THE SHORT TERM STUDY OF POLYLACTIC ACID MICROPLASTICS UPTAKE BY *Eudrilus eugeniae*

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by

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K_{gr}	Growth rate (mg g^{-1} day^{-1}).
M_{org1}	Initial bodyweight of the earthworms (g).
M_{org2}	Final body weight of the earthworms (g).
t	Time
IR_t	Ingestion rate mg cast (g^{-1} $earthworm^{-1}$ day^{-1})
M_c	Weight of the collected cast dry weight (mg).
S_{PL}	The fraction of plastic in the casts.
M_{PL}	The dry weight of the microplastic in the casts(mg).
CF	The cast concentration factor.
S_S	The plastic fraction in the initial substrate.
M_{Tf}	The total feed (g).
M_c	The cast was collected during the 16 days (g).
CR	The consumption rate

LIST OF ABBREVIATIONS

PLA	Polylactic Acid
MPs	Microplastics
C D	Cow dung
LDPE	Low-Density Polyethylene
PS	Polystyrene
NOAA	National Oceanic and Atmospheric Administration
PE	Polyethene
PP	Polypropylene
PS	Polystyrene
PA	Polyamide
PES	Polyester
AC	Acrylic
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PMA	Polymethyl acrylate
PET	Polyethene terephthalate
AKD	Alkyd
PU	Polyurethane
POM	Polyoxymethylene
APM	Airborne particulate matter
USM	Universiti Sains Malaysia
USA	United States America
PPE	personal protective equipment
TAP	Total Available Phosphorus

KAJIAN JANGKA PENDEK PENYERAPAN MIKROPLASTIK POLYLACTIC ACID OLEH *Eudrilus eugeniae*

ABSTRAK

Mikroplastik yang tidak boleh terurai menghalang pertumbuhan hidupan flora dan fauna, dan akan menyebabkan kesan alam sekitar yang serius. Namun kini, terdapat alternatif lain dalam bentuk biopolimer, seperti polylactic acid (PLA), yang akan terurai secara semulajadi dan dijangkakan tidak akan memberi kesan negatif kepada alam sekitar. Dalam kajian ini, prestasi biodegradasi PLA dinilai dengan memerhatikan perubahan berat biomas cacing tanah *Eudrilus eugeniae* dan kepekatan mikroplastik dalam vermikas cacing. Cacing tanah diberi makan tinja lembu yang telah dicampur dengan PLA dalam kepekatan 0%, 10%, 30%, 60%, dan 80% w/w berat kering selama 16 hari. Kadar kematian cacing tanah untuk semua kepekatan PLA selama 16 hari tersebut adalah 0%. Walau bagaimanapun, didapati mikroplastik telah mempengaruhi berat cacing tanah (ANOVA sehala, p=0.00027), pengingsesan (ANOVA sehala, p= 0.037) dan kecekapan (ANOVA sehala, p= 0.0348), terutamanya pada kepekatan 80% PLA. Cacing tanah yang diberi makan 80% PLA menunjukkan kenaikan berat badan (17.74%) dan kadar pertumbuhan (0.47±0.00g/hari) yang paling rendah tetapi mempunyai kadar pengingsesan tertinggi (3.01±0.05g/g. hari). Sementara itu, cacing tanah yang diberi makan campuran pada kadar 30% dan 80% PLA masing-masing menunjukkan kecekapan tertinggi pada nilai 97.48% dan 91.10 %. Ini mungkin disebabkan oleh kesan mikroplastik PLA ke atas mekanisma pencernaan cacing tanah. Faktor kepekatan kas (CF) dan juga kepekatan bahan organik adalah yang tertinggi, masing-masing pada nilai 0.9 dan 90% untuk campuran 10% mikroplastik PLA. Oleh itu, penggunaan bahan organik oleh cacing tanah adalah yang tertinggi (0.79g/hari), menghasilkan kepekatan mikroplastik yang lebih tinggi dalam kas berbanding dengan campuran yang lain. Kesimpulan daripada kajian ini adalah meskipun PLA adalah sejenis biopolimer, ia tidak dapat didegradasi sepenuhnya oleh cacing tanah *Eudrilus eugeniae*. Pengumpulan mikroplastik mempengaruhi mekanisma pencernaan cacing yang akan mengancam kelangsungan hidupnya dalam jangka masa panjang.

THE SHORT TERM STUDY OF POLYLACTIC ACID MICROPLASTICS UPTAKE BY *Eudrilus eugeniae*

ABSTRACT

Non-degradable fossil-based microplastics can inhibit the growth of soil flora and fauna, and can cause serious environmental damage. Potential alternatives to fossil-based plastics are biopolymers, such as polylactic acid (PLA), which degrade over time and does not give negative impact to the environment. In this study, the biodegradation of PLA was evaluated by observing the changes in biomass of earthworms *Eudrilus eugeniae* and the microplastics concentration in the vermicast of the worms. The earthworms were fed with cow dung mixed with PLA for 16 days at concentrations of 0%, 10%, 30%, 60%, and 80% w/w dry weight. A 0% worm mortality rate for all PLA concentrations was obtained during the 16 days feeding period. However, the microplastic had significantly influenced the earthworms' weight (one way ANOVA, p=0.00027), ingestion (one way ANOVA, p= 0.037), and efficiency (one way ANOVA, p= 0.0348), especially at the concentration of 80% PLA. The earthworms fed with 80% PLA had the lowest weight gain (17.74%) and growth rate (0.47±0.00g/day) but the highest ingestion rate (3.01±0.05g/g. day). Meanwhile, the earthworms in 30% and 80% treatments had the highest efficiency of 97.48% and 91.10 %, respectively. This could have been caused by the effects of PLA on the earthworms' digestion mechanisms. The cast concentration factor (CF) and the feed's organic matter concentration were the highest at 0.9 and 90% for the treatment with 10% of PLA microplastics, respectively. Thus, the organic matter consumption by the earthworms was the highest (0.79g/day), resulting in an increasing concentration of microplastics in the cast compared to other treatments. This study concludes that even

though PLA has a biopolymer base, it cannot be degraded by the earthworms. Instead, the microplastic was accumulated within the earthworm's digestive system and influenced the worm's digestive mechanisms that could threaten their long-term survival.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In 2013, the global plastic packaging production has reached 78 million tons from which 98% were produced using virgin materials (Geyer et al., 2017). Although 14% of these plastics were collected for recycling, only 2% were eventually recycled, and about 32% of the produced plastics ended up in oceans or in landfills (Zhu et al., 2006). By 2018, the global production of plastics has reached 8.3 billion tons, from which 6.3 billion tons ended up as plastic waste (Geyer et al., 2017; Parker, 2019). Only 9% of plastics produced were recycled and 12% of the plastics waste was incinerated (Geyer et al., 2017). Approximately 79% of plastics waste ended up accumulating in landfills or leaked into the environment (Geyer et al., 2017). Even though reducing, reusing, and recycling of plastics have been encouraged worldwide, plastics waste still posed a significant problem due to its diversity in terms of properties and the quantities that have exponentially increased over the years.

