amino formaldehyde) (1–5 µm)	–	Spectrofluorimetry	0.4, 0.8, 1.6 mg/L
Varó et al. 2019	NP	PS-NH$_2$ (50 nm)	NSW; sonication (5 min); vortex	TEM; DLS	0.1, 1.0, 10 mg/L; 0.1, 1.0, 3.0 mg/L
Wang et al. 2019a	MP	PS (10 µm)	ASW; sonication (5 min)	FTIR; particle counter	1.0, 10, 10^2, 10^3, 10^4 particles/mL
Sendra et al. 2020	NP	PS (100 nm)	Sonication (10 min)	SEM; TEM; FTIR; DLS	0.006 and 0.6 mg/L
Trestrail et al. 2020	MP	Phenol–formaldehyde (170 ± 147 µm); bio-phenol–formaldehyde (155 ± 56 µm)	–	SEM; optical microscopy; FTIR	1.0 mg/mL; 10, 20, 30, 40, 50 mg/mL (MP leachate)
Eom et al. 2020	MP	PS (1, 3, 6, and 10 µm)	ASW; vortex	–	1.0, 10, 100, 1000 particles/mL
Suman et al. 2020	MP	PS (5 µm)	ASW	IR; DLS	1.0; 25; 50; 75; 100 mg/L
Bour et al. 2020	MP; MF	PE (27–32 µm); polyester (PET) (12 µm × 500 µm)	ASW; sonication	Optical microscopy	100 particles/mL; 500 particles/mL
Han et al. 2021	MP	PS (4–6 µm)	Deionized water; sonication	–	0.2 and 2.0 mg/L
Kim et al. 2021	MF	PP (22.4 µm × 182.76 µm); PET (19.32 µm × 234.43 µm)	–	SEM; FTIR	75, 125, 250, 500, 1000 mg/L
Machado et al. 2021	NP	PS-NH$_2$ (50 nm)	ASW; sonication (10 min); vortex	DLS; electrophoretic mobility	0.005, 0.05, 0.5, 5.0 mg/L
Thiagarajan et al. 2021	MP	PS-COOH; PS-NH$_2$; PS plain (6 µm)	–	SEM; DLS	1.0 mg/L ($\sim 8.93\times 10^9$ particles/mL)
Li et al. 2021	MP	PE (40–220 µm); PS (30–300 µm)	–	FTIR; SEM	100 mg/L (1.6×10^6 items/m^3); 100 mg/L (1.1×10^6 items/m^3)
Albendín et al. 2021	MP	PVC (< 5 µm)	–	–	0.26, 0.69, and 1.6 mg/dm^3
Peixoto et al. 2022	MP	FRM (amino formaldehyde) (1–5 µm)	NSW; sonicated	–	0.4 and 1.6 mg/L
Kim et al. 2022	NP	PS-NH$_2$ (190 nm)	–	SEM; DLS	1 mg/L
Jeyavani et al. 2022	MP	PP (50 µm)	ASW; sonicated (20 min)	FTIR; SEM	1, 25, 50, 75, 100 mg/L

The sign (–) means information not mentioned in the study. *N* = 25 studies

NP nanoplastic, *MP* microplastic, *MF* microfiber, *PS* polystyrene, *PS-COOH* carboxy-functionalized polystyrene nanoparticles, *PS-NH*$_2$ amino-functionalized polystyrene nanoparticles, *PE* polyethylene, *PET* polyethylene terephthalate, *PP* polypropylene, *FMR* red fluorescent polymer microspheres, *PVC* polyvinyl chloride, *NSW* natural seawater, *ASW* artificial seawater, *TEM* transmission electronic microscopy, *SEM* scanning electronic microscopy, *DLS* dynamic light scattering, *IR* infrared spectroscopy, *FTIR* Fourier transform infrared spectroscopy

understand the effects of MFs on zooplanktonic organisms, and the genus *Artemia* can contribute to it.

The plastics used in the studies analyzed here exhibited a broad chemical diversity. We found eight types of polymers: polystyrene (PS), polyethylene (PE), polyethylene terephthalate/polyester (PET), polypropylene (PP), FMR (red fluorescent microspheres; amino formaldehyde), nylon (polyamide), phenol–formaldehyde, and polyvinyl chloride

(PVC) (Table 1). The most studied polymer was PS, with 15 studies, followed by PE (4 studies), PET (3 studies), PP and FMR (2 studies each), and nylon, phenol–formaldehyde, and PVC (1 study each). One study did not report the chemical nature of the polymer (Batel et al. 2016). Concerning NPs, all investigations were carried out with PS.

Four of the polymers cited in the studies (PP, PE, PS, and PET) are among the most produced ones (Plastics - The Facts Europe 2020), and three of them (PS, PP, and PE) are the foremost polymers commonly found in the aquatic ecosystems (Wan et al. 2019; Kedzierski et al. 2022). So the studies agree with the ecotoxicological relevance of the particles investigated: extensive scale use and environmental prevalence.

The present review shows that PS has been the most used polymer in ecotoxicological studies with *Artemia*. Piccardo et al. (2020) reported that around 97% of research on MNPs use PS. From 224 papers analyzed by these authors, only seven were performed with other polymers (Piccardo et al. 2020). The predominance of PS in the studies may be explained by the low cost and the greater availability of PS MNPs in the biotechnology market (Piccardo et al. 2020). Another aspect that contributes to their widespread use is the manufacturing of PS particles in a wide range of sizes (Loos et al. 2014). Our study clearly reveals the need to diversify studies to include other types of plastic polymers for a more comprehensive understanding of the effects of MNPs on the biota.

Our findings indicate that all ecotoxicological studies conducted on *Artemia* using NPs were exclusively focused on PS. This aspect might be related to the reasons previously described, and it also happens in ecotoxicological studies with other taxonomic groups. Shen et al. (Shen et al. 2019) found that 82% of the works about the effects of NPs in different taxa were done with PS. The authors suggest that the manufacturing difficulties of other types of plastic polymers in the nanometric scale might be the main reason for the low number of studies with other NPs.

The functional chemical group present in the particle surface—named particles' functionalization—is another feature addressed by several studies. From the six works with PS found by the present review, five specified the chemical functionalization of the particle ($-NH_2$ and $-COOH$). Two studies used both types, and three used only $-NH_2$. Only one study with MPs mentioned the chemical functionalization, using both $-NH_2$ and $-COOH$.

Functionalized plastic particles are suitable models for the investigations of the effects of NPs' chemical nature on toxicity (Loos et al. 2014). According to the presence or absence of a functional chemical group, PS NPs are classified into three types: cationic ($-NH2$), anionic ($-COOH$), and neutral (Casado et al. 2013). Particles' functionalization poses a relevant aspect to NP toxicity. The effects of NPs are influenced by the chemical characteristics of their surfaces and their interactions with the biological structures, although a more comprehensive understanding of the role of NP functionalization in their toxicity is still required (Loos et al. 2014; Bergami et al. 2016). Della Torre et al. (2014) showed that the functionalization of PS NPs was determinant of the toxic effects on the embryonic development of the Mediterranean sea urchin *Paracentrotus lividus*. The influence of a functional chemical group on NPs' surface was also described on brine shrimp (Bergami et al. 2016). Despite the low number of studies, research with PS NPs on *Artemia* is incorporating the particle's functionalization to understand the toxic effects of the particles.

Several sizes and shapes of the particles were found in the studies here considered. The sphere size ranged from 40 nm to 300 µm (NPs to MPs, respectively), while MFs ranged from 10 to 22.4 µm in diameter versus 40 to 500 µm in length (Table 1). One MP paper showed a different shape: an open-celled honeycomb structure (170 ± 147 µm and 155 ± 56 µm). Considering the PS particles, they ranged from 40 nm to 300 µm. The main sizes were 40 nm (2 studies), 50 nm (3 studies), 100 nm (5 studies), and 10 µm (3 studies). Three studies used the same particle size (100 nm) (Gambardella et al. 2017; Mishra et al. 2019; Sendra et al. 2020), but one considered the particles as MPs, while the other two named them as NP. The other polymers showed less representation and an extensive size variation (from 1 to 250 µm).

The size and the shape of the particles are crucial features for MNP ingestion and influence the post-ingestion effects (Eom et al. 2020; Ma et al. 2020). For this reason, it is essential to inform the size of plastic particles in ecotoxicological studies. Furthermore, it is necessary to investigate different sizes and shapes to better establish a correlation between these parameters and the possible toxic effects caused by exposure to MNPs.

The concept of the term nanoplastic is still under debate in the area. Some authors classify NPs as those particles within the range size from 1 to 100 nm (Koelmans et al. 2015), while others argue that NPs are particles from 1 to 1000 nm (Gigault et al. 2018). Koelmans et al. (2015) suggest using the range 1 to 100 nm because it focuses on specific properties of the nanometric scale, and it includes the NPs produced by the industry. On the other hand, Gigault et al. (2018) propose the classification of NPs as particles ranging between 1 and 1000 nm, which are the sizes of the fragments originating from the degradation of plastic debris. Thus, the particle categorization as NPs depends on the theoretical reference chosen by the authors of each study. The present work assumed the classification proposed by Koelmans et al. (2015) since it englobes NPs independent of their origins (primary or secondary) as both types of NPs pose risks to the ecosystems (Andrady 2011). So we considered the plastic particles used in the studies of Gambardella et al. (2017), Mishra et al. (2019), and Sendra et al. (2020) as NPs.

