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Research article

The comparison effect on earthworms between conventional and biodegradable microplastics

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ABSTRACT

Many studies have reported the toxic effects of microplastics (MPs) on organisms, especially on how conventional plastics affect organisms after short-term exposure. The effects of biodegradable plastics on organisms are, however, largely unexplored, especially concerning their impact after long-term exposure. We perform a series of experiments to examine the effects of conventional (polyethylene (PE)) and biodegradable (polylactic acid (PLA)) microplastics on earthworms at three concentrations (0.5 %, 2 %, and 5 % (w/w)) and particle sizes (149, 28, and 13 μm) over short- (14 d) and long-term (28 d) periods of exposure. Negative effects on earthworms are more pronounced following exposure to PE than PLA, particularly over the shorter term. After longer-term exposure, earthworms may adapt to PE and PLA environments. A close relationship exists between the effects of MPs on earthworms and activities of superoxide dismutase, catalase, and malondialdehyde enzymes, which we use to evaluate the degree of antioxidant damage. We report both PE and PLA to negatively affect earthworms, but for the effects of PLA to be less severe after longer-term exposure. Further investigation is required to more fully assess the potential negative effects of PLA use on soil organisms in agriculture.

1. Introduction

Since the beginning of the 20th century, plastics have been widely used in the development of materials for light, medical, and fisheries industries, and have become indispensable in the daily of people because of their affordability, excellent plasticity, and stability [15]. However, disposal of plastic products represents a significant challenge because many plastic products cannot be effectively recycled. Non-recyclable plastics may take years or decades to degrade in the environment. They break down into smaller particles known as microplastics (MPs)—plastic particles <5 mm—under the influence of factors such as solar exposure, ultraviolet radiation, biodegradation, and mechanical fragmentation [3,15]. The ecological impact of MPs has garnered increasing research attention [13,14,37]. These particles are almost ubiquitous, having been reported from soils and freshwaters, to oceans and even Arctic glaciers [3,41,45].

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The main sources of MPs are agricultural mulch, industrial production, and plastic waste degradation. These MPs occur widely in the environment and enter soils through a series of pathways [6–8,38]. Of these sources, agricultural mulch is a significant contributor to MP pollution in soil, particularly in China, where plastic films are used extensively to enhance agricultural yield [29]. Traditionally, these films are made of polyethylene (PE), a chemically stable plastic that is slow to degrade in soil [25]. The widespread use of PE films in farming, coupled with inadequate recycling mechanisms, has led to the accumulation of plastic residue in agricultural soils. These residues can break down into MPs through mechanical processes, ultraviolet radiation, and biodegradation, and pose a serious threat to farmland soil ecosystems [36]. To address this contamination issue, the agricultural industry has begun using biodegradable plastics such as polylactic acid (PLA), which can be broken down by soil enzymes and microorganisms into harmless substances like carbon dioxide, water, and methane [17]. However, biodegradable plastics can also contribute to environmental MP pollution [35], and because these plastics are more susceptible to degradation than conventional plastics, they may release MPs faster, potentially impacting soil ecosystems [23].

Soil is the primary accumulation site for MPs, and these MPs affect soil physicochemical properties and even ecosystems [43]. Soil animals play important roles in the ecosystem, and are potentially affected by MPs. For example, Kwak and An [19] demonstrated that MPs can be ingested by soil animals, leading to tissue damage, oxidative stress, genotoxicity, reproductive toxicity, neurotoxicity, and metabolic disorders that ultimately affect animal growth, reproduction, and survival. Earthworms, common soil invertebrates, are often referred to as 'soil ecosystem engineers' because of their role in promoting organic matter decomposition, improving soil permeability, and enhancing soil water and fertilizer storage capacity through their feeding and burrowing activities [12,27]. Because earthworms are highly sensitive to some soil pollutants, they are ideal indicator organisms for ecological risk assessments and ecotoxicological studies on soil contamination [11,39]. *Eisenia fetida* is often used in standardized toxicity tests because of its adaptability and short life cycle. Zhang et al. [43] reported that addition of polyethylene MPs (PE-MPs) increased earthworm weight loss rate and mortality. Kwak and An et [19] reported exposure to PE-MPs affected coelomocyte viability and damaged male reproductive organs. Jiang et al. [15] reported exposure to polystyrene MPs caused DNA damage in earthworms. However, each of these studies has focused on the effects of single plastic types on earthworms. Comparative analyses of the effects of different types (conventional and biodegradable), sizes, and concentrations of MPs on earthworms have not been undertaken.

