Interactions between microplastics, cadmium and earthworms

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Abstract

Biodegradable polylactic acid (PLA) mulch has been developed to replace conventional polyethylene (PE) mulch to reduce plastic pollution and the accumulation of plastic mulches derived-microplastics (MPs) in agricultural soil. Cadmium (Cd) is another soil contaminant, and can be adsorbed by MPs. However, the impacts of Cd-MP interactions on earthworm remain highly debated. It is therefore important to assess the ecological risk of the MPs-Cd interaction in farmland.

Laboratory avoidance experiments were carried out to investigate the impacts of plastic mulches derived-MPs and / or Cd on earthworm *Lumbricus terrestris* avoidance and mortality. The results showed realistic levels of MP or Cd and the combination of them in soils did not impact earthworm avoidance or mortality. However earthworms had a slight preference for PLA compared with PE. Exposure experiments were conducted to further investigate the impacts of these MPs and / or Cd on earthworm behaviour. The results showed in addition to mortality realistic levels of MP in soils did not impact earthworm weight change. PLA reduced earthworm exposure to Cd relative to PE by removing it from solution and reducing its bioavailability. Results of the adsorption experiments showed PLA MPs adsorbed significantly more Cd than PE MPs which consistently support the above result of exposure experiment. Furthermore, the abundance of oxygen-containing functional groups in MPs increased and biofilms developed on MPs during weathering leading to increased adsorption of Cd. Field sampling was conducted at an existing experimental site complementing the Cd-free parts of our avoidance experiments.

These results fill the knowledge gap of the impacts of interaction between Cd and mulch-derived MPs on earthworms in agricultural soil and suggest that the replacement of PE by PLA mulches could lead to a reduction in Cd bioavailability in Cd-bearing arable soils in the short term due to increased adsorption onto MPs.

Contents

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I declare that this thesis is a presentation of original work and I am the sole author. All sources are acknowledged as references.

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1 Introduction

1.1 Plastics

Plastics have completely changed human daily lives (Thompson et al., 2009; Sandu et al., 2020), and plastics use is dramatically increasing, with an estimated annual global production of 400.3 million tonnes in 2022 (Plastic Europe, 2023). Plastics are gradually accumulating in the environment due to not only their increasing use but also their resistance to degradation and poor management (Duis and Coors, 2016; Plastic Europe, 2023).

1.2 Microplastics

1.2.1 Microplastics pollution

With time, microplastics (MPs) are generated from the fragmentation and degradation of plastics left in the environment (Rillig et al., 2017; Akdogan and Guven, 2019), acting as a new type of pollution threating organisms and even human health, which have attracted scientific attention worldwide over the last decades (Karbalaei et al., 2018; Huang et al., 2021a; Yan et al., 2022; Ghosh et al., 2023 and Liu et al., 2018; Chen et al., 2020 therein). MPs have been widely observed in marine environments, freshwater bodies (Anderson et al., 2016; Szymańska and Obolewski, 2020; Ding et al., 2021a; Elizalde-Velázquez and Gómez-Oliván, 2021 and Murphy et al., 2016; Enders et al., 2019 therein), and recent studies have reported MPs were also found in soil (Ding et al., 2021b; Huang et al., 2021b; Zhao et al., 2022a). Furthermore, it has been recently estimated that 3 - 10 times greater MPs released into terrestrial environments than that to the marine environments (Earth action, 2023). Notably, agricultural soils are among the most contaminated environmental compartments with plastic debris (Sanchez-Hernandez et al., 2020). Although an increasing number of studies have focused on MPs, most of them focused on the aquatic rather than terrestrial environment, with a particularly low number of studies on agricultural soil (Nizzetto et al., 2016; Ng et al., 2018). Thompson et al., (2024) recently reviewed that the environmental concentrations and bioavailability of MPs will continuously increase and knowledge, however data gaps still exist regarding the assessment of the risks of MPs (Thompson et al., 2024). Therefore, the threats of MPs in farmland, especially their potential impacts on soil biota warrants more research.

1.2.2 Microplastics characteristics in agricultural soil

Generally, MPs are globally detected in agricultural soil, with the majority of studies that detect MPs being conducted in China, probably because the largest part of global plastic production (32 %) occurs there and 27.7 % of that has been poorly managed (Jambeck et al., 2015; Plastic Europe, 2023). The MPs found in the soil have a wide variety of shapes, sizes, and polymer types (Chai et al., 2020; Zhou et al., 2020a). Different polymer types of MPs have a wide variety of properties, affecting their fragmentation rate so that they form different sized and shaped microplastic particles (Harper and Petrie, 2003; Efimova et al., 2018). Shamkhany et al., (2021) found different MPs properties (shape and size) affect the mobility of microplastic particles. MPs move through the environment, and ingested and excreted by animals, which can lead to negative impacts on organisms with the extent of the effects on organisms, potentially depending on the polymer type, size and shape of the MPs (Rochman et al., 2019). It is therefore important to use advanced analytical approaches to characterise MPs.

1.2.3 Microplastics analytical techniques

It is difficult to identify MPs with different sizes, shapes, and polymer using only one analytical method. Therefore, the combination of different analytical techniques has been widely used. MPs characteristics analysis generally includes chemical characterisation (spectroscopy) and physical characterisation (microscopy) to confirm the MPs.

The most commonly used analytical spectroscopy to determine both the presence of MPs and their range of polymers are Fourier transform infrared (FTIR) spectroscopy (Löder and Gerdts, 2015; Primpke et al., 2020) and Raman spectroscopy. FTIR and Raman spectroscopy share the similar approach in that they both identify the polymer types of different MPs by comparing the resulting FTIR or Raman spectra with known plastic polymers in the spectral library (Araujo et al., 2018; Veerasingam et al., 2021). One open and free software OpenSpecy as the spectral library reference can be used for both FTIR and Raman spectroscopy (Cowger et al., 2021). FTIR spectroscopy is able to detect MPs down to 10 μm in size, while Raman spectroscopy is able to detect MPs down to 1 μm in size (Shim et al., 2017).

The most commonly used analytical microscopy to determine the shape and size of MPs are optical microscopy and scanning electron microscopy (SEM) (Shim et al., 2017). SEM provides extremely clear and high magnification images of MPs. Furthermore, crease, cracks, horizontal gaps, flakes, pits, grooves or vermiculite texture on the surface of the MPs as they are weathered can also be detected through SEM high-resolution images (Cooper and Corcoran, 2010).

Once MPs into the soil, they can attach to soil particles and bind them together as stable soil aggregate, so that it is difficult for these analytical methods to identify MPs. Hydrogen peroxide (H_2O_2) can be used to remove soil organic matter to isolate MPs (Li et al., 2019). However, some parts of soil organic matters can not be removed by H_2O_2 and their densities are similar to MPs density, leading to difficulties of distinguishing MPs and impurities (Mikutta et al., 2005; Perie and Ouime, 2008; Hidalgo-Ruz et al., 2012). Zhang et al., (2018) reports that MPs and impurities can be further identified using a heating method (3-5 s at 130 °C), MPs melt but the impurities did not which can be observed by SEM high-resolution images.

As technology evolves, the novel analytical methods and the combinations of analytical methods are gradually developed. Chromatography, such as Pyrolysis coupled with gas chromatographic separation and mass spectrometry detection (Py-GC-MS), is another powerful approach used for MP analysis with high resolution results (Ceccarini et al., 2018; Dehaut et al., 2020; La Nasa et al., 2021). MPs are pyrolysed by pyrolysis system at high temperature (around 650 °C; Toapanta et al., 2021; Lou et al., 2023) and then their pyrolysis products are analysed by GC-MS to provide qualitative (i.e. MPs compositions) and quantitative (i.e. MPs masses) data of microplastics mixtures (Matsui et al., 2020; Roscher et al., 2022). SEM combination with energy dispersive X-ray analysis (SEM-EDX or SEM-EDS) increases potential for analysing chemical elements in MPs and identifying MPs polymer types and estimating their relative abundance (Soursou et al., 2023).

1.2.4 Microplastics leaching additives

Various chemical additives are incorporated into plastics to improve polymer properties such as plasticisers, colorants and stabilisers (Marturano et al., 2017), and these plastic additives can

be released as plastic debris degrades (Tun et al., 2022; Iqbal et al., 2023). Recent research showed that additives leaching from MPs found in marine organisms can pose significant risks to the environment and human health (Fauser et al., 2022; Shafea et al., 2023; Li et al., 2024). However, knowledge of the impacts of additives released from MPs on soils and their associated organisms is lacking to fully evaluate the threats to the terrestrial environment.

1.2.5 Microplastics general sources

MPs are accumulating in soil from a variety of potential sources, such as plastic mulching, the application of sewage sludge, compost, littering, irrigation of wastewater, street runoff and atmospheric deposition (Rillig, 2012; Dris et al., 2015; Duis and Coors, 2016; Horton et al., 2017; Huerta Lwanga et al., 2017; Ng et al., 2018; Huang et al., 2020; Xu et al., 2020; Zhang et al., 2020). Plastic mulching and littering have been identified as two primary input pathways of MPs into the soil environment, while the application of sewage sludge, compost, irrigation of wastewater, street runoff and atmospheric deposition are classified into secondary input pathways of MPs (Praveena et al., 2023; Zhu et al., 2023). Among these sources, the use of plastic mulches has been identified as the major source of MPs in agricultural soil, especially in China (Li et al., 2020; Huang et al., 2021c; Wang et al., 2021; Luo et al., 2022).

1.2.6 Major source of microplastics in agricultural soil - plastic mulches

In agriculture, plastic mulch is a product used in plasticulture that serves the same purpose as mulch for weed suppression and water conservation in crop production and landscaping (Lalitha et al., 2010). Plastic mulches are widely used to increase yields and improve crop quality (Deng et al., 2006), with study more than 1.6 million tonnes / year of plastic mulches used worldwide (Bai et al., 2024). Many plastic mulches are made from durable polymers and most of these are polyethylene (PE), which is not biodegradable and can persist in the environment for decades (Khalid et al., 2023). In response to the growing concerns about plastic pollution, several biodegradable plastics have been developed to replace conventional plastics in a range of applications including as an agricultural mulch material (Sintim and Flury, 2017; Sun et al., 2021). Among these products, biodegradable polylactic acid (PLA) accounts for the largest share of total global biodegradable plastics manufacturing (Haider et al., 2019).

However, under realistic conditions, the ideal rapid degradation of PLA mulches often does not occur and their longevity is also difficult to predict (Hayes et al., 2017; Huang et al., 2023).

PE (Fig. 1.1a) is conventional petroleum-derived plastic and its chemical formular is $(C_2H_4]_n)$, (Doan Tran et al., 2020; Mori, 2023), while PLA (Fig. 1.1b) is a biodegradable, aliphatic polyester derived from lactic acid made from renewable resources, such as corn starch or sugar-cane (Jem et al., 2010; Pang et al., 2010) and its chemical formular is $(C_3H_4O_2)n$ (Nakajima et al., 2017; Su et al., 2024). Furthermore, non-polar PE is made from the polymerisation of ethylene, which is only a long chain of carbon atoms, with two hydrogen atoms attached to each carbon atom (Kempe, 2007; Li et al., 2010), whereas polar PLA contains oxygen-containing functional groups (Dreier et al., 2021; Li et al., 2023). The different chemical structure of conventional mulch-derived PE and biodegradable mulch-derived PLA might lead to different impacts on soil organisms, the research of which is still lacking.

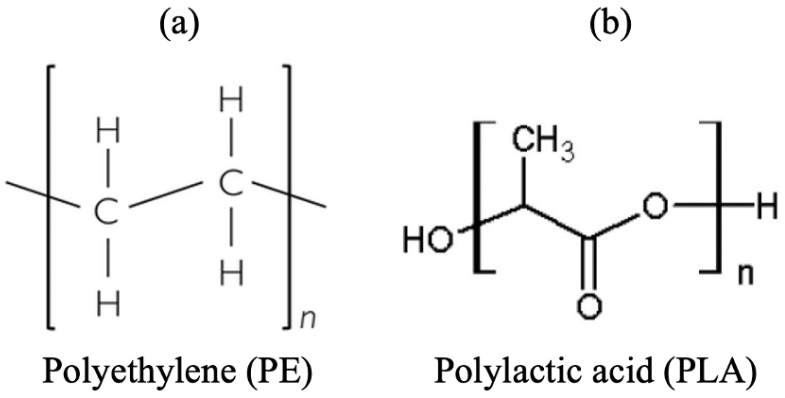


Fig. 1.1. Chemical structure and of (a) PE and (b) PLA.

1.3 Cadmium pollution in soil

Another relatively common contaminant in soil is cadmium (Cd), due largely to the use of Cd-bearing fertilisers (Niño-Savala et al., 2019). Cd pollution in soils is current a global problem (Kubier et al., 2019; Khan et al., 2021), with Cd concentrations in soil being reported up to 50 mg / kg (Huang et al., 2024a). Cd has been considered as a major issue regarding soil organisms and pose an accumulated risk to human health through food chain (Roodbergen et al., 2008;

Sinnett et al., 2009; Wang et al., 2019; Wu et al., 2020). Instead of total soil Cd content, soluble or free Cd in soil solution, such as water-soluble, exchangeable and acid-soluble components (Kubier et al., 2019), provides the most direct and reliable prediction of the bioavailability and toxicity of Cd to soil organisms. Soil pH and organic matter could affect Cd solubility (Sauvé et al., 2000; Vig et al., 2003; Mortensen et al., 2018).

1.4 Earthworms

The roles of soil organisms in maintaining soil health and ecosystem productivity are essential (Neemisha, 2020; Tahat et al., 2020), and they can be used as indicators of soil quality and health due to their sensitive response to changes, correlation with soil functions, important role in ecosystem process (Doran et al., 2000). Earthworms, soil-dwelling organisms, are very sensitive to environmental disturbances, especially environmental pollutants, thus, they have been widely used as test animals in soil ecotoxicological studies (Jager et al., 2005; Zhao et al., 2022b). Anecic species *Lumbricus terrestris*, the common UK earthworm, have widely distributed globally either as native or invasive species (Hendrix et al., 2008; Sizmur et al., 2011). Additionally, *L. terrestris* mode of feeding of pulling plant materials into their vertical burrows which means they are exposed to contaminants present at the soil surface as well as those present in the soil, and this behaviour of *L. terrestris* can also redistribute contaminants through the soil (Tiunov et al., 1999; Sizmur and Hodson, 2009; Sizmur et al., 2011). Although Organization for Economic Co-operation and Development (OECD) recommend *Eisenia fetida* is the test invertebrate species for ecotoxicological assessment (OECD, 1984; OECD, 2004), *E. fetida* is a non-native in the UK and they are less sensitive to metals than other species, such as *L. terrestris* used in this study (Spurgeon et al., 2000; Lukkari, et al., 2005; Chen et al., 2017). Therefore, earthworm, especially *L. terrestris*, is a good example as a bio-indicator to investigate the interaction between MPs, metals and soil organisms.

1.5 Microplastics threat to agricultural ecosystem

MPs pollution pose an ecological risk to soil organism and agricultural ecosystem and because plastics generally degrade very slowly then any MPs will remain in the soil for a long time. The first study about the impacts of MPs on soil organism was Huerta Lwanga et al., (2016) who

pointed out that growth rate of earthworms *L. terrestris* was significantly decreased with increasing concentration of MP which were subsequently concentrated in earthworms casts. Additional, *L. terrestris* was chosen also because it is an anecic earthworm native to Europe and burrowing and ingesting habits of this species lead to MPs could migrate from the soil surface to deeper soil and eventually into groundwater. In a follow up study Huerta Lwanga et al., (2017) went on to demonstrate that MPs can transfer into the terrestrial food chain through earthworms by comparing the MPs concentrations in soil, earthworm casts and chicken feces. Since then the number of studies has increased with academic articles focusing on impacts of MPs on soil health. Recent studies showed that MPs can cause reduced feeding (Besseling et al., 2013), reduced burrowing activity (Huerta Lwanga et al., 2017) and reduced reproduction and avoidance in earthworms (Ding et al., 2021c). MPs can also lead to 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), gene expression (Cheng et al.,2020), and gut microbiota (Cao et al., 2022) of earthworms. A paucity of studies investigated whether replacing conventional plastics with biodegradable plastics reduces the impacts of MPs on soil biology (Ding et al. 2021c; Yu et al., 2022; Han et al,. 2023; Holzinger et al., 2023; Zhao et al., 2023; Zhao et al., 2024). Some studies, published since this PhD began, showed there was no differences in the earthworm avoidance, survival, biomass, reproduction and oxidative stress response to PE and PLA (Ding et al. 2021c; Yu et al., 2022). However, other studies detected that PLA caused more histopathological damages on earthworm compared to PE (Han et al., 2023; Zhao et al., 2023). So also, since there are relatively few studies, it is still hard to know whether on average PLA is less harmful than PE or not.

1.6 Cadmium-microplastics mixture toxicity

Once MPs and non-essential metal Cd are both in the environment, interaction between MPs and Cd occur (Hüffer and Hofmann, 2016; Li et al., 2018; Wang et al., 2018) by adsorption (Alimi et al., 2018; Wen et al., 2018; Zhou et al., 2020b), which could Influence how both impact on earthworms (Kwak and An, 2021; Ding et al., 2021c; Chen et al., 2020). Increases (Zhou et al., 2020c; Huang et al., 2021c; Shang et al., 2023), decreases (Zhang et al., 2024), and no change (Chen et al., 2024) in effects on earthworms have been detected in combined

MPs-Cd studies compared to those observed in MP-only or Cd-only studies. MPs with different characteristics were used in above studies, so that the consequences of Cd-MP interactions still remain highly debated and there is still a lack of information on their interaction mechanisms.

Furthermore, the studies of interaction between MPs and Cd have mainly focused on high concentrations of both MPs and Cd (Holmes et al., 2012; Kim, et. al., 2017; Wen et al., 2018; Godoy et al., 2019; Abbasi et al., 2020; Guo et al., 2020a; Guo et al., 2020b). However, studies about the impacts of MPs-Cd interaction on earthworms under environmentally relative conditions are still lacking, which are also important to assess the ecological risk of the MPs-Cd interaction in agricultural system.

1.7 Microplastics characteristics impact MPs-Cd interactions

Different MPs have various properties, which could impact MPs-Cd interactions. MP particles with different size or shape leading to different specific surface area (Wang et al., 2019). Adsorption is a surface phenomenon and as specific surface area increases more surface is available for sorption per mass of MPs (Zhou et al., 2020d, Li et al., 2022, Chang et al., 2024). Different polymer types of MPs have different capacity of interaction with metals through different mechanisms, due to their different chemical structure (Torres et al., 2021; Huang et al., 2023). Non-specific interaction, such as a weak van der waal force, between non-polar MPs with hydrophobic surface, such as petroleum-based PE (aliphatic polymer) and Cd was the dominant mechanism for their adsorption (Lin et al., 2021; Xu et al., 2021), whereas specific interaction, such as surface complexation, between polar MPs with functional groups, such as biodegradable PLA (containing oxygen-containing function groups) and Cd lead to relative strong adsorption (Fan et al., 2018; Wu et al., 2019). However, the amount of Cd adsorbed by PE and PLA MPs is not exactly clear.

In addition, MPs contain additives (organic chemicals), which can also adsorb metals (Brennecke et al., 2016; Hahladakis et al., 2018). Recent study reported that the leaching additives from MPs could affect adsorption capacity of Cd on polystyrene (PS) MPs (Yu et al., 2021b). PS MPs are aromatic polymer exhibiting π-π interaction with metals. However, it is

still not clear whether leachable additives could affect other mechanisms for interaction between MPs, such as PE or PLA, and Cd.

1.8 Weathering impacts microplastics-Cadmium interactions

Furthermore, weathering of plastics can also impact their adsorption of metals due to changes in their physical and chemical properties (Hüffer et al., 2018; Fu et al., 2021). Biofilm formation on MPs was detected after particularly naturally weathering and the determination of biofilm content using crystal violet method (Barboza et al., 2018; Qi et al;., 2021). These biofilms consist of microorganisms along with extracellular polymeric substance (EPS) (Liu et al., 2023) and EPS are complex molecules consisting of carbohydrates, proteins, uronic acids, nucleic acids, and other substances (Babiak and Krzemińska, 2021). Therefore the presence of functional groups containing oxygen would be expected in biofilms and previous studies reported the new peaks of oxygen functional groups formed on the surface of PE exposed in seawater based on FTIR spectra (Tu et al., 2020; Tu et al., 2021). Furthermore, metals adsorption of MPs was significantly enhanced by these biofilms adhere on MPs due to strengthening of the existing interaction forces and the appearance of additional adsorption mechanisms, such as the enhancement of electrostatic interactions, complexation and surface precipitation, as well as additional ion exchange (He et al., 2022; Stabnikova et al., 2022; Pan et al., 2023 and Qi et al., 2021; Wu et al., 2022 therein). Physiochemical changes were found in artificially weathered MPs (Han et al., 2021, Bhagat et al., 2022, Qiongjie et al., 2022, Huang et al., 2024b), however artificial weathering is unlikely to result in biofilm formation. Therefore naturally weathered-MPs should be more environmental relevant to how MPs interact with Cd relative to artificial weathered-MPs. However natural weathering have rarely been investigated.

1.9 Research questions

Based on the general background above and knowledge gaps in the literature review that follows (Chapter 2), three main research questions are explored in this study.

(1) Do mulch-derived MPs interact with Cd and further alter the responses of earthworms to Cd? If so, why does this altered response occur?

(2) Do interactions between biodegradable mulch-derived MPs (PLA) and Cd have different impacts on earthworms in the soil compared with interactions between conventional MPs (PE) and Cd? If so, what are the driving factors of this difference?

(3) Does naturally weathering lead to the alteration of the surface and physiochemical properties of mulch-derived MPs? If so, how do these changes have further impacts on the interaction between mulch-derived MPs and Cd?

1.10 Aims, objectives and hypotheses

This study aimed to investigate the impacts of interaction between Cd and mulch-derived MPs on earthworms within agricultural soil, and the overall aim can be further divided into the following objectives: (1) to investigate the interactions between Cd and mulch-derived MPs and whether these impact the effects of both Cd and MPs on earthworms, (2) to determine the difference in the above interactions between conventional mulch-derived MPs (PE) and biodegradable mulch-derived MPs (PLA) and (3) to identify the impacts of natural weathering of MPs on their adsorption of Cd. This study provides evidence in support of the overlying hypotheses that (1) mulch-derived MPs would adsorb Cd onto their surfaces so that there would be less Cd in the soil pore water, and less Cd therefore accumulated in the earthworm, (2) biodegradable mulch-derived MPs (PLA) would adsorb more Cd than conventional mulch-derived MPs (PE) so that there would be less Cd accumulated in the earthworm in combination with the presence of PLA MPs compared with PE MPs, (3) natural weathered MPs would adsorb more Cd than pristine MPs.

1.11 Thesis contents

This PhD thesis contains eight chapters to answer the above research questions, including three laboratory experiments (Chapters 4, 5 and 6) and a field experiment (Chapter 7). The contents of each chapter are described briefly below.

Chapter 1 briefly introduces the emerging MPs pollution in agriculture and their impacts on soil, the interactions between MPs and Cd and effects on earthworms. Finally this chapter provides a brief summary of the thesis contents.

Chapter 2 is a literature review of the distribution, abundance, characteristics and major sources of MPs in agricultural soil, impacts of MPs on agroecosystem, and interaction between MPs and metal (Cd) and their impacts on earthworm. The chapter concludes that there are relatively few studies comparing the impacts of biodegradable and conventional plastic mulches derived-MPs on earthworms. Furthermore, this chapter highlights that MPs physiochemical properties alter and biofilms form on MPs after weathering, leading to changes in their interaction with metals. This chapter identifies the knowledge gaps and questions that this thesis aimed to answer.

Chapter 3 is the research methodology used in this study to address the above three research questions. The methodology chapter firstly introduce generally why the method chosen suits the objectives and can answer the research questions, and secondly illustrate the detailed experimental design, material and method used, and thirdly describe data preparation, software used and statistical methods chosen and finally evaluate and justify the methodological choices.

Chapter 4 was set up to investigate single and combined impacts of plastic mulches derived-MPs and Cd on earthworm avoidance behaviour, using the anecic species *Lumbricus terrestris*. The laboratorial avoidance experiments used conventional PE and biodegradable PLA, and / or Cd in plastic boxes filling with the loam soil. A moveable plastic divider was used to separate each soil chamber into the control side (with non-polluted soil) and the test side (with polluted soil). At the end of the 3-day experiment, the number of earthworms in each side of soil was count to calculate avoidance rate. The avoidance of earthworm under different MPs type, different Cd concentrations and their interactions were compared. The avoidance experiments tested the hypotheses that (1) Cd in soil would increase earthworm mortality and avoidance, (2) increasing Cd concentrations in soil would increase earthworm mortality and avoidance, (3) the addition of MPs would decrease the avoidance of earthworms to Cd, and (4) there would be

more decrease in the avoidance of earthworms to Cd in combination with the presence of PLA MPs relative to PE MPs. This was the initial experiment carried out during the PhD and did not yield significant or readily interpretable results. The decision was made to include this chapter as it represented a substantial amount of work. However, rather than repeat the experiment, further, potentially more insightful experiments were developed instead.

Chapter 5 was set up to investigate the impacts of combination between MPs and Cd on the mortality, weight changes and Cd uptake of common earthworm *Lumbricus terrestris*, and whether conventional PE and biodegradable PLA MPs affect earthworm differently. Earthworms were exposed to either or both MPs (conventional PE and biodegradable PLA) and Cd for 28 days. At the end of exposure experiment, the Cd concentrations in soil pore water and earthworm tissues were measured and also earthworm mass change and mortality were assessed. In addition the adsorption of Cd by PE and PLA was determined to aid interpretation of the earthworm exposure experiments. The exposure and adsorption experiments tested the hypotheses that (1) the addition of MPs would reduce Cd concentrations in soil pore water, (2) decrease in Cd bioavailability of pore water would lead to decrease the Cd bioaccumulation in earthworm body, (3) decrease in Cd concentrations in pore waters and earthworms would due to Cd adsorbed onto MPs surfaces, and (4) there would be less Cd uptake of earthworm and lower concentrations in soil pore water in the presence of the MPs generated from biodegradable PLA plastic mulches than MPs generated from conventional PE plastic mulches.

Chapter 6 was set up to investigate the adsorption of Cd on MPs generated from the most commonly used conventional (PE) and biodegradable (PLA) mulching plastics, and whether natural weathering can impact the adsorption capacity of these mulch-derived MPs. Adsorption experiments were conducted using pristine MPs, weathered MPs and weathered MPs washed in ethanol to remove any adhere biofilm that formed during weathering. The adsorption experiments tested the hypotheses that (1) the different chemical structures of PE versus PLA would be the dominant factor causing their different adsorption capacity, (2) natural weathering would increase the adsorption of Cd on MPs, and (3) biofilms would be the main reason impacting the adsorption after natural weathering.

