Assessment and mitigation of human's exposure to microplastics (MPs) from biodegradable plastics

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By

Luming Yang

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Department of Civil, Structural and Environmental Engineering

Trinity College Dublin,

The University of Dublin

Ireland

Declaration

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Abstract

Plastic waste and pollution have become a global environmental challenge due to their detrimental impacts on the environment, ecosystems, and human health. Since 1950s, plastic waste, particularly single-use plastics, has been accumulating in landfills, oceans, and other natural habitats, leading to a range of environmental problems. With the growing awareness on the plastic pollution, biodegradable plastics, with the advantages of high degradability, non-toxic end products and low cost, have been regarded as an ideal alternative of conventional plastics. Biodegradable materials, such as those derived from plants, cellulose, and starch, exhibit the capability of undergoing degradation by microorganisms or within composting environments over a relatively brief period. In recent years, there has been a notable increase in the market share of biodegradable materials, and projections indicate that this trend is expected to continue over the coming decades. Polylactic acid (PLA) is widely recognized as one of the most prevalent biodegradable polymers owing to its advantageous properties such as high hardness, exceptional strength, and cost-effectiveness. Consequently, PLA finds extensive utilization across diverse fields, including medical and packaging applications. While the biodegradable plastics can be degraded within months when the specific conditions are met, including optimal temperature, appropriate humidity levels, and an environment abundant in microorganisms, it could take them two years or longer to decay in an open environment.

Many studies have provided the evidence that biodegradable materials are hard to degrade in two years period, which means the fragmentation and broken apart could occur during the degradation, therefore leading to the release of microplastics (MPs) and other microparticles-

A great number of studies have found that large number of MPs additive microparticles and other impurities could be released from plastic wastes and daily used plastic products. As the results, MPs are ubiquitous in the environment. The presence of MPs in the environment, including in water bodies, soil, and even in the air, has raised concerns about their potential impacts on human health and the environment.

While biodegradable materials exhibit considerable resistance to degradation within a two-year timeframe, the fragmentation and disintegration processes during their degradation could lead to the release of MPs and other microparticles. In fact, there has been an increased emphasis on the potential MPs release from biodegradable plastics and their associated risks on the public health and the environment.

In this study, PLA was taken as the example to conduct the biodegradable plastic research. Firstly, a comprehensive review was conducted to summarize the category, properties and characterization, the additive addition as well as the ecological risk of the biodegradable plastic. Afterwards, a systematic method of determining the plastic-released microparticles (PMPs), including MPs and other microparticles, released from PLA products was developed by simulating the daily use protocol, which can be applied on the determination of various plastic products. Subsequently, the polyethylene (PE) and PLA single-used paper cups were selected as the experiment objects to compare the PMPs release from biodegradable material with the conventional plastic products. This study found that the PMPs from biodegradable cups were 4.2 times higher than that of conventional paper cups, including MPs and other additives. Sequentially, four different forms of PLA material were selected to evaluate the PMPs release impact factor and degradation behavior under alkaline condition. The results indicated that the mechanical stress was the main factor leading to the particle release, while the addition of functional chemical additives caused different degradation degrees. Finally, a lab-scale mitigation method was developed by coating a layer of copper oxide (CuO) film on 3D printed PLA sheet. The CuO can provide a reduction rate of 66.7% on PMPs release. In parallel, a 9-week weathering experiment was conducted and the results showed that the CuO coating still displayed a PMPs release reduction of 36.7%. Furthermore, the CuO coating does not affect the biodegradability of PLA sheet, which is an effective and practical method to apply on the PMPs mitigation research of biodegradable polymers. This study conducted a comprehensive investigation into biodegradable materials, specifically focusing on whether biodegradable plastics, particularly PLA, can serve as environmentally friendly materials in the future. However, the findings of this study suggest that further research and careful consideration are necessary to determine the true environmental viability of biodegradable plastics.

Key words: Microplastics, Biodegradable plastic, Additives, Degradation, Mitigation, Heath risk

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List of acronyms

MPs	Microplastics
PLA	Polylactic acid
PMPS	Plastic-released microparticles
CuO	Copper oxide
PBAT	Polybutylene adipate terephthalate
AFM	Atomic force microscopy
SUPCs	Single-used paper cups
PE	Polyethylene
FTIR	Fourier-transform infrared spectroscopy
BFB	Baby feeding bottle
NMPs	Nano and microplastics
PS	Polystyrene
NPs	Nanoplastics
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrates
PGA	Polyglycolic acid
PBS	Polybutylene Succinate
PLLA	Poly (L-lactide) acid
PCL	Polycaprolactone
PEG	Polyethylene glycol
DBP	Dibutyl phthalate
PHBV	Poly (3-hydroxybutyrate-co3-hydroxyvalerate)
TEC	Triethyl citrate

CMC	Carboxymethylcellulose
CT	Catechin
LNPs	Lignin nanoparticles
PPDO	Poly(p-dioxanone)
HDPE	High density polyethylene
PET	Polyethylene terephthalate
PPC	Polypropylene carbonate
PU	Polyurethane
RT	Room temperature
PC	Polycarbonate
DI water	Deionized water
SEM	Scanning electron microscope
HQI	Hit quality index
GC-MS	Chromatography-mass spectrometry
TGA	Thermogravimetric analysis
NMR	Nuclear magnetic resonance
TPU	Thermoplastic polyurethane
PP	Polypropylene

Chapter 1

Introduction

1 Introduction

1.1 Background

Conventional plastic materials, due to their nonbiodegradable properties, could pose great risk to both human and environment [1, 2]. Biodegradable plastic is regarded as an ideal alternative to conventional plastic. Biodegradable plastic are extracted from natural sources including plants, cellulose and starch, which has the advantages of high degradability, high strength, and low cost [3]. Therefore, the market size of biodegradable plastic is predicted to have an annual growth rate of 9.7% from 2023 to 2030 [4]. Among the range of biodegradable materials, PLA, PBAT, and starch-based plastics emerge as the most extensively employed plastic materials. Their widespread utilization encompasses diverse sectors such as cutlery production, food packaging, agricultural applications, and medical settings [5-7]. Fig 1.1 shows the wide spread plastic necessities of biodegradable plastics in food industries and packaging applications.

Fig 1. 1 Food industry and packaging applications of biodegradable plastics

However, many studies have found that biodegradable plastic are not able to degrade under natural environment in a short timescale [8, 9]. The fully degradation requires specific conditions such as high temperature, humidity and other microbial conditions [10]. Due to the high persistent of biodegradable materials in natural environment, the fragmentation and detachment of nano and micro-sized particles could occur during the degradation process [11]. Studies have revealed that the biodegradable plastic release higher number of microparticles than that of conventional plastic products, including MPs, additive microparticles and other impurities [12, 13]. The ecological effect of biodegradable MPs and additives were sequentially investigated, concluding that the biodegradable MPs have the similar toxicity as conventional MPs [14, 15]. Although a growing number of studies are focusing on the biodegradable material, the potential health risk and mitigation method are still limited.

1.2 Research objectives and thesis layout

The primary objectives of this thesis are twofold: firstly, to conduct a comprehensive comparison of plastic-released microparticles (PMPs) originating from biodegradable plastics (PLA) and conventional plastics (PE); and secondly, to advance the field by developing effective mitigation strategies. Additionally, the research aims to delve into the release mechanisms and degradation behaviors of various forms of PLA materials. Comprising seven interconnected chapters, this thesis unfolds a cohesive narrative. Chapter 1 serves as an introduction, setting the stage for the entire study by elucidating the topic and outlining the thesis structure. Chapter 2 meticulously reviews relevant research and studies pertaining to biodegradable plastics and microparticle release, providing a robust foundation for subsequent investigations. Chapters 3 to 6 present four distinct yet interrelated research papers, each contributing to the overarching theme. Notably, Chapter 3 and Chapter 4 have been published in prestigious journals—JOVE and Chemical Engineering Journal, respectively—signifying the scholarly impact of the research. Finally, Chapter 7 synthesizes the findings, offering conclusive insights and recommendations derived from the comprehensive data collected throughout this study. Fig 1.2 shows the research overview of this thesis:

(1) In order to provide a comprehensive understanding of biodegradable plastics, this study seeks to consolidate and examine background information encompassing material types, properties, the incorporation of additives, microparticle release dynamics, and potential health risks associated with these materials;

(2) This research aims to establish a systematic approach for evaluating plastic products by determining the release of plastic-released microparticles (PMPs). The study outlined an experimental protocol and chemical identification method to facilitate an accurate and thorough assessment;

(3) A crucial objective is the investigation and comparison of PMPs released from conventional plastics (PE) and biodegradable PLA plastics. By scrutinizing and contrasting these two types of plastics, the study aims to uncover insights into their microparticles release mechanisms;

(4) The development of a mitigation strategy takes center stage in this research, specifically targeting the reduction of PMPs released from 3D printed PLA. This involves applying a CuO film as a coating and assessing particle release following prolonged weathering, providing valuable insights into the effectiveness of the mitigation method;

(5) The exploration of PMPs release under elevated temperatures and the chemical composition or of four different forms of PLA material during alkaline decomposition is a key focus. This investigation aims to unveil the potential release mechanisms and additives present in various PLA products, contributing to a nuanced understanding of their environmental implications.

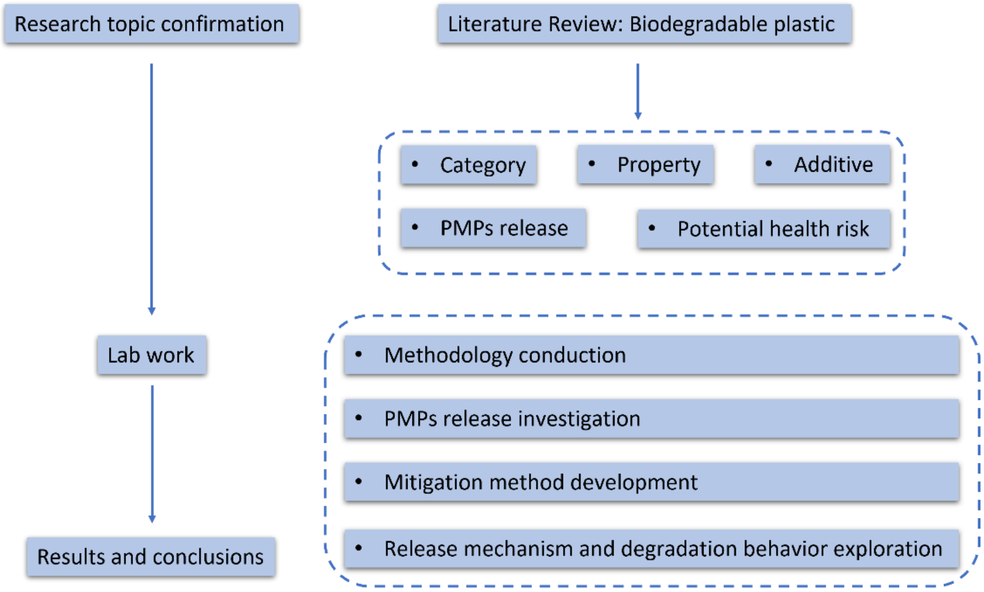


Fig 1. 2 Research overview

Chapter 2

Literature Review

2 Literature Review

A Comprehensive Review on Categories, Properties, Applications, Chemical Additives, and Biological Effects of Biodegradable Plastics

Abstract

Biodegradable plastics are widely recognized as environmentally friendly materials, primarily due to their high degradability and the generation of non-toxic end products after degradation. However, a growing number of studies confirmed that they cannot be fully degraded in natural environments within years due to the specific conditions required. In addition, limited research has been conducted on microplastics (MPs) and chemical additives derived from biodegradable polymers. This review paper systematically summarized biodegradable plastic categories, properties, applications, and commonly used additives and modifiers. In conclusion, the paper presented critical thinking about the growing trend of biodegradable materials and addressed the current gap and limitations of research in this area. The implication of health risks and effects on humans and other organisms are still unclear, which should be a vital direction in the future. Therefore, there is a need for further studies to expand our understanding of the potential impacts and risks associated with these components in order to make informed decisions regarding their use and disposal.

Key words Biodegradable plastics, microplastics, additives, ecological effects, heath risk

2.1 Introduction

Plastics have been the most widely used material for food packaging, and industrial and agricultural applications for decades due to the advantages of low cost, lightweight, high transparency, strong plasticity, etc. However, plastic waste pollution has become a global environmental problems because they usually have long persistence in the environment, which cannot be naturally degraded for decades or even hundreds of years [16]. A report pointed out that from 2000 to 2019, the annual global plastic production rate has significantly increased from 213 million tonnes per year to 470 million tonnes per year, while the world had produced a total of 9.5 billion tonnes of plastic by 2019 [17]. The report also revealed that around 380 million tonnes of plastics was emitted into environment and ocean every year [17]. It was predicted that a large amount of plastic waste would remain on oceans surface and the emission would continue to grow till 2050 [18]. The plastic waste emitted into the open environment could degrade to macro, micro and nano-sized plastic particles (NMPs). Many studies have investigated the release level and potential health risks of NMPs from different types of plastic products. Researchers have indicated that a tea bag could release 11.6 billion microplastic (MPs) at 95℃ [1]. Li et al. found that during formula preparation, up to 16 million of microplastic (MPs) per liter released from polypropylene (PP) baby feeding bottles, which is one of the commonly used food-contacted products in daily life [2]. In addition to the MPs, high levels of additive microparticles could also be released from plastic products [19, 20]. Therefore, more and more studies have focused on the potential health risk of MPs in the environment for human beings and marine animals. MPs have been found in human feces, breast milk, placenta and tissue cells and other organs [21-24]. It has been proved that the MPs can penetrate into human blood streams with a concentration of 1.6μg/ml [25]. In terms of aquatic animals, polystyrene nanoparticles (PS NPs) has been found to cause severe behavior and metabolism effects on fish [26, 27]. In parallel, animal test modules focusing on the MPs' effect on mammalian such as mice have demonstrated that depending on particle size, the MPs could be accumulated in the liver, kidney, and gut of mice, negatively affecting the energy disturbance, lipid metabolism, oxidative stress, and intestinal barrier function [24, 28, 29].

The plastic waste pollution thereby causes an urgent need to replace the plastics with new materials. Biodegradable plastic has become an ideal alternative to conventional plastic due to its biodegradability and environmentally friendly final products like carbon oxide, biomass and water [30]. Biodegradable plastics are synthesized from renewable resources such as cellulose or starch from plants, which are now widely used in food packaging, waste packaging, agricultural and medical areas [31, 32]. By far, the most common degradable plastics include polylactic acid (PLA), polyhydroxyalkanoates (PHA), Polyhydroxybutyrates (PHB), starch blend plastic, polybutylene adipate terephthalate (PBAT), etc [31]. It is projected that the market size of biodegradable plastic will increase from USD 5.4 billion in 2023 to USD 10.0 billion in 2023 with an annual growth of 9.7%[4]. However, the uncertainty of safety and environmental benefit of biodegradable plastics is more and more concerned by experts and researchers. A number of studies showed that the degradation of biodegradable plastics requires specific environments and conditions, such as specific PH conditions, temperature and humidity, and microorganisms [8, 33]. It has been uncovered that biodegradable plastics cannot be degraded in a short time scale in the marine environment [23]. Another environmental issue is the transport and fate of biodegradable NMPs. A great number of research focusing on the NMPs release revealed that a large amount of NMPs formed from biodegradable plastics during the degradation process [24, 25]. Although the study of the potential risk of biodegradable MPs is limited, it has been reported that biodegradable MPs have similar toxicity as conventional MPs [14].

Compared with conventional plastics, biodegradable plastic usually have the disadvantages of poor mechanic property, poor thermal stability, brittleness, low melting point, etc [34]. In order to utilize biodegradable plastics in various fields as much as possible, multiple chemical additives are used in the manufacture for pretreatment to improve the performance of degradable plastics [35, 36]. Additives, similar to those used in conventional plastic [37], such as plasticizers, antioxidants and slip agents, are blended with biodegradable plastics during production. Therefore, similar to conventional plastics, biodegradable plastic products, especially food-contacted products, could release additives in the form of microparticles. However, there are lack of comprehensive reviews on microparticles (MPs and additives) release from biodegradable plastics. This study reviewed 117 papers from different databases including Google Scholar, Science Direct, and JSTOR, and summarized the most commonly used biodegradable plastics in food packaging, agricultural and daily necessities, the different types of chemical additives added in the biodegradable materials, as well as the potential biological effects on both natural environment and ecosystems.

2.2 Biodegradable plastics and applications

Biodegradable plastics are currently playing an important role in replacing conventional plastic in multiple areas [38]. The most significant advantages of biodegradable plastics are their high degradability, low cost and environmentally friendly. Biodegradable plastic made by materials extracted from renewable resources such as plants, starch and fats can be degraded into CO_2, water, and other biomass[30]. Fig 2.1A illustrated the proportion of different types of biodegradable plastics in the market in 2021. By far, the three most widely used biodegradable plastics are PLA, PBAT and starch-based plastics with the proportion of 30%, 30% and 26%, respectively, followed by other biodegradable plastics including PHA, Polyglycolic Acid (PGA), Polycaprolactone (PCL), Polybutylene Succinate (PBS), etc. The application of biodegradable plastics is distributed in many fields based on the types of plastics, mainly including packaging, agricultural, consumer goods and medical materials. Fig 2.1B showed the proportion of different applications of biodegradable plastic in various fields in 2022 [39]. Table 2.1 summarized the widespread biodegradable plastic materials as well as their advantages, disadvantages and applications in different scenarios.

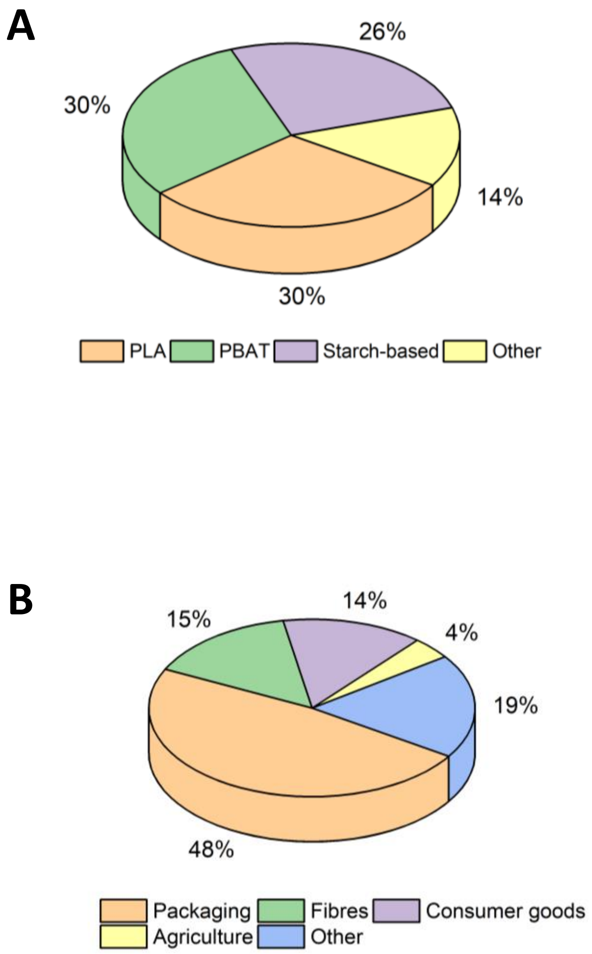


Fig 2. 1 Proportion of biodegradable plastic in categories and applications. (A) Proportion of biodegradable plastic in various types. (B) Proportion of biodegradable plastic in various applications.

Table 2. 1 Biodegradable plastic categories, properties and applications

Biodegradable plastics	Advantages	Disadvantages	Applications	References
PLA	High hardness, high strength, low cost	Poor toughness, low thermal stability, poor blown molded processability, poor gas barrier properties	3D printing, disposable cutleries, food packaging, agricultural covering films, medical devices	[5, 40, 41]

PBAT	High ductility, high heat resistance, strong impact resistance	Poor oxygen and water vapor barrier properties, poor weathering resistance	Agricultural covering films, packaging	[6, 42, 43]
Starch-based	Non-toxicity, Low cost	Poor mechanical properties, poor heat resistance	Packaging, disposable cutleries, food containers, garbage bags	[7, 44]
PHA	High thermoplastic processability, high biocompatibility	Low thermal stability, brittleness, high cost,	Surgical sutures, packaging, planting material	[45, 46]
PGA	High biocompatibility, high heat resistance, high mechanical strength	Poor toughness, high cost	Food packaging, agricultural covering films, surgical sutures	[47, 48]
PCL	High thermal stability, high biocompatibility, high toughness, low cost	Low strength	Medical material	[49]
PBS	High heat resistance, high toughness	Poor mechanical properties	Foam packaging material, daily necessities bottles, medicine bottles, agricultural covering films	[50]

2.2.1 Polylactic acid (PLA)

PLA is one of the most common used biodegradable plastics, which is manufactured through a process called polymerization, where lactic acid molecules are chemically bonded together to form long chains. It is derived from natural resources such as cornstarch, tapioca roots, or sugarcane. PLA is considered an environmentally friendly alternative to traditional petroleum-based plastics because it is produced from renewable resources and has a reduced carbon footprint.

Compared with other biodegradable plastics, PLA exhibits prominent characteristics such as high strength, high hardness, and cost-effectiveness. Consequently, it has found widespread utilization in various applications including food packaging, disposable cutlery, and agricultural mulching [5, 40]. Notably, PLA and PLA composites have emerged as the most prevalent materials in the realm of 3D printing manufacturing, owing to the thermoplastic nature of PLA [51]. In the packaging industry, PLA is often combined with other biopolymers or nanocomposites to enhance its mechanical strength and barrier properties [52, 53]. PLA is commonly utilized in the production of disposable cutlery items such as coffee cups, forks, spoons, and straws, offering a more sustainable alternative. Furthermore, PLA films are extensively employed in agricultural applications to improve crop yields. These plastic covering films, known for their sustainability and durability, are widely embraced in agricultural areas [54]. According to a study, the use of plastic covers has been found to result in an average crop yield increase of 45.5% [55]. Interestingly, in numerous crop types, PLA covering films have shown similar positive effects on soil temperature and crop growth as conventional plastic covers, such as low-density polyethylene [56]. Poly (L-lactide) acid (PLLA), which is a stereoisomer of PLA, is extensively employed in various medical applications, including bioresorbable stents and sutures. PLLA's popularity in the medical field is attributable to its low glass transition temperature (around 60℃), high degradability, and durability [41]. These properties make it a suitable choice for applications where biocompatibility and controlled degradation are crucial factors, allowing for the gradual absorption of the material in the body over time.

2.2.2 Polybutylene adipate terephthalate (PBAT)

PBAT is a fossil-based biodegradable plastic that has gained significant market share comparable to PLA products. It is widely used in packaging products such as rubbish bags and containers, as well as in the agricultural sector like crop plastic covering films. PBAT is favored for its special characteristics and competitive cost, making it a popular choice in various industries [6]. According to Gao et al., the utilization of PLA/PBAT biodegradable covering film presents a viable and sustainable alternative to traditional PE films. The biodegradable film demonstrates a significantly faster degradation rate compared to conventional plastic film, resulting in residual biodegradable films that can permeate the soil. This phenomenon has the potential to enhance soil health in comparison to residual PE film, offering ecological benefits in agricultural practices [43].

While PBAT possesses numerous advantages, it shares a common characteristic with other types of biodegradable plastics: it can only quickly biodegrade and compost under specific conditions, including specific temperature, oxygen levels, and humidity. It takes years or even decades for PBAT to degrade 100% in the natural environment. Consequently, the degradation process of PBAT materials can result in the formation of biodegradable MPs, which can potentially give rise to environmental issues similar to those associated with traditional MPs. It is crucial to consider the potential risks and environmental impacts associated with the use and disposal of biodegradable plastics like PBAT [11].

2.2.3 Starch-based biodegradable plastic

Starch-based biodegradable plastics rank as the third most prevalent biodegradable plastic material, holding a market share of 26%, following PLA and PBAT. The extraction of starch for the production of these plastics primarily involves botanical sources, such as potatoes, various crops, and wheat. The production process of starch-based biodegradable plastics can be categorized into two main methods: the dry process, typically carried out on a laboratory scale, and the wet process, which is employed on an industrial scale [57]. Several studies have been conducted to extract starch from corn and rice in lab-scale experiments. These investigations aimed to assess the mechanical properties, water solubility, and biodegradability of the extracted starch. By evaluating these parameters, researchers sought to understand and characterize the performance and environmental impact of starch-based biodegradable plastics derived from corn and rice sources [58]. Such studies contribute to expanding our knowledge of the potential applications and sustainability of starch-based biodegradable materials in various industries. Starch-based materials are currently extensively employed in the food packaging industry, primarily due to their ability to meet the high demands for mechanical strength, antioxidant properties, and specific barrier properties [7]. The versatility of starch-based materials allows them to be tailored to meet the specific requirements of food packaging, ensuring product quality, safety, and shelf-life extension. Modified starch-based materials have emerged as environmentally friendly packaging solutions that can effectively enhance the shelf life of food products. By blending modified starch with other biopolymers, the material performance can be improved, leading to enhanced barrier properties, mechanical strength, and stability [44]. These materials offer a sustainable and biodegradable alternative to traditional packaging materials, catering to the growing consumer demand for environmentally friendly packaging solutions, which aligns with the growing demand for sustainable packaging options that minimize environmental impact while ensuring product quality and safety.

2.2.4 Other biodegradable plastics

In addition to the three most commonly used biodegradable plastics (PLA, PBAT, and starch-based materials), there are several other biodegradable materials that make up approximately 14% of the overall market share. These materials include PHA, PGA, PCL, PBS, and more. Previous research papers have highlighted the various applications of these materials as sustainable alternatives to fossil-fuel-based thermoplastics. These biodegradable materials are sought after for their potential to reduce environmental impact, promote circular economy principles, and address concerns related to plastic pollution. Their applications span across various industries, including packaging, agriculture, biomedical, etc. Bugnicourt et al. provided a comprehensive overview of the potential applications of PHAs in various sectors, including packaging, molded goods, and additives. The study also highlighted the limitations and challenges associated with improving the properties of this material [45]. PHAs have shown promise as biodegradable alternatives in packaging materials, offering advantages such as flexibility, barrier properties, and compatibility with other polymers. However, there are still areas that require further research and development to enhance the mechanical properties, processability, and cost-effectiveness of PHAs for broader commercial adoption. The paper by Bugnicourt et al. contributes to our understanding of PHAs and highlights areas for future improvement in their application potential. Indeed, PGA and PCL have found wide applications in the pharmaceutical and medical industries, particularly in tissue engineering. PGA offers high versatility for modification and is relatively cost-effective. It is commonly utilized in the development of drug delivery devices and biomedical sutures. On the other hand, PCL and PCL-based materials are frequently employed as substitutes for hard tissues in the human body, owing to their robust mechanical properties. The medical field benefits from the strong structural integrity and compatibility of PCL-based materials, allowing for their successful integration and use in various applications, including tissue engineering [48, 49]. The PBS (Polybutylene Succinate) biopolymer possesses several favorable characteristics that make it an ideal candidate for bioimplants. It exhibits high chemical resistance, which allows it to withstand the harsh physiological conditions within the human body. Additionally, PBS demonstrates high mechanical strength, enabling it to withstand the forces and stresses that may be exerted on the implant. One of the key advantages of PBS is its ability to undergo degradation and absorption within the human body. This property is particularly important for bioimplants, as it allows for the gradual breakdown and replacement of the implant material by natural tissue over time, promoting healing and minimizing the need for subsequent removal surgeries [50]. The unique combination of chemical resistance, mechanical strength, and biodegradability makes PBS a highly attractive option for bioimplant applications.

2.3 Addition of various functional additive categories to the biodegradable plastics performance improvement

Although biodegradable materials tend to be the substitutions of conventional plastics, the drawback of such polymers needs to be modified by the addition of synthetic, natural, or organic additives to reach the high requirement of applications in various fields. Additives are typically added to biodegradable plastics to enhance their properties or to facilitate their biodegradation. Table 2.2 listed the common additives used in biodegradable plastics with the details of function in typical materials, additive names and applications in various areas. It is important to note that the specific additives used in biodegradable plastics can vary depending on the type of plastic, the intended use, and the manufacturing process. Normally, the commonly used additives added into biodegradable plastics are for the purpose of enhancing the properties and strength, mechanical properties, thermal stabilities, etc. The additives are usually categorized into many types based on their functions in different materials including plasticizers, antimicrobial agents, antioxidants, nucleation agents, slip agents, etc. Fig 2.2 presented the different additive types and ratios used in biodegradable plastic. The data in Fig 2.2 was collected from 40 related literature from Google Scholar.

Apart from the chemical additives, biodegradable plastic and polymer nanoparticle are also widely added to improve the mechanical properties of biodegradable plastics. For example, PLA with nucleation agent and polyamide (PA) blends have higher crystallization properties and mechanical properties such as strength, ductility and impact resistance [59]. In addition, lignin nanoparticles/PBS composites showed superior performances of transparency, UV absorption and stiffness with high photo-oxidation stability suitable for outdoor applications [60]. After weathering acceleration, the tensile strength of Lignin/PLA composites slightly decreased, while the water sorption capacity increased [61].

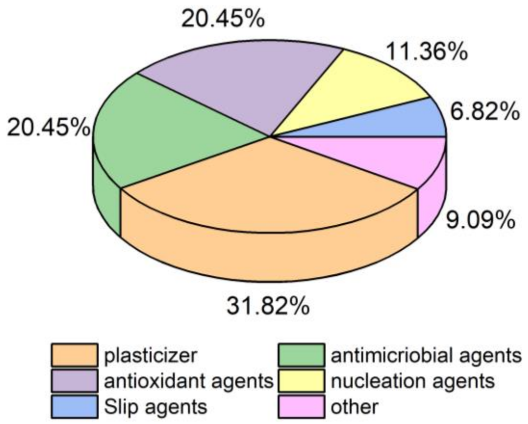


Fig 2. 2 Proportional usage of chemical additives in biodegradable plastics

2.3.1 Plasticizers added in biodegradable plastics

Plasticizers, being the most predominant category of additives, exhibit the highest prevalence in terms of quantity, production volume, and overall utilization among various plastic additives. The primary role of plasticizers lies in their ability to attenuate the van der Waals forces operating between polymer molecules, consequently augmenting the mobility of polymer chains and diminishing the crystalline nature of such chains. This increases the plasticity of polymers, as evidenced by the decrease in the hardness of the polymer while the elongation, flexibility and flexibility increase. Collectively, the common plasticizers added in biodegradable plastics include polyethylene glycol (PEG), butoxytriglycol, dibutyl phthalate (DBP), citrates, glycerol, etc.

PEG is one of the most widely used plasticizers for PLA and other bioplastics [62]. Researchers have found that the mechanical strength and ductile properties have been improved with the addition of 2% of total weight PEG as the plasticizers in PLA and PLA composites [63]. In addition, the compatibility and flexibility of PLA can be better improved by adding plasticizers [64]. Moreover, the efficiency of plasticizers also depends on the chemical structure, molecule weight and concentration of the additive. A published study investigated four different plasticizers added in a biodegradable plastic poly (3-hydroxybutyrate-co3-hydroxyvalerate) (PHBV) with the same blend ratio of 20wt%. The results showed that triethyl citrate (TEC) has the highest effect on thermal properties, flexibility and ductility of PHBV polymer [65]. Furthermore, plasticizers can play the role of performance modification additives of starch-based biodegradable plastics. The starch-based biodegradable plastics change to show the thermoplastic properties by adding specific plasticizers such as citric acid and glycerol through gelatinization [44]. In order to expand the applications of starch-based biodegradable materials, the plasticizers are usually blended into the materials to reach the performance requirements. For example, glycerol and epoxidized soybean oil are usually used as plasticizers to increase the flexibility, extensibility, chain mobility and elongation at break, and accelerate the degradation timescale of starch-based biodegradable materials [7, 66]. Furthermore, butoxytriglycol is used as an active solvent for coatings and an intermediate for ester production, surfactants and plasticizers in PHB [62].

2.3.2 Antimicrobial agents in biodegradable plastics

Antimicrobial agents are chemical substances capable of effectively suppressing the proliferation or reproductive capabilities of specific microorganisms, encompassing bacteria, fungi, yeasts, algae, viruses, and other similar entities, to a level deemed insufficient for sustained growth within a specified temporal span. Normally, antimicrobial agents can be divided into organic, inorganic and natural antimicrobial agents. The main varieties of organic antibacterial agents are vanillin or ethyl vanillin compounds, which are often used in food packaging industries. The inorganic antimicrobial agents can be prepared through physical and chemical processes based on metal ions such as silver, copper, zinc, etc. A research paper demonstrated that cellulose is a proper support material for both organic and inorganic antibacterial agents due to the physical properties of cellulose [67]. Additionally, natural antibacterial agents are mainly derived from natural plants, animals, and bacterial and algae extracts, which can be used in the area of films, coatings and food system [68].

Agustin et al. investigated the antimicrobial activity and biodegradability of a starch-based material by adding chitosan as the antimicrobial agent and glycerol as the plasticizer, indicating that the addition of these additives inhibited the growth of bacterial and accelerated the biodegradability process [69]. Such antibacterial agents can be widely used in the food packaging industries as a novel active packaging system. Many bio-based polymers showed high thermoplastic properties and have good compatibility with natural antibacterial agents extracted from plants such as chitin, mustard, castor oil, etc. In addition, polymers mainly use the matrix of organic (essential oils and organic acids) and inorganic (salt and metals) antimicrobial compounds to reach higher mechanical performances in the application of food packaging [70].

2.3.3 Antioxidant agents added in biodegradable plastics

A previous study has proved that the biodegradable plastics PLA and PBAT have faster UV aging speed than conventional plastics, which indicated that the biodegradable polymers are more sensitive to UV radiation [71]. Therefore, antioxidants are the additives widely used in biodegradable plastics. Antioxidant agents are substances that can process the ability to decompose polymer hydroperoxides produced during the oxidation process, thus effectively terminating the reaction and retarding the oxidative deterioration of polymers. As a consequence, these antioxidants facilitate polymer processing and contribute to an extended operational lifespan.

Generally, the addition of antioxidant agents contributes to the biodegradable plastic film in the application of food packaging and agricultural plastic covering films [72-74]. A study has found that the combination of carboxymethylcellulose (CMC) and/or catechin (CT) with soy protein-based bio-films can be used as an antioxidant food packaging material. The addition of CMC and/or CT as the natural antioxidant agents also showed a significant enhancement of tensile strength and water solubility [75]. Another research focused on characterizing the PLA films incorporated with chitin nanocrystals as an additive, revealing potent properties associated with the antioxidative activity. Simultaneously, the chitin nanocrystals also present an improvement in mechanical strength [76]. In general, antioxidant agents assume a significant role in the context of food packaging by effectively prolonging the shelf life of food products.

2.3.4 Nucleation agents added in biodegradable plastics

The inclusion of a nucleating agent within a polymer matrix can exert substantial influence over its partial crystallization behavior, leading to enhancements in various aspects such as transparency, rigidity, surface gloss, impact toughness, and heat deflection temperature of the final product. Moreover, the nucleating agent has the potential to reduce the molding cycle duration, as well as enhance both the processing efficiency and application performance of the product. Many studies investigated the effect of different substances as nucleation agents in PLA and PBAT, indicating that the effect of nucleation agents depends on the substances type, content proportion and processing temperature [77-79]. In addition, cellulose nanofibers are proved to be functional nucleation agents using PLA materials showing an improvement in the crystallization rate with different fibre loading (1-6 wt.%). The results showed that the PLA with 3% cellulose nanofibers nanocomposites presented the highest crystallinity and crystallization rate [80].

2.3.5 Slip agents and others added in biodegradable plastics

In the process of polymer producing, slip agents need to be added to improve lubricity, reduce friction, and reduce interfacial adhesion. In addition to improving fluidity, slip agents can also play the role of melt accelerator, anti-blocking, antistatic agent, etc.

Normally, the addition of slip agents accounts for 0.5%-1% of plastic manufacturing. Slip agents have a variety of effects in the actual process, such as in mixing and calendaring processing, they can prevent the polymer from sticking to the barrel, suppress frictional heat, reduce the mixing torque and load, and thus prevent the thermal deterioration of the polymer material. Furthermore, slip agents can also improve the appearance and gloss of the plastic film. Cui et al. determined various organic additives in a PBAT covering film, identifying several amides such as erucamide and hexadecanamide, which are the most common slip agents [81]. Another study also detected a quite high concentration of oleic acid in the PLA products, which is usually used as the slip agent in PLA straws, bottles and films [62].

