

Earthworms' Degradable Bioplastic Diet of Polylactic Acid: Easy to Break Down and Slow to Excrete

Lei Wang,* Yawen Peng, Yali Xu, Junjie Zhang, Chunguang Liu, Xuejiao Tang, Yuan Lu, and Hongwen Sun



Cite This: <https://doi.org/10.1021/acs.est.1c08066>



Read Online

ACCESS |

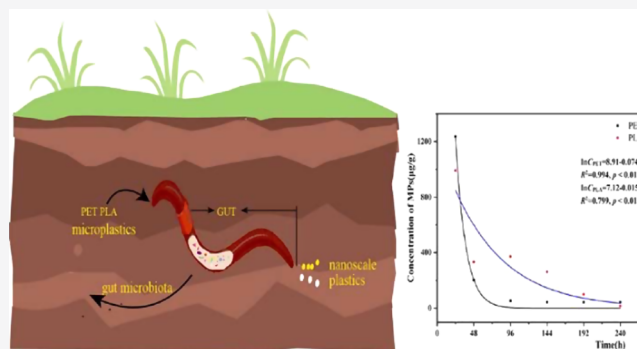
Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Microplastics (MPs) in soils may be ingested by terrestrial animals. While the application of bioplastics is increasing, the ingestion and excretion characteristics of bio-MPs by terrestrial animals are poorly understood as compared to fossil-MPs. Here, the approach–avoidance behavior of adult earthworms *Eisenia fetida* to MP-contaminated soil was assessed. Fossil-based poly(ethylene terephthalate) (PET) and bio-based poly(lactic acid) (PLA) MPs were found to be preferred by the earthworms, which might be due to the odor of polymer monomers. MPs in earthworm casts were analyzed by microscopy counting and liquid chromatography–tandem mass spectrometry. The amount of microscopically recognizable excreted PET and PLA was 553 and 261 items/g, respectively, while a higher proportion of smaller PLA particles also presented. Bio-based PLA is much easy to break down by earthworms than fossil-based PET. Submicron and nanocron PLA accounted for 57 and 13% of the excreted PLA on the 10th day of excretion. MP excretion was well described with the first-order kinetic model, and the elimination half-life was 9.3 (for PET) and 45 h (for PLA). A longer excretion period of PLA may be related to its potential to break down in the earthworms' digestive tract. This not only promotes the environmental degradation of PLA but also suggests the ecological risk caused by nanoparticles.

KEYWORDS: microplastic, earthworm cast, approach–avoidance behavior, excretion kinetics, nanoparticles, ecological risk



INTRODUCTION

Microplastics (MPs) are plastic particles, fibers, or debris with a diameter of <5 mm.¹ MP pollution has become a major global environmental issue due to their environmental persistence and potential biological toxicity.^{2,3} Sewage sludge applications, fragmentation of farmland plastic mulch films, and refuse composting and aerial depositions lead to MP pollution of soil.⁴ For instance, 1000 to more than 4000 particles of MPs per kg of dry mass sludge were found in agricultural sites and landfills in Europe, where the first 10 cm of soil could contain 670 plastic fibers per kg soil.⁵ In soils of the Yellow River delta wetland in China, the abundance of MPs ranged from 136 to 2060 items/kg, while the concentration of poly(ethylene terephthalate) (PET) polymers was 536–660 µg/kg.⁶

Soil fauna are critical to soil health.⁷ Earthworms are one of the most important soil animals and are considered key ecosystem engineers⁸ and bioindicators of environmental quality.⁹ Through their feeding, burrowing, and casting behavior, earthworms break down organic matter and aid in the structural development of soil aggregates.¹⁰ During this process, particulate matter are also ingested by earthworms. For example, silver nanoparticles in soils can be ingested by

earthworms but can only be retained in trace amounts in the tissues after two days of depuration.¹¹ MPs of polyethylene (PE) in soils can also be ingested by the earthworms and lead to a significant difference in particle size distribution between MPs in soils and those in worm casts.¹² A recent study reported the ingestion of PE and polystyrene (PS) MPs by *Eisenia fetida*,¹³ which even led to the transfer of MPs in the terrestrial food chain from the soil to earthworms and chicken.¹⁴

Previous studies of ingestion of MPs by earthworms have only been concerned with fossil-based MPs, most of which are nondegradable plastic polymers. As a growing problem of plastic waste, biodegradable plastics are considered as potential substitutes for traditional plastics and are being gradually applied in daily life.¹⁵ The production of degradable plastics, especially the degradable bioplastics, is rapidly increasing.¹⁵

Received: November 26, 2021

Revised: February 25, 2022

Accepted: March 10, 2022

Poly(lactic acid) (PLA) is the most important degradable bioplastic, with an annual production of 300 000 tons, which accounts for 47% of all degradable plastics.¹⁶ Biodegradable microplastics (BMPs) share some common features with MPs. Similar to conventional MPs, toxic effects of BMPs on animals and plants, such as leading to increases in intracellular reactive oxygen species (ROS) levels and impairment of membrane integrity, were also recorded.^{17,18} Exposure of PLA MPs resulted in elevated respiratory rates in the oyster *Ostrea edulis* and the lugworm *Arenicola marina*.¹⁹ The starch-based BMPs presented a greater negative impact on wheat height and biomass compared to nondegradable fossil-based MPs.²⁰ However, due to the lack of comparative research, it is uncertain whether earthworms have a preference for the ingestion of bio-based MPs. In addition, the existing characterization of bio-MPs excreted by earthworms is insufficient to quantitatively describe their *in vivo* decomposition efficiency and excretion kinetics.

In this study, the approach and avoidance behaviors of earthworms (*E. fetida*) toward a series of bio-based and fossil-based MP polymers were compared. Furthermore, earthworms were exposed to MPs of a typical fossil-based polymer (PET) and a typical bioplastic polymer (PLA), and the quantity and quality of MPs in earthworm casts were measured by stereomicroscopy and mass spectrometry. The abilities of earthworms to decompose PLA and PET MPs *in vivo* were compared, and the excretion characteristics of different MPs in earthworms were described.

MATERIALS AND METHODS

Chemicals and Earthworms. *p*-Phthalic acid (PTA) was purchased from Toronto Research Chemicals, Inc. (North York, ON, Canada). Lactic acid (LA) was purchased from Sigma-Aldrich (St. Louis, MO). D₄-PTA (99%) and ¹³C₁₂-sodium lactate (99%) were purchased from Cambridge Isotope Laboratories (Andover, MA). HPLC grade methanol, acetonitrile (ANPEL Laboratory Technologies Inc., Shanghai, China), and 1-pentanol (Meryer Chemical Technology Co., Ltd., Shanghai, China), as well as analytical grade H₂O₂ (30%), ZnCl₂, and KOH, were used in this study. Solid-phase extraction (SPE) cartridges (MAX, 6 mL/500 mg; HLB, 6 mL/200 mg) were purchased from ANPEL Laboratory Technologies Inc. (Shanghai, China).

Adult earthworms (*E. fetida*) were purchased from the Wangjun Earthworm Farm Company (Jiangsu, China). All earthworms were cultured in the test soil under laboratory conditions for two weeks before the exposure experiments.

Preparation of Soil and MPs. Agricultural soil collected in Tianjin, China, was air-dried and sieved through a 2 mm stainless steel mesh. The physicochemical characteristics of the soil are listed in Table S1. PET, polycarbonate (PC), PLA, and polyhydroxyalkanoate (PHA) particles with the same diameter of 50–125 μm were purchased from Sigma-Aldrich Corp. (Shanghai, China). Carboxymethylcellulose sodium (CMC) and hydroxypropyl methyl cellulose (HPMC) particles of 50–125 μm were purchased from Macklin Inc. (Shanghai, China).

