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# Short-term effect of poly lactic acid microplastics uptake by *Eudrilus eugenia*

Shahad Khaldoon [a], Japareng Lalung [a,*], Mohamad Anuar Kamaruddin [a], Mohd Firdaus Yhaya [b], Mahboob Alam [c], Masoom Raza Siddiqui [d], Mohd Rafatullah [a,*]

[a] School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia
[b] School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[c] Division of Chemistry and Biotechnology, Dongguk University, 123, Dongdaero, Gyeongju-si 780714, Republic of Korea
[d] Chemistry Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

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A B S T R A C T

The present study investigated the uptake of polylactic acid (PLA) microplastics, the most commonly used biodegradable plastic by *Eudrilus eugeniae*. It was done by observing their weight changes, biomass, and microplastics concentration after feeding them with a mixture of PLA and cow dung for 16 days at the ratio of 0%, 10%, 30%, 60%, and 80% w/w dry weight. The mortality rate of the earthworms for all the PLA concentrations during the 16 days of the feeding period was 0%. However, the microplastic significantly affected the earthworms' weight (*P*-value 0.00027), especially at 80% of PLA concentration. The earthworms had the lowest weight gain at 80%, followed by 60%, 30%, and 10% of PLA, respectively. The earthworms subjected to 80% and 10% of PLA had a similar pattern with the control. The PLA microplastics concentration factor (CF) in the vermicast was the highest at the 10% PLA than other treatments because *Eudrilus eugeniae* did not degrade the PLA. This study concludes that even though PLA is biopolymer-based, the earthworm cannot assimilate it. However, in the long run, there is a possibility of further degradation of the PLA MPs ejected in the vermicast due to the rich microbial environment provided by the earthworms and vermicast.


## 1. Introduction

Soil ecosystem provides various services such as carbon sequestration, biogeochemical cycling, and the promotion of biodiversity. Documentation of microplastics polluting soil has shown an increasing trend, with a high potential effect on soil biodiversity and function (de Souza Machado et al., 2019; Helmberger et al., 2020; Wang et al., 2019; Zhou et al., 2020). Microplastics (MPs) can enter the soil ecosystems through a few different ways, such as by mulching materials used in agriculture, during the production of MPs, secondary sources from the breaking down of plastics materials, and sludge produced by wastewater treatment. Despite the widespread MPs inland, long-term or large-scale monitoring data are limited. Sources of terrestrial MPs and their effect on terrestrial microorganisms have been neglected until recent years (de Souza Machado et al., 2018; Rillig et al., 2017a). A few researchers reported the possibilities of surface MPs transported by the earthworms into the more in-depth soil profile (Huerta Lwanga et al., 2016; Rillig et al., 2017b; Yu et al., 2019; Zhang et al., 2018). Worms move particles by the adhesion on their body when it comes to contact with the pollutant and by their cast due to ingestion. Therefore, there is a potential of MPs leaching to the groundwater through the earthworm burrows. The mortality of earthworms caused by petroleum (polystyrene, polystyrene) and bio-based (Polylactic acid, Mater-BI) MPs was observed in a few studies such as (Cao et al., 2017; Chen et al., 2020; Alauzet et al., 2002a; Sforzini et al., 2016; Yu et al., 2019).

Since most biodegradation testing of bioplastic is done in controlled conditions, it is hard to ensure the complete degradation of these materials when they enter the environment. As a result, accumulation and further fragmentation of these plastics material into smaller particles in nature are likely. These fine particles have

* Corresponding authors.
*E-mail addresses:* japareng@usm.my (J. Lalung), mrafatullah@usm.my (M. Rafatullah).

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shown various impacts over ingested organisms (González-Pleiter et al., 2019; Shruti and Kutralam-Muniasamy, 2019; Zuo et al., 2019). Furthermore, these fine particle sizes of microplastics were the main cause of metal accumulation and genotoxicity in earthworms (Huang et al., 2021; Xu et al., 2021a; Xu et al., 2021b; Xu et al., 2022). Nevertheless, less attention has been paid to their impacts on the soil ecosystem (Kooch and Jalilvand, 2008). Thus, the potential of earthworms for plastics degradation and toxicity is underexplored.

