Research article

Ecotoxicology of Low-density Polyethylene (LDPE) Microplastics on Earthworms (*Eisenia foetida* and *Eudrilus eugeniae*)

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Abstract Microplastics are plastic particles smaller than 5 mm. These particles now pollute oceans, rivers, and soil worldwide, threatening ecosystems and adding to environmental contamination. This study investigated the acute toxicity of earthworms (*Eisenia foetida* and *Eudrilus eugeniae*) from soil contaminated with microplastics, specifically low-density polyethylene with a plastic particle size of ≤ 1 mm, (LDPE, ≤ 1 mm). Earthworms were selected related to their role as essential organisms for soil health and function. Earthworms were exposed to four different concentrations (0%, 0.1%, 0.25%, 0.5%, and 1%) of LDPE, ≤ 1 mm with replicates in artificial soil. Results show that the LC_{50} values of LDPE, ≤ 1 mm microplastics at 14 days for *Eisenia foetida* and *Eudrilus eugeniae* were 5.96% and 3.60%, respectively. The microplastics affected both *Eisenia foetida* and *Eudrilus eugeniae* and surface damage was observed at LDPE, ≤ 1 mm concentrations above 0.25% after 14 days of exposure. Greater than 10% mortality was observed at a concentration of 1% in *Eisenia foetida*, and greater than 10% mortality was observed at concentrations above 0.25% in *Eudrilus eugeniae*. Additionally, a decrease in earthworm weight was observed with exposure to increasing microplastic concentrations. This study has determined the concentrations of microplastics that impact earthworms and helping to fill a knowledge gap regarding microplastics' impact on soil ecosystems. This study reveals a toxicity trend related to increasing concentrations of microplastics affecting earthworms. The findings lay a foundation for future research on the long-term impacts on earthworm and soil health as well as broader ecological impacts and potential strategies to reduce plastic pollution in soil.

Keywords toxicology, earthworm, microplastics, pollution

INTRODUCTION

A result of the massive worldwide use of plastics and microplastics is microplastic contamination which is now found in various environments including air, soil, and water, and surprisingly, even in food, animals, and humans. Global plastic production increased from 2 million tons in 1950 to 380 million tons in 2015, a compound annual growth rate (CAGR) of 8.4%, and approximately 2.5 times the CAGR of the global gross domestic product over the same period. Between 1950 and 2015, worldwide primary plastic production generated 8.3 billion tons of plastic. Of this total, 2.5 billion tons (30%) remain in use, while the combined waste from primary and secondary plastics amounted to 6.3 billion tons. Only 12% of this plastic waste has been incinerated and 9% recycled, with 10% of recycled plastic undergoing more than one recycling cycle. Alarmingly, approximately 4.9 billion tons (60%) of all plastic produced are now in landfills or the environment (Geyer et al., 2017). When plastic waste decomposes into particles smaller than 5 mm in diameter, they are referred to as "microplastics." Microplastics have a significant impact on the environment, and over

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the past decade, microplastics have been increasingly recognized as one of the major environmental pollutants that threaten biota and the sustainability of food chain ecosystems (Ding et al., 2021).

Research on microplastics has predominantly centered on marine and freshwater ecosystems, leaving a significant gap in our understanding of microplastics in soil environments. Polyethylene (PE) and polypropylene (PP) are consistently identified as the most common microplastics in soil studies. PE dominates in Swiss floodplain soils (Scheurer and Bigalke, 2018) and comprises 75% of polymers in Hangzhou Bay (Zhou et al., 2020). Piehl et al. (2018) found that soils in Shanghai and Franconia contain the highest levels of both PP and PE. In Xinjiang Province's farmlands, PE is the most prevalent microplastic, likely due to its widespread and global use in agricultural plastic mulch (Huang et al., 2020). Due to PE plastic mulch’s significant contribution to agriculture and its economic benefits including increased harvests, improved water use efficiency, and high-quality produce, the global market for agricultural plastics production including plastic mulch was 4 million tons in 2016 which expected to grow at a rate of 5.6 million tons per year by 2030. It is estimated that 20 million hectares of cropland worldwide will be covered with plastic mulch film, with China having the highest proportion, approximately 90% (Yang et al., 2021). When agricultural harvests are complete, clearing plastic mulch films from fields is a labor-intensive and time-consuming task. As such and whether intentional or unintentional, plastic mulching films often remain on fields, leading to microplastic contamination of the soil which may affect the environment and soil-dwelling organisms.