In addition to the additives mentioned above, there are a variety of other additives in biodegradable plastic used to change and improve the manufactural technology and appearance of plastic materials. For example, an antiblock agent is a substance employed to inhibit the adhesion between plastic films. Typically, this agent is incorporated during the batching process, and at room temperature, it exhibits incompatibility with the polymer. Consequently, the antiblock agent migrates towards the surface of the product, facilitating easy separation of the film. Talc, chalk and silica are commonly used as antiblock agents [82]. The antifog agent is an additive to remove the water vapor on the plastic film, which is widely used in food packaging material. The antifog effect can prevent the condensation of water droplets on the inner wall of the packaging bag, which affects the light transmittance in the packaging bag thereby extending the shelf life of vegetables and fruits. Antifog agents like fatty acids usually used in the bio-based thermal plastic wrapping films under room and low temperatures [83]. Besides, colorant and pigment are added to plastic materials with the effect of beautification, decoration, facile identification and enhanced optical properties. Inorganic pigments such as ferrous metal oxides are employed as the commonly used colorants including zinc dioxide, titanium dioxide, etc. A previous study indicated that natural food colorants are commonly applied in PLA and PBAT food package films [84].

It's worth noting that some additives, such as certain types of plasticizers and stabilizers, can have negative environmental impacts if they are not properly disposed of or if they leach into the environment. As such, it's important to carefully consider the use of additives in biodegradable plastics and choose those that are the most environmentally friendly and suitable for a particular application.

Table 2. 2 Additives used in biodegradable plastics with the details of function in typical materials, additive names and applications in various areas

Category	**Additive name**	**Applications and functions in plastic**	**References**
Plasticizer	Polyethylene glycol (PeG)		[62]
	Butoxytriglycol		[62]
	Dibutyl phthalate (DBP)	PHA	[65]
	Citrates, glycerol	Improve mechanical properties of starch-based plastics	[69, 85]
	Epoxidized vegetable oils	Enhance the stiffness and strength of starch-based plastics	[66]
Antimicrobial agent	cellulose		[67]
	chitosan	Inhabited the growth of bacteria in starch-based plastics	[69]
	Essential oils, salt and metals	Improve the mechanical properties of biodegradable food packaging plastic bags	[70]
Antioxidant	Lignin nanoparticles (LNPs)	PLA	[86]
	Oregano essential oil	PLA, PBAT	[87, 88]
	Sorbic acid	PLA	[89]
Nucleation agent	Zinc PhenylPhosphonate	PLA	[77]
	Polyglycolide	PLA	[78]
	Hydrochar	PBAT	[79]
Slip agent	Oleic acid	PLA straws, bottles and films	[62]
	Fatty acids (Oleamide, erucamide, ethylene-bis-stearylamide), hexadecanamide	PBAT covering film	[81, 82]
Antiblock agent	Talc, chalk, silica		[82]
Antifog agent	Fatty acid ester (glycerol oleates, glycerolstearates)		[82]

Colorant and pigment	Carbon black, titanium dioxide	Plastic covering film, carrier bags	[82]

2.4 Microplastic and additive microparticles abundance in biodegradable plastics

2.4.1 Microplastic released in biodegradable materials

Due to the physicochemical properties of biodegradable materials, it is more likely to be broken into small fragments and MPs during and after use. It is inevitable for biodegradable plastic to continuously breakdown into micro and nano-sized pieces during the degradation process, but it requires a long time scale for the completion of the whole process. Although MPs abundance in conventional plastic products has been extensively investigated in recent years, fewer studies focused on the MPs derived from biodegradable materials. The degradation rate and degree of different types of biodegradable materials highly depend on the polymer properties and chemical structures [11]. Therefore, biodegradable MPs are likely to be derived from the bulk material during the degradation process, thereby causing waste accumulation in different environment.

2.4.2 Biodegradable microplastics abundance in soil environment and marine system

In the natural environment, biodegradable plastics usually degrade by UV irradiation and weathering process [90]. However, the rapid degradation of biodegradable plastics into end productions needs specific conditions such as high temperature, humidity and microbial and composting circumstances [10]. Therefore, biodegradable plastics products such as plastic bags and films are hard to be fully degraded under natural conditions. Several studies were conducted to investigate the degradability of biodegradable materials including bulk materials and plastic films by establishing the experiment with simulated different natural aquatic conditions. All the results presented that such biodegradable could last for 6-12 months with gradual degradation in the marine environment [9, 91, 92].

Due to the incomplete degradation of biodegradable plastics, the small plastic pieces could be formed, and enter the natural ecosystem then remain in the soil, sediment and waterbody for a long timescale. Studies have shown that the degradation rate of biodegradable plastics in aquatic environments is lower than that exposed to other conditions under UV, temperature and mechanical actions [93]. Nazarethet al. proved that

4 of 6 samples showed no observed morphological degradation by conducting an aging experiment to immerse the samples in seawater for 180 days, even though they were claimed as 100% biodegradable [8]. Another study explored the MPs generation of blended biodegradable materials after 90 days' aging process, indicating that the blended material produced massive MPs during the weathering period [94]. The results of these studies lead to a conclusion that biodegradable plastics will have the same environmental issue as MP pollution.

Biodegradable plastics have a wide application in agricultural plastic covers. The UV irradiation and weathering outdoor could cause a fast fragmentation of biodegradable covering films, thereby leading to a large number of biodegradable MPs [95]. The MPs generation rate of different types of agricultural plastic covering films under simulated UV irradiation was determined by Yang et al. The study came to a conclusion that the biodegradable covering films had the highest MPs generation rate, followed by oxo-degradable plastic film and conventional plastic films[96]. In parallel, the thickness effect on the degradability of biodegradable film was examined by Uzamurera et al. The study compared the MPs release rate of both conventional and biodegradable films in agricultural soil with two different thicknesses. The results indicated that both the thin and thick biodegradable films have a faster release rate than that of conventional films, with a 2.55 and 3.72 times higher MPs number respectively. Moreover, the thinner films have a higher release rate compared with thicker films [97]. In addition, the effect of sediment on the degradability of biodegradable plastics has been reported by Beltran-Sanahuja et al., indicating that sediment can promote the degradation of plastic debris due to the abundance of microorganisms[98]. Liao et al. conducted a study that determined the degradation degree of different biodegradable polymers in air and soil by photo- and biodegradation. The research concluded that Poly(p-dioxanone) (PPDO) showed the highest degradation degree due to the enriched microorganisms on its biofilm. Simultaneously, PPDO presented a high level of MPs abundance during the degradation process in both air and soil environment [99]. As a consequence, the natural environment can witness the formation of numerous MPs before the complete degradation of biodegradable polymers occurs.

2.4.3 Microplastics released from biodegradable necessities

Since degradable plastics are considered an ideal alternative to traditional plastics, plastic products, especially for single-use food containers, disposable cups, and single-used takeaway cutleries are gradually replaced by biodegradable materials. Therefore, many studies focus on the MPs and other microparticles released from daily necessities made by biodegradable materials. A previous study investigated the nano-sized plastic level from PLA teabags by the experiments simulating the preparation of a cup of tea. The result showed that about one million PLA nanoplastics per teabag were identified [13]. The hydrolysis process of biodegradable polymer is another way to produce MPs in the natural environment. It was reported in a previous paper that a biodegradable polyester can be rapidly degraded by enzyme hydrolysis, which will release millions of microparticles from only 0.1g of the biodegradable film. The fragmentation formed with various shapes and morphology from the surface of the bulk material, causing a large number of MPs, oligomers, and other microparticles [100].

2.5 Additive microparticles released in biodegradable materials

In order to reach the high-quality requirements of biodegradable plastic applications, as well as to overcome the shortcomings of properties of biodegradable materials, chemical additives were added to these polymers during production and manufacturing. Subsequently, when the commercial products are transported into the market, the additive microparticles are the potential to be released during the transportation and photo- or UV degradation process. The study has proved that the fragmentation of the plastics occurs from the surface of the polymer, thereby causing the microparticles to detach from the bulk material [97].

Studies have revealed that high levels of additive microparticles were detected in both conventional and biodegradable plastic products, which were separated by alcohol treatment and identified by Raman spectroscopy [12, 20]. In parallel, a study was conducted to investigate the additive presence in 9 single-use plastic products, including 8 biodegradable materials (3 PHB and 5 PLA) and 1 conventional material (HDPE). The findings revealed the presence of more than 200 additives in the examined plastic materials, with plasticizers comprising the highest content among all the categories of additives. Notably, the PLA drinking straw exhibited the highest concentrations of additives among the eight bio-based items analyzed [62]. Food packaging is one of the main applications of biodegradable polymers. For example, PLA and PHB are used to replace traditional plastic bags to contain fruits and vegetables. Antioxidant and antimicrobial agents are the major additives in the food packaging material to prolong the shelf life, and storage time and to prevent the quality loss of food. Jariyasakoolroj et al.

summarized several studies focusing on the additive release mechanism and the impact factors that affect the additive release. The study demonstrated that the factors such as the storage temperature and crystallinity of polymers can influence the migration and mobility of additives, so that cause the release of additives from packaging materials during the storage and degradation process [101]. Additionally, another paper revealed that chemical additives such as catalyst stabilizers and Bisphenol A (BPA) were detected in the leachate of several different bioplastics and compostable bags (i.e. Bio-PET, PLA and PBAT) during the degradation in artificial seawater for 14 days [102]. In addition, the release of harmful chemical additives such as dye, pigment, plasticizers and stabilizers could also occur during natural degradation, especially in the soil environment which is rich of microorganisms and composting conditions, which could be another threat and pollutant to both aquatic and terrestrial environment [103].

2.6 Health risk

2.6.1 Toxicological research on biodegradable MPs and additive microparticles to environment and ecosystem

The aged plastic debris during the degradation has become a severe threat to the environment and ecosystem. In terms of conventional plastics, many studies have conducted toxicological research on the released MPs and chemical additive microparticles to different species. Mattsson et al. investigated the toxicological effect of polystyrene (PS) nanoparticles on fish. The study concluded that the PS nanoparticles can enter the food chain and severely affect the behavior and metabolism of fish when the fish were exposed to approximately 1×10^{13} particles [26]. Another study also proved that the 180nm and 53nm NPs with a concentration of 0.1g/L and 0.029g/L reduce survival of fish and have the potential of penetrating the blood barrier transferring in the brain, therefore causing the behavioral disorders [27]. Further study revealed the potential health risk of PE and PS MPs on mice by exposing the mice to PE and PS MPs coupled with an organophosphorus flame retardant environment for 90 days. The results explored that the co-exposure of MPs and organophosphorus flame retardants (10µg/L) has higher health risk than the exposure environment without MPs [28]. Furthermore, the NMPs and additives also present significant hazards to the natural environment and living organisms. A recent review summarized the toxic effect of MNPs particles on different organisms, as well as the adsorption behavior of these particles. Collectively, the NPs can accumulate in the organs of living organisms and cause the change in normal metabolism, organism growth and oxidative stress reaction. Moreover, it is important to highlight that the degree of harm caused by NMPs is directly influenced by their particle size. The smaller the particle size, the greater the potential for adverse effects on living organisms and ecosystems. This is due to the increased likelihood of ingestion by organisms at lower trophic levels, as well as the potential for these particles to penetrate biological tissues and accumulate in vital organs, leading to physiological disruptions and ecological imbalances.

With the development and high attention on biodegradable plastics, a growing number of studies start to focus on the toxicity of released biodegradable NMPs and additive microparticles. Toxicity indicates the level of a substances causing harm to living organisms. Su et al. proposed that the conventional MPs have similar toxicity as biodegradable MPs by conducting an experiment on the effect of PE, PA, PLA and PBS MPs on microalgae growth. The results showed all four types of MPs have an inhibition effect on microalgae growth with slight differences under the concentration of 100mg/L mPE, 100mg/L mPA, 100mg/L mPLA and 100mg/L mPBS, respectively. Besides, PLA MPs showed the strongest effect, which also induce the release of pigments from the bulk material [14]. A similar conclusion was drawn in another research by Ding et al. The biological effect of different concentrations of PE, PLA and polypropylene carbonate (PPC) MPs on earthworms was determined in a lab-scale experiment. They concluded that the MPs concentration instead of MPs types is the main factor in the behavior of earthworms. Both conventional and biodegradable polymers showed similar biological impacts on the species with a critical threshold concentration of 40 g kg^{-1}, which could cause the avoidance behavior and a reduction of cocoons [104]. Xie et al. compared the potential effect on fish by MPs released from conventional and biodegradable plastic bags respectively, indicating that both conventional and biodegradable MPs can be accumulated in gastrointestinal tracts of fish after 21 days, as well as leading to immunity decreased based on a daily ingestion of 0.22g of food per fish [105]. Moreover, the PLA bio-MPs showed higher toxicity than conventional PE MPs in freshwater ecosystems in another study conducted by Chagas et al [106]. The research also compared the biochemical toxicity effect of PE and PLA MPs on dragonfly larvae under 48h exposure at environmentally relevant concentration of 6mg/L. The results did not show the severe metabolism change caused by both PE and PLA MPs, while the PLA MPs present increase oxidative stress processes and a decrease in superoxide dismutase activity.

In addition to the comparative studies, several papers have been published regarding the risk assessment and toxicological study, as well as the adsorption and desorption behavior of chemical additive microparticles on biodegradable NMPs. A previous paper assessed the health effect of PLA Bio-MPs on zebrafish larvae individuals and demonstrated that PLA MPs can lead to a variety of physical dysfunction and behavioral disorder in these freshwater animals including locomotor and exploration activities. The accumulation of PLA MPs in the organs of zebrafish has the potential to inhibit the acetylcholinesterase activity at concentration of 3 and 9 mg/L, therefore decreasing the biological activity of such living organisms [107]. In parallel, PLA MPs (2-3mm with flake shape) were applied to investigate the toxicity to a specific bacteria, drawing the result that 100 mg/L PLA MPs lead to the damage of cell walls and oxidative activity, as well as inhibiting the growth of the bacteria [108]. It is worth noting that the secondary nanoplastic, which naturally degraded from micro and macro plastic with irregular shape and morphology, could cause a higher biological impact on the environment. González-Pleiter probed this issue by investigating the secondary PHB nanoparticles and the PHB leachate. The results showed that the supernatant of PHB leachate has no toxicity effect on the organisms, while the PHB nanoparticles derived from PHB microplastic with an initial concentration of 50mg/mL presented to inhibit the growth of two aquatic organisms with high percentages of 90% and 95%, respectively [109]. Apart from the biodegradable MPs themselves, the microparticles have the potential to absorb the pollutants and transfer them into the natural environment [110]. A study pointed out that the NMPs can be a carrier of other toxic substances such as chemical additives, heavy metals and organic compounds, which become a more severe threat to living organisms [111, 112]. The review paper summarized the studies focused on the adsorption ability of micro-sized biodegradable polymers, as well as the comparative research with conventional MPs. The results from several studies reported that the PLA MPs have the highest adsorption capacity within both petroleum-based and biodegradable MPs [113, 114]. Besides, the adsorption and desorption behavior of PE and PHB MPs were examined by Tong et al. Triclosan was selected as the target substance in this study, which is a highly toxic organic compound used as a disinfectant and antibacterial agent widely used in household and medical products. The results revealed that the adsorption capacity of triclosan of PHB MPs is much higher than that of PE MPs, with the adsorption concentration of 9442.27 μg/g and 3431.85 μg/g, respectively. In addition, the desorption result also suggested that the triclosan can be released from both PE and PHB MPs under physiological conditions, which performs a potential health risk to the environment and ecosystem [115].

Additionally, biodegradable MPs have been proven to be a threat to the soil environment and species [11]. Biodegradable and PP covering films were applied to investigate the sensitivity of tomato and lettuce growth by Serrano-Ruíz et al. The study was conducted by in vitro ecotoxicity test which conclude that bioplastic films could inhibit the germination of both tomato and lettuce, while tomato plants are more sensitive than lettuces to these plastic covering films extracts [103]. Zhou et al. studied the impact of PHA biodegradable MPs in soil, drawing the conclusion that the PHA MPs are likely to change the microbial community structure in soil and have the potential to break the ecological balance of the soil [116]. A review paper explored the effects of biodegradable MPs on the soil environment from several aspects including the physicochemical properties, microorganisms, and plant species and living organisms, demonstrating that compared with traditional MPs, biodegradable MPs have more serious effects and potential risks [90].

To sum up, it is worth noting that the small size of micro and nano plastics allows them to easily enter various environmental compartments, such as water bodies and soil, where they can persist for extended periods. This widespread distribution and persistence amplify the risks they pose to the natural environment, including potential harm to aquatic organisms, soil ecosystems, and overall ecosystem health. Based on the studies conducted previously, the potential risk of these microparticles derived from biodegradable polymers has species specifications and depends on various factors. Consequently, there is a pressing need for further research focused on investigating the biological impacts of biodegradable MPs and their interactions with other microparticles. This research is crucial in order to better understand the potential consequences and ecological implications associated with the presence and behavior of biodegradable MPs in various environmental contexts.

2.6.2 The potential health risk of biodegradable MPs and chemical additive to humans

Health risk refers to the possibility of experiencing health effects as a result of exposure to a specific hazard, combines the likelihood of exposure with the severity of the potential health effects. Currently, only a few papers studied the potential effect of conventional MPs on human organs and gastrointestinal tracts fluid. Ingestion from food, especially seafood, drinking water, and MPs release from daily necessities is one of the main pathways of human exposure to MPs. Schwabl et al. found evidence of MPs existence in human feces for the first time by examining the stool samples of 8 volunteers from different countries. The results showed an average concentration of 20 MPs items per 10g samples with a size range varying from 50-500 μm were detected in human stool, indicating the MPs intake from various sources [21]. Besides, another study was carried out to explore the MPs distribution in the placenta, infant feces, breast milk and infant formula samples from 18 mother-infant participants. In conclusion, 16 different MPs were detected and identified with PA and polyurethane (PU) dominated in type and 20-50 μm dominated in size [23]. On the other hand, another main pathway of MPs ingestion is inhalation from an airborne environment. A published paper revealed that nylon flock work may cause severe lung disease to workers who have been exposed to nylon workplace for a long time, which indicated that nylon can lead to severe respiratory diseases [117].

In addition to the MPs themselves, the chemical additive and the associated contaminants in the biodegradable plastic material are other important implications for human health. As we summarized in previous sections, the used additives could be released at the same time while degradation. For example, BPA has been widely studied as a highly toxic additive in PC plastic products in packaging applications. Human exposure to BPA is mainly due to inhalation and dermal contact [118]. A study has proved that BPA can penetrate the barrier of human tissues entering urine and blood, which could cause a potential risk to human organs [119]. From another aspect, many studies reported the potential health hazards of nano- and micro-sized particles, which has the potential to cause harm to human health. In general, small-sized particles present in the atmosphere have the potential to accumulate within the human gastrointestinal tract and respiratory system when they are inhaled [120].

Although studies have been published focusing on the toxicity of biodegradable MPs on plants and animals, the potential risk of these polymers on human health is still limited. Based on the previous study, biodegradable MPs have similar ingestion pathways to human and additive addition during the manufacturing process. As a result, the persistence of biodegradable polymers in the food chain and ecosystems could lead to similar or even more severe potential health risks to the human body. This is due to the accumulation and prolonged exposure of these polymers, which can have adverse effects on human health, similar to non-biodegradable plastics. Therefore, the potential risks associated with biodegradable polymers should not be overlooked in terms of their impact on human health.

2.7 Conclusion

The review summarized the biodegradable polymers categories, advantages and drawbacks, and applications in different commercial sectors from published papers in recent years. This study also reviewed the common additives used in biodegradable plastic products during production and manufacture, which play the roles of the modifiers of biodegradable materials, in order to improve mechanical properties to reach the higher requirements of such applications. In addition, the toxicological studies of the biodegradable MPs as well as the chemical additives in terrorist, marine environments, plant species, living organisms and humans were revealed respectively. According to the result from published papers, the concept of biodegradable plastic being an ideal alternative to conventional plastic need further consideration. This paper also addressed current gaps and limitations of the research on the biodegradable materials especially on the abundance release of NMPs and additive microparticles.

Chapter 3

Methodology

3 Methodology

Sampling, Identification and Characterization of Microplastics Release from Polypropylene Baby Feeding Bottle during Daily Use

Abstract

Microplastics (MPs) are becoming a global concern due to the potential risk to human health. Case studies of plastic products (i.e., plastic single-use cups and kettles) indicate that MP release during daily use can be extremely high. Precisely determining the MP release level is a crucial step to identify and quantify the exposure source and assess/control the corresponding risks stemming from this exposure. Though protocols for measuring MP levels in marine or freshwater has been well developed, the conditions experienced by household plastic products can vary widely. Many plastic products are exposed to frequent high temperatures (up to 100 °C) and are cooled back to room temperature during daily use. It is therefore crucial to develop a sampling protocol that mimics the actual daily-use scenario for each particular product. This study focused on widely used polypropylene-based baby feeding bottles to develop a cost-effective protocol for MP release studies of many plastic products. The protocol developed here enables: 1) prevention of the potential contamination during sampling and detection; 2) realistic implementation of daily-use scenarios and accurate collection of the MPs released from baby feeding bottles based on WHO guidelines; and 3) cost-effective chemical determination and physical topography mapping of MPs released from baby feeding bottles. Based on this protocol, the recovery percentage using standard polystyrene MP (diameter of 2 µm) was 92.4-101.2% while the detected size was around 102.2% of the designed size. The protocol detailed here provides a reliable and cost-effective method for MP sample preparation and detection, which can substantially benefit future studies of MP release from plastic products.

Keywords: Microplastics, plastic product, daily use, baby feeding bottle, hot water, polypropylene

3.1 Introduction

Most types of plastics are non-biodegradable but can break down into small pieces due to chemical and physical processes such as oxidation and mechanical friction [121, 122]. Plastic pieces smaller than 5 mm are classified as microplastics (MPs). MPs are ubiquitous and found in almost every corner in the world. They have become a global concern due to the potential risk to humans and wildlife [123, 124]. To date, significant accumulations of MPs have been found in fish, birds, insects [125, 126] as well as mammals (mouse, in the gut, kidney and liver [127, 128]). Studies found that the exposure and accumulation of MPs can damage the lipid metabolism of mice [127, 128]. A risk assessment focusing on fish found that sub-micron MPs can penetrate the blood-to-brain barrier and cause brain damage [129]. It should be noted that to date all MP risk results have been obtained from animal studies while the specific risk to human health is still unknown.

In the last 2 years, concerns about the MP threat to human health substantially increased with the confirmation of the levels of human exposure to MPs. The accumulation of MPs has been found in the human colon [130], the placenta of pregnant women [131] and adult stool [132]. A precise determination of MP release levels is crucial to identify exposure sources, assess the health risk and evaluate the efficiency of any potential control measures. In the last few years, some case studies reported that daily-use plastics (i.e., the plastic kettle [133] and single-use cups [134]) can release extremely high quantities of MPs. For example, disposable paper cups (with interiors laminated with polyethylene-PE or copolymer films), released approximately 250 micron-sized MPs and 102 million sub-micron-sized particles into each millilitre of liquid following exposure to 85-90 °C hot water [134]. A study of polypropylene (PP) food containers reported that up to 7.6 mg of plastic particles is released from the container during a single use [135]. Even higher levels were recorded from teabags made from polyethylene terephthalate (PET) and nylon, which released approximately 11.6 billion MPs and 3.1 billion nano-sized MPs into a single cup (10 mL) of the beverage [136]. Given that these daily-use plastic products are designed for food and beverage preparation, the release of high quantities of MPs is likely and their consumption is a potential threat to human health.

Studies on MP release from household plastic products (i.e., the plastic kettle [133] and single-use cups [134]) are at an early stage, but it is expected that this topic will receive increasing attention from researchers and the general public. The methods required in these studies are significantly different from those used in room temperature marine or freshwater studies where well-established protocols already exist [137]. In contrast, studies involving the daily-use of household plastic products involves much higher temperature (up to 100 °C), with in many cases repeated cycling back to room temperature. Previous studies pointed out that plastics in contact with hot water can release millions of MPs [136, 138]. In addition, the daily-use of plastic products may over time change the properties of the plastic itself. It is therefore crucial to develop a sampling protocol that accurately mimics the most common daily-use scenarios. The detection of micro-sized particles is another major challenge. Previous studies pointed out that MPs release from plastic products are smaller than 20 µm [136, 139, 140]. Detection of these types of MPs requires the use of smooth membrane filters with small pore size. In addition, it is necessary to distinguish MPs from possible contaminants captured by the filter. High sensitivity Raman spectroscopy is used for chemical composition analysis, which has the advantage of avoiding the need for high laser power that is known to easily destroy small particles [140]. Hence, the protocol must combine contamination-free handling procedures with the use of optimal membrane filters and for a characterization method that allows fast and accurate MP identification.

The study reported here focused on the PP-based baby feeding bottle (BFB), one of the most commonly used plastic products in daily life. It was found that a high number of MPs are released from plastic BFB during formula preparation [138]. For further study of MP release from daily plastics, the sample preparation and detection method for BFB is detailed here. During sample preparation, the standard formula-preparation process (cleaning, sterilizing and mixing) recommended by the WHO [141] was carefully followed. By designing the protocols around the WHO guidelines, we ensured that the MP release from BFBs mimicked the baby formula preparation process used by parents. The filter process was designed to accurately collect the MPs released from BFBs. For the chemical identification of MPs, the working conditions for Raman spectroscopy were optimized to obtain clean and easily identified spectra of MPs, while at the same time avoiding the possibility of burning the target particles. Finally, the optimum test procedure and applied force to allow accurate 3-dimensional topography mapping of MPs using atomic force microscopy (AFM) was developed. The protocol (Fig 3.1) detailed here provides a reliable and cost-effective method for MP sample preparation and detection, which can substantially benefit future studies of plastic products.

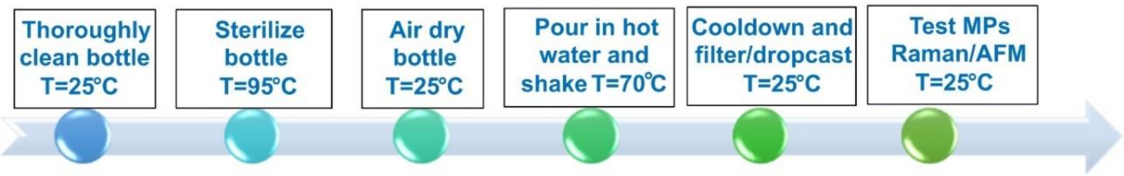


Fig 3. 1 The diagram of sample preparation and test.

3.2 Protocol

3.2.1 Hot water preparation

(1) For all hardware that comes into contact with the samples, use clean glass made of borosilicate 3.3 to prevent any potential contamination. Thoroughly clean all the glassware.

Caution: Pre-existing scratches or imperfection spots on glassware can release particles during the heating and shaking process. We suggest that users check the glassware and avoid the use of the scratched glassware. Our comparison of glassware made of different glasses (such as soda-lime and borosilicate) showed that borosilicate 3.3 releases the lowest quantity of glass particles (can be screened for by Raman spectroscopy), and we recommend the use of borosilicate 3.3 glassware in all tests.

(2) Pour 360 mL of DI water into a glass beaker. Cover the beaker with a clean glass disk. Then move it into a brand-new microwave oven and heat for 2.5 minutes at full oven power. After gently shaking to remove any potential temperature gradients due to uneven heating, the temperature of the water inside of the beaker is 70 °C and ready for sample preparation.

(3) Prepare 95 °C water for BFB sterilization by pouring 1 L of DI water in glassware and heating in a microwave oven for 14 minutes.

Caution: Never use plastic kettles to prepare hot water. The plastic kettle itself releases millions of MPs into the hot water during the boiling process [133]. The released microparticles from plastic kettle will be transferred into baby feeding bottle thereby being exposed by infants.

3.2.2 MP release during formula preparation

NOTE: Carefully following the standard formula-preparation process (cleaning, sterilizing and mixing) recommended by the WHO [141], the MPs released from BFBs during formula preparation is mimicked in the following 3 steps.

(1) Collect brand-new BFB products from pharmacy stores and clean them thoroughly after removing the product from its packaging. Wash each BFB using detergent water (repeat 3 times at room temperature-RT) and distilled water (repeated 3 times, RT). Finally, rinse the BFB 3 times using DI water at RT.

Caution: Do not clean the BFB using sonication. Though sonication is widely used in laboratories for mixing and cleaning, the sonication of BFB can severely damage the bottle surface and cause MP release from PP products within 1 minute.

(2) Soak the BFB in 95 °C DI water (section 1.3) to sterilize the bottle. To avoid the floating of the BFB, slightly press the exterior of the BFB using a stainless-steel tweezer and ensure that the whole bottle body immerses in the water. After 5 minutes, take out the bottle and move it to a clean glass disk. During the air-drying step, invert the bottle on the glass disk until there is no evidence of droplets.

(3) Pour 180 mL of hot DI water (70 °C, from Section 1.2, corresponding to WHO guidelines) into the air-dried bottle. Then cover the bottle immediately using a glass Petri dish and place it into a shaking bed.

To simulate the formula mixing process, shake the bottle at a speed of 180 rpm for 60 seconds. After shaking, move the bottle to a clean glass plate and allow it to cool down.

3.2.3 Sample preparation for MP identification and quantification

(1) Sonicate and thoroughly rinse all parts of the glass filter (diameter of 25 mm, glass funnel, fritted glass support base and receiver flask) using DI water.

1) Place a piece of gold-coated polycarbonate-PC membrane filter (pore size of 0.8 µm, Au coating layer thickness of 40 nm) in the middle of glass base.

2) Assemble the glass funnel and stainless-steel clamp to fix the membrane filter. Finally connect the assembled glass filter to a vacuum pump (Fig 3.2).

Caution: Gold-coated polycarbonate-PC membrane filter was selected in the MPs study due to the flat and smooth surface of the filter membrane, which is benefit for microparticles observation under optical microscope. In addition, the gold coating on the filter membrane can effectively subtract the background interference of PC during the Raman identification. To ensure that the membrane smoothly sticks onto on the surface of the glass base it is important to keep the glass base wet. If necessary, 1-2 drops of DI water should be dropped on the surface of the glass base before placing down the membrane filter.

(2) Carefully mix the cooled water sample in the BFB (from Section 2.3), and then transfer a certain amount of the water sample to the glass funnel using a glass pipette. Switch on the vacuum pump to allow the water sample to filter through the membrane filter slowly.

After filtering, wash the interior of the glass funnel using DI water to ensure that there are no particles sticking on the funnel.

Caution: To avoid the overlap of the particles on the surface of the membrane filter, it is important to carefully choose the correct volume of water that is passed through the filter. BFBs release large number of particles, so that 3-5 membrane filters are needed to filter the entire volume of the water sample.

Disconnect the vacuum pump and disassemble the glass filter. Then carefully take out the membrane filter using a stainless-steel tweezer and move it to a clean cover glass. Fix the membrane filter on the cover glass using a small piece of paper tape. Immediately store the sample in a clean glass Petri dish.

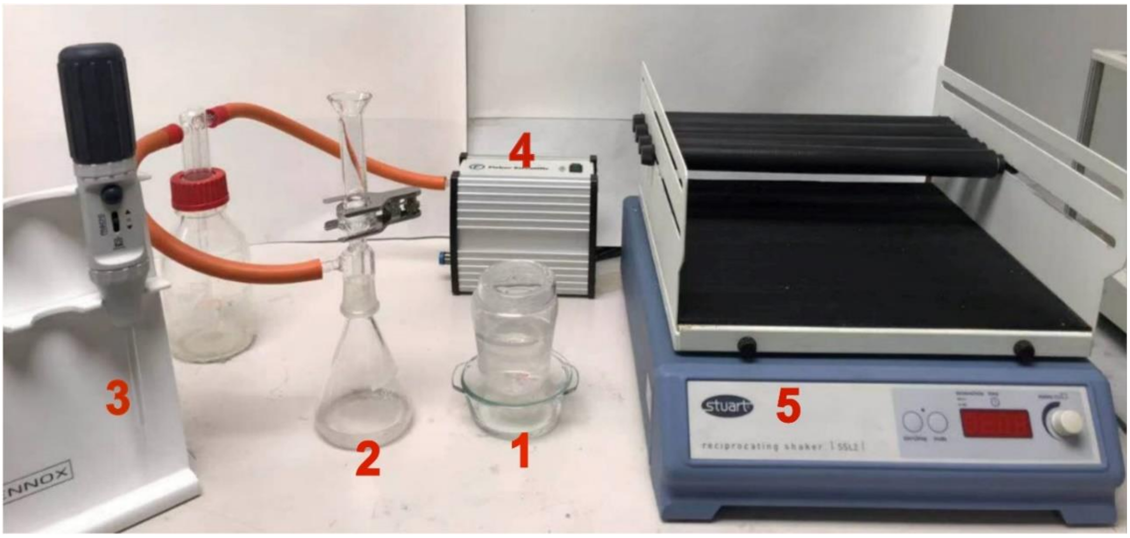


Fig 3. 2 Assembly of the glass filter and pump. 1-cooled water sample in BFB; 2-assembled glass filter; 3-glass transfer pipette; 4-vacuum pump; 5- reciprocal shaker. Assembly of the glass filter and pump. 1-cooled water sample in BFB; 2-assembled glass filter; 3-glass transfer pipette; 4-vacuum pump; 5- reciprocal shaker

3.2.4 Sample preparation for AFM topography characterization

(1) Prepare a clean silicon wafer. Drop a 50 µL water sample (from Section 2.3) on the surface of the silicon wafer and dry it in an oven at a temperature of around 103 °C. Repeat this process if the MP level in the water sample is low.

(2) After 1 hour of drying, move the wafer to a clean glass Petri dish and allow it to cool down in a desiccator.

(3) After the wafer has cooled, store the sample in a dry place.

3.2.5 MP identification and quantification using Raman spectroscopy

(1) Calibrate the Raman system using a zero-order correction and a silicon wafer. Ensure that the peak location of silicon wafer is at 520.7 cm^{-1} and that peak intensity is higher than 6000 a.u. when the laser intensity is at 100%.

(2) Set up the parameters of the Raman system to obtain high signal-to-noise MP spectra while avoiding the burning of MPs. Set the system as follows: 532 nm excitation laser, remove cosmic ray, laser intensity of 10% (laser power of 0.18 mW), spectral resolution of 1.5 cm^{-1}, exposure time of 10-20 seconds, accumulations of 10-40 times and spectral range of 200-3200 cm^{-1}. Fig 3.3 showed typical spectra of MPs with accumulation times from 1 s to 400s.

Caution: Do not test particles using 100% laser directly to avoid the rapid-burning (can be burned in 1 minute if the particle is small). Use low intensity (10-50%) to conduct the test first.

(3) Place the filter sample in the middle of the Raman sample stage. Choose four representative spots (2 spots are in the middle area while other 2 spots are close to the edge of working area, Fig 3.3C) on the membrane filter to conduct the test (total test area around 1.5 mm^2).

(4) Observe and photograph the particles on the surface of the membrane filter using an optical microscope (100x) followed by chemical identification using Raman spectroscopy.

1) Compare the Raman spectrum obtained to the reference standard PP spectra (from bulk material of BFB and previous publication [142]).

2) Determine the particles' chemical property using the intensive peaks in the range of 2780-2980, 1400-1640 and 709-850 cm^{-1}, corresponding to the stretching vibrations of $CH/CH_2/CH_3$ and C-C groups associated with polymer materials (Fig 3.3).

(5) Analyze the size and quantity of the identified MPs using ImageJ.

1) Obtain the MPs concentration in the water sample based on the tested area, total working area (227 mm^2) and the known filtered sample volume.
2) Classify the confirmed MPs into 5 groups in terms of the size: 0.8-5 µm, 5-20 µm, 20-50 µm, 50-100 µm and > 100 µm.
3) Finally, determine the MPs quantity in one litre of water sample based on the filtered sample volume, number of MPs recorded and tested area of the membrane filter. The total MPs amount (particles/L) can be determined by following equations.

$$A_s = \frac{N_t \times \frac{S_f}{S_t}}{V_f} - A_{ctl}$$

$$A_{ctl} = \frac{N_{t-ctl} \times \frac{S_f}{S_{t-ctl}}}{V_{f-ctl}}$$

Where, A_s is the MPs amount in the water sample, particles/L;

N_t is the number of determined MPs in analysed areas, number of particles;

S_t is the total analysed area of the filter membrane;

S_f if the filtered area of the filter membrane;

V_f is the volume of filtered water samples, L;

A_{ctl} is the MPs amount of control sample, which can be calculated by the second equation;

N_{t-ctl} is the number of determined MPs in analysed area of control samples, particles;

S_{t-ctl} is the total analysed area of the filter membrane;

V_{f-ctl} is the filtered volume of control samples, L.

