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Earthworms As An Emerging Biotechnological Intervention in the Mitigation of Microplastics

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IN THE CONTEMPORARY era, the disposal of plastic has emerged as a significant environmental concern, primarily due to the prevalence of non-biodegradable plastics in the environment. The decomposition of plastics through biological means is inherently slow, given the resistant nature of plastic polymers that are hard and insoluble in water. However, certain conducive conditions enable the degradation of microplastics by organisms such as earthworms. Earthworms play a pivotal role in this process through their gut microflora and mucous secretions, which actively contribute to the degradation of microplastics. This involvement triggers the production of microbial exoenzymes, stimulating microbial activity and leading to the depolymerization of plastics. Essentially, earthworms function as eco-engineers, fostering habitat conditions that enhance the rate of plastic biodegradation under specific environmental circumstances. Recent studies have introduced the concept of bioaugmentation as a potential approach to expand biological treatments for waste management, specifically targeting plastic biodegradation. This review comprehensively explores the role of earthworms' gut microflora and the associated microbial metabolic pathways involved in the degradation of plastics. The dire threat of plastic pollution to both human health and the environment is also acknowledged. In light of the current situation surrounding plastic pollution, there is a pressing need for a more critical and scientific approach for the remediation of pollutants. This review is dedicated to addressing this demand, emphasizing the urgency of adopting effective strategies for mitigating the impact of plastic waste on our ecosystems and, consequently, on human well-being.

Keywords: Micro-plastics; Biodegradation; Gut-microflora; Environment; Microbes, Pollutants.

1. Introduction

Earthworms play crucial roles in terrestrial ecosystems, exerting influence on soil physical, chemical, and biological properties, and thereby contributing significantly to ecosystem health and function (AI-Maliki et al., 2021). Preserving and safeguarding earthworm populations is paramount for sustaining soil fertility, biodiversity, and ecosystem resilience across natural and managed landscapes (De Deyn and Kooistra, 2021). Within soil ecosystems, earthworms establish microhabitats that serve as shelters and resources for various soil-dwelling organisms. Through their digestion and casting deposition processes, earthworms facilitate the cycling of essential nutrients like nitrogen, phosphorus, and potassium, rendering them more accessible to plants (Ahmed and AI-Mutairi, 2022).

They also promote microbial decomposition of organic matter by fragmenting it into smaller particles, thereby enhancing microbial colonization and activity on soil surfaces. This fosters microbial diversity and activity, crucial for nutrient cycling and overall soil health (Farooqi et al., 2023). Earthworms serve as indicators of soil health and ecosystem function due to their presence and abundance (Alves et al., 2022).

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Soils with abundant organic matter and robust structure typically harbor diverse earthworm communities. Monitoring earthworm populations aids in evaluating the impacts of soil management practices, such as tillage, fertilization, and pesticide use, on soil health and sustainability (Edward and Arancon, 2022).

In the realm of pollution mitigation, earthworms contribute to the degradation of microplastics by ingesting soil containing these pollutants (Gudeta et al., 2023). Their digestive processes, coupled with gut microbiota, assist in breaking down microplastics. Furthermore, their burrowing and feeding activities enhance soil aeration and microbial activity, accelerating microplastic degradation. Earthworm castings, containing degraded microplastics, integrate into the soil, potentially diminishing the bioavailability and environmental impact of these persistent pollutants (Wang et al., 2022).

Thus, through their ecological roles, earthworms play a critical part in ameliorating microplastic pollution in soil ecosystems, underscoring their importance in environmental remediation endeavors. This review focuses on the ecological and biotechnological roles of earthworm in mitigation the micro-plastics pollution.

2. Why plastics?

Plastic is defined as synthetic polymers comprising silicon, nitrogen, hydrogen, chloride, and oxygen. Its widespread use is attributed to properties such as durability and stability. Annually, global plastic production reaches approximately three hundred million metric tons (Sharma et al., 2016). Surprisingly, terrestrial soils hold a greater mass of microplastics compared to oceanic waters. The generation of plastic waste amounts to about 400 million tons annually, with approximately 175 million tons entering the natural environment through landfills. Projections indicate that plastic accumulation in landfills may reach a staggering 12,000 million tons by the year 2050 (Zheng & Suh, 2019). Anticipated trends suggest that global plastic production could reach up to 1800 million tons by 2050 (Gallo et al., 2018).

The safe and efficient disposal of polymers remains a significant challenge, with potential harmful consequences for ecology and a variety of flora and fauna species. Microplastics, in particular, have wreaked havoc on aquatic and terrestrial life, leading to issues such as plastic bezoars in gills and intestines, posing a lethal threat. The presence of microplastics has been linked to endocrine disruption in animal models like earthworms, resulting in abnormal growth and reproductive complications (Zhang et al., 2018). The detrimental effects extend to the food chain, with microplastics entering it and causing contamination, leading to various health problems upon human ingestion (Elbasiouny et al., 2022). Pollutants leaching into the soil, when ingested by microorganisms, enter food chains, contributing to microbiome alteration, oxidative stress, and energy disturbance (Deng & Zhang, 2019). Microplastics' bioaccumulation across various food chains and webs significantly impacts the ecosystem, contributing to biodiversity loss. Studies have reported that microplastic ingestion and accumulation by organisms can cause inflammation and tissue damage, affecting numerous food chains. In comparison to macro-plastics, microplastics are deemed more harmful due to their adverse effects on environmental components (Rilling et al., 2017).

The escalating production of plastic coupled with inadequate waste management practices is a matter of grave concern, especially considering that only approximately 26% of plastic waste is currently being recycled, presenting a minute fraction of the overall plastic disposal (Alimi et al., 2018). The prevailing scenario reveals that around 18% of plastics undergo recycling, 24% are subjected to incineration, while a staggering 58% find their way into landfills, ultimately entering the natural environment where plastic accumulation becomes persistent and enduring (Geyer et al., 2017). The challenge of plastic degradation is further exacerbated in low landfill compost conditions. The slow degradation results from factors such as limited oxygen availability and reduced penetration of solar UV radiation, impeding photo-degradation activities (Harrison et al., 2011). Microbial decomposition of plastic is also hindered in deeper soils with diminished microbial populations, highlighting the prolonged persistence of microplastics in the soil profile (Fuller et al., 2016). In response to these challenges, bioremediation emerges as a highly effective approach for mitigating the environmental impact of pollutants, including plastics. This method, recognized for its eco-friendly and cost-effective nature, harnesses natural processes to eliminate harmful substances (Das & Adholeya, 2012). Implementing bioremediation techniques presents a promising solution for addressing the burgeoning issue of plastic waste and its persistent environmental consequences.

Plastic degradation is indeed a complex process, and different types of plastics exhibit varying rates of degradation. Sunlight exposure has been identified as a factor that accelerates the degradation of plastics, as demonstrated by the faster degradation of polystyrene when exposed to sunlight (Ward et al., 2019). The degradation of plastics is influenced by environmental factors such as oxygen and moisture. Sufficient oxygen and moisture are essential to enhance and expedite the degradation process. For instance, hydrolysis in the case of certain plastics like polylactic acid or thermoplastics occurs when exposed to temperatures equal to or greater than 60 °C under specific industrial composting conditions. The time required for the degradation of plastics varies significantly. Plastic bags, for example, may take between 10 to 20 years or 500 to 1000 years to degrade, depending on the specific conditions. In contrast, plastic bottles are estimated to have a degradation time ranging from 70 to 450 years (Chamas et al., 2020).