We examine differences in the responses of earthworms to conventional and degradable MPs, and examine their mortality, biomass, and oxidative stress enzymes activities over a 28-day period. Polyethylene, a commonly used traditional plastic, is prevalent in soil [42]. Polylactic acid is a widely used biodegradable plastic known for its strength, biocompatibility, and biodegradability [1,16,26]. These results provide a scientific basis for the use and ecological risk assessment of conventional and degradable MPs.

2. Materials and methods

2.1. Materials

Experimental soil samples were collected from the Yangzhou University campus, China (119°32′28.8″E, 33°16′40.3″N). Soil samples were collected from subsurface depths of 20–30 cm to prevent soil-surface MP contamination from affecting the experiment. Prior to experimentation, soils were air dried, sieved through a 2 mm screen to remove impurities (e.g., gravel, grass roots), and sealed to minimize contamination.

Worms (*Eisenia fetida*) were obtained from a vermiculture farm in Jurong, Jiangsu China province. Healthy, active worms with clear clitella, weighing 400–600 mg, were selected. Before inoculation, worms were acclimatized for one week to experimental soil in laboratory culture conditions. Earthworms underwent a 12-h fasting period prior to inoculation, during which their intestines emptied; surface impurities were also washed off with distilled water. Worms were then wiped dry and placed into culture dishes. Culture conditions were maintained at 25 ± 2 °C and a light cycle of 16 h D: 8 h L, with adequate ventilation.

MPs used in experiments were non-degradable plastic PE and biodegradable plastic PLA powders purchased from a plastic material company (Dongguan City, Guangdong Province). To remove possible contaminants, MP particles were rinsed once with ethanol (70 %), washed twice with deionized water, and dried at 40 °C [32].

2.2. Indoor experimental design

PE and PLA MPs of 149, 28, 13 μm particle size at three concentrations (0.50 %, 2 %, 5 % (w/w)) were used. Each treatment was replicated three times, with six additional blank controls (without MPs). MPs were thoroughly mixed with the soil and packed into culture boxes. Additionally, 120 g of well-decomposed cattle manure was added to each culture box as a nutrient source for the earthworms. Worms that had grown actively, appeared healthy, and weighed 400–600 mg after 24 h of purging, were selected for experiments; each culture box contained 40 individuals.

2.3. Earthworm's biomarkers in response to MPs exposure

To visualize the toxic effects of MPs on earthworms, five earthworms from each culture box were randomly collected and underwent pre-treatment analysis to determine oxidative stress enzyme (superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT)) levels. Enzymes were assessed using a kit (microfabrication) and tested by Suzhou Kemin Biological Company, China (120°44′9.481″E, 31°15′53.971″N).

2.4. Data analysis

Data are expressed as the mean ± standard deviation and were analyzed using Spss 2023 and plotted using R 4.2.3. Statistical differences between treatments were assessed using one-way ANOVA and differences were considered statistically significant when $p < 0.05$.

3. Results

Worm mortality was significantly higher in all treatments compared with controls (Fig. 1). After both short (14 d) and longer-term (28 d) exposure, the mortality rate of worms exposed to PE was higher than those exposed to PLA. However, mortality rate decreased after longer-term exposure (4%–35 %) compared with short-term exposure (10%–47 %).

Following exposure to MPs, the rate of change in biomass trended upwards after 14 d exposure (Fig. 2A and B), particularly with exposure to PLA (Fig. 2B). An increase in PLA increased the biomass change rate, particularly with smaller-sized MP particles (~30 % for 13 μm particles at 5 % (w/w)). However, MP exposure for 28 d negatively affected the biomass change rate (Fig. 2C and D), especially with exposure to PE. Irrespective of the exposure addition and particle size, worm biomass decreased by ~20 % compared with worms in controls.

