THÈSE DE DOCTORAT

PRESENTEE PAR

Wajid Ali

POUR L'OBTENTION DU GRADE DE

DOCTEUR DE L'UNIVERSITE DE LILLE

Ecole Doctorale : Science de la Matière, du Rayonnement et de l'Environnement (ED SMRE)

Discipline du doctorat : Biologie de l'environnement, des organismes, des populations, écologie

Assessing the toxicity of plastic fragments on zooplankton ecology

Evaluation de la toxicité des fragments plastiques sur l'écologie du zooplancton

Soutenance de thèse le 26 Novembre 2024

Composition du Jury

Rapporteur Président du jury	Dr. Isabella Buttino	ISPRA
Rapporteur	Dr. Davide Degli Esposti	UR Riverly, INRAE
Examinateur	Pr. Jean-Marie Raquez	LPCM, Université de Mons
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Abstract

This thesis investigates the effects of plastic fragments from bioplastics on various zooplankton species and is divided into three parts. The first part introduces the background of plastic pollution, focusing on the transition from plastics to microplastics (MPs) in aquatic environments, and discusses eco-friendly alternatives like bioplastics, which are derived from renewable sources or designed to be biodegradable. The second part comprises a compilation of published review and research papers, each presented as a chapter. The final part presents findings from literature reviews and experimental studies, along with future research perspectives.

The mass production of plastics began in the 1950s, with most being synthesized from nonrenewable resources and resistant to degradation. These plastics fragment into MPs through environmental weathering, which can be ingested by aquatic organisms, leading to mechanical damage, gut blockage, increased mortality, and facilitating pollutant transport. In recent years, biobased plastics have emerged as an eco-friendly alternative to fossil fuel-based plastics. Polylactic acid (PLA) is a widely produced biobased plastic that is also biodegradable *in vivo* with diverse applications. However, its degradation in aquatic environments is slow, resulting in the formation of MPs that have shown toxicity to aquatic species, although long term effects remain understudied. To address this, the multigenerational toxicity of PLA MPs was evaluated at lower concentrations on the sentinel estuarine species *Eurytemora affinis*. The findings revealed increased mortality, prolonged naupliar stages, reduced fitness in offspring, and decreased female body size.

A literature review comparing biobased plastics and fossil fuel-based plastics revealed that both contribute to pollution through the formation of MPs and have similar negative effects on biota, challenging the assumption that biobased plastics have a lower environmental impact in that context. Therefore, the potential toxicity of MPs from different sources was evaluated and compared. The *in vivo* and *in vitro* toxicities of poly(butylene adipate-*co*-terephthalate) (PBAT) as a biodegradable plastic, PLA as a biobased plastic, β-cyclodextrin-grafted PLA as a modified biobased plastic, and low-density polyethylene (LDPE) as a nonbiodegradable fossil fuel-based reference were assessed using the brackish water flea *Diaphanosoma celebensis*. The results indicated that MPs exposure led to reproductive decline, redox stress, and altered transporter protein activity across all types, suggesting comparable effects. Since species have varying susceptibilities to contaminants, the toxicity of the four MPs type on the marine rotifer *Brachionus plicatilis* was studied. The results showed that MPs exposure significantly reduced

reproductive output, with reactive oxygen species (ROS) levels, antioxidant response, mitogen-activated protein kinase (MAPK) signaling, and transporter proteins showing MPs type-specific modulation.

Moreover, finding that all MP types showed comparable toxicity and that potentially biodegradable MPs may share a similar fate in terms of their interaction with pollutants in the marine environment as nonbiodegradable MPs, the sorption of environmental pollutants on biodegradable MPs was reviewed together with their toxicity. Biobased plastics vary in biodegradability, with some requiring specific conditions, and their MPs can adsorb pollutants, potentially causing synergistic or antagonistic effects on exposed organisms. Building on this, a comparative study was conducted on the transport of heavy metals, such as methylmercury (MeHg), by PLA and LDPE MPs in *Daphnia magna* at environmentally relevant concentrations. Results showed that PLA MPs, both alone and with MeHg, significantly reduced survival and reproduction while inducing oxidative stress, with stronger effects than LDPE MPs. This suggests that PLA MPs, despite being potentially biodegradable *in vivo*, may pose similar or greater risks than fossil fuel-based MPs, particularly by enhancing the bioaccumulation and toxicity of coexisting pollutants.

Keywords. Zooplankton; (Bio)plastic; Pollution; microplastics; toxicity.

Résumé

Cette thèse examine les effets des fragments de plastique provenant de bioplastiques sur diverses espèces de zooplancton et est divisée en trois parties. La première partie présente le contexte de la pollution plastique, en mettant l'accent sur la transition des plastiques vers les microplastiques (MPs) dans les environnements aquatiques, et discute des alternatives écologiques telles que les bioplastiques, qui sont dérivés de sources renouvelables ou conçus pour être biodégradables. La deuxième partie est composée d'une compilation d'articles de revue et de recherche publiés, chacun présenté sous forme de chapitre. La dernière partie présente les résultats des revues de littérature et des études expérimentales, ainsi que des perspectives de recherche futures.

La production de masse des plastiques a commencé dans les années 1950, la plupart étant synthétisés à partir de ressources non renouvelables et résistants à la dégradation. Ces plastiques se fragmentent en MPs par l'altération environnementale, pouvant être ingérés par les organismes aquatiques, entraînant des dommages mécaniques, des obstructions intestinales, une mortalité accrue et facilitant le transport de polluants. Ces dernières années, les plastiques biosourcés ont émergé comme une alternative écologique aux plastiques d'origine fossile. L'acide polylactique (PLA) est un plastique biosourcé largement produit, qui est également biodégradable *in vivo*, avec diverses applications. Cependant, sa dégradation dans les environnements aquatiques est lente, entraînant la formation de MPs ayant montré des effets toxiques sur les espèces aquatiques, bien que les effets à long terme demeurent sous-étudiés. Pour aborder cette question, la toxicité multigénérationnelle des MPs de PLA a été évaluée à des concentrations plus faibles sur l'espèce sentinelle estuarienne *Eurytemora affinis*. Les résultats ont révélé une mortalité accrue, des stades naupliens prolongés, une réduction de la condition physique des progénitures et une diminution de la taille corporelle des femelles.

Une revue de la littérature comparant les plastiques biosourcés et les plastiques d'origine fossile a révélé que les deux contribuent à la pollution par la formation de MPs et ont des effets négatifs similaires sur le biote, remettant en question l'hypothèse selon laquelle les plastiques biosourcés auraient un impact environnemental moindre dans ce contexte. Par conséquent, la toxicité potentielle des MPs issus de différentes sources a été évaluée et comparée. Les toxicités *in vivo* et *in vitro* du poly(adipate-*co*-téréphtalate de butylène) (PBAT) en tant que plastique biodégradable, du PLA en tant que plastique biosourcé, du PLA greffé avec la β-cyclodextrine en tant que plastique biosourcé modifié, et du polyéthylène basse densité (LDPE) en tant que référence non biodégradable d'origine fossile, ont été évaluées en utilisant la puce d'eau

saumâtre *Diaphanosoma celebensis*. Les résultats ont indiqué que l'exposition aux MPs entraînait un déclin reproductif, un stress oxydant et une altération de l'activité des protéines de transport pour tous les types de MPs, suggérant des effets comparables. Étant donné que les espèces présentent des sensibilités variables aux contaminants, la toxicité des quatre types de MPs a été étudiée chez le rotifère marin *Brachionus plicatilis*. Les résultats ont montré que l'exposition aux MPs réduisait significativement la reproduction, avec une modulation spécifique au type de MPs des niveaux d'espèces réactives de l'oxygène (ROS), de la réponse antioxydante, de la signalisation des protéines kinases activées par les mitogènes (MAPK) et des protéines de transport.

De plus, constatant que tous les types de MPs présentaient une toxicité comparable et que les MPs potentiellement biodégradables pourraient partager un destin similaire aux MPs non biodégradables en termes d'interaction avec les polluants dans le milieu marin, une revue a été réalisée sur la sorption des polluants environnementaux sur les MPs biodégradables ainsi que sur leur toxicité. Les plastiques biosourcés présentent une biodégradabilité variable, certains nécessitant des conditions spécifiques, et leurs MPs peuvent adsorber des polluants, entraînant potentiellement des effets synergiques ou antagonistes sur les organismes exposés. Dans cette optique, une étude comparative a été menée sur le transport des métaux lourds, tels que le méthylmercure (MeHg), par les MPs de PLA et de LDPE chez *Daphnia magna* à des concentrations environnementalement pertinentes. Les résultats ont montré que les MPs de PLA, seuls ou en présence de MeHg, réduisaient significativement la survie et la reproduction tout en induisant un stress oxydatif, avec des effets plus marqués que ceux des MPs de LDPE. Ces résultats suggèrent que les MPs de PLA, bien que potentiellement biodégradables *in vivo*, pourraient poser des risques similaires ou supérieurs à certains MPs d'origine fossile, notamment en favorisant la bioaccumulation et la toxicité des polluants coexistant dans l'environnement.

Mots-clés : Zooplancton; (Bio)plastique; Pollution; Microplastiques; Toxicité.

Contents

Preface

This interdisciplinary Ph.D. thesis was carried out at two laboratories: Unité de Catalyse et Chimie du Solide (UCCS) in Lille and Laboratoire d'Océanologie et de Géosciences (LOG), Station Marine in Wimereux, both of which are part of the University of Lille in France. This work was undertaken to fulfill the requirements for obtaining a Doctoral degree.

The research was conducted from November 2021 to April 2024 under the co-direction of Professor Philippe Zinck from UCCS and Professor Sami Souissi from LOG. Some components of this research were also carried out in collaboration with cross-border laboratories. Work was conducted at the Materials Research and Development Center, University of Mons, in Belgium, in collaboration with Dr. Samira Benali and Professor Jean-Marie Raquez. Additionally, research was performed at the Department of Biological Sciences, College of Science, Sungkyunkwan University, in South Korea, in collaboration with Professor Jae-Seong Lee.

The PhD project was funded by the Program for Early-Stage Researchers in Lille (PEARL), coordinated by the Foundation I-SITE ULNE. Mission grants were also awarded through the French government's "Investissements d'avenir" program (I-SITE ULNE/ANR-16-IDEX-0004 ULNE), managed by the French National Research Agency, and through the PRIORITY COST ACTION CA20101.

This thesis is structured into three main parts. The first part provides an introduction, showing the background from plastics to microplastics in aquatic environment, followed by a brief overview of eco-friendly alternatives, bioplastics, where I put in context the projects carried out during this PhD. The second part comprises a compilation of research papers, presented as individual chapters of this thesis. The final section presents the general conclusions drawn from the literature review and the experiments conducted throughout the projects, along with future perspectives that could further enrich and expand upon this work.

All experimental work was conducted across various laboratories, and the contributions come from researchers in these different institutions. I am the first author of all the papers resulting from this research, having made significant contributions to achieving the objectives of this work.

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Additionally, I would like to extend my heartfelt thanks to Professor Jae-Seong Lee from the Department of Biological Sciences, College of Science, Sungkyunkwan University in South Korea, for welcoming me during my research stay and for partially supporting both my stay and my research activities. During my time there, I had the privilege of working closely with my esteemed colleague, Haksoo Jeong, from whom I gained critical skills. I am thankful for his support, our stimulating discussions, and his valuable ideas, as well as his assistance with administrative tasks.

I also extend my gratitude to all the co-authors who contributed to improving the articles included in this thesis. Special thanks to Professor Hazrat Ali from the University of Malakand, Pakistan, for his valuable suggestions during the article-writing process. I am also profoundly grateful to my thesis co-directors, whose guidance has greatly enhanced my writing skills and deepened my passion for science.

Finally, I would like to express my deepest thanks to my parents and siblings for their unwavering support, understanding, and encouragement throughout this academic journey. A heartfelt thank you to my wife for her immense courage, motivation, and patience throughout the long years of separation during this journey.

List of Publications

Ali, Wajid, Hazrat Ali, Sayed Gillani, Philippe Zinck, and Sami Souissi. "**Polylactic acid synthesis, biodegradability, conversion to microplastics and toxicity: a review**." Environmental Chemistry Letters 21, no. 3 (2023): 1761-1786.

Ali, Wajid, Shagnika Das, Jeremy Thery, Haksoo Jeong, Jae-Seong Lee, Philippe Zinck, and Sami Souissi. "**Acute and multigenerational toxicity of polylactic acid microplastics on a copepod bioindicator.**" Environmental Chemistry Letters (2024).

Ali, Wajid, Hazrat Ali, Sami Souissi, and Philippe Zinck. "**Are bioplastics an ecofriendly alternative to fossil fuel plastics?**" Environmental Chemistry Letters 21, no. 4 (2023): 1991-2002.

Ali, Wajid, Haksoo Jeong, Michaël Lalanne Tisné, Audrey Favrelle-Huret, Wim Thielemans, Philippe Zinck, Sami Souissi, and Jae-Seong Lee. "**Comparing the toxicity of biobased, modified biobased, biodegradable, and petrochemical-based microplastics on the brackish water flea *Diaphanosoma celebensis*.**" Science of the total Environment (2024).

Ali, Wajid, Haksoo Jeong, Jin-Sole Lee, Philippe Zinck, Sami Souissi, and Jae-Seong Lee. "**Adverse effects of environmentally relevant microplastics on *in vivo* endpoints, oxidative stress, and mitogen-activated protein kinase signaling pathway and multixenobiotic resistance system in the marine rotifer *Brachionus plicatilis***" Science of the total Environment (2025).

Ali, Wajid, Haksoo Jeong, Jae-Seong Lee, Philippe Zinck, and Sami Souissi. "**Biodegradable microplastics interaction with pollutants and their potential toxicity for aquatic biota: a review.**" Environmental Chemistry Letters (2024): 1-36.

Ali, Wajid, Haksoo Jeong, Jin-Sole Lee, Philippe Zinck, Sami Souissi, and Jae-Seong Lee. "**Toxicity comparison of polylactic acid and polyethylene microplastics co-exposed with methylmercury on *Daphnia magna***" Marine Pollution Bulletin (2025).

List of Abbreviations

Acetylcholinesterase (AchE)

Cadmium (Cd)

Carbon dioxide (CO_2)

Copper (Cu)

Fourier-transform infrared spectroscopy (FTIR)

Gas chromatography (GC)

Gastrointestinal tracts (GIT)

Greenhouse gases (GHGs)

High density polyethylene (HDPE)

Hydrogen peroxide (H_2O_2)

Infrared (IR)

Lead (Pb)

Life cycle analyses (LCAs)

Low-density polyethylene (LDPE)

Mass spectrometry (MS)

Methane (CH_4)

Methylmercury (MeHg)

Microplastics (MPs)

Mitogen activated protean kinase (MAPK)

Nanoplastics (NPs)

Nickel (Ni)

Nitric acid (HNO_3)

Nonylphenol (NP)

Persistent organic pollutants (POPs)

Poly(butylene adipate-co-terephthalate) (PBAT)

Polyamide (PA)

Polychlorinated biphenyls (PCBs)

Polycyclic aromatic hydrocarbons (PAHs)

Polyethylene (PE)

Polyethylene terephthalate (PET)

Polyhydroxyalkanoates (PHAs)

Polylactic acid (PLA)

Polypropylene (PP)

Polystyrene (PS)

Polyurethanes (PU)

Polyvinyl chloride (PVC)

Potassium hydroxide (KOH)

Reactive oxygen species (ROS)

Roxithromycin (ROX)

Sodium hydroxide (NaOH)

Tetrabromobisphenol A (TBBPA)

Titanium (Ti)

Ultraviolet (UV)

Zinc (Zn)

Popular science abstract

Plastic production exceeded 400 million tons by 2022, with projections estimating an increase to 800 million tons by 2050. By 2015, only 9% of all plastics produced was recycled, 12% incinerated, and 60% landfilled. Due to their slow degradation, plastics fragment into microplastics (MPs), adversely affecting aquatic biota. Bioplastics, designed to mitigate pollution, reached a production of 2.18 million tons in 2023, with forecasts predicting 7.4 million tons by 2028. However, bioplastics, including polylactic acid (PLA), have been shown to fragment into MPs under aquatic settings, potentially leading to toxic effects on aquatic biota. Herein, PLA MPs have shown gender-specific toxicity in copepods, along with effects on marine rotifers and brackish water fleas. Comparisons of various MP types indicated similar toxic effects on zooplankton, with oxidative stress identified as a key mechanism. Polar PLA MPs can transport higher heavy metals concentrations than certain apolar petrochemical MPs, suggesting increased environmental risks.

Résumé de vulgarisation scientifique

La production de plastique a dépassé les 400 millions de tonnes en 2022, avec des projections estimant une augmentation à 800 millions de tonnes d'ici 2050. En 2015, seulement 9 % de tous les plastiques produits étaient recyclés, 12 % incinérés et 60 % enfouis. En raison de leur lente dégradation, les plastiques se fragmentent en microplastiques (MPs), affectant négativement le biote aquatique. Les bioplastiques, conçus pour réduire la pollution, ont atteint une production de 2,18 millions de tonnes en 2023, avec des prévisions de 7,4 millions de tonnes d'ici 2028. Cependant, il a été démontré que les bioplastiques, y compris l'acide polylactique (PLA), se fragmentent en MPs dans les milieux aquatiques, entraînant potentiellement des effets toxiques sur la biota aquatique. Ici, les MPs de PLA ont montré une toxicité spécifique au sexe chez les copépodes, ainsi que des effets sur les rotifères marins et les puces d'eau saumâtre. Les comparaisons de divers types de MPs ont montré des effets toxiques similaires sur le zooplancton, le stress oxydatif étant identifié comme un mécanisme clé. Les microplastiques de PLA polaire peuvent transporter des concentrations plus élevées de métaux lourds que certains microplastiques apolaires d'origine pétrochimique, ce qui suggère des risques environnementaux accrus.

1. Introduction

Plastics are versatile synthetic polymers, primarily derived from petroleum or natural gas, that can be molded or shaped during manufacturing and their performance is enhanced with chemical additives to achieve desirable properties. The development of plastics began with natural materials like shellac and gum, which had inherent plastic properties, followed by chemically modifying natural materials such as rubber, nitrocellulose, and collagen among others. The first fully synthetic plastic, Bakelite, was synthesized by Leo Baekeland in 1907 by reacting phenol and formaldehyde (Mülhaupt, 2013), paving the way for the development of new plastics in the subsequent decades. The 1930s marked the emergence of plastics as an industry, with the commercialization of synthetic materials like polystyrene (PS) and nylon. The commercialization of nylon in 1939 marked a key milestone in synthetic plastics, spurring industrial research that drove innovation and expansion (Lintsen et al. 2017). Until the 1950s, the rapid development of modern plastics led to the synthesis of over 15 new polymer classes, establishing plastics as highly versatile materials in a wide range of types and forms (Andrady and Neal, 2009). The versatility of plastics, combined with their cost-effectiveness, lightweight nature, strength, durability, corrosion resistance, and excellent thermal and electrical insulation properties (Thompson et al. 2009), has made them suitable for a wide range of industrial and consumer applications. Plastics are now ubiquitous in modern infrastructure, serving as the preferred material for products such as electronics, food packaging, and 3D printing, while also reducing food waste, enabling fuel-efficient vehicles, and improving energy efficiency in electrical insulation (Nielsen et al. 2020). Thus, plastics have gained widespread use over traditional materials like wood, ceramics, and metals since the 1950s due to their versatile properties and lower cost, leading to a significant increase in their consumption.

1.1. Production of plastics

Plastic production is closely linked to fossil fuel resources, with approximately 99% of the raw materials used in plastic manufacturing derived from fossil fuels such as oil and natural gas. This dependence significantly contributes to global energy consumption patterns, accounting for around 9% of total global oil and gas usage (Nielsen et al. 2020). Of this, approximately 4% is consumed as feedstock for plastic production, while an additional 3-4% is used to provide the energy necessary for production processes (Hopewell et al. 2009). While global annual plastic production remained below one million tons during the early 1940s (Thompson et al. 2009), their use expanded, driving production to approximately over two million tons by 1950 (Lintsen et al. 2017). By 1960, annual plastic production had surged to 8.2 million tons, and this figure further skyrocketed to 36 million tons by 1970, reflecting the rapid growth of the industry. This rise in production paralleled a sharp increase in per capita plastic consumption, particularly in developed nations. For example, in the Netherlands, each individual used approximately 1.7 kg of plastics per year in 1950. This consumption rose significantly in the following decades, reaching 9.1 kg by 1960 and further increasing to 35 kg by 1971 (Lintsen et al. 2017). This growth was powered by the widespread adoption of plastics across various sectors, including textiles, fashion, toys, and domestic applications, such as the introduction of polyethylene (PE) bags in the 1950s. Global plastic production has since grown

exponentially, with an annual growth rate of 8.5% between 1950 and 2015 (Nizamuddin et al. 2024), reaching 322 million tons in 2015, with China contributing 27.8% and Europe 18.5% (PlasticEurope, 2016). By 2022, global plastic production surpassed 400 million tons, with China's share rising to 32% and that of Europe to 28% (PlasticEurope, 2023). Furthermore, estimates indicate that by 2050, plastic production could exceed 800 million tons (Sardon and Dove, 2018).

In this context, with plastic production on the rise, several key polymers have come to dominate the 21st century global market. For instance, PE emerged as one of the most widely manufactured synthetic polymers by 2022 in Europe, accounting for around 22% of the plastic production of over 58 million tones. Polypropylene (PP) followed closely, contributing 15.4%, while polyvinyl chloride (PVC) accounted for 9.1%. PS and polyethylene terephthalate (PET) each made up approximately 5% of the total production. Preliminary estimations on the conversion of plastic products and parts by European companies reveal that a significant portion is directed towards packaging, accounting for 39% of the total applications. The building and construction sector follows, utilizing 23% of the produced plastics. The automotive industry absorbs 8%, while agriculture represents a smaller share, with only 4% of the plastic being used in this sector (PlasticEurope, 2023). Single-use packaging quickly becomes waste (Nizamuddin et al. 2024), while durable goods in construction and electronics last longer but often generate waste sooner due to their low cost. Thus, with the current production rates and diverse utilization, plastics have become integral to modern life, but their life cycle extends far beyond mere production and use. A critical aspect of managing plastics involves addressing their fate once these synthetic materials reach the end of their useful life. Understanding the end-of-life options for plastics is crucial for a comprehensive evaluation of their overall impact. From recycling to disposal and emerging innovations, various methods for managing plastic waste have been employed since the 20th century. The following section will explore these end-of-life strategies for plastics, offering insights into how these approaches are shaping the future of plastic waste management.

1.2. Management of Plastic wastes

Plastics are classified into two categories: thermosetting plastics and thermoplastics. With a crosslinked molecular structure, thermosetting plastics like certain polyurethanes (PU) offer strength, thermal stability, and chemical resistance. Once cured, they cannot be reshaped, making them durable for extreme conditions. Thermoplastics, such as PE and PP, have flexible, linear molecular chains that can be melted and reshaped, making them versatile and recyclable in various manufacturing processes (Mourshed et al. 2017). Despite their utility, the widespread use of plastics has significantly contributed to the global generation of solid waste. A large share of plastic products is designed for single-use applications, which, although convenient, intensifies environmental challenges by facilitating rapid disposal. As a result, plastics now constitute a substantial portion of municipal solid waste, with significant quantities accumulating in natural environments (Napper and Thompson, 2020). The proportion of plastics in solid waste has significantly increased, rising from less than 1% in 1960 to over 10% by 2005 in economically advanced countries (Jambeck et al. 2015). This increase mirrors the

global growth in waste generation, driven by economic expansion and higher consumption. Consequently, plastic waste management has become a critical issue, with options such as landfilling, recycling, and incineration being the primary end-of-life strategies typically considered (Mousavimehr et al. 2020).

A. Recycling

Recycling is a waste management method that collects waste materials and converts them into reusable raw materials for new products. Due to their non-biodegradable nature, plastics are commonly recycled. The mechanical recycling process involves six steps: collection, sorting, washing to remove impurities, resizing, identifying and separating different plastics, and compounding the materials for reuse (Szostak et al. 2020). Recycling plays a crucial role in mitigating the environmental impacts associated with plastic waste. By diverting plastic materials from landfills and incinerators, recycling reduces the release of harmful pollutants and minimizes the consumption of landfill space. Additionally, recycling plastic is often a more environmentally sustainable alternative to the production of new plastics. This is because it conserves raw materials, reduces energy consumption, and decreases greenhouse gas emissions associated with plastic manufacturing. Furthermore, recycling helps to extend the lifecycle of plastic products, lessening the demand for virgin plastic production and contributing to the reduction of the overall environmental footprint of plastic use. As of 2015, approximately 6300 million tons of plastic waste had been generated globally, with only about 9% of it being recycled (Geyer et al. 2017). However, despite its benefits, the recycling process is not without limitations. During recycling, chemicals, including volatile gases from plastic waste, are released due to the heat required to melt plastics. This process can emit sulfur, carbon, and other gases, contributing to global warming, the greenhouse effect, and acid rain (Evode et al. 2021). Moreover, not all plastics can be mechanically recycled indefinitely, as only 10% of the total plastics being recycled have been recycled more than once (Geyer et al. 2017). Some plastic waste becomes unsuitable for further mechanical recycling after multiple cycles due to degradation in quality, leading it to be sent to landfills rather than being converted into secondary raw materials (Zheng et al. 2005). Furthermore, recycling plastic waste requires sorting due to the incompatibility of different polymers, as each has unique properties like melting points and chemical structures. Traditional recycling is limited by this polymer heterogeneity and the presence of additives such as plasticizers and stabilizers (Argun et al. 2020), which complicate the process and reduce the quality of recycled materials. While advanced methods like chemical recycling offer solutions by breaking down plastics into reusable components, these technologies still face challenges in terms of cost high costs, inefficient sorting, and feedstock quality (Joseph et al. 2021). In this context, pyrolysis has gained increasing attention as an alternative for plastic waste management. Pyrolysis is a thermochemical process that converts plastic waste into usable energy and valuable by-products, with the added benefit of recovering heat for electricity generation (Ofori-Boateng et al. 2013). While it offers notable environmental advantages, such as waste reduction and energy recovery, pyrolysis is not entirely free from environmental impact. The process still results in the emission of greenhouse gases, particularly carbon dioxide (CO_2), which contributes to global warming (Ali et al. 2023).

B. Incineration

Incineration is a widely employed waste management technique involving the controlled combustion of waste materials, including plastics, in an oxygen-rich environment. This process yields three primary by-products: ash, flue gas, and heat. Incineration is notably effective in reducing waste volume by up to 90%, and it prevents methane (CH_4) emissions, a potent greenhouse gas that is typically released during waste decomposition in landfills (Gu et al. 2019). The inert ash generated, which contains minimal hazardous materials, can be safely disposed of or repurposed in construction applications (Mayer et al. 2020). Consequently, incineration offers dual benefits: substantial waste reduction and energy recovery (He and Lin, 2019), making it as a critical component in integrated waste management systems. As of 2015, approximately 12% of global plastic waste was managed through incineration (Geyer et al. 2017). However, despite its effectiveness in minimizing waste volume, incineration is associated with several significant drawbacks. The process incurs high operational costs and leads to the emission of harmful gases, including CO_2, nitrogen oxides, and sulfur dioxide, which contribute to global warming, acid rain, and public health concerns (Evode et al. 2021). Moreover, pollutants such as particulate matter, dioxins, and volatile organic compounds can contaminate air, water, and soil, potentially causing long-term environmental harm (Gebre et al. 2021).

C. Landfills

Landfilling, which involves the deposition of solid waste, including plastics, into large depressions in the ground (Argun et al. 2020), remains one of the most widely used waste disposal methods globally. It is favored for its simplicity, high capacity for handling large volumes of waste, and relatively low investment and operational costs (Yang et al. 2024). As of 2015, approximately 60% of global plastic waste was estimated to have been disposed of in landfills (Geyer et al. 2017). However, rapid population growth and urbanization have led to a decline in available land suitable for landfill development. A significant environmental concern associated with plastic waste in landfills is the release of harmful chemicals. Plastics degrade slowly, and additives like phthalates, commonly used as plasticizers, can leach into the environment due to their non-covalent bonding, particularly in marine environments (Gewert et al. 2015). This leaching contributes to long-term ecological risks and exacerbates environmental pollution. Moreover, plastic waste in landfills contributes to the release of GHGs during decomposition. While the degradation process for plastics is typically slow, it still results in the emission of CO_2 and CH_4, both of which are potent contributors to climate change (Evode et al. 2021). Additionally, plastic waste can escape from landfills through various pathways, including runoff, wind, and flooding, particularly in open dumps or landfills lacking physical barriers to prevent such losses. In landfills, plastics undergo long-term degradation processes, during which notable alterations in their chemical structure occur, leading to a gradual loss of their original properties. Several polymeric characteristics critically influence these degradation processes, including molecular weight, the presence of functional groups, crystallinity, and the additives incorporated into the polymer matrix (Wojnowska-Baryła et al. 2022). Despite growing research in this area, the fate of synthetic plastics in landfills, particularly the timescale

required for their complete mineralization remains poorly understood. Thus, while landfilling remains a prevalent waste management strategy, its environmental drawbacks, particularly concerning plastic waste, pose significant environmental challenges.

1.3. Plastics in the environment and their fragmentation

Plastics have indeed become ubiquitous in modern society, contributing significantly to municipal solid waste. They account for approximately 10–13% of the total inorganic fraction of municipal solid waste (Moharir and Kumar, 2019). The predominant sources of plastic waste include packaging materials, bottles, containers, cups, and bags, which often end up in landfill sites or the marine environment, where they make up about 50% of plastic waste (Duru et al. 2019). Land-based sources are the main contributors to marine pollution, approximately 50% generated by coastal populations (Jambeck et al. 2015), while rivers, tides, and coastal activities carry buoyant plastics into the ocean, where wind and waves push debris into gyres, resulting in high concentrations and subsequent distribution in the marine environment (Eriksen et al. 2014). It is estimated that land-based activities contribute 98% of marine plastics, while only 2% originate from ocean-related activities such as commercial shipping, aquaculture, and fishing (Boucher, 2017; Kibria et al. 2023). Furthermore, persistent plastics rarely undergo complete degradation, instead, they undergo physical, chemical, and biological processes such as weathering, photooxidation, and microbial action, leading to their fragmentation.

Among these processes, photooxidative degradation plays a particularly vital role in aerobic environments, where it acts as a highly significant abiotic degradation pathway for plastics. This process involves a sequence of three primary stages, initiation, propagation, and termination, each of which contributes to the breakdown of polymeric structures (Gewert et al. 2015). In the first step, when plastic waste in the natural environment is exposed to ultraviolet (UV) light (wavelengths 290–400 nm), the absorption of radiation breaks down chemical bonds on the surface of the plastic polymers (Singh and Sharma, 2008). For the initiation process of photodegradation, polymers must contain chromophoric groups (Gijsman et al. 1999), which are capable of absorbing the light spectrum to initiate the breakdown of chemical bonds and the formation of free radicals (Yousif and Haddad, 2013). In the propagation phase, these free radicals react with oxygen in the environment, leading to the formation of peroxy radicals and hydroperoxides (Singh and Sharma, 2008). This oxidative chain reaction progressively weakens the polymer matrix, causing further degradation of the polymer backbone. As the polymer chains break, the molecular weight of the plastic decreases (Wayman and Niemann, 2021), and surface oxidation results in the formation of smaller, oxygenated degradation products, such as ketones, aldehydes, and carboxylic acids. Finally, in the termination stage, the degradation process slows down as reactive species, such as radicals, recombine to form inert products, effectively halting the chain reaction (Gewert et al. 2015). However, the photodegradation process typically results in a mixture of partially degraded polymer fragments that may remain in the environment. The overall effectiveness of photooxidative degradation depends on factors such as polymer type, environmental conditions, and exposure time to UV radiation.

In the marine environment, microorganisms colonize plastics, forming a "plastisphere" that affects buoyancy and accelerates the fragmentation of larger plastics into smaller particles (Kirstein et al. 2019). The influence of plastic characteristics on bacterial selection during early colonization tends to diminish as biofilms mature over time. While the initial surface properties of different polymers play a crucial role in determining which bacterial communities are able to colonize the material (Pompilio et al. 2008), the long-term development of biofilms tends to follow a different dynamic (Dussud et al. 2018). Studies have shown that biofilm formation and maturation can vary based on the polymer type (De Tender et al. 2017). However, as biofilms progress, other environmental factors seem to have a more substantial influence than the initial plastic properties. Although degradation of plastic is a multi-step process that can be summarized in four essential stages as reviewed by Dussud and Ghiglione (2014). It begins with biodeterioration, which occurs as biofilms form on and within the plastic, causing cracks and increased pore size, leading to physical deterioration, while the release of acidic compounds alters the pH, causing chemical deterioration. Next, bio-fragmentation is driven by extracellular enzymes, such as oxygenases, lipases, and depolymerases, which break down the polymer into oligomers and monomers. In the assimilation stage, oligomers are absorbed by microbial cells and used as a carbon source, increasing biomass. Recently, novel thermophilic consortia of *Brevibacillus spp.*, and *Aneurinibacillus sp.*, were identified, demonstrating significant degradation of LDPE, high density polyethylene (HDPE), and PP, with notable weight reductions (Skariyachan et al. 2018). Certain bacterial strains, including *Exiguobacterium sp.*, *Halomonas sp.*, and *Ochrobactrum sp.*, have also shown the ability to degrade PET and PE through various putative plastic-degrading enzymes, facilitating the breakdown of plastics into their constituent monomers (Gao and Sun, 2021). Finally, the mineralization stage completes the process by converting the degraded products into fully oxidized metabolites, including CO_2, CH_4, and H_2O.

Over all, plastic fragmentation typically begins at the surface due to exposure to chemical and enzymatic degradation, with surface cracking exposing the inner material for further degradation, ultimately leading to disintegration. The degradation of plastics in natural environments is a complex, multi-step process influenced by various physical, chemical, and biological factors, but under realistic conditions, the complete mineralization of plastics is exceedingly slow and often incomplete. Although biofilms can contribute to the physical deterioration and fragmentation of plastics, they do not necessarily accelerate the ultimate degradation or mineralization. Given the long-lasting presence of plastic fragments in the aquatic environment, it is crucial to understand their attributes and environmental fate within aquatic ecosystems.

1.4. The concept of Microplastics

The continual weathering of larger plastic debris releases smaller fragments into the environment, driven by degradation processes, as discussed earlier, leading to their widespread distribution and accumulation in aquatic ecosystems. The release of smaller plastic fragments, commonly known as microplastics (MPs), marks a critical stage in the weathering of plastics, contributing to their emergence as a significant environmental pollutant. Although the issue of

marine plastic pollution has been recognized around the mid-20th century, the term "microplastic" is relatively new, first appearing in scientific literature in 2004 (Thompson et al. 2004). This term was introduced to describe the small plastic particles resulting from the fragmentation of larger debris, which have since been identified as a pervasive and concerning form of environmental pollution. Frias and Nash (2019) provided a comprehensive review of the evolving definitions of MPs. They noted that the definition has evolved over time, with Arthur et al. (2009) establishing an upper size limit of 5 mm. Additionally, they highlighted that the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP, 2016) further extended this definition to include particles as small as 1 nm. Notably, this inclusion of a small particle size is now generally recognized as part of the nanoscale plastic category, referred to as nanoplastics (NPs). Moving forward, the authors emphasized that no comprehensive consensus has yet been reached. They also brought attention to the report of Verschoor (2015), which focused on specific properties of MPs but has largely been overlooked internationally. The authors proposed the following definition: "*Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape, and with sizes ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water.*" However, this definition has been expanded, and the commonly accepted definition in the literature refers to plastics with diameters ranging from 1 µm to 5 mm. In this context, the categorization of MPs into two main types is widely recognized in the literature: primary and secondary MPs. Primary MPs are intentionally manufactured to be microscopic in size, with various applications, including cosmetics and, increasingly, as drug vectors in medical treatments. Since the 1980s, MPs of varied shape, size, and composition have replaced natural exfoliants in cosmetic products, with significant increases in their use. Common types include PE, PP, and PS (Cole et al. 2011). Likewise, secondary MPs are formed through the fragmentation and degradation of plastic wastes, as discussed in the previous section. Importantly, a specific type of secondary MP that warrants particular attention is microfibers. Additionally, microfibers, typically characterized as filaments with microscale lengths and diameters reaching the nanometer range, represent a particularly prevalent form of microplastic pollution in aquatic environments. These fibers, commonly originating from textiles, fishing gear, and synthetic clothing, contribute substantially to microplastic contamination in marine ecosystems (De Falco et al. 2018).

1.5. Microplastics Detection techniques

The detection and analysis of MPs begins with robust sampling techniques for extraction/purification, which are critical for collecting representative samples from various environmental compartments. Sampling methods can vary depending on the medium, whether water, sediment, or biological tissues, each requiring specific approaches to ensure accurate capture of MPs. In aquatic environments, surface water sampling is typically conducted using plankton nets with fine mesh sizes, allowing the collection of floating MPs, followed by filtration to concentrate the particles (Li et al. 2018). Sediment samples, on the other hand, are usually obtained through coring or grab sampling, followed by density separation using saline solutions to isolate MPs from heavier sediment particles (Claessens et al. 2011). In biological tissues, the extraction of MPs typically requires digestion using acids, alkalis, or enzymes, as

reviewed by Prata et al. (2019). Acid digestion, such as with nitric acid (HNO_3), effectively breaks down organic matter but can damage polymers, particularly at high temperatures. Alkali digestion, primarily with potassium hydroxide (KOH) or sodium hydroxide (NaOH), is efficient but may degrade or discolor certain plastics and leave residues. Hydrogen peroxide (H_2O_2) serves as an oxidizing agent, efficiently digesting organic matter with minimal polymer degradation, although prolonged exposure or high temperatures may affect some plastics. Enzymatic digestion provides a safer alternative with minimal impact on microplastics, but it is time-consuming and costly, often necessitating supplementary H_2O_2 treatments to remove residual organic matter. Combining these methods can improve efficiency, though caution is needed to prevent underestimation of MPs concentrations due to potential polymer degradation.

The detection and analysis of MPs are critical for understanding their prevalence, environmental impact, and potential risks to ecosystems and human health. Over the years, a variety of analytical techniques have been employed to identify, characterize, and quantify MPs, each designed to address specific aspects of MP properties such as size, shape, polymer composition, and degradation state. These methods, while valuable, come with their own set of advantages and limitations, influencing their suitability for different research purposes and particle size ranges.

a). Visual Identification

Visual identification is one of the earliest and most basic methods for detecting MPs. This technique involves observing large size MPs visually to separate it from other residues, while a dissection microscope could be used for small size MPs, where information about the particle's size, shape, color, and type. Additionally, the degradation stage of MPs can be evaluated visually, providing insight into their environmental exposure and aging. However, this method is constrained by its inability to detect particles smaller than 1 mm, as the resolution becomes insufficient for smaller particles. Moreover, it is both time-consuming and subject to significant human error, with error rates reported to exceed 20% (Hidalgo-Ruz et al. 2012). As a result, while visual identification remains a common method, it is often supplemented by other, more precise techniques for smaller particles.

b). Sieving

Sieving is one of the most straightforward and traditional methods for separating MPs by size. The process involves passing a sample through sieves of varying mesh sizes, allowing for the physical separation of particles into size fractions. Although sieving provides a rough estimation of the size distribution of MPs in a sample, it has significant limitations in terms of precision and resolution. This method struggles to accurately classify MPs smaller than the mesh size of the smallest sieve and can lead to misclassification due to particle aggregation. Additionally, this technique is less effective for separating MPs from organic matter or other debris commonly found in environmental samples, which can lead to contamination (McDermid and McMullen, 2004).

c). Density Separation

Density separation is commonly used to isolate MPs from environmental matrices by exploiting differences in the densities of polymers and surrounding organic or inorganic materials. In this process, samples are placed in a liquid medium (often saline solutions) of known density, and MPs, which typically have lower densities than other materials, float while heavier materials sink. This allows for the effective separation of MPs from sediments, water, or biota. However, smaller MPs, which may not exhibit distinct density differences, can be missed. Additionally, this technique is time-consuming and often requires further chemical analysis, such as C:H: N analysis, to determine polymer types. While C:H analysis is effective for polymer identification, it does not provide a complete chemical profile and requires extensive sample preparation (Morét-Ferguson et al. 2010).

d). Pyrolysis

Pyrolysis coupled with gas chromatography-mass spectrometry (GC/MS) is a more sophisticated analytical technique used to determine the polymer composition of MPs. In pyrolysis-GC/MS, MPs are thermally decomposed into smaller fragments, and these fragments are then analyzed to identify their polymer origins based on combustion products. This method provides highly accurate identification of MPs, especially when compared to density separation techniques. However, its application is limited by the need for manual handling of small particles, which can introduce errors or inefficiencies. Furthermore, the analysis of very small particles (<50 µm) remains challenging, and the method can be time-consuming due to the need for specialized equipment and preparation steps (Fries et al. 2013).

e). Raman Spectroscopy

Raman spectroscopy is a powerful non-destructive technique that relies on the scattering of monochromatic laser light to identify the molecular structure of MPs. By irradiating samples with laser wavelengths typically between 500–800 nm, the technique can detect even the smallest MPs and accurately identify their polymer composition. Raman spectroscopy offers several advantages, including high specificity in polymer identification and minimal sample preparation. However, the technique is not without its limitations. For instance, MPs that fluoresce under laser irradiation can interfere with the detection process, making certain types of polymers undetectable. Additionally, Raman spectroscopy can be time-intensive when analyzing large sample volumes (Song et al. 2015; Imhof et al. 2012).

f). Infrared (IR) Spectroscopy

Infrared spectroscopy, including Fourier-transform infrared spectroscopy (FTIR), is one of the most widely used methods for identifying MPs due to its ability to characterize polymers based on their unique vibrational frequencies. When exposed to IR radiation, MPs absorb specific wavelengths that correspond to their molecular vibrations, producing characteristic spectral bands that can be used to identify different polymer types. IR spectroscopy is particularly effective in distinguishing MPs in environmental samples, even when mixed with

organic materials. However, it has limitations when it comes to detecting black or dark-colored MPs, as these particles absorb a large amount of IR radiation, complicating the analysis. Furthermore, when analyzing secondary MPs (those derived from the breakdown of larger plastic items), the small particle size can lead to reduced resolution, making it difficult to obtain clear results (Harrison et al. 2012; Talvitie et al. 2017).

Thus, the detection and analysis of MPs require a combination of various analytical techniques, each offering unique strengths and limitations. Visual identification and sieving provide basic physical characterization but lack the precision needed for small particles. More advanced methods, such as Pyrolysis-GC/MS, Raman spectroscopy, and IR spectroscopy, offer detailed chemical insights into polymer composition but are often have specific limitations, such as difficulties with certain particle sizes or colors. Therefore, a comprehensive approach, combining multiple techniques, is crucial for obtaining accurate, reliable, and thorough characterization of MPs in environmental samples.

1.6. Microplastics in the aquatic environment

The focus on MPs researches began from the middle of Sargasso Sea in the early 1970s when Carpenter and Smith (1972) identified plastic fragments in the surface waters samples. Their research highlighted two significant findings: first, plastic fragments provided attachment sites for marine organisms such as hydroids and diatoms, establishing an early indication of the ecological impacts of plastic pollution in marine environments. Second, plastic fragments as a potential source of toxic chemical compounds, including plasticizers and polychlorinated biphenyls (PCBs), capable of entering marine food webs and posing direct toxicological risks to marine organisms. In the same year, Carpenter et al. (1972) documented the ingestion of plastic pellets by several fish species, revealing unexpected selective feeding behaviors. Furthermore, these plastic pellets were found to harbor bacterial colonies, now referred to as the "plastisphere," the microbial community that forms on plastic surfaces. The authors also found that plastic pellets could absorb hazardous compounds like PCBs, highlighting their role as vectors for chemical contaminants, a concern that has now become a hot topic in MPs research. Although, the ingestion of plastic in aquatic birds' have been reported in early 1960s, when Kenyon and Kridler (1969) reported plastic in the stomachs of 74% of Laysan Albatross chicks that died before fledging. They proposed that large ingested plastic items could have contributed to mortality by obstructing the birds' digestive tracts. Rothstein (1973) also documented similar cases of plastic ingestion in birds collected in the early 1960s, providing further evidence of the growing presence of plastic pollution in marine ecosystems. Thus, by the 1970s, the scientific community began to pay significant attention to plastic pollution in marine environments, particularly the pervasive presence of small plastic fragments in oceanic waters. These early studies laid the groundwork for the recognition of MPs as an environmental issue, emphasizing their potential physiological impacts on marine life, their role in the formation of the plastisphere, and their function as vectors for persistent organic pollutants (POPs) such as PCBs. However, much of the terminology, such as "microplastics," and the understanding of the ecological threats associated with plastic pollution were still in nascent

stages at the time. This period marked the initial exploration of a problem that would later become a prominent environmental and ecological concern.

Plastic pollution in marine environments is a growing global concern, with an estimated 5.25 trillion plastic particles, weighing approximately 270 tons, are found at depths between 0.5 and 2 meters below the ocean surface (Eriksen et al. 2014). More recent studies indicate that the number of plastic pieces in the upper layers of the world's oceans has significantly increased, with estimates suggesting 24.4 trillion plastic particles, weighing about 57.8×10^4 tons (Isobe et al. 2021). These figures underscore the vast scale of plastic fragments accumulation in marine ecosystems, a trend that is projected to intensify. By the year 2025, it is anticipated that the total weight of plastic debris in the world's oceans could reach to 250 million metric tons, representing a substantial increase in plastic waste contributions to marine environments (Jambeck et al. 2015). In terms of density, the concentration of MPs in ocean surface waters has been found to vary widely, ranging between 5 and 70 particles per m^2, reflecting regional differences in plastic pollution concentrations (Ter Halle et al. 2017). However, these figures may underestimate actual concentrations due to methodological limitations in some sampling techniques. Certain methods, such as the use of nets with larger mesh sizes, are known to miss smaller particles, potentially leading to an underestimation of total densities. Coastal waters, which are often subject to greater human activity and industrial runoff, show an average density of six particles per m^3, a figure that highlights the significant level of MP contamination close to shorelines (Cole et al. 2011). In contrast, the situation in river surface waters, which often serve as conduits for land-based plastic waste entering marine environments, is even more concerning. For instance, the abundance of MPs has been reported to range between 1,580 and 57,665 particles per m^3 in the five rivers feeding into Manila Bay in Philippines, illustrating the critical role that rivers play in transporting plastic pollutants from terrestrial sources to the oceans (Osorio et al. 2021). This substantial variation in particle density across different water bodies reflects the complex dynamics of plastic pollution dispersion and accumulation, which are influenced by factors such as proximity to pollution sources, water currents, and local environmental management practices.

The widespread use of plastics like PE, PP, and PS increases the likelihood of their release as primary MPs and, through weathering, the formation of secondary MPs, contributing to higher detection rates in aquatic ecosystems. These MPs are characterized by their persistence, hydrophobic nature, and unique surface properties, making them highly susceptible to interactions with environmental pollutants. Studies dating back to the 1970s first highlighted the potential for plastic debris to adsorb various contaminants, with Carpenter and Smith (1972) reported early evidence of pollutant associated with plastic fragments. Since then, a growing body of research has reported similar findings. For example, in China, PE and PS MPs have been found to accumulate organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), with measured concentrations ranging from 3,400 to 119,000 ng/g (Mai et al. 2018). Additionally, macroplastic debris composed of PE recovered from beach environments has been observed to contain significant levels of metals, such as lead (Pb) at concentrations of 78 ± 19 μg/g (Nakashima et al. 2012). Similarly, MPs collected from river sediments in Beijing have been found to adsorb metals like nickel (Ni), cadmium (Cd), Pb, copper (Cu), zinc (Zn),

and titanium (Ti) at varying concentrations, highlighting the potential for MPs to act as vectors for contaminant transport in aquatic environments (Wang et al. 2017).

Overall, the early recognition of MPs in aquatic environments, starting with their identification in the 1970s, has evolved into a major area of environmental research. The ability of MPs to absorb hazardous substances and support microbial communities like the "plastisphere" emphasizes their dual role as both physical and chemical agents in ecosystems. With MPs concentrations rapidly increasing in marine and freshwater systems, it is important to understand the potential risks that MP pollution could pose to aquatic organisms at various trophic levels, as these interactions may lead to significant ecological and toxicological consequences.

1.7.Potential effects on aquatic biota

In recent years, the scope of research on plastic pollution has broadened significantly, with numerous studies documenting the ingestion of MPs by almost all trophic levels. Recently, an increasing number of studies have focused on the presence of MPs in the gastrointestinal tracts of aquatic mammals, including those that have been beached, accidentally bycaught, or hunted, demonstrating the widespread occurrence of MPs within these marine mammals, although the reported abundance has varied significantly across different species and geographical regions (Zantis et al. 2021). For instance, large marine mammals, such as whales, dolphins, and seals, can ingest MPs either directly from the marine environment or indirectly through the consumption of prey that have either ingested or absorbed MPs (Guzzetti et al. 2018; Nelms et al. 2018). Among aquatic mammals, whales are estimated to ingest substantial quantities of MPs daily, with estimates ranging from 200,000 to 10 million particles, depending on species and feeding strategies. Approximately 99% of this ingestion is estimated to occur via trophic transfer through their prey (Kahane-Rapport et al. 2022). However, the majority of studies on the effects of plastic pollution have primarily focused on the detection of MPs in the gastrointestinal tracts (GIT) or scats of aquatic mammals (Nabi et al. 2022). More recently, evidence of MP translocation to other tissues and organs has been reported in these species. Merrill et al. (2023) found MPs, primarily PE fibers, in 68% of individuals from 12 marine mammal species, with translocation in fat pad, blubber, lung, and melon tissues, raising concerns about potential health risks. The authors suggested that the large structure of the whale's gastrointestinal system may facilitate the expulsion of most MPs via defecation, but some particles translocate to various tissues or organs. Recently, many review papers have summarized the effects of plastic pollution on aquatic mammals. However, the consequent implications of MPs toxicity are largely inferred from studies on lower trophic organisms and laboratory models. The evidence of such effects in marine mammals remains limited due to the logistical, ethical, and technical challenges involved in studying these large, long-lived animals.

In the aquatic environment, fish are increasingly being contaminated with MPs worldwide. This contamination not only affects fish health but also poses a risk to human health, as MPs could enter the human body through the consumption of contaminated food (Sequeira et al. 2020). Several studies have reported the ingestion of MPs in a wide range of fish species and have reported the consequent effects. For instance, PE MPs have been shown to cause toxic

effects in *Danio rerio* (zebrafish) embryos and larvae. Malafaia et al. (2020) reported that exposure to PE MPs significantly reduced the hatching rate of zebrafish embryos, indicating developmental impairment or delay. In addition to developmental toxicity, the tissue-specific accumulation of MPs is a concern. Lu et al. (2016) found that PS MPs of two sizes (5 µm and 20 µm) accumulated in the gills, liver, and gut of *D. rerio*, with the larger (20 µm) MPs exclusively localized in the liver. This led to inflammation and lipid accumulation in the liver, suggesting potential hepatic toxicity. Similarly, Veneman et al. (2017) exposed zebrafish larvae to 700 nm PS MPs, reporting minimal biodistribution. Despite this limited internalization, the MPs still triggered immune responses and disrupted lipid metabolism, while also inducing oxidative stress. In a broader investigation, Lei et al. (2018) exposed zebrafish to a range of MPs, including polyamide (PA), PE, PP, PVC of ~70 µm in diameter, and PS particles (0.1 µm, 1.0 µm, 5.0 µm). The study reported significant intestinal damage and oxidative stress as the primary adverse effects, further demonstrating the size-dependent and polymer-specific toxicity of MPs on aquatic species. Furthermore, several studies have explored the mechanisms of toxicity induced by MPs in various fish species, with oxidative stress being a common outcome. For example, Espinosa et al. (2019) observed oxidative stress in *Dicentrarchus labrax* exposed to PE and PVC MPs, while *Symphysodon aequifasciatus* exhibited disruptions in oxidative defense mechanisms following exposure to PS MPs (Wen et al. 2018). Similarly, *Pomatoschistus microps* larvae exposed to PE MPs experienced oxidative stress along with neurotoxicity, evidenced by increased EROD activity and DNA damage (Pannetier and Morin, 2020). Immune responses are another key toxicological effect of MP exposure. Liu et al. (2019) demonstrated that both PE and PS MPs triggered immune responses in *Danio rerio*, while *Dicentrarchus labrax* also exhibited immune responses when exposed to PE and PVC MPs (Espinosa et al. 2019). Additionally, *Cyprinus carpio* showed immune responses following exposure to PE MPs (Banaee et al. 2019). Neurotoxicity has also been widely documented. In *Oreochromis niloticus*, exposure to PS MPs resulted in decreased acetylcholinesterase (AchE) activity in the brain (Ding et al. 2018), while *Clarias gariepinus* exposed to PVC MPs showed a decrease in both AchE and antioxidant enzyme activities (Iheanacho et al. 2020). Additionally, *Danio rerio* exposed to PS MPs exhibited both a decline in AchE activity and neurotoxicity (Chen et al. 2017), while PP MPs cause increased apoptosis, enzyme upregulation, DNA damage, and histological changes in *Oreochromis mossambicus* (Jeyavani et al. 2023).

Organisms that utilize deposit and detritus-feeding strategies, such as amphipods, are especially vulnerable due to their direct interaction with sediment, which is known to accumulate significant concentrations of MPs (Ramírez-Olivares et al. 2024). Similarly, filter and suspension feeders like barnacles, copepods, and mussels are prone to MP ingestion as they filter large volumes of water, inadvertently capturing suspended MPs along with their natural prey (Guzzetti et al. 2018). For instance, the ingestion of MPs has been found to reduce the filtration activity of various bivalves and mussels, primarily by causing physical blockages in their gills or digestive tracts. For instance, Rist et al. (2016) reported a decline in the filtration rate of *Perna viridis* exposed to micro-sized PVC particles, while Gardon et al. (2018) reported that MPs obstructed the gills of *Pinctada margaritifera*, reducing filtration efficiency. Similarly, Abidli et al. (2021) found that PE MPs led to blockages in the digestive systems of

mussels, impairing both filtrations and feeding efficiency, and induce oxidative stress. In addition to physical blockages, MPs pose another significant threat to marine organisms through biochemical pathways. These particles, once ingested, can initiate molecular stress responses, particularly oxidative stress, which has been observed in a wide range of marine species. The ingestion of MPs may not only hinder filtration and feeding, as seen in mussels and bivalves, but also induce harmful intracellular effects. These effects are especially prominent in smaller organisms such as copepods, where the accumulation of reactive oxygen species (ROS) triggered by MPs can lead to further physiological disturbances. This biochemical aspect of MP toxicity adds another layer of complexity to how marine species are impacted, as it involves not just mechanical interference but also molecular-level damage. For instance, Jeong et al. (2017) demonstrated that PS microbead exposure in the marine copepod *Paracyclopina nana* led to increased ROS accumulation and the induction of antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and glutathione-S-transferase, suggesting MPs induced oxidative stress. The authors also identified that ROS overproduction activated the mitogen activated protean kinase (MAPK) pathway leading to antioxidant gene expression. However, despite this response, growth retardation and reproductive impairments were observed, and an adverse outcome pathway linking molecular toxicity to fitness decline was proposed. Similarly, Choi et al. (2020) reported that exposure to PS beads in *Tigriopus japonicus* induced significant ROS generation, along with increased transcript levels and enzyme activities of antioxidants like glutathione reductase, further supporting oxidative stress as a key mechanism in MPs toxicity for zooplanktons.

Recent studies have shown that MPs, due to their ability to adsorb environmental pollutants, can cause complex and sometimes unpredictable effects on exposed organisms when combined with these contaminants. MPs through their ability to adsorb environmental pollutants, can lead to a variety of complex and sometimes unexpected interactions with exposed organisms, depending on the type of MPs and species involved. This complexity stems from the dual role that MPs play, as physical entities in ecosystems and as carriers of chemical pollutants, making their biological effects context-dependent. For instance, Guilhermino et al. (2018) found that the combined exposure of red fluorescent polymer MPs and florfenicol resulted in increased neurotoxicity and oxidative damage in *Corbicula fluminea*. Similarly, Zhang et al. (2019) showed that MPs promoted the bioaccumulation of roxithromycin (ROX) in red tilapia while mitigating its oxidative damage and neurotoxicity. These varying outcomes suggest that the interactions between MPs and co-occurring pollutants are highly species-specific and pollutant-dependent, which complicates the prediction of ecological consequences. Yu et al. (2020) also noted that co-exposure to PE MPs and tetrabromobisphenol A (TBBPA) triggered a stronger antioxidative stress response in zebrafish. However, not all studies indicate enhanced toxicity, as Beiras et al. (2019) found no increase in nonylphenol (NP) accumulation or toxicity in zooplankton. Such contrasting results highlight the need for further studies to unravel the nuanced mechanisms governing MP-pollutant interactions and their effects on various organisms across ecosystems.

To sum up, the extensive research on MPs exposure and its impacts on aquatic species demonstrates the serious ecological threat posed by plastic pollution. From large marine

mammals to smaller fish and invertebrates, the toxicological effects of MPs are evident at multiple biological levels, ranging from physical harm to oxidative stress and immune responses. However, these effects only capture a portion of the broader picture. Plastic pollution not only threatens aquatic ecosystems but also interacts with other environmental pollutants, compounding the toxicity of coexisting contaminants. A growing body of evidence suggests that MPs act as vectors for these pollutants, leading to more complex and potentially harmful outcomes. These interactions introduce a new layer of concern, as MPs may facilitate the transport and persistence of various toxic substances in aquatic environments, thereby exacerbating their ecological and health impacts.

1.8. The emergence of sustainable alternatives to persistent plastics

The ever-increasing production of fossil fuel-based plastics, their growing accumulation as waste in the environment, particularly the proliferation of MPs, and the limited availability of non-renewable crude oil resources have highlighted significant challenges associated with plastics. Furthermore, the reliance on crude oil for plastic production accelerates resource depletion and contributes to greenhouse gas emissions, exacerbating climate change. The persistence of plastics, combined with inefficient disposal and recycling processes, has led to escalating environmental pollution, especially in aquatic ecosystems, where MPs pose serious risks to biodiversity and ecological health. These interrelated challenges underscore the urgent need for sustainable alternatives to mitigate the environmental impacts of plastic pollution and fossil fuel dependency. In response to these challenges, bioplastics have emerged since the late 20th century as a viable alternative to fossil fuels plastics, offering a pathway toward sustainability. Bioplastics are considered to address two critical aspects of environmental sustainability: biodegradability and renewability. These concepts have become central to efforts aimed at reducing plastic pollution and mitigating the broader environmental impacts of plastic production and disposal.

Bioplastics represent a diverse family of materials that encompass a wide range of plastics designed for different uses. According to European Bioplastics, a plastic is classified as a bioplastic if it is derived from renewable resources (biobased) and/or biodegradable. Bioplastic alternatives exist for almost every conventional plastic, offering benefits like a reduced carbon footprint and additional waste management options, such as composting (EuropeanBioplastics, 2016). The bioplastics market has been steadily growing over the past two decades, with production continuing to rise significantly. As of 2022, global bioplastic production was 1.8 million tons, and it is projected to increase to 2.18 million tons in 2023, reaching 7.4 million tons by 2028. Despite this growth, as of 2023, bioplastics represent less than 1 percent of the over 400 million tons of global plastic production recorded in 2022. Bioplastics have become part of everyday life, with applications in a variety of sectors, including packaging, food services, agriculture, consumer electronics, automotive, and transport. They are also used in consumer goods, household appliances, construction, coatings, adhesives, and fibers. There remains a strong demand for bioplastic packaging, particularly for wrapping organic food and premium branded products requiring specialized solutions, with 43% (about 973,400 tons) of

total bioplastic production in 2023 allocated to the packaging sector, making it the largest market segment within the industry (EuropeanBioplastic, 2023).

Beyond these characteristics, recent studies have begun to document the formation of MPs from bioplastics, with evidence showing that these MPs have already been detected in the natural environment (Cai et al. 2018; Kazour et al. 2019). While bioplastics are often promoted as a sustainable alternative, their fate in nature and potential ecological and toxicological impacts remain poorly understood, raising growing concerns. As bioplastics degrade, fears persist that they may break down into micro- and nano-bioplastics, which can leak into ecosystems similarly to conventional plastics. These smaller particles have uncertain effects on biodiversity and may pose risks to both marine and terrestrial organisms, potentially entering the food chain. To ensure the sustainability of bioplastic solutions, a comprehensive life cycle analysis (LCA) is required, incorporating an in-depth evaluation of environmental and human health risks. Ecotoxicology plays a crucial role in such assessments, as the impact of MPs and NPs on organisms can significantly influence LCA outcomes. Without integrating scientific evidence with stakeholder action, regulatory frameworks may remain incomplete, potentially exacerbating environmental challenges. Therefore, there is a growing need for research that not only enhances our understanding of the fate of bioplastics in the aquatic environment but also addresses their potential consequences for both ecosystems and human health. Therefore, there is a growing need to focus on gaining a better understanding of the fate of bioplastics in the aquatic environment and their potential consequences on the aquatic biota.

2. Aims and objectives

The aim of this interdisciplinary thesis is to investigate the environmental fate of bioplastics and their impacts on aquatic biota, integrating approaches from both polymer chemistry and ecotoxicology. This thesis is a compilation of seven papers, including three literature reviews and four laboratory studies, each addressing different aspects of bioplastic, with an emphasis on their environmental conversion into MPs and their toxicity to aquatic biota. This thesis firstly investigates the environmental fate of bioplastics, with a particular focus on polylactic acid (PLA), the most commonly used bioplastic. To guide the direction of the research, an initial literature review was conducted to assess the environmental behavior of PLA, including its synthesis, the tunability of its biodegradability, and its conversion into MPs, as well as its effects on aquatic organisms at different trophic levels. This review provided a foundation for the experimental work by offering insights into the environmental degradation pathways of PLA and highlighting its ecological risks, particularly for aquatic organisms. Building on these insights, a laboratory study was designed to specifically test the toxicity of PLA MPs on planktonic copepods, assessing both acute and multigenerational effects. These studies were crucial for establishing whether PLA, as a biodegradable material, poses a significant risk to aquatic organisms.

Following this, a second literature review compared bioplastics with fossil fuel-derived plastics to place their environmental impacts in a broader context. The aim was to understand how the life cycle of bioplastics, their fragmentation into MPs, and their biodegradability differ from fossil fuel-derived plastics, particularly in terms of their fate and toxicity in aquatic ecosystems. This comparison was important to determine whether bioplastics truly offer an environmentally safer alternative or if they exhibit similar or greater risks compared to fossil fuel-based plastics. The comparative insights gained from this review led to laboratory experiments that tested the differential toxicity of MPs from bioplastics and fossil fuel-based plastics on two species of zooplankton. Finally, the thesis explores the shared toxicological concerns associated with both petrochemical and biodegradable MPs, particularly their potential role in adsorbing environmental pollutants and transporting them to aquatic biota, along with the subsequent impacts. A literature review examined the interactions between biodegradable MPs and environmental contaminants, focusing on the combined toxicity of these MPs and associated pollutants to aquatic organisms. Building on these findings, the final experimental study investigated the role of MPs, from both bioplastics and petrochemical-based plastics, as vectors for environmental pollutants, assessing their potential to transport contaminants and the resulting toxicity to zooplankton.

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Paper I

Polylactic acid synthesis, biodegradability, conversion to microplastics and toxicity: a review

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REVIEW ARTICLE

Polylactic acid synthesis, biodegradability, conversion to microplastics and toxicity: a review

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Abstract

Global pollution by plastics derived from petroleum has fostered the development of carbon–neutral, biodegradable bioplastics synthesized from renewable resources such as modern biomass, yet knowledge on the impact of bioplastics on ecosystems is limited. Here we review the polylactic acid plastic with focus on synthesis, biodegradability tuning, environmental conversion to microplastics, and impact on microbes, algae, phytoplankton, zooplankton, annelids, mollusk and fish. Polylactic acid is a low weight semi-crystalline bioplastic used in agriculture, medicine, packaging and textile. Polylactic acid is one of the most widely used biopolymers, accounting for 33% of all bioplastics produced in 2021. Although biodegradable *in vivo,* polylactic acid is not completely degradable under natural environmental conditions, notably under aquatic conditions. Polylactic acid disintegrates into microplastics faster than petroleum-based plastics and may pose severe threats to the exposed biota.

Keywords Biota · Degradation · Microplastics · Petroleum-based plastics · Polylactic acid · Toxicity

Introduction

The production of durable plastics started in 1940s (Chia et al. 2021). Plastics became an integral part of human life and as a result a huge amount is being produced every year, with around 370 million tons in 2021 (EuropeanBioplastics 2022). The non-biodegradable character of most petroleum-based plastics in addition to a low rate of recycling, for example only 9% of the total plastic produced until 2015 has been recycled (Almeshal et al. 2020), are the key factors of its accumulation in the natural environment. Plastic wastes are prone to natural weathering processes including ultraviolet radiations, oxidation, and biodegradation, which result into tiny pieces of 1 µm to 5 mm in size, termed as microplastics (Frias and Nash 2019). The presence of microplastics has been reported in almost all types of environmental media, including the ice blocks of Antarctic region (Kelly et al. 2020; Obbard et al. 2014). Marine organisms accidently take up microplastics from their ambient environment with food (Matijaković Mlinarić et al. 2022). As a result, the presence of microplastics has been reported in a wide range of organisms, ranging from zooplankton (Thery et al. 2022) to fish (Alomar et al. 2021), followed by their accumulation in the food chain via trophic transfer (Sarker et al. 2022). Consequently, a wide range of implications has been reported in the exposed individuals ranging from physical damage (Eltemsah and Bøhn 2019) to hormonal disruption (Ismail et al. 2021) and even mortality (Eom et al. 2020), suggesting that the impact of petroleum-based plastic on aquatic ecosystems is becoming severe (Nandhini et al. 2022).

Bioplastics, biodegradable and or compostable, may be considered as a relevant alternative to petroleum-based plastics. Polylactic acid is one of the most widely produced

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biopolymers which constituted 33% of all the bioplastics produced in 2021 (EuropeanBioplastics 2022). This aliphatic polyester is made from renewable resources (Nanda et al. 2022) and is used in agriculture, medicine and medical devices including temporary implants (Shruti and Kutralam-Muniasamy 2019), as well as for packaging, in automotive, and is now emerging for textile applications. The investigation of its degradation in natural environment is thus of great interest. Although polylactic acid is known for its in vivo biodegradable character, its biodegradation in the natural environment, notably in aquatic, is not so easy. Several review papers have summarized the applications, degradation mechanisms, modifications and ecotoxicological evaluation of polylactic acid over the last few years (Ainali et al. 2022; Chamas et al. 2020; Hubbe et al. 2021; Karamanlioglu et al. 2017; Qin et al. 2021; Ribba et al. 2022; Taib et al. 2022; Zaaba and Jaafar 2020). A comprehensive presentation on polylactic acid including the strategies available to promote biodegradability, the synthesis of microplastics and their impact on aquatic biota is still missing. This is the objective of the present contribution.

Polylactic acid as bioplastic

Polylactic acid is a low weight, in vivo biodegradable and compostable semi-crystalline bio-based polymer, synthesized from natural sources like corn starch, sugarcane or cassava roots (Grigoras 2021). For the very first time polylactic acid was synthesized in 1845 by Théophile-Jules Pelouze (Benninga 1990) and was commercialized in 1990s by Cargill and Dow Chemicals. However, polylactic acid and its copolymers were used as biomedical material during 1970s (Masutani and Kimura 2015). Due to its bio-absorbable and biocompatible nature, polylactic acid has been used in various biomedical applications ranging from instant drug delivery (Hu et al. 2003; Miao et al. 2011) to tissue engineering (Zhang and Ma 2004) (Fig. 1).

Due to its mechanical properties, for example, Young modulus of 1–3 GPa and elongation at break around 6–7% for poly(L-lactide), and biodegradable nature, polylactic acid has also been utilized for short-term use ranging from packing to coating (Garlotta 2001) (Fig. 1), since the mid of 1990s, with the aim to replace petroleum-based plastics. Polylactic acid has a glass transition temperature (T_g) around 60 °C and is relatively brittle. Improvements are being achieved by means of copolymerization (Meimoun et al. 2021), blending, compounding and additives (Kfoury et al. 2013), which reflect the hopes and interests of both the researchers and public for replacing petroleum-based plastics with the bioplastic, polylactic acid.

Synthesis of polylactic acid

The precursor of polylactic acid is lactic acid, 2-hydroxy propionic acid, which exists in two stereo isomeric forms (Fig. 2). They are mainly produced by microbial fermentation and/or chemical synthesis. During chemical synthesis, an equal amount of L-lactic acid and D-lactic acid is produced (Juodeikiene et al. 2015), while during fermentation either type of lactic acid is produced depending on the type of bacteria used. It is then subjected to purification process in which a number of techniques are used, ranging from nanofiltration and electrodialysis to reactive distillation (Msuya et al. 2017). Once purified, an oligo(lactic acid) is synthesized by the process of polycondensation, which gives rise to a cyclic dimer, lactide, by depolymerization reaction (Filachione and Costello 1952; Masutani and Kimura 2015) (Fig. 2). L-lactide, D-lactide and meso lactide are obtained, as shown in Fig. 2. Polylactic acid can be synthesized from these monomers either by ring-opening polymerization or

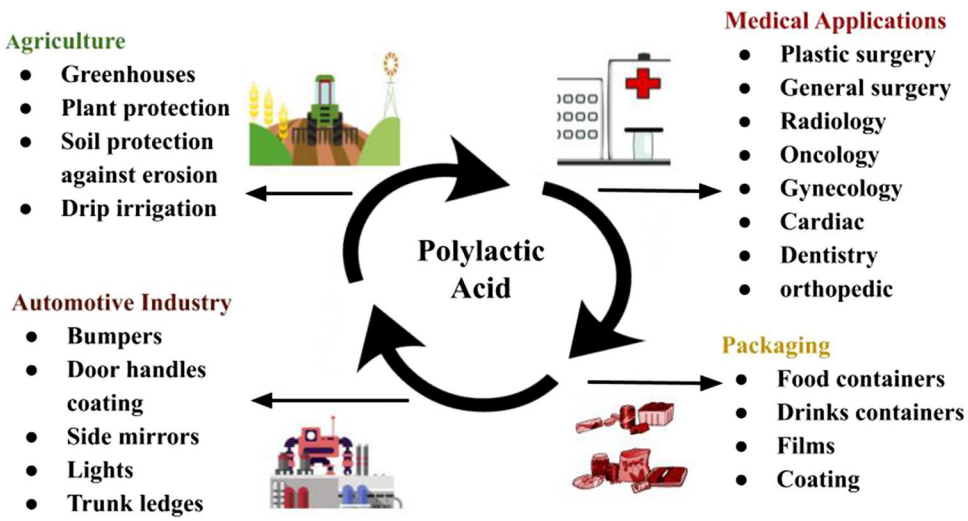

Fig. 1 Polylactic acid has been used as a potential alternative to petroleum-based plastics in many sectors. In agriculture, polylactic acid is used in greenhouses, drip irrigation and as a protective layer against soil erosion. Medical applications of polylactic acid range from surgery to dentistry. It is also used in automotive industry to produce different parts of vehicles. It is also used in increasing amount in packaging to produce cartoons, cups and plastic lined paper bags

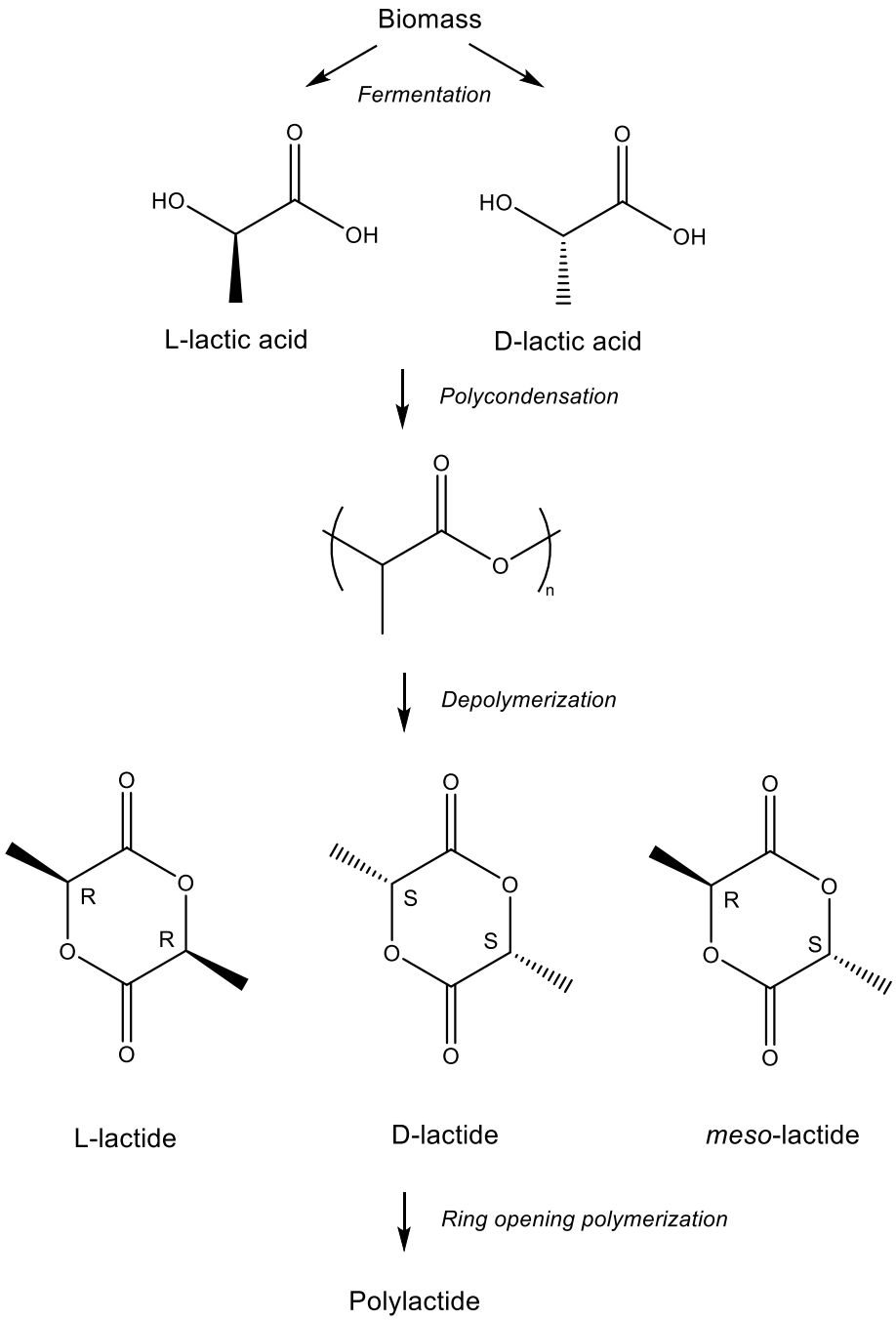

Fig. 2 Synthetic route of stereo forms of polylactic acid from biomass. Stereo forms of lactic acid are synthesized from biomass. Oligo(lactic acid) is formed from lactic acid by polycondensation. Oligo(lactic acid) gives rise to different stereo forms of lactide through depolymerization reaction. Polylactide is mainly synthesized by the ring opening polymerization of lactide in the presence of catalyst

polycondensation, catalyzed by metallic and organic catalysts mainly. However, ring-opening polymerization was the first method of polylactic acid synthesis (Taib et al. 2022) and still being used for the manufacturing of polylactic acid on industrial scale. The resultant stereo forms of polylactic acid, poly(L-lactide), poly(D-lactide), and poly(DL-lactide) (Nair et al. 2013), and their properties depend on the stereo isomeric form of lactide and the catalyst used for their synthesis. For example, the resultant polylactic acid can be either semi-crystalline, poly(L-lactide) or poly(L-lactide), or amorphous, poly(DL lactic acid) (Naser et al. 2021).

