Accepted Manuscript

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Joana Correia Prata, João P. da Costa, Isabel Lopes, Armando C. Duarte, Teresa Rocha-Santos

PII: S0048-9697(19)30618-7
DOI: https://doi.org/10.1016/j.scitotenv.2019.02.132
Reference: STOTEN 30877

To appear in: *Science of the Total Environment*

Received date: 2 January 2019
Revised date: 8 February 2019
Accepted date: 8 February 2019

Please cite this article as: J.C. Prata, J.P. da Costa, I. Lopes, et al., Effects of microplastics on microalgae populations: A critical review, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.02.132

Effects of microplastics on microalgae populations: a critical review

Joana Correia Prata[1], João P. da Costa[1b], Isabel Lopes[2c], Armando C. Duarte[1d], Teresa Rocha-Santos[1e]

[1] Centre for Environmental and Marine Studies (CESAM) & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

[2] Centre for Environmental and Marine Studies (CESAM) & Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal

Corresponding author:

Joana C. Prata, Centre for Environmental and Marine Studies (CESAM) & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Email: pratajc@ua.pt

Contact: +35191545801

b: jpintocosta@ua.pt

c: ilopes@ua.pt

d: aduarte@ua.pt

e: ter.alex@ua.pt

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Abstract

Microplastics are persistent contaminants accumulating in the environment. Aquatic ecosystems have been studied worldwide, revealing ubiquitous contamination with microplastics. Microalgae, one of the most important primary producers in aquatic ecosystems, could suffer from microplastic contamination, leading to larger impacts on aquatic food webs. Nonetheless, little is known about the toxic effects of microplastics on microalgae populations. Thus, the objective of this review was to identify these effects and the impacts of microplastics on microalgae populations based on currently available literature, also identifying knowledge gaps. Even though microplastics seem to have limited effects on parameters such as growth, chlorophyll content, photosynthesis activity and reactive oxygen species (ROS), current environmental concentrations are not expected to induce toxicity. Even so, microplastics could disrupt population regulation mechanisms, by reducing the availability or absorption of nutrients (bottom-up) or reducing the population of predator species (top-down). Microplastics' properties can also influence the effects on microalgae, with smaller sizes and positive surface charges having higher toxicity. Therefore, more research is needed to better understand the effects of microplastics on microalgae, such as adaptation strategies, effects on population dynamics and microplastics properties influencing toxicity.

Keywords: phytoplankton; primary producers; microalgae; microplastics toxicity

1. Introduction

Microplastics, plastics <5 mm, are persistent and ubiquitous contaminants (Thompson *et al.* 2009) originating from an intentional production for products or industries (primary

microplastics; Browne *et al.* 2008) or the fragmentation of larger plastics under environmental conditions (secondary microplastics; Andrady 2011). Microplastics have been reported in seawater from the Artic (Morgana *et al.* 2018) to the Antarctic (Waller *et al.* 2017), as well as in freshwaters, such as rivers (Rodrigues *et al.* 2018) or lakes (Eriksen *et al.* 2013). Highest concentrations have been reported in ocean gyres, such as 20,328 particles km^{-2} in the Atlantic Subtropical Gyre (Law *et al.* 2010), and in industrial ports, such as 102,550 particles m^{-3} in a Swedish port (Norén 2007). Most frequent polymers found in the marine environment are polyethylene (PE), polypropylene, polystyrene (PS), nylon, polyethylene terephthalate, polyvinyl chloride and cellulose acetate (Andrady 2011).