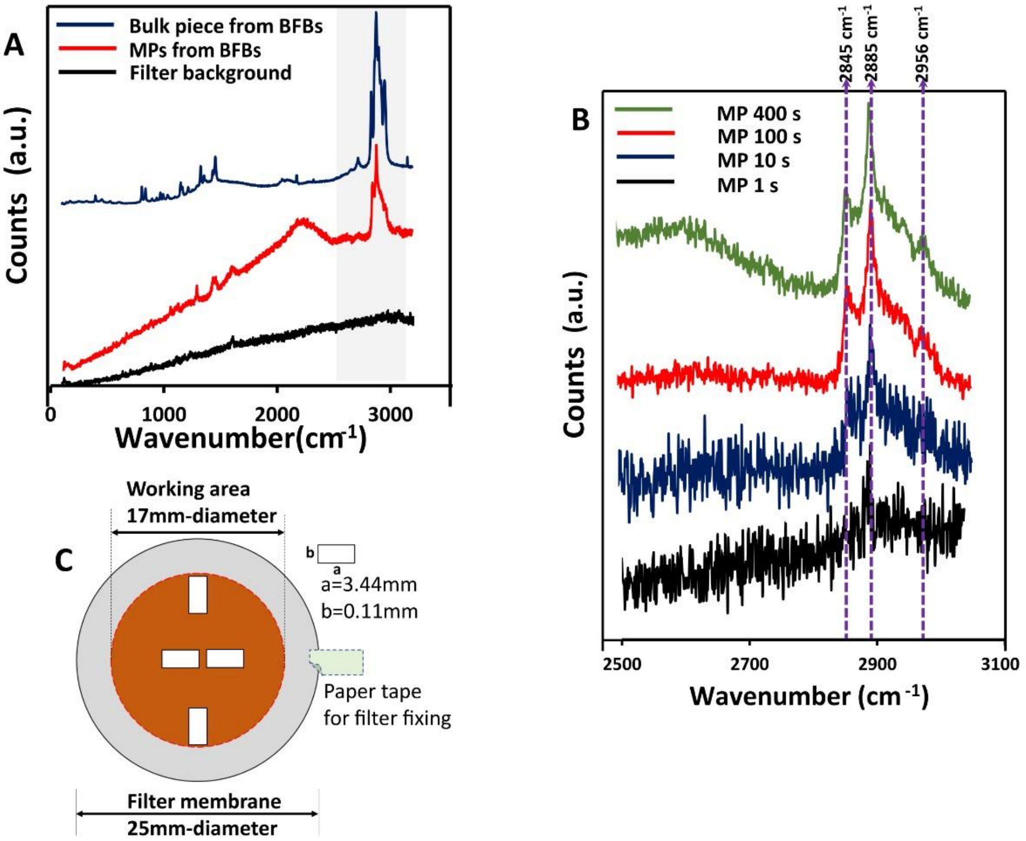


Fig 3. 3 Typical Raman spectra for MPs determination. (A) The Raman spectra of a bulk piece from BFB, membrane filter and MPs on the membrane filter, respectively. (B) The Raman spectra of one potential MP with different acquisition time (1 s, 10 s, 100 s, 400 s). (C) The representative spots tested. The total diameter of filter membrane is 25 mm in diameter with a 17 mm diameter real working area. The four white boxes indicate full representative spots for Raman testing. 2 spots are in the middle area while the other 2 spots are close to the edge of the working area. In total, the tested area of the four spots is 1.5 mm^2.

3.2.6 MP topographic characterization using AFM

(1) Equip the AFM system (NT-MDT) with a tapping mode probe. Calibrate the system using a step height standard (SHS). Set up the system within optimal work conditions: the scan rate is 1 Hz, the scan size is 10-50 µm, the tuning frequency is around 160 kHz, and the scan line is 512 pixels.

(2) Fix the silicon wafer (from Section 4.3) on the AFM sample stage. Observe and photograph the target particles on the surface of the silicon wafer, followed by

chemical identification using the method in Section 5.

(3) Switch the system to AFM mode (the Raman spectroscopy and AFM are assembled in one system) and test the topography of identified MPs.

(4) Analyze the 3d data using Gwyddion 2.54 software. Use the option of *profile* to obtain the particle dimensions and average heights while *3D view* to obtain 3D structure.

3.3 Representative results

To validate this protocol, the water sample was prepared by adding standard polystyrene microplastic spheres (a diameter of 2.0 ± 0.1 µm) to DI water. The MP quantity added corresponded to 4,500,000 particles/L, which is similar to the MP release level from BFBs. The PS spheres were added into DI water and gently shaken until a uniformly dispersion in the DI water. Following protocol sections 2-3, the MPs were successfully collected (Fig 3.4A) on the filter membrane through a vacuum pump filtration system. Based on the equations described above, the recovery rate was determined to be 92.4-101.2%. This recovery rate is comparable to a previous study on MPs [143]. Using ImageJ, the detected diameter of standard MPs was 2.04±0.08 µm (where ± represents standard error of the mean value), which is around 102.2% of the designed size (2.0 ± 0.1 µm). Meanwhile, the potential interference from other types of MPs, such as PP and PE, was also tested for but none was found in these standard PS water samples. Hence, the developed protocol avoids contamination and is a reliable test of MP release from BFBs.

This protocol was used to test the MP release from eight popular BFB products. Fig 3.4B showed the typical MPs collected on the surface of the membrane filter. During the chemical determination using Raman spectroscopy (Fig 3.3), the peaks in the range of 2830-2970 cm^{-1} became more and more significant with the increased accumulation time. These peaks reflect the stretching vibrations of $CH/CH_2/CH_3$ groups, which can be used to identify MPs. A high number of MPs were released during the use of BFBs. The MPs levels ranged from 1.31 million to 16.20 million particles per liter (Fig 3.5). This result is 3-5 orders of magnitude higher than the previously reported levels of MPs in drinking water [144]. It is evident that the babies likely experience high levels of MPs exposure.

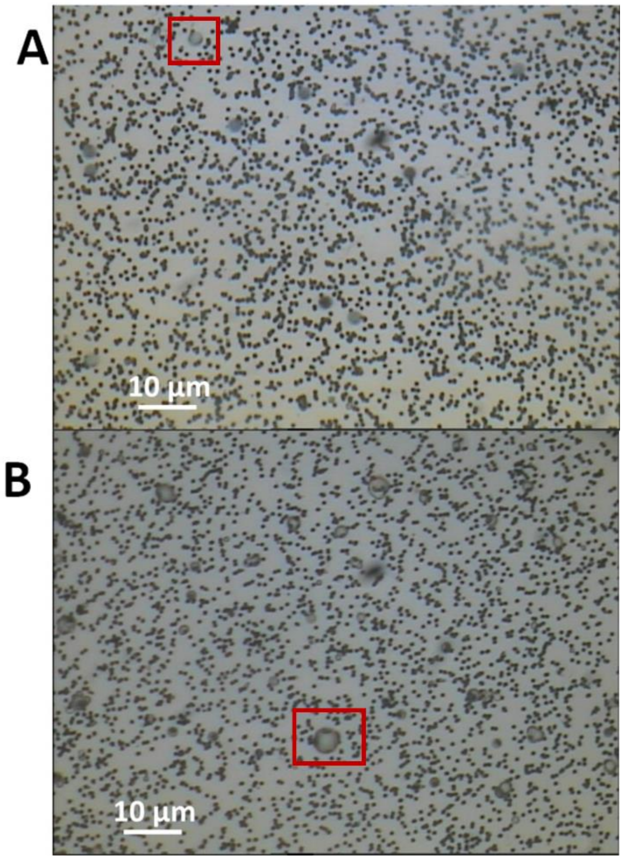


Fig 3. 4 Typical optical image of standard PS MPs and MPs release from BFB, respectively. (A) The optical image of standard PS MPs. The particle inside of the red box was confirmed as typical PS MP. (B) The optical image of MP release from BFBs. The particle inside the red box was confirmed as a typical MP.

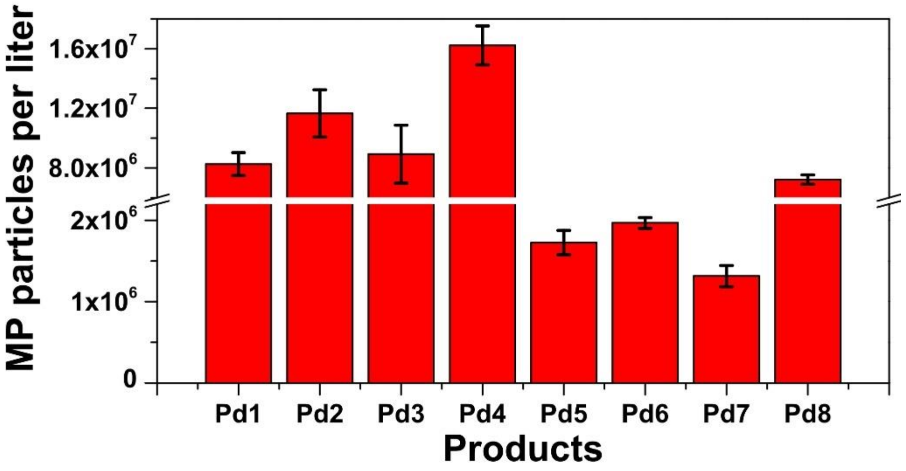


Fig 3. 5 Quantity of MPs released from plastic BFB products. 8 popular products were chosen at the study. The error bar indicates the standard error of the mean value.

Fig 3.6 shows the typical topography maps of MPs recorded using protocol sections 1, 2, 4 and 6. For large MPs of around 8 µm in lateral size (P1 in Fig 3.6), the average thickness is 0.82 µm. For smaller MPs around 3 µm in lateral size (P2 in Fig 3.6), the thickness is close to 0.25 µm. In general, the thickness of the MPs released from BFB is around a tenth of the lateral size. It is also noticeable that the surface texture of MPs is rich with nano-sized bumps and valleys, which can substantially increase their absorption capacity. Previous studies found that MPs are effective carriers for pollutants, such as pesticides[145, 146]. The observed topography of the MPs found here is likely an important contributor to the high carrying capacity of MPs.

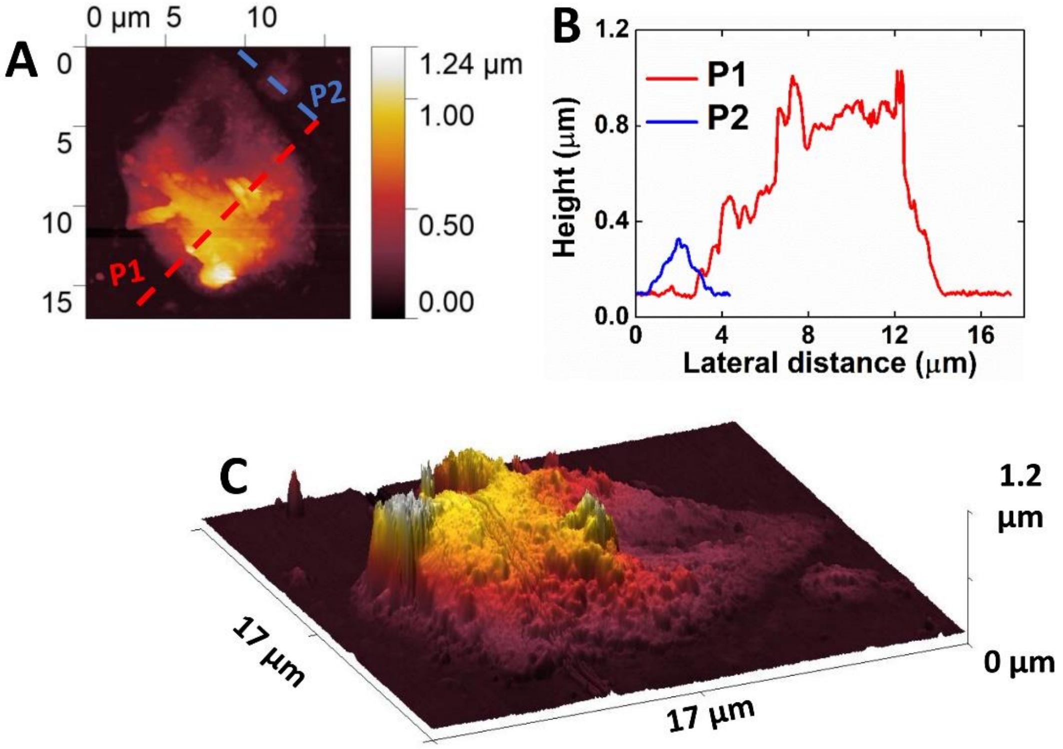


Fig 3. 6 Typical 3D image of MPs release from BFB. (A) AFM image of typical MPs release from BFB. (B) Extracted cross-section profiles of the MPs. (C) The 3D topographic image of the released MPs.

3.4 Discussion

Though the study of MPs in marine and freshwater has been widely reported and the relevant standard protocol has been developed [137], the study of daily-use plastic products is an important emerging research area. The different environmental conditions experienced by household plastic products means that extra care and efforts are needed to obtain reliable results. The study protocol must be consistent with the real daily use scenarios. For example, sonication is widely used in lab-tests to clean samples. However, it was found that 1 minute sonication can severely damage the BFB’s surface, resulting in levels of MP release an order of magnitude higher. Similar polymer breakage due to sonication was also reported previously [147], which indicates that sonication is not a suitable cleaning method for plastic sample preparation in MP studies.

In addition, potential contamination sources must be identified and eliminated. Kettles are widely used to prepare hot water, which is necessary for the BFB test. However, a single boil can generate up to 30 million particles per liter in a plastic kettle [133]. Microwave ovens are a non-contact method to prepare hot water once care is taken to eliminate local heating. For filtration, a glass transfer pipette is recommended rather than the plastic one (usually made of PP). For brand-new PP products, it has been reported that a high quantity of MPs is attached to the surface due to the manufacturing process [135] so care must be taken to properly clean all products before testing begins. During the baby feeding bottle study, a repeated experiment was conducted for 21 days to investigated the MPs release level change during a specific time period. The preliminary results showed that during the 21-day period, the MPs release from the baby feeding bottle has a periodic fluctuation. In summary, the researcher must be vigilant to avoid any procedure that can adversely influence the measured levels of MP release from BFBs.

It should be noted that the protocol cannot account for all types of MP release. Due to the use of a filter with a 0.8 µm pore size, nanoparticles smaller than 0.8 µm are beyond the scope of this method. In addition, individual parents might not follow the WHO guidelines on which the protocol is based so that in real life the MPs level in prepared formula could be significantly different from that reported here.

Chapter 4

High levels of microparticles release from biodegradable polylactic acid paper cups compared with polyethylene-lined cups

4 High levels of microparticles release from biodegradable polylactic acid paper cups compared with polyethylene-lined cups

Abstract

Polylactic acid (PLA) is a popular replacement for conventional fossil-fuel based plastics products such as polyethylene (PE) due to its high biodegradability and recyclability. Previous studies confirmed that PLA microplastics (MPs) and PE MPs pose similar toxicity risks due to that MPs' risk is primarily attributed to physical and indirect nutritional effects. Surprisingly, despite the widespread use, there have been very few studies of microparticles released from daily products made of biodegradable materials. We investigated release levels from eight single-use paper cups (SUPCs) lined with PLA and PE film. Under typical hot-beverage preparation conditions, the total number of particles released from PLA SUPCs was 4.2 times higher than that from PE SUPCs, with total numbers of 180,000±31,000 and 43,000±10,000 particles per litre, respectively. 22,000±6,000 microplastic (MP) particles were released per litre from PLA, which was 3.6 times the level of MPs released from PE SUPCs. In addition, significant levels of cellulose microfibres were released from PLA SUPCs, with quantities of 38,000±31,000 microfibres per litre, while no such fibres were released from PE SUPCs. It should be noted that the levels of these particles may be underestimated due to the exclusion of nanoparticles (<0.8 μm, filter's pore size), unidentified microparticles with high fluorescence backgrounds and attachments between different types of particles. In summary, a proportionately higher level of release of additive microparticles together with the release of cellulose microparticles are the key difference between biodegradable plastics like PLA and the conventional plastic such as PE. Cellulose blending in manufacturing is likely related to higher MPs release from PLA cups than PE cups. Therefore, it is highly recommended to re-assess the manufacturing process, potential health and environmental risks of biodegradable plastic products as well as the formulation of related policies.

Keywords: Microplastics, biodegradable plastics, polylactic acid, polyethylene, single-used paper cups

4.1 Introduction

Microplastics (MPs) released from single-use plastic products are a concern due to the potential risk of high levels of ingestion by consumers [1, 148]. Extremely high levels of MPs and nanoplastics (10^{12} particles per litre, mean size of 30-80 nm) had been reported to be released from polyethylene (PE) lined SUPCs [148]. Conventional SUPCs are comprised of a paperboard substrate with a base weight of 150 to 350 g/m^2 and a plastic liner of 8 to 20 g/m^2 (thickness of around 50 μm) [149]. PE-lined SUPCs are one of the most widely used disposable cups, with daily consumption rates of 136 million, 27.4 million and 7.2 million in the US, China and Germany, respectively [150]. This huge consumption contributes significant plastic pollution to the environment. For instance, in Europe, disposable plastics make up around 70% of marine litter [151]. Currently, there is a significant drive to replace and/or reduce the usage of conventional PE-lined SUPCs. A global survey found that 75% of people across 28 countries agree with the ban of single-use plastics [152]. In the EU, SUPCs made of expanded polystyrene and oxo-degradable plastics will be banned by 2030 [153]. Similarly, the US Secretary of the Interior issued Secretary's Order 3407 in 2022, which aims to identify environmentally preferable alternatives to single-use plastic products, such as compostable or biodegradable materials. Clearly, there is a global drive to reduce and replace conventional SUPCs.

Biodegradable plastic (e.g., polylactic acid-PLA) based SUPCs are widely considered to be the solution [149]. It is believed that these biodegradable plastics will undergo decomposition into carbon dioxide (CO_2), biomass and water within a timescale that is short enough for the plastics not to be harmful to marine life and not to lead to an accumulation of plastics in the environment [30]. PLA has become increasingly used in single use products (e.g., disposable cutleries and paper cups) owing to its variety of advantages, such as being 100% biodegradable and compostable, safe and non-toxic, and good mechanical and physical properties [154]. To date, PLA-lined SUPCs are the most widely used compostable SUPCs [149]. Although having the similar market share of PLA and PBAT, PLA SUPCs are more competitive because of the high strength, long environmental resistance and low cost. The global market for PLA is expected to grow 26.6% from 2022 to 2030, while the market for PLA-lined SUPCs is growing 8.5% annually [155, 156].

However, recent studies have pointed out that biodegradable plastics can release high levels of MPs [100]. The toxicity risk from both conventional PE MPs and PLA MPs is primarily attributed to physical and indirect nutritional effects [157, 158]. It was reported that in marine animals MPs released from biodegradable plastics have similar toxicity to conventional MPs [14]. Furthermore, a previous report published by United Nations Environment Programme (UNEP) indicated that the by-products and litters produced during the degradation of biodegradable plastic can cause the similar or even worse risks as conventional plastics in biodiversity and ecosystem functions [159].

It is also well known that the degradation of biodegradable plastics such as PLA is highly temperature dependent. While at lower temperatures and sub-optimal conditions, PLA had no observable degradation even after 120 days [160], at a temperature of 57 ℃ it can degrade in composting or thermophilic anaerobic conditions in a few weeks [157]. Considering that the most common use of SUPCs is in hot beverage preparation, which involves high temperatures (up to around 100 ℃) and repeated mechanical shaking, we hypothesize that PLA SUPCs may release higher levels of MPs compared with conventional SUPCs. To test this hypothesis, three global leading brands of commercial PE lined SUPCs and five PLA lined SUPCs were selected to mimic hot beverage preparation and to record the levels of MPs release. Raman Spectroscopy combined with multiple other techniques (e.g., FTIR, SEM and AFM) was used to quantify and characterize the released particles. It was found that there were 22,000±6,000 MPs per litre released from PLA SUPCs, which was 3.6 times higher than that released from PE SUPCs. An in-situ ethanol treatment was also conducted to reveal the presence of additive microparticles and their influences on the physicochemical modification of microplastics. Additionally, significant levels of cellulose microfibres were also released from PLA SUPCs while there was no cellulose released from PE SUPCs. SEM images reveal that the new PLA SUPCs have fibre-like particles attached to the inner surface, while no obvious particles observed on the surface of PE SUPC. This study underscores the urgent need to re-assess the potential health and environmental risks of biodegradable plastic products and formulate related policies.

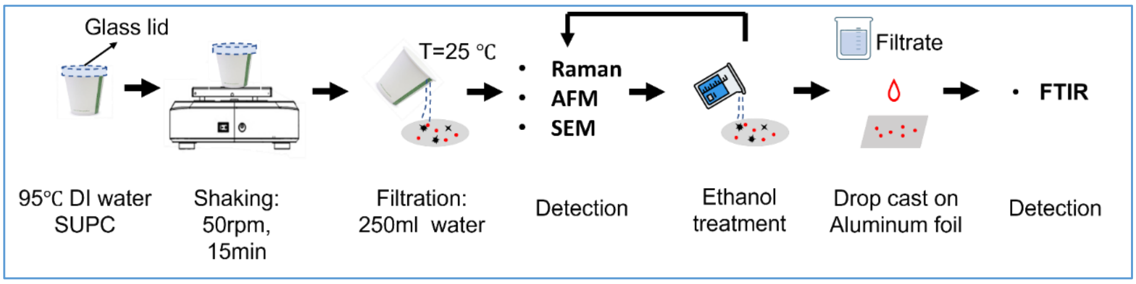


Fig 4. 1 The experiment protocol for preparing SUPC samples

4.2 Materials and methods

4.2.1 Particles release from conventional and compostable SUPCs

In this study, 3 brands of PE and 5 brands of PLA-lined SUPCs were tested for particle release. These PE and PLA SUPCs were purchased from 3 global leading brand retail shops in Dublin, and 5 different manufacturers, all of which have the PE or PLA liners on the inner side of the paper board. The manufacturers selected comply with the FSC certification and have plastic laminated lining thicknesses of 15-20 µm. The details of each cup are summarized in Table 4.1. Referring to established protocols [161, 162], our experiments were conducted to mimic hot beverage preparation and to test particle release levels (Fig 4.1). To eliminate potential interference of particles in the air, all the tested SUPCs were covered with glass lids. For each sample, a brand-new paper SUPC was directly filled with 250ml deionized (DI) water with the temperature of 95 ℃ (typical brewing temperature is 92 ℃-96 ℃ [163]). After covering the cup with a glass lid, it was placed on a shaker and slightly shaken for 15min at a speed of 50 r.p.m to mimic the shaking actions during holding, moving, and drinking activities of the user. The water samples were then allowed to naturally cool to room temperature (~ 30 mins) followed by filtering through a gold-coated polycarbonate membrane (APC, Germany Ltd) with a pore size of 0.8 µm using a vacuum pump. This filter membrane can effectively capture micro-sized particles that are large enough to avoid background spectra during Raman testing. The filter membrane was placed on a dry, clean glass petri dish to await further testing. Raman spectroscopy and AFM were used respectively to determine the type and morphology of the particles released from PE and PLA paper SUPCs. Finally, ImageJ 1.53t software was used to quantitatively determine the total number of released particles (PMPs) and the size distribution of the PLA SUPCs. Firstly, the function “Set scale” was applied in ImageJ to set the known distance of the image based on the screen size of Raman microscope (110 µm *86 µm). Subsequently, the particle number can be counted manually and the length and width of particles can be obtained with the function “Straight”. The experiment was repeated 5 times for each sample to obtain the average result (n=5). After the total number of released particles was determined, the filter membrane was exposed to 20 mL of ethanol to eliminate additive microparticles aggregated by small molecules. The filtrate was collected and drop casted onto aluminum foil and allowed to dry to analyze the dissolved additive particles using FTIR. After ethanol treatment, the filter membrane was dried naturally and the remaining insoluble particles were analyzed using Raman, SEM and AFM. The ethanol treatment can improve the accuracy of Raman detection of the MPs and cellulose microparticles by eliminating the additive particles. The additive-attached MPs and cellulose particles can be better detected. The filtration method and membrane selection in this study are beneficial to avoid potential contaminants and to facilitate Raman detection.

Table 4. 1 Summary of paper SUPCs used in this study

No	Lining Material	Colour	Volume	Weight (g)
1	**PE**	**White**	**12oz**	**14**
2	**PE**	**Red**	**12oz**	**17**
3	**PE**	**Red**	**12oz**	**23**
1	**PLA**	**White**	**12oz**	**10**
2	**PLA**	**Brown**	**12oz**	**14**
3	**PLA**	**Green**	**8oz**	**13**
4	**PLA**	**Orange**	**8oz**	**13**
5	**PLA**	**Brown**	**12oz**	**16**

4.2.2 Control experiments and contamination prevention

Glass beakers and DI water were used in control experiments following the same protocol (Fig 4.1). The DI water was sourced from a Veolia Ultrapure water system, which includes a Thermo Scientific™ Barnstead™ Nanopure unit with a 0.2 μm absolute final filter for dispensing and monitoring water quality. The DI water used in this study had a resistivity of 18.2 MΩ and a conductivity of 1.5 μs/cm. No PLA MPs were found in the control samples, while there were only 300 particles per litre detected from the control samples. These control sample levels were 2-3 orders of magnitude lower than that detected in PE and PLA SUPCs, confirming the reliability of our methods (Fig 4.2A). In addition, the control experiment was repeated using room temperature (around 25 °C) DI water instead of 95 °C DI water to investigate the influence of water temperature on particle release. Particle release levels from PE and PLA SUPCs under room temperature conditions were similar to the control sample (Fig. S4.1). To prevent potential contamination, all glassware was cleaned using an ultrasonic bath for 30 mins before each experiment. Lab coats and gloves were worn throughout, consistent with our reported protocol [162].

4.2.3 Microplastic identification

Multiple techniques were used to characterize the particles released from PE and PLA SUPCs. Gold-coated membrane filters that captured released particles were analyzed using Raman spectroscopy (Renishaw InVia Raman spectrometer) equipped with a 532nm laser (Coherent), a cooled charge-coupled device and a microscope (NT-MDT) with a 100× objective (Mitutoyo, M Plan Apo; numerical aperture = 7.0). The whole Raman system is controlled by the WiRE 3.4 (Renishaw) software. For each sample, four areas were randomly selected for detection at the top, bottom, left, and right sides on the filter membrane. Ten images (40 images in total) with the size of 110 μm *86 μm were captured in each area. The particles in each image were characterized using Raman spectroscopy to obtain the Raman spectra, followed by comparing with the Raman spectra of standard materials (e.g., PE, PLA and cellulose). Referring to previous studies [161, 164, 165], the hit quality index (HQI) value of 0.70 was set as the minimum threshold for identifying particle composition.

AFM system combined with Raman spectroscopy was also used to study the morphology of representative particles. After characterizing the released particles by Raman spectroscopy, the AFM system was used to generate surface morphology images of representative particles from which their shape and size was determined. Gwyddion 2.61 software was used to analyze the AFM image and 3D topographic images. To further investigate the topography of plastic lining and microparticles, scanning electron microscope (SEM) tests were performed. The obtained topography images through AFM and SEM help distinguish different types of microparticles, as well as indicating the surface features of released microparticles. In addition, the certain surface features may facilitate the absorption or aggregation of different microparticles, thereby affecting the behavior and ecological impact of microparticles in aquatic environments. Before the test, small pieces (around 0.5 cm^2) of PE and PLA cup were cut and cleaned using DI water. After drying, each sample was fixed onto a double-sided adhesive tab on an aluminum SEM stub. The fixed samples were sputter coated with a 10-nm Pt layer (Cressington 208HR) and analyzed using SEM (Zeiss Ultra Plus) with an acceleration voltage of 5 kV and magnifications ranging from 50× to 10,000×. Using the same method, microparticles on the filter surface were also analyzed.

4.2.4 In-situ test of PLA microplastics and additive microparticles

A previous study reported the ability of chemical additives to become leached from plastics and absorb onto MPs, and their potential implications for human health [166]. In a recent report we showed that additive microparticles can be dissolved by ethanol treatment [20, 167]. Therefore, an in-situ test was conducted to identify additive microparticles using this ethanol treatment in combination with Raman spectroscopy and AFM analysis. Selected particles were first identified by Raman spectroscopy and imaged by AFM. Subsequently, three drops of alcohol were sequentially dropped onto the filter membrane and the corresponding changes in the Raman spectra and morphology of the particles were recorded. Raman spectra and AFM images acquired after each drop of ethanol tracked the compositional and morphological changes of the selected particles.

4.3 Results

4.3.1 Total microparticles and additive microparticles released from PLA and PE SUPCs

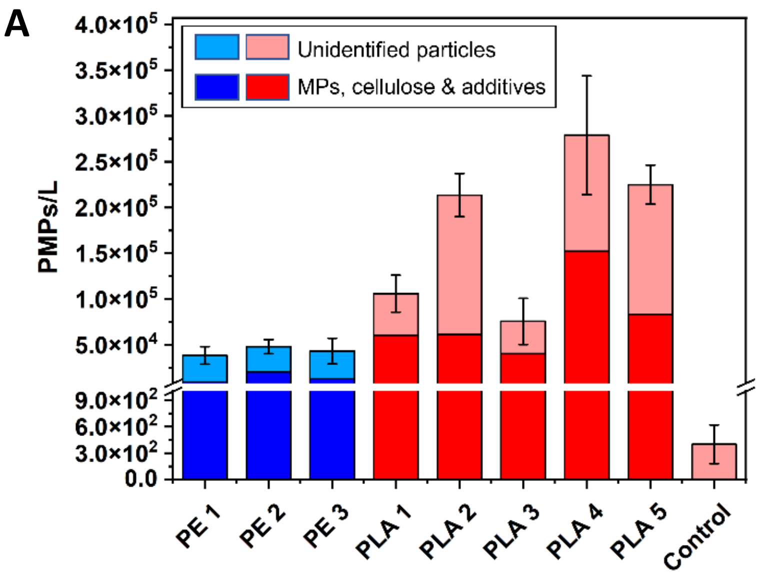
Following the hot beverage protocol described in Fig 4.1 and Method section, the total number of plastic-released microparticles (PMPs) captured on the filter membrane from PE and PLA SUPCs was determined without ethanol treatment. Fig 4.2A shows the total PMPs levels from PE samples 1-3 and PLA samples 1-5, respectively. On average, the total number of PMPs released from PLA SUPCs was 4.2 times higher than that of PE SUPCs, 180,000±31,000 PMPs/L and 43,000±10,000 PMPs/L, respectively. Fig 4.2B shows the types of released PMPs, include MPs, additive microparticles, cellulose microparticles and unidentified particles.

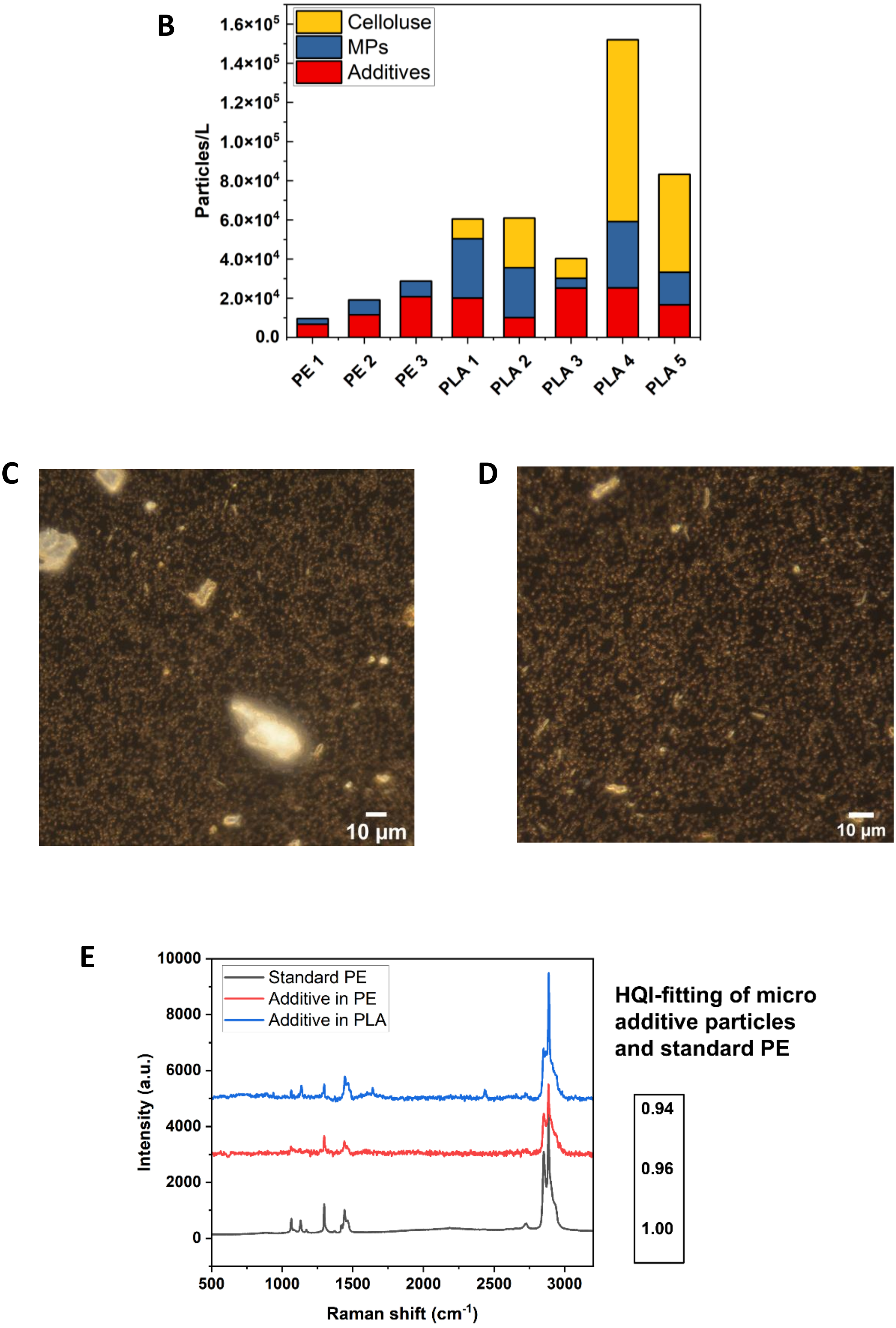
To identify the levels of released additive microparticles, the filter membrane was then placed onto a glass holder and rinsed using 20ml ethanol. Of the 180,000±31,000 particles released per litre, 20,000±6,000 were removed by ethanol and therefore identified as additive microparticles [20], accounting for around 11.1% of the total particles released from the SUPCs. Fig 4.2C-D shows two different locations randomly selected on the filter membrane of a PLA sample before and after ethanol treatment under an optical microscope (dark mode). By reviewing 10 random images captured using the microscope, the calculation result shows that 10.8%±5.2% of the total particles are removed following the ethanol treatment, consistent with our statistical analysis.

Following the same protocol, a large quantity of additive microparticles was also released from PE SUPCs. The total number of particles released was 43,000±10,000 PMPs/L, while the additive microparticles comprised 13,000±6,000 particles/L, accounting for 29.9% of the total.

The data was analyzed using one-way Analysis of Variance (ANOVA) through Original software. A p value of <0.05 showed the significant differences between the PMPs release from PE, PLA and control samples.

The filtrate containing the dissolved additive microparticles was then drop cast onto an aluminum (Al) foil and allowed to air dry. Raman testing found that the additive microparticles released from PE and PLA cups showed near identical spectra to that of PE, with HQIs of 0.94-0.96 compared with standard PE polymer (Fig 4.2E). In contrast to PE, the additive microparticles were ethanol soluble and are again attributed to the precipitation of small molecular additives incorporated into the polymer. FTIR analysis showed significant peaks around 1742 cm^{-1} and 2930 cm^{-1} (Fig 4.2F), which are associated with carbonyl groups (C=O) and hydrocarbon groups (C-H) [168]. Library fitting indicates that they are fatty acid mixtures, which are widely used to facilitate plastic processing and modify plastic properties [169].