Biobased versus biodegradable/degradable nature of polylactic acid

According to the International Union of Pure and Applied Chemistry, biodegradable plastics are macromolecular substances which can be degraded as a result of biological activity, leading to reduction in molecular weight. Not all bioplastics are degradable. For example, biobased polyamides are not degradable (Vardar et al. 2022). The misconception that all bioplastics are biodegradable became the base of their popularity (Reddy et al. 2013).

The biodegradation of polylactic acid depends on several factors including its material properties, first and higher order structure, environmental conditions, ultraviolet radiations, temperature, pH and humidity (Karamanlioglu et al. 2017), catalytic species, microorganisms and/or enzyme (Nampoothiri et al. 2010; Qi et al. 2017). The biodegradation of polylactic acid occurs in two main steps: fragmentation and mineralization. Fragmentation of polylactic acid is achieved by means of hydrolysis which can be biotic or abiotic. For instance, biotic hydrolysis involves microorganisms and/or enzymes, whereas abiotic hydrolysis involves mechanical weathering. The second step, mineralization, is achieved by microbes resulting into CO_2, water and methane (Fig. 3) depending on the presence and/or absence of oxygen in the medium.

Many studies have assessed the biodegradation of polylactic acid and its blends with other polymers. Narancic et al. (2018) studied the biodegradation of polylactic acid and other polymers along with some of their blends using standards of biodegradation. The authors reported that polylactic acid alone or other polylactic acid-based plastics were not degraded in 56 days experiment in a simulated aquatic environment, anaerobic sludge, derived from wastewater treatment plant, under 35 ± 2 °C. The authors concluded that polylactic acid and its blends are similar to non-biodegradable plastics in terms of biodegradation in aquatic environment. Bagheri et al. (2017) studied the degradation of polylactic acid, along with other biopolymers and synthetic polymers, in artificial freshwater and sea water, in a thermostatic chamber at 25 °C and under fluorescence light of 16 h light and 8 h dark cycle. The authors found that polylactic acid did not show any significant degradation as compared to others during a 400 days experiment. Other studies have also reported very low or negligible degradation rate of polylactic acid in the aquatic environment (Martin et al. 2014; Pinto et al. 2015; Tsuji and Suzuyoshi 2002a).

Similarly, Weinstein et al. (2020) conducted a 32-week experiment in intertidal salt marshes, where samples were submerged at high tide for 6 h and exposed to low tide for approximately 6 h too, and compared the degradation of polylactic acid, as a commercial biobased case study polymer, to conventional petro-based plastics. The conventional petro-based plastics included poly(ethylene terephthalate), high density polyethylene and polystyrene, some of which contain additives to enhance the degradation. The authors reported the formation of biofilms and microplastics, and polylactic acid showed the slowest rate of degradation of all polymers tested. Kliem et al. (2020) proposed that low temperatures along with low bacterial density make the sea water unsuitable for the biodegradation of polylactic acid.

It can be concluded from these results that polylactic acid is having no or very low degradability in the natural environment at room temperature. Consequently, like majority of other petroleum-based plastics, polylactic acid will be fragmented by mechanical weathering and due to uncommon microorganisms for polylactic acid degradation and

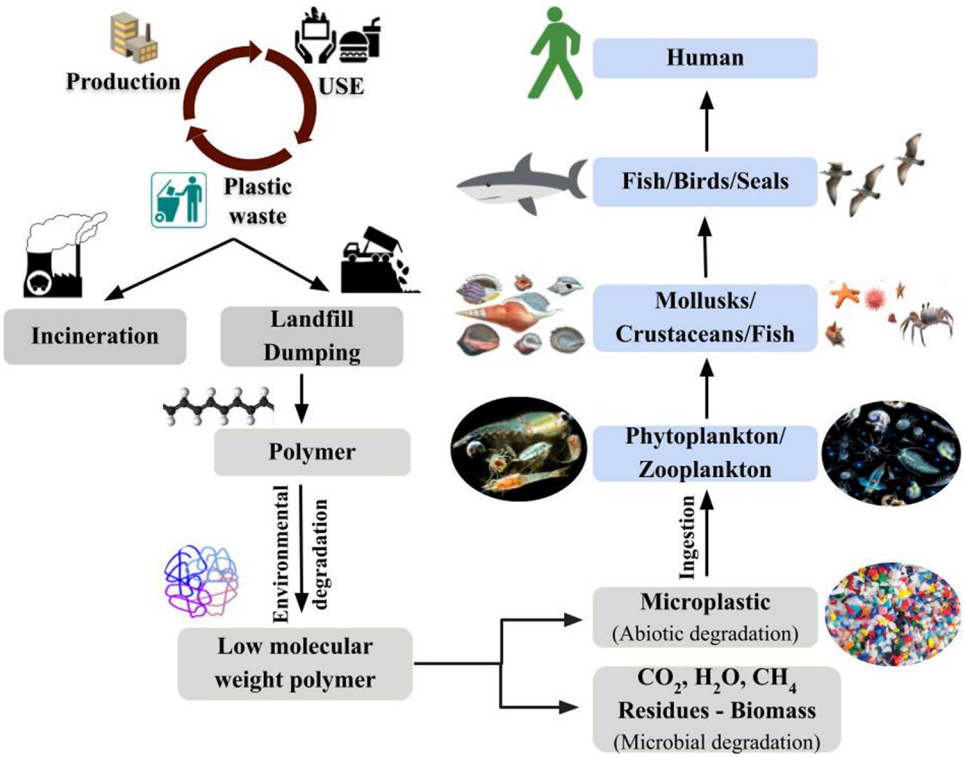

Fig. 3 A huge amount of polylactic acid is produced each year. After its use, a small portion is either recycled or incinerated. The rest of polylactic acid goes to dumping sites where it is prone to environmental degradation to form low molecular weight polymer. The low molecular weight polymer is either degraded by microbes, to form carbon dioxide, water, methane and biomass, or fragmented, to form pieces of less than 5 mm in size termed as microplastics. Microplastics accumulate in phytoplankton and then in zooplankton via ingestion, hence make their way to the aquatic food chain. The prey–predator relationship in aquatic ecosystems makes the microplastics to reach higher trophic levels, including humans

assimilation (Ribba et al. 2022), these fragments will turn to microplastics by the actions of several environmental factors including interaction with biota (Wang et al. 2021), which will have impact on aquatic biota. It should be noted in turn that polylactic acid is a compostable polymer which can be degraded under controlled conditions. In fact, the degradation of polylactic acid requires a high temperature (55–175 °C), which is beyond the limits of the natural environment (Garlotta 2001; Haider et al. 2019).

Modifications strategies for promoting the biodegradability of polylactic acid

Efforts are being made to modify polylactic acid for long term utilities. Literature survey reveals that blending with other polymers in the presence of eventual compatibilizers and the use of additives, such as plasticizers, have a significant effect on the degradability of polylactic acid as shown in Table 1.

Plasticizers

Plasticizers are used as additives which increase elongation at break, the stretching of plastic before it breaks, along with reduction in glass transition temperature and elastic modulus. Many studies have reported the use of different additives as plasticizers which promote degradation of polylactic acid. For example, Arrieta et al. (2014b) reported that the use of polyethylene glycol and acetyl-tri-*n*-butyl citrate, as plasticizer, can enhance polylactic acid degradation, where weight loss was higher than 90% after 28 days of incubation under composting conditions. In another example, Gardella et al. (2017) investigated the effects of a dendrimer like structure, a hyperbranched polyglycerol core poly(D-lactide) arms, as a plasticizer on the degradation rate of polylactic acid under composting conditions. The authors reported a faster degradation and increase in hydrophilicity of the polymer, up to about 30%. The authors attributed the faster degradation to increased hydrophilicity of the modified polylactic acid. Similar results were also reported by many other authors including Xie et al. (2014) and Sharma et al. (2021) while investigating the degradation of plasticized polylactic acid.

The degradation of modified polylactic acid depends on the nature of the additive used. In some cases, the use of plasticizer is shown to have very little or no effects on the degradation of polylactic acid. For example, Balart et al. (2018) reported that the use of epoxidized linseed oil, as plasticizer and or compatibilizer, can delay the degradation of polylactic acid. The authors reported that plasticized polylactic acid showed a weight loss of 61% only, while this value for unplasticized polylactic acid was 86%. Likewise, literature review reveals that other additives, when used as plasticizer, including D-limonene, glucose pentaacetate, sucrose octaacetate and glucose hexanoate esters, are reported to have slight or no effects on polylactic acid in terms of degradation (Fortunati et al. 2014; Nair et al. 2012; Yang and Hakkarainen 2015).

Blends with hydrophilic polymers

Blending with hydrophilic polymers is another approach for the modification of polylactic acid to improve mechanical properties as well as degradability. As polylactic acid is hydrophobic in nature, therefore blending it with hydrophilic polymers/polysaccharide has been shown to increase its degradability, attributed to an increase in hydrophilic characteristic of the polymer. For example, Claro et al. (2016) have shown that polylactic acid/chitosan and polylactic acid/cellulose acetate blends allow rapid biodegradation as compared to neat polylactic acid. Lv et al. (2017) studied the biodegradation of polylactic acid blended with starch and wood flour for 105 days under ambient environmental conditions and reported an accelerated degradation of polylactic acid. The authors concluded that the degradation of starch created holes in the polylactic acid matrix which facilitated the diffusion of water, leading to an enhanced hydrolysis of polylactic acid.

In another example, Wilfred et al. (2018) investigated the biodegradation of polylactic acid/starch blend in a commercial compost and soil for 14 and 28 days. The authors reported that the degradation rate of polylactic acid/starch blend was higher as compared to neat polylactic acid. These results also reveal a positive correlation of starch content and degradation rate, as proposed by Yamano et al. (2014) that biodegradability is correlated with hydrophilicity. Likewise, a number of studies have shown that blending of polylactic acid with hydrophilic polymers and or polysaccharides can make them more susceptible to degradation as shown in Table 1.

Blends with hydrophobic polymers

Chuayjuljit et al. (2017) studied the degradation of polylactic acid blended with poly(butylene succinate) in natural environment, buried in soil under ambient environmental temperature for 90 days. The authors reported faster degradation of blended polylactic acid and attributed it to the faster degradation of poly(butylene succinate). Similar results were also reported for blending of polylactic acid with other hydrophobic polymers, for example poly(β-hydroxybutyrate) (Bonartsev et al. 2012) and poly(vinyl acetate) (Haque et al. 2017).

However, not necessarily the blending of polylactic acid with hydrophobic polymers could contribute to their degradability profile. For example, Luzi et al. (2016) reported a

Table 1 Effects of modifications in polylactic acid on its degradability

Category	Additives	System	Percentage of additive	Degradation conditions	Sample size	Effect on degradation rate	References
Compatibilizer for polymer blend and composite	Poly(ε-caprolactone)	Polylactic acid based composites	5% by weight	20 °C under lake water, soil and compost for 28 days	10 mm^2	Degraded faster in lake water and compost	Olewnik-Kruszkowska et al. (2020)
	Glycerol	Wood filled polylactic acid composite/ starch blend	30% by weight in thermoplastic starch	Thermogravimetric analysis under nitrogen environment	Not applicable	Thermal degradation temperature decreased with increase in thermoplastic starch in the blend	Sun et al. (2021)
	Polymethylmethacrylate	Polylactic acid/wood flour composite	20% by weight	In water bath with pH = 12.5 under 50 °C for 10 days	150 × 10 × 4 mm^3	Degraded faster as compared to neat polylactic acid	Wan and Zhang (2018) and Wan et al. (2019)
	Poly(ethylene glycol)	Polylactic acid/ nanocrystalline cellulose bio-nanocomposites	10–16% by weight	37 °C in buffer solution of pH = 7.4 for up to 60 days	10 × 35 mm^2	Degraded faster as compared to neat polylactic acid	Zhang et al. (2019a, b)
	Epoxidized or maleinized cottonseed oil derivatives	Polylactic acid/ poly(butylene adipate-*co*-terephthalate) binary blends	1 and 7.5% by weight	Buried in compost 6, 8, 9, 10, 11, 14, 16, 21 and 23 days	25 × 25 mm^2	No effects on the degradation	Carbonell-Verdu et al. (2018)
	Wood flour	Polylactic acid/ poly(butylene succinate) blend	5, 10, 15, 20, and 30 parts per hundred	In Soil, 20 cm from the surface, for 30–90 days under ambient temperature	Not applicable	Higher degradation rate as compared to neat polylactic acid	Chuayjuljit et al. (2017)
	Cellulose nanocrystals, Surfactant modified cellulose nanocrystals	Polylactic acid/ poly(butylene succinate) blend	1 or 3% by weight	Buried in organic substrate with 58 °C temperature and 50% of humidity for 1–17 days	15 × 15 × 0.03 mm^3	Degraded slower as compared to neat polylactic acid	Luzi et al. (2016)
	Copolymer of Ethylene vinyl acetate and glycidylmethacrylate	Polylactic acid/cellulose fibers binary composite	10% by weight	58 °C under aerobic conditions in the presence of compost inoculum	20 × 20 × 2 mm^3	Degraded faster	Fortunati et al. (2013a)
	Maleic-anhydride-grafted poly(L-lactide) and maleic-anhydride-grafted poly(butylene succinate)	Polylactic acid/ poly(butylene succinate) blend	1–4% by weight	In test chamber under 50 °C humidity of 50% for 19.8 days	3 × 3 cm^2	Degradation rate was double in the presence of compatibilizer as compared to the blend without compatibilizer	Persenaire et al. (2014)

Table 1 (continued)

Category	Additives	System	Percentage of additive	Degradation conditions	Sample size	Effect on degradation rate	References
	Triallyl isocyanurate	Polylactic acid/lignin blend	3 parts per hundred	In phosphate-buffer solution with pH of 7.1 under 58 °C for 7, 14, 21 and 28 days	Not applicable	Degradation rate was higher in first 2 weeks as compared to neat polylactic acid Degradation rate was lower in the last 2 weeks as compared to neat polylactic acid	Kumar et al. (2019)
Plasticizer	Polyethylene glycol, triethyl citrate and lotader	Polylactic acid	3, 5 and 10% by weight	Buried at a depth of 80 mm in a novel compost with temperature of 40 °C and humidity of 70% for 20, 40 and 60 days	Not applicable	All the samples degraded faster as compared to neat polylactic acid	Sharma et al. (2021)
	Epoxidized linseed oil	Polylactic acid	7.5–22.5% by weight	58 °C and humidity of 55% under compost conditions	20×20×1 mm^3	Degraded slowly as compared to neat polylactic acid	Balart et al. (2018)
	Hyperbranched polyglycerol core poly(D-lactide) arms	Polylactic acid	3.75% by weight	37 °C under aerobic conditions in the presence of proteinase K for 120 h	15×15 mm^2	Degraded faster as compared to neat polylactic acid	Gardella et al. (2017)
	Cellulose nanocrystals, Surfactant modified cellulose nanocrystals	Polylactic acid	1 or 3% by weight	Buried in organic substrate with 58 °C temperature and 50% of humidity for 1–17 days	15×15×0.03 mm^3	Degraded slower as compared to neat polylactic acid	Luzi et al. (2016)
	Glucose pentaacetate, Sucrose octaacetate and Glucose hexanoate esters	Polylactic acid	10 or 20% by weight	37 and 60 °C in water for 1 day to 3 weeks	10 mm	Degradation rate was not affected as compared to neat polylactic acid	Yang and Hakkarainen (2015)
	Acetyl (tributyl citrate)	Polylactic acid	15% by weight	58 °C under compost conditions for 1–14 days	15×15 mm^2	Disintegration rate was faster compared to neat polylactic acid	Arrieta et al. (2015)
	Phenol, 5-methyl-2-(1-methylethyl)	Polylactic acid	6 or 8% by weight	58 °C in aerobic medium under compost conditions for 7–57 days	15×5×2 mm^3	Degradation was little faster as compared to neat polylactic acid	Ramos et al. (2014)

Table 1 (continued)

Category	Additives	System	Percentage of additive	Degradation conditions	Sample size	Effect on degradation rate	References
	Poly (ethylene glycol)	Polylactic acid	5–15% by weight	37 °C in alkaline solution for up to 100 days	Not applicable	Degraded faster as compared to neat polylactic acid	Xie et al. (2014)
	Poly (ethylene glycol) and acetyl-tri-*n*-butyl citrate and D-limonene	Polylactic acid, polylactic acid/polyhydroxybutyrate blend	15% by weight	50 °C under compost conditions for 21 and 35 days	30×30 mm^2	Disintegrated faster as compared to neat polylactic acid	Arrieta et al. (2014a, 2014b)
	D-limonene	Polylactic acid	15, 20 or 25% by weight	58 °C, 50% of humidity and in aerobic medium under compost conditions	15×15×0.05 mm^3	Disintegrated slowly as compared to neat polylactic acid	Fortunati et al. (2014)
	Gum grabic (GA), poly(ethylene glycol)	Polylactic acid	50% by weight	30 °C for 7 days in the presence of soil bacterium (*Lentzea waywayandensis*)	Not applicable	Degradation was faster in the case of gum arabic, while slower in the case of poly (ethylene glycol)	Nair et al. (2012)
Polysaccharides	Starch	Polylactic acid	10 and 20% by weight	Thermogravimetric analysis under nitrogen environment	Not applicable	Thermal degradation temperature decreased with increase in starch in the blend	Sun et al. (2021)
	Starch	Polylactic acid	90, 75, 50, 25 and 10% by weight	45 °C and 55 °C with 40% of humidity under soil and compost	3×1 cm^2 and thickness about 2 mm	Degradation rate was directly proportional to starch content in the blend	Wilfred et al. (2018)
	Starch, wood flour	Polylactic acid	9, 15 and 21% by weight	Buried in soil 15 cm deep under ambient environmental condition	Not applicable	Degradation rate was higher as compared to neat polylactic acid	Lv et al. (2017)
	Chitosan	Polylactic acid	10, 20, and 30% by weight	25 °C and a humidity of 50% for 48 h	Not applicable	Exhibited decrease in onset and degradation temperature	Claro et al. (2016)
Hydrophobic polymers	Poly(vinyl acetate) functionalized with glycidyl methacrylate	Polylactic acid	33% by weight	58 °C, 50% humidity and aerobic conditions with compost inoculum, for 40 days	Not applicable	Degraded faster as compared to other blends but slower as compared to neat polylactic acid	Haque et al. (2017)

Table 1 (continued)

Category	Additives	System	Percentage of additive	Degradation conditions	Sample size	Effect on degradation rate	References
	Poly(butylene succinate)	Polylactic acid	30% by weight	In soil, 20 cm from the surface, for 30–90 days under ambient temperature	Not applicable	Higher degradation rate as compared to neat polylactic acid	Chuayjuljit et al. (2017)
	Poly(butylene succinate)	Polylactic acid	10 and 20% by weight	Buried in organic substrate with 58 °C temperature and 50% of humidity for 1–17 days	15×15×0.03 mm^3	Degraded slower as compared to neat polylactic acid	Luzi et al. (2016)
	Poly(β-hydroxybutyrate)	Polylactic acid	25% by weight	58 °C under compost conditions for 1–14 days	15 mm×15 mm	Disintegration rate was similar to that of neat polylactic acid	Arrieta et al. (2015)
	Poly(β-hydroxybutyrate)	Polylactic acid	25% by weight	58 °C, and in aerobic medium under compost conditions for 35 days	30×30×0.2 mm^3	Disintegrated slowly as compared to neat polylactic acid	Arrieta et al. (2014a)
	Poly(β-hydroxybutyrate)	Polylactic acid	50% by weight	37 and 70 °C in phosphate buffer of pH = 7.4 for 91 days	Not applicable	Degraded faster as compared to neat polylactic acid	Bonartsev et al. (2012)
	Stereo-copolymers of polylactic acid	Polylactic acid	L-Lactide content of 75–95%	37 °C with proteinase K in buffer solution of pH = 8.6, in a rotatory shaker	1×1 cm^2	Degradation of amorphous region was higher	MacDonald et al. (1996)
	Stereo-copolymers of polylactic acid	Polylactic acid	L-Lactide content of 50–99%	37 °C with proteinase K in buffer solution of pH = 8.6, in a rotatory shaker of 100 rpm	25×10 mm^2	Degradation of amorphous region was higher	Reeve et al. (1994)
Composites fillers	Woodfiber, Wollastonite	Polylactic acid	30 parts by weight per hundred of resin	2 and 4 moths under ambient condition in compost	Not applicable	Degraded faster as compared to neat and blended polylactic acid, wood fibre composite degraded faster as compared to Wollastonite	Chaiwutthinan et al. (2019)
	Sisal fibers	Polylactic acid	20 and 40% by weight	Buried in soil with 30% moisture for 98 days	30×30×1 mm^3	Degraded faster as compared to neat polylactic acid	Wu (2012)

Table 1 (continued)

Category	Additives	System	Percentage of additive	Degradation conditions	Sample size	Effect on degradation rate	References
	Halloysite nanotubes (HNT), Ramie fabric	Polylactic acid	5% by weight in the case of HNT and 40 ± 3% in the case of ramie fabric	Buried at a depth of 80 mm in a novel compost with temperature of 40 °C and humidity of 70% for 20, 40 and 60 days	Not applicable	Both the samples degraded faster as compared to neat polylactic acid	Sharma et al. (2021)
	Okra fibres	Polylactic acid	10, 20 and 30% by weight	50 °C and in aerobic medium under modified compost with 50% humidity for 10, 20, 30 and 40 days	Not applicable	Initial disintegration rate of composite (10 and 20% by weight) was higher as compared to neat polylactic acid	Fortunati et al. (2013b)
	Olive husk flour	Polylactic acid	20% by weight	Incubated under 37 °C in the presence of *Bacillus subtilis* for 6 weeks	1×1 cm^2	Disintegrated faster as compared to neat polylactic acid	Hammiche et al. (2019)
	Benzylated pulp and pulping liquor	Polylactic acid	10, 20 and 30% by weight	Buried in agricultural rich soil with humidity of 50–60% under 20–25 °C for 180 days	$2 \times 2 \times 0.1$ cm^3	Considerable biodegradation as compared to neat polylactic acid	Zandi et al. (2019)
	Paddy straw powder	Polylactic acid	5, 10, 15 and 20% by weight	Buried in natural soil for 6 months under ambient environmental condition	Not applicable	Degradation rate was higher in the blends as compared to neat polylactic acid	Yaacob et al. (2016)

decrease in the degradation of polylactic acid blended with poly(butylene succinate), compared to neat polylactic acid, attributed to higher crystallinity induced by poly(butylene succinate). These contradictions in the results, as compared to Chuayjuljit et al. (2017), could be attributed to the difference in the percentage of poly(butylene succinate) content in the blend and composting conditions as well. Furthermore, in some cases the blending of polylactic acid with hydrophobic polymers is shown to have varying effects on the degradation of polylactic acid, as shown in Table 1.

Composites

Compounding of polylactic acid with other materials, having significantly different characteristics, either synthetically or artificially to obtain composites is another approach to improve the degradation rate of polylactic acid. Many studies have reported significant improvements in the degradation rate of the resultant polylactic acid composites. For example, Yaacob et al. (2016) investigated the degradation of paddy straw powder/polylactic acid composite and reported an improved degradation rate of polylactic acid in natural soil burial experiment. The authors attributed the improved degradation rate to the hydrophilic nature of paddy straw. In another work, Zandi et al. (2019) investigated the benzylated pulp (rich in cellulose)/polylactic acid and pulping liquor (rich in lignin)/polylactic acid composites in an indoor soil biodegradation experiment. The authors reported considerable biodegradation of polylactic acid composites, attributed to lower glass transition temperature and higher water absorption, in addition to larger biodegradation of filler. Similar results were also reported by many other authors as shown in Table 1.

Compatibilizers for polymer blend and composite

Many studies have reported that the use of compatibilizers can affect the degradation rate of polylactic acid in blends and composites. For example, Fortunati et al. (2013a) investigated a blend of polylactic acid with ethylene–vinyl acetate-glycidylmethacrylate copolymer, on the degradation rate of polylactic acid. The authors reported a faster degradation of the blend, 71% weight loss, under aerobic conditions in the presence of compost inoculum at 58 °C, as compared to neat polylactic acid. The authors attributed it to the faster diffusion of water in to the polymer mixture. Persenaire et al. (2014) also studied the biodegradation of polylactic acid/poly(butylene succinate) blends in the presence of a compatibilizer, maleic-anhydride-grafted polylactic acid and maleic-anhydride-grafted poly(butylene succinate), under composting conditions, in a test chamber under 50 °C and humidity of 50% for 475 h. The authors reported an enhanced molar mass loss in the presence of compatibilizer as compared to the polylactic acid/poly(butylene succinate) blend. Similar results were also reported by many other studies, including Olewnik-Kruszkowska et al. (2020), Sun et al. (2021), Wan and Zhang (2018) and Wan et al. (2019), while investigating the degradation of polylactic acid after the addition of compatibilizers, as shown in Table 1.

In contrast to these results, a few studies have shown that the use of compatibilizer can also negatively affect the degradation rate of polylactic acid. For example, Carbonell-Verdu et al. (2018) reported that the use of epoxidized cotton seed oil derivatives, as compatibilizer in polylactic acid/poly(butylene adipate-*co*-terephthalate) binary blends, reduced the disintegration ability of polylactic acid. The authors attributed the reduced disintegration rate of the blend to the lower disintegration rate of poly(butylene adipate-*co*-terephthalate). However, it is worthy to note that the compatibilizer do improve some of the mechanical properties of the blend as reported by Luzi et al. (2016).

Conversion of polylactic acid into microplastics

The term microplastic was introduced to the scientific literature by Thompson et al. (2004), and defined it as microscopic plastic pieces, while investigating its accumulation in sediments and water of European territory. This definition was further refined by Arthur et al. (2009) as plastic fragments less than 5 mm in size. In term of size, this is the most used definition in the literature with controversy on lower size limit. However, many authors have set different lower size limits ranging from 1 to 20 µm. To address this issue, Frias and Nash (2019) defined microplastic as any synthetic polymeric particle of 1 µm to 5 mm in size, irrespective of shape and source of origin (primary or secondary).

As discussed earlier, polylactic acid is having no or very low biodegradability in the natural environment; as a result it will remain for a long time in the environment. Like petroleum-based plastics, many biotic and abiotic factors will lead to its fragmentation and consequently microplastics will be generated from it. For example, Lambert and Wagner (2016) reported the release of microscopic size particles into the surrounding solution while investigating the biodegradation of polylactic acid along with polyethylene, polyethylene terephthalate, polystyrene and polypropylene in the weathering chamber. The authors also reported that polylactic acid generated significantly higher rate of particles, 11.6×10^6 particles per milliliter, as compared to other polymers, where polyethylene, polyethylene terephthalate, polystyrene and polypropylene generated 8.0×10^6, 9.4×10^6, 9.9×10^6, and 9.8×10^6 particles per milliliter, respectively. This could be attributed to the fact that bioplastics are more susceptible to degradation factors as compared to petroleum-based plastics

and consequently bioplastics are having high degradation rate (Napper and Thompson 2019; Wei et al. 2021), leading to the generation of huge number of microplastics.

Niu et al. (2022) assessed the disintegration of polylactic acid and its capacity to form microplastics using sea water and accelerated ultraviolet radiations for 18 months. The authors reported that polylactic acid forms almost 18 times fewer microplastics as compared to the petroleum-based plastic, polypropylene. However, the capacity of polylactic acid to form microplastics was double as compared to polypropylene in control. In contrast to Lambert and Wagner (2016), the number of generated microplastics were very less. The possible reason might be difference in the experimental conditions, for example, temperature which is known to have impact on the degradation of polylactic acid, and chemistry of polylactic acid used in both the studies. In a recent study, Le Gall et al. (2022) studied the formation of microplastics from self-reinforced polylactic acid (homocomposite of two comingled grades of polylactic acid fibers) using artificial sea water and accelerated ultraviolet radiations for 18 months. The authors reported the formation of microplastics in both, control (without ultraviolet radiations exposure and kept in dark) and experimental group. However, the experimental group had double number of microplastics (17 ± 18) as compared to control (9 ± 5).

Many recent research studies including Shruti and Kutralam-Muniasamy (2019), Wei et al. (2021) and Wei et al. (2022) show that other bioplastics also give rise to microplastics under laboratory conditions. The findings of these studies indicate that bioplastics including polylactic acid can give rise to microplastics, similar to petroleum-based plastics. Consequently, very recently, a few studies have identified polylactic acid microplastics in sediments (Bancin et al. 2019), marine ecosystems (Kazour et al. 2019) and wastewater treatment plant (Granberg et al. 2019), which further makes it an alarming issue. Yagi et al. (2012) reported that polylactic acid microplastics degradation rate is much slower as compared to large fragments, which reflects their persistent nature similar to petroleum-based microplastics. Polylactic acid microplastics are also resistant to degradation under normal environmental conditions and will persist in ecosystems, where they will pose potential threat to the natural environment and biota.

Environmental factors affecting formation of microplastics from polylactic acid

The degradation of polylactic acid requires both abiotic and biotic processes therefore the term environmental degradation was suggested for the overall mechanisms of degradation (Nampoothiri et al. 2010). Both types of processes are crucial for the degradation of polylactic acid, as there is evidence that polylactic acid can be degraded by biotic factors, for example enzymes, after decrease in its molecular mass by an abiotic mechanism (Stloukal et al. 2015). Like for all other plastics, there are several factors which affect the degradation of polylactic acid and lead to the formation of microplastics.

Ultraviolet radiations

Ultraviolet radiations can cause changes in the polymer microstructure by different pathways including chain scission and/or cross linking (Kijchavengkul et al. 2010). Considering the latter, polymers exposed to ultraviolet radiations were found to have reduced biodegradation because of higher molecular weight which in turn decreases its assimilation by microorganisms (Kijchavengkul et al. 2008). Jeon and Kim (2013) reported that exposure of polylactic acid to ultraviolet radiations for a long period reduced its biodegradation by microorganisms which suggested the formation of poorly assimilated solids or microplastics. However, Stloukal et al. (2012) reported that exposure of polylactic acid to ultraviolet radiations leads to chain scissions and specific surface area is more important factor than photooxidation for degradation. It is very clear from the above discussion that ultraviolet radiations can affect the degradation of polylactic acid and lead to the formation of microplastics.

Temperature

Temperature is another factor which can influence the degradation of polylactic acid. It is shown by many studies that an increase in temperature can accelerate polylactic acid degradation. The possible reason is that high temperature enhances water's affinity for the polymer and increases hydrolysis rate (Goto et al. 2020), resulting in a faster degradation of the polymer. The hydrolysis of polylactic acid starts from its amorphous region (Growney Kalaf et al. 2017), which allows water to diffuse more readily as compared to the crystalline region. Consequently, with the passage of time, portion of crystalline region increases and the rate of degradation decreases (Siparsky et al. 1998). The degradation of polylactic acid was investigated by Le Duigou et al. (2009) considering 20 and 40 °C temperature for 3 months. The authors reported slight change in the molecular weight of polylactic acid at 20 °C and 48% decrease at 40 °C after 3 months.

Lyu et al. (2007) reported significant variations in abiotic hydrolysis of polylactic acid considering a range of temperature from 37 to 90 °C. Similarly, Karamanlioglu et al. (2017) reported that the polylactic acid chains become more flexible at or above glass transition temperature, therefore rate of polylactic acid degradation is higher above this temperature which accelerates both hydrolysis and attachment

of microbes. The attachment of microbes to polylactic acid could be very rare in the aquatic environment, as two studies have reported that there is no evidence of microbial degradation after 10 weeks of polylactic acid immersion in static and dynamic seawater (Tsuji and Suzuyoshi 2002a, 2002b). The degraded portion might be that of the amorphous region of polylactic acid and the crystalline region will persist which could lead to the formation of microplastics.

pH

Hydrolysis of polylactic acid in acidic and basic media occurs by bulk erosion and surface erosion, respectively (Rodriguez et al. 2016). There is evidence that pH can also influence the degradation of polylactic acid. The degradation of polylactic acid has been reported under both, acidic and basic media driven by different mechanisms of depolymerization. For example, chain end scission hydrolyzes polylactic acid under acidic medium where protonation activates hydroxyl group, resulting in the depolymerization of polylactic acid to lactic acid (Codari et al. 2012; Lazzari et al. 2014). It should be noted that the rate of degradation was independent of chain length due to high hydrophilicity of chain end and hydrophobicity of polymer chain.

On the other hand, back biting reaction leads to random chain scission under basic medium, which depolymerizes polylactic acid into lactide followed by hydrolysis (de Jong et al. 2001; van Nostrum et al. 2004), as shown in Fig. 4. During hydrolysis, hydroxide ions catalyzed the cleavage of ester. At higher pH, the concentration of hydroxide ions is higher and therefore enhances the degradation of polylactic acid (Cam et al. 1995; Tsuji and Ikada 1998). However, the complete degradation of polylactic acid in natural environment takes time and the resulting disintegrated fragments or microplastics will aid in microplastic pollution before mineralization. Additionally, the reduction in the size of microplastics makes them likely of ingestion by organisms (Naqash et al. 2020). The question is whether these microplastics will pose the same threat to the ecosystems as petroleum-based microplastics or they will have different effects?

Impact of polylactic acid microplastics on aquatic biota

Recently some studies have shown that polylactic acid microplastics have a prominent impact on marine biota. Due to their compostable nature, polylactic acid microplastics cannot be degraded and are assimilated as all marine biota do not have the specific enzymes responsible for their degradation. In case of petroleum-based microplastics, it is well known that ingestion of these contaminants can cause a number of adverse effects. However, in the case of polylactic acid microplastics, very recently scientific community has shown an interest in their ecotoxicological evaluation. Very few studies have been conducted on the impact of polylactic acid microplastics on limited number of aquatic species which revealed that in some cases these contaminants can negatively affect the exposed individuals analogous to petroleum-based microplastics. The literature survey reveals that Green et al. (2016) is pioneer in assessing the toxic effects of polylactic acid microplastics on aquatic biota. After this, many research studies focused on the ecotoxicological evaluation of polylactic acid microplastics using a range of experimental model species (Table 2).

Microbial communities

Sediment microbial communities consist of large number of the earth's biodiversity and which play a key role in biogeochemical cycling of nutrients (Vincent et al. 2021) and ecological purification of pollutants (John et al. 2022). The presence of microplastics in the environment provides a new habitat to these microbes (McCormick et al. 2014), but on the other hand, the degradation of these polymers can produce toxic substances which will have negative effects on these microbes (Kong et al. 2018). A few species of bacteria will be benefited but the others will be negatively affected (Li et al. 2020), as different species of bacteria respond differently to the presence of microplastics (Wang et al. 2020). The impact of petroleum-based microplastics on microbial communities is well studied. However, studies focusing on the impact of polylactic acid microplastics on microbial communities are scarce.

Seeley et al. (2020) have conducted a 2-week microcosm experiment to investigate the effects of petroleum-based and bio-based microplastics on composition and function of sedimentary microbial communities. They reported a significant alteration in microbial communities exposed to petroleum-based microplastics. Surprisingly, polylactic acid microplastics were found to promote nitrification and denitrification. Based on the results, the authors suggested that the microorganisms might have utilized the microplastics as organic carbon for the energy which facilitated these phenomena. However, degradation and assimilation of polylactic acid microplastics in 2 weeks have not been reported in the literature.

Algae

Microalgae are the primary producer of aquatic ecosystems which play an important role in their functioning (Casado et al. 2013). Being a primary producer, microalgae account for 50% of net production (Barbosa 2009), and therefore, any alteration in microalgae population can have serious effects on food webs. Many studies have been carried out to

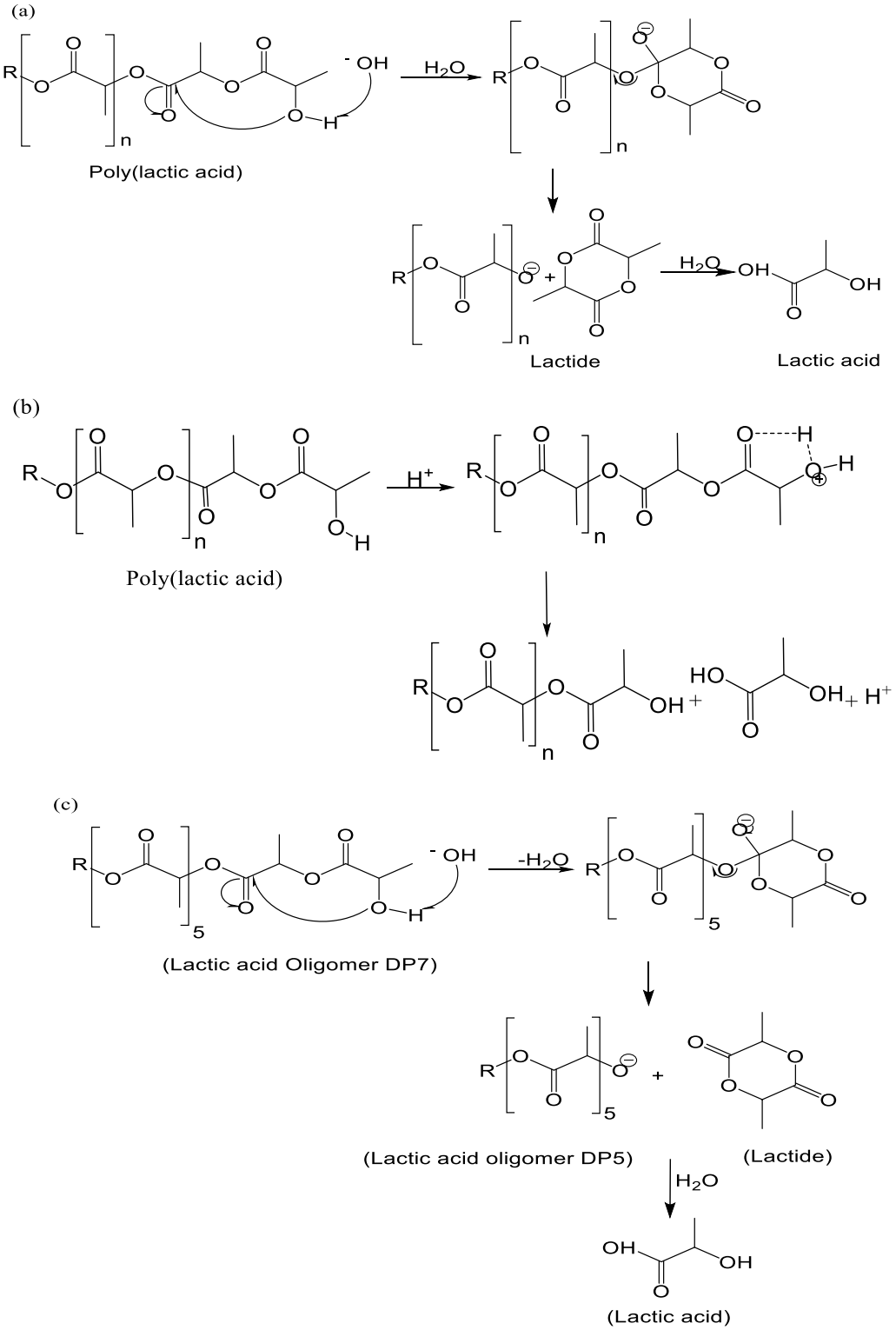

Fig. 4 Mechanism of polylactic acid degradation. **a** Back biting reaction, where polylactic acid is depolymerized into lactide followed by hydrolysis. **b** Chain end scission under acidic environment, where polylactic acid is depolymerized into lactide by the activation of hydroxyl group through protonation. Scheme is adapted from (**c**) and (**d**) [de Jong et al. (2001), used with permission from Elsevier]

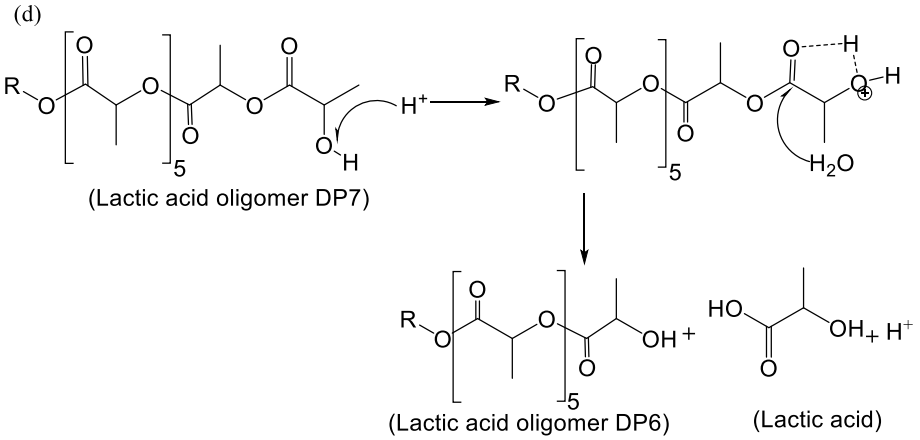

Fig. 4 (continued)

investigate the impact of petroleum-based microplastics on several parameters of microalgae, including growth (Long et al. 2017), morphological changes (Mao et al. 2018), chlorophyll content (Prata et al. 2018), photosynthesis (Zhang et al. 2017) and gene expression (Lagarde et al. 2016). The results of all these studies reveal that petroleum-based microplastics have deleterious effects on microalgae ranging from individual to population level.

The impact of polylactic acid microplastics on microalgae has also been investigated. Su et al. (2022) investigated the effects of petroleum-based microplastics and bio-based microplastics including polylactic acid on marine alga *Chlorella vulgaris*. The authors reported that both types of microplastics inhibited the growth of microalgae. However, polylactic acid microplastics were reported to have severe effects on growth of algae with highest inhibition rate of 47.95%, as compared to other petroleum-based and bio-based microplastics. The authors attributed these effects to the physiochemical properties and chemical changes of microplastics. The authors also reported that microplastics can stimulate pigments content (chlorophyll a, chlorophyll b and carotenoid), attributed to the cellular defense against stress. These findings are very interesting in two domains. Firstly, in the context of adaptation and or defense mechanisms against microplastics pollution, secondly, in understanding the fact that polylactic acid microplastics appear to be more toxic as compared to petroleum-based microplastics.

Phytoplankton community

Being primary producers, phytoplankton plays a key role in maintaining aquatic ecosystems. Due to their key role in food chains/webs, there is a serious concern about the impact of microplastics on phytoplankton (Koenigstein 2020). Any alteration or threat to the primary producers will have significant effects on the food chains/webs and consequently on the entire ecosystems, therefore, assessing the impact of emerging pollutants on primary producers is crucial. Literature survey revealed that petroleum-based microplastics have deleterious effects on various parameters of phytoplankton including photosynthetic capacity and growth (Sánchez-Fortún et al. 2021).

Studies on the impact of polylactic acid microplastics on phytoplankton are scare. Only a few studies have assessed their impact on phytoplankton. For example, Yokota and Mehlrose (2020) have assessed the impact of polylactic acid microplastics, originated from body wash scrub, on natural phytoplankton communities in a 7-days incubation experiment conducted in temperate mesotrophic lake. The authors reported that polylactic acid microplastics eliminated cryptophytes and increased chrysophytes, resulting in the alteration of taxonomic composition of the phytoplankton in the mesocosms. They suggested that chrysophytes contain a protective siliceous loricae against the polylactic acid microplastics whereas cryptophytes do not have any such protection and thereby got affected by the polylactic acid microplastics.

Zooplankton

Zooplanktons are primary consumers and located at the base of food chains/webs, thereby channeling nutrients and energy from the primary producers to higher trophic levels. Most researchers investigating the effects of microplastics on aquatic biota have focused on the primary consumers. As compared to other species, zooplanktons are more prone to microplastics and therefore documented as potential microplastics consumers (Cole and Galloway 2015). Microplastics act as analogues of zooplankton prey (Gambardella et al. 2017) and can have negative impact on different ecological processes. Recently, many studies have reported adverse effects of petroleum-based microplastics on a range of zooplankton species. The results of those studies reveal that

Table 2 Effects of polylactic acid microplastics on aquatic biota

	Model species	Concentration	Size range	Duration	Effects	References
Algae	*Chlorella vulgaris*	100 mg/L	57.41 µm	11 days	Inhibited the growth of algae. Microplastics stimulated pigment contents. Effects were due to physiochemical properties and chemical changes of microplastics	Su et al. (2022)
Microbes	Microbial communities	0.5% by weight	53–300 µm	7–16 days	No effects on microbes. Increased nitrification and denitrification	Seeley et al. (2020)
Phytoplankton	Chrysophytes and Cryptophytes	67 mg/L	–	7 days	Increased chrysophytes and eliminated cryptophytes	Yokota and Mehlrose (2020)
Zooplankton	*Daphnia magma*	10, 50, 100 and 500 mg/L	<59 µm	21 days	Reduced the survival up to 40% at 500 mg/L. Reduced the mean body length and reproductive outputs	Zimmermann et al. (2020)
	Artemia franciscana (larvae), *Aurelia* species (ephyrae)	1, 10 and 100 mg/L	<500 µm	24 h	Microplastics were internalized by both the species. Reduced pulsation in *Aurelia* species at all the concentrations. No effects on the immobility at each concentration	Di Giannantonio et al. (2022)
Annelids	*Lumbriculus variegatus*	1–8.4% dry weight of sediments	≤150 µm	128 days	Chemicals associated with plastic were the driver of toxicity. Survival rate reduced. Microplastics layered on the sediment surface affected less than that mixed with sediments	Klein et al. (2021)
	Arenicola marina	0.02–2% by weight of wet sediments	1.4–707 µm	31 days	At all the concentrations feeding was decreased and at higher concentration microalgae mass was reduced. Ammonia concentration was reduced in pore water	Green et al. (2016)
	Polychaetes and oligochaetes	0.0025 and 0.025 mg/L	0.6–363 µm	50 days	Ammonia concentration in pore water and biomass of cyanobacteria decreased in addition to effects on infauna invertebrate assemblage, with significantly less polychaetes and more oligochaetes	Green et al. (2017)
Mollusks	*Ostrea edulis* and associated macrofauna	0.8 and 80 mg/L	0.6–363 µm	60 days	Minimal effects on *Ostrea edulis* but richness of benthic species decreased. In higher concentration the total number of organisms was 1.2 and 1.5 times less as compared to control	Green (2016)

Table 2 (continued)

	Model species	Concentration	Size range	Duration	Effects	References
	Ostrea edulis and *Mytilus edulis*	0.0025 and 0.025 mg/L	0.6–363 µm	50 days	At higher concentration filtration by *Mytilus edulis* was decreased, while no effects on associated fauna at both concentrations. Filtration by *Ostrea edulis* was increased at both the concentrations	Green et al. (2017)
	Mytilus edulis	0.025 mg/L	0.6–363 µm	52 days	Alteration in immunological profile of hemolymph. No adverse effects on the attachment strength of *Mytilus edulis*	Green et al. (2019)
	Microcosmus exasperates	1 and or 10 particles per day	200–500 µm	28 days	Accumulation rate of PLA microplastics was comparable to petroleum-based plastic, PET. Rate of fertilization was reduced	Anderson and Shenkar (2021)
	Mytilus edulis	0.01 and 0.1 mg/L	0.8–10 µm	8 days	No effects on oxidative stress, neurotoxicity and immunotoxicity	Khalid et al. (2021)
Fish	*Danio rerio*	2.5–5 mg/L	2.34 ± 0.07 µm	30 days	PLA accumulated in carcass, gills, brain, and liver tissues. Increased acetylcholinesterase activity and REDOX imbalance leads to cholinergic changes. Neither anxiety like behavior nor locomotor damages. In shoal behavioral changes	Chagas et al. (2021)
	Danio rerio	3–9 mg/L	< 150 µm	5 days	Inhibition of acetylcholinesterase activity. Induced anxiety. Reduced swimming distance and speed	de Oliveira et al. (2021)
	Danio rerio	0.1, 1, 10 and 25 mg/L	5–50 µm	7 days	Aged PLA bioaccumulation was higher which inhibited skeletal development Pristine PLA had higher efflux and detoxification as compared to aged PLA Apoptosis, fission inhibition, depolymerization and mitochondrial structural damages triggered by oxidative stress	Zhang et al. (2021)

Table 2 (continued)

Model species	Concentration	Size range	Duration	Effects	References
Danio rerio	17.5 mg/L	135.35 ± 37.12 µm	15 days	PLA microplastics were actively ingested and abundance was 170 times in the intestines as compared to PET microplastics. Damaged gastrointestinal tract and induced changes in the microbial diversity of gut. Promoted microbiota linked with energy metabolism, cellular processes and fish diseases	Duan et al. (2022)

petroleum-based microplastics have adverse effects on survival rate and reproduction (Yu et al. 2020; Zhang et al. 2019a), feeding capacity and selectivity (Cole et al. 2019; Coppock et al. 2019) and behavior (Suwaki et al. 2020). However, studies investigating the effects of polylactic acid microplastics on zooplankton are scare and only a few studies are available.

Zimmermann et al. (2020) investigated that how polylactic acid microplastics affected the survival, reproduction, and growth of *Daphnia magna* in a 21-days experiment. They found that polylactic acid microplastics cause the mortality of 60% individuals exposed to 500 mg/L, while in control the mortality was 5%. The authors also reported decrease in reproductive output and body length in the exposed individuals, induced by the microplastics themselves rather than leachates or additives. Similarly, very recently Di Giannantonio et al. (2022) studied the effects of polylactic acid microplastics on uptake of microplastics, immobility, and behavior of two zooplankton species, the crustacean *Artemia franciscana* and the cnidarian *Aurelia* species (common jellyfish) in a 24 h experiment. The authors reported polylactic acid microplastics in the digestive system of *A. franciscana* and in the gelatinous tissue of *Aurelia* species exposed to 100 mg/L, with no effects on the immobility of both the species. However, significant alterations were reported in the swimming behavior (pulsation) of *Aurelia* species at all the exposure concentrations (1, 10 and 100 mg/L), attributed to the direct toxicity of polylactic acid microplastics. It is worthy to note that the concentration of microplastics used in majority of the ecotoxicological studies, to evaluate their potential effects on the exposed organisms, are much higher as compared to their concentrations found in the natural environment.

Annelids

Annelids are invertebrates which play an important role in benthic ecosystems by serving as a link from primary producers to higher trophic levels and in the cycling of minerals (Rafia and Ashok 2014). They are the dominant invertebrates of the deep sea and mostly occupy sediments. Recent studies reported that petroleum-based microplastics can have deleterious effects on various parameters of annelids which include decrease in food intake (Wright et al. 2013), impairment of immune system, physical stress and even death (Browne et al. 2013), leading to a drastic impact on ecological processes (Green et al. 2016). To the best of our knowledge, only three studies have assessed the negative effects of polylactic acid microplastics on annelids. Klein et al. (2021) investigated the impact of polylactic acid microplastics (mixed and or layered on sediment surface) on

freshwater worms (*Lumbriculus variegatus*) under laboratory conditions. The authors reported a significant reduction in the survival of the worms exposed to microplastics mixed with the sediments. However, they attributed the toxicity to the associated chemicals rather than to the polymer.

Similarly, Green et al. (2016) assessed the effects of polylactic acid and petroleum-based microplastics on lugworms (*Arenicola marina*) using concentrations of 0.02, 0.20 and 2% (wet sediment weight) in a 31-days mesocosm experiment with focus on health, biological activity and nitrogen cycling, in addition to the primary productivity of the sediments. The authors reported a significant impact of both types of microplastics on the health and behavior of the exposed individuals, as well as reduction in the primary productivity of the sediments they inhabited. Polylactic acid microplastics exposure not only reduced the feeding activity of the exposed individuals but also reduced the biomass of the algae on the surface of sediments. They also found that polylactic acid microplastics reduced the concentration of ammonia in pore water, which might be due to the potential of carbonyl and hydroxyl groups of polylactic acid to adsorb cations.

In another study, Green et al. (2017), while investigating the ecological impacts of polylactic acid and petroleum-based microplastics, high-density polyethylene, on the biodiversity and ecosystem functioning, found a difference in faunal invertebrate assemblages in the exposed groups, with less polychaetes and more oligochaetes, highlighting the potential of polylactic acid microplastics to affect ecosystem. These results are quite interesting in the context of species-specific response to microplastics (Bai et al. 2021) or other contaminants, as both the species were exposed to the same types and concentrations of microplastics but showed completely different responses.

Mollusks

Mollusks are a diverse group of filter feeders which can be found in a variety of aquatic habitats. They provide ecological services to a number of organisms ranging from habitat to food (Fernández-Pérez et al. 2018). Being filter feeders, mollusks can accumulate and transfer microplastics to higher trophic levels, which will have detrimental effects on their consumers including humans. Therefore, many ecotoxicological studies have used mollusks as bioindicators of pollution (Capillo et al. 2018). However, there are only a few studies available on the impact of polylactic acid microplastics on mollusks. Green et al. (2017) studied the ecological impacts of polylactic acid and petroleum-based microplastics on the biodiversity and ecosystem functioning of bivalve-dominated European flat oysters (*Ostrea edulis*) and blue mussels (*Mytilus edulis*) habitats in outdoor 50-days mesocosms experiment, using two different concentrations of 2.5 and 25 µg/L for each type of microplastics. The authors reported a significant reduction in filtration by *M. edulis* (exposed to 25 µg/L), while no effects were observed on ecosystem functioning or the associated assemblages of invertebrates. On the other hand, the authors reported a significant increase in filtration by *O. edulis* after exposure to 2.5 and 25 µg/L and decrease in the pore water ammonium and biomass of benthic cyanobacteria.

Khalid et al. (2021) also studied the effects of polylactic acid microplastics on blue mussels (*M. edulis*) using two different concentrations, 10 and 100 µg/L, in an 8-days experiment with biochemical endpoints. The authors found no significant effects of polylactic acid microplastics on *M. edulis* in terms of oxidative stress (catalase, glutathione-*S*-transferase, and superoxide dismutase activities), neurotoxicity (acetylcholinesterase), and immunotoxicity (lysosomal membrane stability and acid phosphatase activity). In contrast to these results, Green et al. (2019) found a significant alteration in the immunological profile of haemolymph of *Mytilus edulis* exposed to polylactic acid microplastics in a 52-days mesocosms experiment. However, the authors found no adverse effects of polylactic acid microplastics on the attachment strength of the exposed individuals.

Green (2016) investigated that how polylactic acid and petroleum-based microplastics at low and high concentrations (0.8 and 80 µg/L) affect the health and biological functioning of European flat oysters (*Ostrea edulis*) along with the impact on structure of associated macro faunal assemblages in a 60-days mesocosm experiment. They reported minimal effects on the exposed individuals, but the associated macro faunal assemblages were significantly altered which were ~ 1.2 and 1.5 times reduced as compared to the control. For instance, the biomass of *Scrobicularia plana* (peppery furrow shell clam), the abundance of juvenile *Littorina* sp. (periwinkles) and *Idotea balthica* (an isopod) were decreased 1.5, 2.0 and 8.0 times in groups exposed to either type of microplastics compared to the control.

Beside the mussels, other filter feeder organisms were also used for the ecotoxicological evaluation of microplastics. For example, Anderson and Shenkar (2021) investigated the impact of polyethylene terephthalate and polylactic acid microplastics on the biological and ecological features of a solitary ascidian (*Microcosmus exasperatus*). The authors reported that both polylactic acid and petroleum-based microplastics had similar impact on the exposed individuals; for example, both types of microplastics reduced the fertilization rates in the exposed individuals.

Fish

Fish are a good source of unsaturated fatty acids and proteins; therefore, their consumption is recommended in human diet (Ali et al. 2017). Therefore, assessment of

microplastics and its consequent impact on fish is of major environmental importance. Many ecotoxicological studies have used fish as bioindicator of water quality and ecosystem health. Fish have the potential to accumulate and magnify pollutants which may have potential impacts on their consumers including humans. Many studies have reported the ingestion and accumulation of petroleum-based microplastics in a range of fish species, while studies on polylactic acid microplastics are very few.

Recently, Chagas et al. (2021) studied the bioaccumulation of polylactic acid microplastics, at a concentration of 2.5 and 5 mg/L, in adult zebrafish and its consequent impact on behavioral, biochemical, and morphological parameters in a 30-days experiment. The authors reported the accumulation of microplastics in the liver, brain, gills, and carcass of the exposed group in addition to behavioral and morphological changes. The reported behavioral and biochemical changes were shoals predictive of co-specific social interaction and an anti-predator defense response defect, attributed to cholinergic changes inferred by an increase in the activity of acetylcholinesterase and redox imbalance whereas the morphological changes were alteration In the pigmentation pattern. However, in contrast to de Oliveira et al. (2021), no locomotor damages or anxiety-like behavior was observed in the exposed individuals. The possible reason might be difference in the life stages of the test organism as early life stages are more sensitive to different contaminants.

Most studies, while investigating the effects of various contaminants on aquatic organisms, have focused on early life stages of fish, for example larvae and embryo (Mu et al. 2022), because of their sensitivity to different contaminants (Schweizer et al. 2018), which are critical on individual and population health's point of view. For instance, zebrafish (*Danio rerio*) has been widely used as a biological model and/or as a representative of fish group by many researchers to investigate the toxicological impact of microplastics. de Oliveira et al. (2021) investigated the effects of polylactic acid microplastics (3 and 9 mg/L) on zebrafish larvae in a 5-days exposure experiment with behavioral and biochemical endpoints. The authors reported a decrease in the swimming speed and distance of the exposed individuals in open field test. The authors attributed these outcomes as a consequent impact of microplastics on fish locomotor and exploration activities. They also reported anxiety like behavior and accumulation of microplastics, which inhibited the activity of acetylcholinesterase leading to the reinforcement of neurotoxic action in the exposed group.

Similarly, another study also focused on the impact of polylactic acid microplastics (virgin and degraded) on zebrafish larvae (Zhang et al. 2021). They found a slower efflux and detoxification of degraded polylactic acid, mediated by ABC transporters and P450 enzymes, leading to increase in bioaccumulation of microplastics and thereby inhibiting the skeletal development of larvae. They also pointed the higher toxicity of degraded polylactic acid microplastics by identification of crucial mechanisms, for example, mitochondrial structural damage by oxidative stress, apoptosis, depolarization, and fission inhibition. However, no effects were reported on the hatching rate of larvae when exposed to both types of polylactic acid microplastics. The authors attributed these outcomes to the fact that the size of microplastics was larger as compared to the chorionic pore canals and the resistance of the chorionic barrier to polylactic acid microplastics.

Very recently, Duan et al. (2022) compared the accumulation and toxicity of polylactic acid and poly(ethylene terephthalate) microplastics using zebrafish (*Danio rerio*) as a model organism. The authors reported 170 times higher polylactic acid microplastics in the fish as compared to poly(ethylene terephthalate) microplastics resulting in intestinal epithelial tract damage followed by affecting the diversity of intestinal microbiota. The authors attributed these results to the depolymerization of polylactic acid in the digestive tract of fish, which decreased the intestinal pH and changed the carbon source structure. These results are quite interesting in understanding the toxicity of polylactic acid microplastics. These findings strongly support the concept that polylactic acid microplastics will have severe effects, similar to petroleum-based plastics, on the exposed individuals.

Conclusion

Due to the persistence and non-biodegradability of most petroleum-based plastics, efforts have been made to develop ecofriendly and environmentally safe substitutes. Polylactic acid is considered as a potential substitute of petroleum-based plastics. A comprehensive literature review shows that polylactic acid is compostable rather than biodegradable in the natural environment, leading to the formation of microplastics. Recent research has clearly identified that microplastics originating from polylactic acid are emerging environmental contaminants similar to microplastics from petroleum-based plastics. They are severely toxic to aquatic biota and might be a threat to human population as well through the food chain.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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Paper II

Acute and multigenerational toxicity of polylactic acid microplastics on a copepod bioindicator

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Acute and multigenerational toxicity of polylactic acid microplastics on a copepod bioindicator

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Abstract

Bioplastics such as polylactic acid are actually promoted as eco-friendly alternatives to fossil fuel-derived plastics, yet bioplastic toxicity remains poorly known. Here we studied the acute and multigenerational effects of polylactic acid microplastics on the copepod *Eurytemora affinis*, a bioindicator species of zooplankton. Results on acute toxicity revealed that lethal concentration values are higher for adult males, of 134.6 mg microplastic/L, than for adult females, of 106.9 mg/L. In multigeneration exposure, 400 µg/L polylactic acid microplastics induced higher mortality, production of smaller-sized eggs, elongation of the naupliar phase, and offspring with lower fitness. This led to reduction in female body size, including prosome length, width, and volume. Noteworthy, we also observed a recovery in copepod survival and reproductive parameters in the fifth filial generation.

Keywords: Bioplastics; Polylactic acid; Microplastic; Copepod; Multigeneration; Toxicity.

1.Introduction

Global plastic production increased to over 400 million metric tons in 2022, and if current management trends continue, 12,000 million metric tons of plastic waste will be disposed of by 2050 (John et al. 2022; Ali et al. 2024). Microplastics originating from petrochemical plastics have been reported to exert negative effects on biota, accumulate in the food chain, and have adverse effects on ecosystems (Osman et al. 2023; Eze et al. 2024). Bioplastics have emerged and been promoted as a safe alternative, and their production has reached 2.1 million tons in 2023 (EuropeanBioplastics 2023). Among the bioplastics, polylactic acid, a biobased polymer, constituting approximately 31% of all bioplastics produced in 2023 which has found extensive applications across various sectors, including medical applications, packaging, and now also emerging in textile applications (Ali et al. 2023b; Ferreira et al. 2024).

Polylactic acid has been reported to produce microplastics under aquatic environmental conditions (Le Gall et al. 2022), and these microplastics have been detected in the aquatic environment (Kazour et al. 2019). Recently, we reviewed the negative impacts of these biobased microplastics on the exposed aquatic biota and concluded that these microplastics could be as toxic as petrochemical ones (Ali et al. 2023a). We emphasized the long-term toxicity evaluation of these microplastics, including their multigenerational effects, which have never been studied.

Considering this knowledge gap, this study is the first to investigate polylactic acid microplastics' acute toxicity and assess its intergenerational chronic toxicity on *Eurytemora affinis*, a bioindicator species, for five generations using environmentally relevant concentrations. We focused on exploring rarely addressed biomarkers associated with the morphological and reproductive characteristics of *E. affinis*. Our primary objective was to gain a comprehensive understanding of the organism's responses and the inheritance of developed traits when exposed to next-generation microplastics in successive generations through a multigenerational assay. Additionally, we aimed to examine the reversible vulnerability of the studied biomarkers in a recovery generation to gain an insight to polylactic acid induced toxicity.

2.Experimental

2.1 Microplastics and model organism

In this study, we used Ingeo™ Biopolymer 4032 D polylactic acid (Nature Works LLC, United States) as starting material to synthesis microplastics of the size range 2 to 10 µm (**Fig. S1**). A detailed on the synthesis and characterization are presented in Supplementary Material (**Text S1**).

The model organism used in this study was *E. affinis*, an estuarine zooplankton that transports energy from primary producers to higher trophic levels, supports commercially important fishes, and contributes to the biological carbon pump. Thus, it has been widely used as a model organism for intergenerational toxicity studies of natural and anthropogenic stresses (Souissi et al. 2016a; Souissi et al. 2021; Das et al. 2023; Thery et al. 2023). The selected model organism originated from the wild which were collected at oligohaline zone of the Seine

estuary, France, in September 2014. Since then, this culture is maintained at the Laboratory of Oceanology and Geosciences, Wimereux, using copepods culturing and rearing protocol developed by Souissi et al. (2016a).

2.2 Acute toxicity and multigeneration exposure

The 96-hour lethal concentration (LC50) of polylactic acid microplastics in *E. affinis* adults were determined based on our previous work (Kadiene et al. 2017), with details in the Supplementary Material (**Text S2**). For multigeneration experiment, we exposed copepods to 400 µg/L polylactic acid microplastics for five generations following the protocol of Thery et al. (2023). We chose this concentration considering the highest reported environmental petrochemical microplastic concentration of 2.5 mg/L (Cózar et al. 2014) and the limited data on biomicroplastics in aquatic environments. Although our selected concentration may exceed from those found in the environment but remaining lower than concentrations used in other studies (An et al. 2024; Savva et al. 2023), bringing our experiment closer to realistic contamination conditions.

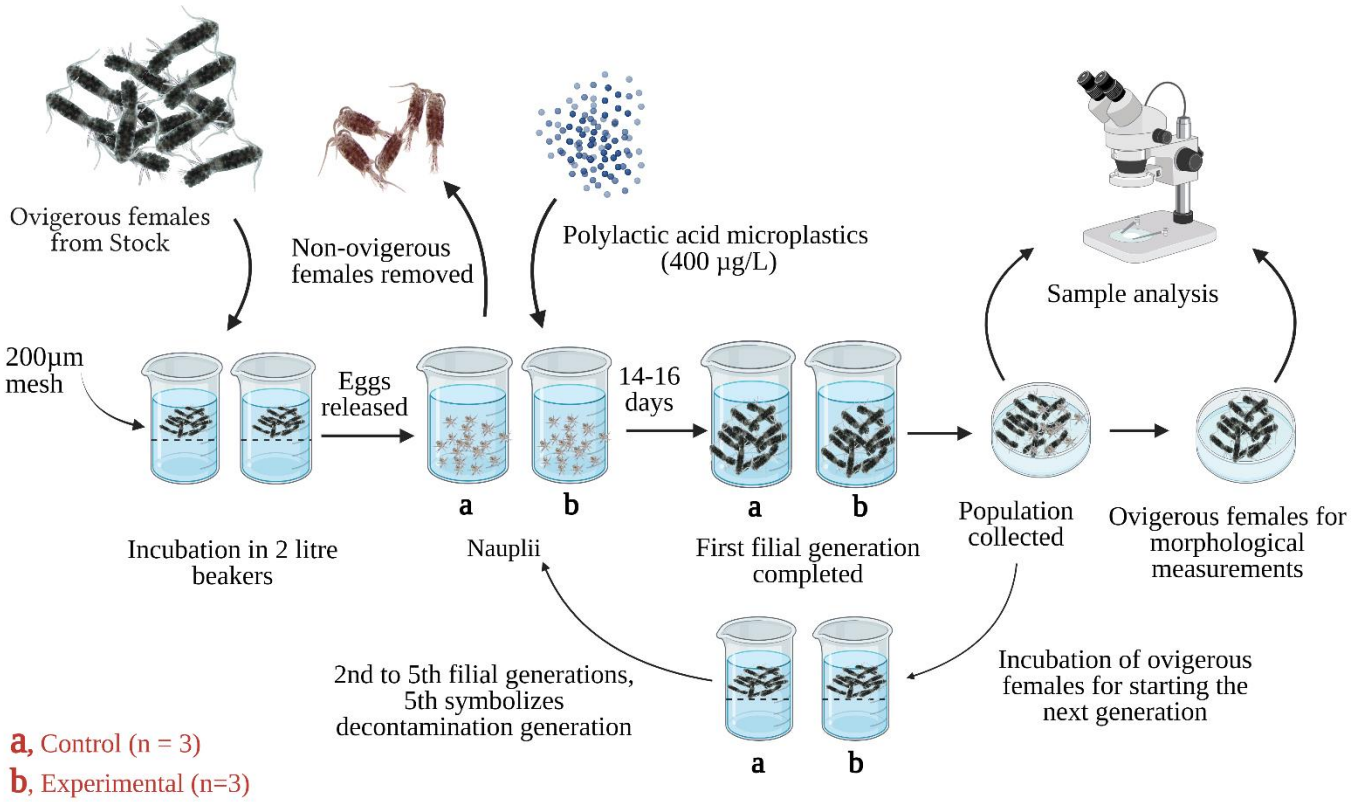

Figure 1. Protocol for multigenerational exposure of *Eurytemora affinis* to polylactic acid microplastics, analyzing morphological and reproductive parameters at the end of each filial generation. Four generations assess transgenerational toxicity, with a fifth generation for recovery potential.

The experiment comprised a control group and a polylactic acid microplastics group, each with three replicates. 30 ovigerous females were initially selected for each replicate, and after egg release, non-ovigerous females were removed. Microplastics were added to the treatment group, and after 14 to 16 days, the population was filtered to complete the first filial generation.

Subsequently, 30 ovigerous females from each replicate were introduced into new beakers for subsequent generations from the second to fifth filial generation. The fifth filial generation served as a recovery group without microplastic exposure. Selected endpoints, including total population, mortality, sex ratio, morphological, and reproductive parameters, were analyzed following established methods (Souissi et al. 2016b; Das et al. 2023), with details provided in the Supplementary Material (**Text S3**). Results were analyzed according to in text Supplementary Material (**Text S4**).

3.Results and discussion

3.1 Acute toxicity and multigenerational effects on sex ratio

We calculated the average 96-hour LC50 value for *E. affinis* as 120.8 mg/L. Gender-specific responses were observed, with adult males exhibiting a significantly higher ($p < 0.05$) tolerance, with LC50 of 134.6 mg/L (95% CI= 60–239 mg/L), compared to adult females who showed more susceptibility, with LC50 of 106.9 mg/L (95% CI = 64–203 mg/L) (**Fig. 2a**). Additionally, multigeneration exposure altered the sex ratio, with males comprising 62 percent of the population compared to 52 percent in the control group (**Fig. 2b**).