*Eudrilus eugeniae* has a natural ability to colonise organic waste, with high resistance, possess tolerance to a wide range of environmental factors and ability to digest and assimilate organic matter (Kooch and Jalilvand, 2008). This research aims to observe the possibility of *Eudrilus eugeniae* earthworms degrading PLA MPs. The investigation was done by examining the effect of different concentrations of PLA on their weight changes, growth rate and cast concentration factor (CF) when the specific concentration of PLA MPs was added into their feed.

## 2. Materials and methods

Twenty-five plastic containers with a diameter of 8.5 cm and a height of 6.0 cm were used for the feeding experiment. Virgin polylactic acid, Grade PLA4032D were imported from Nature Works, the USA by Innovative Pultrusion Sdn. Bhd. The PLA was ground to the required size of between 40 and 106 µm. *Eudrilus eugeniae* were provided by the ECO- PRO laboratory in the School of Industrial Technology Universiti Sains Malaysia (USM). The cow dung was obtained from a cow farm in Balik Pulau, Penang, Malaysia. The same batch of cow manure was used to feed the earthworms throughout this experiment.

### 2.1. Media preparation

Pre-composted cow dung was used as a primary feeding media modified from the methods (Coulibaly et al., 2011; Manyuchi and Whingiri, 2014; Sampedro and Whalen, 2007). The cow dung was homogenised by passing them through a 2 mm and 800 µm sieve and immersed for 24 h in water to remove insects. It was then pressed in a cloth to remove excess water before usage. The PLA MPs were mixed with cow dung at five different percentages; 0%, 10%, 30%, 60%, and 80% of PLA. Finally, a synthetic nylon-based cloth was placed on top of the media to attract the earthworms to cast on the media's top and collect and retain the moisture.

### 2.2. Earthworms selection and adaptation stage

The selection and adaptation methods of *Eudrilus eugeniae* earthworms were modified from Pokarzhevskii et al., 2000. For this experiment, 25 individuals (average weight of 1.041 ± 0.030 g) were selected from the vermicomposting units at the ECO-PRO laboratory in the School of Industrial Technology, Universiti Sains Malaysia. The initial weight of the control was 1.060 ± 0.040 g before gut voiding. The earthworms were adapted to the new environment for seven days, with continuous monitoring of moisture content, feed, and mortality every two days.

### 2.3. Feeding experiment

Round plastic containers with 10.4 cm diameter and 8.0 cm height were used for the feeding treatment. All the containers have six holes for aeration. The cow dung and PLA MPs mixture was placed into the container before adding an earthworm. Five replicates were used per treatment. The container was labelled and placed in a dark, closed box with a 16:8 ratio of light/ drank. The experiment was carried out for 16 days. Every four days, the earthworms were measured for their weight and checked for their feed moisture. After 16 days, the earthworm was subjected to gut voiding for 48 h by placing them into an empty container. Five millilitres of distilled water were sprayed every two days to maintain the moisture. The cast was collected from the containers, placed into a zip-locked bag, and kept at 4C for further analysis.

### 2.4. PLA MPs concentration in the vermicast

The concentration of PLA MPs in the vermicast, in terms of cast concentration factor (CF), was calculated based on the work done by Huerta Lwanga et al. (2016), as shown in Eq. (1):

$$CF = \frac{S_{PL}}{S_s} \tag{1}$$

Where $S_S$ is the plastic fraction in the initial substrate, the fraction of plastic in the casts ($S_{PL}$) was indicated in Eq. (2). $M_{PL}$ and $M_C$ are the dry weight of the microplastic in the casts (mg) and the collected cast's dry weight (mg), respectively.

$$S_{PL} = \frac{M_{PL}}{M_c} \tag{2}$$

### 2.5. Statistical analysis

Statistical analysis such as descriptive analysis, analysis of variance, and correlation analysis was performed using IBM SPSS Statistics 26. A descriptive study was present as mean, standard deviation, maximum and minimum values for different parameters. One-way and two-way analyses of variance were performed to determine any significant differences between the parameters. Finally, correlation analyses using Pearson Correlation Coefficient Formula were conducted to show the relationship between PLA concentration and earthworms' weight change.