B
Celloluse
MPs
Additives
Particles/L
1.6×10^5
1.4×10^5
1.2×10^5
1.0×10^5
8.0×10^4
6.0×10^4
4.0×10^4
2.0×10^4
0.0
PE 1
PE 2
PE 3
PLA 1
PLA 2
PLA 3
PLA 4
PLA 5
C
10 µm
D
10 µm
E
Standard PE
Additive in PE
Additive in PLA
Intensity (a.u.)
10000
8000
6000
4000
2000
0
500
1000
1500
2000
2500
3000
Raman shift (cm-1)
HQI-fitting of micro additive particles and standard PE
0.94
0.96
1.00

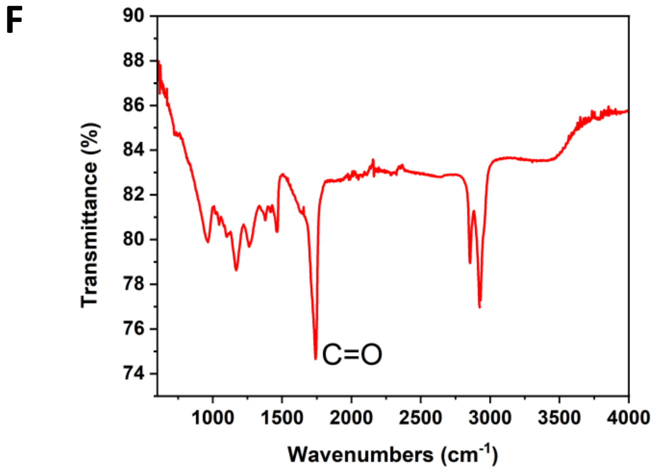
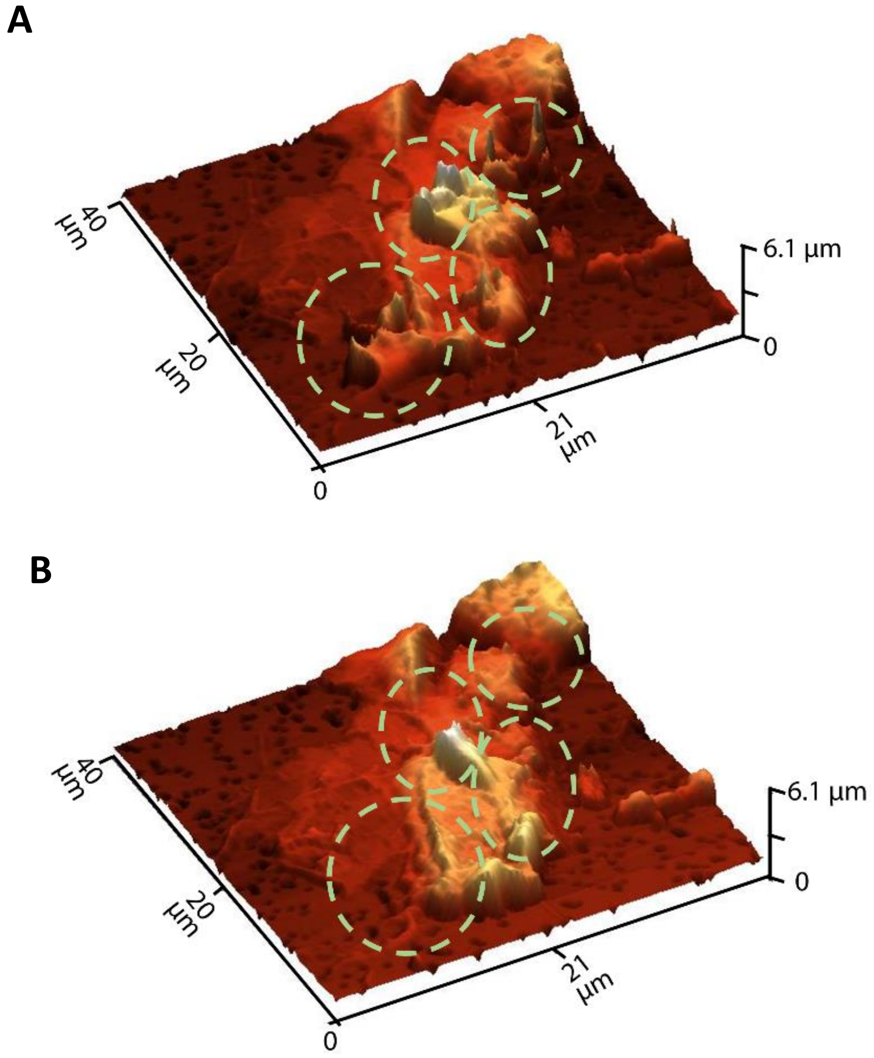


Fig 4. 2 Total particle numbers, types and characterizations from PE and PLA SUPCs before and after ethanol treatment, as well as filtration identification. (A) PMPs released from different brands of PE SUPCs (1-3) and PLA SUPCs (1-5) with DI water background. (B) Particle type and proportion released from PE and PLA SUPCs. Optical microscope image of PLA sample on a gold membrane (C) before and (D) after ethanol treatment (dark mode). (E) Raman spectrum of standard PE and additive microparticles released from PE and PLA SUPCs. (F) FTIR spectrum of filtrate from PLA sample on the aluminum foil.

An in-situ test was conducted to reveal the details of the process whereby ethanol dissolves and separates additives microparticles from MPs. Fig 4.3A shows a representative AFM image of micron-sized particles released from a PLA SUPC captured on the surface of the filter membrane. There are large irregular-shaped particles (green circles in Fig 4.3A) released from the PLA SUPC. The particles were then sequentially exposed to ethanol to further investigate their composition. After exposure to 1 drop of ethanol (Fig 4.3A-C), the particles' topography was substantially changed.

The bottom and right regions of the particles in Fig 4.3B disappeared after 1 drop of ethanol treatment (green circle regions in Fig 4.3B). Further ethanol treatment resulted in no significant change in the particle shape (Fig 4.3C). Raman analysis showed that the major spectral peaks associated with this green circle region originally located around 2880 cm^{-1} are shifted to around 2944 cm^{-1}, the latter being attributable to the asymmetric stretching of CH_3 of PLA polymer [170]. Meanwhile, the peak intensity at low wavelength regions around 1200-1500 cm^{-1} substantially decreased while the peak at 1600 cm^{-1} disappeared. Consistent with this topography change, further ethanol treatment resulted in no significant change in the Raman spectra (Fig 4.3D). HQI of the tested particles compared to standard PLA polymer increased from the original level of 0.27 to 0.84 and then 0.95 after 1 and 3 drops of ethanol treatment, respectively. Comparing the spectra changes of the PLA-MPs in the selected areas, it is reasonable to attribute the peak shift of around 2900 cm^{-1} and high intensity of peaks between 1200-1500 cm^{-1} to attached additives, which showed strong signals at 2880 and 1200-1500 cm^{-1}. A validation test using standard PLA particles confirmed that ethanol does not dissolve or damage PLA polymer (Fig S4.2A-B). Evidently, additive microparticles can be released alone or can attach to the surfaces of PLA MPs. Ethanol treatment can substantially benefit the separation and accurate identification of additive microparticles, enabling an accurate determination of the composition, shape and size of MPs. On the other hand, the ethanol rinse may reduce the size of the mixed particles containing polymers and additives, following some of the MPs captured on membrane filter, which will lead to an underestimation of MPs levels. In addition, the size distribution will be affected so that the true MPs distribution has been shifted to large values due to attached additives.



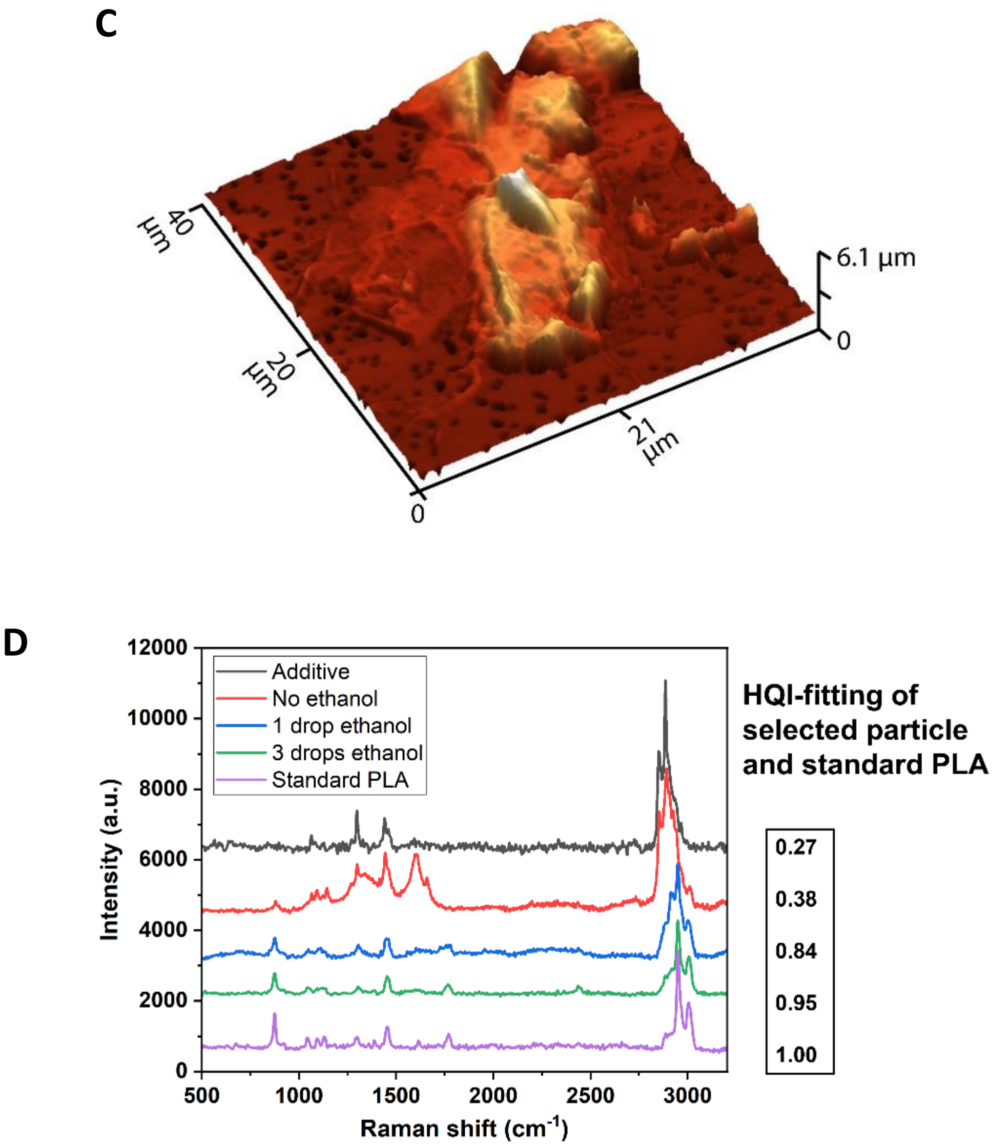


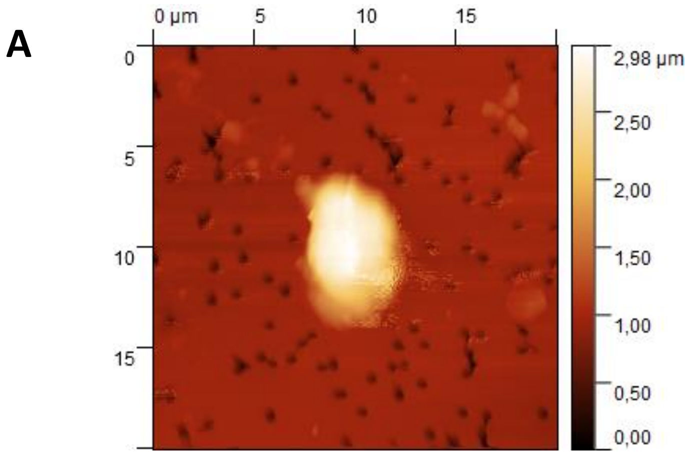
Fig 4. 3 In-situ AFM images and Raman spectra of ethanol treatment on selected particles. (A-C) AFM 3D in-situ images of additive microparticles attached on a PLA MP before and after 1 drop and 3 drops of ethanol. (D) Raman spectra of an in-situ particle before and after 1 drop and 3 drops of ethanol.

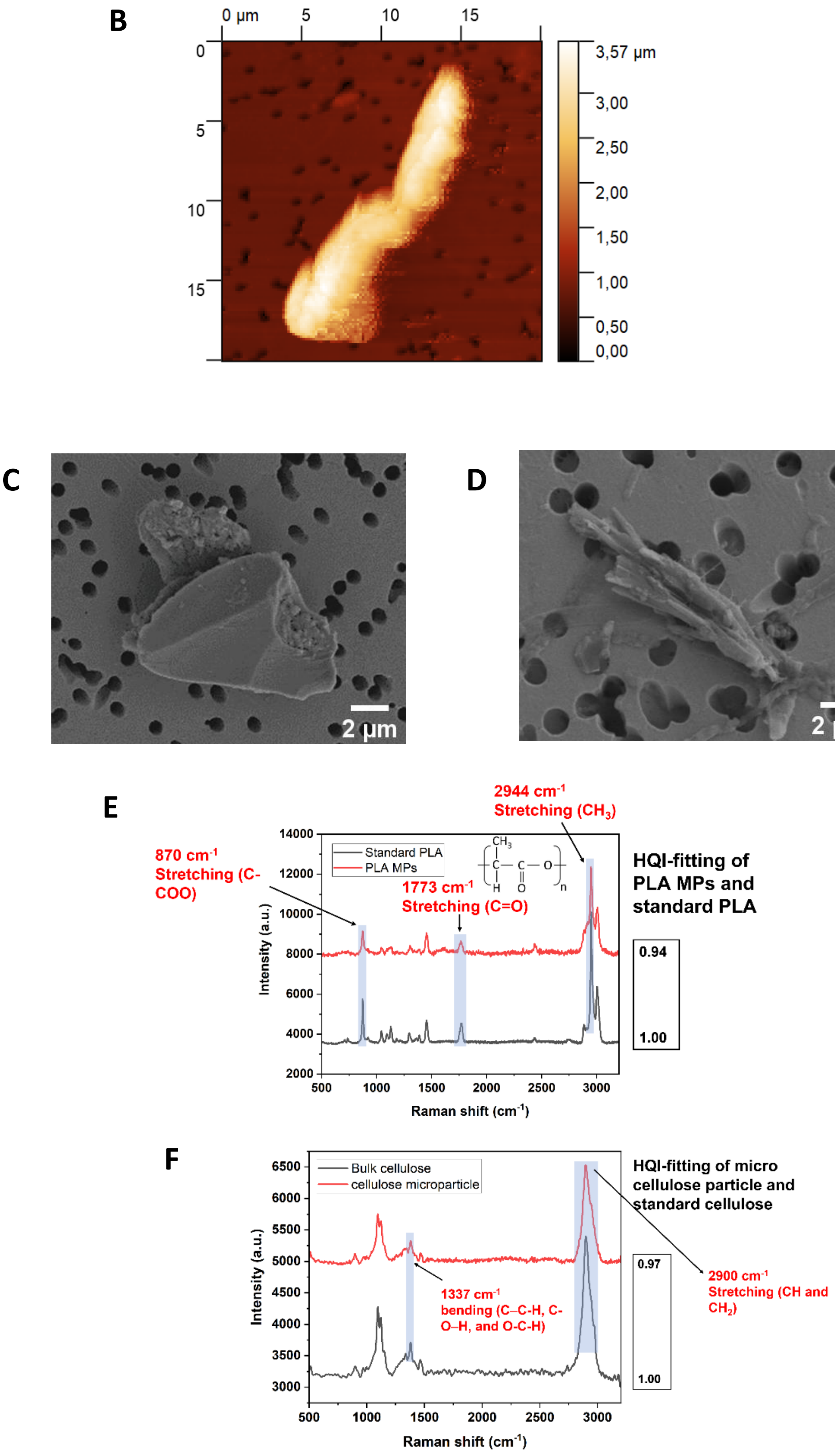
4.3.2 Microplastics and cellulose microparticles release from PLA and PE SUPCs

After ethanol treatment to eliminate additive microparticles, the remaining particles are counted and further analysed. Particles identified as MPs released from PLA ranged from 5,000 to 34,000 particles per litre depending on the brand of PLA SUPCs, with an average value of 22,000±6,000 MPs per litre. Representative AFM and SEM images (Fig 4.4A, 4.4C) reveal that around 79% of MPs are flake-like with a coarse surface and an average thickness of around one-tenth of the lateral dimensions. The surfaces of the particles are rich in bumps and valleys after ethanol treatment, so that MPs have a high capacity to adsorb and attach other pollutants, such as chemical additives and persistent organic pollutants (POPs). Fig 4.4E shows the Raman spectrum of a PLA MP had a HQI index of 0.94 compared with that of standard PLA material. The Raman peaks at around 1773 cm^{-1} and 2944 cm^{-1} are attributed to the stretching vibrations of C=O bond and CH_3 bond, respectively, which closely matches the Raman spectrum of standard PLA material [170, 171].

The level of MPs released from PLA SUPCs was 3.6 times higher than that of PE SUPCs. The PE MPs released from PE SUPCs ranged from 3,000 to 8,000 particles per litre, depending on brand, with an average value of 6,000±2,000 PE MPs per litre. Fig 4.4G shows the Raman spectra of a detected PE MP with a HQI value of around 0.97 compared with standard PE material.

Surprisingly, a significant quantity of cellulose microparticles/fibres were released from PLA SUPCs, ranging from 10,000 to 93,000 particles per litre, depending on the product brand. Fig. 4B, 4D present AFM and SEM images of some representative cellulose microparticles with a width of 2-4 µm, a length of 14-20 µm and a thickness of 2-4 µm. Clearly, these cellulose microparticles are elongated, irregular strips, with coarse surfaces similar to that of MPs, making them also a potential carrier of chemical additives and other contaminants. Fig 4.4F shows the Raman spectra of a cellulose microparticle and cellulose bulk material (fibre). The spectrum comprises two major groups of peaks at 1337 cm^{-1} and 2900 cm^{-1}, which are associated with HCO, HCC and HOC bending, and CH and CH_2 stretching, respectively, both recognised as signature features of the Raman spectrum of cellulose bulk material [172]. The release of cellulose microparticles/fibres was unique to the PLA SUPCs, no such fibres were released from PE SUPCs.




B
0 µm
5
10
15
0
5
10
15
3,57 µm
3,00
2,50
2,00
1,50
1,00
0,50
0,00
C
2 µm
D
2 µm
E
2944 cm-1
Stretching (CH3)
870 cm-1
Stretching (C-COO)
Standard PLA
PLA MPs
CH3
C
C
O
H
O
n
1773 cm-1
Stretching (C=O)
HQI-fitting of PLA MPs and standard PLA
0.94
1.00
14000
12000
10000
8000
6000
4000
2000
Intensity (a.u.)
500
1000
1500
2000
2500
3000
Raman shift (cm-1)
F
Bulk cellulose
cellulose microparticle
HQI-fitting of micro cellulose particle and standard cellulose
0.97
1.00
2900 cm-1
Stretching (CH and CH2)
1337 cm-1
bending (C–C-H, C-O–H, and O-C-H)
6500
6000
5500
5000
4500
4000
3500
3000
Intensity (a.u.)
500
1000
1500
2000
2500
3000
Raman shift (cm-1)

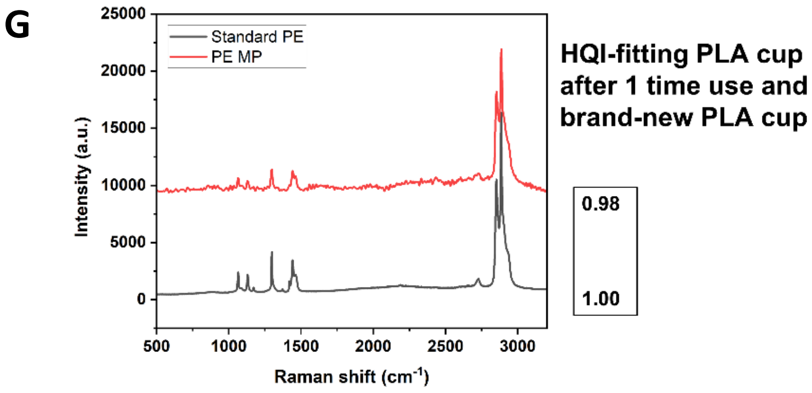


Fig 4. 4 Representative MPs and cellulose microparticles topographic images and Raman spectra. AFM image of (A) PLA MP and (B) cellulose microparticle on the gold filter membrane from PLA sample. SEM images of (C) PLA MP and (D) cellulose microparticles after ethanol treatment. (E) Raman spectra of standard PLA and PLA MPs released from PLA cup. (F) Raman spectra of bulk cellulose material (fibre) and cellulose microparticles released from PLA cup. (G) Raman spectra of standard PE (sheet) and PE MPs released from PE SUPC.

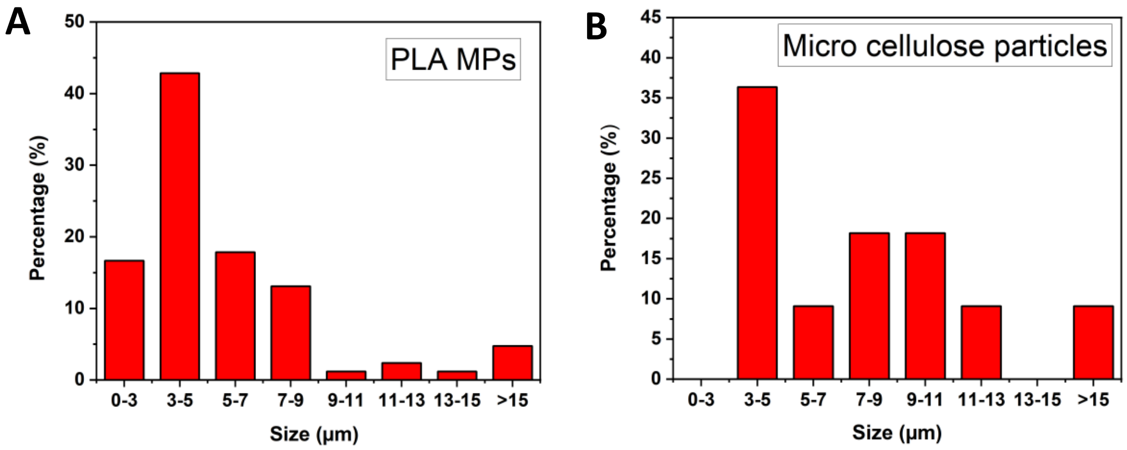


Fig 4. 5 Size distribution. Size distribution of (A) PLA MPs and (B) cellulose microfibres from PLA SUPC

4.3.3 Size distribution of MPs and cellulose microparticles from PE and PLA SUPCs

The size distribution of the particles released from PLA SUPCs following ethanol treatment was determined by optical microscopy (Fig 4.5A, obtained from 418 particles). It was found that 42.9% of the PLA MPs were 3-5 µm in size while 17.9% were in the 5-7 µm range. MPs over 15 µm accounted for less than 5% of the total MPs (Fig 4.5A). On the other hand, the size distribution of cellulose microparticles shows that particles between 3-5 µm account for the highest (36.4%) proportion of particles (Fig 4.5B). Particles in the 9-13 µm range accounted for the second largest number with 36.2%, slightly lower than that of 3-5 µm particles. No cellulose microparticles in the 1-3 µm range were detected (Fig 4.5B). However, it is important to note that due to the pore diameter of the membrane, particles with dimensions less than 1 µm are not detected in the present protocol.

The reported dimensions of the particles are based on the length of their longest axis. In general, the average size of the cellulose microparticles was twice than that of PLA MPs. This is in part due to shape anisotropy of cellulose microparticles that have length-to-width ratios of about 4 to 1 or even higher, while the PLA MPs were irregular ellipsoids with a smaller length-to-width ratio. The PE MPs have a similar size distribution as the PLA MPs, most of them are smaller than 20 µm (Fig S4.3), which is consistent with the previous report [134].

4.3.4 Lining morphology change of PLA and PE SUPCs

It is well known that micro- and nano-sized cellulose particles cannot be digested by humans but could still be a health risk [173-175]. For this reason, SEM testing was used to study the lining of SUPCs and identified the presence of large quantities of fibres attached or buried on/in the inner surface of PLA SUPCs (Fig 4.6A). Although it is difficult to perform in-situ chemical identification of fibers on PLA lining, the size and shape of these fibres perfectly match that of cellulose fibres released and captured on filter membrane, indicating that they are the source of microfibers. In contrast, there was no similar fibres observed on the surfaces of PE linings (Fig 4.6B). AFM images also confirmed these differences, with fibres protruding about 120 nm on the lining surface. After exposure to hot water many fibres were removed, and significant valleys approximately 90 nm in depth were observed. In contrast, only minor changes in surface roughness were observed in the case of PE. The manufacturing and compositional differences between PE and PLA linings is likely to be one of the reasons for the levels of cellulose microfibres released from PLA cups. Another potential source of cellulose fibres is added cellulose as additives or fillers in the bulk PLA materials, which is a common method to enhance the physiochemical properties of biodegradable plastics [63, 176].

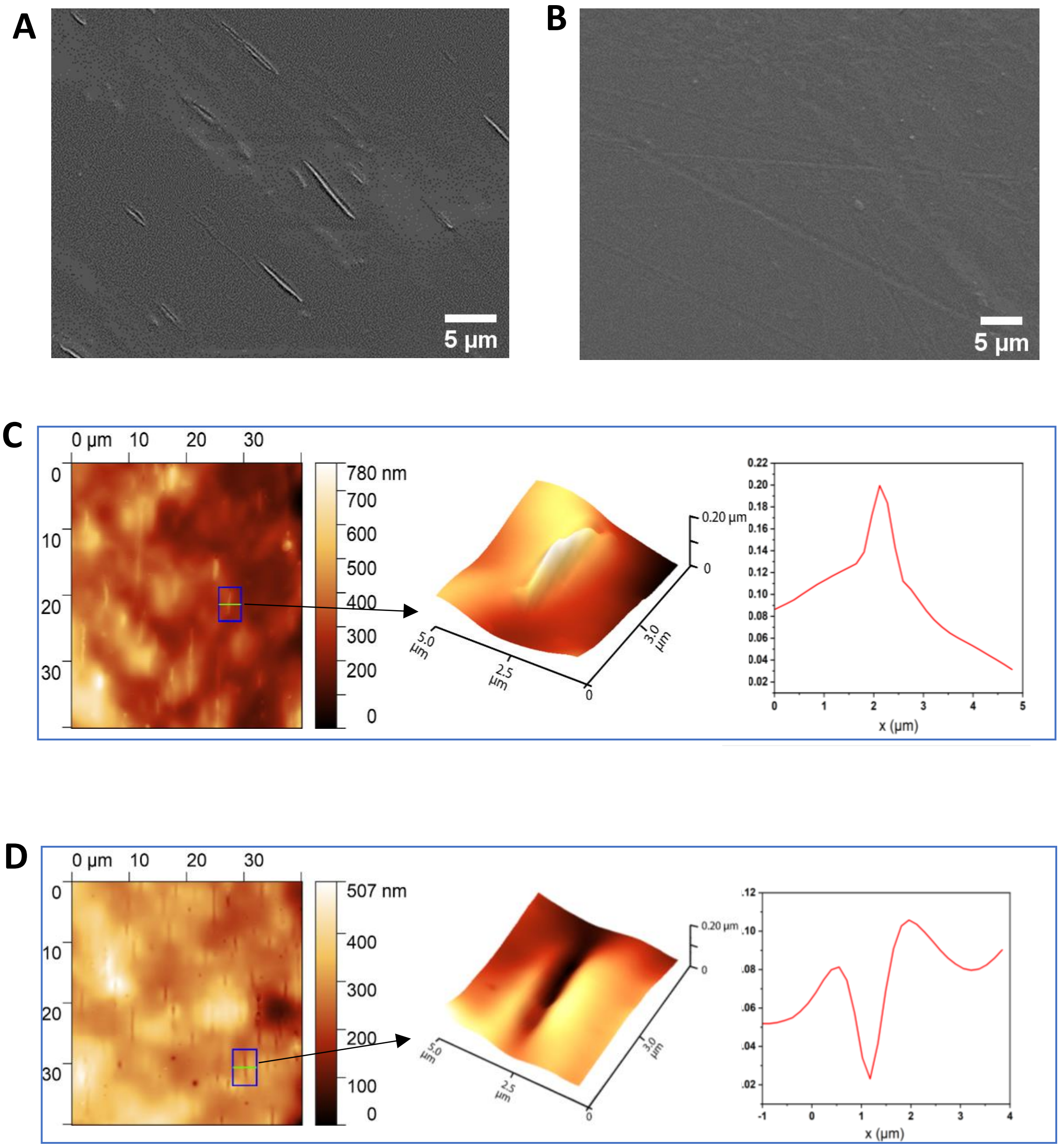


Fig 4. 6 Topographic images of the inner surface of PE and PLA SUPCs. SEM images of the inner surface of new (A) PLA and (B) PE SUPC. 2D AFM images, 3D images in blue box (~5× magnification of 2D image) and cross-section graphs of the inner surface of PLA SUPC (C) before and (D) after exposure to hot water

4.4 Discussion

4.4.1 Evaluation of MPs and cellulose exposure

Human exposure to MPs and cellulose release from SUPCs depends on consumption levels and future policies on single use plastics. It is projected that the demand and consumption of disposable coffee cups will continuously increase after the Covid pandemic [177]. The market share of the PLA SUPCs in 2021 was 3.0%, which will grow by 8.5% per year, assuming that the remaining SUPC market is PE SUPCs [178]. Based on this projection, MP exposure per capita will increase steadily, primarily due to the increased market share of PLA SUPCs and the higher levels of MPs released from PLA SUPCs compared to PE SUPCs. The emergence of new policies that prohibit the sale of PE SUPCs will, in the absence of innovative packaging technologies, result in increased per capita exposure to MPs. The increased human exposure may be a health threat given the widely reported adverse biological effects caused by PLA MPs [106, 179, 180] and micro- and nano- sized cellulose [173-175].

4.4.2 MPs release mechanism and mitigation

To date, little is known about the release mechanism of PLA MPs during daily use scenarios. Previous studies pointed out that PLA polymer has poor heat resistance. It tends to become soft and deforms at temperatures above 55-80℃ [181]. Given the high temperature (up to 95 ℃) and mechanical shaking during tea/coffee brewing and the drinking process, it is understandable that the damage to the inner film results in a high release level of PLA MPs. In addition, the blending of PLA with additional materials may also contribute to the high release. Pure PLA polymer is very brittle with poor thermal resistance and low ductility, which together does not meet the requirement for SUPCs. Generally, materials such as bio-oils are blended to improve the polymer properties [182, 183]. The addition of certain additives or fillers to PLA material can affect its morphology, which refers to the arrangement of the polymer chains in the material. For example, the addition of plasticizers can affect the crystallization bahaviour of the polymer, thereby promoting the formation of amorphous regions and changing the morphology of the material. The more heterogeneous or porous morphology could create more opportunities for MPs to be released as the material degrades or is subjected to abrasion. SEM images clearly showed the presence of blended cellulose fibers in the bulk inner lining of PLA SUPCs and from which cellulose particles/fibres were detected in

PLA water samples (Fig 4.6A). In contrast, there were no cellulose microparticles released from PE SUPCs (Fig 4.6B). From this point of view, based on the different material properties, the manufacturing method used for PE lining of SUPCs if applicable to PLA lining may avoid the excessive release of cellulose fibers.

To reduce the level of MPs release from paper SUPCs, it has been suggested to rinse the SUPC with hot water before use. The industry has also suggested a hot-water pre-treatment of these SUPCs before shipment to the consumers [184]. During the daily use of disposable coffee cups, some users may pre-wash them for hygiene reasons or for repeated use based on environmental and/or personal motivations. However, based on our results involving repeat use experiments, pre-wash and reuse of PLA SUPCs has little impact on the levels of MPs release (Fig S4.4A-B). A similar trend in MP release from reused PE SUPCs was observed in previous research [185]. Alternatively, reusable coffee cups (usually PP based) are recommended in coffee stores to reduce environmental impact. Life cycle assessment found that reusable coffee cups have to be used 150 times to reach a break-even point from the perspective of climate change and non-renewable energy [186]. Additionally, reusable plastic cups need repeated rinsing, disinfection and transport during travelling and represent a real exposure to MPs and additives due to the well-known degradation of the PP in presence of hot water [2]. A separate study compared different materials and types of cups and concluded that ceramic mugs have the lowest environmental impacts [187]. Clearly, the optimal mitigation method has yet to be identified and will likely require novel and cost-effective materials and product design.

4.4.3 Uncertainty of PLA SUPCs and MPs release

The methods of assessment and detection employed in this work have some limitations. In the assessment, the SUPCs market was split into two groups (i.e., PE and PLA lined SUPCs), similar to the current market situation. However, it can be substantially changed if there is a market breakthrough material in the future. In addition, ethanol treatment can effectively remove interference from the additives. Due to technical limitations, some additive-attached MPs were regarded as single particles in the counting process. This could lead to an underestimation of the total particle numbers. The potential interference from other additives is also expected, given that over 400 additives are used in plastic processing [188]. Based on our previous study, there is potential for an unintentional overestimation of MPs release levels in the previous research due to the similarity of the Raman spectra of PE MPs and chemical additives [20]. In addition, the filtration method in this study is unable to provide any information on the numbers of nanoparticles smaller than 0.8 µm, while previous studies using the drop cast method can capture all the particles in the water sample [189], but compositional characterization is very challenging. Consequently, differences in detection and testing methods could cause quantitative differences between the results of present and and previous studies. In addition, the unidentified particles detected in PE and PLA samples exhibited Raman spectra (Fig S4.5) with high fluorescence background, so that their composition could not be determined, but they had a similar size distribution to that of PE and PLA MPs, ranging in size from 3 µm to 15 µm.

It also should be noted that the PMPs release levels obtained using ultrapure DI water in this study may be different from the real beverages. For instance, the pH of DI water used in this study was 6.8-7, which is slightly higher than the actual hot drink (e.g. pH of coffee is around 5, pH of black tea is 4.9-5.5). A previous study has shown that additives particles are more likely to be released from plastics with low pH levels [190]. Additionally, the acidic condition can weaken the bonds between the microplastics and the product surface, making it easier for the microplastics to detach and be released [191]. On the contrary, impurities in hot beverages (organic compounds, caffeine and tennins) may attach to the surface of inner lining, preventing the direct contact between hot liquid and plastic lining and resulting in a slower speed of PMP release. Crucially, ultrapure DI water is a effective tool widely used in previous studies investigating microplastic release from daily use plastic products, such as PE lined cups [134, 148], disposable plastic food containers [135, 192] and teabags [136, 193]. These studies using DI water provide valuable information on MPs and additives released from our daily plastic products and the potential human exposure.

In addition, the pore size of filter membrane used in this study was 0.8µm, which indicates that the nano-sized microparticles and soluble additives can not be detected using current method. To further investigate the nano-sized microparticles, including MPs and soluble additives, the leachate from PLA cup after filtration can be collected and further identify using other technologies such as liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS), which have been proved to be the effective method for MPs leachate detection [194]. The soluble additives in the biodegradable plastic leachate that come into contact with food or are used in medical applications can migrate into the surrounding environment, therefore leading to another health concern. Further research should be conducted to understand the potential health implications.

4.5 Conclusion

This study takes the biodegradable PLA SUPC as an example, compared with the traditional plastic PE SUPCs, investigating the total particle release level of biodegradable and conventional plastic products by mimicking hot beverage preparation and drinking protocol. When exposed to 95℃ water, PLA SUPCs can release up to 4.2 times as many microparticles compared to traditional SUPCs, including a large proportion of additive microparticles. Ethanol treatment was verified as an effective method to separate MPs and additive microparticles. It should be noted that the wide existence of additive-attached MPs and cellulose microparticles could lead to an underestimation of the total particle numbers. Additionally, since 0.8-μm pore size membrane filter was used in the study, nano-sized particles are not captured in our measurement, which substantially contributes to the underestimation of the total particle numbers. Crucially, the approach described here can be used to investigate MPs and additives released from other biodegradable plastic products and assess the potential MPs exposure trends.

This paper has provided an in-depth investigation on degradable plastics with an investigative insight on their candidature to be the best alternative to traditional plastics and the future in terms of new environmentally friendly materials. Clearly, it is too early to conclude that biodegradable SUPCs are a better alternative than conventional SUPCs from both human and environmental health perspectives. Taken together, there is still a large research gap on the potential harm of biodegradable MPs and additives to human health, which should be an important research direction in the future.

4.6 Supplementary Information

For High levels of microparticles release from biodegradable poly(lactic) acid paper cups compared with polyethylene-lined cups

The PMPs release of PE and PLA SUPCs under room temperature was determined following the hot-beverage preparation condition.

