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# Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil

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Abstract. Microplastics (MPs) pollution is widespread in the environment, while the effects of MPs on the soil organisms are poorly understood. In this study, we investigated the fitness of earthworms (E. Foetida) exposed to MPs (Polystyrene, 58 µm) in soils at the concentrations of 0, 0.25, 0.5, 1 and 2% (w/w). The results showed that MPs had little effects on the fitness of earthworms under low exposure concentrations ( $\leq 0.5$  % (w/w)), while MPs exposure with high concentrations (i.e., 1% and 2%) significantly inhibited the growth and increased the mortality of earthworms. The results indicated that the MPs pollution in soils have an adverse effect on the fitness of soil organisms, and implied the ecological risk of MPs in terrestrial ecosystems.

# 1. Introduction

The global production of plastics is around 280 million tons annually and large amount of plastic wastes accumulate in the marine and terrestrial ecosystem [1]. Large plastic items in waters and soils gradually become smaller pieces or particles through various environmental weathering processes, such as mechanical breakdown, microbiological decomposition and photodegradation [2]. When the plastic particles are less than 5 mm in diameter, they are defined as "microplastics (MPs)" by the National Oceanic and Atmospheric Administration (NOAA) [3].

MPs have adverse effects on organisms, mainly due to their great accumulation in the gut or stomach of the organisms [4], which further affect the feeding behavior and development of the organisms [5,6]. However, intense studies have been focused on the effects of MPs on the fitness of aquatic organisms in the marine ecosystem, and little is known about the effects of MPs on soil organisms. It has been reported that the concentration of MPs ranged from 1000 to 4000 particles per kilogram soil in European agricultural soils [7]. Therefore, it is essential to investigate the effects of MPs on soil organisms at a range of concentrations for ecological risk assessments. The objective of this study was to investigate the potential effects of MPs on the survival and growth of earthworms at different MPs exposure concentrations in soils.

# 2. Materials and methods

#### 2.1. Earthworms, MPs and soil

Earthworms (E. Foetida) were obtained from an earthworm farm, located in Jinan, Shandong province, China. The obtained earthworms were acclimated in a culture system (SPX-358, Jiangnan, Ningbo) with a constant temperature of  $24 \pm 1$  °C for 7 days. Then, the healthy earthworms with similar weight  $(0.32 \pm 0.02g)$  were selected and fasted for another 3 days before exposure. The polystyrene

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microspheres (PS-MPs, 58µm) was purchased from Sigma-Aldrich (USA). The soil was collected from an orchard in Jinling Town, Shandong Province, China. The soil was air dried at room temperature (20°C) and thoroughly sieved through 2mm for the exposure experiment. The sea sand was collected from Shilaoren beaches (Qingdao, China). Following the removal of visible gravel, the sea sand was washed with hydrochloric acid several times until its pH reached around 7. Afterwards, PS-MPs was thoroughly incorporated into the mixture of the air-dried sea sand and soil (40% sea sand, and 60% soil) at the rate of 0.25, 0.5, 1 and 2% (w/w), respectively.

# 2.2. Exposure experiment

For PS-MPs exposure, 10 healthy pre-cultured earthworms were randomly selected, and then were moved into every 250-ml glass beaker which already contained PS-MPs (0, 0.25, 0.5, 1 or 2% (w/w)) in the mixed soil (500g) in the culture system under dark. During the exposure, 5g cow dung was uniformly placed on the surface of mixed soils as food in each beaker every 3 days. The moisture of soil mixture was kept at 20% during the experiment. After exposure for 30 days, the earthworms were rinsed for four times with ultrapure water to remove the adsorbed particles (e.g., soil and PS-MPs), dried with filter paper and then weighted by analytical balance to get the growth parameters of earthworms. The mortality of earthworms was assessed by counting the death adults in the beakers. Each treatment was replicated five times.

# 2.3. Statistical analysis

All results were expressed as the mean values (n = 5). Error bars presented in the results represent the standard deviation. Significant differences between the treatments were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple range test (P = 0.05) using Statistical Product and Service Solutions Software 20.0 (SPSS 20.0).

# 3. Results and discussion

The morphology the PS-MPs particles, which were observed using a scanning electronic microscope (SEM) (S-4800, Hitachi, Japan) is shown in figure 1. Imagery of the PS-MPs used in this study showed that they were flaky in nature and the major size of them was between 50  $\mu$ m and 80  $\mu$ m (figure 1).

The effects of PS-MPs on the weight and mortality of earthworms are shown in figure 2. PS-MPs exposure at lower rates (i.e., 0%, 0.25% and 0.5%) had no obvious effects on the weight of earthworms, while PS-MPs exposure at higher rates (i.e., 1% and 2%) caused a significant inhibition effect on the growth of earthworms (P < 0.05, figure 2a), which resulted in 27.6% and 29.8% decreases in the weight of earthworms in the 1% and 2% treatment, respectively, compared to the 0% treatment (figure 2a). Similarly, the PS-MPs exposure elevated the mortality of earthworms with the increasing exposure concentration (except for the 1% treatment) (figure 2b). Especially in the 2% treatment, around 40 % of earthworms were dead due to the PS-MPs exposure, implying that PS-MPs could have a lethal effect on earthworms. These results could be attributed to the serious damage in the self-defense system of earthworms caused by the PS-MPs exposure at the high concentrations ( $\geq 0.5\%$ ) [8].



**Figure 2.** The weight (a) and mortality (b) of earthworms (*E. Foetida*) with PS-MPs concentration of 0%, 0.25%, 0.50%, 1% and 2% (w/w) under a 30-day exposure. The bars represent standard deviation of the means (n = 5). Different letters among different treatments indicate significant differences, which were analyzed by Duncan's test (P = 0.05) using SPSS 20.0.

# 4. Conclusions

The results suggested that PS-MPs could inhibit the growth of earthworms and had an obvious lethal effect on earthworms at the high exposure concentrations ( $\geq 0.5\%$ ), which were possibly explained by the damage in the self-defense system of earthworms. This study will support the evidences that the MPs pollution in soils could pose a great adverse effect on the fitness of earthworms.

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