Two aspects concerning the preparation of particle suspensions are present in the analyzed works: the medium and the mechanical technique used to prepare the suspensions. Regarding the suspension medium, the two most used media were ASW (artificial seawater; 7 studies) and NSW (natural seawater; 5 studies). Only one work used deionized water (Han et al. 2021). In twelve of the analyzed studies, the medium used for preparing the particle suspensions was not reported. Three different techniques were used for the mechanical suspension of the particles: vortex (3 studies), sonication (8 studies), and both methods (2 studies). The most used sonication time was 5 min (3 studies). Ten studies did not provide a description of the mechanical method used for preparing the particle suspensions, while nine works did not describe the process used to prepare the suspensions of the particles.

The preparation of particle suspension is an important procedure for ecotoxicological studies involving plastic particles, but we noted a lack of its description in the studies analyzed here. This pattern also happens in studies with brine shrimp and other nanomaterials (Libralato et al. 2016). The physicochemical properties of a particle suspension—particularly NPs—can influence their physicochemical properties in the medium, such as aggregation, stability, and reactivity (Sharma et al. 2021) (these aspects are further discussed in the "Methodologies of cultures" section). Thus, we strongly recommend a detailed description of the methods used to prepare NP suspensions to ensure accurate and reproducible data.

To a better dispersion of particles in the medium to mimic a realistic scenario, most studies have used mechanical agitation for preparing particle suspensions (Manfra et al. 2017; Vaz et al. 2021). Yet there is still no consensus in the literature about the best method to obtain a homogeneous suspension. According to Della Torre et al. (2014), sonication does not improve the dispersion of particles, and they suggest that this technique is not required. Instead, they recommend only using vortex agitation. Actuality, there is evidence that sonication can alter the toxicity of NPs in microcrustaceans. Vaz et al. (2021) observed a reduction in the toxicity generated by sonicated PS NPs compared to non-sonicated NPs in *Daphnia magna*. They point out that sonication alters the interaction of particles with the medium and thus with animals. So their study brings new reflections on the choice of procedures performed in investigations with plastic and how they can influence the toxicological tests. Here, most studies that reported the method to obtain NP suspension stated the use of sonication (10 of 13 studies). Thereby, it is still necessary to determine—in a standardized way—which method is better to prepare NP suspensions and to what extent this procedure has an influence on the physicochemical properties of the particles.

The studies used different techniques to characterize the size and the physicochemical properties of the particles. Seven different methods were reported. The most used were dynamic light scattering (DLS) (11 studies), electron microscopy (EM) (including SEM and TEM) (12 studies), and infrared spectroscopy (IR) (including IR and FTIR) (8 studies) (Table 1). Other methods were also applied: optical microscopy (3 studies), particle counter (2 studies), spectrofluorimetry (1 study), and electrophoretic mobility (1 study). Most of the studies (17 studies) used more than one technique to characterize the material. According to the size of the particles, different techniques were used: DLS and EM are commonly employed to characterize NPs, while IR is more often applied to analyze MPs. Five works had no data regarding particle characterization.

The physicochemical characteristics of NPs are commonly analyzed since they can directly influence their toxic effect (Gangadoo et al. 2020). DLS is the predominant standard technique for analyzing colloidal systems, and this is the main reason it is used to analyze the physicochemical properties of NP suspensions in aqueous media (Bryant & Thomas 1995; Kim et al. 2019). It measures three significant parameters: *Z*-average, polydispersity index (PDI), and zeta potential (ζ). These parameters represent the average hydrodynamic diameter, size distribution, and surface charge of the nanoparticles, respectively, and are essential for interpreting their physicochemical properties in the medium (Varó et al. 2019). Twelve studies used EM techniques to determine the size and the shape of particles. Some authors suggest the combination of both methods (DLS plus EM) for the characterization of NPs and nanomaterials in general (Boyd et al. 2011; Johari et al. 2019; Kim et al. 2019; Gangadoo et al. 2020). Accordingly, we also suggest the use of both methods for a comprehensive characterization of NPs.

The characterization of MPs is performed by other approaches, as they do not constitute a colloidal system. Studies with MPs focused primarily on confirming the chemical composition of the polymer. The IR was the main technique for this purpose (Schmid et al. 2021). It is also the most adopted method in other studies with MPs, especially those to determine the type of plastic present in the environment (Schmid et al. 2021). Likewise, MP studies used different complementary techniques to obtain more information about the particle's suspensions, such as the number of particles in the medium. Thus, it is notable that studies with NPs are more detailed about particle description and have fewer variations in techniques, indicating a trend toward standardizing the characterization of nanoparticles.

The studies expressed the concentration units of the MNP suspensions in six different ways: mg/L (17 studies), particles/mL (7 studies), mg/mL (1 study), mg/dm^3 (1 study), items/m^3 (1 study), and particles/nauplii (1 study) (Table 1). For the first unit, the concentration range was 0.001 to 1000 mg/L. The concentrations expressed by the number of particles in the medium were only used in studies investigating the effects of MPs or MFs and ranged from 0.1 to 10.000 particles/mL. This approach was only possible due to the larger dimension of microsized particles, which allows their quantification in the medium and the adjustment to the number of particles per nauplii.

Wan et al. (2019) also found differences in the concentration units reported in several studies, such as mass/volume, mass/mass, and particle/volume. The use of different concentration units makes it difficult to compare the effects of plastic particles between studies (Phuong et al. 2016). Wang et al. (2019b) suggest the use of measured concentration (the actual number of particles in the medium) rather than the nominal concentration (related to the particle mass). The authors argue that MP suspensions with different sizes, but with the same nominal concentration, exhibit a difference in orders of magnitude in the number of particles in the medium. Therefore, it can lead to inaccurate results. Therefore, we suggest the standardization of the use of the number of particles in the medium as the concentration units for studies with MPs. However, this approach is only suitable for MPs, which can be easily measured with an optical microscope. The same procedure is not factual for NPs. Due to technical limitations, studies with NPs express particle concentration as nominal concentration (mg/L).

Another aspect to be considered in the design of the ecotoxicological experiment is the use of environmentally realistic concentrations. Several authors have reported that most studies with MNPs use a much higher concentration of these particles than those found in nature (Phuong et al. 2016; Shen et al. 2019; Wan et al. 2019). This discordance can lead to the overestimated results of the plastics' toxic effects. Some studies with brine shrimp tested high plastic particle concentrations for several reasons: technical limitations, to ensure the interaction of particles with organisms (Bour et al. 2020), or to represent severe contamination scenario (Peixoto et al. 2019; Han et al. 2021). Nevertheless, other works have adopted environmentally relevant concentrations (Peixoto et al. 2019; Wang et al. 2019b; a; Han et al. 2021). Since there is a wide difference in published data on the actual MNP concentrations in the environment, it is difficult to realize the relevance of the results obtained in the studies (Phuong et al. 2016). Consequently, a realistic approach in laboratorial investigations is still limited, even those that claim to follow this criterion. It is noteworthy that there are already efforts to determine the environmentally relevant concentration range of plastic particles. Despite that, it is important to keep the focus of the research on smaller MPN concentrations to obtain the results that may reflect the real contamination scenario of ecosystems.

General particularities of brine shrimp

The studies used three *Artemia* species: *A. franciscana* (13 studies), *A. salina* (5 studies), and *A. parthenogenetica* (3 studies). Four studies identified the brine shrimp as *Artemia* sp. (Table 2).

The species of the genus *Artemia* show differences in sensitivity to different compounds (Ruebhart et al. 2008). Nevertheless, there are divergences regarding the nomenclature adopted in the studies. The name *Artemia salina* is widely used but often incorrectly, leading to misunderstandings about which specific species is actually used in the studies (Asem et al. 2010). Ruebhart et al. (2008) found that several studies reported *A. salina* as working species, but according to the geographic origin of the cysts, authors identified the studied species as *A. franciscana* (most from the Great Salt Lake, UT, USA). Since *A. franciscana* originates from North America and *A. salina* from Europe (Vanhaecke et al. 1987; Sainz-Escudero et al. 2021), authors detected an inaccuracy in the followed classification. Therefore, *A. franciscana* has been considered the most studied species (Ruebhart et al. 2008). An example of this fact can be illustrated from one of the studies analyzed here: Mishra et al. (2019) stated that they used the species *A. salina*, but the cysts originated from the Great Salt Lake. Here, it is important to highlight that the correct identification of the species is required to enable a safe cross-comparison between toxicological data obtained from different studies.

Regarding *A. parthenogenetica*, it does not represent a single species, and they arise from four independent origins (Baxevanis et al. 2006). Thus, the use of *A. parthenogenetica* is not recommended for ecotoxicological testing. Consequently, due to the differences in species sensitivity and imprecision in taxonomic classification, *A. franciscana* is the recommended species for ecotoxicological studies (Ruebhart et al. 2008; Kos et al. 2016). Overall, half of the studies analyzed in the present review are in agreement with this recommendation.

Out of the studies analyzed, only three stated the geographic origin of the cysts. A similar result was reported by Libralato (2014). Here, most works only informed the company supplier of the commercial cysts (Table 2). However, it was possible to identify the geographic origins of the cysts based on information provided by the companies on their websites (Table 2). All cysts whose origin could be identified were from Great Salt Lake (8 studies). It was not possible to determine the origin of the cysts from most studies (17).