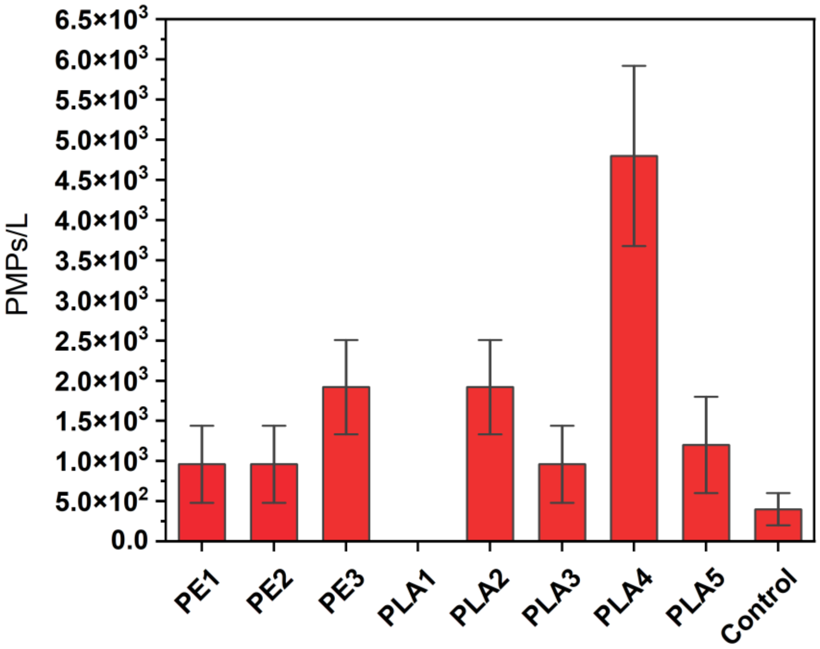
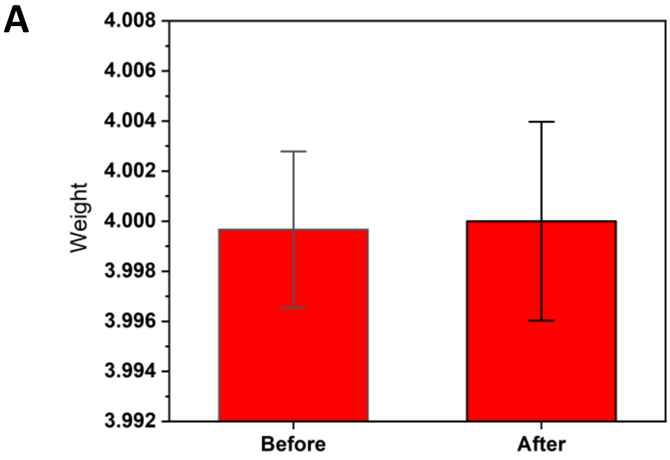


Fig S4. 1 PMP release of SUCPs under room temperature. PMP release of PE (1-3), PLA (1-5) and DI water background under room temperature.

The validation test using 4.00g±0.002g of standard PLA pellets (with the diameter of 3-5mm) confirmed that ethanol will not dissolve or damage PLA polymer. The pellets were settled in a glass beaker soaked in 20ml ethanol for 3 days. Fig S4.2A-B showed the pellets mass change and One-Way ANOVA analysis of the PLA pellets mass before and after soaking in the ethanol. The results indicated that at the 0.05 level, the population means are not significantly different.



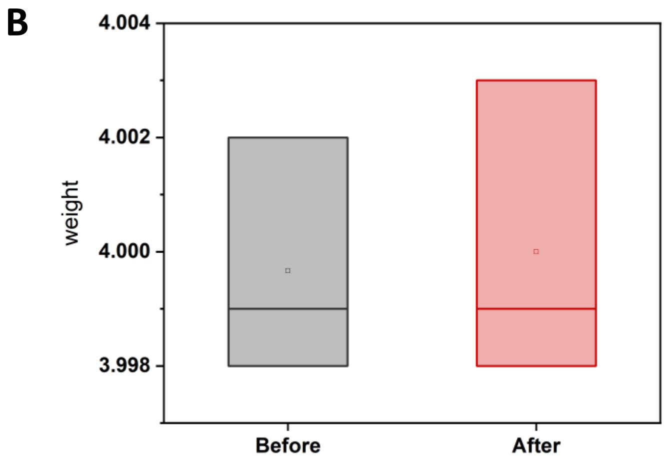


Fig S4. 2 PLA pellet mass change of soaking in ethanol. (A) Mass of PLA pellets before and after soaking in the ethanol. (B) ANOVA analysis of mass of PLA pellet before and after soaking in ethanol.

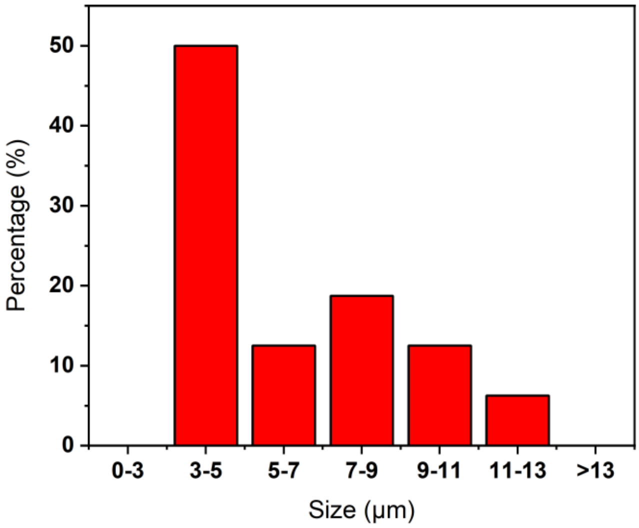
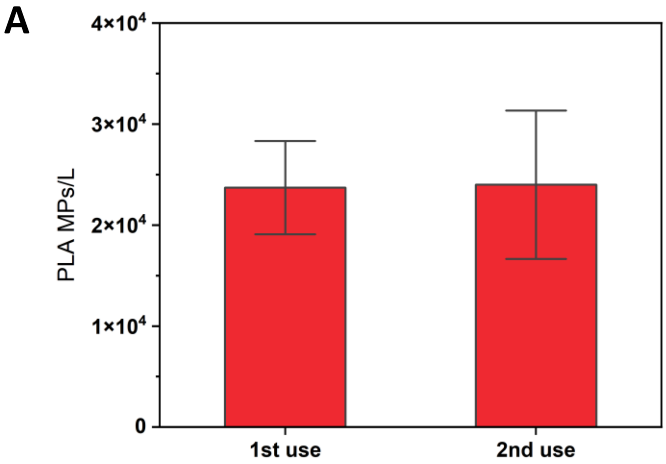


Fig S4. 3 PE MPs size distribution before ethanol treatment



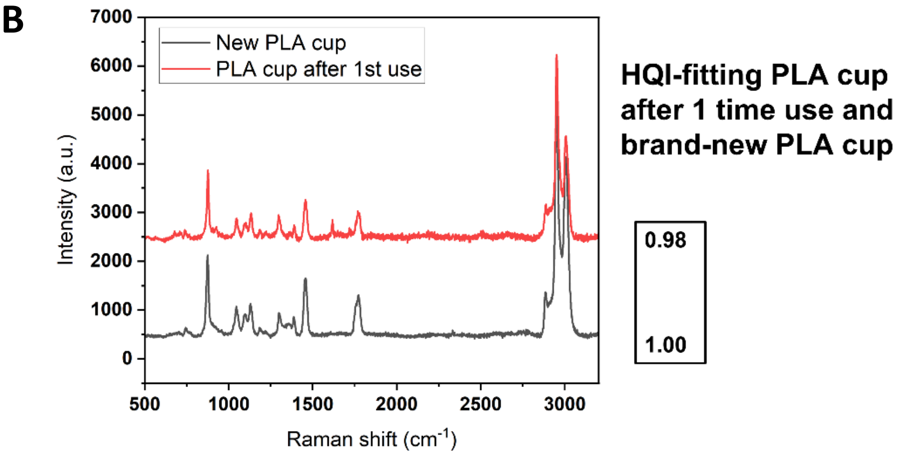


Fig S4. 4 MPs release of repeat use of PLA SUPCs. (A) MPs release of PLA SUPCs after 1st and 2nd use. (B) Raman spectra of PLA cup before and after 1st time use.

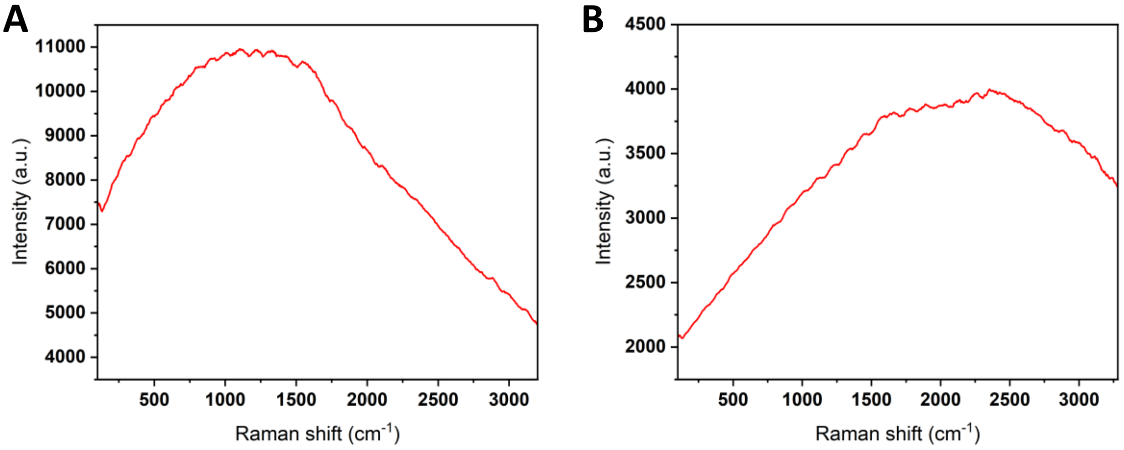


Fig S4. 5 Raman spectra of unidentified particles. Raman spectrum of unidentified particles in (A) PE SUPCs and (B) PLA SUPCs.

Chapter 5

Alkaline decomposition and composition analysis of different forms of polylactic acid material

5 Alkaline decomposition and composition analysis of different forms of polylactic acid material

Abstract

Biodegradable plastics have been regarded as the ideal alternative of conventional plastics especially in the application of food packaging and daily necessities. However, studies have found evidences that the biodegradable plastic product is likely to release high level of microparticles including microplastics (MPs), additive microparticles and other impurities. Chemical additives are normally added in the biodegradable polymers during the production and manufacture in order to improve the mechanical properties of such materials. The addition of organic and inorganic compounds depends on the polymer properties and the purpose of application, which can be released during the degradation process under different conditions. Therefore, this study selected 4 different forms of polylactic acid (PLA) products including standard PLA pellets, PLA straws, PLA spoons and PLA single-used paper cups, determined the total number of plastic-released microparticles (PMPs) through a simulation experiment of exposing in 95°C deionized water (DI water), coupled with chemical identification of bulk material and released particles using Raman spectroscopy. Additionally, the composition analysis of PLA products was also examined under an alkaline (NaOH) condition. The results showed that when exposed into hot water, standard PLA pellets presented a highest PMPs release level of 515.83±38.21 particles/cm^2, followed by PLA-lined disposable paper cup with 373.63±41.25 PMPs/cm^2. The PLA straw showed lowest release potential with the number of 101.89±62.4 PMPs/cm^2. During the alkaline decomposition process, the standard PLA pellet showed the highest alkaline resistance with no visible change before and after soaked into NaOH solution for 3 days, while the PLA lining of the paper cup broke into small pieces in the first 10 minute.

Key words: Biodegradable plastic, microplastic, additives, alkaline decomposition, mechanism

5.1 Introduction

The increasing public awareness of plastic pollution and environmental protection has led to a growing focus on biodegradable materials. These materials are receiving more attention as they offer potential solutions to mitigate the negative impacts of conventional plastics on the environment. Biodegradable materials have the ability to break down naturally through biological processes, reducing their persistence in the environment and minimizing their contribution to pollution [194]. Biodegradable plastic products have gained recognition as potential alternatives to conventional plastic products [195]. As a result, research efforts have been directed towards studying the physicochemical properties and characterizations of biodegradable materials. In our previous study, we conducted an investigation comparing the release of PMPs from compostable paper cups made of PLA and polyethylene (PE) materials. Our findings revealed that the PLA-based compostable paper cup released a significantly higher amount of PMPs compared to the PE-based paper cup [12]. In a recent study, it was found that approximately 1 million nano-sized plastic particles were released from a teabag made of polylactic acid (PLA). Moreover, the study suggested that the nano-sized PLA particles could cause slight disruptions in the body's barrier systems [13]. Additionally, a study has indicated that biodegradable MPs exhibit comparable or even higher toxicity to marine algae when compared to conventional MPs. This finding highlights the potential adverse effects of biodegradable MPs on marine ecosystems [14]. In the study conducted by Almroth et al., the biological effects of both biodegradable and conventional MPs were investigated by examining the leachate from various types of plastic disposable cups and lids. The study focused on polypropylene (PP) cups, polystyrene (PS) lids, and PLA-lined paper cups. The research findings indicated that all these different types of MPs exhibited negative effects on chironomids, which are aquatic insects commonly used as indicators of environmental health [196]. In another toxicological study, it was reported that the compostable PLA cup caused severe brain and gill damage in goldfish [197].

In addition to the release of micro and nano-sized plastic particles, it is worth noting that biodegradable plastics often contain a higher amount of additives during their production and manufacturing processes. These additives are incorporated into the materials to enhance their properties and usability [64]. During the decomposition process of biodegradable polymers, there is a potential for additives and other impurities to be released from these materials. This has led to numerous studies exploring the presence and behavior of additive compounds in biodegradable polymers. For instance, Cui et al. conducted a study where they developed a systematic method to detect organic additives used in poly(butylene adipate-co-terephthalate) (PBAT) covering films by using gas chromatography-mass spectrometry (GC-MS) as the analytical technique [81]. Furthermore, studies have shown that a wide range of additives, numbering over 300, are commonly added to single-use biodegradable plastic products [62]. Indeed, the determination of various functional additive substances added in biodegradable materials is of utmost importance for different applications. This study takes four different forms of PLA materials as examples, including standard PLA pellets, PLA-lined disposable paper cups, PLA straws and PLA spoons, firstly determining the PMPs release of these products using Raman spectroscopy. Sequentially, NaOH solution was applied as an alkaline solution to characterize the chemical composition of four PLA products. In addition, in-situ images of PLA cup lining decomposition process were obtained to present the morphology change of PLA lining. Afterwards, the residual substances of four PLA products were collected and identified using Fourier-transform infrared spectroscopy (FT-IR) as well as the recording of dry weight of four PLA products before and after alkaline decomposition. The study demonstrated that the release of PMPs from biodegradable plastics is influenced by the form of the material, specifically the mechanical forces exerted on it. It was observed that higher mechanical forces resulted in a greater number of PMPs being released. The alkaline decomposition results indicated that the addition of various additives lead to different decomposition behavior in a short timescale, which could accelerate the degradation of biodegradable polymers thereby leading to a higher release of organic compounds in marine and terroir environment. Indeed, while there has been increasing research on the release of MPs from biodegradable materials, there are still significant gaps in our understanding of the underlying mechanisms as well as the biological and health implications associated with these biodegradable MPs. Further investigation into the degradation mechanisms and comprehensive toxicological studies are crucial to fill these knowledge gaps.

5.2 Materials and methods

5.2.1 PMPs release from different forms of PLA products and MPs identification

To avoid any potential contamination, glass beakers and DI water were used for all experiments in this study. All the glassware were deep cleaned using ultrasonic bath for 30min and rinsed by DI water for three times before every experiment. The cotton lab coat and gloves were worn for the entire time of all experiments.

Four different forms of PLA products are used in this study including standard PLA pellets, PLA-lined disposable paper cups, PLA straws and PLA spoons. The standard PLA pellets are purchased from NatureWorks from USA, while other 3 products are ordered from a commercial shopping website. The sample preparation and testing process were followed by a MPs sampling and testing method with minor modification [2, 198].

Fig 5.1 illustrates the experimental protocol employed for the preparation of samples, simulating the rigorous conditions encountered during the daily usage of PLA products. The PLA sample was soaked into 95℃ DI water followed by shaking with the speed of 50 r.p.m. for 15min. Subsequently, the water sample was naturally cooled until room temperature followed by filtering through a gold-coated polycarbonate membrane (APC, Germany Ltd) with the pore size of 0.8µm. Afterwards, the filter membrane was stock into a glass petri dish waiting for Raman identification.

Raman spectroscopy (Renishaw InVia Raman spectrometer) equipped with a 532nm laser (Coherent) and a cooled charge-coupled device and a microscope (NT-MDT) with a 100× objective (Mitutoyo, M Plan Apo; numerical aperture = 7.0) was used to chemical identify the released particles. Sequentially, the number of released particles were counted and calculated using ImageJ software.

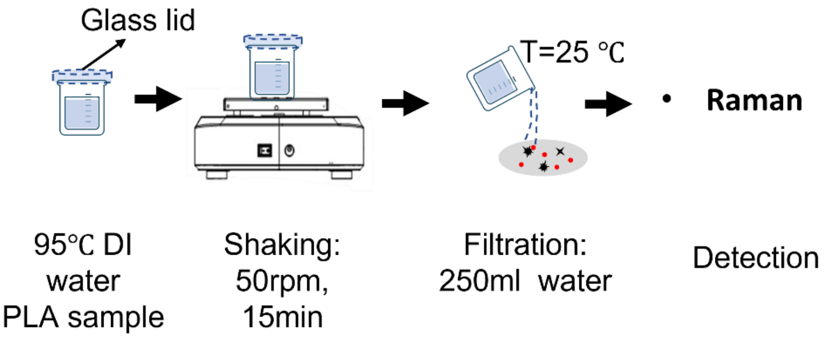


Fig 5. 1 Experiment protocol for PLA sample preparation

5.2.2 Bulk material characterization of PLA products

Multiple instruments were applied to determine the characterization of bulk material of PLA products. Firstly, the bulk material of four PLA products were analyzed by Raman spectroscopy to obtain the Raman spectra of original materials. Subsequently, four PLA samples were cut into small pieces and identified by solid state ^{13}C-nuclear magnetic resonance (NMR) and thermogravimetric analysis (TGA) following the method described by Anton et al. and Sheng et al. [199, 200]. The PLA pellets were regarded as the standard PLA material to compare the obtained spectra and curves. The TGA testing was progressed with the heating temperature from 30℃ to 900℃ at 10℃/min in the nitrogen atmosphere. The Raman and NMR spectra and TGA curves were plotted in Fig 5.3A-3C.

5.2.3 Alkaline decomposition of PLA products

The alkaline digestion experiments involved the utilization of the identical set of four PLA samples. These samples were subjected to the digestion process under controlled conditions to evaluate their breakdown and composition analysis. The consistent use of the same four PLA samples ensured reliability and comparability of the obtained results throughout the basic digestion experiments. 0.1M NaOH solution was prepared using DI water and applied for the decomposition experiment. For this experiment, dry weight was recorded before and after decomposition to each PLA product (Table 5.1). It is important to highlight that the PLA lining of the paper cup was delicately separated and peeled from the paper board prior to the experiment. Subsequently, four PLA samples were immersed in the NaOH solution at room temperature, allowing for subsequent observation and analysis of their behaviour and characteristics. Among the four PLA samples, PLA paper cup lining was taken as an example to conduct an in-situ decomposition experiment. An OLYMPUS DSX1000 Optical microscope coupled with MPlanFL N 10x /0.25 BD P ∞/-/OFN26.5 camera lens was used to observe the decomposition process and obtain the optical images of PLA lining after specific time periods.

5.2.4 Residue identification using Fourier-transform infrared spectroscopy (FTIR) after alkaline decomposition

Four sets of PLA samples were digested using NaOH solution for 3 days. After decomposition, the NaOH was filtered using the same gold-coated filter membrane and the residual substances was remained to be air dried and characterized using FTIR (PerkinElmer). FTIR is particularly sensitive to the presence of specific functional groups in molecules. This makes it well-suited for identifying chemical bonds and molecular structures associated with functional groups. To fully collect the residues, DI water was used to rinse the digestion tube during the filtration process. The dry weight before and after digestion was record in Table 1. The FTIR spectra were analyzed using PerkinElmer Spectrum IR software Version 10.6.2 to match the most ten similar substances from the source spectra.

Table 5. 1 Dry weight of 4 sets of PLA samples before and after alkaline decomposition

	Standard PLA pellet	**PLA cup lining**	**PLA straw**	**PLA spoon**

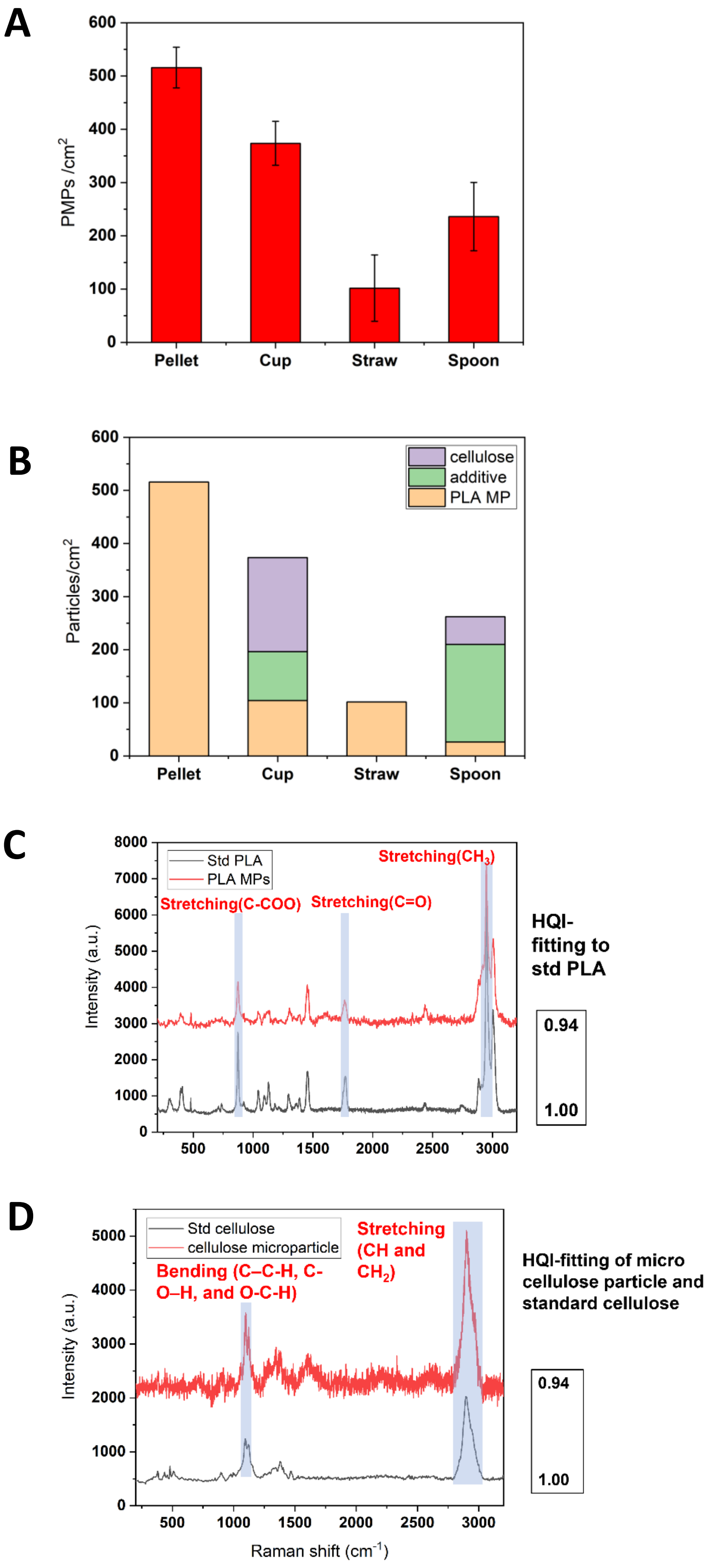
Before	0.0530g ± 0.002g	0.0267g ± 0.0003g	0.1448±0.0001g	0.1298g± 0.0002g
After	0.0516g ± 0.0002g	0.0017g ± 0.0001g	0.0317g±0.0002g	0.0598g± 0.0001g
Mass reduction	2.6%	93.6%	78.1%	53.9%

5.3 Results

5.3.1 Particle release of different forms of PLA products

Following the experiment protocol in Fig 5.1, the total microparticles release was quantitative and qualitative confirmed. Raman spectroscopy was used to chemical identify the particle types, followed by the number calculation using ImageJ software. The Raman spectra of bulk material and released microparticles were obtained and compared with the standard spectra through hit quality index (HQI) with a minimum similarity threshold of 0.7 [2, 12]. Consistent with our previous findings reported in [12], the Raman spectroscopy results revealed that, in addition to the presence of MPs, both additive and cellulose microparticles were detected in PLA-lined paper cups and PLA spoons under 95℃, whereas these particles were not detected in the other two PLA products. Fig 5.2B presents the Raman spectra of MPs, additive microparticles and cellulose microparticle, alongside a comparison with standard reference materials. Based on the calculation results, it was determined that the standard PLA pellets exhibited the highest level of microparticle release among the four PLA products, with a recorded count of 515.83±38.21 particles/cm^2, followed by PLA-lined paper cup which released 373.63±41.25 PMPs/cm^2. PLA straw showed a lowest PMPs release number of 101.89±62.4 PMPs/cm^2. The analysis revealed that 47.5% of the detected microparticles in the PLA cup lining were identified as cellulose microparticles. Similarly, in the case of PLA spoons, cellulose microparticles accounted for 22.2% of the total PMPs detected. On the other hand, the additive microparticles released from the PLA cup lining constituted 24.6% of the total PMPs, while in the PLA spoons, additive microparticles accounted for 66.7% of the total PMPs.

The Raman spectroscopy analysis of the released PLA MPs from the four different PLA products exhibited similar Raman spectra, indicating the presence of typical PLA compounds (Fig 5.2C). The Raman peaks observed in the spectra of PLA MPs, such as around 870 cm^{-1}, 1773 cm^{-1}, and 2944 cm^{-1}, closely aligned with the characteristic stretching vibrations of C-COO, C=H, and CH_3 functional groups, respectively. These peaks closely matched those observed in the Raman spectrum of standard PLA material, providing evidence for the presence of PLA MPs in the released particles [170]. In addition, the Raman spectra of cellulose microparticles released from both the PLA cup lining and PLA spoon presented similar characteristics (Fig 5.2D). The spectra revealed distinct peaks corresponding to C–C-H, C-O–H, and O-C-H bending vibrations, as well as CH and CH_2 stretching vibrations, observed at approximately 1337 cm^{-1} and 2900 cm^{-1}, respectively. These spectral features are indicative of the presence of cellulose in the released microparticles from both the PLA cup lining and PLA spoon. Moreover, the Raman spectra of additive microparticles released from the PLA cup lining and PLA spoon were compared with the spectra of additives available in a public library. The comparison revealed close matches between the additive spectra and the microparticles, suggesting the presence of specific additive types. In particular, the additive microparticles from the PLA cup lining closely resembled the Raman spectra of Oleamide with the HQI-fitting value of 0.96, while those from the PLA spoon showed similarities to the Raman spectra of Stearamide with an HQI value of 0.94 (Fig 5.2E-F). The results are consistent with a previous study that there were over 300 different types of additives added into biodegradable plastic products in order to improve the properties of PLA materials [62].


A
PMPs /cm^2
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HQI-fitting to std PLA
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4000
5000
Std cellulose
cellulose microparticle
Bending (C–C-H, C-O–H, and O-C-H)
Stretching (CH and CH$_2$)
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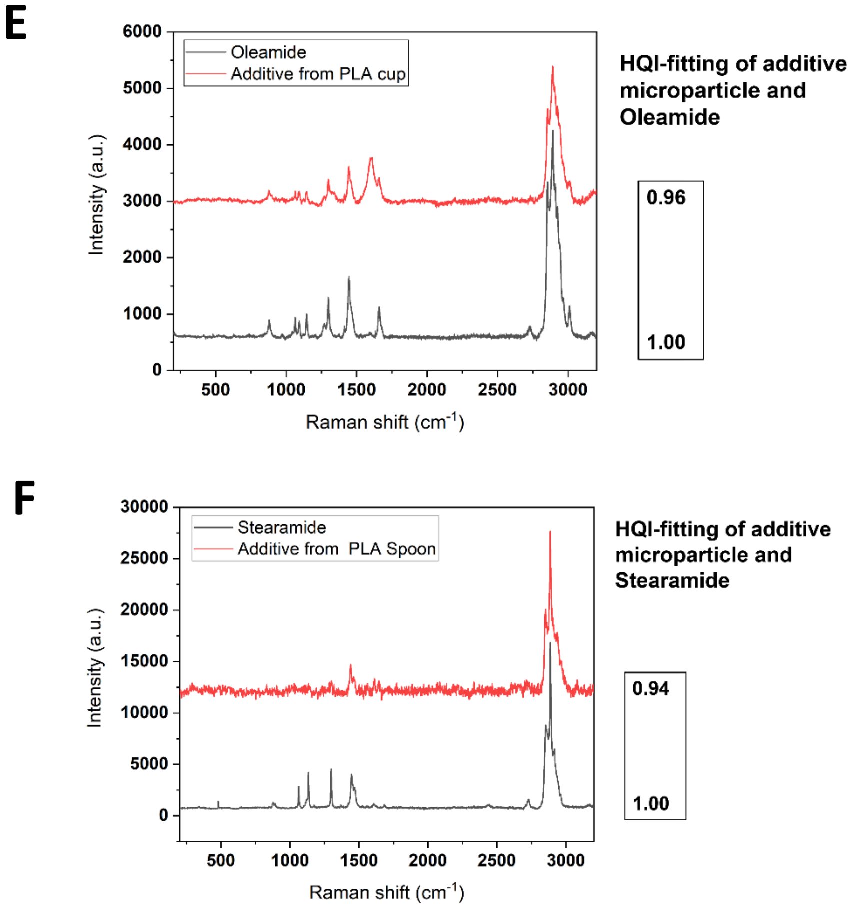

Fig 5. 2 PMPs numbers, types and chemical identification. (A) PMPs numbers released from 4 PLA products. (B) PMPs types released from 4 PLA products. (C) Raman spectrum of PLA MP released from PLA products and standard PLA pellet. (D) Raman spectrum of cellulose microparticle released from PLA cup lining and spoon and standard cellulose. (E) Raman spectrum of additive microparticle released from PLA cup lining and Oleamide bulk material. (F) Raman spectrum of additive microparticle released from PLA spoon and Stearamide bulk material.

5.3.2 Chemical characterization of different forms of PLA products

The bulk material of PLA products were tested using multiple technologies including Raman spectroscopy, Nuclear magnetic resonance (NMR), and Thermogravimetric Analysis (TGA). Both Raman and NMR spectra indicated that the chemical compound of PLA straw is different from other three forms of PLA products. The Raman spectrum of the bulk material from PLA straw exhibited a prominent peak at approximately 1614 cm-1 (Fig 5.3A). Additionally, the NMR spectrum of the PLA straw bulk material displayed distinct peaks at around 26-29 ppm, 65 ppm, and 173 ppm (Fig 5.3B). These peaks suggest the potential presence of additional organic compounds in the raw material used for manufacturing PLA straws. The TGA curves of the four PLA products illustrated a trend where, under a nitrogen atmosphere, the mass loss of the PLA materials initiated at temperatures ranging from 300°C to 330°C. Subsequently, the highest mass loss occurred within the temperature range of 380°C to 400°C (Fig 3C). Following this peak mass loss, the mass remained relatively constant until reaching temperatures as high as 900°C.

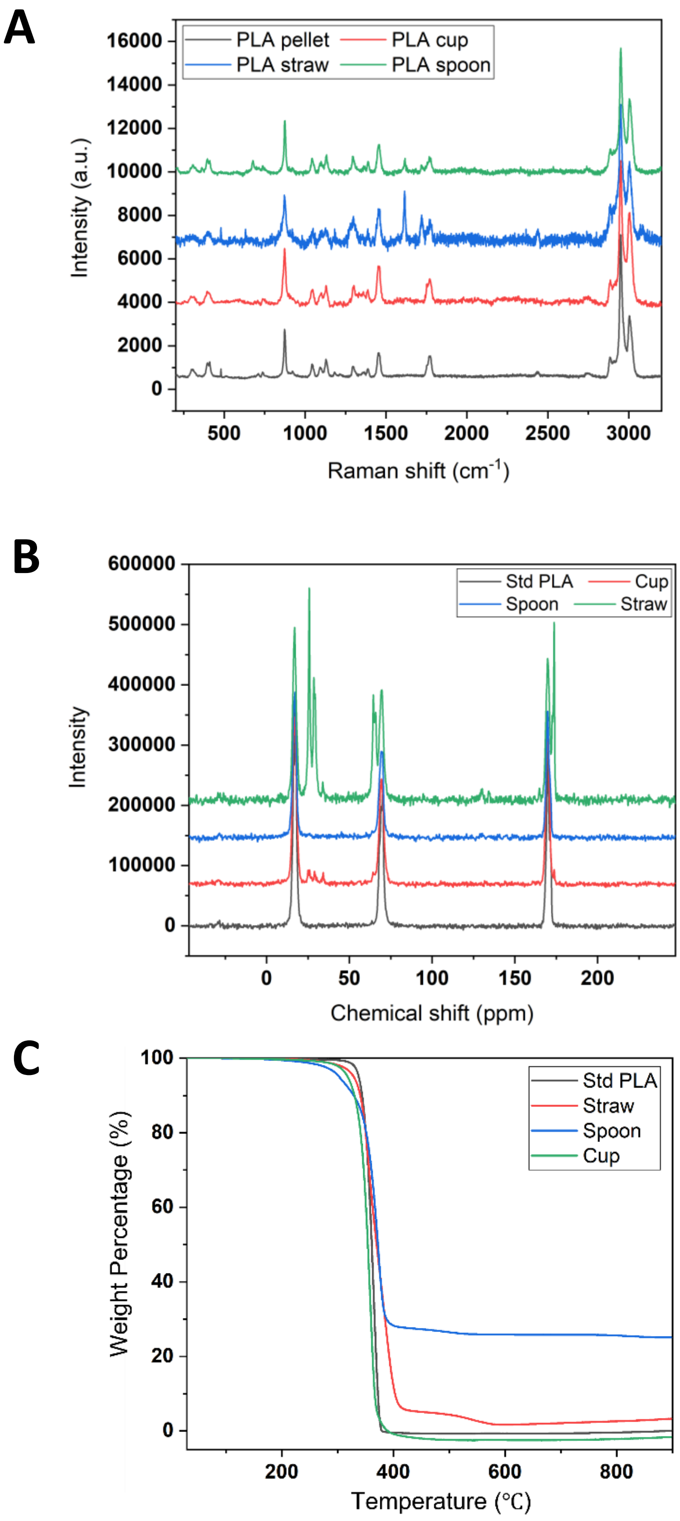

Fig 5. 3 Characterization of bulk material of four PLA products. (A) Raman spectra of PLA pellet, PLA straw, PLA cup lining and PLA spoon. (B) NMR spectra of PLA pellet, PLA straw, PLA cup lining and PLA spoon. (C) TGA curves of PLA pellet, PLA straw, PLA cup lining and PLA spoon.

5.3.3 Alkaline decomposition of various forms of PLA materials

To investigate the chemical composition of the four different forms of PLA products, they were submerged in a 0.1M NaOH solution for a period of 3 days. Notably, the PLA cup lining exhibited the most rapid decomposition rate, with visible signs of digestion occurring within the first 5 minutes of immersion. This decomposition was characterized by the PLA lining breaking into small pieces. Following the PLA cup lining, the PLA straw displayed the second-fastest digestion rate. Within a period of two hours, visible fragmentations of the PLA straw were observed, indicating the breakdown of its structure. On the other hand, the PLA spoon exhibited a slower decomposition process, taking place over a span of three days. Throughout this period, the PLA spoon underwent noticeable morphological changes, transitioning from a solid form to amorphous, clay-like substances. In contrast to the rapid decomposition and visible changes observed in the PLA cup lining, PLA straw, and PLA spoon, no visible evidence of change was observed in the PLA pellets after the 3-day period. The PLA pellets appeared to maintain their original form and structure, suggesting a relatively slower decomposition rate compared to the other PLA products tested. In the same time, the dry weight of four PLA products before and after digestion was recorded in Table 5.1. PLA pellets presented a highest mass reduction ratio of 93.6%, followed by the PLA straw with the reduction of 78.1%. PLA spoon showed a lower mass reduction of 53.9%, while the PLA pellets retained almost the same weight with only 2.6% mass reduction ratio. These results further indicated that the PLA pellets maintained a high resistance in alkaline environment. The various decomposition time of different forms of PLA products also illustrated that under the same alkaline condition, the addition of various additives can significantly affect the decomposition behavior of same material, thereby influence the microparticles released from these products in natural environment.