Earthworms are often studied to measure the effects of microplastics in soil. Earthworms play a crucial role in maintaining soil fertility, improving soil structure and nutrient cycling, decomposing organic matter, and preserving biological diversity, all of which are beneficial to the ecosystem. In assessing environmental impacts, earthworms are used as bioindicators to measure the contamination of various toxins in the soil related to their response to various types of toxins and the biological accumulation that can be analyzed to reflect the results of past residues. They can also serve as indicators of an inappropriate environment (Iwai et al., 2011). Therefore, studying the ecotoxicology of microplastics on earthworms is an important study, which can inform and guide the reduction of environmental degradation and ensure the continued existence of a healthy ecosystem.

OBJECTIVE

The objectives of the study were to analyze various concentrations of LDPE, ≤ 1 mm microplastics, and the effect on two earthworm species, *Eisenia foetida* and *Eudrilus eugeniae,* including changes in their function and behavior.

METHODOLOGY

Microplastics

Low-density polyethylene (LDPE) was obtained from a plastic pellet manufacturing company located in Thailand. The LDPE was crushed by machines in the Department of Soil Science and Environment at Khon Kaen University and then passed through a sieve with a 1.0-0.5 mm hole diameter. The LDPE, ≤ 1 mm microplastic particles were washed twice with 70% ethanol and then rinsed with distilled water before being dried in an oven at 40°C to remove contaminants before their use in our experiments (Chen et al., 2020).

Artificial Soil

We prepared artificial soil by mixing 10% sphagnum peat, 20% kaolinite clay, and 70% industrial quartz sand. We adjusted the pH of the wetted substrate to 6.0 ± 0.5 using calcium carbonate (CaCO3) and maintained the moisture between 40% and 60% with distilled water (ISO 1993).

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Earthworms

The earthworms, *Eisenia foetida, and Eudrilus eugeniae* were selected from Vermitechnology for Sustainable Agriculture and Environment with selected groups of 10 worm-healthy adults with clitellum of similar size and wet mass of individual worms between 300 and 600 mg for *Eisenia foetida* and between 1,000 and 2,500 mg for *Eudrilus eugeniae*. Both species of earthworms were placed in artificial soil for 24 hours to acclimate before starting the experiment (ISO, 1993).

Ecotoxicology Experiments

The study experiments were conducted at the Department of Soil Science and Environment, Faculty of Agriculture, Khon Kaen University, Thailand. Earthworms were raised in artificial soil at 500 g with LDPE, ≤ 1 mm concentrations of 0%, 0.1%, 0.25%, 0.5%, and 1.0% with four replicates for 14 days in plastic boxes (11.5 cm x 17 cm x 6.5 cm) and the test environment, namely controlled moisture at 40–60%, controlled temperature at 20°C ± 2°C, and controlled light/dark (16 hr.: 8 hr.) followed ecotoxicology protocol of Wang et al. (2016)

Statistical Analysis

All results reported in the study were analyzed using a one-way analysis of variance (ANOVA) to determine a significant difference ($P < 0.05$) between all conditions using the Statistix 10.0 program. A multi-comparison of the least significant difference (LSD) was conducted for all measured variables.