After 28 d exposure, a notable difference on egg case (cocoon) numbers occurred between PLA and PE treatments. After exposure to PLA, cocoon numbers exceeded those of worms exposed to PE (Fig. S1A). Additionally, in PLA treatments, cocoon numbers were significantly greater than in controls, particularly those in which small-sized (13 μm) MPs were added, and cocoon numbers increased with increased MP concentrations. In contrast, after exposure to PE and small-sized (13 μm) MPs, cocoon numbers significantly decreased compared with controls (Fig. S1B), but for other MP size classes, cocoon numbers trended upwards with increased MP concentration.

Following MP exposure, changes in SOD levels differed between treatments with different PE and PLA concentrations (Fig. 3). After 14 d exposure, with increased particle size, SOD activity in PE treatments trended upwards from 0.5 % to 2 % (w/w) concentrations, but at 5 % (w/w) SOD activity significantly decreased. The opposite trend was observed with exposure to PLA. In contrast to 14 d exposure, SOD activity in both longer-term PE and PLA treatments decreased significantly. Additionally, with increased MP concentration, smaller particle sizes had marginally lower SOD levels.

After 14 d exposure (Fig. 4), PE did not affect CAT activity, whereas its activity decreased significantly with PLA. However, after 28 d exposure, regardless of MP type, CAT activity trended upwards compared with controls. The increase in CAT activity was lower after exposure to PE than PLA.

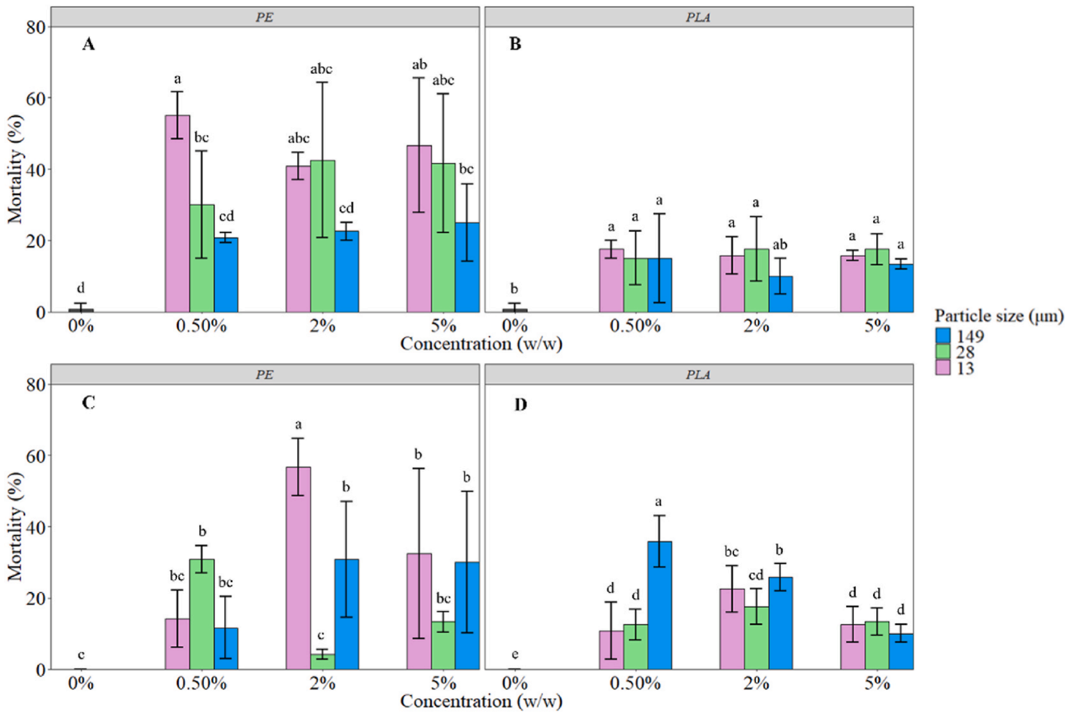

Fig. 1. The mortality in *E.fetida* induced by exposure to MPs. (A) and (B) are the mortality after 14 days of exposure to PE and PLA, respectively; (C) and (D) are the mortality after 28 days of exposure to PE and PLA, respectively. Different alphabet letters indicate significant differences between treatments at the $p < 0.05$ level.