Recently, there has been a growing concern mainly on the accumulation of plastics in the environment, which is detrimental to plants and wildlife. The degradation processes of plastics are extremely slow due to their high resistance towards environmental weathering and the difficulties of recovering them from the environment (Funabashi et al., 2009). In addition, environmental weathering may contribute to the breakdown of plastics into smaller or fine fragments, known as microplastics (MPs). Plastics and microplastic pollutions were first observed in aquatic environments (de Souza Machado *et al.* 2018) and may enter the soil ecosystem through plastics mulching materials that are used in agriculture and as secondary sources from the breaking down of plastics materials and sludge produced by wastewater treatments. A recent study in Europe showed that the amounts of MPs in agricultural soils ranges from 1000 to 4000 MPs per kilogram of soil. Runoffs, soil erosion, and winds contribute to polluting the aquatic environment and improper waste management for plastics (Machado *et al.,* 2018). MPs have been contaminating oceans in the estimation of 14,400 tons to 268,940 tons (Lithner *et al.,* 2011). As a result, the wildlife are severely affected by plastics and MPs in both the aquatic and terrestrial ecosystems (Cadee, 2002). In additions, these MPs are capable of carrying pollutants and heavy metals, negatively affecting wildlife, and can penetrate into the food chain (Rochman, 2013). There has been an increase in the documentation of MPs polluting the soil, resulting in potential detrimental effects on soil biodiversity and functions. Despite the widespread of plastic debris in the environment, information on the effects of MPs on terrestrial fauna is limited, and there is a lack of evidence on their destructive behaviour in the soil (Machado *et al.,* 2018).

To overcome this problem, the use of bioplastics and biodegradable plastics as alternatives to fossil-based plastics are being promoted for microorganism degradation, such as by bacteria and fungi. The biodegradable polymer is converted into carbon dioxide (CO_2) and water (H_2O) as a result of the biodegradation process stimulated by these microorganisms. A widely used biopolymer is based on polylactic acid (PLA), produced from starch tapioca roots or sugarcane. It is biodegradable as well as hydro-degradable, and can be an alternative to traditional petroleum-based plastics such as PE, PP, PS, and PVC (Ahmed, *et al.,* 2018; Liu, *et al.,* 2000). PLA is widely used in agricultural and food packaging applications, with production in the USA reaching 150,000 tons/year. Generally, it has been shown that microorganisms are able to degrade PLA plastics, although the interaction of PLA with earthworms is yet to be characterised.

In general, earthworms are known to improve the soil structure, and the decomposition and mineralization of litter by breaking down organic matter and increasing soil fertility. Known as soil engineers, their presence have a positive impact on soil properties and influences other species' availability, including microorganisms and plants. However, less attention has been paid on their impact to the soil ecosystem (Kooch & Jalilvand, 2008), and the potential of earthworms for plastics degradation and toxicity are yet to be evaluated in detail. Recent studies, such as those by Lwanga *et al.*(2016) and Rillig *et al.* (2017) have observed the possibilities of surface microplastics transported by the earthworms into the more in-depth soil profiles. Earthworms are suitable candidate for investigation into the transportation and effects of plastic and MPs pollution in the soil due to their natural behaviours.

The mortalities of earthworms caused by petroleum and bio-based MPs have been observed in several studies. For example, according to Cao *et al.* (2017), a 2% of polystyrene (PS) mixed with the earthworms feed has caused a significant inhibition to the growth of the earthworms. In comparison, Lwanga *et al.* (2017) have found that for low-density Polyethylene (LDPE) feed mix, the earthworms' mortality rate was between 8-25% when the LDPE concentration was 28 % and 60%, respectively. However, a contradictory result was observed by Zhang *et al.* (2018a), which recorded zero per cent mortality of worms exposed to LDPE films. This result might be caused by the selective behaviour of the earthworms toward their feeds.

PLA plastics have become one of the leading bioplastics for a wide range of industries such as agriculture, food packaging, and medical applications. Like any plastics, the possibility of PLA plastics entering the soil profile is highly likely, due to the limitation of PLA recycling facilities. Some studies have shown the degradability of PLA by earthworms, such as by Alauzet *et al.* (2002). However, in some cases, the PLA material was treated prior to exposure to the earthworms, which has improved the material's degradability due to the reduction in the molecular weight of the PLA. This research attempts to understand the biodegradable nature of PLA MPs by earthworms by determining the health effects on the worms due to exposure to PLA and the extent of PLA biodegradation. To achieve this, the PLA samples would be ingested by the earthworms and enter into their digestive system. It is expected that the earthworm’s digestives enzymes microflora would degrade the PLA, although the PLA may also exhibit its toxic effects. This testing method would simulate the earthworms' natural behaviours as these PLA particles enter the soil profile and mixed with the worm’s feed. Moreover, the findings from this study would contribute to the understanding of the digestibility of PLA MPs' by the earthworms and the side effect of these particles on their health if consumed as a secondary source of carbon, mixed with the main feed of the worms.

1.2 Problem Statement

Polylactic acid (PLA) is categorised as a bio-based polymer. PLA mulching materials are being used in agricultural crop productions to control the soil environment. It is usually buried in the soil after use as it is considered a biodegradable material. Several studies have been conducted on the effects of PLA towards the mortality of worms in the soil, such as the study by Alauzet *et al.*(2002). Qi *et al.* (2018a) have observed the weight loss of earthworms with zero rates of mortality. However, the worms were unable to digest and consume the PLA as a carbon source (Qi *et al.*, 2018). Even though PLA is a plant-based polymer, it shows resistance to degradation without prior exposure to hydrolysis degradation. These past research were conducted using PLA $_{50}$ and PLA$_{96}$, and there is yet to be any analysis on the effect of commercial PLA on the health of *Eudrilus eugeniae*. *Eudrilus eugeniae,* commonly known as African Nightcrawler, is an earthworm species native to tropical west Africa and is now widespread in warm regions under vermicompost. It has a natural ability to colonize organic waste, with high endurance and handling resistance, possess tolerance to a wide range of environmental factors, and capable of digestion and bioassimilation of organic matter (Kooch et al., 2008). However, the PLA material's effect on *Eudrilus eugeniae* earthworms as an organic matter composter, is yet to be evaluated. Thus, in this study, *Eudrilus eugeniae* earthworms are selected as the main subject to determine the growth rate effect and bioassimilation of PLA of the earthworms by observing the effect of different concentrations of PLA MPs on their weight changes and the efficiency of vermicast production. This study aims to determine the effect of PLA MPs on the *Eudrilus eugeniae* and determine the relationship between the MPs concentration and the earthworm’s weight and uptake of PLA.