The impact of microplastics on the environment is closely linked to abiotic components, particularly soil, and is heavily influenced by microbial communities in both soil biota and the gut microflora of earthworms (Lehmann et al., 2019). Earthworms play a crucial role in shaping the diversity of soil microbiota, actively contributing to the biodegradation process. Reports suggest that earthworms contribute to microplastic degradation through the formation of biofilms and the excretion of enzymes from their gut (Auta et al., 2018). However, there are conflicting findings regarding the susceptibility of microplastics to microbial degradation. While some studies indicate resistance of plastics to microbes, others demonstrate that gut microbes can play a significant role in degrading microplastics during gastro-intestinal transit. Certain bacteria within the gut have been found to have the potential to cause up to 60% degradation within a 21-day incubation period. Enzymes present in the gut of earthworms, along with those from surrounding soil microbes, further facilitate the degradation process. Microplastic pollution is a significant concern both ecologically and in the field of material science. However, the current literature on the degradation of microplastics under various environmental conditions is relatively limited. To address this issue, comprehensive studies and diverse approaches are needed to enhance our understanding of the nature and processes of microplastic biodegradation. This knowledge is essential for developing effective technologies and strategies to reduce microplastic pollution and address the associated environmental challenges. The Bio-augmentation process includes addition of microbes like bacteria and fungi which fastens the process of plastic biodegradation. Microbes are tiny organisms which are not visible through a naked eye and generally they include classes such as bacteria and fungi if we talk about the earthworm’s gut microflora and surrounding soil. They are known to be helpful in the decaying and degradation process.

Catalysts and enzymes, in conjunction with chemically complex substances, play a crucial role in catalyzing the breakdown of carbon during the microbial degradation process of plastic waste. The mechanism of degradation, however, is highly dependent on the type of polymer and the specific environmental conditions to which it is exposed.

Biodegradation encompasses a range of physical and chemical changes in a material, influenced by environmental factors such as temperature, moisture, pH, and the presence of microbes. One potential solution to the plastic waste problem is to enhance the biodegradation rate of plastics. In this context, earthworms are employed to promote the biodegradation of plastics by creating a favorable habitat for microbial degraders of plastic. Earthworms, acting as bio vectors of plastic degradation, contribute to a practical approach that not only aids in plastic breakdown but also enhances soil quality, particularly under plastic mulching. This innovative strategy accelerates the rate of biodegradation, addressing both plastic pollution concerns and soil health. By leveraging the natural capabilities of earthworms and creating an environment conducive to microbial activity, this approach offers a sustainable and environmentally friendly method for managing plastic waste. It aligns with the broader goal of developing effective strategies to mitigate the impact of plastic pollution while simultaneously improving soil conditions in agricultural settings.

The plastic era commenced in 1869 with the synthesis of the first plastic polymer known as 'celluloid' by John Hyatt. Over the years, a diverse array of plastic polymers has been developed to overcome limitations associated with other materials, contributing to advancements in the field of material science. In 2019, the plastic market reached a valuation of 568.9 billion USD, and it is anticipated to grow at a rate of 3.2% between 2020 and 2027. Plastics are derived from various sources, including oil and coal, and exhibit a wide range of applications. The synthesis of polymers occurs through two main routes:

- **Polymerization of Olefins**: This process involves the addition polymerization of carbon double bonds in olefins, leading to the formation of new C-C bonds and the creation of polymers along the carbon chain. Polyolefin production, which includes polybutene, polyethylene, and polypropylene, constitutes over 60% of the total production (Krupa, 2016).
- **Condensation Reactions**: Polymers are also synthesized through condensation reactions, where carboxylic acids react with amines or alcohol groups to form polyesters or polyamides. This reaction is fundamental to polyurethane production, involving the condensation of isocyanate and polyol molecules (Akindoyo et al., 2016).

The term "microplastics" was introduced by Thompson in 2004, marking a significant development in the understanding and identification of minute plastic particles. In 2009, an upper size limit was established for the term, encompassing plastic particles with dimensions smaller than 5mm. This definition recognizes microplastics as a distinct category of plastic pollution, emphasizing their pervasive presence and potential environmental threats. Microplastics are considered revolutionary materials due to their widespread distribution and persistent nature, posing a significant risk to both terrestrial and aquatic ecosystems. The slow degradation and fragmentation of larger plastic items within these environments contribute to the formation of tiny particles referred to as microplastics, as highlighted in research by Nash et al. in 2019. The factors and conditions within ecosystems play a crucial role in the breakdown of plastics into microplastics. Environmental processes, such as weathering, sunlight exposure, and mechanical forces, contribute to the gradual disintegration of plastic items into smaller fragments. The ubiquity of microplastics in various environmental compartments, including water bodies and soil, raises concerns about their potential impact on wildlife, ecosystems, and even human health.

The versatility of plastics, stemming from their diverse chemical compositions and synthesis methods, has led to their widespread use in various industries. As the plastic market continues to grow, ongoing research and development in the field aim to address environmental concerns and promote sustainable practices in plastic production and usage.

3.Generation of microplastic

The production and usage of plastics have led to a substantial environmental issue, with approximately 79% of plastic waste, totaling 4900 to 6300 million tons, accumulating in landfills within terrestrial and aquatic ecosystems (Geyer et al., 2017). Plastic litter undergoes degradation and disintegration through weathering processes, both physical and chemical, resulting in the formation of microplastics—considered a secondary form of plastics compared to the primary forms found in various industrial and cosmetic products (Rillig, 2012).

Plastic polymers like polyethylene and polypropylene are extensively used in agriculture, contributing to increased crop productivity through applications such as mulching and greenhouse cultivation (Kasirajan and Ngouajio, 2012). However, concerns arise as microplastics and pesticides can accumulate on plastic films, subsequently migrating into the soil and atmosphere, posing adverse effects on biota (Ramos et al., 2015).

In 2011, microplastics were categorized based on their origin into primary and secondary forms (Cole et al., 2013). Primary microplastics are commercially produced microscopic particles, while secondary microplastics result from the fragmentation of micro and meso-waste under various environmental conditions, including temperature fluctuations and UV exposure (Horton et al., 2017).

Microplastics, defined as plastic particles with diameters less than 5mm, including nano-sized particles of 1nm, have become a significant concern. Literature on microplastics is growing exponentially, highlighting issues related to plastic pollution. Some countries, such as the USA, Canada, and the UK, have implemented bans on microplastics, while others, like Taiwan, Italy, and Korea, are in the process of drafting bills addressing microplastic pollution. The global impact of microplastics has prompted considerations in international policies, recognizing the need to address this pollution issue on a global scale (Bergman et al., 2015).

3.1 The two main types of pastics

1. Thermoplastics: The plastics which melt on heating and gets hardened on cooling are termed as Thermoplastics. These include Polypropylene (PE), Polystyrene (PS), Polyvinyl chloride (PVC), Polyethylene Terephthalate (PET), Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Polypropylene (PP), Polyhydroxyalkanoates (PHA).
2. Thermosets: The plastics which on heating undergoes a chemical change and leads to formation of 3D structures which further can neither be melted or reformed, are termed as Thermosets. These include Phenolic resins, Polyurethane, Vinyl ester, Silicone, Urea formaldehyde.

