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Daphnia magna as a model animal for assessing microplastic toxicity

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Abstract
Microplastics, originating directly from household and industrial products or from large plastic degradation, are currently of extreme global concern. The coronavirus pandemic also increased the use of microplastics because all the protective equipment like face masks, full face shields and gloves are made up of different plastics such as polyurethane, PET, polyester and polypropylene. The face masks generally contain a lot of polypropylene in it which produces toxicity in ocean beds and great lakes during the weathering process. Actually, weathering processes lead to the fragmentation of plastics into microplastics by affecting their physicochemical properties. Microplastics are pervasive in the environment due to the delayed disposal of plastic wastes, a lack of detection tools and particular removal procedures, and a gradual disposal rate. Due to their small size (ranging from 1 µm to 5 mm) microplastics can easily be ingested. Consequently, living organisms living in the water column as well as those found in benthic zones are threatened by the presence of microplastics. In this paper, we have discussed the effects of microplastics on *Daphnia magna*. The filter feeder D. *magna* ingest microplastics and are not able to differentiate between particles of different nature. Microplastics decreased the survival rates, body growth, reproduction and immune responses in *Daphnia*. Sinking microplastics also decreased the swimming velocity of *Daphnia* during cruising and vertical swimming trajectories. In conclusion, these findings highlight the health risks of contamination of microplastics in aquatic environments and present *Daphnia* as a good model animal for research in the field of microplastic toxicity.

Keywords: *Daphnia magna*; Microplastics; Toxicity; Pollution; hazard

Introduction
The use of plastic is increasing day by day. Slow decomposition of plastic leads to increased microplastic concentrations in the environment (Singh, 2022). The plastics entering the coastal environment may remain for millions of years after they get busted due to the mechanical and photochemical processes resulting in the formation of microplastics. Microplastic particles are usually divided by their origin into primary and secondary microplastics. The primary microplastics are generated as such and appear in marine environments either by chance or with waste waters (like residuals of used cosmetics, scrubbers, etc.). The secondary microplastics come from damage of larger objects which conclude in the marine environment. The large amount of macroplastics which enter the environment is generally the secondary microplastics (Efimova et al., 2018). Because plastic waste initially degrades into meso particles and macroparticles, it is a major source of secondary microplastics found in the ocean and soil. Ultraviolet (UV) radiation from the sun and various physical forces results in the fragmentation of meso and macro plastic debris including Polystyrene coffee cup lids, disposable plates and PS foams into microplastics. A study irradiated plastic debris with simulated UV light to determine the degradation mechanism (Hwang et al., 2020). The floating microplastics over the oceans are misinterpreted as food by various marine animals and get ingested. This results in enormous health impacts on aquatic organisms (Sheng et al., 2021). Humans are also exposed to microplastics. The presence of microplastics in products like hand cleansers, foodstuffs and scrubbers in cosmetics, is the source of exposure. Microplastic exposure in different biological systems may cause microplastic toxicity causing various ailments including inflammatory lesions.

The lack of immunity against these synthetic particles leads to an increase in abnormal growth or neoplasia (Prata et al., 2019). Marine species contain a heavy concentration of microplastics in their body and when humans consume seafood, these are likely to cause many harmful effects such as infertility, obesity and even cancer (Chatterjee and Sharma, 2017).