The geographical origin of the cysts is a relevant parameter for the analysis of the toxicological effect of a contaminant (Vanhaecke et al. 1980). Sorgeloos et al. (1978) demonstrated that nauplii hatched from cysts of different

Table 2 Characteristics of microcrustaceans of the genus *Artemia* used in studies with plastic micro- and nanoparticles

Reference	Species	Origin	Stage	Feeding	Density	Immobilization method
Bergami et al. 2016	*Artemia franciscana*	MicroBioTests (Ghent, Belgium)	Instar II	No	10 nauplii/2 mL	–
Batel et al. 2016	*Artemia* sp.	Sanders (cysts from the Great Salt Lake, USA)	Instar II	–	~20,000 nauplii/70 mL	Ethanol 96%
Cole 2016	*Artemia* sp.	–	Instar II	–	~10 nauplii/mL	Formaldehyde 4%
Bergami et al. 2017	*Artemia franciscana*	MicroBioTests (Ghent, Belgium)	Instar I	No; yes (*Dunaliella tertiolecta*)	~200 nauplii/100 mL; 10 nauplii/30 mL	–
Gambardella et al. 2017	*Artemia franciscana*	MicroBioTests (Ghent, Belgium)	Instar I	–	10–20 nauplii/mL	Paraformaldehyde 4%
Kokalj et al. 2018	*Artemia franciscana*	JBL Artemio Bur	Instar I	No; yes (JBL Artemio Fluid)	10 nauplii/2 mL	–
Wang et al. 2019b	*Artemia parthenogenetica*	Tianjin Ocean Pal Carol Biotech Co., Ltd., China	Instar I; Instar II	No; yes (*Chaetoceros muelleri*)	10 nauplii/100 mL; 10 nauplii/20 mL	Formaldehyde 4%
Mishra et al. 2019	*Artemia salina*	Ocean Star International, Inc., Snowville, EUA (cysts from the Great Salt Lake, USA)	Adults	Yes	–	–
Peixoto et al. 2019	*Artemia franciscana*	San Francisco Bay Brand, (CA, USA) (company)	12 DAH	Yes (*Phaeodactylum tricornutum*)	15 individuals/200 mL	–
Varó et al. 2019	*Artemia franciscana*	INVE Company, Belgium	Instar I	No; yes (*Tetraselmis suecica*)	~200 nauplii/50 mL; 100 nauplii/100 to 150 mL	Solution of distilled water saturated with chloroform (a few drops)
Wang et al. 2019a	*Artemia parthenogenetica*	Tianjin Ocean Pal Carol Biotech Co., Ltd., China	Instar II	No; yes (*Chaetoceros muelleri*)	13 nauplii/100 mL	Formaldehyde 4%
Sendra et al. 2020	*Artemia franciscana*	Cysts from the Great Salt Lake, USA	3-week-old adults	No; yes (*Phaeodactylum tricornutum*)	20 individuals/10 mL; 10 individuals/10 mL	–
Trestrail et al. 2020	*Artemia* sp.	Southern Biological, Australia	30 h (Instar II-III)	No	10 individuals	–
Eom et al. 2020	*Artemia franciscana*	INVE Aquaculture, Belgium	Instar I; juveniles	No; yes (*Tetraselmis suecica*)	60–70 individuals/1600 mL	–
Suman et al. 2020	*Artemia salina*	Tianjin, China	1, 2, 7 and 14 DAH	No; yes (*Chlorella vulgaris*)	10 nauplii/well; 10 individuals/50 mL; 10 individuals/100 mL; 10 nauplii/30 mL	Formaldehyde 4%;
Bour et al. 2020	*Artemia* sp.	HOBBY Aquaristik, Germany	3-week-old adults	No; yes (phytoplankton powder, HOBBY Aquaristik)	10 individuals/100 mL; 6 individuals/15 mL	–
Han et al. 2021	*Artemia franciscana*	Cysts from the Great Salt Lake, USA	Newly hatched nauplii	Yes (*Dunaliella salina*)	100 nauplii/200 mL; 100 individuals/L	–

Table 2 (continued)

Reference	Species	Origin	Stage	Feeding	Density	Immobilization method
Kim et al. 2021	*Artemia franciscana*	Ocean Star International, (UT, USA) (cysts from the Great Salt Lake, USA)	Instar II	–	4 nauplii/4 mL	Formaldehyde (30 min)
Machado et al. 2021	*Artemia franciscana*	Artemia International LLC, (TX, USA) (Cysts from The Great Salt Lake, USA)	Instar II; instar III	No	10 nauplii/2 mL	KCl (0.5 M; 10–15 min)
Thiagarajan et al. 2021	*Artemia salina*	Ocean Star International, (UT, USA) (cysts from the Great Salt Lake, USA)	Instar II	No/yes (*Chlorella* sp.)	10 nauplii/10 mL	Glutaraldehyde 2%
Li et al. 2021	*Artemia parthenogenetica*	Tianjin Haiyoujiayin Biotechnology Co., China	Newly hatched nauplii	Yes (*Chlorella* sp.)	–	–
Albendín et al. 2021	*Artemia salina*	Marine Culture Laboratory (Marine and Environmental Sciences Faculty (University of Cádiz))	4-week-old adults	No	10 individuals/25 mL	Ice immersion
Peixoto et al. 2022	*Artemia franciscana*	INVE Company, Belgium	Instar II-III; 12 DAH	No; yes (*Tetraselmis suecica*)	5 nauplii/mL (200 mL); 1200 individuals/200 mL	–
Kim et al. 2022	*Artemia franciscana*	–	Instar II	–	50 mg/80 mL	–
Jeyavani et al. 2022	*Artemia salina*	Ocean Star International, (UT, USA) (cysts from the Great Salt Lake, USA)	2, 7, and 14 DAH	–	10 nauplii/2 mL; 10 individuals/50 mL; 10 individuals/100 mL	–

Individuals = adult, juvenile, metanauplii. The sign (–) means information not mentioned in the study. $N=25$ studies

DAH days after hatching, *KCl* potassium chloride geographic origins showed different sensitivities to the same compound. This also applies to organisms of the same species but with different origins (Ruebhart et al. 2008). Consequently, it is desired that studies accurately inform the origin of the cysts, indicating both the supplier and the geographical location. Moreover, considering that the most popular origin of commercial cysts is the Great Salt Lake (Dvorak et al. 2012), we suggest their use for assays with MNPs to achieve the standardization of the studies.

Investigations regarding the toxic effects of plastic particles were conducted with the larval form and adult individuals. The development stages were divided into four main groups: instar I (~ 24 h after hatching), instar II (~ 48 h after hatching), days after hatching (DAH), and adults (Table 2). The instar I larval stage (9 studies) and instar II larval stage (13 studies) were the most used. Some of these studies were short-term (18 studies), while others started exposure in the larval period carrying long-term experiments until adulthood (9 studies: 1 study lasting for 5 days, 6 studies lasting for 14 days, and 2 studies lasting for 45 days). Four studies used the DAH to inform the life stage of organisms, with 7, 12, and 14 days of duration. Four studies used adult individuals, ranging from 3 to 4 weeks old. All adult subjects were for short-term studies with a time ranging from 5 min to 48 h.

The larval stage has a direct impact on the outcome of toxicological tests. Therefore, it is important to start exposure from the same phase of the developmental stage (Libralato et al. 2016). Previous studies have shown that instar I stage is less sensitive to toxic compounds than instar II stage (Sorgeloos et al. 1978; Vanhaecke et al. 1980). Thus, the restriction of tests to instar I stage can lead to misinterpretations about the toxicity of MNPs. The difference in sensitivity in the initial stages of the larvae is because the gastrointestinal tract opens in the instar II stage. From this stage on, the intestinal epithelium comes into direct contact with the external environment (Sorgeloos et al. 1978). Due to these morphological alterations, the larva starts to filtrate the medium from this developmental stage (Treece 2000), which helps to ingest the plastic particles. Recent studies have highlighted the differential sensitivity between larval developmental stages (Lish et al. 2019). Thus, the present study suggests that investigations on the exposure of brine shrimp to plastic particles should be conducted with the instar II stage onwards since oral ingestion acts as the main route of MNP accumulation (Wan et al. 2019). This observation is relevant for short-term studies, having fewer implications for long-term tests that start exposure to a contaminant from the instar I stage. Despite this, some authors recommend that assays should start at the instar II stage, regardless of the duration of the tests (Manfra et al. 2012).

Another parameter analyzed in the present study was the availability of food during the toxicological assays. Fourteen studies were performed in the absence of a feeding source, while 15 works were conducted in the presence of food during the tests. In some studies, the two patterns were available: presence and absence of feeding (10 studies). In six studies, it is not clear whether food was available or not (Table 2). The principal food source offered to the organisms was microalgae (12 studies). Six species were cited: *Tetraselmis suecica* (3 studies), *Chlorella* sp. (2 studies), *Chaetoceros muelleri* (2 studies), *Phaeodactylum tricornutum* (2 studies), *Dunaliella tertiolecta* (1 study), *Dunaliella salina* (1 study), and *Chlorella vulgaris* (1 study). Two studies provided commercial food (Kokalj et al. 2018; Bour et al. 2020), and one did not mention which food was offered (Mishra et al. 2019).

The supply of food is related to the developmental stage and the duration of the test. In general, short-term assays with larvae do not require feeding, as nauplii have energy reserves and can feed on their yolk for up to 5 days (instar V stage) (Treece 2000). In contrast, for long-term tests that start in the larval stage, it is compulsory to feed the organisms to preserve larvae survival. Experiments with juvenile and adult individuals also require feeding.

Food supply might be considered essential for an experimental protocol based on a realistic exposure scenario. For example, Bergami and colleagues (Bergami et al. 2017) showed that the presence of microalgae influences the physicochemical properties of particles in the medium. However, Johari et al. (2019) observed that most studies with short-term tests in the genus *Artemia* and nanomaterials did not feed the larvae. These authors do not recommend feeding the nauplii for assays lasting up to 48 h. The supply of a diet might contaminate the experiment with substances and metabolites that are not part of the study (Dvorak et al. 2012). Moreover, the absence of food can be relevant to identify the direct consequences of plastic particles per se. Therefore, the decision for feeding or not feeding the larvae should be based on the experimental design and its relevance to the objective of the investigation.

