

The effect of micro-plastics on the growth and development of *Eisenia fetida* (Savigny, 1826) in vermiculture

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Abstract

The effect of different concentrations of e-waste plastic (acrylonitrilebutadiene styrene) in vermiculture on the growth and development of the compost earthworm *E. fetida* was studied. It was shown that at low concentrations of microplastics (1%) in vermiculture, earthworms practically do not reduce their growth and development indicators, while with an increase in the plastic concentration to 5–10%, the number of cocoons and juveniles decreases, and the growth of earthworm biomass slows down. Avoidance of the substrate and death of worms are not observed even with a relatively high (10%) proportion of plastic in the composted mixture.

Keywords

Microplastics, acrylonitrilebutadiene styrene, earthworms, *Eisenia fetida*, fertility, biomass dynamic

Introduction

Currently, there is an increasing interest in the use of biological agents for the disposal of plastic waste. A significant proportion of plastic waste accumulates on the surface and in the upper layers of the soil cover. In this regard, special attention was paid to soil invertebrates in the search for biological agents for the disposal of microplastics (MPs). Previously, it was shown that the presence of MPs in the

soil negatively affects nematodes, collembolus, enchytraeids, and earthworms. The mechanisms of plastic's effect on animals are often associated with damage or disruption of the digestive system. On the other hand, soil animals can contribute to mechanical destruction and stimulate microbial degradation of plastic in the soil (Atyieh et al. 2000; Fan et al. 2022; Huang et al. 2023).

Earthworms are among the most common animals in most global ecosystems, they absorb and process large amounts of organic material (Petersen et al. 2000). Earthworms can change the soil environment due to their ability to influence its physical properties, in particular the stability of soil aggregates, porosity, permeability, density and hydraulic conductivity (Atyieh et al. 2001; Ojha et al. 2014).

The widespread practice of vermicomposting is the processing of organic waste using the culture of some types of earthworms. In temperate climates, composting earthworms *Eisenia fetida* (Savigny, 1826) (Oligochaeta, Lumbricidae) are most often used for vermicomposting. This species has a worldwide distribution, a relatively short life cycle and the ability to consume a wide range of organic materials, including agricultural and household waste. They are also resistant to changes in hydrothermal conditions and tolerate an unfavorable period in the diapause state well. In addition, these earthworms multiply rapidly and create a populations that are successfully adapted to local conditions (Mitchell 1997; Whalen et al. 2000; De Souza et al. 2018).

The reason for conducting experiments on the use of vermiculture for the disposal of plastic electronic waste was previously published data on the impact of plastic waste on various types of earthworms and on the potential use of earthworms for the disposal of MPs. In particular, it was noted that the consumption of earthworms *E. fetida* and *Eisenia andrei* (Bouche, 1972) of five types of plastics (polyethylene, nylon, polypropylene, ethylene vinyl acetate and linear low-density polyethylene) led to a decrease in the mass of these polymers. It is hypothesized that this is due to ingestion and accumulation of MPs by earthworms (Kim et al. 2016).

Other researchers have shown that when a low-density polyethylene MPs passed through the intestines of *Lumbricus terrestris* Linnaeus, 1758, the size of its particles decreased. However, there was no decrease in the mass of plastic, which indicates that earthworms were not able to decompose low-density polyethylene MPs during the feeding period, affecting only their physical properties. Thus, this shows that the digestive tract of earthworms can split MPs into smaller particles; however, its complete degradation does not occur (Lwanga et al. 2017; Meng et al. 2023).

A preliminary study was also conducted on the potential of the earthworm *L. terrestris* to reduce soil contamination with MPs (20–648 microns) made of low-density polyethylene (LDPE), polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT). The results obtained indicate that ingested MPs can undergo fragmentation and depolymerization (for some polymers) in the intestine of an earthworm. It was noted, that further research was needed to identify the mechanisms of polymer depolymerization in the earthworm intestine and to assess the possibility of bioremediation of MPs using earthworms (Meng et al. 2023).