1.3 Research Questions and Hypotheses

This study aims to address the following questions:

a) Will *Eudrilus eugeniae* earthworms be capable of biodegrading PLA?

b) How significant will the growth rate and the ingest rate be affected by the PLA in the worms' diet?

c) Will there be any actual effect on *Eudrilus eugeniae* efficiency in producing vermicast and MPs' concentration in the vermicast due to the different concentrations of PLA MPs in the worms' diet?

This study proposes the following hypotheses:

a) Worms enhance the biodegradation of polylactic acid (PLA) microplastics by providing suitable conditions (pH, nutrients, and moisture) for microbial degradation.

b) *Eudrilus eugeniae* growth rate and ingestion rate will show no noticeable significant effect with the increase of PLA MPs in their diet.

c) PLA MPs will not affect *Eudrilus eugeniae* efficiency in producing vermicast and lower MPs' concentration in the vermicast since PLA is a biodegradable plastic.

1.4 Objectives

Generally, the aim of the study is to assess the short-term biodegradation of PLA MPs by *Eudrilus eugeniae* earthworms. This is achieved by addressing these specific objectives:

a) To assess the ability of *Eudrilus eugeniae* worms to degrade PLA MPs.

b) To estimate the growth rates of *Eudrilus eugeniae* earthworms upon ingestion of PLA MPs.

c) To determine the cast concentration factor (CF) and the earthworm’s efficiency in producing vermicast.

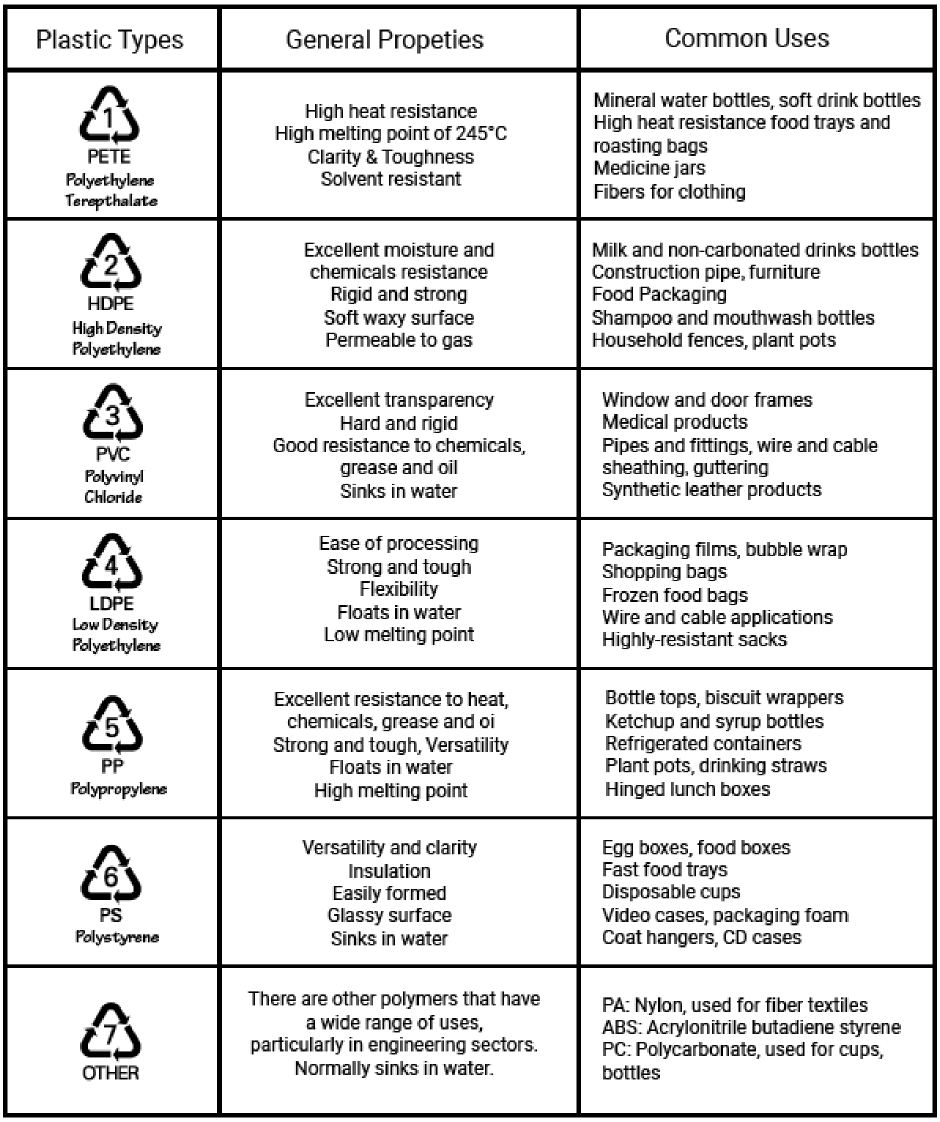
CHAPTER 2

LITERATURE REVIEW

2.1 Types of Plastics Waste

Plastics are defined as semi-organic materials having the physical behaviour and chemical properties of polymers. Polymers are subcategories of long-chained material and long-chained molecules, having very high average molecular weights. They can be produced either naturally or synthetically, moulded into shapes, and they include both resin pellets and virgin plastics (GESAMP, 2015; Narayan, 1993; Scott, 1999).

Synthetic polymer materials are extensively used in various segments of everyday life due to their high plasticity characteristics, as shown in Figure 2.1. Most of these plastics are produced through the polymerization of monomers (low molecular weight) into polymers (high molecular weight) which are known as thermoplastics polymers (Yang *et al.* 2011). The global production of plastics has increased from 275 million metric tons of plastic in 2010 to 380 million metric tons in 2015, as shown in Figure 2.2. From these amounts, 4.8 to 12.7 million metric tons have entered the ocean (Liu *et al.* (2017). Unfortunately the recovery or recycling of plastics still remains very low, with around 79% of plastics produced usually end up in landfills and natural systems (Geyer, Jambeck, & Law, 2017b). Approximately 32% of the waste plastics are being accumulated in the soil or continental aquatic ecosystem (Jambeck, 2015). The recent COVID-19 crisis from 2019 has increased the dependency on single used plastics for safety, such as in the production of personal protective equipment (PPE), bottled water, plastic bags, and packaging. However, with no proper disposal and shut downs of recycling facilities, used gloves and face mask are being littered on the streets and public spaces, as well as adding up to the domestic household wastes which are sent landfills and incineration facilities (Rob Picheta, 2020; Roberts, Keiron ; Stringfellow, Anne; Williams, 2020).



