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Review

Progress in Research on the Bioavailability and Toxicity of Nanoplastics to Freshwater Plankton

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Abstract: The present review critically examines the advancements in the past 5 years regarding research on the bioavailability and toxicity of the nanoplastics (NPLs) to freshwater plankton. We discuss the recent progress in the understanding of adsorption, absorption, trophic transfer, and biological effects in phyto- and zooplankton induced by NPLs exposure. The influence of plankton on NPLs' bioavailability via the excretion of biomolecules and formation of eco-corona is also examined. Despite important research developments, there are still considerable knowledge gaps with respect to NPLs' bioavailability and trophic transfer by plankton as well as a potential adverse effect in natural aquatic systems. As plankton play a critical role in primary production, nutrient cycling, and food web structure, understanding the interactions between NPLs and plankton is essential in assessing the potential implications of NPLs pollution for aquatic ecosystem biodiversity and services.

Keywords: nanoplastics; uptake; toxicity; trophic transfer; algae; cyanobacteria; *Daphnia*; extracellular polymeric substances; eco-corona

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1. Introduction

The use of various plastic materials is continuously growing, leading to an increase in plastic waste. Consequently, the concentrations of micro- and nanoplastics (MPLs/NPLs) in the environment are increasing [1–3]. In parallel, the concerns about the environmental implications of NPLs are rising [4,5]. Plastic particles would be defined as nanomaterials if 50% or more of the plastic particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm [6]. However, most frequently, the term NPLs is used for materials within the size range 1–1000 nm [7].

Currently, NPLs, originating from primary or secondary sources, are the least studied area of plastic pollution; however, they are potentially the most hazardous given their small size and high specific surface area [4,5]. Indeed, under environmental conditions, various plastic debris can release a large amount of NPLs through various physical and chemical degradation pathways, as comprehensively reviewed by [8]. NPLs are characterized by a very small size and high reactivity, which distinguishes them from microplastics [9]. NPLs interact with different abiotic and biotic components in aquatic environments, and thus, they are transformed by various interconnected processes involving aggregation, sedimentation, chemical and physical alterations, etc., which greatly affect the particles' properties, reactivity, fate, and impact [3,5,8,10,11].

Given the limitations of existing analytical techniques, the concentrations of NPLs in the environment have not yet been measured. However, estimates show that more than 5 trillion pieces with a size between 300 µm and 5 mm are floating in the ocean [12]. As the smaller particles are usually more abundant in number, it can be assumed that a comparable

or larger number of NPLs will be present in the aquatic environment. Multi-media model estimations give average concentrations of NPLs in surface water of 280 μg L^{-1} [13]. More recently, NPLs' abundance has been estimated to be in the range 0.3–488 μg L^{-1} in freshwater environments, which are higher than those for marine environments (2.7–67 μg L^{-1}) [14]. These concentrations are lower as compared to the predicted no-effect concentrations (PNECs) derived from probabilistic species sensitivity distributions, resulting in values of 99 μg L^{-1} and 72 μg L^{-1} for freshwater and marine datasets [15]. They are also below the estimated hazard concentration affecting 5% of the species (HC5) of 410 μg L^{-1} for marine plankton for two types of materials, polymethylmethacrylate (PMMA) and polystyrene (PS) [16], as well as HC5 for NPLs with a size of 50 nm in freshwater, 187.9 (8.0–2978.3) μg L^{-1} [17]. Nevertheless, some hotspots of NPLs pollution can be of risk for aquatic biota. Critical gaps in NPLs research and their connection to risk assessment show numerous open questions that are vital to assessing risk and the necessity of considering the smallest plastic particles, namely, their sources, fate and transport, exposure measures, toxicity, and effects [18].

Significant attention is paid to NPLs' bioaccumulation and adverse effects at high levels of trophic chains [14]. Advances in the understanding of their absorption, distribution, metabolization, and excretion within organisms' bodies have been recently put into perspective and revealed many challenges [19]. The accumulation of NPLs in aquatic organisms results in adverse effects on different freshwater organisms [3–5,8,10,14,20–26]. A plethora of such effects, including oxidative stress and damage, inflammation, altered development, reduced growth, energy and movement, genotoxicity, etc., have been reported, as recently reviewed in [14,22,25,27–30]. Different factors, such as particle nature, concentration and size, exposure time, and co-factors such as contaminants, food availability, species, developmental stage, and environmental conditions, were also thoroughly discussed in [22,28,30,31].

In the present review paper, we comprehensively review the progress in the past 5 years concerning the bioavailability and toxicity of NPLs to freshwater plankton. Biological availability (or bioavailability) is understood as "the extent of absorption of a substance by a living organism compared to a standard system" [32]. Bioavailability is considered a key concept allowing one to quantitatively relate changes in (dissolved or particulate) pollutant concentrations, including nanoparticles, with the intensity of the biological response in biota [33,34].

Plankton, consisting of free-floating phytoplankton and zooplankton, represent a critical component of aquatic ecosystems, playing a central role in driving primary production, shaping food webs, and influencing nutrient cycling [35]. Therefore, understanding the interaction between NPLs and plankton is essential in assessing the potential consequences for aquatic ecosystems. Their contributions to primary production, nutrient cycling, and trophic interactions are pivotal to maintaining biodiversity and ecosystem services.

In this paper, we especially focus on NPLs uptake and toxicity, which can be considered results of several key processes (Figure 1). (i) First, there is adsorption on plankton: NPLs diffuse towards and can adsorb onto the surfaces of plankton, including both phytoplankton and zooplankton. (ii) NPLs adsorbed by plankton can penetrate (or not) the cell wall and membrane of phytoplankton species. Zooplankton can ingest NPLs along with their regular diet of suspended particles. Once ingested, the NPLs may be retained within the digestive tracts or tissues. (iii) There is also trophic transfer from phytoplankton to zooplankton: NPLs enter the food web at lower trophic levels, such as phytoplankton, and subsequently could be consumed by higher-trophic-level organisms, including zooplankton. (iv) Biological effects and NPLs' transformations. NPLs can degrade within the bodies of planktonic organisms, releasing smaller plastic fragments or chemical components that can be further absorbed and retained by the organisms. (v) Lastly, there is excretion of the NPLs. In addition to the above-mentioned processes, in the present review, we will discuss how the plankton species can affect the bioavailability of the NPLs by secreting different biomolecules.

