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Microplastics and metals: Microplastics generated from biodegradable polylactic acid mulch reduce bioaccumulation of cadmium in earthworms compared to those generated from polyethylene

X. Xiao [*], J.B. Sallach, M.E. Hodson

Department of Environment and Geography, University of York, York YO10 5NG, United Kingdom

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ABSTRACT

Biodegradable polylactic acid (PLA) mulch has been developed to replace conventional polyethylene (PE) mulch in agriculture as a response to growing concerns about recalcitrant plastic pollution and the accumulation of microplastics (MPs) in soil. Cadmium is a significant soil pollutant in China. MPs have been shown to adsorb metals. In this study the earthworm *Lumbricus terrestris* was exposed to either Cd (1.0–100 mg / kg) or MPs (PE and PLA, 0.1–3 % w / w), or a combination of the two, for 28 days. Cd bioavailability significantly decreased in the presence of MPs. In particular, at the end of the experiment, PLA treatments had lower measured Cd concentrations in both earthworms (2.127–29.24 mg / kg) and pore water (below detection limits - 0.1384 mg /L) relative to PE treatments (2.720–33.77 mg / kg and below detection limits - 0.2489 mg / L). In our adsorption experiment PLA MPs adsorbed significantly more Cd than PE MPs with maximum adsorption capacities of 126.0 and 23.2 mg / kg respectively. These results suggest that the PLA MPs reduce earthworm exposure to Cd relative to PE by removing it from solution and reducing its bioavailability.

1. Introduction

In agriculture, mulches are used around the globe to increase yields and improve crop quality by increasing soil temperature and enhancing water use efficiency (Deng et al., 2006). Plastic mulches have been developed for use (Kasirajan and Ngouajio, 2012), and these are most commonly made from polyethylene (PE) (Khalid et al., 2023). Plastic mulch usage is increasing globally but the biggest usage is seen in Asia (FAO, 2021). According to the China Agricultural Statistical Yearbook (National Bureau of Statistics of China, 1982 - 2012), the amount of plastic mulch used in China increased from 319,000 tons to 1,245,000 tons from 1991 to 2011 and has been growing at an annual rate of 7.1 %; more recent estimates put usage of plastic mulches at 3 000 000 tonnes per year (FAO, 2021). Under some management practices mulches are ploughed into the soil at the end of their use which can lead to the production of plastic fragments including microplastics (MPs) (Piehl et al., 2018; Tang, 2023). Microplastic mulch residues can also form following degradation of the mulches due to exposure to UV radiation and the weather (Luo et al., 2022). Liu et al., (2022) found about 99 % of MPs in arable land was PE.

Due to the small particle size of MPs, soil organisms are able to ingest them (Zhang et al., 2020). Whilst not all earthworm MP exposure investigations observe negative impacts (e.g. Hodson et al., 2017; Prendergast-Miller et al., 2019; Wang et al., 2019b; Mondal et al., 2023; Shang et al., 2023) many studies, particularly those that use high concentrations of MPs, do. The presence of MPs has led to observations of decreased growth and increased mortality (Huerta Lwanga et al., 2016), reduced feeding (Besseling et al., 2013), reduced burrowing activity (Huerta Lwanga et al., 2017) and, reduced reproduction and avoidance of MP-bearing soil (Ding et al., 2021). MPs can also cause histopathological damage (Jiang et al., 2020) and adversely affect the immune system (Xu and Yu, 2021), oxidative response (Cheng et al., 2020, Jiang et al., 2020), CRT, ANN, TCTP, and Hsp70 gene expression (Cheng et al., 2020), and gut microbiota (Cao et al., 2022) of earthworms.

To date, several biodegradable plastics have been developed to replace conventional PE in a range of applications including as an agricultural mulch material as a response to growing concerns about plastic pollution (Sintim and Flury, 2017; Sun et al., 2021). Among these products, biodegradable polylactic acid (PLA) accounts for the largest share (24 %) of total global biodegradable plastics manufacturing (Haider et al., 2019). PLA initially degrades via hydrolysis and then by microbial degradation (Kale et al., 2007; Watanabe et al., 2007; Saadi

* Corresponding author.
E-mail address: xx1135@york.ac.uk (X. Xiao).

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et al., 2012). There is a paucity of studies that determine whether use of biodegradable plastics reduces the impacts of MPs on soil biology. Ding et al. (2021) and Yu et al., (2022) found no differences in the avoidance, survival, biomass, reproduction and oxidative stress response of *Eisenia fetida* to PE and PLA and Zhao et al., (2024) reported that exposure to both PLA and PE MPs led to increases in earthworm weight. However, Han et al., (2023) and Zhao et al., (2023) found that PLA caused more histopathological damage to *Eisenia fetida* in comparison to PE whereas Holzinger et al., (2023) found that exposure to PLA increased reproduction of *Eisenia fetida* relative to conventional plastics (polystyrene, PP; polyethylene terephthalate PET and polypropylene PS). Inconsistency in the results of studies comparing the impacts of biodegradable and conventional MPs on earthworms suggest that more research is warranted.

Mulches are commonly used in China, where cadmium (Cd) contamination of agricultural soils is also an ongoing challenge due to the historic use of Cd-bearing phosphate fertilisers (Niño-Savala et al., 2019). Cd ranks at the top of lists of metal pollutants in farmland exceeding Chinese soil environmental quality standards (Zhao et al., 2015). Cd contamination of soils is however a global issue (e.g. New Zealand, Gray and Cavanagh, 2023; Switzerland, Park et al., 2010; Quezada-Hinojosa et al., 2009; Burau, 1981). It is already known that MPs can interact with metals and this can impact on how both MPs and metals affect soil biota (Wen et al., 2018; Hodson et al., 2017; Lian et al., 2020). However, impacts on earthworms are rather varied and the majority of them are additive. Huang et al., (2021) found that the combined effects of Cd and MPs reduced *E. fetida* biomass and reproduction more than the effect of either PE or Cd alone. Similarly, Zhou et al. (2020a) found that the combination of polypropylene (PP) and Cd led to more severe oxidative damage of *E. fetida* after both 14 and 28 days exposure than exposure to Cd or PP alone. However, Liang et al. (2022) found that effects were time dependent with exposure to PE and Cd having an antagonistic impact on oxidative damage of *E. fetida* after 10 days but a synergistic impact after 30 days. Studies comparing differences in the impacts of biodegradable and conventional MPs when they interact with Cd on earthworms are scarce. Shang et al., (2023) found that bioaccumulation and toxicity to earthworms was greater for PLA and Cd than for PS and Cd based on an Integrated Biomarkers Response index. In contrast, Chen et al. (2024) found no significant differences in the antioxidant defense responses of earthworms to Cd in combination with either PE or PLA.

