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Research article

The Effect of Polyethylene Terephthalate and Low-density Polyethylene Microplastics in Organic Material on Vermicomposting Process

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Abstract

Despite extensive research on microplastics (MPs) present in solid organic waste, the precise impact of MPs on the vermicomposting process remains poorly understood. The objective of this research was to assess the influence of particular MP polymers on the vermicomposting procedure. To investigate the effects of MP particles on vermicomposting, low-density polyethylene (LDPE) and polyethylene terephthalate (PET) particles were added to organic material. As vermicomposting process indicators, the germination index (GI), carbon-to-nitrogen (C/N) ratio, survival rate, pH, and electrical conductivity (EC) were identified. In this study, the survival rate, pH, C/N, EC, and GI values indicated that the addition of different varieties of MP polymers had a detrimental effect on composting that was direct and proportional. PET and LDPE significantly reduced earthworm survival rates by 10.51% and 14.52%, respectively. The addition of LDPE resulted in a substantial decrease in pH, likely attributable to its chemical constituents. Furthermore, treatments involving LDPE exhibited elevated electrical conductivity (EC) and carbon-to-nitrogen (C/N) ratio values. Nevertheless, the germination index (GI) effect of LDPE was markedly lower than that of PET. The findings of this research will contribute to the comprehension of the ecotoxicological impacts that polymer MPs have on the process of vermicomposting.

Keywords: microplastics; solid organic waste; vermicomposting; earthworm

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1. Introduction

In recent years, developed countries have been implementing organic farming (Reganold & Wachter, 2016). Consequently, substantial quantities of organic fertilizers are being applied to organic agricultural lands (Li & Shen, 2021). However, it has been observed that organic fertilizers or composts, in addition to being rich in organic matter and nutrients, also act as vectors for microplastic (MP) contamination in agricultural soils (Zhang et al., 2022; Iswahyudi et al., 2023; Garfansa et al., 2024; Iswahyudi et al., 2024c; Iswahyudi et al., 2024d; Iswahyudi et al., 2024e;). Generally, composting processes do not entirely eliminate MPs contaminations (Zhou et al., 2023). Vermicomposting, regarded as an environmentally sustainable approach to compost production, presents a promising alternative method for generating MPs-free compost (Sanchez-Hernandez et al., 2020). This method leverages the synergistic interaction between earthworms and microorganisms to produce high-quality vermicompost (Devi & Khwairakpam, 2023). Researchers have highlighted that the efficiency of vermicomposting is influenced by factors such as temperature, humidity, earthworm species, and population density (Ratnasari et al., 2023). Additionally, the presence of toxic substances and heavy metals are critical determinants affecting the vermicomposting process (Ducasse et al., 2022).

Previous study has demonstrated that MPs can inhibit the activities of both microorganisms and earthworms in the soil (Xu et al., 2021a). Vermicomposting of sludge contaminated with MPs has been shown to increase the quantity and decrease the size of MPs within the treated sludge (Cui et al., 2022). However, the presence of MPs does not significantly alter the chemical properties of the vermicomposting process or the physical activities of earthworms (Ragoobur et al., 2022; Iswahyudi et al., 2024a). Elevated levels of MPs introduced during the vermicomposting process can potentially diminish the efficiency of organic matter degradation, adversely affect composting parameters such as pH, C/N ratio, electrical conductivity, and germination index, and induce neurotoxicity and oxidative stress in earthworms (Zhong et al., 2021). Consequently, exposure to environmentally relevant concentrations of PE can inhibit earthworm reproduction and cause DNA damage (Sobhani et al., 2022).

Low-density polyethylene (LDPE) and polyethylene terephthalate (PET) are the two types of MPs most commonly found in the environment (Borriello et al., 2023), particularly in the form of films and fibers, due to their extensive use in plastic packaging, drinking water bottles, and various other consumer products (Nisticò, 2020; Zangmeister et al., 2022). These MPs exhibit distinct properties regarding degradation and bioavailability (Wang et al., 2020; Chen et al., 2022; Xu et al., 2023), potentially influencing the vermicomposting process. Moreover, the increasing influx of LDPE and PET into the environment raises concerns about contamination in agricultural systems, including vermicomposting processes. Understanding the impact of LDPE and PET on vermicomposting is crucial for safeguarding environmental sustainability and the efficacy of organic farming practices. Research on LDPE and PET in vermicomposting can identify risks to plants and the environment, facilitating the development of effective mitigation strategies. Therefore, comprehensive investigations into LDPE and PET within the vermicomposting context are essential to guide policymaking, promote sustainable agricultural practices, and enhance environmental protection. Currently, there is limited documentation regarding the detrimental impacts of MPs on earthworms within vermicomposting systems. Sáez et al. (2022) reported the morphological effects of agricultural plastic waste on the vermicomposting process can be detrimental to the survival and body biomass of earthworms. Earthworms increase their antioxidant activities