The density of organisms exposed to the particles varied significantly throughout the works. Twenty-five different densities were noted in the studies, ranging from 10 nauplii/2 mL to 100 individuals/L (Table 2). The only recurrent pattern was 10 nauplii/2 mL (5 studies). Three studies did not report the density of organisms. Densities were mainly expressed as nauplii/mL or individual/mL. This variation depends on whether the study started with the larval stage or not, with the term "individual" related to metanauplii, juveniles, or adults. Regarding studies with nauplii, the difference in density is explained by the extent of the experiment, as long-term exposure needs a lower density of organisms due to larval growth over time.

Kos et al. (2016) showed that nauplii density did not interfere with Ag nanoparticle toxicity using four different densities: 10 nauplii/200 μL, 10 nauplii/2 mL, 10 nauplii/4 mL, and 10 nauplii/8 mL. Lish et al. (2019) also investigated the influence of larva density on the toxicity of Ag nanoparticles, adopting two conditions: 10 nauplii/10 mL and 10 nauplii/100 mL. The authors observed a difference in the toxicity of nanoparticles, whose effect was more significant in the lower density. It indicates that a larger volume of treatment provides more contact with the particles (Lish et al. 2019). Thus, given the relevance of treatment volume to MNP toxicity tests, we recommend that studies report the density of larvae/individuals used in the experimental protocols.

Due to the swimming behavior of brine shrimp, its immobilization becomes necessary for proper observation under the microscope or stereomicroscope and photomicrographic records. The main objective of this analysis is to identify the presence of plastic particles in the body of the organisms, as well as to investigate possible morphological changes. Seven different methods were reported in the analyzed studies (Table 2). Among them, formaldehyde was the most used compound (4 studies). The use of paraformaldehyde, glutaraldehyde, ethanol, potassium chloride (KCl), distilled water with chloroform, and ice immersion (1 study each) was also noted. Thirteen studies did not describe whether they used any immobilization method to observe the brine shrimp, even presenting results referring to microscopy.

The choice of immobilization method may depend on the species, strain, or origin of the brine shrimp used in toxicological tests. For example, the *A. franciscana* from the Great Salt Lake employed by Machado et al. (2021) is not completely immobilized with formaldehyde (up to 16%; unpublished data). Depending on the analysis to be performed, the crushing of brine shrimp by coverslip can be an alternative method to replace fixatives or other compounds that promote the immobility of the larvae, such as KCl. It causes the contraction of the appendages and can interfere with morphological analyses, depending on the structure to be considered. Thus, due to the lack of standardization and the different responses of animals to the compounds, it is necessary to inform the method used in the immobilization of these microcrustaceans, consequently allowing future standardization in the studies.

Methodologies of cultures

Two culture media were used in the toxicity assays: ASW (18 studies) and NSW (7 studies) (Table 3). Only one study did not report the culture media used. Works analyzed here agree with the fact that artificial culture medium is predominantly used in ecotoxicological studies (Manfra et al. 2017). Artificial seawater has advantages that explain its widespread use: greater standardization of experiments, commercially available ASW ready-made formulations, easy laboratory preparation, and low cost (Libralato et al. 2016; Manfra et al. 2017). Since ASW is a controlled medium, it also provides a more reproducible response to the MNP effects on brine shrimp. Nevertheless, there are some criticisms about the composition of artificial media, which can vary from study to study, and it is not always appropriately described in the works (Libralato 2014; Kos et al. 2016).

Besides the ASW convenience, it is important to discuss the environmental parameters of NSW. Different environmental parameters, such as pH, salinity, mineral composition, and natural organic matter (NOM), are known to influence the physicochemical properties of plastic particles and, consequently, their effect on biological systems (Sharma et al. 2021). In this regard, the use of ASW becomes a disadvantage, as it fails to assess the influence of NOM on the possible toxic effect of the particles. In contrast, the use of NSW aligns with a realistic exposure scenario (Manfra et al. 2017). In their study, Manfra et al. (2017) observed different toxicities for rotifers exposed to PS nanoparticles in ASW and NSW, showing that the medium can influence the toxicity of the particles. Thus, since both media have advantages and disadvantages, the choice of the culture medium must be in coherence with the aim of each study.

Regarding the physicochemical characteristics of the culture media, two parameters were evaluated: pH and salinity. The pH of the culture medium ranged from 7.49 to 8.5, but this information was not found in thirteen studies (Table 3). The pH values of the culture medium used in the studies showed a small amplitude and are within the recommended range (7.5 to 9) (Libralato et al. 2016). Despite that, it is important to emphasize that more than 50% of the studies did not provide information about this parameter. The pH is a crucial factor that can modify the physicochemical properties of plastic particles in the medium (Sharma et al. 2021). It was reported that a pH above 9 provides a drastic change in PS nanoparticles, causing an increase in the degree of aggregation and a decrease in the charge (Ramirez et al. 2019). So pH can potentially influence plastic's interaction and consequently its effect on biological systems. Besides that, changes in pH can cause consequences to nauplii since a pH below 7 is harmful to their development (Kos et al. 2016). Therefore, the pH of the culture media must be monitored and reported in the works that investigate the effect of MNPs.

Nine different salinities were identified, ranging from 15 to 43.2. The most used salinities were 30 (6 studies) and 38 (3 studies). Six studies did not mention any information about the salinity of the medium (Table 3). The amplitude of the salinity range adopted in the analyzed studies was higher than that found in other studies with *Artemia* (15 to 43.2 against 25 to 38; Table 3 and Johari et al. 2019). Brine shrimps are euryhaline animals and can tolerate wide variations of salinity (Persoone & Wells 1987). Despite

Table 3 Methodological parameters evaluated from publications of microplastics and nanoplastics with the genus *Artemia*

Reference	Culture medium; pH; salinity	Temperature	Agitation of the medium	Photoperiods	Exposure time	Positive control	Protocol
Bergami et al. 2016	NSW; 8.3; 38	25 ± 1 °C	–	Absence of light	24 and 48 h	$K_2Cr_2O_7$	APAT CNR-IRSA 8060 (2003)
Batel et al. 2016	ASW; –; 20	–	Yes	–	3, 6, 9, and 24 h	–	–
Cole 2016	ASW;–; 15	28 °C	Yes (< 5 rpm)	–	2 h	–	–
Bergami et al. 2017	NSW; 8.3; 38	25 ± 1 °C	–	Absence of light; 16:8 h light:dark	48 h; 14 days	–	Bergami et al. (2016); Savorelli et al. (2007); Manfra et al. (2012); Artoxkit® (2014)
Gambardella et al. 2017	NSW; –; 37	25 °C	–	Absence of light	24 and 48 h	$K_2Cr_2O_7$	Gambardella et al. 2015; APAT CNR-IRSA CNR 8060 (2003)
Kokalj et al. 2018	ASW; –; –	21 ± 1 °C	No	16:8 h light:dark	48 h	–	Kos et al. (2016)
Wang et al. 2019b	ASW; 8.1 ± 0.1; 30 ± 1	25 ± 1 °C	–	12:12 h light:dark	24 h; 14 days	–	Lee et al. (2013)
Mishra et al. 2019	NSW; –; –	–	–	–	24 h	–	OECD 2024
Peixoto et al. 2019	ASW; –; 35	25 °C	–	14:10 h light:dark	44 days	–	Varó et al. (19982015)
Varó et al. 2019	NSW; 8.25; 38.3	25 ± 0.5 °C	–	Absence of light; 16:8 h light:dark	48 h; 14 days	–	Varó et al. (2015), Comeche et al. (2017)
Wang et al. 2019a	ASW; 8.1 ± 0,1; 30 ± 1	25 ± 1 °C	–	12:12 h light:dark	1, 3, 6, 9, 12, and 24 h; 14 days	–	Wang et al. (2019a)
Sendra et al. 2020	ASW; –; –	–	–	Absence of light	24 h; 3.5 h	–	–
Trestrail et al. 2020	NSW; –; 43.2; ASW; –; 37.5	28 ± 0.5 °C	No	Absence of light	2 h; 24 h	–	Artoxkit® (2014)
Eom et al. 2020	ASW; 7.9–8.1; 32	20 °C	–	16:8 h light:dark	1 h; 96 h; 30 days	–	–
Suman et al. 2020	ASW; –; –	27 ± 0.2 °C	–	–	24 and 48 h; 14 days	–	Madhav et al. (2017); Bergami et al. (2017)
Bour et al. 2020	ASW; –; 30	–	–	–	5 min; 2 h	–	–
Han et al. 2021	ASW; –; 30	22; 26 and 30 °C	–	14:10 h light:dark	14 days	–	Bergami et al. 2017
Kim et al. 2021	ASW; 8.0–8.5; 30 ± 1	20 ± 1 °C	–	12:12 h light:dark	48 h	$K_2Cr_2O_7$	ISO/TS 20787 (2017)
Machado et al. 2021	ASW;8; 33	25 ± 1 °C	Yes (50 rpm); no	Absence of light	24 and 48 h	$K_2Cr_2O_7$	APAT CNR-IRSA 8060 (2003); ISO/TS 20787 (2017)
Thiagarajan et al. 2021	ASW; –; –	–	No	Continuous light	48 h	–	–
Li et al. 2021	ASW; 8.5 ± 0.5; 32.3 ± 0.5	–	–	–	45 days	–	–
Albendín et al. 2021	–; 7.49–7.63; –	20.55–21.22 °C	–	16:8 h light:dark	48 h	$K_2Cr_2O_7$	OECD 2004
Peixoto et al. 2022	NSW; –; –	25 °C	–	16:8 h light:dark	48 h; 5 days	–	Varó et al. (2019)
Kim et al. 2022	ASW; 8–8.5; 30 ± 1	–	–	–	1 h	–	–
Jeyavani et al. 2022	ASW; 7.8–8; 32	30 °C	–	16:8 h light:dark	48 h	–	Madhav et al. (2017);

The sign (–) means information not mentioned in the study. $N = 25$ studies

NSW natural seawater, *ASW* artificial seawater, *h* hour, $K_2Cr2_2O_7$ potassium dichromate, *rpm* revolutions per minute, *APAT* Italian Agency for Environmental Protection and Technical Services, *ISO* International Organization of Standardization, *OECD* Organisation for Economic Co-operation and Development this characteristic, there is evidence that salinity influences toxicological tests, especially in studies involving nanoparticles, since the particles' physicochemical properties can be affected by this parameter. The increase in ionic strength, which is related to the number of ions in the medium, decreases the stability of plastic particles, contributing to their aggregation (Sharma et al. 2021). Thereby, as salinity is a relevant parameter for toxicological studies with MNPs, it must be clearly reported in the experimental designs of the studies. The present review shows that—in addition to the lack of standardization of the salinity in experiments—some works did not show this information, making difficult a comparative analysis between the obtained data.