Chapter 7 was set up to investigate an existing small field experiment planned and carried out by colleagues at the University of Bangor. This opportunity was offered and it was a way of experiencing some field sampling. This chapter was included here as it represented effort during the PhD despite the results being inconclusive, potentially due to the experimental design, over which I had no control. This field sampling was in a multi-year site with maize planted and covered by plastic mulches (conventional PE and biodegradable PLA types matched with the Cd-free treatments of the laboratory experiments) that started in 2021. Soil and earthworm were sampled and then transported back to York for the further measurement. Soil physicochemical properties (soil pH, moisture, organic matter, texture) were measured and earthworms (abundance and biomass) were identified. The field work tested the hypothesis that earthworm population would be more abundant in biodegradable PLA plastic mulch-treated plots than control plots and conventional PE plastic mulch-treated plots within the agricultural soil.

Chapter 8 summarises the main findings, provides critical evaluation of the methodology used and the environmental relevance implications of this study. It also considers the limitations of this study and suggests future research that are recommended.

2 Literature Review

2.1 Introduction

Microplastics (MPs) have been globally found in agricultural soil (Zhang and Liu, 2018; Zhou et al., 2019a; van der Berg et al., 2020; Cusworth et al., 2023; Berenstein et al., 2024) and plastic mulches have been identified as the major source of them (Li et al., 2020; Huang et al., 2021; Wang et al., 2021; Luo et al., 2022). Once MPs are in the environment, interaction between MPs and metals, such as non-essential metal cadmium (Cd), occur (Hüffer and Hofmann, 2016; Li et al., 2018; Wang et al., 2018) by adsorption (Alimi et al., 2018; Wen et al., 2018; Zhou et al., 2020b), which could have further impacts on earthworms (Kwak and An, 2021; Ding et al., 2021; Chen et al., 2020). To better understand the mechanisms of interaction between Cd and mulch-derived MPs and their impacts on earthworms in soil, the next is important to make a comprehensive summary and find the current knowledge gaps based on previous studies.

2.2 Literature review strategy

This literature review followed the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 flow diagram (Page et al., 2021). During February 2024, a database search was carried out using Google Scholar, ScienceDirect and Web of Science search engines. The purpose of this literature search was to identify peer-reviewed academic journal articles on plastic mulch usage, MPs generated from plastic mulches, difference between conventional and biodegradable MPs, MPs-metals interaction and their impacts on earthworm in soil. This search therefore was conducted using the following search terms: (“plastics” OR “microplastics” OR “plastic films” OR “mulching”) AND (“soil” OR “farmland” OR “agricultural” OR “terrestrial”) AND (“cadmium” OR “metals”) AND (“earthworm” OR “soil organisms”) AND (“adsorption” OR “interaction”).

Literature search results was performed in four steps (Fig. 2.1). The earliest paper returned was from 1972, whilst the latest was from February 2024 (thesis submission data was 2nd May

2024). Only English language publications were incorporated and peer-reviewed journal articles of original research and review papers together with early access papers were included. Additionally, grey literature, two reports from government organisations were included. The initial yielded 651 articles were identified in the databases (331, 262 and 58 in the Google Scholar, ScienceDirect and Web of Science databases, respectively), and a reference manager Zotero was used to organise the articles. Initial screening was done by removing replicates, leaving 621 papers to be further screened for suitable titles, key works or abstracts; 401 articles that did not contain sufficient information were screened and eliminated. The remaining 220 articles were assessed by the eligibility. Certain exclusion criteria were set based on this study (Fig. 2.1); articles meeting the exclusion criteria (n = 24) were eliminated from this review, and 196 papers included. Additionally, 3 records were included via two other methods, one from websites (The PRISMA 2020 statement) and two from government organisations (National Bureau of Statistics of China and National Health Commission). Altogether, a total of 199 articles were considered, comprehensively evaluated and summarised.

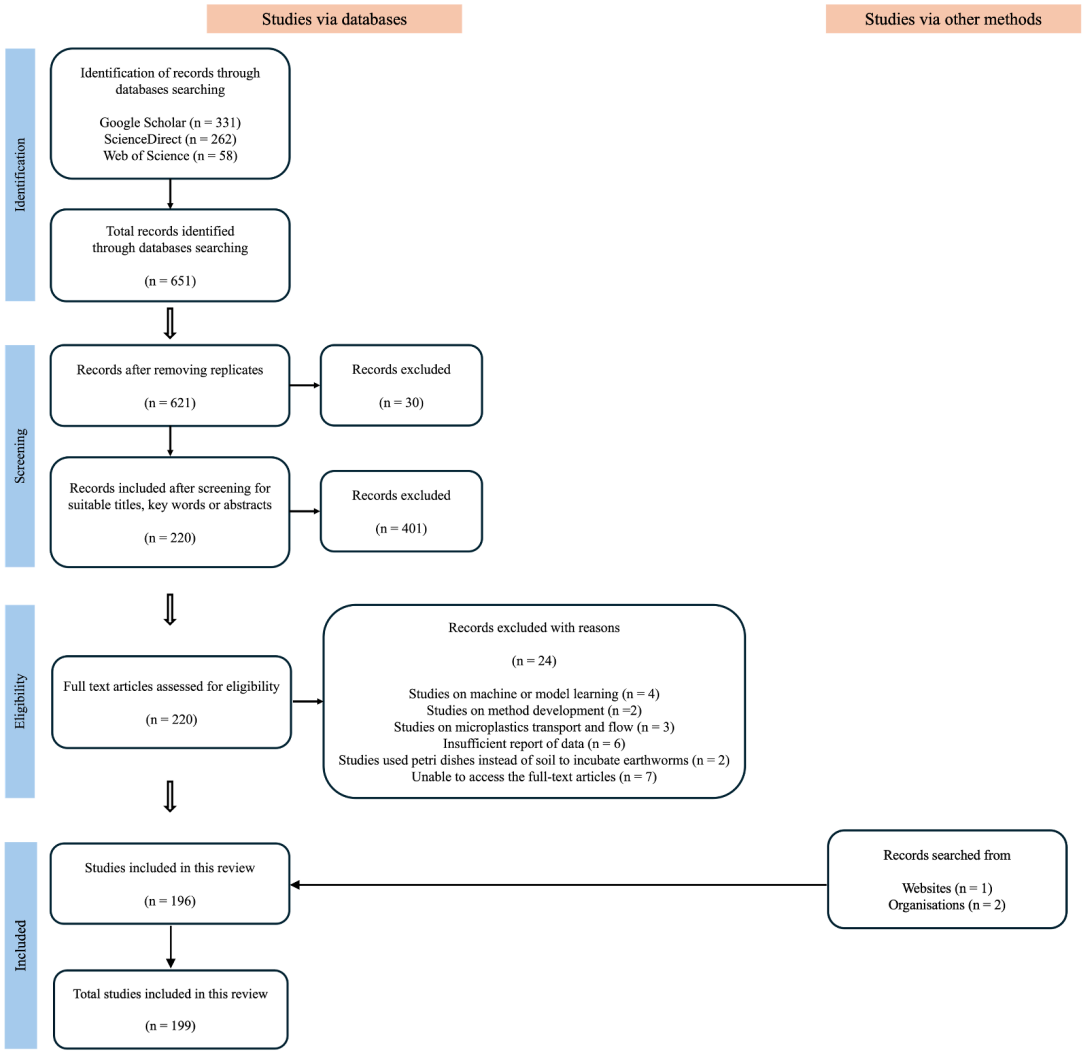


Fig. 2.1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram of this literature review identification, screening and eligibility. "n" = "number of studies".

2.3 Microplastics pollution in the environment

2.3.1 Microplastics as an emerging pollutants

The earliest plastic material was invented in the mid-19th century, and since then plastics have been widely used to provide us convenience; their global production was 400.3 million tons in 2022 (Plastic Europe, 2023). At first, big pieces of plastics were focused on as environmental pollutants. However, due to exposure to ultraviolet (UV) radiation and photo-oxidation, plastics can break down into formation of large sheets and then fragmentation of smaller pieces (Akdogan and Guven, 2019). The presence of small plastic particles in the marine environment

was previously reported in the early 1970s (Carpenter and Smith, 1972). The concept of "microplastics" as plastic debris and particles < 5 mm were first proposed by Thompson et al., (2004). After that, MPs are commonly defined as < 5 mm particles (Rillig et al., 2017, Akdogan and Guven, 2019), which act as an emerging threat to the environment (Liu et al., 2018; Lv et al., 2019; Chen et al., 2020). MPs have been increasingly detected and quantified in marine and freshwater environment (Anderson et al., 2016). Recent studies have been turning to terrestrial environment, as Horton et al., (2017) estimated that annual release of plastics on the land is 4 - 23 times greater than those in the ocean. Furthermore, terrestrial ecosystems are a major source of plastic residues in water (Xu et al., 2020). However, fewer studies about MPs in terrestrial system have been carried out, particularly in agricultural soil (Nizzetto et al., 2016; Ng et al., 2018). Therefore, based on the available literatures, the situation of MPs in agricultural soils is discussed next.

2.3.2 Distribution and abundance of microplastics in soil

MPs are an important contaminant (Huerta Lwanga et al., 2016; Horton et al., 2017) and agricultural soils are a major receptor of them (Cusworth et al., 2023), so that it is particularly crucial to understand the abundance and distribution of MPs in soil. Although field investigations on the occurrence and distribution of MPs in soil have not been widely undertaken, the summarized available data (39 worldwide sampling sites) indicates that MPs does globally occur in soil (Fig. 2.2, Table S2.1). MPs have been found in both the eastern and western hemispheres. There have been more published studies looking at MPs in the field for eastern hemisphere sites than those in the western hemisphere sites, and fewer published studies of detecting MPs have been found in the southern hemisphere sites (Fig. 2.2). In Asia, MPs abundance was relatively high compared with other regions (Fig. 2.2). Furthermore, the abundance of detected MPs in soil is greater in developing countries compared with developed countries (Fig. 2.3). From the continental perspective, the number of studies that detected MPs were dominant in Asia (68 %), followed by North America (11 %), Europe (9 %), Latin America (6 %), Africa and Oceania (both 3 %). Although the MPs in Antarctica soil have not

been detected so far, MPs were found in other parts of Antarctic terrestrial such as snow on Ross island (Aves et al., 2022 and sediments from Fildes Bay (Perfetti-Bolaño et al., 2022). The abundance of MPs in soil of China was significantly greater than other countries (Figs. 2.2, 2.3, Table S2.1), which is not surprising. The production of plastics reached 400.3 million tons, the largest part of which (32 %) came from China in 2022 (Plastic Europe, 2023). Additionally, the management of plastic waste has been poor and 27.7 % of that has not been properly managed in China (Jambeck et al., 2015).

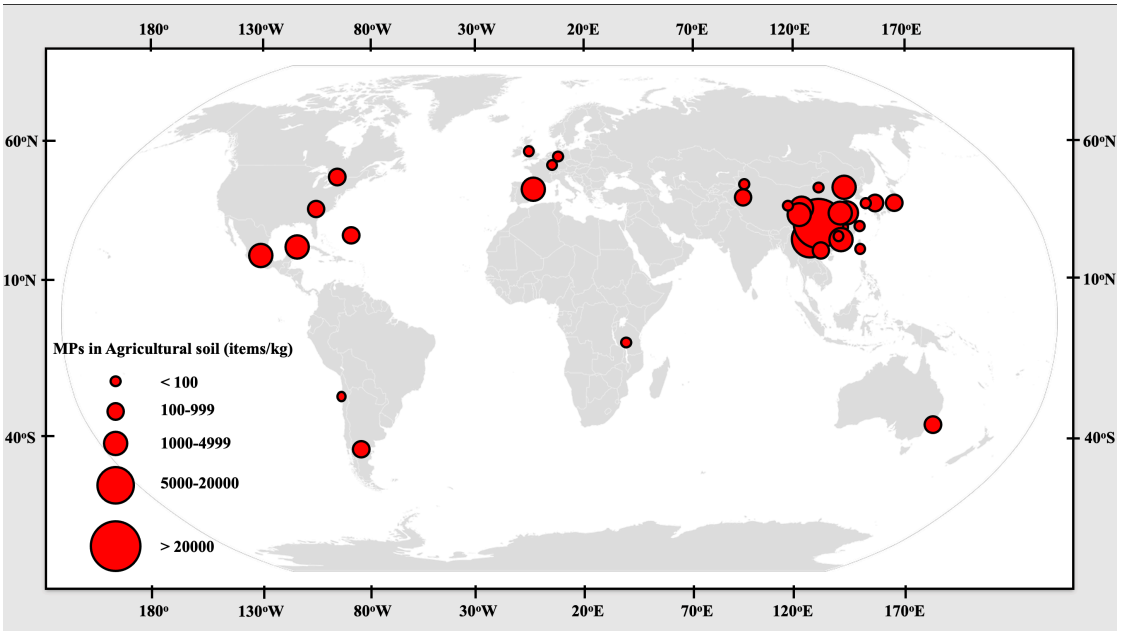


Fig. 2.2. Summary of the global distribution of MPs detected in soil from published academic papers (n = 35). Each spot represents the concentration of MPs detected by a single study in a particular country.

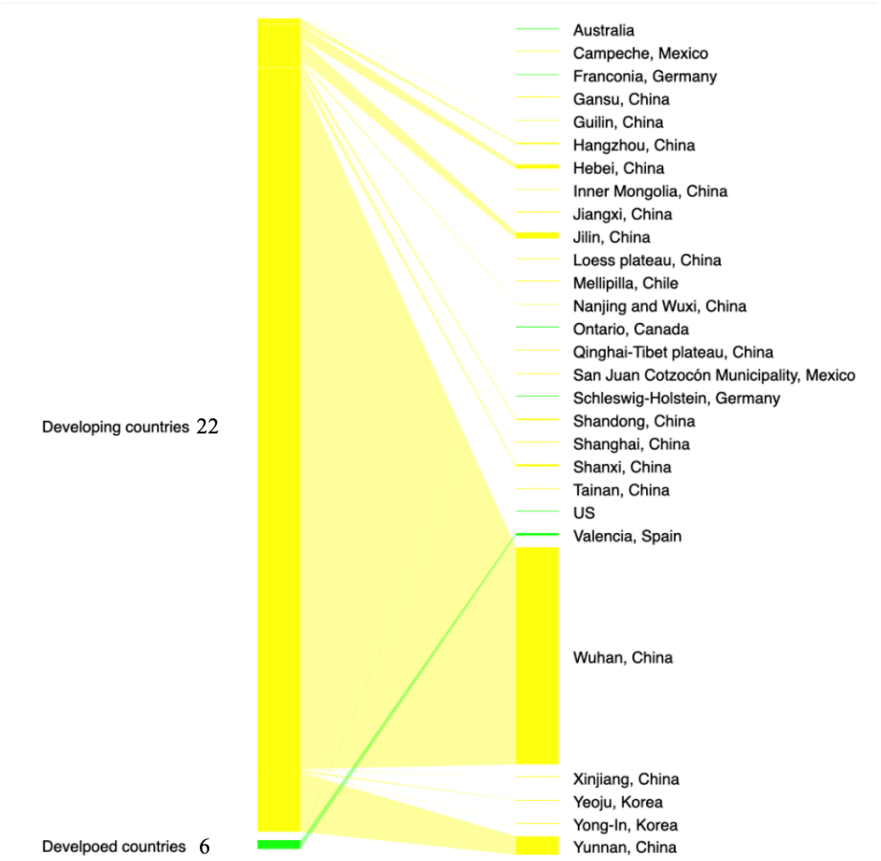
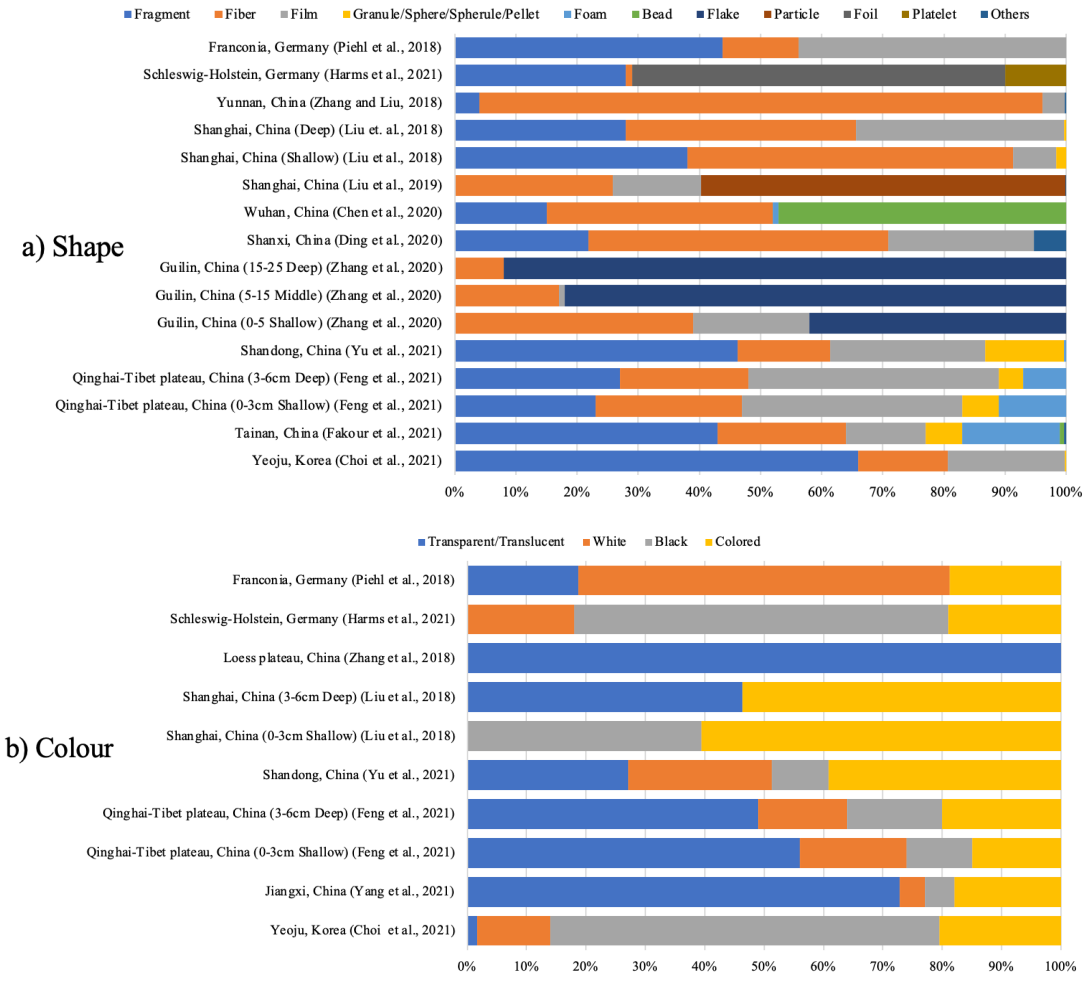


Fig. 2.3. Distribution of studies about MPs in soil in terms of the developed and developing countries. The numbers represent the number of published studies looking at MPs in the field.

The relative abundance of MPs in soils varies widely, spanning 1 - 5 orders of magnitude in different regions globally (Table S2.1). Maximum concertation of MPs was detected in farmland up to 690000 items / kg dry soil in Wuhan, China (Zhou et al., 2019a), followed by 42960 items / kg dry soil in Yunnan, China (Zhang and Liu, 2018). In other regions, the MPs abundance detected in soil was relative lower, with the concentration range of 1320 - 8190 items / kg dry soil in UK (Cusworth et al., 2023), 862.5 - 4162.5 items / kg dry soil in Spain (van der Berg et al., 2020) and 2383 - 3815 items / m^2 soil area in Argentina (Berenstein et al., 2024). To meet the needs of production requirements, different colours, sizes and compositions are used in the plastics processing (Hahladakis et al., 2018; Qiu et al., 2022). The various properties of generated MPs are worthy summarised next.

2.3.3 Characteristics of microplastics in agricultural soil

MPs in soil vary in shape, colour, size and polymer type (Chai et al., 2020; Zhou et al., 2020a). There have been 10 definitive shapes and other shapes, 4 different types of colours (transparent, black, white and coloured), 2 wide classes of sizes (< 1.0mm and 1.0 - 5.0 mm) and 8 different polymer types of MPs summarized from the previous studies (Fig. 2.4).



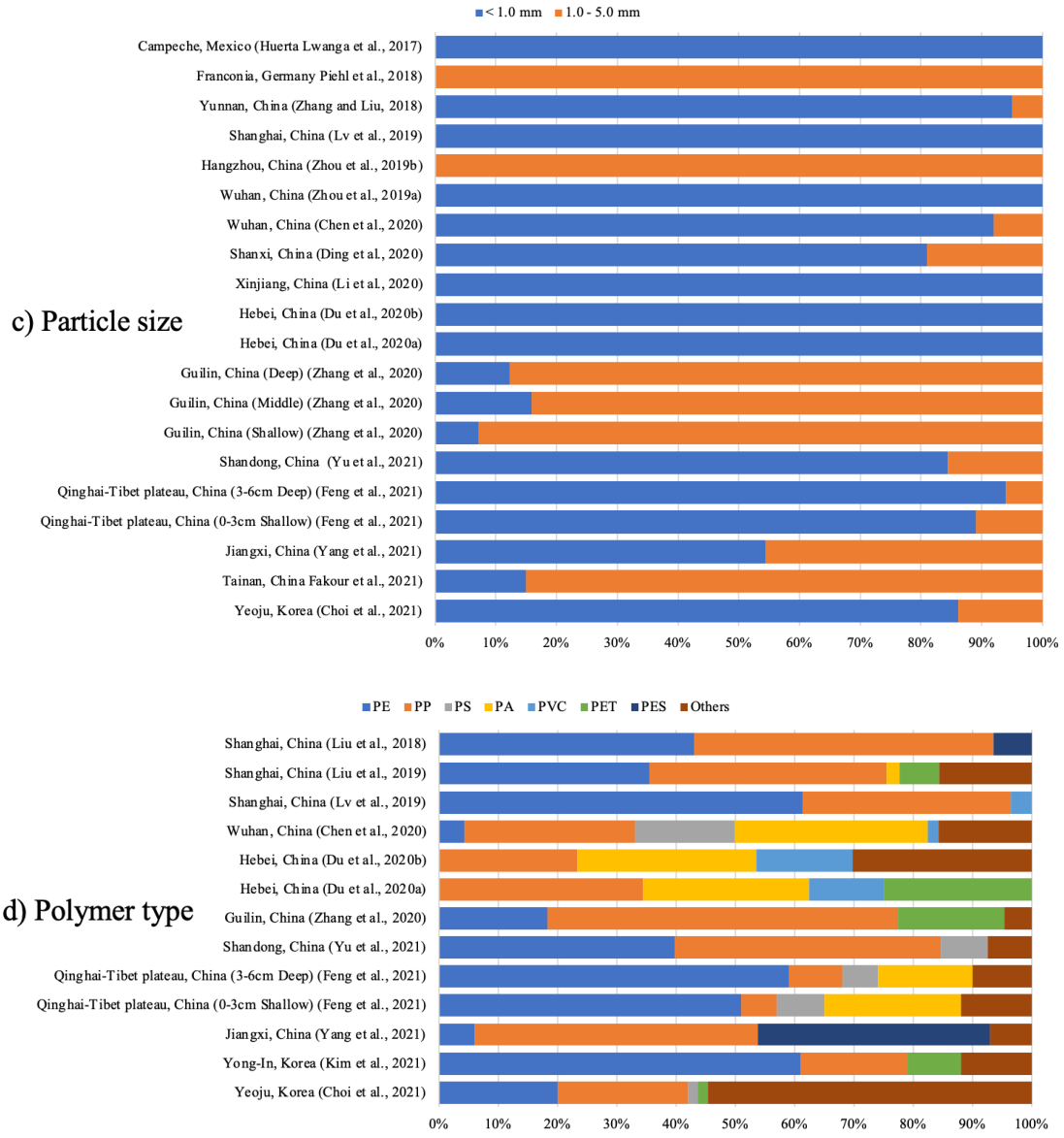


Fig. 2.4. Reported MPs properties (a) shape, (b) colour, (c) particle size and (d) polymer type found in soil from the published academic studies (n = 21). The percentages in each figure represents the proportion of different components from each study.

A wide range of MPs shapes in the farmland soils was reported, including fragments, fibres, films, granule / sphere / spherule / pellets, foams, beads, flakes, particles, foils, platelets and other forms with a negligible percentage (Fig. 2.4a). Fragments, fibres and films were the most common shape of MPs in soil (Fig. 2.4a) and these different shapes of MPs were identified by Shi et al., (2022) using scanning electron microscopy (SEM) (Fig. 2.5).

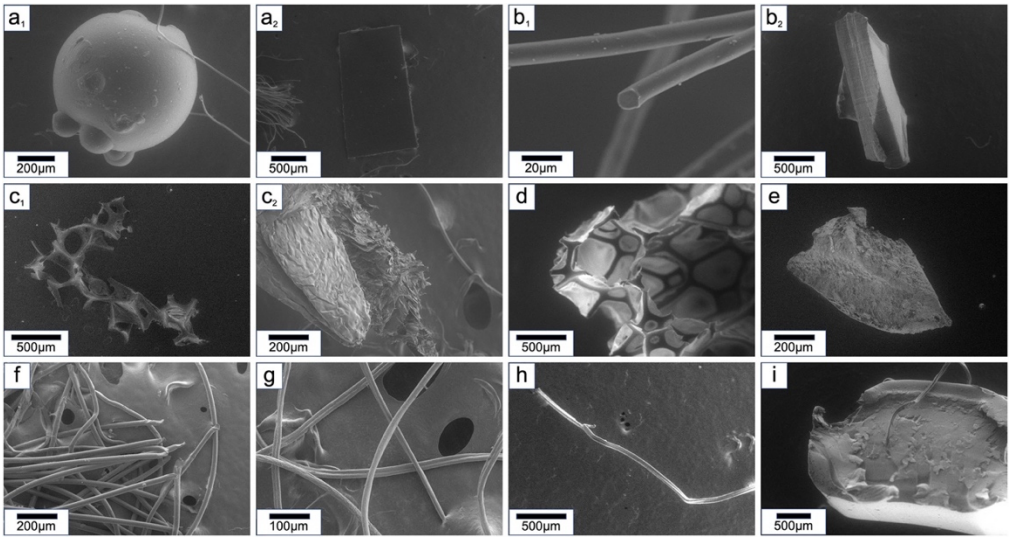


Fig. 2.5. SEM images of microplastics in different chemical compositions and shapes: (a) polyethylene (PE) bead / film, (b) polyethylene terephthalate (PET) fibre / fragment, (c) polystyrene (PS) fragment / foam, (d) polyurethane (PUR) foam, (e) polycarbonate (PC) fragment, (f) polypropylene (PP) fibre, (g) polyacrylonitrile (PAN) fibre, (h) polyamide (PA) fibre, (i) polyvinyl chloride (PVC) fragment (Images and caption taken from Shi et al., 2022).

White, black, and transparent MPs are dominant (70 %) in the soil (Qiu et al., 2022). Other colours of MPs such as red, green, blue, yellow and grey are also present and their total percentage was about 30 % (Harms et al., 2021; Feng et al., 2021; Yang et al., 2021a). Thus, MPs were grouped more generally into transparent / translucent (average about 34 %), white (average about 15 %), black (average about 21 %) and coloured (average about 30 %) in our summary (Fig. 2.4b). White, black, and transparent MPs were the mainly type of MPs found in soil in China, especially transparent MPs (maximum 100 %), consistent with the research of Qiu et al., (2022) and Zhang et al., (2022). White and black MPs were also detected a significant proportion (> 50 %) in soil in Germany (Piehl et al., 2018; Harms et al., 2021).