as a defensive mechanism against the deleterious impacts of MPs (Xu et al., 2021b). Guts of earthworms may break up and depolymerize MPs that are ingested (Fard et al., 2023; Iswahyudi et al., 2024b).

It is notable that while previous research has explored the impact of MPs on vermicomposting using diverse organic substrates, investigations specifically targeting solid household organic waste with varying types of MPs polymers are lacking. Hence, the effects of specific MP polymers on the vermicomposting process were investigated in this study. This research introduces a novel perspective by shedding light on the diverse array of MPs polymers capable of influencing vermicomposting dynamics, thereby facilitating measures to mitigate the adverse effects of those polymers.

2. Materials and Methods

2.1 Materials

Organic materials derived from livestock waste sourced from Rahayu Farm, Pamekasan, were employed as experimental substrates. To reduce the toxicity for earthworms, a partial composting process was carried out. The stabilized organic material was obtained following a 60-day aerobic turning process, utilizing a volumetric mixture of four substances, including cow dung (CD) and vegetable waste (VW). For a detailed description of the procedure, see Marco et al. (2023). The selection of these materials for experimentation was based on the absence of plastic components in both the primary materials and the stabilization process.

The earthworm species used in the experiment was *Eisenia fetida*, sourced from the Kastubi worm farm in Kasembon village, Bululawang subdistrict, Malang Regency. The earthworms were acclimated to the feed ingredients and composted for 30 days under laboratory conditions. Before introducing the laboratory-reared earthworms into the composting reactor, the earthworms' surfaces were cleansed with ultrapure water and subsequently dried overnight on moist filter paper to facilitate intestinal emptying (Zhong et al., 2021).

LDPE and PET MPs were obtained from PT. Sinergy Inti Plastindo Tbk. These MPs underwent a size reduction process through blending until a smooth texture was achieved, followed by filtration using a stainless steel sieve with a mesh <250 μm. The LDPE and PET samples that passed through the sieve were washed with ethanol (70%), then cleaned with ultrapure water once and dried at 40^{o}C for 12 h (Rodriguez-Seijo et al., 2017).

2.2 Experimental design

In this experiment, four vermicomposting reactors (vermibins) were set up, and 1 kg organic material without MPs was put into each vermibins. Edo et al. (2022) investigated compost from municipal solid waste and found that the content of MPs ranged from 5000-20,000 particles/kg (dry weight) dominated by PET and LDPE. Considering that more and more MP particles in the compost, MPs were weighed using an analytical balance (KERN ABS 220-4) and mixed with 1 kg of organic material in vermibin. the concentrations of the MPs added into three vermicomposting reactors were PET (1 g/kg), LDPE (1 g/kg), and control (0 g/kg). In that order, fifty mature earthworms with an average weight of 0.5 to 0.7 g each were arbitrarily selected and introduced into the vermibin receptacles during the final stage. Finally, to impede the ingress of earthworms and MPs into the decomposition

reactor, the entire apparatus was enveloped in shade cloth. The study was carried out under standard environmental conditions (26°C-34°C), and ultrapure water was sprayed three times a week to maintain the relative humidity at around 65% (Marco et al., 2023).

2.3 Experimental methods

The determination of vermicompost waste's pH, electrical conductivity (EC), C, and N was conducted in accordance with Standard Methods (Zhong et al., 2021). The seed germination index (GI) of the vermicompost samples was computed by Kong et al. (2022). Each vermicompost sample examination was performed at ambient temperature (25± 2°C). The mean value was recorded after three evaluations of each sample were conducted.