## 3. Results and discussion

### 3.1. pH values for the vermicast

Table 1 shows the pH value for vermicast produced by the worms with different PLA MPs concentrations after 16 days. The initial pH value of the cow dung was 6.7 ± 0.005. The treatment of pH values for all concentrations did not vary much from the control, as degradations could occur to the PLA MPs. However, the ANOVA test showed that treatment with 60% of PLA was significantly different from all other treatments with a P-value of <0.05. The significant difference was due to the very little variation or identical values between the replicates of the pH of all the different concentrations of PLA (Table1). In reality, although ANOVA test showed a significant difference between pH 6.85 and 6.96, the two values are very similar.

**Table 1**
The pH value of vermicast produced by worms after 16 days of exposure to 10%, 30%, 60%, and 80% concentrations of PLA MPs.

| Percentage of cow dung | Percentage of PLA | pH value (1:10) |
|---|---|---|
| 100 | 0 | 6.96 ± 0.017$^b$ |
| 90 | 10 | 6.97 ± 0.008$^b$ |
| 70 | 30 | 6.92 ± 0.005$^b$ |
| 40 | 60 | 6.85 ± 0.005$^a$ |
| 20 | 80 | 6.98 ± 0.048$^b$ |

Different letters indicate significant differences amongst treatment, a > b > c (Anova, $P < 0.05$).

The pH of vermicast produced by worms after the 16th day was neutral. A drop in pH value indicates the occurrence of degradation due to lactic acid formation (Goto et al., 2020; Rodríguez-Morgado et al., 2017). In addition, earthworms are less tolerant to extreme acidic and alkaline soil conditions, causing them to escape from their growth medium. This factor may lead to their death. However, adding granulated limestone would increase the pH and reduce the mortality of the earthworms (Anderson et al., 2013). In addition, the availability of certain nutrients such as carbon, nitrogen, potassium, and calcium are strongly dependent on pH (Viljoen and Reinecke, 1989). Thus, the pH of the media used in this experiment was 6.7 ± 0.005, as the cow dung was composted before use. In this experiment, the temperature and moisture content was kept constant within the range of the earthworms' tolerance (temperature of 28 °C, moisture content of 67.16 ± 0.327%). It is also known that moisture content, pH, and temperature are considered important environmental factors that influence the biodegradation of plastic materials and affect earthworms' health. In the biodegradation process, moisture content affects the microbial activities and hydrolysis process, which is crucial in polymers degradation (Müller, 2002). Earthworms' activities and behaviour are highly influenced by moisture content because their body weight consists of about 90% water content (Dalby et al., 1996). Similarly, temperature and pH also affects the activities of both microbes and earthworms. The temperature has a big role in the growth of earthworms. For example, the growth rate of *Lumbricidae* sp. was increased by 1.5 times when the temperature was raised from 15 to 20 °C (Wever et al., 2001). The pH level affects the growth of microbes by causing the inhibition of certain enzymes. For example, the activities of enzymes released by microbes to break down polymers may be affected by lower pH and therefore influences the survival of the microbes and the degradation of the plastics materials (Gupta et al., 2016; Jin and Kirk, 2018).

### 3.2. PLA MPs in the vermicast

The PLA MPs were recovered from the vermicast after 16 days of feeding. The concentration of the cast MPs was significantly higher in the 10% of PLA and the lowest at 30% of PLA at 0.9 and 0.48, respectively, as shown in Fig. 1. The concentration factor (CF) is expressed as the MPs' ratio in the vermicast to MPs in the feed. The P-value was 0.049 showing a low effect between the ratios. Thus, a lower concentration of PLA MPs in the cast than in the feed would result in a higher CF value. This result is related to the digestion of organic matter and MPs' excretion in the cast under different feeding treatments. A significant effect was observed between the CF and different PLA concentrations, as shown in Table 2.