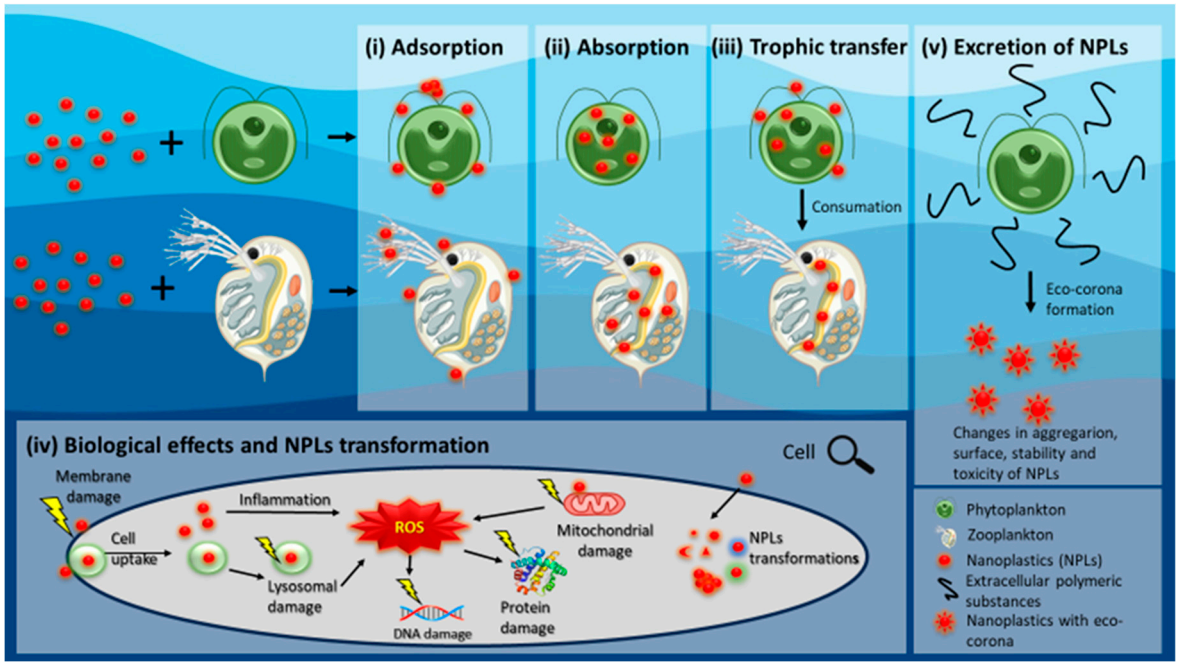

Figure 1. Key processes involved in the bioavailability and toxicity of NPLs by phyto- and zooplankton.

Similarly to the other nano-sized materials, the bioavailability and toxicity of NPLs to plankton depend on various factors, including (i) the type and characteristics of the NPLs, such as their chemical composition, size, shape, surface functionalization, etc.; (ii) the physicochemical parameters of the aquatic environment, including pH, water hardness and alkalinity; (iii) the presence and concentrations of other dissolved, nanoparticulate and colloidal forms of contaminants; (iv) the presence and concentrations of different ligands from natural and anthropogenic sources, which could absorb onto the NPLs, influencing their fate and effect; (v) plankton characteristics, such as type, cell wall composition, differentiation stage and pathways of particle uptake and cellular processing.

2. Interactions of NPLs with Freshwater Phytoplankton Species

Table 1 summarizes the most recent studies published in the last 5 years focusing on the interactions between NPLs and microalgae and cyanobacteria. The progress in this field is discuss below.

2.1. Advances in Research on the Bioavailability of NPLs to Phytoplankton

A recent review delved into the uptake and effects of NPLs on microalgae [27]. Like engineered nanoparticles, potential mechanisms governing their bioavailability might encompass absorption onto microalgae, penetration into the cell via endocytosis or physical damage, and the shading and blocking of substance and energy exchange with the surrounding medium [27].

However, scientific evidence regarding the bioavailability of NPLs to phytoplankton remain considerably limited. Both amidine and carboxyl-functionalized polystyrene NPLs (PS-COOH) were adsorbed onto the marine diatom *Dunaliella tertiolecta*. However, only amidine PS induced the inhibition of algal growth, with an effective concentration for 50% of the algal population, EC50 of 12.97 μg mL^{-1} [36]. In another study, fluorescent-blue 50 nm amino-modified polystyrene (PS-NH_2) adsorbed onto the diatom *Chaetoceros neogracile*, causing impairment to the photosynthetic machinery and an increase in reactive oxygen species (ROS) production at both low (0.05 μg mL^{-1}) and high (5 μg mL^{-1}) exposure concentrations [37]. The adsorption onto the cells of green alga *Pseudokirchneriella subcapitata* was significantly higher for neutral and positively charged PS-NH_2 at a concentration of 100 mg L^{-1}. Conversely, negatively charged PS-COOH exhibited minimal adsorption

onto the algal cell wall, but this increased with water hardness [38]. These examples underscore the important role of the surface charge of primary NPLs particles and the specificity of interactions with different algal species. The rapid ad/absorption of PS onto/in *Phaeodactylum tricornutum* was evidenced through an observed increase in cell complexity, size and microalgae fluorescence induced by 100 nm fluoresbrite [39].

Fluorescent 51 nm PS attached to and penetrated the outer layer of *Chlamydomonas reinhardtii* during cell division [40]. In a recent study involving metal-doped PS, it was revealed that more than 60% of Fe-PS or Eu-PS remained associated with algal cells of *P. subcapitata* after 72 h [41]. A pioneer study unveiled that fluorescent aggregation-induced emission fluorogens-incorporated nanoparticles (AIE-NPs) sized at 40, 70, and 85 nm were internalized intracellularly via clathrine-dependent endocytosis in *C. reinhardtii*, while the 140 nm AIE-NPs remained attached to the surface [42]. Importantly, the authors demonstrated the involvement of endocytosis, algal cell membrane permeability, and exopolymer substance thickness in these processes and their cell cycle dependence [42].

2.2. Advances in Research on the Toxicity of NPLs to Phytoplankton

A recent review paper systematically presented the toxicological effects of MPLs/NPLs on phytoplankton and aquatic environment [43]. Additionally, the behavior and adverse effects of PS NPLs with positive and negative surface charges to marine plankton were reviewed [44]. For example, exposure of the marine green microalga *Platymonas helgolandica* to 70 nm PS beads at concentrations of 20, 200, and 2000 μg L^{-1} resulted in inhibited algal growth after 3 days, which was followed by stimulation after 5 days of exposure. Higher concentrations (200 and 2000 μg L^{-1}) of PS led to increased membrane permeability and mitochondrial membrane potential, reduced light energy utilization in photochemical processes of microalgae, and caused damage to cell morphology and organelle function [45]. Exposure to 100 nm PS at concentrations ranging from 10 to 200 mg L^{-1} resulted in the stimulation of growth in the green alga *Scenedesmus quadricauda*. At the higher concentration of 200 mg L^{-1}, PS induced an increase in chlorophyll content, soluble proteins, and polysaccharides and an enhancement in the antioxidant enzyme activities [46]. Exposure to 10–100 mg L^{-1} of 100 nm PS led to dose-dependent adverse effects on *Chlorella pyrenoidosa* growth, which was observed from the lag to the earlier logarithmic phases. However, during the transition from the end of the logarithmic to the stationary phase, *C. pyrenoidosa* demonstrated resilience to the adverse effects of NPLs. This was achieved through mechanisms such as cell wall thickening, algae homo-aggregation, and algae-NPLs hetero-aggregation, consequently triggering an increase in algal photosynthetic activity and promoting growth. As a result, the cell structures reverted to a normal state [47]. Further studies have documented that the exposure of various algae and cyanobacteria to different NPLs led to oxidative stress, membrane damages, and alterations in photosynthesis. Specifically, 50 and 100 nm PS beads induced increased oxidative stress biomarkers, damage to the photosynthetic apparatus, DNA damage, and the depolarization of mitochondrial and cell membranes in the marine diatom *P. tricornutum* after 24 h exposure to concentrations starting from 5 mg L^{-1} [39].