Plastic Types	General Propeties	Common Uses
1 PETE Polyethylene Terepthalate	High heat resistance High melting point of 245°C Clarity & Toughness Solvent resistant	Mineral water bottles, soft drink bottles High heat resistance food trays and roasting bags Medicine jars Fibers for clothing
2 HDPE High Density Polyethylene	Excellent moisture and chemicals resistance Rigid and strong Soft waxy surface Permeable to gas	Milk and non-carbonated drinks bottles Construction pipe, furniture Food Packaging Shampoo and mouthwash bottles Household fences, plant pots
3 PVC Polyvinyl Chloride	Excellent transparency Hard and rigid Good resistance to chemicals, grease and oil Sinks in water	Window and door frames Medical products Pipes and fittings, wire and cable sheathing, guttering Synthetic leather products
4 LDPE Low Density Polyethylene	Ease of processing Strong and tough Flexibility Floats in water Low melting point	Packaging films, bubble wrap Shopping bags Frozen food bags Wire and cable applications Highly-resistant sacks
5 PP Polypropylene	Excellent resistance to heat, chemicals, grease and oi Strong and tough, Versatility Floats in water High melting point	Bottle tops, biscuit wrappers Ketchup and syrup bottles Refrigerated containers Plant pots, drinking straws Hinged lunch boxes
6 PS Polystyrene	Versatility and clarity Insulation Easily formed Glassy surface Sinks in water	Egg boxes, food boxes Fast food trays Disposable cups Video cases, packaging foam Coat hangers, CD cases
7 OTHER	There are other polymers that have a wide range of uses, particularly in engineering sectors. Normally sinks in water.	PA: Nylon, used for fiber textiles ABS: Acrylonitrile butadiene styrene PC: Polycarbonate, used for cups, bottles

Figure 2.1 Most commonly used types of plastics and their application in daily life.

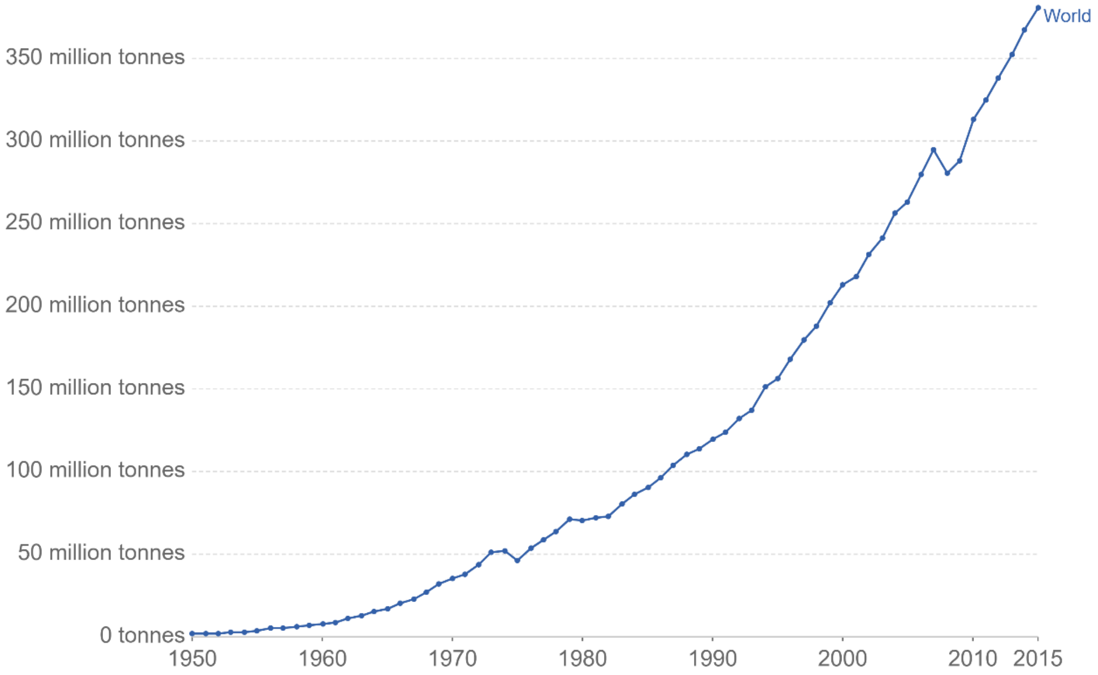


Figure 2.2 the global plastics production 1950 to 2015 (Geyer *et al.* 2017)

*Annual global polymer resin and fibre production (plastics production) measured in metric tonnes per year.

High volume plastic production, coupled with non-systematic waste management would result in significant accumulation of plastics in the environment. A large portion of waste plastics ends up in marine and terrestrial ecosystems, as shown in Figure 2.3. The plastic pollution of the marine ecosystem is well acknowledged and observable as the waste can float due to the differences in densities. However, as the plastic degrade and fragment over time, they are transformed into micro-sized microplastics (MPs). MPs are causing long-lasting pollution in both the marine and terrestrial ecosystems. However, the presence of microplastic in the terrestrial ecosystem was brought into the limelight only recently. It is harder to detect microplastic in the soil, as the disintegration of plastics or biodegradation is easier in soil media as compared to in water.

Most plastics, including biodegradable plastics, tend to disintegrate rather than being degraded (Whitacre,2014). Fragmentation into smaller sizes creates a bigger problem and challenge for scientists to solve. MPs pollution is more hazardous to the environment, as shown in Figure 2.4. To overcome this problem, new plant-based plastic materials are being introduced as an alternative to regular petroleum-based plastics. These bioplastics seek to be more environmentally friendly by being able to biodegrade into simpler polymer forms.