2.4 Statistical analysis

For each treatment, the mean value and standard deviation of pH, EC, GI, and C/N ratio were computed and displayed. The data underwent statistical analysis using IBM SPSS Statistics 24. The results were presented in the form of mean standard deviation (SD). Utilizing one-way analysis of variance (ANOVA) and Duncan's test ($P<0.05$), statistical differences between the treatments were examined.

3. Results and Discussion

3.1 Survival rate

Figure 1 illustrates the survival rates observed in this study. The control treatment exhibited a survival rate of 93.12%, whereas the rates for the PET and LDPE treatments were 83.33% and 79.59%, respectively. Notably, the lowest survival rate was recorded in the LDPE treatment. Substrates conducive to the habitat of *E. fetida* earthworms (control) successfully maintained earthworm survival by ensuring the availability of food/nutrients, an undisturbed feeding rate, and stable burrowing activity. In contrast, the presence of MPs (PET and LDPE) likely contributed to elevated earthworm mortality. This increase in mortality may be attributed to biotic factors disrupting survival, leading to stress in the physiological activities of the earthworms, culminating in weight loss and eventual death.

The results of this study were in line with Marco et al. (2023), which stated that PET and LDPE MPs decreased survival rates *E. fetida* by less than 10% compared to control treatments. LDPE concentrations of ≥1% were found to significantly decrease the survival rate of *E. fetida* (Angmo et al., 2023). The critical threshold for MP contamination in soil, including LDPE, was found to be 40 g kg^{-1}, above which earthworms exhibited avoidance behavior (Palansooriya et al., 2022). Furthermore, Sáez et al. (2022) noted that resistance to PET MPS was associated with a 10% and 15% reduction in the average body weight and survival of *E. fetida*, respectively. These findings suggested that PET MPs and LDPE MPs could have a detrimental effect on the survival rate of *E. fetida*, with higher concentrations leading to increased negative impacts.

3.2 pH

The pH level plays a critical role in regulating the vermicomposting process. Figure 2 illustrates the pH changes in organic matter undergoing vermicomposting with MPs. Over time, the pH levels of the three vermibins exhibited a decreasing trend, with the pH decreasing

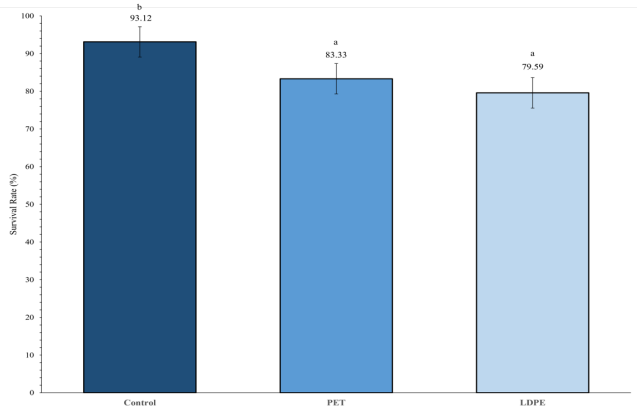


Figure 1. Survival rate in *E. fetida*. The data are the average of three replicates± standard deviation (SD). Different letters indicate a significant difference, and similar letters indicate no difference between the samples based on the comparison of means using Duncan's test at the probability level of P<0.05.

