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REVIEW

Ecotoxicological effects of microplastics and adsorbed contaminants on aquatic organisms

Efectos ecotoxicológicos de los microplásticos y contaminantes adsorbidos en organismos acuáticos

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Abstract
Microplastic (< 5 mm) pollution have raised concern on behalf of the scientific community and the general public. Microplastic occurrence in aquatic environments and organisms have been well documented. However, it is in recent years that the ecotoxicological effects of microplastics have begun to be studied. The aim of the present study was to review, evaluate and discuss the current state of art regarding microplastic and related contaminants ecotoxicological effects in microalgae, crustaceans, molluscs and fish. The results of previous studies have proven growth inhibition and chlorophyll-a decrease in microalgae. Ingestion by small crustaceans and population reduction have been evidenced. Biomarkers in bivalves and fish have shown neurotoxic effects and oxidative stress, along with abnormal behavior. The current state of art lacks realistic parameters and microplastic concentrations to assess environmental pollution. The need for further research was discussed.

Keywords: ecotoxicology; microplastics; aquatic; marine; organisms.

Resumen
La contaminación por microplásticos (< 5 mm) ha generado preocupación por parte de la comunidad científica y el público en general. La presencia de microplásticos en ambientes y organismos acuáticos ha sido bien documentada. Sin embargo, es en los últimos años que los efectos ecotoxicológicos de los microplásticos han comenzado a estudiarse. El objetivo del presente estudio fue resumir, evaluar y discutir el estado del arte actual con respecto a los efectos ecotoxicológicos de los microplásticos y contaminantes relacionados en microalgas, crustáceos, moluscos y peces. Los resultados de estudios previos han demostrado la inhibición del crecimiento y la disminución de la clorofila-a en las microalgas. Se ha evidenciado la ingestión en pequeños crustáceos y la reducción de la población. Los biomarcadores en bivalvos y peces han mostrado efectos neurotóxicos y estrés oxidativo, junto con un comportamiento anormal. El estado del arte actual carece de parámetros y concentraciones de microplásticos realistas para evaluar la contaminación ambiental. Se discutió la necesidad de más investigación.

Palabras clave: ecotoxicología; microplásticos; acuático; marino; organismos.

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Introduction

Plastics are lightweight, strong and durable synthetic organic polymers derived from petroleum (**Andrady, 2011**; **Rios *et al.*, 2007**). In 2016, the production of plastic products reached more than 355 million tons globally (**PlasticsEurope, 2018**). **Avio *et al.*** (**2017**) estimates that at least 10% of the annual production of plastic end up in the oceans. The impacts of plastic waste have been subject of research for a long time (**Cole *et al.* 2011**). Entanglement, ingestion and transportation of invasive non-native species adhered to plastic surfaces have been identified as the major impacts (**Barnes, 2002**; **Derraik, 2002**).

Microplastics are defined as small plastic particles, smaller than 5 mm in diameter (**Andrady, 2017**) and are divided in two categories. While microplastics commercially manufactured as small particles are called primary microplastics, the result of the breakdown and fragmentation of larger plastics (macroplastics) are known as secondary microplastics (**Cole *et al.*, 2011**; **Piehl *et al.* 2018**). Macroplastic breakdown occurs mainly due to mechanical and photolytic fragmentation and biological degradation (**Browne *et al.*, 2007**). Most microplastics are less dense than seawater and travel long distances by the ocean currents or wind (**Maximenko *et al.*, 2012**). However, some denser polymers or biofouled particles may sink and reach the sediment (**Andrady, 2011**; **Kooi *et al.*, 2017**). In recent studies microplastics have been found in the deep-sea sediments (**Kanhai *et al.*, 2019**), water column (**Dai *et al.*, 2018**), water surface (**Ding *et al.*, 2019**) and sandy beaches (**Piñon-Colin *et al.*, 2018**; **Purca and Henostroza, 2017**), evidencing microplastics have become ubiquitous in aquatic environments.

Due to their physical characteristics and ubiquity in the environment, microplastics are highly bioavailable to aquatic organisms. Microplastics have been reported in zooplankton (**Sun *et al.*, 2018**), molluscs (**Naji *et al.*, 2018**), birds (**Provencher *et al.*, 2018**), turtles (**Duncan *et al.*, 2018**), fish (**Hossain *et al.* 2019**; **Zhu *et al.*, 2019**), mammals (**Lusher *et al.*, 2018**) and other aquatic organisms (**Mohsen *et al.*, 2019**).