Exposing *C. pyrenoidosa* to 80 nm PS at concentrations of 5–50 mg L^{-1} for 24–48 h resulted in decreased algal growth, chlorophyll a level, and Fv/Fm by 27.73%, 29.64%, and 11.76%, respectively. However, these effects decreased after an exposure duration of 96 h. Transcriptomic analysis revealed that NPLs inhibited the gene expression of aminoacyl tRNA synthetase and the synthesis of related enzymes and proteins at low concentration (10 mg L^{-1}), while at high concentration (50 mg L^{-1}), they affected DNA damage repair and hindered photosynthesis [48].

A 28-day exposure *of Chlorella vulgaris* to carboxyl-functionalized and non-functionalized PS sized at 20 and 50 nm, at a concentration of 250 mg L^{-1}, resulted in reduced algal cell viability and chlorophyll a concentration. Additionally, it led to an increase in lactate dehydrogenase activity and ROS concentration. These exposures also caused an increase in cell size, deformed the cell wall, and increased the volume of starch grains [49]. In

another study, exposure of *Euglena gracilis* to 100 nm PS at 50 mg L^{-1} inhibited the growth of *E. gracilis* by 35.5% over a 96 h period. This effect was significantly higher than the impact of 5 µm PS (27.9%) within the same exposure duration. Both sizes of PS significantly reduced pigment contents, altered superoxide dismutase (SOD) and peroxidase (POD) activities, and dysregulated the expression of genes involved in cellular processes, genetic information processing, organismal systems, and metabolisms [50].

In contrast, 5 µm PS at 1 mg L^{-1} exhibited stronger growth inhibition and physiological toxicity compared to 100 nm PS during 96 h of exposure to *E. gracilis* [51]. Positively charged PS-NH_2 sized at 50 nm induced growth inhibition in the cyanobacterium *Synechococcus elongatus* with a 48 h EC50 of 3.81 mg L^{-1}. The main toxicity mechanisms included oxidative stress, disruption of glutathione metabolism, and damage to membrane integrity [52]. Conversely, negatively charged PS-NH_2 at a size of 500 nm and a concentration of 2.5 µg mL^{-1} significantly reduced cellular esterase activity and neutral lipid content, indicating a cellular adaptation of energy metabolism in response to stress [53]. The exposure of *C. reinhardtii* to increasing concentrations (5, 25, 50 and 100 mg L^{-1}) of 300–600 nm PS resulted in decreased chlorophyll a fluorescence yields and photosynthetic activities. The PS beads adhered to the surface of microalgae, causing membrane damage [54]. Pd-doped PS-NPLs influenced the growth of both the filamentous cyanobacterium *Anabaena* sp. (72 h EC50 of 151.3 ± 22.5 mg L^{-1}) and the green alga *C. reinhardtii* (72 h EC50 of 247.8 ± 32.7 mg L^{-1}), indicating the higher sensitivity of the cyanobacterium to PS compared to the green alga. Both algae exhibited a dose-dependent overproduction of ROS, membrane damage, and metabolic alterations. However, ROS overproduction and damages were less pronounced in *C. reinhardtii* [55]. A 96 h exposure to PMMA resulted in a species-specific reduction in the growth rate of several marine microalgae: *Tetraselmis chuii* (EC50 of 132.5 mg L^{-1}), *Nannochloropsis gaditana* (EC50 of 116.5 mg L^{-1}), *Isochrysis galbana* (EC50 of 123.8 mg L^{-1}) and *Thalassiosira weissflogii* (EC50 of 83.4 mg L^{-1}) [16]. Additionally, both PMMA and PMMA-COOH induced the overproduction of pigments, loss of membrane integrity, hyperpolarization of the mitochondrial membrane, increased production of ROS and lipid peroxidation, decreased DNA content and reduced photosynthetic capacity in the red marine alga *Rhodomonas baltica*. This interaction with cell walls was suggested to lead to the formation of small holes in the lipid layer by PMMA, potentially resulting in the permeabilization and internalization of small PMMA aggregates.

Exposure to PMMA-COOH resulted in reduced algal growth, which was attributed to alterations of cell cycle leading to decreased cell viability, metabolic activity, and photosynthetic performance [56]. However, no specific findings were provided regarding the possible uptake or cell association to the algae. PS-NPLs were internalized in the *Anabaena* sp., triggering an excessive generation of ROS, lipid peroxidation, membrane disruptions, intracellular acidification, and a decrease in photosynthetic activity. When exposed in combination with poly(amidoamine) dendrimers of generation 7 (G7), the cellular internalization of PS decreased, subsequently lowering their adverse effects [57]. Recently, a significant increase in the teratological frequency was observed in the diatom *Cocconeis placentula* when exposed to 0.1 µg L^{-1} of poly(styrene-co-methyl methacrylate) (P(S-co-MMA)) with an average size of 425.70 ± 175.02 nm. However, no effect on diatom growth was observed within the concentration ranges of 0.1, 1, 100, or 10,000 µg L^{-1} for 28 days [58].

New evidence has emerged, shedding light on the effects of secondary NPLs to phytoplankton species. A 48 h exposure to 1 µg L^{-1}–10 mg L^{-1} of reference polyethylene (PE) or NPLs derived from PE collected in the North Atlantic gyre (PEN) was conducted on two microalgae: the green alga *Scenedesmus subspicatus* and the diatom *Thalassiosira weissflogii*. Interestingly, this exposure had no discernible influence on the cell growth of *T. weissflogii*, while it resulted in the growth inhibition of *S. subspicatus* upon PEN exposure [59].

Table 1. Selected examples of the most recent studies (<5 years) researching phytoplankton interactions with NPLs.

Species	Type of NPLs	Size of NPLs	Concentration	Duration	Observed Effects	Reference
Alexandrium tamarense (marine dinoflagellate)	PS (plain)	100 nm 1 µm	0, 0.1, 1, 5, 10, 50, 100 mg L^{-1}	4 days	Inhibition of growth, photosynthetic production, and extracellular carbonic anhydrase activities stronger in MPLs than in NPLs. Intracellular paralytic shellfish toxins production stimulated by NPLs and decreased by MPLs.	[60]
Anabaena sp. (freshwater cyanobacteria) *C. reinhardtii* (freshwater green algae)	PHB (polyhydroxybutyrate, mechanically broken-down)	200 nm	0, 50 mg L^{-1}	3 days	Decrease in growth, increase in ROS production and membrane damage, secondary NPLs may be more toxic than primary. Biodegradable plastics show the same toxic effects to organisms as non-biodegradable.	[61]
Chlorella sp. (freshwater green algae)	PS (plain) PS-NH_2 PS-COOH	200 nm	0, 1 mg L^{-1}	3 days	EPS aged NPLs significantly lowered the oxidative stress and cytotoxic impact, eco-corona may change the way NPLs interact with the organisms.	[62]
Chlorella vulgaris (freshwater green algae)	PS (plain) PS-COOH	20, 50, 500 nm	0, 250 mg L^{-1}	28 days	Smaller NPLs have a higher impact—decrease in algal viability and pigments; increase in ROS, lactate dehydrogenase activity and starch grains content; shrinkage in cell wall. Bigger PS could aggregate and sediment making them non-bioavailable.	[49]
Chlorella pyrenoidosa (freshwater green algae)	PS (plain)	100 nm 1 µm	0, 10, 50, 100 mg L^{-1}	30 days	Hetero- and homoaggregation observed, EPS production increased, during the first phase, growth rate and photosynthesis decreased, while in the second phase, growth and photosynthesis recovered.	[47]
	PS (plain)	80 nm	0, 5, 10, 20, 30, 40, 50 mg L^{-1}	4 days	Strong inhibition of growth, photosynthetic pigments and efficiency after 24–48 h, after 96 h inhibition lowered. Heteroaggregation, ROS production, gene expression changes, membrane and DNA damage observed.	[48]
Chlamydomonas reinhardtii (freshwater green algae)	PS (plane)	300–600 nm	0, 5, 25, 50, 100 mg L^{-1}	10 days	A decrease in growth, photosynthetic activity and EPS follows an increase in concentration, observed higher soluble proteins and membrane damage.	[54]
	PS (fluorescent)	51 nm	0, 20, 40, 60, 80, 100 mg L^{-1}	2 days	Adsorbed to the surface of algae, passing into the outer layer when the cell is dividing.	[40]