Given the increased adoption of biodegradable plastics such as PLA, the substantial use of plastic mulches in China and the high levels of Cd in some Chinese agricultural soils together with the paucity of data that compares the impacts on soil biota of interactions between Cd and conventional versus biodegradable plastics, further research in this topic is warranted. The aim of this study was therefore to better understand the impacts of interaction between conventional versus biodegradable MPs and Cd on earthworms. To achieve this we exposed the common European earthworm *L. terrestris* to either or both MPs (conventional PE and biodegradable PLA) and Cd, measured Cd concentrations in soil pore water and earthworm tissues and assessed earthworm mass change and mortality. In addition the adsorption of Cd by PE and PLA was determined to aid interpretation of our earthworm exposure experiments. As far as we are aware this is the first study to consider the impacts of the interactions of Cd and PE versus PLA on Cd uptake in earthworms and to present accompanying adsorption data to support the findings.

2. Materials and methods

2.1. Soils

Because our study was designed to investigate mechanisms and to avoid biosecurity issues associated with overseas soil use, we used uncontaminated natural topsoil purchased from Garden Topsoil Direct, Northamptonshire, UK (Cranford Road, Burton Latimer, Kettering, Northamptonshire, NN16 8UN, U.K.) rather than obtaining (and potentially importing) Cd-contaminated soil from either the UK or overseas. The soils were air-dried to constant weight, sieved to < 2 mm and characterised before use. The soil was a clay loam soil (12.12 ± 1.19 % clay, 50.15 ± 4.49 % silt, 37.73 ± 5.64 % sand) (n = 3, ± standard deviation) with a pH of 7.51 ± 0.02, water holding capacity (WHC) of 76.15 ± 0.58 %, organic matter content of 11.24 ± 0.13 g / 100 g over-dry soil and effective cation exchange capacity (ECEC) of 1.86 ± 0.04 $cmol_c$ / kg, (n = 5, ± standard deviation). Characterisation methods are given in the Supporting Information (Text S1). Background concentrations of MPs in the soil were determined using a 30 % H_2O_2 organic matter digestion step followed by a density separation (Li et al., 2019). The concentration of material separated by this method from the < 2 mm fraction was 0.37 ± 0.26 (n = 3, ± standard deviation) wt%. The FTIR spectrum of the separate did not match that of PE or PLA (Fig. S1a) and was a close fit to lignin (Fig. S1b) (Liu et al., 2008). Furthermore, no melting of the particles was observed when the samples were heated at 130 °C (Fig. S2) (Zhang et al., 2018a). Thus the separated particles appear to be largely recalcitrant organic matter and the soil appears to contain a negligible concentration of MPs. Background Cd concentrations in the soil were determined by an aqua regia digestion (Text S3, digestion method of British Standard BS, 7755, 1995) followed by analysis by inductively coupled optical emission spectroscopy (ICP-OES); measured Cd concentrations were below detection limits (Table S4).

2.2. Microplastics

Two types of plastic mulches, non-biodegradable PE and biodegradable PLA, were purchased from the BEIJUANDEOZXIAODIAN storefront on Amazon. Details of their characterisation are given in the Supplementary Information (Text S2, S3, Figs. S3–5, Tables S1-3, 5). The MPs were confirmed to be PE and PLA using Fourier transform infrared (FTIR) spectroscopy (Text S2, Fig. S3, Table S1). The two types of mulch were cut into small pieces (2 cm^2) using scissors, and then shredded to generate MPs using a Ninja kitchen blender (↱BN495UK), which was purchased from NINJA store on Amazon; the shredded material was sieved to less than 2 mm. The PE formed planar particles whilst the PLA formed fibres. Images of the MPs are given in Fig. S4 and their sizes and shapes, as assessed by optical microscopy and use of ImageJ 2.3.0 / 1.53r, are provided in Fig. S5 and Tables S2 and S3. No Cd was detected in solutions obtained by digestion of the PLA and PE in aqua regia (Text S3; Table S4).

2.3. Earthworms

We used the common UK earthworm *Lumbricus terrestris* in our study; in addition to *L. terrestris* being found in the UK, Europe, the US and Australasia, either as native or invasive species, Lumbricidae more widely are found globally (Hendrix et al., 2008), thus it is a good example earthworm to investigate the impacts of Cd-MP interactions and avoids biosecurity issues associated with the use of non-native earthworms. Adult, that is clitellate, *Lumbricus terrestris* were purchased from Worms Direct, Ulting, UK (Drylands, Ulting, Nr Maldon, Essex, CM9 6QS, U.K.). Earthworms were rinsed with deionised water and cultivated for 1 week in the same soil type used in the following experiment to acclimatise them to ambient laboratory conditions. Approximately 48 h prior to use, the earthworms were removed from the soil, gently rinsed to clean off adhering soil particles with deionised water and subsequently depurated on moist blue paper towel in petri dishes in the dark at 12 °C to void their gut contents for 2 days (Arnold et al., 2007). During earthworm depuration, the moist papers were changed twice per day and afterwards each earthworm was weighed.