**Table 2**
One-way Anova analysis of variance of the CF and the different concentrations of PLA MPs.

| PLA MPs concentration | P-Value | | |
|---|---|---|---|
| | 10% | 30% | 60% |
| 30% | 3.62E−06 | | |
| 60% | 2.28E−06 | 5.18E−06 | |
| 80% | 2.26E−07 | 3.10E−07 | 4.3E−06 |

In this study, the highest CF value of 0.9 was obtained for earthworms placed in 10% of PLA, where the cast contained the lowest concentration of PLA MPs and the highest organic matter compared to other treatments. A similar pattern was observed by Huerta Lwanga et al. (2016), where the CF for *L. terrestris* was the highest when the earthworms were placed in the lowest concentration of LDPE MPs. The other treatments showed lower increments of CF, as shown in Fig. 1. This observation was due to the higher concentration ratio of MPs in the feed, which is not digestible as organic matters. Therefore, it was assumed that the treatment of 80% of PLA would result in the lowest CF value since it contained the most concentration of PLA MPs in the feed. However, 30% of PLA MPs resulted in the lowest CF value among all the treatments. This observation suggests that some of the organic matter ingested by the earthworms were used for their metabolism process. Therefore, the organic matter content excreted in the cast was lower than in the feed.

All the PLA ingested by the earthworms were excreted, resulting in a similar amount of PLA content in the feed and the cast. Thus, the concentration of PLA in the cast was higher than the feed since the amount of organic matter excreted reduced while the PLA amount remained constant. This might explain the lowest CF of the worms placed in 30% PLA, where some of 70% of organic matter is available for ingestion while avoiding the ingestion of the PLA MPs, causing the low concentration of PLA in their cast. While the worms placed in the 80% PLA only have 20% organic matter in their feed, leaving them with no option but to ingest the PLA, causing the increase of PLA concentration in their cast.

### 3.3. The effect of PLA MPs concentration towards earthworms weight

The earthworms in this experiment were starved for 48 h. After the starvation, they were fed with PLA for 16 days and starved again for 48 h. During the 16 days of feeding, the earthworms were subjected to 10%, and 80% of PLA had a similar weight gain curve to the control. However, their weight changes significantly depending on the PLA concentration, with a *P*-value of 0.00107 (Table 3). It was positively correlated with the PLA concentration, as shown

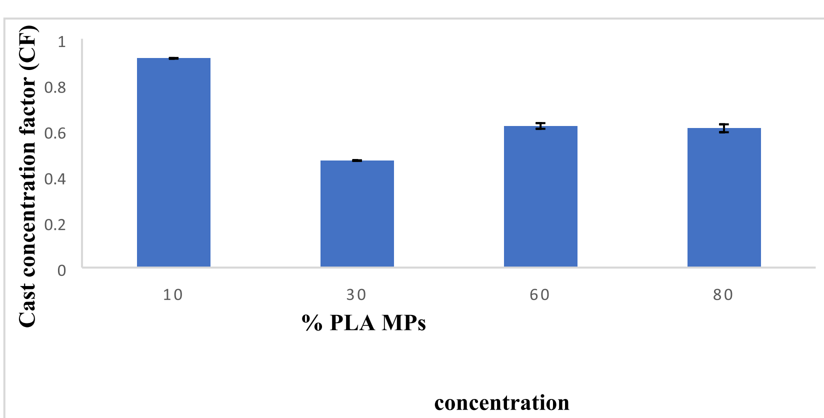


**Fig. 1.** Cast concentration factor (CF) for different concentrations of PLA MPs.

**Table 3**
Two-way ANOVA analysis of variance of weight change for 16 days without any starvation and weight change for 16 days before and after 48 h of starvation for the different concentrations.

| | Source of Variation | *df* | *F* | *P*-value |
|---|---|---|---|---|
| Weight change within 16 days of feeding | within the group | 4 | 143.4384 | 1.64E-40 |
| | between group | 16 | 2.761366 | 0.00107 |
| Weight change with 16 days of feed and two days of starvation | within the group | 6 | 143.4384 | 3.66E-65 |
| | between group | 24 | 2.761366 | 0.00027 |

Significant difference at $P < 0.05$.