Earthworm Avoidance Test. The earthworm approach and avoidance tests were conducted in a four-compartment test chamber according to the ISO method²¹ under the starvation and satiety states. MPs were weighed and spiked into sieved soil (<2 mm) to obtain a level of 1.0% (w/w). The humidity of the soil was raised to 25%. The test chamber (25 cm × 25 cm × 15 cm) was divided into four zones by glass

panels (Figure S1), including zone A (blank control), zone B (spiked with the fossil-based MPs, PET, or PC), zone C (spiked with fully degradable bio-based MPs, PLA, or PHA), and zone D (spiked with semisynthetic MPs, CMC, or HPMC). The separating lines refer to the lines that divide the test chamber into four zones, which are shown in Figure S1. Soils (870 g) were added to each zone of the chamber. Then, the panels in the middle of the chamber were removed, and 20 earthworms (in the satiety or starvation states) were placed in the middle of the chamber on the surface of soil.

The chambers were then closed with transparent perforated lids. The tests were conducted in the dark in incubators at 20 ± 1 °C for 48 h. The earthworms were not fed during the test. At the end of the 48 h test period, the sections of the control and contaminated soil were carefully separated by inserting panels, and the number of earthworms in each section was counted. The worms found on the separating line were counted by locating the direction of the head. All experiments were conducted in three replicates.

The approach–avoidance behavior of earthworms was judged by the approach index (*R*) according to the following equation

$$R = \frac{T_i - T_C}{T_C} \quad (1)$$

where *T_C* is the number of earthworms in the control section and *T_i* is the number of earthworms in sections spiked with different MPs.

As supplementary experiments, a two-compartment test (Figure S1) was also conducted to further identify the approach–avoidance behavior of earthworms toward PET and PLA monomers (PTA and LA) as well as toward semisynthetic plastics (CMC and HPMC). The earthworms were also directly exposed to soils spiked with CMC and HPMC to prove their adhesion to the earthworm skin. Details of these supplementary experiments are described in Text S1 in the Supporting Information.

Ingestion and Excretion of MPs by Earthworms. Four earthworms were placed into a 2 L glass beaker with 500 g of artificial soil (dry weight) containing 5 g of PET or PLA MPs. Before spiking, the MP particles were washed three times with methanol under 1 h ultrasonication and filtered by a PP membrane (50 μm) to remove the soluble chemicals and smaller particles in polymers. The beakers wrapped with gauze were then incubated at 20 ± 1 °C and 25% humidity for 2 days. A treatment without MPs was set as the blank control. Then, the earthworms were placed individually on filter papers (Whatman No. 540) moistened with ultrapure deionized water in 9 cm diameter Petri dishes. The moisturized filter paper was changed every 5 h to reduce the earthworm repeatedly ingesting casts. Three replicates were set up for each experiment. Earthworm casts were sampled on the 1st, 2nd, 4th, 6th, 8th, and 10th days.

Sample Pretreatment. *Extraction of MPs in Earthworm Casts.* A frequently used digestion–flotation method²² was applied to separate MPs from earthworm casts collected on the 1st, 2nd, 4th, 6th, 8th, and 10th days of excretion. To obtain micron (>1 μm), submicron (0.1–1 μm), and nanocron (<0.1 μm) plastics, three membranes, i.e., 0.8 μm, 0.1 μm, and 20 nm, were selected to isolate the plastics from the earthworm casts. Details of the process are described in the Supporting Information (Text S2).

Depolymerization of PET and PLA Polymers in the Samples. Membranes containing PET or PLA particles of three sizes ($>0.8 \mu\text{m}$, $0.1\text{--}0.8 \mu\text{m}$, and $20\text{--}100 \text{ nm}$) were depolymerized to the corresponding monomers, i.e., PTA and LA, in a heated alkaline 1-pentanol system, according to the method developed in a previous study with minor modifications.^{23–26} The depolymerization process and efficiency of PLA are provided in Text S3, Figure S2, and Table S2. Before depolymerization, the backgrounds of the free-form PTA and LA in solids were extracted from the sample by a methanol/water mixture solution (5:3, v/v). The extraction ability of the methanol–water mixture for LA is shown in Text S4. The membrane with MPs was digested by heating in a stirring heating mantle for 30 min. The newly formed PTA and LA in the depolymerization system were extracted by HLB and MAX SPE cartridges, respectively, with the detailed process described in Text S5. Then, the monomers PTA and LA were extracted for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, as described in our previous study.²³ Theoretically, oligomers will depolymerize to produce the same monomers (e.g., PTA and LA in this study), resulting in overestimation of polymer quantification. We did not distinguish them from the polymers because not all oligomer standards are available for their MS analysis. Besides, the amount of oligomers is usually negligible compared with polymers.²⁷ Therefore, the occurrence of insoluble oligomers can be considered together with polymers when conducting MP pollution investigation.

MP Analysis. Microscopy Observation. The separated particles on the glass fiber filter ($>0.8 \mu\text{m}$) were observed and counted using a continuous zoom stereomicroscope (SZN71, Sunny Optical Technology Co., Ltd, China) with an instrumental resolution of $0.5 \mu\text{m}$. The number of MPs in two size ranges ($0.8\text{--}50 \mu\text{m}$ and $>50 \mu\text{m}$) was recorded.

SEM Analysis. To directly observe the MPs of various particle sizes in casts, the cast samples were dispersed in 2 mL of methanol after digestion and flotation and then drawn up and dried in a critical point dryer (Tousimis Samdri-795). Subsequently, samples were sputter-coated with 5 nm platinum and visualized on a Merlin Compact field emission scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany) operating at an accelerating voltage of 15 kV. It was found that digestion by H_2O_2 did not change the size of fresh MP particles (Figure S3).

Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) Analysis. PTA and LA were analyzed by a triple quadrupole mass spectrometer G6460C coupled with an Agilent 1260 system for chromatographic separation (Agilent Technologies, Inc., Santa Clara, CA). An Ultra AQ C_{18} column ($100 \text{ mm} \times 2.1 \text{ mm}$, $3.0 \mu\text{m}$, Restek Corporation, Bellefonte, PA) and an Ultimate HILIC silica column ($150 \text{ mm} \times 4.6 \text{ mm}$, $3 \mu\text{m}$, Welch Materials, Shanghai, China) were used for chromatographic separation of PTA and LA, respectively.^{25,28} More detailed information regarding chromatographic separation and the tandem mass system is provided in Tables S3 and S4. The concentrations of PTA and LA were calibrated with the corresponding internal standards of $\text{D}_4\text{-PTA}$ and $^{13}\text{C}_{12}\text{-sodium lactate}$. The concentrations of PET and PLA polymers in earthworm casts were calculated according to eqs 2 and 3

$$\text{PET}_{\text{amount}} = \frac{\text{PTA}_{\text{depolym}} \times \text{MW}_{\text{PTA-H}_2\text{O}_2} / \text{MW}_{\text{PTA}}}{f_{(\text{PTA-H}_2\text{O}_2)}} \quad (2)$$

$$\text{PLA}_{\text{amount}} = \text{LA}_{\text{depolym}} \times \frac{\text{MW}_{\text{IPLA}}}{\text{MW}_{\text{LA}}} \quad (3)$$

where $\text{PTA}_{\text{depolym}}$ and $\text{LA}_{\text{depolym}}$ are the masses of the newly formed PTA and LA from the depolymerization of PET or PLA polymers ($\mu\text{g}/\text{kg}$), MW is the molecular weight, MW_{PTA} (166 g/mol) and $\text{MW}_{\text{PTA-H}_2\text{O}_2}$ (132 g/mol) represent the molecular weights of PTA and $[-\text{C}(\text{O})-\text{C}_6\text{H}_4-\text{C}(\text{O})-]$, f ($f = 77.4\%$) is the mass percentage of $\text{PTA-H}_2\text{O}_2$ in the polymer, and MW_{IPLA} and MW_{LA} of 72 and 90 g/mol represent the molecular weights of $[-\text{O}-\text{CH}(\text{CH}_3)-\text{CO}-]$ and LA, respectively.