3.2 Microplastic accumulation in soil

Plastic waste infiltrates soil through various pathways, including erosion, agricultural practices, and the use of sewage sludge in soil fertilization, contributing to the accumulation of microplastics in the soil (Rochman, 2018). Microplastics, characterized by their limited biodegradability, persist in the soil for extended periods, posing environmental challenges and adversely affecting terrestrial ecosystems. While microplastics are well-known for their harmful effects on aquatic life, terrestrial habitats, particularly soil, bear the brunt of their impact on flora and fauna (Horton et al., 2017). The entry of microplastics into the soil occurs through multiple routes, such as the application of plastic mulch films, biosolid use, and atmospheric deposition, posing a threat to ecosystems (de Souza Machado et al., 2018). Earthworms play a significant role in addressing the issue of plastic pollution in soil. These organisms act as facilitators of biodegradation by creating optimal habitats for microbial proliferation in their guts. The gut microflora of earthworms contributes to the breakdown of polymers, accelerating the biodegradation rate. Additionally, the introduction of specific microbial strains can further enhance the biodegradation of polymers. Leveraging earthworms as model organisms due to their unique gut microflora presents an effective and sustainable strategy for plastic remediation in the soil. In conclusion, understanding the pathways of microplastic entry into the soil and recognizing the pivotal role of earthworms in biodegradation are crucial steps in mitigating the environmental impact of plastic pollution. Implementing strategies that harness the natural processes facilitated by earthworms and microbes holds promise for addressing the persistent issue of microplastic accumulation in terrestrial ecosystems.

3.3 Possibility of microplastic degradation

Earthworms play a crucial role in soil ecosystems, contributing significantly to both the number and biomass of fauna in the soil. Their activities stimulate soil microbes, enhancing the digestion of organic matter. The release of energy-enriched mucus by earthworms not only primes microbial action but also contains molecules with hormone-like effects, influencing gene expression (Freitas and Blouin, 2015). In experiments exposing earthworms to different concentrations of microplastics, a notable decrease in the size of microplastics was observed in the feces and gut of earthworms. This reduction was attributed to the action of microbes and enzymes present in the earthworm's gut (Lwanga et al., 2017). Earthworms, considered model organisms in terrestrial ecosystems, exhibited resilience when exposed to 0.25 to 0.5% polystyrene microplastics, showing no significant effects on adaptation or growth. However, concentrations exceeding 1% led to growth inhibition (Cao et al., 2017).

Microorganisms in the soil are vital for terrestrial ecosystems, contributing to geochemical cycles, pollutant degradation, and adaptive changes. Studies found no major changes in soil microbial communities when exposed to microplastics. Introduction of low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polyvinyl chloride (PVC) did not alter the α diversity or structure of the microbial soil community (Huang et al., 2019; Judy et al., 2019). Different types of earthworms have varying capacities for ingesting dry matter, with epigeic earthworms capable of ingesting 3-50 mg per day, while geophagous worms can ingest 200-6,700 mg per day (Curry and Schmidt, 2007).

The "Sleeping Beauty Paradox" describes the mutual interaction between soil microbiota and earthworms (Brown et al., 2000), highlighting the intricate dynamics between these essential components of terrestrial ecosystems.

4. Plastic biodegradation: A slow process

Decomposition processes tend to be slower in deeper soils due to reduced microbial populations. This characteristic has significant implications for microplastics, as their inherent slow decomposition in the environment can result in prolonged persistence in the soil profile (Fuller et al., 2016). Figure 1 and 2 illustrates various factors that influence the biodegradation process.

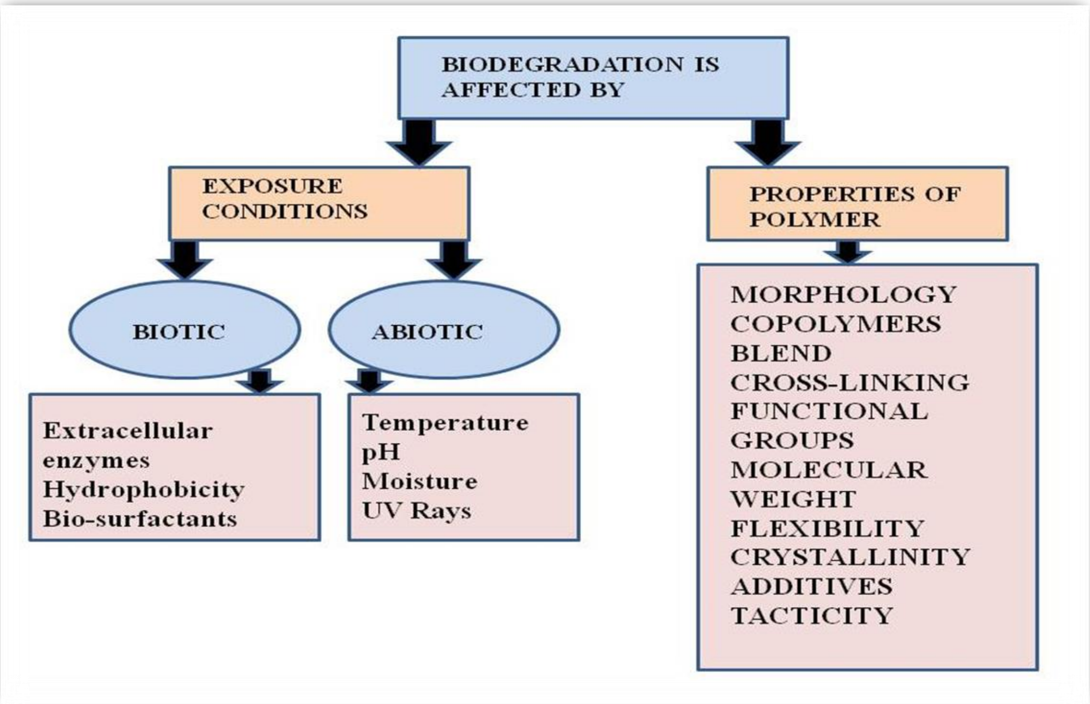

Fig. 1. Factors affecting the biodegradation process.