Concentrations of microplastics are expected to increase in aquatic ecosystems, leading to a growing effort to understand their toxic effects. Toxicity assays in organisms reveal the potential of microplastics to cause oxidative stress, impairment of the immune system and general decrease in fitness (e.g. reduced survival and fecundity) (Anbumani and Kakkar 2018; Guzzetti *et al.* 2018; Strungaru *et al.* 2019). For instance, chronic exposure (21 days) of *Daphnia magna* to 1 — 5 µm microplastics (unknown polymer) in concentrations of 0.02 and 0.2 mg L^{-1} resulted in mortality and reduced reproductive fitness (Pacheco *et al.* 2018), whereas *Artemia franciscana* larvae exposed to 40 nm anionic carboxylated and cationic amino PS up to 100 mg L^{-1} for 48 hour showed microplastic accumulation in the gut, potentially limiting food intake, and to adsorption to the body surface, impairing mobility (Bergami *et al.* 2016). However, ingestion of microplastics was lower when *Chironomus riparius*, *Gammarus pulex*, *Daphnia magna* were co-exposed to natural prey, except in cases where microplastics adhered to the food's surface (Scherer *et al.* 2017). On the other hand, microplastics could suffer biomagnification through trophic transfer. However, lack of confirmatory field data and the capacity for organisms to egest microplastics from the digestive system do not support the idea of trophic transfer, except in cases where there is translocation to other tissues (Burns and Boxall 2018), such as in crabs *Uca*

rapax exposed to 180 — 250 µm PS where these fragments were found not only in the stomach and gills, but also in the hepatopancreas suggesting translocation (Brennecke *et al.* 2015).

Microalgae may suffer toxic effects as inhabitants of pelagic areas contaminated with microplastics. Furthermore, as primary producers essential to the functioning of aquatic ecosystems (Casado *et al.* 2013), small disruptions of microalgae populations may lead to serious repercussions on food webs. Even so, the effects and toxicity of microplastics have seldom been determined in microalgae and current experimental results offer no consensus. A review on the topic is needed to identify the potential mechanisms of toxicity as well as to guide further inquiries. Thus, this review was conducted with the objective of summarizing current literature on the effects of microplastics on microalgae and their potential underlying mechanisms, attempting to assess the impacts on ecosystems and factors involved in their toxicity, while simultaneously identifying knowledge gaps in these areas. Thus, the following sections offer a perspective on the toxicity of microplastics in microalgae, the potential for disturbing microalgae populations and ecosystems, factors determining their interaction, as well as recommendations for further research.

2. What effects do microplastics have on microalgae?

Microplastics seem to have little effect on the growth of microalgae (**Table 1**). Some authors have reported that these materials may induce growth inhibition, although significant results were detailed in only four reports (Casado *et al*. 2013; Besseling *et al*. 2014; Bergami *et al*. 2017; Lyakurwa 2017), with only two works reporting EC_{50}: Bergami *et al.* (2017) reporting EC_{50}=12.97 mg L^{-1} for 0.04 µm polystyrene (PS) of and Casado *et al.* (2013) reporting EC_{50}=0.58 mg L^{-1} and 0.54 mg L^{-1} for polyethyleneimine polystyrene (PS-PEI), for sizes of 0.05 and 0.1 µm respectively. An increase in growth was also reported and attributed to the use of microplastics as substrate by some species (Yokota *et al*. 2017; Canniff and Hoang 2018). Nonetheless, most works are

unable to find EC_{50} values for microplastics due to the high concentrations needed to induce significant toxicity; and EC_{50} values may vary depending on the characteristics of specific microplastics used in the assay (e.g. surface charge, size, additives), restricting generalizations even by polymer type.

The presence of microplastics has been shown to lead to a decrease in chlorophyll content (Besseling *et al*. 2014; Zhang *et al*. 2017; Prata *et al*. 2018) and photosynthetic activity (Bhattacharya *et al*. 2010; Mao *et al*. 2018; Zhang *et al*. 2017), independent from growth inhibition (Besseling *et al*. 2014) and shading effect (Besseling *et al*. 2014; Zhang *et al*. 2017), and possibly related to a decrease in the expression of photosynthesis genes (Lagarde *et al*. 2016), interference in substance exchange and increase in energy demand for motility due to surface adsorption of microplastics (Bhattacharya *et al*. 2010). Furthermore, microplastics may hinder photosynthesis by affecting the electron donor site, the reaction center of photosystem II (responsible for energy conversion) and the electron transport chains, also leading to electron accumulation and the production of reactive oxygen species (ROS) responsible for oxidative stress (Bhattacharya *et al*. 2010; Mao *et al*. 2018).