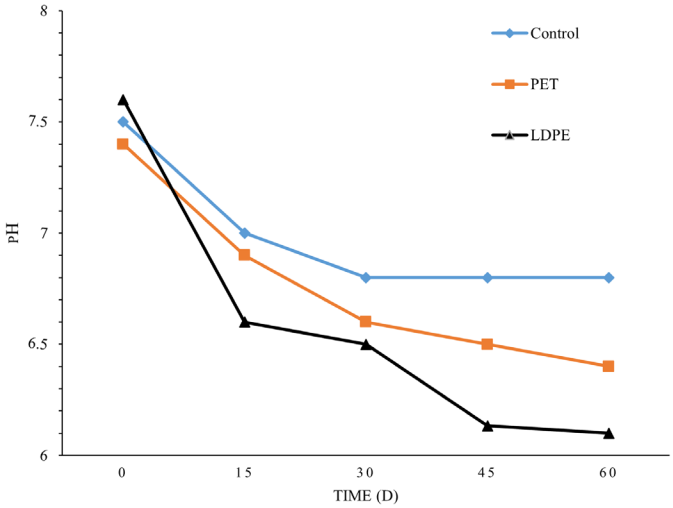


Figure 2. Effects of MPs on pH in vermicomposting

to 6.80 (Control), 6.40 (PET), and 6.10 (LDPE) after sixty days. The pH variations in the vermibins occurred in two distinct phases. Initially, there was a substantial decline to below 7.0 within the first two weeks, followed by a relatively constant pH level for the subsequent sixty days. Optimal conditions for *E. fetida* were identified to be within a pH range of 6.5 to 7.0. To facilitate the growth of active vermibin, *E. fetida*, the pH was adjusted to a slightly acidic state. During this period, the pH of the three vermibins did not exhibit significant variation. Consequently, the influence of MPs on pH was minimal during the initial stages of vermicomposting, corroborating the findings of previous research. In addition, there was a decrease in soil pH over time in all treatments. This may be due the decomposition process of organic matter by microorganisms and earthworms. Various organic acids, including acetic acid, lactic acid, and citric acid were produced (Wei et al., 2018; Li et al.,

2022). These acids contribute to a decrease in the pH of the compost medium as they dissociate, releasing hydrogen ions (H^+) into the solution (In et al., 2013).

Contamination of organic waste with polymeric MPs, including LDPE and PET, impacts the process's pH value. PET exhibits a lower pH impact compared to LDPE may be due to its slower decomposition rate in vermicomposting conditions. This slower rate is attributed to PET's chemical and structural properties, which render it more inert and resistant to decomposition (Barnard et al., 2021). In contrast, LDPE degrades more quickly, thus contributing more to the pH reduction than PET. This is because LDPE degradation releases organic acid compounds that counteract pH elevation (Jebashalomi et al., 2024). The chemical structure of LDPE, characterized by long chains of hydrocarbon polymers with minimal branching, facilitates the development of microbial biofilms on its surface.

These biofilms secrete a range of degradation enzymes, which expedite the generation of organic acids (Jiang et al., 2021), thereby exacerbating the reduction in pH levels. These variations influence the environmental equilibrium of vermicomposting and can affect fertilizer quality. The pH of the decomposition process was directly impacted by the addition of MPs to vermicompost (Katiyar et al., 2023). In the abstract by Zhong et al. (2021), it was stated that the pH of the decomposition system was directly proportional to the number of MPs added to it. An increase in the concentration of MPs had an adverse impact on the decomposition process, as evidenced by the observed reduction in pH. Furthermore, the abstract authored by Hénault-Ethier et al. (2016) noted that the pH of vermicompost had a substantial impact on the long-term composition of cultured bacteria. Consequently, a reduction in pH was observed upon the introduction of MPs into vermicompost; this, in turn, impacted the microbial composition and overall composting stability.

3.3 Electrical conductivity

Figure 3 illustrates the trajectory of EC among the three vermibins. Figure 3 showed that MPs did not significantly impact EC during vermicomposting ($P>0.05$). EC, indicative of salinity and inorganic ion content in organic material, showed a distinctive pattern. Initially, the EC values of the vermibins increased, peaking on the thirty-first day with maximum values of 1100 µs/cm (Control), 1300 µs/cm (PET), and 1400 µs/cm (LDPE). Subsequently, the EC values decreased steadily, lasting from 45 to 60 days, yet they never fell below the initial organic material's EC (488 µs/cm). The EC increased gradually and decreased rapidly, influenced by the type of MPs introduced into the vermibins. The addition of MPs evidently promoted EC growth, enhancing the total dissolved ions in the sludge. This rise in EC during the first 40 days can be attributed to the metabolic activities of earthworms and microorganisms, which increased the salinity of the organic material and released mineral salts such as phosphate, potassium, ammonium, and inorganic ions. (Gupta & Garg, 2008; Raza et al., 2022).

LDPE exhibited a more substantial reduction in EC values compared to PET during the vermicomposting process. This phenomenon may attribute to the chemical nature of LDPE, which is inert and challenging to biodegrade, resulting in a denser and less decomposable residue (Datta & Halder, 2019). This residue can inhibit EC in a vermicomposting environment. Conversely, PET tends to increase EC values. The degradation of PET generates more biologically active organic compounds (Jabłońska et al., 2019), elevating ion concentrations in solution, thus enhancing electrical conductivity. Furthermore, the differences in chemical structure and degradation rate between LDPE and PET influence the quantity of MP particles released into the solution, subsequently impacting electrical conductivity.