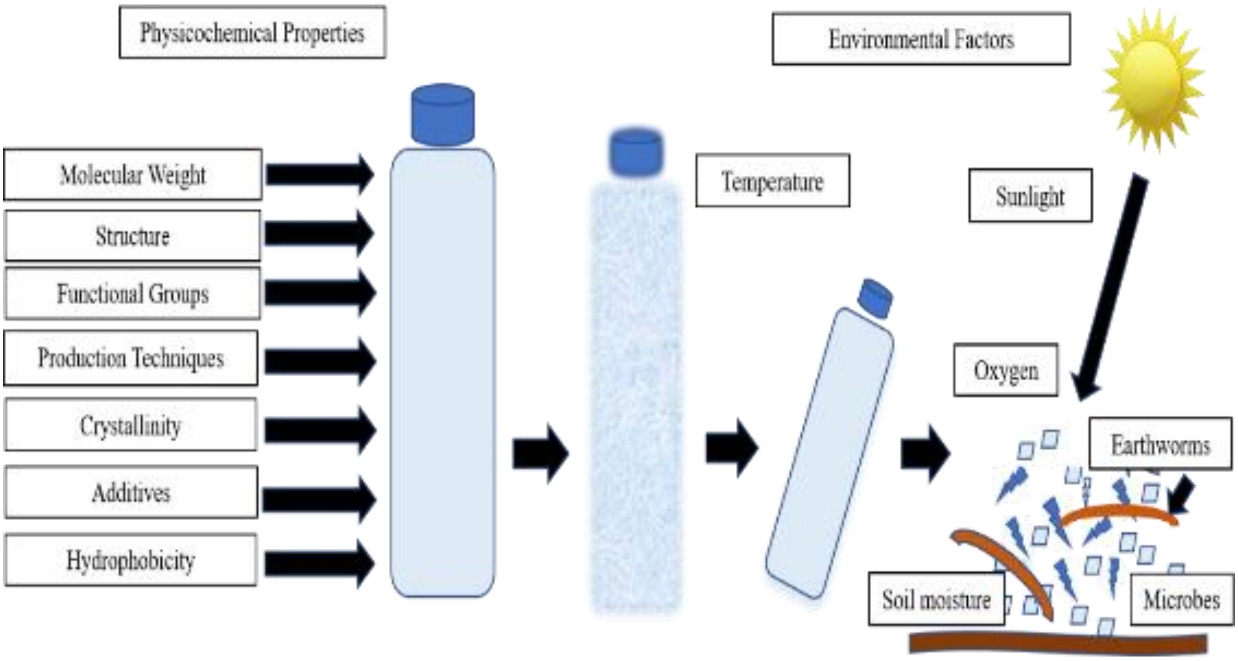

Fig. 2. Physiochemical properties and environmental factors affecting the process of plastic degradation.

4.1 Conditions required for micro-plastic biodegradation process in the presence of soil engineers

According to the studies and research done, essential conditions occurring during biodegradation of microplastics in the presence of earthworms have been shown in the following (Table 1):

4.2 Phenomenon of microbial plastic degradation

Microbial plastic degradation refers to the process of breaking down plastics and microplastics with the involvement of microbes present in the earthworm's gut microflora and the surrounding soil biota. This degradation occurs through biochemical transformation, where complex compounds are broken down into simpler forms by microbial action. The study of biodegradation in plastic polymers involves examining various changes in the physical properties of the polymers. Several processes form the basis of the biochemical pathways involved in the biodegradation of plastics and microplastics. These processes include bio-fragmentation, mineralization, assimilation, and biodeterioration (Pathak et al., 2017). Biofragmentation involves the breaking down of complex compounds into smaller fragments, while mineralization converts organic compounds into inorganic substances. Assimilation refers to the incorporation of degraded products into microbial biomass, and biodeterioration involves the deterioration of the plastic's physical structure by microbial action. Observations of cracks, pits, and the presence of fungal and bacterial spores on the surface of microplastics confirm the degradation process. These visible signs indicate various chemical, physical, and biological changes, ultimately resulting in the weathering of microplastics (Huang et al., 2020).

4.3 Biochemical pathways for bio-degradation

The degradation of plastics and microplastics involves a series of processes influenced by bacterial and fungal diversity, enzymes produced by them, and various biotic and abiotic factors. The stages of biodegradation include bio-deterioration, bio-fragmentation, assimilation, and mineralization.

a) Bio-deterioration

Bio-deterioration is initiated by the physical and chemical activities of microbes present in the gut microflora of earthworms, causing superficial degradation of plastic polymers (Anjana et al., 2020). Prolonged exposure to external conditions enhances changes during biodegradation. Biodeterioration involves microbial adhesion, leading to surface degradation and alterations in physio-chemical properties. Bacterial biofilm formation increases interaction with polymeric surfaces. Some bacteria in earthworm guts cause a significant decrease in microplastic mass in soil. Bacterial strains like Bacillus, Streptomyces, and Pseudomonas show efficiency in plastic biodegradation (Yoshida et al., 2016). Pseudomonas, a biofilm-forming bacterium, efficiently adheres and degrades low-density polymers (Tribedi et al., 2015). Exopolysaccharides play a crucial role in attachment and biodeterioration (Anjana et al., 2020).

b) Bio-fragmentation

Bio-fragmentation involves depolymerization, cleaving bio-deteriorated plastic polymers into tiny units using extracellular enzymes and free radicals produced by microbes. Decrease in molecular weight and oxidation of low molecular weight polymers occur, facilitating microbial enzymatic activities. Some inorganic compounds and organic acids scavenge cations to induce erosion and fragmentation (Krause et al., 2020).

c) Assimilation

Bio-fragmentation releases low-weight molecular compounds, transported into microbial cytoplasm or other organisms like earthworms in the assimilation process. Pseudomonas species takes up Octadecane, a product of plastic degradation, through facilitated passive transportation at high concentrations and active transportation at low concentrations. Specific transporters, like terephthalic acid in Comamonas bacterial species, facilitate assimilation. Porins move degradative plastic products into the cytoplasm for further processing (Duret & Delcour, 2010).

d) Mineralization

Mineralization, either aerobic or anaerobic, involves enzymes such as lipases, peroxidases, esterases, and cutinases. Plastic derivatives are transported into cells, leading to enzymatic reactions and complete degradation, resulting in the formation of oxidized metabolites like nitrogen oxide, carbon dioxide, methane, and water. Earthworms stimulate nitrogen mineralization in the soil. Mineralization rates vary between old and newly formed earthworm casts. The earthworm gut serves as a microenvironment for bacteria releasing N2O from soil-originated denitrifiers. Techniques like isotope tracing and carbon dioxide release quantification are employed for complete plastic polymer mineralization (Yang et al., 2020).

4.4 Microbial enzymes present in earthworm gut influence plastic biodegradation

Enzymes crucial for plastic degradation are broadly categorized into two main types:

a) Extracellular Enzymes:

Functionality: Extracellular enzymes exhibit a wide reactivity range, encompassing both oxidative and hydrolytic functionalities (Glaser et al., 2019).

Role in De-polymerization: These enzymes play a vital role in the de-polymerization of long carbon chains found in plastic polymers.

Microbial Secretions: The extracellular enzymes are often part of microbial secretions, which contain substances that enhance penetration rates. They also exhibit effects on both hydrophobic and hydrophilic phases, contributing to pollutant accumulation, microbial growth, and the biodegradation process (Zanardini et al., 2000).

b) Intracellular Enzymes:

Aerobic and Anaerobic Processes: A significant portion of intracellular enzymes is involved in both aerobic and anaerobic processes. These enzymes are essential for converting intermediates generated during plastic degradation into compounds that can be assimilated by microbes (Pathak, 2017).

Understanding the roles of extracellular and intracellular enzymes is crucial for comprehending the biochemical pathways involved in the degradation of plastic polymers. This knowledge is instrumental in developing strategies to enhance the efficiency of plastic degradation processes, contributing to the mitigation of plastic pollution.

4.5 Enzymatic degradation

Intracellular and extracellular degradation processes in the context of polymer breakdown have distinct definitions. Intracellular degradation involves the hydrolysis of endogenous carbon reservoirs accompanied by bacteria accumulation. On the other hand, extracellular degradation refers to the utilization of exogenous carbon where microbe accumulation is not mandatory (Kumaravel et al., 2010).