Microplastics may also cause direct physical damage, nutrient depletion, increased osmotic pressure, and the release of toxic chemicals (Besseling *et al*. 2014; Nolte *et al*. 2017; Zhang *et al*. 2017; Chae *et al*. 2018). Moreover, microplastics can induce morphological changes in microalgae (unclear pyrenoid, plasma detached from the cell wall, deformed thylakoids, cell wall thickening) (Mao *et al*. 2018), be internalized during cell division (Chae *et al*. 2018) or by mixotrophic organisms (Long *et al*. 2017).

However, all these effects seem to be temporary, with an initial period of vulnerability followed by adaptative responses leading to recovery (Yokota *et al*. 2017; Zhang *et al*. 2017; Mao *et al*. 2018), such as membrane thickening, homoaggregation (to reduce surface exposure) and heteroaggregation (Mao *et al*. 2014). The latter results from the production of exopolymeric

substances (EPS) by microalgae, with assemblage promoted by the hydrophobic domains of microplastics, overexpression of biosynthesis of sugars and debris from the stationary growth phase (Chen *et al.*2011; Casado *et al*. 2013; Lagarde *et al*. 2016; Lyakurwa 2017; Prata *et al*. 2018). Microplastics can accumulate in EPS, reducing light availability and substance exchange, changing the bioavailability of carbon and microbial communities, and increasing the frequency and severity of harmful algae blooms (HAB) (Chen *et al*. 2011; Long *et al*. 2015; Yokota *et al*. 2017; Mao *et al*. 2018).

As highlighted in **Table 1**, current understanding of the effects of microplastics in microalgae is scarce. It is not yet understood how microplastics properties, microalgae species, and adaptative responses play a role in toxicity. Thus, there is a need to further clarify the mechanisms of action, toxic properties of microplastics, susceptible algae species and adaptative responses based on environmentally relevant concentrations.

3. How can microplastics disturb microalgae populations and the ecosystem?

Since microalgae are responsible for 50% of the primary net production (Barbosa 2009), toxic effects from contaminants may have wider impacts on the ecosystem. Current concentrations of microplastics in the environment (e.g. mean 0.00168 mg L^{-1} in the Mediterranean Sea (Suaria et al. 2016)) are not expected to have significant impacts on microalgae (**Table 1**). However, microplastics may interfere with microalgae through mechanisms of population regulation.

Bottom-up regulation is based on growth rates and nutrient limitation, whereas top-down regulation is based on the regulation of abundance by predators (Barbosa 2009). Microplastics can potentially affect both bottom-up and top-down regulation mechanisms, thus disturbing the ecosystem. Microplastics can adsorb essential nutrients, such as vitamin B12 (Croft *et al*. 2005) or decrease their absorption and thus reduce the growth of microalgae through bottom-up regulation. Toxicity or preferential ingestion of microplastics by primary consumers could

decrease predation, leading to an increase in microalgae population due to the lack of top-down regulation. For instance, new evidence suggests that zooplankton is highly susceptible to the toxicity of microplastics (Foley *et al*. 2018) and the effects may span generations (Martins and Guilhermino 2018). However, the effects on population regulation are dynamic and dependent on environmental factors, therefore hard to predict (Menge 2000).

Ecosystems may also be affected by the increase in growth of microalgae stemming from the availability of microplastics as growth substrates, which may lead to higher occurrences of HAB with negative impacts to other organisms (Yokota *et al*. 2017; Canniff and Hoang 2018). Thus, there is the need to understand the complex effects of microplastics on microalgae populations, especially through nutrient availability, predation and density of primary consumers, and role as a substrate.