Table 1. *Cont.*

Species	Type of NPLs	Size of NPLs	Concentration	Duration	Observed Effects	Reference
Cocconeis placentula var. lineata (freshwater diatom)	P(Sco-MMA) (poly(styrene-co-methyl methacrylate))	100–2800 nm	0, 0.0001, 0.001, 0.1, 10 mg L^{-1}	28 days	Significant increase in deformed valve outlines, changes in characteristics of longitudinal and central area, and mixed type of aberration changes in the lowest concentration.	[58]
Dunaliella tertiolecta (marine green algae)	PS-COOH (fluorescent)	40 nm	0, 0.5, 1, 5, 10, 25, 50 mg L^{-1}	3 days	Aggregation, adsorbed on the surface of algae, potential trophic transfer.	[36]
	$PS-NH_2$	50 nm			Aggregation, inhibition of algal growth.	
Euglena gracilis (freshwater euglena)	PS (fluorescent)	100 nm, 5 µm	1 mg L^{-1} (NPLs or MPLs) + 0.5 mg L^{-1} (Cd^{2+})	4 days	MPLs alone inhibits the growth while mixture with Cd^{2+} increases it. NPLs shows lower toxicity than MPLs, while in mixture with Cd^{2+}, it acts synergistically and exceeds toxic effects.	[51]
Microcystis aeruginosa (freshwater cyanobacteria)	PS (plain)	60 nm	0, 25, 50, 100 mg L^{-1}	30 days	Growth inhibited at the beginning while aggregation rates were high. After 10 days, growth increases, while aggregation decreases, indicating a connection between growth rate and aggregation. Negative effect on photosynthetic activity, SOD and MDA affected in the beginning, then mitigated. Production of microcystin increased with the concentration increase.	[63]
Phaeodactylum tricornutum (marine diatom)	PS (plain and fluorescent)	50, 100 nm	0, 0.1, 1, 5, 10, 20, 50 mg L^{-1}	3 days	Hetero- and homo-aggregation observed, during the first 24 h changes in oxidative stress, photosynthesis, membrane integrity and DNA damage, while after 48 h, these responses were mitigated. Growth, chlorophyll a levels and fluorescence and protein content negatively influenced after 72 h.	[39]
	PS-COOH	60 nm	0, 1, 5, 50, 100 mg L^{-1}	3 days	EPS reduces aggregation and ROS production, toxicity of NPLs not observed with or without EPS.	[64]
Platymonas helgolandica (marine green algae)	PS (plain)	70 nm	0, 0.02, 0.2, 2 mg L^{-1}	6 days	Observed morphological changes, inhibition of growth during the first 4 days, increase in growth (after 5 days) and membrane permeability, disturbance in mitochondrial and chloroplast functions.	[45]

Table 1. *Cont.*

Species	Type of NPLs	Size of NPLs	Concentration	Duration	Observed Effects	Reference
Rhodomonas baltica (marine red algae)	PMMA PMMA-COOH	50 nm	0, 0.5, 1, 5, 10, 25, 50, 100 mg L^{-1}	3 days	PMMA aggregated, impacted cell viability and size, pigments, membrane integrity, ROS formation, lipid peroxidation, DNA content and photosynthetic capacity, while PMMA-COOH influenced viability, metabolic activity, photosynthetic performance, and algal growth changes. PMMA physicochemical characteristics important in response to interaction with cells.	[56]
Scenedesmus subspicatus (freshwater green algae)	PE (plain) PE (from Atlantic Gyre, mechanically broken down)	<450 nm	0, 0.001, 0.01, 0.1, 1, 10 mg L^{-1}	2 days	PE from the Atlantic gyre negatively influencing algal growth more than plain PE, may be due to presence of other contaminants like metals.	[59]
Scenedesmus quadricauda (freshwater green algae)	PS (plain)	100 nm	0, 10, 25, 50, 100, 200 mg L^{-1}	14 days	Increase in growth, antioxidant enzyme activity, pigments, soluble proteins, and soluble polysaccharides. Observed strong defensive and recovery response to stress.	[46]
Synechococcus elongatus (freshwater cyanobacteria)	$PS\text{-}NH_2$	50 nm	2–9 mg L^{-1}	2 days	$PS\text{-}NH_2$ negatively impacted growth rate, $PS\text{-}SO_3H$ had no effect. $PS\text{-}NH_2$ induced oxidative stress and membrane permeability which led to damage.	[52]
	$PS\text{-}SO_3H$	52.03 nm				
Tetraselmis chuii, *Nannochloropsis gaditana*, *Isochrysis galbana*, *Thalassiosira weissflogii* (marine algae)	PMMA	40 nm	0–304.1 mg L^{-1}	3 days	Growth rates inhibited at higher concentrations with *T. weissflogii* being the most affected. Big aggregates observed which could explain higher tolerance to PMMA.	[16]

The nano-sized fraction (<100 nm) resulting from the degradation of polycaprolactone (PCL-plastics + PCL oligomers) triggered an overproduction of ROS, altered intracellular pH and affected metabolic activity in the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp., and the unicellular cyanobacterium *Synechococcus* sp. Additionally, it inhibited nitrogen fixation in *Anabaena* sp. [65]. Secondary NPLs originating from the degradation of polyhydroxybutyrate (PHB) significantly reduced the growth of both *Anabaena* sp. and *C. reinhardtii* by 90 and 95%, respectively. These secondary NPLs increased intracellular ROS levels and induced membrane damage with more pronounced effects observed in *Anabaena* sp. [61].