There are no standard methods to classify particles into different size groups. Fakour et al., (2021) divided MPs into four different size ranges, namely < 1 mm, 1 - 3 mm, 3 - 5 mm and > 5 mm. While Yu et al., (2021a) considered different MPs size groups as < 0.5 mm, 0.5 - 1.0 mm, 1.0 - 2.0 mm and 2.0 - 5.0 mm. Others have classified MPs size into just two groups, < 1

mm and > 1 mm (Piehl et al., 2018; Lv et al., 2019; Zhou et al., 2019b; Álvarez-Lopeztello et al., 2021; Choi et al., 2021), which was used in this review. The majority of MPs detected in soil were small particles less than 1mm in diameter (Fig. 2.4c).

Advanced technology, such as Fourier Transform Infrared (FTIR) Spectroscopy is crucial to identify MPs based on their unique structures (Duis and Coors, 2016; Mai et al., 2018). The identification of MPs types is important to trace their sources (Yang et al., 2021a). Eight types of MPs polymers were reported as detected in the soil, and polyethylene (PE) and polypropylene (PP) were the dominant (23.3 % - 96.5 %) MPs in soil (Fig. 2.4d).

2.3.4 Source classification of microplastics in agricultural soil

To identify the characteristics of MPs, it is also important to trace the sources of their entry into the soil (Xu et al., 2020; Yang et al., 2021a). MPs can enter into the soil through multiple pathways, mostly linked to anthropogenic activities (Zhang and Chen, 2020). MPs in soil could come from agricultural practices (use of plastic film, application of sewage sludge and compost and wastewater irrigation), and other sources like atmospheric input, littering, flooding and street runoff (Fig. 2.6, Table S2.2). These sources can be classified as primary or secondary MPs (Rillig, 2012; Horton et al., 2017; Xu et al., 2020). Primary MPs are commonly defined as MPs manufactured and released to the environment in a micro-size range (Koelmans et al., 2014), mainly industry and domestic abrasives (Duis and Coors, 2016). Secondary MPs result from the fragmentation of larger plastic materials (Koelmans et al., 2014), specifically refers to degraded plastic materials (Huang et al., 2020) and weathered plastic materials (Yang et al., 2022).

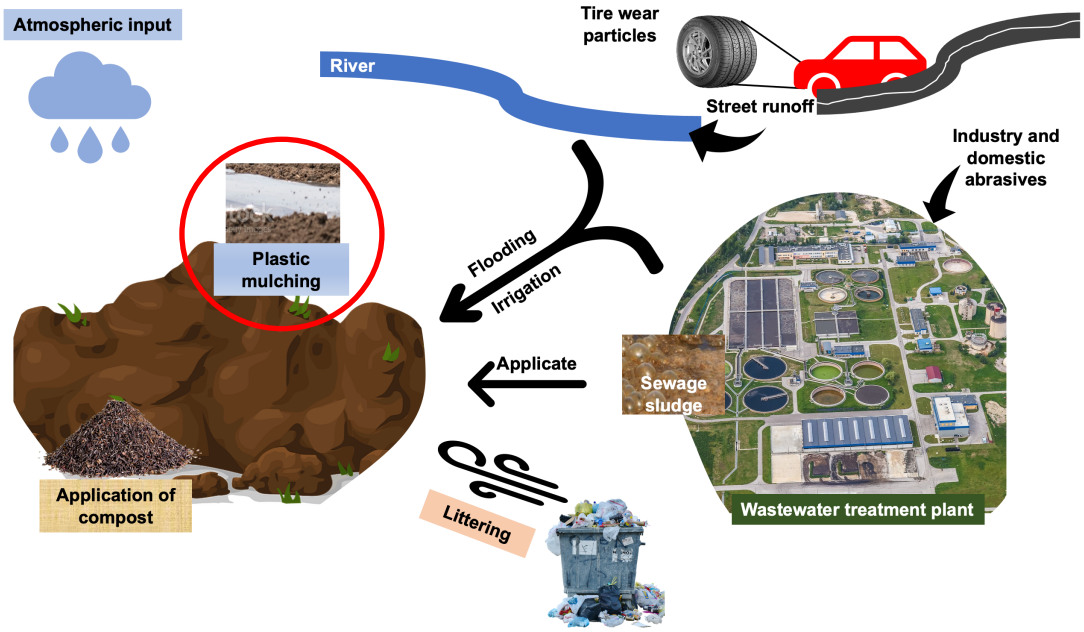


Fig. 2.6. The main possible sources of MPs in soil.

Primary MPs could directly or indirectly enter into the environment as waste (Cole et al., 2011; Rillig, 2012; Ng et al., 2018). Fragmented plastic mulches directly enter into the soil, besides primary MPs also directly transferred into the soil from littering, street runoff and atmospheric input (Dris et al., 2015; Huerta Lwanga et al., 2017a). Furthermore, primary MPs in treated wastewater and sewage sludge from wastewater treatment plants (WWTPs) could be indirectly released into the soil (Horton et al., 2017; Ng et al., 2018). Degraded and weathered plastic productions as secondary MPs (Huang et al., 2020; Yang et al., 2022) could be directly or indirectly carried into the soil through the above sources (He et al., 2018; Qi et al., 2020a).

2.3.5 Microplastics mostly derived from plastic mulches

MPs in farmland are mainly from plastic mulches, sewage sludge, wastewater irrigation, littering and atmospheric input (Dris et al., 2015; Liu et al., 2018; Zhang and Liu, 2018; Zhou et al., 2019a; Huang et al.,2020; Cusworth et al., 2023), the most studies identified plastic mulch as a source (Piehl et al.,2018; Li et al.,2020a; Huang et al.,2020; Cusworth et al., 2023) (Table S2.2). In agriculture, plastic mulches are used to increase yields and improve crop quality by increasing soil temperature and enhancing water use efficiency (Deng et al., 2006; Zhao et al.,

2016; Huang et al., 2020). More than 1.6 million tons of plastic film are used worldwide annually (Bai et al., 2024), covering approximately 20 million hectares of agricultural land (Kasirajan and Ngouajio 2012; Dai and Dong 2014). Widespread presence of plastic mulches has led to mulch-derived MPs pollution. Cusworth et al., (2023) carried out a national survey in the UK and found that where plastic mulches had been used in the last decade, the average number of MPs particles was 4689 ± 147.1 particles / kg, whereas where plastic mulches had not been used, the average number of MPs particles was 2667 ± 84.1 particles / kg. These findings suggest that the use of plastic mulches in agriculture leads to an increase in MPs in the soil.

China accounts for the largest proportion of absolute amount of plastic mulch globally (Steinmetz et al., 2016). 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 %. It takes a long time and lots of effort to remove all the mulches in farmlands. During removal, mulches residues were left intentionally or unintentionally in soil (Yang et al., 2021b). Furthermore, 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 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). MPs were detected in agricultural soil in many provinces of China (Li et al., 2020a; Huang et al., 2021a; Wang et al., 2021). The concentrations of these MPs were in the range from 2283 to 6360 items / kg (Wang et al., 2021).

2.3.6 Biodegradable mulches developed to replace conventional mulches

To date, several biodegradable plastics have been developed to replace conventional plastics 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). After use,

biodegradable mulches can be directly incorporated into the soil, where they can be converted by microorganisms into carbon dioxide or methane, water and biomass (Kasirajan and Ngouajio, 2012). Since biodegradable materials do not produce waste that needs to be disposed, it has been suggested that they can be used as a sustainable and ecological alternative to conventional plastic mulches, especially PE (Liu et al., 2022). Thus, the manufacture and application of biodegradable mulches has caught the interest of researchers (Qin et al., 2022). However, it takes years for biodegradable plastics to in-situ degrade under environmental conditions and their mineralization process has been shown to be incomplete (Sintim et al., 2020). Due to the lack of suitable disposal methods, the biodegradable plastic wastes may accumulate in the environment and subsequently fragment into new types of microplastics (Qin et al., 2021). Thus, the ecotoxicological safety of biodegradable plastics is still being evaluated (Qin et al., 2022). Among these products, biodegradable polylactic acid (PLA) accounts for the largest share (24 %) of total global biodegradable plastics manufacturing (Haider et al., 2019). Biodegradable PLA structure has oxygen-containing functional groups, whereas conventional PE structure only contain groups made of carbon and hydrogen atoms (Fu et al., 2021; Sun et al., 2021; Wang et al., 2022).

2.3.7 Impacts of microplastics on earthworms

Microplastics generated from plastic mulches can enter into the agricultural soil and have further effects on agroecosystem (Fig. 2.7). The impacts of MPs on agricultural soil health include soil physicochemical characteristics (Rillig, 2012; Liu et al., 2017; Zhang and Zhang, 2020), soil organisms (de Souza Machado et al., 2018a; de Souza Machado et al., 2018b) and plants (Qi et al., 2018; Rillig et al., 2019a; Qi et al., 2020b) (Table S2.3). Concentrations of MPs used in the previous studies were from 0.1 % wt to 60 % wt, and higher concentration of MPs cause more damage on earthworms (Chen et al., 2020; Baeza et al., 2020).

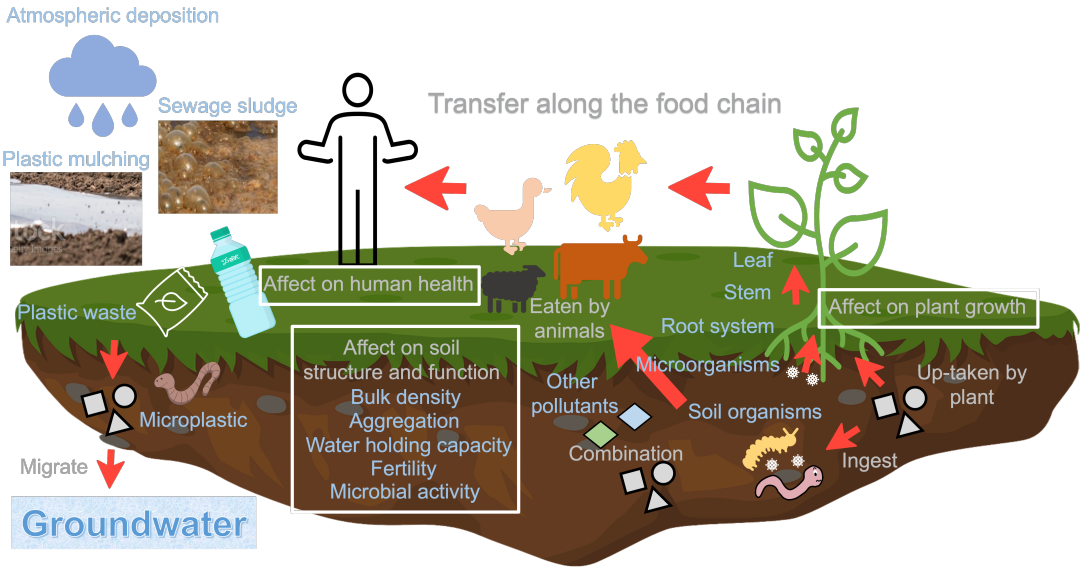


Fig. 2.7. Conceptual diagram about the impacts of MPs on agroecosystem, in terms of soil properties, soil organisms and plants and the possible movements (red arrows) of soil MPs.

Soil fauna is very important for the soil healthy (Li et al., 2024). Among soil fauna, earthworms are one of the most important animals in the soil and are considered to be key engineers of soil ecosystems and bioindicators of environmental quality (Blouin et al., 2013; Singh et al., 2016; Xiao et al., 2022). Earthworm can be affected by MPs in soil in terms of many aspects (Fig. 2.8). Due to the small particle size of MPs, soil organisms are able to ingest them (Wang et al., 2019a; Zhang et al., 2020; Li et al., 2021a). Whilst not all earthworm was detected negative impacts by exposure to MPs (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 feeding (Besseling et al., 2013), reduced burrowing activity (Huerta Lwanga et al., 2017b), reduced growth rate (Huerta Lwanga et al., 2016; Cao et al., 2017;), reduced biomass (Huerta Lwanga et al., 2016; Rillig et al., 2017; Boots et al., 2019; Prendergast-Miller et al., 2019; Jiang et al., 2020; Ding et al., 2021), reduced reproduction (Huerta Lwanga et al., 2016; Ding et al., 2021; Kwak and An, 2021; Sobhani et al., 2021), reduced avoidance and increased mortality (Huerta Lwanga et al., 2016; Cao et al., 2017; Jiang et al., 2020; Ding et al., 2021) in MP-bearing soil (Ding et al., 2021). MPs can also cause histopathological damage (Baeza et al., 2020; Chen et

al., 2020; Jiang et al., 2020) and adversely affect the immune (Xu and Yu, 2021) and metabolic system (Rodriguez-Seijo, et al., 2018; Kwak and An, 2021; Xu and Yu, 2021), such as increased oxidative response (Rodriguez-Seijo, et al., 2018; Chen et al., 2020; Jiang et al., 2020; Boughattas et al., 2021; Cheng et al., 2021; Li et al., 2021a; Xu and Yu, 2021), increased lipid peroxidation (Rodriguez-Seijo, et al., 2018; Boughattas et al., 2021), decreased CRT, ANN, TCTP, and Hsp70 gene expression (Cheng et al., 2020), and disturbed gut microbiota (Cao et al., 2022) of earthworms.

Few studies investigated on whether biodegradable plastics as an alternative to conventional plastics can mitigate the effects of derived-MPs on soil organisms. Studies showed there was no differences in the avoidance, survival, biomass, reproduction and oxidative stress response of earthworms to PE and PLA (Ding et al. 2021; Yu et al., 2022) and Zhao et al., (2024) found that earthworm weight increases after exposure to both PLA and PE MPs. However, other research detected that PLA caused more histopathological damages on earthworms relative to PE (Han et al., 2023; Zhao et al., 2023a). Holzinger et al., (2023) found that exposure to PLA increased reproduction of earthworms in comparison 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.

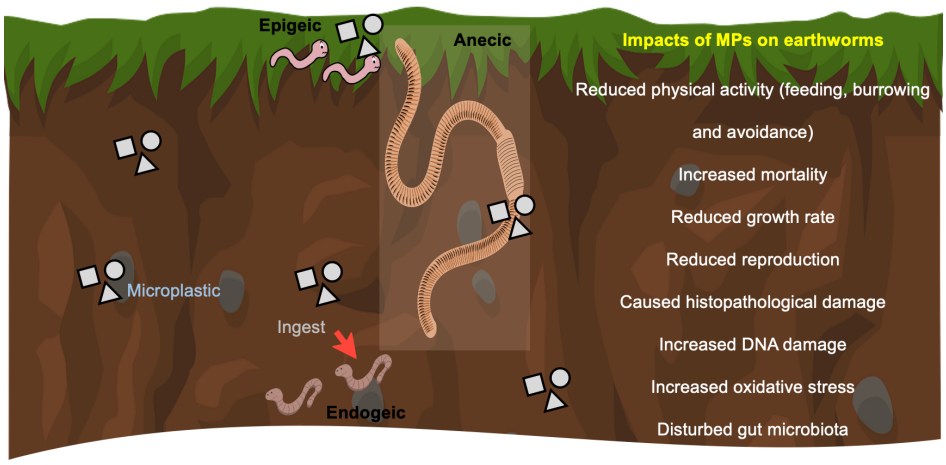


Fig. 2.8. Impacts of MPs on earthworms.

Recent studies showed that different polymer types of MPs can affect earthworms (Boots et al., 2019; Judy et al., 2019; Wang et al., 2019a; Baeza et al., 2020), however, there was no consistent trends between earthworms performance and different polymer types of MPs (Table S2.3). Apart from polymer types, MP shapes vary in characteristics like surface area and thickness (Rillig et al., 2019a). However, there is no studies investigating on how different shapes of MPs affect earthworms.

In summary, since the increasing growth of plastics production, especially in China, derived-MPs have caused widespread contamination in the soil (Figs. 2.2, 2.3, Table S2.1). All these MPs are various shapes, colours, sizes, and polymer types (Fig. 2.4), which come from wide ranges of sources in farmland (Fig. 2.6, Table S2.2). Plastic mulches are the dominant source of MPs. Once mulch-derived MPs enter into the soil, they can have impacts on earthworms (Fig. 2.8, Table S2.3). Furthermore, biodegradable plastic mulches has been developed to replace conventional plastic mulches, such as the most used PE. Inconsistent results investigating the impacts of conventional mulches-derived MPs on earthworm and lack of studies comparing the impacts of biodegradable MPs and conventional MPs on earthworms suggest more research are warranted.

2.4 Interaction between microplastic and cadmium

2.4.1 Cadmium contamination in agricultural soil

Cd is an important contamination of agricultural soils in China (and elsewhere in the world) due to the use of Cd-containing phosphate fertilisers (Niño-Savala et al., 2019). Cd ranks first among the metal pollutants in agricultural soils that exceed China's soil environmental quality standards (Zhao et al., 2015). Cd occurs ubiquitously in Chinese soils, its contaminated concentrations range from 0.003 to 9.57 mg / kg (Wang et al., 2015). Given Cd is readily absorbed by plants, the Cd contamination in soil can lead to an increase in the Cd concentration in plant-derived food stuffs, such as rice (Smolders and Mertens, 2012). Therefore, Cd has been

considered as a major issue regarding human health after intake from contaminated rice caused itai-itai disease in Japan in 1970 (Kobayashi, 1978). Wang et al., (2019c) found that 2.2 - 10 % of the surveyed rice samples exceeding the maximum permissible level of Cd in rice (0.2 mg / kg) established by the National Health Commission (NHC, 2022). If the increase in dietary intake of Cd continues, it will pose accumulated risk to human health (Wang et al., 2019c). Furthermore, Cd in soil can also be transferred to earthworms via their food (Roodbergen et al., 2008; Sinnett et al., 2009) and gradually bioaccumulated in earthworms (Ernst et al., 2008; Wu et al., 2020). Žaltauskaitė and Sodienė, (2014) found that with increasing time of Cd exposure earthworm *Eisenia fetida* mortality increased and its body weight and cocoon production decreased. The activity of antioxidant enzymes (superoxide dismutase, SOD) in the earthworm *Eisenia fetida* increased at low Cd concentrations and decreased at high Cd concentrations (Zhang et al., 2009). Cd also caused physiological harm to earthworm cells and reduced their burrowing ability (Liu et al., 2020a). Accumulation of Cd in soil animals and plants eventually leads to toxic impacts on human via food chain (Dai et al., 2004; De Vries et al., 2007; Gall et al., 2015). Therefore, it is crucial to carry out studies on Cd toxicity in agricultural soils. Since Cd and MPs both exist in soil, their interactions are necessary to discuss next.

2.4.2 Microplastic interacted with cadmium by adsorption

Once microplastics and cadmium are in the environment, they can interact with each other by adsorption (Alimi et al., 2018; Wen et al., 2018; Zhou et al., 2020b) rather than behaving alone. Therefore, MPs can accumulate and transport Cd, altering the ecological risk of both (Lu et al., 2018; Li et al., 2021b; Zhao et al., 2023) for example, their impacts on soil fauna (Fig. 2.8). To date, studies about interactions between Cd and MPs is still lacking, so that investigating the adsorption behavior between MPs and Cd is important to understand their comprehensive impacts on agricultural soil.

Potential mechanisms of the interaction between MPs and metals were summarized and there are two sorption mechanisms, non-specific and specific interaction (Table S2.4). Metals can be

either as cations directly adsorbed on the charged sites of the MPs surface or as complexes adsorbed on the neutral regions of MPs surface (Ashton et al., 2010; Holmes et al., 2012; Holmes et al., 2014; Davranche et al., 2019). Electrostatic interaction between non-polar MPs with hydrophobic surface and metals was the dominant mechanism for their adsorption (Dong et al., 2020; Guo et al., 2020a; Li et al., 2020a; Mao et al., 2020; Zuo et al., 2020; Fu et al., 2021; Liu et al., 2021), whereas polar MPs with functional groups can adsorb metals by specific interaction (Dong et al., 2020; Tang et al., 2021). Non-specific interactions were found between non-polar MPs and metals, such as PE (aliphatic polymer) exhibiting van der Waals interactions (Lin et al., 2021) and PS (aromatic polymer) exhibiting π-π interactions (Li et al., 2020b). Specific interactions were identified between polar MPs and metals, such as PSMPs (polymer with the carboxyl group) (Dong et al., 2020) and PTFE (polymer with the hydroxyl group) (Dong et al., 2019). Furthermore, more than one adsorption mechanisms can exist together during adsorption process (Fu et al., 2021), due to the physical and chemical characteristics of the MPs and metals (Torres et al., 2020). Zuo et al., (2020) found that metals adsorption on MPs occurred through electrostatic interactions between bivalent cations (such as Cu^{2+}, Cd^{2+} and Pb^{2+}) and the charged or polar regions of the MPs surface or via surface complexation onto the MPs surface. In all, different MPs have different structure of carbon chain, crystallinity, polarity and surface functional groups, resulting in different mechanism of adsorption behavior with metals.

The adsorption isotherms are generally used to calculate the potential adsorption capacity between the MPs and metals (Turner et al., 2015; Zou et al., 2020; Zhou et al., 2022). The suitable isotherm models are mainly Langmuir model and Freundlich model. These two main isotherms vary in different sorption experiments due to various properties of MPs and metals (Table S2.5). In summary, the equilibrium data are sometimes fitted better into Langmuir model compared with Freundlich model, which indicated the adsorption process had monolayer coverage (Godoy et al., 2019; Wang et al., 2019a; Li et al., 2020b; Fu et al., 2021). Whereas, if the Freundlich model fitted the data better than Langmuir model, this indicates that the

interactions between MPs and metals were multi-layer adsorption on heterogeneous surfaces of MPs (Andersson et al., 2011; Qiao et al., 2019; Mao et al., 2020; Zuo et al., 2020). There were also other studies showed both Langmuir and Freundlich fitted the data well (Dong et al., 2020; Tang et al., 2021). Other isotherm models, such as Temkin and BET models, were only used in few studies (Guo et al., 2020a; Lin, et al., 2021). In terms of MPs-Cd adsorption, previous studies showed the data fit better into Langmuir model (Yu et al., 2021b; Li et al., 2022; Ma et al., 2022), Freundlich model (Guo et al., 2020b; Zhou et al., 2020b; Huang et al., 2021b) or both of them (Wang et al., 2019d). Generally, there is no clear rule as to which model is the best suitable for different adsorption processes, which might mean that adsorption is a complex process affected by multiple factors, such as the polymer types, concentrations of MPs and the concentrations of Cd. Environment is a complex compartment, so that many factors could affect the MPs-Cd interaction which is important to discuss next.

2.4.3 Main factors that impact microplastic-cadmium interaction

The factors that could influence adsorption of metals onto MPs (Table S2.6) are mainly the characteristics of MPs (i.e. particle size, surface area, shape and functional groups) (Qiao et al., 2019; Wang, et. al., 2019a; Dong et al., 2020; Mao, et. al., 2020; Yuan et al., 2020; Zuo et al., 2020; Fu et al., 2021) and weathering (Wijesekara et al., 2018; Godoy et al., 2019; Qiao et al., 2019; Wang et al., 2019a; Tang et al., 2021). Additional, the initial concentration of MPs and initial concentration of metals could also have effects on MPs-metal interaction (Barboza et al., 2018; Öz et al., 2019; Fu et al., 2021).

Particle size is considered a key factor influencing the adsorption capacity of MPs (Fu et al., 2021). MPs with smaller particles have higher specific surface area and more adsorption sites leading to greater adsorption capacity (Brennecke et al., 2016). Wang et al., (2019d) found smaller PE with higher specific surface area led to greater adsorption capacity of Cd. This was consistent with the study of Li et al., (2022) who showed the adsorption capacity of Cd on both PP and PS decreases significantly as their particle size increases. These studies only compared

adsorption per MPs mass, however it is also worthy to compare adsorption capacity of MPs on a surface area bases in the future studies. MPs exists in various shapes such as films, fibres, fragments, spheres and pellets (Fig. 2.4) (Waldschläger et. al., 2019; Lozano et al., 2021; Shi et al., 2022), and these shapes can have varying characteristics such as specific surface area or thickness (Rillig et al., 2019b). Therefore, MPs shapes can play an important role in their environmental behaviours (Lozano et al., 2023). Although no study comparing adsorption of metals onto different shapes of MPs so far, Yu et al., (2020) found PE microbeads and particles had significantly greater adsorption of organic compound (tetrabromobisphenol, TBBPA) relative to reference PE spheres, and specific surface areas of PE microbeads and particles were larger than reference PE MPs, indicating different adsorption capacities of MPs with different shapes might mainly result from their specific surface areas. The functional groups on MPs could also influence the sorption capacity (Torres et al., 2021). Huang et al., (2023) found that PLA MPs had higher adsorption capacity of divalent metal Pb compare to PS MPs due to oxygen-containing functional groups on PLA MPs. Jiang et al., (2024) indicated that oxygen functional groups were the major and preferential binding sites of PLA MPs, resulting in their high Cd adsorption capacities. PLA with functional groups adsorbed metals more likely via specific interaction, whereas PS might exhibit non-specific interaction. Only few studies showed that MPs particle size (specific surface area), shapes and functional groups can impacts their adsorption, therefore these properties of MPs should be considered in the future adsorption research.

Weathering of plastics can also impact their adsorption of metals due to changes in their physical and chemical properties (Hüffer et al., 2018; Fu et al., 2021). After weathering, MPs size decreased (Wang et al., 2018a; Wang et al., 2018b; Liu et al., 2019b), MPs roughness increased and MPs became cracked (Ye et al., 2020; Wang et al., 2023) resulting in an increase in the specific surface area of MPs providing more adsorption sites for metal capture (Mao et al., 2020; Ye et al., 2020). Oxygen-containing functional groups (including hydroxyl, carbonyl and carbon-oxygen) (Liu et. al., 2019a; Liu et al., 2020b) formed on MPs surface after

weathering leading to increased polarity and transfer from non-specific interaction to specific interaction, which potentially enhances the adsorption of metals on MPs (Wang and Wang, 2018; Liu et al., 2019b). Previous studies found that the adsorption capacity of conventional MPs (PE, PS, PP, PET, PA and PVC) of divalent metals increased with weathering due to new functional groups generated (Chen et al., 2021; Gao et al., 2021; Wang et al., 2023). However, these studies compare pristine with artificially-aged (UV-aged and H_2O_2-aged) MPs (Gao et al., 2021; Wang et al., 2023) which may not be good representatives of naturally weathered MPs.

Biofilms can form on weathered MPs (Kaiser et al., 2017; Rummel et al., 2017; Bhagwat et. al., 2021; Hanun et al., 2021; Alimi et al., 2022; Luo et al., 2022; Lozano et al., 2023) and affect the MPs properties leading to have further impacts on their sorption behaviour (Nauendorf et al., 2016; Paço et al., 2017; Tu et al., 2020). Biofilms formation can decrease MPs hydrophobicity (Rummel et al., 2017; Johansen et al., 2019; Tu et al., 2020) and formed biofilm contains carbon-oxygen functional groups (Paço et al., 2017; Guan et al., 2020; Tu et al., 2020). Qiongjie et al., (2022) found that the adsorption capacity of Pb and Cu on biofilm-developed PS was higher than that of virgin PS due to the complicated composition of biofilm formed on the PS surface.