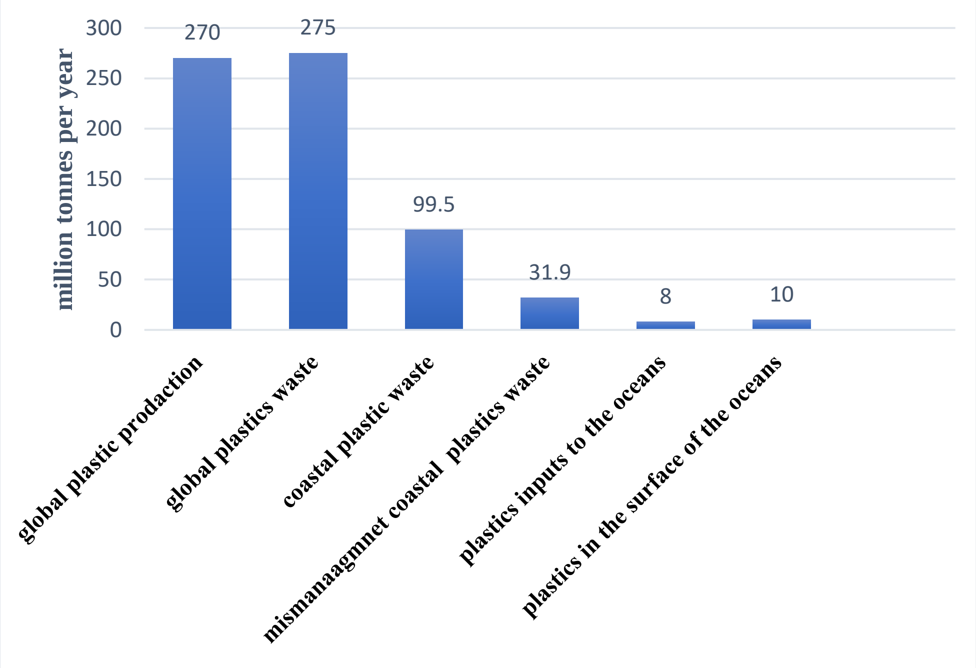


Figure 2.3 The amount of plastics waste entrance the different ecosystems (Eriksen *et al.* 2014).

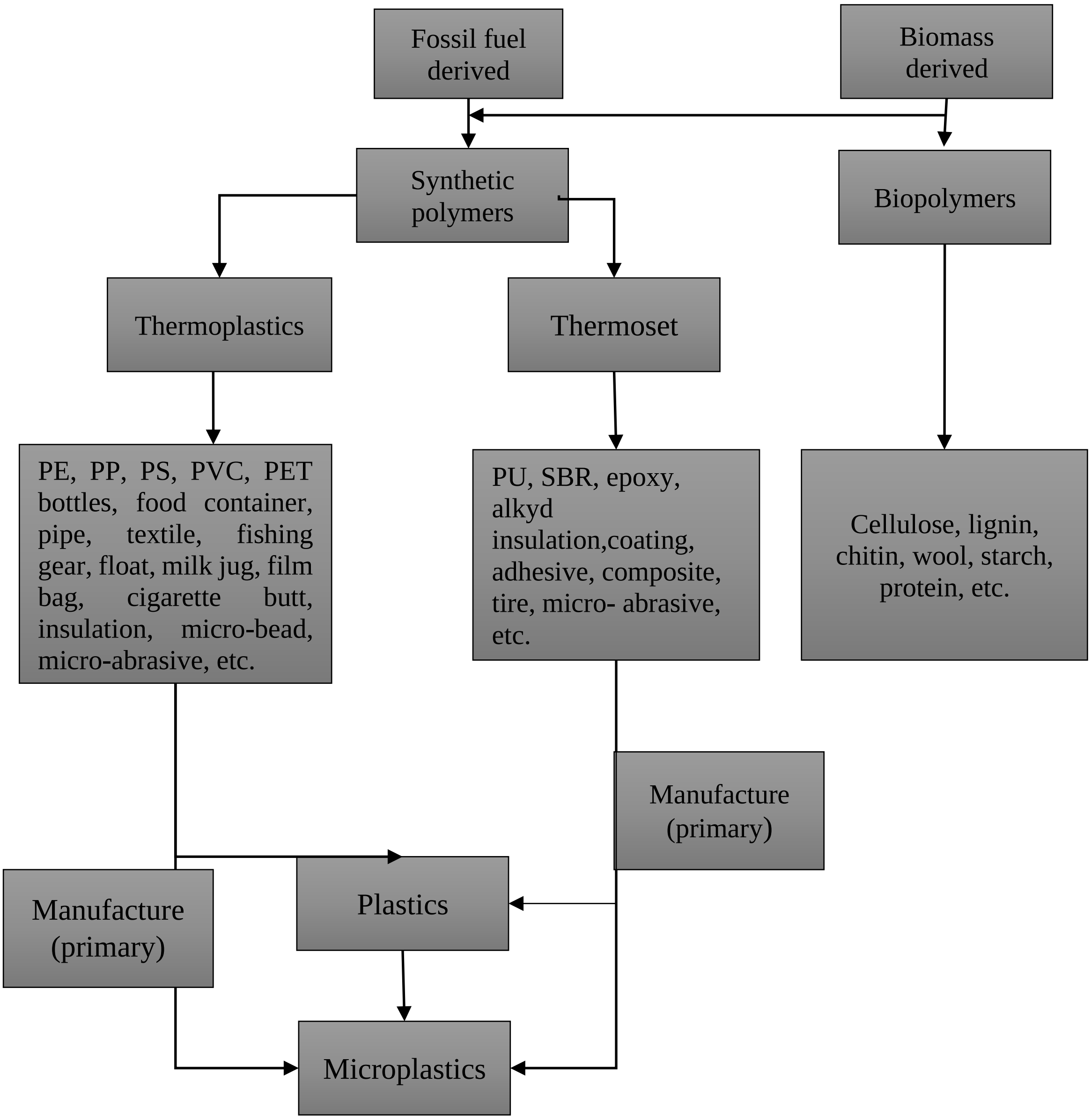


Figure 2.4 Illustrations shows the relation between primary material source, synthetic and natural polymers, thermoplastics, and thermoset plastics(GESAMP, 2015; Narayan, 1993; Scott, 1999)

2.1.1 The Emerging of Bioplastics as Sustainable Plastics

Bioplastics are produced from feedstocks such as plants and crop waste, cellulose, starch, and protein. They are available in both biodegradable and non-biodegradable forms (Byun & Kim, 2013; Gonçalves de Moura *et al.*, 2017; Laycock & Halley, 2014). However, to be classified as bio-based plastics, they are required to be produced from renewable resources and be biodegradable and compostable based on the standard of compostability and biodegradability. The usage of biodegradable plastics is rising in both industrial and consumer markets due to the increasing concerns of sustainability and environmentally friendly solutions for the problem caused by the accumulation of plastic waste in the environment.

In recent years, significant consumption of biodegradable plastics have been observed, especially in food packaging, material hygiene product, and agricultural tools. However, non-degradable plastics still dominate the market due to their practical properties and lower production costs (Gross, 2012; Sudesh et al., 2008).

Bioplastics are renewable and sustainable resources, known primarily by aliphatic bio-polyester, as shown in Figure 2.5 (Altaf M, Venkateshwar M, Srijana M, 2007; Carus, 2012). Their compostability and biodegradation properties means that the quantity of waste sent to landfills can be reduced (Barnett, 2011). Bioplastic usage in the United Kingdom has enabled the reduction of 7.1 million tons of waste from being sent to landfills in 2010 (Byun et al., 2013). Globally, bioplastics production has reached 1.7 million metric tons in 2014 and it expected to grow by 20-30% annually. There are several types of bio-based plastics, with Polylactic Acid (PLA) being one of the leading type of bioplastics (Jamshidian *et al.* 2010).