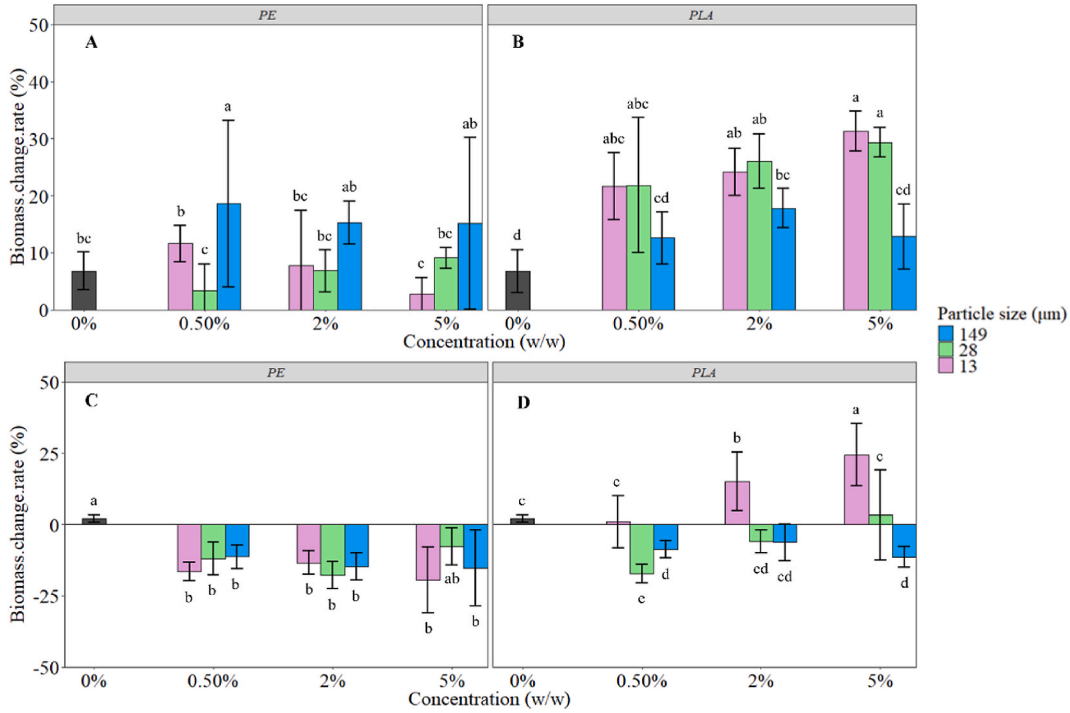

Fig. 2. The biomass change rate in *E.fetida* induced by exposure to MPs. (A) and (B) are the biomass change rate after 14 days of exposure to PE and PLA, respectively; (C) and (D) are the biomass change rate after 28 days of exposure to PE and PLA, respectively. Different alphabet letters indicate significant differences between treatments at the $p < 0.05$ level.