Model Fitting of Elimination Kinetics. Excretion of PET and PLA MPs in earthworms was described by detecting the mass concentrations of PET and PLA in casts and fitting with a one-compartmental first-order model (eq 4),²⁹

$$\ln C_t = \ln C_0 - kt \quad (4)$$

where C_0 and C_t are the mass concentrations ($\mu\text{g}/\text{g}$) of PET or PLA polymers in casts at the initial stage of excretion and at time t (h), respectively, and k (h^{-1}) is the kinetic rate constant. Besides, the half-life time of elimination ($t_{1/2}$, time required for MP concentrations in casts to decrease to 50% of C_0) was calculated as $t_{1/2} = \ln(2)/k$.³⁰

Quality Assurance and Quality Control (QA/QC). Any plastic containers were avoided in the experiments, except PP tubes. All experimental vessels were rinsed twice with Milli-Q water and HPLC grade methanol, dried in a fume hood, and then wrapped with aluminum foil before use. ZnCl_2 solution was filtered through a $0.45 \mu\text{m}$ polytetrafluoroethylene (PTFE) filter membrane before the flotation process. The round-bottom flasks were heated at $500 \text{ }^\circ\text{C}$ for 2 h in a muffle furnace to remove the possible MP background. The depolymerization process was performed in a fume hood to avoid external pollution of indoor dust.

The isotope dilution method was used to calibrate the PTA and LA concentrations, and the recoveries in spiked earthworm casts were 83.4–96.9 and 89.3–94.0%, respectively (Table S5).

Standard curves ranged from 0.2 to 200 ng/mL for PTA and LA, and the regression coefficients of the calibration curves (r) were >0.99 . For samples with concentrations above the calibration range, extracts were diluted and reanalyzed. A calibration standard sample was injected after every 20 samples to check the instrument drift sensitivity, and a pure solvent was injected into the LC-MS/MS as a check for carryover of target chemicals from sample to sample. The blank of each batch of 15 samples was carried through the whole analysis procedure (including the alkaline depolymerization step). After applying a 10:1 signal-to-noise ratio (SNR), the limits of quantitation (LOQs) for PET and PLA were 180 and 148 ng/g, respectively.

Statistical Analysis. MP counting was conducted using Capture 2.0 software (Sunny Optical Technology Co., Ltd., Zhejiang, China). Statistical analyses were performed using SPSS 25 statistics software (IBM, NY). The differences among samples were tested by one-way analysis of variance (ANOVA). A nonparametric Kolmogorov–Smirnov test was used to check whether the data were normally distributed. Linear regression and Pearson correlation analysis were performed using OriginPro 2018 software (OriginLab Corporation, MA). A p value of <0.05 was regarded as statistically significant.

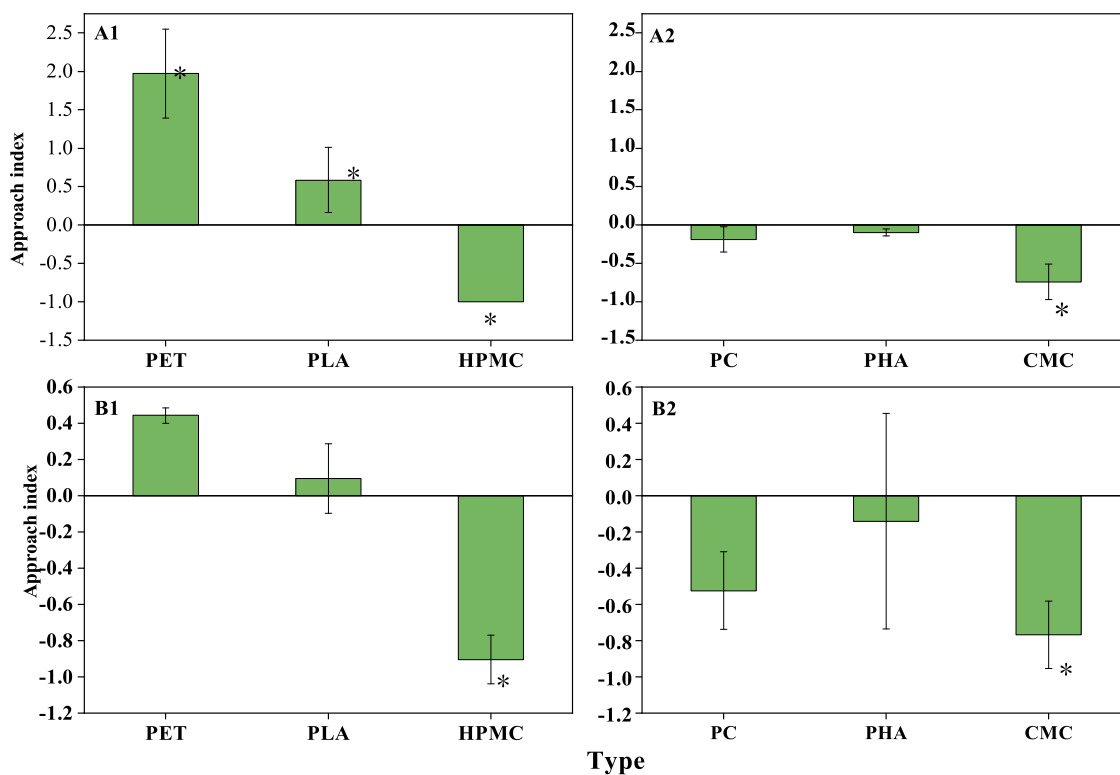


Figure 1. Approach–avoidance behavior of earthworms to different MP polymers in the four-compartment experiment, under the state of starvation (A1, A2) and satiety (B1, B2). The approach index was estimated as $R = (T_i - T_c)/T_c$, while the values of >0 indicated an approach behavior. “*” indicates there is a significant difference compared with the control group at $\alpha < 0.05$.