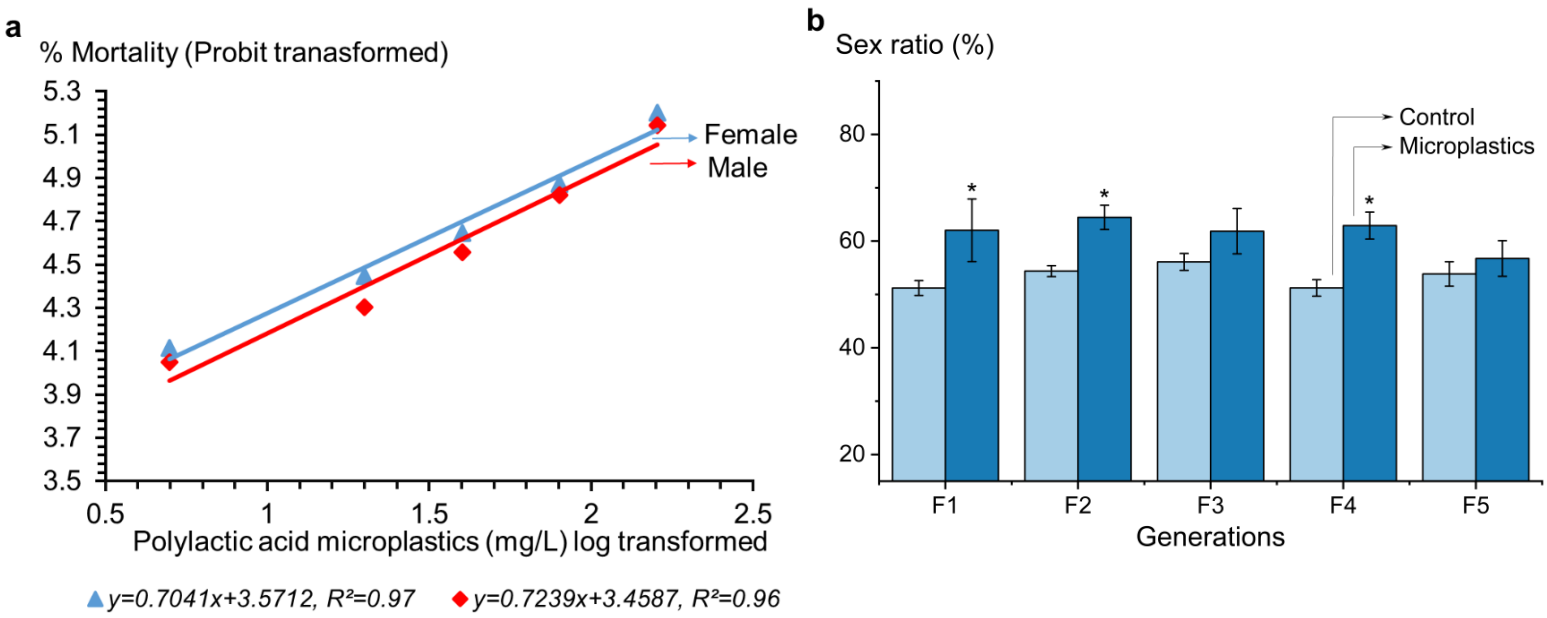

Figure 2. 96-hour concentration to mortality curves for male and female *Eurytemora affinis* exposed to polylactic acid microplastics **(a)**. Sex ratio (% of male) at the end of generations from the first filial (F1) to the fifth filial (F5) with mean ± SE **(b)**. Asterisks (*) represent statistical significance ($p < 0.05$). Standard error (SE).

Gender-specific responses to contaminants have been reported in crustacean populations (Kadiene et al. 2017, 2019). While other studies showed no lethal effects of polylactic acid microplastics on jellyfish and water flea with an effective concentration 50% (EC50) values of 77.4 and 16.4 mg/L (Di Giannantonio et al. 2022; An et al. 2024), our study demonstrates distinct LC50 values for male and female *E. affinis*. Similar to our findings, Kadiene et al.

(2017) reported that *E. affinis* females are more sensitive to cadmium (LC50: 90.04 µg/L) than male (LC50: 127.75 µg/L). Sroda and Cossu-Leguille (2011) observed higher sensitivity to copper in females of *Gammarus roeseli* and *Dikerogammarus villosus* compared to males. Furthermore, we propose that the toxicity is caused by microplastics rather than the associated chemicals, aligning with Zimmermann et al. (2020) and Jemec et al. (2016).

Exposure of *Daphnia magna* to polylactic acid and polyethylene terephthalate microplastics increased the male offspring ratio (An et al. 2024). In our study, the increase in the male ratio may be attributed to the higher sensitivity of females, indicated by a lower LC50 value. The observed effects on the sex ratio in the exposed group could be linked to the larger size of *E. affinis* females, prompting increased energy demand for foraging and a higher likelihood of encountering microplastics. Higher grazing rates in female copepods have been associated with size differences (Gréve et al. 2017). Moreover, our findings contribute to the understanding of microplastic-induced alterations in sex ratios and their potential ecological consequences. If female copepods are more susceptible to microplastic toxicity than males, it could lead to increased female mortality and decreased recruitment production, potentially disrupting the aquatic food chain due to lower food supply at higher trophic levels.

3.2 Effects on total population and mortality

In the multigeneration exposure, we found that the exposed group had a significantly lower population density ($p < 0.05$) than the control group, with 202 to 317 individuals per 1.8L versus 393 to 498 individuals in the control group (**Fig. 3a**). Notably, the decontamination generation, the fifth filial generation, had lower mortality (47.68%) and a greater total population than the exposed groups, suggesting a reversible vulnerability of reproductive parameters. The relative difference in total population between the control and exposed groups varied across the generations, ranging from 35.72 to 50.04% (**Fig. 3b**). Mortality data consistently showed higher rates in the exposed group across all five generations (**Fig. S4**). Similarly, a significant relationship was observed between the total population and successive generations in the control group ($r^2 = 0.59$, $p = 0.016$), but not in the exposed groups ($r^2 = 0.41$, $p = 0.16$) (**Fig. S5a**). Thus, the multigenerational exposure resulted in lower population density and consistently higher mortality rates, indicating disrupted reproductive patterns with potential significant ecological impact.

The ingestion of microplastics by zooplankton has been linked to increase bioconcentration and oxidative stress, resulting in gastrointestinal tract obstruction and mortality (Na et al. 2023; Jemec et al. 2016; Eltemsah and Bøhn 2019). In our study, the higher mortality in the exposed could be attributed to the accumulation of microplastics in the gut, followed by the associated implications. The increasing trend for mortality observed in the first and second generations aligns with previous research on microplastics exposure (Schür et al. 2020), indicating a substantial adverse impact during the initial exposure. Furthermore, fluctuations in resources availability can significantly impact fitness (Hämäläinen et al. 2017). The decline in the total population in subsequent generations could be attributed to the production of offspring with lower fitness. In the third generation, a decrease in mortality and an increase in the total population could be linked to the increased egg diameter in the second generation. These results support the findings of Jamieson et al. (2003) who reported that large eggs in the copepod

Cyclops kolensis produce offspring with high fitness. The decontamination generation, the fifth generation, displayed lower mortality and the highest total population, which could be attributed to their proteomic plasticity, leading to an increase in energy metabolism and stress defense. In conclusion, the exposure of *E. affinis* to polylactic acid microplastics has led to increased mortality with transgenerational effects, affecting the population of subsequent generations and highlighting the potential implications of these microplastics for the aquatic food web.

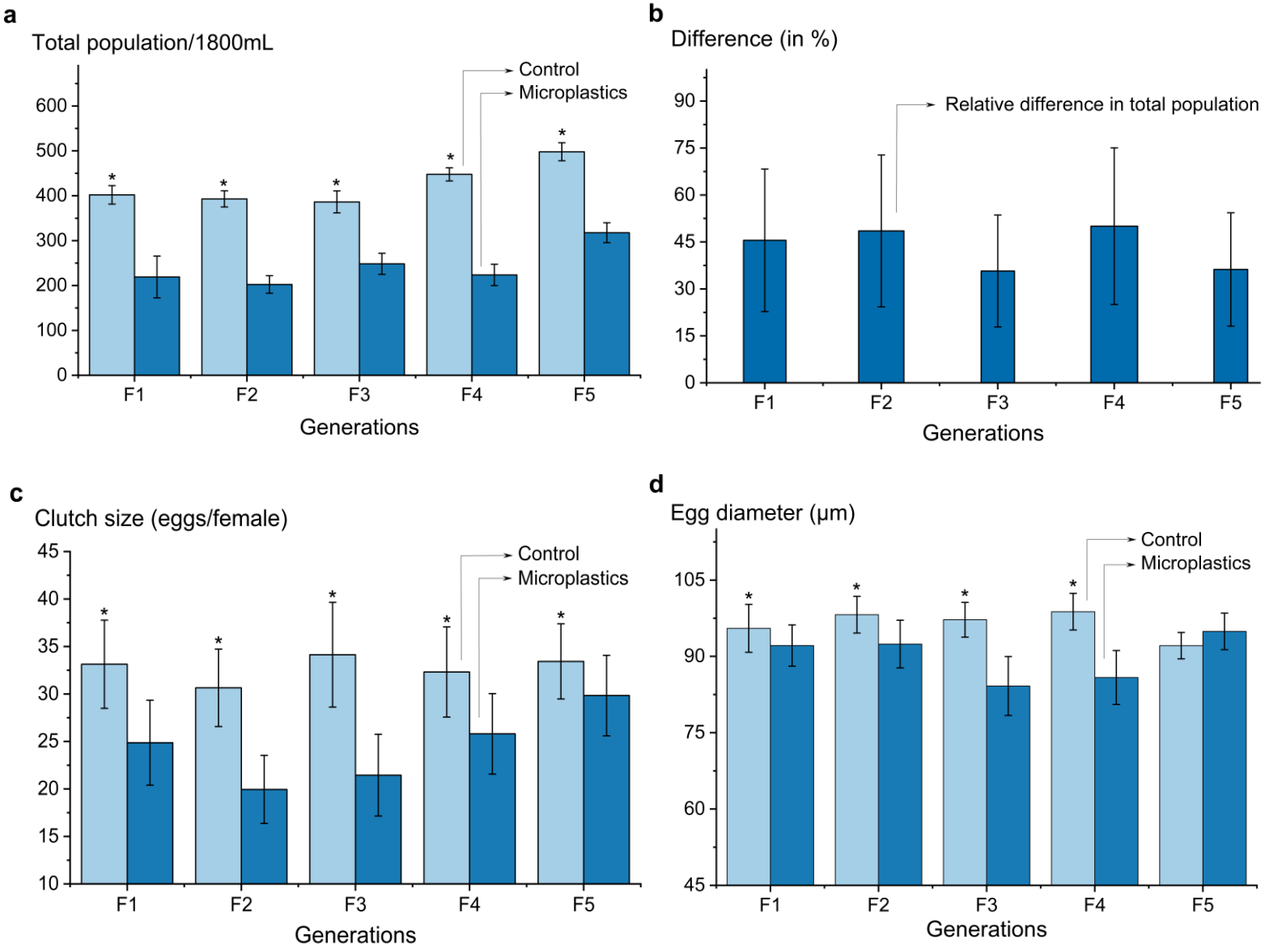

Figure 3. Total population **(a)**, relative difference in total population **(b)**, clutch size **(c)**, mean egg diameter **(d)** of *Eurytemora affinis* in the control and polylactic acid microplastics-exposed groups at the end of generations from the first filial (F1) to the fifth filial (F5) with Mean ± SE. Asterisks (*) represent statistical significance ($p < 0.05$). Standard error (SE).

3.3 Clutch size and egg diameter

In the multigeneration exposure, we found that clutch size significantly ($p < 0.05$) decreased compared to the control group, with the lowest value (19.5) in the second generation (**Fig. 3c**). However, from the second to the fifth generations, the clutch size showed an increasing trend, suggesting a tradeoff between fecundity and egg diameter. Moreover, mean egg diameter in the exposed groups also significantly ($p < 0.05$) decreased, with a notable decline from the second to third generation (92.41 to 84.17 µm), followed by an increase from the third to the fifth generations (**Fig. 3d**). The control group exhibited an average of 32.7 eggs per female with an average egg diameter of 96.35 µm, while the exposed groups had an average of 24 eggs per female with an average egg diameter of 89.89 µm. No significant correlation was observed between clutch size and subsequent generations in the control ($r^2 = 0.02$, $p = 0.99$) or exposed ($r^2 = 0.37$, $p = 0.61$) groups **(Fig. S5e**). In the control group, egg diameter showed a moderately positive correlation with subsequent generations ($r^2 = 0.27$, $p = 0.02$), while the exposed group showed a negative correlation ($r^2 = 0.51$, $p = 0.00$) (**Fig. S5f**).

Microplastics, despite reports of fragmentation in some species' digestive tracts, such as rotifers (Zhao et al. 2023), cannot be digested or absorbed by aquatic filter feeders. This may result in energy depletion and consequently a decline in reproductive outputs. Exposure to polystyrene microplastics in copepods resulted in reduced fecundity and smaller egg size, attributed to an energy deficit and oxidative stress (Cole et al. 2013, 2015). These findings are consistent with our results, where clutch size consistently decreased from the first to the second generation. Our results also showed an increasing trend in clutch size and a decreasing egg diameter from the third to the fourth generation, attributed to the tradeoff between clutch size and egg size, as previously reported in *E. affinis* under environmental stress (Souissi and Souissi 2021). Microplastic exposure in marine medaka led to reduced fecundity, with offspring from exposed parents exhibiting compromised antioxidant enzyme activities, resulting in decreased fertility and hatching rates (Wang et al. 2019). Thus, the ingestion of polylactic acid microplastics by *E. affinis* presumably leads to similar consequences as those observed for petroleum-based microplastics, resulting in a reduction in food intake, which, in turn, prioritized energy allocation for growth and survival, ultimately leading to reduced reproduction.

3.4 Effects on the life stages *Eurytemora affinis*

The copepod life stages of *E. affinis* in control and microplastics-exposed groups at the end of each generation are shown in Table S1. The number of nauplii in the exposed groups was significantly higher ($p < 0.05$) than in the control group in most generations, with the control group averaging 15.6 nauplii, while the exposed group averaged 33 nauplii. In contrast, the number of all other copepod life stages, including copepodite females and males, and adult females and males, was significantly higher ($p < 0.05$), except for the adult males in the fifth generation and copepodite males in the third to fourth generations, where the difference was not significant ($p < 0.05$). Polylactic acid microplastics have resulted in premature hatching, leading to the production of substandard nauplii, followed by an increase in the naupliar phase and subsequent high mortality.

Microplastics ingestion in copepods could decrease food uptake, potentially causing an energy deficit and impacting growth and development to adulthood (Botterell et al. 2019). *Daphnia pulex* exposed to polystyrene microplastics significantly reduced the total number of offspring (Liu et al. 2019). The development of *Tigriopus japonicus* from naupliar to copepodite was delayed when exposed to polyamides and polyethylene microplastics (Yu et al. 2020). Exposure to polystyrene microbeads resulted in slower growth in the larvae of the marine gastropod *Crepidula onyx* (Lo and Chan, 2018). Exposure of *Acartia tonsa* copepods to polystyrene microbeads resulted in reduced nauplii survival (Shore et al. 2021). Microplastics exposure in copepods increases the naupliar phase and leads to reduced egg hatching and premature hatching (Lee et al. 2013; Beiras et al. 2018). Consistent with these results, we found that exposure to microplastics from polylactic acid affects reproduction by reducing egg hatching, increasing the nauplii phase, and consequently reducing the transition to copepod stages.

3.5 Prosome dimensions and number of lipid droplets

Over generations, the exposed group exhibited significant changes in prosome length, width, and volume compared to the control group, indicating considerable impact on copepod morphology (**Fig. 4 a–c**). We also found that the prosome dimensions of the control group showed fluctuations, likely due to higher density. The control group demonstrated an average prosome length and width of 781.5 and 277.7 μm, respectively. In contrast, the exposed groups exhibited a reduced prosome size, with average lengths and widths of 768.8 and 263.4 μm. The regression results for the prosome dimensions show a stable trend in successive generations for both the control and exposed groups, with all *p*-values for the tests being greater than 0.05 (**Fig. S5 b–d**). Furthermore, the number of lipid droplets in the exposed group varied across generations, with reductions in the early generations (first to second) and subsequent increases in the third to fifth generations, suggesting potential stress responses (**Fig. 4d**). The observed changes in copepod morphology and lipid droplet dynamics suggest adaptive responses to microplastic exposure over successive generations. The fluctuations in prosome dimensions in the control group highlight the influence of population density on zooplankton morphology.

The body size of zooplankton is a useful ecological indicator however it could also be influenced by the density of the population (Corona et al. 2021), which explain the variation in the prosome dimensions we found in the control group. *Daphnia magna* exposure to microplastics resulted in significantly reduced body length and produced offspring with small body size (Martins and Guilhermino, 2018). The body size of offspring produced by microplastic-exposed mothers was significantly smaller compared to the control (Eltemsah and Bøhn 2019), which is consistent with our findings. An increase in the prosome dimension of the exposed group during the second generation could be attributed to a tradeoff between population size and individual body size, which is in line with the findings of Souissi and Souissi (2021).

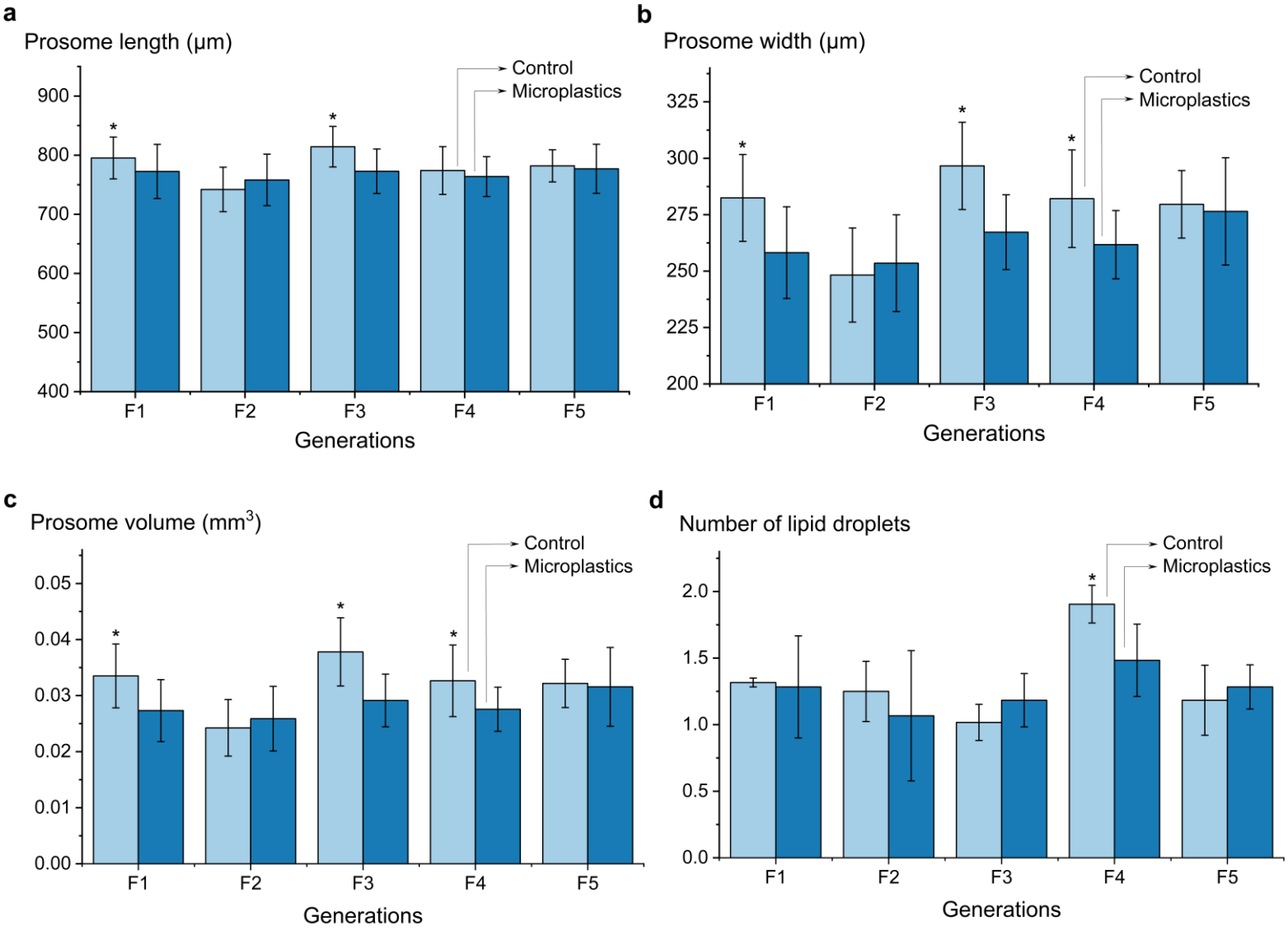

Figure 4. Prosome length **(a)**, prosome width **(b)**, prosome volume **(c)**, and number of lipid droplets **(d)** of *Eurytemora affinis* in the control and polylactic acid microplastics-exposed groups at the end of generations from the first filial (F1) to the fifth filial (F5) with Mean ± SE. Asterisks (*) represent statistical significance ($p < 0.05$). Standard error (SE).

Lipid droplets maintain energy reserves when food availability is low however, copepods are unable to utilize lipid droplets under stressful conditions (Das et al. 2023). In our study, an increase in the number of lipid droplets in the third and preceding generations could be attributed to the stress response. The decrease in the number of lipid droplets observed in the decontaminated generation is in line with the findings of Souissi et al. (2016b), where survival exhibited a negative correlation with the number of lipid droplets. Overall, we found that polylactic acid microplastics induced effects on the morphological parameters of *E. affinis* that are consistent with those observed with other contaminants and, notably, with other types of microplastics.

4.Conclusion

Together, using *Eurytemora affinis* as an experimental model, we investigated the acute and multigenerational effects of effect of polylactic acid microplastics. Key findings of this research indicate that polylactic acid microplastics induced gender-specific acute toxicity, while multigenerational exposure, at lower environmentally relevant concentration, resulted in reduced survival, altered reproductive parameters, and morphological changes. This study highlights the potential ecological risks posed by microplastics, generated from bioplastics, in estuarine and marine environments and emphasizes the importance of understanding the long-term consequences on marine organisms. As the use of bioplastics like polylactic acid continues to rise, it becomes crucial to assess their environmental impacts comprehensively and identify strategies to address the challenges associated with their persistence and accumulation in marine ecosystems.

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Authors' contributions

WA contributed to the experiments, data collection, analysis, and writing of the original draft. SD and JT contributed to the experimental design and editing. HJ contributed to the experiment, data analysis, and revision. J-.SL was involved in supervising and editing. PZ and SS contributed to supervising, editing, and funding acquisition.

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Supplementary material

Text S1. Polylactic acid microplastics

Microplastics were synthesized using cryogrinding and cryomilling techniques at the Materials Research and Development Centre, University of Mons, which had a diameter ranging from 2 to 10 µm. The size distribution of the microplastics were determined using Dynamic Light Scattering (Mastersizer 2000, Malvern P analytical, Malvern, UK) with distilled water as the solvent, and the average size range was obtained from 10 consecutive measurements (**Fig. S1**). Zeta potential was determined using an electrophoretic light scattering spectrophotometer (ELS-Z, Otsuka Electronics, Osaka, Japan) in three consecutive measurements (**Fig. S3**).

Text S2. Acute toxicity test

Twenty-five active adults male and female *Eurytemora affinis* individuals from the acclimation column were placed individually in 100 mL beakers, each containing 90 mL of water with a salinity of 15 practical salinity units. For each sex, these beakers were divided into six groups, each with three replicates. Group one was designated as the control, while groups 2, 3, 4, 5, and 6 were exposed to 5, 20, 40, 80, and 160 mg/L of polylactic acid microplastics, as illustrated in **Fig. S2**. The beakers were covered with aluminum foil, and copepods were not fed during the experiment. The entire setup was maintained in a constant temperature incubator at 18 °C, under a photoperiod of 12-hour dark and 12-hour light. Mortality rates were recorded every 24 hours using a stereomicroscope (SZX9; Olympus, Tokyo, Japan) until the 96-hour mark.

Text S3. Sample analysis

Population counts, mortality, sex ratio, and morphological measurements

Copepod counts were determined using a stereomicroscope (SZX9; Olympus, Tokyo, Japan). The stored samples were manually stirred to ensure equal distribution. One drop of the sample was taken, and copepods in various stages were identified and counted. This process was repeated until all copepods were counted in each triplicate.

For each generation, the mortality percentage was calculated according to ***Equation 1***. The theoretical production is determined by multiplying the average number of eggs per female in each generation by the total number of females, indicating the theoretical recruitment for the next generation. Population density is the summation of all the stages of copepod development, from larvae to adult.

$$Mortality\% = 100 \times \frac{theoretical\ production - population\ density}{theoritical\ production} \quad \textbf{(Eq. 1)}$$

The sex ratio was calculated according to Equation 2, where C5 male refers to copepodite stage 5 and C5 female refers to copepodite stage 5 female.

$$Sex\ ratio = \frac{C5\ male + adult\ male}{C5\ male + C5\ female + adult\ male + adult\ female + ovigerous\ female} \quad \textbf{(Eq. 2)}$$

For morphological measurements, twenty *E. affinis* females from each replicate of each group in every generation were photographed using an inverted microscope (OLYMPUS IX71, Tokyo, Japan) equipped with a camera. Image J 1.41 software was employed to measure prosome length, prosome width, prosome volume, and egg diameter. For instance, prosome length was measured from the frontal part of the cephalosome to the end of the fifth body segment, and prosome width was measured between the dorsal line and the line connecting the appendages. Additionally, all ovigerous females were used to detect and count the number of lipid droplets in each generation.

Text S4. Statistical analysis

The acute toxicity test, median lethal concentration (LC50), with 95% confidence interval (CI) were calculated from the mortality data through Probit analysis for males and females separately. SPSS software, version 21, was used to conduct. Levene's Test for homogeneity of variance ($p < 0.05$) and an independent sample t-test ($p < 0.05$) was used to compute the differences in the selected endpoints between the control and experimental groups of each generation. Tukey's test was then employed to identify statistically distinct groups for the morphological and reproductive endpoints. The Pearson correlation coefficient was computed to assess the relationship between various morphological and reproductive indices. Significant differences were considered at two levels: $p < 0.05$ and $p < 0.01$.

Text S5. Relationship between morphological and reproductive traits of *Eurytemora affinis*

The correlation analysis revealed interesting patterns between different parameters **(Table S2)**. In the control group, a weak negative correlation ($r = -0.44$, $p = 0.09$) between population and prosome length was observed within the whole dataset from first filial generation to fifth filial generation. Similar trends were found within the first to second filial generation generations ($r = -0.93$, $p = 0.00$) and the third to fourth filial generations ($r = -0.43$, $p = 0.38$), thereby suggesting a correlation between larger populations and shorter prosome lengths. Conversely, within the exposed groups, correlation between population and prosome length consistently exhibited reduced magnitudes across all subsets ($r = -0.16$ to -0.52), however, none of these correlations attained statistical significance ($p = 0.28$ to 0.55). The correlation between population and clutch size displayed weak correlations in both groups across all generations, with none being statistically significant ($p > 0.05$). Regarding prosome volume and clutch volume, a weak yet statistically significant positive correlation was noted ($r = 0.121$) in the control within the whole dataset, first filial generation to fifth. This correlation weakened in the first to second and third to fourth filial generations. In contrast, the experimental group showed a positive relationship between prosome volume and clutch volume within the whole dataset and within the first to second filial generations. Lastly, a significant positive correlation surfaced within the control population between prosome length and clutch size ($r = 0.29$, $p = 0.00$) within whole dataset, extending its statistical significance to all generations. In contrast, the exposed group exhibited a moderately strong positive correlation between prosome length and clutch size which attained statistical significance in some generations.

The body size and fecundity of calanoid copepods are interrelated, influenced by food availability, positively correlated with food concentration, and commonly observed to be directly affected by high population density, often attributed to competition for available food resources (Medina and Barata 2004; Peck and Holste 2006). This is evidenced by an inverse correlation between the population density of copepods and clutch size and prosome length (Maly 1973; Corona et al. 2021). This aligns with our correlation results for all generations of the control group, where a negative correlation was found between population and prosome length. However, in the exposed groups, this negative correlation was disturbed, and a positive correlation was observed in the third and fourth filial generations, consistent with the results of Kadiene et al. (2022), who reported a similar trend when exposing copepods to cadmium in a multigeneration experiment. Furthermore, in *E. affinis*, the prosome length correlates positively with clutch size (Souissi et al. 2021), which is in line with our control group results. Unlike the control group, the correlation between prosome length and clutch size was negative during the third and fourth filial generations of exposed groups. This suggests that the correlation between body size and clutch size was disturbed due to the inheritance of compromised antioxidant systems from mothers exposed to microplastics during the initial generations, first and second filial generations. These findings confirm previous studies indicating that offspring from exposed parents exhibit compromised antioxidant enzyme activities (Wang et al. 2019). Our findings align with previous studies, revealing negative correlations between population size and clutch size, and positive correlations between prosome length and clutch size, as well as prosome volume and clutch volume (Souissi and Souissi 2021; Kadiene et al. 2022).

We further report that polylactic acid microplastics exerted reversible alterations in the morphological and reproductive indices of *E. affinis*. This study highlights the recovery of the morphological and reproductive parameters showed in the decontaminated generation, which could be attributed to the adaptive response of copepod when exposed to any pollutant. Planktons in general are unable to recover when exposed to a combination of two or three pollutants, while they are resilient to a single stressor (Das et al. 2023). These findings highlight the dynamic nature of the morphological and reproductive responses in copepods, suggesting potential adaptability to the presence of microplastics over successive generations. However, the observed variations in morphological and reproductive indices underscore the complex and species-specific nature of copepod responses to microplastics exposure. These findings indicate that exposure to polylactic acid microplastics can exert significant morphological and reproductive alterations in copepods, potentially influencing their physiological functions and overall fitness.

Table S1. Copepod life stages in control and the groups exposed to 400 µg/L polylactic acid microplastics across five filial generations.

Filial Generation	Groups	Nauplii	Copepodites	Copepodite stage 5 male	Copepodite stage 5 Female	Male	Female	Ovigerous female
1	Control	15 ± 4	50 ± 11*	88 ± 2.64	58 ± 4.58	95.66 ± 11.06*	79 ± 9.16*	31.33 ± 7.50*
	Exposed	35 ± 6*	21.66 ± 4.04	61 ± 15.87	29.33 ± 14.97	61 ± 13.11	29.33 ± 7.02	16.66 ± 4.04
2	Control	16.33 ± 5.50	44.66 ± 10.50*	80.33 ± 4.72*	60.33±3.21*	102 ± 9.64*	71.33 ± 15.30*	34.33 ± 4.16*
	Exposed	34 ± 7*	39 ± 8	44.66 ± 5.50	18.33±2.51	70 ± 7.18	33.33 ± 7.37	17.66 ± 11
3	Control	15.33 ± 2.51	36.66 ± 6.11*	91.33 ± 11.59	64.33±11.15*	91 ± 8.66*	72.33 ± 4.16*	30.33 ± 8.50
	Exposed	31.66 ± 8.32	16 ± 5	72.33 ± 4.16	36.33±5.07	71.33 ± 8.50	31 ± 9.16	21.33 ± 4.50
4	Control	16.34 ± 3.05	43 ± 7.21*	98.33 ± 14.01	71.33±6.02*	109 ± 13.74*	84.66 ± 12.34*	41.33 ± 4.50*
	Exposed	34.33 ± 4.04*	19 ± 2	73 ± 17.08	35±11.53	56 ± 7.64	22.66 ± 10.44	18.66 ± 5
5	Control	15.70 ± 4.14	47.33 ± 13.57	116.33 ± 14.57*	92.66±14.04*	117.33 ± 11.50	90.66 ± 9.07*	33.66 ± 7.50
	Exposed	30 ± 9.53*	29.66 ± 7.02	70 ± 11.52	43.66±7.02	93.33 ± 13.31	53.33 ± 9.50	27.67 ± 5.50

Results are shown as mean ± SE. *Eurytemora affinis* in the control and polylactic acid microplastics exposed groups at the end of each filial generation. Asterisks (*) represent statistical significance ($p < 0.05$) between control and polylactic acid microplastics exposed group. Standard error (SE).

Table S2. Relationship (r) between population *versus* prosome length, population *versus* clutch size, prosome volume *versus* clutch volume, and prosome length *versus* clutch size.

		F1–F5		**F1–F2**		**F3–F4**	
		Control	**Exposed**	**Control**	**Exposed**	**Control**	**Exposed**
Population *versus* prosome length	r	−0.44	−0.16	−0.93**	−0.52	−0.43	0.01
	p-value	0.09	0.55	0.00	0.28	0.38	0.48
Population *versus* clutch size	r	−0.04	−0.06	0.13	−0.51	−0.60	−0.36
	p-value	0.87	0.81	0.79	0.29	0.20	0.47
Prosome volume *versus* clutch volume	r	0.12*	0.25**	0.08	0.32**	0.06	0.04
	p-value	0.03	0.000	0.34	0.00	0.50	0.65
Prosome length *versus* clutch size	r	0.29**	0.17*	0.32*	0.27*	0.32**	−0.02
	p-value	0.000	0.003	0.000	0.00	0.00	0.79

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).
Filial generation (F)

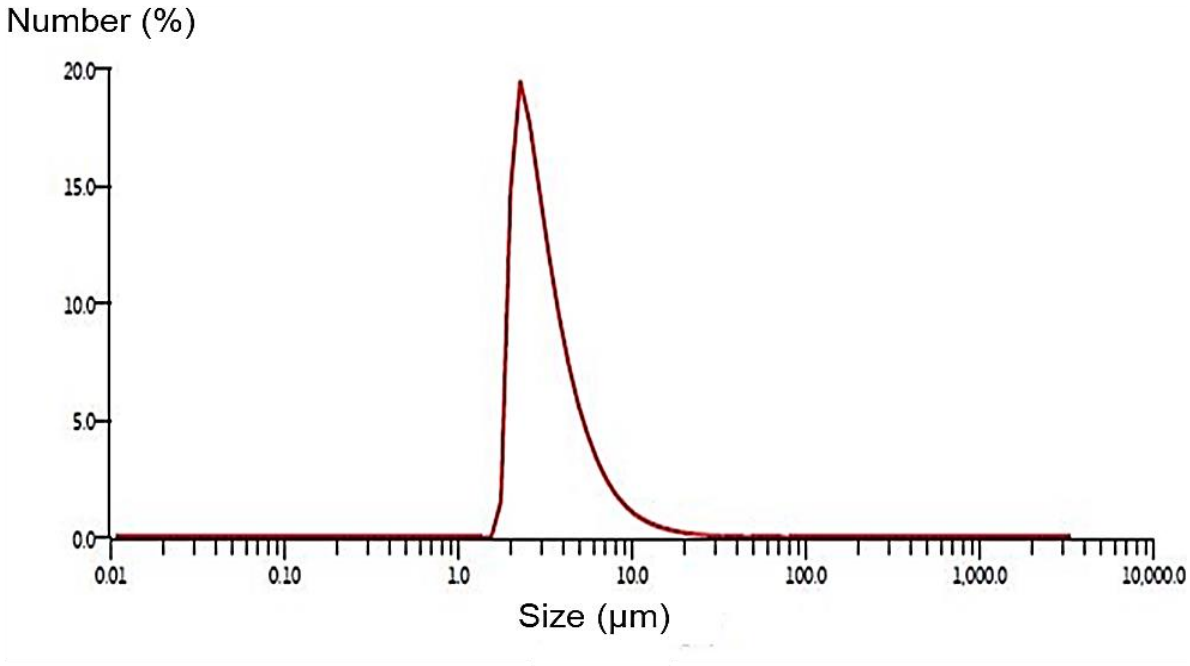


Figure S1. Size distribution analysis of polylactic acid microplastics using Dynamic Light Scattering in distilled water. The average size range was derived from 10 consecutive measurements.

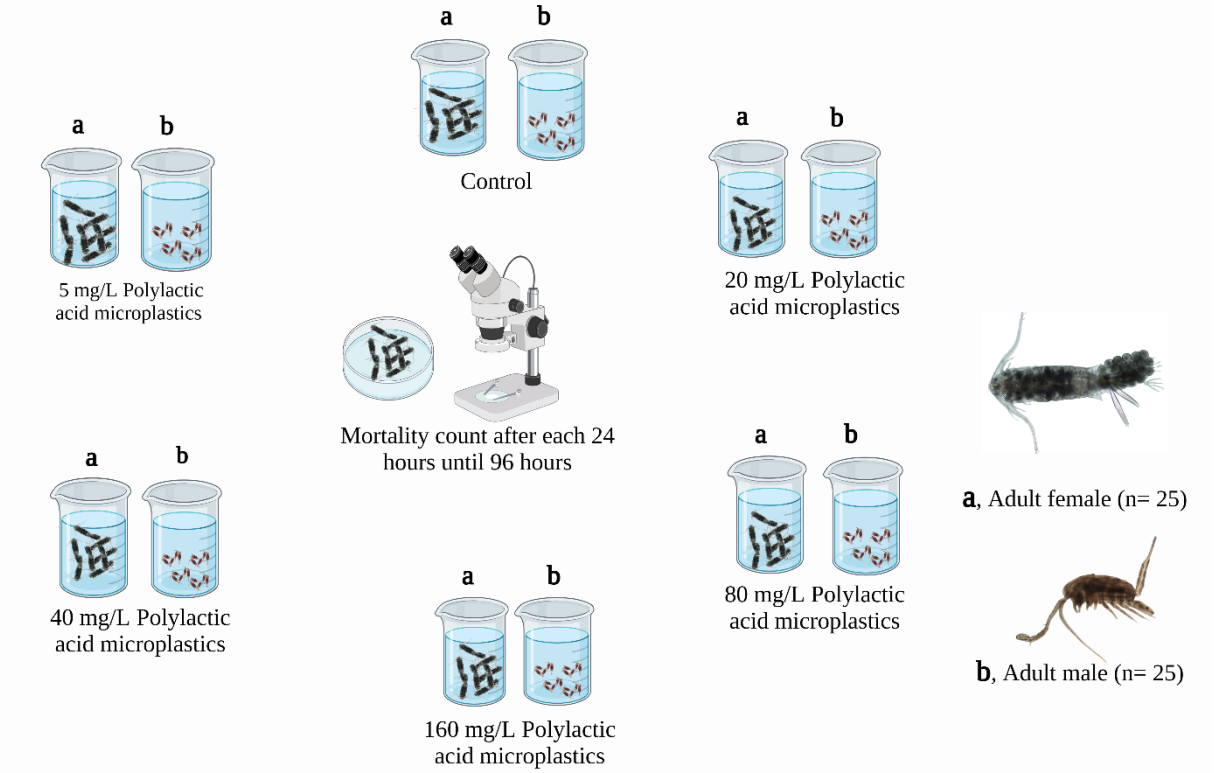


Figure S2. The experimental setup for the 96-hour lethal concentration (LC50) of polylactic acid microplastics in *Eurytemora affinis* consisted of 25 active adult male and female copepods,

each individually kept in 100 mL beakers containing 90 mL of water at 15 practical salinity units. Each sex was allocated into six groups with three replicates per group. Group one served as the control, while groups 2-6 were exposed to ascending concentrations of microplastics. The entire setup was incubated at a constant 18°C temperature with a 12-hour light-dark cycle. Mortality rates were documented every 24 hours using a stereomicroscope until the conclusion of the 96-hour experiment.

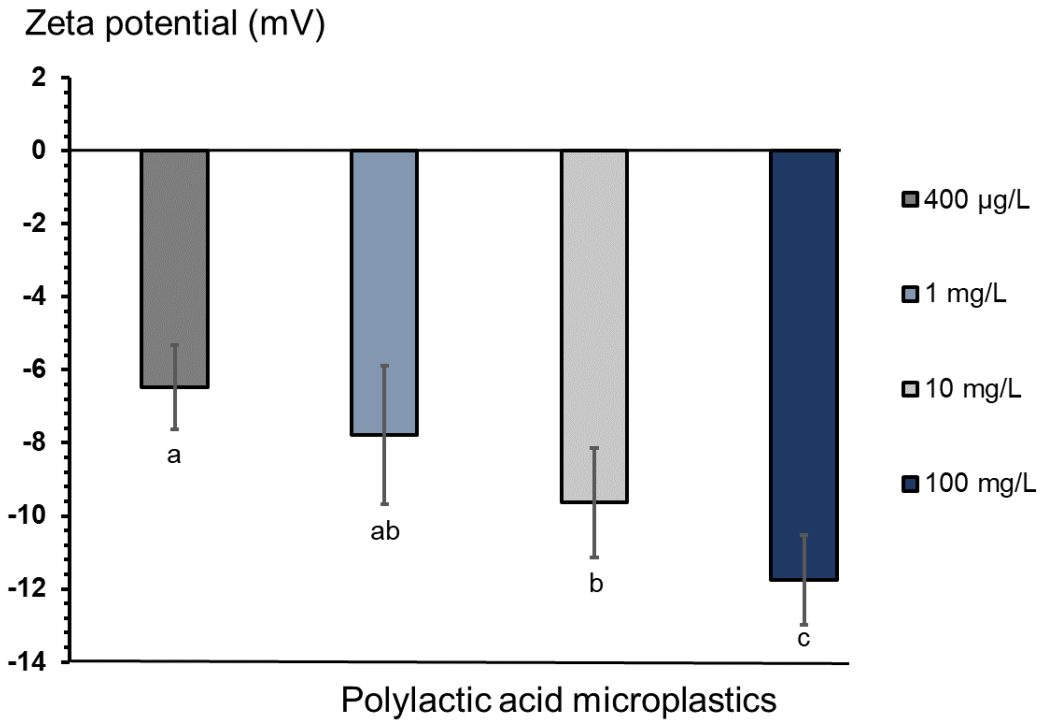


Figure S3. Zeta potentials of polylactic acid microplastics (400 µg/L, 1, 10 and 100 mg/L) using an electrophoretic light scattering spectrophotometer in practical salinity units 15 water. The average values were derived from three consecutive measurements.

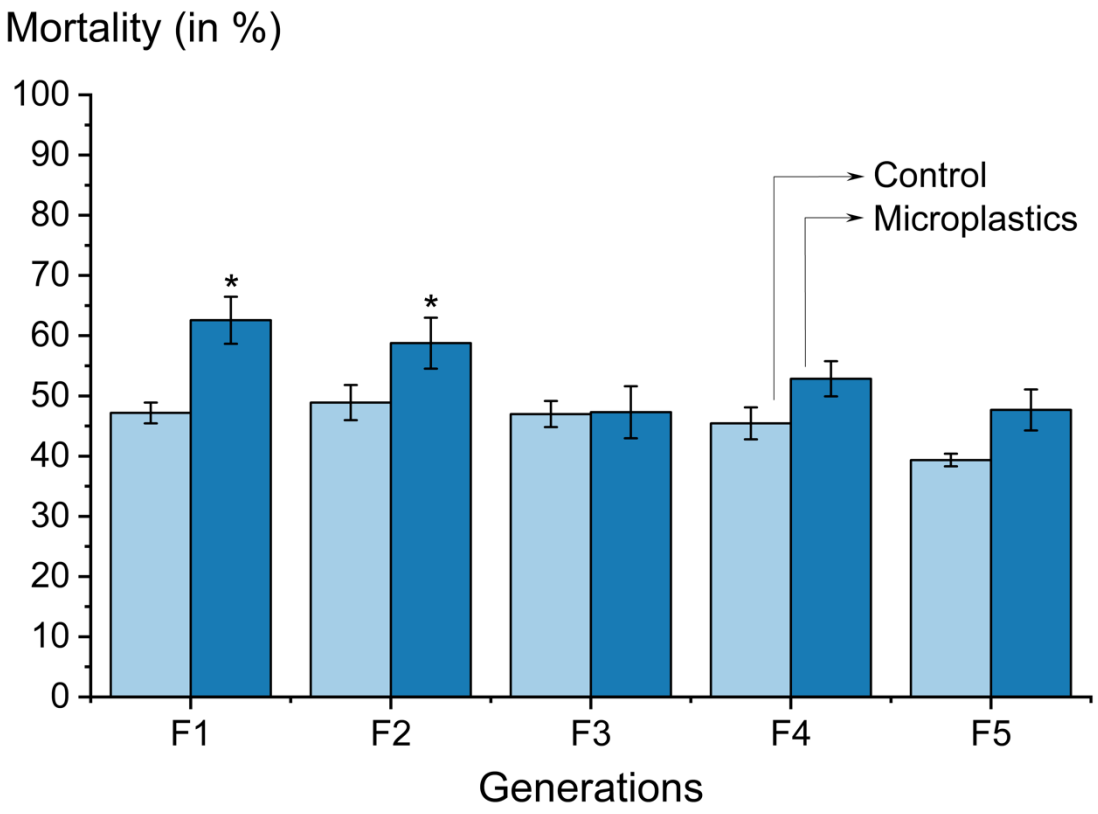
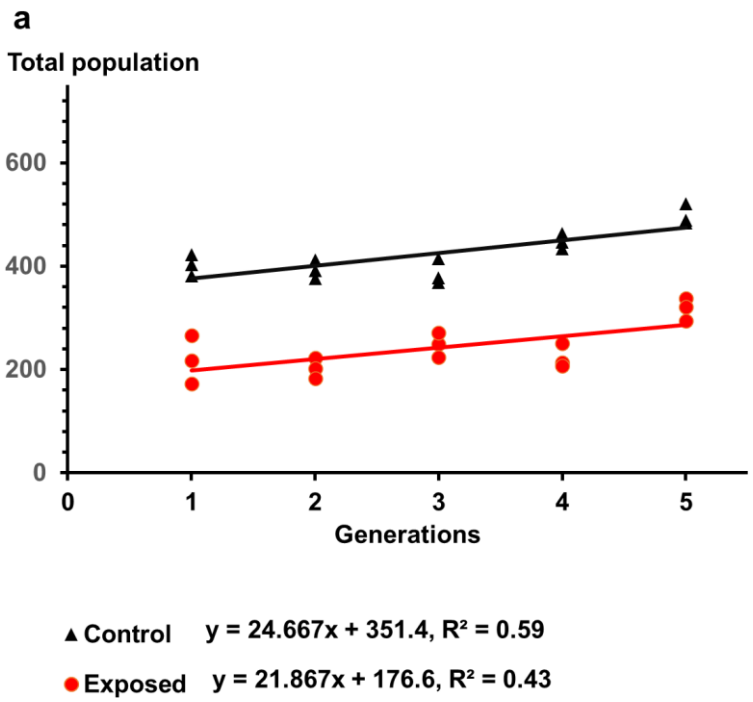
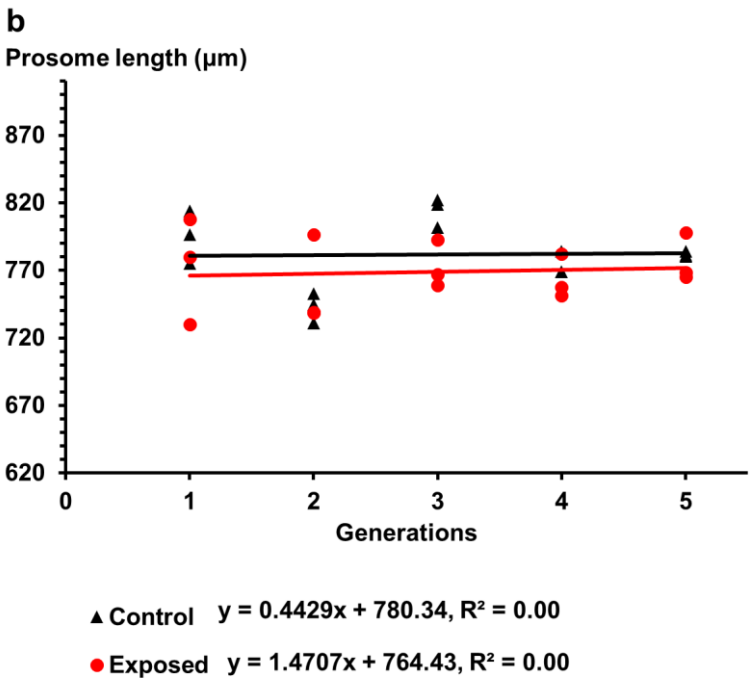
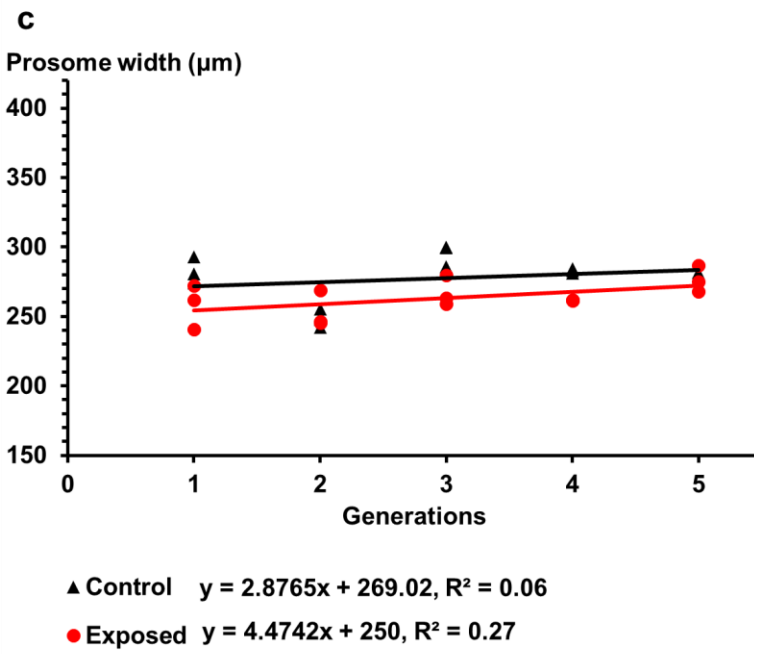
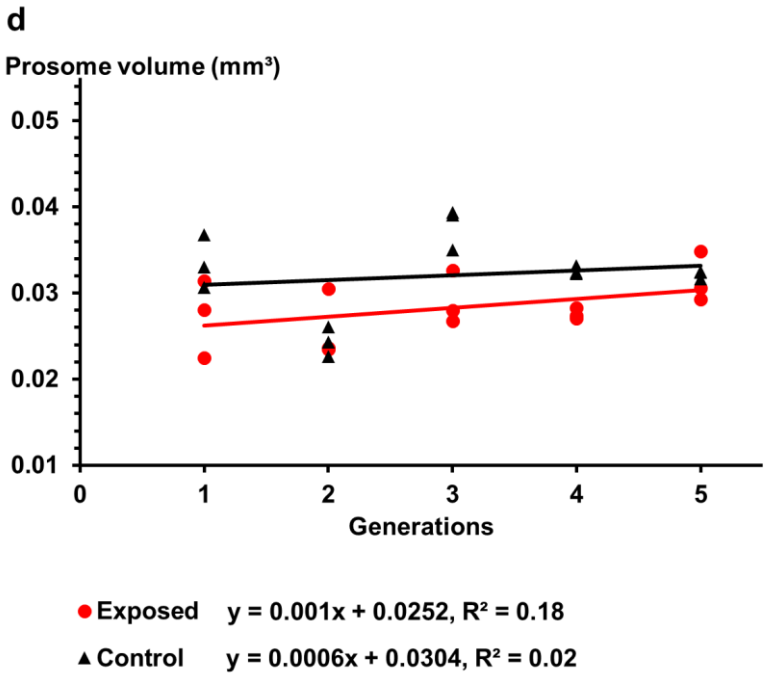


Figure S4. Mortality percentage with mean ± SE of control and polylactic acid microplastics exposed groups at the end of generations from the first filial (F1) to the fifth filial (F5) with Mean ± SE. Asterisks (*) represent statistical significance ($p < 0.05$). Standard error (SE).


a
Total population
600
400
200
0
0
1
2
3
4
5
Generations
Control y = 24.667x + 351.4, R² = 0.59
Exposed y = 21.867x + 176.6, R² = 0.43


b
Prosome length (µm)
870
820
770
720
670
620
0
1
2
3
4
5
Generations
Control y = 0.4429x + 780.34, R² = 0.00
Exposed y = 1.4707x + 764.43, R² = 0.00


c
Prosome width (µm)
400
350
300
250
200
150
0
1
2
3
4
5
Generations
Control y = 2.8765x + 269.02, R² = 0.06
Exposed y = 4.4742x + 250, R² = 0.27


d
Prosome volume (mm³)
0.05
0.04
0.03
0.02
0.01
0
1
2
3
4
5
Generations
Exposed y = 0.001x + 0.0252, R² = 0.18
Control y = 0.0006x + 0.0304, R² = 0.02

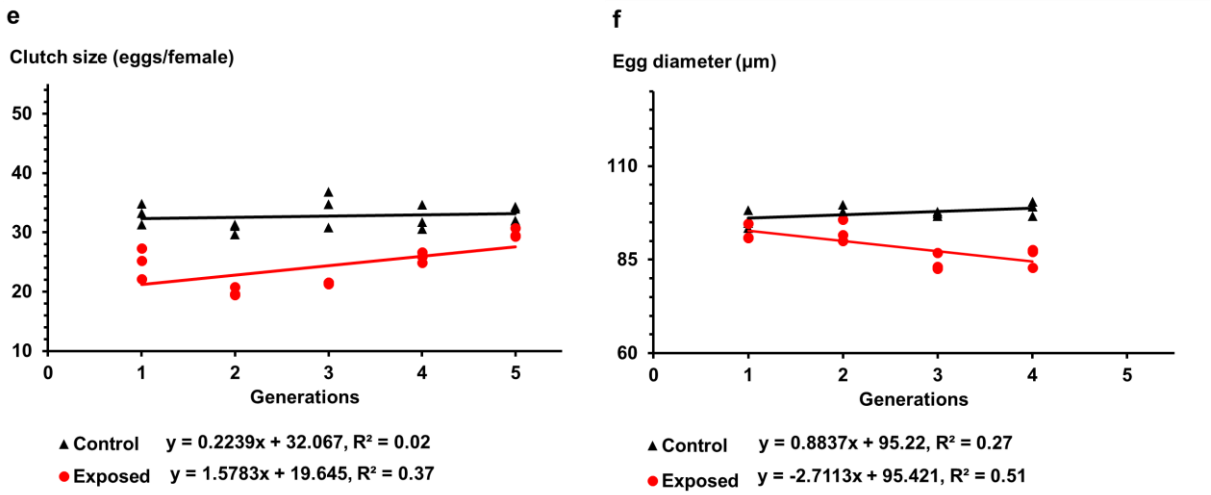


Figure S5. Relationship between the total population of control and exposed (400 µg/L polylactic acid microplastics) groups with different generations **(a)**, prosome length **(b)**, prosome width **(c)**, prosome volume **(d)**, clutch size **(e)**, and egg diameter **(f)** of female *Eurytemora affinis* across five filial generations.

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Paper III

Are bioplastics an ecofriendly alternative to fossil fuel plastics?

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Are bioplastics an ecofriendly alternative to fossil fuel plastics?

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Abstract

More than 390 million tons of fossil fuel plastics have been produced in 2022, and plastic pollution has become a major health issue for humans and ecosystems. Fossil fuel plastics are commonly recalcitrant, thus accumulating in the environment. Fossil fuel plastics also contains toxic chemicals that leach out to waters and are ingested by living organisms. Moreover, recent research has revealed that plastic fragmentation has led to an unprecedented global contamination by microplastics and nanoplastics, with yet poorly adverse impacts on life. In this context, bioplastics, that we will refer to as biobased plastics in this contribution, appear as a promising alternative because bioplastics are carbon neutral and most of them are biodegradable. As a consequence, the world bioplastic production has increased from 1.8 million tons in 2021 to 2.22 million tons in 2022. However, the complete degradation of most bioplastics in the environment remains difficult. Here we compare bioplastics with fossil fuel plastics with focus on life cycle assessment, biodegradability and compostability, end-of-life options, fragmentation into microplastics, and microplastic pollution of soil and aquatic systems. Overall, we observe that bioplastics are not necessarily more ecofriendly than fossil fuel plastics. Technical and cost limitations still prevent bioplastics from fully substituting fossil fuel plastics. For instance, there is still no widely accepted methodology to compare the potential environmental footprint between fossil fuel plastics and bioplastics. Some microorganisms can degrade plastics under laboratory conditions, but both fossil fuel plastics and bioplastics degradation can be limited under environmental conditions. End-of-life options for both fossil fuel and bioplastics are similar, except for composting. Both fossil fuel plastics and bioplastics contribute to environmental pollution by forming microplastics and acting as vectors for environmental pollutants. Overall, the study found that bioplastics and fossil fuel plastics have similar negative effects and both are comparable in terms of their vector role, formation of microplastics, and toxicity to biota. To conclude, the common belief that bioplastics have less or no negative impact on the environment may not be entirely accurate.

Keywords: Biodegradability, bioplastics, ecologically friendly, microplastics, petroleum-based plastics, and toxicity.

1. Introduction

Pollution of the environment with fossil fuel plastics has become a major environmental issue. About 6300 metric tons of plastic waste was produced until 2015 (Dhaka et al. 2022). Their ecological impacts originate from their smallest fractions, 1 to 5000 μm (microplastics) and below 1 μm (nanoplastics) (Sharma et al. 2022). Bioplastics that we will consider as biobased plastics in this contribution have been considered as a promising substitute to petrochemical-based plastics, notably regarding the finite petroleum feedstock available on earth. Research on bioplastics is achieving a leading position for the replacement of fossil fuel plastics and their production is increasing exponentially. For example, in 2021, world bioplastics production was around 1.8 million tons which was raised to 2.22 million tons in 2022. It is estimated that global bioplastic production will reach to approximately 6.3 million tons by 2027. On the other hand, world fossil fuel plastics production was more than 390 million tons in 2021, which accounts for more than 99 % compared to bioplastics production in 2022. Fossil fuel plastics have become ubiquitous in the modern society and are used in a wide range of applications, including packaging, consumer goods, construction, transportation, and more, while majority, almost half, of the bioplastics (1.1 million ton) have been used in packaging, and their use in automotive and construction is also increasing (EuropeanBioplastics 2023).

The accumulation of plastics in the natural environment is a consequence of the widespread use and poor management, such as lower rate of recycling, leading to only 9% recycling of total plastic produced until 2015 (Naser et al. 2021). Once accumulated in the environment, plastics are fragmented by natural weathering processes leading to the formation of microplastics, and even nanoplastics. Microplastics from fossil fuel plastics have been shown to have toxic effects on a range of wildlife. Ingestion of microplastics can lead to physical harm, such as blockages in the digestive system, and chemical harm, as toxic substances from the plastic can leach into the animal body. On the other hand, fate of bioplastics in the natural environment and their potential ecological and toxicological impact, are not well understood, but they are a growing area of concern. Fears are increasing concerning their leakage into the environment in the form of micro-bioplastics and even nano-bioplastics. Currently, research on the fate of bioplastics in the natural environment and their ecotoxicological evaluation is limited. An important question is whether bioplastics are environmentally friendly substitute to fossil fuel plastics. The current article is an attempt to address this question. In the following sections, we provide a comprehensive review of recent research comparing fossil fuel plastics and bioplastics with regards to their potential substitution, life cycle assessments, biodegradability, end-of-life options, microplastics synthesis, and their impact on environmental ecosystems.

2. Bioplastics as substitutes for fossil fuel plastics

Fossil fuel plastics production started in 1940s. Since then, they have been widely used in almost every aspect of human life. Many advantageous features such as durability, lightweight, versatility, resistance and low cost have made them widely used in many applications, including packaging, construction, automotive, and electronic industries. However, many of their disadvantages including high degree of chemical stability, formation of microplastics, leaching of toxic chemicals and additives, have been linked to a variety of health problems and have

resulted in environmental damage (Yee et al. 2021). Biobased plastics have raised interest as a sustainable alternative in the last decades, notably regarding the finite petroleum feedstock. There has been some confusion about the term bioplastic, often used also for biodegradable / compostable plastics. It should be noted that biobased plastics are not necessarily biodegradable or compostable (Nanda et al. 2022), but many of them are, such as polylactic acid, polyhydroxyalkanoates, polybutylene succinate, as well as carbohydrate derivatives and blends based on starch, cellulose and chitin.

Bioplastics can partially replace fossil fuel plastics in selected applications. For example, polyethylene terephthalate, low density polyethylene and high-density polyethylene are being substituted by polylactic acid in packaging applications. However, bioplastics could not fully substitute fossil fuel plastics due to their higher costs, as well as some chemical, mechanical, and technical drawbacks, such as processing performance, brittleness and lower gas barrier. For example, starch is a low cost and one of the most widely available polymers, but a few disadvantages including low mechanical strength and water resistance are hindering its use. Starch can thus be blended with other bioplastics to enhance its mechanical properties, and has been used in packing application. However, the release of a higher number of microplastics, specifically 20,348 ± 5857 items per gram, has been reported after 90 days of under long term aging when blended with poly(butylene adipate-*co*-terephatalate) (Bao et al. 2022). Likewise, polylactic acid was considered biodegradable, as it is the case *in vivo*, but its degradation under natural environment remains difficult (Ali et al. 2023; Weinstein et al. 2020). Similarly, other limitations, for example stiffness, thermal instability and brittleness, have also been reported for other bioplastics including polyhydroxyalkanoates and in particular polyhydroxy butyrate (Jabeen et al. 2015). Over all we found that, in recent years, the production and use of bioplastics have gained attention as a sustainable alternative to fossil fuel plastics, particularly for packaging applications. However, the technical and cost limitations of bioplastics, along with the formation of microplastics, still present challenges that prevent them from fully replacing fossil fuel plastics. Despite these limitations, ongoing research and development in the field of bioplastics hold promise for further progress towards sustainable and environmentally-friendly materials.

3. Life cycle assessment

Fossil fuel plastics are synthesized from non-renewable resources. Their production leads to the depletion of these resources, while their wastes contribute to environmental pollution. Addressing these environmental concerns is a critical challenge, and requires the development of more sustainable and eco-friendly alternatives. On the other hand, bioplastics are produced from renewable feedstock, such as plant-based materials, which is one of the aims of green chemistry. Both fossil fuel plastics and bioplastics have been evaluated for comparison with focus on energy use, global warming potential, acidification potential, carbon footprints and so on. However, this comparison is complex and currently there are no well accepted rigorous and reliable tools and or methodologies available to assess potential environmental footprint differences between biobased and fossil fuel plastics.

Life cycle assessment (LCA), a method designed to quantify the environmental impacts of a product from cradle to grave, for example is barely usable for that purpose in its current state (Bishop et al. 2021; Walker and Rothman 2020). Walker and Rothman (2020) compared life cycle assessment studies on fossil fuel plastics and bioplastics across selected impact categories. The authors reported significant variation between the types of plastics and between fossil fuel plastics and bioplastics, and as a result, were unable to declare any polymer as having a minimum environmental impact. Furthermore, it is a very general approach in the life cycle assessment of plastics to only consider microorganisms as a life form, in terms of their involvement in biodegradation. Certainly, the bioaccumulation and biomagnification of microplastics along the food chain, as well as their biotransformation during this process, may impact life cycle assessment of plastics. However, accounting for these factors is difficult. Besides the challenges in comparing the environmental footprints of these two types of plastics, bioplastics are considered a green approach for achieving a sustainable environment. Although we found that progress has been made in the comparison of environmental impacts between fossil fuel plastics and bioplastics, there is still no widely accepted methodology that accounts for all the factors while comparing the potential environmental footprint differences between the two. As a result, declaring any of the polymers as safe or eco-friendly will be difficult.

4. Biodegradability and compostability

In general, fossil fuel plastics such as polyolefins possess unique structural features, such as hydrophobic chains, and lack of bio-accessible organic chemical groups, as well as high mechanical and fluid barrier properties, achieved by packing their chains into specific crystalline and amorphous regions. The unique packing of these chains contributes to the material strength and rigidity, while the amorphous regions provide flexibility and allow for the material to be molded into different shapes. These structural features on one hand make the plastic durable but on the other hand limit the ability of microorganisms to break them down. Many factors contribute to the persistence of fossil fuel plastics, for example, cross-linked structure, morphology (crystallinity and physical structure) and hydrophobicity, which result in their low bioavailability to microorganisms. Recent research has identified many bacteria, fungi and microbial consortia, as reviewed by Mohanan et al. (2020), capable of degrading fossil fuel plastics, mostly under laboratory conditions. There are however a few fossil fuel polyesters that have the potential for biodegradation, for example, poly(ε-caprolactone) and poly(butylene adipate-*co*-terephatalate).

In general, the long chains of biodegradable bioplastics are initially broken-down by chain scission as a result of environmental factors such as water, sun-light, temperature and so on, leading to the formation of shorter units of oligomers, dimers and monomers. These smaller units are small enough to pass through the outer semipermeable membrane of microorganisms, making them available as substrates for microbial metabolism (Shah et al. 2008). However, it should be noted that while the degradation of bioplastics could be easily achieved under controlled environment, it is not so easy under natural environmental conditions. The biodegradability of bioplastics depends on their material properties and environmental conditions. For example, several polyhydroxyalkanoates are home-compostable, while

polylactic acid requires the presence of heat, moisture, and microbial activity to break down. This means that they are best suited for industrial composting facilities, where these conditions can be carefully controlled to ensure proper biodegradation (Ali et al. 2023). In the natural environments or in landfills, where these conditions may not be present, the biodegradation of compostable bioplastics can be slower, leading to plastic pollution. Bioplastics have the advantage of being biodegradable or compostable, but they still face limitations in terms of the required environmental conditions for proper degradation. Overall, we found that recent research has identified several microorganisms capable of degrading fossil fuel plastics, primarily under laboratory conditions, while the biodegradability of bioplastics depends on their nature and the environmental conditions. Without these conditions, they may end up having a similar fate as fossil fuel plastics.

5. End-of-life options

The environmental impact of a polymer is notably determined by its end-of-life options. The major disposal strategies for polymers currently include landfill, incineration, recycling and composting. However, the later applies only to plastics which are designed to be compostable, mostly bioplastics, for example polylactic acid as shown in Figure 1. Among the other end-of-life options, 22 to 43 % plastic wastes ended up in landfills until 2015 (Naser et al. 2021). Fossil fuel plastics are durable and resistant to degradation, leading to pollution when land filled. They also contain chemicals that can leach out, in addition to the formation of microplastics, and thus cause harm to ecosystems and human health. Similarly, a major portion of bioplastics end up in landfills due to many reasons including lack of sorting and composting infrastructure.

Incineration is an older method of waste management that is still used today as a possible end-of-life option for certain types of waste materials including plastics. It is one of the major end-of-life options for plastics, as around 12 % of plastic waste has been incinerated until 2015 (Naser et al. 2021). However, the burning of plastics releases toxic gases on one hand and produces airborne particulates and ash on the other hand. Similarly, incineration of bioplastics can produce greenhouse gases and toxic emissions. This not only implies a loss of energy but also contributes to air pollution, which is a cause for human health concerns. Currently, pyrolysis, a method to convert wastes into energy, is thought to address the issue of landfilling. However, the production of greenhouse gases, for example, carbon dioxide, will contribute to global warming.

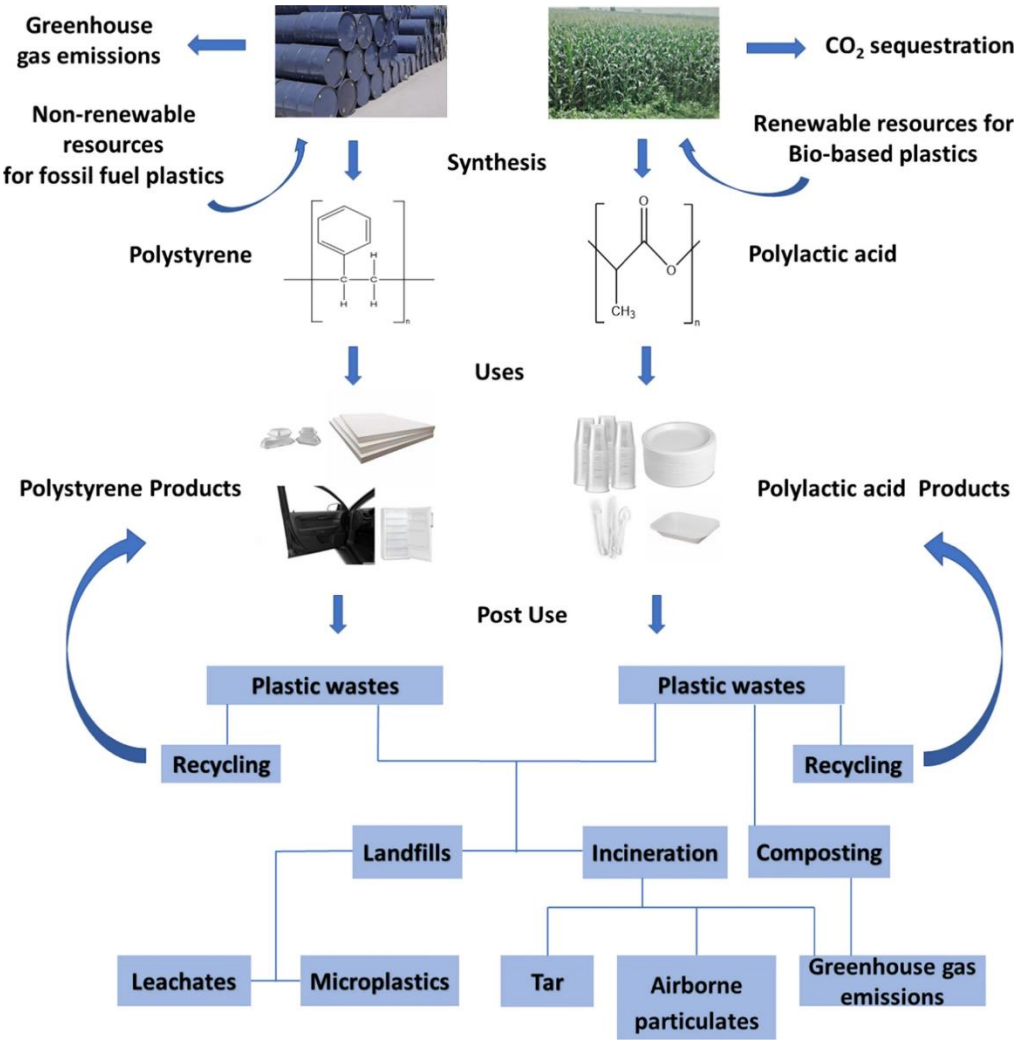

Fig. 1. Comparative life-cycles of fossil fuel plastics and bioplastics. Fossil fuel plastics are produced from a non-renewable resource whose processing contributes to greenhouse gas emissions and environmental pollution, while bioplastics are produced from renewable feedstock, e.g., plants which contribute to the sequestration of CO_2, a major greenhouse gas, through photosynthesis. Both types of plastics contribute to microplastic pollution in the environment.

Recycling of plastic waste, both mechanical and chemical, is on increase, for example, in 2020 more than 10 million tons of plastic waste were sent for recycling, while around 5.5 million tons recycled plastic was introduced in 2021, 20 % more compared to 2020 (Plastic Europe 2022). Recycling not only helps to divert plastic waste from landfills and incinerators, which can have negative environmental impacts, but it can also be a more environmentally friendly option than the production of new plastic. In Europe, the Green Deal and the Circular Plastics Alliance aim to bring the market of recycled plastics to 10 million tons in 2025 and to make all packaging recyclable by 2030. In particular, chemical recycling appears to be an economically viable route (European Commission 2023). Recycling of plastic waste requires sorting due to incompatibility among different polymers, and the current lower quantity of

bioplastics produced versus fossil fuel ones may not be an advantage at short term. Overall, we found that, the end-of-life options for both types of plastics are almost the same, except composting, which is specific to bioplastics. Progress in end-of-life options has led to an increase in plastic waste recycling, which not only helps to divert plastic waste from landfills and incinerators but is also a circular solution to plastic wastes that will reduce the plastic pollution.

6. Fragmentation of plastics into microplastics

Fossil fuel plastics are known to be very resistant to degradation, particularly by natural processes such as biodegradation. However, they can still undergo a slow process of fragmentation by environmental weathering processes, such as exposure to sunlight, oxygen, temperature, and moisture. For example, fossil fuel plastics absorb ultraviolet radiations when exposed to sunlight, which can lead to the formation of free radicals within the polymer chains. These free radicals are highly reactive and can trigger a chain reaction that results in the breakdown of the polymer chains, leading to the fragmentation of the plastic material and consequently formation of microplastics. The exact mechanisms of degradation can vary depending on the specific plastic material and the environmental conditions. On the other hand, bioplastics, especially biodegradable, are very susceptible to weathering process and microbial degradation. Theoretically, degradation of bioplastics involves two steps, fragmentation and biodegradation. Fragmentation is achieved by environmental factors, physical, chemical and even biological processes, for initial breakdown of the main chain to small size polymeric structures. The second step, biodegradation, involves the mineralization of small-sized polymeric structures such as monomers, dimers, and oligomers by microorganisms. However, it should be noted that the degradation of plastics, both fossil fuel and bio-based, depends on the type of plastic material and the environmental conditions.