Besides the physiological effects, microplastic ingestion pose a chemical hazard due to adsorbed contaminants and plastic industrial additives (**Gallo *et al.*, 2018**). Heavy metals, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine pesticides (OCP), and pharmaceuticals are known to be adsorbed by microplastics in trace concentrations (**Brennecke *et al.*, 2016**; **Camacho *et al.*, 2019**; **Li *et al.*, 2018a**; **Rochman *et al.*, 2014**). Leaching industrials additives, such as polybrominated diphenyl ethers (PBDEs), lead heat stabilizers and phthalate plasticizers (**Lithner *et al.*, 2011**) exacerbate microplastic toxicity.

Trophic-level transfer of microplastics may result in commercial seafood contaminated with microplastics. Previous studies have evidenced the presence of microplastic in seafood from markets (**Cho *et al.*, 2019**; **Li *et al.*, 2018b**; **Teng *et al.*, 2019**), suggesting potential risks to human health through contaminated food consumption.

Risk assessments and bioassays of microplastics and related contaminants are needed to fully understand the effects on aquatic biota. Although some studies have suggested prerequisites, considerations and identified gaps in the ecotoxicological impact assessment of microplastics (**Karami, 2017**; **Lambert *et al.*, 2017**; **Potthoff *et al.*, 2017**), there still no standard protocol under reproducible laboratory conditions for this matter.

Considering the importance of ecotoxicological impact assessment knowledge regarding microplastics and adsorbed contaminants, the aim of the present study was to review, evaluate and discuss the current state of art regarding microplastic and related contaminants ecotoxicological effects in four types of organisms.

Microalgae

Microalgae are autotrophic organisms that play a fundamental role in the network of the marine ecosystem as a source of food for other animals (**Demirbas, 2010**). Microalgae are key to the proper functioning of aquatic ecosystems, as they transform large amounts of inorganic compounds into biomass (**Ogburn, 2017**). Most microalgae are found inhabiting pelagic areas, many of which are contaminated with microplastics (**Casado *et al.*, 2013**). Microalgae populations is affected, although it may minimally have a serious impact on the food chain and the global nitrogen cycles (**Prata *et al.*, 2019**; **Bergman *et al.*, 2013**), however, the toxicity effects on the part of microplastics, the results do not offer consensus. A review on the subject is needed to identify possible toxicity mechanisms, as well as to guide new consultations (**Prata *et al.*, 2019**).

Besseling *et al.* (**2014**) investigated the effects of nano-polystyrene (nano-PS), of ~70 nm in diameter, on the growth and production of chlorophyll a (Chl-a) of microalgae *Scenedesmus obliquus* in three 72-h bioassays. Concentrations are not specified. Results indicated significant growth inhibition in the 3 test sets performed (2-way ANOVA, significant plastic treatment, p-value = 0.013) and was proportional to nano-PS concentrations (1 $g.L^{-1}$ there were approximately 2.5 % growth inhibition), likewise, it was found that the production of Chl-a falls significantly in function of the increase in nano-PS concentration, however below 100 $mg.L^{-1}$ there is no reduced concentration of Chl-a, but is expected to occur in the long run.

Bergami *et al.* (**2017**) conducted a 72-h growth inhibition test exposing *Dunaliella tertiolecta* to PS nanoparticles (NP). Two tests with anionic carboxylated PS (PS-COOH, 40 nm) NP and cationic amino-modified PS (PS-NH_2) NP under six concentrations (0.5, 1, 5, 10, 25, and 50 $\mu g.mL^{-1}$). PS-COOH did not significantly affect the growth of *D. tertiolecta* (EC_{50} = > 50 $\mu g.mL^{-1}$); however, they were absorbed and accumulated in microalgae cell surface, suggesting a possible trophic transfer from prey to predator. On the other hand, PS-NH_2 caused a significant inhibition in algal growth (EC_{50} = 12.87 $\mu g.mL^{-1}$).

Zhang *et al.* (**2017**) carried out a 96-h microalgae growth inhibition test using pristine pure polyvinyl chloride spherical powder (mPVC; ~1 μm) and bulk plastic cut in blocks (bPVC; 1 mm). Microalgae *Skeletonema costatum* was exposed to 1, 5, 10 and 50 $mg.L^{-1}$ of mPVC and 50, 500, 1000 and 2000 $mg.L^{-1}$ of bPVC. Growth inhibition ration (IR) was calculated and chlorophyll content and photosynthetic efficiency (ΦPSII) were determined. Algae-microplastic interaction was observed by scanning electron microscopy (SEM). It was found that mPVC did inhibit microalgae growth; the maximum rate of growth inhibition (IR) reached up to 39.7% after 96-h of exposure. However, bPVC did not significantly inhibit growth. High concentrations (50 $mg.L^{-1}$) of mPVC decreased chlorophyll content in 20% from 25-h to 96-h of exposure. Regarding ΦPSII, it decreased 5% under 5 $mg.L^{-1}$ at 1 and 24-h of exposure. For both chlorophyll content and ΦPSII, higher concentrations caused significant effects. SEM images evidenced the formation of mPVC aggregations, mPVC adsorption by *S. costatum* and physical damage due to algae-mPVC interaction.