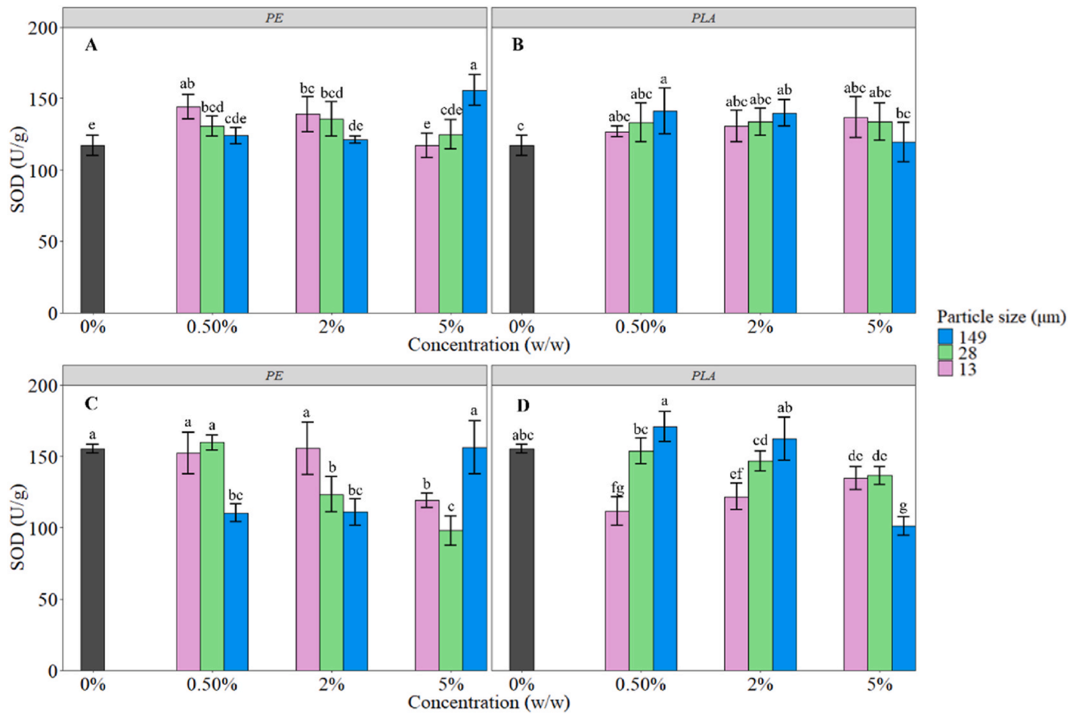

Fig. 3. The SOD level in *E.fetida* induced by exposure to MPs. (A) and (B) are the SOD level after 14 days of exposure to PE and PLA, respectively; (C) and (D) are the SOD level after 28 days of exposure to PE and PLA, respectively. Different alphabet letters indicate significant differences between treatments at the $p < 0.05$ level.