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This condition also provokes stress, high immune responses, and results in developmental and reproductive toxicity (Blackburn and Green, 2021). In the automotive industry, demand for bakelite increases due to the big ask for lightweight materials to ensure fuel efficiency in vehicles. Due to increased abrasion and mechanical pressure, the bakelite is speedily degraded into microplastic. However, these microplastics have a high environmental concern. On ingestion and absorption, microplastics affect freshwater organisms with mechanical as well as chemical stress because of unreacted monomers, additive leaching and by-products of polymerisation (Klun et al., 2022). The expansion of microplastic entering oceans is black rubber identified as tire debris (Cunningham et al., 2022). Fibres are the most influential microplastic in the freshwater ecosystem. The textiles fragmentation during laundry and successive discharge in the environment as untreated and treated wastewaters add up to the major pathway of fibres. Microplastic fibres do not contain a single compound and are of different shapes and sizes, so ingestion chances of fibres may differ in freshwater organisms. Spherical particles have different ingestion rates as compared to the longer fibrous material. The tire particles arise from tire friction on roadways and flow into the environment from different sources such as wastewater, and runoff into soils and ocean beds. An estimated range from 0.23 to 1.9 kg/yr of tire particles flow in (China, Japan, India, Sweden, Norway and Denmark) (Schell et al., 2022). Tire particles are composed of vulcanisation agents, oils, synthetic rubbers (styrene), filling agents and other additives. floating plastics present on the surface of oceans are buoyant plastic, while microplastic with high-density sinks beneath the floor of oceans. A recent report suggests that most of the plastic comes from municipal waste. The granules, fibres and small plastic pieces are well-known ocean plastic debris forms. This involves synthetic particles like in cosmetics microbeads used, in drug delivery, in medicines and other applications. Biodegradable plastic produces much of the microplastic in agricultural fields and mainly affects the fertility of soils (Bhatt et al., 2021).

Table 1. Studies revealing toxic effects of microplastics

Sr. No.	Author	Year	Animal model	Inference
1.	Grassl et al.	2021	*Daphnia magna*	Elevated temperatures lead to increased ingestion of microplastics.
2.	Aljaibachi and Callaghan	2018	*Daphnia magna*	Uptake of microplastics decreased in presence of algae.
3.	Scherer et al.	2017	*Daphnia magna*	Rate of microplastic uptake depends upon biotic and abiotic factors.
4.	Guilhermino et al.	2021	*Daphnia magna*	At increased water temperature, the total number of broods produced decreases.
5.	Laforsch et al.	2021	*Daphnia magna*	Shape, size, type and age of particles might influence the toxicity.
6.	Arnott et al.	2022	*Daphnia magna*	Transgenerational impacts of microplastics on reproduction.
7.	Miloloža et al.	2021	*Daphnia magna*	Small microplastic particles caused higher growth inhibition.
8.	Parolini et al.	2022	*Daphnia magna*	Long term exposure of microplastics effects at molecular and biochemical level.
9.	Wagner et al.	2021	*Daphnia magna*	wastewater-incubated microplastics resulted in a lower mortality than pristine microplastics.
10.	Schell et al.	2022	*Daphnia magna*	D. *magna* did not ingest fibres but ingest tire particles.
11.	Rozman et al.	2022	*Daphnia magna*	Bakelite microplastics had a low impact on *Daphnia*.
12.	Xiao et al.	2022	*Daphnia magna*	Polyethylene microplastics affect trophic cascade strength and reduced stability.
13.	Barcelona et al.	2021	*Daphnia magna*	Lethal and sub-lethal effects like reduced body growth rate, swimming velocity and survival.

Effects of microplastics on Daphnia magna

Contamination with microplastic in freshwater habitats is threatening the aquatic community particularly to suspension feeders (filter feeders) and their pollution have serious ecological consequences (Grassl et al., 2021). Cladoceran D. *magna* is a keystone species in numerous freshwater habitats all over the world. *Daphnia* can eat particles between 1 to 70 µm in size and typically eat algae. They can also eat bacteria (Aljaibachi and Callaghan, 2018). It occupies an important position in aquatic food webs as it is the highly effective suspension feeder which regulates algal and bacterial growth (Scherer et al., 2017). Preferential habitats for this species are shallow water ecosystems due to reduced pressure of predation but are vulnerable to effects of microplastic pollution and climate changes (Guilhermino et al., 2021).