Fig 5.4A suggested the in-situ experiment of the decomposition process of PLA cup lining in a NaOH solution over a 10min timescale. In the first 5min of the in-situ experiment, distinct cracks and fragmentations can be observed in the PLA cup lining. These visual changes indicate the initial stages of decomposition. However, it is important to note that the subsequent images in the series may not focus on the exact same location due to the significant breakdown and deterioration of the PLA lining after the 10min duration. The in-situ experiment performed a more intuitive change of PLA lining breakdown in a short time.

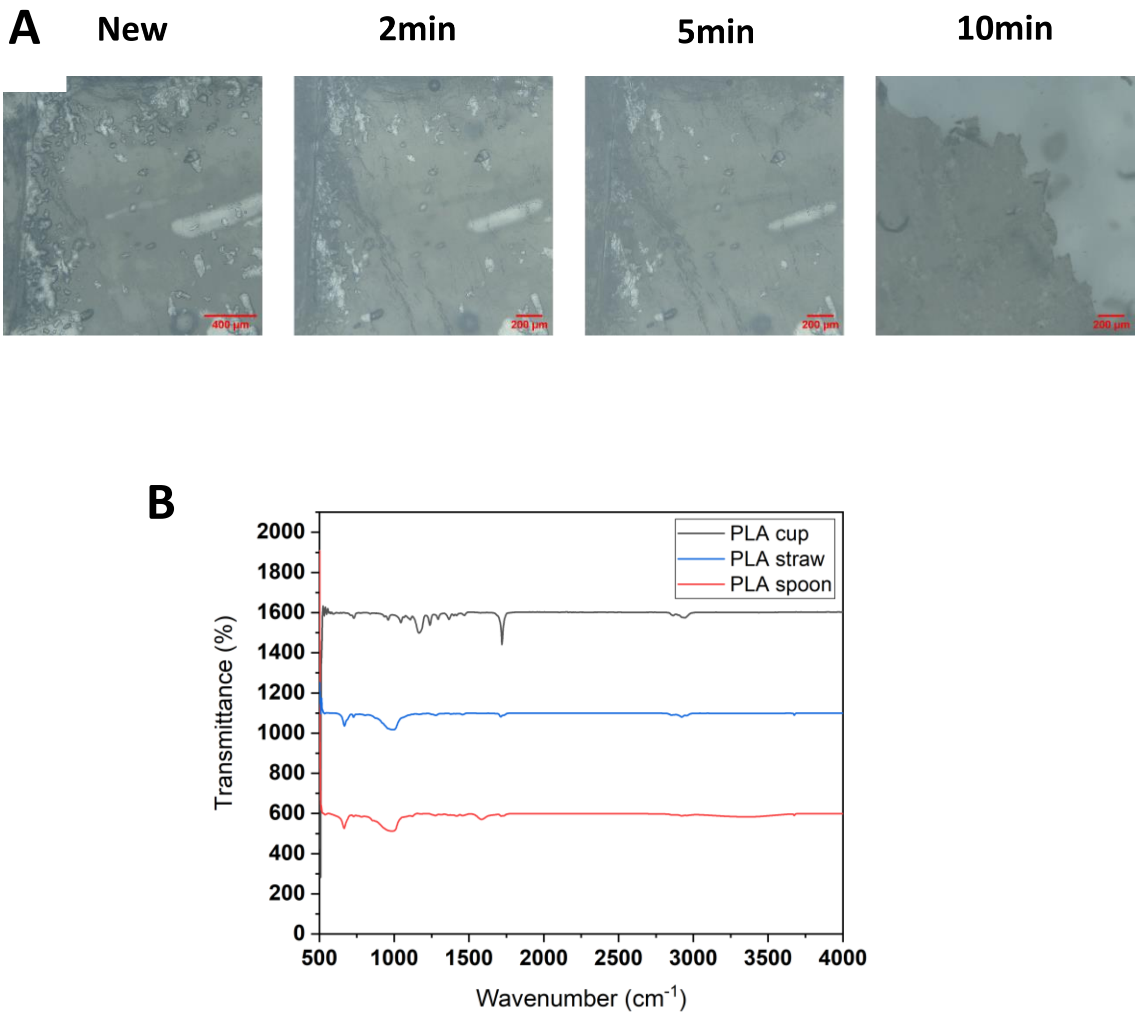

Fig 5. 4 In-situ alkaline decomposition and residue identification. (A) In-situ optical images of alkaline decomposition of PLA cup. (B) FTIR spectra of PLA cup lining, PLA spoon and PLA straw residue after 3-day alkaline decomposition.

5.3.4 FTIR identification of PLA sample residue

The dry weight before and after alkaline decomposition of four PLA products indicated that the PLA pellets presented a very less decomposition degree. Therefore, after alkaline decomposition for 3 days, the residue of PLA cup lining, PLA spoon and PLA straw were collected and identified by FTIR. Fig 5.4B illustrated the FTIR spectra of residue of PLA cup lining, PLA spoon and PLA straw. The PerkinElmer Spectrum IR software automatically matched the most similar substances with the tested sample. The FTIR analysis of the residue from the PLA cup lining revealed the presence of chemical compounds resembling polycaprolactone (PCL) with a similarity of 75%. PCL is a commonly used chemical additive in the polymer industry, known for its ability to enhance the processing characteristics of polymers. A study conducted by Semba et al. reported that the brittle behavior was significantly improved by blending the PLA with PCL [201]. The testing results of the PLA spoon residue revealed a spectrum that closely matches 3-(2-Imidazolin-1-yl) propyltriethoxysilane, with a similarity of 73% compared to the reference spectrum. 3-(2-Imidazolin-1-yl) propyltriethoxysilane is commonly used as an additive for the inorganic modification of block copolymers and as a catalyst for condensation reactions [202]. In parallel, the FTIR spectrum of PLA straw residue demonstrated that the tested substances was identified as modified silicate complex with 75% similarity. In addition, the result also presented the detected substances should be the antiblock agent used in the raw material. The FTIR results of these residue samples provided the evidence of the addition of various additives in the PLA products during different production processes.

5.4 Discussion

5.4.1 Addition of chemical substances into PLA product

PLA material has gained popularity as a substitute for conventional plastic products in various applications, including medical, agricultural, and food packaging fields [5]. However, PLA does have certain drawbacks, such as low thermal stability and poor toughness, which can limit its performance in certain scenarios [203]. To overcome these limitations, chemical additives are often incorporated into PLA to modify its properties [204]. The released additive microparticles and the different decomposition behavior of four PLA products indicated the addition of various additives during different manufacture process. The detected additive compounds from FTIR results indicated the organic and inorganic additives are used as functional additives to improve properties of biodegradable polymers. Previous studies also showed the evidence of the addition of additives. A study published previously reported that the thermal stability and brittleness were significant improved by combining with plasticizer, modifier and nanoclay particles to replace the conventional PP products in automotive applications [205]. A study conducted by Eichers et al. revealed that the cellulose nanocrystals and polyethylene glycol were able to use as the reinforcing agent and plasticizer to enhance the mechanical and ductile properties of PLA materials [63].

Indeed, the addition of additives in biodegradable materials can raise potential environmental concerns. As biodegradable material needs similar or even more plastic additives for the property modification, the chemical additives in biodegradable materials may lead to similar or higher risk with that in conventional plastics. A review paper summarized the potential risk of plastic additives, indicating that the toxic plastic additives such as bisphenol A and phthalates are likely to be released in the leachate of plastics and are hazardous to organisms in environment. In addition, the chemical compounds contained in the additives cause metabolism and oxidative stress to marine animals. Furthermore, the MPs and additives are likely to be aggregated together leading to further biological risk to the aquatic and terroir species [206]. In deed, toxicological studies of MPs, chemical additives, and other impurities present in biodegradable materials are crucial for a comprehensive understanding of their potential impacts on human health and the environment. These studies help to assess the toxicity, bioaccumulation, and long-term effects of these substances, aiding in the development of appropriate risk assessment and management strategies.

5.4.2 Release mechanism of MPs and other additives

Compared with the conventional petroleum-based plastic, although the biodegradable materials are able to be degraded with non-toxic and environmental friendly by products [30], it is worth noting that the specific conditions such as temperature, humidity and composting environment are required for biodegradable polymers to be fully degraded [10]. Therefore, the biodegradable MPs are more likely to be released in the ecosystem. As indicated in the results from section 5.3.1, it was suggested that the elevated level of particle release observed can be primarily attributed to the mechanical forces generated by the collisions of PLA pellets during the shaking process. These mechanical interactions between the pellets could induce the detachment and subsequent release of particles, thereby contributing to the higher levels of particle emissions observed. The level of PMPs release depends on various impact factors such as polymer type, stress situation and physical and chemical environment condition during the decomposition process. For instance, a study was conducted to investigated the MPs release level of two polymers PE and thermoplastic polyurethane (TPU) under UV exposure, mechanical abrasion and UV and mechanical abrasion combination conditions. The results concluded that in terms of the experiment condition, the UV combined with mechanical abrasion led to the highest release of MPs, followed by mechanical abrasion and UV themselves. On the other hand, the PE film released more MPs than that of TPU under same conditions, which indicated that the polymer type also affects the release level [207].

Other release impact factors including biodegradation and chemical oxidation and corrosion such as alkaline decomposition in this study were also regarded as the mechanism of the abundance of PMPs from plastic materials. A studies has summarized the possible natural degradation factors during aging process related to the mechanism of MPs release, as well as the adsorption to the contaminants in natural environment. The review showed that the MPs during natural degradation have more coarse surfaces thereby are more likely to absorb other chemical compounds in the environment [208].

While biodegradable polymers offer potential benefits in terms of reducing plastic pollution and environmental impact, there are still many aspects that need to be explored and understood. To sum up, the understanding of the complex impact factors and degradation mechanisms of biodegradable polymers is still limited and requires further investigation.

5.5 Conclusion

PLA is widely recognized as one of the most commonly used biodegradable polymers in a variety of applications across different industries. In this study, the release of PMPs from four different forms of materials was investigated, along with their chemical composition analysis under alkaline conditions. The results of the study revealed that the release of particles from the investigated PLA materials was particularly influenced by mechanical stress, such as abrasion. The data showed that higher mechanical forces resulted in a greater number of particles being released from the PLA materials. In addition, the study employed Raman spectroscopy and FTIR analysis to identify and determine the presence of various functional additives in different products made from the same material after alkaline decomposition. Furthermore, the study observed distinct decomposition behaviors among the four sets of PLA products over different timescales, which provided insights into the relative ratios of additives used in these products.

Building upon previous research and the findings of this study, an in-depth investigation was conducted to explore the impact factors and mechanisms underlying the release of MPs, additives, and other impurity compounds in biodegradable materials. Nevertheless, there are still important considerations that need to be further explored on the potential environmental concern of these released particles, such as the absorption of heavy metals, toxic substances and other contaminants, along with the ecological risk of released particles. Therefore, this study has contributed valuable insights into the release of PMPs and highlighted the future research directions. Moreover, raising awareness about the issue of biodegradable MPs is crucial for driving positive change and promoting sustainable practices. It is important for individuals, industries, and policymakers to understand the potential environmental impacts associated with the use and disposal of biodegradable materials.

Chapter 6

Mitigation of microplastic release from polylactic acid material

1. Mitigation of microplastic release from polylactic acid material

Abstract

Biodegradable plastic is considered an ideal alternative to conventional plastic because of the growing concern of plastic waste accumulation in the environment. Polylactic acid (PLA) is one of the most used biodegradable plastic in hospital, industry and food packaging sectors. However, studies have shown that biodegradable plastic has a higher level of microplastic (MPs) release than that of conventional plastic. Therefore, it is necessary to develop systematic methods to mitigate the release of MPs from biodegradable plastic products and keep the degradation features for long term in the meantime. This study proposed that coating a layer of metal oxide on the surface of biodegradable plastics could reduce the MPs release. In addition, the coating material has very less influence on the degradability after a long time of weathering. To test this hypothesis, 3D printed PLA sheets were used as the specimens, and the copper oxide (CuO) was coated on the specimens' surfaces using wet coating process. The PMPs release of PLA sheets before and after CuO coating were assessed. Although most of the plastic products are designed for single use, in the natural environment, the weathering behavior is continuously occurring from the manufacture, transportation and waste management stage. Therefore, a long-term experiment was also conducted to assess the stability and duration of the coating. Raman spectroscopy, atomic force microscopy (AFM), nuclear magnetic resonance (NMR) and thermogravimetric analysis (TGA) were used to characterize the bulk materials, released PMPs and surface morphology of PLA sheet before and after CuO coating. The results show that after CuO coating, the PMPs release of PLA sheet reduced from 100±40.8 particles/cm^2 to 33.3±7.5 particles/cm^2 under 95°C, which accounted for the reduction of 66.7%. After 9 weeks, the CuO coating still showing an average reduction of 36.7%. The surface change of PLA sheet after 9 weeks weathering process revealed that the CuO coating has very less influence on the degradability. The coating protocol of heating and cooling process can be regarded as the annealing process thereby improved the heat resistance of PLA sheet.

Key words: Biodegradable plastic, microplastic, mitigation, copper oxide, coating, heat resistance

6.1 Introduction

Microplastics (MPs) has become a hot spot and global concern for the recent years because of the high-level release and potential risk to human and environment. Many studies reported that there were million even trillion levels of MPs released from the food contacted plastic products such as teabags [1], baby feeding bottles [2], plastic kettles [209], single used paper cups [185] even human feces [21, 210]. Although there were few studies investigating the toxicity and health risk of MPs released from plastic, it has been proved that the micro and nano-sized plastics particles can cause the potential risk in human cells such as hypersensitivity, immune response and hemolysis response [211]. Luo et al. indicated that the mice during gestation and lactation exposure to polystyrene (PS) MPs have the disease of metabolic disorder. The MPs exposure also has the negative effect on the next two generations of the mice [212]. In addition, a previous study also tested several types of plastics in human blood, indicating that nano-sized plastic particles can penetrate in human bloodstream [25]. Many consistent results show that the MPs in smaller size will be easier to be ingested and transferred into human cells and liver tissues [213, 214].

Biodegradable plastic has been regarded as the ideal alternatives of the petrol-based conventional plastics due to its renewability, high degradability and the environmental friendliness of the final product [30, 31]. There is a significant increase trend of the biodegradable plastic products in the plastic market. It is reported that the market value of biodegradable plastics has an annually growing rate of 24.9% from 2021 to 2026 [215]. Poly(lactic) acid (PLA) is a most commonly used biodegradable plastic because of the advantages of biodegradability, high transparency, thermoplasticity and ductility [40]. PLA has been widely used in biomedical, industry and food packaging areas combined with three-dimensional (3D) printing technology [5, 216]. However, many previous studies pointed out that there was a high level of MPs released from biodegradable plastics [217, 218]. The MPs released from biodegradable plastics have a similar toxicity risk as conventional plastics, such as the potential ability to be the carrier of toxic chemicals and the ability to enter animal tissues due to their micro and nanoscale size [14]. Toxicity risk demonstrates the likelihood or the possibility of that exposure to a substance will result in harmful effects, depending on the concentration of the substance, the duration and route of exposure, and the sensitivity of the exposed population. Therefore, it is urgent to develop a systematic method to mitigate the release of MPs and additive microparticles.

Currently, most of the methods for MPs mitigation focus on the establishment of regulations and strategies, such as reducing the use of plastic products and charging for plastic shopping bags [219]. A literature review paper summarized the current policies and regulations of reducing MPs pollutions from the aspect of waste management, including reducing the plastic waste emission and increasing the plastic waste recyclability [220]. On the experimental side, Ward invented and patented a polymer-coated elongated mesh screen, which is a MPs removal tool to remove microplastics from drinking water [221]. Very few studies have focused on reducing the release of microplastics from plastic daily necessities. A previous study indicated that the $CaCO_3$, Fe_2O_3 and CuO particles formed by the ions in drinking water can reduce the MPs release from plastic kettles during daily use [222]. In addition, CuO can be used as an antibacterial agent in the application of plastic and coatings [223]. Therefore, in this study, CuO was used as the coating material to investigate the mitigation effect for 3D printed PLA sheets. The CuO particles were formed by the chemical reaction of heating $CuSO_4$ and NaOH. The 3D printed PLA sheet was coated during heating process by soaked in the chemical solution. The MPs release of the PLA material before and after coating was determined using Raman spectroscopy and ImageJ. Afterwards, atomic force microscopy (AFM) was applied to present the surface morphology of PLA sheets before and after coating. Moreover, a 9-week long-term experiment was conducted to investigate the MPs release level and degradability of the PLA sheets during weathering process with and without CuO coating. Based on the previous study, it is proved that biodegradable plastic may release higher MPs, additive microparticles and other impurities [12]. This lab-scale experiment could become a novel mitigation method for the particles release of biodegradable materials. It is also necessary to develop a modular method of coating process to inhibit the release of MPs and other harmful by-products during the degradation process of biodegradable plastics.

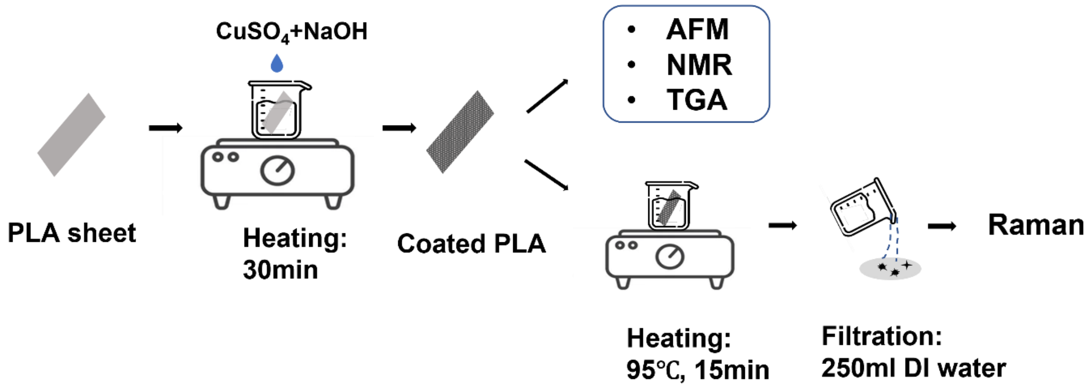

Fig 6. 1 Experiment protocol for CuO coating on PLA and sample preparation

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6.2 Materials and methods

6.2.1 Contamination prevention and control experiment conduction

To prevent any potential contaminant and pollutant involved during the experiment, lab coat and gloves were worn at all times. All the containers used in the experiment process were glassware and sonicated for 30min before experiment [2, 198]. Deionized water with resistivity of 18.2 MΩ was used to rinse the glassware and prepare the coating solution and all water samples. Blank control experiment was conducted to investigate the particles in original DI water. 250ml of DI water was heated to 95°C and naturally cooled to room temperature, followed by filtration process and Raman identification. The result shows that the PMPs in blank DI water was around 499.93 particles/L, which was 2 orders of magnitude lower of that in PLA sheet without coating under 95°C exposure.

6.2.2 PLA sheet preparation and CuO coating process

The PLA sheets were manufactured using dark grey Prusament PLA filament and a 3D printer (Original Prusa i3 ML3S+ 3D printer) from Prusa Research, with the dimension of 30 mm x 100 mm x 1 mm.

The $CuSO_4$ and NaOH used to prepare the coating solution were purchased from Sigma-Aldric. The $CuSO_4$ solution was prepared by dissolving a certain amount of $CuSO_4$

powder in deionized water to make the concentration of 1000mg/L. Subsequently, the PH value was adjusted using 1M NaOH to around 9.0 waiting for further experiment.

As described in Fig 6.1, the PLA sheet was roughly rinsed using DI water to clean the surface of the sample followed by soaking in the $CuSO_4$ solution. The $CuSO_4$ solution with PLA sample was heated from room temperature until boiling and keep boiling for 15min. The PLA sheet was then collected from the solution and stock in a dry glass beaker after cooling down to the room temperature waiting for further experiment.

6.2.3 Characterization of PLA materials with or without CuO coating

Multiple technologies were used to determine the characterization of the PLA sheet before and after CuO coating. Raman spectroscopy (Renishaw InVia Raman spectrometer) system equipped with a 532nm laser (Coherent), a cooled charge-coupled device and a microscope (NT-MDT) with a 100× objective (Mitutoyo, M Plan Apo; numerical aperture = 7.0) and combined with WiRE 3.4 (Renishaw) software was used to identify the PLA and CuO particles before and after coating. To characterize the CuO particles, the black particles on the coated PLA sheet are gently scraped and collected, and their Raman spectra are compared with standard CuO Raman spectra (Fig 6.2E). In parallel, the PLA sheets and standard PLA pellets were prepared to conduct the solid state ^{13}C-NMR measurement [200]. Additionally, the PLA samples and standard PLA pellets were also prepared for TGA test with the heating temperature from 30℃ to 900℃ at 10℃/min in the nitrogen atmosphere following the previous method [199]. The Raman, NMR spectra and TGA curves were obtained and plotted in graphs to present the characterization of standard PLA and PLA samples used in this study (Fig 6.2A, 6.2C-D).

The PLA sheet before and after CuO coating was prepared to determine the surface morphology using Olympus optical microscope and AFM. An OLYMPUS DSX1000 Optical microscope was applied to present the CuO particles onto the surface of PLA sheet. MPlanFL N 10x /0.25 BD P ∞/-/OFN26.5 camera lens was equipped with the microscope to capture the images. The images were collected under bright mode from random locations on the samples (Fig 6.3A). AFM height and phase images were analyzed using Gwyddion 6.21 software to demonstrate the surface morphology of PLA sheet before and after CuO coating (Fig 6.3C).

6.2.4 Particles release and identification from coated/uncoated PLA samples during weathering process

Following the experiment protocol described in Fig 6.1, the PMPs release of PLA sheets with and without CuO coating were investigated under room temperature and 95℃, respectively. The PLA sheets were soaked in the DI water with the room temperature and 95 ℃, respectively, and gently shaken for 15min at the speed of 50 rpm. The DI water was then filtered through a gold-coated polycarbonate membrane (APC, Germany Ltd) with the pore size of 0.8μm using a vacuum pump. After filtration, the gold-coated membrane was stock in a glass petri dish to naturally dry waiting for Raman spectroscopy testing.

Although the main applications of the PLA material are designed for single use, the PMPs released during manufacturing, transportation, and after-use treatment are potential to migrate into natural environment and ecosystem. Therefore, a long-term experiment was conducted to investigate the stability and durability of the coating. Five groups of coated and uncoated PLA sheets were placed into a weathering chamber with a 40℃ degree and 60% humidity combined with UV process. The UV effect of exposing the samples in the weathering chamber for three weeks is equivalent to one year outside. The PLA sheets were collected in the 3rd, 6th, 7th, 8th and 9th week to determine the PMPs release, which equivalent to 1, 2 and 3 years outdoor environment.

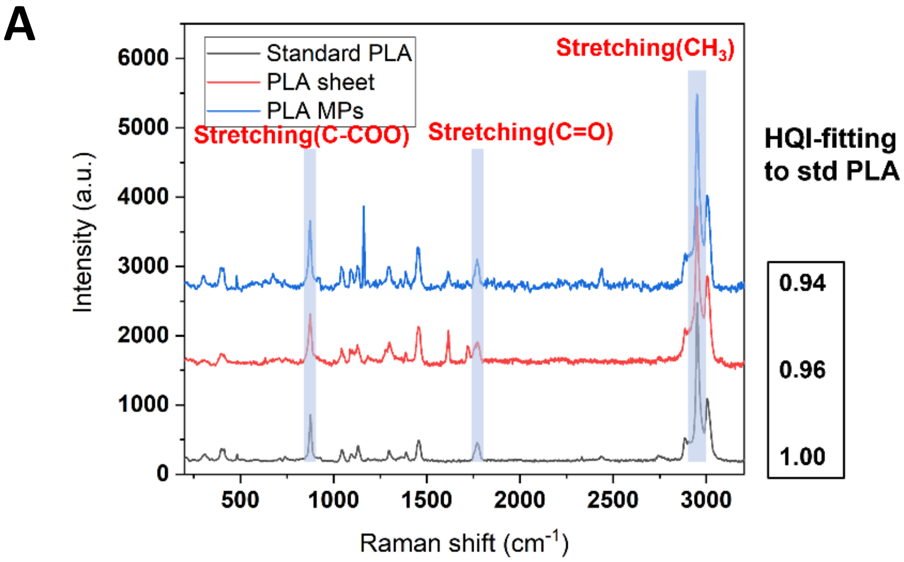
Raman spectroscopy system mentioned above was used to identify the particles released from the PLA sheet with or without CuO coating. Subsequently, ImageJ software was used to determine the number of particles released from relative samples. The obtained Raman spectra of MPs and additive microparticles were collected and compared with the standard Raman spectra of PLA and additive bulk material collected under same conditions. To analyze the Raman spectra, high quality index (HQI) value was used as the parameter to characterize the similarity of the Raman spectra with a minimum threshold of 0.7 [20, 222].

6.3 Results

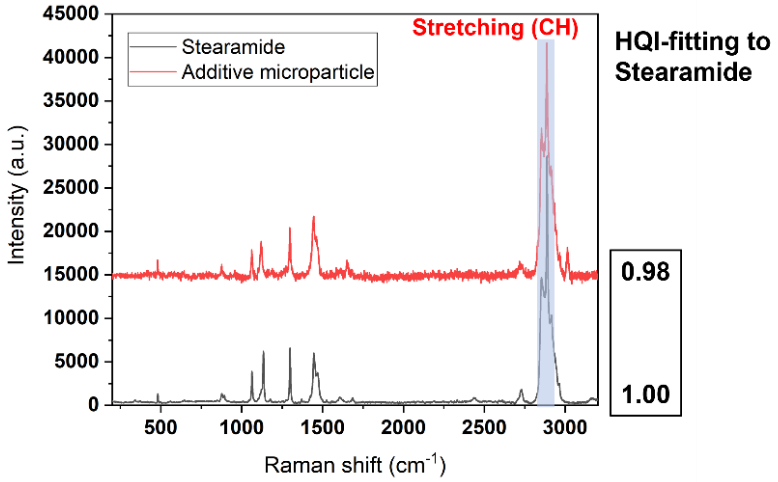
6.3.1 Characterization of PLA sheet before and after CuO coating

The PLA sheet was analyzed using optical microscope, Raman spectroscopy, nuclear magnetic resonance (NMR) and thermogravimetric analysis (TGA) before CuO coating. Fig 6.2A shows the Raman spectra of standard PLA pellets and PLA sheet. The HQI fitting of the PLA sheet to standard PLA pellet was 0.96 with the typical peak of C=O and CH_3 stretching around 1773cm^{-1} and 2944 m^{-1}. Fig 6.2C presented the NMR spectra of standard PLA and PLA sheet, showing the peaks in 17.33ppm, 70ppm and 170.1ppm correspond to CH_3, CH and CO carbons [200]. TGA curve showed in Fig 2D indicated that in the nitrogen atmosphere, the mass of standard PLA and 3D printed PLA started 5% loss at 338.6℃ and 328.4℃, respectively. At 378.7℃, both standard PLA and 3D printed PLA reached the maximum mass loss, following by a relatively constant mass. The residue of standard PLA and 3D printed PLA sheet was 7.66% and 0.53% after 500℃. The Raman spectra of formed particles and standard CuO showed the qualitative identification of CuO coated on the PLA sheet, which the HQI fitting was 0.92.

Fig 6.3B shows the microscope images of 3D printed PLA sheet before and after CuO coating under bright light mode. The image on the right indicated that the CuO particles coated on the surface of PLA sheet, showing the dark brown spots in the image. Because of the filament structure of 3D printed PLA, CuO particles are not fully coated on the surface of PLA sheet. Although the 3D printed PLA sheet was only partially coated, the CuO material has a high effect on MPs mitigation. Subsequently, AFM was applied to determine the surface change of PLA sheet with and without CuO coating. Fig 6.2C shows the AFM height and 3D images of PLA sheet before and after CuO coating. The 3D images clearly indicated that the PLA surface coated with CuO particles.



B


Stretching (CH)
HQI-fitting to Stearamide
Stearamide
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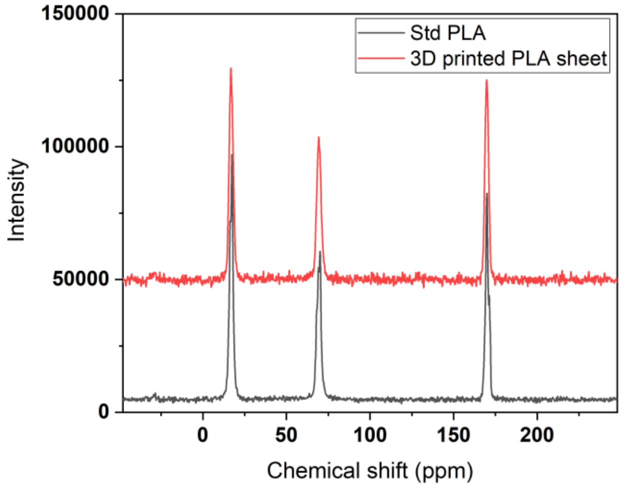
Raman shift (cm^{-1})

C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3D printed PLA sheet
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Chemical shift (ppm)

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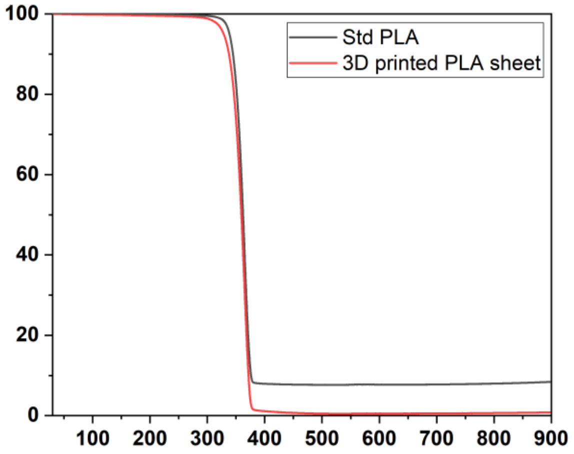
Std PLA
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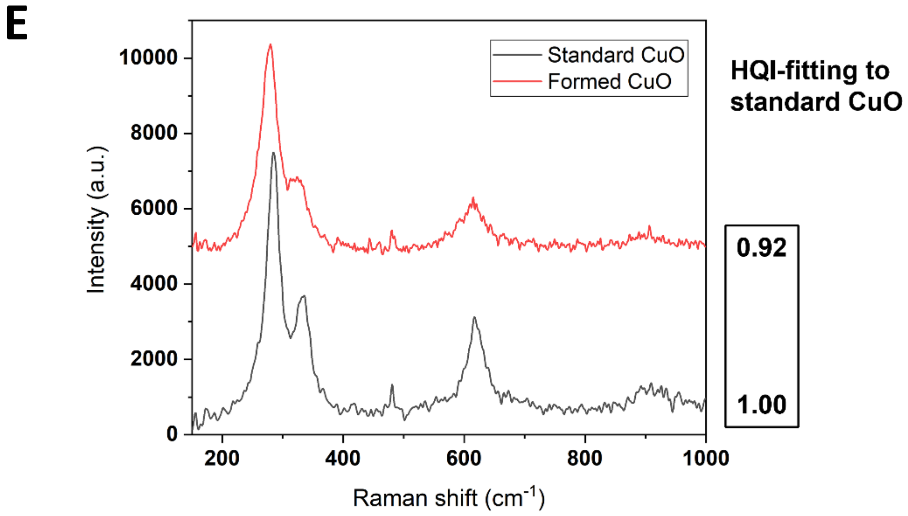
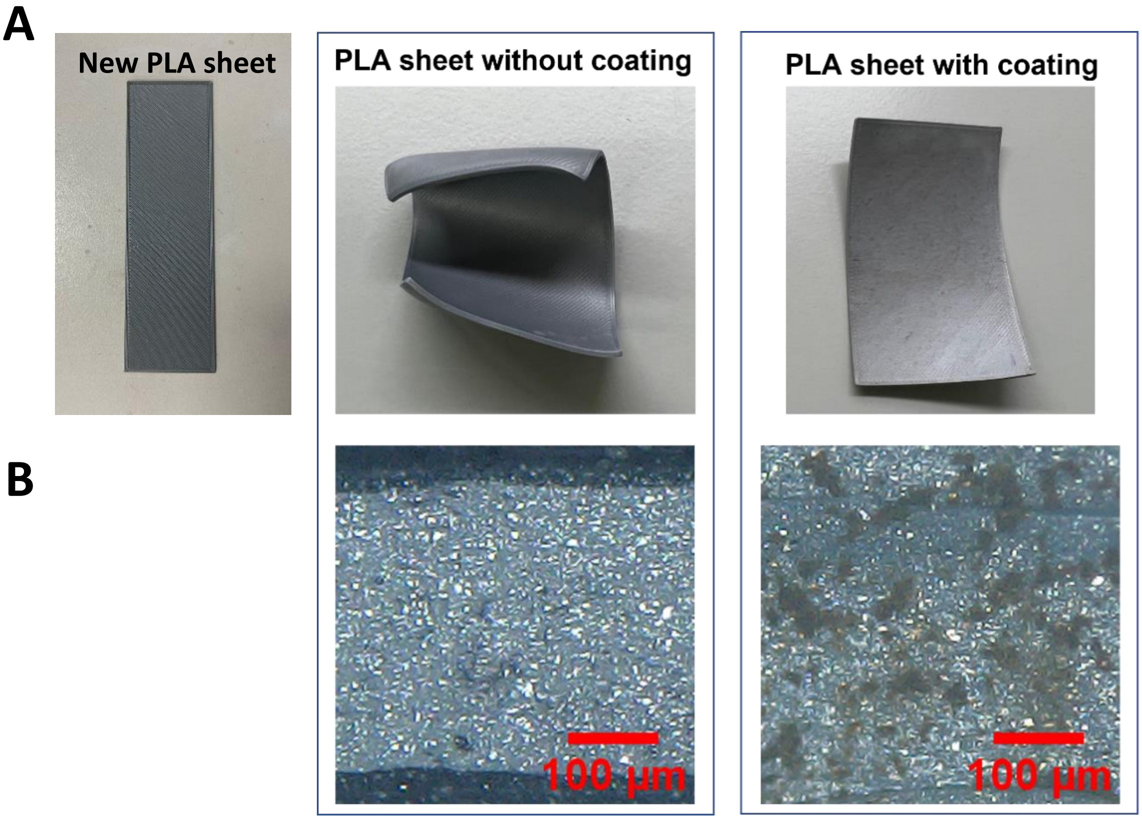


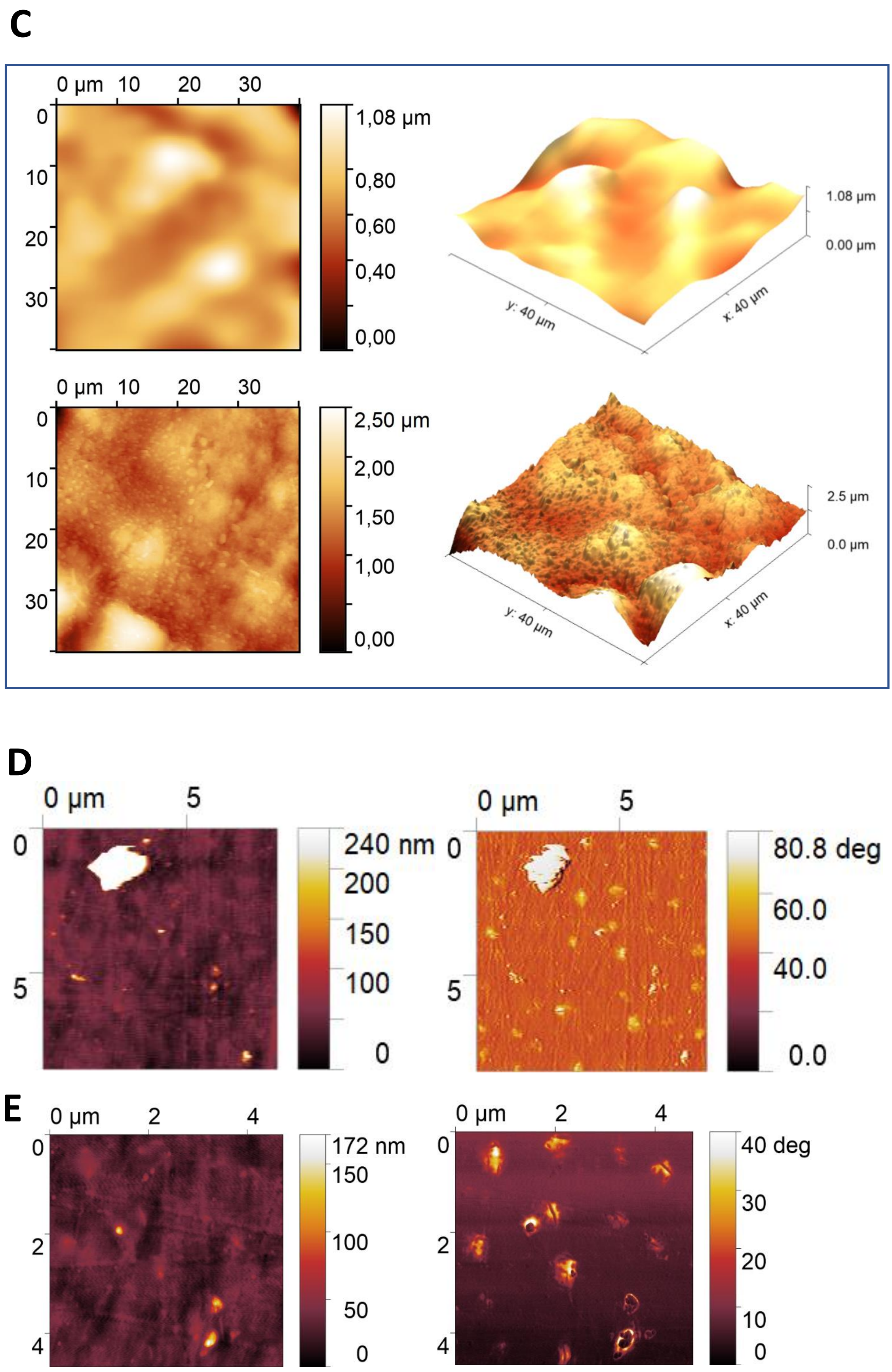
Fig 6. 2 Characterization of 3D printed PLA sheet. (A) Raman spectra of standard PLA, PLA sheet and PLA MPs released from PLA sheet. (B) Raman spectra of stearamide bulk material and additive microparticle released from PLA sheet. (C) ^{13}C NMR spectra of standard PLA and 3D printed PLA sheet. (D) TGA spectra of standard PLA and 3D printed PLA sheet. (E) Raman spectra of standard CuO and CuO particle coated on the PLA sheet.