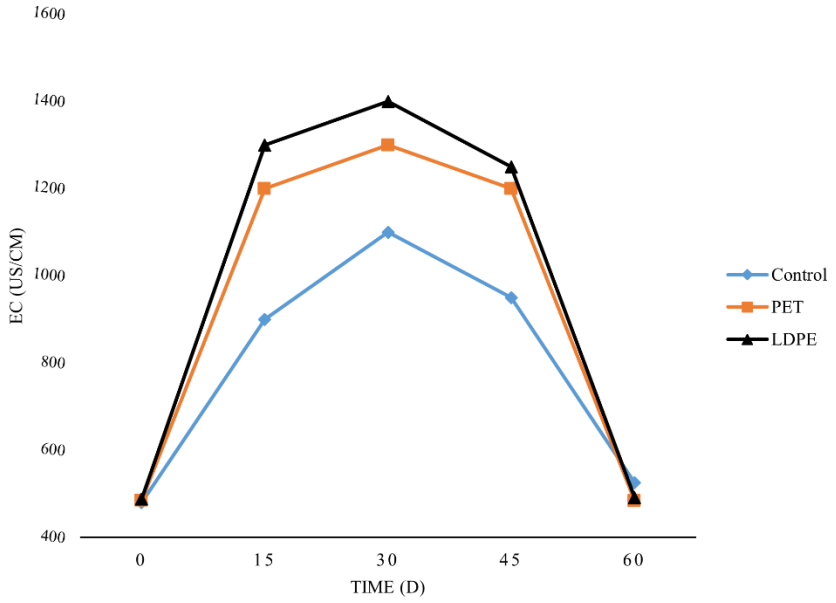


Figure 3. Effects of MPs on EC in vermicomposting

The contaminant of vermicompost with MPs had a significant effect on the EC of the final product (Oyege & Balaji, 2023). High MP addition in vermicomposting resulted in an increase in EC (Zhong et al., 2021). Additionally, the type or origin of the vermicompost used also influenced EC, with teas extracted from grazing vermicompost and sheep and cattle manures showing higher EC values (Xing et al., 2015). The vermicomposting itself can also affect EC, as vermicomposting has been found to decrease EC values in sodium salt soils (Tammam et al., 2023). Overall, the addition of MPs and the type of vermicompost used can influence the EC of vermicompost, with higher MP addition and certain types of vermicompost leading to increased EC values.

3.4 Seed germination index

The maturity and toxicity of vermicomposting organic material containing MPs were evaluated using the germination index (GI). Figure 4 presents the GI values recorded in this study. On the 60th day, the GI values peaked, with the control, PET, and LDPE samples achieving maximum values of 95%, 90%, and 65%, respectively. Except for the LDPE sample, the control and PET vermibins reached maturity on the 13th day, attaining full maturity by the 60th day. Generally, a GI value exceeding 80% is indicative of mature compost or non-phytotoxic material (Bhat et al., 2017; Karapantzou et al., 2023; Miao et al., 2023). MP contamination was observed to hinder compost maturity, with the type of MP polymer significantly impacting the vermicomposting process. This effect may be due to the degradation of some MPs during vermicomposting, which produces phytotoxic compounds that inhibit plant growth (Gao et al., 2019). Consequently, a high concentration of MPs in vermicomposting led to a reduced GI, reflecting a diminished capacity for seed germination and successful plant growth (Zhong et al., 2021).