Contrary to most studies, **Canniff and Hoang** (**2018**) found a growth enhancement of algae *Raphidocelis subcapitata* when exposed to polyethylene (PE) microbeads (63 – 75 μm). It is suggested microbeads could serve as a substrate for *R. subcapitata* growth.

The ingestion of microplastics in different species of freshwater and marine microalgae has been reported and demonstrated. It was found that microplastics negatively affect and could pose a threat to microalgae in terms of population stability, growth, chlorophyll content and photosynthetic efficiency. Likewise, microalgae may server microplastic bioaccumulators, thus representing a threat to organisms to higher trophic levels after ingestion.

Crustaceans

As zooplankton are basic primary consumers of the aquatic food chain, they have an essential role in the marine ecosystem (**Chatterjee and Sharma, 2019**). They are an important source of food for secondary producers, like commercially important fish and cetaceans (**Botterell *et al.,* 2019**). Their exposure to microplastic ingestion is due to feeding behavior, as they predominately feed in surface waters where microplastics are abundant (**Cózar *et al.,* 2014**).
Several studies have investigated micro-plastic ingestion by zooplankton and evaluated ecotoxicological effects. **Jeong *et al.*** (**2017**) exposed copepod *Paracyclopina nana* to nanosized (0.05 µm) and microsized (0.5 µm and 6 µm) PS microbeads and evaluated ingestion, egestion, growth rate and fecundity. *P. nana* ingested the three different sizes of microbeads, although 6 µm microbeads were egested and disappeared after 24-h post-ingestion observations. *P. nana* exposed to 0.05 µm microbeads developed a delay and reduced fecundity, while those exposed to 0.5 µm microbeads delayed molting without a significant retardation.
Coppock *et al.* (**2019**) investigated feeding selectivity and faecal density in copepod *Calanus helgolandicus* exposed to nylon fibers and fragments, low-density poly-ethylene (LDPE) and high-density poly-ethylene terephthalate (HDPET). Results indicated a decrease in ingestion of chain-forming and unicellular algae that were similar to nylon fibers and fragments respectively. Faeces containing LDPE sank significantly slower than control, while sinking rates increased in faeces containing HDPET.
Bosker *et al.* (**2019**) conducted an assay investigating the impact of PS (1 – 5 µm) on a population of cladoceran *Daphnia magna*. Populations exposed to 10^5 MP.mL^{-1} were reduced significantly, representing 21% in reduction of the total biomass. On the contrary, **Canniff and Hoang** (**2018**) reported no significant effect on survival and reproduction although *D. magna* had ingested PE microbeads (63 – 75 µm). The effects of microbeads in *D. magna* may be conditioned by the particle size. Further research regarding behavioral effects by De **Felice *et al.*** (**2019**), showed an increased swimming activity in terms of distance moved and velocity in *D. magna* after a 21 days' exposure to 1 and 10 µm PS micro-plastics.
Importantly, trophic transfer along the planktonic food web has been also investigated. **Setälä *et al.*** (**2014**) fed mysid shrimps with zooplankton that had ingested PS microbeads. Three hours after incubation, microscopy of the mysid intestines showed the presence of zooplankton prey and microbeads, thus showing a potential microbead transfer between planktonic organisms from a trophic level to a higher level.
Zhang *et al.* (**2019**) investigated the single and combined effects of 1 µm and 10 µm PS particles and roxithromycin (ROX) on *D. magna*. The EC_{50}-48-h of 1 µm and 10 µm particles were 66.97 mg.L^{-1} and 199.94 mg.L^{-1} respectively, while 20.28 mg.L^{-1} for ROX. Co-exposure to 1 µm PS and ROX decreased the responses of glutathione peroxidase (GPx) and malondialdehyde (MDA) compared to ROX alone, while co-exposure to 10 µm PS decreased glutathione S-transferase (GST) and MDA responses.
Larger crustaceans have also been subject of ecotoxicological research. **Watts *et al.*** (**2015**) reported a significant reduction in energy available for growth and food consumption after exposing *Carcinus maenas* to polypropylene rope microfibers (1 – 5 mm) for four weeks.
Microplastic ingestion by crustacean species have been reported. Indeed, microplastics could pose a threat to crustaceans in terms of population stability, reproduction and growth depending on the type, size and concentration of exposure. Sublethal effects of enzymatic biomarkers activities indicate oxidative stress. Lastly, crustaceans may be subject to changes in swimming and feeding behavior.