2.4. Exposure experiment

Individual adult *L. terrestris* earthworms weighing 4.87 ± 0.80 g (n = 228, ± standard deviation) were exposed to either or both MPs and Cd (Fig. S6). 1200 g of < 2 mm, air-dry soil either mixed with MPs by mass or without MPs were moistened to 50 % water holding capacity (WHC) using either 456.92 g deionised water or Cd solution; the water was added in small volumes with mixing between additions to ensure a homogeneous distribution of water and Cd. The moist soil was divided into four equally weighted subsamples which were placed in 0.47 L (1 pint), 94 mm diameter, transparent beer cups to give four replicates per treatment. MPs of either PE or PLA were added to MP exposure soils in small quantities with mixing between additions to ensure a homogeneous distribution of MPs until concentrations of 0.1, 0.3 and 3 % w / w were obtained, which covers environmentally relevant exposure levels reported in previous studies (Fuller and Gautam, 2016; Corradini et al., 2019; Dierkes et al., 2019; Zhou et al., 2020a; Ding et al., 2021). Cadmium solution was made by dissolving $Cd(NO_3)_2 \cdot 4\ H_2O$ in deionized water to produce a 0.6006 g / L stock solution of $Cd(NO_3)_2$. This solution was either added undiluted to the soil giving a nominal soil concentration of 100 mg / kg or diluted to give nominal soil Cd concentrations of 1.0, 5.0, 10, 15, 30 and 50 mg / kg. This concentration range, together with the 0 mg / kg Cd control was selected to span the ambient contaminant level of Cd in agricultural lands (Huang et al., 2024; Wang et al., 2015). Below, treatments are referred to in the format XYCdZ where X identifies the plastic type (PE or PLA), Y the plastic concentration (0.1, 0.3 or 1.0 wt%) and, Z the nominal Cd concentration (1.0–100 mg / kg), e.g. PLA0.3Cd10 indicates the 0.3 wt% PLA treatment with a nominal Cd concentration of 10 mg / kg. The soils were left for 1 day to allow the Cd to equilibrate with the soil then an individual *L. terrestris* was added to each pot. The cups were covered with fleece which was secured with rubber bands to prevent earthworm escape (and deposition of any MPs present in the air) and placed in a 12 °C controlled temperature (CT) room for 28 days. The temperature was chosen because it falls within the range of temperatures appropriate for culturing *L. terrestris* (Lowe and Butt, 2005). The exposure period was chosen as it is the duration of time used in standardised OECD earthworm exposure tests (OECD, 2004). The mass of each individual treatment was weighed every day and any mass loss of more than 5 % made up by addition of deionised water to maintain constant water concentrations. Earthworms were not fed during the experiment. After 28 days exposure, earthworms were removed (with mortality being noted) from the pots and depurated in petri dishes on moist blue paper towels for 2 days. During earthworm depuration, the moist papers were changed twice per day (Arnold et al., 2007) and afterwards each earthworm was weighed. Earthworm weight change in each replicate was calculated by difference between mass of the depurated individual at the end of the 28 day exposure period and before being added to the soil. The earthworm depurate was collected, air-dried and weighed. Earthworms were euthanised by freezing then defrosted and digested in nitric acid after the method of Davies et al. (2002); details are given in Text S3. Pore waters were extracted from the moist soils by centrifugation following the method of Carter et al., (2014); details are given in Text S4. In brief, 30 g of moist experimental soil was centrifuged for 15 mins at 4000 RPM to separate the pore water. The remaining soils were air-dried and subsamples digested in aqua regia (British Standard BS, 7755, 1995); details are given in Text S3. Where Cd concentrations were above detection limits, the distribution coefficient, $K_{d\text{-exposure}}$, for the partitioning of Cd between the bulk soil and pore water was determined (Sauvé et al., 2000). Bioaccumulation factors (BAF), were calculated as the ratio between the concentration of Cd in the earthworm and concentration in both the soil (BAF_{soil}, Wang et al., 2018a) and porewater (BAF_{pw}, Palladini et al., 2022) when Cd concentrations were above detection limits.

2.5. Adsorption experiment

An adsorption experiment was carried out to compare the adsorption ability of the same non-biodegradable PE MP particles and biodegradable PLA MP fibres for Cd as used in the earthworm exposure experiments. Approximately 0.2 g of MPs were accurately weighed and added to 30 mL of a Cd solution obtained by dissolving $Cd(NO_3)_2 \cdot 4\ H_2O$ in a background electrolyte of 0.01 mol / L $NaNO_3$ to give a constant solution ionic strength (Hodson et al., 2017). Initial target concentrations were 0.1, 0.5, 1, 5, 10, 50 and 100 mg / L with triplicate controls and MPs treatments at each concentration. Samples were shaken in 50 mL centrifuge tubes at 220 rpm on a flatbed shaker in the 12 °C controlled temperature room for 24 h. A number of previous studies indicate that 24 h should be sufficient for adsorption to reach equilibrium (Hodson et al., 2017; Li et al., 2022). After 24 h, suspensions were centrifuged at 4000 RPM for 15 mins and filtered through Whatman no. 540 12.5 cm diameter filter paper. Controls were used to measure actual concentrations of initial Cd solution concentrations. Cd adsorption by the MPs was calculated by difference between the average Cd concentrations in the controls and MPs treatments at the end of adsorption period. Linear, Langmuir and Freundlich isotherms were used to fit the adsorption data. For the linear isotherm the regression line was constrained to pass through the origin.

2.6. Data analysis and quality control

All solutions were analysed for Cd using a Thermo Scientific iCAP 7000 inductively coupled plasma-optical emission spectrometer (ICP-OES). Quality control data are presented in Table S4; accuracy was in the range 91–103 %, precision 1.90–3.06 % and detection limits were 0.0010–0.0015 mg / L for the pore water, 0.0130 mg / kg for the soil digests and 0.0286–0.0400 mg / kg for the earthworm digests.

Statistical analyses were run using SigmaPlot version 15.0, SPSS version 28.0.1.1 (15) and Excel version 16.72. When Cd concentrations were below the detection limit (DL) they were set to a value equal to the DL divided by the square root of two when used in statistical analysis (Croghan and Egeghy, 2003). Data sets (earthworm mortality, mass of earthworm depurate, earthworm weight change, pore water and earthworm Cd concentration, $K_{d\text{-exposure}}$, BAF_{soil}, BAF_{pw}) were analysed to compare responses to the different Cd and plastic treatments. Normality and equal variance were assessed using the Shapiro–Wilks and Brown–Forsythe tests respectively. Of the data sets investigated none of the above data sets were normally distributed ($P \leq 0.05$) and six (pore water Cd concentration, $K_{d\text{-exposure}}$, earthworm Cd concentration, BAF_{soil}, BAF_{pw}, mass of earthworm depurate) did not have equal variance ($P \leq 0.05$). Where possible data were transformed using a log (earthworm Cd concentration and BAF_{soil},) or square root (mass of earthworm depurate) transform to obtain normal distributions and equal variance. Data were then analysed using two way analysis of variance (ANOVA) followed by Holm-Sidak post hoc tests. It was not possible to transform five of the data sets to both a normal distribution and equal variance (pore water Cd concentration, $K_{d\text{-exposure}}$, earthworm weight change, earthworm mortality, BAF_{pw}). These data were analysed using the non-parametric Scheirer Ray Hare test (Holmes et al., 2017) followed by Games-Howell post hoc tests (Field, 2013). To further investigate variation in pore water Cd concentration, $K_{d\text{-exposure}}$ and BAF_{pw} between plastic treatments a 3 way Scheirer Ray Hare test was used as it proved not possible to transform the data sets to obtain a normal distribution and equal variance. Cd concentration, plastic type, and plastic concentration were the factors (the plastic-free treatments were excluded from this analysis), and Games-Howell post hoc tests (Field, 2013) were carried out. Full statistical outputs are provided in the Supporting information. Independent t tests were used to compare the mass of Cd in the soil and in the soil plus earthworms.