**Table 4**
The correlation analysis for the weight changes in different concentration of PLA MPs with time.

| | 1st day | 4th day | 8th day | 12th day | 16th day |
|---|---|---|---|---|---|
| 1st day | 1 | | | | |
| 4th day | 0.157557 | 1 | | | |
| 8th day | 0.211906 | 0.671728 | 1 | | |
| 12th day | 0.292728 | 0.773144 | 0.847879 | 1 | |
| 16th day | 0.39303 | 0.587778 | 0.710034 | 0.846246 | 1 |

in Table 4. During the 16 days, the earthworms showed a steady weight increase for all treatments (Fig. 2). That may be because PLA MPs contributed to the earthworm's weight. A second gut voiding was conducted after the 16 days of feeding to conclude the effect of PLA on the earthworm's net weight. ANOVA test shows a significant difference between the different concentrations of PLA with time on the earthworm's weight gain within the same group, with a *P*-value of 1.64E−40 (Table 3).

After the 16 days of feeding and observations were completed, the earthworms were subjected to 48 h of guts voiding. After the second gut voiding, their weights were compared to their weight after the first starvation. From the second gut voiding test, the earthworms for the 0% and 60% PLA treatments have shown the highest weight gain percentage at 45.178% and 31.385%, respectively. Based on these results, the sequence of weight changes follows the sequence of 0% > 60% > 10% > 30% > 80% PLA. The highest weight gain among all other PLA treatments was obtained for 0% PLA, suggesting that the cow dung was the earthworm's primary source of organic matter. In contrast, the weight of earthworms for the 80%, 10% and 30% treatment of PLA declined after the guts voiding treatment (Table 5), following the sequence of 0% > 60% > 10% > 30% > 80% PLA. The earthworms' weight gain for 80% treatment was the third-highest before gut void treatments. However, the weight value dropped to become the lowest after the gutting process, which could have been influenced by the presence of the MPs in their digestive systems. Therefore, it can be concluded that the net weight gain was due to the ingestion of the cow dung and not the PLA.

However, the worms in the 60% PLA treatment had the highest weight (31.385%) compared to all the other treatments. In theory, the earthworms placed in 60% of PLA should have less weight than both earthworms fed with 10% or 30% PLA after the gut voiding because the 60% PLA treatment had more MPs and less cow dung content as compared to the 10% and 30% PLA concentrations. This irregular weight loss pattern of earthworms fed with 60% of PLA may be caused by insufficient time for the earthworms to clear their gut content, leading to extra weight due to the remaining PLA MPs in the earthworm's gut. A similar observation by (Jager et al., 2003) stated that the earthworm's gut emptying process is influenced by the time factor and the earthworm's diet media properties.

Similarly, in a study by Alauzet et al. (2002b), a different earthworm species, *Eisenia andrei*, could not digest PLA (PLA 96 and PLA 50). However, a study was done by Huerta Lwanga et al., (2018)

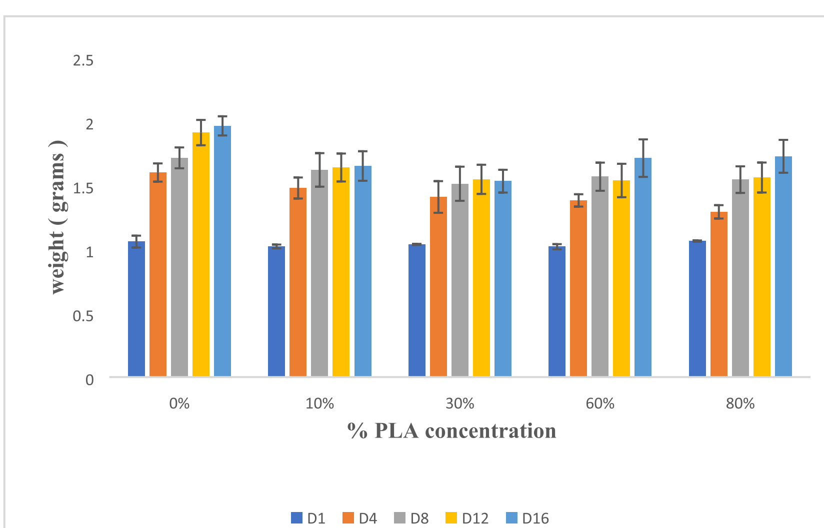


**Fig. 2.** The effect of the different concentrations of PLA MPs on the earthworm weight over time. The weight was measured every four days during the 16 days of the feeding experiment.