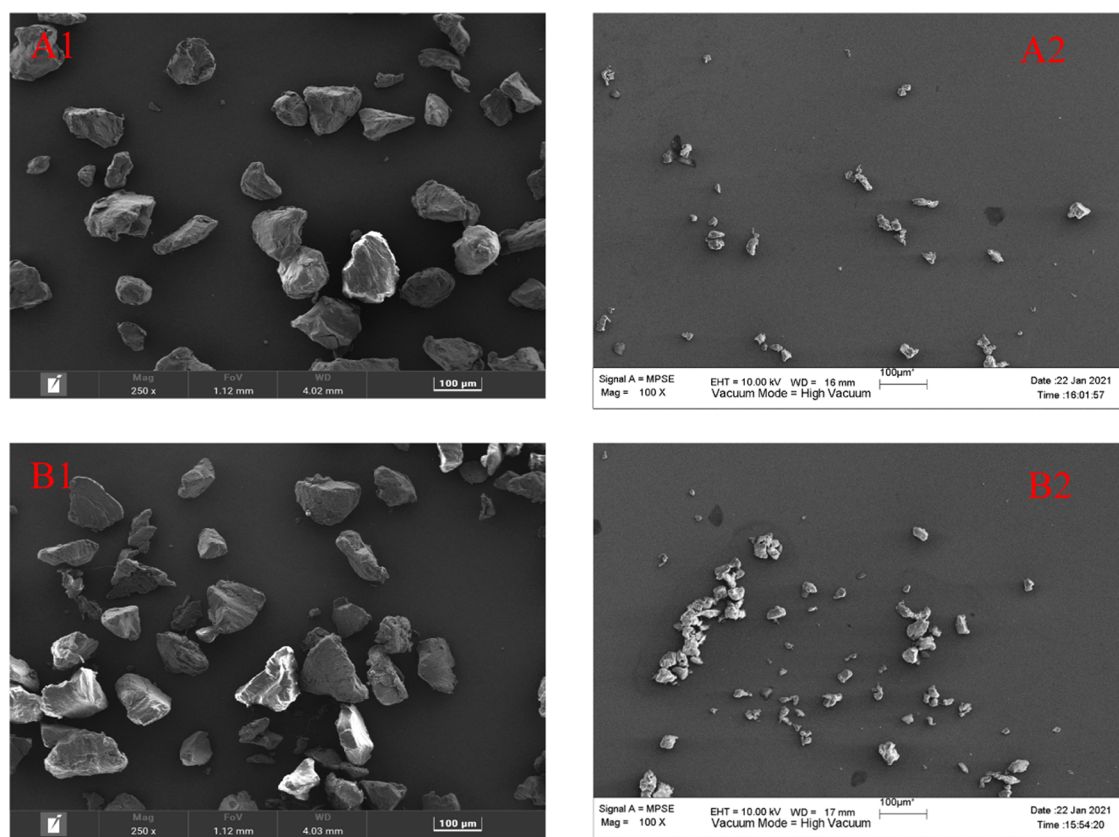


Figure 2. SEM images of the fresh PET (A1) and PLA (B1) MPs, as well as PET (A2) and PLA (B2) MPs in earthworm casts.

RESULTS AND DISCUSSION

Approach–Avoidance Behavior of Earthworms to Different MP Polymers. After 2 days of experiment, the earthworms showed a significant approach–avoidance behavior in the four-compartment experiment. The earthworms in a starvation state showed an obvious approach to PET and PLA MPs, which accounted for an approach index of 1.97 (PET) and 0.59 (PLA) (Figure 1). More earthworms moved to areas spiked with PET or PLA MPs, especially in the starvation state. This may be due to their preference for free monomers in PET and PLA, i.e., PTA and LA, as they have a sour odor.^{31,32} Odors are potential cues for earthworms to detect food.^{33,34} The two-compartment approach–avoidance experiment was conducted to identify the preference of earthworms to PTA and LA. It was found that earthworms also had an approach behavior to the areas spiked with PTA or LA (Figure S4). The earthworms seem to prefer PET to PLA. However, earthworms have no significant difference in their preference for monomer compounds. The ingestion behavior of earthworms is influenced by many factors. We speculate that this may be due to the taste difference caused by the different mechanical strength and flexibility of the particles, or that the harder PET particles can help earthworms grind food in the gizzard. This is not discussed in the text because we did not find evidence to support it. Besides, PLA MPs may be decomposed by gut microorganisms. This may lead to the digestion and absorption of part of the ingested PLA particles.

In comparison, earthworms showed a significant avoidance behavior to CMC and HPMC in both the starvation and satiated states, with the approach indexes of -0.741 and -0.768 for CMC and -0.904 and -1.02 for HPMC, respectively (Figure 1). This was further confirmed in the two-compartment experiment, while 70 and 75% of the earthworms moved to the control area rather than the CMC- or HPMC-contaminated area (Figure S4). CMC and HPMC are hygroscopic and used as adhesion materials.^{35,36} It was found that CMC and HPMC were easy to adhere to the surface of earthworms when the earthworms were exposed to soils spiked with CMC and HPMC (Figure S5). This is suspected to cause discomfort to earthworms and leads to the avoidance behavior. In comparison, the earthworms did not show an approach or avoidance behavior to PC and PHA MPs in both the starvation and satiated states. Compared with the satiated state, the approach behavior of earthworms to MPs (PET and PLA) is more obvious, and the avoidance behavior of earthworms to MPs (CMC and HPMC) is weakened in the starvation state.

Evidence of MP Ingestion and Breakage by Earthworms. As earthworms tended to approach PET and PLA, SEM was used to observe the MP fragments in earthworm casts to prove their ingestion by earthworms exposed to PET or PLA MPs. Occurrence of PET and PLA MPs can be found in the digested earthworm casts, proving the ingestion behavior to these MPs by earthworms. Although only particles $>50 \mu\text{m}$ were exposed to the earthworms (Figure 2A1,B1), some smaller MPs were observed in the casts (Figure 2A2,B2). This may be attributed to the grinding action of the earthworm gizzard and gut system, which plays an important role in particle breakage.³⁷ When the initially coarser mineral grains pass through the earthworm gut, muscle action obviously comminute the particles.³⁸

In addition, submicron and nanocron PLA particles of 300–500 nm were observed from casts (Figure S6). This suggests that smaller PLA particles can be generated from larger PLA MPs. Compared with PET, the proportion of small particles of PLA in earthworm casts seems to be higher (Figure 2A2,B2), indicating that this degradable bioplastic is much easier to break in the gastrointestinal tract of earthworms. The following count results after grading filtration and MS data also reflect the same conclusions.

Quantity and Mass Abundance of Micron MPs in the Earthworm Casts. As MPs $>0.8 \mu\text{m}$ in earthworm casts can be separated and observed under a stereomicroscope, the excreted micron PET and PLA MPs, which were divided into two particle sizes, i.e., the original size ($50\text{--}125 \mu\text{m}$) and the smaller size ($0.8\text{--}50 \mu\text{m}$), were counted, respectively (Figure 3). In the casts collected on the 1st day after exposure, a great number of PET particles, i.e., 368 items/g of $50\text{--}125 \mu\text{m}$ particles and 55 items/g of $0.8\text{--}50 \mu\text{m}$ particles, were