A complete degradation of plastic polymer, from macroplastics to mineralization, is a very slow process which can take years, while micro-bioplastics and nano-bioplastics can be produced during all this process (González-Pleiter et al. 2019). For example, Lambert and Wagner (2016) compared the release of microplastics from fossil fuel plastics and bioplastics in a weathering chamber under 30 °C temperature and ultraviolet radiations. The authors found that both types of plastics released micron sized fragments, where polylactic acid and polypropylene exhibited higher concentration, such as 11.6×10^6 and 8.0×10^6 particles per milliliter, respectively. Similarly, Wei et al. (2021) and Napper and Thompson (2019) also reported the formation of microplastics from fossil fuel plastics and bioplastics under laboratory conditions. Very recently, Yang et al. (2022) compared the formation of microplastics from fossil fuel plastics and biodegradable plastics mulch films in soil under ultraviolet irradiation. The authors reported that biodegradable plastics mulch films, polylactic acid, generated more microplastics, 475 particles/cm^2, compared to fossil fuel plastics mulch films, such as polyethylene which generated around 150/160 particles/cm^2, respectively. These microplastics, if persisted in the environment could adsorb toxic metals on their surface, will interact with the biota and more likely will have similar or more negative impact like microplastics from fossil fuel plastics. Also, the presence of several additives, to improve the mechanical properties of

bioplastics, could leach into the environment and affect biota and physiochemical properties of the environmental medium. We found that both fossil fuel plastics and bioplastics can exhibit similar tendencies to form microplastics and contribute to environmental pollution. Despite being more susceptible to biodegradation, bioplastics still produce microplastics and release additives during the degradation process, which can have detrimental effects on the environment similar to those of fossil fuel plastics.

7. Microplastics as vectors for environmental pollutants

In general, microplastics have been detected in almost all types of environments, where heavy metals and organic pollutants are also present. Microplastics are durable, rather hydrophobic and possess specific surface, therefore they can interact with other coexisting contaminants (Figure 2). These interactions, for example, adsorption of heavy metals and persistent organic pollutants, on microplastics can change their transport, accumulation and toxicity. Fossil fuel plastics and bioplastics both have been reported to possess high affinities for adsorption of environmental pollutants. Very recently, Shi et al. (2023) have compared the adsorption capacities of biodegradable plastics, polylactic acid, poly (butylene adipate-*co*-terephthalate), and poly(ε-caprolactone), with polyethylene as a non-biodegradable plastic, for 3 to 12 months in a field experiment. The results showed that both types of plastics could adsorb heavy metals, where adsorption of copper (Cu), arsenic (As), and lead (Pb) were higher on biodegradable plastics compared to that on fossil fuel plastics. In another field study, it was found that biodegradation of poly (butylene adipate-*co*-terephthalate) in soil changed its surface functional groups, leading to higher adsorption of heavy metals as compared to polyethylene (Liu et al. 2022). These results show that microplastics from bioplastics not only act as a vector for heavy metals, but may also magnify their potential toxicity to biota, compared to fossil fuel plastics.

There is also evidence that both types of microplastics could adsorb organic pollutants. Gong et al. (2019) compared the adsorption of the pesticide, fipronil, on biodegradable microplastics (polylactic acid, polybutylene succinate) with non-degradable microplastics (polyethylene, polystyrene, polyvinyl chloride and polypropylene). The authors reported that the adsorption capacity on biodegradable microplastics was higher compared to that on non-biodegradable microplastics. In another example, Zuo et al. (2019) found that the affinity of poly (butylene adipate-*co*-terephthalate) to adsorb phenanthrene was 40 times higher compared to that of polystyrene and 3 times higher compared to that of polyethylene. Similarly, other studies have also reported high affinities of bioplastics for adsorption of environmental pollutants (Černá et al. 2021; Fan et al. 2021; Zhao et al. 2020). Microplastics present in the environment can transfer adsorbed pollutants to biota in higher doses, but not necessarily, depending on the type of environmental medium. For example, Jang et al. (2022) reported that bioplastics could not only transfer higher amount of heavy metals from aquatic environment to fish but also affected the abundance of their intestinal microbiota. On the other hand, Wang et al. (2019) reported decrease in the accumulation of organic pollutants in earthworm in the presence of microplastics. In conclusion, the high affinity for adsorption of environmental pollutants is exhibited by both bioplastics and fossil fuel plastics. Microplastics generated from

bioplastics can interact with other pollutants, similar to those generated from fossil fuel plastics. Overall, we found that, both bioplastics and fossil fuel plastics can act as vectors for environmental pollutants, including heavy metals and organic pollutants, and can potentially increasee their toxicity to biota. While certain bioplastics may have a higher affinity for adsorption of certain pollutants, the ultimate impact on the environment and biota is highly dependent on various factors such as the type of pollutant, environmental medium, and biota.

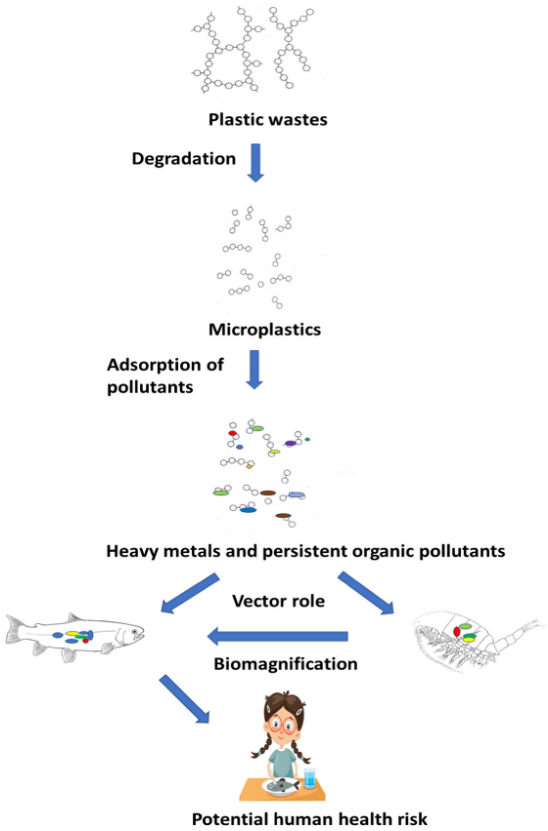

Fig. 2. Biodegradable plastics generate more microplastics compared to conventional polyolefins. These microplastics in the environment can adsorb various environmental pollutants such as toxic metals, pesticides and other persistent organic pollutants on their surface. The pollutant-loaded microplastics will interact with aquatic and terrestrial biota and more likely will have similar or even more negative impact than microplastics from polyolefins. The interaction of microplastics with environmental pollutants and their role as a vector for such chemicals may increase the bioaccumulation of pollutants in biota with subsequent risk to wildlife and human health.

8. Effects of microplastics on soil ecosystems

The different sources of microplastics in soil, for example use of plastic mulch and sewage sludge in agriculture (Chia et al. 2021), cause their interaction with the physicochemical parameters of soil, which can have a negative impact. For example, Koskei et al. (2021) investigated the effects of low-density polyethylene and polylactic acid on different properties

of soil for a period of two years. The authors reported an increase in bulk density (1.19 to 1.31 g/cm^3) and water storage (30.1% and 26.9%), while decrease in porosity (2.4% and 1.8%) and carbon/nitrogen ratio (1.3% and 1.2%) of soil amended with both low-density polyethylene and polylactic acid. In another example, Boots et al. (2019) assessed the impact of polylactic acid and high-density polyethylene microplastics (concentration of 0.1 % weight/weight and sizes of 65 µm and 102.6 µm) on biophysical response of soil in a mesocosm experiment. The authors reported that both types of microplastics decreased macro-aggregates (>2000 µm) and increased micro-aggregates (250–63 µm) of the soil. Similar trend was also reported by Zhao et al. (2021), where an increase in micro-aggregates (0.1–0.25 mm) and silt aggregates (<0.01 mm) were found in the presence of low-density polyethylene and polylactic acid, respectively. In long term, these changes in physicochemical parameters of soil could affect geophysical processes leading to alterations in community structures. However, the impact of microplastics on soil properties strongly depends on their shape, size, composition and doses (Qi et al. 2020), therefore, it is hard to generalize these effects.

Microplastics not only affect the physicochemical properties of soil but also affect the associated biota, which play a key role in the biochemical activity of the soil and in the biodegradation of bioplastics. Recent studies have reported that microplastics, from both fossil fuel plastics and bioplastics, could affect the abundance of soil microbiota. For example, Qi et al. (2020) studied and compared the effects of traditional (low density polyethylene) and biodegradable plastics (starch-based) residues, with different sizes, on wheat rhizosphere bacterial communities. The authors reported that both types of microplastics significantly affected the abundance of rhizosphere bacterial communities, where high relative abundance of *Bacillus* and *Variovorax* genera, among the others, were found in groups with bioplastics residues and genus *Saccharibacteria* in groups with fossil fuel plastics. Similarly, Sun et al. (2022) have also reported negative effects of both types of microplastics on microbial communities. However, it should be noted that the abundance of microbiota in soil is also affected by environmental factors like moisture, temperature, presence and or absence of oxygen, and physiochemical properties of soil.

The effects of fossil fuel plastics and bioplastics on plants, under different ecotoxicological endpoints, significantly vary. For example, Boots et al. (2019) compared the effects of polylactic acid and high-density polyethylene microplastics on *Lolium perenne*. The authors reported the germination of fewer seeds and reduction in shoot height of plants when exposed to polylactic acid microplastics, and an increased root biomass when exposed to high-density polyethylene microplastics. Similar results were also reported by Wang et al. (2020), where reduction in chlorophyll content, root and shoot biomass were found for treatments with polylactic acid microplastics as compared to those for polyethylene. Similarly, a few other studies have also compared phytotoxicity of both types of plastics and have reported negative effects (Liu et al. 2022; Menicagli et al. 2019a; Menicagli et al. 2019b). In contrast to these results, Meng et al. (2021) found that soil amended with polylactic acid and poly (butylene adipate-*co*-terephthalate) blend had significantly increased specific root length and nodules as compared to low density polyethylene.

Recent studies have investigated the effects of fossil fuel plastics and bioplastics on soil fauna (Figure 3), and have reported negative impacts on their health. For example, Yu et al. (2022) investigated and compared the effects of conventional (polyethylene) and biobased (polylactic acid) microplastics exposure on earthworm, *Eisenia fetida*, for 14 and 28 days. The authors reported an increase in malondialdehyde levels and decrease in the activities of superoxide dismutase, catalase, peroxidase, glutathione S-transferase, and acetylcholinesterase after 14 days of exposure, where the toxicity of polyethylene microplastics was generally higher compared to polylactic acid microplastics. On the other hand, after 28 days of exposure, decrease in malondialdehyde levels and an increase in all the mentioned activities were reported, where no significant difference was found in the toxicity of both types of microplastics. In another example, Zhao et al. (2023) compared the toxicity of conventional (polyvinylchloride and low-density polyethylene) and biodegradable (polylactic acid) microplastics on earthworm, *Eisenia fetida*, after 28 days of exposure. The authors reported that microplastics of both, low-density polyethylene and polylactic acid, exhibited similar ecotoxicity. Similarly, other studies have also reported negative effects of both types of plastics on terrestrial fauna (Ding et al. 2021; Schöpfer et al. 2020). Thus, bioplastics as well as fossil fuel plastics in the terrestrial environment can potentially affect not only the physicochemical properties of soil but also the associated biota.

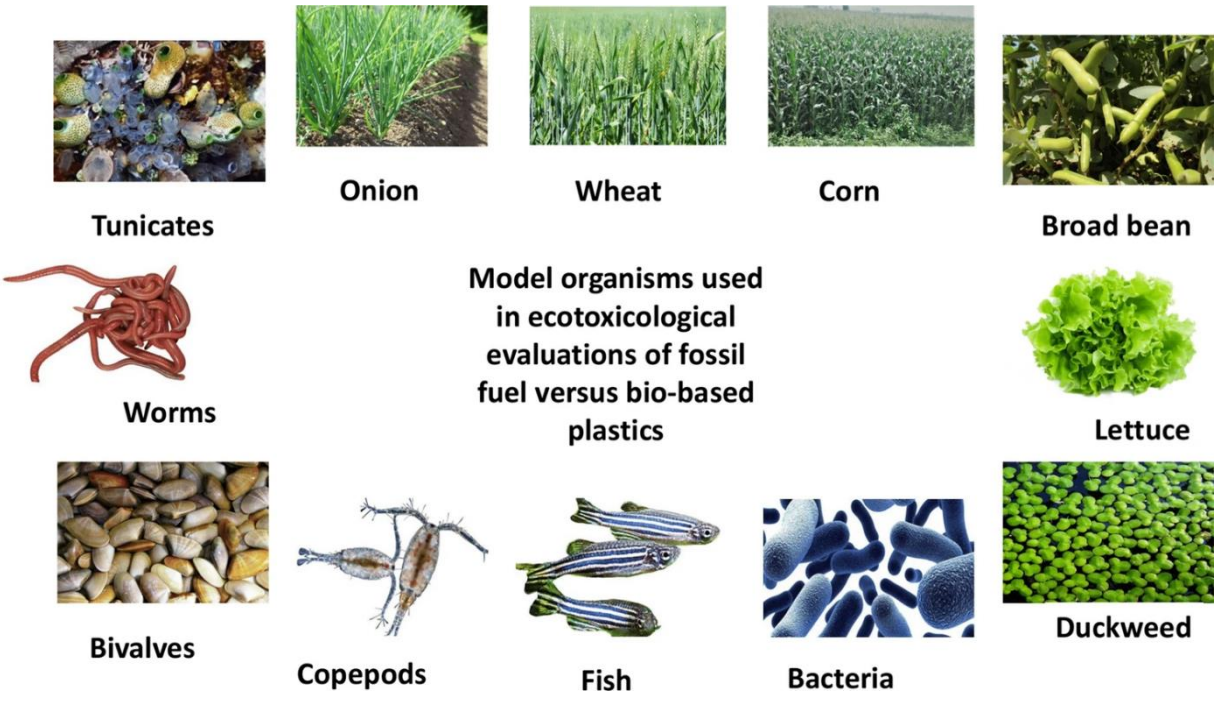

Fig. 3. Microorganisms (bacteria), plants (duckweed, lettuce, broad bean, wheat, green onion, and corn), and animals (bivalves, worms, copepods, tunicates, and fish) that have been used as model organisms in ecotoxicological evaluation of fossil fuel plastics and bioplastics.

In summary, we found that the physicochemical properties of soil, including bulk density, porosity, and carbon/nitrogen ratio, can be negatively affected by the interaction with bioplastics, similar to fossil fuel plastics, which may alter community structures and affect

geophysical processes. Microplastics from both fossil fuel plastics and bioplastics can also affect the abundance of soil microbiota, which can impact soil biochemical activity and biodegradation of bioplastics. Furthermore, the effects of bioplastics on plants and soil fauna can also vary, suggesting that bioplastics are not necessarily more eco-friendly than fossil fuel plastics in this regard, as they can also cause negative impacts on soil, microbiota, plants, and soil fauna.

9. Effects of microplastics on aquatic ecosystems

Aquatic environment is a sink for plastic wastes, introduced by a number of sources ranging from waste-water treatment effluents to household decors (John et al. 2022). Bioplastics, like other plastics, can alter the physical and chemical characteristics of the marine environment. During the degradation process, bioplastics can also release chemicals into the matrices where they end up and can affect the pH, dissolved oxygen, and nutrient levels, and consequently disrupting the delicate balance of the marine ecosystem. For example, Green et al. (2015) investigated the effects of carrier bags made from conventional plastics (high density polyethylene) and biodegradable plastic (manufactured from corn starch) litters on biogeochemical processes on an intertidal shore near Dublin, Ireland. The authors reported negative redox potential and reduction in organic matter, while an increase in ammonium and biogenic silicate of the sediments under both types of plastics. In another example, Balestri et al. (2017) reported that bioplastic, starch-based plastic, altered the oxygen concentration and temperature of marine sediments in a six-month experiment. Any alteration in temperature can influence the degradation rate of plastics under aquatic conditions. In the long term, the impact of both types of plastics can be parallel which may indirectly affect the functioning of aquatic ecosystems.

In fact, the functioning of aquatic ecosystems can be affected by changes in community structures, originating from population and individual level effects of microplastics pollution. Both types of plastics have been shown to negatively affect aquatic microbial communities. For example, Cristina et al. (2022) compared the bioavailability and effects of leachates, from fossil fuel plastics and biobased plastics, on the richness and abundance of bacteria in freshwater sediments. The authors reported an increase in bacterial abundance in the leachates of low-density polyethylene, expanded polystyrene, and polylactic acid. Similarly, Zimmermann et al. (2019) investigated the effects of plastic extracts from eight major polymer types including polylactic acid and polyvinyl chloride, among the others, using microtox assay with the bioluminescent bacterium *Aliivibrio fischeri*. The authors reported that polyurethane, polylactic acid and polyvinyl chloride were similarly effective in their baseline toxicity and cytotoxicity. In another study, Zimmermann et al. (2020) studied the effects of bioplastics extracts, from 43 different products, and compared their toxicity to previously studied fossil fuel plastics extracts by using the bacterium *Aliivibrio fischeri*. The authors reported that same proportion of both samples, bioplastics and fossil fuel plastics, induced toxicity, where baseline toxicity and endocrine activity, based on yeast-based reporter gene assay, were higher for bioplastics and fossil fuel plastics, respectively.

Similarly, both types of plastics have been shown to have negative impacts on aquatic primary producers. For example, Cui et al. (2022) found that both types of microfibers increased the energy requirements for photosynthesis in an acute experiment, whereas growth and photosynthesis were affected in multi-generation experiments using polypropylene and polyethylene terephthalate versus lyocell and viscose. In another example, Yokota and Mehlrose (2020) conducted one week incubation experiment in mesotrophic lake to assess the toxic effects of polylactic acid and polystyrene microplastics on natural phytoplankton assemblages. The authors reported that polylactic acid potentially altered the taxonomic composition in the mesocosm by eliminating cryptophytes and increasing chrysophytes, while polystyrene had no such effects. Green et al. (2016) reported that high doses (2 % of wet sediments weight) of both types of microplastics, polyvinyl chloride and polylactic acid, decreased algal biomass compared to low concentration and control. Thus, alterations in the taxonomic composition of the microenvironment caused by exposure to microplastics suggest that both types of plastics can reduce the primary productivity of aquatic ecosystems.

The toxicity of microplastics towards aquatic biota has been intensively studied, while such studies on bioplastics are scarce. However, a few studies have compared the toxicity of biobased plastics to that of fossil fuel plastics (Figure 3). For example, Straub et al. (2017) found that both types of microplastics negatively affected freshwater amphipods, polymethylmethacrylate significantly reduced assimilation efficiency and net wet weight gain, but overall difference in comparison with polyhydroxy butyrate was small. Both types of plastics could affect physiological activities of the exposed organisms at cellular level. Magara et al. (2019) reported significant decreases in digestive gland catalase activity of blue mussel, *Mytilus edulis*, exposed to polyethylene, and decrease in gills catalase activity and digestive gland superoxide dismutase activity, when exposed to polyhydroxy butyrate. Similarly, Green et al. (2016) reported that polyvinyl chloride microplastics pose stronger effects compared to polylactic acid microplastics on the ecosystem engineer, *Arenicola marina*. Furthermore, it has been reported that oysters, when exposed to high levels (80 mg/L) of polylactic acid, showed 2.6-fold higher respiration rates than in high density polyethylene exposure, though no statistical differences were found between the two polymers in terms of filtration rates and shell growth (Green 2016). Similarly, other studies have reported effects of both types of plastic on aquatic biota (Anderson and Shenkar 2021; Magni et al. 2020), which conveys the idea that the toxicity of bioplastics may not be lower than that of fossil fuel plastics. Thus, exposure to microplastics, irrespective of their type, has the potential to affect individual organisms, which can be translated to population or even ecosystem level effects.

In summary, the results of all these studies reflect that bioplastics waste are not necessarily more ecofriendly than fossil fuel plastics waste. This fact may question the impression that bioplastics have less or no negative impact on the environment. Microplastics originating from bioplastics possess almost all the characteristics of microplastics from fossil fuel plastics before complete mineralization. The size of microplastics produced from bioplastics enables them to physically interact with biota. For example, micro-bioplastics can be ingested by zooplankton and or filter feeders, which will have an impact on various aspects of the exposed organisms, ranging from biomarker content to species abundance and even mortality. The effect of

microplastics on microbiota of organisms, which has been documented recently, should be carefully studied in the future, as they could also have an impact on humans. Furthermore, the application of bioplastics in textile industry could generate as fossil fuel plastics do microfibers during production and consumer use. Therefore, more research is needed to understand the toxicity of bioplastics, including new industrialized ones, to both, aquatic and terrestrial life. It is important to study their environmental impacts upstream and ultimately the risk to human health. Further studies will help to determine the potential impacts of bioplastics on the environment and the efficacy of bioplastics as an alternative to fossil fuel plastics. It is important to continue evaluating and improving the sustainability of bioplastics to reduce the overall impact of plastics on the environment.

10. Conclusion

Although bioplastics have the potential to substitute fossil fuel plastics as an environmentally friendly alternative, their benefits are not universal and depend on various factors. Their renewable character is a clearly an asset. However, bioplastics waste like fossil fuel plastics waste can break down into microplastics and contribute to environmental pollution, which can have potential impacts on both soil and aquatic ecosystems. It is important to assess if bioplastics will significantly increase the number of micro- and nano-plastics in the natural environment. This point is crucial because toxicity should be integrated into the criteria for the industrialization of bioplastics. The continuous development of bioplastics that are biodegradable, compostable and notably home-compostable by citizens, and non-toxic, is important in order to provide a more sustainable alternative to traditional fossil fuel plastics. This has to be done while maintaining their high level of applicative properties, and providing low cost. Plastic recycling, for both fossil fuel and bio-based plastics, is furthermore a circular solution to plastic wastes, notably as it will go with the increase and development of collection systems, thereby reducing the overall extent of plastic pollution.

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Paper IV

The comparative toxicity of biobased, modified biobased, biodegradable, and petrochemical-based microplastics on the brackish water flea *Diaphanosoma celebensis*

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The comparative toxicity of biobased, modified biobased, biodegradable, and petrochemical-based microplastics on the brackish water flea *Diaphanosoma celebensis*

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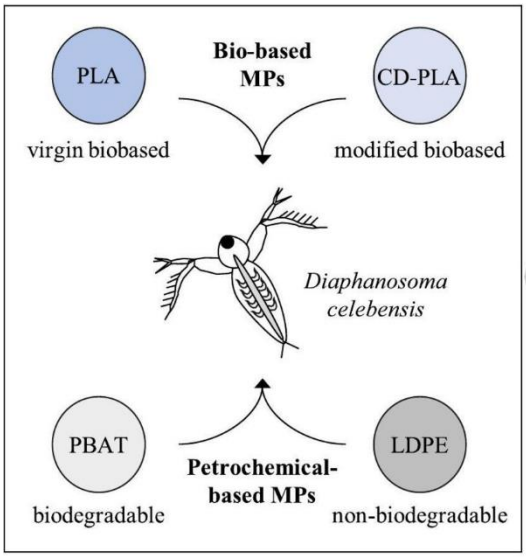
Abstract

The escalating production and improper disposal of petrochemical-based plastics have led to a global pollution issue with microplastics (MPs), which pose a significant ecological threat. Biobased and biodegradable plastics are believed to mitigate plastic pollution. However, their environmental fate and toxicity remain poorly understood. This study compares the *in vivo* effects of different types of MPs, poly(butylene adipate-co-terephthalate) as a biodegradable plastic, polylactic acid (PLA) as a biobased plastic, β-cyclodextrin-grafted PLA as a modified biobased plastic, and low density polyethylene as the reference petrochemical-based plastic, on the key aquatic primary consumer *Diaphanosoma celebensis*. Exposure to MPs resulted in significant reproductive decline, with comparable effects observed irrespective of MP type or concentration. Exposure to MPs induced distinct responses in redox stress, with transcriptional profiling revealing differential gene expression patterns that indicate varied cellular responses to different types of MPs. ATP-binding cassette transporter activity assays demonstrated altered efflux activity, mainly in response to modified biobased and biodegradable MPs. Overall, this study highlights the comparable *in vivo* and *in vitro* effects of biobased, biodegradable, and petrochemical-based MPs on aquatic primary consumers, highlighting their potential ecological implications.

Keywords: PLA, PBAT, LDPE, *Diaphanosoma celebensis*, Redox stress, ABC transporter

Graphical abstract



	PLA	CD-PLA	PBAT	LDPE
Reproduction	↓	↓	↓	↓
Life span	▭	▭	▭	▭
ROS	↓	↓	↓	↓
SOD/CAT activity	↑↑	↓↓	↓↓	▭▭
SOD/CAT expression	↑▭	↑↑	↑↓	↓↓
P-gp/MRP activity	↓▭	↓↓	↓↓	↓↑
P-gp/MRP expression	↓↑	↑↑	↑↑	↓↑

1. Introduction

The use of plastic products is exponentially increasing due to their excellent physico-chemical properties and cost-effectiveness. As a result, the global plastics production increased from approximately 370 million tons in 2018 to over 400 million tons in 2022 and is expected to increase to 500 million tons by 2030. Among these, over 90% of the plastics are synthesized from fossil fuels (PlasticsEurope 2023) and the majority of them are non-biodegradable. After their use, the choice of end-of-life option drives the ecological impact of plastic waste (Ali et al. 2023a). Notably, a significant portion of plastic waste ends up in the natural environment, resulting in approximately 60% of all plastic ever produced accumulating in landfills, the environment and in oceans (Geyer et al. 2017). Plastic waste is prone to environmental factors that contribute to degradation and the generation of microplastics (MPs), with a size less than 5 mm, and even nanoplastics (NPs), with a size less than 1 μm. Complete mineralization is a very slow or an unlikely process, especially in an aquatic environment (Maddison et al. 2023), thereby providing an ideal ground for the formation of MPs.

MPs were included in the list of contaminants of emerging concern in the Clean Water Act of 2021 approved during the 117th Congress (Gatz 2021). The presence of petrochemical MPs has been documented in nearly every type of environment, encompassing freshwater bodies, deep oceans, and even the Arctic and Antarctic regions (Parsaeimehr et al. 2023). Among these petrochemical-based MPs, as an example, polyethylene (PE) is one of the most frequently detected in aquatic environments, exhibiting variations in abundance across different regions (Erni-Cassola et al. 2019). In the aquatic environment, these MPs could be ingested by biota either along with their food (Matijaković Mlinarić et al. 2022) or directly, depending on the feeding mode and the size of the organisms (Sulaiman et al. 2023). A handful of studies have reported on the interaction and/or ingestion of PE MPs and the consequent implications in several aquatic species of phytoplankton (Chae et al. 2019; Baudrimont et al. 2020), zooplankton (Castro et al. 2020; Wang et al. 2022), corals (Jiang et al. 2020; Chen et al. 2022), shrimp (Leads et al. 2019; Gray et al. 2022), mussels (Abidli et al. 2021), and fish (Bobori et al. 2022; Lee et al. 2023), among others.

Considering the detrimental ecological impacts of petrochemical plastics, there has been a growing focus on biobased and biodegradable plastics, collectively referred to as bioplastics. It is an umbrella term that encompasses plastics that are either biobased, biodegradable, or both (EuropeanBioplastics 2024). However, the use of the term “bioplastics” is still debatable, as it overlaps different terminologies, including biobased, compostable, biodegradable, and even polymer blends. This issue has been raised by many authors (Zimmermann et al. 2020a; Goel et al. 2021; Nandakumar et al. 2021), and some have recommended not using this term (Aubin et al. 2020). Therefore, we propose that the terms 'biobased' and 'bioplastics' should be used specifically for polymers originating from renewable sources, irrespective of their degradability. Likewise, the term 'biodegradable' should be reserved for polymers capable of degradation under natural environmental conditions, regardless of their source of origin. We will refer to bioplastics and biodegradable plastics according to these definition in this contribution.

The combined production of biobased and biodegradable plastics grew from 1.8 million tons in 2022 to 2.1 million tons in 2023 and is anticipated to further surge to 7.4 million tons by 2028 (EuropeanBioplastics 2023). Among these, poly(butylene adipate-*co*-terephthalate) (PBAT) is a petrochemical-based biodegradable plastic that contributed 4.6% to the total production of biobased and biodegradable plastics in 2023 (Ali et al. 2024). It finds applications in coatings, agriculture, consumer goods, and packaging. Despite its biodegradable nature, its degradation rate in aquatic environments is very slow (De Monte et al. 2022), and it can generate MPs in both marine and freshwater environments (Wei et al. 2021). However, the environmental fate of these micro-bioplastics remains largely unexplored with only a few studies conducted so far. For instance, PBAT MPs have been reported to affect copepod microbiota diversity (Thery et al. 2023) and reduce the abundance of denitrifying and anammox bacteria in freshwater sediments (Nie et al. 2022). Meanwhile, minimal effects on the growth and survival of fish have also been reported (Xie et al. 2022). However, their toxicological impact, such as effects on key ecological traits as well as molecular responses has not been studied in primary consumers.

Biobased plastics play a key role in reducing carbon dioxide (CO_2) emissions and resilience on fossil fuels resource. However, there is a concern for their contribution to the emerging environmental issue of MP pollution. For example, polylactic acid (PLA) is widely used as biobased plastics, constituting 31% of the total biobased and biodegradable plastics produced in 2023. It finds extensive applications in many sectors ranging from medical to packaging and beyond (Pang et al. 2010). While PLA is biodegradable *in vivo*, its degradation in the natural environment can be challenging, potentially leading to accumulation and the formation of MPs (Ali et al. 2024). The fragmentation into MPs and even NPs have been reported under laboratory conditions (Le Gall et al. 2022; Tong et al. 2022). Concerns about such accumulation and MP formation have been proven by reports of their presence in various water bodies and effluents (Granberg et al. 2019; Kazour et al. 2019). Alarmingly, PLA MPs have been found in herbivorous limpets (*Lottia subrugosa*) collected from the Santos Estuarine System, Brazil (Ribeiro et al. 2024). The toxic effects of PLA MPs on various aquatic organisms, ranging from phytoplankton to fish, have been reported, while initiatives aimed at enhancing their biodegradability through various modification methods have been undertaken (Ali et al. 2023b). For example, cyclodextrin, a cyclic oligosaccharide derived from starch and recognized as safe for contact with foods (Friné et al. 2019), is widely employed for the modification of PLA for various applications. Its advantages, such as possessing different cavity sizes, an external hydrophilic surface, as well as excellent biocompatibility and biodegradability (Gao et al. 2005; Lu et al. 2008), make it an ideal modifier of PLA, especially for food packaging and biomedical applications (Hu et al. 2018; Friné et al. 2019). However, it is currently unknown whether modified versions of PLA, together with virgin PLA, produce the same effects or exhibit differential effects on primary consumers, such as brackish water fleas.

Diaphanosoma celebensis is a key species of water fleas found in the estuarine zone of tropical Asia, where environmental conditions are highly variable (Kim et al. 2024a). As a primary consumer, this species plays a key role in the trophic transfer of nutrients within the aquatic food web (Kim et al. 2021) and is also used as live feed in aquaculture. *D. celebensis*, with its small adult size (>300 µm), high sensitivity to environmental pollutants, short generation cycle (4–5 days), and ability for parthenogenetic reproduction, is an ideal species

for ecotoxicological studies (Yoo et al. 2021a). Therefore, it has been widely utilized in assessing the ecotoxicological impact of various pollutants, including heavy metals (Bae et al. 2018; Yoo et al. 2021a), MPs and NPs (Jeon et al. 2023; Kim et al. 2024a), as well as polycyclic aromatic hydrocarbons (PAHs) (Han and Lee 2021). However, to the best of our knowledge, there is no study on the toxicological effects of biobased and biodegradable MPs on *D. celebensis*. To address these research gaps concerning the toxicological impact of biodegradable, biobased, and modified biobased MPs on primary consumers, the present study increases our understanding of the toxicity of these MPs. We used PBAT as a representative of biodegradable plastics, PLA as a representative of biobased plastics, and modified PLA by grafting PLA onto β-cyclodextrin to form β-cyclodextrin grafted PLA (CD-PLA) as a representative of modified biobased plastics. Low density polyethylene (LDPE) was used as a reference of petrochemical-based plastics. We studied the reproduction, lifespan, first breeding day, oxidative stress, antioxidant activity, cellular efflux activity, and the expression of associated genes in *D. celebensis*. This analysis aimed to deepen our understanding and to facilitate a comparison of how different categories of MPs may influence physiological and biochemical processes in the exposed organisms.

2. Materials and methods

2.1. Modification of PLA

PLA was grafted onto β-cyclodextrin following the methodology of Miao et al. (2011). Here, we briefly restate the main features. β-Cyclodextrin, 4-dimethylaminopyridine (DMAP), and L-Lactide were purchased from Sigma Aldrich and purified by co-evaporation with toluene (three times). DMAP and L-lactide were further sublimated under vacuum at 85°C. β-Cyclodextrin and L-Lactide were then dried several days at room temperature under ultra-high vacuum (5.10^{-6} mbar). For the polymerization run, L-Lactide (4000.02 mg), β-Cyclodextrin (300 mg), and DMAP (1356.26 mg) were weighed into a flask in a glove box, followed by magnetic stirring at 120 °C for one hour. The product was dissolved in dichloromethane, precipitated in cold diethyl ether, and dried under vacuum for 24 hours. The formation of PLA was confirmed by ^{1}H NMR (300.13 MHz, DMSO-d6) (ppm) 5.21 (-OC*H*(CH_3)CO- PLA) ; 4.22 (COOC*H*(CH_3)-OH PLA end group); 1.46 (-OCH(C*H*$_3$)CO- PLA); 1.29 (-COOCH(C*H*$_3$)-OH PLA end group).Traces of DMAP were removed from the product by a series of sonications in diethyl ether (*ca.* 15 min) and removal of the solvent by a syringe until a ratio DMAP/L-lactide molar ratio of 1/127 was achieved. Final yield : 3g (68,8%).

2.2. Synthesis and characterization of MPs

We used PBAT (product code: Ecoflex F blend C 1200, distributed by B-Plast 2000, BASF, Ludwigshafen, Germany), PLA (Ingeo™ Biopolymer 4032 D from Nature Works LLC, United States), CD-PLA, and LDPE (product code: BM50, EXXONMOBIL™, Dallas, United States) to synthesize MPs. These MPs, with diameters ranging from 2 to 10 µm, were prepared through cryogrinding using a cryomill (CRYOMILL, RETSCH ®, Hann, Germany) at the Materials Research and Development Centre, University of Mons, Belgium. Ten consecutive measurements were conducted using distilled water as the solvent to determine the size distribution of MPs by Dynamic Light Scattering (Mastersizer 2000, Malvern Instruments,

Malvern, UK). Scanning Electron Microscopy (SEM) was used to examine the surface morphology of all the MPs. Additionally, three consecutive measurements were performed in distilled water to determine the Zeta potential using an electrophoretic light scattering spectrophotometer (ELS-Z, Otsuka Electronics, Osaka, Japan).

2.3. Animal culture

The brackish water flea, *D. celebensis*, population generously provided by Professor Atsushi Hagiwara from Nagasaki University, Japan, was maintained in transparent 6-liter aquariums at the Marine Molecular and Environmental Bioscience Laboratory, Department of Biological Sciences, Sungkyunkwan University in Suwon, South Korea. The individuals were cultured in artificial seawater (ASW) with a salinity of 15 practical salinity units (psu) at a temperature of 23 °C, following a light-dark cycle of 12/12 hours. The 15 psu ASW was prepared using artificial sea salt (Instant Ocean; Aquarium System, Sarrebourg, France) and sterilized distilled water, followed by filtration with a 0.2-µm filter paper (Whatman). As a food source, green algae (*Tetraselmis suecica*) at approximately 6×10^4 cells/mL were provided once daily. The identification of the experimental species was previously confirmed by Choi et al. (2020) using sequence analysis, via cytochrome oxidase 1 as a barcoding gene, of mitochondrial DNA.

2.4. Life history traits of D. Celebensis

To compare the toxicity of PLA, CD-PLA, PBAT, and LDPE MPs on the life history traits of *D. celebensis*, one neonate (<2 h post-hatching) was exposed to 10 mL of artificial seawater (ASW) with a salinity of 15 psu, treated with MPs and algae. Different concentrations of each type of MPs (0, 0.5, 1, 5, and 10 mg/L) and 6×10^4 cells/mL of *T. suecica* were used in 10 replicates. The exposure took place in 12-well cell culture plates (SPL, Seoul, South Korea) maintained at a constant temperature of 23 °C under a light-dark cycle of 12/12 hours inside an incubator (MIR-554, Sanyo, Osaka, Japan). Throughout this experiment, half of the exposure media was renewed every 24 hours.

Every 24 hours, all replicates of each MPs-treated group were observed under a stereomicroscope (SZX-ILLK200, Olympus, Tokyo, Japan) for breeding, neonate count, and mortality. To determine the first breeding day, the initial day of reproduction was noted for each replicate of each group. For measuring total reproduction, all newborn neonates were counted and removed for the entire life of each individual *D. celebensis*. To calculate the mean lifespan, the times of death for the adults were recorded until all had perished.

2.5. Oxidative stress and enzymatic antioxidant activity

To compare the differences in oxidative stress induced by PLA, CD-PLA, PBAT, and LDPE MPs, we conducted two sets of experiments. Firstly, we exposed adult *D. celebensis* (>300 µm) to 0.5 mg/L of each type of MPs for 24 hours at a constant temperature of 23 °C under a light-dark cycle of 12/12 hours inside an incubator (MIR-554, Sanyo, Osaka, Japan). Secondly, we exposed adult *D. celebensis* (>300 µm) to the same concentration of each type of MPs under the same conditions for 6, 12, and 24 hours to estimate and compare the time-dependent oxidative stress induced by these microplastics. The exposed organisms were filtered and

washed with ASW and phosphate-buffered saline (PBS), homogenized in lysis buffer (40 mM Tris–HCl, 0.1% NP-40, 120 mM NaCl) containing proteinase inhibitor with a Teflon mini pestle, and then centrifuged at 4 °C at 10,000 g for 10 minutes, after which the supernatant was collected. To estimate the intracellular reactive oxygen species (ROS) level, a reaction mixture was prepared with 10 µl of supernatants, 170 µL PBS, and 20 µl of 400 µM 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA). For each sample, a 200 µl mixture was loaded onto a 96-well black plate (SPL, Seoul, South Korea) in triplicate, followed by incubation at 37 °C for 20 min. The ROS level was measured with a spectrophotometer (Thermo™ Varioskan Flash, Thermo Electron, Vantaa, Finland) at 485/520 nm (excitation/emission).

The activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) was estimated using commercially available kits following the given instructions. SOD activity was measured using the Abcam kit (Cambridge, United Kingdom), which is based on the reaction between water-soluble tetrazolium salt (WST) and superoxide anion, forming a water-soluble formazan dye. A 200 µl formazan dye (n=3) was loaded onto a 96-well white plate and measured in a spectrophotometer at 450 nm. CAT activity was measured using the Sigma-Aldrich kit, which is based on a red quinoneimine dye produced by the decomposition of hydrogen peroxide (H_2O_2). A 200 µl sample of the assay was loaded onto a 96-well white plate and measured with a spectrophotometer at 520 nm. All values for ROS, SOD, and CAT were normalized by the quantified concentration of proteins.

2.6. Efflux Activity of ATP-Binding Cassette Transporter

To compare changes in ATP-binding cassette (ABC) transporter activity induced by PLA, CD-PLA, PBAT, and LDPE MPs, we estimated P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) in adult *D. celebensis* (>300 µm) following the method described by Yoo et al. (2024) with modifications. Adults were exposed to 0.5 mg/L of each type of MPs in 200 mL beakers for 24 hours, followed by transfer to clean ASW for a 2-hour dark incubation with 0.5 µM rhodamine B (Sigma-Aldrich) and 1 µM calcein AM (Sigma-Aldrich) as substrates for P-gp and MRP. After 2 hours, all individuals were washed with ASW and exposed again to PLA, CD-PLA, PBAT, and LDPE MPs for 1 hour to maximize the effects on the transporter proteins (Wang et al. 2023). After one-hour, exposed organisms were washed with ASW, and *D. celebensis* from each group were fixed in 4% paraformaldehyde and imaged using a fluorescence microscope (Olympus IX71, Olympus Corporation, Tokyo, Japan). Subsequently, the rest of the population was homogenized with 600 µL PBS, and centrifuged at 10,000 g for 10 minutes at 25 °C. The fluorescence intensity for each dye was determined by transferring 200 µL of supernatants (n=3) into 96-well black plates and measured on a spectrophotometer at 535/590 (excitation/emission; in nm) for rhodamine B and 485/535 (excitation/emission; in nm) for calcein AM. All values were normalized by the quantified concentration of proteins in each sample.

2.7. Quantification of protein concentration

The protein concentration was determined using the Bradford assay (Bradford, 1976) with Bio-Rad's Protein Assay Dye Reagent. A 0.1% Bovine Serum Albumin (BSA) solution was

used to create a standard curve with five different concentrations, and the absorbance was measured at 595 nm using a spectrophotometer (Thermo™ Varioskan Flash, Thermo Electron, Vantaa, Finland).

2.8. mRNA expression of antioxidant and ABC transporter genes

2.8.1. RNA extraction and cDNA synthesis

To compare the differences in mRNA expression of antioxidant and ABC transporter genes induced by PLA, CD-PLA, PBAT, and LDPE MPs, adults of *D. celebensis* (>300 µm) were exposed to 0.5 mg/L of each type of MPs for 24 hours at a constant temperature of 23 °C under a light-dark cycle of 12/12 hours. The total RNA of *D. celebensis* was extracted using TRIzol® Reagent (Invitrogen, Carlsbad, CA, U.S.A.) as per the given instructions, followed by estimation of quality and quantity based on A260/280 ratio using a Nanodrop (QIAxpert, QIAGEN GmbH, Hilden, Germany). The samples with the A260/280 ratios in the range of 1.9–2.0 were used for single-stranded cDNA synthesis. cDNA was synthesized using a commercial superscription kit (SuperScript III reverse transcriptase kit, Invitrogen, Carlsbad, CA, U.S.A.), considering a sample volume of 2 µg of RNA from each, as per the manufacturer's instructions.

2.8.2. Quantitative Real-time polymerase chain reaction (qRT-PCR)

To confirm the differences in mRNA expression of antioxidant genes, SOD (n=4) and CAT (n=2), as well as ABC transporter genes, ABCB (n=6) and ABCC (n=5), in *D. celebensis* induced by various MPs, we used a set of primers (forward and reverse) specific to these genes, as presented in **Tables S1 and S2**, for quantitative real-time polymerase chain reaction (qRT-PCR). The qRT-PCR was conducted using a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, U.S.A.) with the following conditions: initial denaturation at 95 °C for 3 minutes, followed by 35 amplification cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 10 minutes. SYBR Green was employed as the fluorescent dye (Molecular Probes Inc., Eugene, OR, U.S.A.). To validate proper target gene amplification, melting curves were obtained with a temperature profile of 95 °C for 1 minute, 55 °C for 1 minute, and 80 cycles of 55 °C for 10 seconds, with a 0.5 °C increase per cycle. Confirmation of the transcriptional levels was achieved using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.9. Statistical analysis

We used SPSS software (version 22) to conduct Levene's Test for assessing homogeneity of variance. It is important to note that this test is needed to satisfy the condition required for applying parametric ANOVA and related tests. Since this condition was satisfied in all experiments, we applied one-way ANOVA and an independent sample t-test ($p < 0.05$) to determine differences between the control group and groups exposed to different types of MPs. Tukey's test was then applied for post hoc analysis to identify statistically distinct groups. Statistical significance was set at $P < 0.05$. The results are presented as mean values with standard deviations.

3. Results

3.1. MPs characterization

The SEM images of PLA, CD-PLA, PBAT, and LDPE MPs are shown in **Fig. 1**. All types of MPs exhibit atypical and irregular shapes, as well as rough surfaces with fractures and cracks, which are similar to the common morphology of MPs found in the natural environment. Next, to access the surface morphology changes in ASW, the zeta potential values were determined for all four types of MPs in ASW. The zeta potential values of PLA, CD-PLA, PBAT, and LDPE MPs in PSU 15 water are depicted in **Fig. S1**. Notably, all MPs demonstrated a negative charge, with PLA exhibiting the least negative zeta potential, followed by CD-PLA, PBAT, and LDPE, indicating a potential difference in surface charge characteristics. This difference could represent one of the potential factors contributing to differences in toxicity that different MPs may pose within aquatic ecosystems.

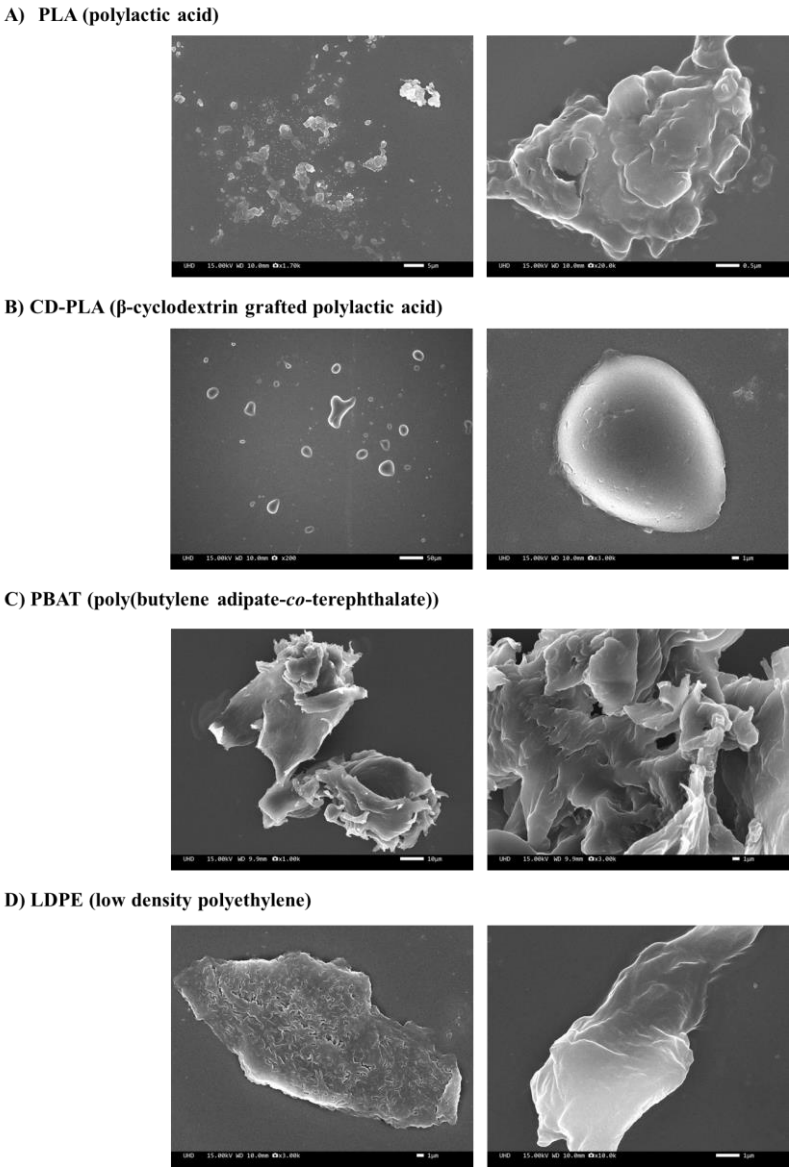


Fig. 1. Scanning electron microscopy image of A) polylactic acid (PLA), B) β-cyclodextrin grafted polylactic acid (CD-PLA), C) poly(butylene adipate-*co*-terephthalate) (PBAT), and D) low density polyethylene (LDPE) MPs.

3.2. In vivo effects comparison of different types and concentrations of MPs

In our study, all concentrations (0.5, 1, 5, and 10 mg/L) of PLA, CD-PLA, PBAT, and LDPE MPs exhibited a significant reduction ($P < 0.05$) in the reproduction of *D. celebensis* compared to the control, as illustrated in **Fig. 2A**. However, no significant differences ($P < 0.05$) in the reproduction of *D. celebensis* were observed among the various concentrations of MPs.

The mean lifespan of *D. celebensis*, when exposed to different concentrations (0.5, 1, 5, and 10 mg/L) of PLA, CD-PLA, PBAT, and LDPE MPs, decreased after exposure to each concentration of MPs, with significance ($P < 0.05$) observed only in the case of 10 mg/L LDPE MPs **(Fig. 2B)**. Similarly, the first breeding (spawning) did not exhibit significant changes ($P < 0.05$) with exposure to any of the concentrations and type of MPs, as shown in **Fig. 2C**. Thus, our findings suggest that biobased, modified biobased, biodegradable, and petrochemical-based MPs may demonstrate comparable *in vivo* effects, highlighting the potential for similar ecological consequences irrespective of their chemical nature.

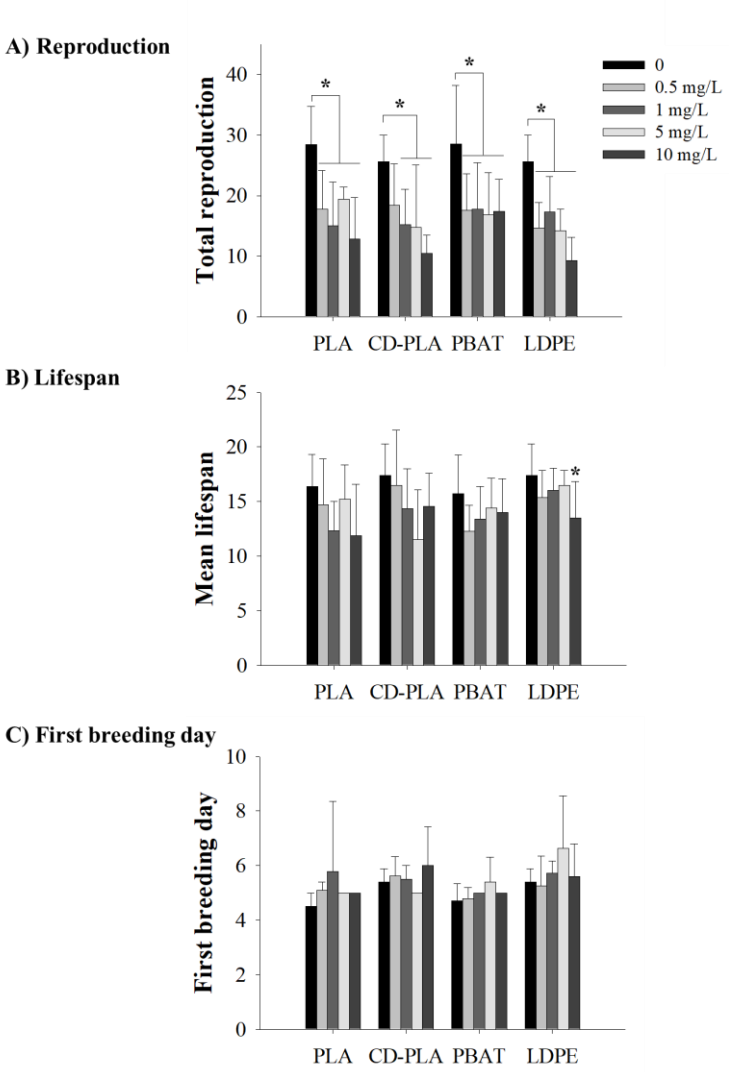


Fig. 2. *In vivo* effects of different concentrations of polylactic acid (PLA), β-cyclodextrin grafted polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics on A) reproduction, B) lifespan, and C) first

breeding day of brackish water flea *Diaphanosoma celebensis*. Asterisk indicate a significant difference ($P < 0.05$) between experimental groups and control.

3.3. Oxidative stress, antioxidant response, and gene expression comparison

ROS levels significantly ($P < 0.05$) decreased in all exposed groups, with the lowest level observed in the LDPE MP exposed group compared to the control **(Fig. 3A)**. In antioxidant enzymatic activity, SOD and CAT exhibited distinct patterns, with both antioxidant enzymes significantly ($P < 0.05$) increased in groups exposed to either PLA or LDPE MPs, while they decreased in groups exposed to CD-PLA or PBAT MPs, with the PBAT MPs-exposed groups showing the lowest activity **(Figs. 3B and 3C)**. The distinct patterns in SOD and CAT activities highlight the varied antioxidant responses that could be elicited by different types of MPs in aquatic organisms.

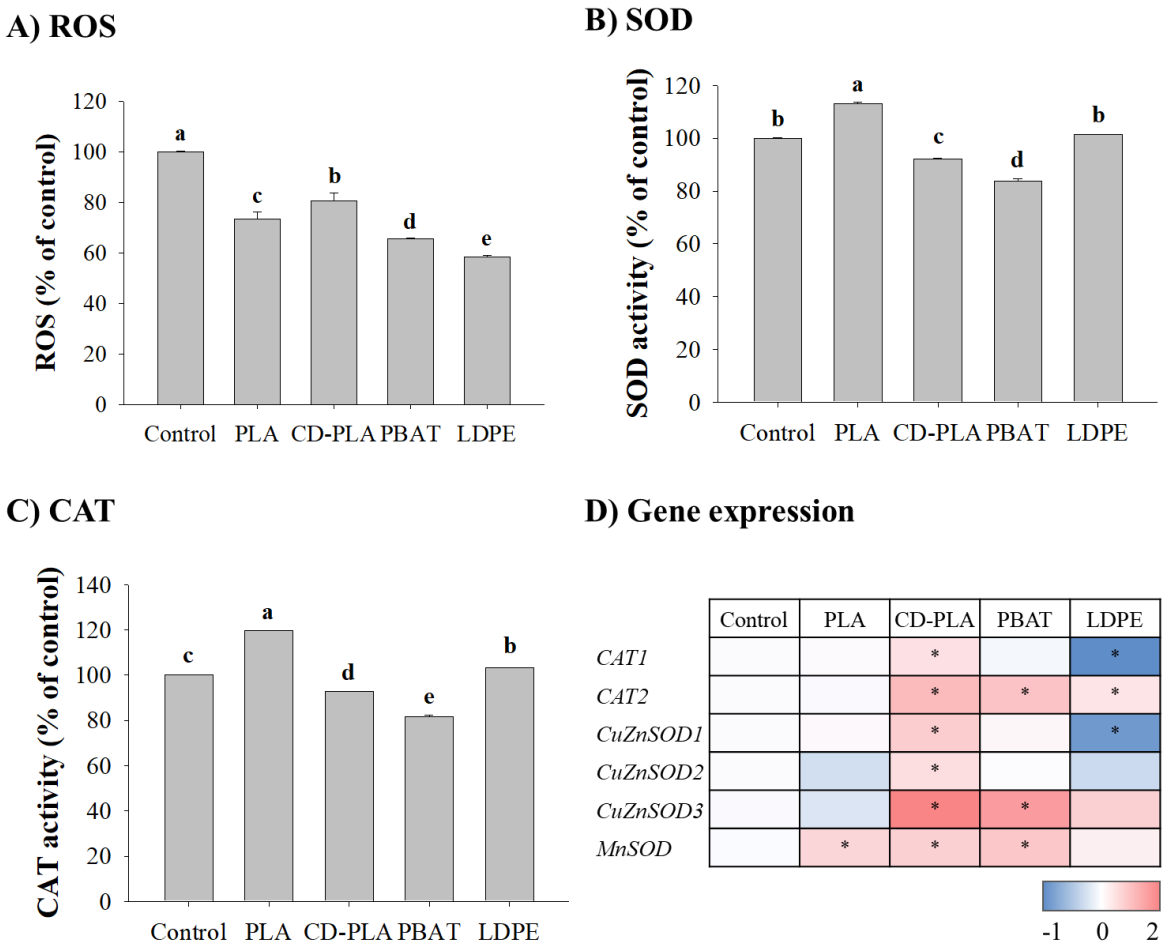


Fig. 3. Effects of polylactic acid (PLA), β-cyclodextrin graft polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics on A) intracellular reactive oxygen species (ROS) levels, B) superoxide dismutase (SOD) activity, C) catalase (CAT) activity, and D) the transcriptional level of antioxidant genes in brackish water flea *Diaphanosoma celebensis*. The bars represent the mean ± standard deviation of experiment results, and different letters above each column indicate a significant difference between groups, as examined using one-way ANOVA with

Tukey's test ($P < 0.05$; n = 3). Asterisk indicate a significant difference ($P < 0.05$) between experimental groups and control. Detailed expression values are noted in **Table S3**.

The mRNA expression profiles of genes related to antioxidant, *SOD* and *CAT*, exhibited distinct patterns in response to exposure to PLA, CD-PLA, PBAT, and LDPE MPs **(Fig. 3D)**. Notably, the expression of all CAT and SOD genes was significantly upregulated ($P < 0.05$) in the group exposed to CD-PLA. The group exposed to PBAT MPs demonstrated upregulation in only three genes, *CAT2*, *CuZnSOD3*, and *MnSOD*, similar to the expression patterns observed in the CD-PLA group. The observed similarities in gene expression profiles between CD-PLA and PBAT, despite their distinct chemical compositions, suggest that the biodegradability of polymers may influence their behavior within the gut environment. Interestingly, exposure to PLA MPs resulted in the upregulation of a single gene, *MnSOD*, suggesting a distinct cellular response compared to CD-PLA and PBAT. In contrast, exposure to LDPE MPs led to the downregulation of two genes, *CAT1* and *CuZnSOD1*, and the upregulation of one gene, *CAT2*. The variations in gene expression suggest that the toxicity induced by different MPs may be comparable, but the cellular response varies significantly.

3.4. ABC transporter activity and transcriptional response comparison

The fluorescence intensity of rhodamine B significantly increased ($P < 0.05$) in all the groups exposed to different types of MPs. PLA and CD-PLA MPs exhibited the highest fluorescence intensity of rhodamine B, followed by PBAT and LDPE **(Fig. 4A)**. In contrast, a significant ($P < 0.05$) increase in fluorescence intensity of calcein AM was observed in the groups exposed to CD-PLA and PBAT MPs, while a significant ($P < 0.05$) decrease was observed in the group exposed to LDPE MPs. The intensity of the group exposed to PLA MPs remained unchanged compared to the control **(Fig. 4B)**. The differential patterns of fluorescence intensity observed for rhodamine B and calcein AM across different exposed groups highlight the complexity of cellular responses to different types of MPs, suggesting potential variations in their interactions with biological systems.

The transcriptional modulation of MXR-related genes in the ABCB subfamily showed significant variation in response to different types of MPs **(Fig. 5)**. In PLA-exposed groups, two genes, *ABCB1-1* and *ABCB1-2*, were downregulated, while only one gene, *ABCB7*, was upregulated. No downregulation of the ABCB subfamily genes was found in the groups exposed to CD-PLA and PBAT MPs, instead, five genes, *ABCB1-1*, *ABCB1-2*, *ABCB7*, *ABCB8*, and *ABCB10*, were upregulated. The similarity in transcriptional response of CD-PLA and PBAT MPs could indeed be related to their shared characteristic of enhanced biodegradability compared to PLA MPs. This shared property might result in similar metabolic pathways within the gastrointestinal tract, leading to convergent molecular responses in the exposed organisms. Furthermore, the LDPE-exposed group showed the down and up regulation of *ABCB1-1* and *ABCB1-2*, respectively. Furthermore, in the subfamily *ABCC*, the transcriptional response of P-gp activity was consistent, with four genes, *ABCC1-1*, *ABCC1-2*, *ABCC4-1*, and *ABCC4-2*, being upregulated in all the groups exposed to MPs, except for ABCC4-1, which showed no change compared to the control in the LDPE-exposed group. Only one gene, *ABCC9*, of the subfamily ABCC, remained unchanged across all the MP-exposed groups. Thus, while transcriptional responses to different types of MPs varied, the shared

upregulation patterns in ABCB and ABCC subfamily genes suggest comparable molecular impacts on the exposed organisms.

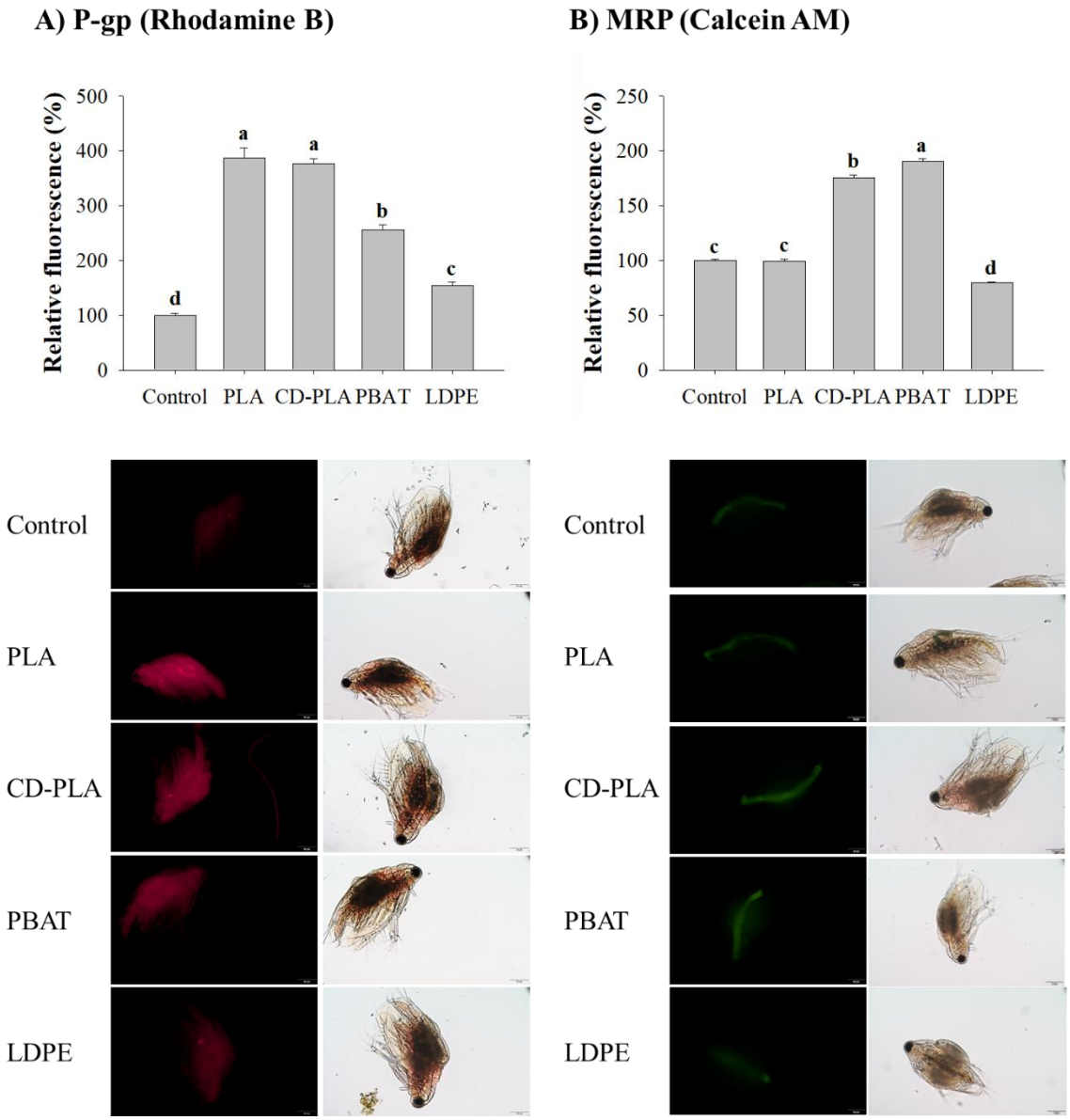


Fig. 4. The activity of multixenobiotic resistance ATP-binding cassette (ABC) proteins after exposure to polylactic acid (PLA), β-cyclodextrin graft polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics. A) P-glycoprotein (P-gp) and B) multidrug resistance–associated protein (MRP). Increased fluorescence in a marine water flea implies decreased activity of ABC proteins, and decreased fluorescence indicates the opposite. The bar represents the relative fluorescence of accumulated substrates, and letters indicate a significant difference ($P < 0.05$) between experimental groups.

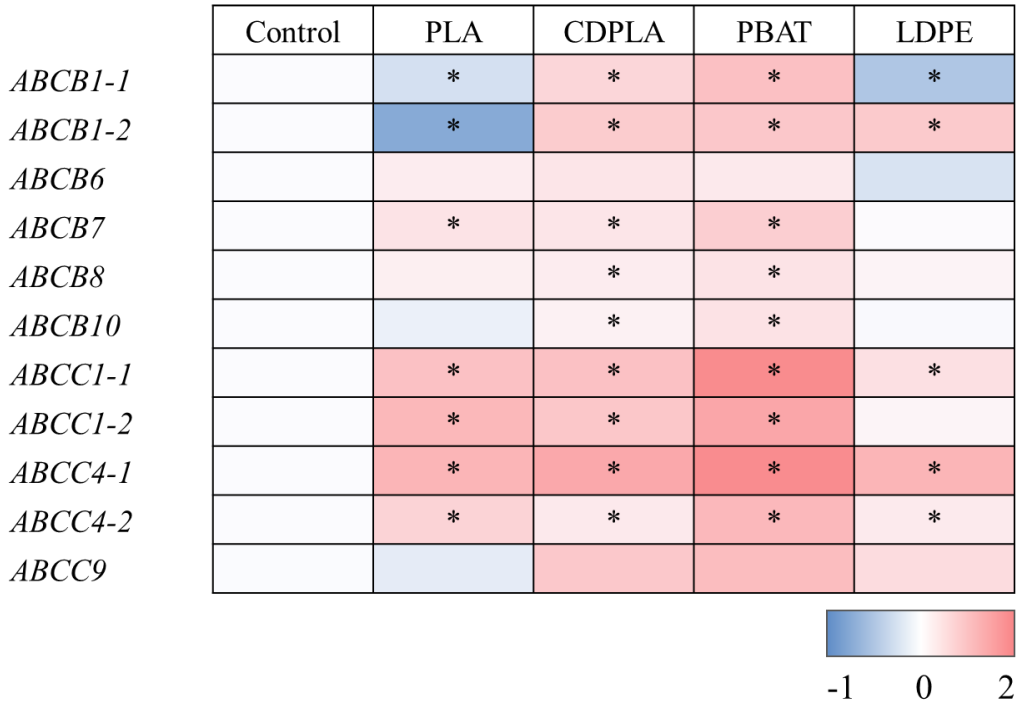


Fig. 5. The activity of ATP-binding cassette (ABC) proteins related gene expression levels in brackish water flea *Diaphanosoma celebensis* after exposed to polylactic acid (PLA), β-cyclodextrin grafted polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) MPs. Asterisk indicate a significant difference ($P < 0.05$) between experimental groups and control. Detailed expression values are noted in **Table S3**.

4. Discussion

From a toxicological perspective, biodegradable plastics, both biobased and petroleum-based, have been introduced to address issues related to the persistence of plastics, their conversion into MPs, and the resulting toxicity to living organisms. Biobased polyesters, such as PLA, are commonly employed in biomedical applications, single-use items, as well as textiles. However, due to its limited mechanical properties and low biodegradability, PLA is often modified by various means to enhance both its mechanical properties and biodegradability (Ali et al. 2023a). Biodegradable polyester, such as PBAT, is utilized in biomedical, food packaging, and agriculture applications (Ali et al. 2024). PBAT degrades in a natural environment but may produce MPs. Recent studies on the biodegradation of biobased and biodegradable polymers show that these polymers could generate MPs in the natural environment (Wei et al. 2021; Le Gall et al. 2022; Tong et al. 2022). The potential effects of MPs originating from these polyesters on the environment and organisms may be detrimental. For the hazards assessment of MPs, it is crucial to consider the diverse array of polymers, including the newly emerging ones entering the environment. In this scenario, this study compares and evaluates the toxicity of MPs synthesized from four different categories of plastics, i.e., biobased, modified biobased, biodegradable, and petrochemical-based plastics.

To evaluate and compare the toxicity of different types of MPs on the brackish water flea, *D. celebensis*, we synthesized MPs by grinding the pellets of the respective polymer. The size range distribution was similar to those observed in the natural environment (Mai et al. 2018), as well as those that could be generated from biobased and biodegradable plastics during the degradation process (Lambert and Wagner, 2016; Tong et al. 2022). Likewise, the shape and surface morphology of the MPs used in this study were comparable to the MPs reported in the natural environment (Nayeri et al. 2023). Many studies on *D. celebensis* have used spherical-shaped MPs (Jeon et al. 2023; Kim et al. 2024a; Yoo et al. 2024). However, to the best of our knowledge, no studies have evaluated the toxicity of irregularly shaped MPs on this species, beside the fact that MP shape has been reported to affect their ecotoxicity (Rozman et al. 2023). Moreover, for *in vitro* toxicity comparison, a lower exposure concentration of 0.5 mg/L was selected compared to the environmental MPs concentration of 2.5 mg/L (Cózar et al. 2014; Pencik et al. 2023) due to bioplastics production being less than 1 percent compared to petrochemical plastics. These concentrations align with those employed for assessing PBAT and LDPE MPs (Thery et al. 2023) but are lower than the concentration used for PLA MPs toxicity assessment (Malafaia et al. 2021; Savva et al. 2023; An et al. 2024). Thus, the characterization of different types of MPs used in this study shows that the morphology, size, and concentration of the selected MPs bringing the current experiment closer to realistic environmental scenario.

In the present study, exposure to biobased, modified biobased, biodegradable, and petrochemical-based MPs resulted in similar *in vivo* effects on *D. celebensis*, and in general these effects were independent of the type and concentration of MPs studied. No significant effects on first breeding day and mean lifespan were induced by any type of MPs at concentrations of 0.5, 1, 5, and 10 mg/L, except for LDPE, which significantly reduced the lifespan at the higher concentration of 10 mg/L. Similar to our results, comparative studies on the toxicity comparison of different types of MPs reported no negative effects on the survival of exposed organisms. PLA and polyvinylidene difluoride (PVDF) have been reported to have no effects on the survival of marine zooplankton, *Artemia franciscana* and the cnidarian *Aurelia* sp. (Di Giannantonio et al. 2022). PLA, high density polyethylene (HDPE), and polyvinyl chloride (PVC) at a concentration range of 0.2 to 2% weight of sediments had no effects on the survival of *Arenicola marina* (Green et al. 2016). PE and polyethylene terephthalate (PET) MPs, at a concentration of 100 mg/L, demonstrated no impact on the survival of *Daphnia magna* and *A. franciscana* (Kokalj et al. 2018). Degraded PLA induced severe reproductive toxicity, delayed hatching, and diminished zebrafish offspring survival compared to undegraded PLA at a 5 mg/L concentration (Zhang et al. 2024). In contrast, other comparative studies have reported negative and differential effects of various MPs types on the survival and development of the exposed organisms. A blend of PLA with polyhydroxyalkanoate (PHA) has been reported to have no effects at a concentration of 3.3 to 33.3% on the development of sea urchin embryos, while PVC and polyhydroxybutyrate (PHB) did show effects (Uribe-Echeverría and Beiras, 2022). PLA MPs pose significantly higher acute toxicity to *D. magna* compared to PE MPs at a concentration of 5, 7.5, and 10 mg/L (Luangrath et al. 2024). Thus, due to the variability in exposure concentrations in previous studies, it is impossible to elucidate the reasons for differences in toxicity outcomes among various MPs. Nevertheless, in our study,

D. celebensis were exposed to a variety of MPs, and no effects were found on lifespan or the first breeding day.

We observed a decrease in the total reproduction of groups exposed to PLA, CD-PLA, PBAT, and LDPE MPs compared to the control; however, there were no significant differences among the groups exposed to different types of MPs at any concentrations of 0.5, 1, 5, and 10 mg/L. To date, a few studies have reported on the adverse effects of PLA, modified PLA and LDPE MPs on the reproduction of different aquatic organisms (Chisada et al. 2021; Uribe-Echeverría and Beiras, 2022; Kim et al. 2024a; Zhang et al. 2024), although, to the best of our knowledge there are no such studies on PBAT MPs. Similar to our study, exposure to PLA and PET MPs induced comparable negative effects on the reproduction of *Microcosmus exasperates* (Anderson and Shenkar 2021). A reduction in the total reproduction of *D. magna* exposed to PLA and PET MPs, irrespective of whether they were biobased or petrochemical-based, was reported (An et al. 2024). The authors also reported that 1 and 5 mg/L of PLA MPs significantly reduced the total number of offspring per female, with no difference between the two concentrations. The analogous impacts of these different MPs suggest that their toxicity is more likely to be mechanical than chemical, consistent with previous studies (Jemec et al. 2016; Zimmermann et al. 2020b) highlighting that MP toxicity is predominantly linked to polymer particles or fibers rather than specific chemicals. Furthermore, exposure to MPs has been reported to significantly reduce food ingestion by zooplankton due to the blockage of the gut, leading to energy depletion (Malinowski et al. 2023). As reproduction is an energy-consuming process, and reproductive success has been reported to be dependent on food quantity and quality (Bi and Sommer 2020; Traboni et al. 2020), the observed negative effects of MPs in the *in vivo* endpoints, only in reproductive outputs, could suggest a possible strategy of *D. celebensis* to reduce energy consumption in order to maintenance other life history traits.