The enzymatic degradation process of polymers occurs in two steps:

Enzyme Substrate Binding: Enzymes bind to the substrate, initiating the degradation process.

Catalysis Process: The catalysis process involves the formation of enzyme-substrate complexes, with colonization of microbial species being the initial stage. Microbes grow, leading to biofilm formation and subsequent damage to the polymer. Proteins and polysaccharides produced during microbial adhesion contribute to the formation of enzyme-substrate complexes within the polymer, altering its size (Capitelli et al., 2006).

Increased hydrolysis rates and challenging enzyme penetration result in surface erosion, reducing the thickness of the polymer. Enzymes such as lipases, esterases, proteases, and ureases released by microbes affect the crystalline nature of the polymer (Yoshida et al., 2016).

During the breakdown of polymers, water-soluble monomers undergo metabolism through β-oxidation and the tricarboxylic acid cycle (TCA) under aerobic conditions, producing CO_2 and H_2O. Under anaerobic conditions, methane is produced (Scott, 1990; Luzier, 1992).

The release of digestive enzymes through exocytosis facilitates the breakdown of large, complex macro and organic molecules into simpler, smaller organic compounds, releasing CO_2 and H_2O under suitable aerobic and anaerobic conditions (Pathak & Kumar, 2017).

Microbial biodegradation of polymers involves several steps:

- Microorganisms attach to the polymer surface.
- Polymers serve as a carbon source, enhancing microbe growth.
- Extracellular enzymes are released, causing cleavage and fragment formation, resulting in oligomers, dimers, and monomers with lower molecular weight. Microbes utilize these fragments for energy (Azevedo & Reis, 2005).

These steps are depicted in Figure 3.

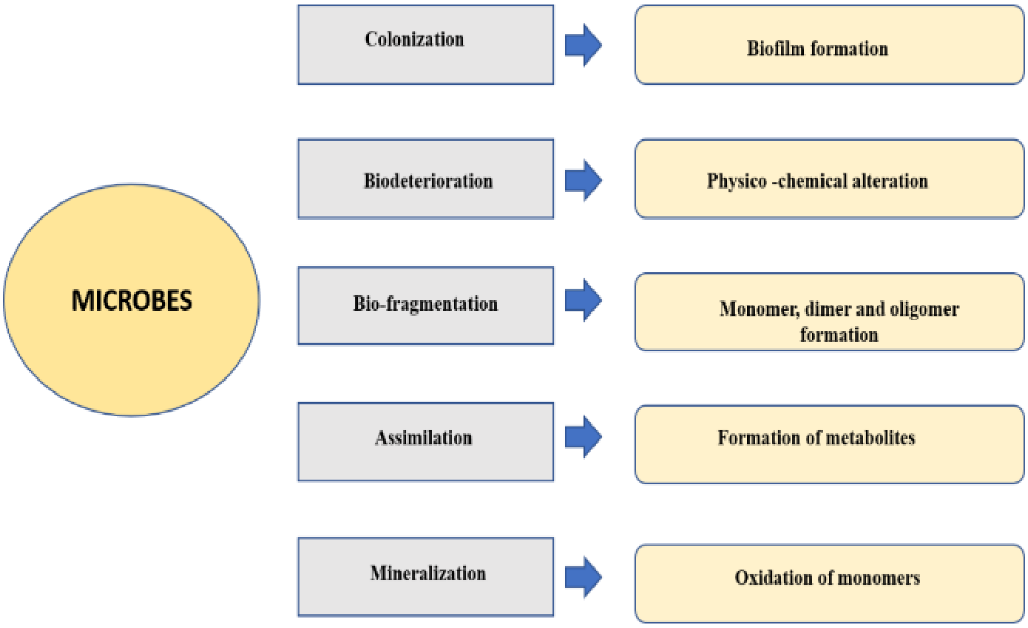

Fig. 3. Stages showing various changes that occur as a result of microbial enzymatic degradation.

5. Earthworm's gut playing an essential role in degradation of micro plastics

Earthworms help in shaping the structure of soil and have impact upon the matter dynamics of the soil along with microbes associated with its gut and cast. Gut of earthworm provides an essential micro-environment for accumulation of microplastic while the microbes present in the earthworm's gut are known to show a wide and efficient potential for carrying out the degradation process (Wang et al., 2019).

Earthworm gut act as a micro eco-zone and presence of intestinal bacterial community act as an essential component which helps in maintaining the stability as well as sustainability of microbial community existing in soil (Sun et al., 2020). It has been described in figure 4. Earthworm species like *Lumbricus terrestris*, *Eisenia andrei*, *E. fetida* and *Metaphire californica* have shown the potential in microplastic degradation (Sun et al., 2020).

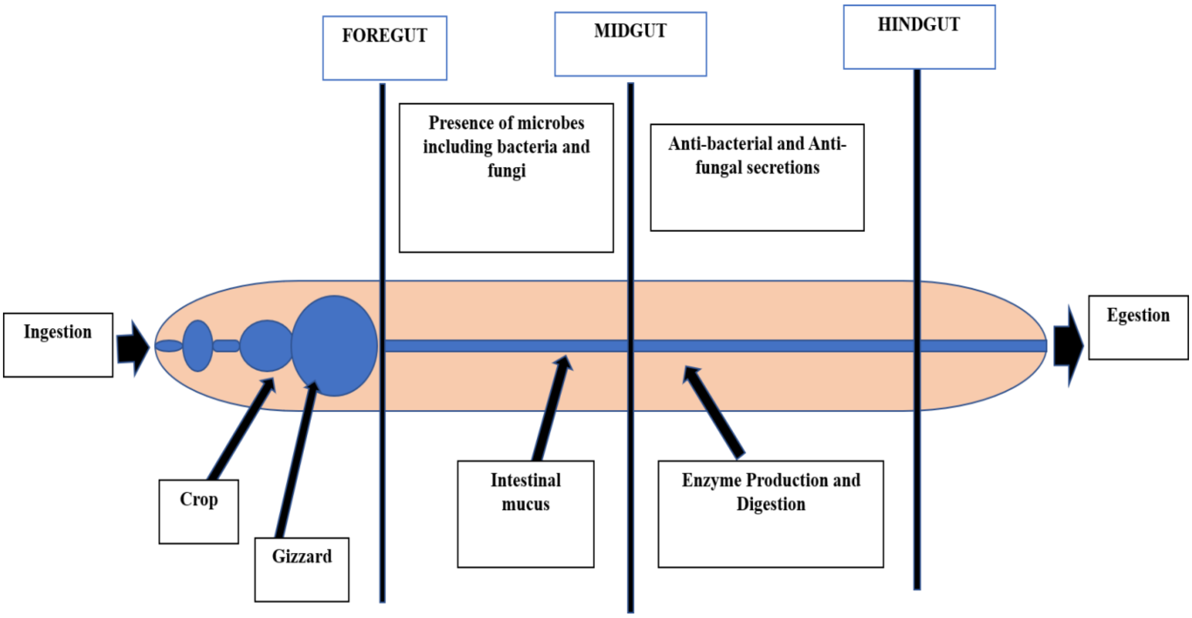

Fig. 4. Activities associated with earthworm's gut micro-flora.