Regarding the conditions of culture maintenance, three parameters were evaluated: temperature, medium agitation, and photoperiod. The temperature of the experiments ranged from 20 to 30 °C (Table 3), but the most used was 25 °C (9 studies). In seven works, there was no information about the temperature of the cultures. The temperature is a relevant variable because it can clearly influence toxicity (Libralato et al. 2016), and an elevated temperature can raise the mortality of larvae or adult individuals (Nunes et al. 2006). Studies showed that higher temperatures contribute to the deleterious effect of MPs on microcrustacean growth and survival as an additional stress for animal development (Han et al. 2021). Although the present review shows that the temperature range of the analyzed studies is within the recommended by the literature (Johari et al. 2019), about 20% of the analyzed works did not mention the adopted temperature. So we strongly recommend that temperature be stated in all ecotoxicological studies.

To mimic the aquatic environment, which is in constant movement, the agitation of the culture medium has been evaluated in some studies involving nanoparticles (Lish et al. 2019; Machado et al. 2021). This parameter is reported in only three evaluated studies (Table 3). Of the remaining twenty-one studies, four works did not use agitation of the medium, while the others did not mention this aspect. The strong tendency of plastic particles to aggregate, especially NPs, can cause their precipitation in the culture medium, influencing their interaction with biological systems. For this reason, it is interesting to assess whether the agitation of the medium can help to maintain the suspensions of NPs in the water column. This method might favor the ingestion of NPs by filtering feeders with swimming habits, which are in constant displacement in the water column. The studies that evaluated the agitation of the medium, however, reported that it was not relevant to the toxicity of the tested nanoparticles (Ag and PS nanoparticles) (Lish et al. 2019; Machado et al. 2021). We suggest that further studies assessing this parameter, which is still poorly addressed, must be conducted.

Five different photoperiods were used in the studies: absence of light (7 studies), 12:12 h light:dark (3 studies), 14:10 h light:dark (2 studies), 16:8 h light:dark (7 studies), and continuous light (1 study). In two studies, both protocols (absence and presence of light) were performed. Seven works have no information regarding photoperiod (Table 3). Some studies suggest the use of the 16:8 h photoperiod (light:dark) for short-term assays (Kos et al. 2016; Johari et al. 2019). But the use of photoperiod must be according to the studied particle. Results obtained with Ag nanoparticles showed that the photoperiod influences their physicochemical properties in the medium, changing their toxicity (Kos et al. 2016). We did not find any studies evaluating the effect of photoperiod on the toxicity of plastic particles. However, the photoperiod is important to brine shrimp growth (Asil et al. 2012), and it should be used for long-term assays. In these tests, the microalgae used to feed the brine shrimp must determine the light period, adapting the culture conditions to those that also satisfy the phytoplankton culture (Manfra et al. 2012). In the studies analyzed here, a diversity of microalgae species was noticed, which can explain the variance of photoperiods adopted. When we consider acute exposure scenarios, protocols elaborated by national and international environmental agencies such as APAT CNR-IRSA 8060 (APAT; CNR-IRSA 2003) and ISO/TS 20787 (International Organization for Standardization 2017) recommend the absence of light during nauplii exposure. This statement makes sense as light tends to increase the phototactic behavior of brine shrimp larvae (Dojmi Di Delupis & Rotondo 1988).

The exposure time of larvae to MNPs ranged from 5 min to 45 days, having two main categories: short- (22 studies) and long-term (10 studies) assays. Seven studies used both types of exposure. Short-term investigations ranged from 5 min to 96 h, with 24 h (10 studies) and 48 h (12 studies) as the most utilized time intervals. Long-term assays used the time intervals of 14 (6 studies), 30, 44, and 45 days (1 study each) (Table 3). Short-term tests (≤ 96 h) are the most used and better-established toxicological tests; on the other hand, the use of long-term tests (usually 7 to 14 days) has increased in the last decade (Manfra et al. 2015; Libralato et al. 2016). Regarding short-term tests, 24-h exposure tests are more commonly used (Libralato et al. 2016). As well as reported in the literature, this was the most used exposure time in the works analyzed in the present review. The choice of 24 h as the exposure period may be correlated to the period suggested in the first protocol about toxicological standardization for studies with brine shrimp (Vanhaecke et al. 1980, 1981). In addition, 24-h assays provide more homogeneous results, are simple to perform, and, from a technical point of view, do not require sophisticated equipment or a high degree of staff trained (Vanhaecke et al. 1980; Nunes et al. 2006). Currently, ISO/TS 20787 (2017) also suggests a 24-h exposure time for toxicological tests with brine shrimp, although there is an additional indication for the use of a 48-h interval of exposure.

Long-term investigations are suitable to increase knowledge about the sensitivity of zooplankton to contaminants. This category of tests can reveal toxic effects that are not observed in short-term assays, mimicking a realistic environmental scenario as it addresses the chronic effect of the contaminant throughout the animal's life. Long-term exposure to PS nanoparticles has shown toxic effects on brine shrimp that were not noticeable in short-term tests (Bergami et al. 2016, 2017). Thus, it is relevant to emphasize that studies should, whenever possible, develop protocols that allow the assessment of both acute and chronic effects of MNP exposure.

The use of reference toxicants as the positive control was also evaluated in the present review. Positive controls are a fundamental part of an experimental design. For the validation of the effect caused by a compound/particle, and the reliability of the test, it is necessary to verify the organism's sensitivity to a reference toxicant (Vanhaecke et al. 1980). For toxicological studies using the genus *Artemia*, positive control is particularly relevant: different geographical origins and strains of the cysts can lead to different responses to compounds. So the sensitivity of the strain used in the ecotoxicological tests needs to be monitored. In addition, the use of positive control makes it possible to compare results between the studies (Silva et al. 2003).

In the works analyzed here, only five studies reported a reference toxicant as a positive control (potassium dichromate, $K_2Cr_2O_7$; Table 3). The lack of information about the use of a reference toxicant as a positive control was also recurrent in the studies with brine shrimp analyzed by Libralato (2014). Other authors have also highlighted the importance of using a positive control in toxicological tests with *Artemia* (Kos et al. 2016; Johari et al. 2019). We emphasize the need to use an experimental group as a positive control in all studies regarding the toxic effects of MNPs in brine shrimp.

Regarding the protocols used in the experiments, three groups were identified: manuals produced by national environmental agencies or organizations, protocols prepared by a company operating in the (eco)toxicological area, and those established in works published in scientific journals (Table 3). In the first group, three protocols were cited: APAT CNR-IRSA CNR 8060 (3 studies), ISO/TS 20787 (2 studies), and OECD Test No. 202 (2 studies; OECD 2004). In the second group, there is the Artoxkit® (1 study), a protocol developed by the company MicroBioTests. Finally, eleven studies referred to protocols established by other scientific studies, and eight studies did not mention the protocol used.

As discussed above, several parameters can influence toxicological tests with MNPs with the genus *Artemia*. In the last 40 years, different efforts have been aimed to standardize ecotoxicological tests with this microcrustacean (Vanhaecke et al. 1980; Persoone & Wells 1987; Libralato 2014; Manfra et al. 2015; Kos et al. 2016; Libralato et al. 2016; Johari et al. 2019; Lish et al. 2019). Although as evidenced by the present review, this goal has not yet been achieved.

Recently the publication of ISO/TS 20787 (2017) gave directions about the use of the genus *Artemia* in toxicological studies with nanomaterials. It came to fill a significant gap in the area: the absence of a protocol prepared by an international standardization agency exclusively for studies with nanomaterials (Libralato 2014). Thus, only the most recent articles have adopted this protocol. This fact reflects the variety of methodologies adopted in the studies discussed here. Yet according to the purpose of the study or laboratory conditions, it is not always possible, or even relevant, to adopt standard protocols. Nonetheless, all experimental conditions must be detailed in the methodology section of the manuscripts (Libralato 2014).

Toxicological parameters analyzed

The present review analyzed the test performed, the endpoints investigated, and the toxic effects (Table 4). Among the main toxic effects induced by MNPs in brine shrimp, the following stood out: mortality/immobility, development/growth modifications, biochemical profile alterations, gene expression alterations, and intestinal damage. Other endpoints and effects were also described but less representatively. Next, the studies will be discussed, in detail, according to two categories: (i) tests related to toxicity and (ii) tests related to realistic exposure conditions.

Toxicity tests

Among the studies, tests related to toxicity were more representative (19 studies). In this group, the acute (16 studies) and chronic toxicity tests (9 studies) stand out, and the lethal and sublethal effects of the particles were analyzed. Five studies used both acute and chronic tests. The foremost sublethal endpoint was particle ingestion. Besides that, some studies carried out additional tests such as particle elimination, histological analysis of the gut, and transcriptomic analysis (Table 4).