Exposure to NPLs has been demonstrated to influence the production of certain toxins by algae and the release of the extracellular polymeric substances (EPS). For example, both 100 nm and 1 µm-sized PS inhibited the growth, photosynthetic parameters, nutrients uptake and extracellular carbonic anhydrase activities (CAext) in the harmful algal blooms, causing dinoflagellate *Alexandrium tamarense*. Notably, the inhibitory effects were more pronounced when exposed to 1 µm-sized NPLs compared to 100 nm PS exposure [60]. Interestingly, while 100 nm PS increased the concentrations of intracellular paralytic shellfish toxins in this alga, 1 µm PS exposure decreases these toxin levels [60]. The increase in PS concentration from 25, 50, and 100 mg L^{-1} promoted the production and release of microcystine in *M. aeruginosa*, resulting in concentrations of 199.1 for intracellular microcystine and 166. 5 µg L^{-1} for extracellular microcystine concentrations at 100 mg L^{-1} PS [63]. Finally, exposure to NPLs was observed to affect the production of EPS, such as an increased release of EPS and alterations of the protein-to-carbohydrate ratio. These effects were evident in marine species *T. pseudonana*, *Skeletonema grethae*, *P. tricornutum,* and *D. tertiolecta* when exposed to 1 mg L^{-1} PS [66]. Carboxyl-functionalized and non-functionalized PS particles sized at 20 and 50 nm adsorbed to the cell wall of *C. vulgaris,* inducing the generation of a substantial amount of EPS [49].

The examples above highlight the significance of the composition, size, and surface functionalization of NPLs in their interactions with phytoplankton species, underscoring their phytoplankton species specificity. Studies have indicated that PS particles tend to exhibit higher toxicity, whereas PE and PMMA show fewer effects [28]. Previous discussions have emphasized the central role of the particle size in biological interactions and the physicochemical behavior of plastics in the environment [67]. However, an analysis using probabilistic species sensitivity distributions of available data did not reveal any substantial variance in ecotoxicity among NPLs of different sizes [15].

2.3. The Phytoplankton Feedback on NPLs Bioavailability and Toxicity

Recent reviews have highlighted how phytoplankton influence the bioavailability of NPLs through the excretion of various EPS, forming the eco-corona [68,69]. EPS, ubiquitous in the environment [70], can account for up to 25% of natural organic matter in freshwater ecosystems, particularly during algal blooms [71]. While polysaccharides and proteins typically dominate EPS composition, their specific components vary according to species and environmental factors [72]. Studies indicate that marine phytoplankton-produced EPS contribute to the formation of an eco-corona on various NPLs, thereby influencing their reactivity [68]. For example, the EPS derived from the diatom *P. tricornutum*, containing proteins with molecular weight ranging from 30 to 100 kDa and high molecular weight carbohydrates, formed an eco-corona on PS-COOH sized at 60 nm, effectively reducing NPLs' aggregation [64]. However, when EPS from *P. tricornutum*, *Ankistrodesmus angustus*, and *Amphora* sp. interacted with 23 nm PS, it led to the formation of gel-like micrometer aggregates, which was presumably driven by hydrophobic interactions [73]. The formation of the eco-corona has been found to be contingent upon NPLs size, charges, and incubation duration [68]. Alginate, serving as a model polysaccharide, formed an eco-corona on amidine functionalized PS, modifying the surface charge, although aggregation remained minimal [74,75]. Additionally, aminated, carboxylated and plain NPLs aged in EPS reduced the oxidative stress and mitigated toxic effects in the marine alga *Chlorella* sp. [62].

The capacity of phytoplankton species, such as diatoms and cyanobacteria, to transform NPLs has also been documented. Observations include the biodegradation of plastic materials through processes like fouling, corrosion, hydrolysis, and penetration, as along with the degradation of leaching components and the diffusion of pigment coloration into the polymers [76,77]. Furthermore, the activity of the algal PETaze enzyme in *C. reinhardtii* when acting on PET plastic demonstrated the potential for biological degradation with a high conversion rate [78]. The precise influence of phytoplankton on NPLs degradation and transformation warrants further in-depth research in the future.

3. NPLs Interactions with Freshwater Zooplankton Species

Numerous research papers have demonstrated the uptake of NPLs and their aggregates by various zooplankton species through both waterborne and foodborne exposure [79–83]. Among these species, daphnids have been extensively studied [84], particularly *Daphnia magna*, which constituted 79% of the studied cases, followed by *Daphnia pulex* (18%), and *Daphnia galeata* (3%) [85]. *Daphnia* sp. serve as filter feeders and act as primary consumers, forming a crucial link between primary producers and higher trophic levels in freshwater ecosystems [86]. Studies have examined both "apical endpoints"—including mortality/immobility, growth, feeding and egestion, swimming behavior, reproduction, embryonic development, body adsorption—and mechanistic endpoints such as oxidative stress, detoxification, immune-related processes, neurotoxicity, energy metabolism, heart rate, changes in gut epithelium, and processes related to moulting [85].

A meta-analysis examining the impact of micro- and nanoplastics on the mortality and immobilization rates revealed insights into the influence of particle properties (size, density, shape, surface coating, additives), *Daphnia* species characteristics (body size, clone, and the chosen brood), variations in food supply quality/quantity and temperature on toxicity outcome [87]. In *D. magna*, a bioconcentration factor (BCF) ranging from 12 to 363 was calculated for fluorescent PS with a primary size of 1000 and 20 nm. However, these values were comparatively lower than the BCF estimated for other carbonaceous nanoparticles like fullerene, carbon nanotubes and graphene [88].

Here, we will exclusively present the recent advances from the past five years (Table 2) concerning the uptake and toxicity of NPLs.

3.1. Advances in Research on the Bioavailability from Waterborne Exposure

Advances in understanding and quantifying the bioavailability have been achieved through the utilization of fluorescent, luminescent and metal-doped NPLs. For example, the exposure of *D. magna* to 100 nm fluorescent PS resulted in a 21% decrease in feeding rates and lower egestion; however, no observable effect on reproduction was found over a 21 d period [89]. The fluorescent PS penetrated the inner gut of *D. magna*, causing histological damage to intestinal walls (squashed and torn-out microvilli). Nevertheless, little or no acute toxicity was observed within the tested concentration range of 1–10 mg L^{-1} [40]. Fluorescently labeled 75 nm PS were found in the digestive organs of *D. pulex* [90]. A comparison between the exposure of *D. magna* to the fluorescent PS (F-PS, palmitic acid-functionalized PS (PA-PS), and Al_2O_3 metal-core PS, all sized between 90 and 95 nm, revealed that their ingestion by *D. magna* was higher by factors of 2.8 and 3.0 for PA-PS and 1.9 and 1.7 for F-PS when compared to PS [91]. A very recent study has demonstrated that *D. magna* rapidly ingested aggregated-induced emission (AIE) microplastic fluorogenic plastics with sizes of 20 µm and 200 nm (reaching 50% of the steady-state amount within 1 h). Larger-sized particles with positive charge were ingested in higher but egested in lower amounts [92]. The presence of algae had a significant negative impact on the uptake and depuration of NPLs [92]. Comparison between the positively charged AIE-NH_2-NPs and negatively charged AIE-COOH-NPs, approximately 230 nm and 21 µm, respectively, demonstrated that the size and surface charge of the AIE significantly altered the selectivity of *D. magna*. The daphnids exhibited a selective ingestion of larger, positively charged plastics, which accumulated in the middle and posterior gut [93]. Fluorescent PS-

COOH accumulated and retained in the gut of other zooplankton species, rotifer *Brachionus plicatilis*. The retention was still present after the recovery period, suggesting that PS-COOH can be retained in the gut of the larvae for a long time [94]. Amidine- and carboxyl-functionalized PS were ingested by three zooplankton species and primarily accumulated in the gut of *D. magna* and *Thamnocephalus platyurus* as well as in the stomach of *Brachionus calyciflorus* [74]. This accumulation correlated with the exposure concentration and the surface functionalization of the PS. However, further studies are needed to explore whether NPLs could traverse the gut epithelial cells.