In zooplanktons, the ingestion of MPs may lead to gastrointestinal tract obstruction (Jemec et al. 2016). The ingestion of MPs has also been reported to result in energy depletion (Watts et al. 2015) and change in the redox environment (Martyniuk et al. 2024), consequently causing disruptions in the homeostasis and impaired cellular function of the exposed organisms. The malfunctioning related to the dramatic change in the redox environment originates from both high levels of ROS and excessive low levels of ROS (Zorov et al. 2014). Cellular redox balance is crucial in signaling pathways, which are essential for life history traits; thus, any alteration in ROS levels can lead to cytotoxicity as well as have an impact on physiological functions (Schieber and Chandel 2014; Sies and Jones 2020). Therefore, the estimation of ROS levels is a valuable indicator for assessing stress conditions, as ROS such as H_2O_2 and O_2 fluctuate from their normal levels under stress conditions. However, determining the exact mechanism resulting in fluctuations in ROS levels after exposure to MPs is challenging (Alaraby et al. 2024). It is generally reported that MPs, irrespective of their origin whether biobased or petrochemical-based, may leads to alterations in ROS levels of the exposed organisms. Kim et al. (2024b) found elevated ROS levels in *Microcystis aeruginosa* exposed to PLA, polycaprolactone (PCL), and polystyrene (PS) MPs at concentrations of 0.1, 1, and 5 mg/L for 4 days, but after 12 days, the ROS levels in the PCL and PS exposed groups were similar to the control group. On the other hand, Liu et al. (2022) found that while exposure to PBAT MPs increased ROS levels in *Arabidopsis thaliana* after 14 days, no significant change was observed with LDPE MPs; however, after 28 days, both PBAT and LDPE MPs exposure led to

significantly higher ROS levels compared to the control. Nevertheless, exposure of *D. magna* to PE and PLA MPs at a concentration of 1 mg/L resulted in an increase in ROS levels, with no significant difference observed between the two types of MPs (Luangrath et al. 2024). Our findings are consistent with prior studies, suggesting that MPs can induce stress in exposed organisms regardless of their origin, whether biobased, biodegradable, or petrochemical-based. However, our results revealed a distinct stress response in *D. celebensis*, where the level of ROS significantly decreased regardless of the type of MPs when compared to the control group. This reduction could be either attributed to a redox imbalance or an overcompensated antioxidant response, which, in turn, could induce reductive stress. Our results support the findings of previous studies, indicating a decrease in ROS levels in rotifers and fish (Yamashita et al. 2013; Han et al. 2023; Jeong et al. 2024), as well as reductive stress in mollusks when exposed to different pollutants (Martyniuk et al. 2024), which could have severe toxic effects.

The antioxidant enzyme system, such as SOD and CAT, plays a key role in regulating the equilibrium between ROS generation and scavenging. This system is tuned by redox stress and serves as a significant biomarker for toxicity of different pollutants. Several studies have reported that exposure to different types of MPs, whether biobased, biodegradable, or petrochemical-based, could modulate the activity of antioxidant enzymes in the organisms. For instance, an increase in SOD and CAT activity has been observed in *Physalaemus cuvieri* tadpoles when exposed to PLA MPs (Malafaia et al. 2021). *D. magna* exhibited an increase in SOD and CAT activities when exposed to PLA MPs compared to PE MPs (Luangrath et al. 2024). *M. aeruginosa* exhibited elevated SOD and CAT activities when exposed to PLA MPs compared to PS and PCL MPs (Kim et al. 2024b). Conversely, Yoo et al. (2021b) found a reduction in SOD activity in *D. celebensis* exposed to PS MPs of different sizes (0.05 and 0.5 μm) at a concentration of 10 mg/L. Similarly, *A. thaliana* demonstrated increased SOD activity when exposed to LDPE or PBAT MPs, with no significant difference observed between the groups exposed to either type of MPs (Liu et al. 2022). Magara et al. (2019) reported differential effects of PE and PHB MPs at a concentration of 1000 MPs/mL on the antioxidant response of *M. edulis*, wherein the activity levels of CAT in the gills and SOD in the digestive glands were reduced in the presence of PHB, while increased in PE MPs exposed groups. In line with these results, in the present study, the activities of SOD and CAT were found to increase in the group exposed to PLA MPs, suggesting a potential adaptive response to MPs-induced stress. Conversely, inhibitory effects on both SOD and CAT were observed in the group exposed to CD-PLA and PBAT MPs, indicating severe damage to the antioxidant system. SOD serves as a first line defense molecule; its reduction may serve as a signal for redox stress, ultimately resulting in the disruption of cellular protection ability. The observed variation in modulations of antioxidant activities in *D. celebensis* exposed to different types of MPs suggests that while the specific mechanisms underlying the toxicity of different types of MPs may vary, all types of MPs exert adverse physiological and biochemical effects. Moreover, energy is vital for both the antioxidant system and activities like reproduction, and ingestion of MPs can cause energy deficits (Watts et al. 2015; Malinowski et al. 2023), prompting organisms to tradeoff between self-maintenance and reproduction. Thus, it is possible that exposure to MPs, irrespective of their type, alters redox balance, leading to changes in gene expression in *D. celebensis*, which could result in a decrease in reproductive capacity.

In general, organisms possess a variety of defense mechanisms to cope with stress induced by exposure to different environmental pollutants. These defense mechanisms can involve a range of strategies, such as activating detoxification pathways (Jeon et al. 2023), as well as enhancing antioxidative defenses (Jeong et al. 2024), driven by the expression of antioxidant genes. In the present study, we observed that the expression patterns of both SOD and CAT genes were influenced by the type of MP **(Fig. 3D)**. The CAT and SOD enzyme activities showed a complex trend when compared to their associated gene expression. In the PLA MPs-exposed group, the CAT and SOD activities increased, but the expression of associated genes remained unchanged when compared to the control. In the case of CD-PLA MP, the CAT and SOD activities decreased, with all genes showing upregulation. However, when exposed to LDPE MPs, some genes were upregulated while others were downregulated. Our results are in line with the findings of Wang et al. (2023), where exposure of *Brachionus plicatilis* to a combination of nanoplastics and heavy metals led to a decrease in the expression of CAT genes while an increase in CAT protein activity was observed. Nevertheless, changes in the expression levels of antioxidant genes have been used as biomarkers to assess the toxicity of different aspects of MPs exposure, including size, dose, and type, on aquatic organisms. Yoo et al. (2021b) found that exposure of *D. celebensis* to PS MPs can lead to alterations in the expression of antioxidant genes depending on the size of the MPs, indicating a strong association between particle size and toxicity. The SOD gene exhibited downregulation in *Eriocheir sinensis* when exposed to a high concentration (40,000 μg/L) of PS MPs, whereas exposure to lower concentrations (40, 400, and 4000 μg/L) led to an upregulation of SOD gene expression (Yu et al. 2018). Viel et al. (2023) observed downregulation of stress and detoxification genes in *Paracentrotus lividus* embryos exposed to biobased PLA, PHB, and PCL MPs, with CAT affected solely by PCL MPs. An et al. (2024) found that exposure of *D. magna* to PLA and PET MPs at a concentration of 5 mg/L had differential effects on SOD gene expression, with PLA significantly downregulating SOD gene expression while PET showed no significant differences compared to the control. In line with these results, our findings support these reports, demonstrating that the expression patterns of SOD and CAT genes are differentially influenced by the type of MPs which could lead to oxidative damage as well as membrane dysfunction.

The cell membrane regulates the entry and exit of substances, including toxic compounds, to maintain cellular homeostasis and protect the cell from damage. The direct interaction between plastic particle and membranes may induce membrane damage as well as alterations in the transmembrane efflux pumps, such as ABC proteins (Jeong et al. 2018), which are pivotal in detoxifying environmental contaminants. Within the family of ABC proteins, ABCB have a role in multi-xenobiotic resistance (MXR) as P-glycoprotein (P-gp), while ABCC have a role as multidrug-resistance protein (MRP) (Sturm et al. 2009). Both P-gps and MRPs, which play roles in MXR, are conserved among various species of aquatic invertebrates, including *Daphnia pulex* (Sturm et al. 2009), *B. plicatilis* (Kang et al. 2021), and *D. celebensis* (Yoo et al. 2024), among others. Several studies have reported alterations in the MXR activities of P-gps and MRPs in various aquatic organisms after exposure to environmental pollutants. For instance, when *Mytilus galloprovincialis* were exposed to PS MPs, a significant reduction in MXR activity and down-regulation of ABCB and ABCC transcripts encoding the P-gps and MRPs were reported (Franzellitti et al. 2019). Disruption in the ABC efflux capacity of *Oryzias latipes*

was observed when exposed to PS nanoparticles (NPs) at concentrations higher than 0.01 µg/mL (Yu et al. 2022). Likewise, a significant reduction in MXR activity and up and downregulation of ABCB and ABCC transcripts encoding the P-gps and MRPs were reported in *D. celebensis* after being co-exposed to PS beads and methylmercury (Yoo et al. 2024). Similarly, we observed higher intensity fluorescence for P-gp and MRP substrates in all groups exposed to either type of MPs compared to the control group, except for the MRP substrate in the PLA and LDPE MPs exposed groups. The intensity of fluorescence varied among the MPs exposed groups, with significantly higher fluorescence for P-gp substrate found in the PLA and CD-PLA exposed groups, whereas significantly higher fluorescence for the MRP substrate was observed in the PBAT exposed groups. Thus, the degree of inhibition of P-gp and MRP activity depend on the type of MPs which is supported by our finding of suppressed P-gp and MRP protein expression. The transcriptional expression in the MPs exposed group well matched to the activities of P-gp and MRP, where PLA, CDPLA, and PBAT MPs exposed groups tended to increase overall, whereas in the LDPE MPs exposed groups, transcriptional expression tended to decrease. Our results are consistent with those reported by Liu et al. (2016), wherein the activity patterns of P-gp and MRP corresponded well with the expression of ABCB and ABCC genes in *D. magna* exposed to chromium and PS MPs. We propose that different types of MPs can induce cell damage by inhibiting P-gp and MRP activity, with the severity of the damage depending on the type of MPs, ultimately leading to cellular toxicity and potential hazards to exposed biota. Therefore, our results support the notion that the common belief of less to no negative environmental impact of bioplastics may not be entirely accurate. Thus, our study on comparing the effects of MPs of different origin shows similar effects which emphasizes the need to increase public awareness regarding the appropriate waste management of bioplastic products.

5. Conclusions

In conclusion, our study provides valuable insights into the toxicological implications of MPs derived from various types of plastics on aquatic organisms, focusing on the brackish water flea, *D. celebensis*. We found that despite the diverse origins of MPs, their effects on *D. celebensis* were largely similar across different types and concentrations. The underlying mechanisms found were alterations in ROS levels and antioxidant enzyme activities in *D. celebensis* exposed to MPs. Although variations in gene expression patterns and antioxidant activities were observed among different MP types, all MPs induced adverse physiological and biochemical effects, suggesting a disruption of cellular homeostasis and oxidative stress. Furthermore, our study highlighted the influence of MPs on the expression and activity of MXR, such as P-gp and MRP, which play crucial roles in detoxification processes. The differential modulation of these proteins by various MP types underscores the complexity of MP toxicity and its potential implications for cellular function and organismal health. Overall, our findings contribute to a deeper understanding of the environmental impacts of MPs and highlight the comparable toxicity of biodegradable, biobased, modified biobased, and petrochemical-based plastics, urging comprehensive assessments to understand their environmental implications.

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Supplementary material

Table S1. Information on the primers targeting the antioxidant genes, superoxide dismutase (SOD), and catalase (CAT), of *Diaphanosoma celebensis* used in real-time polymerase chain reaction.

Subfamily	Gene	Real-Time RT-PCR primer (5' → 3')	Amplicon size (bp)
SOD	*DC-CuZnSOD1*	F: CGTCATGTGGGAGATCTTG R: CCACCATAGTTCTTCCGATT	121
	DC-CuZnSOD2	F: GGTATGCTCAATTTGACCCA R: GCCTTTCGTGTGGATGT	120
	DC-CuZnSOD3	F: CGACGTTATTTCCTTGTGTC R: GAATTCCCGTGGATAAGGC	120
	DC-MnSOD	F: ACATCATGCAGCTTACGTT R: CCACCATTAAAACGAAGAGC	120
CAT	*DC-CAT1*	F: GTATTCCTGATGGCTACCG R: TACGAATGCCTTGATCTGAC	120
	DC-CAT2	F: CAATCCTGTTCCCAAGTTTC R: AGAGTAGTTGGTGCATAGTC	120
Reference	*DC-ELFA*	F: CAACGTTAAAAACGTTTCAGTC R: CCTGGATGGTTCAATACAATTAC	119

Table S2. Information on the primers of the ATP-binding cassette (ABC) transporter genes of *Diaphanosoma celebensis* used in real-time polymerase chain reaction (qPCR).

Subfamily	Gene	Real-Time RT-PCR primer (*5' → 3'*)	Amplicon size (bp)
ABC transporter family B	*Dc_ABCB1-1*	F: ACAGGACCATAGCCGATAAC	111
		R: GAGGCAACGACTGGATAAAGC	
	Dc_ABCB1-2	F: AGGTCGCATCTGAGTATTGTG	160
		R: GTGGTAGGGACTGGATAAACG	
	Dc_ABCB6	F: TGGTTGGTCCGTCTGGTG	127
		R: CGAACTGAGGCTTGCTTTAC	
	Dc_ABCB7	F: TGCAAGTTATTCGCTCCAT	120
		R: GGACACCGACATTGAGTAAT	
	Dc_ABCB8	F: TGTCTCAAATGTCGCTCCTC	92
		R: AATCGTCGGCTCCAAGTTAC	
	Dc_ABCB10	F: CCCCGAAGGATTGAACACG	93
		R: TTGATGATGGCTCTGGCG	
ABC transporter family C	*Dc_ABCC1-1*	F: CGTTCTTCTGGTCATACTTGC	166
		R: TTGAATACCTCTCTTCTTGCTGC	
	Dc_ABCC1-2	F: GCAACATCGGTAATGGTAGCG	104
		R: ATGGGTAAGCGAAGCACAC	
	Dc_ABCC4-1	F: FCGAGACGATGCTGGAGAAAC	134
		R: GGGGAACGAAGAGTAAGGTG	
	Dc_ABCC4-2	F: GCTGCTCAGAAGAGGCTAAC	191
		R: ACAAACAACCGCCGATAAAAC	
	Dc_ABCC9	F: CACCGCTTTCACTCTCACTG	195
		R: GTCCTCTTCTTGCTCGCTG	
Reference	*Dc_ELFA*	F: ACATCAACATCGTCGTCATT	119
		R: GGCTTCCTTCTCGAACTTT	

Table S3. Fold-change of superoxide dismutase (SOD), catalase (CAT), and ATP-binding cassette (ABC) transporter genes in *Diaphanosoma celebensis* after exposure to 0.5 mg/L polylactic acid (PLA), β-cyclodextrin grafted polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics (MPs). Each letter indicates a significant ($p < 0.05$) difference in the expression of target genes.

Gene family	Gene	Control	PLA	CD-PLA	PBAT	LDPE
SOD	*CuZnSOD1*	1.000±0.038^{a}	1.044±0.021^{a}	1.568±0.049^{b}	1.061±0.022^{a}	0.553±0.053^{c}
	CuZnSOD2	1.000±0.031^{a}	0.844±0.102^{a}	1.337±0.038^{b}	1.005±0.090^{a}	0.814±0.026^{a}
	CuZnSOD3	1.000±0.116^{a}	0.875±0.060^{a}	3.119±0.485^{b}	2.480±0.171^{b}	1.525±0.077^{a}
	MnSOD	1.000±0.098^{a}	1.462±0.259^{ab}	1.516±0.047^{ab}	1.698±0.348 ^{b}	1.144±0.090^{ab}
CAT	*CAT1*	1.000±0.014^{a}	1.025±0.070^{a}	1.321±0.175^{b}	0.971±0.047^{a}	0.511±0.010^{c}
	CAT2	1.000±0.019^{a}	0.997±0.094^{a}	1.863±0.082^{b}	1.717±0.039^{b}	1.263±0.059^{c}
ABC	*ABCB1-1*	1.000±0.016^{a}	0.852±0.114^{ab}	1.438±0.068^{c}	1.761±0.040^{d}	0.722±0.020^{b}
transporter	*ABCB1-2*	1.000±0.025^{a}	0.727±0.346^{a}	1.592±0.130^{b}	1.659±0.077^{b}	1.616±0.104^{b}
family B	*ABCB6*	1.000±0.488^{a}	1.570±0.063^{a}	1.679±0.066^{a}	1.619±0.088^{a}	1.1568±0.017^{a}
	ABCB7	1.000±0.031^{a}	1.268±0.027^{b}	1.247±0.081^{b}	1.546±0.102^{c}	1.027±0.008^{a}
	ABCB8	1.000±0.008^{a}	1.141±0.042^{abc}	1.170±0.030^{bc}	1.270±0.070^{c}	1.095±0.046^{ab}
	ABCB10	1.000±0.036^{ab}	0.934±0.087^{a}	1.119±0.013^{b}	1.282±0.026^{c}	0.988±0.040^{ab}
ABC	*ABCC1-1*	1.000±0.060^{a}	1.747±0.084^{b}	1.778±0.287^{b}	2.890±0.090^{c}	1.308±0.022^{ab}
transporter	*ABCC1-2*	1.000±0.056^{a}	1.916±0.062^{c}	1.660±0.038^{b}	2.257±0.026^{d}	1.083±0.052^{a}
family C	*ABCC4-1*	1.000±0.055^{a}	1.967±0.094^{b}	2.212±0.076^{b}	2.882±0.179^{c}	1.978±0.184^{b}
	ABCC4-2	1.000±0.016^{a}	1.474±0.02^{b}	1.206±0.050^{c}	1.901±0.046^{c}	1.186±0.016^{d}
	ABCC9	1.000±0.330^{a}	1.707±1.178^{a}	2.052±0.445^{a}	2.261±0.391^{a}	1.669±0.125^{a}

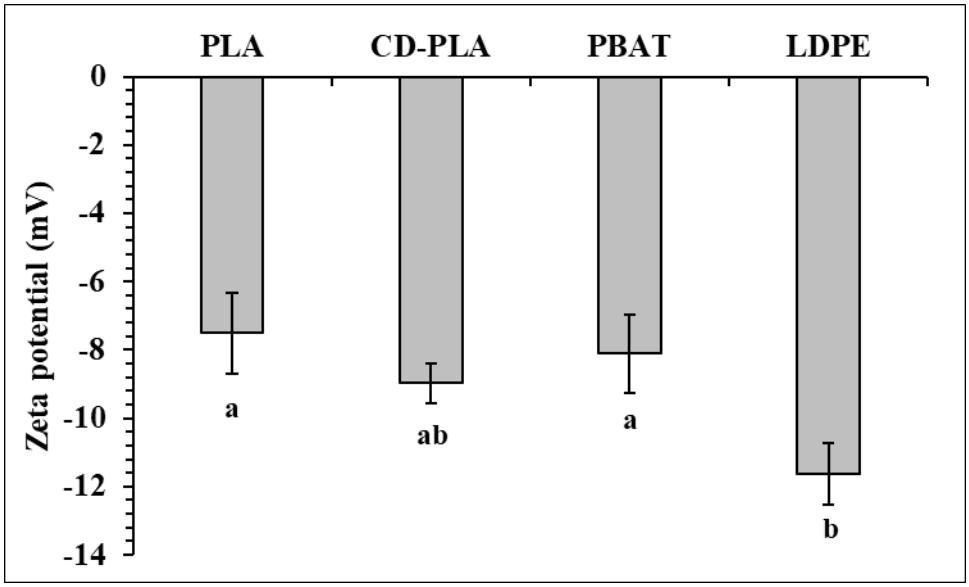

Fig. S1. The zeta potentials of 0.5 mg/L polylactic acid (PLA), β-cyclodextrin grafted polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics (MPs) in artificial seawater with a salinity of 15 Practical salinity unit (PSU) at 23°C.

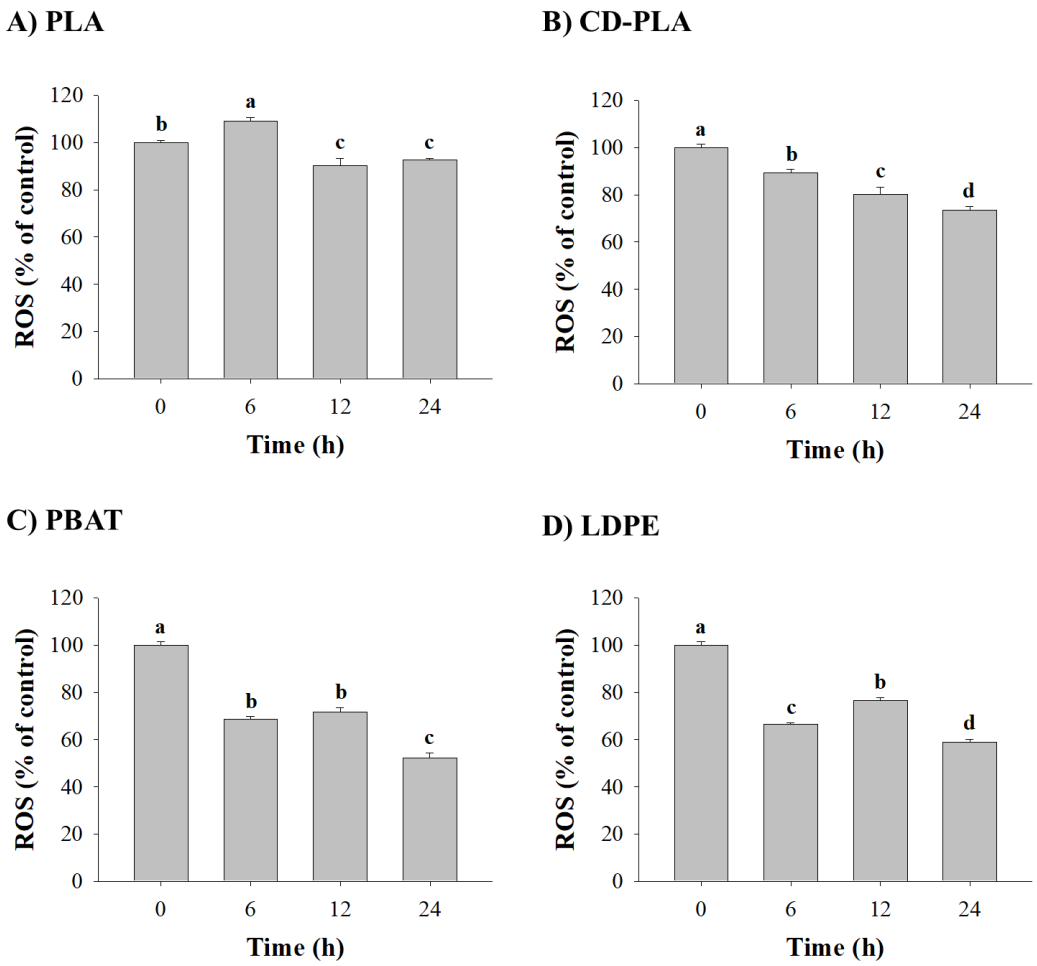

Fig. S2. Time-dependent effects of polylactic acid (PLA), β-cyclodextrin graft polylactic acid (CD-PLA), poly(butylene adipate-*co*-terephthalate) (PBAT), and low density polyethylene (LDPE) microplastics on intracellular reactive oxygen species (ROS). The bars represent the mean ± standard deviation of experiment results, and different letters above each column indicate a significant difference between groups ($P < 0.05$), as examined using one-way ANOVA with Tukey's test.

Paper V

Adverse effects of environmental relevant microplastics on *in vivo* endpoints, oxidative stress, and mitogen-activated protein kinase signaling pathway and multixenobiotic resistance system in the marine rotifer *Brachionus plicatilis*

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Adverse effects of environmental relevant microplastics on *in vivo* endpoints, oxidative stress, and mitogen-activated protein kinase signaling pathway and multixenobiotic resistance system in the marine rotifer *Brachionus plicatilis*

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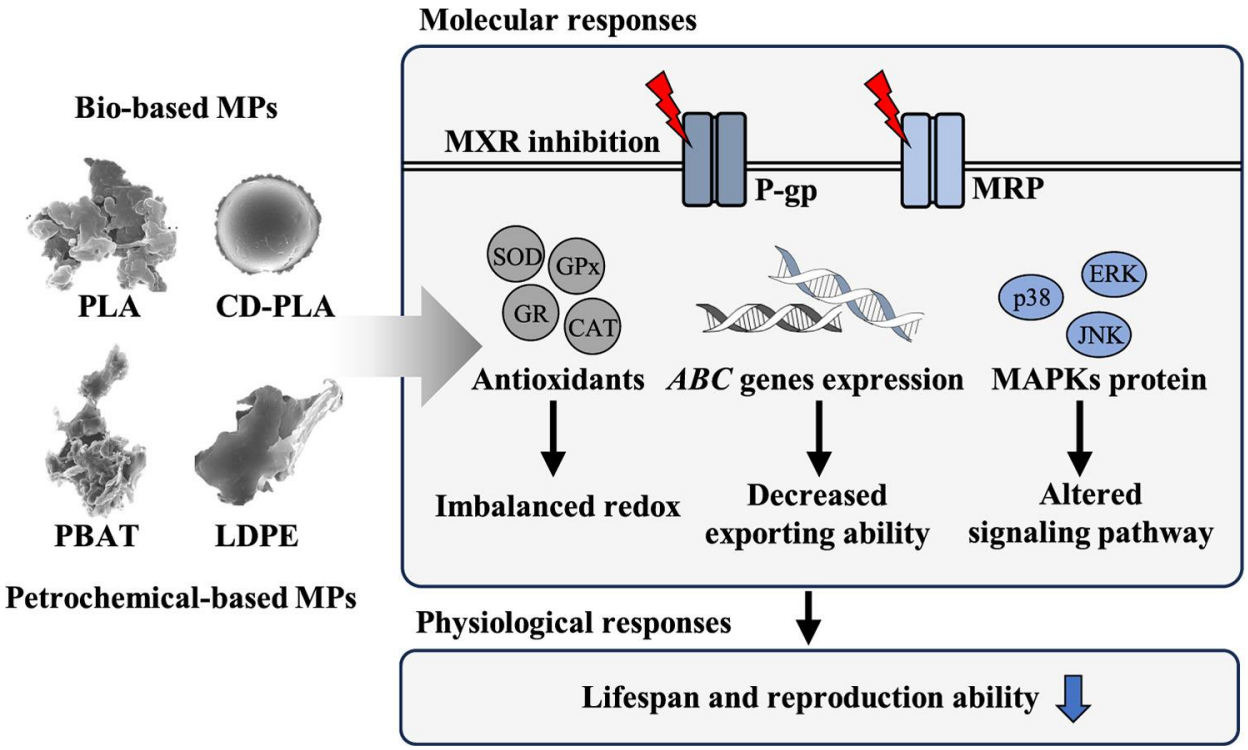
Abstract

This study compared the toxicological effects of environmentally relevant microplastics (MPs) on the marine rotifer *Brachionus plicatilis*, focusing on MPs derived from various sources, including fossil fuel-based low-density polyethylene, biobased polylactic acid (PLA), biodegradable poly(butylene adipate-*co*-terephthalate), and a novel PLA modified with β-cyclodextrin. We assessed *in vivo* effects such as reproductive output and mortality, alongside *in vitro* oxidative stress responses, including reactive oxygen species, antioxidant enzyme activities, and activation of the mitogen-activated protein kinase (MAPK) signaling pathway and multixenobiotic resistance (MXR) system. Reproductive output and lifespan reduced significantly across all MP types, ranging from 0.5 to 10 mg/L, indicating compromised reproductive fitness and life maintenance. At an environmentally relevant concentration of 0.5 mg/L, *in vitro* assessments revealed differential modulation of redox levels and antioxidant enzyme activities, contingent upon the specific MP type. Moreover, the MAPK signaling pathway and MXR assays showed changes in phosphorylation and detoxification proteins depending on the type of MPs. This study highlights the comparable ecological risks that various MPs, including biobased, biodegradable, and petrochemical-based MPs, could pose in marine environments.

Keywords Biobased biodegradable MPs; oxidative stress; antioxidant enzyme activity; MAPK signaling pathway; multixenobiotic resistance.

Graphical abstract



1. Introduction

Plastic pollution is one of the many challenges in the 21st century. In 2022, plastics contributed 362.3 million tons to the global production of 400.3 million tons, largely from fossil fuels. Only 5 million tons of plastic waste were recycled (PlasticsEurope, 2023). The majority of these plastics are durable and non-biodegradable, leading to their persistent accumulation in the aquatic environment, where they are fragmented by environmental weathering processes (Wang et al. 2024). In aquatic ecosystems, plastics break down slowly or not at all (Maddison et al. 2023), promoting the generation of microplastics (MPs) and nanoplastics (NPs). MPs refer to plastic particles less than 5 mm in size, while NPs are characterized as particles smaller than 1 μm (Ali et al. 2024c). Studies have identified a lot of MPs in aquatic environments (Novotna et al. 2019). These range from deep-sea sediments (Tsuchiya et al. 2024) to coastal waters (Pasquier et al. 2024), and from estuaries (Sawan et al. 2024) to the surface of freshwater bodies (Semmouri et al. 2023). MPs have been observed in nearly all trophic levels of the aquatic food chain, from zooplankton (Iannilli et al. 2019) to fish (Horton et al. 2024), marine mammals (Merrill et al. 2023) and seabirds (De Pascalis, 2022). MPs also disrupt biological processes, threatening the health and survival of exposed species (Osman et al. 2024), highlighting the worldwide plastic impact.

Among fossil fuel-based plastics, polyethylene (PE) is one of the most widely produced plastic types (PlasticsEurope, 2023). Its durability and lightweight properties make it a common choice for daily use, particularly in packaging, which has increasingly become a significant source of MP pollution (Sruthy and Ramasamy, 2017; Strungaru et al. 2019; Wang et al. 2019b). PE MPs have been widely detected in aquatic environments and are among the most abundant (Pasquier et al. 2024; Tsuchiya et al. 2024). Previous studies have investigated their ingestion (Tongo et al. 2022), egestion (Xiong et al. 2019), and the consequent effects on different exposed biota (Liu et al. 2023; Savuca et al. 2023). PE MPs have been found to induce several toxic effects, including oxidative damage (Silva et al. 2021; Savuca et al. 2024a, 2024b), reproductive toxicity (Xia et al. 2023), impaired development (Malafaia et al. 2020), disrupted physiological functions (Tongo et al. 2022), and mortality (Jacob et al. 2021). Additionally, PE MPs ingested by lower trophic levels, like zooplankton, can be transferred to higher levels, posing environmental concerns and potential risks to humans (Costa et al. 2020; da Costa Araújo et al. 2020).

Bioplastics have emerged and been promoted as an eco-friendly alternative to petrochemical plastics, which has led to a significant increase in their production. Bioplastics are used in packaging, consumer goods, electronics, automotive parts, and textiles. The bioplastics market's largest segment (43%) in 2023 was packaging (EuropeanBioplastics, 2023). Even if bioplastics are growing more common, the term "bioplastic" is still ambiguous, comprising biobased, biodegradable, compostable, and doped plastics (Ali et al. 2024c). Importantly, biodegradable polymers are not always biobased, and biobased plastics are not always biodegradable (Nanda et al. 2022). There is insufficient scientific evidence that bioplastics are better than petrochemical plastics, especially regarding their toxicity.

Among biodegradable polymers, poly(butylene adipate-*co*-terephthalate) (PBAT) is a commercially available aliphatic-aromatic copolyester (Ali et al. 2024b). In 2023, PBAT

accounted for 4.6% of bioplastics production, encompassing both biobased and biodegradable plastics. Although biodegradable, PBAT poses significant environmental concerns as its biodegradation is particularly slow, with negligible weight loss in sterile and microbe-containing water even over prolonged durations (Wang et al. 2019a). PBAT decomposing can produce MPs, especially in coastal conditions and under UV radiation, in greater quantities than other plastics (Wei et al. 2021; Bao et al. 2022). PBAT MPs have been discovered in nature (Okoffo et al. 2022; Qian et al. 2023), calling for urgent ecotoxicological study. PBAT MPs have been shown to impair fertilization and development in *Mytilus galloprovincialis* (Capolupo et al. 2023), accumulate in *Lates calcarifer* (Xie et al. 2022), and alter microbiota in copepod *Eurytemora affinis* (Thery et al. 2023). Their toxicological effects on aquatic biota are unknown and need further investigation (Ali et al. 2024b).

Among biobased plastics, polylactic acid (PLA) is the most extensively produced lightweight, semi-crystalline polymer, primarily synthesized from renewable resources. PLA is regarded as a promising alternative to fossil fuel-based plastics (Ali et al. 2023b). In 2023, PLA contributed 31.0% of all biobased and biodegradable plastics produced (EuropeanBioplastics, 2023). PLA is compostable, but its degradation in natural settings, particularly aquatic settings, could lead to MP pollution (Lambert and Wagner, 2016; Tong et al. 2022; Ali et al. 2023a). Recently, PLA bio-MPs have been detected in various natural environments (Lerebours et al. 2022) and organisms (Ribeiro et al. 2024). Moreover, previous studies have reported that PLA bio-MPs pose toxicity to exposed aquatic biota (Kardgar et al. 2023; Khosrovyan et al. 2023; Ali et al. 2024a), with some research indicating that their toxicity is comparable to that of fossil fuel-based MPs (Su et al. 2022; Tamayo-Belda et al. 2023; Zhong et al. 2024). Consequently, research is focused on modifying PLA to enhance biodegradation and improve mechanical properties by blending with other polymers which significantly impact PLA degradability (Hu et al. 2018; Friné et al. 2019; Yu et al. 2020; Ali et al. 2023a). Considering the increasing amount of PLA modification, it is essential to evaluate the toxicity of modified bio-MPs to their pristine counterparts and other biodegradable and non-biodegradable MPs.

The marine rotifer *Brachionus plicatilis* Müller, 1786 is a tiny marine zooplankton, that plays a crucial role in energy transfer within the marine food web (Jeong et al. 2024). This species, characterized by its small size, short life cycle, high fecundity, and ease of laboratory maintenance, is an ideal model organism for research in ecotoxicology (Yoon et al. 2024). *B. plicatilis* is frequently used as a bioindicator to evaluate the ecotoxicological impacts of different environmental stressors (Han et al. 2020; Liang et al. 2022; Han et al. 2023; Seong et al. 2024). There are no studies comparing the toxicological effects of MPs from different sources on *B. plicatilis*. Therefore, this study aimed to evaluate and compare the toxic effects of MPs from different origins, including fossil fuel-based, biobased, biodegradable, and modified biobased MPs, on *B. plicatilis*. In this study, the toxicological effects of several plastics were examined: PBAT (representing biodegradable plastics), PLA (a biobased plastic), CD-PLA (PLA grafted onto β-cyclodextrin, a modified biobased plastic), and LDPE (a reference fossil fuel-based plastic) (Ali et al. 2024a). We examined effects on reproduction, lifespan, oxidative stress, antioxidant activity, cellular efflux, gene expression, and MAPK signaling pathways in *B. plicatilis*. The findings of this study will offer valuable insights into

the differential toxicological effects of various MPs, contributing to a deeper understanding of how these emerging pollutants may impact marine ecosystems.

2. Materials and methods

2.1. MPs preparation and characterization

To produce three distinct types of MPs, PLA (Ingeo™ Biopolymer 4032D, NatureWorks LLC, United States), PBAT (Ecoflex F blend C 1200, BASF, Ludwigshafen, Germany), and LDPE (BM50, EXXONMOBIL™, Dallas, United States) beads were utilized. Additionally, a modified form of PLA, grafted onto β-cyclodextrin, was synthesized at the Laboratory of Catalysis and Solid-State Chemistry, University of Lille, France, to create modified bio-MPs. This modification followed the protocol established by Miao et al. (2011) and further elaborated in a recent study (Ali et al. 2024c). All four types of MPs were generated by performing cryogenic grinding using a cryomill (CRYOMILL, RETSCH®, Hann, Germany). To determine the particle size distribution, ranging from 2 to 10 µm, ten consecutive dynamic light scattering analyses were performed using a Mastersizer 2000 (Malvern Instruments, Malvern, UK) with distilled water serving as the dispersing medium. Surface morphology of the MPs was examined using scanning electron microscopy (SEM).

2.2. Marine rotifer culture

Generously supplied by Prof. Atsushi Hagiwara from Nagasaki University, Japan, the NH1L strain of the marine rotifer *B. plicatilis* was identified through mitochondrial DNA cytochrome oxidase I (*CO1*) gene sequencing and morphological analysis (Suga et al. 2008; Mills et al. 2017). Since arriving, optimal culture and maintenance of the strain have taken place at Sungkyunkwan University's Department of Biological Sciences in Suwon, South Korea. Conditions for culturing include artificial seawater (ASW) with a salinity of 15 practical salinity units, prepared using Tetra Marine Salt Pro (Tetra, Cincinnati, OH, USA), and a 12-h light/12-h dark photoperiod at 23 °C. Daily feedings consist of the green microalga *Tetraselmis suecica* (Kylin) Butcher, 1959 at a concentration of 6×10^4 cells/mL, while the medium is changed twice each week.

2.3. Life history traits of *B. plicatilis*

Neonates of *B. plicatilis* (<2 h post-hatching) were individually exposed to 2 mL of 15 psu ASW, and treated with either type of MPs including PBAT, CD-PLA, PLA, or LDPE to evaluate their effects on life history traits until rotifers are dead. A range of MPs concentrations (0.5, 1, 5, and 10 mg/L) were used alongside a control group, with *T. suecica* provided at a concentration of 6×10^4 cells/L as a food. The concentrations of MPs were selected based on environmental estimated concentrations and laboratory-used concentrations (Thornton Hampton et al. 2022; Rakib et al. 2023). Each condition was replicated six times in 24-well culture plates and conducted at 23 °C under a 12-h light-dark cycle. Observations were conducted every 12 h on all replicates of the MPs exposed groups using a microscope (SZX-ILLK200, Olympus, Tokyo, Japan) to monitor breeding activity, count neonates, and assess mortality rates. Reproductive output was quantified by systematically counting and removing all neonates produced throughout the lifespan of each individual *B. plicatilis*. The mean

lifespan was calculated by recording the time of death for each adult until all individuals had perished.

2.4. Oxidative stress and antioxidant enzymatic activity

To compare the *in vitro* effects of different types of MPs, we used a concentration of 0.5 mg/L, selected based on our previous work (Ali et al. 2024a,c; Ali et al. 2025). This concentration was determined with reference to the highest reported environmental concentration of petrochemical MPs (2.5 mg/L) and accounts for the limited data available on bio-MPs in aquatic environments. Although this concentration may exceed levels typically found in natural settings, it is significantly lower than those used in other studies, thereby aligning the experimental conditions more closely with realistic contamination scenarios (Ali et al. 2024c). Rotifers were exposed to control, PBAT, CD-PLA, PLA, and LDPE MPs at a concentration of 0.5 mg/L for 24 h under optimal conditions of 23 °C to compare intracellular ROS levels and antioxidant enzyme activity. A parallel experiment exposed rotifers to the same MPs concentrations for 6, 12, and 24 h to assess oxidative stress in a time-dependent manner. Following the exposure, rotifers were collected, washed, homogenized, and the supernatants were used for biochemical assays. ROS levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) activities were measured using established protocols. Detailed methods are provided in the **Supplementary material (Text S1)**.

2.5. P-glycoprotein and multidrug resistance-associated protein activity

To investigate the impact of various MPs on ATP-binding cassette (ABC) transporters, the expression levels of P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) were assessed following the protocol outlined by Yoo et al. (2024). Rotifers were exposed to 0.5 mg/L of various MPs and a control at 23 °C for 24 h in 100 mL beakers. Afterward, they were incubated in darkness with rhodamine B (0.5 μM) and calcein AM (1 μM), which act as P-gp and MRP substrates. This was followed by a re-exposure to MPs or the control for 1 h to enhance transporter activity (Wang et al. 2023). Post-exposure, rotifers were filtered, rinsed, and either fixed in paraformaldehyde or homogenized for fluorescence analysis using a spectrophotometer. The fluorescence intensity was measured and normalized to total protein content. Full experimental details are available in the **Supplementary material (Text S2)**.

2.6. Expression profiles of genes related to antioxidant defenses and ABC transporters

Gene expression profiles of antioxidant enzymes (*CAT* and *SOD*) and *ABC* transporters were analyzed in rotifers exposed to four types of MPs (0.5 mg/L) and a control group under controlled conditions (23 °C, 12-h light-dark cycle) for 24 h. After exposure, total RNA was extracted using TRIzol® reagent and checked for quality. cDNA synthesis was performed, and primer information is detailed in **Tables S1 and S2**. qPCR was conducted using specific primers for *SOD*, *CAT*, *ABCB*, and *ABCC*, with gene expression differences calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Full details, including expression levels, are available in **Tables S3 and S4**, with methods provided in the **Supplementary material (Text S3)**.

2.7. Western blotting of mitogen-activated protein kinase signaling proteins

Cell Signaling Technology provided the antibodies for phospho-ERK, p38, SAPK/c-JNK, and phospho-SAPK/JNK, while those for phospho-p38 and β-actin were sourced from Santa Cruz Biotechnology. Secondary antibodies (HRP-conjugated) were purchased from Novus Biologicals. After 24 h of exposure to 0.5 mg/L of MPs, rotifers were homogenized, and the supernatants were collected. Proteins (30 μg per sample) were resolved via SDS-PAGE, transferred onto nitrocellulose membranes, and blocked with BSA. The membranes were then incubated with primary and secondary antibodies before the protein expression was detected using HRP substrates. Detailed protocols are available in the **Supplementary material (Text S4)**.

2.8. Statistical analysis

Variance homogeneity was checked using Levene's test, followed by a one-way analysis of variance to determine group differences. Independent samples t-tests were used to compare the control group with the MPs-exposed groups. Tukey's test was applied for post hoc analysis to explore significant differences among the groups. Statistical significance was set at $P < 0.05$ for all analyses, and the results are presented as mean ± standard deviation, indicating both central tendency and variability. All statistical analyses were performed using SPSS software (version 22).

3. Results and discussion

3.1. Characterization of MPs

The surface morphology of MPs synthesized from PLA, CD-PLA, PBAT, and LDPE is depicted in the SEM images **(Fig. 1A-D)**. The images show that all MP types possess irregular shapes with sharp edges and rough, uneven surfaces. These surfaces are marked by prominent fractures, cracks, and sharp edges. The observed morphological features, including angular structures and particle sizes ranging from 2 to 10 μm, could potentially reflect the characteristics of secondary MPs formed from plastic waste in natural environments. Importantly, no significant differences in surface morphology were detected among the different types of MPs analyzed.

The physical characteristics, such as shape and size, of MPs could significantly influence their accumulation and residence time in the gut and consequent toxicity. MPs fragments accumulated at higher concentrations than beads (An et al. 2021). For instance, less than 1% of the water flea *Daphnia magna* could egest MP fragments compared to 49% for MP beads within 24 h (Frydkjær et al. 2017). Grass shrimps accumulated more MP fragments than beads, likely due to the longer gut residence time of fragments (Gray and Weinstein, 2017), and zebrafish showed higher concentrations of PS fragments than PS beads in their gut (Qiao et al. 2019). This increased accumulation and prolonged retention time of fragmented MPs could lead to higher toxicity, as fragmented PE MPs have been reported to exhibit nearly 80 times greater acute toxicity in zooplankton than their bead counterparts (Na et al. 2021). However,

much of the previous research on MP toxicity assessments has predominantly used beads or uniformly shaped particles. This approach lacks environmental relevance, as plastic fragmentation in natural environments produces asymmetrical and heterogeneous mixtures, leading to results that may not accurately reflect the risks posed by secondary MPs (Barrick et al. 2024). Therefore, in this study, we synthesized MPs from representative plastics, including PLA (biobased), PBAT (biodegradable), and LDPE (fossil fuel-based), by grinding their commercially available pellets to produce particles similar in shape (Nayeri et al. 2023) and those reported to be produced from them under laboratory conditions (Lambert and Wagner, 2016; Tong et al. 2022), using an environmentally relevant concentration of 0.5 mg/L (Ali et al. 2024c) for *in vitro* experiments to reflect MPs that may exist in the aquatic environment.

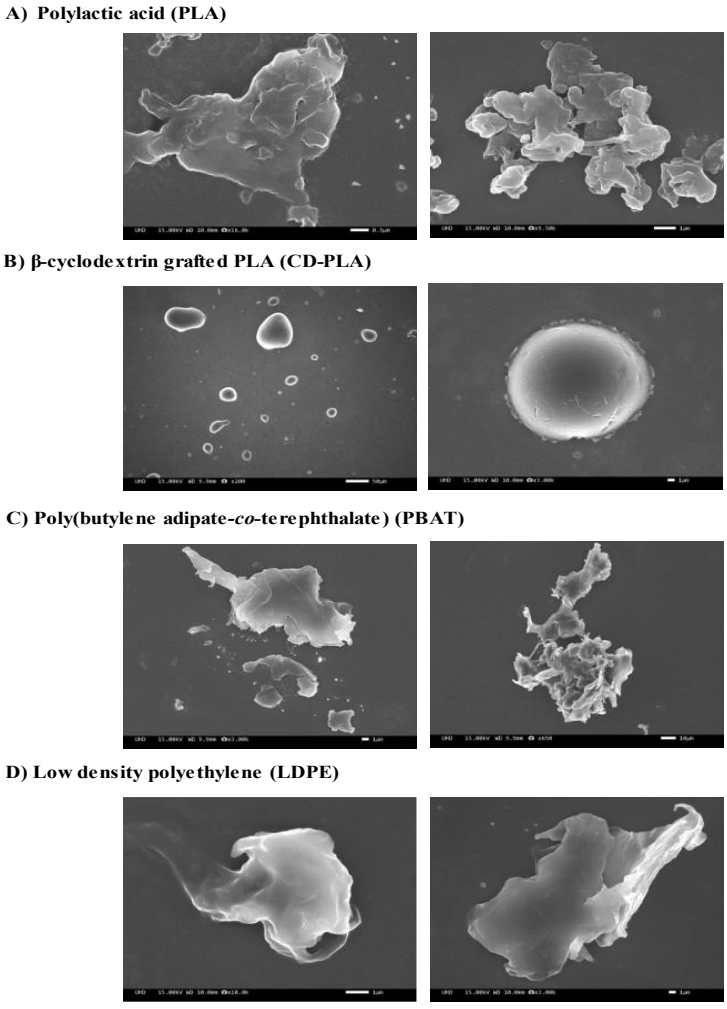


Fig. 1. Image of microplastics (scanning electron microscopy), which display the surface morphology.

3.2. *In vivo* effects of MPs

To assess the toxicity of various types of MPs, including PLA, PBAT, LDPE, and CD-PLA, we evaluated their individual effects across a range of concentrations on *in vivo* endpoints such as reproduction and lifespan. Exposure to all concentrations (0.5, 1, 5, and 10 mg/L) of PLA, CD-PLA, PBAT, and LDPE MPs resulted in a significant reduction ($P < 0.05$) in the

total reproduction of *B. plicatilis* compared to the control group **(Fig. 2A)**. This consistent reduction indicates that all types of MPs tested exert a significant negative impact on reproductive output, regardless of the concentration. Reproduction did not show any significant differences ($P > 0.05$) across the different MP concentrations. Similarly, all types of MP exposure shortened the lifespan, especially at 5 and 10 mg/L **(Fig. 2B)**, showing that the type of MP has an effect that depends on the concentration. PLA bio-MPs also caused a detrimental effect at a concentration of 1 mg/L. At the lowest concentration, however, there were no significant modifications in lifespan except for PBAT MPs.

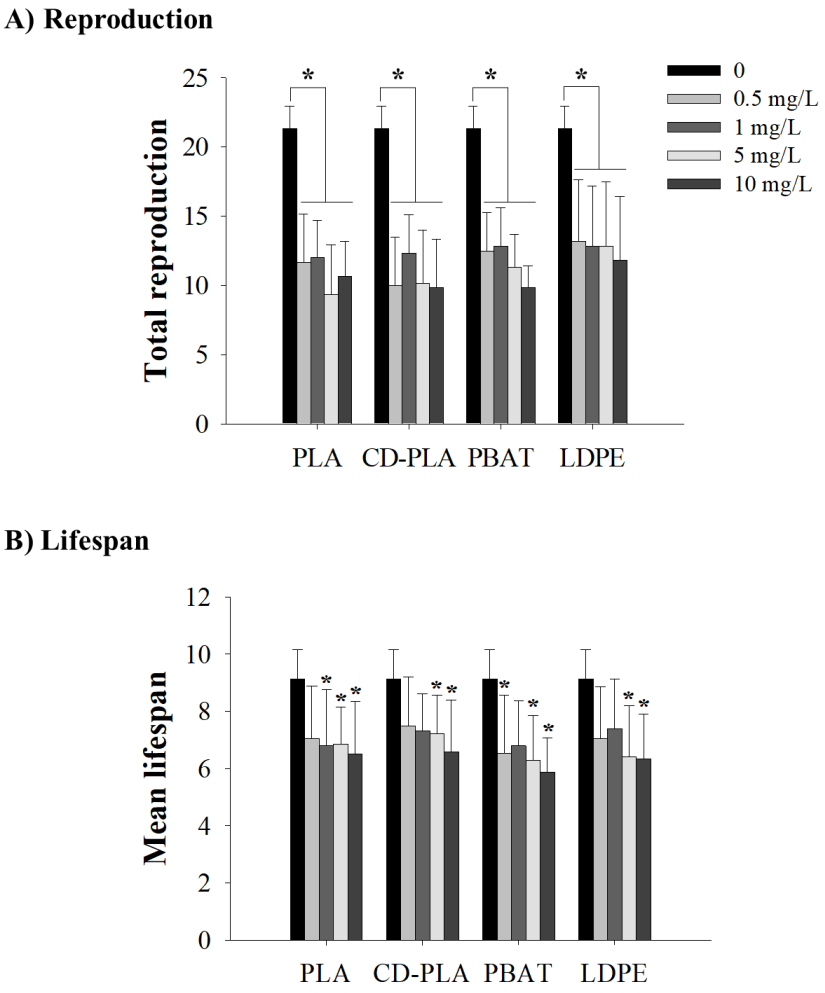


Fig. 2. The *in vivo* effects of various concentrations (0.5, 1, 5, and 10 mg/L) of microplastics (MPs). A) reproduction, B) lifespan. Significant differences ($P < 0.05$) between the treated groups and the control are indicated with asterisks.

Overall, our results show that all of the MPs we tested—whether they were biobased, biodegradable, derived from fossil fuels, or modified biobased—had a big impact on the lifespan of *B. plicatilis* at high concentrations (5 and 10 mg/L). This suggests that irregular shapes of MPs might be much more harmful to life than bead shapes, which are commonly used in laboratory studies. In an earlier study, it was found that PLA, PP, PS, and LDPE MPs with broken shapes caused more deaths in freshwater cnidarian *Hydra viridissima* when concentrations were higher (10 and 100 mg/L). Although some studies showed no effect on survival in green mussel *Perna viridis* exposed to polyethylene terephthalate (PET) and PLA

MPs (135–140 particles/L) (Joyce and Falkenberg, 2022) and brine shrimp *Artemia franciscana* treated with PLA and polyvinylidene difluoride (PVDF) MPs (1 to 100 mg/L) (Di Giannantonio et al. 2022), our result can support that potentially increased environmental concentration of MPs, regardless of type, even in bioplastics, would have to impact aquatic organisms. Furthermore, uniform shapes and functional groups of MPs may pose a greater risk to aquatic organisms. For instance, Xie et al. (2024) reported that PBAT MPs exhibit higher toxicity to zebrafish embryos compared to modified PBAT MPs. This suggests that the chemical modifications of MPs can significantly influence their toxicity profiles, potentially altering their interaction with biological systems.

Given that reproduction is a highly energy-demanding physiological process, it may be particularly vulnerable to the stress induced by accumulated MPs. In our study, statistically significant reductions in the reproductive output of *B. plicatilis* were observed at each concentration tested: 0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L. This suggests that they may substantially impair reproductive fitness. Similar to our findings, PLA and PET MPs have been shown to reduce the reproduction of the tunicate *Microcosmus exasperatus* (Anderson and Shenkar, 2021) and *D. magna* (An et al. 2024), with the effects likely being mechanical rather than chemical as supported by Jemec et al. (2016). This shift likely contributes to the observed decline in reproductive output, potentially linked to reduced feeding rates caused by the ingestion of particles, as seen in *D. magna* (Malinowski et al. 2023), where a reduction in feeding rates was reported. Zimmermann et al. (2020) found that biobased PLA MPs exhibited similar toxicity to non-biodegradable PVC and PU MPs in *D. magna*, with the toxic effects mainly attributed to their small size and irregular shape. The similar effects of all these MP types on survival and reproduction could be attributed to their similarity in shape and size, as fragmented MPs tend to have a longer gut residence time (Gray and Weinstein, 2017), leading to easier accumulation and, consequently, a more severe impact on food ingestion. These findings highlight the potential chronic risks posed by MPs, regardless of their type, particularly concerning reproductive fitness, which could have significant ecological implications for populations and consequently for the aquatic food web.

3.3. Oxidative stress, antioxidant enzymatic activity, and gene expression

In all MP-exposed groups, ROS levels were significantly lower compared to the control **(Fig. 3A)**. PLA bio-MPs and PBAT MPs exhibited similar trends in ROS reduction, both showing significant decreases ($P < 0.05$). CD-PLA bio-MPs and LDPE MPs also showed significant reductions ($P < 0.05$) with comparable trends. Likewise, ROS levels showed a consistent decreasing trend over time across all MP types **(Fig. S1)**. In terms of enzymatic activity, SOD activity did not differ significantly between the control, SOD levels showed no significant change in the PLA and CD-PLA groups but were notably higher in the LDPE group ($P < 0.05$) and lower in the PBAT group **(Fig. 3B)**. CAT activity was significantly increased in the PBAT group ($P < 0.05$) compared to the control, while the PLA group displayed a marked reduction in CAT activity ($P < 0.05$) **(Fig. 3C)**. GPx activity was decreased at all types MP exposure **(Fig. 3D)**. GR activity was decreased in PLA group compared to PBAT and LDPE groups **(Fig. 3E)**. Overall, our results indicate that while all MPs effectively reduced ROS levels, the response of antioxidant enzymes was more dependent on the specific type of

MP, with LDPE eliciting a distinct response compared to the biobased or biodegradable MPs (PLA, CD-PLA, and PBAT).

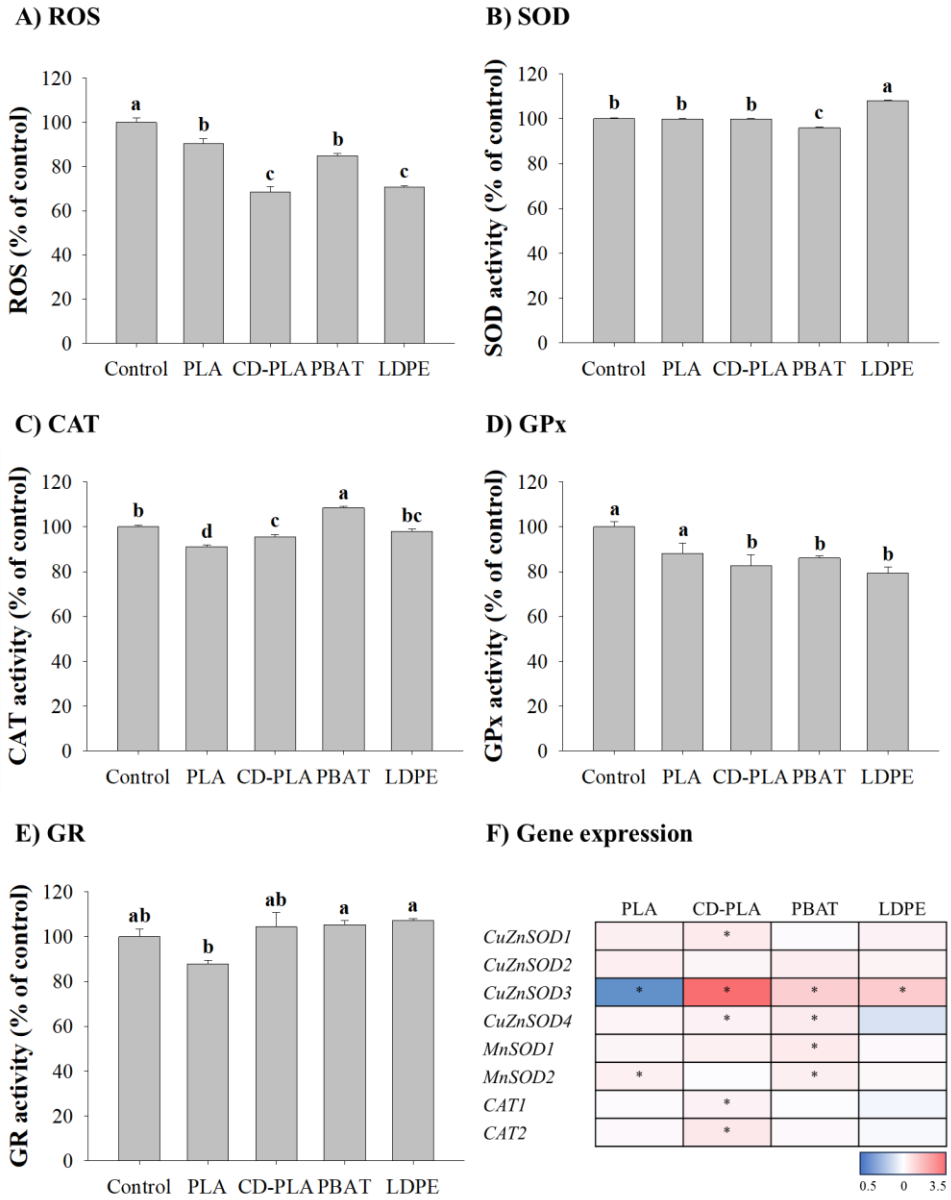


Fig. 3. Antioxidant responses and related gene expression after exposure to microplastics (MPs). Data are expressed as mean ± standard deviation, with significant differences between groups indicated by different letters above columns ($P < 0.05$; $n = 3$). Asterisks denote significant differences ($P < 0.05$). Detailed expression values are listed in **Table S3**.

The mRNA expression levels of *SOD* and *CAT* genes showed variations similar to the patterns seen in antioxidant enzyme activity across the different MP exposure groups **(Fig. 3F)**. In the PLA group, one *SOD* gene (*MnSOD2*) was upregulated, while another (*CuZnSOD3*) was downregulated, indicating a complex oxidative stress response. The CD-PLA exposure group showed a significant increase ($P < 0.05$) in the expression of five genes (*CuZnSOD1*, *CuZnSOD3*, *CuZnSOD4*, *CAT1*, and *CAT2*), indicating a robust oxidative stress response involving both SOD and CAT pathways. In contrast, the PBAT exposure group resulted in the

significant upregulation ($P < 0.05$) of four *SOD* genes (*CuZnSOD3*, *CuZnSOD4*, *MnSOD1*, and *MnSOD2*) without affecting *CAT* genes, suggesting a more specific response focused on the SOD pathway. Although the specific genes involved differ, the overall upregulation of oxidative stress-related genes in both CD-PLA and PBAT groups suggests that the biodegradable nature of these ingested MPs may cause them to undergo rapid changes in the gut environment, leading to similar oxidative stress responses compared to compostable or non-biodegradable MPs.

Oxidative stress is a common biochemical response in aquatic organisms exposed to environmental contaminants, including the ingestion of MPs, which can result in gastrointestinal tract obstruction, energy depletion, and disruption of the redox balance (Watts et al. 2015; Jemec et al. 2016; Martyniuk et al. 2024; Jeong et al. 2024). These effects, caused by both excessively high and low ROS levels, ultimately lead to homeostasis disruption and impaired cellular function (Zorov et al. 2014; Ali et al. 2024c). Imbalanced redox homeostasis and disruption of antioxidant systems have been associated with MP exposure in various organisms, including rotifers (Jeong et al. 2016). MPs from different sources have been shown to induce altered ROS levels in exposed biota. For example, *Microcystis aeruginosa* showed an increase in ROS levels after 4 days of exposure to PLA, PCL, or PS MPs (0.1, 1, 5 mg/L), with levels normalizing by day 12 (Kim et al. 2024). *Arabidopsis thaliana* exhibited elevated ROS levels after 14 days of exposure to PBAT MPs, while LDPE caused no change; however, both types resulted in higher ROS levels after 28 days (Liu et al. 2022). Similarly, *D. magna* displayed increased ROS levels following exposure to PE or PLA MPs (1 mg/L), with no significant differences observed (Luangrath et al. 2024). In our study, rotifers exposed to various types of MPs exhibited altered ROS levels regardless of MP type, but these levels were significantly lower compared to those in the control group. Consistent with our findings, Seong et al. (2024) have also reported reduced ROS levels in rotifers exposed to MPs, attributing this reduction either to a decrease in metabolic activity induced by MP exposure or to the upregulation of antioxidant enzymes as a protective response. A recent study reported that *B. plicatilis* exposed to copper (Cu) and PS NPs, either individually or in combination, showed decreased ROS levels, with the most significant reduction observed when NPs were combined with Cu at higher concentrations (Wang et al. 2023). In *Anabaena spp*., after exposure to poly(ε-caprolactone) (PCL) oligomers alone or co-exposure to PCL NPs and PCL oligomers, there was no significant difference in ROS production between treatments; however, both treatments led to a marked reduction in ROS levels from 24 to 72 h (Tamayo-Belda et al. 2022). These results, including ours, suggest that MPs can disrupt redox homeostasis in exposed organisms. However, the observed decrease in ROS levels across all MP types in *B. plicatilis* may indicate either an imbalance in the redox system or an excessive activation of antioxidant defenses, potentially leading to reductive stress. However, a comprehensive assessment of intracellular oxidation necessitates an integrated approach that extends beyond merely measuring ROS levels; incorporating the evaluation of antioxidant activities and other intracellular signaling pathways is crucial for understanding the oxidative status and its broader implications (Lee et al. 2021; Wang et al. 2023).

The SOD and CAT antioxidant enzymes are crucial in maintaining the balance between ROS production and scavenging, serving as key indicators of pollutant toxicity by reflecting the impact of redox stress (Ali et al. 2024c). Environmental pollutants, including MPs, are

endogenous sources of ROS (Sussarellu et al. 2016), and alterations in antioxidant activities indicate shifts in cellular antioxidant balance (Trestrail et al. 2021; Tuncelli et al. 2024). The enzymatic activities of SOD and CAT have been widely shown to change in response to exposure to environmental MPs. For example, *Physalaemus cuvieri*, when exposed to PLA MPs tadpoles, exhibited higher levels of both SOD and CAT (Malafaia et al. 2021). Additionally, oxidative stress responses can vary depending on the type of MPs, as seen in *D. magna*, where no significant difference in CAT activity was observed when exposed to pristine PLA or PE MPs at a concentration of 1 mg/L for four weeks compared to the control, although SOD activity significantly increased only with PLA MPs exposure (Luangrath et al. 2024). In a similar pattern, *M. aeruginosa* showed greater SOD and CAT activity when exposed to PLA MPs compared to PS or PCL MPs (Kim et al. 2024). On the other hand, *A. thaliana* exhibited elevated SOD activity in response to LDPE or PBAT MPs, with no notable differences between the two (Liu et al. 2022). Likewise, *C. vulgaris* responded to PE, PA, PLA, and PBS MPs with increased SOD activity (Su et al. 2022). Additionally, *M. edulis* exhibited varying antioxidant responses to PE and PHB MPs, including reduced CAT activity in the gills and lower SOD activity in the digestive glands when exposed to PHB (Magara et al. 2019). In thick-shelled mussels, *Mytilus coruscus*, exposed to PLA bio-MPs and PS MPs, SOD activity remained unchanged, while CAT activity showed no significant increase with PLA but increased with PS MPs (Zhong et al. 2024). In this study, significant SOD alterations were observed in the PBAT and LDPE MPs exposed groups, while CAT activity was altered in all MPs exposed groups except LDPE, suggesting that MPs exposure can differential regulation of redox homeostasis by changing antioxidant enzyme activities. Overall, the general decreasing trend of antioxidant enzymes suggests that exposure to MPs may have disrupted redox balance, thereby inhibiting CAT activity. As SOD activity is a major cellular defense against ROS, converting superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide, which is then decomposed by CAT into water and more oxygen (Cheng et al. 2017), low CAT activity indicates a potential disruption in the enzymatic stress response. Moreover, the observed differences in SOD and CAT activities across studies indicate that while these enzymes serve as useful biomarkers for assessing oxidative stress, their responses may not always be consistent or predictable. Furthermore, our results showed that all types of MPs influenced GPx activity, while different MP types showed different responses to GR activity, suggesting that MPs could inhibit the detoxification of ROS. Specifically, PLA exhibited a decrease in GR activity when compared to PBAT and LDPE. Because GPx and GR enzymes were associated with the hydrogen peroxide (H_2O_2) elimination pathway (Dong et al. 2022), these results suggest that biobased and biodegradable MPs are toxic to the redox regulation system by affecting protein.

In this context, given that antioxidant enzyme activity is widely used as a biomarker to evaluate the stress effects of environmental pollutants, and considering that changes in gene expression often constitute the earliest molecular response (Bernard et al. 2015), the antioxidant response genes, particularly those related to *SOD* and *CAT*, were evaluated. In our results, the level of *SOD* and *CAT* genes were more dependent on the type of MPs. Exposure to both CDPLA and PBAT MPs resulted in significant alterations in the expression of many genes. The pronounced response to CDPLA and PBAT MPs may be attributed to their ability to biodegrade, which likely leads to more dynamic interactions with biological systems. Similar

to our findings, differential modulation of antioxidant-related genes has been observed with exposure to various types of MPs; for example, exposure of *D. magna* to 5 mg/L PLA MPs significantly downregulated *SOD* gene expression, whereas PET MPs showed no significant effect (An et al. 2024). Similarly, exposure of *Litopenaeus vannamei* to HDPE MPs at concentrations of 0.5, 1, and 3% over 28 days significantly downregulated *SOD* gene expression (Niemcharoen et al. 2022), while exposure of Chinese mitten crabs, *Eriocheir sinensis*, to PS MPs at 40,000 μg/L downregulated *SOD* and *CAT* expression after 21 days, with upregulation at lower concentrations (Yu et al. 2018). Furthermore, a study on *B. plicatilis* exposed to heavy metals and NPs reported a decrease in *CAT* gene expression, accompanied by an increase in CAT protein activity (Wang et al. 2023). According to Lee et al. (2022), the regulation of mRNA levels and antioxidant activity *in B. plicatilis* may involve distinct mechanisms when responding to environmental stressors. This differential regulation was further supported by our results, where an increase in mRNA levels, indicated by the upregulation of gene expression, was observed in response to different types of MPs exposure. However, the corresponding antioxidant enzymatic activities did not exhibit a parallel increase, reinforcing the notion that gene expression and enzymatic activity may either lack a direct correlation (Koussounadis et al. 2015) or be regulated through distinct mechanisms. Furthermore, the alterations in the regulation of the gene expression profile in response to different types of MPs exposure, together with our results, suggest the presence of redox imbalance. Previous research has shown that shifts in cellular redox balance are crucial in modulating the structure and activity of signaling proteins, which in turn affect gene expression under cellular stress conditions (Regoli et al. 2011). This finding is consistent with the results of the present study.

3.4. Multixenobiotic resistance activity and related genes expression

The intensity of rhodamine B and calcein AM varied according to the type of MPs. In the CD-PLA bio-MP and PBAT MP groups, rhodamine B intensity significantly increased ($P < 0.05$), indicating inhibition of P-gp activity compared to the control. In contrast, no significant difference ($P > 0.05$) was found between the control and the LDPE MP group. However, the PLA bio-MP group exhibited a significant reduction in fluorescence intensity ($P < 0.05$), suggesting enhanced P-gp activity **(Fig. 4A)**. Likewise, the intensity of calcein AM varied among the groups. Significant increases in fluorescence ($P < 0.05$) were noted in the CD-PLA bio-MP, PBAT, and LDPE MP groups, suggesting inhibited MRP activity compared to the control. In contrast, the fluorescence intensity in the PLA bio-MPs exposed group did not differ from the control, as shown in **Fig. 4B**. Overall, our results showed that CD-PLA and PBAT disrupt both P-gp and MRP activities, while PLA affects P-gp and LDPE impacts MRP in marine rotifers.

The regulation of gene expression associated with multixenobiotic resistance (MXR) within the ABCB and ABCC subfamilies showed varied and distinct responses based on the type of MP exposure **(Fig. 5)**. This variability reveals both commonalities and unique regulatory patterns among different MPs. In the PLA-exposed group, a mixed response was observed: four ABCB subfamily genes were significantly downregulated ($P < 0.05$), while one was upregulated, and in the ABCC subfamily, three genes were upregulated ($P < 0.05$) and one was downregulated. This pattern suggests that PLA exerts a dual effect on gene expression,

suppressing certain ABC transporters while upregulating others, indicating a complex interaction with the cellular environment. In contrast, the CD-PLA group showed more consistent and pronounced changes in gene expression, with nine genes from the ABCB subfamily exhibiting a significant increase in expression ($P < 0.05$) and only two showing decreased expression.

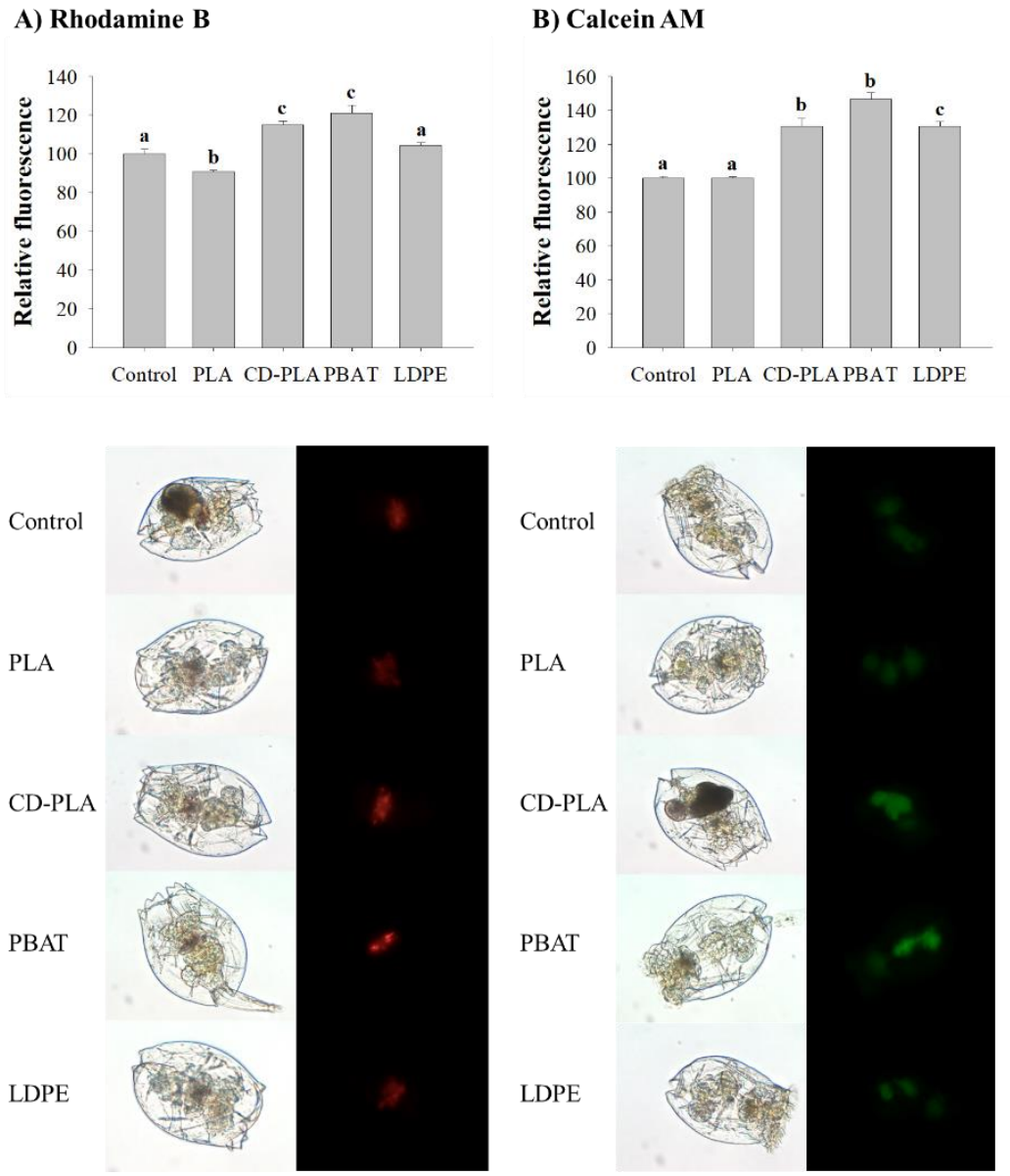


Fig. 4. The activity of multixenobiotic resistance receptor (ATP-binding cassette proteins) in the marine rotifer *Brachionus plicatilis* following exposure to various types of microplastics (MPs). Panels A and B depict the activity of P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP), respectively. An increase in fluorescence intensity observed in marine rotifers shows reduced activity of the ABC proteins, whereas a decrease in fluorescence intensity shows enhanced activity. The bars illustrate the relative levels of fluorescence intensity, with letters denoting statistically significant differences ($P < 0.05$) between the various groups exposed to MPs and control.