3. Results

3.1. Cd distribution in soil and pore water after 28 days exposure

Cd concentrations in the bulk soil were checked at the end of the experiment and found to be 66–88 % lower than the nominal concentrations though they still gave the desired trend of increasing concentration (Table S5). For convenience treatments are still referred to as their nominal concentrations but measured values were used for any subsequent calculations.

Cd concentrations in pore water ranged from below detection to 0.416 ± 0.0359 mg / L (n = 3–4, ± standard deviation) (Fig. 1, Table S6), increasing significantly with increasing concentrations of Cd in the soil (Fig. 1) ($P \leq 0.001$, 2 way Scheirer Ray Hare Test followed by Games - Howell post hoc test). Cd pore water concentrations increased with increasing measured bulk soil Cd concentrations for the different MP treatments (R^2 = 0.88–0.95, $P \leq 0.001$, linear regressions) (Table S7). For a given nominal soil Cd concentration, pore water concentrations were significantly greater in the absence of MPs than in their presence ($P \leq 0.001$, 2 way Scheirer Ray Hare Test) and were lower in the presence of PLA than in the presence of PE although this difference was not significant ($P \geq 0.05$, 3 way Scheirer Ray Hare Test). Increasing concentrations of MP at a given nominal soil Cd concentration resulted in lower pore water concentrations ($P \leq 0.05$, 3 way Scheirer Ray Hare Test followed by Games - Howell post hoc test).

$K_{d\text{-exposure}}$ values were determined for all treatments for which Cd was detected in both the bulk soil and soil pore water (Fig. 1, Table S5 and S6); values ranged from 68.70 ± 9.3 to 4154 ± 808.5 L / kg (n = 3–4, ± standard deviation) (Fig. 2, Table S8). For a given nominal bulk soil Cd concentration, the $K_{d\text{-exposure}}$ values were significantly greater in the presence than in the absence of MP ($P \leq 0.001$, 2 way Scheirer Ray Hare Test) and were significantly greater in the presence of PLA than in the presence of PE ($P \leq 0.05$, 3 way Scheirer Ray Hare Test). Increasing concentrations of MP at a given nominal bulk soil Cd concentration resulted in greater $K_{d\text{-exposure}}$ values (Fig. 2, Table S8) though increases from one MP concentration to the next higher one were not always significant (3 way Scheirer Ray Hare Test followed by Game - Howell post hoc test; Table S8). Although there were some significant differences between $K_{d\text{-exposure}}$ values for different Cd concentrations at a given MP treatment ($P \leq 0.05$, 3 way Scheirer Ray Hare Test followed by Game - Howell post hoc test) there was no systematic variation in these values (Fig. 2, Table S8), consistent with the linear relationship between bulk soil and pore water Cd concentrations (Table S7).

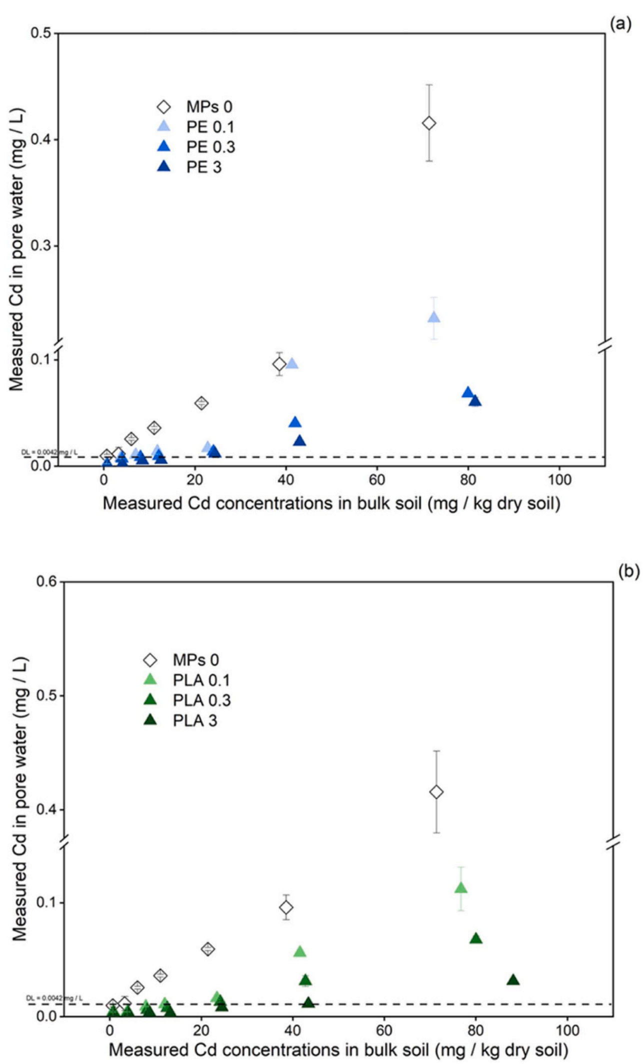

Fig. 1. Mean Cd concentrations in pore water following exposure to Cd with and without MPs over 28 days. (a) PE treatments, (b) PLA treatments. Results are mean ± standard deviation, n = 3–4 (for some replicates we failed to extract pore water). Values that were below detection are expressed as the detection limit divided by the square root of two (Croghan and Egeghy, 2003). Dashed horizontal line on the figures indicates the detection limit.

3.2. Earthworm mortality and weight change

By the end of the exposure experiment, all surviving earthworms had burrowed to the bottom of the soils. There were 13 dead earthworms (out of a total of 228) and none missing. One dead earthworm was found in a single replicate of the following treatments: MPs0Cd30, PE0.1Cd30, PE0.1Cd100, PE0.3Cd10, PE3Cd1, PE3Cd100, PLA0.1Cd50, PLA0.3Cd10, PLA0.3Cd50. Individual dead earthworms were also found in two replicates of the PE3Cd5 and PLA0.3Cd100 treatments. There was no significant difference in mortality between treatments ($P \geq 0.05$, 2 way Scheirer Ray Hare Test). Weight change of the surviving earthworms was 0.13 ± 0.40 g (n = 215, ± standard deviation) and showed no systematic variation between treatments ($P \geq 0.05$, 2 way Scheirer Ray Hare Test) (Fig. S7). Mortality and weight change of < 10 % and < 20 % respectively conforms to the validity criteria of the OECD 317 test for bioaccumulation in earthworms (OECD, 2010).