6.3.2 Heat resistant improvement of PLA sheet after coating and confirmation of CuO particles incorporated into the plastic sheet

Following the experiment protocol in Fig 6.1A, when preparing the samples for PMPs release test, the PLA sheet without coating was significantly deformed soaking in 95℃ DI water, while the PLA sheet with CuO coating remained the same shape. The deformation was because of the poor heat resistance property of PLA material [34]. It was improved after coating with CuO particles. Fig 6.3A presented the image of a new 3D printed PLA sheet and the deformation degree of PLA sheet with and without CuO coating when soaking into hot water. The PLA with coating showed a slight deformation when exposed in 95℃ DI water, while the pure PLA sheet underwent severe deformation under the same condition.

In order to investigate the connection situation of the CuO particles and plastic material, an experiment was conducted with standard PP sheet as the surface of the PP sheet was smooth and flat. The PP sheet was coated with CuO particles following the same protocol as described in Fig 6.1A, followed by obtaining the AFM height and phase images. For the confirmed CuO particle bulged on the PP surface (Particle in blue box of Fig 6.3D), AFM images of height and phase showed that the CuO particle is around 20-40° higher than that of bulk PP sheet. Sequentially, the same PP sheet was thoroughly cleaned using DI water to keep the CuO particle level to a minimum level (no microscopic visible particles). The following test using AFM showed that for many flat spot of the cleaned CuO-PP, the significant phase angle differences were observed (Fig 3E). Cross section analysis showed that though the height is lower than the surrounding area, the phase degree is around 20° higher than that of bulk PP (Fig 6.3F). This angle difference is consistence with the angle difference between CuO particle and PP bulk material, indicating that it is likely a CuO incorporated with PP bulk during exposure process.

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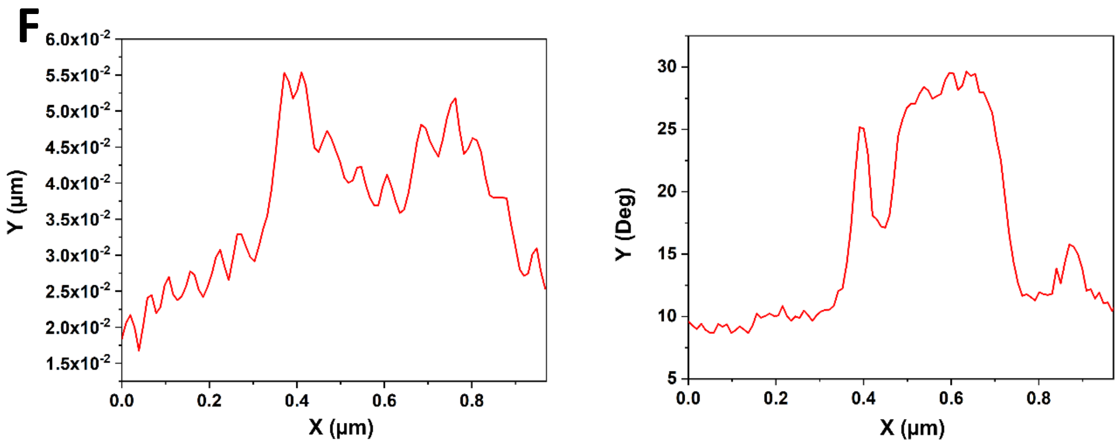


Fig 6. 3 Microscope and AFM image of PLA and PP sheets before and after coating. (A-B) Images of new PLA sheet and PLA sheets exposed in 95°C DI water before and after CuO coating under camera and optical microscope. (C) AFM height and phase images of PLA sheet before (up) and after (down) CuO coating. (D-E) AFM height and phase images of PP sheets with CuO coating before and after cleaning the surface. (F) AFM height and phase cross section images on the same point of CuO-PP sheet after cleaning the surface.

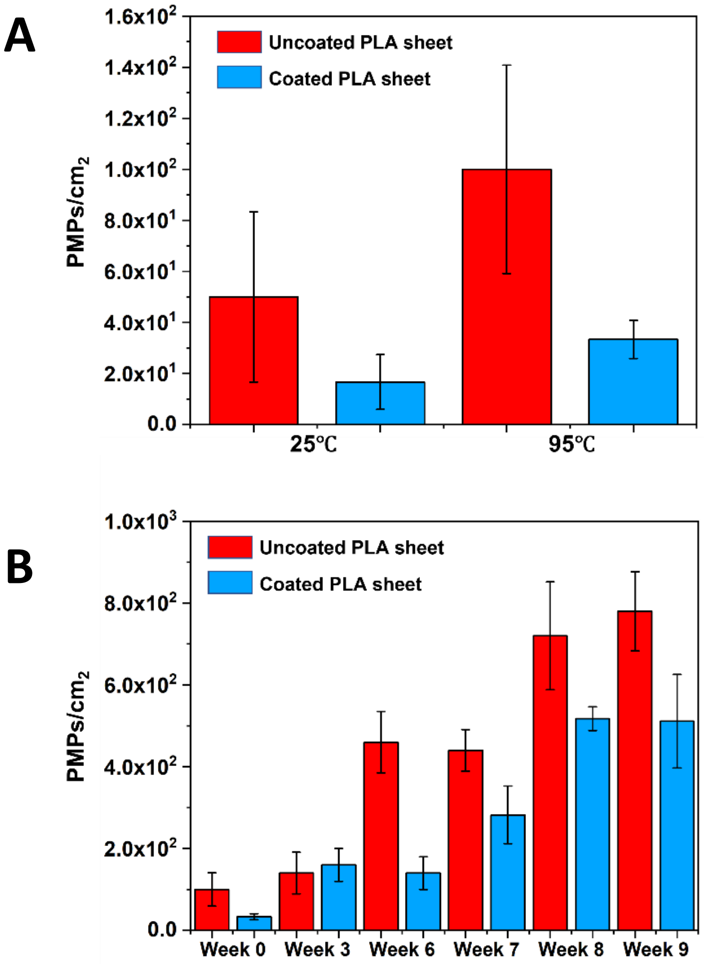
6.3.3 PMPs release mitigation by CuO coating

After the CuO coating on the surface of the PLA sheet, PMPs release before and after CuO coating was determined under room temperature and 95°C respectively following the protocol in Fig 6.1A. The particles were qualitative and quantitative identified by Raman spectroscopy and ImageJ software. It was determined that the PMPs release levels of PLA sheet raw material were around 49.99±33.33 and 99.98±40.82 particles/cm^2 under room temperature and 95°C DI water, respectively. After coated with CuO particles, the PMPs release number was decreased to 16.66±10.97 and 33.32±7.45 particles/cm^2 under room temperature and 95°C DI water, respectively. The results indicated that the CuO particles coated on the PLA sheet lead to the PMPs reduction of 66.7% in both room temperature and high temperature condition.

Statistical analysis was addressed using one-way Analysis of Variance (ANOVA) through Original software. A p value of <0.05 was regarded as the threshold of the significant differences. Based on the result, the ANOVA test to showed a significant difference which indicated that the CuO coating being an effective method to inhibit the release of PMPs from 3D printed PLA sheet.

Among the released particles, there were PLA MPs, additive microparticles and other impurities detected using Raman spectroscopy Fig 6.4C. Fig 6.2A shows the Raman spectrum of a PLA MP with a HQI index of 0.94 compared with that of standard PLA material. The peaks at around 1773 cm-1 and 2944 cm-1 are related to the stretching C=O and CH_3 bond, respectively, which perfectly matched with the Raman spectrum of standard PLA material [170, 171]. Fig 6.2B shows the Raman spectra of a typical additive microparticle released in the PLA sheet and stearamide bulk material, which is one of the common slip additives used in plastic materials [3]. The HQI-fitting of the additive microparticle and stearamide is as high as 0.98, with the well-matched CH stretching peak at 2855-2886 cm^{-1}.

The PMPs release during the weathering process was investigated and plotted in Fig 6.4B. From Week 0 to Week 9, the result illustrated that the PMPs release of PLA sheet without CuO coating was increased from 99.98±40.82 $particles/cm^2$ to 779.86±96.94 $particles/cm^2$. The PLA sheets with CuO coating were tested at the same time showing the result that the PMPs release has an average reduction of 36.7%.



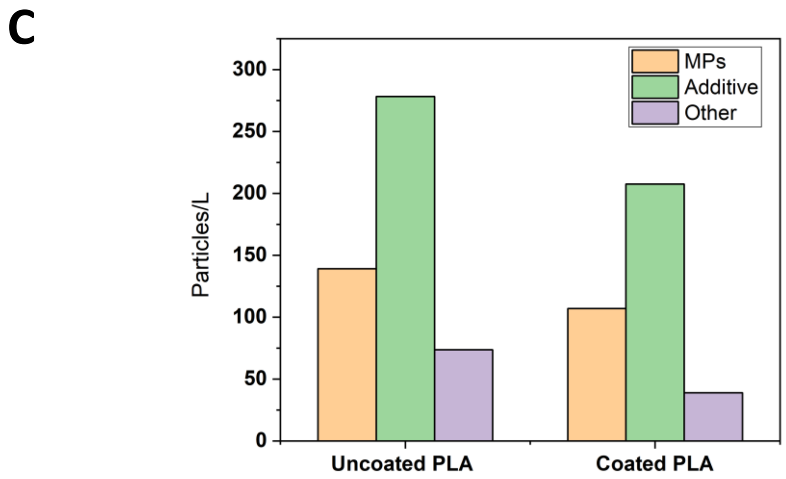


Fig 6. 4 PMPs release of 3D printed PLA sheet. (A) PMPs release of PLA sheet with/without CuO coating under 25°C and 95°C. (B) PMPs release of PLA sheet with/without CuO coating after 9-week weathering process under 95°C. (C) Particle types released from 3D printed PLA sample with or without CuO coating under 95°C.

6.4 Discussion

6.4.1 Improvement of CuO coating effect on plastics

The CuO coating on plastics usually provides several advantages such as improved conductivity, enhanced durability, increased heat resistance, and antimicrobial properties [224, 225]. CuO is a semi-conductive material. By coating plastics with CuO, the surface becomes conductive, enabling the dissipation of static electricity. In addition, CuO coating enhances the durability and scratch resistance of plastic surfaces. It provides a protective layer that can withstand mechanical stress, environmental factors, and chemical exposure, thereby extending the lifespan of plastic components while reducing the release of PMPs. Furthermore, CuO coatings can potentially improve the recyclability of plastic materials by enhancing their conductivity and eliminating the need for separate conductive layers, which can simplify the recycling process. Normally, various coating process have been applied during the manufacture and production including chemical vapor deposition [226], electroplating [227], and thermal spray [225]. Shi et al. found that CuO-based film is easy to form on the surface of plastic products when exposed to natural tap water due to the existence of Cu^{2+}. The study also proved that the CuO coating has the high effect for MPs mitigation, and CuO particles do not tend to leach into the surrounding water environment [228].

In this study, it was observed that the coating effect could be further enhanced by adjusting various experimental conditions, including the concentration of Cu^{2+}, pH value, coating time, and other relevant parameters. By optimizing these factors, researchers can achieve a higher coating effect, which in turn contributes to better mitigation of MPs release. The coating process plays a crucial role in reducing the release of MPs and improving their stability within the environment. Therefore, by fine-tuning the experimental conditions, researchers can potentially enhance the effectiveness of the coating method and achieve more efficient mitigation of MPs release [50]. Further investigations and adjustments are necessary to optimize the coating process and its impact on reducing MPs in the environment.

6.4.2 Heat resistance improvement of PLA by CuO coating

The PLA material has a low heat resistance due to the low crystallization rate and low heat deflection temperature of 55°C-65°C. It has been summarized that one of the most effective methods to enhance the heat performance and crystallinity is the addition of the nucleation agents and heat resistant filler. Metal oxides is one of the commonly used inorganic nucleation agents. Chen et al. found that the crystallinity and the crystallization rate have significant increased by using zinc oxide combined with phenylphosphonic acid zinc salt as a composite nucleating agent, thereby improving the heat resistance of PLA [229]. In addition to this, the incorporated particles in the plastic material can enhance the physical crosslinking of polymer molecules and chain firmness, which leads to the improvement of the mobility resistance and the stability under high temperature [230]. Therefore, the CuO particles incorporated in plastic sheet in this experiment showed the same effect of strengthening the heat resistance of PLA material.

Moreover, the addition of nucleation agents is often in combination with annealing process in order to better improve the performance of PLA [64]. Zhou et al. annealed pure polyethylene (PP) and a mixture of PP and high density polyethylene (HDPE) at 130°C for 12 hours. The results showed that the impact strength of pure PP and PP/PE mixture increased by 336.7% and 1107%, respectively, which greatly enhanced the toughness and crystallinity of polymer [231]. A study also revealed that the PLA specimens showed best nuclei and spherulite generation when annealing at low temperature (100°C and 110°C), while the nuclei gradually decreased when the temperature increase [232]. In this study, the coating process of gradually heating the solution with PLA sheet and then cooling the PLA sample under room temperature shown in Fig 6.1 can be regarded as the annealing process of PLA, thus explaining the phenomenon of increased heat resistance of PLA.

Additionally, the results in this experiment show that the CuO coating can lead to the reduce of PMPs release from PLA 3D printed material. In addition to the mitigation of MPs release, the CuO particles incorporated in the PLA sheet also have the potential to inhibit the foodborne pathogens [230]. Currently, biodegradable plastic films are widely used in the agriculture field such as plastic covers on different soils [233]. Therefore, the antibacterial effect is necessary for use. Researchers have found that the CuO nanoparticles embedded in the polypropylene matrix have the antimicrobial activity, which reached the antibacterial effect as high as 95%. This finding indicated that the CuO nanoparticles combined with plastic materials can be used as a novel antimicrobial agent [234].

To date, there were only a few papers focusing on the mitigation of MPs release from the experimental aspect. The coating method in this study also has many limitations, such as the coverage and uniformity of CuO particles and the stability of the coating need to be further strengthened. At the same time, the exploration of different types of coating material are in need to be developed to reduce MPs release and the effectiveness of the coating is also necessary to be investigated. Therefore, this study also points out a future research direction for microplastic reduction, including inhibiting the release of microplastics from plastic products and the practical application of this technology.

6.4.3 CuO coating influence on PLA degradability and potential release of CuO

Following the 9-week weathering process, the morphological analysis of the 3D printed PLA sheet, comparing samples with and without a CuO coating, revealed that the presence of CuO did not exhibit any discernible influence on the degradability of the PLA material. Additionally, the AFM 3D image of CuO coated PLA sheet indicated that the CuO forms a thin layer on the surface of plastic, which is not expected to significantly alter the inherent degradability of the plastic substrate. In most cases, surface coatings are relatively thin compared to the bulk of the plastic material. As a result, the coating's presence is unlikely to affect the overall degradation behavior of the plastic substrate. In another hand, the degradability of plastics is influenced by factors such as polymer composition, molecular weight, environmental conditions, and exposure to specific degradation agents (e.g., heat, light, enzymes) [235]. These factors primarily affect the polymer matrix rather than the surface coating. Consequently, based on these findings, the application of metal oxide coatings can be regarded as a viable and efficient approach for mitigating the release of MPs, while minimally impacting the degradability of the plastic. However, further investigations are warranted to explore alternative coating materials of a similar nature, building upon the insights obtained from this laboratory-scale study.

On the other hand, while the thesis primarily focuses on a specific subject, it is crucial to acknowledge the potential release of CuO particles and their associated toxic effects, even if not directly measured within the study. CuO nanoparticles, often employed in various industrial applications, can pose environmental and health risks due to their small size and increased surface area, potentially leading to enhanced reactivity. Moreover, their introduction into the environment may have ecological consequences. Although the current research may not directly measure the release and impact of CuO particles, recognizing their potential presence and effects is essential for a comprehensive understanding of the broader implications of the studied processes or materials. Further investigations into the fate and toxicity of metal oxide particles should be taken more consideration to the overall scope of the research.

6.5 Conclusion

In this study, 3D printed PLA specimens were utilized as the primary materials of interest. PLA is widely recognized as one of the most commonly employed biodegradable materials. The objective of the study was to examine the effectiveness of a wet coating method in inhibiting the release of PMPs from the PLA specimens. CuO particles formed by heating $CuSO_4$ and NaOH solution was used as the coating material to attach on the surface of PLA sheet. The CuO coating showed a great mitigation effect of PMPs release from 3D printed PLA sheet both under 25℃ and 95℃. After exposure to a weathering chamber for 9 weeks (equivalent to 3 years outdoor environment), the average PMPs release of coated PLA sheets was still 36.7% lower than that of the uncoated PLA sheets.

The deformation occurred in this experiment when soaking the new PLA sheet in the hot water was due to the poor heat resistance of the PLA material. However, after the coating process, the stability of PLA sheet has increased. One of the possible reasons is that the wet coating process including heating and cooling process can be also regarded as the annealing process, which is a practical method to improve the heat performance of PLA.

In addition to this, the incorporation of the CuO particles and PP sheets shown in Fig 6.3E also indicated the CuO increased the crosslinking structure of the polymer. The incorporated CuO particles can be regarded as the nucleation agent, which played an auxiliary role in improving the heat resistance. Moreover, the analysis of the coated CuO particles demonstrated no discernible evidence of impacting the degradability of the PLA sheets. This additional observation further highlights the significance of considering the implementation of CuO coatings as a viable strategy, as it does not compromise the inherent degradability of the PLA material.

In conclusion, this study successfully identified and developed a practical method for effectively mitigating the release of PMPs from PLA products. However, further research is required to enhance the efficacy of the coating method and explore alternative metal oxide coating materials for the mitigation of microplastics. These future investigations will contribute to the advancement of strategies aimed at reducing the environmental impact associated with microplastic pollution.

Chapter 7

Conclusions and Recommendations

7 Conclusions and recommendation

7.1 Conclusions

This thesis delved into the release of MPs from biodegradable plastics, particularly PLA, encompassing a comprehensive examination of experimental protocols, analysis methods, and degradation processes. Through simulation experiments utilizing SUCPs, the abundance of biodegradable MPs was investigated, shedding light on the decomposition process of various forms of PLA materials in alkaline solutions. Furthermore, the thesis proposed a method for mitigating MPs release through metal oxide coating.

By reviewing previous publications, the categories and market size of biodegradable materials, their advantages and disadvantages, as well as the applications of biodegradable polymers in different sectors were summarized. Previous studies relevant to the addition of functional additives and the MPs and additive microparticles released from biodegradable plastics were reviewed. This review also collated the previous studies which figured out the potential risk of biodegradable MPs to both environment and human bodies. The review pointed out the current state of art of the biodegradable polymers research as well as the limitations and gaps of this field, which indicated a research direction of the PhD study.

According to the preliminary study, the experiment protocol for investigating the MPs release from different biodegradable plastic daily necessities was conducted. The protocol was developed to simulate the real use process of different plastic necessities during daily scenarios. After the sample preparation, the total number of released microparticles can be calculated using ImageJ software. Raman spectroscopy was applied to identify the released microparticles in the collected water sample, which can be combined with various technology to further investigate the morphology of the particles such as AFM, SEM, etc. The developed methodology provided an effective and practical method to the PMPs study from daily used plastic products. The results were published in journal JoVE [198].

PE and PLA-lined SUPCs were selected as the specimens to compare the total particles and MPs released from the conventional and biodegradable materials following the developed method in Chapter 3. Raman spectroscopy and ImageJ were used to qualitatively and quantitatively identify the released PMPs, combined with the AFM and SEM analysis. The results showed that the total number of PMPs released from PLA SUPCs is 4.2 times higher than that from PE SUPCs when exposed to 95℃. A large number of additive microparticles were detected from both PE and PLA SUPCs, which can be separated by ethanol treatment. The study concluded that more consideration is needed on the replacement of conventional plastics by biodegradable materials as the high level of MPs release and potential health to environment and human bodies. The results were published in journal Chemical Engineering Journal.

The analysis of the PMPs release of different forms of PLA materials revealed that the mechanical force such as abrasion significantly affect the PMPs abundance. The results indicated that the PLA pellets has the highest PMPs release level among the five different forms of PLA materials with the number of 515.83±38.21 PMPs/cm^2, followed by PLA-lined paper cup which released 373.63±41.25 PMPs/cm^2. PLA straw showed a lowest PMPs release number of 101.89±62.4 PMPs/cm^2. In addition, the study of alkaline decomposition behavior also varied from the forms of PLA materials. The PLA cup lining showed the highest mass loss of 93.6%, while the PLA pellets exhibit only 2.6% mass reduction. The FTIR results proved that the addition of additive can affect the degradation behavior of biodegradable polymers, thereby influencing the PMPs release of different products.

The mitigation study of 3D printed PLA demonstrated that the surface coating of CuO can inhibit the release of 66.7% of PLA MPs both under room temperature and 95℃ exposure. After 9-week weathering process, the CuO coating still have the effect of reducing 36.7% of PLA MPs release. The results also illustrated that the CuO coating can improve the heat resistance of the 3D printed PLA sheet, exhibiting only very slight deformation when exposed to hot water. Although the mitigation study on 3D printed PLA is still in the lab scale, such metal oxide coating method should be one a research directions to reduce the MPs release and MPs pollution.

In conclusion, this thesis contributes valuable insights into understanding and mitigating the release of PMPs from biodegradable plastics, emphasizing the need for careful consideration and development of strategies to address this emerging environmental challenge.

7.2 Recommendations

The research has completed the investigation of PMPs and MPs release from different biodegradable products. However, further studies regarding to the release mechanism and mitigation method are still limited and urgently needed. Therefore, the following research aspects are recommended in the future study.

The various alkaline degradation behaviours indicated that the ratio of chemical additives existed in the biodegradable materials played a significant role to the results. Therefore, in-depth study can be conducted to investigated the leachate of degradation solution of PLA materials, identifying the chemical compounds of the additives, thereby further understanding the MPs and additive microparticles release mechanism.

In addition, more metal oxide materials need to be developed such as titanium dioxide to investigate the mitigation effect to PMPs release. Furthermore, the wet coating process is potential to be modified to improve the coating uniformity and efficiency. After the development of the standard protocol, large-scale experiments are worth implementing as a practical mitigation method to plastic pollution.

Reference

1. Hernandez, L.M., et al., *Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea.* Environ Sci Technol, 2019. **53**(21): p. 12300-12310.
2. Li, D., et al., *Microplastic release from the degradation of polypropylene feeding bottles during infant formula preparation.* Nature Food, 2020. **1**(11): p. 746-754.
3. Hahladakis, J.N., et al., *An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling.* J Hazard Mater, 2018. **344**: p. 179-199.
4. Research, G.v. *GVR Report coverBiodegradable Plastic Market Size, Share & Trends Report*

Biodegradable Plastic Market Size, Share & Trends Analysis Report By Product (Starch Based, PLA, PHA, PBAT, PBS), By Application (Packaging, Consumer Goods, Agriculture), By Region, And Segment Forecasts, 2023 - 2030. 2018; Available from: https://www.grandviewresearch.com/industry-analysis/biodegradable-plastics-market.

5. Farah, S., D.G. Anderson, and R. Langer, *Physical and mechanical properties of PLA, and their functions in widespread applications - A comprehensive review.* Adv Drug Deliv Rev, 2016. **107**: p. 367-392.
6. Jian, J., Z. Xiangbin, and H. Xianbo, *An overview on synthesis, properties and applications of poly(butylene-adipate-co-terephthalate)–PBAT.* Advanced Industrial and Engineering Polymer Research, 2020. **3**(1): p. 19-26.
7. Cheng, H., et al., *Starch-based biodegradable packaging materials: A review of their preparation, characterization and diverse applications in the food industry.* Trends in Food Science & Technology, 2021. **114**: p. 70-82.
8. Nazareth, M., et al., *Commercial plastics claiming biodegradable status: Is this also accurate for marine environments?* J Hazard Mater, 2019. **366**: p. 714-722.
9. Deroiné, M., et al., *Accelerated ageing of polylactide in aqueous environments: Comparative study between distilled water and seawater.* Polymer Degradation and Stability, 2014. **108**: p. 319-329.
10. Adhikari, D., et al., *Degradation of Bioplastics in Soil and Their Degradation Effects on Environmental Microorganisms.* Journal of Agricultural Chemistry and Environment, 2016. **05**(01): p. 23-34.
11. Qin, M., et al., *A review of biodegradable plastics to biodegradable microplastics: Another ecological threat to soil environments?* Journal of Cleaner Production, 2021. **312**.
12. Yang, L., et al., *High levels of microparticles release from biodegradable polylactic acid paper cups compared with polyethylene-lined cups.* Chemical Engineering Journal, 2023: p. 143620.
13. Banaei, G., et al., *The release of polylactic acid nanoplastics (PLA-NPLs) from commercial teabags. Obtention, characterization, and hazard effects of true-to-life PLA-NPLs.* J Hazard Mater, 2023. **458**: p. 131899.
14. Su, Y., et al., *Biodegradable and conventional microplastics posed similar toxicity to marine algae Chlorella vulgaris.* Aquatic Toxicology, 2022. **244**: p. 106097.
15. Liu, X., et al., *Effects comparison between the secondary nanoplastics released from biodegradable and conventional plastics on the transfer of antibiotic resistance genes between bacteria.* Environ Pollut, 2023. **317**: p. 120680.
16. Wei, R. and W. Zimmermann, *Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we?* Microb Biotechnol, 2017. **10**(6): p. 1308-1322.
17. Roser, H.R.a.M. *Plastic pollution*. 2018; Available from: https://ourworldindata.org/plastic-pollution.
18. Lebreton, L., M. Egger, and B. Slat, *A global mass budget for positively buoyant macroplastic debris in the ocean.* Sci Rep, 2019. **9**(1): p. 12922.

19. Qin, J., et al., *Generation of micro(nano)plastics and migration of plastic additives from Poly(vinyl chloride) in water under radiation-free ambient conditions.* Chemosphere, 2022. **299**: p. 134399.
20. Li, D., et al., *Alcohol Pretreatment to Eliminate the Interference of Micro Additive Particles in the Identification of Microplastics Using Raman Spectroscopy.* Environmental Science & Technology, 2022.
21. Schwabl, P., et al., *Detection of Various Microplastics in Human Stool: A Prospective Case Series.* Ann Intern Med, 2019. **171**(7): p. 453-457.
22. Ragusa, A., et al., *Plasticenta: First evidence of microplastics in human placenta.* Environ Int, 2021. **146**: p. 106274.
23. Liu, S., et al., *Detection of various microplastics in placentas, meconium, infant feces, breastmilk and infant formula: A pilot prospective study.* Science of The Total Environment, 2022: p. 158699.
24. Deng, Y., et al., *Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure.* Sci Rep, 2017. **7**: p. 46687.
25. Leslie, H.A., et al., *Discovery and quantification of plastic particle pollution in human blood.* Environment international, 2022. **163**: p. 107199.
26. Mattsson, K., et al., *Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles.* Environ Sci Technol, 2015. **49**(1): p. 553-61.
27. Mattsson, K., et al., *Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain.* Sci Rep, 2017. **7**(1): p. 11452.
28. Deng, Y., et al., *Evidence that microplastics aggravate the toxicity of organophosphorus flame retardants in mice (Mus musculus).* J Hazard Mater, 2018. **357**: p. 348-354.
29. Jin, Y., et al., *Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice.* Sci Total Environ, 2019. **649**: p. 308-317.
30. Van der Linden, A. and R. Almut, *European Environment Agency: Copenhagen*. 2020, Denmark.
31. Rujnic-Sokele, M. and A. Pilipovic, *Challenges and opportunities of biodegradable plastics: A mini review.* Waste Manag Res, 2017. **35**(2): p. 132-140.
32. Cho, H.S., et al., *Biodegradability and biodegradation rate of poly(caprolactone)-starch blend and poly(butylene succinate) biodegradable polymer under aerobic and anaerobic environment.* Waste Manag, 2011. **31**(3): p. 475-80.
33. Shen, M., et al., *Are biodegradable plastics a promising solution to solve the global plastic pollution?* Environ Pollut, 2020. **263**(Pt A): p. 114469.
34. Peelman, N., et al., *Heat resistance of new biobased polymeric materials, focusing on starch, cellulose, PLA, and PHA.* Journal of applied polymer science, 2015. **132**(48).
35. Mohammadi, M., M.C. Heuzey, and P.J. Carreau, *Morphological, thermal, and mechanical properties of cellulose nanocrystal reinforced poly(lactic acid) and poly(butylene adipate‐co‐terephthalate): A comparative study on common and novel solvent casting methods.* Polymer Composites, 2021. **42**(12): p. 6688-6703.
36. Gbadeyan, O.J., et al., *Cellulose nanocrystals and snail shell‐reinforced polyvinyl alcohol bioplastic films: Additive concentration optimization and mechanical properties assessment.* Journal of Applied Polymer Science, 2022. **139**(36).
37. Wiesinger, H., Z. Wang, and S. Hellweg, *Deep Dive into Plastic Monomers, Additives, and Processing Aids.* Environ Sci Technol, 2021. **55**(13): p. 9339-9351.
38. Hubbe, M.A., et al., *Formulating Bioplastic Composites for Biodegradability, Recycling, and Performance: A Review.* BioResources, 2021. **16**(1).
39. Bioplastics, E. *Applications for bioplastics*. 2022; Available from: https://www.european-bioplastics.org/market/applications-sectors/.
40. Domenek, S. and V. Ducruet, *Characteristics and applications of PLA.* Biodegradable and biobased polymers for environmental and biomedical applications, 2016: p. 171-224.
41. Bergstrom, J.S. and D. Hayman, *An Overview of Mechanical Properties and Material Modeling of Polylactide (PLA) for Medical Applications.* Ann Biomed Eng, 2016. **44**(2): p. 330-40.

42. Ferreira, F.V., et al., *An overview on properties and applications of poly(butylene adipate-co-terephthalate)-PBAT based composites.* Polymer Engineering & Science, 2019. **59**(s2): p. E7-E15.
43. Gao, X., D. Xie, and C. Yang, *Effects of a PLA/PBAT biodegradable film mulch as a replacement of polyethylene film and their residues on crop and soil environment.* Agricultural Water Management, 2021. **255**.
44. García-Guzmán, L., et al., *Progress in Starch-Based Materials for Food Packaging Applications.* Polysaccharides, 2022. **3**(1): p. 136-177.
45. Bugnicourt, E., et al., *Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging.* Express Polymer Letters, 2014. **8**(11): p. 791-808.
46. Williams, S.F., et al., *PHA applications: addressing the price performance issue: I. Tissue engineering.* International journal of biological macromolecules, 1999. **25**(1-3): p. 111-121.
47. Najar, I. and S. Das, *Poly-glutamic acid (PGA)-Structure, synthesis, genomic organization and its application: A Review.* International Journal of Pharmaceutical Sciences and Research, 2015. **6**(6): p. 2258.
48. Samantaray, P.K., et al., *Poly(glycolic acid) (PGA): a versatile building block expanding high performance and sustainable bioplastic applications.* Green Chemistry, 2020. **22**(13): p. 4055-4081.
49. Malikmammadov, E., et al., *PCL and PCL-based materials in biomedical applications.* J Biomater Sci Polym Ed, 2018. **29**(7-9): p. 863-893.
50. Barletta, M., et al., *Poly(butylene succinate) (PBS): Materials, processing, and industrial applications.* Progress in Polymer Science, 2022. **132**.
51. Van den Eynde, M. and P. Van Puyvelde, *3D Printing of Poly (lactic acid).* Industrial Applications of Poly (lactic acid), 2018: p. 139-158.
52. Arrieta, M.P., et al., *Characterization of PLA-limonene blends for food packaging applications.* Polymer Testing, 2013. **32**(4): p. 760-768.
53. Arrieta, M.P., et al., *On the Use of PLA-PHB Blends for Sustainable Food Packaging Applications.* Materials (Basel), 2017. **10**(9).
54. Thompson, A.A., et al., *Degradation Rate of Bio-based Agricultural Mulch is Influenced by Mulch Composition and Biostimulant Application.* Journal of Polymers and the Environment, 2019. **27**(3): p. 498-509.
55. Sun, D., et al., *An overview of the use of plastic-film mulching in China to increase crop yield and water-use efficiency.* Natl Sci Rev, 2020. **7**(10): p. 1523-1526.
56. Kasirajan, S. and M. Ngouajio, *Polyethylene and biodegradable mulches for agricultural applications: a review.* Agronomy for Sustainable Development, 2012. **32**(2): p. 501-529.
57. do Val Siqueira, L., et al., *Starch-based biodegradable plastics: methods of production, challenges and future perspectives.* Current Opinion in Food Science, 2021. **38**: p. 122-130.
58. Marichelvam, Jawaid, and Asim, *Corn and Rice Starch-Based Bio-Plastics as Alternative Packaging Materials.* Fibers, 2019. **7**(4).
59. Murariu, M., et al., *Engineered polylactide (PLA) - polyamide (PA) blends for durable applications: PLA with high crystallization ability to tune up the properties of PLA/PA12 blends.* European Journal of Materials, 2022: p. 1-23.
60. Hararak, B., et al., *Physicochemical properties of lignin nanoparticles from softwood and their potential application in sustainable pre-harvest bagging as transparent UV-shielding films.* Int J Biol Macromol, 2022.
61. Spiridon, I., et al., *Evaluation of PLA–lignin bioplastics properties before and after accelerated weathering.* Composites Part B: Engineering, 2015. **69**: p. 342-349.
62. Savva, K., et al., *Cytotoxicity assessment and suspected screening of PLASTIC ADDITIVES in bioplastics of single-use household items.* Chemosphere, 2023. **313**: p. 137494.
63. Eichers, M., et al., *Biobased plasticizer and cellulose nanocrystals improve mechanical properties of polylactic acid composites.* Industrial Crops and Products, 2022. **183**.