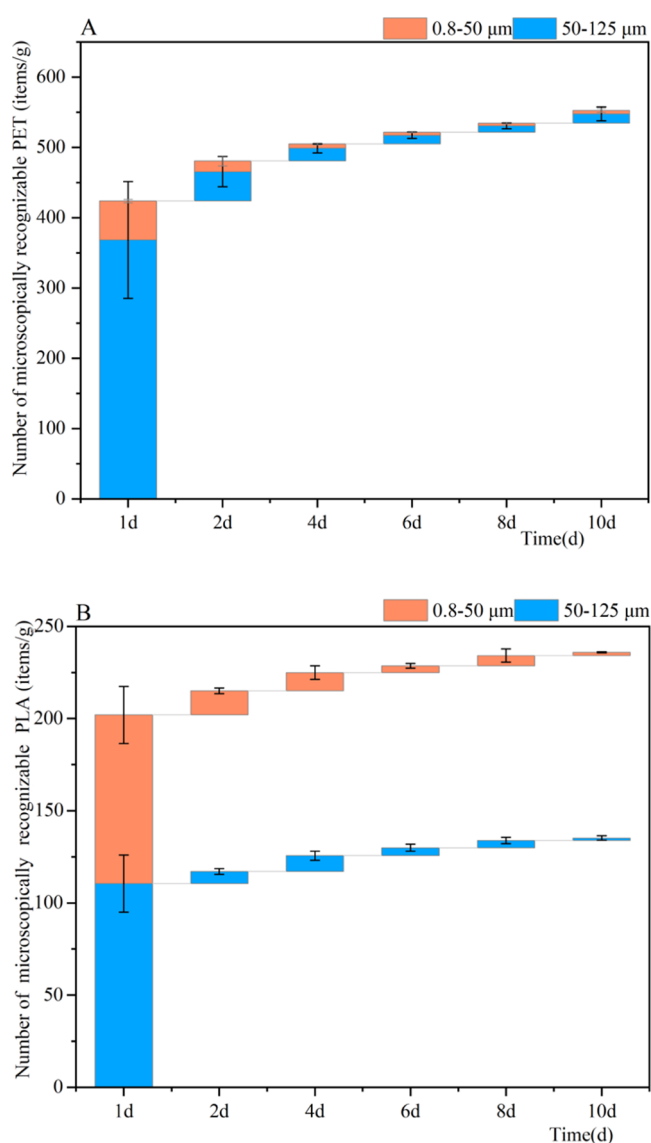


Figure 3. Particle number of microscopically recognizable PET (A) and PLA (B) ($>0.8 \mu\text{m}$) in the casts of the excretion experiment of earthworms exposed to soils containing PET or PLA plastics (1% w/w).

observed. After that, the number of 50–125 μm PET particles in the casts generally decreased from 41 items/g on the 2nd day to 13 items/g on the 10th day, while the number of 0.8–50 μm particles decreased from 15 to 5 items/g (Figure 3A).

The number of PLA particles ($>0.8 \mu\text{m}$) in the casts was significantly lower. Although 110 items/g of 50–125 μm PLA particles and 91 items/g of 0.8–50 μm particles were observed in earthworm casts on the 1st day of excretion, the number of original-size particles (50–125 μm) decreased from 6 items/g (on the 2nd day) to 1 item/g (on the 10th day), and the number of smaller-size particles (0.8–50 μm) decreased from 13 to 2 items/g during the same period (Figure 3B).

During the 10th day of excretion, the proportion of smaller PET particles in PET MPs (0.8–50 μm) separated from the cast increased from 14% on the 1st day to 30% on the 10th day (Figure S7A). Differently, the smaller PLA particles (0.8–50 μm) in the casts of the 1st day accounted for 45% of the PLA MPs (0.8–125 μm), and the proportion further increased to 69% on the 10th day (Figure S7B). The digestive tract of the earthworms seems to be more effective in breaking PLA polymers. This might be related to the physical properties of the polymers, as the hardness of PLA is much lower than that of PET.³⁹ Similarly, the higher ability of earthworms to break down low-density polyethylene (LDPE) than PE plastics was reported.^{12,40} In addition, the ingested MPs may also undergo biochemical transformation mediated by enzymes in the digestive tract of earthworms.⁴¹ Biodegradable PLA plastics can be decomposed by gastrointestinal microorganisms and enzymes, e.g., carboxylesterase, in earthworms.^{42,43} This may also contribute to fragmentation of the ingested PLA MPs in earthworms.⁴⁴

To further describe the excretion of MPs by earthworms, the mass concentrations of PET and PLA polymers in earthworm casts were measured by quantifying the monomers of PET and PLA produced by depolymerization (Figure S8). A significant positive correlation was obtained between the results of stereomicroscope counting and LC-MS/MS analysis for both PET and PLA polymers ($>0.8 \mu\text{m}$) (Figure 4). Consistent with the results obtained by stereomicroscopy, the mass concentration of PET polymers with particles ($>0.8 \mu\text{m}$) in the casts collected on the 1st day of excretion (1225 $\mu\text{g/g}$) was much higher than that of PLA (699 $\mu\text{g/g}$) (Figure 5). This difference in quality is roughly equivalent to that in quantity, that is, 423 items of PET and 201 items of PLA MPs ($>0.8 \mu\text{m}$) were counted on the 1st day. The concentrations of PET and PLA ($>0.8 \mu\text{m}$) in earthworm casts gradually decreased in the following days. On the 10th day, micron-sized PET and PLA could still be detected by LC-MS/MS in the casts (Figure 5A,B).

Mass Concentrations of Submicron PET and PLA Polymers in the Earthworm Casts. Although submicron (0.1–0.8 μm) and nanocron (20–100 nm) particles were hard to identify by microscopy, their occurrence could be measured by LC-MS/MS. In comparison to the mean concentration of microscopically recognizable ($>0.8 \mu\text{m}$) PET in the casts during the 10-day excretion (260 $\mu\text{g/g}$), the amounts of the submicron (mean: 3.71 $\mu\text{g/g}$) and nanocron PET (mean: 5.78 $\mu\text{g/g}$) polymers were much lower (Figure 5A). Differently, the mean concentration of submicron PLA (192 $\mu\text{g/g}$) was even higher than that of the microscopically recognizable PLA (139 $\mu\text{g/g}$) (Figure 5B). This verified that PLA in earthworms is more likely to break down than PET, and a large number of smaller PLA particles might be formed and released by

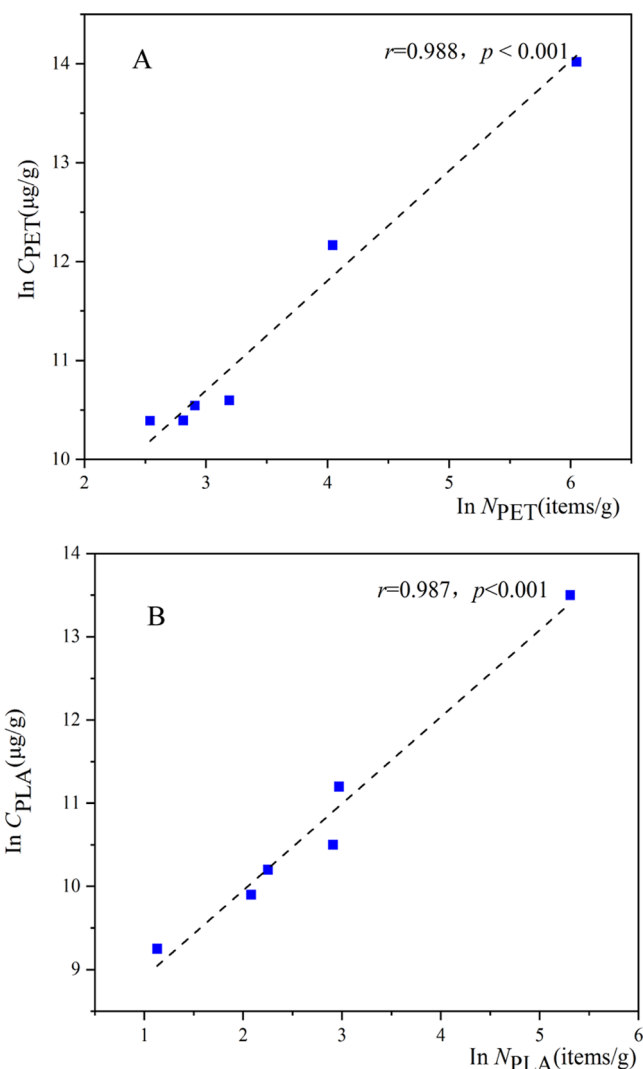


Figure 4. Linear correlation between the natural logarithm of the concentration ($\mu\text{g/g}$) and abundance (items/g) of the PET (A) and PLA (B) MPs with a size of $>0.8 \mu\text{m}$.

earthworms. Although the mean concentration of microscopically recognizable PLA in earthworm casts was much lower than that of PET (Figure 5), the concentrations of submicron and nanocron PLA particles detected in the earthworm casts were even one order of magnitude higher than that of PET (Figure 5).