4. What factors determine the interaction between microplastics and microalgae?

Several factors affect the toxicity of microplastics, including their concentrations in the environment, polymer type, size, presence of additives, surface chemistry and charge. Most of the concentrations tested in the available literature largely exceed those found in the environment; yet, most of these studies show little to no significant effects on microalgae. Nonetheless, direct comparison of the data is made difficult by the reporting of tested concentrations in toxicity assays in mg L^{-1}, as opposed to particle L^{-1} in which most environmental concentrations are reported. Comparison of data would be facilitated by reporting results in both units.

Polymer type and size are also factors that may contribute to microalgae toxicity. Lagarde *et al*. (2016) reported growth inhibition for polypropylene but not for high density polyethylene in *Chlamydomonas reinhardtii.* Furthermore, polymer type and size have a direct impact on its distribution across the water column and may lead to sedimentation (Davarpanah and

Guilhermino 2015) or buoyancy (Zhang *et al*. 2017), modulating exposure. Regarding size, generally, smaller microplastics are more toxic to microalgae. For instance, PS of 0.05 µm induced a more marked decrease in cell density than 0.5 and 6 µm (Sjollema *et al.* 2016) and polyvinyl chloride (PVC) caused negative effects on growth, chlorophyll and photosynthesis at the size of 1 µm, but not of 1 mm (Zhang *et al.* 2017). Polymer and size also determine surface chemistry, leading to variations in toxicity (Casado *et al*. 2013).

Positively charged microplastics have higher interaction and toxicity to microalgae than negatively charged microplastics (Bhattacharya *et al*. 2010; Casado *et al*. 2013; Nolte *et al*. 2017). This probably results from the anionic cellulose in the cell wall, with carboxyl and sulfate groups repelling negatively charged microplastics and adsorbing positively charged microplastics through electrostatic interaction, hydrogen bonds and hydrophobic interaction, varying with algae morphology (Bhattacharya *et al*. 2010).

The adsorption of substances is also related to the properties of microplastics. For instance, polyethylene did not affect the toxicity of copper (Davarpanah and Guilhermino 2015) but increased the toxicity of two pharmaceuticals, procainamide and doxycycline (Prata *et al*. 2018). Indeed, adsorption of substances to microplastics could increase exposure (Besseling *et al*. 2014). On the other hand, most microplastics used in toxicity assays are marked with fluorescent labels to aid identification and quantification (e.g. fluorescence microscopy, fluorometry), which could impact toxicity results when the label's toxicity is not considered. Thus, there is a need to further explore how different properties of microplastics affect their toxicity to microalgae and how they interact with other substances in the environment.

5. Recommendations for further research

As discussed in previous sections, the effects of microplastics on microalgae require further research. Along with the reporting of tested concentrations under various units to allow

comparison with environmental concentrations, we recommend the study of the following research topics based on the knowledge gaps identified in the previous sections:

- Clarification of mechanisms of toxicity of microplastics in microalgae, possibly by an *-omics* approach;
- Determination of the toxic effects of microplastics in microalgae over time to better understand adaptative responses (e.g. by daily cell counts);
- Testing of environmentally relevant concentrations in non-lethal end-points;
- Evaluation of the effects of microplastics on the intake of essential nutrients (i.e. by adsorbing these nutrients or by adsorbing to the cell wall reducing the microalgae surface available for nutrient absorption);
- Evaluation of the effect of microplastics on microalgae' predation, such as toxicity and preferential ingestion by predators (e.g. mesocosm assay);
- Evaluation of the differences in microplastics' effects due to microalgae species (e.g. use of microplastics as substrate, cell wall constitution) and to microplastics' properties, such as polymer type, chemical composition, weathering condition, surface charge and size.