The toxicity of MPNs can be evaluated through acute toxicity tests, which measure the effects of short-term exposure (up to 96 h), or chronic toxicity tests, which assess the toxicity over a longer period, usually lasting between 7 and 14 days. As previously discussed in the "Methodologies of cultures" section, acute toxicity tests were the most used, as they are short-term experiments.

Nevertheless, the development of the ecotoxicology research area has shown that assessing the chronic toxicity of plastic particles can bring new knowledge to the field. In fact, chronic tests can reveal more sensitive results (Manfra et al. 2012). No deleterious effect of PS nanoparticles on larval survival was observed by Bergami et al. (2016) using the acute toxicity test. Nonetheless, authors reported an increased mortality/immobility rate of brine shrimp caused by PS nanoparticles after chronic exposure (Bergami et al. 2017). Hence, both tests are appropriate to elucidate the ecotoxicological effect of plastic on brine shrimp and are complementary to answer different scientific questions.

The most investigated endpoints in the short-term tests are cyst hatching, survival/immobilization, enzymatic alterations, and consequences in swimming (Libralato et al. 2016). Meanwhile, reproduction and immobilization are the most used endpoints in long-term tests (Libralato et al. 2016). The selection of endpoints for the acute tests aligns with the recommendation put forth in ISO/TS 20787 (2017), which indicates survival/immobilization of nauplii for short-term tests. Nevertheless, the toxicological studies of plastic particles and brine shrimp expanded the endpoints, including, as mentioned above, particle ingestion, mortality/immobility, developmental alterations, enzymatic alterations, alterations in gene expression, and intestinal damage.

Twenty-two studies addressed the ingestion of MNPs, ranging from the examination of particle ingestion to their elimination/purification and accumulation. Most studies showed the accumulation of particles. Only seven studies observed partial elimination of particles, and one work indicated total elimination of them. However, it is needed to consider the period of analysis in each study. Besides the accumulation in the digestive tract, two studies reported the adhesion of plastic nanoparticles to the external surface of larvae (Table 4).

The ingestion is the main entry route for NPs in non-selective zooplanktonic organisms, so its assessment is truly relevant (Wan et al. 2019). Interaction with nanoparticles can lead to harmful effects on these animals, with implications for survival, reproduction, development, and growth (Lee et al. 2013; Foley et al. 2018; Botterell et al. 2019). Furthermore, as they represent a key link to the food web, microcrustaceans can act as vectors of contaminants and their transfer to higher trophic levels, making MNP biomagnification possible (Cole et al. 2013; Setälä et al. 2014; Sun et al. 2017; Foley et al. 2018). Hence, the accumulation of these particles can also bring implications for ecosystems. The present study highlights the need to perform trophic transfer studies of MNPs for a better understanding of the consequences of the accumulation of plastic particles by zooplankton.

The second most evaluated endpoint was mortality/immobility (17 studies). Fourteen studies used the term mortality, two works used the term immobility, and one study used both. It is necessary to clarify the terms mortality/survival and immobility since some authors argue that the larvae cannot be considered dead just by observing the lack of mobility (Nunes et al. 2006; Libralato et al. 2016). Even immobile, some nauplii or adult individuals may still be alive and present little movement of the appendages or in the gastrointestinal tract, in some cases (Nunes et al. 2006). Thus, the present review adopts the term "immobility" because we consider it as a more appropriate term regarding the technical view (Kos et al. 2016; Libralato et al. 2016; Johari et al. 2019).

Regardless of the terminology adopted for the endpoint (mortality or immobility), we grouped the two forms since the methodology for its analysis is the same: 10–15 s without movement. From all seventeen studies that used immobility as an endpoint, twelve tested MPs, while five were carried out with NPs. Concerning the studies performed with MPs, only six observed immobility, all using chronic exposure tests except for Peixoto et al. (2022). On the other hand, immobility was reported in 80% of the studies with NPs (Table 4). These data pose NPs as more toxic than MPs, corroborating the hypothesis that smaller plastic particles are potentially more harmful to the biota (Koelmans et al. 2015; da Costa et al. 2016). Nevertheless, the studies using NPs and brine shrimp are subject to variable parameters. Therefore, the performance of new studies, following a greater standardization, such as proposed by the ISO/TS 20787 (2017), can provide a better understating of the impacts of NPs on the microcrustaceans.

The development/growth of individuals exposed to MNPs was the third most examined endpoint (10 studies; Table 4). Six studies reported changes in development/growth, two with NPs (acute and chronic tests) and four with MPs (acute and chronic tests). The other studies did not observe alterations, including three with MPs (acute and chronic tests) and one with NPs (acute test). The measurement of the animal length was done using stereomicroscope or optical microscopy, and it was based on the distance from the cephalic region to the anus for all analyzed studies.

The ingestion of plastic particles can occupy/accumulate in the animal gut. It might harm the feeding and, thus, impact the development of organisms. Although feeding is crucial for brine shrimp development from the instar V stage, the short or long exposure tests showed no difference in the impact of the ingestion of plastic particles for this parameter. At the same time, no correlation was observed between particle type/size on the development/growth of brine shrimp. Nevertheless, there are few studies to affirm that time exposure and particle size do not influence the harmful effect of plastic particles on the development/growth of the microcrustaceans.

Table 4 Tests, endpoints, and observed effects obtained from publications on microplastics and nanoplastics using the genus *Artemia*

Reference	Test/exposure time	Concentration range	Endpoint	Effects
Bergami et al. 2016	Acute toxicity; recovery assay (NPs)/24 and 48 h	5.0 to 100 mg/L	Mortality; ingestion; accumulation; adhesion to the body externally; ecdysis; elimination of NPs	No mortality (PS-COOH and PS-NH_2); ingestion/accumulation (PS-COOH and PS-NH_2); adhesion to the body externally (PS-NH_2); molts present in the medium (PS-NH_2); PS-COOH present in the gut after recovery; PS-NH_2 not observed in the gut after recovery
Batel et al. 2016	Exposure to MPs/3, 6, 9, and 24 h	0.5 and 2.5 mg (~ 1.2×10^6 particles/20,000 nauplii)	Ingestion; trophic transfer of MPs in the presence or absence of POP (BaP)	Ingestion; trophic transfer of MPs to the fish; trophic transfer of BaP (POP) from brine shrimp to the fish
Cole 2016	Exposure to MFs/2 h	100 particles/mL	Ingestion/accumulation of MFs	MF ingestion
Bergami et al. 2017	Acute toxicity; Chronic toxicity (NPs)/48 h; 14 days	0.1–5.0 mg/L	Expression of developmental-related genes; mortality; accumulation; development	*clap* and *cstb* up-regulated (acute); no mortality (PS-COOH, chronic); presence of mortality (PS-NH_2, LC_{50} 0.83 mg/L, chronic); PS-COOH in the gut and feces (chronic); growth affected (PS-NH_2, chronic)
Gambardella et al. 2017	Acute toxicity (MPs)/24 and 48 h	0.001 to 10 mg/L	Mortality; accumulation; enzymatic activity: AChE, PChE, and CAT; swimming behavior	No mortality; ingestion, partial elimination, and accumulation in the gut; enzymatic activity affected; inhibition of swimming speed (24 h); increased swimming speed (48 h);
Kokalj et al. 2018	Acute toxicity; recovery assay (MPs) / 48 h	100 mg/L	Immobility; accumulation; elimination; development	No immobility; accumulation in the gut; elimination; development affected
Wang et al. 2019b	Acute toxicity; chronic toxicity; gut histological analysis (MPs)/24 h; 14 days	0.1 to 10,000 particles/mL	Mortality; ingestion level of MPs; cellular alterations on the gut; development	Ingestion (24 h and 14 d); lower concentrations were 12 particles/mL and 1.1 particles/mL (24 h and 14 d, respectively); no mortality; no development effects; ultrastructure alterations on gut epithelial cells
Mishra et al. 2019	Acute toxicity in adults (NPs)/24 h	0.5 to 200 mg/L	Mortality; biochemical profile (total protein, lipid peroxidation, oxidative stress)	Presence of mortality ($LC_{50} = 4.82$ mg/L; $LC_{50} = 8.79$ mg/L); morphological damage; decrease in total protein and levels of glutathione and catalase; increased lipid peroxidation
Peixoto et al. 2019	Chronic toxicity (MPs)/44 days	0.4 to 1.6 mg/L	Accumulation; mortality; growth; reproduction rate	Ingestion, partial elimination, and accumulation in the gut; no mortality; growth not affected; decrease in the reproductive (offspring number)

Table 4 (continued)