Some studies have shown the inability of earthworms to survive in soils contaminated with certain types of plastics and MPs in certain concentrations. Some types of plastic have a great impact on the mortality of earthworms, even in low concentrations. In particular, polystyrene and low-density polyethylene are classified as dangerous substances of the second stage, capable of causing irritation and harm to living beings (Sharma et al. 2029; Wang et al. 2019).

In general, it has been shown that vermicomposting is one of the best options for processing organic substances, since it offers an environmentally and economically sound strategy for obtaining a premium product enriched with all types of biologically active compounds (Hazarika and Khwairakpam 2022). Earthworms have a high potential for use as a major factor contributing to the decomposition of MPs. They can become one of the future methods for detecting the effects of MPs on soil biota and serve as a biodegradable agent (Sanchez-Hernandez et al. 2020).

Figuratively speaking, earthworms have the ability to act as a grinding machine that can physically change the size of MPs using its skin-muscle sac surrounding elements of the digestive system with a high surface area for microbial and enzymatic activity. In addition, the ability of earthworms to mineralize complex inorganic compounds and organic substances gives them a high potential to reduce decomposition time by providing optimal conditions for microbial activity that can break down MPs to a simple form. In general, the presence of earthworms in the soil increases the moisture content of organic matter and microbial populations, increasing the presence of auxins and gibberellin-like substances (Sanfilippo et al. 1990). Earthworms also play an important role in the degradation of organic matter and soil metabolism through nutrition, fragmentation, aeration, cycling and dispersion (Kiyasudeen et al. 2018).

The purpose of our work was to study the effect of MPs growth and development on earthworms *E. fetida*. These earthworms belong to the epigenic type; they prefer to live in compost or decomposing plant residues, rather than in the mineral layers of the soil.

Materials and methods

To conduct research on waste vermicultivation, together with MPs, a series of organic substrates based on a peat – manure mixture containing one of the types of plastic of electronic waste – plastic of computer monitors: ABS (acrylonitrilebutadiene styrene) was prepared. ABS plastic, and therefore its waste, are non-toxic materials. Plastic does not contain any known carcinogens, and so far there have been no serious health problems associated with the use of this plastic. ABS contains 50% styrene, and the remaining 50% butadiene and acrylonitrile.

The studies were carried out in 500 ml plastic containers, where 10 worms of sexually mature *E. fetida* (total average weight 3.9 ± 0.5 g) with an organic substrate of 250 ml were placed.

The culture of *E. fetida* was obtained from the Department of Agricultural Biology of the National Research Tomsk State University.

During the experiments, containers with vermiculture were placed in the laboratory in shaded conditions at a temperature of 20–25 °C, the humidity of the substrate was 75–80%. MPs were added to each of the containers in an amount of 1%, 5% and 10% by weight, respectively, to the total compostable mass of the organic mixture. When choosing the moisture parameters of the substrate during the vermicomposting of plastic waste, the composted mixture was brought to the desired humidity by adding distilled water.

All variants of the experiments were carried out in three repetitions. Mathematical data processing was performed using the MS Office 2010 software package.

During the experiments, vermiculture was tested three times. 24 hours after the start of the experiment, worms placed in containers with different concentrations of MPs were tested for their avoidance of the substrate. Normally, earthworms are always inside the substrate and avoid lighting. An attempt to get worms to the surface indicates unsuitable conditions for their existence inside the compostable mixture.

14 days after the start of the experiment, the survival rate of earthworms in vermiculture was evaluated. The number of worms in containers and their changes in condition (decreased motor activity, contraction of the musculoskeletal sac, folding into a ring) were estimated. 60 days after the start of the experiment, the population was assessed (reproduction and growth indicators). The number of cocoons, mature and juvenile individuals, as well as the total biomass of worms in containers were taken into account.