Molluscs

Molluscs are ecologically and commercially important aquatic and terrestrial macro-invertebrates. Due to their feeding ecology, molluscs are susceptible to microplastic ingestion. They include a large number of filter-feeding organisms (**de Sá *et al.,* 2018**), like most bivalves, and marine grazers, such as most gastropods and polyplacophorans.

The majority of studies assessing micro-plastic ecotoxicological effects in molluscs have focused in bivalves. **Rist *et al.*** (**2016**) exposed the Asian green mussel (*Perna viridis*) to polyvinylchloride (PVC) particles (1 – 50 µm) for 91 days in two 2-hour-time-periods per day. Results indicate a survival decline with increasing concentrations of PVC. However, the concentrations used (0 mg.L^{-1}, 21.6 mg.L^{-1}, 216 mg.L^{-1} and 2160 mg.L^{-1}) exceed the pollution levels of microplastic in most coastal ecosystems by far.

Avio *et al.* (**2015**) investigated the adsorption of pyrene by microplastics (PE and PS) and its tissue localization, cellular effects and gene expression profile in mussel *Mytilus galloprovincialis* after a 7-day exposure. Microplastics and pyrene bioaccumulate in the haemolymph, gills and digestive tissues. Alterations of immunological responses, lysosomal compartment, peroxisomal proliferation, antioxidant system, neurotoxic effects and start of genotoxicity was observed. Microplastic exposure caused alterations in gene expression profile. Another research (**Capolupo *et al.,* 2018**) evidenced microplastic (PS) uptake by *M. galloprovincialis* in larval stages. Similar transcriptional effects were identified. Despite this, no significant increase in macroscopical abnormalities were noted in *M. galloprovincialis* embryos, suggesting a normal larval development.

Van Cauwenberghe *et al.* (**2015**) reported an increase in energy consumption by *Mytilus edulis* exposed to PS (110 MP.mL^{-1}), although it was not reflected in the energy reserves of the exposed mussel.

Oliveira *et al.* (**2018**) exposed *Corbicula fluminea* to microplastics (unknown composition; 0.13 mg.L^{-1}), mercury (30 µg.L^{-1}) and co-exposure (same concentrations) treatments in an 8 days and 14 days bioassays, followed by 6 day post-exposure recovery in a clean medium. Bioconcentration factors were smaller in the co-exposure treatment bivalves than in the mercury only treatment, thus microplastics may reduce mercury concentration when mixed. Results also indicate antagonism between microplastics and mercury in post-exposure filtration rate (FR), cholinesterase enzymes activity (ChE), GST activity and levels of lipid peroxidation (LPO). Bivalves exposed to any of the treatments showed a significant decrease in FR and LPO. Exposure to microplastics alone caused a significant reduction of the adductor muscle ChE activity. Lastly, the 6-day post-exposure recovery deemed not sufficient to completely reverse the toxic effects induced by the treatments nor to fully eliminate the mercury from the organisms' body.

Gastropod *Littorina littorea* have been found to ingest microplastic contaminated seaweed in laboratory experiments (**Gutow *et al.,* 2016**). However, most microplastics were released with the faeces. Further research by **Gutow *et al.*** (**2019**) indicated that gastropod pedal mucus retains suspended microplastics, thus promoting uptake by other organisms.

Bivalves have been studied more than any other mollusc due to the filter-feeding behavior, which enables them to breath more microplastics than other species (**Setälä *et al.,* 2016**). Microplastic exposure in extremely high concentrations significantly compromise the survival of certain bivalves. Biomarkers have shown possible oxidative damage and neurotoxicity. Little is known regarding the ecotoxicological effects of microplastics with adsorbed contaminants. The mixture of microplastics and other bioavailable contaminants should be further researched to determine synergism or antagonism to a survival and molecular level.

Fish

Due to their interactions in the food chain, also to its significance for human consumption (**Barboza *et al.*, 2018**), fish have vital importance in the functionality of the marine ecosystem. Nevertheless, they are exposed to contaminants and microplastic ingestion, bioaccumulation, and biomagnification (**Rochman *et al.*, 2013**).