Reference	Test/exposure time	Concentration range	Endpoint	Effects
Varó et al. 2019	Acute toxicity; chronic toxicity (NPs)/48 h; 14 days	0.1 to 10 mg/L	Mortality; growth; alimentary behavior; enzymatic activity (ChE, CbE, CAT, GST, HSP70, lipid peroxidation); expression of developmental-related genes (*cstb*, *clap*, *tcp*, *hsp70*, and *hsp26*)	Presence of mortality and body size reduction (acute); presence of mortality, but growth not affected (chronic); feeding not affected (chronic); enzymatic activity affected (48 h and 14 d); gene modulation (48 h and 14 d)
Wang et al. 2019a	Acute toxicity; chronic toxicity; exposure to different concentrations, exposure time, and food supply; gut histological analysis (MPs)/1, 3, 6, 9, 12, and 24 h, 14 days	1.0 to 10^4 particles/mL	MP ingestion: influence of concentration, exposure time, and food supply; MP elimination; gut damage	The increase in concentrations and exposure time increased MP ingestion; the food supply decreased MP ingestion; partial elimination; gut alterations (24 h and 14 d)
Sendra et al. 2020	Exposure to NPS; post-exposure feeding; recovery assay (NPs)/3.5 and 24 h	0.006 to 0.6 mg/L	Ingestion of microalgae and NPs; post-exposure feeding behavior; accumulation; elimination	NP ingestion; microalgae ingestion not affected by NPs; NP ingestion was lower in the presence of microalgae; post-exposure feeding behavior not affected; NP accumulation (mandible, gut, and appendages); partial elimination
Trestrail et al. 2020	Exposure to MPs; toxicity of MP leachate (MPs)/2 and 24 h	1.0 mg/mL; 10 to 50 mg/mL (MP leachate)	Ingestion of two types of MPs; Mortality	Ingestion of both MPs types; presence of mortality; same level of toxicity ($LC_{50}=27.4$ mg/mL, regular MP; $LC_{50}=22.8$ mg/mL, bioMP)
Eom et al. 2020	Exposure to MPs; recovery assay; acute toxicity; chronic toxicity; exposure to different MP sizes; (MPs)/1 and 96 h; 30 days	1.0 to 1000 particles/mL	Influence of particle size; ingestion and elimination; enzymatic activity (*hsp70*, AChE, CAT, SOD); mortality	Ingestion of all MPs sizes; partial elimination; increased CAT and SOD activity; mortality affected by the particle size (30 days); inhibition of AChE activity
Suman et al. 2020	Acute toxicity; exposure to different development stage; chronic toxicity; gut histological analysis; transcriptomic analysis (MPs)/24 and 48 h; 14 days	1.0 to 100 mg/L	Mortality; development; accumulation; influence of the development stage; apoptosis; enzymatic activity; gut damage; differentially expressed genes related to metabolic and physiological processes	No mortality, accumulation, and growth alterations (acute); presence of mortality ($LC_{50}=16.995$ mg/L), accumulation and growth alterations (chronic); presence of apoptosis (48 h and 14 days); enzymatic activity alterations; gut alterations (acute and chronic); alterations in gene expression in important pathways (immune response, oxidative stress, and apoptosis)
Bour et al. 2020	Exposure to MPs/MFs; exposure to different particle shapes and food supply; recovery assay (MPs and MFs)/5 min; 2 h	100 to 500 particles/mL	Influence of shape and feeding in the ingestion and elimination; trophic transfer	No food: little ingestion of MFs and fast elimination; large ingestion of MPs and slow elimination; food supply: no ingestion of MFs, little ingestion of MP and fast elimination; trophic transfer of MPs to the fish

Table 4 (continued)

Reference	Test/exposure time	Concentration range	Endpoint	Effects
Han et al. 2021	Chronic toxicity; exposure to different temperatures; gut histological analysis (MPs)/14 days	0.2 and 2.0 mg/L	Mortality; growth; influence of temperature on the effect of MPs; enzymatic activity: ACP and CAT; expression of apoptosis-related genes (ADRA1B and *CREB3*) gut damage	MPs and temperature affected the mortality and decreased growth; ACP activity increased; *ADRA1B* and *CREB3* modulation; gut alterations;
Kim et al. 2021	Acute toxicity; gut permeability analysis (MFs)/48 h	75 to 1000 mg/L	Mortality; ingestion; gut damage	Ingestion; presence of mortality (PET > PP); no mortality with natural MF; severe gut damage (PET); decreased gut width (all MFs); increased permeability of the intestinal layer in the head region; PET was more harmful than PP
Machado et al. 2021	Acute toxicity; exposure to different agitation methods and development stage; co-exposure assay with the reference toxicants $K_2Cr_2O_7$ and $CuSO_4$ (NPs)/24 and 48 h	0.005 to 5.0 mg/L	Immobility; accumulation; influence of medium agitation and development stage; development; effect of the co-exposure on the toxicity	Presence of immobility; accumulation in the gut; adhesion to the body externally; no influence of agitation; instar III: the most sensitive stage; development not affected; co-exposure $K_2Cr_2O_7$ + NPs: increased immobilization rate (48 h); co-exposure $CuSO_4$ + NPs: decreased immobilization rate (48 h)
Thiagarajan et al. 2021	Acute toxicity; co-exposure assay with TiO_2 nanoparticles/48 h	1.0 mg/L (~ 8.93×10^9 particles/mL)	Mortality; enzymatic activity (ROS); effect of the co-exposure on the toxicity Ti accumulation	No mortality; enzymatic activity not affected; co-exposure: no toxicity alterations and in Ti accumulation;
Li et al. 2021	Chronic toxicity/45 days	100 mg/L (1.6×10^6 items/m^3) and 100 mg/L (1.1×10^6 items/m^3)	ingestion; growth; effect on the structure of gut microbiota	Ingestion of both MPs types; accumulation; MPs exposure decreased growth rate; both MPs increased the diversity and richness of gut microbiota
Albendín et al. 2021	Acute toxicity co-exposure assay with pesticides (triclosan and chlorpyrifos) and pharmaceutical products (simvastatin and carbamazepine)/48 h	0.26 to 1.6 mg/dm^3	Mortality/immobility; ingestion; effect of the co-exposure on the toxicity; enzymatic activity: ChE	Ingestion; no mortality; co-exposure carbamazepine + MPs: alteration in mobility and decrease in ChE activity
Peixoto et al. 2022	Acute toxicity; chronic toxicity/48 h; 5 days	0.4 and 1.6 mg/L	Mortality; growth; ingestion; feeding behavior; enzymatic activity: ChE, CbE, GST, CAT, and GR	No mortality, growth, and feeding behavior alterations (nauplii); survival decrease (juveniles); ingestion, accumulation, and adhesion to the body externally; increased enzymatic activity (nauplii); ChE inhibition and antioxidant enzymes decrease (juveniles)
Kim et al. 2022	Exposure to NPs/1 h	1 mg/L	Ingestion; trophic transfer of NPs	Trophic transfer of NPs to the fish

Table 4 (continued)

Reference	Test/exposure time	Concentration range	Endpoint	Effects
Jeyavani et al. 2022	Acute toxicity; gut histological analysis (MPs)/48 h	1 to 100 mg/L	Mortality; ingestion/accumulation; gut damage; enzymatic activity (CAT, SOD, GST, and AChE)	Mortality (nauplii); ingestion/accumulation; swimming behavior alteration (juveniles); enzymatic activity alterations; presence of gut damage

$N = 25$ studies

BaP benzo[a]pyrene, *POP* persistent organic pollutants, CL_{50} lethal concentration 50%, *AChE* acetylcholinesterase, *PChE* propionylcholinesterase, *CAT* catalase, *ChE* cholinesterase, *CbE* carboxylesterase, *GST* glutathione-S-transferase, *HSP70* heat shock protein 70, *ACP* acid phosphatase, *SOD* superoxide dismutase, *ROS* reactive oxygen species, $K_2Cr_2O_7$ potassium dichromate, $CuSO_4$ copper sulfate, TiO_2 titanium dioxide nanoparticles, *Ti* titanium, *GR* glutathione reductase

Enzymatic alterations were also another frequent endpoint (10 studies). The foremost results were related to oxidative stress and neurotoxicity, mainly the activity of the enzymes catalase (CAT) and acetylcholinesterase (AChE), respectively. Virtually all studies using this endpoint reported biochemical alterations in the organisms: three studies with NPs (2 acute studies and 1 acute and chronic study) and six with MPs (4 chronic studies and 3 acute and chronic studies). The only exception was an acute test study with MPs. It is noticeable that enzymatic activity is a sensitive endpoint to MNP exposure, especially in short-term assays, in which other effects are not always evident (Gambardella et al. 2017; Suman et al. 2020). For these reasons, the present review suggests further exploration of this endpoint in studies on the toxicity of MNPs in brine shrimp. Furthermore, to better understand the deleterious effect of MNPs on brine shrimp, we suggest carrying out further molecular and biochemical investigations to elucidate the toxicological pathway underlying MNP toxicity.

Five studies used gene expression as an endpoint: three with MPs (acute and chronic tests) and two with NPs (acute and chronic tests). Investigations were performed with genes related to ecdysis/development, apoptosis, and oxidative stress. The transcriptomic analysis also investigated differentially expressed genes related to metabolic and physiological processes. Genetic alterations were observed in all studies. Hence, these results can help to confirm, at the molecular level, the findings from other endpoints, such as development, oxidative stress, and apoptosis (the latter will be discussed at the end of the current topic).

As well as the enzymatic activity, gene expression showed to be a sensitive endpoint since the alterations were also observed in short-exposure assays. Furthermore, the investigation of gene expression, combined with enzymatic activity, seems to be an interesting tool for elucidating the mechanisms of MNP toxicity. Using molecular approaches, Varó et al. (2019) contributed to the understanding of the mechanism involved in the toxic effect of NPs on the ecdysis and development of brine shrimp previously reported by the same group (Bergami et al. 2016, 2017). Despite the technical and budgetary constraints associated with molecular approaches in toxicological studies, the inclusion of molecular endpoints is encouraged whenever possible to support the physiological and morphological endpoints.

Gut damage was an endpoint investigated in seven studies. The principal assessments were the histological analysis of the intestine (5 studies) and analysis of gut permeability (1 study). Six studies showed intestinal alterations, five using MPs (acute and chronic exposures) and one with MFs (acute exposure). Alterations in epithelial cells (Wang et al. 2019b; Han et al. 2021) and the decomposition of enterocytes (Wang et al. 2019a) were the most damage described. Wang et al. (2019a) reported gut damage despite low amounts of plastic particles in the intestine or particle elimination. Studies also mention harmful effects on the gut in acute exposure assays, even when larval immobility was not observed (Wang et al. 2019b; Suman et al. 2020). Exposure to PS and PE MPs also increased gut microbiota diversity and richness (Li et al. 2021). Since most studies reported the accumulation of plastic particles in the intestine of brine shrimp, the investigation of the anatomical and physiological integrity of the gastrointestinal tract is essential to assess the risk of exposure to plastics in these organisms. It is especially relevant to long-exposure tests using NPs since studies on damage to the gastrointestinal tract are still restricted to MPs.