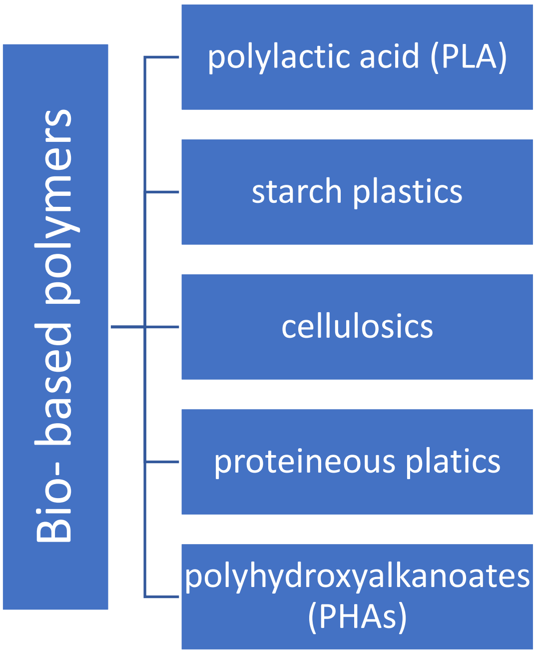


Figure 2.5 Types of renewable-based plastics(Song *et al.* 2009)

2.1.1(a) Polylactic acid (PLA) as Biodegradable Plastics

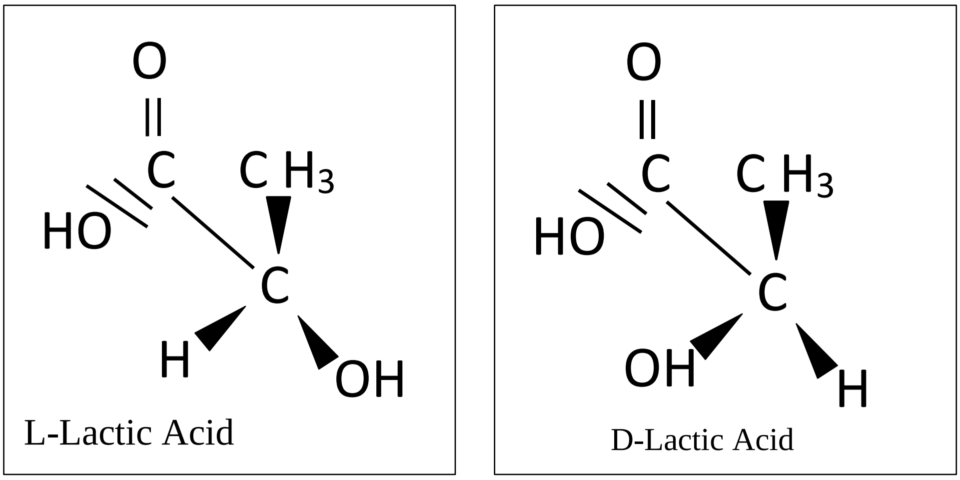
Polylactic acid (PLA) is a biodegradable thermoplastic polymer derived from biomass resources through carbohydrate fermentation or chemical synthesis. Lactic acid is the main unit for polylactic acid. It can be produced by carbohydrate formation of the simple form of sugar or chemical synthesis by condensation polymerization. PLA is considered the best replacement for commercially produced plastics such as HDPE, LDPE, PET, and PS (Peelman *et al.* 2013).

The advantage of using PLA over other types of material is the possibility of obtaining PLA from renewable agriculture sources, its production captures CO_2, and it is also recyclable and compostable (Drumright, Gruber and Henton, 2000; Whiteman, N.F., 2000; Gonçalves de Moura *et al.* 2017). The primary manufacturers with bioplastics patents in the US between 1993 to 2012 are Nissei, Cereplast, Kimberly Clark, Biotic, Novamont, Metabolix, and Cargill (NatureWorks). These companies accounts for 43% of PLA plastics production in the US. The main application of PLA is tabulated in Table 2.1. In the USA, PLA production has reached 140,000 metric tons per year by Blair facility only (Jamshidian *et al.* 2010; Gonçalves de Moura *et al.* 2017). For commercial purposes, it is highly common to blend PLA with other polymers to reduce the production cost. These polymers include polyethene oxide (PEO), polyvinyl alcohol (PVA), and polyethene glycol (Gajria *et al.* 1996; Nijenhuis *et al.* 1996; Sheth *et al.* 1997). These polymer mixtures are still considered bio-based polymers. Their chemical structures and molecular weights play a major role in changing the mechanical and physical properties of the material, increasing their resistance to environmental conditions. In general, Polylactic acid properties are influenced by the backbone's stereoisomer structure (Tokiwa *et al.*, 2009), as shown in Figure 2.6. The stereoisomers influence the crystallization rate and the mechanical properties of PLA. In commercial applications, the mechanical properties of PLA are more favourable compared to polystyrene and polyethylene terephthalate. Commonly, there are three main production methods for PLA with high molecular mass. They are direct condensation polymerization, azeotropic dehydrative condensation, and polymerization through lactide formation, as mentioned by Auras *et al.*(2004).

Theoretically, blending PLA with other types of polymers increases their degradability. However, microorganisms, which are the main cause of biodegradation, are more attracted to glucose sources rather than the carbon sources contained in plastics. Even assuming that plastics are attracting microorganisms, their complexity still reduces the possibilities for degradation. Although PLA are widely used in packaging applications, only few studies have shown that PLA would only degrade under specific conditions with certain types of microbes, as listed in Table 2.2. These studies have shown the possibility of PLA degradation by microorganisms (Tokiwa *et* *al.*, 2009). However, PLA degraders are scarcely found in every environments, thus soil degradation still remains low (Ohkita & Lee, 2006; Urayama *et al.*, 2002).

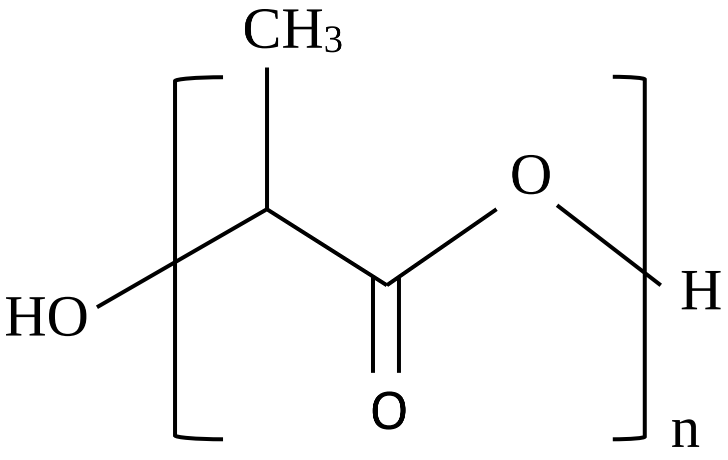
Table 2.1 The polylactic acid plastics application (Gonçalves de Moura *et al.* 2017).