Earthworm digestion changed the particle size distribution of MPs, especially for PLA. In the cast of earthworms, the mass proportion of submicron and nanocron PET plastics increased from 1% on the 1st day to 26% on the 8th day (Figure S9A). In contrast, submicron and nanocron PLA plastics accounted for 30% of total PLA on the 1st day, which further increased to 92% on the 4th day (Figure S9B).

Elimination Kinetics of the Ingested MPs in Earthworms. Without considering the particle size distribution, excretion of PET and PLA in earthworms could be well-described by first-order kinetics according to the total mass of polymers in the earthworm casts (Figure 6). The elimination rate constants for PET and PLA in earthworms were 0.0746 and 0.0156 h^{-1} , with the calculated elimination half-life of PET and PLA of 9.3 and 45 h, respectively, indicating that PET MPs were eliminated more quickly than PLA. Accordingly, the

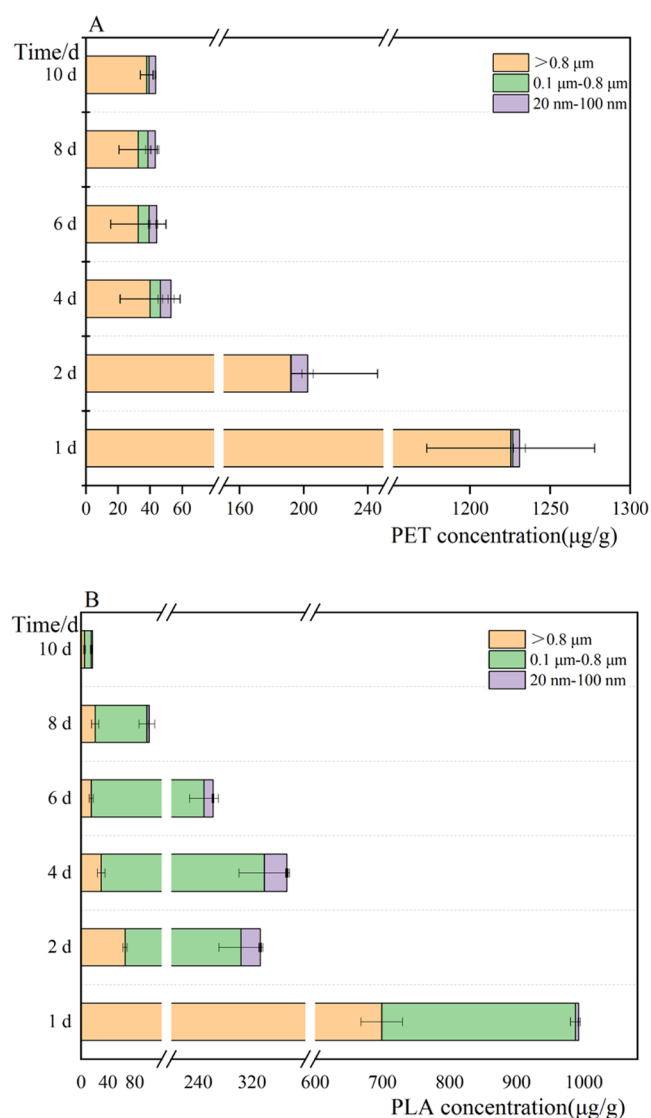


Figure 5. Concentration of PET (A) and PLA (B) MPs ($\mu\text{g/g}$) with different particle sizes in the earthworm casts during the elimination period (1, 2, 4, 6, 8, and 10 days).

calculated elimination half-life of PLA, i.e., 45 h, was longer than that of PET (9.3 h, see Table S6). This timescale of elimination (1–2 days) has also been observed for small PS MPs ($<10\ \mu\text{m}$) in fish and mussels previously.^{45,46} Similar to our results, the half-life of nanocron PS particles in earthworms was estimated to be 1.6 days.⁴⁷ The longer excretion cycle of PLA may be because they are easier to decompose into small particles in earthworms. The small particles can become trapped in the invaginations of the alimentary canal, which extend the egestion time of these small particles.^{48,49} Similar to our finding, smaller PS microspheres ($0.1\ \mu\text{m}$) have a longer gut retention time than larger ones ($10\ \mu\text{m}$) in mussels (*Mytilus edulis*) and oysters (*C. virginica*).⁵⁰

Implications. Earthworms *E. fetida* showed active ingestion of some MP polymers, e.g., PET and PLA, which may be due to the odor of the polymer monomers. Biodegradable PLA was much easier to break down than PET MPs by earthworms, which results in the emission of submicron and nanocron PLA particles into soils.

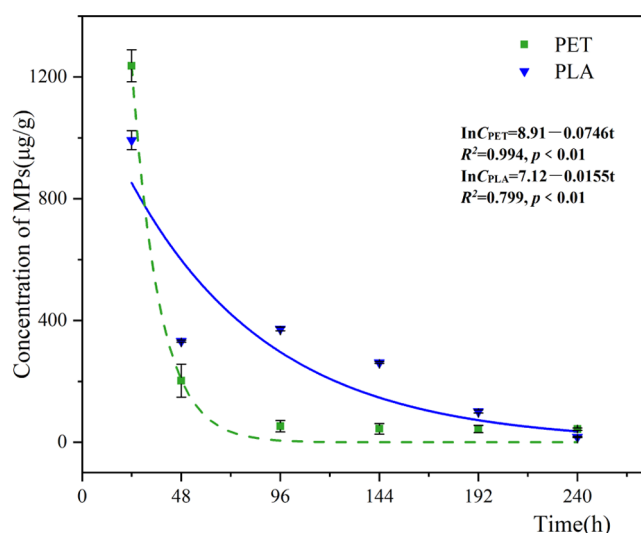


Figure 6. Fitting curves of the first-order kinetic model for the elimination of PET and PLA by earthworms.

The application of the biodegradable plastics increases rapidly,¹⁵ which will lead to increased distribution of biodegradable MPs in the environment.⁵¹ If biodegradable MPs such as PLA can be digested by earthworms, it may promote biodegradable MP decomposition in the terrestrial environment. However, the potential physiological impacts of PLA ingestion as food on earthworms are still unknown. In addition to the environmental emission of nanoplastics, due to the long residence time in earthworms, the nanoparticles formed by PLA decomposition in vivo and the carried pollutants are more likely to be absorbed.^{52–54} Therefore, the ecological impacts of PLA MPs deserve further attention.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.1c08066>.