The above results showed the possible reasons why the weathered MPs can adsorb more metal relative to the pristine MPs. After weathering, biofilm-formed MPs might have more significant potential as the metal carriers and change the fate of metals in the environment compared with pristine MPs. However, the relationship between the amount of metals adsorption and weathered MPs with biofilm attached has not been investigated, therefore studies on comparing the adsorption of weathered and pristine MPs are warranted. MPs and Cd are in soil and single and combined impacts of them on soil dwelling earthworms are summarised next.

2.4.4 Impacts of microplastic-cadmium interaction on earthworm

It is already known that MPs can interact with Cd (Wang et al., 2019d; Li et al., 2022; Ma et al., 2022; Huang et al., 2021b). Although less studies reported, the interaction between MPs and Cd can impact on how both MPs and metals affect earthworm (Fig. 2.9, Table S2.7) (Zhou et al., 2020c; Huang et al., 2021a; Zhang et al., 2024). The co-exposure to MPs and Cd led to higher negative effects on earthworms than exposure to Cd or MPs alone, in terms of earthworms growth rate (Zhou et al., 2020c; Huang et. al., 2021a), mortality (Zhou et al., 2020c), reproduction (Huang. Et. al., 2021a), oxidative damage (Zhou et al., 2020c), histopathological damage (Chen et al., 2024) and DNA damage (Huang et al., 2021a; Shang et. al., 2023). In contrast, Zhang et al., (2024) found PE alleviated histopathological and DNA damage to earthworms caused by Cd relative to PE or Cd alone. The additive effect is when the combined toxicity of two or more chemicals is equal to the sum of these chemicals toxicity. The synergistic or antagonistic is when the combined toxicity of two or more chemicals is greater than or less than the sum of these chemicals toxicity, respectively (Preston et al., 2000). The above studies results are rather varied and the most of them are antagonistic. However, Liang et al. (2022) found time-dependent effects on earthworms after co-exposure of PE and Cd, with an antagonistic effects on oxidative damage in earthworms after 10 days exposure, but a synergistic effect was detected after 30 days exposure.

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. Explanations of why MPs-Cd combined impacts on earthworm differed from their single impacts might occur are discussed next.

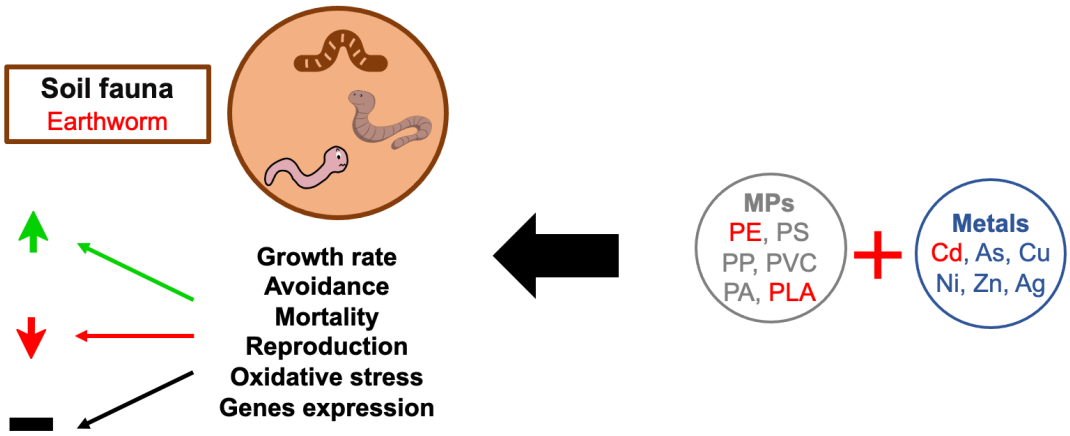


Fig. 2.9. A diagram summarising the possible impacts of MPs-metals interaction on soil fauna, earthworm. Green upward-pointing arrow "↑" indicates a positive correlation; Red downward-pointing arrow "↓" indicates a negative correlation; Black short horizontal line "-" indicates no correlation.

Impacts of MPs-Cd interactions on earthworm can result from MPs acting as carriers for Cd, so that MPs could also change the Cd accumulation in earthworms (Zhou et al., 2020c; Shang et. al., 2023). Zhou et al., (2020c) found the presence of PP MPs increase Cd accumulation in earthworms. Similarly PLA and PS both aggravate bioaccumulation of Cd to earthworms (Shang et al., 2023). However, Zhang et al., (2024) found PE did not affect the accumulation of Cd in earthworms. Same earthworm specie was used in these studies and determination of the total Cd content of earthworm was all carried out using HNO_3-H_2O_2 digestion and measured by inductively coupled plasma (ICP) mass spectrometry. Different MPs types used might be the reason leading to inconsistency in these results on impacts of MPs-Cd interaction on earthworms, suggesting that more research on comparing different MP types is warranted.

The studies of interaction between MPs and Cd have just been started to investigate and mainly focused on the experiments with high concentrations (Table S2.5, S2.6, S2.7) (Holmes et al., 2012; Kim, et. al., 2017; Wen et al., 2018; Godoy et al., 2019; Abbasi et al., 2020; Guo et al., 2020a; Guo et al., 2020b). Under relevant environmental conditions, the mean concentrations of contaminants in the real environment lower than those used in the laboratory experiments

(Wang et al., 2015; Chen et al., 2020; Baeza et al., 2020; Huang et al., 2021a; Yu and Flury, 2021; Chen et al., 2024). Therefore, impacts of MPs-Cd interaction on earthworms may be negligible in the environment. Thus, the impacts of MPs-Cd interaction might be overestimated in the laboratory experiments, so that laboratory experiments using environmentally realistic concentrations are warranted.

2.5 Summary

MPs are widespread contaminants in soil due to the continued growth of plastics production. This literature review summarised the current understanding of the major distribution and abundance and sources of MPs, their interaction with metals, and impacts of their interaction on earthworms. MPs come from a wide range of sources with various characteristics in agricultural soil, especially in China. Among all the sources of MPs in farmland, plastic mulches are the most significant. Once mulch-derived MPs enter into the soil, they have impacts on earthworms in terms of the reduction in growth and reproduction, increase in mortality, causing histopathological damage, oxidative stress and genetic damage. Furthermore MPs in soil can interact with metals further affecting earthworms behaviour. However, inconsistent findings of these impacts indicate more future studies are needed. In addition, currently biodegradable plastics mulches are developing to replace conventional plastics. However, lack of studies comparing the impacts of biodegradable and conventional MPs on earthworms suggest that more research are warranted. During MPs deriving from mulches with time, plastics can be weathered. MPs physiochemical properties alter and biofilms formation on MPs occur, leading to changes in their interaction with metals. As most of the current studies are conducted in laboratory and the concentrations used are higher than real nature, experiments using realistic environmental concentrations are also needed to be considered.

Based on these knowledge gaps, the next contents of the thesis are the findings of experiments. Three laboratory experiments were conducted to investigate single and combined impacts of plastic mulches derived-MPs and Cd on earthworms, whether difference in these impacts

between conventional and biodegradation MPs, and how adsorption affects Cd bioaccumulation in earthworms. A small field experiment was conducted to compare the differences in the impacts of conventional and biodegradable MPs on earthworm populations.

3 Materials and methods

3.1 Introduction

This chapter presents the materials and analytical methods used that are common between chapters. The experimental methods and materials, data analysis, quality control and ethical concerns are primary components of this chapter, including source of soils, chemical and physical characterisation of soils, source of earthworms, source of plastics, plastics identification, microplastics generation methods, chemical and physical characterisation of microplastics, source of Cd solution, statistical analysis and ethical concerns.

3.2. Soils

Since this study was designed to investigate mechanisms and to avoid biosecurity issues associated with overseas soil use, uncontaminated natural topsoil purchased from Garden Topsoil Direct Northamptonshire, UK (Cranford Road, Burton Latimer, Kettering, Northamptonshire, NN16 8UN, UK) was used rather than obtaining (and potentially importing) Cd-contaminated soil from either the UK or overseas. Given the large quantities of soil required, it was deemed more appropriate to buy rather than collect soil. The purchased soil has been used in the previous earthworm ecotoxicology studies (Davies et al., 2003; Langdon et al., 2005; Ellis et al., 2010), and has similar properties to the model soils described in the standard OECD earthworm toxicology test protocols (OECD, 1984; OECD, 2004).

The soils were air-dried to constant weight and sieved to < 2 mm, which was repeated until sufficient air dried 2 mm sieved soil was obtained for subsequent use. All of this soil was homogenously mixed and then characterised before use for soil pH, soil organic matter, soil water holding capacity (WHC), soil effective cation exchange capacity (ECEC), soil texture, soil microplastic and Cd background concentrations. The results of soil properties are shown in Table 3.1.

Table 3.1. Summary of soil characteristics.

Soil characteristics lists	Results
Soil pH	7.51 ± 0.02 (n = 5, ± standard deviation)
Soil organic matter	11.24 ± 0.13 g / 100 g oven-dry soil (n = 5, ± standard deviation)
Soil water holding capacity (WHC)	76.15 ± 0.58 % (n = 5, ± standard deviation).
Soil effective cation exchange capacity (ECEC)	1.86 ± 0.04 $cmol_c$ / kg, (n = 5, ± standard deviation)
Soil texture by feel	clay loam
Particle size distribution	12.12 ± 1.19 % clay, 50.15 ± 4.49 % silt and 37.73 ± 5.64 % sand (n = 5, ± standard deviation)
Soil microplastic background concentration	A negligible concentration of MPs
Soil Cd background concentration	< detection limit 0.0130 mg / kg (section 3.6.2.4)

3.2.1 Soil pH

Ten grammes of air dried 2 mm sieved soil was added to 25 mL deionized water and shaken for 15 minutes. The suspension was allowed to settle and pH was measured using a Thermo Fisher Scientific Orion Model 420 A+ pH meter calibrated with pH 4.01 and 7.00 buffers (Rowell, 1994).

3.2.2 Soil organic matter

Ten grammes of air dried 2 mm sieved soil were heated over night at 105 °C, reweighed and then combusted at 350 °C over night. The sample was reweighed and the loss of mass was calculated and used as a measure for organic matter content in the soil (Rowell, 1994).

3.2.3 Soil WHC

Approximately 50 g of air dried 2 mm sieved soil were added to a plastic container with mesh on the bottom. The containers were placed in a dish of water, with the water level above that of the soil but below the level of the containers to allow the soil to become saturated overnight.

The containers were removed from the water and loosely covered with cling-film and suspended on a retort stand to allow drainage for about three hours. Then the wet soils were removed from each container and placed in oven at 105 °C overnight. Afterwards, the WHC was calculated based on the mass of drained soil and oven dried soil (Klute, 1986).

3.2.4 Soil effective cation exchange capacity (ECEC)

$BaCl_2$ method was used to measure the soil ECEC. In this method, soil ECEC was calculated as the sum of exchangeable cations (Cd, Mg, K, Na, Al, Fe and Mn). 0.5 g of air dried 2 mm sieved soil was added to 30 mL 0.1 M $BaCl_2$ solution in a 50 mL centrifuge tube made of polypropylene and shaken on a flatbed shaker for 2 h. The sample was centrifuged for 15 mins at 4000 RPM, the supernatant filtered through Whatman no.540 12.5 cm diameter fiter paper and then analysed for all of the above cations via a Thermo Scientific iCAP 7000 inductively coupled plasma-optical emission spectrometer (ICP-OES) (Carter and Gregorich, 2007) and the quality control is in Section 3.6.2.

3.2.5 Soil texture

Soil texture was measured by feel, primarily with tactile manipulation with fingers, of a moist soil sample (Thien, 1979).

Additional soil particle size distribution was determined using a Malvern Mastersizer Hydro2000 MU laser granulometer (Wanogho et al., 1987). Since the clay soil contains more than 3.5 % organic matter, 30 % w / v hydrogen peroxide (H_2O_2) solution (Fisher Scientific) was used to remove soil organic matter so as to improve this measurement. Specifically speaking, 10.0 g of air dried 2 mm sieved soil were weighed and then transferred to a 500 mL glass beaker. In a fume cupboard, 10 mL 30 % w / v H_2O_2 solution (Fisher Scientific) was gradually added to the beaker and left for 1 h. The braker was transferred on a hot plate at 50 °C, and then another 10 mL 30 % w / v H_2O_2 solution was added to the beaker and left for 2 h. Afterwards, the suspension was dries down in the oven and then oven-dried soil was used to make measurements on the laser granulometer. The laser granulometer was rinsed three times with deionised water in a 1 L beaker placed in the measurement position and the pump speed

set to 4000 rpm prior to use. Around 700 mL deionised water was added to a 1 L beaker and put in the measurement position on the laser granulometer. The pump speed was set to 1500 RPM and 0.5 g of oven dried soil was added into the breaker. The ultrasonic probe set as 20 W was turned on for 5 s to allow complete homogenisation of the soil and the obscuration rate was maintained at about 4 %. The soil was with 12.12 ± 1.19 % clay, 50.15 ± 4.49 % silt and 37.73 ± 5.64 % sand (n = 3, ± standard deviation), which was loam according to the a texture triangle.

These two different methods gave different results of soil texture. The result of texture by feel was clay loam soil, while the texture obtained by the laser granulometer and a texture triangle was loam. Generally, the soil was loamy and met the standard OECD earthworm toxicology test protocols (OECD, 1984; OECD, 2004).

3.2.6 Soil microplastic background concentration

A method detailed in Li et al., (2019) was used to measure soil microplastic background concentration. 3.0 g air dried 2 mm sieved soil were weighed (M_{soil}) and then transferred to a 200 mL glass beaker. Approximately 30 mL 30 % w / v H_2O_2 solution (Fisher Scientific) was gradually added to the beaker on a hot plate at 50 °C and left for 24 h to remove soil organic matter so as to improve the MPs separation efficiencies. After cooling down, saturated NaCl solution (density: 1.18 g / cm^3) was added and the suspension was centrifuged at 3500 RMP for 5 mins. The supernatant was vacuum filtered through a pre-weighed (M_{fp1}) Whatman Grade 54 filter paper (2.5 μm mesh). The filter paper was oven-dried at 50 °C to a constant weight (M_{fp2}). The microplastic concentration C_{MP} was calculated as follows:

$$C_{MP}\ (\%) = \frac{M_{fp2} - M_{fp1}}{M_{soil}} \times 100$$

The samples on the filter paper were identified by Fourier transform infrared (FTIR) spectroscopy carried out using a Bruker Alpha FTIR with a platinum ATR unit (Li et al., 2019). The spectra were analysed using, OpenSpecy (Cowger et al., 2021). Furthermore, the samples on the filter paper were heated at 130 °C for 5 seconds and any exist MPs would melt to be

identified (Zhang et al., 2018).

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 (Fig. 3.1a) was identified (Pearson's r = 0.92) as organic matter via FTIR reference libraries of OpenSpecy (Data from OpenSpecy, 2024), and its spectrum (Fig. 3.1a) was matched to lignin based on the peaks at around 3400, 2930 and 1050 cm^{-1} from previous study (Fig. 3.1b; Liu et al., 2008). The FTIR spectrum of the samples on the filter paper was not a match to the FTIR spectra of PE and PLA in this study observed by eyes (Fig. 3.1a). Furthermore, no melting of the particles was observed when the samples were heated at 130 °C (Fig. 3.1c) (Zhang et al., 2018). Thus the separated particles appear to be largely recalcitrant organic matter and the soil appears to contain a negligible concentration of MPs.

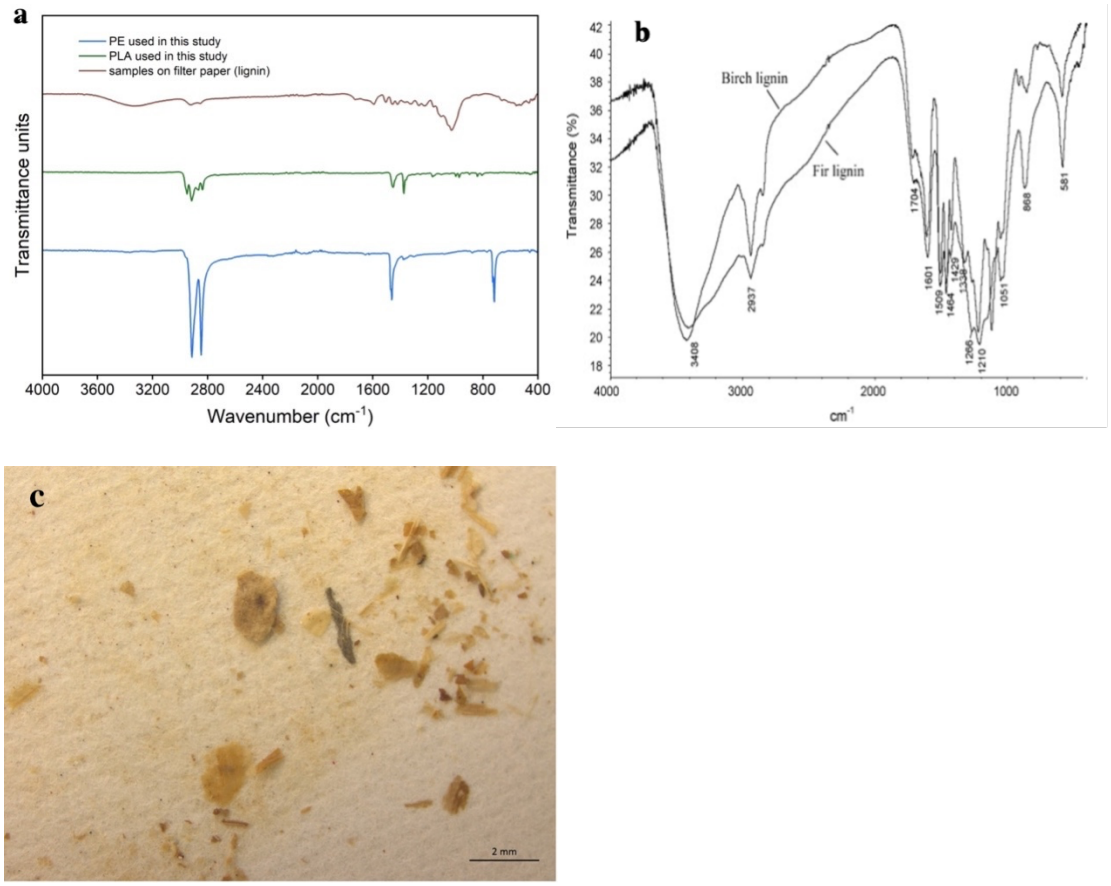


Fig. 3.1. (a) FTIR spectra of samples separated from the initial soil and the PE and PLA used in this study; (b) FTIR spectra of lignins from Liu et al., (2008); (c) optical microscope image of samples separated from initial soil by density flotation after digestion of organic matter on filter paper after 5 second heating at 130 °C.

3.2.7 Soil Cd background concentration

Soil digestions for pseudo-total Cd concentration followed the digestion method of British Standard BS 7755, (1995). 1.5 g air dried 2 mm sieved soil were weighed and transferred to a 100 ml Kjeldahl digestion tube made of borosilicate glass. Four round glass balls were added to the tube as the anti-bumping granules. 10.5 ml of concentrated AnalaR hydrochloric acid was added to each tube followed by 3.5 ml concentrated AnalaR nitric acid (aqua regia, 3:1 HCl-HNO_3). A glass bubble was placed on top of each tube and the tubes were left to stand overnight in a fume cupboard. The next day, the tubes were heated to 50 °C, the temperature was then gradually increased to 140 °C at a rate of 1 °C per minute. The soils were digested for 2 - 2.5 hours. After cooling down, the digestions were filtered through a Whatman no. 540 12.5 cm

diameter filter paper and diluted with 0.5M nitric acid into a 100 ml glass volumetric flask prior to analysis by ICP-OES (quality control in Section 3.6.2). The measured Cd concentrations were below detection limits (Section 3.6.2.4) indicating a negligible concentration of Cd in soil.

3.3 Earthworms

The anecic earthworm *Lumbricus terrestris* was used as a model species in our study because it's a common UK earthworm and more widely is found globally (Hendrix et al., 2008) and their behaviour can reallocates contaminants in the soil (Sizmur and Hodson, 2009), thus it is a good example earthworm to investigate the interactions between MPs, Cd and earthworms (Section 1.3) 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, UK) and confirmed as being *L. terrestris* by reference to the identification (Sherlock, 2012). Earthworms were rinsed with deionised water and cultivated for 1 week in the same soil type used in the following experiments 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 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.

3.4 Microplastics

3.4.1 Plastic mulch selection

Two types of black plastic mulches, non-biodegradable PE (sold as "YYOBK Mo garden PE weeding film vegetable mulch film, 5 hole mulch film for plant cultivation greenhouse agriculture") and biodegradable PLA (sold as "GBNHGYP Mo agricultural ground cloth orchard grass cover cloth durable degradable breathable moisturizing mulch, biodegradable ecological PLA mulch"), were purchased from the BEIJUANDEOZXIAODIAN storefront on Amazon. Black plastic mulches are the most widely used types of mulches in the production of vegetables and other crops because of their significant impact on increasing soil heat by

absorbing high amount of radiation (Gordon et al., 2010; Amare et al., 2021). The most common non-biodegradable mulch is PE whilst the most common biodegradable mulch is PLA (Section 1.2.6). The polymer types and densities of these two types of plastics were measured.

3.4.1.1 Plastic polymer identification

FTIR spectroscopy was carried out using a Bruker Alpha FTIR with a platinum ATR unit (Primpke, et al., 2018). The spectra were recorded in absorbance mode within the wave number range 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} and 24 scans per point. The spectra were normalised and corrected with respect to the baseline. The absorption unit data was transformed to transmittance units using the OPUS spectroscopy 7.5.18 software. The spectra were analysed using OpenSpecy (Cowger et al., 2021).

OpenSpecy identified the spectra of PE (Fig. 3.2a, Table 3.2) via their reference libraries (Pearson's r = 0.99), while there were not matches to PLA spectra but matches to methandriol spectra (Pearson's r = 0.75) in the reference libraries of OpenSpecy (Data from OpenSpecy, 2024). However, the peaks at 2916, 1166, 997 and 972 cm^{-1} (Fig. 3.2b, Table 3.2) suggest that the material was PLA as reported in previous studies (Primpke et al., 2018; Circelli et al., 2024).

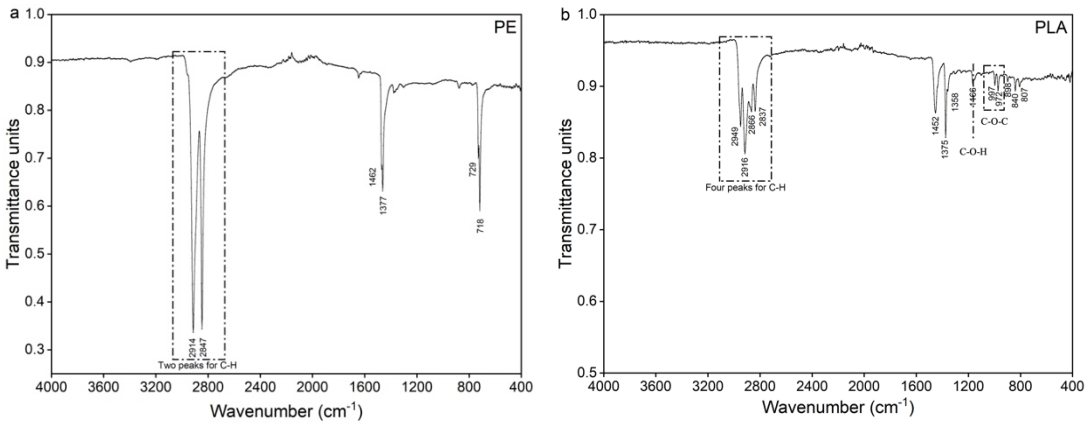


Fig. 3.2. FTIR spectra of (a) polyethylene (PE) and (b) polylactic acid (PLA) used in this study.

Table 3.2. Main observed peaks and associated wavenumbers (cm^{-1}) in the FTIR spectra of the pristine MPs (PE and PLA) used in this study.

Wavenumber (cm^{-1})		Peak assignment
PE	**PLA**	
2914, 2847	2949, 2916, 2866, 2837	C-H stretching vibrations
1462, 1377	1452, 1375, 1358	C-C stretching vibrations
	1166	C-O-H stretching vibrations
	997, 972	C-O-C vibrations
876, 729, 718	898, 840, 807	C-H bending vibrations

3.4.1.2 Relative intensity of functional groups on plastics

Triplicate samples of the different plastics were characterised by FTIR to determine the presence and relative intensity of functional groups. Based on the main observed peaks and associated wavenumbers (cm^{-1}) in the FTIR spectra (Table 3.2), the peak areas of the FTIR spectra in the ranges 1300 to 800 cm^{-1} and 3200 to 2600 cm^{-1} were used to calculate relative intensity of their functional groups using OriginPro 2023b 10.0.5.157 (Table 3.3). These ranges covered the main oxygen containing functional group and the highest intensity peaks for non-polar groups (C-H) in the spectra.

Table 3.3. Integral area of the peaks from 1 300 to 800 cm^{-1} and 3 200 to 2 600 cm^{-1} in FTIR spectra of different MPs types (Section 3.4.2), and their ratio representing the relative intensity of peaks (n = 3).

MPs type	C-O (begin from 1 300 to 800 cm^{-1})					C-H (from 3 200 to 2 600 cm^{-1})					C-O / C-H ratio	Std. dv
	Max height	X at max height	Integral area	Rectangular area of the integration interval	peak area	Max height	X at max height	Integral area	Rectangular area of the integration interval	peak area		
PPE	0	0	0	0	0	1	2995.9	533.71	599.58	65.875	0^{a}	0
WPE	0.9992	813.72	488.77	497.22	8.447	0.99544	3002	568.02	596.85	28.831	0.2930^{b}	≤ 0.001
WWPE	0.9251	825.96	452.09	460.35	8.261	0.93788	3018.3	522.52	562.34	39.823	0.2074^{c}	≤ 0.001
PPLAF	0.9961	919.77	491.38	495.66	4.281	1	3006.1	573.58	599.58	26.003	0.1646^{d}	≤ 0.001
WPLAF	0.9924	862.67	488.42	493.83	5.403	0.999	3016.3	573.29	599.01	25.718	0.2101^{e}	≤ 0.001
WWPLAF	0.9879	913.65	487.1	491.58	4.487	0.998	3014.2	573.16	598.4	25.243	0.1778^{f}	≤ 0.001
PPLAP	0.9866	917.73	486.94	490.96	4.018	1	3024.4	573.5	599.58	26.083	0.1541^{g}	≤ 0.001
WPLAP	0.9921	907.54	488.89	493.68	4.791	0.9984	3020.4	574.69	598.6	23.909	0.2004^{h}	≤ 0.001
WWPLAP	0.9688	913.65	477.92	482.06	4.146	1	3006.1	573.73	599.58	25.858	0.1603^{i}	≤ 0.001

Note: Values with different superscript letters (a, b and c) are significantly different at $P \leq 0.001$ within PE group (PPE, WPE and WWPE).

Values with different superscript letters (d, e and g) are significantly different at $P \leq 0.001$ within PLAF group (PPLAF, WPLAF and WWPLAF).

Values with different superscript letters (h, i and h) are significantly different at $P \leq 0.001$ within PLAP group (PPLAP, WPLAP and WWPLAP).