Recently, the uptake and excretion kinetics PS NPLs, specifically labeled with lanthanide up-conversion luminescence (UCNPs@PS) and sizes between 49 and 58 nm were observed during direct exposure at a concentration of 500 µg L^{-1} in *D. magna* over 24 h period. The study revealed a bi-phasic uptake pattern: an initial rapid uptake via filter feeding, followed by quick diffusion within the intestine of *D. magna*, leading to subsequent deposition on the carapace and within the body tissue over several hours. Similarly, a bi-phasic excretion pattern was observed, although UCNPs@PS were still retained even after 48 h of depuration [95]. Palladium-doped PS accumulated in the digestive tract and were deposited on the carapace surface, forming aggregates attached to the chitin that covered the entire body of *D. magna* [55].

New insights have emerged regarding the transgenerational transfer of NPLs. A study demonstrated that Eu-doped PS of 640 nm can be transferred from parents to offspring [41]. This finding aligns with the observed transgenerational effects on *D. magna*: minimal impacts on the first generation but subsequent generations exposed to the same NPL concentration vanished after two generations [96]. Parental exposure to NPLs has also been shown to result in transgenerational transfer and toxicity. For example, when parental rotifers *Brachionus koreanus* were exposed to fluorescently labeled non-functionalized 50 nm PS, the particles were transferred to offspring, leading to adverse effects on life-cycle parameters, such as development and reproduction in the offspring rotifers. These effects were associated with oxidative stress [97].

3.2. Advances in Research on the Bioavailability from Foodborne Exposure

Comprehensive discussions have addressed significant progress and key research gaps concerning the exposure, uptake, and propagation of microplastics in aquatic food webs [98,99]. Recent reviews have highlighted the trophic transfer of NPLs along the food webs [27]. Nevertheless, only a few laboratory studies with simplified food webs have clearly demonstrated the transfer of NPLs across various trophic levels [40,41,100]. In experiments involving a four-trophic-level chain, it was shown that 51 nm green fluorescence PS were transferred from alga *C. reinhardtii* through each trophic level up to the fish *Zacco temminckii* [40]. Similarly, a study involving two- and three-trophic-level food chains (algae–crustacean–fish) revealed the accumulation of fluorescent 80 nm PS in *Chlorella pyrenoidosa* or *D. magna* and transferred to fish *Micropterus salmoides*, illustrating biomagnification along the food chain. The trophic transfer elicited antioxidant responses, histopathological damage, and disturbances in the lipid metabolism in *M. salmoides* [101]. In another study, 90 nm sized PS-NH_2 were shown to adsorb to the cell walls of microalga *Dunaliella salina* and subsequently transfer to crustaceans *Artemia*, inducing alterations in gut permeability. In addition, PS-NPs gradually transferred through the three-level food chain to small yellow croakers; *Larimichthys polyactis,* leading to the inhibition of digestive enzyme activity [102].

Using Fe-PS and Eu-PS NPLs, quantitative evidence was presented for the first time, demonstrating their transfer from alga *P. subcapitata* to *D. magna,* notably with a higher transfer rate observed for particles with smaller size [41]. This discovery suggests that NPLs of smaller sizes might exhibit a greater tendency to travel along the food webs. Another study highlighted the importance of the feeding strategy and type of algal food (*Nannochloropsis gaditana* and *Tetraselmis chuii*) alongside waterborne exposure in influencing the effects of NPLs on the rotifer *Brachionus plicatilis* when exposed to PMMA [100].

The progress and novel insights in the understanding regarding the dynamics of uptake and trophic transfer of NPLs, discussed earlier, have been achieved through the utilization of fluorescent or metal-doped NPLs. However, this raised questions about their stability and the potential contribution of metal or fluorophore leaching to the experimental observations. Only a few studies have actually assessed the stability and the possible leaching of metals or fluorophores from NPLs over time. For example, research revealed that the concentration of Pd in Pd-doped PS remained unchanged, indicating their stability and suitability for exposure use [103]. In addition, metal-doped NPLs might offer more reliability compared to commercially bought fluorescence ones, where the leaching of fluorophores was observed [104].

3.3. Advances in Research on the Toxicity of NPLs to Zooplankton

Chronic exposures of *D. magna* to concentrations of 0.1 mg L^{-1} and 50 mg L^{-1} of 20 nm PS resulted in impacted growth, molting, and reproduction, without affecting survival at both tested sizes, and at a particle concentrations of 0.1 mg L^{-1} [105]. In a 21-day chronic toxicity test, dose- and time-dependent relationships were observed for *D. pulex* body length. The time to clutch was delayed, and the total offspring per female, number of clutches and offspring per clutch significantly decreased at a concentration of 0.1 mg L^{-1} [90]. The importance of inter-clonal variability along with the PS particle size has been explored, demonstrating that a twofold decrease in PS particle size from 100 to 50 nm resulted in up to a 100-fold increase in toxicity with a 48 h EC(10), while the inter-clonal variability among three genotypically different clones of the *D. longispina* was approximately tenfold [106].

Further evidence from biochemical, genomic and transcriptomic studies support the idea that oxidative stress is a major toxicity mechanism of NPLs. For example, the exposure of *D. magna* to 75 nm PS concentrations ranging from 0.1 to 2 mg L^{-1} induced and subsequently inhibited the expression of stress defense genes (SOD, glutathione S-transferase (GST), glutathione peroxidase (GPx), and catalase (CAT)) [90]. Similarly in *D. pulex*, exposure led to a significant decrease in the activities of antioxidant enzymes (CAT, total SOD, and CuZn SOD) along with an overproduction of ROS [107]. Exposure to PS concentrations of 0.1 and 0.5 mg L^{-1} significantly increased the expressions of genes associated with the MAPK (mitogen-activated protein kinases) pathway, the HIF-1 pathway, SOD and GST. However, the expressions of these genes decreased at 2 mg L^{-1} PS [107]. However, the protein expression ratio of ERK, JNK, AKT, HIF1α, and NFkBp65 (nuclear transcription factor-kB p65), as well as the phosphorylation of ERK and NFkBp65 increased in a dose-dependent manner. Underpinning these findings, RNA Seq analyses revealed that the exposure of neonates of *D. pulex* to 71 nm PS induced oxidative stress, immune suppression, and affected glycometabolism [108]. In *D. magna*, exposure to 80 mg L^{-1} Pd-doped NPLs resulted in ROS overproduction and an alteration in cellular membrane integrity, while a slight increase in mitochondrial membrane depolarization in the gut was observed at 10 mg L^{-1} [55]. A 48-h exposure of *D. magna* to secondary NPLs of PHB (<100 nm) induced excessive ROS and severe membrane damage [61]. Organism age has been shown to influence daphnids' sensitivity to PS, as evidenced by the variations in expression levels of genes encoding for key stress defense enzymes and proteins (SOD, CAT, GST, GPx, HSP70, and HSP90) and the energy-sensing enzyme AMPK (adenosine monophosphate-activated protein kinase) in 7-day-old and 21-day-old *D. pulex* [109].