Table 1. Showing different conditions for biodegradation of microplastics in presence of different earthworm species.

Earthworm Species	Types of Polymer	Presence of microplastic concentration	Conditions present at the time of exposure	Ref.
Lumbricus terrestris	Low density polyethylene	7%,28%,45%,60% (dry mass in plant litter)	Soil Temp. 16-18 °C Soil Moisture 20% (w/w) Exposure time 14days (mortality) 60 days (change in weight and reproduction rate)	Huerta et al. (2016)
Lumbricus terrestris	High density Polyethylene (plastic bags pieces)	Microplastics 0.7g containing Zn concentration +236mg/kg (mixed in dry soil)	Soil Temp 16°C-18°C Soil Moisture 21% Exposure time 28+days (they persist for longer durations)	Hodson et al. (2017)
Lumbricus terrestris	Low density Polyethylene PBAT	Varying concentrations	(Environmental realistic concentrations, microplastics did not have adverse effects on the earthworm)	Adhikari et al. (2023)
Lumbricus terrestris	Low density Polyethylene and PLA	5-7% 30-80%	Soil Temp 16°C-18°C Soil Moisture 21% Exposure time 35 days (The earthworms survived in less microplastic concentrations but showed mortality when concentration was increased)	Meng et al. (2023)
Eisenia fetida	Low density Polyethylene and Polystyrene	0%,1%,5%,10% and 20%(w/w)	Soil Temp 25 °C Soil Moisture 40% Exposure time 14 days (for ingestion of microplastic and toxicity assays) 28 days (for bioaccumulation assay)	Rodriguez et al. (2018)
Eisenia fetida	High density polyethylene, polyethylene terephthalate, polyvinyl chloride	0.5g microplastic and 100g of soil (incubated for 0,3,9 months)	Soil Temp 23.5 ± 2.8 °C Soil Moisture 60% Exposure time 24hrs (avoidance test), 28days (acute test), 56days (chronic test)	Rodriguez et al., (2019)
Eisenia fetida	PE	0.5%, 1%, 2%, 5%, 7%, and 14% w/w	Soil Temp 25 °C Soil Moisture % Exposure time 28 days	Yu et al., (2022)
Eisenia fetida	PET and PLA	0.1 and 1% of soil weight	Soil Temp 25 °C Soil Moisture % Exposure time 7,14,21,28 days	Parolini et al., (2024)
Eisenia Andrei	Low density polyethylene	62.5,125,250,500mg microplastic particles (per kg of dry soil)	Soil Temp 20 ± 2 °C Soil Moisture 40% Exposure time 28 days and 56 days (rate of reproduction)	Rodriguez et al., (2017)
Metaphire californica	PVC particles	2000 particles per Kg dry soil containing 40 mg As	Soil Temp 20± 2°C Soil Moisture 30% Exposure time 28 days	Wang et al. (2019)

Anecic earthworm species drag ingested microplastic and litter mixture to deeper layers of soil by a process called soil bioturbation (Lwanga et al 2017). Thus, anecic earthworms facilitate the accessibility of plastic to microbial communities and soil mesofauna for surface biodeterioration and degradation of plastic. It was reported that *L. terrestris* an anecic earthworm transported LDPE particles to the deep soil by dragging and ingesting litter mixed meso- and microplastics. Organic composites made from mucus, organic matter and plastic debris in burrow walls of anecic earthworms provide favorable conditions for polymer biodegradation to be carried out by microorganisms (Sanchez-Hernandez et al., 2020).

Earthworm casts deposited on the soil surface or in burrow walls are microplastics reservoirs where microorganisms and exoenzymes can depolymerize microplastics. The intestinal mucus in *L. terrestris* is quite rich in the enzymes as well as microflora which protects it from the direct effect of substances like microplastics. Earthworms are even known to produce mucus in large quantities when they ingest substances which are less enriched with the fresh organic matter as in case of plastics. Gastrointestinal lumen of earthworms also plays significant role in biodegradation of plastic. Gut act as a micro inhabitant constituting of low oxygen levels along with high carbon and nitrogen content in comparison to surroundings which makes gut capable for anaerobic microbe colonization. It was also reported that gut flora of *L. terrestris* degraded microplastics. *Aporrectodea caliginosa* is also known to depolymerize ingested biodegradable microplastics and nanoplastics (Sanchez-Hernandez et al., 2014). When gastrointestinal transit of micro-plastic is exposed to Gram-positive bacteria like Actinobacteria and Firmicutes, it degraded LDPE microplastics by approximately 60% of in 21 days. Plastics like LDPE, PVC and PP are known to be metabolized by various bacterial types like *Bacillus, Brevibacillus borstelensis, Firmicutes* and *Actinobacteria.* These bacteria are inhabitant the intestine of the earthworm (Lwanga et al., 2018). Some researchers showed that earthworm gut is favorable for anoxic bacterial colonization which includes bacteria species like *Staphylococcus or Aeromonas* (Hong et al., 2011).

Soil along with litter microflora is enhanced inside the earthworm's gut by the action of priming effect which thereafter produce a positive effect involving symbiotic relationship and nutrient uptake. Biodegradation processes of plastic can be examined by the growth of microbes and changes occurring in the polymer. Various enzyme assays, BOD, along with production of carbon dioxide and biomass under aerobic conditions. Changes in the molecular weight, tensile properties, fragmentation along with the functional groups present upon the surface of the plastics show indication of degradation process (Restrepo- Flórez et al., 2014). The description of various microbes including bacteria and fungi are mentioned in **Table 2** and **Table 3** respectively.

Table 2. Various types of bacterial genus used for plastic biodegradation process.

Polymer Type	Bacterial Genus Used	Activity	Reference
Polyethylene	*Brevibacillus* *Pseudomonas* and *Rhodococcus*	Pseudomonas show about 40.5% biodegradability.	(Nanda et al., 2010) (Amobonye et al., 2021) (Jadaun et al., 2022)
	Rodococcus ruber	It helps in biofilm formation and has hydrophobic nature.	(Sivan et al., 2006)
	Pseudomonas,Bacillus, Staphylococcus	-	(Usha et al., 2011) (Malik et al., 2023)
	Bacillus cereus Strain	Pretreatment with UV rays along with thermal oxidation enhances biodegradation process	(Suresh et al.,2011)
Polythene	*Micrococcus,S.aureus, S.pyogenes,P.aeruginosa and B.subtilis*	Fadama soil along with poultry droppings, inorganic fertilizer and cow dung was used in an expirement.	(Abdullahi & Saidu et al., 2013)
	Bacillus subtilis, Staphlococcus aureus,Streptococcus lactis	-	(Priyanka & Archana,2011)
LDPE	*Microbacterium, Pseudomonas putida, Pseudomonas aeruginosa*	Degradation is accelerated by the combination of bacterial strains.	(Negi et al., 2011)
	Staphylococcus epidermis	Pretreatment in the presence of UV rays and thermal oxidation fastens the biodegradation process.	(Suresh et al., 2011)
HDPE	*Arthrobactor and Pseudomonas*	-	(Balasubramanian et al., 2010)
Low density polyethene and polypropylene	*Pseudomonas stutzeri*	-	(Sharma & Sharma,2004)
Branched low-density polyethylene	*Brevibacillus borstelensis*	-	(Hadad et al., 2005)

Table 3. Various types of fungal genus used for plastic biodegradation process.