3.4.1.3 Plastic density measurement

The different floating and sinking states of MPs in a density gradient solution can be used to measure their density (Barnett et al., 2021). Usually, ethanol (0.8 g / cm^3), water (1.0 g / cm^3) and saturated NaI (1.8 g / cm^3) are considered as the best choices for the density gradients solutions (Li et al., 2018; Barnett et al., 2021). Water and saturated NaI are more polar than ethanol (methyl functional group) so that the MP dispersion system exhibited the best suspension performance in ethanol (Qin et al., 2023). Therefore, ethanol was used for plastic density measurement in this study.

Using modified previous research (Pořízka et al., 2023), 5 mL pycnometers (Pomex Glassware Co., Ltd, Beijing, China) both empty and containing deionised water of known density (0.998 g / cm^3) were weighed (accurate to four decimal places) on an analytical balance at 20 °C to determine their inner volumes (Equation 3.1). Using the known volume of pycnometers, the density of absolute ethanol (0.789 g / cm^3) that acted as a dispersant at 20 °C could be obtained using pycnometers (Equation 3.2).

$$V_p = \frac{M_{pw} - M_p}{\rho_w\ (20\ ^\circ C)} \qquad (3.1)$$

Where V_p is the volume of pycnometer (cm^3); M_{pw} is the mass of pycnometer fully filled with deionised water (g); M_p is the mass of empty pycnometer (g); ρ_w is the density of deionised water at 20 °C.

$$\rho_e = \frac{M_{pe} - M_p}{V_p} \qquad (3.2)$$

Where ρ_e is the density of ethanol (g / cm^3) at 20 °C; M_{pe} is total mass of pycnometer fully filled with ethanol (g); M_p is the mass of empty pycnometer (g); V_p is the volume of pycnometer.

The mass of ethanol solution replaced by the sample in the pycnometer was then measured in order to calculate the volume of MPs, which is equal to the excluded volume of ethanol, and to determine the precise particle density of MPs. Specifically, the precise mass of the empty

pycnometer and a pycnometer containing 0.5 g of oven-dried MPs were weighed and recorded. Ethanol was added until the MPs samples and ethanol fully filled the pycnometer after the installation of the stopper. The mass of pycnometer filled with ethanol and MPs sample was precisely measured to calculate the particle density of MPs according to equation (3.3).

$$\rho_{MPs} = \frac{\rho_e \times (M_{ps} - M_p)}{(M_{ps} - M_p) + (M_{pe} - M_{pse})} \quad (3.3)$$

Where ρ_{MPs} is the measured particle density of MPs sample (g / cm^3); ρ_e is the density of ethanol (g / cm^3) at 20 °C; M_p is the mass of empty pycnometer (g); M_{ps} is the total mass of pycnometer and MPs sample (g); M_{pe} is total mass of pycnometer fully filled with ethanol (g); M_{pse} is the mass of pycnometer fully filled with sample and ethanol (g).

PE density was measured as 0.917 ± 0.0003 g / cm^3 and PLA as 0.924 ± 0.0002 g / cm^3 (n = 5, ± standard deviation), which were in the range of typical MPs densities reported in previous research (Li et al., 2018, Borges-Ramírez et al., 2020, Meng et al., 2023).

3.4.2 Mulch weathering and microplastic generation

Plastic mulches as a row cover provides earlier and higher yield of plants by protecting them from high radiation without affecting soil moisture, reducing the incidence of pests and reducing the use of insecticide (Ruíz-Machuca et al., 2015). Therefore, to mimic the natural weathering during mulches usage, large metre scale sheets of PE and PLA mulches were cut from the purchased mulching materials and hung outside to naturally age from 3rd April 2022 to 2nd August 2023 (Fig. 3.3); local mean monthly weather data for this period are shown in Fig. 3.4. At the end of the exposure period, the weathered mulching sheets were rinsed with deionised water to clean dust from them, and air-dried.

Fig. 3.3. Large sheets of PE and PLA mulches cut from the purchased mulching materials were hung outside to naturally weather from 3rd April in 2022 to 2nd August in 2023.

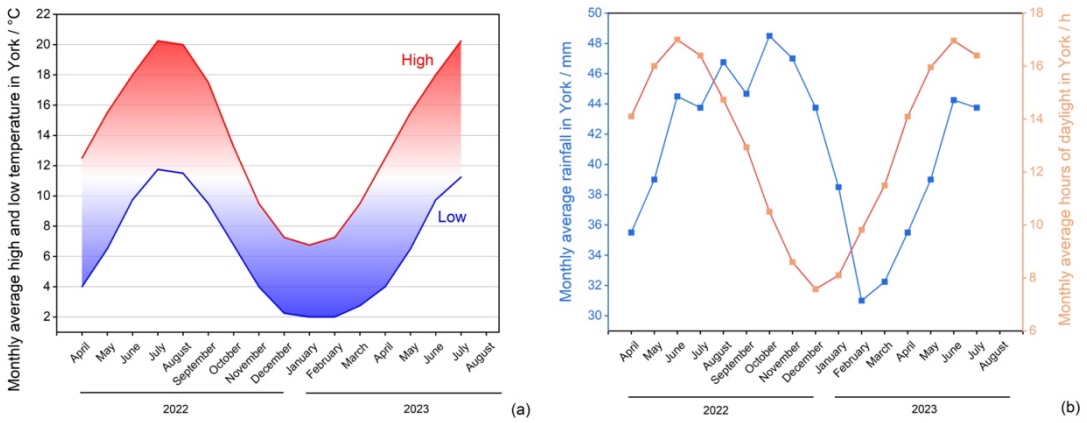


Fig. 3.4. Weather data in York for the period during which plastic mulches were weathered outside from 3rd April in 2022 to 2nd August in 2023, (a) monthly average high and low temperature / °C; (b) monthly average rainfall / mm and hours of daylight / h (Data from Weather Spark, 2024).

Afterwards, both pristine and weathered mulches were cut into small pieces (2 cm^2) using scissors, shredded using a Ninja blender (BN495UK), which was purchased from the NINJA store on Amazon and sieved to less than 2 mm. The PE formed planar particles whilst the PLA formed fibres. This generated the following sample types: pristine PE pieces (PPE), weathered PE pieces (WPE), pristine PLA fibre (PPLAF), and weathered PLA fibre (WPLAF). Pristine and weathered PLA mulches were also cut to produce similar shaped particles to the PE using scissors (generating pristine PLA pieces, PPLAP, and weathered PLA pieces, WPLAP). Finally subsamples of the weathered PE and PLA MPs were washed in absolute ethanol (vwrc20821.330) in an ultrasonic bath (Grant XUBA 3) for 15 mins to remove possible adhering biofilm (Tu et al., 2020, Tarafdar et al., 2021) to generate: ethanol-washed weathered

PE pieces (WWPE), ethanol-washed weathered PLA fibre (WWPLAF) and ethanol-washed weathered PLA pieces (WWPLAP). All of these MPs were characterized prior to use including MPs size (Section 3.4.3.1), MPs shape (Section 3.4.3.1), MPs specific surface area (Section 3.4.3.2), relative intensity of functional groups on MPs (Section 3.4.3.3), biofilm adhere on MPs (Section 3.4.3.4), MPs Cd background concentration (Section 3.4.3.5).

3.4.3 Microplastic characterisation

3.4.3.1 MPs size and shape

Images of the particles (Figs. 3.5, 3.6, 3.7, 3.8) were taken using an Axio Zoom V16 Microscope equipped with an Axiocam 105 colour camera and Zen 2 (blue version) software and a scanning electron microscopy (SEM, HITACHI TM4000Plus Tabletop Microscope) operated at an electron accelerating voltage of 5 kV. Additionally, the size and shape of the particles (n = 50 for each particle type) were analysed using ImageJ 2.3.0 / 151 1.53r (Fig. 3.9 and Tables 3.4, 3.5, 3.6). The SEM images indicate that the PE mulch is initially a smooth sheet whereas for the PLA a fibrous texture is present, though it is not know whether the PLA was initially produced as fibres which were then woven together to form the PLA film or not. The MPs showed no obvious optically-visible changes in their appearance before and after weathering or washing in ethanol (Fig. 3.5). However, SEM images showed that biofilms formed on MPs surface after naturally weathering and were removed after being ethanol-washed (Figs. 3.6, 3.7, 3.8). SEM observations showed that the surfaces of weathered and weathered then washed MPs were rougher and had creases (PE) or were more uneven (PLA) compared with those of the MPs (Figs. 3.6, 3.7, 3.8).

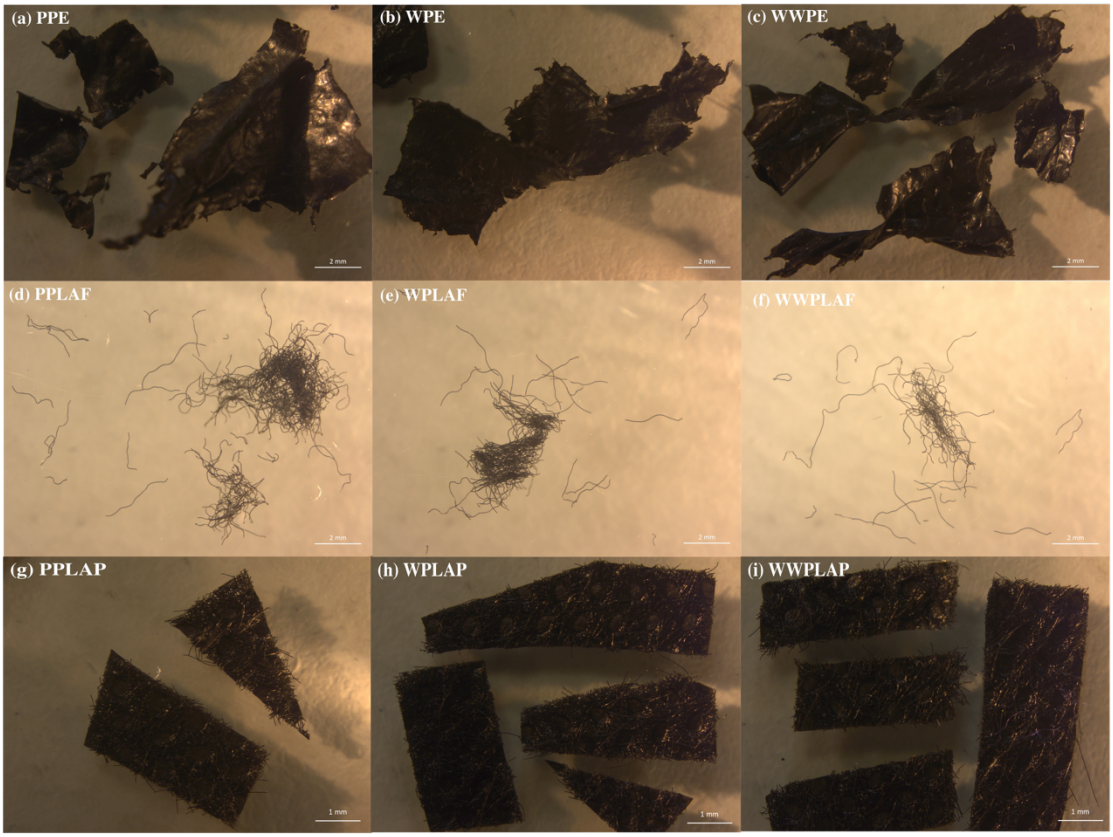


Fig. 3.5. Optical microscope images of the MPs particles used in the experiments: (a) pristine polyethylene (PPE), (b) weathered polyethylene (WPE), (c) ethanol-washed weathered polyethylene (WWPE) particles, (d) pristine polylactic acid fibres (PPLAF), (e) weathered polylactic acid fibres (WPLAF), (f) ethanol-washed weathered polylactic acid fibres (WWPLAF), (g) pristine polylactic acid pieces (PPLAP), (h) weathered polylactic acid pieces (WPLAP) and (i) ethanol-washed weathered polylactic acid pieces (WWPLAP). In the cases of a, b, c, d, e, f, g the scale bar represents 2 mm and in the cases of g, h, i the scale bar represents 1 mm.

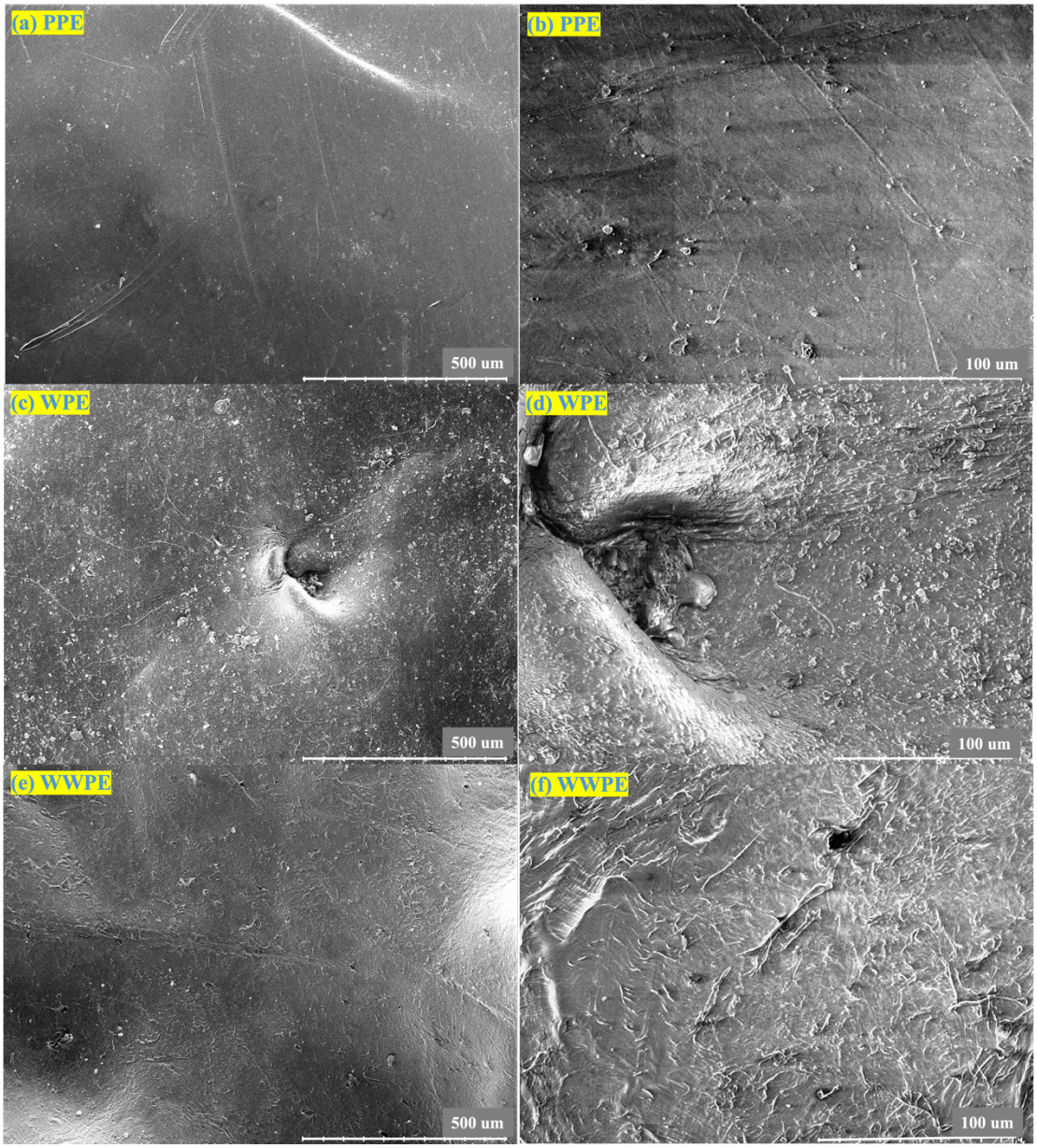


Fig. 3.6. Typical SEM images of the polyethylene (PE) pieces MPs used in the experiments: (a), (c) and (e) are the pristine (PPE), weathered (WPE) and weathered and washed PE (WWPE) pieces at low magnificent (scale bar equal to 500 µm) whilst (b), (d) and (f) are the pristine (PPE), weathered (WPE) and weathered and washed PE (WWPE) pieces at higher magnification (scale bar equal to 100 µm). The PPE samples show a smooth topography; particles interpreted to be biofilm are present on the WPE samples; these particles are not present on the WWPE samples which show a rougher topography than the PPE samples.

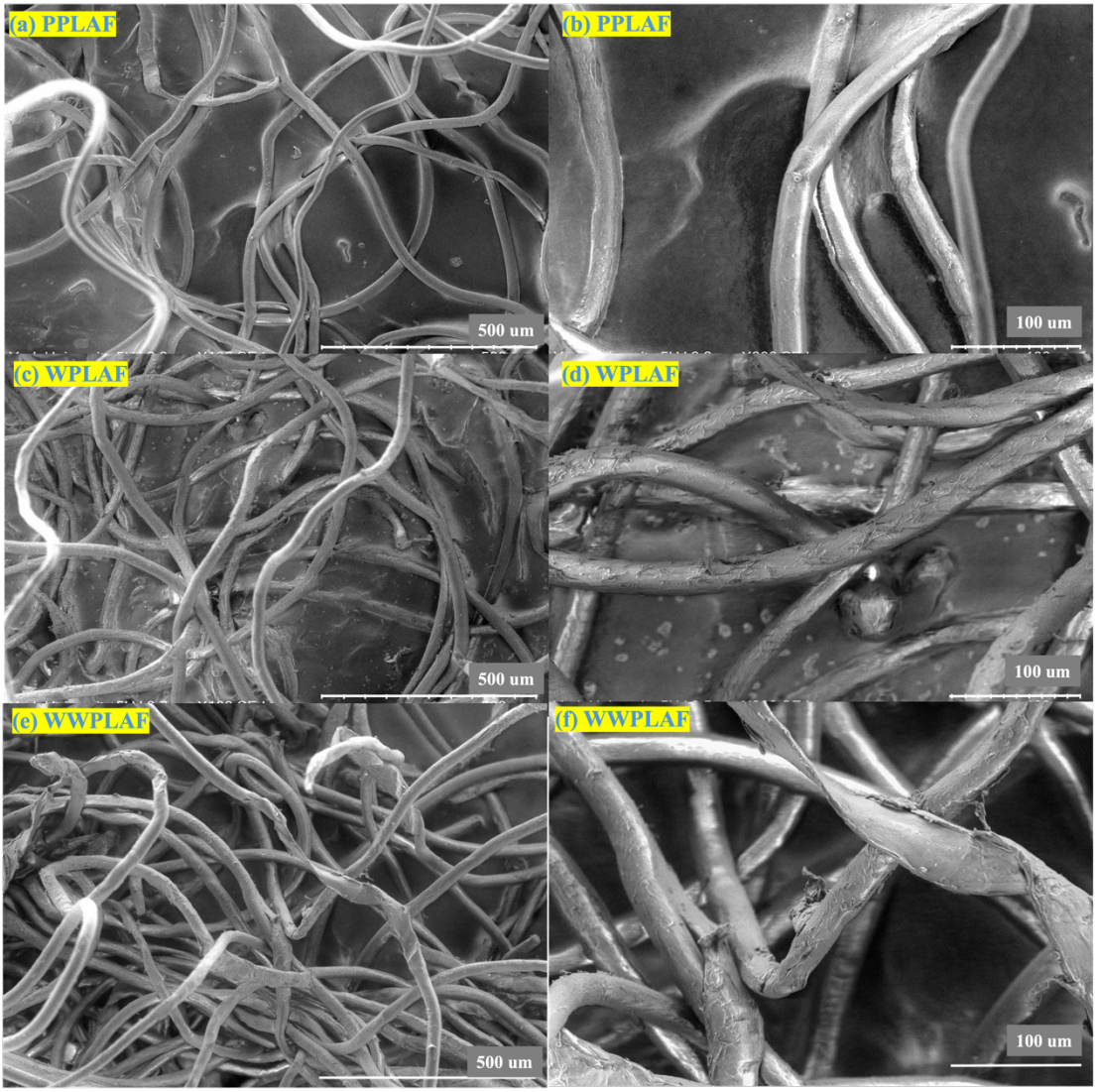


Fig. 3.7. Typical SEM images of the polylactic acid fibres (PLAF) MPs used in the experiments: (a), (c) and (e) are the pristine (PPLAF), weathered (WPLAF) and weathered and washed PLAF (WWPLAF) at low magnificent (scale bar equal to 500 µm) whilst (b), (d) and (f) are the pristine (PPLAF), weathered (WPLAF) and weathered and washed PLAF (WWPLAF) at higher magnification (scale bar equal to 100 µm). The PPLAF samples show a smooth topography; particles interpreted to be biofilm are present on the WPLAF samples; these particles are not present on the WWPLAF samples which show a rougher topography than the PPLAF samples.

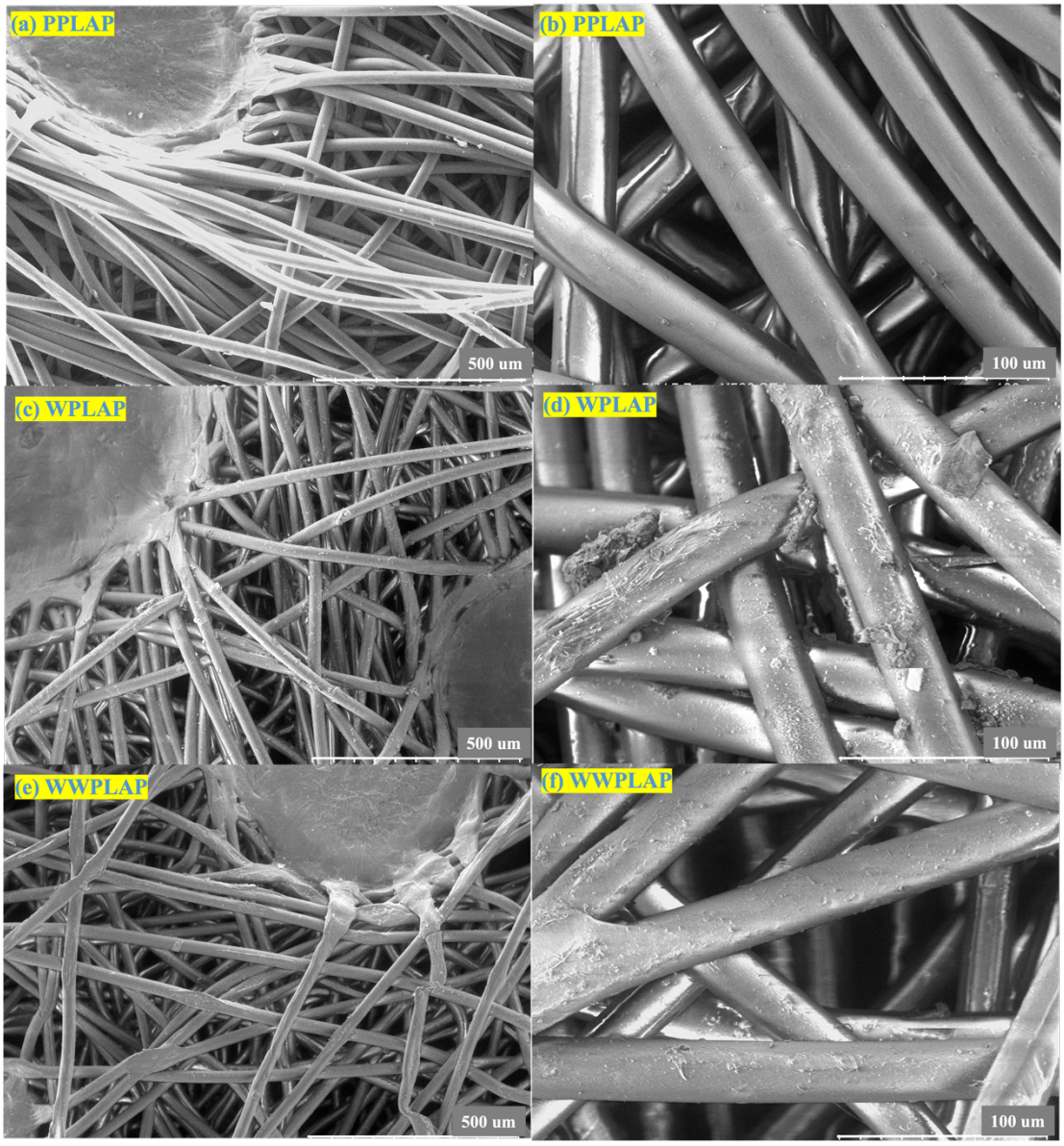


Fig. 3.8. Typical SEM images of the polylactic acid pieces (PLAP) MPs used in the experiments: (a), (c) and (e) are the pristine (PPLAP), weathered (WPLAP) and weathered and washed PLAP (WWPLAP) at low magnificent (scale bar equal to 500 µm) whilst (b), (d) and (f) are the pristine (PPLAP), weathered (WPLAP) and weathered and washed PLAP (WWPLAP) at higher magnification (scale bar equal to 100 µm). The PPLAP samples show a smooth topography; particles interpreted to be biofilm are present on the WPLAP samples; these particles are not present on the WWPLAP samples which show a rougher topography than the PPLAP samples.

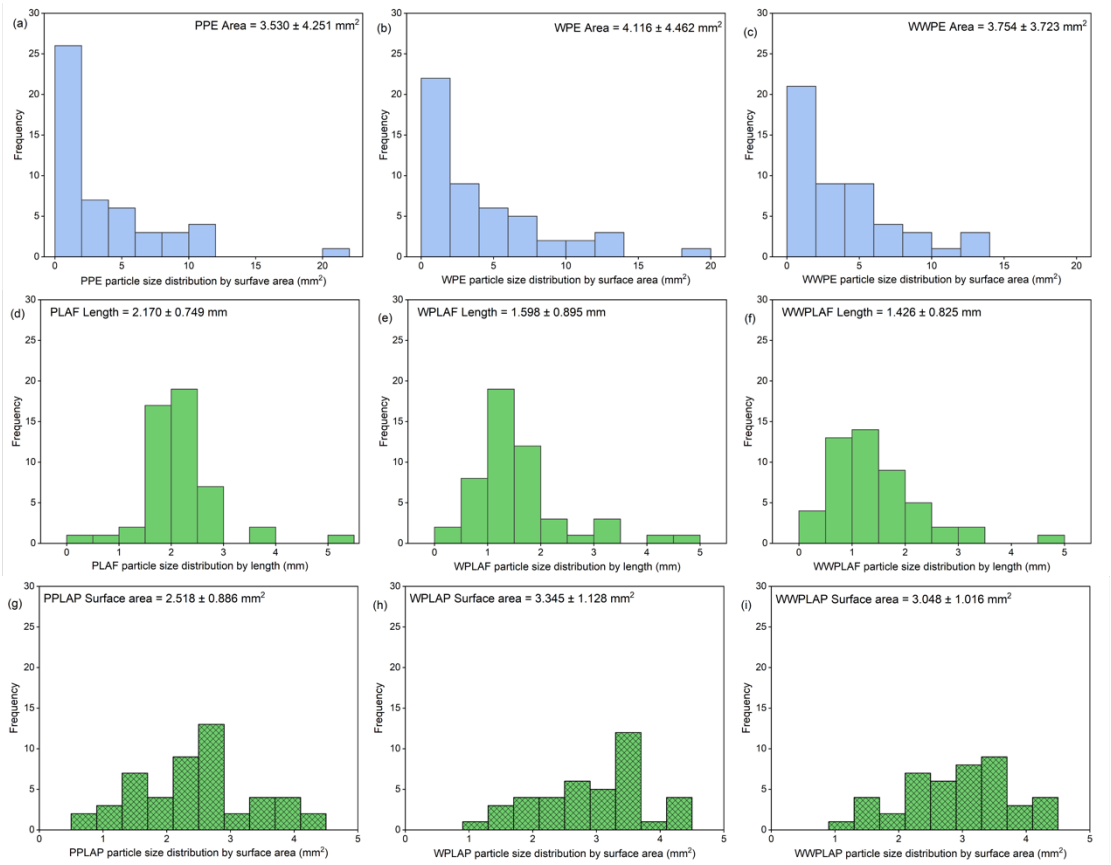


Fig. 3.9. Size distribution of (a) pristine polyethylene (PPE), (b) weathered polyethylene (WPE), (c) ethanol-washed weathered polyethylene (WWPE) particle areas, (d) pristine polylactic acid fibres (PPLAF), (e) weathered polylactic acid fibres (WPLAF), (f) ethanol-washed weathered polylactic acid fibres (WWPLAF) particle lengths, (g) pristine polylactic acid pieces (PPLAP), (h) weathered polylactic acid pieces (WPLAP) and (i) ethanol-washed weathered polylactic acid pieces (WWPLAP) particle areas as determined by microscopy and ImageJ (n = 50).