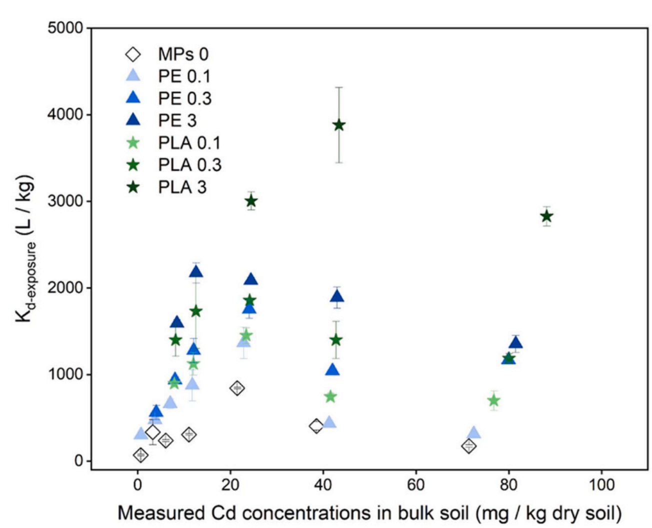

Fig. 2. Mean soil-pore water distribution coefficients ($K_{d\text{-exposure}}$) as a function of soil Cd concentration for the different treatments at the end of the 28 day experiment (n = 3–4, for some replicates we failed to extract pore water). Error bars represent standard deviation; where not visible values are smaller than the symbols.

3.3. Cd bioaccumulation in earthworm after 28 days exposure

Cadmium concentrations in earthworms at the end of the exposure experiment ranged from 3.124 ± 1.031 to 31.363 ± 9.872 mg / kg (n = 2–4, ± standard deviation) (Fig. 3, Table S6) increasing with soil concentrations ($P \leq 0.001$, 2 way ANOVA followed by Holm-Sidak post hoc test). Earthworm Cd concentration was greater in the MP-free treatments ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc tests). In the MP treatments, earthworm Cd concentration decreased with increasing MP concentration, but none of these differences were significant ($P \geq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc tests). Cd concentrations in earthworms exposed to Cd were significantly lower in the 3 % PLA treatments compared with the equivalent PE treatments ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test); at other plastic concentrations although the Cd concentrations were lower for the PLA, the differences were not significant ($P \geq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc tests).

Bioaccumulation factors (BAF) were calculated for treatments where concentrations of Cd in earthworms and bulk soil / pore water were above detection limits (Figs. 1 and 3, Table S5 and S6). BAF_s and BAF_{pw} values were in the range 0.2736 ± 0.028 to 8.777 ± 1.961 and 76.51 ± 26.6 to 4658 ± 583.1 respectively (n = 2–4, ± standard deviation) (Fig. 4, S8). The BAF values decreased exponentially with increasing measured concentrations of Cd in the soil (Fig. 4); the trends were well described by log10-log10 relationships ($R^2 = 0.93$ to 0.97, $P \leq 0.001$, Table S9). The BAF values decreased with increasing MP concentrations ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test) and were significantly higher in PE treatments relative to PLA treatments with the same MP concentrations ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test).

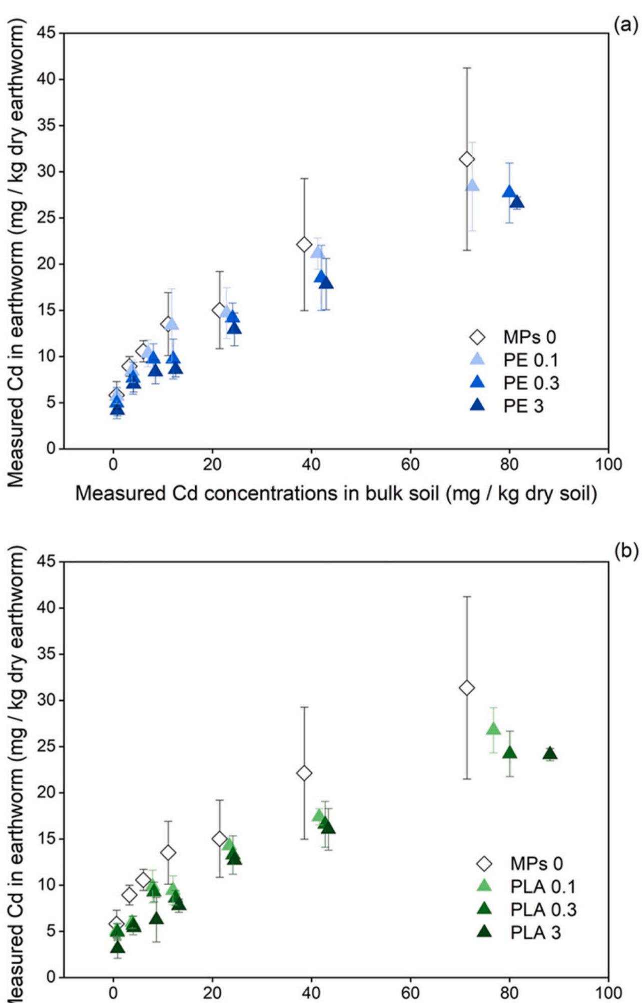

Fig. 3. Mean of measured Cd concentrations in earthworm bodies following exposure to Cd with and without MP over 28 days, (a) PE treatments, (b) PLA treatments. Results are mean ± standard deviation, n = 2–4 (earthworm mortality in some treatments reduced the number of replicates).

3.4. Earthworm depurate recovered after 28 days exposure and MPs still retained in earthworm after depuration

The average mass of earthworm depurate recovered was 0.15 ± 0.10 g (n = 215, ± standard deviation) (Table S10). There was no significant difference between the mass of depurate recovered between Cd treatments ($P \geq 0.05$, two way ANOVA). Mass of earthworm depurate was greater in the MP-free treatments than in the MP treatments; there were significant differences between MP-free treatments, all the PE and the 3 % PLA treatments (Fig. 5, Table S10) ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test). Mass of earthworm depurate decreased with increasing MP concentration (Fig. 5, Table S10); significant differences were found between the 0.1 % and 3 %, and the 0.3 % and 3 % MP treatments ($P \leq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test). For a given MP concentration, mass of earthworm depurate was greater in the presence of PLA than in the presence of PE (Fig. 5, Table S10), however, there were no significant differences between PLA and equivalent PE treatments ($P \geq 0.05$, 2 way ANOVA followed by Holm-Sidak post hoc test).