Multi-generational effects of low concentrations of NPL have been documented. For example, *D. pulex* reproduction was affected in offspring from exposed parents. The exposure of F0 and F1 generations of *D. pulex* to 75 nm PS at 1 μg L^{-1} resulted in a significant increase in the expression of antioxidant genes encoding Mn SOD, CuZn SOD, GCL, HO1, CYP4C33, and CYP4C34 and enhanced enzyme activity of GST and CAT. However, these effects were inhibited in F2 generation. Conversely, AMPK was further increased in the F2 generation [110]. The exposure of *D. magna* to Pd-doped 200 nm PS showed no impact at 0.1 mg L^{-1}. However, at 1 mg L^{-1}, it significantly increased fertility in the F3 generation while decreasing the size and lipid content in F3 offspring [104]. The authors pointed out that the effects of NPLs on *D. magna* adults and offspring occurred only after multi-generational exposure, despite "similar body burden values between the adults and offspring of different generations" [104].

Table 2. Selected examples of the most recent studies (<5 years) on freshwater zooplankton interactions with NPLs.

Species	Type of NP	Size of NP	Concentration	Duration	Exposure to NPLs	Observed Effects	Reference
Artemia franciscana (marine)	PS-COOH (fluorescent)	40 nm	0.5, 1, 1.5, 2.5, 5 mg L^{-1}	14 days	waterborne	Aggregation, accumulation, and excretion noticed, potential trophic transfer.	[36]
	PS-NH_2	190 nm	0, 1 mg L^{-1} 0–200 mg L^{-1}	14 days 2 days	waterborne and foodborne—*D. salina*	Found in the gut, higher levels by direct uptake than through trophic transfer, observed damage to the digestive tract, no difference in mortality and immobilization in short-term exposure.	[102]
	PS (amine) PS (sulfate)	100 nm	0, 1, 10, 100 mg L^{-1}	2 days	waterborne, different levels of temperature, salinity and humic acid and bentonite	Amine NPLs produced additional toxic effects at high salinity, while at low temperatures, HA and bentonite reduced toxicity. Multi-stressor experiment showed that toxicity depends on the physicochemical characteristics of the water.	[111]
Brachionus koreanus	PS (plain)	50 nm	10 mg L^{-1}	1 day	pre-exposed to NPLs, waterborne to POPs	Pre-exposure to NPLs leads to oxidative damage of membranes and disruption of multixenobiotic resistance (MXR) functions, NPLs subsequently enhanced the toxicity of persistent organic pollutants (POPs).	[112]
	PS (plain and fluorescent)	50 nm	0, 1, 10 mg L^{-1}	~1.5 days	maternal transfer to unexposed neonates	Maturation time and reproduction negatively impacted at higher concentration. Bioaccumulated maternally transferred NPLs in offspring. Parent exposures induces an increase in ROS production in offspring.	[97]
Brachionus plicatilis	PMMA	40 nm	4.7, 9.4, 18.9, 37.5, 75.0 mg L^{-1}	2 days	waterborne	Mortality increased after exposure, especially in higher concentrations.	[16]
Daphnia galeata × *longispina*	PS (fluorescent)	100 nm	0, 5, 20 mg L^{-1}	29 days	waterborne with/without inoculated spores of parasite *Metschnikowia bicuspidata*	Increased number of infected hosts in the presence of NPLs, lifespan and reproduction ability are reduced. Parasite reproduction is three times lower in high NPLs concentration. NPLs have a hormetic effect on the host, increasing its fitness.	[113]
Daphnia longispina	PS (fluorescent)	50 nm, 100 nm	0, 0.01, 0.1, 1, 2, 10, 20, 100 mg L^{-1}	4 days	waterborne	Smaller NPLs may be more toxic due to higher bioavailability and particle toxicity.	[106]

Table 2. *Cont.*

Species	Type of NP	Size of NP	Concentration	Duration	Exposure to NPLs	Observed Effects	Reference
Daphnia magna	PS (plain)	50 nm	0.05, 0.5 mg L^{-1}	21 days	waterborne	Increase in energy reserves, no changes in oxidative stress and swimming activity.	[114]
	HDPE-(mechanically broken-down)	90–200 nm	High/low mix of fractions	98/134 days	waterborne and in mixture with smaller fractions	HDPE was not toxic, but the fraction of leached additives and short-chain HDPE cause toxicity.	[115]
	PS (fluorescent)	51 nm	0, 20, 40, 60, 80, 100 mg L^{-1}	3 days	foodborne—*C. reinhardtii*	Presence in the gut and damage to the intestinal walls, trophic transfer detected.	[40]
	PS (plain)	100 nm	1 mg L^{-1}	2 days	waterborne	Plain PS had the highest acute toxicity and ROS production, activated MAPKs but did not influence AChE changes, while PS-COOH, PS-n-NH_2 and PS-p-NH_2 activated antioxidant system and lowered ROS production.	[81]
	PS-p-NH_2	50–100 nm					
	PS-COOH	300 nm					
	PS-n-NH_2	110 nm					
	PS-NH_2	53 nm	0, 0.0032, 0.032. 0.32 mg L^{-1}	64.3 ± 32.5 days	waterborne	Highest concentration increased mortality, long-term exposure to low concentrations leads to a decrease in survival, offspring, and delay in first brood.	[80]
	PS-COOH	26 nm, 62 nm					
	Eu-PS NPD (NPLs debris) Fe-PS NPD	640 nm	0, 1, 7 mg L^{-1}	21 days	foodborne—*P. subcapitata*	Fe-PS-NPD impacted the reproduction time, increased mortality, and decreased the number of neonates. Eu-PS-NPD lowered number of neonates per brood. Smaller NPD (Fe-PS-NPD) have a higher impact on the reproduction than the larger NPD (Eu-PS-NPD).	[41]
	PS-COOH (fluorescent)	20 nm, 200 nm	0, 0.1, 50 mg L^{-1}	21 days	waterborne	Molting and time to first brood prolonged, changes in the body length, neonate production in 200 nm may be higher because of hormesis.	[105]
	PS (fluorescent)	80 nm	0, 5 mg L^{-1}	28 days	foodborne—*C. pyrenoidosa*	Trophic transfer observed, higher accumulation through direct exposure than foodborne. Histopathological damages in the intestinal.	[101]
	Amidine PS	20, 40, 60, and 100 nm	0.5 to 30 mg L^{-1} (0.5 to 100 mg L^{-1} for 100 nm NPLs)	2 days	waterborne	Exposure in lake water. The effect depended on the primary size of PS, with 20 and 40 nm size PS NPLs inducing a stronger effect.	[75]