Polymer Type	Fungal Genus Used	Activity	Reference
Polyurethane	*Aspergillus, Penicillium, Paecelomyces,Alternia, fusariumtrichoderma DIA-T*	Urease and protease activity is seen in most strains	(Loredo et al., 2011)
	Pestalotiopsis	Enzyme of serine hydrolase family is present.	(Russell et al., 2011)
Polylactide	*Amycolatopsis*	-	(Pranamuda et al., 1997)
Polythene	*Aspergillus niger, A.flavus,A.fumigates, Penicillium,Fusarium, Mucor*	-	(Abdullahi &Saidu,2013) (Sankhla et al., 2020)
	Proteus vulgaris, Penicillium,Pseudomonas	-	(Priyanka & Archana et al., 2011)
	Aspergillus niger, Aspergillus nidulance, Aspergillus flavus, Aspergillus glaucus, Micrococcus luteus		(Ekanayaka et al., 2022)
Polyethylene	*Streptomyces, Aspergillusnidulans, Aspergillus flavus*	-	(Usha et al., 2011)
LDPE	*Aspergillus niger and A.flavus*	-	(Deepika & Jaya et al., 2015)
	Aspergillus versicolor and Aspergillus	-	(Pramila & Ramesh,2011)
	Aspergillus and Fusarium	-	(Das & Kumar,2015)
	Aspergillus terreus	-	(Zeghal et al., 2021)
	Aspergillus niger and Penicillium pinophilum	-	(Volke-Sepúlveda et al., 2002)
	Streptomyces	High degradation capacity is shown by Streptomyces.	(Deepika & Jaya,2015)
HDPE	*Aspergillus niger,Aspergillus flavus,Aspergillus oryzae*	-	(Konduri et al., 2010)
Disposable polyethylene	*Streptomyces,Aspergillus flavus and Mucor rouxii*	-	(Shafei et al., 1998)

5.1 Earthworm affecting bacterial diversity modification of microbial soil communities

The type of food consumed by earthworms has a discernible impact on the bacterial communities present in their casts, particularly in epigeic species like E. andrei (Aira et al., 2016). Epigeic earthworm species, such as E. fetida and P. excavatus, are known to influence both the biomass and composition of the microbial community in the soil. This influence can be assessed through techniques like phospholipid fatty acid (PLFA) analysis and bacterial counts (Koubová et al., 2015). Certain earthworm species, including E. fetida and P. excavatus, are recognized for their ability to enhance the efficiency of decomposition processes (Singh et al., 2015). The decrease in soil microbial diversity, induced by the presence of earthworm casts, leads to an increase in various bacterial groups within the casts. These bacteria play a crucial role in the enrichment and degradation of benzoic and aromatic compounds. Vermicomposting, a process that involves earthworms in composting, is commonly utilized to study the impact of earthworms on soil microbial diversity. Epigeic species like *E. fetida* have been shown to positively increase the diversity of bacteria during vermicomposting (Gopal et al., 2017). However, the effects of earthworms on bacterial diversity can vary, with some species and microhabitats showing positive effects, negative effects, or neutral effects. Positive effects on bacterial diversity have been reported in certain epigeic and anecic species belonging to the genus Lumbricus (Hoeffner et al., 2018).

Understanding the intricate relationships between earthworms, their diet, and microbial communities in the soil contributes to our knowledge of ecosystem dynamics and highlights the diverse roles that earthworms play in shaping soil ecology.

5.2 Earthworms affecting fungal diversity modification of microbial soil communities

Microorganisms, including protozoa and fungi, constitute a significant portion of an earthworm's diet. Reports indicate that the gut and cast contents of earthworms contain large amounts of soil fungi, particularly in the intestinal area. The presence of fungal spores in higher numbers in earthworm casts compared to the surrounding soil suggests a potential for selective feeding. Competition for nutrients between earthworms and microorganisms is observed, with different responses to food resource manipulation. Despite a decrease in microbial biomass passing through the earthworm's gut, it indicates that earthworms are not solely dependent on microbes for food (Tiunov & Scheu, 2000). Fungi in the earthworm's gut microflora and the surrounding soil play a crucial role in the mineralization and degradation of pollutants like microplastics through various chemical reactions (Črešnar and Petrič, 2011). Some fungal strains are reported to degrade low-density polyethylene (LDPE) by decreasing the length of polyethylene chains through fungal enzymes, suggesting a potential role in plastic degradation (Restrepo-Flórez et al., 2014). Fungi, in comparison to bacteria, have demonstrated better degradation capabilities for polyethylene (PE) and polyurethane (Muhonja et al., 2018). Fungi possess the ability to counteract various complex compounds, including toxic and pollutant substances (Olicón-Hernández et al., 2017). Research indicates the involvement of fungi in plastic degradation, with a decrease in the number of carbonyl groups reported in products like esters or ketones, resulting from oxidation during fungal growth. The biodegradation of plastics can be assessed by examining microbial growth, changes in polymer structure, various enzyme assays, biochemical oxygen demand (BOD), carbon dioxide production, and biomass under aerobic conditions. Alterations in molecular weight, tensile properties, fragmentation, and the presence of functional groups on the surface of plastics provide insights into the plastic biodegradation process (Restrepo-Flórez et al., 2014). Understanding the role of fungi in these processes contributes to developing effective strategies for managing plastic pollution.

6. Ideal models for investigating environmental fate of microplastics

Earthworms are recognized as crucial biochemical and autogenic engineers within ecosystems. In Plasticulture, the presence of microplastics and nano-plastics in composts and biosolids, coupled with the use of non-biodegradable plastics, poses a threat to soil quality, impacting the health of agricultural crops. Addressing the issue of non-biodegradable plastics requires systemic improvements (Calabro & Grosso et al., 2018). Further research is essential to comprehensively study and understand the environmental fate and adverse effects of both biodegradable and non-biodegradable plastics. Microplastics exhibit various physicochemical properties that can have diverse physical and chemical effects on human health and the environment. Ecotoxicological studies have reported that biodegradable plastics may be harmful to aquatic and terrestrial life, similar to non-biodegradable plastics (González et al., 2019), as illustrated in Table 4.

Table 4. Different types of plastics cause various harmful effects upon human health.