Detailed procedure of the approach—avoidance experiments conducted (Text S1); pretreatment procedure of the earthworm casts (Text S2); depolymerization information of PLA MPs (Text S3); removal of free LA from the samples (Text S4); solid-phase extraction of PTA and LA (Text S5); physicochemical characteristics of soil used in the experiment (Table S1); depolymerization efficiency of PLA under three doses (Table S2); instrument performance and MS/MS parameters (Tables S3 and S4); recoveries of the spiked MPs in casts (Table S5); first-order kinetic fitting results of PET and PLA excretions by earthworms (Table S6); device design of earthworm's approach and avoidance experiment (Figure S1); depolymerization efficiency of PLA at different reaction times (Figure S2); SEM images of PET (A) and PLA (B) before and after H_2O_2 digestion (Figure S3); number of earthworms in different zones of the two-compartment test chamber after 2-day exposure (Figure S4); particulate matter attached to the surface of earthworms exposed to different treatment groups (Figure S5); SEM images of the submicron and nanocron PLA plastics separated from the earthworm casts (Figure S6); size distribution proportion of PET (A) and PLA (B) particles in casts at

different excretion periods (Figures S7 and S9); and chromatograms of PET (A) and PLA (B) particles of different sizes in casts excreted by earthworms on the 1st day (Figure S8) (PDF)

AUTHOR INFORMATION

Corresponding Author

Lei Wang – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China; orcid.org/0000-0002-8193-9954; Phone: +86-22-2350-1117; Email: wang2007@nankai.edu.cn

Authors

Yawen Peng – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

Yali Xu – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

Junjie Zhang – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China; orcid.org/0000-0002-4383-1096

Chunguang Liu – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China; orcid.org/0000-0001-5207-3752

Xuejiao Tang – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

Yuan Lu – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China

Hongwen Sun – MOE Key Laboratory of Pollution Processes and Environmental Criteria/Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China; orcid.org/0000-0003-1948-5047

Complete contact information is available at: <https://pubs.acs.org/10.1021/acs.est.1c08066>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Key Research and Development Project of China (2019YFC1804403) and the National Natural Science Foundation of China (41722304). The study was funded in part by the National Natural Science Foundation of China (42077336 and 41773109), Tianjin Municipal Science and Technology Bureau (18JJCJC47100 and 19JCYBJC24000), Ministry of Education, China (T2017002), and Tianjin Research Innovation Project for Postgraduate Students (2020YJSB061).

REFERENCES

- (1) Horton, A. A.; Walton, A.; Spurgeon, D. J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141.
- (2) Amaral-Zettler, L. A.; Erik, R. Z.; Beth, S.; Gregory, D. B.; Donald, W. M.; Clare, E. M.; Giora, P.; Tracy, J. M. The biogeography of the plastisphere: implications for policy. *Front. Ecol. Environ.* **2015**, *13*, 541–546.
- (3) Mei, W.; Chen, G.; Bao, J.; Song, M.; Li, Y.; Luo, C. Interactions between microplastics and organic compounds in aquatic environments: a mini review. *Sci. Total Environ.* **2020**, *736*, No. 139472.
- (4) Sarker, A.; Deepo, D. M.; Nandi, R.; Rana, J.; Islam, S.; Rahman, S.; Hossain, M. N.; Islam, M. S.; Baroi, A.; Kim, J.-E. A review of microplastics pollution in the soil and terrestrial ecosystems: a global and Bangladesh perspective. *Sci. Total Environ.* **2020**, *733*, No. 139296.
- (5) Barnes, D. K. A.; Galgani, F.; Thompson Richard, C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans.: Biol. Sci.* **2009**, *364*, 1985–1998.
- (6) Duan, Z.; Zhao, S.; Zhao, L.; Duan, X.; Xie, S.; Zhang, H.; Liu, Y.; Peng, Y.; Liu, C.; Wang, L. Microplastics in Yellow River Delta wetland: occurrence, characteristics, human influences, and marker. *Environ. Pollut.* **2020**, *258*, No. 113232.
- (7) Altieri, M. A. Invertebrate biodiversity as bioindicators of sustainable landscapes. Practical use of invertebrates to assess sustainable land use. In *The Ecological Role of Biodiversity in Agroecosystems*; Elsevier, 1999; Vol. 74, pp 19–31.
- (8) Jones, C. G.; Lawton, J. H.; Schachak, M. Organisms as ecosystem engineers. *Oikos* **1994**, *69*, 373–386.
- (9) Fusaro, S.; Gavinelli, F.; Lazzarini, F.; Paoletti, M. G. Soil biological quality index based on earthworms (qbs-e). A new way to use earthworms as bioindicators in agroecosystems. *Ecol. Indic.* **2018**, *93*, 1276–1292.
- (10) Adhikari, K.; Hartemink, A. E. Linking soils to ecosystem services—a global review. *Geoderma* **2016**, *262*, 101–111.
- (11) Coutris, C.; Hertel-Aas, T.; Lapied, E.; Joner, E. J.; Oughton, D. H. Bioavailability of cobalt and silver nanoparticles to the earthworm *Eisenia fetida*. *Nanotoxicology* **2012**, *6*, 186–195.
- (12) Huerta Lwanga, E.; Gertsen, H.; Gooren, H.; Peters, P.; Salánki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A. A.; Geissen, V. Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, lumbricidae). *Environ. Sci. Technol.* **2016**, *50*, 2685–2691.
- (13) Cheng, Y.; Song, W.; Tian, H.; Zhang, K.; Li, B.; Du, Z.; Zhang, W.; Wang, J.; Wang, J.; Zhu, L. The effects of high-density polyethylene and polypropylene microplastics on the soil and earthworm *Metaphire guillelmi* gut microbiota. *Chemosphere* **2021**, *267*, No. 129219.
- (14) Huerta Lwanga, E.; Mendoza Vega, J.; Ku Quej, V.; Chi, J. A.; Sanchez del Cid, L.; Chi, C.; Escalona Segura, G.; Gertsen, H.; van der Ploeg, M.; Geissen, V.; Salánki, T.; Koelmans, A. A. Field evidence for transfer of plastic debris along a terrestrial food chain. *Sci. Rep.* **2017**, *7*, No. 14071.