Table 2. *Cont.*

Species	Type of NP	Size of NP	Concentration	Duration	Exposure to NPLs	Observed Effects	Reference
Daphnia pulex	PS (fluorescent)	75 nm	0, 0.1, 0.5, 1 and 2 mg L^{-1}	21 days	waterborne	Growth inhibition, reproduction time longer while number of neonates reduced, heat shot proteins (HSP70 and HSP90) increased in the higher concentrations.	[90]
	PS (plain)	75 nm	0, 0.1, 0.5, 1, 2 mg L^{-1}	21 days	waterborne	Increase in concentration of NPLs stimulates increase in ROS production, which leads to an increase in antioxidative gene expression and enzyme activity, possible negative effects on cell survival and proliferation via MAPK pathways.	[107]
	PS (plain)	71.18 ± 6.03 nm	0, 1 mg L^{-1}	4 days	waterborne	208 differentially expressed genes analyzed—changes in the expression for oxidative stress, immune defense and glycometabolism pathways.	[108]
Daphnia magna, larvae Thamnocephalus platyurus, and rotifer Brachionus calyciflorus	Amidine PS	226.0 ± 8.6 nm	0 to 400 mg L^{-1}	1 day and 2 days	waterborne	The toxicity decreased in the order *D. magna* (48 h immobilization) > *B. calyciflorus* (24 h lethality) > *T. platyurus* (24 h lethality). Amidine PS was more toxic than carboxyl PS. Alginate and humic acid formed eco-corona on amidine PS nanospheres and reduced toxicity to zooplankton.	[74]
	Carboxyl PS	220.1 ± 9.1 nm	0 to 400 mg L^{-1}				

3.4. Effect of Zooplankton on NPLs Bioavailability and Toxicity

Zooplankton species such as *D. magna* release various biological material into their surroundings. These include kairomones, enzymes and proteins expelled from their gut, chitin-based carbohydrates from molting, as well as digestive enzymes and undigested or partially digested matter [79,116,117]. Extensive reviews have explored the potential consequences of the formed eco-corona, which serves as a modulator of NPLs' bioavailability and toxicity to *D. magna* [116]. These consequences encompass (i) altering the stability and uptake of NPLs; (ii) influencing residency time and the absorption of nutrients; (iii) affecting biomolecules released by gut bacteria; and (iv) impacting signaling through binding to key molecules such as kairomones. These findings confirm the significance of eco-corona formation in modulating the uptake and effects of NPLs. They also underscore the necessity of conducting bioassays under conditions more relevant for the aquatic environment, such as using NPLs coated by EPS.

4. Conclusions and Perspectives

The increasing evidence demonstrates that NPLs are bioavailable and can cause harm to the planktonic organisms when present at concentrations much higher than expected in the aquatic environment. Recent advances in toxicity studies based on biochemical, genomic, and transcriptomic approaches with model NPLs and plankton organisms have demonstrated that high concentrations of NPLs could induce oxidative stress and damage, DNA damage and the depolarization of mitochondrial and cell membranes in various plankton species. Additionally, alteration of the photosynthetic activity in phytoplankton species has been identified as a major response to NPLs-induced stress. Furthermore, multigenerational effects of low concentrations of NPLs have also been observed in zooplankton.

Despite these recent advances, debates persist regarding the bioavailability and toxicity of NPLs in aquatic environment. The absence of reference materials and standardized testing procedures limits the results' comparability and the repeatability of bioassays across different laboratories. Recently, proposals have emerged for standardized short- and long-term toxicity tests on aquatic organisms as well as the top–down production of more realistic NPLs and their comprehensive characterization [118]. Consequently, assessing the biological responses of environmentally relevant materials, such as secondary NPLs and aged NPLs, at concentrations closer to those expected in the aquatic environments could offer further insights into the responses in the natural environment.

Most of the bioavailability and toxicity data are derived from commercially available NPLs, primarily PS, and should be considered cautiously (i) due to a lack of representation of the diversity in NPLs, including variations in size, shapes, and composition [15] and (ii) because certain preservatives present in these products might influence their toxicity. Consequently, it is crucial to prioritize research assessing the responses of planktonic species to NPLs under environmentally realistic conditions. Moreover, when assessing NPL effects, the co-existence of other environmental pollutants capable of adsorbing to NPLs and potentially causing synergistic effects and antagonistic responses in aquatic plankton need to be taken into consideration [119].

Regardless of significant progress, gaps persist in understanding the accumulation patterns and translocation mechanisms of NPLs within planktonic organisms. Key ecotoxicological parameters—bioaccumulation and trophic transfer factors—necessary for accurately assessing the potential impacts of NPLs on aquatic organisms remain unquantified. Considering that many food webs rely on phytoplankton species, the association of NPLs to the cell surface or their penetration via endocytosis inevitably introduces them into the food webs. However, existing research predominantly focuses on the toxicity of NPLs on individual zooplankton species, overlooking trophic transfer at the base of food webs and the contributions of different exposure pathways. Further studies are necessary to address uptake and clearance rates, determine the mechanisms involved, and differentiate the contribution of the waterborne and foodborne exposures to NPLs accumulation in freshwater zooplankton. It is worth noting that such studies pose significant challenges

due to the limitations in current analytical techniques for detecting and quantifying NPLs in complex biological samples.

Most of the current knowledge concerning the uptake and toxicity of NPLs originates from studies conducted with model NPLs, primarily PS nanospheres. However, these models differ significantly from secondary heterogeneous NPLs and cannot be expected to behave similarly [120,121]. Incidentally produced NPLs exhibit diverse compositions, morphologies and heterogeneity absent in model NPLs and engineered nanomaterials [9]. Quantifying NPLs in environmental and biological samples poses a challenge [122], leading to qualitative observations of bioaccumulation and trophic transfer using fluorescence/luminescence labeled NPLs or metal-doped particles [103]. Even if not fully representing the diversity of incidental NPLs in the environment, these models offer novel insights into NPLs interactions with phyto- and zooplankton and the potential trophic transfer of NPLs. Bioassays involving secondary NPLs will provide further insights into the effects caused by naturally fragmentated and aged NPLs found in the environment. Assessing their potentially higher toxicity, eco-corona formation and the acute exposure of organisms becomes crucial.

Furthermore, it is anticipated that aquatic plankton play a role in NPLs transformations. However, it remains unclear if this is general phenomena and if it is of relevance to the natural environments. Plankton species have the potential to influence NPLs by releasing biomolecules capable of modifying NPL surfaces, thereby affecting their stability. This aspect needs consideration for an improved understanding of their interaction with aquatic organisms.

While advances have been made in understanding the role of EPS produced by marine phytoplankton in NPL bioavailability and effects, similar studies focusing on EPS released by freshwater phytoplankton have yet to carried out. There is a scarcity of studies available for the zooplankton species. Given the existing knowledge about the role of freshwater EPS in the fate and transformations of metal-containing nanoparticles [123], it is anticipated that they could significantly affect the bioreactivity of NPLs. Indeed, the need to incorporate the concept of the biomolecular corona into a broader framework that considers interactions and feedback between phyto- and zooplankton in response to nanoparticles exposure has been highlighted [69].

The trophic transfer of NPLs in aquatic food chains has been demonstrated in studies involving model NPLs and artificial two- to four-level trophic chains. However, confirming this transfer necessitates comprehensive in situ surveys. Moreover, evaluating the transfer and fate of biologically ingested plastics within the food chain required careful evaluation. In this context, detecting, quantifying, and determining key characteristics of the secondary NPLs generated in the environment becomes necessary. These aspects serve as cornerstones for understanding NPL fate and impact, enabling scientifically sound and quantitative risk assessment, defining environmental quality standards, and enabling effective monitoring and management.

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