Results

Experiments have shown that earthworms successfully adapt to life in their usual organic substrate when MPs are added to the latter. The worms did not have a reaction of avoiding the substrate (reaching its surface) when applying MPs in all concentrations used in the experiment (from 1 to 10% of the total weight of the substrate).

The second test, 14 days after the start of the experiment, showed that all worms, both in experimental and control, remained alive, however, at a concentration of 5–10% MPs individual earthworms (5–6%) were inactive. However, successfully mating earthworms were noted in the containers, which indicates a favorable external background for the existence of this species.

Based on the results of the third test (60 days after the start of vermicomposting of mixtures with the addition of MPs, an analysis of the state of population indicators of earthworms was carried out. The fertility of worms (the number of cocoons deposited) and the dynamics of biomass (the total weight of all worms) in containers were taken into account. Adult (sexually mature) individuals of earthworms and young (juvenile, immature individuals) were also taken into account separately. As a result, it was noted that for *E. fetida*, as the concentration of MPs in the vermicom-

postable substrate increases, earthworms generally decrease their fertility. For *E. fetida*, at a concentration of MPs in the compostable mixture of 1%, fertility remained at the same level as in the control. The fertility of worms of this species decreased by 10% at a MPs concentration of 5% and by 25% at a maximum (10%) concentration of MPs in the compostable mixture (Fig. 1).

Analysis of changes in the number of juvenile individuals during vermicomposting showed the same increase in the number of young earthworms in the control and at a minimum (1%) concentration of MPs. At the same time, the number of young individuals decreased by 20% under conditions of high MPs content in the composted substrate 60 days after the start of the experiment (Fig. 2).

The same trend was observed when analyzing changes in the growth of earthworm biomass depending on the concentration of MPs in the compostable substrate. As the concentration of MPs in the substrate increases, the total number of deposited cocoons decreases compared to the control (Fig. 3). In general, the condition of *E. fetida* populations in vermiculture can be considered satisfactory at low concentrations of MPs in the compostable mixture.

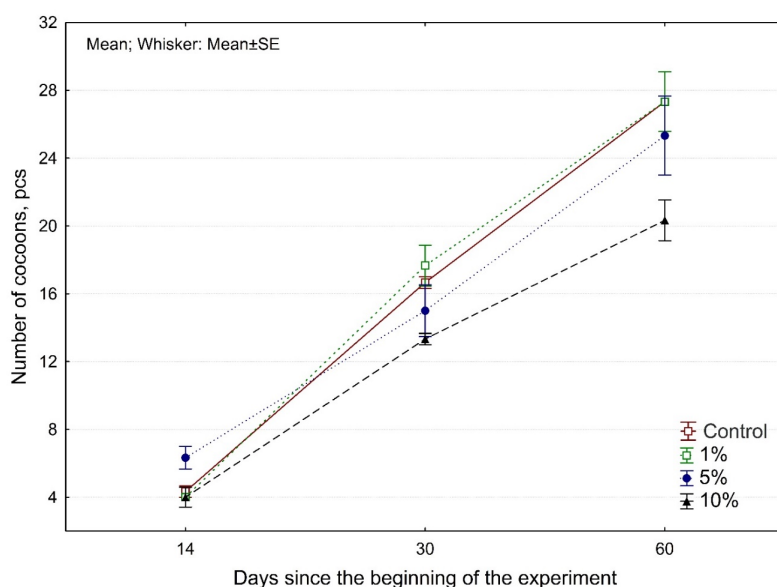


Figure 1. Changes in the number of cocoons in the *E. fetida* populations during composting at different MPs concentrations.

Discussions

Vermicompost produced by earthworms is widely used as an organic fertilizer for growing crops, so the question inevitably arises about the prospects of its use in the presence of MPs in the compostable mixture. We have shown that at low concen-

trations of MPs in vermiculture, earthworms practically do not reduce growth and development indicators, and avoidance of substrate and worm death is not observed even with a relatively high (10%) proportion of plastic in the compostable mixture. Since the probability of high MPs concentrations in agricultural soils is low, it can be assumed that the studied type of plastic (ABS) does not have a significant impact on the growth and development of *E. fetida*.