The least represented endpoints were swimming (1 study; MP, acute test), reproductive rate (1 study; MP, chronic test), apoptosis (1 study; MP, acute and chronic test), and feeding behavior (1 study; MP, acute and chronic test). Swimming and reproduction have been addressed by different authors as relevant endpoints for toxicological studies with brine shrimp (Manfra et al. 2015, 2016; Kos et al. 2016; Libralato et al. 2016). Nonetheless, these endpoints are still not well explored in studies with plastic particles. Gambardella et al. (2017) demonstrated that MPs affected the nauplii swimming, indicating its use in studies about the effect of plastic on zooplankton. Peixoto et al. (2019) showed the negative impacts of MPs on the reproduction of brine shrimp, which is another significant point to evaluate. The inclusion of swimming and reproduction as endpoints should be encouraged, as they represent relevant processes for brine shrimp's physiology. It is especially valid for studies with NPs, given the absence of data concerning the effect of particles on these vital processes for microcrustaceans.

Apoptosis analysis may represent a viable strategy for ecotoxicological investigations. Recently, Suman et al. (2020) observed apoptosis in brine shrimp exposed to MPs using acridine orange dye, both in acute and chronic exposures. The use of apoptosis as an endpoint is an experimental alternative for determining the survival of brine shrimp, solving doubts about the issue of immobility/survival inherent to the observation tests of larvae motility.

Realistic exposure scenarios

Most ecotoxicological studies have investigated the effects of contaminants/pollutants under controlled laboratory conditions (Bour et al. 2015). Nevertheless, to understand the actual effect of these particles on the biota, it is essential to carry out investigations that consider the complexity of the natural environment (Bour et al. 2015; Lenz et al. 2016; Han et al. 2021).

Some works involving plastic particles and the genus *Artemia* aim to establish experimental protocols that mimic the natural environment. We identified nine studies that intended to replicate realistic exposure scenarios through their protocols. Three approaches were used: (i) effect of different parameters (3 studies), (ii) co-exposure of plastic particles with other compounds (5 studies), and (iii) trophic transfer (3 studies) (Table 4).

The investigation on the effect of different parameters contributes to the understanding of the toxic effects of plastic particles in a realistic scenario. The following parameters were tested: particle concentrations (1 study), exposure times (1 study), food availability (2 studies), temperatures (1 study), agitation of the medium (1 study), particle sizes (1 study), and particle shapes (1 study) (Table 4). Except for particle size and medium agitation, all other aspects influenced the ingestion of plastic particles or their toxic effect.

The influence of these parameters on the toxicity of MNPs appears in the previous sections of this work, where we discussed how these factors modulate the physicochemical properties of the particles and the animal behavior. It is possible to notice that studies focusing on realistic scenarios are recent and still not very representative in the area. Nonetheless, the trend is that ecotoxicological studies increasingly include realistic scenarios of exposure to MNPs in their experimental designs, allowing a more reliable analysis of the effects of plastic particles on ecosystems.

Five works investigated the effect of the co-exposure of plastic particles with other compounds, each one combining different categories of contaminant: persistent organic pollutant (POP), metals, TiO_2 nanoparticles, MP leachate, pesticides, and pharmaceutical products. Plastic particles have a high potential for adsorption of inorganic, organic, or even pathogenic contaminants on their surfaces (Montagner et al. 2021; Cholewińska et al. 2022; do Prado Leite et al. 2022; Nugnes et al. 2022). Among them, we can highlight contaminants such as metals, POPs, and other nanomaterials (Mato et al. 2001; Holmes et al. 2012; Amelia et al. 2021; Thiagarajan et al. 2021). Furthermore, additives and monomers present in plastics, which are substances potentially harmful to the environment, can leach from the particles (Fred-Ahmadu et al. 2020). Considering the high interaction of plastic particles with biota, MNPs can become vectors of these materials, increasing their accumulation in organisms (Mato et al. 2001; Shen et al. 2019). Four of the five studies presented here showed that plastic particles might be harmful to biota when in a mixture with other potentially toxic compounds, such as metals (Machado et al. 2021), pesticides, and pharmaceutical products (Albendín et al. 2021). The study on the combination with TiO_2 nanoparticles was the only one that showed no changes in toxicity after mixing. Therefore, it is needed to expand the co-exposure studies of MNPs with other contaminants/pollutants to increase the knowledge about the potential risk of plastic particles as vectors of potentially toxic compounds.

Only three studies investigated trophic transfer. Two studies used MPs and one was carried out with NPs. All papers showed that plastic particles transfer to the next trophic level in the food chain (Batel et al. 2016; Bour et al. 2020; Kim et al. 2022). One of the studies also investigated the trophic transfer of POP via MPs (Batel et al. 2016). The authors demonstrated the transfer of benzo[a]pyrene (POP) present in the MPs from brine shrimp to fish. Thus, it confirms the implications of the effect of plastic particles as vectors of other pollutants. It is interesting to emphasize that MNPs, besides the ingestion by brine shrimp, can be adhered to the surface of their body, as discussed above. It also contributes to the ingestion of plastic particles by constituents of higher levels of the trophic chain.

Thus, even if some plastic particles do not show direct toxicity to microcrustaceans, these particles may still be harmful to the ecosystems. Our review points out that MNPs can cause negative impacts on other organisms through their transfer along the food chain or by combination with toxic compounds present in the environment. Therefore, we strongly encourage further research about the effects of MNPs in realistic exposure scenarios for a better understanding of their consequences to the environment.

Conclusion, main gaps, and future recommendations

The present work carried out a critical bibliographic review of the toxicological studies of MNPs in the genus *Artemia*, presenting the main methodological aspects used and the toxic effects reported in the literature. From these datasets, it was possible to realize the main limitations of the field as well as suggest the directions for future research.

The lack of standardized protocols and incomplete descriptions of methodological procedures significantly limit the quality and reliability of toxicological studies of plastic particles with brine shrimp. There were several attempts to standardize toxicological studies with the genus *Artemia* over the years, from protocols by different environmental agencies to scientific papers. Despite that, the problem is still persistent in the area. It becomes even more urgent given the peculiarities of the chemical nature and physicochemical properties of MNPs in aquatic environments.

The lack of standardization makes it difficult to compare the results obtained by different studies. The problems range from differences in procedures related to the preparation of plastic particle suspensions to issues already well discussed in the literature, such as larvae culture conditions and the stage of development at which the toxicological tests are initiated.

In part, the lack of standardization of experimental procedures can be attributed to the fact that toxicological studies with MNPs and brine shrimp represent a recent area that is still expanding. The publication of the ISO/TS 20787 (2017) may change this trend if the different research groups adhere to it. It is also up to the scientific community, during the process of scientific article review, to demand that experimental procedures be described in a very detailed manner in the methodology sections of the scientific papers. Only in this way it will be possible, over time, to build a more unified knowledge about the effects of these polymers on brine shrimp and, consequently, contribute to the understanding of the impact of MNPs on ecosystems more precisely.

An important aspect to highlight is the development of studies under realistic exposure scenarios. Despite reports of the toxic effects of MNPs, and that they pose a potential risk to ecosystems, further progress is needed. Few studies conducted investigations focusing on a realistic exposure scenario. Thus, the actual effect of plastic particles on ecosystems is still hard to estimate. Conducting studies focusing on the complexity found in the environment, both in terms of exposure conditions and the flow of these particles through the food web, can more accurately reveal the impact of MNPs on ecosystems.

The design of ecotoxicological studies focusing on environmentally relevant exposure conditions, considering temperature, salinity, and the characteristics, concentrations, and interactions of plastic particles in the environment, including mixtures with other contaminants/pollutants, is crucial to advance the knowledge in the area.

The flow of plastic particles through the food web has been the main environmental consequence cited by studies that report the accumulation of plastic particles in aquatic organisms. Studies with brine shrimp and the flow of plastic particles in the food chain need to be extended for a better understanding of this phenomenon of biomagnification and its consequences for ecosystems. *Artemia* has proven to be an excellent experimental model and very suitable for answering questions inherent to the flow of plastics along the food chain. Thus, it is expected that more studies will address the trophic transfer of plastic particles in the coming years.

The lack of diversity in the chemical composition of the plastic particles studied was another gap observed by the present review, mainly due to the ease of obtaining PS particles. Although few studies have been carried out with other polymers, they have already shown the damage these polymers can cause to the biota (Batel et al. 2016; Cole 2016; Kokalj et al. 2018; Peixoto et al. 2019, 2022; Bour et al. 2020; Trestrail et al. 2020; Kim et al. 2021; Li et al. 2021; Jeyavani et al. 2022). Thus, future studies should investigate the toxicological effect of MNPs with different chemical natures, including, above all, those with a high prevalence in the environment.

Briefly, plastic particles are currently considered to be relevant stressors for zooplankton. Thus, plastic can generate harmful consequences for ecosystems. Due to their ecological position at the base of the food chain, ease of use in laboratories, extensive knowledge of their biology, and wide use in toxicological studies, brine shrimp are strategic animals for ecotoxicological research about the effects of plastic particles on the biota. We encourage the development of studies with other microcrustaceans to address a deeper understanding of the effect of plastic particles in the group. The expansion of studies in the area is promising and can contribute mainly from the standardization of protocols to a better understanding of the environmental impact of plastic particles. Moreover, these investigations can collaborate with public policies for the handling and disposal of plastic materials.

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