3.5. Adsorption experiment

In our adsorption experiments, the amount of Cd adsorbed on MPs ranged from 4.67 to 875 mg / kg (Fig. 6a). The adsorption data for initial Cd concentrations of 100 mg / L Cd concentration appear to plot slightly off trend. However, fits of the isotherms to the data showed no significant differences based on the 95 % confidence intervals regardless of whether the 100 mg / L data were included or not (Table 1 and S11). Therefore the 100 mg / L Cd data were included in the analysis reported here. The linear, Freundlich and Langmuir isotherms all described the data well with the fits to the Langmuir and Freundlich isotherms being the best (Fig. 6, Table 1). Isotherm fit parameters indicate greater adsorption of Cd to PLA than PE.

4. Discussion

Our results indicate that MPs generated from plastic mulches and present in soil can decrease earthworm exposure to Cd via adsorption which removes the Cd from pore water thereby reducing its bioavailability. Furthermore, the data from our exposure and adsorption experiments consistently suggest that the PLA MPs adsorb more Cd than PE MPs leading to a greater reduction in Cd bioavailability.

The mismatch between the nominal and measured Cd concentrations in the soil at the end of the experiment (Table S5) is most likely due to the fact that the aqua regia digestions represent pseudo totals (Meers et al., 2007) together with the hygroscopic nature of $Cd(NO_3)_2$ (Lewis, 2004). If $Cd(NO_3)_2.4\,H_2O$ used to make our Cd solutions contained additional water we would have added less Cd to the soil than anticipated. Although earthworms accumulated Cd, mass balance calculations using earthworm tissue concentrations and mass, and soil concentrations and mass indicate that Cd uptake by the earthworms was insufficient to account for the "missing" Cd between the nominal and measured soil concentrations ($P \geq 0.05$, t test) (Table S12, S13). However, because we measured Cd concentrations rather than assuming them, the mismatch between the nominal and measured concentrations does not affect our study.

The total amount of Cd in the pore water indicates that in the MP-free treatments Cd was adsorbed onto the soil particles (Table S14).

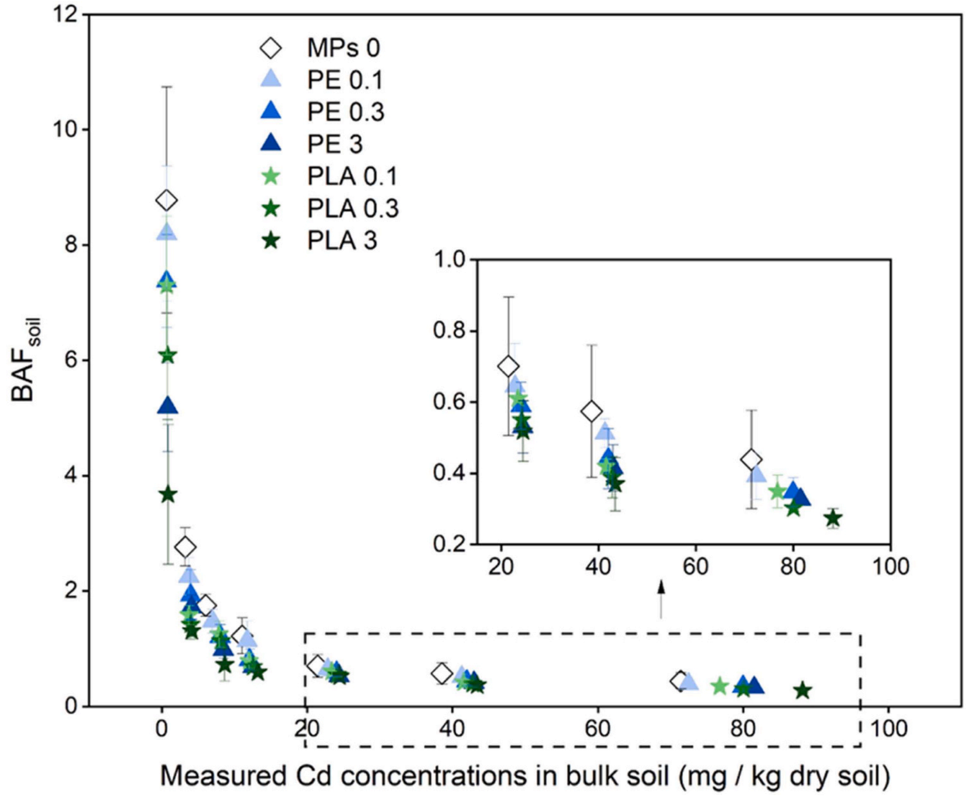

Fig. 4. Earthworm-soil bioaccumulation factors (BAF_s) of the Cd following exposure to Cd with and without MP over 28 days (n = 2–4, earthworm mortality in some treatments reduced the number of replicates). Error bars represent standard deviation.

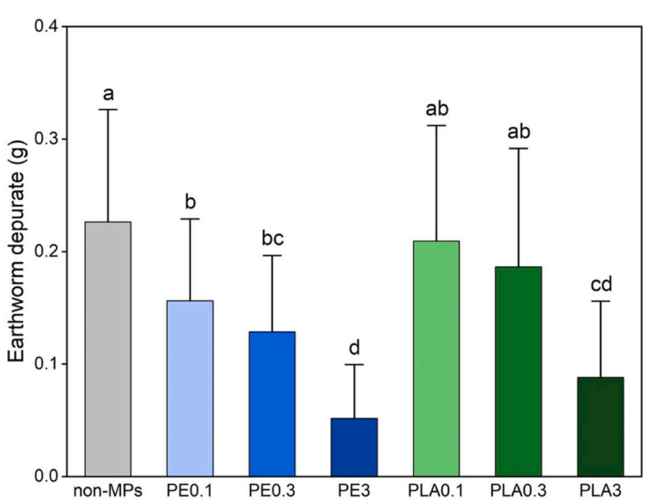

Fig. 5. Mean of earthworm depurates recovered after 28 days exposure between different MP treatments. Error bars represent standard deviation. Different letters above the bars indicate significant differences at P ≤ 0.05 (2 way ANOVA followed by Holm Sidak post hoc tests).

Reductions in pore water concentration in the presence of the MPs (Fig. 1) and our adsorption experiments (Fig. 5) suggest that the MPs increased the amount of adsorption. The K_D values calculated from the exposure experiment (Fig. 2) and our calculated adsorption parameters (Table 1) are similar to those found in other studies (Tables S15 and S16 respectively, Sauvé et al., 2000, Yan et al., 2008, Karapinar and Donat, 2009, Lukman, et al., 2013, Wang, et al., 2019a, Zhou et al., 2020b, Li et al., 2022, Sun et al., 2022, Wang et al., 2022, Coulombe et al., 2023). The $K_{d\text{-exposure}}$, C_m and K_F values suggest that PLA is more sorptive than PE on a per mass basis.