Table 3.4. Summary data for PE particle size and shape descriptors (n = 50).

MPs type		Area (mm^2)	Perimeter (mm)	Major axis (mm)	Minor axis (mm)	Circularity	Aspect ratio
Pristine PE / PPE	Average	3.530	7.857	2.379	1.291	0.526	2.168
	Std. dev	4.251	6.079	1.602	1.028	0.155	0.821
	Maximum	20.340	25.487	6.090	4.560	0.811	4.782
	Minimum	0.015	0.668	0.236	0.076	0.209	1.076
Weathered PE / WPE	Average	4.116	8.276	2.684	1.463	0.603	2.059
	Std. dev	4.462	5.097	1.505	1,015	0.164	0.698
	Maximum	19.552	18.580	5.829	4.271	0.844	4.231
	Minimum	0.029	0.936	0.335	0.112	0.161	1.105
Weathered-washed PE / WWPE	Average	3.754	7.740	2.531	1.420	0.640	1.886
	Std. dev	3.723	4.866	1.524	0.927	0.168	0.556
	Maximum	12.852	19.482	6.154	3.397	0.963	3.869
	Minimum	0.050	0.870	0.302	0.211	0.255	1.142

Note: Major and minor axes are the lengths of the major and minor axes of the smallest fit ellipse that can enclose a particle; Aspect ratio is the ratio of the major and minor axes; Circularity is a measure of how closely the particle has a circular geometry and is calculated as 4π x Area / $(\text{perimeter})^2$.

Table 3.5. Summary data for PLA fibres (PLAF) particle size (n = 50).

MPs type		Length (mm)	Diameter (mm)
Pristine PLA fibre / PPLAF	**Average**	2.170	0.012
	Std. dev	0.749	0.0006
	Maximum	5.254	0.013
	Minimum	0.368	0.010
Weathered PLA fibre / WPLAF	**Average**	1.598	0.012
	Std. dev	0.895	0.0006
	Maximum	4.516	0.013
	Minimum	0.337	0.010
Weathered-washed PLA fibre / WWPLAF	**Average**	1.426	0.012
	Std. dev	0.825	0.0006
	Maximum	4.615	0.013
	Minimum	0.288	0.010

Table 3.6. Summary data for PLA pieces (PLAP) particle size and shape descriptors (n = 50).

MPs type		Area (mm^2)	Perimeter (mm)	Major axis (mm)	Minor axis (mm)	Circularity	Aspect ratio
Pristine PLA piece / PPLAP	**Average**	2.518	6.273	1.941	1.605	0.7803	1.222
	Std. dev	0.886	1.164	0.366	0.314	0.0367	0.160
	Maximum	4.389	8.251	2.602	2.192	0.8600	1.633
	Minimum	0.830	3.669	1.292	0.804	0.700	1.015
Weathered PLA piece / WPLAP	**Average**	3.345	8.437	2.985	1.451	0.586	2.366
	Std. dev	1.128	1.330	0.708	0.448	0.136	1.360
	Maximum	5.376	10.969	4.518	2.324	0.785	7.419
	Minimum	1.211	5.373	1.660	0.600	0.309	1.053
Weathered-washed PLA piece / WWPLAP	**Average**	3.048	7.887	2.841	1.357	0.604	2.231
	Std. dev	1.016	1.402	0.670	0.357	0.122	0.769
	Maximum	4.956	10.526	4.443	2.120	0.781	4.208
	Minimum	0.443	3.847	1.342	0.420	0.376	1.138

Note: Major and minor axes are the lengths of the major and minor axes of the smallest fit ellipse that can enclose a particle; Aspect ratio is the ratio of the major and minor axes; Circularity is a measure of how closely the particle has a circular geometry and is calculated as 4π x Area / $(\text{perimeter})^2$.

3.4.3.2 Specific surface area (SSA)

Ideally SSA would be measured using gas adsorption and application of the BET isotherm (Brunauer et al, 1938). The BET surface area of MPs samples was attempted to be measured using a Micromeritics Porosimeters ASAP 2020 & Tristar with N_2 as the adsorbate following over-night degassing at 60 °C under a constant flow of N_2 using a VacPrep 061 (this temperature was chosen to avoid any melting of the MPs). Only 0.0182 g for PE, 0.0760 g for PLAF and 0.0182 g for PLAP of materials were able to be added into the sample before they were full. Unfortunately this mass of material was insufficient for measuring the surface area to a

meaningful level of accuracy or precision.

Therefore geometric SSAs were calculated instead, i.e. the particles were assumed to have regular geometries. Such calculations will be approximations as they assume smooth surfaced particles and do not account for the surface roughness which is visible on the MPs (Figs. 3.6, 3.7, 3.8). For the PE and PLAP a cuboidal geometry was assumed using average areas obtained from ImageJ (Tables 3.4, 3.6). Furthermore the thickness of the sheets was measured using calipers as 0.0012 and 0.005 mm for PE and PLA respectively. Their thicknesses were several orders of magnitude less than the area of the particles (Table 3.4, 3.6) and therefore it was assumed that total surface area of the particles was equal to two times the ImageJ measured surface (the top and bottom of the particles, 2A; Fig. 3.10) and that the edges contributed negligible surface area (Fig. 3.10). Particle volume (V) was calculated as the surface area (A) multiplied by the thickness (l). Mass of particles was calculated as the volume (V) multiplied by the measured density (ρ_{MPs}). Specific surface area was then calculated as total surface (2A) divided by mass ($Al\rho_{MPs}$) = 2 / $l\rho_{MPs}$. In contrast, to calculate the SSAs of the PLAF, a cylindrical particle shape was assumed and the mean particle length and diameter as determined using ImageJ was used in calculations (Table 3.5). Densities used in this calculations were determined using pycnometers (Section 3.4.1.2). The calculated SSAs were 1.82, 0.217 and 0.433 m^2 / g for the PE, PLAP and PLAF respectively. The presence of biofilms on the weathered material and the increased surface roughness of the weathered and the weathered and washed particles should result in higher SSA than for the pristine materials but our geometric assumptions can not take this into account.

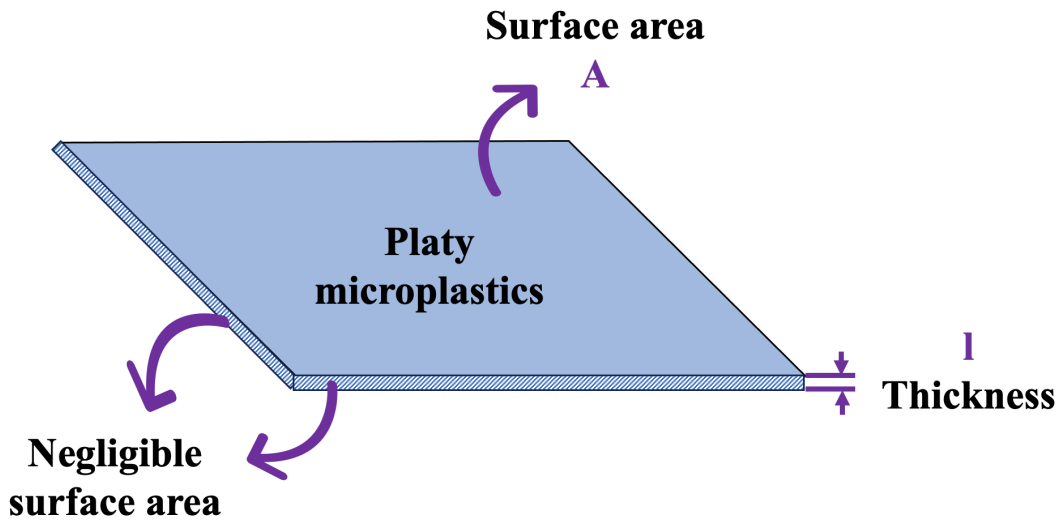


Fig. 3.10. Platy MPs (PE and PLAP) diagram for calculating their specific surface area.

3.4.3.3 Biofilm adhere on MPs

The crystal violet staining method was used to determine the amount of biofilm present on the surface of the weathered MPs through a modification of the method of Bhagwat et al. (2021). Four pieces of MPs (same batch of nine different types of particles as used in the adsorption experiments, Chapter 6) were placed into a sterile Petri dish and carefully rinsed three times with 2 mL of deionised water. The particles were allowed to dry at room temperature for 45 min, and then 0.5 mL of 1 % (w / v) crystal violet solution was added to submerge the MPs. After a further 45 min at room temperature the excess dye solution was carefully discarded. The microplastic samples were washed 3 times with 5 mL of deionised water, allowed to dry for 45 min at room temperature and then placed in a 2 mL polypropylene centrifuge tube to which 1 mL of 95 % ethanol solution was added and left to stand for 10 min. The solution was transferred to a cuvette, and the absorbance was measured at 595 nm using a Shimadzu UV - 1800 UV / Visible Scanning Spectrophotometer to give a measure of the amount of biofilm present (OD_{595}). MP-free treatments of the 95 % ethanol solution served as a blank control to zero the spectrophotometer. The pristine microplastic pieces also served as a control group. All extractions were carried out on triplicate subsamples of MPs. After the extractions the MPs were thoroughly rinsed with deionised water to remove any residual chemicals, air-dried, and weighed with a 5 decimal place balance (Kern, ABT 100-5M, WB14G0048). The surface areas

of the particles were determined as described above before staining (Table 3.7). The amount of biofilm detected was normalised to the mass of MP (g) and the MP surface area (m^2).

Table 3.7. Total surface area of the MP particles (four particles for each replicate) used in crystal violet staining method.

MPs type	Surface area (mm^2)		
	Replicate a	Replicate b	Replicate c
pristine PE / PPE	16.245	15.95	15.94
pristine PLA fibres / PPLAF	7.141	7.073	7.096
pristine PLA pieces / PPLAP	10.46	10.46	9.944
weathered PE / WPE	11.59	12.40	12.37
weathered PLA fibres / WPLAF	8.557	8.408	8.525
weathered PLA pieces / WPLAP	10.01	10.47	10.72
washed weathered PE - washed / WWPE	11.81	11.72	11.65
washed weathered PLA fibres-washed / WWPLAF	9.963	10.37	10.32
washed weathered PLA pieces-washed / WWPLAP	10.49	9.674	10.05

3.4.3.4 MPs Cd background concentration

PE and PLA MPs were digested following the above soil digestion procedures (Section 3.2.7; British Standard BS 7755, 1995). Instead of using 1.5 g air-dried soil, an amount of MPs were added to the digestion tube until MPs were no longer submerged by digestion solution aqua regia followed by analysis by ICP-OES, and its quality control was calculated (Section 3.6.2). The measured Cd concentrations of solutions were below detection limits (Section 3.6.2.4), indicating Cd of MPs is negligible.

3.5 Cd solution

$Cd(NO_3)_2$ solution made from $Cd(NO_3)_2 \cdot 4H_2O$ (98.5 %, Sigma Aldrich) was used as the Cd source in this study, as Cd-nitrate is more soluble than Cd-chloride and Cd-sulfate (Stevens, et al., 2003; Kubier, et al., 2019). Additionally, in earthworm metal toxicity experiments nitrate

salts are commonly used (Reinecke et al., 2002; Schlich et al., 2013). Previous studies found LC_{50}s for nitrate salt with different cations in earthworm was different (Spurgeon et al., 1994; Žaltauskaitė and Sodienė, 2014; Dada et al., 2016), suggesting the toxicity to earthworms was not directly related to the nitrate (Demuynck et al., 2016). The concentration range of the Cd solution was selected from 0 mg / L up to 600.6 mg / L, so that, when moistened to a moisture content of 50 % (Chapter 5 exposure experiments) or 60 % (Chapter 4 avoidance tests), the soil Cd concentration would be in the range 0 - 100 mg / kg to span the ambient contaminant level of Cd in agricultural lands (Huang et al., 2024; Wang et al., 2015).

3.6 Data analysis and quality control

3.6.1 Statistical analysis

Statistical analysis was carried out using SigmaPlot version 15.0 software, SPSS version 28.0.1.1 (15) and Excel version 16.72. Normality and equal variance were assessed using the Shapiro-Wilks and Brown-Forsythe tests respectively. If data sets have a normal distribution and equal variance or data sets could be transformed using transformations (including log 10 , square, square root, 1/X) to obtain a normal distribution and equal variance, these data sets were then analysed using an analysis of variance (ANOVA) followed by Holm-sidak post hoc tests (Underwood, 1997). If it was not possible to transform data sets to have both a normal distribution and equal variance, these data sets were then analysed using the non-parametric Scheirer Ray Hare tests by ranked data (Holms et al., 2017) followed by Games-Howell post hoc tests (Field, 2013). Details of the statistical analysis are given in each chapter.

3.6.2 Quality control

All solutions, including soil digestions (Chapter 5), earthworm digestions (Chapter 5), soil pore waters (Chapter 5) and solutions from MPs adsorption experiment (Chapter 5 and 6), were analysed for Cd using ICP-OES, and their quality control data were calculated, which are presented in Table 3.8.

Table 3.8. Quality control for Cd analyses of soils, earthworms, pore water and microplastics.

Measurement	Accuracy (%)	CRM	Precision (%)	Detection limit
Cd content of $Cd(NO_3)_2$ amended soils	102.9	Fluka analytical trace metals - loamy sand 4, Lot 020829, CRM036 -50Gb; Cd 86.3 ± 1.66 mg / kg	3.06	0.0014 mg / L equivalent to 0.0130 mg / kg
Cd content of earthworms	104.6 ± 3.0	Mussel tissue No. 0347, ERM - CE278k; Cd 0.336 ± 0.025 mg / kg	1.03 ± 0.46	0.0012 ± 0.0001 mg / L equivalent to 0.048 ± 0.020 mg / kg
Cd content of pore waters	101.2 ± 3.0	In house 0.5 mg / L standard	3.82 ± 2.51	0.0012 ± 0.0002 mg / L
Cd content of solutions from microplastics adsorption experiment	100.4 ± 1.1	In house 0.5 mg / L standard	1.91 ± 0.27	0.0055 ± 0.003 mg / L

3.6.2.1 Certified reference material (CRM)

The CRM for measuring Cd content of $Cd(NO_3)_2$ amended soils was used Fluka analytical trace metals - loamy sand 4, Lot 020829, CRM036 -50G containing Cd 86.3 ± 1.66 mg / kg. The CRM for measuring Cd content of earthworms used mussel tissue No. 0347, ERM - CE278k containing Cd 0.336 ± 0.025 mg / kg. The CRM for measuring Cd content of pore waters and Cd content of solutions from MPs adsorption experiment used in house 0.5 mg / L standard.

3.6.2.2 Accuracy

Accuracy was calculated by dividing the measured CRM concentration by the reported CRM concentration and multiplying by 100. The calculated accuracy was 102.9 % for the soil digestions. The calculated accuracy was 104.6 ± 3 % (n = 7, ± standard deviation) for the earthworm digestions. The calculated accuracy was 101.2 ± 3 % (n = 6, ± standard deviation) for the pore waters. The calculated accuracy was 100.4 ± 1.1 % (n = 3, ± standard deviation)

for the adsorption solutions.

3.6.2.3 Precision

Precision was assessed by measurement of paired samples and expressed as the coefficient of variation (Gill and Ramsey, 1997). Specifically speaking, make duplicate measurements on 10 % of samples that are at least 100 times higher than detection limits (Section 3.6.2.4). Calculate the mean of each pair. Then calculate the modulus of the difference for each pair. Express this difference as percentage of the mean. The median percentage represented coefficient of variance, which was precision.

The calculated precision was 3.06 for the soil digestions. The calculated precision was 1.03 ± 0.46 (n = 7, ± standard deviation) for the earthworm digestions. The calculated precision was 3.82 ± 2.51 (n = 6, ± standard deviation) for the pore waters. The calculated precision was 1.91 ± 0.27 (n = 3, ± standard deviation) for the analysis of for the adsorption solutions.

3.6.2.4 Detection limit (DL)

DL was calculated using the mean value plus six times of the standard deviation of the 10 blank values (Walsh, 1997; Gill, 1997). The calculated DL from the blank standards used for the soil digestions was 0.0014 mg / L, which was converted to 0.09 mg / kg Cd concentration in the soil. The calculated DL from the blank standards used for the earthworm digestions was 0.0012 ± 0.0001 mg / L, which was converted to 0.048 ± 0.020 mg / kg Cd concentration in the earthworm. The calculated DL from the blank standards used for the pore waters was 0.0012 ± 0.0002 mg / L. The calculated DL from the blank standards used for the adsorption solutions was 0.0055 ± 0.003 mg / L.

The values that were below the DL were set to DL divided by the square root of 2, and then these converted valued were used in further statistical analysis (Croghan and Egeghy, 2003).

3.7 Ethical consideration

The anecic earthworms *L. terrestris* were involved for this study in the avoidance test (Chapter

4) and the exposure experiment (Chapter 5). The code of ethical practice and principles for the animal research were considered.

At the end of the avoidance test, there was no need to analyse the earthworms further, so they have been properly disposed of as non-polluted and polluted earthworms. Non-polluted earthworms in all controls (non-treated soils) treatments were released into the environment. polluted earthworms in treated-soils treatments were put into labelled bags and frozen, afterwards technicians disposed of them as medical waste.

During exposure experiments, earthworms died when exposed to the MPs and / or Cd. Additionally at the end of the experiments, alive earthworms were quickly and humanely killed by freezing to further determine Cd and microplastic uptake.

During experiments, as few earthworms as possible were used in order to obtain valid statistical results. Generally earthworms were bought from commercial providers in UK. It is not currently possible to obtain the information that this project provides via a theoretical approach, but obtaining this data could yield useful results that would support management interventions which could reduce the risk of harm to far larger natural populations of earthworms.

4 An investigation into the avoidance of Cd and MPs individually and in combination by the earthworm *Lumbricus terrestris*

4.1 Introduction

This chapter is concerned with avoidance experiments. It begins with a brief review of behavioural ecotoxicology and the use and value of avoidance tests before going on to provide experimental details, results and discussion of an avoidance test pilot study carried out to investigate the impact of combinations of Cd and MPs.

4.1.1 Significance of behavioural ecotoxicology

There is a growing awareness that anthropogenic chemicals are ubiquitous in the environment and these pollutants impact various behaviours of aquatic and terrestrial organisms and pose a serious threat to human health (Little, 1990; Zala and Penn, 2004; Rodríguez-Jorquera et al., 2017; Rodríguez-Estival and Mateo, 2019). The disturbed behaviour of organisms therefore can be used to evaluate how these contaminants affect the health of organisms, populations and ecosystems, contributing to the understanding of biological conservation and toxicology (Bünemann et al., 2006; Hellou, 2011; Tourinho et al., 2012; Peterson et al., 2017). Behavioural ecotoxicology determines the potential impacts of the contaminants on organisms by studying their behavioural responses (Peterson et al., 2017; Araújo and Blasco, 2019; Grunst et al., 2023). As concentrations of contaminants that impact behaviour can be far lower than those that impact mortality (Natal-da-Luz et al., 2008; Ge et al., 2018), regulating pollutants using behavioural ecotoxicology has the potential to safeguard the environment more effectively than other forms of ecotoxicology (Ågerstrand et al., 2020).

4.1.2 Laboratory-based research on behavioural ecotoxicology

In behavioural ecotoxicology, laboratory-based studies have been widely used to investigate the behaviour of organisms. Experimental research in the laboratory can isolate and control the multitude of interacting variables that exist in complex and changing natural systems, and simplify tracking of individual behaviour (Campbell et al., 2009; Ford et al., 2021). Furthermore, behavioural responses measured in the laboratory can be a useful way to predict the behaviour of organisms in the field (Spurgeon and Hopkin, 1995; Ho et al., 2009). However, the most development of laboratory-based research on behavioural ecotoxicology has been in aquatic ecosystems rather than terrestrial ecosystems probably because soil environments are more complex than aquatic environments (Likens and Bormann, 1974; Suedel et al., 1994; Bardgett et al., 2001; Edwards, 2002). Therefore, more studies on soil ecotoxicological risk assessment using behavioural endpoints are warranted.

4.1.3 Use of earthworms in soil behavioural ecotoxicology

In soil ecosystems, earthworms are the largest component of animal biomass and are typical "ecosystem engineers" (Blouin et al., 2013; Sivakumar, 2015). Furthermore, the sensitive response of earthworms to contaminants, makes them one of the most suitable animals for use as bioindicators to assess soil quality and risk assessment (Nahmani et al., 2003; Loureiro et al., 2005; Lukkari et al., 2005). In soil ecotoxicology, earthworms therefore are widely used (Schaefer, 2003; Lowe and Butt, 2007; Lee et al., 2008). Furthermore, for these reasons earthworms have been adopted as model species or as standard test organisms for evaluating the toxicity of contaminants in international ecotoxicological testing standards (ISO, 1993; ISO, 1998; OECD, 1984; OECD, 2004). Contaminants in soil can affect the behaviour of earthworms, such as avoidance behaviour, burrowing behaviour, secretion of mucus and cast production (Yasmin and D' Souza, 2010; Roubalová et al., 2015; Sivakumar, 2015; Shi et al., 2017; Gainer et al., 2022; Khaldoon et al., 2022; Zhao et al., 2022). Therefore, earthworms can be used as an appropriate bioindicator in soil behavioural ecotoxicology tests.

4.1.4 Earthworms avoidance behaviour assessing soil ecotoxicology

An avoidance behaviour test used to assess soil ecotoxicology is a test where organisms have the ability to choose or avoid a soil (Loureiro et al., 2005). Previous research reported that avoidance has been a more sensitive indicator of environmental impacts than lethal responses (West and Ankley, 1998; Lopes et al., 2004; Loureiro et al., 2005). Avoidance test with earthworms is a standard test and a sensitive tool for early detection of potential effects of exposure to certain contaminants (ISO, 2008). The presence of chemoreceptors on earthworm body make them highly sensitive to contaminants in their environment, allowing them to avoid toxic environments (Lukkari et al., 2005; Curry and Schmidt, 2007; Udovic and Lestan, 2010). The avoidance behaviour of earthworms is a survival mechanism that prevents their death by reducing exposure to the toxic pollutants (Gainer et al., 2022). If earthworms avoid the particular area this could result in overcrowding, causing intra-specific competition due to restricted resource and living space (Mathieu et al., 2010). Intense competition could further lead to a decrease in earthworm populations (Blouin et al., 2013). Therefore avoidance test has been considered as a valuable tool in the screening evaluation of soil contaminations when assessing ecological risks. Furthermore, they are relatively quick compared to more traditional acute and chronic ecotoxicology tests (Lukkari et al., 2005; Moreira‐Santos et al., 2008).

In laboratory-based avoidance test, the earthworms are exposed to a vessel (box or petri dish) divided into two sections. One section is filled with uncontaminated soil and the other is filled with the same amount of test soils (treated with pollutants) (Loureiro et al., 2009; Morcillo et

al., 2013; Zhang et al., 2013; Yang et al., 2018). Typical behaviours observed in avoidance tests include avoidance, attraction or failure to avoid (non-avoidance or non-attraction) (Gainer et al., 2022). An avoidance response of earthworm greater than 80 % indicates that the soil is severely contaminated and not suitable for habitation (Hund-Rinke et al., 2003). Lukkari and Haimi, (2005) showed that three ecologically different earthworm species (*Aporrectodea tuberculata*, *Lumbricus rubellus*, and *Dendrobaena octaedra*) clearly avoided soil polluted with Cd and Zn, however, the sensitivity between these species was different: *D. octaedra* was the most sensitive species, whereas *L. rubellus* was the least sensitive species. The most commonly used earthworm in avoidance tests is *Eisenia fetida* though this species was found to be less sensitive to the contaminants than *Metaphire guillelmi*, *L. rubellus* and *Aporrectodea caliginosa* (Chen et al., 2017; Spurgeon et al., 2000). As earthworms have been shown to avoid contaminant-bearing soils, this shows that earthworm avoidance behaviour can be used as an ecologically parameter to assess the toxicity of pollutants in soil ecotoxicology. It is also important to consider the specific species to be used in the earthworm avoidance tests.

4.1.5 MPs contaminants affecting earthworm behaviour

MPs are a newly recognised type of pollutant, which can pose a risk to organisms (Sections 1.2.1 and 2.3.7) and also have been detected throughout the human body (Blackburn et al., 2022; Wu et al., 2022; Li and Liu, 2024). Due to the small particle size of MPs, they are able to be ingested by earthworms so that could pose the risks to earthworms (Lahive et al., 2022; Hattab et al., 2024; Parolini et al., 2024). MPs were found to have negative impacts on earthworm behaviour such as survival, growth and reproduction (Zhang et al., 2020a; Chang et al., 2022; Cui et al., 2022). Furthermore, Ding et al., (2021) reported that earthworms *E. fetida* clearly showed avoidance behaviour at MPs (PE, PLA, and polypropylene carbonate PPC) concentrations above 40 g / kg (equivalent to 4 % w / w), and afterwards increased avoidance with the increasing concentrations of these MPs. MPs can cause burns and lesions on the surface of earthworms (Baeza et al., 2020) which may be the reason of their avoidance behaviour (Cui et al., 2022). However, previous research found that there was no avoidance behaviour of earthworms *Lumbricus terrestris* at the MPs (PP, PE, polystyrene PS, and polyethylene terephthalate PET) concentrations of 2.5, 5, and 7 % w / w (Baeza et al., 2020).

The majority of the research on earthworm behaviour has focused on purchased MPs (creating more artificial scenarios and decreasing the ecological relevance), while avoidance experiments using field-relevant plastics types (e.g. MPs derived from plastic mulch films) have not been carried out. To date only two existing studies have investigated the impacts of plastic films-derived MPs on other earthworm behaviour. Zhang et al., (2018a) showed that field-weathered biodegradable plastic mulch pieces were partly ingested by earthworms, *L. terrestris*, but non-

biodegradable PE mulch pieces were not. Forsell et al., (2024) found biodegradable PBAT plastic films-derived MPs significantly increased earthworm *Eisenia andrei* growth, whereas PE plastic films-derived MPs had no significant impacts on earthworm growth (although a trend of decreasing growth with increasing concentration was detected), but no possible explanation reported. However, no research on the impacts of plastic films-derived MPs on earthworm avoidance was found.