To date, the question remains open as to what happens to MPs when they pass through the digestive system of an earthworm. We assume that MPs are used by earthworms as the central part ("nucleolus") of the future coprolite, which is covered with a layer of recyclable organic matter as it passes through the food tract. The analysis of coprolites carried out at the end of the experiments showed the presence of MPs particles inside them; they, like mineral particles, become smoother and, possibly, decrease in size under the influence of microorganisms inside the earthworm.

Our further research will be aimed at tracking the fate of MPs particles during their passage through the intestines of an earthworm, from ingestion to coprolites (defecation). This will allow us to assess the real contribution of vermiculture to the transformation of MPs and assess the economic feasibility of this method of recycling plastic waste.

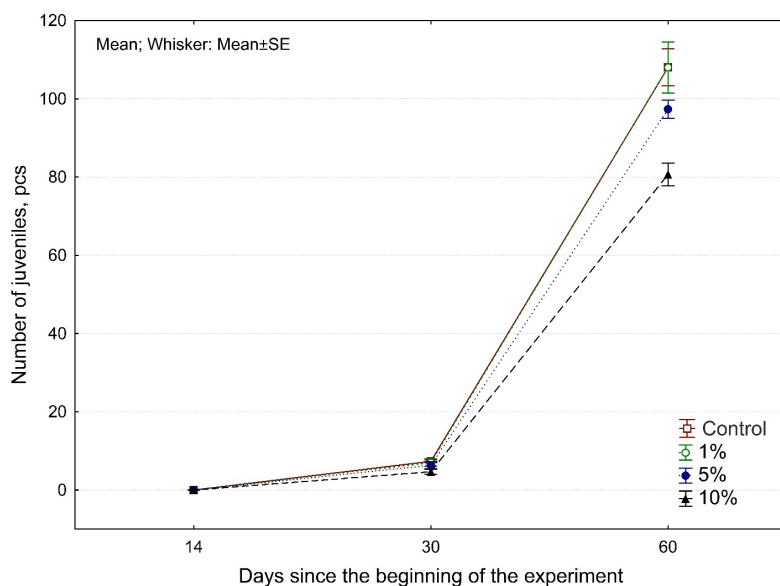


Figure 2. Changes in the number of *E. fetida* juvenile individuals during composting at different MPs concentrations.

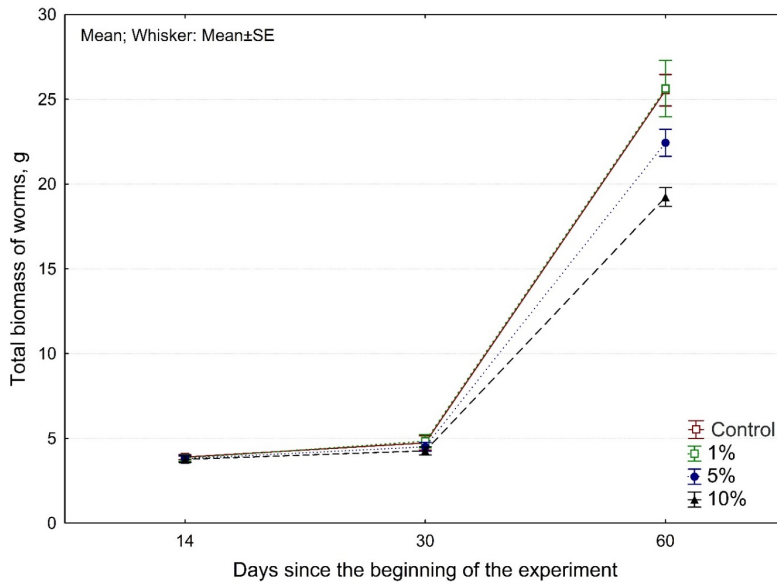


Figure 3. Changes in *E. fetida* biomass during composting at different MPs concentrations.

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