The microplastic poses deleterious effects on *Daphnia* at morphological, anatomical and molecular level. Microplastic releases harmful substances into the digestive tract of the animal and poses major threats (Laforsch et al., 2021). Together with the microplastics, main driving pressures such as increase of water temperature and light intensity act on the biota. Some microplastics are excreted but others are internalized which cause toxic effects like mortality, reduced population fitness, reduced daphnid survival and decreased reproduction over generations (Guilhermino et al., 2021); increased inflammation, altered behaviour, altered fat and energy metabolism, changes in the microbiome and change in the body length, width and tail spine length in offsprings (Arnott et al., 2022); impaired filtering activity and compromising gut activity (Miloloža et al., 2021); abnormal embryonic development, obstruction of digestive tract and the onset of oxidative stress (Parolini et al., 2022). Microplastic ingestion was found to delay intestinal function (Grassl et al., 2021). After reduced food supply and exposure to microplastic, body lengths of adults are affected (Wagner et al., 2021). Fibres are not ingested in *Daphnia* whereas ingestion of tire particles increased with increasing exposure concentrations. The prolonged exposure period and increase in body size of the adults allowed them to consume greater sizes of particles, which led to the increased intake (Schell et al., 2022).

Reduced fitness in *Daphnia magna*

Microplastics in D. *magna* enter into gills, gut, and attach to the surface of the body including appendages. It reduced the efficiency of other gill functions and efficiency of respiration in both parental females and juveniles, contributing to a decrease in fitness (Guilhermino et al., 2021). Egestion is also influenced by microplastics like total absence or very low egestion, leading to reduced intake of food and ultimately starving (Miloloža et al., 2021). Toxicity is caused inside the body by several ways, leading to exposure of eggs when entering into the brood chamber, appendices may compromise swimming. Microalgae ingestion was decreased and reduced filtration was seen with microplastics. Less energy was available due to decreased food ingestion. Need of energy allotment to face microplastic induced stress, basic functions maintenance and need of tissue repair was seen to increase (Guilhermino et al., 2021). Sharp edges on irregularly shaped microplastics can harm internal organs, and these build up in the digestive system of *Daphnia* and ultimately prevent the digestion of fresh food (Rozman et al., 2022). Microplastic induced reproduction toxicity was observed as seen by production of fewer clutch or offsprings and decreased average brood production (Arnott et al., 2022). Polyethylene microplastics reduced the hopping frequency, heart rate, grazing rate and stability (Xiao et al., 2022).

Swimming velocity in *Daphnia magna*

The swimming velocity of water flea D. *magna* is mainly dependent on its body size. Therefore, environmental factors which control growth also affect swimming velocity (Blust et al., 1998). Differences in swimming behaviours can possibly be caused by smaller body lengths arising from reduced growth rates. Changes of the mean velocity of swimming indicates a significant change in swimming behaviour following an exposure to the toxic substance. Other parameters, like fractal dimension and speed distribution did not change (Noss et al., 2013). In D. *magna* individuals are seen with different swimming patterns based on vertical trajectory, cruising trajectory and hopping and sinking based on net displacement. Swimming velocity in D. *magna* could be correlated with body length. Bigger *Daphnia* individuals swim faster. Sublethal effects are produced by microplastics; hopping and sinking (successive ascending and descending short pathways followed by individuals) movements are predominantly exhibited by D. *magna* individuals. Vertical velocity (individuals swam in quasi-straight vertical trajectory with overall angle more than 45° with horizontal axis) is slightly lesser than cruising velocity (near straight trajectory in direction below 45° to horizontal axis). To follow a vertical trajectory, energy is required to overcome the pressure gradient excreted by water. So, this movement was sustained only when favourable conditions were available (system without microplastic/with food) (Barcelona et al., 2021).

Conclusion

Microplastic pollution in marine environments has become a serious and global pollution incident. At present, there is a lack of effective treatment methods. *Daphnia magna* has been used as a model animal to assess microplastic toxicity in several studies. For proper functioning, the stability of D. *magna* under natural conditions is important for the freshwater ecosystem, as they are key food sources for predators as well as important grazers of phytoplankton. Microplastics have been seen to affect D. *magna* populations as these harms reproductive health, trigger a series of immune responses, affect brood amounts and growth rates. There were significant adverse impacts of microplastics on both total number of individuals and total biomass of D. *magna a*s well as a significant reduction in total amount of adult daphnids. Microplastics caused release of immobile juveniles, reduced size of first brood and increased somatic growth. Consumption of microplastic was not seen to affect locomotory speeds D. *magna*. Conclusively, this study reveals that exposure to microplastics induce toxicity to D. *magna* at different biological levels.

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ZS conceived the concept. SK, HG and ZS wrote and approved the manuscript.

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