4.1.6 Cd contaminant affecting earthworm behaviour

In addition to MPs, Cd is another major contaminant in agricultural ecosystems (Sections 1.3 and 2.4.1). Previous studies found that Cd caused histological alterations (such as oozing of coelomic fluid, clitellar swelling and cuticle rupture) of earthworms and Cd bioaccumulated in earthworms via direct skin contact or ingestion (Sivakumar, 2015; Yadav et al., 2023). Cd can impact various behaviour of earthworms such as decrease in burrowing behaviour, feeding rate, survival, growth and reproduction (Lapinski and Rosciszewska, 2008; Garg et al., 2009; Žaltauskaitė and Sodienė, 2010; Domínguez-Crespo et al., 2012; Duan et al., 2020). Metals not only leads to above behavioural and histological changes in earthworms but previous research also showed that earthworms avoided metal polluted-soils (Shoults-Wilson et al., 2011; Yang et al., 2018; Aguzie et al., 2021). Only two published studies have investigated the avoidance of Cd by earthworm *E. fetida* but using different experimental media, and their findings were not consistent (Rodriguez et al., 2013; Demuynck et al., 2016). Demuynck et al., (2016) found earthworm *E. fetida* exhibited significant avoidance behaviour when exposure to 30 mg / L concentrations of Cd using filter paper tests, whereas Rodriguez et al., (2013) found no significant avoidance behaviour of earthworm *E. fetida* when exposure to soils polluted with Cd (8, 16, 48 and 150 mg / kg) suggesting the toxicity of Cd might lead to a decrease in the locomotor ability of earthworms.

4.1.7 MPs-Cd interactions affecting earthworm behaviour

Once in the environment, the interactions between MPs and Cd occur (Wen et al., 2018) and these interactions might influence the impacts of the MPs and Cd on earthworms (Lian et al., 2020). Laboratory exposures, using the earthworm *E. fetida* suggested the toxic effects of the combination of conventional MPs and Cd increased relative to the effects of either MPs or Cd alone. Specifically, Huang et al., (2021) compared Cd-only exposure and MPs-Cd combination exposure and found that both 2 mg / kg and 10 mg / kg Cd led to greater weight loss of earthworm with the addition of 7 - 30 % PE than exposure to Cd only. Similarly, Zhou et al., (2020) found that the combined impacts of 300 - 9000 mg / kg (equivalent to 0.03 - 0.9 % w / w) PP and 8 mg / kg Cd induced higher oxidative damage on earthworms than PP or Cd only. Simple calculations (the effects of PP-only and Cd-only on oxidative damage in earthworms

were summed and then compared to the combined effects of PP and Cd) suggest that the combined impacts of PP and Cd had an antagonistic impact on oxidative damage on earthworms in Zhou et al., (2020) study. Huang et al., (2021) and Zhou et al., (2020) indicated their findings were probably due to the increase in Cd availability in the soil and Cd accumulation in earthworms with the presence of MPs rather than the additional toxic impact of MPs, although a mechanism for this was not presented. However, Liang et al. (2022) found that effects were time dependent with exposure to 0 - 2000 mg / kg (equivalent to 0 - 0.2 % w / w) PE and 0 - 250 mg / kg Cd having an antagonistic impact on oxidative damage of earthworm after 10 days but a synergistic impact after 30 days, but no explanation was presented. In terms of avoidance behaviour of earthworms affected by MPs-Cd interactions, 30 % (extreme high) concentrations of PE MPs increased the avoidance rates of *E. fetida* caused by both 2 mg / kg and 10 mg / kg Cd, but 20 % (extreme high) of PE MPs in the presence of the same Cd concentrations decreased avoidance rates, possibly attributed to the great soil porosity and permeability provided by MPs under short-term exposure (Huang et al., 2021).

4.1.8 Rationale, aims and hypotheses

To date, the existing studies on avoidance behaviour of earthworms to MPs indicate that there is potentially avoidance behaviour, however, there is no research to investigate the impacts of plastic films-derived MPs on earthworm avoidance (Section 4.1.5). Furthermore, only two published studies on earthworm avoidance of Cd have only used *E. fetida* and their results were inconsistent (Section 4.1.6). It is realistic to assume an interaction between MPs and Cd and that their interactions further affect earthworm avoidance (Section 4.1.7). However, there are barely any studies and even fewer with environmentally relevant species such as earthworm *L. terrestris* (Sections 1.4, 2.4.4 and 3.3). Therefore, the experiments in this chapter are warranted to understand how MPs derived from plastic mulches might impact earthworm *L. terrestris* avoidance and the impacts on earthworm *L. terrestris* of interactions between Cd and MPs, particularly under realistic concentrations of both MPs and Cd. Additionally, higher levels of Cd and MPs should also be considered to mimic the accumulated concentrations over decades. Studies that compare the differences in combined impacts mainly focused on the most commonly used conventional PE MPs. Studies on biodegradable PLA MPs interacting with Cd on earthworms avoidance behaviour are lacking. As relatively quick experiments, these are appropriate pilot experiments to give an initial idea of whether there might be differences in these impacts of MPs generated from conventional PE vs biodegradable PLA plastic mulches. It is therefore reasonable to investigate the single and combined effects of plastic mulches-derived MPs and Cd on the response of soil organisms, particularly under realistic concentrations of both MPs and Cd.

This avoidance chapter therefore aimed to compare the avoidance behaviour of earthworms maintained in soil treated with $Cd(NO_3)_2$ solution and / or two different types of plastic mulches-generated MPs (conventional PE and biodegradable PLA). To achieve this the earthworms *L. terrestris* were exposed to either or both MPs (PE or PLA) and Cd for 3 days (Section 4.1.4); earthworm avoidance rate was then assessed. Based on the previous research, we hypothesised that (1) Cd in soil would cause earthworm avoidance, (2) increasing Cd concentrations would lead to an increase in earthworm avoidance and (3) the addition of MPs (both PLA and PE MPs) would decrease the avoidance of earthworms to Cd.

4.2 Materials and Methods

4.2.1 Test organism

In this avoidance study, the anecic earthworm *L. terrestris* was chosen as the test species. The justification of the use of this species are presented in Sections 1.4 and 3.3.

4.2.2 Test soil

The source and characteristics of the purchased natural topsoil are provided in Section 3.2.

4.2.3 Test chemicals

The source of the conventional PE and biodegradable PLA plastic films used as the mulches in agriculture is provided in Section 3.4.1. The identification and functional groups of the PE and PLA samples are presented in Sections 3.4.1.1 and 3.4.1.2, respectively. The MPs were generated from PE and PLA plastic films (Section 3.4.2). The size and shape of PE and PLA MPs were identified (Section 3.4.3.1) and their specific surface areas (SSAs) were calculated (Section 3.4.3.2). The avoidance tests were conducted at an environmentally relevant exposure level of 0.30 % MPs by mass, as reported in previous studies (Zhou et al., 2020a; Ding et al., 2021).

$Cd(NO_3)_2$ solution was used as the Cd source in this avoidance experiment (Section 3.5). A 0.6006 g / L stock solution of $Cd(NO_3)_2$ was prepared, which was equivalent to the soil Cd concentrations of 100 mg / kg when 182.75 g stock solution was added into 400 g air dried 2 mm sieved soil which would raise the soil to 60 % of its water holding capacity (WHC). The stock solution was diluted in a gradient to different nominal soil Cd concentrations of 10, 1.0 and 0.1 mg / kg. The range of five treatments, including the four Cd solution concentrations (0.1, 1.0, 10 and 100 mg / kg) and a control Cd concentration (0 mg / kg), was selected to span the ambient contaminated level of Cd in agricultural lands (Wang et al., 2015; Xu et al., 2017; Huang et al., 2024).

4.2.4 Avoidance test

In the avoidance test, a split mesocosm approach was used (Fig. 4.1). Treatments comprised Cd-only (0, 0.1, 1.0, 10 and 100 mg / kg) and MP-only treatments (0 and 0.30 % w / w) and then Cd-MP treatments with combinations of these same concentrations of Cd and MP. Test chambers involved separating a rectangular box (18 × 18 × 7 cm depth) into two equal sections using a removable plastic divider (Hund-Rinke and Wiechering, 2001; ISO, 2008; Morcillo et al., 2013).

182.75 g deionised water was added to moisten 400 g dry soil to achieve 60 % of the WHC (Elliston and Oliver, 2020), which was then added to the half of the container that was the unamended control side (with no MPs and Cd). For the test side including single contaminated and co-existence MPs + Cd contaminated soils, MPs (PE or PLA) were thoroughly mixed and homogenised by spatula through the 400 g uncontaminated dry soils. MPs were mixed so that they were homogeneously distributed within the soil because ploughing and harvesting are expected to homogenise the distribution of MPs through soil present in the plough layer (Ng et al., 2018). The mixture was then moistened with 182.75 g Cd solution or only deionised water to get the moistened soil, which was placed in the other half of the container that was the treated test side (with MPs and / or Cd). Treatments are referred to in the format XYCdZ where X identifies the plastic type (PE or PLA), Y the plastic concentration (0 or 0.3 wt %) and Z the nominal Cd concentration (0 - 100 mg / kg), e.g. PLA0.3Cd100 indicates the 0.3 wt % PLA treatment with the Cd concentration of 100 mg / kg. Additionally, the format for the Cd-only treatments (Y = 0) is CdZ and the format for the MPs-only treatments (Z= 0) is XY. Treatments with no plastic and Cd (Y = 0 and Z = 0) are control treatments. All treatments were allowed to equilibrate for one day under the same conditions as those used for the avoidance experiments. Four replications were set for each treatment. The separator was removed, and six earthworms were quickly placed in the middle of each container, which was then incubated at 12 °C in a controlled temperature (CT) room for 72 h (EC, 2004). After 72 h exposure, the test and control soil was separated again by quickly inserting the divider. The contents of each test compartment were gently removed with a spatula and placed into a container, and the number of live and dead earthworms was immediately counted and recorded. Avoidance was calculated as follows:

$$Avoidance\ (\%) = \frac{no.\,earthworms\ in\ control - no.\,earthworms\ in\ test}{total\ no.\,earthwoms} \times 100$$

where, “no. earthworms in control” is the number of live earthworms found in the control soil at the end of test; “no. earthworms in test” is the number of live earthworms found in the amended soil at the end of test; “total no. earthworms” is the total number of live earthworms found in all compartments at the end of test.

Given mortality might be observed in some treatments, earthworms were touched gently on their anterior end with a spatula and were regarded as dead if they showed no response (Langdon et al., 1999). Missing earthworms were counted as dead (EC, 2004). If the earthworms were partly on the control side and partly on the test side, the earthworms were allocated to a side in direct percentage to the length of them on each side of the divide.

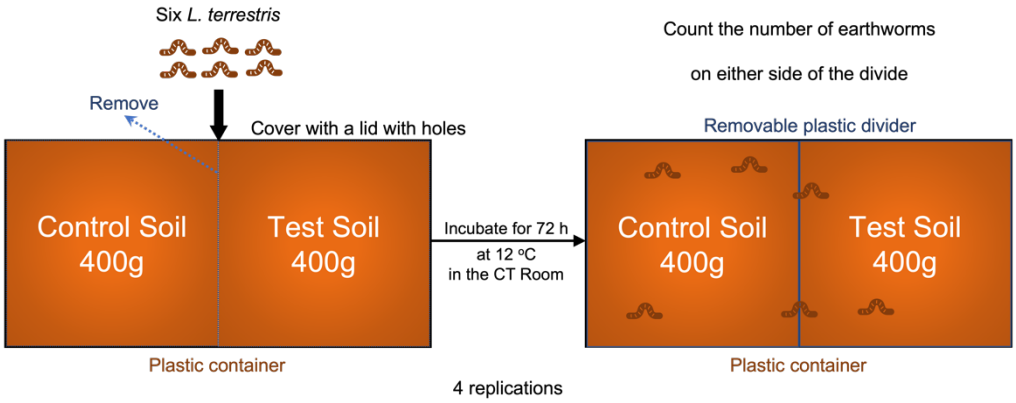


Fig. 4.1. Experimental design of the avoidance test setup illustration.

4.2.5 Statistical analysis

Current guidance on the interpretation of avoidance tests is that soils are considered to have limited habitat function if more than 80 % of earthworms are in the control soil (or less than 20 % of earthworms are in the test soil) (Hund-Rinke and Wiechering, 2001; IOS, 2004). Given a total of 6 earthworms, it would mean that 4.8 (6 × 80 %) individuals are in the control side or 1.2 (6 × 20 %) are individuals in the test side, corresponding to the 60 % avoidance, which is the trigger value of indication that contaminated soil has an impact on earthworm avoidance.

A 2-way analysis of variance (ANOVA) followed by Holm-Sidak post hoc test was run using SigmaPlot version 14.5 software, with Cd concentrations (0, 0.1, 1.0, 10 and 100 mg / kg) and plastic types (non-MPs, PE and PLA) as factors and avoidance exhibited by earthworm as the variable. Normality and equal variance were assessed using the Shapiro-Wilks and Brown-Forsythe tests respectively. Of the data investigated, the avoidance data sets were normally distributed ($P = 0.739$) and had equal variance ($P = 0.099$). However, the mortality experienced by earthworm was normally distributed ($P = 0.438$) but did not have equal variance ($P \leq 0.05$), in addition, it was not possible to transform the mortality data using log 10 , square, square root and 1/X transformation to have equal variance. Thus, the non-parametric 2 way Scheirer Ray Hare test (Holmes et al., 2016) followed by Games - Howell post hoc test was used (Field, 2013). Earthworm mortality data were ranked in Excel version 16.72 software, and then 2 way ANOVA was run using SigmaPlot version 14.5 software with these ranked data. The Scheirer

Ray Hare H factors were calculated using the sum of squares and total sum of mean variances, and significance P values were calculated using the Scheirer Ray Hare H factors and the degrees of freedom. The statistic results are presented in the appendix - Chapter 4.

Independent t tests were used to compare the number of earthworm (both dead and alive) in the control soil and in the test soil for individual treatments.

4. 3 Results

By the end of the experiment there was a single unaccounted for earthworm from a replicate soil of each of the Cd0.1, PE0.3Cd10, PE0.3Cd100 and PLA0.3Cd0.1 treatments (Table 4.1). For all four missing earthworms, it was uncertain whether they had died or simply escaped during the experimental period, so they were excluded from the calculation of mortality and avoidance rate.

4.3.1 Earthworm mortality

Earthworm mortality was observed in all four replicates of Cd100 and PE0.3Cd100, three replicates of PLA0.3Cd100 and a single replicate of PE0.3Cd1 and PLA0.3Cd10 treatments by the end of the experiments (Table 4.1). Two way Scheirer Ray Hare Test (Table S4.1) indicated a significant difference in mortality with Cd concentration ($H_{4,45}$ = 48.7, P ≤ 0.001, 2 way Scheirer Ray Hare Test) but no significant difference with MPs type ($H_{2,45}$ = 0.718, P = 0.689, 2 way Scheirer Ray Hare Test) and no significant interaction between the two ($H_{8,45}$ = 2.17, P = 0.975, 2 way Scheirer Ray Hare Test).

Mortality of earthworms was significantly greater in the Cd100 treatments relative to the other Cd treatments (0, 0.1, 1.0 and 10 mg / kg, Table 4.1; P ≤ 0.001, 2 way Scheirer Ray Hare Test). Mortality of 100 mg / kg Cd treatments were 83.3 ± 23.6 % (n = 4, ± standard deviation) with no MPs added, 29.2 ± 21.0 % (n = 4, ± standard deviation) for PE and 54.2 ± 28.5 % (n = 4, ± standard deviation) for PLA.

The majority of dead earthworms were found on the control soil side in individual tests (Table 4.1). Additionally there were significantly more dead earthworms in the control side than test side at the Cd concentrations of 100 mg / kg with or without MPs (t_{11} = 2.74, P = 0.019, t test) (Table 4.1).

Table 4.1. Number of live / dead earthworms found on each side of the test containers and calculated mortality and avoidance rate at the end of avoidance test. Mortality was represented by percentage of total dead earthworms found in both control and test soils. A positive (+) avoidance response indicates avoidance of the chemical contaminated test soil and a negative (-) avoidance response indicates a non-response (or attraction) to the chemical contaminated test soil.

Treatments		**Number of earthworms in control soil**		**Number of earthworm in test soil**		**Number of missing earthworms**	**Mortality (%)**	**Avoidance (%)**
		Live	**Dead**	**Live**	**Dead**			
control	1-a	3	0	3	0		0	0
	1-b	3.6	0	2.4	0		0	20
	1-c	3	0	3	0		0	0
	1-d	4	0	2	0		0	33
PE0.3	2-a	5	0	1	0		0	67
	2-b	4	0	2	0		0	33
	2-c	5.4	0	0.6	0		0	80
	2-d	4	0	2	0		0	33
PLA0.3	3-a	5	0	1	0		0	67
	3-b	3	0	3	0		0	0
	3-c	2.5	0	3.5	0		0	-17
	3-d	4	0	2	0		0	33
Cd0.1	4-a	3.5	0	2.5	0		0	17
	4-b	3.5	0	2.5	0		0	17
	4-c	5	0	1	0		0	67
	4-d	5	0	0	0	1	0	100
Cd1	5-a	4	0	2	0		0	33
	5-b	3	0	3	0		0	0
	5-c	4	0	2	0		0	33
	5-d	4.5	0	1.5	0		0	50
Cd10	6-a	4.5	0	1.5	0		0	50
	6-b	4	0	2	0		0	33
	6-c	5	0	1	0		0	67
	6-d	4.6	0	1.4	0		0	56
Cd100	7-a	0	5.3	0	0.7		100	/
	7-b	2	2	1	1		50	33

	7-c	0	6	0	0		100	/
	7-d	0.5	5	0.5	0		83	0
PE0.3Cd0.1	8-a	3	0	3	0		0	0
	8-b	4	0	2	0		0	33
	8-c	4	0	2	0		0	33
	8-d	4.4	0	1.6	0		0	46
PE0.3Cd1	9-a	1.4	1	3.6	0		17	-47
	9-b	4.6	0	1.4	0		0	56
	9-c	3	0	3	0		0	0
	9-d	4.4	0	1.6	0		0	46
PE0.3Cd10	10-a	3	0	3	0		0	0
	10-b	4	0	2	0		0	33
	10-c	2.4	0	2.6	0	1	0	-10
	10-d	4.4	0	1.6	0		0	46
PE0.3Cd100	11-a	2.4	0	3.6	0		0	-22
	11-b	0	1	3	2		50	-100
	11-c	1.5	1.5	2.5	0.5		33	-25
	11-d	0.4	2	2.6	0	1	33	-44
PLA0.3Cd0.1	12-a	4.4	0	1.6	0		0	46
	12-b	4	0	2	0		0	33
	12-c	4	0	4	0		0	33
	12-d	3	0	2	0	1	0	20
PLA0.3Cd1	13-a	4.75	0	1.25	0		0	58
	13-b	5	0	1	0		0	67
	13-c	5	0	1	0		0	67
	13-d	4.4	0	1.6	0		0	47
PLA0.3Cd10	14-a	4.5	0	0.5	1		17	80
	14-b	6	0	0	0		0	100
	14-c	4.5	0	1.5	0		0	50
	14-d	4.25	0	1.75	0		0	42
PLA0.3Cd100	15-a	2	1	3	0		17	-20
	15-b	1	2.5	2	0.5		50	-33
	15-c	0	3	1	2		83	-100
	15-d	1.25	2.25	0.75	1.75		67	25

4.3.2 Earthworm avoidance

By the end of the experiment all the earthworms were found burrowed down at the bottom of the soils. There was no significant difference in *L. terrestris* distribution in the soil compartments between the two sides of control treatments (control / control) (Table 4.1). Earthworm avoidance is shown in Fig. 4.2. Two-way ANOVA (Table S4.2) indicated significant differences in avoidance with Cd concentrations ($F_{4,43} = 8.520$, $P \leq 0.001$) and MPs types ($F_{2,43} = 3.279$, $P = 0.047$) and a significant interaction between the two ($F_{8,43} = 2.583$, $P = 0.020$).

Despite the 2-way ANOVA results of significant differences with MPs type (but subtle difference, $P = 0.047$) and significant interactions between Cd concentrations and plastic type, post hoc analyses failed to determine significant differences between the plastic treatments (MP-free treatments vs PE-bearing treatments, MP-free treatments vs PLA-bearing treatments and PE-bearing treatments vs PLA-bearing treatments; $P \geq 0.05$, Holm-Sidak post hoc test; Table S4.3) regardless of the presence or absence of Cd.

Differences of avoidance between different Cd concentrations were only present between the 100 mg / kg treatments and the other Cd concentrations ($P \leq 0.001$, Holm-Sidak post hoc test; Table S4.3). Avoidance was significantly less for the 100 mg / kg Cd concentration treatments (considering both the MP-free and MP-bearing treatments) compared to the other Cd concentrations but only in the presence of PE or PLA ($P \leq 0.05$, Holm-Sidak post hoc test; Table S4.3). Furthermore, within the 100 mg / kg treatments avoidance was only significantly different between treatments in the absence of MPs and the presence of PE ($P \leq 0.05$, Holm-Sidak post hoc test; Table S4.3).

In the MP-only treatments, earthworm avoidance was more in the presence than in the absence of MPs and was more in the presence of PE (blue square at Cd0 in Fig. 4.2) than in the presence of PLA (green square at Cd0 in Fig. 4.2), but the differences were not significant ($P = 0.586$, Holm-Sidak post hoc test; Table S4.3). Although earthworm showed avoidance with the presence of MPs, avoidance was less than the 80 % threshold that indicates limited habitat function (dash line in the Fig. 4.2).

In the Cd-only treatments, earthworms showed some avoidance of Cd regardless of the concentrations of Cd (red outlines in Fig. 4.2). However, earthworm avoidance showed no systematic variation with increasing Cd concentrations. Furthermore, by the end of the avoidance test no avoidance reached the 80% critical threshold indicating inhospitable soil (dash line in the Fig. 4.2).

Within the Cd treatments, earthworm avoidance was lower in the presence PE than in the absence of MPs (Fig. 4.2). However, there were significant differences only between PE0.3Cd100 treatments and Cd100 treatments (P = 0.02, Holm-Sidak post hoc test; Table S4.3). Furthermore, in the PE treatments earthworm avoidance generally decreased with increasing Cd concentrations (blue squares at Cd0.1, Cd1, Cd10 and Cd100 in Fig. 4.2). Whereas, in the PLA treatments, earthworm avoidance rate showed no systematic variation with increasing Cd concentrations (green squares at Cd0.1, Cd1, Cd10 and Cd100 in Fig. 4.2). Furthermore, by the end of avoidance test, avoidance of just reaching the 80 % critical threshold of earthworms was observed at a concentration of 10 mg / kg and 1 mg / kg Cd of soil with the presence of PLA (dash line in Fig. 4.2).

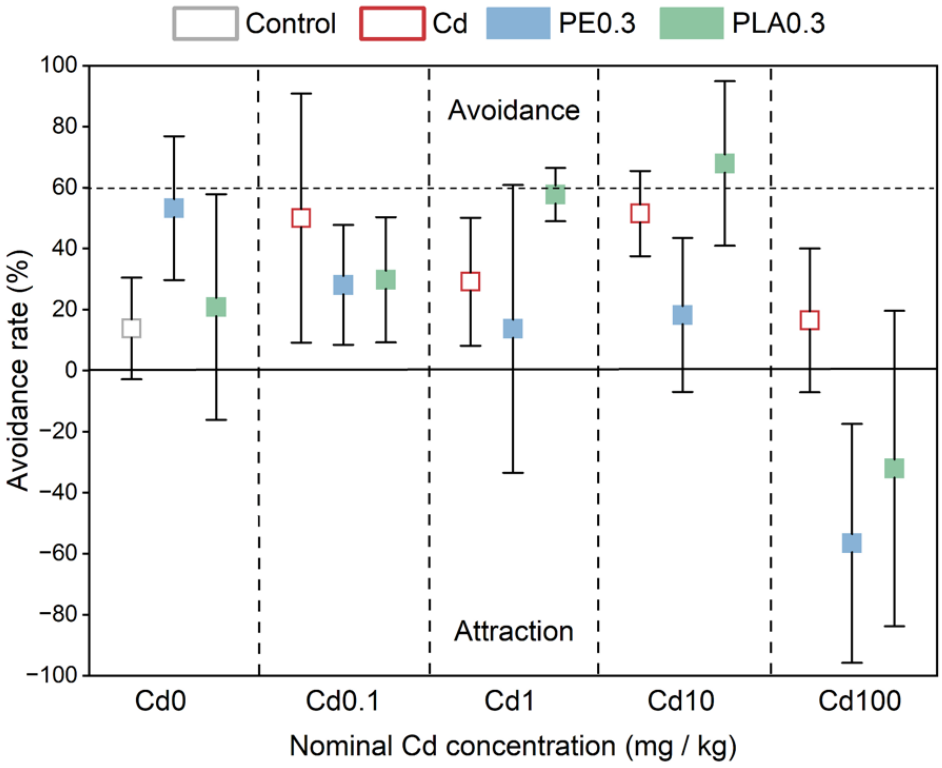


Fig. 4.2. Avoidance (%) of earthworms in the combined MP and Cd treatments (control = 0 % w /w MPs and 0 mg / kg Cd; PE0.3 = 0.3 % w / w PE MPs; PLA0.3 = 0.3 % w / w PLA MPs; Cd0.1 = 0.1 mg / kg Cd; Cd10 = 10 mg / kg Cd; Cd100 = 100 mg / kg Cd) after 72 h avoidance test. Results are mean ± standard deviation, n = 4. Grey outline represent control treatments, red outlines represent Cd-only treatments, blue squares represent with the presence of PE treatments, green squares represent with the presence of PLA treatments. Dash line indicates the threshold value of 80 % earthworms, which corresponds to 60 % avoidance, considered as the habitat function limit.

4.4 Discussion

The avoidance tests were pilot studies trying to find the impacts of Cd-only, MPs-only and MPs-Cd interactions on earthworms avoidance; mortality was also observed in some of the tests. The majority of the earthworms died in the 100 mg / kg Cd treatments and there was some avoidance of earthworms at all concentrations of Cd (Table 4.1, Fig. 4.2). Single MP treatments, both PE and PLA, did not have significant impacts on earthworm mortality and avoidance (Table 4.1, Fig. 4.2). Mortality and avoidance of earthworms caused by 100 mg / kg Cd reduced with the presence of MPs, though there were only significantly decrease in avoidance by PE (Fig. 4.2).