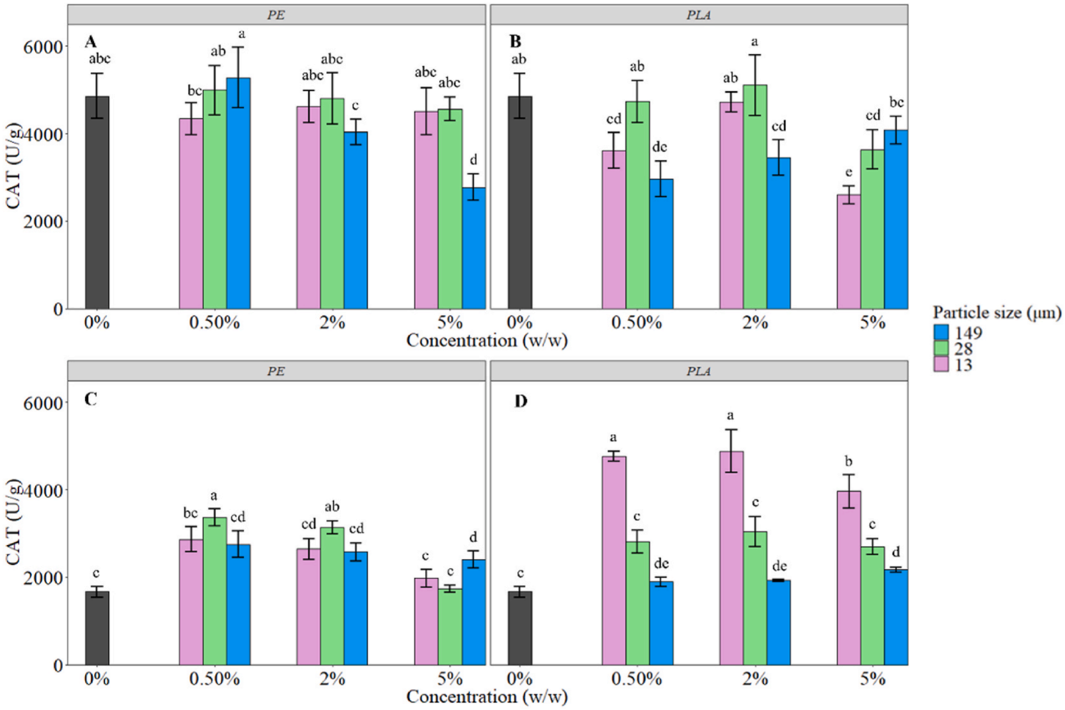

Fig. 4. The CAT level in *E.fetida* induced by exposure to MPs. (A) and (B) are the CAT level after 14 days of exposure to PE and PLA, respectively; (C) and (D) are the CAT level after 28 days of exposure to PE and PLA, respectively. Different alphabet letters indicate significant differences between treatments at the $p < 0.05$ level.

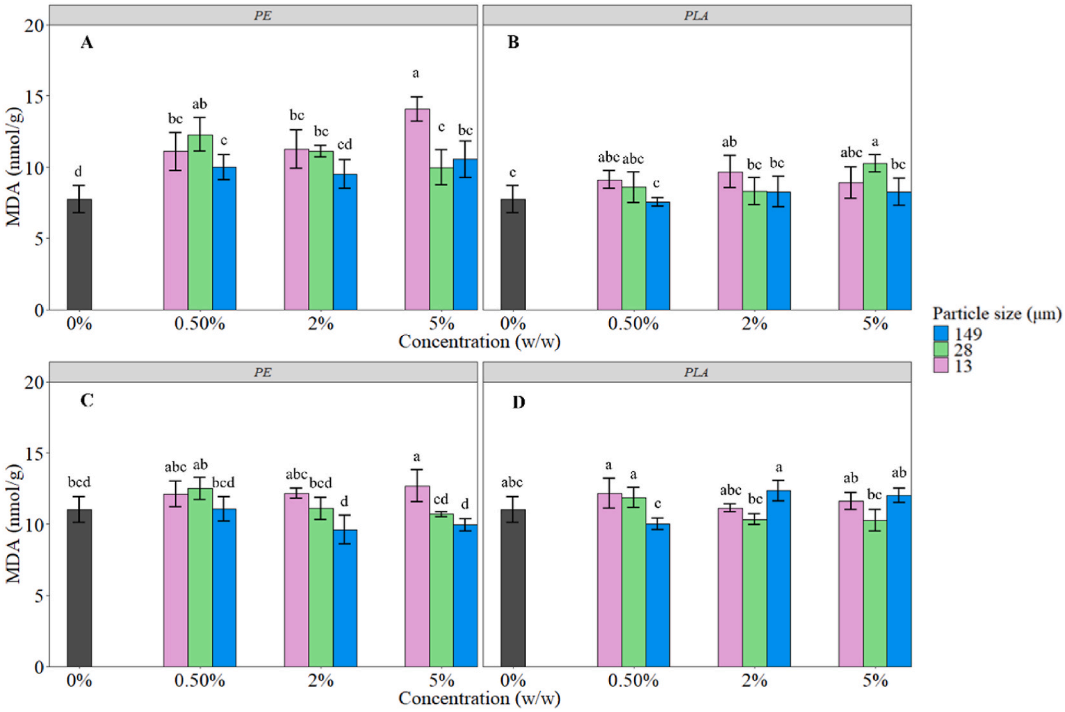

Fig. 5. The MDA level in *E.fetida* induced by exposure to MPs. (A) and (B) are the MDA level after 14 days of exposure to PE and PLA, respectively; (C) and (D) are the MDA level after 28 days of exposure to PE and PLA, respectively. Different alphabet letters indicate significant differences between treatments at the $p < 0.05$ level.

After 14 d of PE exposure, MDA activity significantly increased (Fig. 5) compared with controls. However, after 28 d of PE exposure, MDA activity was not significantly affected. In contrast, PLA exposure did not affect MDA activity after 14 d or 28 d exposure.