Application	Biopolymer
Coffee and tea	Cardboard cups coated with PLA
Beverages	PLA cups
Fresh salads	PLA bowls
Carbonated water, fresh juices, and dairy drinks	PLA bottles
Freshly cut fruits, whole fruit vegetables, bakery goods, and salads	Rigid PLA trays and packs
Organic pretzels and potato chips	PLA bags
Yoghurt	PLA jars
Frozen fries	PLA films (Bio-Flex)
Organic fruit and vegetables	PLA packaging
Pasta	PLA packaging
Herbs	PLA packaging
Prepared sandwiches and pasta salads	PLA bowls, packaging
Bread	Paper bags with PLA window
Organic poultry	PLA bowls and absorbs pads



L-Lactic Acid

D-Lactic Acid



D, L- Lactic acid

Figure 2.6 Chemical structure of L-Lactic Acid, D-lactic Acid, and D, L- Lactic Acid (Tokiwa et al., 2006).

Table 2.2 The studies that reported the degradation of PLA by employing microbial Enzymatic

Type of microbes/ enzymes used in the degradation of PLA	Reported by
Amycolatopsis sp. & Saccharotrix	Pranamuda *et al.* (1997)
α-chymotrypsin	Lim *et al.*(2005)
Several esterase-type enzymes, especially Rhizopus Delmar lipase	Fukuzaki *et al.*(1989)
Proteinase K, bromelain and pronase enzymes.	Williams (1981)

2.2 Microplastics in The Environment

One of the severe global pollution in this era is plastics and microplastics (MPs) pollution. The first report of small fragments of plastics floating on the sea surface was in the early 1970s by Carpenter & Smith (1972). The term microplastics was initially used for marine debris. Ryan, P.G., Moloney (1990) were the first to describe the distribution of plastics fragments in seawater in the Sea Education Association report in the 1990s. In the early years of discovery of microplastics, there was no formal size definition, as the term was generally implied as material that can only be detected with the aid of a microscope. Since then, the term ‘microplastics’ are used to describe small pieces of plastics in the millimetre to the sub-millimetre size range. In 2008, the National Oceanic and Atmospheric Administration (NOAA) was the first to acknowledge MPs' effect and fate in the marine ecosystem (GESAMP, 2015). They recommended the upper breaking point size of microplastic of 5mm. The particles may incorporate a broad scope of small particles that could promptly be ingested by biota.

Such particles may have various types of increased risk than bigger-sized plastics (GESAMP, 2015). MPs are produced through a deterioration of larger plastics or industrially produced as small particles, typically defined as plastic particles < 5mm as mentioned by numerous researchers (Rillig, Ingraffia, et al., 2017; Rillig, Ziersch, et al., 2017).

Small plastic particle sizes behave differently than large plastic pieces in the environment. Due to their microscopic sizes, they can be ingested and accumulated in the food chain. The enlargement of the particle's surface area increases their absorption of pollutants on the surface and release of toxic chemicals. Upon entering the ecosystem, MPs can become highly resistant to degradation (US National Library of Medicine, 2017; Rogers, 2019).

The impact of plastics and microplastics on marine systems have been the focus of most researchers and environmental advocates for many years. In contrast, the knowledge of the impact of microplastics towards the terrestrial ecosystem is still lacking. In addition, it is understood that plastics in the soil are more wide-ranging with different polymers such as Polyethylene, Polypropylene, Polystyrene, Polyamide, Polyester, Acrylic, Polyvinyl alcohol, Polyvinylchloride, Polymethyl acrylate, Polyethylene terephthalate Alkyd, Polyurethane, and Polyoxymethylene. Horton et al. (2017) stated that the amounts of MPs in terrestrial ecosystem might be 4-32 times larger than in the ocean. Plastic particles are easily detected in the ocean because of the density differences between water and plastics, causing them to float. However, the damage can be more severe in the ocean as compared to a terrestrial ecosystem because the plastics in the ocean can easily be ingested by animals such as fish and turtles. In terrestrial ecosystems, MPs are hard to detect without testing the soil samples. In Australia, a study has introduced a new indication of microplastic presence in the terrestrial environment, which were able to measure the microplastic's concentrations in the industrial area using pressurized fluid extraction combined with FTIR spectral matching (Fuller & Gautam, 2016)

Nonetheless, MPs are becoming a major concern in agroecosystems in the mainland, as mentioned by Nizzetto *et al.*(2016); Steinmetz *et al.* (2016). For example, synthetic fibres were found in the US soil, where organic wastewater sludge was released. Through polarized light microscopy examinations, the presence of these fibres was noticeable in field site soils which can be traced back to 15 years after applications, maintaining their sludge products' characteristics (Zubris and Richards, 2005). Nizzetto, Langaas, & Futter (2016) estimated that between 63,000– 430,000 and 44,000–300,000 tons of MPs could have been added annually to farmlands in Europe and North America, respectively. These figures exceed the estimated global burden of MPs in oceanic surface waters of 93,000–236,000 tons. These microplastics present a danger to the soil biota.

MPs are classified based on their two sources of entering the ecosystem, as in Figure 2.7. The first category consists of manufactured microplastics. Usually, manufactured MPs are used in personal hygiene and cleaning products. This usage of MPs is mainly related to their ability to scrape the surface that comes in contact due to their relatively high surface area and high capacity to absorb chemicals and substances. However, some countries such as Canada, France, New Zealand, Sweden, Taiwan, and the United Kingdom have banned the manufacturing of MPs (GESAMP, 2015). The ban was due to their destructive impact on the wildlife, the persistent particles that are toxic on the environment, difficulties of detecting these MPs and removing them upon entering the environment. Secondary source of MPs comes from the disintegration of bigger plastic pieces that are already in the environment, which includes textiles, paints, and tires that are thrown away into the ecosystem. The fragmentation rates are dependable on various variables based on the resistance characteristics of these materials. Plastics materials that have a short lifetime are usually utilised for single-use plastics. They are made to be waterproof, durable, and resistant to wear and biodegradation, which makes them tremendously persistent in the environment.