RESULTS AND DISCUSSION

Effects of Microplastics on Mortality of *E. foetida* and *E. eugeniae*

The results showed that no mortality rate exceeding 50% was observed in earthworms exposed to LDPE, ≤ 1 mm microplastics at concentrations of 0%, 0.1%, 0.25%, 0.5%, and 1% after 14 days for *E. foetida* and *E. eugeniae*. However, the study found that the LC_{50} values of LDPE, ≤ 1 mm microplastics on *Eisenia foetida* and *Eudrilus eugeniae* were 5.96% and 3.60%, respectively (Fig. 1). While no statistically significant differences were found in the mortality of *E. foetida* ($P > 0.05$), greater than 10% mortality was observed at concentrations of 1% in *E. foetida* and at concentrations above 0.25% in *E. eugeniae*. *E. eugeniae* exhibited statistically significant differences in mortality ($P < 0.05$), with the highest mortality observed in LDPE, ≤ 1 mm microplastics at a concentration of 1%, followed by concentrations of 0.5%, 0.25%, 0.1%, and 0%, which were 17.5%, 15%, 12.5%, 7.5%, and 5%, respectively (Fig. 2). This aligns with the findings of Ding et al. (2021), who compared the toxicity of conventional (PE and PPC) and biodegradable (PLA) microplastics on earthworms. They found that earthworm mortality increased with increasing microplastic concentration. Our results suggest that microplastic concentration influences the mortality of both earthworm species.

Weight and Behavior Observed in *E. foetida* and *E. eugeniae*

During the 14-day test period, a decrease in the weight of both earthworm species was observed, with a statistically significant decrease in *E. eugeniae* ($P < 0.05$). Fig. 2 illustrates the decrease in weight of *E. foetida* in all experimental conditions with different concentrations of microplastics resulting in a decrease in the weight of earthworms, but no significant difference was observed when compared to the control ($P > 0.05$). However, in the experimental condition with LDPE, ≤ 1 mm microplastic concentrations of 0%, 0.1%, 0.25%, 0.5%, and 1.0%, *E. eugeniae* exhibited the most significant decreases in weight at concentrations of 1% of LDPE, ≤ 1 mm microplastic, followed by 0.5%, 0.25%, 0.1%, and 0%, which were 48.72%, 37.01%, 32.24%, 31.68%, and 28.70%,

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respectively (Fig. 3). Tests revealed that LDPE, ≤ 1 mm microplastic caused surface damage to *E. foetida* and *E. eugeniae* at concentrations above 0.25%, with earthworms exhibiting lost segments (Fig. 4). This is consistent with the findings of Chen et al. (2020), who observed that at microplastic concentrations lower than 0.1 and 1.0 g/kg LDPE, ≤ 1 mm, no damage was found on the surface of *E. foetida* while tearing of setae and epidermal damage were observed at a concentration of 1.5 g/kg LDPE, ≤ 1 mm. This mortality and decrease in weight may be related to the ingestion of microplastics by the earthworms, which can cause gastrointestinal blockage, leading earthworms to eat less food and/or lose energy trying to expel the foreign microplastics.

The damage to the surface of the earthworm may be related to the erratic shape and sharp irregularities of the microplastics rub against the surface of the earthworm causing irritation and damage which was also found by Chen et al. (2020).