4. Discussion

Exposure to PE and PLA increases earthworm mortality rates, possibly because MPs lack any nutritional benefit [4], damage worm intestines [15] and skin [42], and negatively affect normal feeding behavior [42]. The impact of MPs on worms was closely related to their concentration, particle size, and type. Regardless of type, however, the highest mortality rate was observed after exposure to MPs of 13 μm size at 5 % (w/w), possibly because smaller particle sizes are more easily ingested. Higher concentrations led to accumulation of smaller particles in intestines, and more pronounced negative impacts [18,30,44].

Mortality rates of worms exposed to PE were greater than those exposed to PLA. However, weight loss in worms exposed to PLA was lower than in worms exposed to PE. This suggests that PE more strongly, negatively affected worms than PLA [5]. Whereas PE resists environmental degradation [20], PLA can be enzymatically degraded by microbes into lactic acid oligomers [37] and its degradation by-products are non-toxic to earthworms [2]. Accordingly, PLA exposure is likely to cause less harm to earthworms than PE.

Exposure duration affects MP ecotoxicity. We report (especially for PE) earthworm mortality rates to be higher after 14 d compared with 28 d, but for worm weight to increase after 14 d and decrease after 28 d. The weight increase following 14 d of exposure may be because of a short-term stress 'stimulation effect.' For instance, Gao et al. [10] reported the microalga Chlorella vulgaris to increase absorption and nutrient use as a cellular defense mechanism against MP-petroleum exposure stress. Thus, organisms may allocate more energy towards defense mechanisms such as increased food uptake when faced with short-term stresses. This heightened function may not be sustained with longer-term exposure.

Although worm weight decreased after 28 d of MP exposure, the mortality rate was lower than that following 14 d exposure. This suggests that with an increase in exposure time, earthworms may adapt to MPs. Earthworms have a 'trade-off' strategy to survive long-term PE exposure [40]—a strategy that involves allocation of more energy towards survival than growth and development, potentially to prepare themselves for the next generation [31]. For instance, following 28 d exposure to large-sized (149 μm and 28 μm) PE particles, numbers of cocoons trended upward with increased MP concentration, supporting the theory that worms allocated more energy to reproductive output following PE exposure. However, numbers of cocoons were significantly lower in treatments with small-sized PE (13 μm) compared with controls, suggesting that a pronounced negative effect existed on worms caused by small-sized PE MPs. With PLA exposure, cocoon numbers significantly increased compared with controls, suggesting that negative effects on earthworms following PLA exposure were lower than those following exposure to PE. Thus, after longer-term PE exposure, worm mortality rate decreased, and the negative biomass change rate became more pronounced compared with worms exposed to a shorter period of time. In contrast, although worm weight increased after 14 d exposure to PLA and decreased by 28 d, mortality did not differ significantly between the two time periods. This observation aligns with our suggestion that negative effects on worms with PLA exposure were less severe than those following PE exposure. Although PLA can harm earthworms, its effects are less severe, resulting in minor differences in earthworm mortality rates between shorter and longer term exposures.

Organisms protect themselves from oxidative stress by using antioxidant enzymes to deal with stress-induced free radicals [28]. We assess potential levels of biological damage through enzymes activities [28]. Compared with controls, SOD activity after 14 d exposure was significantly higher in worms exposed to PE, but not after exposure to PLA, possibly because short-term exposure to stress stimulate the earthworm with O^{2-} generation. Excessive accumulation of O^{2-} can, however, cause damage, prompting activation of SOD as the first line of defense against oxidative stress [24]. SOD is an essential antioxidant enzyme that eliminates excessive O^{2-} by catalyzing its conversion to H_2O_2 and O_2. Thus, short-term PE exposure might activate SOD to protect worms from the harmful effects of excessive O^{2-}. Although SOD levels increased after 14 d PLA exposure compared with controls, there was no significant difference between them. This suggests that while SOD was involved in the removal of O^{2-}, the negative effects following PLA exposure were minor (characterized by a low mortality rate and a positive biomass change rate) and less pronounced than those observed after PE exposure. This minor difference in SOD levels between PLA and control values following 14 d exposure indicated a reduced impact of PLA on worms. In contrast to 14 d exposure, we report a downward trend in SOD levels at 28 d exposure to both PE and PLA. The earthworm's SOD antioxidant defense system was gradually triggered by 14 d, with O^{2-} converted to H_2O_2 and O_2, but H_2O_2 accumulation may have partially inactivated SOD in the longer term. Consequently, SOD enzymes may not fully function with long-term exposure [33].