Adsorption is a surface phenomenon and it is possible that the differences in adsorption between the two MPs are due to differences in their shape. The PE MPs used in this study were platy whereas the PLA was fibrous (Fig. S4). Their approximate specific surface areas (SSAs), calculated assuming that the PE were flat particles with a measured thickness and that the PLA fibres were cylindrical, were 0.882 and 0.269 m^2 / g, respectively (Table S17). Thus for a given mass of MP more PE surface would be present than PLA surface suggesting that it is the composition rather than shape of the MPs that resulted in the greater adsorption of Cd to the PLA. The adsorption of metal ions onto MPs is influenced by the surface polarity of the MPs (Brennecke et al., 2016). Given the non-polar nature of PE (Xu et al., 2021) and the charged nature of Cd^{2+} ions the main adsorption mechanism is likely to be a non-specific interaction such as weak van der waal forces. However, biodegradable plastics usually contain polar groups (Sintim and Flury, 2017) and Xu et al. (2021) found that polar MPs showed greater adsorption capacities than nonpolar MPs. PLA contains polar, oxygen-containing ester functional groups (Fig. S3, Table S1) and the Cd^{2+} could bind to these by surface complexation (Fan et al., 2018, Wu et al., 2019). Thus, consistent with our findings, polar PLA would be expected to have more adsorption sites and a larger adsorption capacity than non-polar PE (Chen et al., 2021; Tang et al., 2020; Tang et al., 2023).

BAF_s values in the MP-free treatments were higher than those in the MP treatments and were in the range 0.322–11.7. These values are generally lower than those reported in the literature (3.60–59.7, Table S16, Nannoni et al., 2011, Wang et al., 2018a, Xiao et al., 2020, Ge et al., 2023). However, this is not surprising as different species of earthworm were used in these studies and BAF_s of Cd in earthworms is species dependent (Xiao et al., 2020). Also, soil properties vary between studies. In particular, the soil in our study had an organic matter content of 12.67 ± 0.17 % (n = 5, ± standard deviation), much greater than

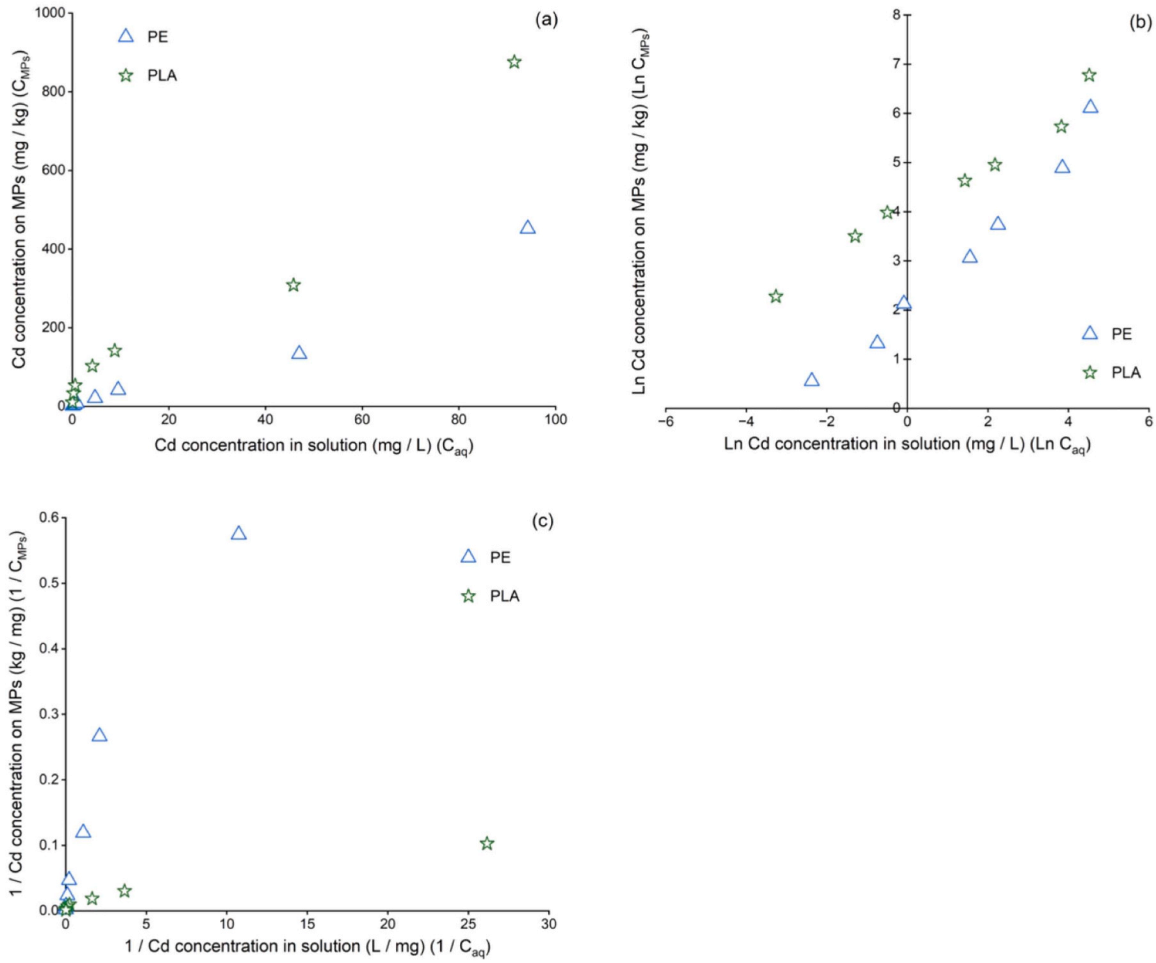

Fig. 6. (a) Adsorption data for PE and PLA MPs. (b) Freundlich isotherms for PE and PLA MPs. (c) Langmuir isotherms for PE and PLA MPs.

Table 1
Linear, Freundlich and Langmuir isotherm parameters for Cd adsorption to the PE and PLA MPs, 95 % confidence intervals are given in brackets. Initial Cd concentrations range from 0.1 to 100 mg / L. The linear adsorption model is expressed as $C_{MPs} = K_{d\text{-}adsorption}C_{aq}$ where $K_{d\text{-}adsorption}$ is the adsorption coefficient, L / kg. The Freundlich model is expressed as $C_{MPs} = K_F C_{aq}^{1/n}$ where K_F is the distribution coefficient constant, $(L / mg)^{1/n}$. The Langmuir model is expressed as $C_{MPs} = C_m K_L C_{aq} / (1+K_L C_{aq})$ where K_L is the binding coefficient, mg / L and C_m is maximum concentration adsorbed to MPs, mg / kg. C_{MPs} is the concentration adsorbed to MPs at equilibrium, mg / kg, C_{aq} is the concentration in solution at equilibrium, mg / L.