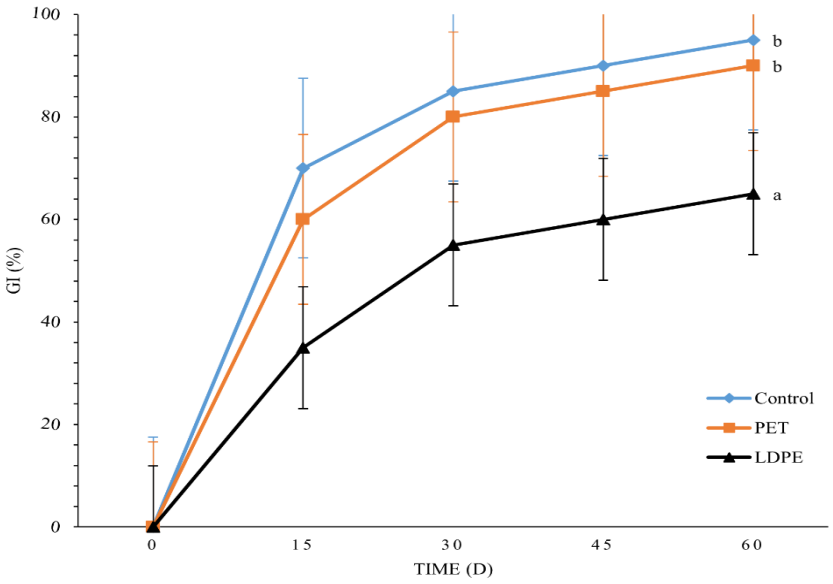


Figure 4. Effects of MPs on GI in vermicomposting. The data are the average of three replicates± standard deviation (SD). Different letters indicate a significant difference, and similar letters indicate no difference between the samples based on the comparison of means using Duncan's test at the probability level of $P<0.05$.

3.5 Carbon or nitrogen ratio

As demonstrated in this study, Figure 5 illustrates the C/N ratio, a critical indicator of organic matter stability. Figure 5 shows that MPs did not significantly impact the C/N ratio ($P>0.05$). However, there was a declining trend of the C/N ratio among the three vermibins that followed the order: LDPE > PET > control. This discrepancy became particularly pronounced between days 15 and 60. After 15 days, the C/N ratio began to decrease, and the rate of decrease accelerated with different types of MPs. Consequently, MPs compromised the stability of newly formed biological matter. During vermicomposting, the incorporation of MP particles altered the C/N ratio. Substantial quantities of MPs in vermicompost resulted in a higher C/N ratio compared to lower MP addition (Zhong et al., 2021). Furthermore, the C/N ratio of vermicompost can be influenced by additives with a high C/N ratio, such as cattle manure (Ragoobur et al., 2022). The C/N ratio of the final products was not significantly impacted by the additives used in vermicomposting (Xing et al., 2015). Overall, the incorporation of MPs and other additives substantially affected the C/N ratio during the vermicomposting process.

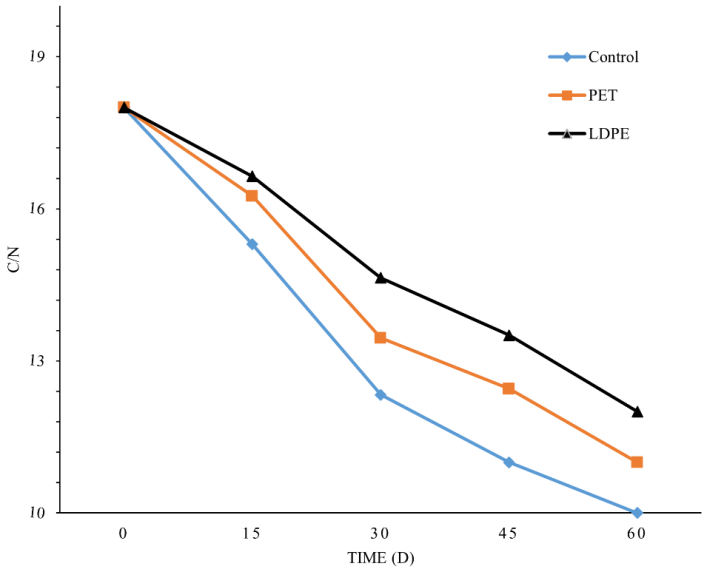


Figure 5. Effects of MPs on C/N ratio in vermicomposting.

4. Conclusions

The aim of this study was to evaluate the impact of several MP polymers (PET and LDPE) on the vermicomposting process. The incorporation of these polymer MPs impeded organic matter degradation and stabilization during vermicomposting. PET and LDPE significantly reduced earthworm survival rates by 10.51% and 14.52%, respectively. The addition of LDPE resulted in a substantial decrease in pH, likely attributable to its chemical constituents. Furthermore, treatments involving LDPE exhibited elevated electrical conductivity (EC) and carbon-to-nitrogen (C/N) ratio values. Nevertheless, the germination index (GI) effect of LDPE was markedly lower compared to that of PET. Future studies should be conducted to determine the impact and the specific effects of MPs type, size, and concentration, as well as the influence of the overall composition of the vermicomposting system.

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6. Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

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