We report a distinct pattern in CAT levels following MP exposure. Regardless of MP type, CAT levels trended downwards after 14 d of exposure compared with controls, and upwards after 28 d of exposure. CAT is an important antioxidant enzyme that converts H_2O_2 into H_2O and O_2 [22]. While stress typically stimulates CAT activity, we report CAT levels to trend downwards after 14 d of exposure. This decline may be because the reactive oxygen species balance in worms was disrupted after 14 d exposure, affecting normal physiological functions and possibly inhibiting production of certain antioxidant enzymes such as CAT [41]. However, this limitation in CAT function is reversible with prolonged exposure time [9]. Moreover, worms may have adapted to MPs after longer-term exposure (decreased mortality rate), and the CAT antioxidant defense system may gradually become more active. This increased CAT activity likely contributes to long-term worm survival in MP-polluted environments.

MDA levels in PE treatments after 14 d exposure exceeded those of controls. MDA is a product of free radicals induced by lipid oxidation [21], which is an important marker of the degree of lipid oxidative damage. When organisms are stressed, which includes exposure to MPs, free radicals are generated, leading to lipid peroxidation and ultimately MDA generation [34]. Without stress, MDA levels are relatively low. We report greater increases in MDA levels after 14 d exposure to PE than PLA, and for no significant difference in MDA levels between 14 d PLA exposure and control values. This further suggests that PLA is less toxic to earthworms than PE (as indicated by reduced mortality rates after exposure to PLA than to PE). However, with prolonged exposure, MDA activity was not further affected by MPs, likely because free radicals induced by MPs exposure had been eliminated by antioxidant enzymes such as increased CAT levels in long-term PE and PLA exposure. Lipid peroxidation generates few free radicals, so regardless of which MPs a worm was exposed to, MDA levels did not differ significantly from controls. Additionally, earthworms may adapt to long-term PE and PLA exposure, explaining why damage caused by lipid peroxidation is weak after long-term exposure.

5. Conclusion

We examined the effects of different types of MPs on earthworms by assessing mortality, change in biomass, and antioxidant enzymes activities. We report both conventional and biodegraded MPs to exert negative effects on these worms, especially after 14 d of exposure. Conventional MPs, however, pose a greater threat to soil ecosystems. Through comparison of MPs types, the negative effects of PE were more pronounced than those of PLA (such as higher mortality and negative biomass change rate). Thus, although PLA is less toxic, the potential risks of using biodegradable plastics in agriculture to soil organisms must still be considered. Interestingly, with increased exposure time, earthworms may have adapted to MPs. Therefore, we recommend that future studies should increase exposure duration to identify any adaptations, and their implications. Additionally, to better understand MPs ecotoxicity and its implications for soil ecosystems, molecular-level investigations would improve understanding of any genetic change in soil organisms resulting from MPs exposure.

Data availability statement

We uploaded our raw data to *Figshare* respiratory. The DOI of our available data is 10.6084/m9.figshare.25529962.

Ethics statement

The study described has been carried out in accordance with either the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive 2010/63/EU or the National Institutes of Health – Office of Laboratory Animal Welfare policies and laws. And The study complies with the ARRIVE guidelines.

CRediT authorship contribution statement

Hailong Lai: Writing – original draft, Investigation, Formal analysis, Data curation. **Shuwen Han:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Jinyu Sun:** Formal analysis, Data curation. **Yujing Fang:** Formal analysis. **Ping Liu:** Conceptualization. **Haitao Zhao:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37308.

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