MP type	Linear		
	$K_{d\text{-}adsorption}$	R^2	P
PE	4.41 (3.63–5.18)	0.80	< 0.001
PLA	9.08 (7.55–10.6)	0.81	< 0.001

MP type	Freundlich			
	LnK_F	1/n	R^2	P
PE	2.12 (1.77–2.46)	0.779 (0.649–0.909)	0.97	< 0.001
PLA	4.04 (3.76–4.32)	0.517 (0.416–0.619)	0.97	< 0.001

MP type	Langmuir			
	K_L	C_m	R^2	P
PE	0.836 (-0.657–1.62)	23.2 (-42.7–9.12)	0.92	< 0.001
PLA	2.16 (0.499–3.32)	126 (69.7–660)	0.97	< 0.001

those from the above reported studies (1–5.3 %, Table S18), suggesting that adsorption to organic matter could explain the lower BAF_s in this study.

Bioaccumulation factors significantly decreased with increasing measured concentrations of Cd in the soil (Fig. 5) and pore water (Fig. S8). This is consistent with the findings of previous studies in which low Cd concentrations in soil resulted in relatively high BAF of Cd in earthworms and BAF decreased sharply as bulk soil Cd concentration increased (Chapman et al., 1996; Demuynck et al., 2007; Zhang et al., 2018b, Richardson et al., 2020). Our data are well described by Log10-Log10 relationships (Fig. S10; Tables S9, S19) similar to the results of Li et al. (2009), and Wang et al., (2018b). When metals accumulate in earthworms they do so in a number of compartments, with precipitation as insoluble granules and binding to metallothionein-like proteins serving as detoxification mechanisms (Sinkakarimi et al., 2020; Vijver et al., 2006; Andre et al., 2009; Morgan et al., 1989; Stürzenbaum et al., 2001; Arnold et al., 2008). Metabolic regulation of metal uptake to control for toxicity as bulk soil concentrations increase would result in the decreasing values of BAF_s and BAF_{pw} observed in our study.

For a given nominal soil Cd concentration the BAF_s decreased in the presence of MPs. BAF_s decreased with increasing concentrations of MP and for a given concentration of MP were lower for PLA than the PE treatments (Fig. 5). This was accompanied by a reduction in pore water concentrations that showed the same trends, i.e. reduced pore water concentrations with increasing MP concentration and, consistent with the adsorption experiment, lower pore water concentrations in the PLA than the PE treatments. This suggests that it was the reduction in pore water concentrations of Cd that reduced Cd bioavailability, and thus decreased Cd uptake by the earthworms in the presence of MPs. This is consistent with other studies (Crommentuijn et al., 1997, Becquer et al., 2005, Hobbelen et al., 2006, Lee et al., 2013) that have reported that the soluble metal fraction in soils controls metal uptake by earthworms. Uptake of metals by earthworms is assumed to be due to diffusion across the earthworm skin and via ingestion (Vijver et al., 2003). Our data showed earthworm depurate mass decreased with increased MP concentration, suggesting that soil ingestion also decreased. Thus it is not possible to uniquely attribute the decrease in Cd uptake to either reduced diffusion across the earthworm skin due to the lower pore water concentrations or reduced ingestion of Cd-bearing soil. Although adsorption of Cd by the MPs reduced pore water Cd concentrations and thus Cd bioavailability, PLA biodegrades (Kale et al., 2007; Watanabe et al., 2007; Saadi et al., 2012). Whilst the majority of PLA degradation studies have been carried out in compost (e.g. Karamanlioglu and Robson, 2013; Karamanlioglu et al., 2014; Vasile et al., 2018) PLA does degrade, albeit relatively slowly in soil (Adhikari et al., 2016). Thus it is likely that over time, any reductions in Cd bioavaiability due to adsorption on PLA will be reversed as the PLA degrades.

Although our depurate data suggest that the presence of MPs reduced soil ingestion, we did not detect any systematic earthworm mortality or weight change. This lack of any distinctive patterns in mortality with increased Cd concentration was perhaps not surprising. Burgos et al., (2005) found no correlation between earthworm mortality and Cd concentrations (5–200 mg / kg dry weight). Other studies where mortality due to Cd has been reported used higher Cd concentrations, or far longer exposure periods with reported LC_{50}s of 344 to > 1000 mg / kg (Table S20) than our study. At the end of our exposure experiment, the weight change of the earthworms did not show a significant pattern, which is typical of many earthworm exposure studies (Langdon et al., 2005, Yang et al., 2016, Wang et al., 2019b). The presence of MPs in the residues left after earthworm digestion (Fig. S11) suggests that the earthworms ingested MPs with soil and that at least some of the MPs accumulated in the earthworms. However, insufficient pieces of MP were collected to allow for any meaningful analyses of these MPs. Our results suggest that at field-realistic concentrations, MPs do not have a substantive toxic effect on earthworms, at least in terms of mortality and weight change.

5. Conclusion

Perhaps counter intuitively our results suggest that MP pollution could reduce the impact of Cd contamination in soil, by removing Cd from solution and thus reducing its bioavailability. Furthermore the move to biodegradable mulches could lead to greater reductions in bioavailability, at least in part because biodegradable plastics such as PLA are able to adsorb more Cd than conventional plastics such as PE. Thus in the short term the move from conventional to biodegradable plastic mulch use on Cd-bearing soils, such as those found in China, could reduce the risk of Cd accumulation in earthworms and further up the food chain by biomagnification. However, this reduction may be only short term as the PLA is degradable and, once degraded any sorbed Cd will presumably be released back into the soil pore water. If steps have not been taken in the mean time to prevent further addition of Cd to the soils this could result in higher available Cd concentrations in the soil in the longer term.

As conventional MPs weather their surface properties may evolve and become more sorptive (Tu, et al., 2020; Wang et al., 2020; Khan and Hodson, 2024) so over time weathering of conventional MPs and the degradation of biodegradable MPs may lead to the reverse situation to the one found in this study, i.e. conventional plastics may reduce Cd bioavailability to a greater extent than biodegradable plastics. Therefore, the mechanisms involved in the adsorption of Cd on naturally weathered and pristine microplastics warrant further study.

CRediT authorship contribution statement

Brett Sallach: Supervision, Conceptualization. **Mark E Hodson**: Writing – review & editing, Supervision, Project administration, Conceptualization. **Xiao Xiao**: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xiao Xiao reports financial support was provided by China Scholarship Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116746.

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