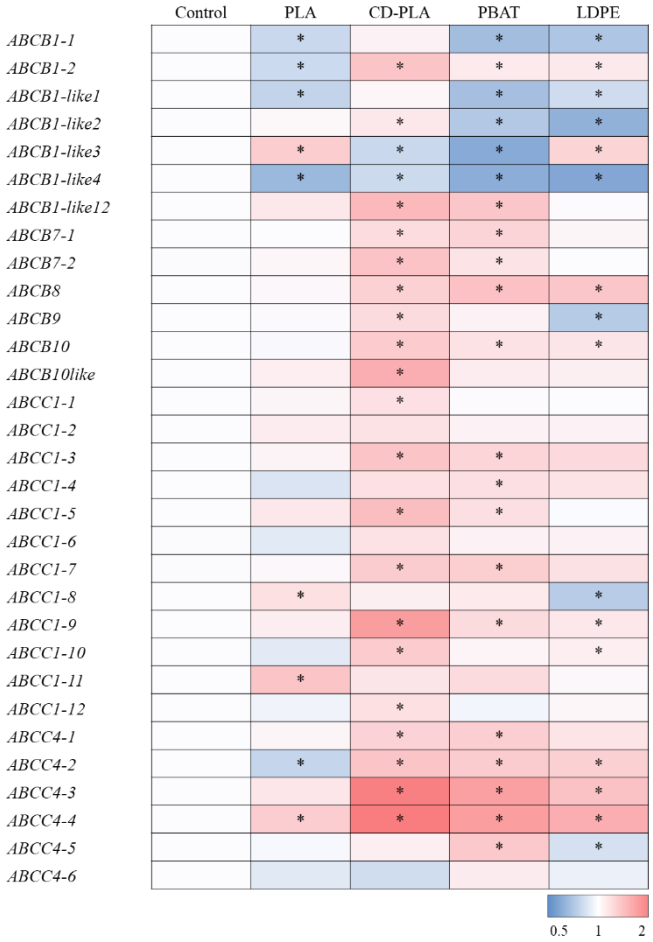


Fig. 5. Gene expression levels of ATP-binding cassette proteins (multixenobiotic resistance; MXR) in marine rotifers *Brachionus plicatilis* exposed to various microplastics (MPs). Asterisks (*) denote significant differences ($P < 0.05$). Detailed expression values are provided in **Table S4**.

Furthermore, 11 genes from the ABCC subfamily demonstrated a marked increase in expression ($P < 0.05$), with no downregulation detected. This consistent upregulation across both subfamilies suggests that CD-PLA may induce a broad activation of MXR-related genes, potentially due to its unique chemical structure and enhanced biodegradability. Similarly, the PBAT-exposed group showed significant upregulation ($P < 0.05$) of six ABCB subfamily genes and downregulation ($P < 0.05$) of five, along with the upregulation of ten ABCC subfamily genes without any downregulation. The parallel upregulation patterns observed in both the CD-PLA and PBAT groups suggest that these biodegradable MPs may trigger similar cellular stress responses, leading to comparable transcriptional activations. In contrast, the LDPE-exposed group exhibited a more diverse and less predictable pattern of gene expression. Five ABCB subfamily genes were significantly downregulated ($P < 0.05$), while four were upregulated. Within the ABCC subfamily, five genes were upregulated, and two were downregulated. This indicates that LDPE, a non-biodegradable plastic, may interact with cellular mechanisms differently, resulting in a more variable transcriptional response.

The MXR system serves as a critical defense mechanism in organisms, activated in response to various environmental stressors and mediated by ABC transporter proteins (Huang et al.

2021), which function as transmembrane efflux pumps. These efflux pumps are located on both the cell membrane and internal organelle membranes, playing a crucial role in maintaining cellular homeostasis by actively transporting endogenous chemicals and xenobiotics out of the cells, thereby preventing their accumulation and mitigating potential toxic effects (Bard, 2000; Franzellitti et al. 2019). P-gp, encoded by the ABCB gene, and MRP, encoded by the ABCC gene, are key members of the ABC transporter proteins family that contribute to the MXR mechanism. A number of studies have documented changes in the activities of both P-gp and MRP, as well as the expression of their encoding genes, in aquatic species after exposure to environmental contaminants, including MPs. For example, exposure of *B. plicatilis* to PS MPs did not lead to significant changes in MXR activity, although P-gps and MRPs showed both upregulation and downregulation (Kang et al. 2021). Similarly, in *Mytilus galloprovincialis*, PS MPs exposure exhibited a marked decrease in MXR expression (Franzellitti et al. 2019). These studies indicate that MP exposure can elicit complex and sometimes contradictory responses within the MXR system, dependent on the species and environmental contaminants. Our results support these findings, showing an increase in P-gp activity and a decrease in MRP activity in *B. plicatilis* exposed to PLA MPs, while only a decrease in MRP activity without changes in P-gp activity was observed, suggesting distinct regulatory responses of the MXR system. Exposure of 5 mg/L PS MPs to *D. magna* resulted in decreased P-gp activity and increased MRP activity, suggesting distinct regulatory mechanisms for these proteins in response to MP exposure (Jeong et al. 2022). However, our results also showed differential MXR activity in *B. plicatilis* depending on the types of MPs to which they were exposed. In contrast to PLA and LDPE MPs exposure, the MXR activity, including both P-gp and MRP proteins, decreased in *B. plicatilis* exposed to CDPLA and PBAT MPs. Similarly, the expression of a few genes was altered in both PLA and LDPE MPs, with CDPLA exposure leading to the upregulation of 9 out of 13 P-gp encoding genes and 11 out of 18 MRP encoding genes, while PBAT exposure resulted in the downregulation of 6 out of 13 P-gp encoding genes and the upregulation of 10 out of 18 MRP encoding genes. In line with our findings, the activities and gene expression of ABC transporters were reported to differ in the rotifer *B. plicatilis* exposed to PS MPs, while a consistent match was observed in *D. magna* exposed to PS MPs and chromium (Kang et al. 2021; Jeong et al. 2022). Our findings reveal that MPs cause significant toxicity in exposed organisms, and the severity of this toxicity varies among different types of MPs. This variability indicates that not all MPs have the same effects on biota.

3.5 Phosphorylation status of the MAPK signaling pathway

The impact of various MPs on stress-related signaling pathways was investigated by examining the activation of ERK, JNK, and p38 kinases in marine rotifers exposed to 0.5 mg/L of PLA, CD-PLA, PBAT, and LDPE **(Fig. 6)**. In the LDPE-exposed group, a marked increase in ERK phosphorylation (p-ERK) was observed, suggesting potent activation of the ERK pathway. However, across all other MP-exposed groups, p-ERK levels remained unchanged compared to the control. The phosphorylation of p38 (p-p38) showed an increase across all MP-exposed groups, though this rise was not statistically significant ($P > 0.05$), except in the LDPE group, where a significant decrease was observed ($P < 0.05$). This suggests that p38 pathway modulation varies depending on the MP type. Phosphorylation of JNK (p-JNK) was significantly higher in the LDPE group ($P < 0.05$) and increased in the PBAT group, though

the latter was not statistically significant. No significant alterations in p-JNK levels were found in the PLA and CD-PLA groups.

The MAPK family consists of protein kinases that relay extracellular signals to the nucleus through phosphorylation cascades (Boutros et al. 2008; Han et al. 2019). These kinases are divided into three main subfamilies: extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38 mitogen-activated protein kinases (p38), which drive the expression of genes linked to various physiological processes (Cowan and Storey, 2003). As a key pathway in stress response (Jeong et al. 2024), MAPK signaling has been widely studied for its role in mediating the toxic effects of environmental pollutants. For instance, Liu et al. (2020) found that PS NPs exposure in *Daphnia pulex* increased ROS levels and activated antioxidant genes via the MAPK pathway. Similarly, Hu et al. (2021) observed that PS NPs triggered oxidative stress and inflammation, leading to p38 MAPK activation and cell apoptosis. Interestingly, in *Tigriopus japonicus*, exposure to multi-walled carbon nanotubes activated MAPK without ROS involvement, indicating potential direct interactions with cell surface receptors (Lee et al. 2016). In another study, Wang et al. (2023) reported that co-exposure of *B. plicatilis* to PS NPs and copper disrupted redox homeostasis and simultaneously activated the ERK signaling pathway, leading to reduced biological activity. These results suggest that ERK induction can occur without an increase in ROS.

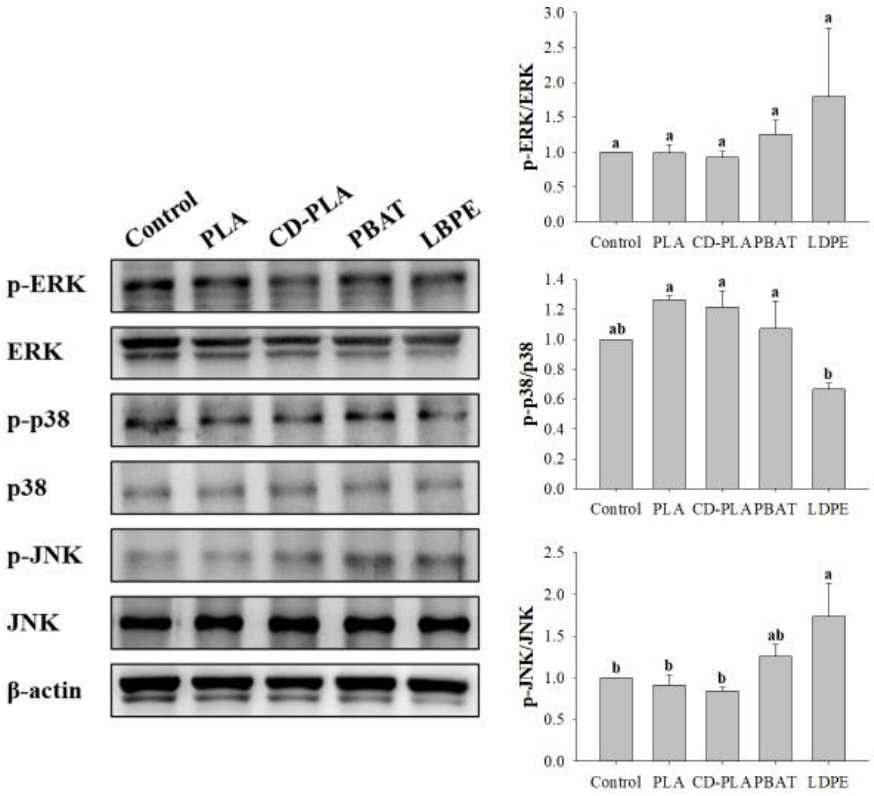


Fig. 6. Phosphorylation levels of MAPK signaling proteins (ERK, JNK, and p38) were measured in marine rotifers (*Brachionus plicatilis*) exposed to microplastics (MPs) (PLA, CD-PLA, PBAT, and LDPE), with β-actin used as the control. Results are displayed as mean ± standard deviation, with significant differences between exposure groups and the control indicated by letters (one-way analysis of variance, Tukey's test, $P < 0.05$). All experiments were conducted in triplicate (n = 3).

However, our findings demonstrate a different trend: we observed a significant decrease in ROS levels alongside an alteration in MAPK phosphorylation. Notably, we found that ERK, p38, and JNK phosphorylation levels varied depending on the type of MPs involved, with both decreases and increases in p38 phosphorylation observed depending on the specific type of MP exposure. Given that p38 and JNK are typically associated with the promotion of apoptosis and proliferation of cells (Wada and Penninger, 2004; Coulthard et al. 2009), the observed alterations in the regulation of JNK level alongside the regulation of ERK level suggest differential effects of MP types on apoptotic activity and proliferative activity within the cell. Overall, our findings reveal that biota respond differently to exposure depending on the specific type of MPs encountered, indicating that the characteristics of each type of MP play a crucial role in shaping the biological response.

4. Conclusion

The growing environmental concerns over plastic pollution have gained significant attention, leading to extensive research on various aspects, ranging from the detection of MPs in the environment to their toxicological profiles in model organisms. A large portion of these studies has centered on MPs originating from fossil fuel-based plastics. Recently, biodegradable and biobased polymers have been developed as eco-friendly alternatives to traditional plastics. However, their rapid expansion has raised concerns regarding the environmental effects of bio-MPs. Despite the growing diversity of plastics entering the environment, limited attention has been given to comparing the toxicity of environmentally relevant MP types that have been reported in aquatic environments. In this study, we aimed to bridge this gap by comparing the toxicity of representative environmentally relevant MPs, including a novel MP derived from a modified version of PLA, chosen to represent the various ways PLA is tailored for enhanced performance and degradability.

In conclusion, this study highlights the differential toxicological impacts of environmentally relevant MPs derived from various sources, including biobased, biodegradable, and fossil fuel-derived polymers, as well as novel MPs on *B. plicatilis*. Even at environmentally relevant concentrations, all MPs significantly reduced reproductive output, indicating a potential impact on our reproductive fitness. All types of MPs also shortened the lifespan of rotifers. The alterations in oxidative stress markers, including ROS levels and antioxidant enzyme activities, highlight the complexity of MP-induced toxicity, with varied responses depending on the type of MP. Additionally, this research underscores the differential effects of MPs on cellular signaling pathways, such as the MAPK pathway, and on the MXR system, particularly in the expression and activity of key detoxification proteins like P-gps and MRPs. These findings suggest that MPs can disrupt cellular redox homeostasis, with specific impacts depending on their chemical and physical properties. These findings underscore the need for a more comprehensive evaluation of the environmental impacts of biobased and biodegradable MPs, particularly in aquatic environments, given the increasing diversity of plastics in these ecosystems.

Although this study focuses on the toxic effects of MPs, the potential long-term behavior and degradation products of biodegradable MPs should be further investigated. Degradation dynamics in marine environments are influenced by factors such as salinity, temperature, UV

exposure, and microbial activity, which can alter the physicochemical properties and toxicity of MPs over time (Ali et al. 2023b; Ali et al. 2024b). These degradation products might interact with aquatic organisms differently than intact MPs, potentially leading to cumulative or distinct toxic effects. Future studies should explore the long-term ecological implications of biodegradable MPs, including their breakdown products, to provide a more comprehensive understanding of their environmental risks.

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Supplementary material

Text S1. Oxidative stress and antioxidant enzymatic activity

To compare intracellular ROS levels and antioxidant enzymatic activity, rotifers were exposed to five different treatments: control, PBAT, CD-PLA, PLA, or LDPE MPs at a concentration of 0.5 mg/L for each type, under optimal conditions of 23 °C and a 12-hour light-dark cycle in an incubator for 24 h. In a parallel experiment, rotifers were exposed to the same MPs concentrations under identical conditions for 6, 12, and 24 h to assess and compare the time-dependent oxidative stress induced by each type of MP. After 24 h, rotifers from each treatment and the control group were collected, filtered, rinsed with artificial seawater (ASW) and phosphate-buffered saline (PBS), and homogenized using a Teflon mini pestle in lysis buffer (40 mM Tris-HCl, 0.1% NP-40, 120 mM NaCl) with an added protease inhibitor cocktail (Roche, Basel, Switzerland). The homogenates were then centrifuged at 4°C and 10,000 g for 10 minutes, and the supernatants were used for subsequent biochemical analyses. Briefly, intracellular reactive oxygen species (ROS) levels were evaluated using a reaction mixture of 10 µL supernatant, 170 µL PBS, and 20 µL of 400 µM 2′,7′-dichlorodihydrofluorescein diacetate. The 200 µL mixture (n=3) was loaded onto a 96-well black plate (SPL, Seoul, South Korea) and incubated at 37°C for 20 minutes. ROS levels were measured with a spectrophotometer (Thermo™ Varioskan Flash, Thermo Electron, Vantaa, Finland) at 485/520 nm. Likewise, the activity of superoxide dismutase (SOD) was measured using an Abcam kit (Cambridge, UK), which is based on the reaction between water-soluble tetrazolium salt and superoxide anions, resulting in the formation of a water-soluble formazan dye. A 200 µL aliquot of this dye (n=3) was transferred to a white 96-well plate, and absorbance was measured at 450 nm using a spectrophotometer. Catalase (CAT) activity was assessed with a Sigma-Aldrich kit, where the decomposition of hydrogen peroxide (H_2O_2) produces a red quinoneimine dye. A 200 µL sample was transferred to a white 96-well plate, and absorbance was recorded at 520 nm. All results were normalized by the quantified protein concentration, determined using the Bradford method (Bradford, 1976). By measuring NADPH consumption, the GR and GPx activities were indirectly detected. Tert-butyl hydroperoxide (t-Bu−OOH) was used as a substrate for GPx analysis. In the process of reducing t-Bu−OOH by GPx, oxidized glutathione (GSH) is produced. This GSH is subsequently reduced to two GSHs using NADPH. Spectrophotometric measurements were utilized to measure the decrease in NADPH absorbance at 340 nm. The oxidation of NADPH during the reduction of oxidized GSH by GR was measured at 340 nm in the case of GR, showing a decreasing absorbance.

Text S2. P-glycoprotein and multidrug resistance-associated protein activity

To evaluate the changes in ATP-binding cassette (ABC) transporter activity in response to different MPs, the levels of P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) were quantified according to the method of Yoo et al. (2024). Rotifers were first exposed to 0.5 mg/L of various types of MPs, as well as a control, at 23 °C for 24 h in a 100 mL beaker. Subsequently, they were transferred to clean ASW and incubated in darkness for 2 h with 0.5 µM rhodamine B and 1 µM calcein AM (both from Sigma-Aldrich), which serve as substrates for P-gp and MRP, respectively. After incubation, the rotifers were rinsed with clean ASW and then subjected to another 1-hour exposure to the MPs, as well as a control, to enhance

the effects on transporter protein activity (Wang et al. 2023). After re-exposure, rotifers were filtered and washed with ASW, followed by fixing a subset from each group in 4% paraformaldehyde. Images were then captured using a fluorescence microscope (Olympus IX71, Olympus Corporation, Tokyo, Japan). The remaining population was rinsed with PBS to remove any residual substances, followed by homogenization in 600 μL of lysis buffer. The homogenates were centrifuged at 10,000g for 10 minutes at 25 °C, and the supernatant was collected. A 200 μL sample of the supernatant was loaded in triplicate onto a black plate, and fluorescence intensity was measured using a spectrophotometer. The following settings were used: for rhodamine B, excitation at 535 nm and emission at 590 nm; for calcein AM, excitation at 485 nm and emission at 535 nm. The fluorescence values were normalized against total protein concentration to ensure accurate comparison.

Text S3. Expression profiles of genes related to antioxidant defenses and ABC transporters

To assess the gene expression profiles of antioxidant enzymes (*CAT* and *SOD*) and the ABC transporter in rotifers exposed to four different types of MPs, rotifers were exposed to each type of MP at a concentration of 0.5 mg/L, alongside a control group, under controlled conditions of 23 °C and a 12-hour light-dark cycle for 24 h. Following the exposure period, rotifers were collected, and total RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The quantity and quality of the extracted RNA were assessed using a Nanodrop spectrophotometer (QIAxpert, QIAGEN GmbH, Hilden, Germany) by measuring the A260/280 ratio. Only samples with A260/280 ratios between 1.9 and 2.0 were selected for downstream applications. cDNA was synthesized from 2 μg of RNA per sample using the SuperScript III reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Specific primer information was noted in **Table S1** and **Table S2**.

For quantitative real-time polymerase chain reaction (qRT-PCR), we used specific primer sets (forward and reverse) for *SOD* (n=6), *CAT* (n=2), *ABCB* (n=13), and *ABCC* (n=18) to confirm differences in the gene profiles of rotifers exposed to different types of MPs and a control group. The qRT-PCR was performed using a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) with the following thermal cycling conditions: initial denaturation at 95 °C for 3 minutes, followed by 35 amplification cycles of denaturation at 95 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 10 minutes. SYBR Green (Molecular Probes Inc., Eugene, OR, USA) was used as the fluorescent dye. Target gene amplification was validated by a melting curve analysis with the following steps: 95 °C for 1 minute, 55 °C for 1 minute, and 80 cycles of 55 °C for 10 seconds with a 0.5 °C increase per cycle. The values were calculated using the $2^{-\Delta\Delta C}$T method (Livak and Schmittgen, 2001). Expression levels were demonstrated in **Table S3** and **Table S4**.

Text S4. Western blotting of mitogen-activated protein kinase signaling proteins

Antibodies against phospho-ERK, p38, SAPK/c-JNK, and phospho-SAPK/JNK (all anti-rabbit) were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies

against phospho-p38 and beta-actin were acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while secondary antibodies (HRP-conjugated goat anti-mouse and anti-rabbit IgG-Fc) were procured from Novus Biologicals (Minneapolis, MN, USA). Rotifers exposed to mg/L of various types of MPs and a control at 23 °C for 24 h were homogenized in lysis buffer (40 mM Tris-HCl, 0.1% NP-40, 120 mM NaCl) with an added protease inhibitor cocktail using a Teflon mini pestle, followed by the collection of the supernatant. Each sample, containing 30 μg of protein, was separated on 8% sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes. These membranes were blocked overnight at 4 °C with 2.5% bovine serum albumin to prevent non-specific binding. The membranes were incubated overnight at 4 °C with primary antibodies (1:1,000 dilution in blocking solution), followed by a two-hour incubation at room temperature with secondary antibodies (1:10,000 dilution in 1X Tris-buffered saline [TBS] with 0.2% Tween 20). The membranes were rinsed six times for 10 minutes in TBS-T before and after the secondary antibody incubation. Protein expression was detected using Immobilon Forte Western HRP substrates, following the manufacturer's instructions.

Table S1. Information about the primers targeting the antioxidant genes superoxide dismutase (*SOD*) and catalase (*CAT*) of *Brachionus plicatilis* used in the real-time polymerase chain reaction.

Subfamily	Gene	Real-Time RT-PCR primer (5'→3')	Amplicon size (bp)	Accession No.
SOD	*BP-CuZnSOD1*	F: TCTGGTGGCATGATCGAT	120	OR644278
		R: ATTACCTAAGTCTCCGGCAT		
	BP-CuZnSOD2	F: TATCACAACTACCAGCGATG	120	OR644279
		R: CCATAGTCGCCTACATGTC		
	BP-CuZnSOD3	F: ATGTGGTTAGTGACGCAAAT	120	OR644280
		R: AATCATCTTCGCCCTCATG		
	BP-CuZnSOD4	F: ACGAAGAAAGACATGTTGGT	120	OR644281
		R: TTCGACCGATCACAGAGTA		
	BP-MnSOD1	F: AGCACCAACTGGAACTTTGA	150	MN836845
		R: CTGATGGCCAGAGATCCGTC		
	BP-MnSOD2	F: AAGCATTCTCTTCCAGATTTG	199	MN836844
		R: TGAACTTCAAGGCTGATGTTA		
CAT	*BP-CAT1*	F: TGCCACTTACTGGTGAAGCA	150	MN836846
		R: ATTCGGCAGCACCACTCAAA		
	BP-CAT2	F: GGAATCGAGCCATCACCAGA	150	MN836847
		R: GCATTGTGGACCATCACGTT		
Reference	*BP-ELFA*	F: CAACGTTAAAAACGTTTCAGTC	123	AB513493
		R: CCTGGATGGTTCAATACAATTAC		

Table S2. Information about the primers targeting the ATP-binding cassette (*ABC*) transporter genes of *Brachionus plicatilis* used for real-time polymerase chain reaction.

Subfamily	Gene	Real-Time RT-PCR primer (5'→3')	Amplicon size (bp)	Accession No.
	Bp-Abcb1-1	F: GCAACATACGAACTGTAGCCA R: CTCCAAGAGCAAAAGCAGC	177	MT524872
	Bp-Abcb1-2	F: GGAGCAGTTGCCGAAGAA R: GCCATACCAAAATCCAAGC	201	MT524873
	Bp-Abcb1-like1	F: CAATGGTCAGCTAGAAATGG R: AGCTAAGAGATTGTTCCTCCG	226	MT524874
	Bp-Abcb1-like2	F: CCAAGCGTTGCCATATCTA R: GCAGGAATCCTCAGACTCAATC	219	MT524875
	Bp-Abcb1-like3	F: GCCATCATTCAACCAGCA R: TGAACATGAGAGTGCGGAT	214	MT524876
ABCB	*Bp-Abcb1-like4*	F: TAGTGAGGCTTCTTTGGGTA R: GGGACATGGAGCTTAAATAATC	171	MT524877
	Bp-Abcb1-like12	F: TCTGCCTCCCGATGAACA R: CTGGGTGGCTAGAATGCTC	226	MT524878
	Bp-Abcb7-1	F: ATAATCACGCCATCCAAGTC R: TATCAATACCCTGCCTTCCA	204	MT524879
	Bp-Abcb7-2	F: GATTCAAGTATCAAGAGGGAGC R: TGCGGTACAATGCCAATC	226	MT524880
	Bp-Abcb8	F: GAGTGTTGTATGCTGGTGGC R: CATCAACTGGAATGCTTGGC	201	MT524881
	Bp-Abcb9	F: TACACTGGACTGATGAACGCA R: AAGCGATTATTTCTCCTGGC	211	MT524882
	Bp-Abcb10	F: AGTGGAAAGAGCACATTGGC R: TGGATTTGGCACACCGTAA	195	MT524883
	Bp-Abcb10-like	F: AGTGTTGTAGCCGAAGAGACC R: TTTCCGCCATACCATAAGAC	207	MT524884
	Bp-Abcc1-1	F: GCAATACTCCACAAGAAGCC R: CAGTTCTGCCAACTATTCCAA	185	MT524885
	Bp-Abcc1-2	F: TTGTAGCCGGTGATAGAACAG R: AACATGAGCATCAACTGCAC	149	MT524886
	Bp-Abcc1-3	F: TTACTTGGATTCGCTCAATAC R: ATTGATGACATCCTTGCTAAAT	243	MT524887
	Bp-Abcc1-4	F: TCCTTGAGGCATTGGAACAT R: CTCATCTGTGTTCGGGTCAA	203	MT524888
	Bp-Abcc1-5	F: CTGCGAGACACCACATGAG R: TTCCTGCTCCAGTTCTTCCA	197	MT524889
	Bp-Abcc1-6	F: AAGACTTTCGTCCCAATCAC R: ATGACCATAACACCGACTCC	201	MT524890
	Bp-Abcc1-7	F: ATTCTATGGCTGGGAAGTATC R: CTTGGATGATGTAGGGTAAA	266	MT524891
ABCC	*Bp-Abcc1-8*	F: CATCGGCACCGTTCATT R: TCGTCCATCAGTAACAAGCG	211	MT524892

	Bp-Abcc1-9	F: GGTTCAACTGCTTATGTATCGC R: AGCCTTGGCATACACAGAAC	243	MT524893
	Bp-Abcc1-10	F: AGCGTGATAATGATAATGAATTC R: ATTGATGACATCCTTGCTAAAT	138	MT524894
	Bp-Abcc1-11	F: ACTCGGGTCGGCTATGAT R: TTGTGCCATGCTTCTACG	164	MT524895
	Bp-Abcc1-12	F: TGACTCGTGACTTGTTAGATGT R: TCTGGTTCGTACTCCTGCC	191	MT524896
	Bp-Abcc4-1	F: CCGTCGAGTCTGTCAGTCTAA R: ATAGTCCACATTGGCAGTTG	183	MT524897
	Bp-Abcc4-2	F: TATTCCAAAAGAGTTGTTGAGTG R: TCATTATCATTCTCAATCGGA	156	MT524898
	Bp-Abcc4-3	F: AAGGCAGCGTGTTCTATGTA R: GCTCTGGCAACACTCACTCTA	229	MT524899
	Bp-Abcc4-4	F: CCGAATCCATTGAACGAGG R: CGGAACATTGCATTGAACA	215	MT524900
	Bp-Abcc4-6	F: ATCGGGACCAAGGAATCA R: TCCAGTCTAAGAGCAAACCAT	382	MT524902
Reference	*BP-ELFA*	F: CAACGTTAAAAACGTTTCAGTC R: CCTGGATGGTTCAATACAATTAC	123	AB513493

Table S3. Fold-changes in catalase (*CAT*) and superoxide dismutase (*SOD*) genes in *Brachionus plicatilis* exposed to 0.5 mg/L polylactic acid (PLA), poly(butylene adipate-co-terephthalate) (PBAT), β-cyclodextrin grafted polylactic acid (CD-PLA), or low density polyethylene (LDPE) microplastics (MPs). Each letter indicates a significant ($P < 0.05$) difference in the expression of the target genes.

Gene Family	Gene	Control	PLA	CDPLA	PBAT	LDPE
CAT	*CAT1*	1.00 ± 0.02^{a}	1.03 ± 0.03^{a}	1.17 ± 0.03^{b}	1.03 ± 0.06^{a}	0.98 ± 0.02^{a}
	CAT2	1.00 ± 0.11^{a}	1.08 ± 0.04^{a}	1.34 ± 0.01^{b}	1.06 ± 0.08^{a}	0.99 ± 0.02^{a}
SOD	*CuZnSOD1*	1.00 ± 0.06^{a}	1.21 ± 0.06^{ab}	1.29 ± 0.14^{b}	1.04 ± 0.04^{ab}	1.16 ± 0.08^{ab}
	CuZnSOD2	1.00 ± 0.08^{a}	1.23 ± 0.08^{a}	1.12 ± 0.05^{a}	1.24 ± 0.11^{a}	1.16 ± 0.03^{a}
	CuZnSOD3	1.00 ± 0.03^{a}	0.62 ± 0.03^{b}	3.21 ± 0.15^{c}	1.71 ± 0.03^{d}	1.78 ± 0.10^{d}
	CuZnSOD4	1.00 ± 0.06^{ab}	1.13 ± 0.06^{bc}	1.18 ± 0.03^{c}	1.27 ± 0.05^{c}	0.91 ± 0.05^{a}
	MnSOD1	1.00 ± 0.06^{a}	1.12 ± 0.10^{ab}	1.19 ± 0.02^{ab}	1.28 ± 0.08^{b}	1.08 ± 0.05^{ab}
	MnSOD2	1.00 ± 0.03^{a}	1.20 ± 0.01^{b}	1.01 ± 0.08^{a}	1.21 ± 0.03^{b}	1.09 ± 0.02^{ab}

Table S4. Fold-changes in ATP-binding cassette (*ABC*) transporter genes in *Brachionus plicatilis* exposed to 0.5 mg/L polylactic acid (PLA), poly(butylene adipate-co-terephthalate) (PBAT), β-cyclodextrin grafted polylactic acid (CD-PLA), or low density polyethylene (LDPE) microplastics (MPs). Each letter indicates a significant ($P < 0.05$) difference in the expression of the target genes.

Gene family	Gene	Control	PLA	CDPLA	PBAT	LDPE
ABC transporter family B	*ABCB1-1*	1.00±0.03^{b}	0.85±0.01^{a}	1.07±0.06^{b}	0.73±0.06^{a}	0.76±0.01^{a}
	ABCB1-2	1.00±0.03^{a}	0.85±0.03^{b}	1.38±0.06^{c}	1.12±0.02^{d}	1.13±0.03^{d}
	ABCB1-like1	1.00±0.03^{a}	0.82±0.05^{bc}	1.05±0.03^{a}	0.74±0.05^{b}	0.86±0.02^{c}
	ABCB1-like2	1.00±0.04^{b}	1.04±0.05^{bc}	1.14±0.06^{c}	0.78±0.01^{a}	0.67±0.04^{a}
	ABCB1-like3	1.00±0.03^{c}	1.32±0.03^{b}	0.85±0.01^{d}	0.64±0.03^{a}	1.28±0.06^{b}
	ABCB1-like4	1.00±0.04^{c}	0.70±0.05^{ab}	0.85±0.05^{bc}	0.66±0.03^{a}	0.63±0.05^{a}
	ABCB1-like12	1.00±0.04^{a}	1.14±0.06^{ab}	1.46±0.06^{c}	1.37±0.14^{bc}	1.02±0.02^{a}
	ABCB7-1	1.00±0.05^{a}	1.01±0.01^{a}	1.22±0.02^{bc}	1.27±0.08^{c}	1.05±0.06^{ab}
	ABCB7-2	1.00±0.03^{a}	1.04±0.05^{ab}	1.39±0.03^{c}	1.17±0.05^{b}	1.00±0.08^{a}
	ABCB8	1.00±0.06^{a}	1.04±0.05^{a}	1.30±0.09^{b}	1.41±0.08^{b}	1.37±0.01^{b}
	ABCB9	1.00±0.04^{a}	1.02±0.01^{a}	1.23±0.10^{b}	1.07±0.02^{ab}	0.79±0.02^{c}
	ABCB10	1.00±0.03^{a}	0.99±0.04^{a}	1.34±0.08^{b}	1.18±0.03^{c}	1.16±0.03^{c}
	ABCB10like	1.00±0.06^{a}	1.10±0.06^{a}	1.53±0.06^{b}	1.11±0.02^{a}	1.09±0.06^{a}
ABC transporter family C	*ABCC1-1*	1.00±0.03^{a}	1.05±0.02^{a}	1.19±0.03^{b}	1.02±0.04^{a}	1.01±0.02^{a}
	ABCC1-2	1.00±0.01^{a}	1.11±0.03^{a}	1.18±0.08^{a}	1.08±0.07^{a}	1.07±0.06^{a}
	ABCC1-3	1.00±0.09^{a}	1.06±0.04^{a}	1.39±0.04^{b}	1.27±0.10^{ab}	1.24±0.12^{ab}
	ABCC1-4	1.00±0.03^{ab}	0.90±0.02^{a}	1.19±0.08^{bc}	1.20±0.05^{c}	1.17±0.08^{bc}
	ABCC1-5	1.00±0.01^{a}	1.15±0.07^{ab}	1.43±0.06^{c}	1.20±0.03^{b}	1.00±0.06^{a}
	ABCC1-6	1.00±0.08^{a}	0.92±0.06^{a}	1.18±0.09^{a}	1.07±0.09^{a}	1.07±0.12^{a}
	ABCC1-7	1.00±0.07^{a}	1.04±0.03^{a}	1.33±0.05^{b}	1.32±0.08^{b}	1.19±0.07^{ab}
	ABCC1-8	1.00±0.04^{a}	1.20±0.03^{b}	1.09±0.06^{ab}	1.13±0.07^{ab}	0.79±0.04^{c}
	ABCC1-9	1.00±0.01^{a}	1.10±0.01^{ab}	1.65±0.06^{c}	1.23±0.04^{b}	1.14±0.05^{b}
	ABCC1-10	1.00±0.02^{ab}	0.92±0.01^{a}	1.34±0.03^{d}	1.05±0.02^{bc}	1.10±0.04^{c}
	ABCC1-11	1.00±0.13^{a}	1.38±0.05^{b}	1.16±0.09^{ab}	1.23±0.12^{ab}	1.09±0.06^{a}
	ABCC1-12	1.00±0.07^{a}	0.96±0.03^{a}	1.20±0.07^{b}	0.97±0.07^{a}	1.10±0.10^{ab}
	ABCC4-1	1.00±0.05^{a}	1.05±0.03^{a}	1.29±0.09^{b}	1.31±0.06^{b}	1.09±0.12^{ab}
	ABCC4-2	1.00±0.05^{a}	0.83±0.07^{b}	1.38±0.02^{c}	1.33±0.02^{c}	1.12±0.15^{c}
	ABCC4-3	1.00±0.04^{a}	1.15±0.02^{a}	1.85±0.10^{c}	1.63±0.04^{d}	1.30±0.04^{b}
	ABCC4-4	1.00±0.07^{a}	1.32±0.02^{b}	1.87±0.04^{c}	1.64±0.04^{d}	1.53±0.07^{d}
	ABCC4-5	1.00±0.09^{a}	0.99±0.01^{a}	1.10±0.12^{ab}	1.35±0.05^{b}	0.88±0.07^{a}
	ABCC4-6	1.00±0.12^{a}	0.92±0.09^{a}	0.87±0.12^{a}	1.12±0.15^{a}	0.95±0.06^{a}

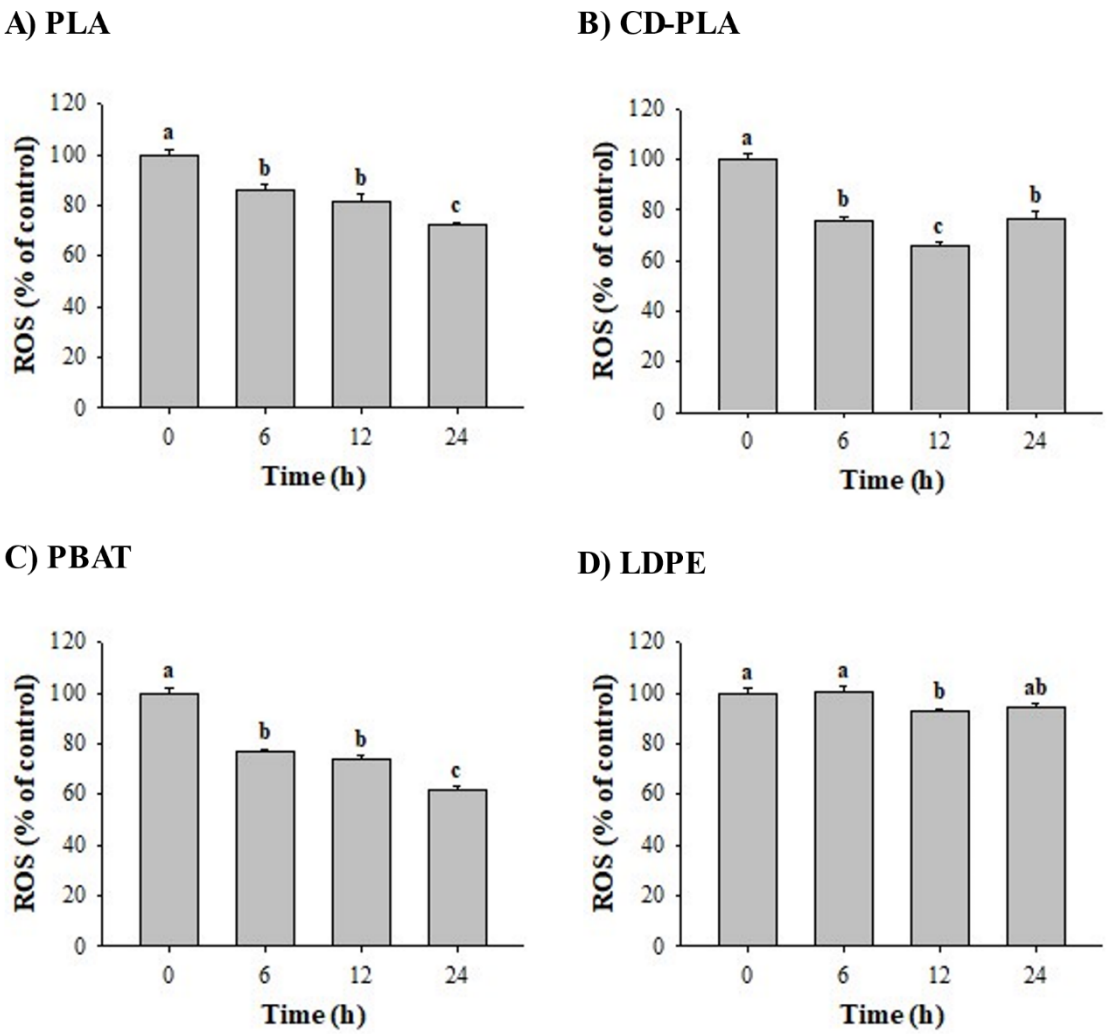

Fig. S1. Time-dependent effects of 0.5 mg/L polylactic acid (PLA), poly(butylene adipate-co-terephthalate) (PBAT), β-cyclodextrin grafted polylactic acid (CD-PLA), or low density polyethylene (LDPE) microplastics (MPs) on intracellular reactive oxygen species (ROS) of *Brachionus plicatilis*. The bars represent the mean ± standard deviation of experimental results, and different letters above each column indicate a significant difference between groups ($P < 0.05$), using one-way ANOVA with Tukey's test.

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Paper VI

Biodegradable microplastics interaction with pollutants and their potential toxicity for aquatic biota: a review

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Biodegradable microplastics interaction with pollutants and their potential toxicity for aquatic biota: a review

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Abstract

The global plastic production has steadily increased from 1.7 million tons in 1950 to over 400 million tons in 2022, with about 60% of plastic ultimately ending up in landfills and oceans. There is also growing evidence that microplastics exert negative effects on biota and ecosystems. Biodegradable plastics may represent a safe alternative, yet their potential adverse effects have not been comprehensively analyzed. Here, we reviewed biodegradable plastics, with focus on their conversion into microplastics, their interactions with pollutants, and their combined toxicity for aquatic biota. Biodegradable plastics include polylactic acid, polyhydroxyalkanoates, polybutylene succinate, poly(butylene adipate-*co*-terephthalate), and poly(ε-caprolactone). We found that some biobased plastics are hardly biodegradable. Some biobased plastics are compostable but require specific environmental conditions for their biodegradation. Biobased plastics can generate microplastics when released into the environment, which can impact biota. Contrary to the common public belief, biodegradable plastics may not only originate from biosources but can be synthesized from fossil fuels. Microplastics originating from biodegradable plastics can interact with pollutants, adsorbing and transporting these pollutants, resulting in synergistic or antagonistic effects on exposed organisms. Biofilm formation on microplastics impacts their degradation and pollutant interactions.

Keywords. Bioplastics synthesis and degradation; Polylactic acid toxicity; Adsorption of pollutants by microplastic; Co-exposure of microparticles and heavy metal; Biofilm formation on microplastic; Biobased versus petrochemical based plastics.

1. Introduction

Since the industrial manufacturing of plastic in the 1940s, their annual production is rising with a growth rate of 9% worldwide (Chen et al. 2021). For instance, global plastic production surged from 1.7 million tons in 1950 (Geyer et al. 2017) to over 400 million tons in 2022 (PlasticsEurope 2023). Approximately 60% of all plastic ever produced accumulates in landfills and oceans (Geyer et al. 2017). Once in the natural environment, plastic waste is susceptible to weathering processes, leading to its fragmentation into microplastics (1–5000 µm) and nanoplastics (less than 1 µm) (Atugoda et al. 2023; Ali et al. 2023a). Microplastics have been detected in nearly all types of environmental media, with most recently, within high-altitude clouds and snow (Wang et al. 2023; Babaei et al. 2023). Due to their small size and persistent nature, microplastics remain available to interact with biota. In this context, the accumulation of microplastics in aquatic organisms has been documented in a wide range of species, spanning from zooplankton (Sun et al. 2018; Thery et al. 2022) to fish (Shabaka et al. 2020; Hamed et al. 2023), and even through bioaccumulation within the food chain via trophic transfer (Costa et al. 2020; Mariani et al. 2023). Consequently, a variety of implications have been reported in exposed individuals, including physical damage (Eltemsah et al. 2019), immune system obstruction (Chen et al. 2022), and even mortality (Eom et al. 2020). These implications raise concerns about the accumulation and biomagnification of microplastics and the subsequent risks they may pose to environment and human health.

Microplastics are known for their durability, hydrophobic nature, and specific surface characteristics, which make them prone to interact with other coexisting pollutants (Osman et al. 2023; Ali et al. 2023a). The adsorption of diverse pollutants onto microplastics, including polycyclic aromatic hydrocarbons (José and Jordao, 2022; Kong et al. 2023) and heavy metals (Gao et al. 2021; Santos-Echeandía et al. 2020), has been recently reported, indicating the high affinity of microplastics for coexisting pollutants. Moreover, environmental weathering processes further increase the susceptibility of microplastics to adsorb pollutants. For instance, it has been observed that the weathering of microplastics enhances the adsorption of heavy metals onto their surfaces (Aghilinasrollahabadi et al. 2021; Gao et al. 2021; Santos-Echeandía et al. 2020). Once pollutants are adsorbed onto microplastic surfaces, they become more bioavailable to certain organisms (Costigan et al. 2022), potentially leading to biomagnification at higher trophic levels when ingested. Microplastics can increase the accumulation of specific coexisting pollutants (Wang et al. 2022; Zhang et al. 2019; Yan et al. 2020) in exposed organisms, highlighting microplastics as vehicles for the transport of different pollutants.

Considering the detrimental ecological impacts of non-biodegradable petrochemical plastics, there has been a growing focus on plastics considered as biodegradable as an alternative to mitigate the issue of plastic pollution (Nandhini et al. 2023). They are used in many applications notably in the packaging and biomedical fields. According to EuropeanBioplastics (2023), biobased and biodegradable plastics are set to increase from two 2.1 million tons in 2023 to approximately 7.4 million tons in 2028. Among them, one should also distinguish natural polymer derivatives, such as polysaccharides like cellulose and starch, from purely synthetic biobased and biodegradable polymers. Indeed, the former may be able to degrade in natural environment (Zambrano et al. 2019), while some of the latter are known to be persistent. Thus, those persistent fully synthetic biodegradable plastics can further fragment

into microplastics and, as non-biodegradable fossil fuel plastics, act as vectors for pollutants. Several review papers have summarized various aspects of biodegradable microplastics, including their formation (Qin et al. 2021), their toxic effects on biota (Malafeev et al. 2023; Ribba et al. 2022), as well as the adsorption of pollutants (Torres et al. 2021). However, a comprehensive presentation on the interaction of microplastics, originating from biodegradable plastics made chemically and those produced by microorganisms, with co-occurring pollutants and their environmental fate, such as their impact on aquatic biota has not yet been documented, which is the objective of the present contribution.

2. Synthetic biodegradable plastics

Biodegradable plastics are polymeric materials capable of undergoing natural degradation primarily due to biological activity. Ideally, these polymers break down into natural substances, such as CO_2, H_2O, CH_4, and biomass through microbial action. This process leaves no toxic residues and allows for seamless integration with nature as part of the carbon cycle. Environmental factors like oxygen, nutrient availability, temperature, humidity, microorganisms, and pH play a role in determining the biodegradability of plastics (Elsawy et al. 2017). It is important to recognize that biodegradable plastics can be synthesized from various sources, including both renewable, such as biobased, and non-renewable, like fossil fuel-based, feedstocks. They share the common characteristic of being capable of natural degradation and or under specific conditions.

A polymer's biodegradability in a specific environment depends on its molecular structure, including factors such as crystallinity, molecular weight, stereochemistry, hydrophilicity, susceptibility to enzymatic breakdown, chain flexibility, and amorphous region size (Polman et al. 2021). In general, biodegradation involves two main steps: firstly, long chains are cleaved into small molecules through hydrolysis. Hydrolysis may occur through microbial hydrolytic enzymes, referred to as biotic hydrolysis, or through environmental factors, known as abiotic hydrolysis. Secondly, mineralization occurs, where these molecules are further breakdown by microbes and eventually mineralized into environmentally benign compounds such as water and carbon dioxide (Suzuki et al. 2021). Furthermore, the rate of mineralization depends on environmental factors, chemical structure of the polymer (Federici et al. 2022) as well as the abundance of polymer degrading microorganisms in the medium. The complex nature of biodegradation and its dependence to various factors is certainly linked to the fact that these plastics biodegrade under conditions that are not always found in natural environments.

In this regard, considering biodegradable plastics as panacea may be questionable. The escalating production of biodegradable plastics has raised concerns regarding their potential contribution to microplastic pollution. Recently, microplastics derived from biodegradable plastics have already been documented in the natural environment (Granberg et al. 2019; Kazour et al. 2019), and their consequent impact on various aquatic organisms has been reported (Yokota and Mehlrose 2020; Duan et al. 2022; Khalid et al. 2021). In the following section, we will discuss the generation of microplastics from synthetic biodegradable plastics (**Fig. 1**), sorption of different pollutants on their surfaces followed by their combined toxicity, with focus on aquatic biota. The structure of these polyesters is given in **Table 1.**

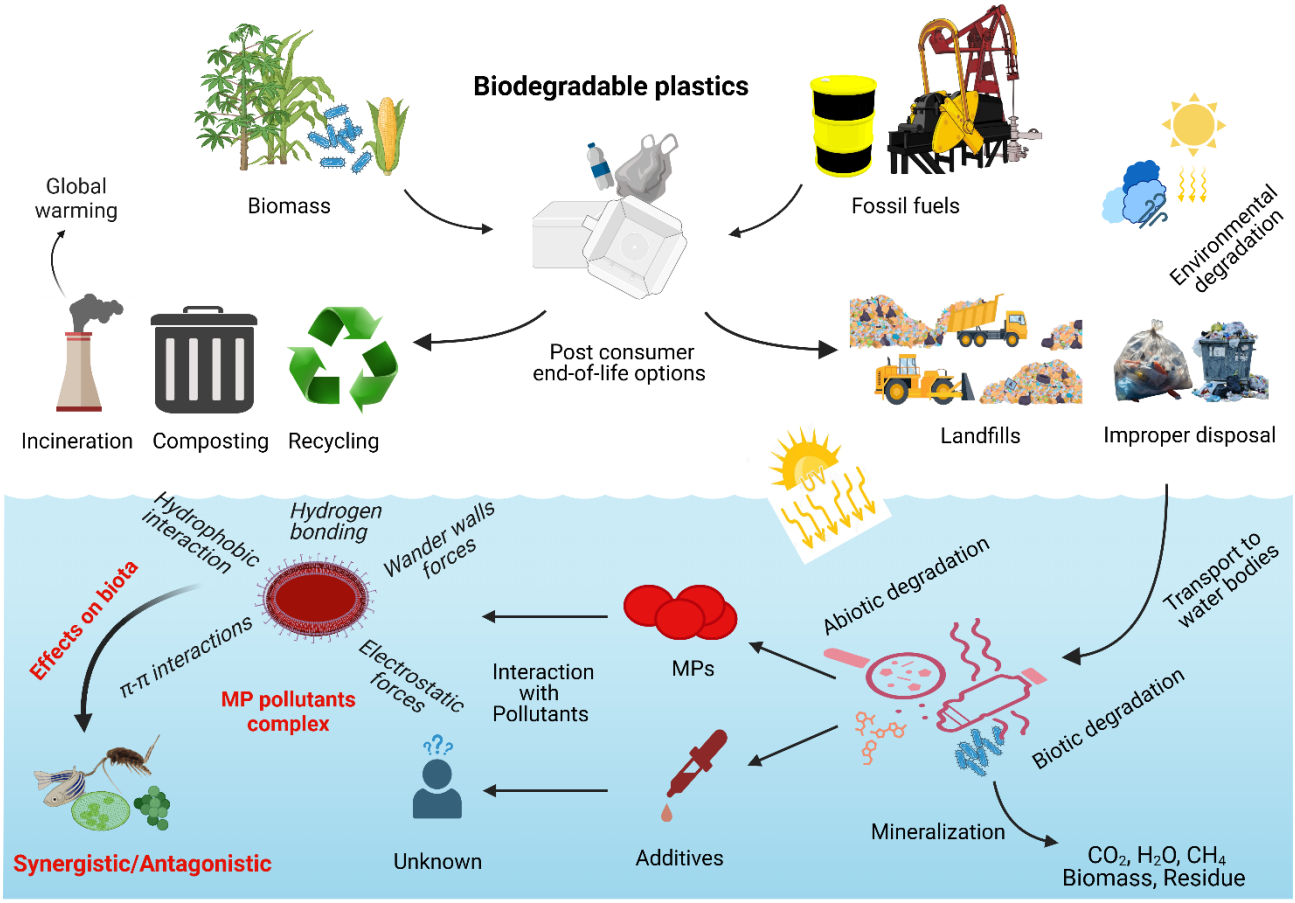


Fig. 1: Biodegradable plastics from production to end of life and environmental fate. Biodegradable plastics are synthesized from both biomass and fossil fuels. After use, major end-of-life options include incineration, recycling, composting, and landfills. The latter dominate the available end-of-life options for plastics, where necessary conditions for biodegradation, such as specific temperature, moisture, and microbes, may not necessarily be present. This can lead to the formation of microplastics and the release of leachates, which could then be leaked into the aquatic environment. Additionally, mismanaged and inadequate disposal, especially of single-use items like bottles and bags, significantly contributes to large amounts of plastic finding its way into aquatic bodies. They have the potential to release leachates and produce microplastics under aquatic environment. The interaction of these leachates with other pollutants and their subsequent fate has not yet been studied. Meanwhile, the interaction of their microplastics with coexisting pollutants demonstrates a strong adsorption ability. The effects of microplastics loaded with pollutants have been studied to a limited extent and have shown both synergistic and antagonistic effects on aquatic biota.

3. Biodegradable microplastics and their toxicity

3.1 Polylactic acid (PLA)

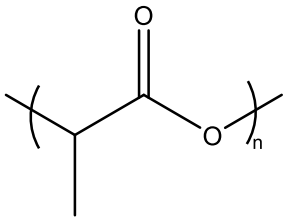
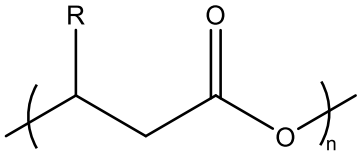
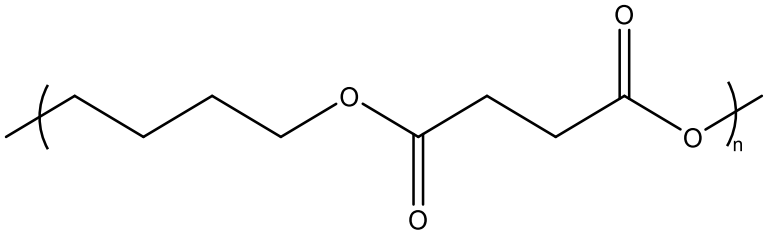
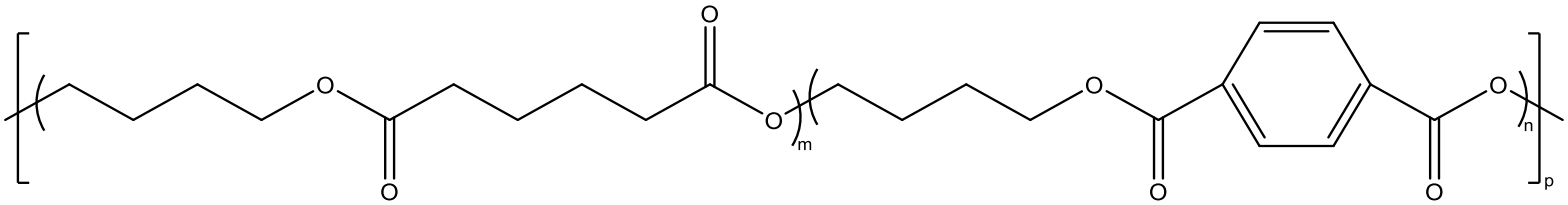
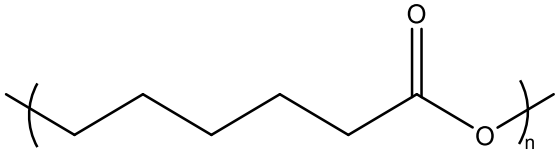
Polylactic acid is one of the most popular low-weight, semicrystalline, biobased polymer synthesized from renewable resources. It is typically produced through the ring-opening polymerization of diastereomeric lactides, although polycondensation of lactic acid is also a viable method (Meimoun et al. 2020). Polylactic acid exhibits a Young's modulus of 1–3 GPa, an elongation at break of 6–7%, and a glass transition temperature (Tg) ranging from 55 to 60

°C. It is one of the most widely produced bioplastics, representing 31.0% of all biobased and biodegradable plastics in 2023 and projected to increase to 43.6% by 2028 (EuropeanBioplastics 2023). This thermoplastic polyester is found on the market since the 1990s and finds applications ranging from single-use packaging to biomedical and textiles (Meimoun et al. 2022; Meimoun et al. 2021). It is also utilized in 3D printing technologies (Chacón et al. 2017). However, its predominant market segments include food packaging and single-use items (Jem and Tan, 2020; Ali et al. 2023b), while broader adoption in other sectors has been hindered primarily by its inferior mechanical properties compared to fossil fuel-based plastics.

Polylactic acid is considered as a promising candidate for replacing fossil fuel-based plastics, particularly in reducing reliance on fossil fuels and decreasing carbon dioxide emissions. However, its efficacy in reducing environmental pollution may be compromised due to reports of microplastic formation during its degradation in simulated aquatic conditions (Le Gall et al. 2022). For example, Lambert and Wagner (2016) studied the degradation of polylactic acid within a weathering chamber for 16 weeks. Their findings revealed a notably high microplastic release rate, amounting to 11.6×10^6 particles per milliliter. These microplastics could be further degraded to form secondary microplastics and even nanoplastics. Another example, is provided by Tong et al. (2022) who studied the degradation of polylactic acid microplastics (3-mm) along with other biodegradable and non-biodegradable microplastics under simulated mechanical abrasion and UV aging for 21 days. The authors reported that 96.7% of the microplastics formed secondary microplastics with an average size of 97.8 ± 21.3 nm under mechanical abrasion and 69.0 ± 8.5 nm under UV aging. These results indicate that the viability of polylactic acid must be critically assessed in terms of the environmental concerns related to microplastic and nanoplastic pollution.

In recent years, polylactic acid microplastics have been detected in various environmental compartments (Bancin et al. 2019; Kazour et al. 2019), including wastewater (Granberg et al. 2019; Wagstaff and Petrie, 2022). Furthermore, numerous laboratory studies have confirmed their toxicological effects on various aquatic model organisms. For example, polylactic acid microplastics have been reported to cause high mortality and decrease in reproductive output in zooplankton *Daphnia magna* (Zimmermann et al. 2020), reduce survival in worms (*Lumbriculus variegatus* and *Arenicola marina*) (Klein et al. 2021), as well as alteration in the immunological profile of hemolymph and reduction in filtration of blue mussels (*Mytilus edulis*) (Green et al. 2019; Green et al. 2017). Furthermore, exposure to polylactic microplastics induce oxidative stress and decrease superoxide dismutase (SOD) activity in dragonfly larvae (*Aphylla williamsoni*) (Chagas et al. 2021), neurotoxic effects in tadpoles (*Physalaemus cuvieri*) and fish (*Danio rerio*) (Malafaia et al. 2021; de oliveira et al. 2021), reproductive toxicity in tunicates (*Microcosmus exasperatus*) (Anderson and Shenkar, 2021), and skeletal development of fish (*D. rerio*) (Zhang et al. 2021). These findings challenge the ecofriendly nature of polylactic acid, particularly concerning a potential role in reducing microplastic pollution, as they indicate both its conversion into microplastics and its potential adverse effects on aquatic organisms and ecosystems.

Table 1. Structures of the selected synthetic biodegradable polyesters.

Polyester	Structure
Polylactic acid (PLA)	
Polyhydroxyalkanoates (PHA)	 R= CH_3 or CH_2CH_3
Polybutylene succinate (PBS)	
Poly(butylene adipate-*co*-terephthalate) (PBAT)	
Poly(ε-caprolactone) (PCL)	

3.2 Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates are a class of natural aliphatic polyesters known for their *in vivo* production by microorganisms. They are synthesized by many bacterial strains as storage molecules for energy sources, followed by biological extraction and chemical extractions (Boey et al. 2021). For instance, in biological extractions, polyhydroxyalkanoates rich cells are consumed by living organisms, such as insects, which can excrete up to 90% of the undigested biopolymer, followed by a simple purification process (Chee et al. 2010). On the other hand, chemical extractions involve the use of chemicals coupled with a range of techniques to extract polyhydroxyalkanoates from the intracellular granules of bacteria (Kunasundari and Sudesh 2011). The high cost and low volume of the microbial fermentation pathway hinder its widespread applications. Therefore, efficient chemical synthesis of polyhydroxyalkanoates has been a focus since the 1960s. Until now, two main strategies have been developed, including the ring-opening polymerization of lactones such as β-butyrolactone and eight-membered cyclic diolide (Zhang et al. 1990; Tang et al. 2019). Furthermore, recent research has been investigating the use of waste biomass as carbon sources to produce polyhydroxyalkanoates (Hierro-Iglesias et al. 2022). These biopolyesters are categorized into three groups: short-chain (3 to 5 carbon atoms), medium-chain (6 to 14 carbon atoms), and long chain (15 or more carbon atoms) based on their structural characteristics (Mahato et al. 2023). These biopolyesters exhibit variable glass transition temperatures (−50 to 4 °C) and melting temperatures (40 to 180 °C) based on composition and chain length. These properties, along with degradation kinetics, vary significantly depending on polyhydroxyalkanoates type, composition, and molecular weight (Raza et al. 2018). These biopolyesters accounted for 4.8% of the total biobased and biodegradable plastics production in 2023 and have been projected to increase to 13.5% by 2028 (EuropeanBioplastics 2023), more than a twofold increase.

Polyhydroxyalkanoates have been considered as alternatives to fossil fuel-based plastics, primarily due to their biobased nature, coupled with biocompatible and biodegradable properties. For example, in the landscape of biodegradable polyesters, they show along with poly(ε-caprolactone) higher composting rate at room temperature than polylactic acid and polybutylene succinate (Mouhoubi et al. 2022). They have found applications in various biomedical fields (Gheibi et al. 2016), single-use items (EuropeanBioplastics 2023), and agricultural film mulching to replace fossil fuel-based plastics, such as polyethylene (Corbin et al. 2013). However, their mechanical properties, such as low impact strength, and the use of solvents for their extraction, which cost around 50% of the product for recovery (Fiorese et al. 2009), are their major drawbacks. According to Dilkes-Hoffman et al. (2019), a polyhydroxyalkanoate bottle with a wall thickness of 800-µm could take one and a half to three and a half years for complete biodegradation in the marine environment, suggesting the potential for microplastic generation before complete mineralization. For instance, Shruti et al. (2019) investigated the degradation of polyhydroxyalkanoates in tap water and drinking water under 23 ± 4 °C for a duration of 30 days and reported the formation of microplastics having size range of 25-µm to 1-mm, respectively. Furthermore, polyhydroxyalkanoates microplastics have been reported to produce more secondary nanoplastics upon their degradation compared to other biobased and petrochemical microplastics (Liu et al. 2023a).

In this context, the toxicity of polyhydroxyalkanoates microplastics has been assessed by

various aquatic species. The reported results show that these microplastics affect the assimilation efficiency of the amphipod *Gammarus fossarum* (Straub et al. 2017), induce changes in physiological parameters of *D. magna*, *Anabaena* sp., and *Chlamydomonas reinhardtii* (González-Pleiter et al. 2019), reduced growth of *Paracentrotus lividus* larva (Uribe-Echeverría and Beiras 2022), alteration in heart, gills and muscles tissues of *Lates calcarifer* (Sai et al. 2022), cause delays and malformations in *P. lividus* embryos (Viel et al. 2023), effect behavior and feeding of water flea, *D. magna* (Savva et al. 2023), as well as inhibition of the denitrification process of *Paracoccus denitrificans* (Pang et al. 2023). These findings underscore the necessity for further research to explore the environmental impact of polyhydroxyalkanoates on aquatic biota. Indeed, while polyhydroxyalkanoates offer promising biodegradable alternatives to petrochemical plastics, their potential environmental impact, including microplastic generation and toxicity to biota, necessitates careful consideration and additional investigation.

3.3 Polybutylene succinate (PBS)

Polybutylene succinate is a biodegradable polymer that can be biobased and fossil-based. It is synthesized through a condensation polymerization process via esterification, in which 1,4-butanediol reacts with succinic acid, or transesterification, where 1,4-butanediol reacts with dimethyl succinate, to produce oligomers followed by the polycondensation of these oligomers to form high molecular weight polybutylene succinate polymer (Barletta et al. 2022). Commercial production of polybutylene succinate, primarily fossil fuel-based, initiated in Japan in the 1930s, with a transition to biobased production inaugurated in Thailand in 2015, followed by China and Korea (Platnieks et al. 2021). Due to its mechanical properties, for example, tensile strength of 36 MPa, melting point of 115 °C, tensile elongation at break of 210% coupled with heat deflection temperature of 95 °C (Kato et al. 2023), these are some of the properties which make it a sustainable alternative to petrochemical plastics, for example polyethylene. For instance, polybutylene succinate finds applications in various fields, including mulching films, compostable bags, nonwoven sheets and garments, catering goods, and foams, among others (Bi et al. 2018). However, poor toughness and high crystallization limit their widespread application and development (Jin et al. 2022), in addition to a higher cost when fully biobased.

The degradation of polybutylene succinate under marine conditions is significantly challenging. Kong et al. (2014) reported a slow degradation rate of 3.5% for polybutylene succinate over a span of 12 days, employing a lipase enzyme produced by *Pseudomonas cepacia*. In a more recent study, Kim et al. (2023) investigated polybutylene succinate's degradation within a simulated marine sedimentary environment, noting a degradation rate of 27% over a six-month period. This reduced rate of polybutylene succinate degradation can be attributed to its high crystallinity, coupled with the limited abundance of polybutylene succinate degrading bacteria in the marine environment (Urbanek et al. 2020). In this context, Tong et al. (2022) examined the effects of weathering factors, including UV radiation and mechanical forces, on the formation of secondary microplastics and nanoplastics from polybutylene succinate, as well as different types of biodegradable and non-biodegradable plastic pellets, over a 21-day period under laboratory conditions. The authors reported that polybutylene succinate released a high number of microplastics, specifically 437.5 ± 4.0, compared to the

other materials. The slow degradation of polybutylene succinate in marine environments poses significant concerns, notably regarding the release of microplastics.

Furthermore, the release of microplastics during the degradation of polybutylene succinate raises concerns about potential harm to aquatic organisms and humans. For instance, polybutylene succinate microplastics have recently been detected in human placenta (Zhu et al. 2023) and ranked as the third most abundant microplastic after polyvinyl chloride and polypropylene. However, research on the potential toxicity of microplastics in humans is in its early stages and requires further investigation. On the other hand, polybutylene succinate microplastics have been reported to induce stress and inhibit the growth of algae (*Chlorella vulgaris*) (Su et al. 2022) and even cause mortality in brine shrimp (*Artemia franciscana*), with mortality rates reaching up to 53.3% after 24 hours of exposure and up to 76.6% after 48 hours of exposure to a concentration of 100 μg/mL (Charoeythornkhajhornchai et al. 2023). In contrast, Viel et al. (2023) reported that polybutylene succinate microplastics in concentrations ranging from 1 mg/L to 10 mg/L had no significant effects on sea urchin larvae, *P. lividus*. Therefore, the slow degradation of polybutylene succinate in marine environments and its potential to release microplastics raise concerns about potential harm to aquatic organisms, emphasizing the need for further research into its toxicity and environmental impact.

3.4 Poly(butylene adipate-*co*-terephthalate) (PBAT)

Poly(butylene adipate-*co*-terephthalate) is a biodegradable aliphatic-aromatic co-polyester synthesized by polycondensation of butanediol, adipic acid and terephthalic acid using metallic catalysts such as zinc, tin or titanium under high vacuum and temperatures. The presence of aromatic component enhances mechanical properties, making it comparable to polyethylene (Kanwal et al. 2022). Poly(butylene adipate-*co*-terephthalate) typically displays desirable mechanical properties, including tensile strength of 21-36 MPa, Young's modulus of 20-30 MPa, elongation at break 650-700%, and a melting point ranging from 115 to 125 °C (Jian et al. 2020). These properties could be optimized by adjusting terephthalate content and molecular weight. For example, higher terephthalate content increases Young's modulus, while increased molecular weight enhances tensile strength (Burford et al. 2023). Furthermore, poly(butylene adipate-*co*-terephthalate) has made a notable impact in the world of bioplastics, contributing 4.6% of the total production of biobased and biodegradable plastics in 2023, and it finds application across various industries, including coatings, agriculture, consumer goods, and packaging (EuropeanBioplastics 2023).

However, despite its biodegradability and promising mechanical characteristics, the use of poly(butylene adipate-*co*-terephthalate) is not without environmental challenges. Firstly, the biodegradation of poly(butylene adipate-*co*-terephthalate) in aquatic environment is very slow (De Monte et al. 2022) and produce residues. For example, Wang et al. (2019) studied poly(butylene adipate-*co*-terephthalate) degradation over 56 weeks in different water bodies. The authors reported that poly(butylene adipate-*co*-terephthalate) showed minimal weight loss with 4.7% in microbe-containing water and less than 1.5% in sterilized water, while molecular weights and mechanical properties decreased significantly. The decrease in molecular weights during hydrolysis yields monomers such as adipic acid, 1,4-butanediol, and terephthalic acid, alongside low molecular weight molecules, including carboxylic acids, that are toxic prior to

undergoing metabolization (Wei et al. 2021; Kim et al. 2001). Another concerning aspect is the potential generation of microplastics during its degradation process. For instance, Wei et al. (2021) investigated the biodegradation of commercially available poly(butylene adipate-*co*-terephthalate) extruded films, simulating diverse water mediums both in the presence and absence of UV radiation at 23 °C over a period of 10 weeks. The authors reported that poly(butylene adipate-*co*-terephthalate) films in seawater exhibited accelerated microplastic formation compared to freshwater, particularly when exposed to UV treatment. These microplastics exhibited varied shapes and were more abundant than those formed from low density polyethylene. Similarly, Bao et al. (2022) found that poly(butylene adipate-*co*-terephthalate) generated a higher quantity of microplastics (428.6 ± 300.4 per gram) over a 90-day period of aging in seawater when compared to polyvinyl chloride (185.5 ± 85.7 per gram).

The slow biodegradation of poly(butylene adipate-*co*-terephthalate) in aquatic environments and its potential leachates coupled with generation of microplastics may present significant challenges for aquatic biota and the ecosystem. For instance, Capolupo et al. (2023) investigated the *in vivo* and *in vitro* toxicity of a 0.6% concentration of leachates from poly(butylene adipate-*co*-terephthalate) and polylactic acid composite plastic bags on the mussel (*Mytilus galloprovincialis*). The authors reported adverse effects on egg fertilization, larvae development, as well as lysosome membrane stability and increased lysosome volume in adult mussels. In another study, Xie et al. (2022) studied the effects of bioplastic bags containing 30% polylactic acid and 70% poly(butylene adipate-*co*-terephthalate) microplastics on fish (*L. calcarifer*) for 21 days. They found that the microplastics accumulated in the fish, leading to minimal effects on growth or survival, minor antioxidant changes, decreased Proteobacteria, and altered liver proteins inhibiting immune homeostasis, while increasing intestinal microbial diversity and protein alterations. Very recently, Thery et al. (2023) studied the effects of virgin and aged poly(butylene adipate-*co*-terephthalate) microplastics on copepod microbiota diversity, comparing them with petrochemical-based low-density polyethylene microplastics in a five-generation experiment. The authors reported that both types of microplastics rapidly and continuously impacted copepod microbiota from the first to the last exposed generation, regardless of plastic origin, type, and aging conditions. Likewise, Nie et al. (2022) also reported that poly(butylene adipate-*co*-terephthalate) microplastics reduced the abundance of nirS (cytochrome cd_1 nitrate reductase gene) denitrifying and anammox bacteria in the freshwater sediments after 30 days of exposure. In summary, although poly(butylene adipate-*co*-terephthalate) offers promising mechanical properties, its slow biodegradation in aquatic environments and potential to generate microplastics raise substantial environmental concerns. The limited research on its impact on aquatic biota underscores the urgent need for comprehensive studies to evaluate its ecological consequences.