64. Zhao, X., et al., *Strategies and techniques for improving heat resistance and mechanical performances of poly(lactic acid) (PLA) biodegradable materials.* Int J Biol Macromol, 2022. **218**: p. 115-134.
65. Choi, J.S. and W.H. Park, *Effect of biodegradable plasticizers on thermal and mechanical properties of poly(3-hydroxybutyrate).* Polymer Testing, 2004. **23**(4): p. 455-460.
66. Belhassen, R., et al., *Thermoplasticized starch modified by reactive blending with epoxidized soybean oil.* Industrial Crops and Products, 2014. **53**: p. 261-267.
67. Ahmad, H., *Celluloses as support materials for antibacterial agents: a review.* Cellulose, 2021. **28**(5): p. 2715-2761.
68. Gyawali, R. and S.A. Ibrahim, *Natural products as antimicrobial agents.* Food Control, 2014. **46**: p. 412-429.
69. Agustin, Y.E. and K.S. Padmawijaya. *Effect of glycerol and zinc oxide addition on antibacterial activity of biodegradable bioplastics from chitosan-kepok banana peel starch*. in *IOP Conference Series: Materials Science and Engineering*. 2017. IOP Publishing.
70. Mousavi Khaneghah, A., S.M.B. Hashemi, and S. Limbo, *Antimicrobial agents and packaging systems in antimicrobial active food packaging: An overview of approaches and interactions.* Food and Bioproducts Processing, 2018. **111**: p. 1-19.
71. Wang, Z., et al., *Aging of poly (lactic acid)/poly (butylene adipate-co-terephthalate) blends under different conditions: Environmental concerns on biodegradable plastic.* Sci Total Environ, 2023. **855**: p. 158921.
72. Domenek, S., et al., *Potential of Lignins as Antioxidant Additive in Active Biodegradable Packaging Materials.* Journal of Polymers and the Environment, 2013. **21**(3): p. 692-701.
73. Woranuch, S., R. Yoksan, and M. Akashi, *Ferulic acid-coupled chitosan: thermal stability and utilization as an antioxidant for biodegradable active packaging film.* Carbohydr Polym, 2015. **115**: p. 744-51.
74. Hayes, D.G., et al., *Biodegradable plastic mulch films for sustainable specialty crop production.* Polymers for agri-food applications, 2019: p. 183-213.
75. Han, J., et al., *Characterization of physical, mechanical, and antioxidant properties of soy protein-based bioplastic films containing carboxymethylcellulose and catechin.* Food Science and Biotechnology, 2015. **24**(3): p. 939-945.
76. Yanat, M., I. Colijn, and K. Schroen, *Chitin Nanocrystals Provide Antioxidant Activity to Polylactic Acid Films.* Polymers (Basel), 2022. **14**(14).
77. Ageyeva, T., J.G. Kovács, and T. Tábi, *Comparison of the efficiency of the most effective heterogeneous nucleating agents for Poly(lactic acid).* Journal of Thermal Analysis and Calorimetry, 2021. **147**(15): p. 8199-8211.
78. Tsuji, H., et al., *Polyglycolide as a Biodegradable Nucleating Agent for Poly(L-lactide).* Macromolecular Materials and Engineering, 2008. **293**(12): p. 947-951.
79. Ye, C., et al., *Hydrochar as an environment-friendly additive to improve the performance of biodegradable plastics.* Sci Total Environ, 2022. **832**: p. 155124.
80. Shazleen, S.S., et al., *Functionality of Cellulose Nanofiber as Bio-Based Nucleating Agent and Nano-Reinforcement Material to Enhance Crystallization and Mechanical Properties of Polylactic Acid Nanocomposite.* Polymers (Basel), 2021. **13**(3).
81. Cui, H., et al., *Development of microwave-assisted extraction and dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry for the determination of organic additives in biodegradable mulch films.* Microchemical Journal, 2021. **160**: p. 105722.
82. Siegenthaler, K., et al., *Ecoflex® and Ecovio®: biodegradable, performance-enabling plastics.* Synthetic biodegradable polymers, 2012: p. 91-136.
83. Zhong, Q., et al., *Adopting Intrinsic Hydrophilic Thermoplastic Starch Composites to Fabricate Antifogging Sustainable Films with High Antibiosis and Transparency.* ACS Sustainable Chemistry & Engineering, 2022. **10**(11): p. 3661-3672.
84. Latos-Brozio, M. and A. Masek, *The application of natural food colorants as indicator substances in intelligent biodegradable packaging materials.* Food Chem Toxicol, 2020. **135**: p. 110975.

85. Sagnelli, D., et al., *Cross-Linked Amylose Bio-Plastic: A Transgenic-Based Compostable Plastic Alternative.* Int J Mol Sci, 2017. **18**(10).
86. Yang, W., et al., *Effect of cellulose and lignin on disintegration, antimicrobial and antioxidant properties of PLA active films.* Int J Biol Macromol, 2016. **89**: p. 360-8.
87. Cardoso, L.G., et al., *Development of active films poly (butylene adipate co-terephthalate) – PBAT incorporated with oregano essential oil and application in fish fillet preservation.* Industrial Crops and Products, 2017. **108**: p. 388-397.
88. Liu, D., et al., *Characterization of Active Packaging Films Made from Poly(Lactic Acid)/Poly(Trimethylene Carbonate) Incorporated with Oregano Essential Oil.* Molecules, 2016. **21**(6).
89. Rodríguez-Martínez, A.V., et al., *Migration kinetics of sorbic acid from polylactic acid and seaweed based films into food simulants.* LWT - Food Science and Technology, 2016. **65**: p. 630-636.
90. Fan, P., et al., *A review on the occurrence and influence of biodegradable microplastics in soil ecosystems: Are biodegradable plastics substitute or threat?* Environ Int, 2022. **163**: p. 107244.
91. Tosin, M., et al., *Laboratory test methods to determine the degradation of plastics in marine environmental conditions.* Front Microbiol, 2012. **3**: p. 225.
92. Niaounakis, M., et al., *Aging of packaging films in the marine environment.* Polymer Engineering & Science, 2019. **59**(s2): p. E432-E441.
93. Manfra, L., et al., *Biodegradable polymers: A real opportunity to solve marine plastic pollution?* J Hazard Mater, 2021. **416**: p. 125763.
94. Bao, R., et al., *Aging of biodegradable blended plastic generates microplastics and attached bacterial communities in air and aqueous environments.* J Hazard Mater, 2022. **434**: p. 128891.
95. Tang, K.H.D., *Microplastics in agricultural soils in China: Sources, impacts and solutions.* Environ Pollut, 2023. **322**: p. 121235.
96. Yang, Y., et al., *Kinetics of microplastic generation from different types of mulch films in agricultural soil.* Sci Total Environ, 2022. **814**: p. 152572.
97. Uzamurera, A.G., et al., *Thickness-dependent release of microplastics and phthalic acid esters from polythene and biodegradable residual films in agricultural soils and its related productivity effects.* J Hazard Mater, 2023. **448**: p. 130897.
98. Beltran-Sanahuja, A., et al., *Monitoring polymer degradation under different conditions in the marine environment.* Environ Pollut, 2020. **259**: p. 113836.
99. Liao, J. and Q. Chen, *Biodegradable plastics in the air and soil environment: Low degradation rate and high microplastics formation.* J Hazard Mater, 2021. **418**: p. 126329.
100. Wei, X.F., et al., *Millions of microplastics released from a biodegradable polymer during biodegradation/enzymatic hydrolysis.* Water Res, 2022. **211**: p. 118068.
101. Jariyasakoolroj, P., P. Leelaphiwat, and N. Harnkarnsujarit, *Advances in research and development of bioplastic for food packaging.* J Sci Food Agric, 2020. **100**(14): p. 5032-5045.
102. Capolupo, M., et al., *Bioplastic leachates characterization and impacts on early larval stages and adult mussel cellular, biochemical and physiological responses.* Environ Pollut, 2022: p. 120951.
103. Serrano-Ruíz, H., L. Martín-Closas, and A.M. Pelacho, *Application of an in vitro plant ecotoxicity test to unused biodegradable mulches.* Polymer Degradation and Stability, 2018. **158**: p. 102-110.
104. Ding, W., et al., *Effect thresholds for the earthworm Eisenia fetida: Toxicity comparison between conventional and biodegradable microplastics.* Science of The Total Environment, 2021. **781**.
105. Xie, M., et al., *Impacts of conventional and biodegradable microplastics on juvenile Lates calcarifer: Bioaccumulation, antioxidant response, microbiome, and proteome alteration.* Mar Pollut Bull, 2022. **179**: p. 113744.

106. Chagas, T.Q., A.P. da Costa Araújo, and G. Malafaia, *Biomicroplastics versus conventional microplastics: An insight on the toxicity of these polymers in dragonfly larvae.* Science of The Total Environment, 2021. **761**: p. 143231.
107. de Oliveira, J.P.J., et al., *Behavioral and biochemical consequences of Danio rerio larvae exposure to polylactic acid bioplastic.* Journal of hazardous materials, 2021. **404**: p. 124152.
108. Li, R., et al., *Investigating the effects of biodegradable microplastics and copper ions on probiotic (Bacillus amyloliquefaciens): Toxicity and application.* J Hazard Mater, 2023. **443**(Pt A): p. 130081.
109. González-Pleiter, M., et al., *Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments.* Environmental Science: Nano, 2019. **6**(5): p. 1382-1392.
110. Ainali, N.M., et al., *Do poly(lactic acid) microplastics instigate a threat? A perception for their dynamic towards environmental pollution and toxicity.* Sci Total Environ, 2022. **832**: p. 155014.
111. Shen, M., et al., *Recent advances in toxicological research of nanoplastics in the environment: A review.* Environ Pollut, 2019. **252**(Pt A): p. 511-521.
112. Torres, F.G., et al., *Sorption of chemical contaminants on degradable and non-degradable microplastics: Recent progress and research trends.* Sci Total Environ, 2021. **757**: p. 143875.
113. Fan, X., et al., *Investigation on the adsorption and desorption behaviors of antibiotics by degradable MPs with or without UV ageing process.* J Hazard Mater, 2021. **401**: p. 123363.
114. Ateia, M., et al., *Sorption behavior of real microplastics (MPs): Insights for organic micropollutants adsorption on a large set of well-characterized MPs.* Sci Total Environ, 2020. **720**: p. 137634.
115. Tong, H., et al., *Adsorption and Desorption of Triclosan on Biodegradable Polyhydroxybutyrate Microplastics.* Environ Toxicol Chem, 2021. **40**(1): p. 72-78.
116. Zhou, J., et al., *The microplastisphere: Biodegradable microplastics addition alters soil microbial community structure and function.* Soil Biology and Biochemistry, 2021. **156**.
117. Eschenbacher, W.L., et al., *Nylon flock–associated interstitial lung disease.* American journal of respiratory and critical care medicine, 1999. **159**(6): p. 2003-2008.
118. Thompson, R.C., et al., *Plastics, the environment and human health: current consensus and future trends.* Philosophical transactions of the royal society B: biological sciences, 2009. **364**(1526): p. 2153-2166.
119. Halden, R.U., *Plastics and health risks.* Annual review of public health, 2010. **31**: p. 179-194.
120. Rist, S., et al., *A critical perspective on early communications concerning human health aspects of microplastics.* Sci Total Environ, 2018. **626**: p. 720-726.
121. Law, K.L. and R.C. Thompson, *Microplastics in the seas.* Science, 2014. **345**(6193): p. 144-145.
122. Thompson, R.C., et al., *Lost at sea: where is all the plastic?* Science, 2004. **304**(5672): p. 838.
123. Coburn, C., *Microplastics and gastrointestinal health: how big is the problem?* The Lancet Gastroenterology & hepatology, 2019. **4**(12): p. 907.
124. The, L.P.H., *Microplastics and human health-an urgent problem.* The Lancet Planetary health, 2017. **1**(7): p. e254.
125. Foley, C.J., et al., *A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates.* Science of the total environment, 2018. **631**: p. 550-559.
126. Chae, Y. and Y.-J. An, *Effects of micro-and nanoplastics on aquatic ecosystems: Current research trends and perspectives.* Marine pollution bulletin, 2017. **124**(2): p. 624-632.
127. Lu, L., et al., *Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice.* Science of the total environment, 2018. **631**: p. 449-458.

128. Yang, Y.-F., et al., *Toxicity-based toxicokinetic/toxicodynamic assessment for bioaccumulation of polystyrene microplastics in mice.* Journal of hazardous materials, 2019. **366**: p. 703-713.
129. Mattsson, K., et al., *Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain.* Scientific reports, 2017. **7**(1): p. 11452.
130. Ibrahim, Y.S., et al., *Detection of microplastics in human colectomy specimens.* JGH Open.
131. Ragusa, A., et al., *Plasticenta: First evidence of microplastics in human placenta.* Environment International. **146**: p. 106274.
132. Schwabl, P., et al., *Detection of various microplastics in human stool: a prospective case Series.* Annals of internal medicine, 2019. **171**(7): p. 453-457.
133. Sturm, M.T., et al., *Determination of particles produced during boiling in differenz plastic and glass kettles via comparative dynamic image analysis using FlowCam®.* 2019.
134. Ranjan, V.P., A. Joseph, and S. Goel, *Microplastics and other harmful substances released from disposable paper cups into hot water.* Journal of Hazardous Materials, 2020. **404**: p. 124118.
135. Fadare, O.O., et al., *Microplastics from consumer plastic food containers: Are we consuming it?* Chemosphere, 2020. **253**: p. 126787.
136. Hernandez, L.M., et al., *Plastic teabags release billions of microparticles and nanoparticles into tea.* Environmental science & technology, 2019. **53**(21): p. 12300-12310.
137. Frias, J., et al., *Standardised protocol for monitoring microplastics in sediments. Deliverable 4.2.* 2018.
138. Li, D., et al., *Microplastic release from the degradation of polypropylene feeding bottles during infant formula preparation.* Nature Food, 2020.
139. Imhof, H.K., et al., *Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes.* Water research, 2016. **98**: p. 64-74.
140. Oßmann, B.E., et al., *Small-sized microplastics and pigmented particles in bottled mineral water.* Water research, 2018. **141**: p. 307-316.
141. Organization, W.H., *World Health Organization. How to prepare formula for bottle-feeding at home* 2007.
142. Käppler, A., et al., *Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both?* Analytical and bioanalytical chemistry, 2016. **408**(29): p. 8377-8391.
143. Zhao, S., et al., *An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy.* Analytical methods, 2017. **9**(9): p. 1470-1478.
144. Organization, W.H., *World Health Organization. Microplastics in drinking-water.* 2019.
145. Šunta, U., et al., *Adsorption of acetamiprid, chlorantraniliprole and flubendiamide on different type of microplastics present in alluvial soil.* Chemosphere, 2020. **261**: p. 127762.
146. Gong, W., et al., *Comparative analysis on the sorption kinetics and isotherms of fipronil on nondegradable and biodegradable microplastics.* Environmental Pollution, 2019. **254**: p. 112927.
147. Wong, M., et al., *Study of surface damage of polypropylene under progressive loading.* Journal of materials science, 2004. **39**(10): p. 3293-3308.
148. Zangmeister, C.D., et al., *Common Single-Use Consumer Plastic Products Release Trillions of Sub-100 nm Nanoparticles per Liter into Water during Normal Use.* Environmental Science & Technology, 2022.
149. Triantafillopoulos, N. and A.A. Koukoulas, *The future of single-use paper coffee cups: Current progress and outlook.* BioResources, 2020. **15**(3): p. 7260-7287.
150. Yuhui, M., *Problems and resolutions in dealing with waste disposable paper cups.* Science progress, 2018. **101**(1): p. 1-7.
151. Bourguignon, D., *Single-use plastics and fishing gear.* European Parliament, 2019. **12**.

152. Ipsos. *Three quarters of people in global survey want single-use plastics banned*. 2022; Available from: https://www.ipsos.com/en/attitudes-towards-single-use-plastics.
153. Commission, E. *EU restrictions on certain single-use plastics*. 2021; Available from: https://environment.ec.europa.eu/topics/plastics/single-use-plastics/eu-restrictions-certain-single-use-plastics_en.
154. Shao, L., Y. Xi, and Y. Weng, *Recent Advances in PLA-Based Antibacterial Food Packaging and Its Applications.* Molecules, 2022. **27**(18): p. 5953.
155. Markets, R.a. *Polylactic Acid Market Size, Share And Trends Analysis Report By End Use (Packaging, Agriculture, Transport, Electronics, Textile, Others), By Region, And Segment Forecasts, 2022 - 2030*. 2022; Available from: https://www.researchandmarkets.com/reports/5186217/polylactic-acid-market-size-share-and-trends.
156. Reaserch, M.M.R. *PLA Cup Market- Industry Analysis and Forecast (2022-2027) by Type, Application and Regions.* 2022; Available from: https://www.maximizemarketresearch.com/market-report/pla-cup-market/146528/
157. Wei, X.-F., et al., *Millions of microplastics released from a biodegradable polymer during biodegradation/enzymatic hydrolysis.* Water Research, 2022: p. 118068.
158. Schöpfer, L., et al., *Microplastics effects on reproduction and body length of the soil-dwelling nematode Caenorhabditis elegans.* Frontiers in Environmental Science, 2020. **8**: p. 41.
159. Maes, T., et al., *From pollution to solution: A global assessment of marine litter and plastic pollution*. 2021: United Nations Environment Programme.
160. Kamiya, M., S. Asakawa, and M. Kimura, *Molecular analysis of fungal communities of biodegradable plastics in two Japanese soils.* Soil science and plant nutrition, 2007. **53**(5): p. 568-574.
161. Shi, Y., et al., *The influence of drinking water constituents on the level of microplastic release from plastic kettles.* Journal of Hazardous Materials, 2021: p. 127997.
162. Li, D., et al., *Sampling, Identification and Characterization of Microplastics Release from Polypropylene Baby Feeding Bottle during Daily Use.* JoVE, 2021: p. e62545.
163. Lingle, T.R., *The coffee brewing handbook: a systematic guide to coffee preparation*. 2011: Specialty Coffee Association of America Santa Ana.
164. Karami, A., et al., *The presence of microplastics in commercial salts from different countries.* Scientific Reports, 2017. **7**(1): p. 1-11.
165. Shi, Y., et al., *Real-world natural passivation phenomena can limit microplastic generation in water.* Chemical Engineering Journal, 2022. **428**: p. 132466.
166. Catrouillet, C., et al., *Metals in microplastics: determining which are additive, adsorbed, and bioavailable.* Environ Sci Process Impacts, 2021. **23**(4): p. 553-558.
167. Dunzhu Li, E.D.S., Yunhong Shi, Liwen Xiao, Luming Yang, John J. Boland, Jing Jing Wang, *Alcohol pre-treatment to eliminate the interference of micro additive particles in the identification of microplastics using Raman spectroscopy* Environmental Science & Technology, 2022.
168. Weigel, S., et al., *Identification and quantification of additives in bituminous binders based on FTIR spectroscopy and multivariate analysis methods.* Materials and Structures, 2021. **54**(4): p. 1-9.
169. Almasi, H., et al., *Novel nanocomposites based on fatty acid modified cellulose nanofibers/poly (lactic acid): Morphological and physical properties.* Food Packaging and Shelf Life, 2015. **5**: p. 21-31.
170. Cuiffo, M.A., et al., *Impact of the fused deposition (FDM) printing process on polylactic acid (PLA) chemistry and structure.* Applied Sciences, 2017. **7**(6): p. 579.
171. Kister, G., G. Cassanas, and M. Vert, *Effects of morphology, conformation and configuration on the IR and Raman spectra of various poly (lactic acid) s.* Polymer, 1998. **39**(2): p. 267-273.
172. Wiley, J.H. and R.H. Atalla, *Band assignments in the Raman spectra of celluloses.* Carbohydrate Research, 1987. **160**: p. 113-129.

173. Farcas, M.T., et al., *Pulmonary exposure to cellulose nanocrystals caused deleterious effects to reproductive system in male mice.* Journal of Toxicology and Environmental Health, Part A, 2016. **79**(21): p. 984-997.
174. Brand, W., et al., *Overview of potential adverse health effects of oral exposure to nanocellulose.* Nanotoxicology, 2022: p. 1-30.
175. Endes, C., et al., *A critical review of the current knowledge regarding the biological impact of nanocellulose.* Journal of nanobiotechnology, 2016. **14**(1): p. 1-14.
176. Rogovina, S., et al., *Biodegradable blends of cellulose with synthetic polymers and some other polysaccharides.* European Polymer Journal, 2013. **49**(1): p. 194-202.
177. Reaserch, G.V. *Disposable Cups Market Size, Share & Trends Analysis Report By Product (Paper, Plastic, Foam), By End-use (Commercial, Institutional, Household), By Region, And Segment Forecasts, 2021 - 2028*. 2020; Available from: https://www.grandviewresearch.com/industry-analysis/disposable-cups-market.
178. Digvijay P, O.S. *Biodegradable Cups Market By Type (Single walled, Double Walled, Triple Walled), By Application (Food, Beverages), By Capacity (Up to 7 oz, 8 to 14 oz, 14 to 20 oz): Global Opportunity Analysis and Industry Forecast, 2021-2031*. 2022; Available from: https://www.alliedmarketresearch.com/biodegradable-cups-market-A08475.
179. Savva, K., et al., *Cytotoxicity Assessment and Suspected Screening of Plastic Additives in Bioplastics of Single-Use Household Items.* Available at SSRN 4188439.
180. de Oliveira, J.P.J., et al., *Behavioral and biochemical consequences of Danio rerio larvae exposure to polylactic acid bioplastic.* J Hazard Mater, 2021. **404**(Pt A): p. 124152.
181. Peelman, N., et al., *Application of bioplastics for food packaging.* Trends in Food Science & Technology, 2013. **32**(2): p. 128-141.
182. Nofar, M., A. Tabatabaei, and C.B. Park, *Effects of nano-/micro-sized additives on the crystallization behaviors of PLA and PLA/CO2 mixtures.* Polymer, 2013. **54**(9): p. 2382-2391.
183. Atarés, L. and A. Chiralt, *Essential oils as additives in biodegradable films and coatings for active food packaging.* Trends in Food Science & Technology, 2016. **48**: p. 51-62.
184. HealthDay. *Your Take-Out Coffee Cup May Shed Trillions of Plastic 'Nanoparticles'*. 2022 May 03, 2022; Available from: https://consumer.healthday.com/5-2-your-take-out-coffee-cup-may-shed-trillions-of-plastic-nanoparticles-2657224727.html.
185. Chen, H., et al., *Release of microplastics from disposable cups in daily use.* Sci Total Environ, 2022. **854**: p. 158606.
186. Cottafava, D., et al., *Assessment of the environmental break-even point for deposit return systems through an LCA analysis of single-use and reusable cups.* Sustainable Production and Consumption, 2021. **27**: p. 228-241.
187. The International Reference Center for Life Cycle of Products, S.a.S., *Life cycle assessment (LCA) of reusable and single-use coffee cups*. 2015.
188. ECHA, *Plastic additives initiative*. 2022.
189. Ranjan, V.P., A. Joseph, and S. Goel, *Microplastics and other harmful substances released from disposable paper cups into hot water.* J Hazard Mater, 2021. **404**(Pt B): p. 124118.
190. Luo, H., et al., *Effects of accelerated aging on characteristics, leaching, and toxicity of commercial lead chromate pigmented microplastics.* Environ Pollut, 2020. **257**: p. 113475.
191. Lin, Z., et al., *Current progress on plastic/microplastic degradation: Fact influences and mechanism.* Environ Pollut, 2022. **304**: p. 119159.
192. Liu, G., et al., *Disposable plastic materials release microplastics and harmful substances in hot water.* Science of The Total Environment, 2021: p. 151685.
193. Xu, J.-L., et al., *Spectral imaging for characterization and detection of plastic substances in branded teabags.* Journal of Hazardous Materials, 2021. **418**: p. 126328.
194. Huang, J.-C., A.S. Shetty, and M.-S. Wang, *Biodegradable plastics: A review.* Advances in Polymer Technology, 1990. **10**(1): p. 23-30.
195. Perera, K.Y., A.K. Jaiswal, and S. Jaiswal, *Biopolymer-Based Sustainable Food Packaging Materials: Challenges, Solutions, and Applications.* Foods, 2023. **12**(12).

196. Almroth, B.C., et al., *Single-use take-away cups of paper are as toxic to aquatic midge larvae as plastic cups.* Environmental Pollution, 2023.

197. Khosrovyan, A., et al., *Polylactic Acid-Based Microplastic Particles Induced Oxidative Damage in Brain and Gills of Goldfish Carassius auratus.* Water, 2023. **15**(11).

198. Li, D., et al., *Sampling, Identification and Characterization of Microplastics Release from Polypropylene Baby Feeding Bottle during Daily Use.* J Vis Exp, 2021(173).

199. Xiang, S., et al., *Evaluation of PLA content in PLA/PBAT blends using TGA.* Polymer Testing, 2020. **81**.

200. Baran, A., et al., *Solid state13C-NMR study of a plasticized PLA/PHB polymer blend.* Journal of Applied Polymer Science, 2018. **135**(21).

201. Semba, T., et al., *The effect of crosslinking on the mechanical properties of polylactic acid/polycaprolactone blends.* Journal of Applied Polymer Science, 2006. **101**(3): p. 1816-1825.

202. *Presence of microplastics and nanoplastics in food, with particular focus on seafood.* EFSA Journal, 2016. **14**(6).

203. Cheng, Y., et al., *Polylactic acid (PLA) synthesis and modifications: a review.* Frontiers of Chemistry in China, 2009. **4**(3): p. 259-264.

204. Wootthikanokkhan, J., et al., *Crystallization and thermomechanical properties of PLA composites: Effects of additive types and heat treatment.* Journal of Applied Polymer Science, 2013. **129**(1): p. 215-223.

205. Notta-Cuvier, D., et al., *Tailoring polylactide (PLA) properties for automotive applications: Effect of addition of designed additives on main mechanical properties.* Polymer Testing, 2014. **36**: p. 1-9.

206. Barrick, A., et al., *Plastic additives: challenges in ecotox hazard assessment.* PeerJ, 2021. **9**: p. e11300.

207. Sun, J., et al., *The surface degradation and release of microplastics from plastic films studied by UV radiation and mechanical abrasion.* Sci Total Environ, 2022. **838**(Pt 3): p. 156369.

208. He, W., et al., *Recent advances on microplastic aging: Identification, mechanism, influence factors, and additives release.* Sci Total Environ, 2023. **889**: p. 164035.

209. Sturm, M.T., et al., *Determination of particles produced during boiling in differenz plastic and glass kettles via comparative dynamic image analysis using FlowCam®.* Analytik News, 2019. **14**.

210. Zhang, J., et al., *Occurrence of Polyethylene Terephthalate and Polycarbonate Microplastics in Infant and Adult Feces.* Environmental Science & Technology Letters, 2021. **8**(11): p. 989-994.

211. Hwang, J., et al., *An assessment of the toxicity of polypropylene microplastics in human derived cells.* Science of the Total Environment, 2019. **684**: p. 657-669.

212. Luo, T., et al., *Maternal Polystyrene Microplastic Exposure during Gestation and Lactation Altered Metabolic Homeostasis in the Dams and Their F1 and F2 Offspring.* Environ Sci Technol, 2019. **53**(18): p. 10978-10992.

213. Stock, V., et al., *Uptake and effects of orally ingested polystyrene microplastic particles in vitro and in vivo.* Arch Toxicol, 2019. **93**(7): p. 1817-1833.

214. Jani, P., et al., *The uptake and translocation of latex nanospheres and microspheres after oral administration to rats.* J Pharm Pharmacol, 1989. **41**(12): p. 809-12.

215. Markets, M.a., *Biodegradable Plastics Market by Type (PLA, Starch Blends, PHA, Biodegradable Polyesters), End Use Industry (Packaging, Consumer Goods, Textile, Agriculture & Horticulture), and Region (APAC, Europe, North America & RoW) - Global Forecast to 2026*. 2023.

216. Tyler, B., et al., *Polylactic acid (PLA) controlled delivery carriers for biomedical applications.* Adv Drug Deliv Rev, 2016. **107**: p. 163-175.

217. Wei, X.-F., et al., *Microplastics generated from a biodegradable plastic in freshwater and seawater.* Water Research, 2021. **198**: p. 117123.

218. Weinstein, J.E., et al., *Degradation of bio-based and biodegradable plastics in a salt marsh habitat: Another potential source of microplastics in coastal waters.* Mar Pollut Bull, 2020. **160**: p. 111518.
219. Patil, S., et al., *Environmental prevalence, fate, impacts, and mitigation of microplastics-a critical review on present understanding and future research scope.* Environ Sci Pollut Res Int, 2021. **28**(5): p. 4951-4974.
220. Prata, J.C., et al., *Solutions and Integrated Strategies for the Control and Mitigation of Plastic and Microplastic Pollution.* Int J Environ Res Public Health, 2019. **16**(13).
221. Ward, M., *MARINE MICROPLASTIC REMOVAL TOOL*, U.S. Patent, Editor. 2015: US. p. 9.
222. Shi, Y., et al., *The influence of drinking water constituents on the level of microplastic release from plastic kettles.* J Hazard Mater, 2022. **425**: p. 127997.
223. Rabee, B.H. and B.A. Al-Kareem, *Study of optical properties of (PMMA-CuO) nanocomposites.* Int J Sci Res, 2016. **5**: p. 879-883.
224. V, B., V. D, and L. S, *Green synthesis of copper oxide nanoparticles for biological applications.* Microbiology: Current Research, 2018. **02**(01).
225. Popescu, C., et al., *Thermal spray copper-based coatings against contamination of thermoplastic surfaces: A systematic review.* Engineering Science and Technology, an International Journal, 2022. **35**.
226. Cheng, C. and M. Gupta, *Surface functionalization of 3D-printed plastics via initiated chemical vapor deposition.* Beilstein J Nanotechnol, 2017. **8**: p. 1629-1636.
227. Vaškelis, A., et al., *New Methods of Electroless Plating and Direct Electroplating of Plastics.* Transactions of the IMF, 2017. **75**(1): p. 1-3.
228. Shi, Y., et al., *Real-world natural passivation phenomena can limit microplastic generation in water.* Chemical Engineering Journal, 2022. **428**.
229. Chen, P., et al., *The synergistic effect of zinc oxide and phenylphosphonic acid zinc salt on the crystallization behavior of poly (lactic acid).* Polymer Degradation and Stability, 2015. **122**: p. 25-35.
230. Mulla, M.Z., et al., *Poly Lactic Acid (PLA) Nanocomposites: Effect of inorganic nanoparticles reinforcement on its performance and food packaging applications.* Molecules, 2021. **26**(7): p. 1967.
231. Zhou, X., et al., *Synergistic improvement of toughness of isotactic polypropylene: The introduction of high density polyethylene and annealing treatment.* Materials & Design, 2013. **49**: p. 502-510.
232. Ma, B., et al., *Effect of poly(lactic acid) crystallization on its mechanical and heat resistance performances.* Polymer, 2021. **212**.
233. Zhang, M., et al., *Biodegradable PLA/PBAT mulch on microbial community structure in different soils.* International Biodeterioration & Biodegradation, 2019. **145**.
234. Delgado, K., et al., *Polypropylene with embedded copper metal or copper oxide nanoparticles as a novel plastic antimicrobial agent.* Lett Appl Microbiol, 2011. **53**(1): p. 50-4.
235. Liu, L., et al., *On the degradation of (micro)plastics: Degradation methods, influencing factors, environmental impacts.* Sci Total Environ, 2022. **806**(Pt 3): p. 151312.

Published Papers:

1. Li, D., Yang, L., Kavanagh, R., Xiao, L., Shi, Y., Kehoe, D.K., Sheerin, E.D., Gun'ko, Y.K., Boland, J.J. and Wang, J.J., 2021. Sampling, identification and

characterization of microplastics release from polypropylene baby feeding bottle during daily use. JoVE (Journal of Visualized Experiments), (173), p.e62545.

2. Yang, L., Li, D., Shi, Y., Hill, C., Pilliadugula, R., Page, L., Wang, J.J., Boland, J. and Xiao, L., 2023. High levels of microparticles release from biodegradable polylactic acid paper cups compared with polyethylene-lined cups. Chemical Engineering Journal, p.143620.

TITLE:

Sampling, Identification and Characterization of Microplastics Release from Polypropylene Baby Feeding Bottle during Daily Use

AUTHORS AND AFFILIATIONS:

Dunzhu Li[1,2,†], Luming Yang[2,1,†], Rachel Kavanagh[1], Liwen Xiao[2,3*], Yunhong Shi[1,2], Daniel K. Kehoe[1], Yurii K. Gun'ko[4,5], John J. Boland[1,4*], Jing Jing Wang[1*]

[1]AMBER Research Centre and Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, Dublin, Ireland
[2]Department of Civil, Structural and Environmental Engineering, Trinity College Dublin, Dublin, Ireland
[3]TrinityHaus, Trinity College Dublin, Dublin, Ireland
[4]School of Chemistry, Trinity College Dublin, Dublin, Ireland
[5]BEACON, Bioeconomy SFI Research Centre, University College Dublin, Dublin, Ireland

†These authors contributed equally.

CORRESPONDING AUTHORS
Jing Jing Wang, jjwang@tcd.ie
John J. Boland, jboland@tcd.ie
Liwen Xiao, liwen.xiao@tcd.ie

Email of other authors
Dunzhu Li lidu@tcd.ie
Luming Yang YANGLU@tcd.ie
Rachel Kavanagh kavanara@tcd.ie
Yunhong Shi YUSHI@tcd.ie
Daniel K. Kehoe KEHOEDA@tcd.ie
Yurii K. Gun'ko IGOUNKO@tcd.ie

KEYWORDS:
Microplastics, plastic product, daily use, baby feeding bottle, hot water, polypropylene

SUMMARY:

This study detailed a reliable and cost-effective protocol for microplastics collection and detection from the daily use of plastic products.

ABSTRACT:

Microplastics (MPs) are becoming a global concern due to the potential risk to human health. Case studies of plastic products (i.e., plastic single-use cups and kettles) indicate that MP release during daily use can be extremely high. Precisely determining the MP release level is a crucial step to identify and quantify the exposure source and assess/control the corresponding risks stemming from this exposure. Though protocols for measuring MP levels

High levels of microparticles release from biodegradable polylactic acid paper cups compared with polyethylene-lined cups

Luming Yang[a,b]†, Dunzhu Li[b,a]†, Yunhong Shi[a,b], Christopher Hill[b,c], Rekha Pilliadugula[b,c], Laura Page[b,c], Jing Jing Wang[b,]*, John Boland[b,c,]*, Liwen Xiao[a,d,]*

[a] Department of Civil, Structural and Environmental Engineering, Trinity College Dublin, Dublin 2, Ireland.
[b] AMBER Research Centre and Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, Dublin 2, Ireland.
[c] School of Chemistry, Trinity College Dublin, Dublin 2, Ireland.
[d] TrinityHaus, Trinity College Dublin, Dublin 2, Ireland.

*Correspondence to: Jing Jing Wang, jjwang@tcd.ie; John J. Boland, jboland@tcd.ie;Liwen Xiao, liwen.xiao@tcd.ie
†For equal contributions

ABSTRACT

Polylactic acid (PLA) is a popular replacement for conventional fossil-fuel based plastics products such as polyethylene (PE) due to its high biodegradability and recyclability. Previous studies confirmed that PLA microplastics (MPs) and PE MPs pose similar toxicity risks due to that MPs' risk is primarily attributed to physical and indirect nutritional effects. Surprisingly, despite the widespread use, there have been very few studies of microparticles released from daily products made of biodegradable materials. We investigated release levels from eight single-use paper cups (SUPCs) lined with PLA and PE film. Under typical hot-beverage preparation conditions, the total number of particles released from PLA SUPCs was 4.2 times higher than that from PE SUPCs, with total numbers of 180,000±31,000 and 43,000±10,000 particles per litre, respectively. 22,000±6,000 microplastic (MP) particles were released per litre from PLA, which was 3.6 times the level of MPs released from PE SUPCs. In addition, significant levels of cellulose microfibres were released from PLA SUPCs, with quantities of 38,000±31,000 microfibres per litre, while no such fibres were released from PE SUPCs. It should be noted that the levels of these particles may be underestimated due to the exclusion of nanoparticles (<0.8 μm, filter's pore size), unidentified microparticles with high fluorescence backgrounds and attachments between different types of particles. In summary, a proportionately higher level of release of additive microparticles together with the release of cellulose microparticles are the key difference between biodegradable plastics like PLA and the conventional plastic such as PE. Cellulose blending in manufacturing is likely related to higher MPs release from PLA cups than PE cups. Therefore, it is highly recommended to re-assess the manufacturing process, potential health and environmental risks of biodegradable plastic products as well as the formulation of related policies.

Keywords: Microplastics, biodegradable plastics, polylactic acid, polyethylene, single-used paper cups

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