Plastic Type	Application	Impact on the health	Ref.
Polyethylene	Film former, emulsion, cosmetics, packing	Endocrine disorders, Instabilities of chromosome in lymphocytes, and Reproduction	(Çobanoğlu et al., 2021)
Polypropylene	Automobiles, packaging, Consumer products	Disorders associated with repiration, Induced hypersensitivity	(Hwang et al., 2019)
Polyvinylchloride	Toys, teethers, pipes, Luggage	Human carcinogen	(Nicholson et al., 1975)
Polystyrene	Lids, bottles, trays	Affect CNS	(Waring et al., 2018)
Polytetrafluoroethylene	Tape, films	Tumors, Neo-natal death, Toxicity of liver, immune and endocrine system	(Steenland et al.,2010)

7. Microplastics have negligible impact upon geophagous earthworms

Microplastics have been studied, and it has been found that under favorable environmental conditions, they have a negligible effect on earthworms. A long-term soil experiment involving contamination with PVC (Polyvinyl Chloride), HDPE (High Density Polyethylene), and PET (Polyethylene terephthalate) microplastics showed no significant impact on the growth, mortality, and avoidance behavior of earthworms (Judy et al., 2019). The type and size of microplastics play a role in the dose effect, with dose-dependent actions resulting in the ingestion of LDPE microplastics (Chen et al., 2020). Exposure of earthworms to 0.25% (w/w) HDPE with a size of 25 or PP microplastics with a size of 13 did not induce gut microbiota dysbiosis. Most earthworm species have mouth sizes of about 3 mm, making it easy for them to ingest microplastics with sizes less than 3 mm. Size-selective egestion of PE microplastics with smaller sizes was observed in the excreted casts (Cheng et al., 2021). Earthworms play a role in facilitating the microplastic degradation process by mediating microbes associated with degradation. A study found that earthworms induce the generation of nano-plastics from PE microplastics through digestive action, suggesting that earthworm ingestion may contribute to microplastics fragmentation. Various microbes, including fungi, bacteria, and mixed microbial biofilms, can participate in the degradation of microplastics (Kwak & An, 2021).

Understanding the interactions between earthworms and microplastics is crucial for assessing the potential ecological impacts and developing strategies for mitigating the effects of microplastics in soil ecosystems.

Future Perspectives

As earthworms possess various microbes and enzymes in their gut biota, they are capable of breaking down the organic and inorganic matter including certain types of microplastic. Harnessing such natural potential provides a sustainable and environment friendly solution for the mitigation of microplastic pollution. Trending biotechnological, nano-technological and biochar advances could offer a synergetic response in addition with the earthworms for developing promising solutions with context to the microplastic degradation.

Biotechnological approach

Combining earthworm-based biodegradation with other biotechnological or physicochemical methods may enhance overall efficiency in microplastic remediation. For instance, coupling earthworms with microbial consortia or employing techniques like sonication or photodegradation alongside earthworm activity could accelerate microplastic breakdown and removal. Earthworms could also serve as bioindicators for assessing microplastic pollution levels in various ecosystems. By studying earthworms' responses to microplastic exposure, researchers can gain insights into the extent of contamination and potential ecological impacts, aiding in the development of targeted remediation strategies. Earthworms could also be integrated into bioremediation systems designed for treating environments contaminated with microplastics. These systems could utilize earthworms to break down microplastics in soil, sediment, or wastewater, offering a cost-effective and environmentally friendly approach compared to conventional methods such as incineration or landfilling. There is a scope for enhancing the earthworm’s ability to degrade microplastics through bioaugmentation. This process involves introducing specific microorganisms into their gut microbiota that are particularly effective at breaking down plastic polymers. By selecting or engineering these microorganisms, plastic degradation efficiency of earthworms can be boosted. However, continued research, technological development, and regulatory oversight would be absolutely essential to unlock the full potential of this biotechnological approach for ensuring its safe and responsible application. Thus, the biotechnological future of microplastic degradation by earthworms holds promise for addressing one of the most pressing environmental challenges of the time, offering sustainable solutions that leverage nature's own mechanisms for plastic waste management.

Nanotechnological approach

Leveraging nanotechnology with the earthworms could enhance their abilities to degrade microplastics more efficiently and on much larger scale.

Creation of engineered nanoparticles would attract earthworms ensuring microplastic consumption as a part of their diet. Moreover, when they adhere to microplastics, they could make them palatable and easier for digestion. Nanotechnology could also be used for stimulating earthworms to secrete specific enzymes for targeting the chemical bonds of plastic. By employing genetic engineering and molecular techniques, the genetic makeup of the earthworms may be modified to get specific and selective breed with enhanced degradation potential. Moreover, by understanding the genetic mechanisms responsible for enzyme production in earthworms, researchers could engineer nanoparticles to interact with these pathways, boosting enzyme activity responsible for microplastic breakdown.

Creating habitats embedded with nanomaterials could encourage earthworm colonization in highly pollutant contaminated areas. These nanomaterials could provide essential nutrients or signalling cues to attract earthworms, enhancing their presence and action in polluted environments.

Nano sensors could also be deployed to monitor earthworm activity and the degradation process at the nanoscale level. This real-time data could inform adjustments to environmental conditions or the introduction of additional nanoparticles to optimize microplastic degradation rates.

Earthworms themselves could be engineered to synthesize biogenic nanoparticles capable of degrading microplastics. By harnessing the natural processes within earthworms, researchers could develop eco-friendly and sustainable methods for producing nanoparticle catalysts. Nanotechnology could revolutionize waste management practices by enabling the development of earthworm-based bioremediation systems at industrial scales. By incorporating nanomaterials into soil amendments or bioreactor designs, earthworms could be deployed effectively to degrade microplastics in contaminated soils or wastewater treatment plants.

Thus, the nanotechnological approaches to microplastic degradation by earthworms could offer innovative solutions to one of the most pressing environmental challenges of the time.

Biochar mediated approach

Biochar is a form of charcoal produced by heating organic material in the absence of oxygen. It is known for its high surface area and adsorption capacity. It has ability to adsorb various contaminants like microplastics because of its porous structure. When earthworms are introduced into the soil system containing biochar, the matter breakdown potential is enhanced. Biochar also offers a conducive habitat for soil microbes including bacteria and fungi. An increase in the microbial action stimulated by earthworms aids into microplastic degradation. The synergistic effect occurring due to adsorption ability of biochar and biological action of earthworm would lead to an enhancement in efficieny of microplastic breakdown. This synergistic approach needs to be properly understood for understanding the effectivenesss as well as long term implication with context to the microplastic degradation.

7. Conclusion

In the future, further research can be conducted to investigate additional earthworm species, considering both agronomical and ecological aspects.

- Endogeic species, in particular, are the most abundant in agroecosystems, and their interactions with microplastics may have synergistic effects, shedding light on the fate of plastic biodegradation.
- The degradation of plastics involves macromolecular changes through physical or chemical processes, collectively referred to as bio-deterioration.
- Physical parameters include the formation of biofilm cracks and pits, while chemical parameters involve the secretion of acids and other substances, including various enzymes, that weaken the plastic structure.
- Subsequently, the secreted enzymes lead to the breakdown of polymers, resulting in the formation of oligomers with low molecular weight. This step is known as bio-fragmentation and is considered the rate-limiting step in the process.
- Following bio-fragmentation, bacteria play a crucial role by assimilating the bio-fragmented oligomers.
- This assimilation step is essential for the bacteria to extract energy through various biochemical pathways, contributing to the secretion of oxidized waste products into the environment. This overall process is termed mineralization.
- Mineralized byproducts, such as water, nitrogen, carbon dioxide, and methane, re-enter various biogeochemical cycles.
- The microbiota present in the gut of earthworms may actively contribute to both the fragmentation and decomposition of microplastics, providing a potential avenue for remediating soils contaminated with microplastics.
- Understanding the intricate processes involved in plastic degradation by earthworms and their associated microbiota is critical for developing effective strategies to address plastic pollution in soil ecosystems.

- This research not only expands our knowledge of earthworm-mediated plastic biodegradation but also offers insights into sustainable approaches for managing plastic contamination in agricultural and ecological settings.

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