3.5 Poly(ε-caprolactone) (PCL)

Poly(ε-caprolactone) is a fossil fuel-based, semi-crystalline, biodegradable aliphatic polyester synthesized through the ring-opening polymerization of ε-caprolactone. Poly(ε-caprolactone) has a melting point of about 56–65 °C, Young's modulus of 251.9–440 MPa, tensile strength of 10.5–27.3 MPa, elongation at break of 80–800% (Bartnikowski et al. 2019), and a glass transition temperature of about −60 °C. According to the International Market Analysis Research and Consulting Group (2022), it represents a global market size of 556

million US dollars in 2022, and expected to increase to 1,001 million US dollars by 2028. It is one of the widely used polymers in the medical industry, finding applications in medical devices, drug delivery, tissue engineering, and wound healing due to its excellent biocompatibility, high permeability to different drugs, and complete excretion from the body (Ali Akbari Ghavimi et al. 2015). There is currently a growing trend in its application in the food sector for compostable bags and bottles (Khan et al. 2013; Thakur et al. 2021), in agriculture as a carrier for fertilizers and herbicides (Cesari et al. 2020; Pouladchang et al. 2022), and also in agricultural film mulching.

Despite its biodegradable profile, the degradation of poly(ε-caprolactone) in the natural environment is very slow. De Falco et al. (2021) investigated the degradation of a 300 µm thick poly(ε-caprolactone) film under simulated marine environment conditions over 38 weeks. The authors found that the weight loss of poly(ε-caprolactone) was very slow, reaching 13% after 38 weeks of sand burial, wherein the initial poly(ε-caprolactone) weight of 0.35 mg decreased to only 0.31 mg. In another example, Lu et al. (2018) studied the degradation of poly(ε-caprolactone) in seawater collected from Bohai Bay, China, for one year under ambient temperature. The authors reported a decrease in the mechanical strength, numerous depressions on the surface of the specimens, and a weight loss of 29.8% compared to the initial weight. The authors hypothesized that poly(ε-caprolactone) degradation primarily occurs at the sample surface, leading to gradual shedding, exposure of crystal structures, and slowed weight loss, potentially hindering further degradation. For instance, Wei et al. (2022) investigated the biodegradation of a 0.5-mm poly(ε-caprolactone) film in a phosphate-buffered saline enzymatic solution with a lipase concentration of 0.5 mg/ml at 40 °C and a shaking rate of 200 rpm for one to six days. They reported estimated microplastic counts of 391,000, 441,000, and 342,000 after the first, second, and third days, respectively, from 0.1 g of poly(ε-caprolactone) film. The average microplastic size was 15-µm for those formed in the initial two days, decreasing to 12-µm and 9.5-µm in the subsequent days. Likewise, Tamayo-Belda et al. (2022) also reported the formation of microplastics, nanoplastics, and oligomers after 132 days of abiotic degradation of 3-mm poly(ε-caprolactone) microbeads.

Recently, poly(ε-caprolactone) microplastics have been reported from different water bodies. For example, Cai et al. (2018) found that poly(ε-caprolactone) accounted for 20.9%, making it the second most abundant polymer in their study among all 21 types collected from the South China Sea. Suaria et al. (2016) reported poly(ε-caprolactone) microplastics from Mediterranean offshore water, highlighting that certain biodegradable plastics may not effectively degrade in natural conditions and questioning their efficacy in mitigating marine litter. Despite the presence of poly(ε-caprolactone) microplastics in various water bodies, research on their effects on aquatic biota is notably scarce. Very recently, Viel et al. (2023) investigated the toxicity of poly(ε-caprolactone) microplastics at concentrations of 1, 5, and 10 mg/L on the embryos of *P. lividus*. The authors reported that exposure to microplastics led to delayed development, malformations, affected gene expression, and changes in detoxification genes. In another example, Tamayo-Belda et al. (2022) studied the toxicity of suspension of 91 mg/L poly(ε-caprolactone) nanoplastics and oligomers, and 238 mg/L oligomer alone on two cyanobacteria strains, *Anabaena* and *Synechococcus* spp., at 28 °C on a rotary shaker at 135 rpm for 72 hours. The authors found that poly(ε-caprolactone) nanoplastics and oligomers,

alone or in combination, induced reactive oxygen species (ROS) overproduction and altered metabolism in both cyanobacteria, with combine exposure causing membrane damage and morphological changes, and both fractions inhibiting nitrogen fixation in *Anabaena* sp. Similarly, the degradation products of poly(ε-caprolactone) have been reported to reduce the survival and reproduction of *D. magna* at varied concentrations ranging from 0.1 mg/L to 100 mg/L (Matsumoto et al. 2023). In conclusion, the evidence from these studies emphasizes the prevalence of poly(ε-caprolactone) microplastics in aquatic environments and their potential adverse effects on marine organisms. The reported negative effects highlight the need for comprehensive research, considering other model aquatic organisms, to address the ecological implications of these biodegradable plastics.

4. Sorption of coexisting pollutants on biodegradable microplastics and their combined toxicity

Microplastics, characterized by their small size, large surface area, and hydrophobic nature, possess the capability to adsorb a wide spectrum of pollutants onto their surfaces. They have been detected in almost all types of environments where various organic and inorganic pollutants are present. Recent research indicates that different types of microplastics exhibit distinct affinities for the adsorption of various organic and inorganic pollutants (Guo et al. 2020, Godoy et al. 2019; Cui et al. 2023), which could not only potentially alter the behavior and toxicity of microplastics itself but also the coexisting pollutants. Microplastics have been reported to transport the adsorbed pollutants into the bodies of exposed organisms, where these pollutants can subsequently desorb from the microplastics into the organisms' digestive tracts (Zhou et al. 2020), potentially exacerbating these threats.

In recent years, there has been a growing body of research focusing on the combined effects of microplastics, including nanoplastics, and various environmental stressors, such as heavy metals (Lee et al. 2021; Kang et al. 2021), polycyclic aromatic hydrocarbons (Byeon et al. 2024; Sun et al. 2021), as well as temperature and acidification (Lee et al. 2023; Guilhermino et al. 2021), on aquatic biota. The results of these studies consistently suggest that the combined exposure has more severe effects on biota compared to their single toxicity. However, the majority of such studies have focused on fossil fuels-based microplastics, while research on the combined effects of biodegradable microplastics and coexisting pollutants remains scarce. In the following section, we will first discuss the sorption of different pollutants on the synthetic biodegradable microplastics previously mentioned. Secondly, we will explore their combined toxicity, with a focus on aquatic biota.

4.1 Polylactic acid

Polylactic acid microplastics have shown strong pollutant sorption capabilities, with faster equilibrium and higher adsorption for various contaminants compared to fossil fuel-based microplastics (Table 2). For instance, Gong et al. (2019) found that polylactic acid microplastics sorbed pesticide fipronil efficiently. Yan et al. (2023) observed that these microplastics had high adsorption for heavy metals, particularly chromium, and superior desorption capacity at pH 2.5. Other studies, including Fan et al. (2021) and Lang and Xue (2022), also reported enhanced sorption of pollutants on polylactic acid microplastics compared to non-biodegradable petrochemical based microplastics. However, contrary findings indicate that

polylactic acid microplastics have lower sorption capacities compared to certain other polymers. For example, Lončarski et al. (2021) found that in the case of polyaromatic hydrocarbons, polylactic acid had the lowest adsorption affinity, ranging from 30 µg/g to 50 µg/g, while fossil fuel-based microplastics, especially polypropylene, exhibited higher adsorption capacities. This difference may be due to functional group variations. Tubić et al. (2019) reported lower adsorption of chlorinated phenols onto polylactic acid microplastics, especially in the presence of natural organic matter in water. Additional studies, like Liao and Yang (2020), have also reported reduced adsorption of pollutants by polylactic acid microplastics compared to non-biodegradable petrochemical based microplastics.

The adsorption behavior of polylactic acid microplastics toward pollutants is a complex phenomenon influenced by various factors, including the pollutant type, concentration, microplastic surface morphology, and environmental conditions like salinity, pH, and temperature (Jeong et al. 2023). Additionally, studies suggest that aging of polylactic acid microplastics can significantly increase their adsorption capacity for coexisting pollutants. For example, Fan et al. (2021) found that aged polylactic acid had 1.1 to 2.1 times higher adsorption capacity compared to virgin microplastics, possibly due to surface morphology changes. This suggests that aged polylactic acid microplastics may pose greater threats to the aquatic ecosystem. Furthermore, according to Liao and Yang (2020), chromium adsorbed on polylactic acid microplastics is more bio-accessible than when adsorbed on petrochemical-based microplastics, as observed in an *in vitro* simulation of the human digestive tract. These results suggests that microplastics could potentially magnify pollutant concentrations in exposed organisms. There are also examples where fossil fuel-based non-biodegradable microplastics, such as polyethylene and polypropylene, were reported to release more than 86% and 32% of adsorbed heavy metals, chromium and lead, in an *in vitro* simulation of the gastrointestinal tract (Godoy et al. 2020). Therefore, there are indeed risks for polylactic acid microplastics as vectors for environmental pollutants, but these risks are similar to those associated with petrochemical microplastics.

Ayala-Silva and Al-Hamdani (1997) were the first to study how polylactic acid and heavy metals combined affect aquatic organisms. They examined the effects of polylactic acid at a concentration of 1 µg/L along with different levels of aluminum (between 2–16 mg/L) on *Azolla caroliniana*. They measured various factors, such as growth, chlorophyll content, carotenoid levels, anthocyanin presence, and carbohydrate accumulation. The results showed that polylactic acid helped reduce the negative impacts of aluminum on these factors. This improvement was likely because polylactic acid bound to aluminum, making it less harmful to the plant. Li et al. (2022c) found similar results when studying the combined effects of polylactic acid microplastics and sulfamethoxazole on marine algae (*Skeletonema costatum*). The combined effects were less harmful than the individual toxicities.

In another study, Verdú et al. (2021) studied the toxicity of polylactic acid microplastics loaded with triclosan on the growth and chlorophyll content of a freshwater cyanobacterium (*Anabaena* sp.) over 72 hours. They found that the triclosan-loaded polylactic acid microplastics did not significantly affect growth but did slightly decrease chlorophyll a content compared to the control group. It's worth noting that the triclosan did not release from the polylactic acid microplastics. This suggests that the microplastics reduced the availability of

triclosan in the surrounding environment, making it less toxic to the organisms (Deng et al. 2017). Li et al. (2023a) examined the combined effects of 100 mg/L polylactic acid microplastics and 10 mg/L copper on *Bacillus amyloliquefaciens* bacteria. Polylactic acid microplastics didn't significantly increase copper toxicity; instead, they triggered resistance mechanisms. In contrast, Jang et al. (2022) found that polylactic acid microplastics effectively transported heavy metals, such as copper and lead, as well as bacteria to catfish tissues. This transport resulted in significant heavy metal bioaccumulation, reaching up to four times higher than the control group. This caused dysbiosis in the catfish, weakening their immunity (**Table. 3**). In conclusion, despite the individual potential hazards of both polylactic acid microplastics and other pollutants, the combined effects can be less detrimental than the individual effects in some cases.

4.2 Polyhydroxyalkanoates

Polyhydroxyalkanoates, a class of biodegradable polymers, have limited research on their interactions with pollutants in aquatic ecosystems. In a study by Tong et al. (2021), triclosan sorption on polyhydroxybutyrate reached equilibrium within 24 hours, whereas it took 120 hours on polyethylene microplastics. The adsorption capacity on polyhydroxybutyrate (9442.2 μg/g) greatly exceeded that onto polyethylene (3431.8 μg/g) based on pseudo-first-order model. Polyhydroxybutyrate exhibited faster triclosan release with an initial concentration of 5 mg/L. In another study by Li et al. (2022a), the sorption of polybrominated diphenyl ethers (BDE-47 and BDE-209) onto polyhydroxyalkanoates microplastics exceeded that onto petrochemical-based microplastics, such as polyethylene and polystyrene. Polyhydroxyalkanoates exhibited the highest equilibrium sorption capacity for BDE-47 and BDE-209, at 0.86 and 0.54 mg/g, respectively. In a study by Li et al. (2023b), the adsorption of sulfamethoxazole onto both virgin and aged polyhydroxyalkanoates microplastics and polyethylene microplastics in artificial seawater at 25 °C reached equilibrium around 24 hours. Both aged and virgin polyhydroxyalkanoates exhibited higher adsorption capacity compared to polyethylene. Similarly, Catarci Carteny et al. (2023) also found that polyhydroxyalkanoates microplastics exhibited higher adsorption capacity for certain pollutants compared to polyethylene microplastics (**Table 2**).

Collectively, these findings emphasize the substantial role of polyhydroxyalkanoates, including polyhydroxybutyrate, as vectors for various pollutants in aquatic environments. Their efficient adsorption and potential release of pollutants can impact pollutant distribution in aquatic ecosystems. Studies on the combined effects of polyhydroxyalkanoates microplastics and coexisting pollutants are limited (**Table. 3**). Li et al. (2022a) recently investigated how the combination of polybrominated diphenyl ethers (BDE-47 and BDE-209) with polyhydroxyalkanoates and petrochemical microplastics affected fish (*Epinephelus moara*). The results showed that combined exposure had differential effects, affecting catalase activity and glutathione content differently, and leading to significant changes in gene expression. In another study, Li et al. (2023b) explored the release of sulfamethoxazole from both virgin and aged polyhydroxyalkanoates microplastics, as well as polyethylene microplastics, under simulated digestive fluids. The desorption pattern varied based on the type of microplastic and the simulated biological fluid. Polyethylene microplastics had a higher desorption rate of sulfamethoxazole in simulated fish intestinal fluids, while polyhydroxyalkanoates microplastics

exhibited a greater release in simulated mammalian gastric fluids. These findings underscore the dynamic nature of microplastic interactions with coexisting pollutants and suggest that these interactions can differ depending on the specific conditions and species involved.

In contrast, Magara et al. (2019) studied the combined exposure of polyhydroxybutyrate microplastics and fluoranthene on blue mussels (*M. edulis*) for 96 hours. They found that the combined exposure did not have any synergistic effects on the antioxidant enzymatic activities of the mussels. In another study, Pang et al. (2023) investigated the individual and combined effects of nano copper oxide (between 0.05–0.15 mg/L) and polyhydroxyalkanoates, along with other petrochemical-based microplastics (500 mg/L), on the denitrification process of *P. denitrificans* over 16 hours. They reported that the combined exposure to microplastics and copper nanoparticles had an antagonistic effect and alleviated the individual effects of copper nanoparticles. Genes related to denitrification and metabolic pathways were initially down-regulated by nano copper oxide or microplastics, but they recovered when both were present. This was attributed to microplastics reducing the release of copper ions from nano copper oxide and inducing lower oxidative stress levels through agglomeration. Overall, the findings from these studies indicate that polyhydroxyalkanoates can play a significant role as vectors for pollutants, with efficient adsorption and potential desorption capabilities. These interactions have the potential to impact the distribution of pollutants within aquatic environments which will have an impact on aquatic food web.

4.3 Polybutylene succinate

The interaction between microplastics and other pollutants in the aquatic environment depends on their physicochemical properties. Research on polybutylene succinate microplastics has revealed distinct sorption characteristics (**Table 2**). Catarci Carteny et al. (2023) studied the sorption of various pollutants on polybutylene succinate, polyhydroxyalkanoates, and polyethylene microplastics in the port of Zeebrugge, Belgium, over 64 days. They found that polybutylene succinate and polyhydroxyalkanoates exhibited higher adsorption capacity for certain pollutants compared to polyethylene microplastics. It should be noted that, in some cases, the adsorption capacity of certain pollutants is also higher for polyethylene microplastics. For example, polyethylene has been shown, both under laboratory conditions and in field experiments, to adsorb a high amount of polychlorinated biphenyls compared to polyvinyl chloride, polystyrene, and polyethylene terephthalate (Pascall et al. 2005; Rochman et al. 2013). The high sorption ability of polyethylene was attributed to its large surface area and free volume between polymer chains. Conversely, Sun et al. (2022) investigated the sorption of norfloxacin antibiotics on polybutylene succinate microplastics and non-biodegradable microplastics for 72 hours at 25 °C. They reported that polybutylene succinate microplastics had higher adsorption capacity than polyethylene but lower than polystyrene microplastics, primarily due to polystyrene's unique properties. Similar studies have also demonstrated elevated adsorption capacities of polybutylene succinate microplastics for coexisting pollutants (Gong et al. 2019; Jiang et al. 2020). These results suggests that the interplay of microplastics and pollutants in aquatic environments is influenced by their properties and can be further altered by environmental factors, adding complexity to their interactions.

Environmental factors, including salinity, dissolved organic matter, temperature, and

weathering processes, can modify the physicochemical properties of microplastics and influence their sorption of coexisting pollutants. Li et al. (2022b) investigated the sorption of lead iodide on different polybutylene succinate microplastics types (virgin, biofilm, and biodegraded) at 25 °C, emphasizing pH. They found that the adsorption of lead iodide was significantly pH-dependent, with biofilm polybutylene succinate showing substantially higher adsorption compared to virgin microplastics. Fan et al. (2023) studied the sorption of sulfamethoxazole antibiotics on virgin and laboratory-aged polybutylene succinate and polypropylene microplastics. Polybutylene succinate microplastics, both virgin and aged, exhibited higher adsorption and desorption capacities compared to polypropylene microplastics, attributed to their distinct physicochemical properties. However, Jiang et al. (2020) explored the sorption behavior of fungicides on polybutylene succinate microplastics and reported higher adsorption capacities influenced by the characteristics of the sorbates and the stable nature of the microplastics, demonstrating insensitivity to environmental factors like pH, temperature, and salinity. These results shows that the adsorption behavior of pollutants by polybutylene succinate microplastics is intricately influenced by environmental factors such as pH, temperature, and salinity, where the stability of the sorbate and the surface charge of the sorbent play pivotal roles, highlighting the complexity of microplastic interactions with pollutants in aquatic environments.

While research on the toxicity of polybutylene succinate microplastics in aquatic ecosystems is still emerging, it is essential to consider the potential combined toxicity when these microplastics coexist with other pollutants. The interactions between polybutylene succinate microplastics and other pollutants may create a complex web of toxic effects on aquatic organisms. For instance, the increased sorption of certain pollutants onto polybutylene succinate microplastics, as demonstrated in previous studies, can potentially lead to higher exposure levels for aquatic organisms. Additionally, the presence of these microplastics may alter the transport and bioavailability of coexisting pollutants. To comprehensively evaluate the ecological impact of polybutylene succinate microplastics in aquatic environments, future studies should investigate the combined toxicity of these microplastics and coexisting pollutants, considering the potential synergistic or antagonistic effects on the health and survival of aquatic species. Such research will be critical in addressing the complex challenges of next generation microplastic pollution.

Table 2. Selected studies on the adsorption of pollutants on biodegradable vs. non-biodegradable microplastics.

Biodegradable microplastics	Non-biodegradable microplastics	Characteristics and size of microplastics	Sorption				Reference
			Adsorbate	Experimental conditions	Model	Kinetic	
PLA	PE and PP	3-mm	4-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol	Up to 96 hours with an adsorbate concentration of 100 µg/L with MPs mixture agitated at 150 rpm	pseudo-second-order	Adsorption equilibrium was achieved within 48 hours. PP had the highest uptake for more hydrophobic CPs. PP adsorbed 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol (126-144 µg/g), while PLA adsorbed 4-chlorophenol (85-101 µg/g).	Tubić et al. (2019)
PLA and PBS	PE, PS, PVC and PP	Size range of 75 to 150-µm	Fipronil	Up to 96 hours with 0 to 300 µg/L adsorbate under 25 °C	pseudo-second-order	PLA and PBS achieved equilibrium within 12 hours, with significantly higher sorbed concentrations of 78.5 µg/g and 158.7 µg/g, and sorption efficiencies of 39.3% and 79.0%. Nondegradable microplastics achieved equilibrium within 48 hours, resulting in sorbed concentrations from 40.1 µg/g to 55.0 µg/g, with sorption efficiencies between 20.1 to 27.5%.	Gong et al. (2019)
PLA	PE, PP and PVC	125-µm	Cd^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+},	10 mg/L adsorbate and 1	pseudo-second-	PLA reached equilibrium in 5 hours, while it took 10 hours for PE and PVC and 24 hours for	Yan et al. (2023)

			Pb^{2+}, and Zn^{2+}	gram of MPs agitated at 160 rpm for 1 to 48 hours	order	PP. Cd^{2+} had the highest adsorption capacity for all MPs except PLA. Cr^{3+} had the highest adsorption capacity, with PLA showing maximum sorption capacity of 0.8 mg/g. PLA exhibited superior adsorption for 5 heavy metals compared to PVC and PP.	
PLA	PVC	Virgin and aged with the sizes of 250 to 550-µm.for PLA and 75 to 150-µm for PVC	Tetracycline and ciprofloxacin	Up to 96 hours with an adsorbate concentration of 100 µg/L in both natural and synthetic water MPs mixture using a digital mixture at 150 rpm	pseudo-second-order model	Aged MPs exhibited increased adsorption of antibiotics, tetracycline adsorption by PLA rose from 0.9 to 1.9 mg/g, and by PVC, from 0.7 to 1.3 mg/g. The aging process increased the number of adsorption sites on the surface of aged PLA MPs due to the formation of cracks and pits during aging.	Fan et al. (2021)
PLA	PE, PET, PP	Granulated with size of 3-mm, while 49.7 to 358-µm for powdered PE.	Naphthalene, fluorene, fluoranthene and pyrene	2 to 96 hours with adsorbate concentration of 100 µg/L in both natural and synthetic water in a digital mixture (150 rpm) under 25 °C.	pseudo-second-order model	The adsorption equilibrium reached after 24 hours for powdered and 48 hours for granulated MPs. PP exhibited the highest adsorption affinity at equilibrium (between 115–160 µg/g), while PLA (between 30–50 µg/g) was much lower which could be attributed to the carbonyl group forming cluster with water and reduce the adsorption capacity. The adsorption affinity decreases as follows: PP > PET > PE > PLA in	Lončarski et al. (2021)

						both water matrices.	
PLA	PE, PVC, PP, and PS	Average diameter of around 150-µm	Cr	100 µg/L Cr solution with MPs (1:300) ratio agitated at 150 rpm under 22 ± 2 °C for 24 to 96 hours	NA	Adsorption equilibrium reached after 48 hours. PS had the highest Cr uptake (5.07 µg/g), while PLA had the lowest Cr adsorption (2.88 µg/g).	Liao and Yang (2020)
PLA	PS, PVC and PE	The size ranges of 75 to 150-µm	Triclosan	8 mg/L triclosan and 15 mg microplastics shaken at 190 rpm under no light (dark) for up to 36 hours	pseudo-second order model	The adsorption equilibrium reached in 24 hours. PLA exhibited the highest adsorption capacity, influenced by its biodegradability. The adsorption of triclosan by MPS followed the order PLA > PE > PVC > PS. MPs size has no effects on PLA adsorption capacity.	Lang and Xue (2022)
PLA	PVC, PS, PET	Virgin size of 100-µm	Tetramethylthiuram disulfide	5 mg/L adsorbate and 1 mg/mL MPs on shaker (150 rpm) in dark under 25 °C	pseudo-second-order model	The sorption equilibrium was approximately 8 hours. PLA and PS has stronger adsorption sites for tetramethylthiuram disulfide. PET MPs had fast adsorption but low capacity for tetramethylthiuram disulfide due to high crystallinity and smaller surface area.	Liu et al. (2024)
PHB	PE	Average diameter of 1255 ± 14	Triclosan	10 mg of MPs and 10 mg/L triclosan in a thermostatic	pseudo-first-order model	The sorption equilibrium reached after 24 and 120 hours for PHB and PE. The rate constant K1 and equilibrium capacity qe were much higher for PHB (K1 = 0.32, qe =	Tong et al. (2021)

		4-µm for PHB and 1255 ± 14 4-µm for PE		shaker (150 rpm) under 30 °C up to 216 hours		9442.2) than polyethylene (K1 = 0.02, qe = 3431.8).	
PHA and PHB	PE, PS	6.5-µm	2,2′,4,4′-tetrabromo diphenyl ether and decabromo diphenyl ether	1 mg/L adsorbate with 0.3 g MPs on thermostatic oscillator (170 rpm) under 25 °C for 24 hours	pseudo-first-order model	The sorption equilibrium reached in 18 hours. The equilibrium sorption capacities of 2,2′,4,4′-tetrabromodiphenyl ether and decabromodiphenyl ether on PE, PS, PHA and PHB were 0.26, 0.28, 0.86 and 0.59 mg/g, and 0.54, 0.22, 0.54 and 0.40 mg/g. Surface roughness and chemical compatibility make PHA and PHB effective at sorbing organic compounds.	Li et al. (2022a)
PHA	PE	Virgin and UV-aged with the size of 6.5-µm	Sulfamethoxazole	40 mg of MPs and 20 mL of sulfamethoxazole (2 mg/L) on shaking incubator (200 rpm) under 25 °C	pseudo-second-order	The sorption equilibrium reached around in 24 hours. PHA showed greater antibiotic adsorption compared to PE, in both states, virgin and aged. The UV aging process increases the adsorption capacity onto PHA MPs by boosting surface area, adsorption sites, and hydrophilicity.	Li et al. (2023)
PHA and PBS	PE	Median diameter of 5-µm	Polycyclic aromatic hydrocarbons, polychlorinated	10 g of MP in stainless-steel mesh in sea water of Belgian Naval Base in	NA	PBS MPs had the highest total polycyclic aromatic hydrocarbons (0.7 nmol/g) compared to PHA MPs (0.4 nmol/g). Organophosphorus flame retardants accumulation increased in PHA and PBS MPs suggesting potential adsorption from the sea water. PHA exhibited	Catarci Carteny et al. (2023)

			biphenyls, organophosphorus flame retardants, phthalates, and plasticizers	Zeebrugge for 64 days		the most significant increase in plasticizers (phthalates and alternatives combined) at 4.2 nmol/g, followed by PBS at 2.06 nmol/g.	
PBS	NA	Virgin and aged with sizes of 10 to 100-μm	Pb (II)	10 mg/L adsorbate and 5 g/L MPs on saker (180 rpm) under 25 °C	pseudo-second-order model for virgin PBS and Elovich Model for aged PBS	Both materials exhibited similar adsorption trends. Aged PBS MPs displayed significantly enhanced adsorption of Pb (II), attributed to the increased heterogeneity and active reaction sites provided by the adhered biofilm.	Li et al. (2022b)
PBS	PP	Virgin and aged (under different conditions) with average size of 40-μm	Sulfamethoxazole	50 mg/L adsorbate and 50 mg MPs in air bath thermostatic shaker (180 rpm) in dark under 25 °C for up to 48 hours	pseudo-second-order model	The adsorption by PBS increased from 4.5 to 5.7 mg/g, whereas that of PP increased from 2.8 to 3.4 mg/g. PBS demonstrated a higher adsorption capacity for sulfamethoxazole due to several factors including an increase in crystallinity during aging, a larger number of oxygen-containing functional groups on its surface, and a greater specific surface area.	Fan et al. (2023)

PBS	PE, PVC	Size range of 75–150	Triadimefon and difenoconazole	200 µg/L adsorbent with 10 mg MPs and 10mL background solution (0.01 M CaCl2) on thermostatic shaker (200 rpm) at 25 °C up to 120 hours	Pseudo-second-order	Sorption equilibrium was reached within approximately 12 hours for PBS, while it took nearly twice longer time for PE and PVC. PBS MPs exhibited stronger sorption, (104.2 ± 4.8 µg/) for triadimefon, which was 1.8 and 4.4-fold higher than that of PE and PVC. The sorption capacity of difenoconazole on PBS (192.8 ± 2.3 µg/g) was 1.3 and 7.4-fold that on PE and PVC.	Jiang et al. (2020)
PBS	PS, PE	25-µm	norfloxacin	15 mg/L norfloxacin and 5 g/L MPs on in an air bath oscillator (150 rpm) under 25 °C for up to 72 hours	pseudo-second-order kinetic	The sorption equilibrium reached around at 48 hours. PBS exhibited an adsorption capacity approximately twice that of PE (0.3> 0.1) and lower than that of PS (0.6) based on the fitted model. The higher adsorption capacity of PS is likely due to its benzene ring structure.	Sun et al. (2022)
PBAT	PS, PE, PP	Virgin, UV aged, and $K_2S_2O_8$ aged with size range of 69.2 to 121.8-µm	Tetracycline	10 ml tetracycline (10 mg/L) and 10 mg MPs on oscillator (150 rpm) under 25 °C	pseudo-second-order	The sorption equilibrium was achieved within 6 hours for PBAT, whereas it took 10 hours for the other. Aging altered both physical and chemical properties of MPs including increased oxygen containing functional group and roughness, and formation of short polymers chains which favored an increase in adsorption capacity.	Guo et al. (2023)

PBAT	PS	Virgin and UV aged with size range of 75 to 150-µm	Diclofenac sodium	20 ppm adsorbate and 10 mg MPs shaken (160 rpm) under 25 ± 1 °C up to 36 hours	pseudo-second-order	Sorption equilibrium was reached at 12 hours. UV-aged MPs showed significantly increased adsorption capacity for diclofenac sodium, with aged PBAT (27.6 mg/g) > aged PS (23.9 mg/g) > virgin PBAT (9.3 mg/g) > virgin PS (9.2 mg/g). The higher adsorption onto aged PBAT MPs is due to changes in surface morphology, increased binding sites, enhanced hydrophilicity, and stronger chemisorption.	Liang et al. (2022)
PLA and PBAT	PP	Virgin and aged with average sizes of 107 to 139-µm	Chlorpyrifos	0.25 to 48 hours with adsorbate concentration of 1 mg/L on rotating shaker at 150 rpm and 25 ◦C	pseudo-second-order model	Adsorption by PBAT (9.6 mg/g) was 20 times higher than that of PLA (0.5 mg/g). PLA's adsorption capacity was similar to PP (0.5 mg/g). The higher adsorption capacities onto PBAT were mainly attributed to crystal structures, small particles size, and rubber or glass states. The lower sorption capacity of PLA was attributed to decrease in surface area.	Zhang et al. (2023)
PLA and PBAT	NA	Virgin and aged microplastics with sizes <150-nm	Atrazine	20 mg/L atrazine and 50 mg of MPs in glass centrifuge tube for 0.5 to 48 hours	pseudo-second-order model	PLA and PBAT reached equilibrium in 24 and 5 hours, respectively. PLA's higher atrazine adsorption compared to PBAT is due to its surface properties (more micropores) that encourage microbial attachment. Microbial aging increased PBAT's crystallinity, reducing atrazine diffusion and adsorption. The loss of amorphous regions in PBAT after aging decreased its atrazine sorption capacity.	Sun et al. (2023)
PBAT	PE, PS	PBAT MPs	PHEN	0.178 mg/L to 0.623 mg/L of	pseudo-second-	The sorption equilibrium reached in 12 hours. Sorption capacity of PBAT were higher than	Zuo et al. (2019)

		produced from plastic bags and size was 2.3-mm. PS and PE sizes were 250 and 400-µm		adsorbate concentration in orbital shaker at 150 rpm for 72 hours under 25 °C	order model	PE and PS. The thickness of PBAT and PE (12 and 16-µm) was significantly smaller than that of PS, indicating that thickness is a crucial factor in determining diffusion on microplastics. The molecular chains of PBAT have free volume which could adsorb more pollutants.	
PBAT, PBST	NA	Virgin (less than 150-µm and 250 to 600-µm) and UV Aged (less than 150-µm and 250 to 600-µm)	atrazine	2 mg/L adsorbate with 0.5 g/L MPs on a rotary shaker (60 rpm) in the dark at 25 °C for up to 96 hours	pseudo-first-order model for PBAT and pseudo-second-order model for PBST	Sorption equilibrium was achieved within 12 hours. UV aging significantly reduced atrazine sorption capacity. For PBAT MPs, capacity decreased from 333.2 mg/kg to 193.0 mg/kg (250 to 600-µm) and from 317.2 to 178 mg/kg (<150-µm). For PBST, it decreased from 231.5 to 174.4 mg/kg (250 to 600-µm) and remained stable at 221.7 to 217 mg/kg (<150-µm). Virgin MPs had a higher atrazine sorption capacity compared to aged, attributed to differences in crystallinity and amorphous regions.	Cao et al. (2023)
PBAT	PS	Size range of 75 to 150-µm	Diclofenac sodium	20 mg/L adsorbate and 10 mg MPs shaken (160 rpm) under 25 ±	pseudo-second-order model	The sorption equilibrium reached after 24 hours. The adsorption capacity of diclofenac sodium onto the PBAT (9.26 mg/g) was almost similar to that on PS (9.03 mg/g).	Liang et al. (2023)

				1 °C up to 36 hours		PBAT and PS exhibit minor differences in specific surface area which could explain the difference.	
PCL and PBS	PE, LDPE	Size range of 40 to 850-µm	Thiamethoxam	20 mL adsorbate (10 mg/L Concentration) and 2 g soil containing 10% MPs on shaking incubator (210 rpm) under 25 °C	pseudo-second-order model	The sorption equilibrium reached after 12 hours. Both MPs significantly enhanced the adsorption efficiency of thiamethoxam onto the soil from 14% to 27% and 21% in the presence of PBS and PCL, attributed to their polarity and potential for forming hydrogen bonds.	Hu et al. (2023)
PCL and PBS	PU, PS	Size range of 150 to 200-µm	Phenanthrene, pyrene, 1-nitronaphthalene, 1-napthylamine, atrazine	120 to 168 hours with adsorbate concentrations ranging from 50 µg/L to 10 mg/L in the dark at 25 °C at 180 rpm	Pseudo first order model and pseudo-second-order model	The sorption equilibrium reached in 120 hours. The higher sorption capacities of PBS and PCL for various chemicals are attributed to their higher crystallinity, indicating that crystallinity is a key factor controlling the sorption of organic pollutants on microplastics.	Zhao et al. (2020)
PCL, PLA, and PBAT	PE	NA	Mn, Cr, Pb, As, Cu, Co	In a nylon mesh bag (1-mm pore size) within a stainless-steel case (1.2-mm	NA	The adsorption of heavy metals on all MPs was variable, with high variability among the biodegradable MPs. In general, the adsorption of As, Pb, and Cu was higher onto biodegradable MPs, while Mn, Cr, and Co	Shi et al. (2023)

				pore size) inside a large cage (49 × 36 × 21 cm3) at a water depth of 0.5 meters in the Pearl River Estuary for 3 to 12 months.		showed higher adsorption onto PE. The variability among biodegradable MPs was attributed to the shedding of the aged outer layer and the loss of oxygen-containing groups during the aging process.	

Polylactic acid (PLA), polybutylene succinate (PBS), polyhydroxy butyrate (PHB), polyhydroxy alkanoates (PHA), poly(butylene adipate-co-terephthalate) (PBAT), poly(ε-caprolactone) (PCL), polyurethane (PU), polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), low density polyethylene (LDPE)
Mercury (Hg), Cadmium (Cd^{2+}), Copper (Cu^{2+}), Chromium (Cr^{3+}), Nickel (Ni^{2+}), Lead (Pb^{2+}), Zinc (Zn^{2+}), Manganese (Mn), Arsenic (As), Cobalt (Co)
Not applicable (NA).

4.4 Poly(butylene adipate-*co*-terephthalate)

Poly(butylene adipate-*co*-terephthalate), due to their susceptibility to degradation and potential generation of microplastics, will inevitably interact with other pollutants in the aquatic environment. The adsorption of pollutants is largely affected by factors such as the type, size, aging degree, and the properties of the pollutant. Considering the type, Sun et al. (2023a) investigated the adsorption of atrazine by virgin and aged poly(butylene adipate-*co*-terephthalate) and polylactic acid microplastics. The authors reported that poly(butylene adipate-*co*-terephthalate) exhibited a lower atrazine sorption capacity compared to both virgin and aged polylactic acid microplastics (**Table 2**). In another study, Liang et al. (2023) investigated the adsorption behavior of diclofenac by poly(butylene adipate-*co*-terephthalate) and polypropylene microplastics under conditions of 25 ± 1 °C for up to 36 hours. The authors found that poly(butylene adipate-*co*-terephthalate) and polypropylene exhibited almost similar adsorption capacities for diclofenac, 9.3 mg/g and 9.2 mg/g, reaching equilibrium in 24 hours, with a non-homogeneous chemisorption mechanism confirmed by fitting pseudo-second-order kinetics model. The authors attributed the adsorption of higher amount of diclofenac onto poly(butylene adipate-*co*-terephthalate) due to their hydrophilic nature and larger pore volume. These results show that the adsorption behavior of poly(butylene adipate-*co*-terephthalate) microplastics in aquatic environments can vary depending on the specific pollutant and its properties.

Considering the aging of microplastics, Guo et al. (2023) studied the adsorption of tetracycline onto virgin and aged poly(butylene adipate-*co*-terephthalate) microplastics and compared with polystyrene, polypropylene, and polyethylene microplastics. The authors reported that aged microplastics exhibited higher adsorption capacity to tetracycline and attributed it to the increased oxygen containing functional group and roughness, and formation of short polymers chains in aged microplastics. Similarly, Liang et al. (2022) also reported higher adsorption capacity of aged poly(butylene adipate-*co*-terephthalate) and polystyrene microplastics to diclofenac due to changes in surface morphology, when compared to virgin microplastics. In contrast, Cao et al. (2023) reported a decrease in the sorption capacity of aged poly(butylene adipate-*co*-terephthalate) microplastics, along with poly(butylene succinate *co*-terephthalate microplastics). The authors also noticed that aging increased the crystallinity, and that the decreased atrazine sorption capacity may be attributed to the loss of amorphous regions. The impact of aging on poly(butylene adipate-*co*-terephthalate) microplastics adsorption behavior appears to be complex and dependent on the chemical properties of pollutant. These findings emphasize the need for further research to understand the effects of aging on microplastic-pollutant interactions.

Studies on the combined effects of poly(butylene adipate-*co*-terephthalate) microplastics and coexisting pollutants are limited on aquatic biota (**Table 3**). To the best of our knowledge only one study has investigated the combined effects of poly(butylene adipate-*co*-terephthalate) microplastics and coexisting pollutants on aquatic biota. Tong et al. (2022) studied the acute toxicity of secondary microplastics derived from poly(butylene adipate-*co*-terephthalate) and polylactic acid blended carrier bags and triclosan on *Tigriopus japonicus* over 48 hours, considering two microplastic concentrations (20 mg/L and 500 mg/L) and four triclosan

concentrations (between 90.5–732.0 μg/L). The authors reported that the toxicity of triclosan remained unchanged when combined with secondary microplastics of poly(butylene adipate-*co*-terephthalate) and polylactic acid blend. The 48-hour lethal concentration, 50% (LC50) values were found to be 234.9 μg/L and 229.0 μg/L, respectively. The authors attributed it to the weak adsorption of triclosan by secondary poly(butylene adipate-*co*-terephthalate) and polylactic acid microplastics. These findings, revealing unchanged toxicity levels despite the combination, highlight the complex nature of microplastic-pollutant interactions and emphasize the need for more comprehensive studies to understand their environmental impact.

4.5 Poly(ε-caprolactone)

Poly(ε-caprolactone), owing to its slow degradation and high potential for the generation of microplastics, will interact with other pollutants in the natural environment. However, studies on these interactions with other pollutants are very limited. Zhao et al. (2020) investigated the sorption behavior of pollutants such as phenanthrene, pyrene, 1-nitronapthalene, 1-napthylamine, and atrazine, on poly(ε-caprolactone) microplastics, as well as on polybutylene succinate, polyurethane, and polystyrene for 120 to 168 hours at 25 °C. The authors found that poly(ε-caprolactone) microplastics demonstrated superior sorption capacity for various pollutants over all the studied microplastics, except for 1-nitronaphthalene and pyrene, where polybutylene succinate and polystyrene microplastics showed higher sorption capacity, respectively. The enhanced sorption was attributed to factors including hydrogen bonding, the rubbery nature (low glass transition temperature), and the polar characteristics of poly(ε-caprolactone) microplastics. In another example, Shi et al. (2023) conducted a field experiment to investigate the adsorption of heavy metals from the surface seawater of the Pearl River Estuary, China, onto poly(ε-caprolactone) microplastics, along with polylactic acid and poly(butylene adipate-*co*-terephthalate), and compared with polyethylene microplastics over a duration ranging from 3 to 12 months. The authors reported that the adsorption of heavy metals onto all microplastics was variable, with high variability among the biodegradable microplastics. For instance, the adsorption of arsenic, lead, and copper was higher for biodegradable microplastics, while manganese, chromium, and cobalt were higher for polyethylene. The authors attributed the variability among the biodegradable microplastics to the shedding of the aged outer layer and the loss of oxygen-containing groups during the aging process.

While research on the interaction of pollutants with poly(ε-caprolactone) microplastics in aquatic ecosystems is still emerging, similar studies are also scarce when considering the terrestrial environment (**Table. 2**). For example, Hu et al. (2023) reported a significant enhancement in the adsorption efficiency of thiamethoxam onto the soil, increasing from 14% to 21% due to the presence of poly(ε-caprolactone) microplastics. The ability of poly(ε-caprolactone) microplastics to influence the fate and availability of pollutants raises concerns, especially considering their slow degradation rate. It is essential to evaluate the combined toxicity of poly(ε-caprolactone) microplastics and other pollutants on model organisms to understand their vector role. Understanding these interactions is crucial for assessing their potential environmental impact. Furthermore, the interactions between poly(ε-caprolactone) microplastics and other pollutants may pose significant toxicity to exposed biota. The increased sorption of certain pollutants onto these microplastics, as mentioned above, can potentially lead

to higher exposure levels for the exposed biota. Addressing these knowledge gaps is essential for developing strategies to mitigate the future environmental impact of biodegradable microplastics.

5. Interaction of plastisphere with coexisting pollutants

In the aquatic environment, microplastics have the potential to be colonized by various microbial communities, resulting in the development of a biofilm commonly referred to as the plastisphere. The formation of the plastisphere is linked to the physicochemical properties of the plastics. To illustrate, in a study conducted by Kirstein et al. (2018), it was found that after an incubation period of 15 months, polylactic acid displayed a distinctive microbial community primarily dominated by *Leptobacterium* sp. Furthermore, Odobel et al. (2021) reported that *Planctomycetaceae* sp. were the dominant microbes on polylactic acid, while *Saprospiraceae* sp. predominated on polystyrene. In freshwater environments, polyhydroxybutyrate microplastics exhibited dominance by the *Moraxellaceae* sp., while polyethylene was primarily dominated by *Erythromicrobium* spp., (González-Pleiter et al. 2021b). Furthermore, biofilm formation has been reported to facilitate the biodegradation of microplastics (Mercier et al. 2017), with plastisphere biofilm bacteria demonstrating superior plastic degradation compared to planktonic bacteria (Wilkes and Aristilde 2017). However, it is important to note that formation of biofilm on microplastics does not necessarily means an increase in the degradation process, in fact it could also delay the degradation process due to plastisphere community succession (Wright et al. 2019) resulting in shielding from photodegradation (Weinstein et al. 2016).

The formation of biofilm on the surface of microplastics is of potential concern due to its susceptibility to colonization by pathogenic bacteria and the associated risk of interactions with other pollutants. The attachment of pathogenic microbes to microplastics has become a significant concern, as studies have demonstrated their ability to adhere to microplastic surfaces, thereby raising concerns about potential associated risks and hazards (Frère et al. 2018). Biofilm formation can enhance the heterogeneity of microplastics, creating multiple active sites for coexisting pollutants. Sun et al. (2023b) reported the highest copper ion adsorption (192.1 μg/g) onto biofilm-covered poly(butylene adipate-*co*-terephthalate), attributing it to the presence of oxygen and nitrogen-containing functional groups. Similarly, Cui et al. (2023) documented increased levels of organic and metallic contaminants on biofilm-covered high-density polyethylene. Furthermore, the physical and chemical properties of film-covered microplastics differ from those of both pristine and photo-aged, such as UV-aged. These properties can also vary depending on the type of microplastics, leading to variations in pollutant sorption. For instance, Sun et al. (2023a) found that microbial aging increased the crystallinity of polylactic acid and poly(butylene adipate-*co*-terephthalate), leading to alterations in their surface properties. Atrazine sorption increased by 11.1% on polylactic acid but decreased by 4.9% on poly(butylene adipate-*co*-terephthalate) due to microbial influences on polymer surfaces. In conclusion, biofilm formation on microplastics induces changes in their properties and significantly influences interactions with pollutants.

Table 3. Summary of the studies on the combined effects of biodegradable microplastics on aquatic biota.

Biodegradable microplastics	Characteristics	Associated pollutant	Model species	Experimental conditions	Potential impact	Reference
PLA	less than 5-µm	Sulfamethoxazole	*S. costatum*	Sulfamethoxazole at 0.3 mg/L and MPs at 50 mg/L were assessed for their acute effects over 96 hours	Combined exposure displayed antagonistic effects, reducing toxicity compared to single pollutant exposure, evidenced by decreased inhibition rate, increased chlorophyll content, MDA levels, and ROS, along with reduced SOD activity. PLA and sulfamethoxazole showed antagonistic effects on algae, attributed to the adsorption of sulfamethoxazole onto PLA surfaces.	Li et al. (2022c)
PHA and PHB	6.5-µm	2,2′,4,4′-tetrabromodiphenyl ether and decabromodiphenyl ether	*E. moara*	2,2′,4,4′-tetrabromodiphenyl ether and decabromodiphenyl ether at 1 mg/L and MPs at 750 mg/L for 96 hours.	Combined exposure decreased SOD activity, glutathione content, and CAT activity, while increasing MDA content, attributed to the release of adsorbed pollutants in the digestive tract.	Li et al. (2022a)
PLA	28.66-µm	Ciprofloxacin	*Shewanella* sp.	150 mg/L or 800 mg/L MPs and 15 mg/L ciprofloxacin for 6 and 8 hours	PLA and ciprofloxacin showed an antagonistic effect on phosphorus removal inhibition and reduced the promotion of SOD enzyme activity suggesting lowered oxidative stress and weakened the inhibition of phosphorous removal, attributed to reduced ciprofloxacin toxicity by PLA.	Yang et al. (2022)
PLA	NA	Al	*A. caroliniana*	1 µg/L PLA and between 2–16 mg/L Al for 14 days	PLA mitigated the increase in anthocyanin concentration and carbohydrate accumulation caused by Al. The negative effects of Al on growth, chlorophyll, and carotenoid concentrations were reduced, attributed to the binding of PLA with aluminum.	Ayala-Silva and Al-Hamdani (1997)

PLA	3 to 4-mm	Cu	*B. amyloliquefaciens*	100 mg/L PLA and 10 mg/L Cu for three days in the incubator under 37 °C	PLA did not alter the effects of Cu on bacterial growth. Both PLA and copper induced oxidative stress but didn't influence each other. Antioxidant system displayed differences in CAT and SOD activity compared with no significant synergistic effect. PLA didn't intensify copper toxicity but triggered resistance mechanisms suggesting complex interactions.	Li et al. (2023a)
PLA/PBAT	10 to 40-µm	Triclosan	*T. japonicas*	20 mg/L MPs and between 90.50–732.08 µg/L triclosan under the light-dark cycle of 14 and 10 hours at 25 °C for 48 hours	Triclosan toxicity remained unchanged with PLA and PBAT blend (48-hour LC50: 234.9 µg/L) due to weak adsorption.	Tong et al. (2022)
PLA	3 to 5-mm	Triclosan	*Anabaena* sp.	MPs (3 particles per 20 mL) loaded with triclosan for 72 hours	PLA loaded with triclosan had no significant impact on growth and chlorophyll a content, likely because PLA alone had no significant effects, sorbed less triclosan, and didn't show any desorption.	Verdú et al. (2021)
PLA	3 to 5-mm	Azithromycin and clarithromycin	*Anabaena* sp.	1 g/20mL Antibiotic loaded MPs, either AZI or CLA, for 72 hours	PLA could adsorb and desorb both azithromycin and clarithromycin. PLA loaded with both pollutants significantly reduced growth and chlorophyll a content.	González-Pleiter et al. (2021)
PLA	4-mm × 4-mm × 3-mm	Cu and Pb	*C. gariepinus*	Fish were fed for 3 months with MP loaded with Cu and Pb (285 g MPs treated with 0.050 mg/L and 0.060 mg/L of Cu and	The presence of MPs laden with metals increased the transfer of metals, attributed to the high partition coefficient of the adsorbed metals. Fish exposed to PLA exhibited a high level of *Vibrio* sp in their gut, indicating dysbiosis.	Jang et al. (2022)

				Pb) mixed with feed at a 10% ratio.		
PHB	10 to 90-µm	Fluoranthene	*M. edulis*	1000 MPs/ml and 100 µg/L fluoranthene (incubated and co-exposure) for 96 hours at 10 ± 1 °C and a photoperiod of 16- and 8-hours light/dark.	Catalase and glutathione S-transferase levels significantly decreased in gills (except when PHB incubated with fluoranthene), while superoxide dismutase, glutathione peroxidase, and glutathione reductase activities remained unchanged. In digestive glands, superoxide dismutase, glutathione peroxidases, and catalase (except when PHB incubated with fluoranthene in the case of glutathione peroxidases and catalase) activities decreased, while glutathione S-transferase and glutathione reductase increased. Co-exposure had a similar impact to fluoranthene or MPs alone, attributed to the sink role of MPs for pollutants.	Magara et al. (2019)
PHA	500-nm	nano-CuO	*P. denitrificans*	500 mg/L MPs and 0.05 mg/L or 0.15 mg/L nano-CuO in a shaker (120 rpm) under 30 °C	Inhibit denitrification by altering gene expression, impacted nitrogen metabolism pathways, and reduced the expression of denitrification-related genes, with 23 up-regulated and 26 down-regulated genes.	Pang et al. (2023)
PLA and PHA	100-nm	*In vivo* antibiotic resistance genes	*E. coli* DH5α and *E. coli* HB101	*E. coli* DH5α cultured in antibiotics contaminated medium (*E. coli* DH5α in antibiotics-ampicillin, kanamycin and tetracycline with concentrations of 100	Antibiotic resistance genes transfer into *E. coli* was confirmed. NPs significantly affected bacterial conjugative transfer frequency, with PLA having a stronger influence than PHA. Upregulated membrane protein genes (ompA and ompC) and repressed regulatory genes (korA, korB, trbA), subsequently activated downstream genes (trfAp and trbBp).	Liu et al. (2023)

				mg/L, 50 mg/L and 40 mg/L, and *E. coli* HB101 in streptomycin 30mg/L) were suspended with NPs for 8 hours under 37 °C		

Polylactic acid (PLA), polyhydroxy butyrate (PHB), polyhydroxy alkanoates (PHA), poly(butylene adipate-*co*-terephthalate) (PBAT), microplastics (MPs), Aluminium (Al), copper (Cu), lead (Pb), nano copper oxide (nano-CuO).
Lethal Concentration, 50% (LC50) Not applicable (NA

6. Conclusion

The rapidly expanding body of literature investigating the vector role of microplastics for coexisting pollutants provides valuable insights into their complex interactions and consequent toxicity. On the one hand, biodegradable microplastics show a higher adsorption for several type of pollutants, which might be linked to their polar nature, in comparison with, for example, apolar polyolefins. On the other hand, apolar pollutants such as polyaromatic hydrocarbons leads to a higher adsorption onto apolar microplastics, for example, polypropylene, compared to biodegradable plastics. This highlights that specific pollutant and microplastic interactions have to be considered. The ability of microplastics to transport these pollutants into the exposed biota raises concerns about the fate of biodegradable microplastics in aquatic ecosystems, as it is the case for fossil fuel-based ones. These microplastics-pollutant complexes could be also colonized by microorganisms, including pathogenic and plastics degrading bacteria, forming biofilms within the plastisphere. As the degradation process is not instantaneous, these biofilms may serve as a source of food for primary consumers, such as zooplankton. Being a source and sink of pollutants, this ingestion can have adverse effects on their physiological processes due to microplastics themselves, the leaching of their additives as a result of weathering, and coexisting pollutants already carried. For instance, a study conducted by Vroom et al. (2017) demonstrated that the aging of polystyrene microplastics in seawater led to increased ingestion by zooplankton due to the formation of biofilms, enhancing their attractiveness. This ingestion by primary consumers can also serve as a channel for the transfer of multiple pollutants to higher trophic levels through predator-prey relationships. This process may facilitate the transport of pollutants, potentially increasing the risk to the aquatic ecosystem. Nevertheless, there remains a lack of research addressing the fate of polluted biobased microplastics within the aquatic food chain, leaving unanswered the question of whether they contribute to biomagnification along the food chain and pose potential ecological hazards.

In conclusion, the exponential increase in global plastic production has led to a widespread distribution of microplastics in various environmental compartments. Microplastics, recognized for their high adsorption capacity of co-occurring contaminants, have raised concerns regarding their potential ecological and human health impacts. The emergence of biodegradable plastics as eco-friendly alternative holds promises; however, the disintegration of these materials into microplastics and their interactions with concurrent pollutants and their fate in the environment and their potential impacts on aquatic biota is an alarming issue, as it is for fossil fuel plastics. Their interactions with a variety of pollutants in aquatic environments are intricate and dynamic. These microplastics have exhibited the capacity to efficiently adsorb pollutants, potentially impacting the distribution and bioavailability of contaminants within aquatic ecosystems. However, the combined effects of biobased plastics and concurrent pollutants on aquatic biota remain a subject of ongoing research, characterized by complex and variable outcomes. Further research is needed to fully comprehend the environmental implications of these interactions and to develop effective strategies for mitigating potential risks to aquatic ecosystems and biota. The scarcity of research on their toxicity and ecological consequences for aquatic organisms exacerbates these challenges. As the demand for these plastics continues to grow, it is important

to undertake comprehensive studies to better understand and address their environmental implications and to formulate strategies for enhancing their sustainability in various applications.

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Contributions

WA contributed to the data collection, analysis, and writing of the original draft. HJ contributed to the methodology, visualization, and review. JS Lee was involved in supervising, reviewing, editing, and formatting. PZ and SS contributed to supervising, reviewing, editing, and funding acquisition.

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Paper VII

Toxicity comparison of polylactic acid and polyethylene microplastics co-exposed with methylmercury on *Daphnia magna*

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Toxicity comparison of polylactic acid and polyethylene microplastics co-exposed with methylmercury on *Daphnia magna*

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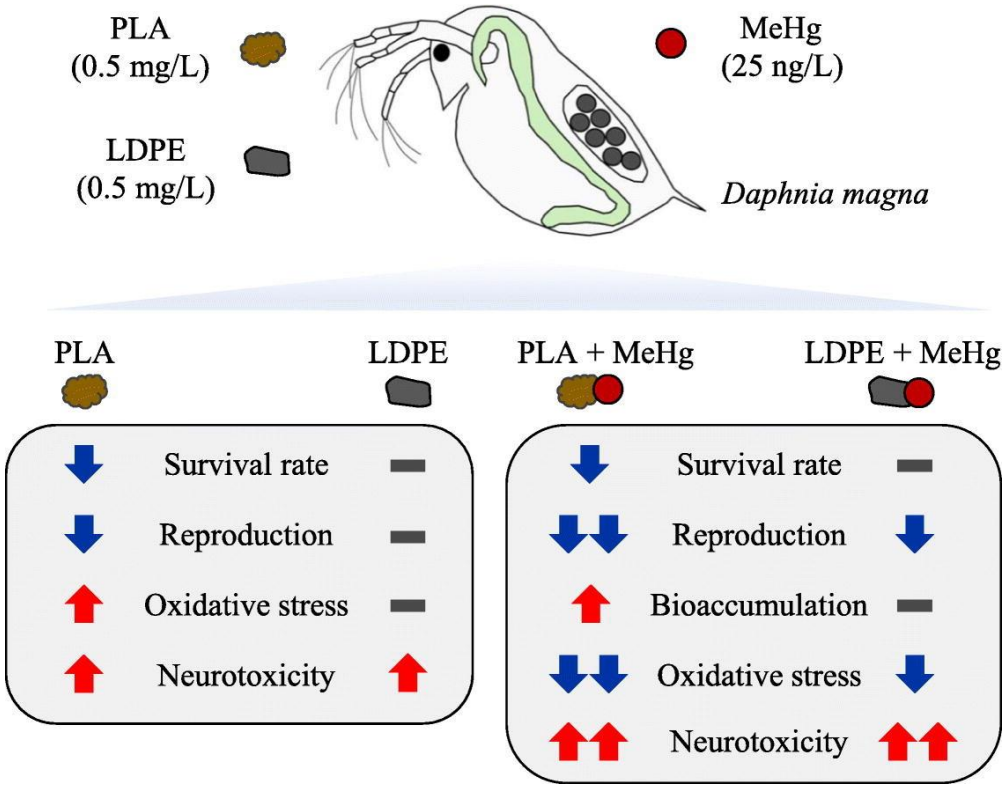
Abstract

The prevalence of microplastics (MPs) in aquatic ecosystems has become a significant environmental concern due to their persistence and potential toxicity. Although bioplastics, such as polylactic acid (PLA), are promoted as eco-friendly alternatives to conventional plastics, their toxicity remains poorly understood. This study compares the toxicity and pollutant vector roles of polar PLA-derived bio-microplastics (bio-MPs) with apolar low-density polyethylene (LDPE) MPs, both individually and in combination with methylmercury (MeHg), in *Daphnia magna*. PLA bio-MPs, both alone and in combination with MeHg, significantly reduced survival rates and reproduction while inducing oxidative stress. Additionally, PLA bio-MPs increased Hg accumulation and negatively impacted acetylcholinesterase activity and vitellogenin gene expression compared to LDPE MPs. The findings of this study suggest that PLA bio-MPs, despite being *in vivo* biodegradable, may pose similar or even greater environmental risks than fossil fuel-based MPs, particularly due to their potential to enhance the bioaccumulation and toxicity of coexisting pollutants.

Keywords: Polylactic acid; Low-density polyethylene; Methylmercury; Oxidative stress; Bioaccumulation

Graphical abstract



1. Introduction

Plastics, synthetic polymers primarily derived from fossil fuels, are known for their lightweight, elastic, durable, and cost-effective properties, which have led to their widespread use across various sectors. Ranging from household to industrial applications, plastics have become an integral part of daily life (Hassan et al. 2022). As of 2022, global plastic production has flowed to over 400 million tons, with projections indicating an increase to 500 million tons by 2030 (Ali et al. 2024a). The main end-of-life options for plastic waste are recycling, biodegradation, landfilling, and incineration (Ali et al. 2023a). Despite these options, plastic waste management poses significant environmental challenges, with about 22% of plastic waste escaping formal management systems and ending up in landfills, being openly burned, or accumulating in natural environments (OECD, 2022). In the natural environment, weathering processes fragment plastics into smaller particles, known as microplastics (MPs) (less than 5 mm) and nanoplastics (less than 100 nm). These synthetic wastes have since become the most numerically abundant form of solid waste on Earth (Eriksen et al. 2014), being detected in nearly all environmental matrices, ranging from the deepest oceans (Peng et al. 2018) to near the peak of the highest mountain, Everest (Napper et al. 2020).

Fossil fuel-based MPs pollution poses significant environmental challenges, notably due to their persistence in aquatic ecosystems. Ingestion of MPs has been reported at almost all trophic levels of the aquatic food web, ranging from the base, such as the plankton community (Sun et al. 2017; Carlotti et al. 2023) and predator-prey interaction (Sun et al. 2019; Liu et al. 2022), to higher trophic levels, including fish (Barboza et al. 2020; Horton et al. 2024) and even birds (Essoufi et al. 2024; Benjaminsen et al. 2024). This ingestion leads to bioaccumulation, allowing MPs to move up the food chain via trophic transfer, suggesting an indirect but potentially significant pathway of MPs ingestion in species at higher trophic levels (Nelms et al. 2018). For example, polyethylene (PE) is one of the most widely produced petrochemical-based plastics and is commonly found in aquatic environments (Erni-Cassola et al. 2019; Wu et al. 2020); it has also been reported as the most-ingested polymer compared to other types of MPs in various aquatic species, including zooplankton (Kosore et al. 2018; Traboni et al. 2023) and fish (Merga et al. 2020; Huang et al. 2020). Furthermore, trophic transfer of PE MPs through contaminated prey has been shown to lead to greater accumulation in predators compared to direct ingestion from the environment (Athey et al. 2020), which highlights the capability of MPs to bioaccumulate and even biomagnify in higher trophic levels. Such accumulation is concerning because exposure to MPs leads to oxidative stress, reproductive toxicity, and even mortality in the exposed biota (Kang et al. 2021; Masud and Cable, 2023; Kim et al. 2022), which will have severe impact on the overall fitness of the ecosystem.

In recent decades, bioplastics have emerged and been promoted as an alternative to fossil fuel-based plastics, primarily to reduce carbon dioxide emissions and dependence on fossil fuels. For instance, in 2019, fossil fuel-based plastics were responsible for 3.4% of global greenhouse gas emissions, with 90% of these emissions originating from their production and conversion from fossil fuels (OECD, 2022). In contrast, bioplastics can significantly reduce carbon emissions; producing one metric ton of bioplastics can decrease CO_2 emissions by 0.8

to 3.2 times compared to fossil fuel-based plastics (Abraham et al. 2021). There has been a significant increase in bioplastics production annually, with 1.8 million tons produced in 2022 and 2.18 million tons in 2023 (European Bioplastics, 2023). Polylactic acid (PLA), a bioplastic derived from natural sources like corn and starch, is used in various applications ranging from medical applications to packaging (European Bioplastics, 2023). This semi-crystalline linear aliphatic polyester consists of lactic acid monomers linked by ester bonds. Its degradation primarily involves fragmentation through hydrolysis, which cleaves the macromolecule's backbone or side chains, followed by mineralization into simpler compounds (Ali et al. 2023b). Thus, they have been marketed as more sustainable compared to petrochemical plastics, but there is limited scientific evidence supporting this notion, especially regarding their toxicity. For example, PLA is an *in vivo* degradable polymer, it has been reported to produce MPs under aquatic environmental conditions (Le Gall et al. 2022). These bio-MPs have been detected in the aquatic environment (Kazour et al. 2019) and most recently in herbivorous limpets (*Lottia subrugosa*) collected from the Santos Estuarine System, Brazil (Ribeiro et al. 2024).

Several experimental studies shows that PLA bio-MPs could leads to high mortality and decrease in reproductive output (Zimmermann et al. 2020; Ali et al. 2024b), oxidative stress (Chagas et al. 2021), neurotoxicity (Malafaia et al. 2021; de oliveira et al. 2021) and even skeletal development inhibition (Zhang et al. 2021) in the exposed model organisms. Recent literature reviews indicate that PLA bio-MPs can be as toxic to aquatic biota as fossil fuel-based MPs (Ali et al. 2023b). Recently, there has been growing interest in comparing the toxicity of bio-MPs with fossil fuel-based MPs. For example, biobased polyhydroxybutyrate (PHB) and fossil fuel-based polymethylmethacrylate (PMMA) MPs have been shown to significantly reduce assimilation efficiency and impact the physiological activities of freshwater amphipods (Straub et al. 2017). Similarly, exposure to PE MPs and PHB bio-MPs led to significant decreases in antioxidant enzyme activities in blue mussels (Magara et al. 2019). Furthermore, PLA bio-MPs have been reported to alter the taxonomic composition of natural phytoplankton assemblages in a mesotrophic lake, whereas fossil fuel-based polystyrene (PS) MPs had no such effects (Yokota and Mehlrose, 2020). These comparisons between bioplastics and fossil fuel-based MPs suggest that bioplastics may not be as environmentally benign as previously thought (Ali et al. 2023a), highlighting the need for further exploration and comparison of their toxicological effects.

There has been growing interest in the role of MPs in the sorption of co-existing pollutants, with the majority of studies focusing on comparing the sorption of these pollutants between bio-MPs and fossil fuel-based MPs (Liao and Yang, 2020; Shi et al. 2023). Recently, we reviewed the adsorption of coexisting pollutants on biobased and biodegradable MPs compared to fossil fuel-based MPs and concluded that these bio-MPs can also adsorb pollutants and their consequent toxicity could be either synergistic or antagonistic (Ali et al. 2024a). Although research on the transport of environmental pollutants by bio-MPs to aquatic biota and their potential toxicity is limited. Understanding the role of bio-MPs, such as PLA bio-MPs, in the transport and toxicity of coexisting pollutants is crucial for evaluating their environmental impact.

Heavy metals, another group of pollutants of concern, are defined as metals with a density greater than 5 g/cm³ (Ali and Khan, 2018), originating from both natural and anthropogenic sources, including industrial activities and agricultural practices (Ali et al. 2019). They persist in the environment, contaminate food chains, and pose toxic risks to those exposed (Waqas et al. 2024). Among heavy metals, mercury (Hg) is recognized as one of the most toxic pollutants and poses significant threats to aquatic biota, making it a global contaminant of concern (Li and Tse, 2015). In the natural environment, anaerobic bacteria convert Hg to mercuric sulfide (HgS) and then methylate it to produce methylmercury (MeHg) (Janssen et al. 2016). MeHg can easily penetrate cell membranes by forming a complex with sulfhydryl groups (Jeong et al. 2024a). MeHg has been reported to have severe effects on exposed biota, ranging from oxidative stress and genotoxicity to neurotoxicity and even mortality (Raihan et al. 2020; Zeid et al. 2021; Carvalho et al. 2023). Moreover, the interaction between MPs and MeHg can adversely affect aquatic organisms. MPs can adsorb MeHg onto their surfaces, potentially increasing the concentration of MeHg upon ingestion by aquatic organisms and thereby exacerbating toxicity (Zhu et al. 2022). The physical damage induced by MPs, coupled with the chemical toxicity of MeHg, may exacerbate stress responses and disrupt the immune system, ultimately compromising health outcomes in aquatic organisms (Barboza et al. 2018). In addition, MeHg bound to MPs may persist longer within the bodies of aquatic organisms, potentially facilitating biomagnification from phytoplankton to higher trophic levels along the food chain, thereby posing a higher risk to the aquatic ecosystem (Ogorek et al. 2021). Recently, Yoo et al. (2024) found that the size of MPs also affects the toxicity of MeHg in exposed organisms. However, there is still a lack of research addressing whether the bioaccumulation and toxicity of MeHg is mitigated or enhanced depending on the type of MPs.

To address this knowledge gap, this study investigates and compares the toxicity and vector roles of low-density polyethylene (LDPE) MPs, an apolar fossil fuel-based MPs with PLA bio-MPs, which contain polar ester functional groups capable to interact with metals in their repeating unit. The objectives of this study were to examine their potential role as vectors influencing the bioaccumulation of MeHg and to assess their synergistic or antagonistic effects on primary consumers, using *Daphnia magna* as our experimental model. We studied chronic survival, reproduction, oxidative stress, antioxidant activity, neurotoxicity, and expression of their related genes following exposure to each type of MP alone and combinations of MeHg with either LDPE MPs or PLA bio-MPs. Our goal was to deepen our understanding and compare how fossil fuel-based MPs and bio-MPs, as well as their vector role in transporting coexisting pollutants, influence the overall health and ecological function of aquatic ecosystems.

2. Materials and Methods

2.1. Chemicals and reagents

Methylmercury (II) chloride (MeHg, CH_3HgCl; CAS: 115-09-3) was obtained as a standard solution in water from Alfa Aesar (Ward Hill, Massachusetts, USA). PLA (Ingeo™ Biopolymer 4032D from NatureWorks LLC, United States) and LDPE (product code: BM50, EXXONMOBIL™, Dallas, United States) beads were utilized to produce MPs with a size range

of 2 to 10 µm, as described in our recent studies (Ali et al. 2024b, Ali et al. 2024c). Briefly, PLA and LDPE beads were converted into MPs using cryogrinding techniques with a cryomill (CRYOMILL, RETSCH®, Haan, Germany) at the Materials Research and Development Centre, University of Mons, Belgium. The size distribution of each type of MPs was estimated by conducting ten consecutive measurements using distal water as the solvent with Dynamic Light Scattering (Mastersizer 2000, Malvern Panalytical, UK). The surface morphology of both types of MPs was examined using scanning electron microscope (SEM). To estimate the zeta potential of MPs, both alone and in combination with MeHg, they were incubated in 50 mL of M4 medium for 24 hours at a constant temperature of 23°C with a 12-hour light-dark cycle inside an incubator (MIR-554, Sanyo, Osaka, Japan). After incubation, three consecutive measurements of each sample were taken using an electrophoretic light scattering spectrophotometer (ELS-Z, Otsuka Electronics, Osaka, Japan). N-acetyl-L-cysteine (NAC), with a purity greater than 99%, was used in reactive oxygen species (ROS) inhibitor test and was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Model organism

In this study, we used a KIT strain of the freshwater crustacean, *D. magna*, as our model organism. As primary consumers, *D. magna* play a crucial role in channeling energy from primary producers to higher trophic levels and are widely used as model species for risk assessment of environmental pollutants, including heavy metals and MPs (Zimmermann et al. 2020; Yuan et al. 2020), among others. *D. magna* possesses numerous characteristics that make it an excellent model species, including ease of handling, its ability to reproduce parthenogenetically, phenotypic plasticity, high sensitivity to environmental variations, and the availability of extensive genomic data (Lee et al. 2019; Jeong et al. 2022). The population of our selected model organism, sourced from Carolina Science and Math (Burlington, NC, USA) via the Korean Institute of Toxicology (Daejeon, South Korea), is maintained in plastic tanks containing ADaM media, fed daily with the green alga *Chlorella vulgaris* at approximately 1×10^6 cells/mL, and kept at a constant temperature of 23°C under a 12-hour light/12-hour dark photoperiod (Klüttgen et al. 1994; Sanpradit et al. 2024). To initiate our experiment, *D. magna* were transferred to and acclimated in M4 medium, which was also used as a medium during the experiment.

2.3. *In vivo* chronic toxicity experiments

To compare the toxicity of PLA bio-MPs and LDPE MPs, both alone and in combination with MeHg, a chronic toxicity experiment was conducted following the Organization for Economic Cooperation and Development (OECD) Guideline 211 (OECD, 2012). Six neonate *D. magna* (< 24 h old) were incubated in a glass jar with 600 ml of M4 medium, fed daily with *C. vulgaris* at a concentration of 5×10^5 cells/mL, and maintained at a constant temperature of 23°C under a 12-hour light/12-hour dark photoperiod, considering three replicates for each group. Briefly, daphnid were divided into six groups (n=3): control (M4 medium only), PLA bio-MPs (0.5 mg/L), LDPE MPs (0.5 mg/L), MeHg (25 ng/L), PLA bio-MPs (0.5 mg/L) plus MeHg (25 ng/L), and LDPE MPs (0.5 mg/L) plus MeHg (25 ng/L), respectively. Tween 80 at a concentration of 0.001% v/v was added to each group to prevent MPs aggregation, with no

observed impact on toxicity, oxidative stress, or bioconcentration compared to the control group (Luangrath et al. 2024). The selected concentrations of both PLA bio-MPs and LDPE MPs were based on our previous study, mirroring significant toxicity (Ali et al. 2024c), and the concentration of bio-MPs was chosen with the understanding that it might approximate realistic environmental levels (Ali et al. 2024b). Furthermore, given that the 48-hour Lethal Concentration 50% (LC50) of *D. magna* exposed to MeHg in the presence of feed is 7.1 µg/L (Hylton et al. 2021), the 25 ng/L concentration used in this chronic study is significantly lower, facilitating the evaluation of chronic effects that may not be evident in acute toxicity tests. The chronic toxicity experiment lasted for 21 days, during which the M4 media was renewed every second day and the organisms were fed daily. Mortality and the total number of offspring per female, including live, dead, and unhatched eggs, for each replicate of each group were evaluated every 24 hours.

2.4. Accumulation of methyl mercury

To compare the vector roles of PLA bio-MPs and LDPE MPs in the transport of MeHg, 150 daphnids (14 days old) per group were incubated with MeHg (25 ng/L), MeHg + PLA bio-MPs (25 ng/L + 0.5 mg/L), and MeHg + LDPE MPs (25 ng/L + 0.5 mg/L), for 24 hours at 23°C. Following exposure, the daphnids were collected, rinsed with a 25 mM ethylenediaminetetraacetic acid (EDTA) solution to remove external contaminants, including metals, and then freeze-dried for 24 hours, according to the method detailed in Byeon et al. (2020). The dried samples were then digested with 1 mL of 70% nitric acid and a few drops of hydrogen peroxide, followed by digestion on a graphite block at 200°C for 16 hours. After digestion, the samples were cooled, evaporated, re-dissolved, and diluted to 10 mL with Milli-Q water. The concentration of MeHg was assessed by inductively coupled plasma optical emission spectroscopy.