6. Conclusion

Microplastics at concentrations in the low ppm range have negative effects on microalgae by inhibiting growth, reducing chlorophyll and photosynthesis, inducing oxidative stress, causing changes in morphology and promoting the production of heteroaggregates. However, microalgae seem to recover from these changes through adaptative responses and current environmental concentrations are unlikely to cause harm. Nonetheless, microplastics may disturb microalgae populations by reducing the available nutrients, by inhibiting primary consumers or by acting as a substrate. All these changes are dependent on specific properties

of microplastics, such as polymer type, size and surface charge, that are still not well understood and thus require further research.

Funding

This work was supported by national funds through FCT/MEC (PIDDAC) under project IF/00407/2013/CP1162/CT0023. Thanks are also due, for the financial support, to CESAM (UID/AMB/50017), to FCT/MEC through national funds, and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020 (POCI-01-0145-FEDER-028740). This work was also funded by Portuguese Science Foundation (FCT) through scholarships SFRH/BPD/122538/2016 and PD/BD/135581/2018 under POCH funds, co-financed by the European Social Fund and Portuguese National Funds from MEC.

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Table 1. Effects of microplastics exposure to microalgae based on several effect criteria.

Species environment	Species	Polymer	Size (µm)	Tested concentration (mg L^{-1})	Effect Criteria	Result	Test duration (h)	Reference
Freshwater	*Chlorella* sp.	PS (+, -)	0.02	0.08 – 0.58	Adsorption Photosynthesis ROS production	Yes, higher in (+) Significant decrease >1.8 mg L^{-1} Higher, especially in (+)	4	Battacharya *et al*. 2010
Freshwater	*Scenedesmus* sp.				Adsorption Photosynthesis ROS production	Yes, higher in (+) Decrease up to 40% Higher		
Freshwater	*Scenedesmus obliquus*	PS	0.07	44 – 1100	Growth inhibition Chlorophyll content	2.5% at 1000 mg L^{-1} Decrease > 100 mg L^{-1}	72	Besseling *et al*. 2014
Saltwater	*Dunaliella tertiolecta*	PS (+)	0.04	0.5 – 50	Growth inhibition Aggregation	EC_{50}: 12.97 mg L^{-1} Yes	72	Bergami *et al*. 2017
		PS (-)	0.05		Growth inhibition Aggregation	Up to 25.4% Yes		
Freshwater	*Raphidocelis subcapitata*	PE	63 – 75	130	Growth inhibition	Significant increase in growth	120	Canniff and Hoang 2018
Freshwater	*Pseudokirchneriella subcapitata*	PS-PEI (+)	0.05	0.1 – 1	Growth inhibition	EC_{50}: 0.58 mg L^{-1}	72	Casado *et al*. 2013
			0.1	0.1 – 0.8	Growth inhibition	EC_{50}: 0.54 mg L^{-1}		
Freshwater	*Chlamydomonas reinhardtii*	PS (-)	0.05	0 – 100	Growth inhibition Internalization	No effect Yes, in cell division	72	Chae *et al*. 2018
Freshwater	*Amphora* sp.	PS	0.02	10 – 100 *	Heteroaggregation	Induce assembly		Chen *et al*. 2011
Freshwater	*Ankistrodesmus angustus*				Heteroaggregation	Accelerate assembly		
Freshwater	*Phaeodactylum tricornutum*				Heteroaggregation	Accelerate assembly		
Saltwater	*Tetraselmis chuii*	PE	1 – 5	0.046 – 1.472	Growth inhibition	No effect	96	Davarpanah and Guilhermino 2015