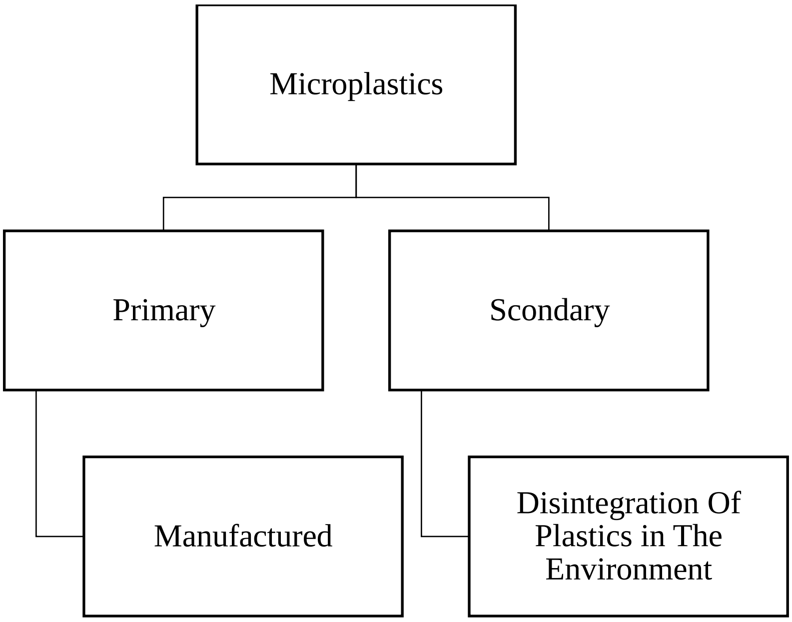


Figure 2.7 Illustrate the sources of MPs in the natural environment

2.2.1 Impact of Microplastics on The Environment

MPs pollution is the most extensive and long-lasting anthropogenic threat to the planet and biodiversity conservation (Barnes *et al.*, 2009). The possibilities of plastics waste ending up in the soil and aquatic ecosystems are high due to the low recovery, which only reaches up to 32% (Sutherland *et al.* 2010, Jambeck *et al.* 2015a). In addition, Galloway *et al.* (2017) and Lusher *et al.* (2017) have reported the direct and indirect harmful effects of MPs on the coastal and ocean biota.

Consequently, the aquatics ecosystem is highly threatened by MPs pollution. Jambeck *et al.*(2015b) and Nizzetto *et al.* (2016) have also mentioned that particles that end up in the ocean were mainly produced, used, and disposed of on the land, either in their original form or after undergoing partial environmental degradation. This problem creates high possibilities for MPs to affect not only marine but also terrestrial biota. Theoretically, environmental biophysical and geochemistry changes can cause environmental toxicity (Duis et al., 2016).

Terrestrial microplastics pollution, especially in agricultural soil was found to be higher than in the ocean basins (Horton *et al.,* 2017; Nizzetto *et al.*, 2016). In the ocean, MPs have the possibility of being digested, transported, and degraded by marine life (Barnes DK, Galgani F, Thompson RC, 2009; Rehse, S., Kloas, W., & Zarfl, 2016; Zhan *et al.* 2016). In other words, the damage caused by MPs on the land biota is similar to the ocean. Generally, upon entering the environment, the MPs can turn into potentially lethal materials, regardless of whether it was pure or coated with an additive substance. In addition, the high surface area of microplastic enables them to accumulate toxic material easily.

Table 2.3 shows examples of the impact of MPs on biota. There is also the possibility that the presence of these MPs would change the soil composition. This effect is due to the physical-chemical changes in the soil structure and texture due to the effect of microplastics which would significantly interact with the plant and soil, the biogeochemical cycle (De Souza Machado *et al.* 2019; Zheng *et al.*2016), and the biodiversity of the microbial communities such as Mycorrhizal (Hallett *et al.*2009).

Big plastics particles limit gas exchanges, affecting environmental health and causing entanglement for the animals (Barnes *et al.*, 2009; Steinmetz *et al.*, 2016). The studies conducted by Barnes *et al.* (2009), and Rehse *et al.* (2016) have found that ingestion of the small MPs particles lead to false satiation and blockage of the digestive system or affects the mucosa by causing irritation and abrasion. In addition, leaching of chemicals from the plastics such as additives, plasticizers, and components of the polymer matrix, can occurs during usage, in the environment, or within the organisms (EPOCITFC, 2016; Whitacre, 2014). As these particles enter the environment, environmental factors like UV and high temperature will stimulate the release of these toxic chemicals (Andrady, 2011). Furthermore, the smaller the MPs particles are, the lower their dissolvability, allowing them to interact with biological membranes, organelles, and molecules. Since the additive chemicals in the MPs are weakly bound or even not bound to the polymer's molecule, they may eventually leach into the environment.

Table 2.3 Studies that illustrate the impact of certain types of MPs on animals (Zhang *et al.* 2019)

Categories		Negative impact	MPs type	Reference
Animal	Echinoderms	Fertilization (insemination)	PS microspheres; HDPE fluff exposure	Martínez-Gómez *et al.*(2017)
	Molluscs	ChE activity inhibition	Red fluorescent polymer	Guilhermino *et al.*(2018)
		Indirect neurotoxic effect	Virgin polystyrene, microbeads	(Magni *et al.* 2018)
		Energy Balance and Gametogenesis	PS exposure	Gardon *et al.*(2018)
		Reduce the energy intake	PS exposure	Xu *et al.* (2017)
		Lysosomal membrane destabilization	HDPE exposure	Von Moos, Burkhardt-Holm and Köhler,(2012)
	Arthropods	Immobilization	PE Exposure	Rehse, Kloas and Zarfl, (2016)
	Annulate	Weight reduction Higher mortality	LDPE exposure	Huerta Lwanga *et al.*(2017a)
	Cnidaria	Bleaching Bleaching Tissue necrosis Tissue necrosis Bleaching	PE	Reichert, Schellenberg, Schubert, & Wilke,(2018)
		Significant impact the feeding	PE flakes ingesting exposure	Murphy & Quinn (2018)
		Growth and ingest	PS	Redondo *et al.* (2018)
	Mammals	bioaccumulation	PS and PVC Feeding, drinking, inhalation;	Carbery *et al.* (2018)
		Potential toxicological effect	Compositive Feeding	Fossi *et al.* (2012)
		Modify the gut microbiota composition and induce hepatic lipid disorder	PS exposed	Lu, Wan, Luo, Fu, & Jin (2018)
	Birds	Contaminants substance	Natural PE resin pellets Feeding	Teuten *et al.* (2009)
	Amphibians	Reproduction and development	Almost all commercially available plastic products Exposed	Yang *et al.* (2011); Ziková *et al.* (2017)
	Fish	Immune gene expression	PS Exposed	Cao *et al.* (2018)
		Behavioural responses and reduction of swimming velocity and resistance time	Fluorescent red polymer microspheres Exposed	Barboza *et al.* (2018)
		Localized thickening of the mucosal epithelium and histology and protease activity	Pristine PVC fragments	Bui *et al.* (2016)