- (15) Zhang, M.; Thomas, N. L. Blending polylactic acid with polyhydroxybutyrate: The effect on thermal, mechanical, and biodegradation properties. *Adv. Polym. Technol.* **2011**, *30*, 67–79.
- (16) Ncube, L. K.; Ude, A. U.; Ogunmuyiwa, E. N.; Zulkifli, R.; Beas, I. N. Environmental impact of food packaging materials: a review of contemporary development from conventional plastics to polylactic acid based materials. *Materials* **2020**, *13*, No. 4994.
- (17) Langdon, C. J.; Hodson, M. E.; Arnold, R. E.; Black, S. Survival, Pb-uptake and behaviour of three species of earthworm in Pb treated soils determined using an OECD-style toxicity test and a soil avoidance test. *Environ. Pollut.* **2005**, *138*, 368–375.
- (18) Ge, J.; Xiao, Y.; Chai, Y.; Yan, H.; Wu, R.; Xin, X.; Wang, D.; Yu, X. Sub-lethal effects of six neonicotinoids on avoidance behavior and reproduction of earthworms (*Eisenia fetida*). *Ecotoxicol. Environ. Saf.* **2018**, *162*, 423–429.
- (19) Lowe, C. N.; Butt, K. R.; Cheynier, K. Y.-M. Assessment of avoidance behaviour by earthworms (*Lumbricus rubellus* and *Octolasion cyaneum*) in linear pollution gradients. *Ecotoxicol. Environ. Saf.* **2016**, *124*, 324–328.
- (20) Garcia, M.; Römbke, J.; de Brito, M. T.; Scheffczyk, A. Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. *Environ. Pollut.* **2008**, *153*, 450–456.
- (21) Langdon, C. J.; Hodson, M. E.; Arnold, R. E.; Black, S. Survival, Pb-uptake and behaviour of three species of earthworm in Pb treated soils determined using an OECD-style toxicity test and a soil avoidance test. *Environ. Pollut.* **2005**, *138*, 368–375.
- (22) Horton, A. A.; Svendsen, C.; Williams, R. J.; Spurgeon, D. J.; Lahive, E. Large microplastic particles in sediments of tributaries of the river Thames, UK - abundance, sources and methods for effective quantification. *Mar. Pollut. Bull.* **2017**, *114*, 218–226.
- (23) Zhang, J.; Wang, L.; Kannan, K. Polyethylene terephthalate and polycarbonate microplastics in PET food and casts from the United States. *Environ. Sci. Technol.* **2019**, *53*, 12035–12042.
- (24) Zhang, J.; Wang, L.; Kannan, K. Microplastics in house dust from 12 countries and associated human exposure. *Environ. Int.* **2019**, *134*, No. 105314.
- (25) Wang, L.; Zhang, J.; Hou, S.; Sun, H. A simple method for quantifying polycarbonate and polyethylene terephthalate microplastics in environmental samples by liquid chromatography-tandem mass spectrometry. *Environ. Sci. Technol. Lett.* **2017**, *4*, 530–534.
- (26) Piemonte, V.; Gironi, F. Lactic acid production by hydrolysis of poly(lactic acid) in aqueous solutions: an experimental and kinetic study. *J. Polym. Environ.* **2013**, *21*, 275–279.
- (27) Tsochatzis, E. D.; Alberto Lopes, J.; Kappenstein, O.; Tietz, T.; Hoekstra, E. J. Quantification of PET cyclic and linear oligomers in teabags by a validated LC-MS method – In silico toxicity assessment and consumer's exposure. *Food Chem.* **2020**, *317*, No. 126427.
- (28) Bi, W.; Jun, Z.; Kyung Ho, R. O. W. Solid phase extraction of lactic acid from fermentation broth by anion-exchangeable silica confined ionic liquids. *Talanta* **2011**, *83*, 974–979.
- (29) González-Alcaraz, M. N.; Loureiro, S.; Van Gestel, C. A. M. Toxicokinetics of Zn and Cd in the earthworm *Eisenia andrei* exposed to metal-contaminated soils under different combinations of air temperature and soil moisture content. *Chemosphere* **2018**, *197*, 26–32.
- (30) Bednarska, A. J.; Choczyski, M.; Laskowski, R.; Walczak, M. Combined effects of chlorpyrifos, copper and temperature on acetylcholinesterase activity and toxicokinetics of the chemicals in the earthworm *Eisenia fetida*. *Environ. Pollut.* **2017**, *220*, 567–576.
- (31) Wendland, R. T.; Wheeler, D. H. A System of Characterization of Common Organic Acids. *Anal. Chem.* **1954**, *26*, 1469–1474.
- (32) Ganzevles, P. G. J.; Kroeze, J. H. A. The sour taste of acids. The hydrogen ion and the undissociated acid as sour agents. *Chem. Senses* **1987**, *12*, 563–576.
- (33) Mcmanus, F. E.; Wyers, E. J. Olfaction and selective association in the earthworm *Lumbricus terrestris*. *Behav. Neural Biol.* **1979**, *25*, 39–57.
- (34) Zirbes, L.; Mescher, M.; Vrancken, V.; Wathélet, J.-P.; Verheggen, F. J.; Thonart, P.; Haubruge, E. Earthworms use odor cues to locate and feed on microorganisms in soil. *PLoS One* **2011**, *6*, No. e21927.
- (35) Miao, C.; Chen, X.; Pelton, R. Adhesion of poly(vinylamine) microgels to wet cellulose. *Ind. Eng. Chem. Res.* **2007**, *46*, 6486–6493.
- (36) Javanbakht, S.; Shaabani, A. Carboxymethyl cellulose-based oral delivery systems. *Int. J. Biol. Macromol.* **2019**, *133*, 21–29.
- (37) Curry, J. P.; Schmidt, O. The feeding ecology of earthworms – a review. *Pedobiologia* **2007**, *50*, 463–477.
- (38) Edwards, C. A.; Bohlen, P. J. Biology and ecology of earthworms. *Agric., Ecosyst. Environ.* **1996**, *64*, No. 426.
- (39) Gong, W.; Jiang, M.; Han, P.; Liang, G.; Zhang, T.; Liu, G. Comparative analysis on the sorption kinetics and isotherms of fipronil on nondegradable and biodegradable microplastics. *Environ. Pollut.* **2019**, *254*, No. 112927.
- (40) Chen, Y.; Liu, X.; Leng, Y.; Wang, J. Defense responses in earthworms (*Eisenia fetida*) exposed to low-density polyethylene microplastics in soils. *Ecotoxicol. Environ. Saf.* **2020**, *187*, No. 109788.
- (41) Soobhany, N. Insight into the recovery of nutrients from organic solid waste through biochemical conversion processes for fertilizer production: A review. *J. Cleaner Prod.* **2019**, *241*, No. 118413.
- (42) Sanchez-Hernandez, J. C.; Mazzia, C.; Capowiez, Y.; Rault, M. Carboxylesterase activity in earthworm gut contents: potential (eco)toxicological implications. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2009**, *150*, 503–511.
- (43) Sanchez-Hernandez, J. C.; Aira, M.; Dominguez, J. Extracellular pesticide detoxification in the gastrointestinal tract of the earthworm *Aporrectodea caliginosa*. *Soil Biol. Biochem.* **2014**, *79*, 1–4.
- (44) Sanchez-Hernandez, J. C.; Capowiez, Y.; Ro, K. S. Potential use of earthworms to enhance decaying of biodegradable plastics. *ACS Sustainable Chem. Eng.* **2020**, *8*, 4292–4316.
- (45) Hu, L.; Su, L.; Xue, Y.; Mu, J.; Zhu, J.; Xu, J.; Shi, H. Uptake, accumulation and elimination of polystyrene microspheres in tadpoles of *Xenopus tropicalis*. *Chemosphere* **2016**, *164*, 611–617.
- (46) Kinjo, A.; Mizukawa, K.; Takada, H.; Inoue, K. Size-dependent elimination of ingested microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. *Mar. Pollut. Bull.* **2019**, *149*, No. 110512.
- (47) Lahive, E.; Cross, R.; Saarloos, A. I.; Horton, A. A.; Svendsen, C.; Hufenus, R.; Mitrano, D. M. Earthworms ingest microplastic fibres and nanoplastics with effects on egestion rate and long-term retention. *Sci. Total Environ.* **2021**, *807*, No. 151022.
- (48) Dalby, P. R.; Baker, G. H.; Smith, S. E. Filter paper method to remove soil from earthworm intestines and to standardise the water content of earthworm tissue. *Soil Biol. Biochem.* **1996**, *28*, 685–687.
- (49) Arnold, R. E.; Hodson, M. E. Effect of time and mode of depuration on tissue copper concentrations of the earthworms *Eisenia andrei*, *Lumbricus rubellus* and *Lumbricus terrestris*. *Environ. Pollut.* **2007**, *148*, 21–30.
- (50) Ward, J. E.; Kach, D. J. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. *Mar. Environ. Res.* **2009**, *68*, 137–142.
- (51) Sanchez-Hernandez, J. C.; Capowiez, Y.; Ro, K. S. Potential use of earthworms to enhance decaying of biodegradable plastics. *ACS Sustainable Chem. Eng.* **2020**, *8*, 4292–4316.
- (52) Holmes, L. A.; Turner, A.; Thompson, R. C. Adsorption of trace metals to plastic resin pellets in the marine environment. *Environ. Pollut.* **2012**, *160*, 42–48.
- (53) Bouwmeester, H.; Hollman, P. C. H.; Peters, R. J. B. Potential health impact of environmentally released micro- and nanoplastics in the human food production chain: Experiences from nanotoxicology. *Environ. Sci. Technol.* **2015**, *49*, 8932–8947.
- (54) Lehner, R.; Weder, C.; Petri-Fink, A.; Rothen-Rutishauser, B. Emergence of nanoplastic in the environment and possible impact on human health. *Environ. Sci. Technol.* **2019**, *53*, 1748–1765.