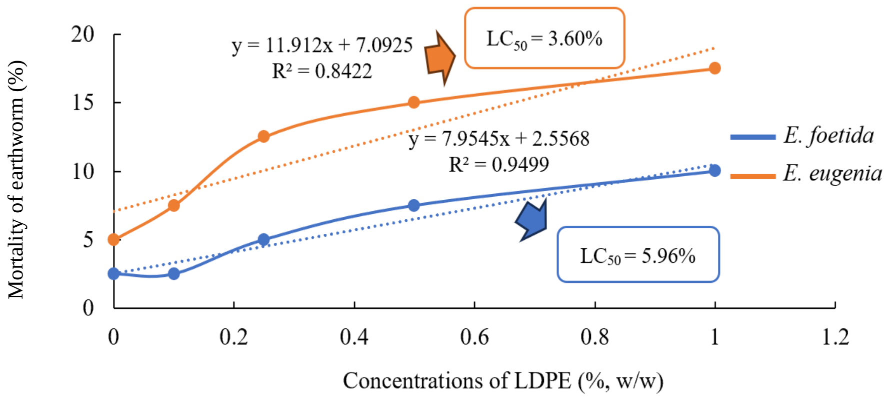

Fig. 1 Lethal concentration fifty of earthworm at 14 days

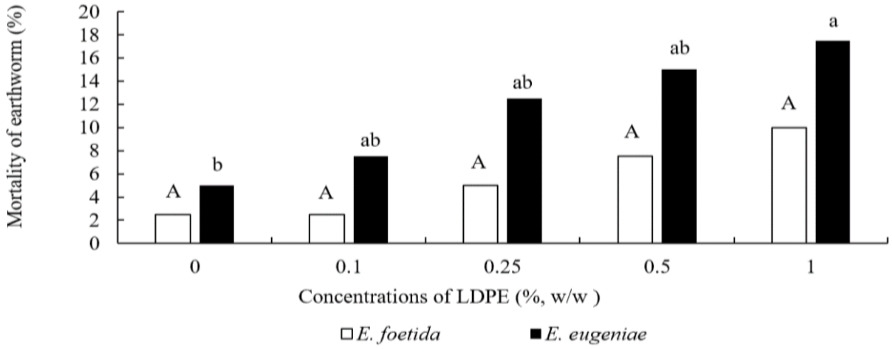

Fig. 2 The mortality of *E. foetida* and *E. eugeniae* at 14 days

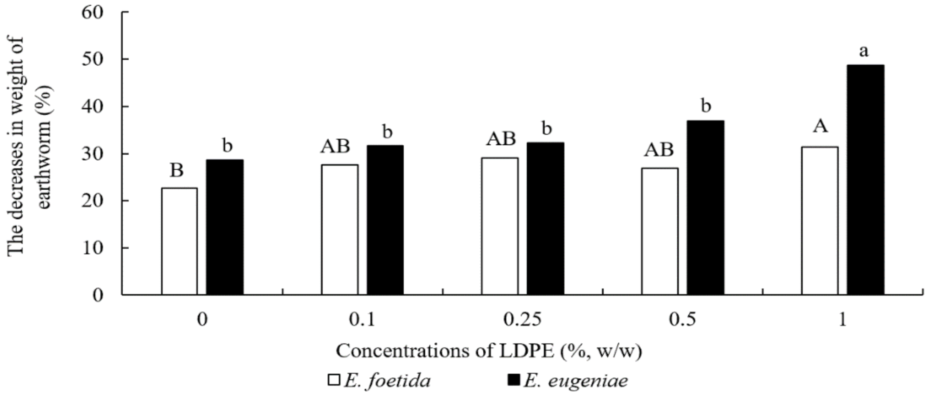

Fig. 3 The decrease in weight of the earthworm at 14 days

(a) (b)

Fig.4 The effect of microplastic observed in earthworms (a) *E. foetida* and (b) *E. eugeniae*

CONCLUSION

This study shows that LDPE microplastics with particle size ≤ 1 mm, can damage *E. foetida* and *E. eugeniae* skin and lead to decreased weight, as well as contribute to the mortality of these earthworm species. When exposed to microplastics for 14 days, the impact on the earthworms and the results indicates that microplastics may be an environmental pollutant that affects living biota in soil ecosystems. Therefore, microplastic-contaminated soil needs to be managed and rehabilitated for a sustainable ecosystem and food safety. Further studies and research should be conducted to identify and develop methods to reduce microplastic residue in the soil. Reduction of microplastic contamination of soil and its negative consequences will lead to improved soil and earthworm health reducing the impact of microplastic contamination. Based on our experimental results, earthworms can survive in soil contaminated with microplastics. This suggests that using earthworms for managing and restoring areas contaminated with microplastics is feasible. The further research on the bioremediation of contaminated soil by using earthworm could be one alternative solution as an environmentally friendly biotechnologies for cleaning up microplastic-contaminated soil.

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