Freshwater	*Chlamydomas reinhardtii*	PP	400 – 1000	400	Heteroaggregation	Yes	1872	Largade *et al*. 2016
					Growth inhibition	18% after 78 days		
					Stress response genes	No effect		
					Apoptosis genes	No effect		
					Sugar biosynthesis genes	Increase		
					Photosynthesis genes	Non-significant decrease		
		HDPE				No		
					Heteroaggregation	No effect		
					Growth inhibition	No effect		
					Stress response genes	No effect		
					Apoptosis genes	Increase		
					Sugar biosynthesis genes	Non-significant decrease		
					Photosynthesis genes			
Saltwater	*Tisochrysis lutea*	PS	2	0.004	Growth inhibition	No effect	840	Long *et al*. 2017
					Chlorophyll content	No effect		
					Heteroaggregation	No		
Saltwater	*Heterocapsa triquetra*				Growth inhibition	No effect		
					Chlorophyll content	No effect		
					Heteroaggregation	No		
Saltwater	*Chaetoceros neogracile*				Growth inhibition	No effect		
					Chlorophyll content	No effect		
					Heteroaggregation	Yes		
Saltwater	*Rhodomonas baltica*	PS	10	75 – 7500 **	Cell count	Significant inhibition	264	Lyakurwa 2017
					Chlorophyll content	Significant decrease		
Freshwater	*Chlorella pyrenoidosa*	PS	0.1, 1	10 – 100	Growth inhibition	Inhibition until day 22, recovery	720	Mao *et al*. 2018
					Photosynthesis	Inhibition until day 6-8, recovery		
					Morphology	Unclear pyrenoid, damaged membrane, distorted thylakoid, wall thickening at day 13, recovery		
Freshwater	*Raphidocelis subcapitata*	PS (-)	0.11	1 – 100	Growth inhibition	Inhibition, EC_{50} not found	72	Nolte *et al*. 2017
Saltwater	*Tetraselmis chuii*	PE	1 – 5	0.75 – 48	Growth inhibition	No effect	96	Prata *et al*. 2018

					Chlorophyll content	Decreased at 0.9 and 2.1 mg L^{-1}		
Saltwater	*Dunaliella tertiolecta*	PS (0)	0.5 – 6	25, 250	Growth inhibition	57% at 250 mg L^{-1}	72	Sjollema *et al*. 2016
					Photosynthesis	No effect		
			0.5					
		PS (-)			Growth inhibition	Inhibition		
Saltwater	*Dunaliella tertiolecta*				Photosynthesis	No effect		
			0.5					
		PS (-)			Photosynthesis	No effect		
Freshwater	*Chlorella vulgaris*	PS (-)	0.5		Photosynthesis	No effect		
Saltwater	*Thalassiosira pseudonana*							
Freshwater	*Microcystis aeruginosa*	PS	20 – 350	66.7	Cell count	Increase (inconsistent)	504	Yokota *et al*. 2017
					Algae size	Smaller (inconsistent)		
					Growth inhibition	No effect		
					Biomass	No effect		
					Colonization	No effect		
Freshwater	*Dolichospermum flos-aquae*				Cell count	Increase (inconsistent)		
					Algae size	Smaller (inconsistent)		
					Filament elongation	Decrease		
					Growth inhibition	No effect		
					Biomass	No effect		
					Colonization	Yes		
Saltwater	*Skeletonema costatum*	PVC	1	0 – 50	Growth inhibition	Up to 39.7%	96	Zhang *et al*. 2017
					Chlorophyll content	Decrease up to 20%		
					Photosynthesis	Decreased up to 20%		
		PVC	1000	0 – 2000	Growth inhibition	No effect		

Polymer: PS – polystyrene, PS-PEI – polyethyleneimine polystyrene, PE – polyethylene, HDPE – high density polyethylene, PP – polypropylene, PVC – polyvinyl chloride; when available, particle charge is presented as (+) positive, (0) neutral or (-) negative when available; ROS –reactive oxygen species; Concentration: *ppb, **particle L^{-1}.

Highlights

- Microplastics' current concentrations are not expected to directly harm microalgae;
- Microplastics may inhibit predation or reduce nutrient availability in microalgae;
- Factors responsible for species sensitivity and microplastics toxicity are unclear.