A variety of studies have investigated the interaction between microplastics and fish, evaluating the ecotoxicological effects and endpoints. **Lei *et al.*** (**2018**) exposed freshwater fish *Danio rerio* to common types of microplastics: polyamides (PA), PE, polypropylene (PP), PVC and PS particles; survival rates and histopathological changes were evaluated. A group of sixteen *D. renio* were exposed to four concentration of each microplastic type in suspension (0.001, 0.01, 0.1, 1.0 and 10.0 $mg.L^{-1}$) diluted in dechlorinated water. Then, 15 fish were selected randomly in each group of a single concentration (1 $mg.L^{-1}$) for histopathological analysis. The results prove the non-lethal significance of microplastic effects on *D. renio*, in spite of this result, the investigation shows histological alterations on the intestine of this species.

Planktonic organisms are often confused with microplastics by organisms from higher trophic levels. **Ory *et al.*** (**2018**) exposed *Seriolella violacea* to microplastics in different color groups (black, blue, translucid and yellow) to determine whether color influenced microplastic ingestion. *S. violacea* specimens were put in tanks filled by fresh seawater and fed with food pellets mixed with microplastics (in ⅕ ratio). Results showed black microplastics to be the most ingested particles. It was also found that microplastics are more common to remain a long period of time in the digestive tract, meaning they are not easily egested compared to fish food pellets.

Microplastic exposure into marine biota is also subject to non-lethal effects and behavior alterations. **Qiang and Cheng** (**2019**) studied the effects of microplastics (468-508 nm PS microspheres) on embryos and larval *D. rerio*. The embryos of *D. rerio* were exposed to microplastics (100 and 1000 $\mu g.L^{-1}$) starting from 4 hours post-fertilization, the analysis shows that microplastics first adhered to the embryo chorion and then entered the digestive tract. In spite of the analysis, the results indicated that microplastics do not have significant effects on the growth of *D. rerio* embryos. On the other hand, the study also analyses the effect of microplastics on the swimming competency of larval *D. renio*. A significant decrease in swimming and speed, as a consequence of the inflammation and oxidative stress-related to genes, expressed at the molecular level was evidenced.

Mak *et al.* (**2019**) studied the effects of PE microplastics in five size ranges (10-22 µm, 45-53 µm, 90-106 µm, 212-250 µm, and 500-600 µm) at 2 $mg.L^{-1}$ (treatment A) and a second set of three size ranges and colors (45–53 µm [blue], 90–106 µm [green], and 212–250 µm [clear]) in a high (1100 $MP.L^{-1}$), medium (110 $MP.L^{-1}$) and low (11 $MP.L^{-1}$) concentrations (treatment B) on 4 month old *D. rerio*. Ingestion, interaction with the aryl hydrocarbon receptor (AHR), the disruption of the oogenesis process and neurotoxicity were assessed. Microplastic exposure was carried out through their feed for a 96-h period, following visual inspection of the fish organs and gene expression analysis. No deaths were identified and no morphological differences in the liver. Abnormal behavior, like erratic movement, seizures, and tail bending, were exhibited in medium to high concentration tanks. *D. rerio* intestine *cyp1a* expression showed upregulations when exposed to medium concentrations of microplastics, while liver *vtg1* expression showed upregulations under medium and high concentrations. In addition, the authors proposed that sickness behaviors may be caused by acute exposure to microplastics as a hypothesis to further investigations.

Fish are among the most studied species. In general terms, microplastics have not shown lethal effects over fish. Common effects are abnormal behavior or slight morphological changes. Biomarkers have proven neurotoxicity, oxidative stress and oogenesis process disruption.

Conclusions and further research

The amount of microplastics in aquatic environments have raised concern regarding their effects on aquatic biota. Microplastics and adsorbed contaminants exposure to aquatic organisms is an undeniable fact that could threat the survival of some species. Several studies from recent years have investigated the effects of microplastics on aquatic organisms, assessing survival, growth, behavior and biomarkers.

We have identified three major issues regarding the current state of art. First, studies have investigated *in vitro* microplastic effects with unrealistic concentrations. Many treatment concentrations surpass by far that of the test organism's natural environment. Consequently, giving results that are less likely to apply in a real scenario. Second, laboratory studies tend to choose PP, PE, PS or PVC as the microplastic contaminant, although fibres are the most common microplastic type found in aquatic environments. And third, very few studies have assessed microplastic-adsorbed contaminant effects in co-exposure bioassays. Additionally, as microplastics have been proven to scale from prey to predator through ingestion (**Welden *et al.*, 2018**), it would be recommended to further investigate the effects and biomagnification on higher trophic level organisms after ingestion of contaminated natural prey. Further research should consider

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