**Table 5**
Weight of the earthworm throughout gut voiding and feeding on PLA.

| The percentage of PLA (%) | The initial weight of the earthworm (g) A | The weight of earthworm after the first 48 h of gut voiding (g) B | The weight of the earthworm after the first 48 of gut voiding followed by feeding on PLA (g) for 16 days C | The weight of earthworm after the first 48 h gut voiding followed by 16 days of feeding and a second 48-hour gut voiding (g) D | The weight of the guts load after the second gut voiding (g) E | % weight gain based on the initial weight of the earthworm after the 16 days of feeding. C-A | % weight gain by the earthworm after gut voiding D-B |
|---|---|---|---|---|---|---|---|
| 0 | 1.060 ± 0.040[b] | 1.016 ± 0.072[a] | 1.965 ± 0.070[ab] | 1.475 ± 0.104[a] | 0.190 ± 0.127 | 85.39[ab] | 45.18[b] |
| 10 | 1.022 ± 0.015[b] | 1.004 ± 0.024[a] | 1.651 ± 0.110[ab] | 1.279 ± 0.073[b] | 0.372 ± 0.106 | 61.56[a] | 27.41[ab] |
| 30 | 1.038 ± 0.004[b] | 0.966 ± 0.036[a] | 1.533 ± 0.081[a] | 1.211 ± 0.086[b] | 0.322 ± 0.014 | 47.64[ab] | 25.36[ab] |
| 60 | 1.020 ± 0.020[b] | 0.962 ± 0.018[a] | 1.713 ± 0.142[ab] | 1.264 ± 0.086[b] | 0.449 ± 0.084 | 67.91[b] | 31.39[ab] |
| 80 | 1.064 ± 0.005[a] | 1.036 ± 0.022[a] | 1.727 ± 0.122[b] | 1.220 ± 0.098[b] | 0.507 ± 0.193 | 62.31[ab] | 17.74[a] |

*Different letters indicate significant differences amongst treatment, a > b > c (Anova, $P < 0.05$).

found that *Eisenia andrei* was able to digest low-density polyethylene (LDPE). These studies have shown that although the LDPE is a petroleum-based plastic, the earthworms could break them upon digestion of the MPs. In contrast, although the PLA is plant-based, the earthworms could not break them after digestion.

## 4. Conclusions

In this study, different PLA MPs concentrations were added in the feeding media for *Eudrilus eugeniae*. The mortality was 0% for all treatments, even though they were able to ingest the MPs and egest in their cast. The worms could not bioassimilate the MPs, perhaps because their feed content is a simpler form of organic matter, which is easier to digest than the microplastic. The PLA has affected the earthworm's weight and may even be the digestive system or the guts load. They could adapt to the existing PLA MPs in the media within the short-term exposure, even if they could not degrade and use it as their organic matter source. Thus, they had a high vermicast production when the PLA microplastic in their diets increased due to the MPs' indigestibility. The highest CF was (0.9) at the lower concentration (10%) of PLA MPs in the feed. In conclusion, the earthworm could uptake the MPs and excrement them in their cast. However, *Eudrilus eugenae* did not degrade PLA. Therefore, this study concluded that the PLA MPs eaten by the earthworms were indigested particles and could not be used as carbon sources. Thus, the plant-based PLA microplastic is not biodegradable using earthworms, *Eudrilus eugeniae*. That indicated that biodegradable plastics might not necessarily be a better alternative for conventional plastics.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Declaration

Ethics approval and consent to participate.

All institutional and national guidelines for the care and use of laboratory animals were followed.

## Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102111.

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