2.5. Swimming behavior

For the assessment of *D. magna's* behavioral response to bio-MPs versus MPs, MeHg, and their combinations, we exposed 5-day-old daphnids to the following conditions: PLA (0.5 mg/L), LDPE (0.5 mg/L), MeHg (25 ng/L), PLA (0.5 mg/L) plus MeHg (25 ng/L), and LDPE (0.5 mg/L) plus MeHg (25 ng/L), for 24 hours in the M4 medium following the protocol of Bownik et al. (2020) with modification. Briefly, post-incubation, *Daphnia* were carefully transferred, along with 6 mL of exposure medium, into small round plates and allowed to acclimate for 5 minutes. Subsequently, a 30-second video of the *Daphnia* was recorded using a Samsung Galaxy S22 Ultra at a frame rate of 30 frames per second under consistent lighting conditions. The recorded videos were then analyzed using Tracker® 5.1.3 software to quantify movement parameters such as speed and distance traveled.

2.6. Oxidative Stress and enzymatic activity assay

To investigate the oxidative stress induced by bio-MPs versus MPs, MeHg, and their combinations, we exposed 5-day-old daphnids to the following conditions: PLA (0.5 mg/L), LDPE (0.5 mg/L), MeHg (25 ng/L), PLA (0.5 mg/L) plus MeHg (25 ng/L), and LDPE (0.5

mg/L) plus MeHg (25 ng/L). The exposure was conducted for 24 hours under controlled conditions, with a constant temperature of 23 °C and a 12-hour light-dark cycle, within an incubator (MIR-554, Sanyo, Osaka, Japan). Post-exposure, daphnids were filtered, rinsed with M4 medium and phosphate-buffered saline (PBS), and homogenized using a Teflon mini pestle in lysis buffer (40 mM Tris–HCl, 0.1% NP-40, 120 mM NaCl) supplemented with a protease inhibitor. The homogenates were then centrifuged at 10,000 g for 10 minutes at 4 °C, and the supernatant was collected for estimation of ROS level, superoxide dismutase (SOD), catalase (CAT), and acetylcholinesterase (AChE) activity. The details regarding the estimation of ROS, SOD, CAT, and AChE activities are provided in the Supplementary Materials (**Text S1).**

2.7. Total protein concentration

The total protein concentration, determined using the Bradford assay (Bradford, 1976) with Bio-Rad's protein assay dye reagent, was used to normalize the results for reactive oxygen species (ROS) and antioxidant enzymes. A standard curve was constructed with a 0.1% bovine serum albumin (BSA) solution across five different concentrations. Absorbance was measured at 595 nm using a spectrophotometer.

2.8. RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction

To compare the mRNA expression, 5-day-old *Daphnia* were exposed to the following conditions: PLA (0.5 mg/L), LDPE (0.5 mg/L), MeHg (25 ng/L), PLA (0.5 mg/L) plus MeHg (25 ng/L), and LDPE (0.5 mg/L) plus MeHg (25 ng/L). The exposure lasted for 24 hours under controlled conditions, with a constant temperature of 23°C and a 12-hour light-dark cycle, maintained in an incubator (MIR-554, Sanyo, Osaka, Japan). Total RNA from each group was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA quality and quantity were assessed using a Nanodrop (QIAxpert, QIAGEN GmbH, Hilden, Germany), with an A260/280 ratio ranging from 1.9 to 2.0. For cDNA synthesis, 1 µg of RNA from each sample was reverse transcribed using the SuperScript III Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's instructions. The details regarding the quantitative reverse transcription polymerase chain reaction (qRT-PCR) are provided in the Supplementary Materials (**Text S2**).

2.9. Statistical analysis

SPSS software (version 22) was used to perform Levene's test for assessing the homogeneity of variances across different groups. Given that the assumption of homogeneity of variances was satisfied, we proceeded with one-way analysis of variance and independent sample t-tests ($P < 0.05$) to evaluate differences between the control and exposed groups. Post hoc analysis was conducted using Tukey's HSD test to pinpoint statistically significant differences between specific groups. Statistical significance was set at a threshold of $P < 0.05$. Results are reported as mean values with standard deviations.

3. Results

3.1 Characterization of MPs

The scanning electron microscope (SEM) images of both PLA bio-MPs and LDPE MPs are presented in **Fig. 1**. These images show that both types of MPs have rough and uneven surfaces, characterized by prominent fractures, cracks, and various irregular shapes. The irregular morphology of these particles is further highlighted by the presence of sharp edges and angular features, with sizes ranging from 2 to 10 μm. The SEM images also showed no evident difference in the surface morphology between the PLA bio-MPs and LDPE MPs. The morphology of these MPs indicates the typical characteristics commonly observed in MPs found in the natural environment.

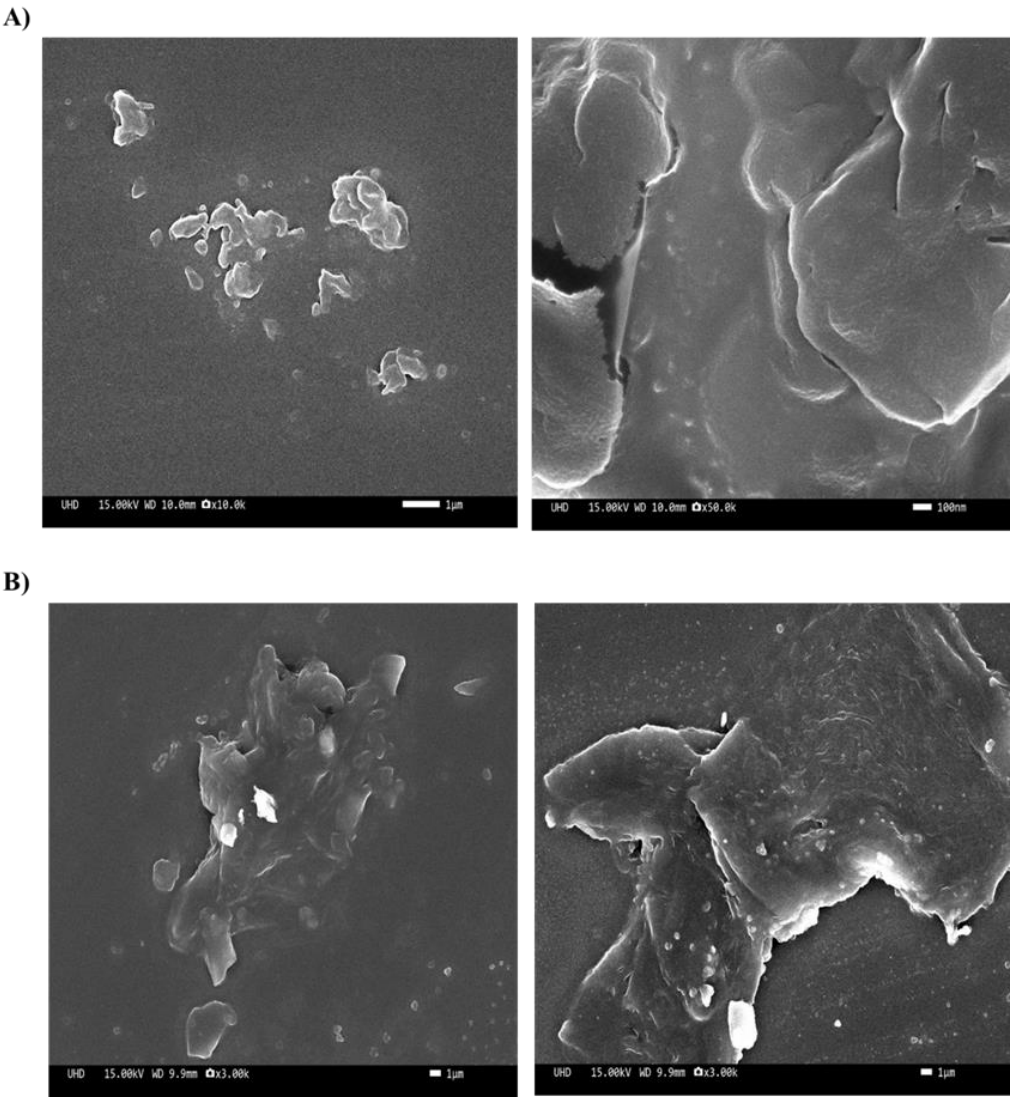


Fig. 1. Scanning electron microscope images of A) polylactic acid bio-microplastics (PLA bio-MPs) and B) low density polyethylene microplastics (LDPE MPs).

Next, to assess alterations in the properties of both types of MPs in M4 medium, with or without MeHg, we determined the zeta potential values. The zeta potential of PLA bio-MPs

and LDPE MPs in M4 medium reveal that both types of MPs exhibit a net negative surface charge, with PLA bio-MPs showed a zeta potential of −13.59 ± 0.88 mV and LDPE MPs −13.46 ± 1.76 mV. In the presence of MeHg, the zeta potential for PLA and LDPE MPs decreased to −16.17 ± 1.84 mV and −15.73±0.69 mV, as shown in **Fig 2A**. This suggests that MeHg further decreased the negative surface charge of both types of MPs, likely through adsorption, which alters their surface properties. These changes could potentially impact the intensity of toxicity that the MPs might pose within aquatic ecosystems.

3.2 *In Vivo* Chronic Effects on Survival and Reproduction

The chronic survival rate of *D. magna* exposed to bio-MPs and MPs, with or without MeHg, over 21 days is illustrated in **Fig. 2B**. The survival data revealed distinct differences between the effects of PLA bio-MPs and LDPE MPs. In the group exposed to PLA bio-MPs, a significant ($P < 0.05$) decline in survival was observed, with the survival rate decreasing to 61.11% by day 21, indicating a pronounced toxic effect of PLA bio-MPs on *D. magna*. Conversely, the group exposed to LDPE MPs maintained a considerably higher survival rate of 94.44%, suggesting a lower toxicity profile for LDPE MPs under the same conditions. When *D. magna* were co-exposed to PLA bio-MPs and MeHg, the survival rate exhibited a similar decline as seen with PLA bio-MPs alone, reaching 61.11% by day 21. This indicates that the presence of MeHg did not further exacerbate the toxicity of PLA bio-MPs on survival rate. In contrast, the survival rate of *D. magna* exposed to the combination of LDPE MPs and MeHg remained relatively stable, with only a slight decrease to 94.44% by day 21, mirroring the survival rate observed in the LDPE MPs alone group. This study found that PLA bio-MPs exert a negative impact on the survival of *D. magna* compared to LDPE MPs. Additionally, the presence of MeHg does not seem to modify the toxic effects associated with either type of MPs on the survival rate.

The results from the 21-day chronic *in vivo* experiment also demonstrate significant inhibited reproductive performance in *D. magna* under various treatments, as shown in **Fig. 2C**. Exposure to PLA bio-MPs (0.5 mg/L) led to a significant ($P < 0.05$) reduction in live neonates and an increase in dead neonates, reflecting a pronounced toxic effect on reproduction. In contrast, LDPE MPs (0.5 mg/L) did not result in significant ($P < 0.05$) alterations in reproductive outcomes compared to the control. This suggests that LDPE MPs at this concentration do not have a noticeable impact on the reproduction of *D. magna*. MeHg exposure (25 ng/L) alone significantly ($P < 0.05$) impaired reproduction, as evidenced by fewer live neonates and increased mortality. When combined with PLA bio-MPs, MeHg did not exacerbate the toxic effects beyond those observed with PLA alone, suggesting a lack of synergistic toxicity. A similar trend was observed with the combination of LDPE MPs and MeHg, where reproductive outcomes were comparable to those seen with either MeHg or LDPE MPs alone. Overall, PLA bio-MPs and MeHg each individually impaired reproduction in *D. magna*. PLA bio-MPs have a more pronounced effect than LDPE MPs, which did not affect reproduction at the tested concentration. The addition of MeHg does not significantly alter the reproductive toxicity of LDPE MPs or enhance the effect of PLA bio-MPs.

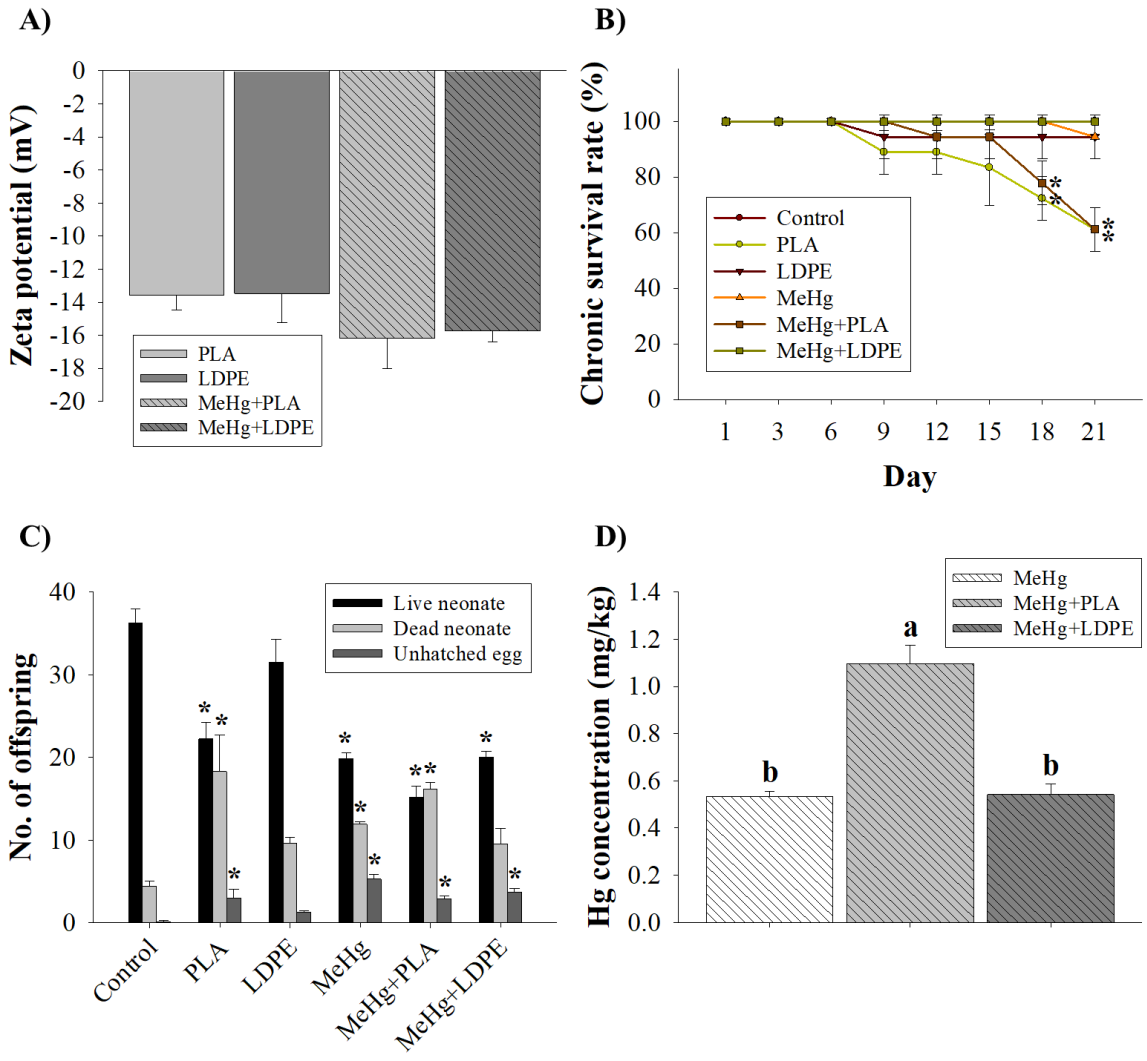


Fig. 2. Zeta potential values of polylactic acid bio-microplastics (PLA bio-MPs) and low-density polyethylene microplastics (LDPE MPs) with and without methylmercury (MeHg) (A). *In vivo* effects of 21-day exposure to PLA bio-MPs, LDPE MPs, and MeHg and the combinations of either type of MPs with MeHg on the survival (B) and reproduction (C) of *Daphnia magna*. Total mercury (Hg) concentrations following co-exposure to MeHg with either polar PLA bio-MPs or apolar LDPE MPs (D). Statistically significant differences ($P < 0.05$) are indicated by different letters and asterisks.

3.3 Mercury concentration

The mean concentration of mercury (Hg) after exposure to MeHg alone or in combination with PLA bio-MPs or LDPE MPs is shown in **Fig. 2D**. The results showed that when *D. magna* was exposed to MeHg alone, the mean concentration of Hg was 0.534 mg/kg. However, when exposed to MeHg in combination with PLA bio-MPs, the mean concentration increased significantly ($P < 0.05$) to 1.09575 mg/kg. This indicates that PLA bio-MPs may enhance the bioaccumulation of MeHg in *D. magna*, likely by facilitating its uptake via adsorption. On the other hand, when *D. magna* was exposed to MeHg in the presence of LDPE MPs, the mean

concentration remained relatively unchanged at 0.542 mg/kg, similar to the concentration observed with MeHg alone.

3.4 Oxidative stress, antioxidant enzymatic activity, and gene expression profiles

ROS and antioxidant enzyme activities were evaluated to compare oxidative stress induced by PLA bio-MPs and LDPE MPs, both alone and in combination with MeHg as illustrated in **Fig. 3A**. PLA bio-MPs significantly ($P < 0.05$) increased ROS levels compared to the control, indicating a high oxidative stress. LDPE MPs did not significantly alter ROS levels, while MeHg alone significantly ($P < 0.05$) reduced ROS compared to the control. Combined exposure to MeHg with either PLA bio-MPs or LDPE MPs significantly ($P < 0.05$) decreased ROS levels compared to the control. Moreover, ROS levels in *D. magna* treated with NAC showed a significant ($P < 0.05$) decrease in the PLA bio-MPs exposed group compared to the control group, indicating that the induced ROS was effectively scavenged by NAC (**Fig. S1**). SOD activity was significantly higher ($P < 0.05$) in both the PLA bio-MPs and LDPE MPs groups compared to the control. However, co-exposure to PLA bio-MPs and MeHg resulted in a decrease in SOD activity compared to exposure to PLA bio-MPs alone **(Fig. 3B)**. Co-exposure to LDPE MPs and MeHg did not significantly affect SOD activity compared to the control or MeHg alone, but it was significantly ($P < 0.05$) reduced compared to LDPE MPs alone, suggesting antagonistic effects. CAT activity in *D. magna* varied, with PLA bio-MPs alone reducing CAT activity, indicating potential suppression of oxidative stress defenses, whereas LDPE MPs and MeHg individually increased CAT activity **(Fig. 3C)**, suggesting induced oxidative stress. Notably, co-exposure to PLA bio-MPs and MeHg significantly ($P < 0.05$) increased CAT activity, indicating a synergistic effect, while co-exposure to LDPE MPs with MeHg reduced CAT activity compared to their individual exposures.

The gene expression data shows distinct effects on oxidative stress response genes in *D. magna* **(Fig. 3D)**. Exposure to PLA bio-MPs induced a significant upregulation of *MnSOD1*, *CuZnSOD4*, *6*, *7*, and *CAT*, indicating an enhanced oxidative stress response. Conversely, exposure to LDPE MPs alone resulted in the downregulation of most *SOD* genes, including *CuZnSOD2*, *CuZnSOD6*, and *CuZnSOD7*, while *MnSOD2* was upregulated. MeHg alone consistently downregulated multiple *SOD* genes and *CAT*. Co-exposure to PLA bio-MPs and MeHg resulted in less pronounced changes in expression levels, characterized by the downregulation of *CuZnSOD7* and *CAT*, and the upregulation of *CuZnSOD6*, suggesting potential antagonistic effects. In contrast, co-exposure to LDPE MPs and MeHg resulted in downregulation of all genes examined, except for *MnSOD*, *CuZnSOD3*, and *CuZnSOD8*, which remained unaffected.

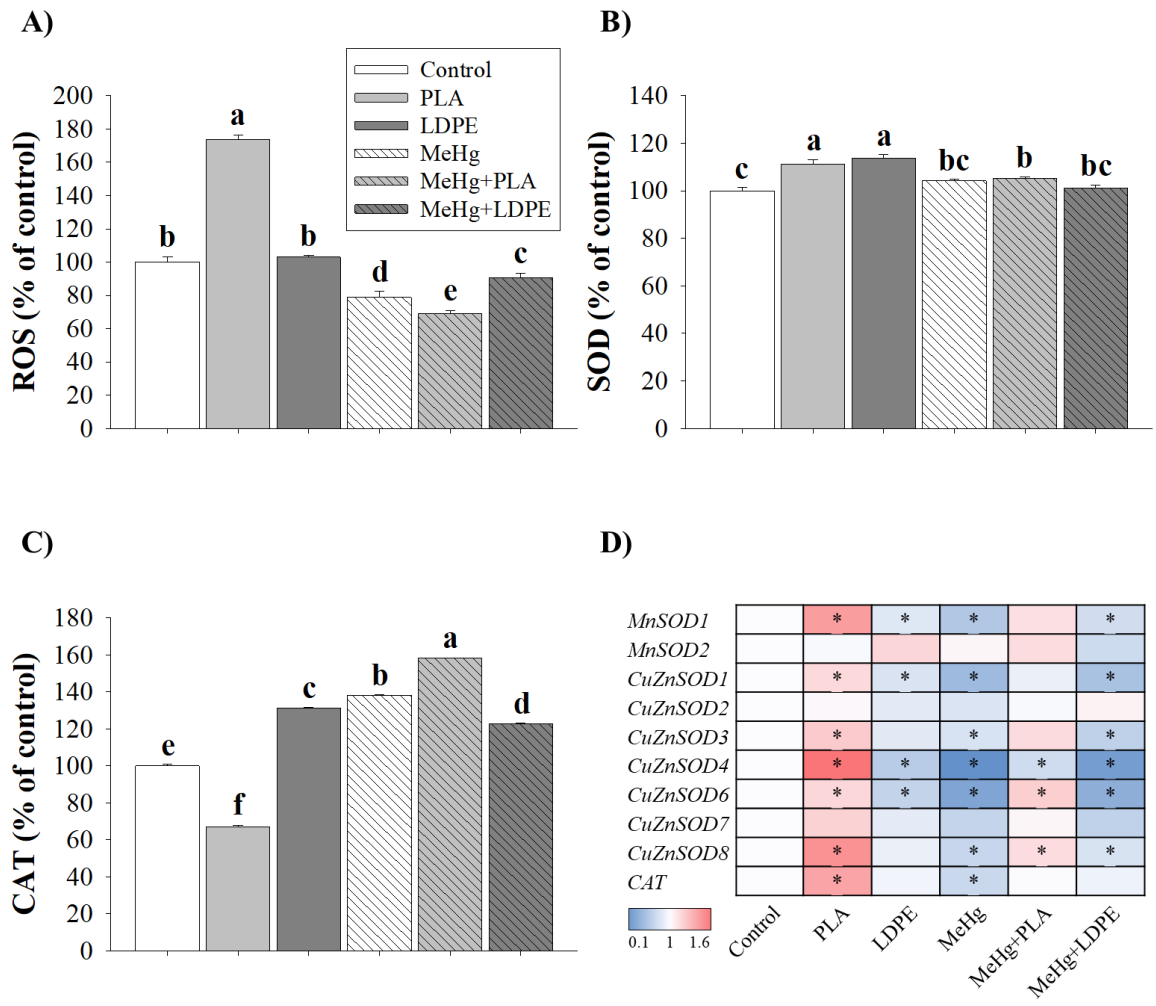


Fig. 3. Effects of polylactic acid bio-microplastics (PLA bio-MPs), low density polyethylene microplastics (LDPE MPs), methylmercury (MeHg) and their combined effects on A) intracellular reactive oxygen species (ROS) levels, B) superoxide dismutase (SOD) activity, C) catalase (CAT) activity, and D) the expression of antioxidant genes in *Daphnia magna*. The alphabets and asterisk (*) indicate significant differences ($P < 0.05$). Results represent the mean ± standard deviations of three replicates.

3.5 Swimming speed and distance

The swimming speed and distance covered by *D. magna* exposed to PLA bio-MPs, LDPE MPs, MeHg, and their combinations with MeHg, compared to the control group, are illustrated in **Fig. 4**. Exposure to PLA bio-MPs or MeHg alone resulted in alterations in swimming speed and distance, although these changes were not statistically significant, while LDPE MPs had no effect on either parameter compared to the control. Exposure to MeHg alone resulted in a slight increase in swimming speed and distance, suggesting a potential stimulatory or stress-induced response. In contrast, exposure to PLA bio-MPs alone led to a slight decrease in both

swimming speed and distance relative to the control. Interestingly, co-exposure to MeHg and LDPE MPs led to a reduction in both swimming speed and distance compared to MeHg alone, while the combination of MeHg with PLA bio-MPs did not significantly alter the behavior relative to PLA bio-MPs alone, though it did reduce these parameters compared to MeHg exposure alone.

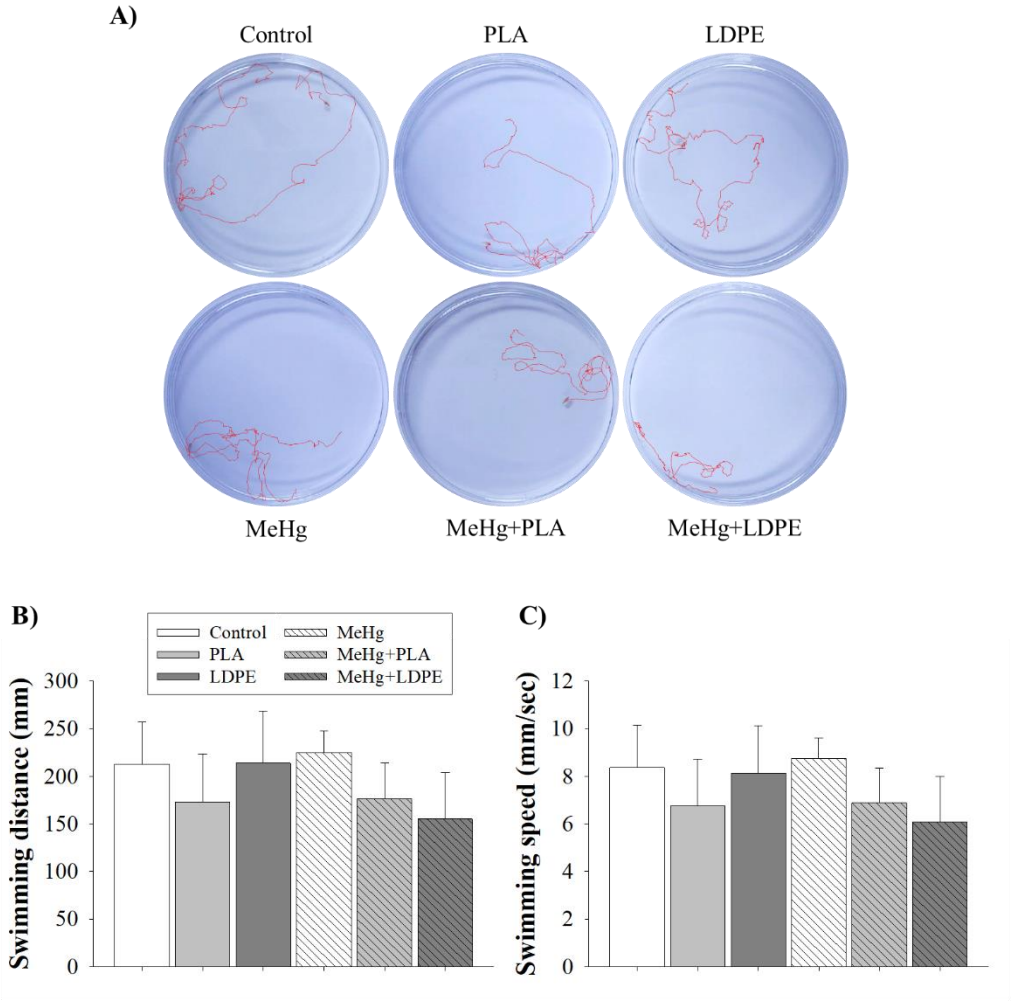


Fig. 4. Effects of exposure to polylactic acid bio-microplastics (PLA bio-MPs), low density polyethylene microplastics (LDPE MPs), methylmercury (MeHg) and their combined effects on the swimming behavior of *Daphnia magna*. Representative swimming pathway (A), Swimming distance (mm) (B), and Swimming speed (mm/sec) (C). Data are presented as mean ± standard deviation (SD).

3.6 AChE activity and gene expression

The AChE activity of *D. magna* exposed to PLA bio-MPs, LDPE MPs, and MeHg, alone or in combination are illustrated in **Fig. 5A**. AChE activity was significantly ($P < 0.05$) reduced in all treatments compared to the control, with PLA, LDPE, and MeHg each reducing AchE activity, co-exposure to LDPE and MeHg showing a greater reduction than LDPE alone, suggesting a synergistic effect. *AChE* gene expression was slightly downregulated in the group exposed to PLA bio-MPs and LDPE MPs alone, though not significantly compared to the

control **(Fig. 5B)**. MeHg alone significantly upregulated *AChE* gene expression. However, co-exposure to MeHg either with PLA bio-MPs or LDPE MPs significantly downregulated *AChE* gene expression compared to MeHg alone, suggesting an antagonistic interaction.

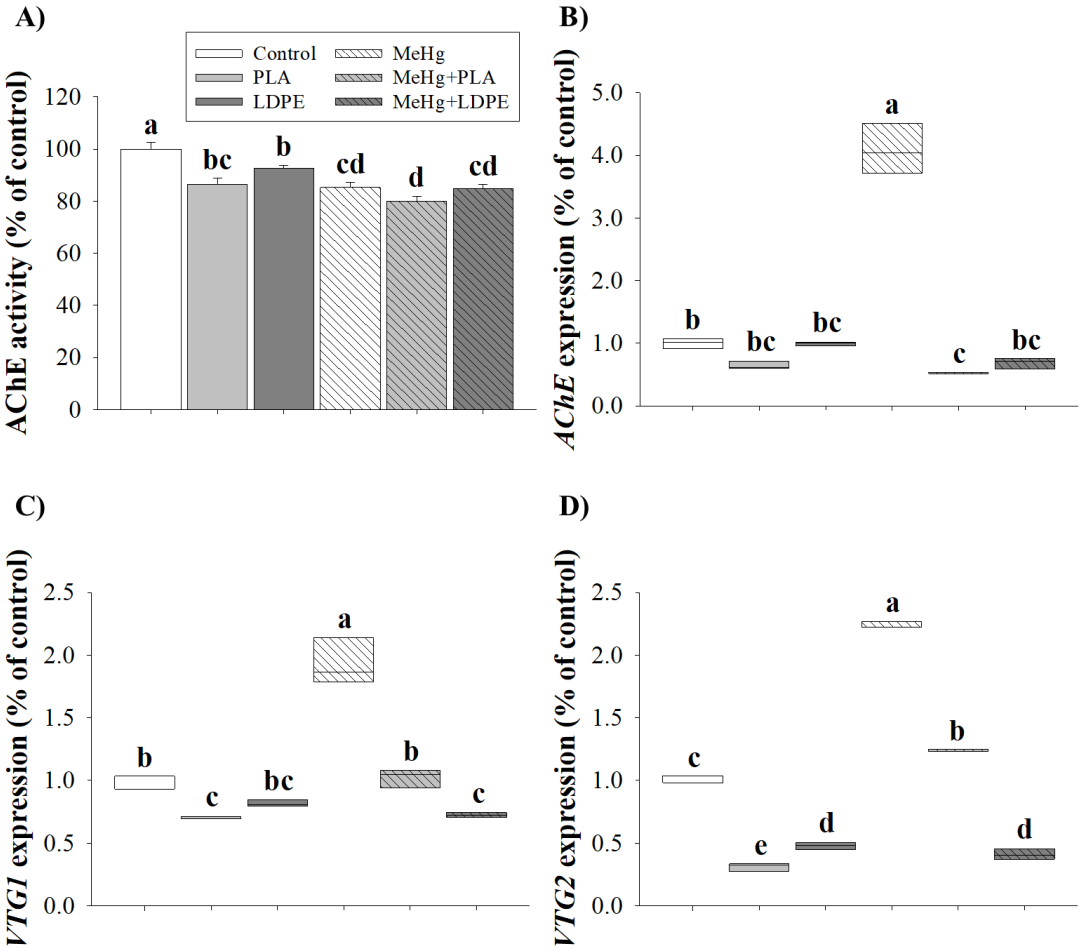


Fig. 5. Effects of polylactic acid bio-microplastics (PLA bio-MPs), low density polyethylene microplastics (LDPE MPs), methylmercury (MeHg) and their combined effects on A) Acetylcholinesterase (AChE) activity, B) *AChE* gene level, C) vitellogenin-1 (*VTG1*) gene expression level, and D) vitellogenin-2 (*VTG2*) gene expression level in *Daphnia magna*. The alphabets indicate significant differences ($P < 0.05$). Results represent the mean ± standard deviations of three replicates.

3.7 Reproduction related genes expression

The expression of vitellogenin-1 (*VTG1*) gene was significantly downregulated in PLA bio-MPs compared to the control ($P < 0.05$), while LDPE MPs did not, as shown in **Fig. 5C**. MeHg upregulated ($P < 0.05$) the expression of *VTG1* gene compared to the control. Co-exposure to MeHg and PLA bio-MPs had no significant effect ($P > 0.05$) on *VTG1* expression, indicating an antagonistic interaction. In contrast, co-exposure to LDPE MPs and MeHg significantly downregulated *VTG1* expression compared to both the control and MeHg alone; however, this effect was not significantly different from that of LDPE MPs alone, suggesting that the co-exposure does not produce a synergistic effect. *VTG2* gene expression was downregulated following exposure to PLA bio-MPs or LDPE MPs compared to the control group **(Fig. 5D)**.

Methylmercury (MeHg) significantly upregulated *VTG2* gene expression. Upon co-exposure to MeHg, PLA bio-MPs further increased *VTG2* expression, whereas co-exposure to LDPE MPs and MeHg resulted in significant downregulation of *VTG2* gene expression, consistent with the effect observed with LDPE MPs alone.

4. Discussion

MPs pollution has emerged as a global environmental concern, with particles small enough to be edible, MPs pollution threatens aquatic biota worldwide (Au et al. 2017). Ingestion occurs accidentally (Germanov et al. 2018) or through active selection, as many species mistake MPs for prey (de Sá et al. 2015), and indirectly via trophic transfer (Santana et al. 2017). Once ingested, MPs cannot be digested or absorbed by aquatic organisms, despite reports of fragmentation occurring within the digestive tracts of some species, such as Antarctic krill and rotifers (Dawson et al. 2018; Zhao et al. 2023). This may result in energy depletion, metabolism disorder, impairing growth and reproduction in organisms (Trestrail et al. 2021; Tuncelli et al. 2024). In addition, MPs can adsorb pollutants like heavy metals due to the presence of polar functional groups (Chen et al. 2023), and aging processes can introduce oxygen-containing groups on apolar MPs, enhancing adsorption capacity (Gao et al. 2021), which in turn affects their transport, accumulation, and toxicity (Ali et al. 2023a). Biobased polymers, such as PLA, are being promoted as eco-friendly alternatives to fossil fuel-based plastics and are widely used in applications including biomedical devices, single-use items, and textiles. However, PLA has been reported to generate MPs under aquatic conditions that are toxic to aquatic biota (Ali et al. 2023b) and to adsorb coexisting pollutants, potentially altering their subsequent toxicity (Ali et al. 2024a). Thus, it is important to study the vector role of bio-MPs and compare them with fossil fuel-derived MPs. Therefore, we compared PLA bio-MPs, both alone and in combination with MeHg, to widely produced fossil fuel-based LDPE.

4.1 Morphology and surface properties of PLA bio-MPs and LDPE MPs

To compare the toxicity and vector role of bio-MPs versus fossil fuel-derived MPs on *D. magna*, we synthesized MPs by grinding PLA and LDPE pellets. Both types of MPs were irregular in shape, with numerous cracks and fractures on their surfaces. The toxicity of MPs has been reported to depend on their size and shape (Park et al. 2024). MPs in the environment are primarily found as fragments, films, pellets, fibers, and foams, while ideal spherical MPs, though rare in nature, are most commonly used in research (Rozman et al. 2023). The occurrence of MPs with irregular shapes in the natural environment have been reported (Nayeri et al. 2023), aligning the shape of the MPs used in this study with realistic environmental scenarios. Similarly, the sizes of both types of MPs used in this study, ranging from 2 to 10 μm, are consistent with those reportedly produced from PLA and PE under laboratory conditions (Tong et al. 2022), including the ingestible range for *D. magna* (Rehse et al. 2016). This study provides a more ecologically relevant evaluation of bio-based and fossil fuel-derived MPs on *D. magna*, improving environmental understanding.

Zeta potential, a key physical property associated with the net charge at the particle surface, reflects the distribution of ions and non-ions in the interfacial region and forms electrostatic

layers that interact with the surrounding environment (Lage et al. 2012). In this study, the zeta potential values of PLA bio-MPs and LDPE ranged from −13 to −15 mV. The addition of MeHg slightly decreased the mean of zeta potential values to −15 to −17 mV ($P > 0.05$), which could suggest a potential interaction between MPs and MeHg, and altered surface charge. Charge neutralization is fundamental to adsorption processes, where alterations in surface charge can significantly impact the adsorption rate and the capacity of MPs to adsorb pollutants (Fan et al. 2021). For instance, a decrease in the zeta potential of PLA bio-MPs has been reported to increase the adsorption of lead (Pb) (Guan et al. 2022). An increase in negative surface charges on MPs could enhance the electrostatic adsorption of heavy metals (Xi et al. 2022), making changes in surface charge critical, as they directly influence the adsorption capacity of MPs and, consequently, their toxicity to biota.

4.2 Toxicity comparison on *in vivo* endpoints of *D. magna*

In chronic survival tests with *D. magna*, we found that PLA bio-MPs, alone or in combination with MeHg, significantly reduced the survival rate. In contrast, LDPE MPs, whether alone or with MeHg, did not affect survival, consistent with previous findings indicating that PE MPs (63–75 μm) at concentrations of 25, 50, and 100 μg/mL over a 21-day exposure did not induce mortality in *D. magna* (Canniff and Hoang, 2018). Thus, our results show that PLA bio-MPs had a greater negative impact on the chronic survival of *D. magna* compared to LDPE MPs. Similarly, a few studies comparing the toxicity of bio-MPs versus fossil fuels MPs have reported that PLA bio-MPs negatively impact the survival of exposed organisms. Recently, An et al. (2024) found that PLA bio-MPs significantly reduced the chronic survival rate of *D. magna* compared to polyethylene terephthalate (PET) MPs, with no significant difference between the control and PET MPs groups. Likewise, Luangrath et al. (2024) reported that PLA bio-MPs exhibited greater acute toxicity to *D. magna* compared to PE MPs, and Zimmermann et al. (2020) also observed a decreased survival rate in *D. magna* exposed to PLA bio-MPs compared to polyvinyl chloride (PVC) and polyurethane (PU) MPs. Furthermore, co-exposure to MeHg and either PLA bio-MPs or LDPE MPs did not significantly alter survival rates compared to single exposure, indicating no synergistic or antagonistic effects on *D. magna*. Consistent with our findings, Zhu et al. (2022) reported decreased survival in zebrafish larvae exposed to MPs alone or in combination with MeHg, with no significant difference between the single and combined exposures. Co-exposure of *Acartia tonsa* to oxidized MPs (0.25 mg/L) and Hg (0.5 μg/L) showed no impact on survival at these concentrations, but survival was significantly reduced at higher concentrations (Pinto et al. 2024). Thus, the lack of synergistic or antagonistic effects of both types of MPs and MeHg on survival, despite findings from other studies, could be attributed to the lower concentrations of MeHg used, suggesting that the potential toxicity of combined exposure is concentration-dependent.

MPs are increasingly recognized for impacting the reproduction of marine invertebrates (Jeong et al. 2022; Kim et al. 2022). Our study reveals that the type of MPs strongly influences their reproductive toxicity. Notably, PLA bio-MPs significantly reduced total reproduction by increasing mortality and the number of unhatched eggs during 21 days of chronic exposure,

while there was no significant difference in the LDPE MPs group, aligning with previous studies (Zimmermann et al. 2020; An et al. 2024). Zimmermann et al. (2020) found that all tested MPs decreased the reproductive output of *D. magna*, with PLA among the more toxic. Anderson and Shenkar (2021) similarly observed reduced fertilization rates in *Microcosmus exasperatus* with PLA bio-MPs and PET MPs, with no significant difference between the two MP types. However, the environmental risks of MPs are further complicated by their capacity to adsorb and accumulate heavy metals, such as MeHg. Our results showed that MeHg exposure significantly reduced total reproduction, and co-exposure with either type of MP had a similar effect as MeHg alone, suggesting that neither type of MPs altered the reproductive toxicity of MeHg. Previous studies have presented varied findings on the combined toxicity of MPs and heavy metals. Cheng et al. (2024) found that biodegradable polybutylene adipate terephthalate (PBAT) MPs mitigated the toxicity of heavy metals (Cu and Pb) in zebrafish embryos by adsorbing the metals and reducing their bioavailability. The combined exposure to PS MPs and zinc (Zn) in *D. magna* resulted in significant reductions in total fecundity and delayed the time to the first brood, indicating potential reproductive impairment (Lee et al. 2021). Conversely, Zhu et al. (2022) reported no significant difference in hatching rates when zebrafish were exposed to PS MPs, MeHg, and their combination. These results, together with ours, suggest that the interaction between MPs and heavy metals may vary depending on the types of MPs and contaminants involved, as well as the exposed organism, highlighting the complexity of assessing the combined ecological risks of MPs and associated pollutants.

4.3 Vector role of PLA bio-MPs versus LDPE MPs

MPs have gained significant attention not only as potential toxic substances but also as vectors for heavy metals in the environment (Kutralam-Muniasamy et al. 2021). Metals can easily adsorb onto MPs, but they may desorb when exposed to changing aqueous environments, potentially posing risks to the health of organisms (Li et al. 2024). In our study, PLA bio-MPs significantly increased Hg accumulation compared to exposure to MeHg alone or co-exposure with LDPE MPs and MeHg, suggesting that PLA bio-MPs may act as carriers of MeHg, highlighting MP type-specific interactions. Similar to our results, PLA bio-MPs have been reported to transport Cu and Pb, leading to up to four times higher bioaccumulation in fish, compared to polyamide (PA) (Jang et al. 2022). Co-exposure of fish to 20–200 μg/L PS MPs and 10 μg/L $CdCl_2$ resulted in increased cadmium (Cd) bioaccumulation (Lu et al. 2018). Exposure of fish to the metal alone resulted in significantly lower Hg concentrations in the gills compared to those exposed to the same concentration of Hg in combination with MPs (Barboza et al. 2018). According to Verdú et al. (2021), the extent to which MPs adsorb and desorb pollutants varies among different MPs type, primarily depending on the physicochemical properties of both the sorbate and the sorbent. Furthermore, *in vitro* simulations of the human digestive tract have shown that chromium (Cr) adsorbed on PLA bio-MPs is more bioaccessible than when adsorbed on petrochemical-based MPs (Liao and Yang, 2020), indicating that the bioavailability of heavy metals can differ significantly based on the type of MPs, affecting the consequent toxicity. Our results highlight the complex interaction between MPs and heavy metals, where the nature of the MP plays a crucial role in determining the environmental and biological fate of the adsorbed metals.

4.4 Oxidative stress response comparison

Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) production and the antioxidant defenses, is a well-established response to pollutants (Valavanidis et al. 2006), which can disrupt homeostasis and lead to cellular dysfunction, as both excessive and low ROS levels can be detrimental (Zorov et al. 2014; Ali et al. 2024c). In our study, distinct oxidative stress responses were observed when *D. magna* were exposed to MPs and MeHg, either individually or in combination. Specifically, exposure to PLA bio-MPs significantly increased ROS levels, which was accompanied by an elevation in SOD activity and a reduction in CAT activity, indicating a partial mitigation of oxidative stress. In contrast, LDPE MPs did not significantly alter ROS levels but did activate the antioxidant activities. The crucial roles of these enzymes in the antioxidant defense system (Sanpradit eong et al. 2024) suggest that their activities may partially alleviate oxidative stress induced by PLA bio-MPs. Consistent with our findings, Kim et al. (2024) reported that ROS levels were higher in *Microcystis aeruginosa* exposed to PLA bio-MPs compared to polycaprolactone (PCL), with SOD activity elevated in both groups after 12 days of exposure.

In addition, the combined exposure to MeHg with either PLA bio-MPs or LDPE MPs resulted in reduced ROS levels but increased CAT activity, implying that cells were undergoing oxidative stress despite the lower ROS levels, possibly due to a compensatory detoxification response. Similarly, Jeong et al. (2022) reported that exposure to a combination of MPs and Cr reduced ROS and induced antioxidant enzyme activities in *D. magna* as a response to oxidative stress. These findings highlight the role of antioxidant enzyme activation as a critical defense mechanism under combined pollutant exposure, which may occur even when ROS levels are not elevated. For example, Jeong et al. (2024b) found decreased ROS levels in both neonate and adult stages of *Brachionus plicatilis* exposed to MeHg, attributing this reduction to disrupted redox homeostasis. These findings are supported by several studies that have documented reduced ROS levels in aquatic species following exposure to various MPs or heavy metals (Han et al. 2023; Martyniuk et al. 2024), linking this phenomenon to either disrupted redox homeostasis or an overcompensated antioxidant response (Ali et al. 2024c).

Under stressful conditions, organisms expend significant energy to activate defense mechanisms and modify their physiological status (De Felice et al. 2024), including the activation of detoxification pathways and antioxidative defenses driven by the expression of antioxidant genes (Ali et al. 2024c). Exposure to PLA bio-MPs resulted in a significant upregulation of key oxidative stress-related genes, indicating a robust oxidative stress response. In contrast, LDPE MPs caused a downregulation of several SOD genes, suggesting the involvement of an alternative oxidative mechanism. Similarly, MeHg exposure led to the downregulation of SOD and CAT genes, indicating a potential suppression of the oxidative stress response. These findings are consistent with previous research. For instance, An et al. (2024) reported a significant upregulation of SOD gene expression in *D. magna* following exposure to PLA bio-MPs, while Yu et al. (2018) observed a downregulation of SOD gene expression in *Eriocheir sinensis* following exposure to high concentrations of PS MPs. In the combined exposure of PLA bio-MPs and MeHg, the expression levels of SOD and CAT genes

were downregulated compared to PLA bio-MPs alone, suggesting potential interactive effects on the antioxidant transcription process. Similarly, Wang et al. (2023) reported that co-exposure to NPs and heavy metals in *B. plicatilis* led to a significant alternation in SOD and CAT gene expression. These results suggest that the oxidative stress responses induced by MPs and heavy metals may vary significantly depending on the type of MPs, highlighting the complexity of environmental stress responses in aquatic organisms.

4.5 Neurotoxic effects comparison

It has been suggested that oxidative stress could contribute to the inhibition of AChE (Wyse et al. 2004; Aboul Ezz et al. 2015) by causing damage to cellular structures, which may impair the enzyme's synthesis and secretion (Banaee et al. 2019). MeHg is known for causing irreversible damage to the central nervous system and is recognized as a significant risk factor for inducing neuronal degeneration (Ramos et al. 2020). Acetylcholinesterase (AChE) is an essential enzyme in neurotransmission, responsible for rapidly breaking down the neurotransmitter acetylcholine in synapses (Barreto et al. 2023). PLA bio-MPs, LDPE MPs, and MeHg, either alone or in co-exposure with both types of MPs, significantly inhibited AChE activity in *D. magna*, supporting the findings of other studies regarding their potential neurotoxic effects, such as inhibition of AChE activity (de oliveira et al. 2021; Henriques et al. 2023; Jeyavani et al. 2023; Zhong et al. 2024). For instance, Luís et al. (2015) demonstrated that exposure to MPs, either alone or in combination with the pesticide pyrene, as well as exposure to Cr, significantly reduced AChE activity in the common goby, *Pomatoschistus microps*. Similarly, Santos et al. (2021) reported that MPs and Cu, whether alone or in combination, induced neurotoxicity in zebrafish larvae by disrupting cholinergic neurotransmission, suggesting that heavy metals and MPs could compromise the cholinergic system. Moreover, co-exposure of MeHg with PLA bio-MPs or LDPE MPs led to a further reduction in both AChE activity in *D. magna* compared to single MPs exposures. AChE is essential for proper neurotransmission, and its inhibition is known to impair motor functions, including swimming behavior. Ren et al. (2015) indicated inhibition of AChE can cause a stepwise reduction in swimming performance, beginning with hyperactivity and potentially leading to paralysis as AChE activity decreases. Meanwhile, co-exposure to MeHg with PLA bio-MPs or LDPE MPs significantly downregulated AChE gene expression, counteracting MeHg's upregulatory effect. Similarly, Umamaheswari et al. (2021) found that zebrafish exposed to PS MPs exhibited an oxidative stress response, characterized by reduced expression of genes related to neurotransmission. Sun et al. (2024) reported that co-exposure to PS MPs and thiamethoxam significantly altered mRNA expression associated with neurotransmission pathways. Although in our result, swimming performance did not significantly change despite the effects of PLA bio-MPs, LDPE MPs, and MeHg on AChE activity and gene expression, swimming behavior slightly decreased in both PLA bio-MPs alone and both MPs with MeHg, suggesting potential differences in the mechanisms of toxicity and interactions with MeHg between these MP types in regarding the neuronal system.

4.6 Comparison of reproductive gene expression

MPs can impact the expression of genes related to reproduction, including vitellogenin (VTG), in *D. magna*, leading to a decrease in reproductive output (Liu et al. 2021). VTG serves both as a precursor to lipoproteins and phosphoproteins, which are components of egg yolk, and as a vital nutritional reserve necessary for the normal development of the embryo (Kim et al. 2011; Lyu et al. 2021). Several studies have reported alterations in VTG expression in *D. magna* following exposure to various pollutants (Liu et al. 2019; Aksakal and Arslan, 2020). We observed that PLA bio-MPs and LDPE MPs downregulated VTG expression in *Daphnia*, while MeHg upregulated it, confirming that egg production is particularly sensitive to both MPs and MeHg. Our results support the findings of other studies where *D. magna* exposed to PLA bio-MPs and PET MPs exhibited significantly downregulated VTG gene expression (An et al. 2024). Interestingly, the combined exposure of *D. magna* to MeHg with LDPE MPs did not further alter VTG expression compared to LDPE alone. These results are consistent with the findings of Yoo et al. (2023), where co-exposure to PS MPs reduced the reproductive toxicity of MeHg, suggesting that combined exposure to MPs and MeHg results in less severe reproductive toxicity compared to MeHg alone. However, in this study the combined exposure of PLA bio-MPs and MeHg led to a distinct downregulation of VTG, despite MeHg alone causing an upregulation. This suggests that PLA bio-MPs may exert a dominant inhibitory effect on VTG expression, which counteracts the upregulation of genes induced by MeHg. This indicates that the interaction between MPs and MeHg is complex, with the specific type of MP playing a crucial role in determining the overall impact on gene expression and reproductive outcomes.

In summary, this study demonstrated that PLA bio-MPs exhibited greater toxicity to *D. magna* than LDPE MPs in terms of both survival and reproduction. Polar PLA bio-MPs promoted MeHg bioaccumulation, compared to MeHg exposure alone or in combination with LDPE MPs. Additionally, PLA bio-MPs and LDPE MPs elicited different oxidative stress responses, with PLA bio-MPs enhancing ROS production and altering antioxidant enzyme activity, resulting in a higher oxidative stress response than LDPE MPs. Furthermore, AChE activity was significantly decreased by PLA bio-MPs, both alone and in combination with MeHg, indicating neurotoxic effects in *D. magna*. Overall, this study underscores the significant ecological risks that PLA bio-MPs could pose, despite being marketed as eco-friendly.

5. Conclusions

In conclusion, our study demonstrates that both PLA bio-MPs and fossil fuel-based LDPE MPs induce significant physiological and biochemical alterations in *D. magna*, including oxidative stress, impaired reproduction, and disruptions in detoxification pathways. The investigation of the combined effects of both types of MPs with MeHg highlights the complexity of MP toxicity, as evidenced by both synergistic and antagonistic interactions depending on the specific MP type. PLA bio-MPs, despite their *in vivo* biodegradable nature, demonstrated a more pronounced negative impact on *D. magna*, with and without co-exposure with MeHg, compared to fossil fuel-based LDPE MPs. Polar bio-based MPs can enhance the bioaccumulation and toxicity of coexisting pollutants, thereby posing similar, if not greater,

environmental risks than apolar fossil fuel-based MPs. These findings emphasize the need for a comprehensive evaluation of the ecological risks associated with bio-based and biodegradable plastics. Although they lead to a lower carbon footprint at source, their potential to contribute to pollution and exacerbate the toxicity of other contaminants cannot be overlooked. Future research should focus on the long-term environmental implications of bio-based MPs, particularly in complex ecosystems where they may interact with various pollutants.

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Supplementary materials

Text S1. Oxidative Stress and enzymatic activity assay

To estimate intracellular reactive oxygen species (ROS) levels, a reaction mixture was prepared comprising 10 µL of supernatant, 170 µL of PBS, and 20 µL of 400 µM 2′,7′-dichlorodihydrofluorescein diacetate. The mixture was then loaded on to a 96-well black plate (SPL, Seoul, South Korea) in triplicate for each experimental group and incubated at 37 °C for 20 minutes. ROS levels were measured using a spectrophotometer (Thermo™ Varioskan Flash, Thermo Electron, Vantaa, Finland) at excitation/emission wavelengths of 485/520 nm. The antioxidant enzymatic activity of SOD was estimated using an Abcam kit (Cambridge, United Kingdom) according to the manufacturer's instructions. In this assay, a water-soluble tetrazolium salt reacts with the superoxide anion to form a water-soluble formazan dye. The reaction mixture was loaded as 200 µL per well (n = 3) onto a white 96-well plate, and the absorbance was measured at 450 nm using a spectrophotometer. Similarly, CAT activity was measured using a Sigma-Aldrich kit, which is based on the formation of a red quinoneimine dye resulting from the decomposition of hydrogen peroxide (H_2O_2). After following the kit instructions, 200 µL of the reaction mixture was loaded onto a white 96-well plate, and the absorbance was measured at 520 nm with a spectrophotometer. Finally, acetylcholinesterase (AChE) activity was measured using a commercially available kit, following the manufacturer's instructions. The reaction mixture (200 µL per well, n = 3) was loaded onto a white 96-well plate, and absorbance was recorded at 410 nm using a spectrophotometer.

Text S2. RNA extraction, cDNA synthesis, and quantitative real-time polymerase chain reaction

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted using forward and reverse primers specific for the targeted genes (Table. S1) on a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA). The amplification was carried out under the following thermal cycling conditions: an initial denaturation at 95°C for 4 minutes, followed by 35 cycles consisting of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. To ensure accurate amplification of the target gene, melting curves were generated using a temperature profile consisting of an initial phase at 95°C for 1 minute, followed by 55°C for 1 minute, and 80 cycles of 55°C for 10 seconds with a 0.5°C increment per cycle. The validation of transcriptional levels was performed employing the $2^{-\Delta\Delta C}$T method, as described by Livak and Schmittgen (2001).

Table S1. Information on the primers targeting the superoxide dismutase (*SOD*), catalase (*CAT*), acetylcholinesterase (*AChE*) and Vitellogenin (*VTG*) of *Daphnia magna* used in real-time polymerase chain reaction.

Gene	Amplicon size (bp)	Real-time PCR primer (5′ -> 3′)	Accession number
MnSOD1	100	F: AACAAGGATTATGGGTCATTAGA R: TCTTATTATATCCTAGCCAACCC	OP087632
MnSOD2	100	F: CTTTGGCAGAATTTTTCTCCTAA R: GGCCATTCTTCAAAGTATCGAAT	OP087633
CuZnSOD1	100	F: CCTCGATTTGACCGAATCAG R: GAATTGATGAACGTGGAAGC	OP087622
CuZnSOD2	100	F: GGATTGCAAACATTGACATATTAG R: AGATCATCCTCATTGGCATG	OP087623
CuZnSOD3	100	F: GGTGATCTTGGAAACATTGTAG R: GACCTATTATTCCATTAGCACCA	OP087624
CuZnSOD4	100	F: ATTTCTTCGACAACCGTTATTC R: AAGTGAATTTGACCGCTAATG	OP087625
CuZnSOD6	102	F: CACGAGCTGGTTCCATATAC R: ATGCCTGGTTCGTGACTTGG	OP087627
CuZnSOD7	119	F: TGCTTGATGACACTTACTGG R: CCAGCATTACCTGTGACTT	OP087628
CuZnSOD8	90	F: CGACTGGTCCGCATTACAA R: CGTTACCCAAGTCACCGA	OP087629
CAT	100	F: TGTATACATTGATGAAACAGCTC R: AGTAACCTCAAAGTATCCAAAGG	OP087621
VTG1	120	F: CCAAGGCTATCAAGACCATC R: TGATCTGCTCTCCGTAAATG	AB114859.1
VTG2	120	F: CTGATCCTCTCGAACAAAGT R: TTGTTCTCCGCTCAAGATAC	AB252738.1
AChE	120	F: CCATCTACAATAATGCGGGA R: GTAGAAGCGTGTTGGGAATA	XM_032936630.2

Table S2. Fold-changes in superoxide dismutase (SOD), catalase (CAT), acetylcholinesterase (AChE), and vitellogenin (VTG) genes in *Daphnia magna* exposed to 0.5 mg/L polylactic acid bio-microplastics (PLA bio-MPs) and low-density polyethylene microplastics (LDPE MPs) with and without methylmercury (MeHg). Each letter indicates a significant ($P < 0.05$) difference in the expression of the target genes.

Gene Family	Gene	Control	PLA	LDPE	MeHg	MeHg+PLA	MeHg+LDPE
SOD	*MnSOD1*	1.00 ± 0.05^{c}	1.39 ± 0.03^{d}	0.83 ± 0.03^{b}	0.58 ± 0.03^{a}	1.12 ± 0.02^{c}	0.76 ± 0.05^{b}
	MnSOD2	1.00 ± 0.11^{ab}	0.98 ± 0.15^{ab}	1.16 ± 0.02^{b}	1.03 ± 0.06^{ab}	1.13 ± 0.07^{b}	0.74 ± 0.07^{a}
	CuZnSOD1	1.00 ± 0.04^{c}	1.15 ± 0.05^{d}	0.81 ± 0.01^{b}	0.48 ± 0.03^{a}	0.90 ± 0.00^{bc}	0.55 ± 0.02^{a}
	CuZnSOD2	1.00 ± 0.18^{a}	1.02 ± 0.08^{a}	0.86 ± 0.03^{a}	0.81 ± 0.09^{a}	0.98 ± 0.09^{a}	1.04 ± 0.13^{a}
	CuZnSOD3	1.00 ± 0.02^{cd}	1.21 ± 0.11^{e}	0.86 ± 0.05^{bc}	0.80 ± 0.02^{ab}	1.14 ± 0.01^{de}	0.66 ± 0.03^{a}
	CuZnSOD4	1.00 ± 0.07^{c}	1.55 ± 0.04^{d}	0.62 ± 0.02^{b}	0.16 ± 0.01^{a}	0.75 ± 0.08^{b}	0.25 ± 0.00^{a}
	CuZnSOD6	1.00 ± 0.03^{c}	1.15 ± 0.06^{d}	0.68 ± 0.01^{b}	0.31 ± 0.01^{a}	1.19 ± 0.07^{d}	0.39 ± 0.01^{a}
	CuZnSOD7	1.00 ± 0.07^{a}	1.18 ± 0.01^{a}	0.87 ± 0.03^{a}	0.69 ± 0.04^{a}	1.03 ± 0.09^{a}	0.67 ± 0.04^{a}
	CuZnSOD8	1.00 ± 0.02^{c}	1.44 ± 0.06^{e}	0.90 ± 0.02^{bc}	0.71 ± 0.01^{a}	1.13 ± 0.05^{d}	0.80 ± 0.03^{ab}
CAT	*CAT*	1.00 ± 0.02^{b}	1.36 ± 0.02^{c}	0.94 ± 0.01^{b}	0.72 ± 0.02^{a}	1.00 ± 0.06^{b}	0.92 ± 0.03^{b}
AChE	*AChE*	1.00 ± 0.06^{b}	0.64 ± 0.05^{bc}	0.99 ± 0.02^{bc}	4.09 ± 0.32^{a}	0.53 ± 0.01^{c}	0.69 ± 0.07^{bc}
VTG	*VTG1*	1.00 ± 0.05^{b}	0.71 ± 0.01^{a}	0.82 ± 0.03^{ab}	1.93 ± 0.15^{c}	1.02 ± 0.06^{b}	0.73 ± 0.02^{a}
	VTG2	1.00 ± 0.03^{c}	0.31 ± 0.03^{a}	0.48 ± 0.02^{b}	2.24 ± 0.02^{e}	1.24 ± 0.01^{d}	0.41 ± 0.04^{b}

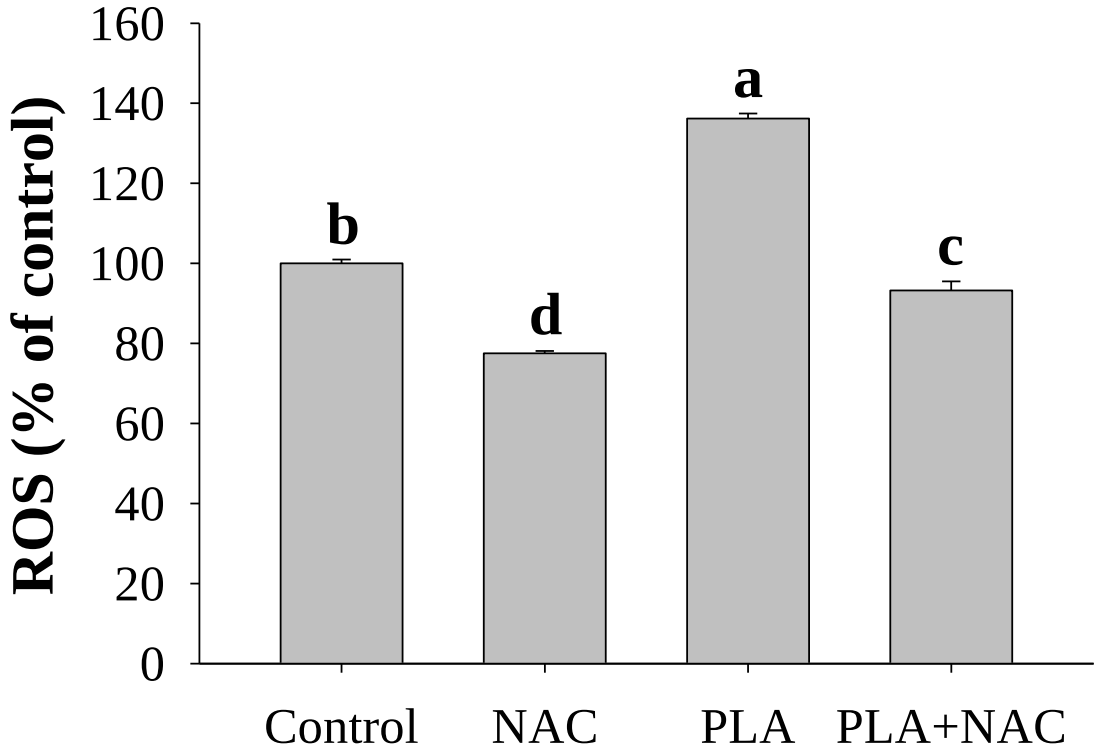

Fig. S1. Intracellular reactive oxygen species (ROS) levels in *Daphnia magna* relative to the control group following exposure to polylactic acid bio-microplastics (PLA bio-MPs) with or without N-acetyl-L-cysteine (NAC). NAC, a known ROS scavenger, was used to assess its effectiveness in mitigating ROS generated in response to PLA bio-MPs exposure. Data are presented as mean ± standard error. Different letters above the bars indicate statistically significant differences between treatments ($P < 0.05$).

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5. General conclusions

Despite the increasing research on MPs pollution, our understanding of the effects of MPs derived from bioplastics remains limited, particularly concerning their fate and impact in marine environments. Although bioplastics are often presented as a sustainable alternative to petrochemical plastics due to their renewable nature, they still contribute to environmental pollution as they degrade into MPs, posing risks to aquatic ecosystems. This thesis has provided important insights into the fate of bioplastics in aquatic ecosystems and experimentally evaluated their impacts on various zooplankton species.

Firstly, the thesis presents valuable data on PLA, a commonly used bioplastic, focusing on its synthesis, tuning of biodegradability, environmental conversion to MPs, and its effects on aquatic biota. While PLA is biodegradable under specific conditions, it does not fully degrade in natural aquatic environments and fragments into MPs more rapidly than petroleum-based plastics. These PLA-derived MPs are identified as emerging contaminants, with potential risks comparable to those posed by MPs from fossil fuel-based plastics. Through experimental studies, this thesis demonstrated the long-term adverse effects of low concentrations of PLA MPs on copepods, a key bioindicator species. The results show that PLA MPs negatively affect morphological and reproductive traits, reducing total population size. Furthermore, gender-specific toxicity was observed, which may have broader ecological impacts by disrupting copepod recruitment and thus altering the dynamics of the food web. When comparing these findings with other studies on environmental contaminants, the toxic effects of PLA MPs were found to be similar, indicating that these MPs may not be less harmful than their petrochemical counterparts.

Secondly, the thesis explored comparative studies on bioplastics and petrochemical plastics, examining their eco-friendliness in terms of biodegradability, compostability, fragmentation into MPs, and pollution in soil and aquatic systems. The findings suggest that the environmental behavior of bioplastics, particularly in terms of their toxicity to biota, may not be necessarily more eco-friendly than that of petrochemical plastics. Experimental comparisons of different MPs types, including biobased PLA, biodegradable PBAT, and petrochemical-derived LDPE, as well as a novel modified CD-PLA, revealed comparable *in vivo* and *in vitro* effects in primary consumer, *Diaphanosoma celebensis*, across MPs categories. Oxidative stress was identified as a key mechanism underlying the observed toxicological effects. This thesis also documented species-specific responses to various MPs types, focusing on the marine rotifer *Brachionus plicatilis*. Different MPs types were found to modulate oxidative stress responses, protein phosphorylation in the MAPK signaling pathway, and detoxification mechanisms through the multixenobiotic resistance system, indicating that the toxicological impacts of MPs are dependent on their specific physicochemical properties.

Finally, the thesis emphasizes the shared toxicological concerns associated with biobased and biodegradable MPs, particularly regarding their ability to adsorb environmental pollutants and their subsequent impact on aquatic organisms. Once in the environment, these plastics degrade into MPs that can adsorb pollutants, leading to combined toxic effects on aquatic life.

Notably, the study found that PLA MPs, when combined with MeHg, became more toxic to *Daphnia magna*, increasing pollutant accumulation, inducing oxidative stress, and severely impacting survival and reproduction. In contrast, LDPE MPs exhibited different toxicological interactions under similar conditions, demonstrating that the effects of MPs on organisms are influenced by both the type of MPs and the nature of the associated pollutants. The results highlight the potential for both synergistic and antagonistic effects, with PLA MPs showing more pronounced adverse outcomes compared to LDPE MPs, despite their biodegradable properties. In conclusion, this thesis contributes to the growing body of research on bioplastics by providing experimental evidence on their environmental fate and toxicity. While bioplastics offer certain sustainable advantages over petrochemical plastics, their fragmentation into microplastics and interactions with pollutants present significant ecological risks. The findings underscore the need for further research on bioplastic-derived MPs and their potential environmental impacts, particularly in aquatic ecosystems.

6. Future Perspectives

The research conducted in this thesis has provided significant insights into the ecotoxicological impact of MPs originating from bioplastics on zooplanktons, a key component in aquatic ecosystems which support the food chain/web. Based on the findings of my research, an effort has been made to understand the ecological and toxicological effects of bioplastic MPs on aquatic environments. The work presented in this thesis also highlights areas where further advancements are necessary to address current environmental challenges. These perspectives aim to extend the scope of current knowledge and propose future perspective based on the results presented herein this thesis.

The findings of several studies consistently showed that bioplastics like PLA fragment into MPs and do not fully degrade under natural environmental conditions. While certain bioplastics, such as polyhydroxyalkanoates (PHAs), have been shown to be biodegradable, they often require longer timeframes to degrade, particularly in marine environments, and do not yet represent a universal solution to the problem of MPs pollution, as they could fragment into MPs before complete mineralization. Therefore, the development of bioplastics with tailored degradation profiles suited to specific environmental compartments (e.g., marine, freshwater, and soil) is a necessary first step. However, because the final environmental destination of plastics is often unpredictable, a significant future goal should be the creation of universally biodegradable plastics. These materials would need to degrade completely within a defined timeframe under a variety of natural environmental conditions, without forming MPs. This would involve designing materials that degrade completely within a defined timeframe and under natural environmental conditions without forming MPs. Engineering bioplastics with advanced degradation pathways, triggered by environmental factors such as temperature, salinity, or pH, could be a promising solution to ensure bioplastics do not persist in unintended environments. While bioplastics have often been marketed as eco-friendly, which is truly the case (lower carbon footprint for biobased plastics, and biodegradable plastic such as PHAs degrade in the environment) my research shows that the majority contribute to MPs pollution, which complicates their environmental credentials. While PHAs demonstrate biodegradability in the marine environment, which could be further enhanced, their broader adoption and functionality can be achieved by improving their material properties. A significant future direction would be to expand current life cycle analyses (LCAs) of bioplastics to comprehensively account for MPs formation during their degradation. This would ensure that MPs generation and its ecological impacts are integrated into the environmental assessments of bioplastics. Future bioplastic development should aim to balance material properties such as strength and durability with the need to minimize MPs formation at the end of life.

My results have highlighted the potential for biodegradable MPs to act as vectors for environmental pollutants. To mitigate this issue, future research should focus on the development of bioplastics that have reduced affinity for pollutant adsorption. This could involve altering the surface chemistry of bioplastic materials to minimize interactions with environmental contaminants. The creation of bioplastics with anti-fouling properties, for example, could limit the formation of biofilms and reduce the adsorption of toxic pollutants,

thereby minimizing the risks of pollutant transfer along the food chain. Given the need to create more bioplastics that degrade more efficiently and avoid MPs formation, bioinspired designs could offer new solutions. Drawing inspiration from natural polymers, such as those found in plant cell walls, e.g cellulose, or insect cuticles, could lead to the development of bioplastics that exhibit better environmental performance. Indeed, previous work in the field of textile has shown that the fate of cellulose-based fibers differs significantly from synthetic fibers in aquatic environments. Zambrano et al. (2019) found that cellulose-based fabrics, such as cotton and rayon, release more fibers during laundering than polyester. However, these cellulose fibers biodegrade rapidly, with cotton showing 75.9% biodegradation after 243 days, while polyester fibers, which persisted in the environment, degraded only 4.05%. This highlights the environmental advantage of cellulose fibers, which degrade readily and reduce long-term pollution risks, unlike polyester fibers that persist and contribute to plastic pollution. Future research could explore bioinspired materials that naturally degrade under ambient environmental conditions without releasing MPs or harmful by-products. These materials could be designed to mimic the degradation processes of organic matter in ecosystems, providing a more sustainable alternative to current bioplastics.

Moving forward, the production of bioplastics should prioritize environmental safety alongside performance properties. Developing industrial standards that integrate environmental toxicity assessments during the production phase is crucial. Manufacturers should adopt stringent environmental safety protocols, ensuring that new bioplastic formulations undergo thorough testing for their potential to form MPs and interact with environmental pollutants before they are widely adopted. While this thesis provides critical evidence of the risks associated with bioplastics, there remains a gap in public understanding and regulatory frameworks. It is essential that future efforts focus on educating consumers and influencing policies toward responsible bioplastic use and disposal. This includes raising awareness about the conditions required for proper biodegradation and encouraging consumer behavior that supports composting and recycling of bioplastics. In this regard, developing more research toward plastic recycling is crucial. On the policy side, governments and regulatory agencies need to adopt clear guidelines for labeling and marketing bioplastics, ensuring that only those materials that meet stringent environmental criteria are promoted as sustainable alternatives. Overall, my research was an attempt to contribute to understanding the complex environmental interactions of bioplastic MPs. The future directions proposed here build directly upon my findings, emphasizing the development of bioplastics with improved environmental degradation profiles, reduced MPs formation potential, and limited pollutant interactions. Integrating environmental safety into bioplastic design and industrial production, optimizing recycling processes, and influencing consumer behavior through policy and education will be key to realizing the potential of bioplastics as truly sustainable alternatives to fossil fuel-derived plastics.