4.4.1 Cd-only exposure and earthworms mortality

The majority of earthworms *L. terrestris* died in the 100 mg / kg Cd (in the form of $Cd(NO_3)_2$) treatments (pH = 7.51 ± 0.2) in this study, however, Rodriguez et al., (2013) found no mortality of earthworms *E. fetida* occurred in the Cd-bearing (up to 120 mg / kg Cd in the form of $CdCl_2$) artificial soils (pH = 6.0 ± 0.5; OECD, 1984). This is similar to a study by Žaltauskaitė and Sodienė (2014) who detected the mortality of the earthworm *E. fetida* in soil (pH = 6.0 ± 0.5) was just 23 % when exposure to 250 mg / kg Cd (in the form of $CdCl_2$), indicating either *E. fetida* was less sensitive than *L. terrestris* (Pelosi et al., 2013) and / or soil pH and the speciation of the Cd may have impacts on earthworm mortality (Bradham et al., 2006; Liu et al., 2018). At lower soil pH (previous studies, e.g. Rodriguez et al., 2013; Žaltauskaitė and Sodienė 2014) the concentration of H^+ ions increases, leading to an antagonism between the H^+ and Cd^{2+} and a reduction in the number of available negative charge sites to bind the Cd^{2+} (Spurgeon and Hopkin, 1996; Wu et al., 2020). At the highest Cd concentration (100 mg / kg) the pH of the soil was still 7.5, i.e. neutral, so the mortality observed in this study was not due to a low pH. Furthermore, Zhang and Van Gestel, (2017) indicated there is no significant difference in mortality of earthworms between NO_3^- and Cl^- salts. This suggests that it was differing sensitivity to Cd between species that account for the differences in mortality in the above studies.

Žaltauskaitė and Sodienė (2014) also found when expose to 500 mg / kg Cd earthworm mortality was 50 % after 3 weeks and increased to 97 % after 14 weeks. Zhang et al., (2018b) also recorded that the mortality of *P. guillelmi* and *M. guillelmi* increased to 100% in soils with Cd concentrations above 60 mg / kg throughout the 14 days exposure period. Žaltauskaitė and Sodienė (2014) detected more dead earthworms with increasing time of exposure and Zhang et al., (2018b) used lower Cd level but longer exposure time compared with those used in this study, suggesting mortality can occur at lower Cd concentrations but only with longer exposure period, perhaps allowing for accumulation of Cd to reach a critical threshold. Thus, exposure

experiments with longer time to investigate earthworms mortality rather than 3-day exposure are warranted.

4.4.2 Exposure with the presence of MPs and earthworms mortality

This study used environmentally relevant concentration (0.30 % w / w) of MPs based on previous studies (0.2, 0.4, 0.5, and 1.2 % of Huerta Lwanga et al., 2016, 1.25 % of Ding et al., 2021 and 0.01, 0.025, 0.05, 0.1 and 0.15 % of Liu et al., 2022). At these MPs concentrations no mortality was observed. More dead earthworms were found in the 100 mg / kg Cd with the absence of MPs than with the presence of MPs (83.3 ± 23.6 % vs 41.7 ± 26.7 %, Table 4.1).

4.4.3 Cd-only exposure and earthworms avoidance

Holm-Sidak post hoc tests (Fig. S4.3) show that, in the absence of MPs there were no significant differences in avoidance of earthworms *L. terrestris* between Cd concentrations (0.1 - 100 mg / kg). The results were consistent with other studies even when in those studies different Cd salts were used. Stander et al., (2019) and Rodriguez et al., (2013) showed 50 mg / kg Cd (in the form of $CdSO_4$) and 120 mg / kg Cd (in the form of $CdCl_2$), respectively, were both not high enough for earthworms *E. fetida* to show significant avoidance behaviour. Furthermore, the earthworm avoidance towards Cd did not increase in a regular fashion with the increasing Cd concentrations up to 100 mg / kg Cd (Fig. 4.2). This finding is similar to that of Rodriguez et al., (2013) who also did not find concentration dependence in the avoidance behaviour of earthworms *E. fetida* over the concentration range from 5 to 120 mg / kg. These suggest that as far as avoidance behaviour is concerned, earthworms exhibit tolerance at similar concentrations of Cd, regardless of species in terms of mortality to the Cd and the form of the Cd solution used.

4.4.4 MPs-only exposure and earthworms avoidance

Although 2-way ANOVA indicated significant differences in avoidance with MPs types ($F_{2,43}$ = 3.279, P = 0.047; Table S4.2), differences between non-MPs, PE and PLA treatments were not detected by the Holm-Sidak post hoc test ($P \geq 0.05$; Table S4.3). It could be the case that 2-way ANOVA indicates significant difference between the MPs types but that these were not sufficiently large for Holm-Sidak post hoc tests to determine where these differences lie. These different statistical results are just down to the different assumptions made by the different tests and their resultant sensitivity to differences between groups.

The results in the absence of Cd showed there was no significant difference in avoidance between the absence of MPs and the presence of 0.3 % MPs. This supports the previous study of Baeza et al., (2020) who showed that *L. terrestris* did not exhibit significant avoidance behaviour in the 7 % MPs treatments. However, Ding et al., (2021) showed that earthworms *E.*

fetida significantly showed avoidance behaviour in the 4 % MPs treatments. The avoidance of *E. fetida* but not *L. terrestris* is opposite to the reported different sensitivities to MPs in general mentioned above (*E fetida* was less sensitive than *L. terrestris*; Section 4.4.2). Possible reasons for these conflicting results could be the different characteristics of the used soils. Baeza et al., (2020) detected *L. terrestris* did not avoid 7 % MPs in natural soil sampled from an organic farm (organic matter = 25.29 %), while Ding et al., (2021) found *E. fetida* avoided 4 % MPs in artificial soil mixed with peat, kaolinite clay and quartz sand in a ratio (w / w) of 1:2:7 (organic matter = 5 %). Earthworms prefer organic matter-enriched diet given that they extract food energy from decaying organic matter (Jager et al., 2003; Huang et al., 2020). Therefore the natural soil with high organic matter level was not sterilised to maintain their natural condition in Baeza et al., (2020) study as well as in this study, which might conceal any repulsive effects of MPs on earthworms. However, the artificial soil with low organic matter content used in Ding et al., (2021) might not inhibit MPs-induced avoidance of earthworms. Therefore, artificial soils may not be a good representation of environmental soil compartment given that natural soil is the most complicated biomaterial on the planet and is constantly changing (Young and Crawford, 2004; Lehmann and Kleber, 2015). Furthermore, previous studies have shown that there were significant differences (underestimation or overestimation) in the toxicological responses of earthworms between natural and artificial soils, leading to erroneous risk assessments caused by the use of artificial soils (Zhu et al., 2020; Li et al., 2023; Reis et al., 2003). Natural soil therefore should be used in the laboratory experiments instead of artificial soil which might need to be cautiously used for the prediction of fate and behaviour in natural soils (Hofman et al., 2008).

Avoidance of the PE was greater than avoidance of the PLA although this difference wasn't statistically significant perhaps suggesting that PLA might be more environment-friendly at realistic concentration than PE. Consistent with this finding, Ding et al., (2021) found the avoidance behaviour of earthworms *E. fetid*a was relatively more sensitive to PE in comparison to PLA. The difference of earthworm avoidance between PLA and PE may relate to their polymer composition and physiochemical properties. PLA is a biodegradable biopolymer material obtained by polymerisation of lactic acid (Taib, et. al., 2023), which is generally derived from starch or sugar (Chen and Patel, 2012). As PLA degrades this may release organic compounds into the soil that are digestible and attract earthworms (Ding et al., 2021). Additionally, the biodegradation of PLA may lead to increases in microbial populations (Brunšek et al., 2023) which then attract earthworms as it has been suggested (Bonkowski et al., 2000) that earthworms feed on soil bacteria and fungi. In contrast to this PE is a petroleum-based plastic and unlikely to biodegrade (Wei and Zimmermann, 2017).

PE and PLA MPs were different shapes in this study, they were films and fibres respectively (Section 3.4.3.1). Previous studies found both fibre and film could cause burns and lesions on the surface of earthworms, which can lead to avoidance behaviour (Baeza et al., 2020; Khalid et al., 2023). Qiao et al., (2019) found that PP MPs fibres resulted in more severe intestinal toxicity of zebrafish than PS films did, probably due to PP fibres of relatively higher ratio of the length and diameter may be more able to embed in tissue relative to MPs films (Hurley et al., 2017). However, the results of the current study showed slightly more avoidance of PE films compared to PLA fibres. Besides different polymer types, the calculated SSAs of PE films and PLA fibres were 1.82 and 0.433 m^2 / g respectively (Section 3.4.3.2). The higher SSAs of PE suggest more PE surfaces than PLA surfaces per mass. More surfaces of PE may lead to release more plastic additives (such as plasticisers and dyes) relative to PLA, which may contribute to the greater avoidance of the PE.

4.4.5 MPs-Cd interactions affecting earthworms behaviour

Dead earthworms and avoidance of earthworms increased in the order PE0.3Cd100 < PLA0.3Cd100 < Cd100 (Table 4.1, Fig. 4.2) although the difference was only statistically significant in avoidance between PE0.3Cd100 and Cd100. A possible explanation for these is Cd from the soil might be adsorbed onto the MPs (Wang et al., 2019; Zhang et al., 2020b; Zhou et al., 2020b), resulting in a lower concentration of Cd in the soil pore water than treatments with no presence of MPs. Additionally, PE might adsorbed more Cd than PLA. However, previous studies showed PLA were able to adsorb more metal ions than PE, due to polar oxygen-containing groups on PLA compared to non-polar PE (Gao et al., 2023; Shi et al., 2023). Oxygen-containing functional groups (C-O) were detected in the PLA (Section 3.4.1.1), it would be expected that PLA could adsorb more Cd per unit mass / area than PE because of its polar groups. This effect might be present, but given the higher SSAs, more surface is available for adsorption per mass of PE relative to PLA (Li et al., 2022; Chang et al., 2024), greater adsorption by PE than PLA might be expected. Therefore, further MPs adsorption experiments (Chapter 6) are warranted to explore if there any difference of adsorption capacities between PLA and PE and their mechanisms.

Further to the above discussion, almost all the dead earthworms were found in the control side of 100 mg / kg Cd-only treatments (Table 4.1). This is counter intuitive. A possible explanation is that earthworms initially moved into the Cd side soil, got poisoned, and then moved to the control side soil because of avoidance, afterwards they died because they had already experienced Cd toxicity. Previous research have reported the presence of resistance or tolerance to Cd in earthworms (Reinecke et al., 1999; Liang et al., 2011; Aigner et al., 2023), therefore earthworms do not die immediately and could have sufficient time and ability to move away

from the polluted soil when exposure to Cd (Rożen, 2006; Duan et al., 2020; Prabha et al., 2024). Earthworms finally reach death endpoint with Cd concentration in the detoxification proteins (such as a specific Cd-binding metallothionein isoform, Cd-MT) overloading (Chabicovsky et al., 2004). Furthermore, the few remaining live earthworms randomly distributed in the control and test sides of the 100 mg / kg Cd treatments (Table 4.1). The possible reason is earthworms might not be killed, but they may have been on the point of death and had insufficient energy to move away from the polluted soils. Some earthworms might still have energy to move away from the contaminated soils.

4.5 Conclusions

4.5.1 Summary

These avoidance tests were a pilot study. MPs and Cd at field-relevant concentrations (0.3 % w / w and up to 10 mg / kg respectively) did not cause earthworm mortality. Significant more earthworms were killed under the high Cd concentration of 100 mg / kg. Some avoidance of earthworms was found at all concentrations of Cd. However earthworm avoidance did not significantly increase with the increasing Cd concentrations, possibly avoidance was hard to detect due to the death of the earthworms. Earthworm mortality and avoidance detected in the 100 mg / kg Cd-only treatments decreased in the presence of MPs, although a significant decrease was only found in avoidance by PE.

4.5.2 Environmental implications

There was no evidence for the presence of the MPs / Cd at the environmentally relevant concentrations to impact earthworm avoidance behaviour indicating that it could be safe for earthworms to remain in soil. However, although earthworms do not avoid the MPs / Cd, it might have more subtle negative effects on other earthworms behaviours with longer term implications such as feeding, growth, reproduction and burrowing (Sections 4.1.5, 4.1.6). These effects on earthworm may have implications and consequently on earthworm population and, therefore, on the role of earthworms on soil structure and fertility. Furthermore, these impacts may also have potential implications on human health by the accumulation of Cd / MPs through the food chain. The data also showed that MPs might mitigate the impacts of Cd toxicity on earthworm suggesting MPs pollution generated from plastic films could reduce the impacts of Cd contamination in soil. This study therefore provides evidence for the accumulation of MPs in the Cd-polluted agricultural soils might mitigate the negative effect of Cd on agroecosystems, using an earthworm avoidance experiment.

4.5.3 Limitations and recommendations

At the end of experiments, soil properties such as pH and organic matter were not measured and all of the used soils were properly disposed of so that the measurements are not possible. The organic matter in the soils of each treatments would be the same at the start and end of the avoidance experiment since the same batch of homogeneously mixed soil was used (Section 4.2.4) and earthworms are unlikely to have digested significant amounts of organic matters with 72 h (Curry and Schmidt, 2007). Soil pH might have changed due to the Cd treatments. Langdon et al., (2005) found there was no increase in avoidance of earthworms in the Pb-bearing soils as pH significantly decreased from 5.78 to 4.56 with increasing in Pb concentrations from 0 mg / kg to 10000 mg / kg. Furthermore, Langdon et al., (2005) measured the pH of soils treated with $Pb(NO_3)_2$ over the 28 day experimental period and there was no significant differences in pH between 0 mg Pb / kg treatments (pH = 5.79 ± 0.03) and 1000 mg Pb / kg treatments (pH = 5.83 ± 0.02). As both Pb and Cd are divalent metals and added into soil as nitrates, it is reasonable to hypothesis that soil pH would remain stable over the avoidance test period after being treated with 100 mg / kg Cd (far more lower than 1000 mg / kg used in Langdon's study), Therefore, soil pH seems unlikely to change as well as soil organic matter and they would not have impacts on earthworm avoidance behaviour in this study.

Although mortality was not observed, nor avoidance, at the lower concentrations of Cd and / or MPs, this could not rule out other sublethal effects that might occur over longer time scales (Yasmin and D' Souza, 2010). The chronic tests, targeting sublethal effects, are more sensitive and are the more realistic approaches to predict environmental impacts as exposure concentrations of contaminants are usually quite low in the field (Römbke et al., 2007). In this case earthworm avoidance response therefore was chosen as a more sensitive indicator of environmental impacts than lethal responses (West and Ankley, 1998; Lopes et al., 2004; Loureiro et al., 2005). Previous research reported noisy avoidance data without clear patterns (Martínez Morcillo et al., 2013) same as this study. However there were suggestions of trends that the toxic effects of MPs led to earthworm avoidance behaviour and more avoidance after exposure to PE compared with PLA in the Cd-free treatments.

As we discussed before (Section 4.4.6), some earthworms might not be killed in the 100 mg / kg Cd treatments, but they were on the point of death and unable to move away from the Cd-treated side. These earthworms were not counted into the calculation of avoidance ratios, which might bring variability into avoidance results. Previous studies used eight (Martínez Morcillo et al., 2013) or ten earthworms *L. terrestris* (Dittbrenner et al., 2012) to conduct avoidance experiments. The number of earthworms (n = 6, 1 earthworm = 16.7 %) used in this avoidance chapter might lead to the avoidance results very sensitive to individual response. More

earthworms *L. terrestris* therefore may need to be used in the avoidance tests to decrease the variability in avoidance results.

In this avoidance chapter, the noisy data indicate it may need to repeat the experiment using more earthworms to see if results with trends can be obtained. However, due to time constraints and the fact that the avoidance tests were just the preliminary scoping experiments, the part aim of these was to provide valuable laboratory experience. This study suggested other interesting lines of research with defensible hypotheses: (1) there was significantly lower earthworm avoidance in PE0.3Cd100 treatments and lower earthworm avoidance in PLA0.3Cd100 treatments but not significant relative to Cd100 treatments, indicating MPs might decrease Cd bioavailability in soil pore water and there might be different impacts between PE and PLA (Chapter 5), (2) the decrease in Cd of soil pore water with the presence of MPs suggesting there might be the potential adsorption of Cd by MPs (Chapter 5) and (3) earthworms showed different avoidance behaviour towards PE and PLA probably due to the differences of adsorption capacities between PLA and PE (Chapter 6). The decision therefore was made to pursue the above hypotheses rather than simply repeat the avoidance experiments given that often in the literatures (Martínez Morcillo et al., 2013) the avoidance data are noisy. Together, these will provide a more detailed assessment of the safety of plastic film mulches use in farmland.

5 An investigation into the impacts of MPs on the bioaccumulation of Cd in earthworms *Lumbricus terrestris*

5.1 Introduction

The pilot avoidance tests in Chapter 4 suggested two interesting lines of research, MPs might decrease Cd bioavailability in soil pore water and therefore Cd bioaccumulation in earthworm body, and this reduction might due to the potential adsorption of Cd by MPs. This chapter therefore concerns an exposure experiment that investigated the impacts of combinations of MPs and Cd on Cd concentrations in soil pore water and Cd bioaccumulation in earthworm body. This study has been published in Ecotoxicology and Environmental Safety (Xiao et al., 2024). As the exposure chapter has been published, it has been presented in much the same form here. A front introduction has been added before the published paper to introduce this work. Repetition in the contents of Materials and Methods has been removed because those contents are presented in Chapter 3 Materials and Methods. Additionally, the numbers of the figures and tables in the published manuscript have been updated, such as Fig. 1 was changed into Fig. 5.1 and Table 1 into Table 5.1 to keep the format of figures and tables numbers consistent in the whole thesis. Additional details of statistical parameters e.g. N, degrees of freedom and F or H values that were not required for publication have also been added throughout the text. Furthermore, a paragraph discussing no impacts of asymmetry of the test design (two sets of controls) in this experiment on statistical results has been added in the Data analysis and quality control. Finally, a paragraph discussing the lack of impacts of particle shape on earthworm response in the experiments has been added in the final Discussion in this particular experiment. The supplementary information includes two sections, SIa supporting figures and tables and SIb statistical results, which have also been published in the Ecotoxicology and Environmental Safety paper (Xiao et al., 2024). This introduction summarises the existing research into the topics of Cd bioavailability in soil, Cd bioaccumulation in earthworm and MPs impacts on Cd partitioning in soil, to contextualise this study.

5.1.1 Cd bioavailability in soil

Cadmium has been considered as a serious threat in soil (Sections 1.3 and 4.1). A major factor influencing the toxicity of Cd in soil is its bioavailability that is the amount of Cd that can be taken up by organisms and have an effect rather than the bulk Cd concentration (Vig et al., 2003; Reeves and Chaney, 2008; Shahid et al., 2017). Cd bioavailability depends on the fraction of the soil solution Cd (i.e. free Cd) in the soil (Vig et al., 2003; Kim et al., 2015; Riaz et al., 2021). The International Organisation for Standardisation also defined environmental bioavailability as the fraction of dissolved metal species (such as Cd in this case) in the pore water, which can be taken up by plant roots or other soil organisms (ISO, 2008). The high mobility of dissolved Cd in the soil lead to readily accumulate in soil organisms (Shahid et al., 2017; Zhang and Reynolds, 2019). Furthermore, Cd bioavailability can be estimated by modelling using the soil-liquid partitioning to assess the environmental risks of Cd (Sauvé et al., 2000a; Sheppard et al., 2007; Degryse et al., 2009). Solid-liquid partitioning is usually evaluated using the ratio between the total solid concentrations of metal (in bulk soil, mg / kg) and the concentration of metal in solution (in soil pore water, mg / L) (Sauvé et al., 2003). Previous studies reported various values of metals partitioning coefficients K_d that could be used to predict metals mobility and bioavailability (Luo et al., 2006; Zhang et al., 2018; Liao et al., 2021). The K_d values for Cd varied by orders of magnitude among soils covering a range of 0.44 - 192000 L / mg (Table S5a.11; Sauvé et al., 2000b; Yan et al., 2008; Karapinar and Donat, 2009; Lukman, et. al., 2013; Coulombe et al., 2023). Furthermore, the K_d values for Cd are related to soil properties, pH and organic matter content (McBride et al., 1997; Sauvé et al., 2000b; Degryse et al., 2009; Palleiro et al., 2013). Generally, K_d values increase with the increasing pH (range from 3 to 9) and a significantly positive correlation between pH and log transformed K_d ($\log K_d = a + b\ pH$) (Sauvé et al., 2000b; Degryse et al., 2009), whereas there was no clear relationship between organic matter content and K_d values (Sauvé et al., 2000b). However, Cd complexation by soil organic matter (Krishnamurti and Naidu, 2003) leads to decrease in soil solution Cd (Filipović et al., 2018). Therefore, K_d values for Cd increase to a certain extent with increasing soil organic matter content (Sauvé et al., 2000b).

5.1.2 Cd bioaccumulation in earthworm

If Cd is highly bioavailable in soil, a high tendency to bioaccumulate in soil organisms occurs (Spurgeon and Hopkin, 1996; Khanam et al., 2020). Soil-dwelling earthworms, as important soil organism in ecosystem processes (Sections 1.4 and 2.3.7), are exposed to Cd in a variety of ways due to they live in the soil and come into direct contact with Cd in both soil particles and soil pore water. Earthworms uptake contaminants from soil solid particles and soil pore water by ingestion (oral) and through their skin (dermal) (Vijver et al., 2003; Duarte et al., 2012). Earthworms take up pollutants adsorbed on soil particles due to their burrowing behaviour and digestion of soil organic components as well as leaf litter (Vijver et al., 2003; Li et al., 2009). Given the exchange of water across earthworm skin (Carley, 1975), soluble metal ions in soil pore water can enter into earthworm body by passing through its body wall (Carley, 1978; Oste et al., 2001; Veltman et al., 2007). Previous studies indicated that metals have to be in a dissolved state to be bioavailable to earthworms and dermal uptake via soil pore water is the main exposure route (Veltman et al., 2007; Saxe et al., 2001). The bioaccumulation factor (BAF) of earthworm has been mainly used to express the level of Cd bioaccumulation in earthworm (Li et al., 2010; Nannoni et al., 2011; Huang et al., 2021a), which can be calculated as the ratio of earthworms to soil metal concentrations (Nahmani et al., 2009; Vermeulen et al., 2009; Wang et al., 2018). The reported BAF values are in the range 3.60 - 59.7 (Table S5a.13, Nannoni et al., 2011, Wang et al., 2018, Xiao et al., 2020, Ge et al., 2023). The BAF values vary with soil pH and soil organic matter content, generally greater pH and soil organic matter content can explain the lower BAF values in the above reported studies. Furthermore, different species of earthworms were used in these studies, indicating BAF in earthworms varies with earthworm species (Xiao et al., 2020).

Bioaccumulation of metals can be affected by environmental factors such as the impact of soil pH on metals solubility (Sauvé et al., 2000a; Sauvé et al., 2000b; Lock and Janssen, 2003) and the effect of soil organic matter on metals complexation with soil (Nahmani et al., 2007). Protons are one of the main competitors for binding of metal ions in soil, possibly leading to increase in metal desorption from soil particles so that increase in bioavailable metal in solution and then increase in dermal uptake by earthworm with decreasing pH (more protons) (Kiewiet

and Ma, 1991; Spurgeon and Hopkin, 1996; Peijnenburg et al., 1999). Organic matters are an important metal sorbent in soils, as well as oxide-type components and clay minerals, through surface complexation (Weng et al., 2002; Gustafsson et al., 2003; Zhang et al., 2022). Therefore, increasing organic matter leads to more metals adsorption by organic matter, so that decrease in metals bioaccumulation (Mortensen et al., 2018).

5.1.3 MPs impacts on Cd partitioning in soil

The partitioning of Cd between the fraction bound to soil solids and the part that is dissolved in the soil solution is a dynamic process. An equilibrium steady state is reached when the concentrations of the two phases are substantially stable (Luo et al., 1971; Hatti-Kaul, 2001). MPs have become an emerging pollutants in soil (Sections 1.2.1 and 2.3.1). Once MPs and Cd are both in the environment, interactions between MPs and Cd occur by adsorption (Sections 1.6 and 2.4.2). MPs-Cd interactions could break the balance of the Cd soil-liquid partitioning (Wang et al., 2020a; Zhang et al., 2020; Huang et al., 2023). MPs interactions with Cd therefore could have further impacts on Cd bioavailability in soil and Cd bioaccumulation in earthworm (Zhou et al., 2020; Huang et al., 2021b; Liang et al., 2022). However, there are only a few studies focused on the interactions between MPs and Cd and how this affects Cd partitioning in soil. Huang et al., (2021b) found that purchased PE MPs particles (≤ 300 μm) increased the Cd bioaccumulation in *E. fetida* by enhancing the Cd availability in the co-exposure soil, and they explained that Cd might be absorbed on the MPs surface and then released again through the internal digestion by earthworms after the earthworms had ingested MPs. More recently a study indicated PE MPs (< 500 μm) derived from a commercial agricultural film also increased Cd accumulation in *E. fetida*, however no mechanism was presented (Jiang et al., 2024). In contrast, purchased LDPE MPs particles (< 1 mm) did not affect the accumulation of Cd in earthworms *E. fetida* (Zhang et al., 2024). Higher pH and soil organic matter content were used in Huang et al., (2021b) study than those used in Zhang et al., (2024) study (Table 5.1). Also, increase in Cd bioaccumulation in earthworm *E. fetida* was found by Huang et al., (2021b), whereas no impacts on earthworm bioaccumulation was found by Zhang et al., (2024). This is perhaps counter the above mentioned that higher pH and organic matter content lead to decrease in metals bioaccumulation in earthworms (Section 5.1.2), indicating in this case probably MPs

size might be the main factor dominating the Cd bioaccumulation in earthworm, which might conceal any opposite effects of soil properties on Cd bioaccumulation in earthworm. Here it probably due to that more Cd is adsorbed on smaller size of MPs (≤ 300 μm particles; Huang et al., 2021b) with greater specific surface areas (Yu et al., 2019) and then Cd-bearing MPs with smaller size more easily enter earthworm body and retain in earthworm (Wang et al., 2020b) causing increase in Cd bioaccumulation in earthworm (Huang et al., 2021b).

Table 5.1 Soil characteristics and use of Cd solution in three studies on MPs affecting Cd partitioning in soil.

Soil source	Soil pH	Soil organic matter	Cd solution	References
Field surface soil	7.24	13.6 %	$CdCl_2$	Huang et al., (2021b)
Artificial soil (20 % kaolinite clay, 70 % quartz sand and 10 % peat)	/	5 %	/	Jiang et al., (2024)
Artificial soil (20 % kaolinite clay, 69 % quartz sand, 10 % peat and 1 % $CaCO_3$)	6.5	5 %	$CdCl_2$	Zhang et al., (2024)

5.1.4 Rational, aim and hypotheses

So far, research on investigating the impacts of MPs generated from plastic mulches on Cd bioaccumulation in earthworm *L. terrestris* (Sections 1.4 and 3.3) is lacking. Therefore, exposure experiments in this chapter to investigate the impacts of interaction between plastic mulch-derived MPs and Cd on earthworm *L. terrestris* are warranted.

The experiment documented in this chapter aimed to investigate the effects of MPs on Cd bioavailability in soils and Cd bioaccumulation in earthworms. To achieve this, the earthworm *L. terrestris* was exposed to soil treated with $Cd(NO_3)_2$ solution and / or two different types of plastic mulch-derived MPs (conventional PE and biodegradable PLA; Sections 1.2.6 and 2.3.6) for 28 days. At the end of exposure, Cd in bulk soil, soil pore water and earthworm body were

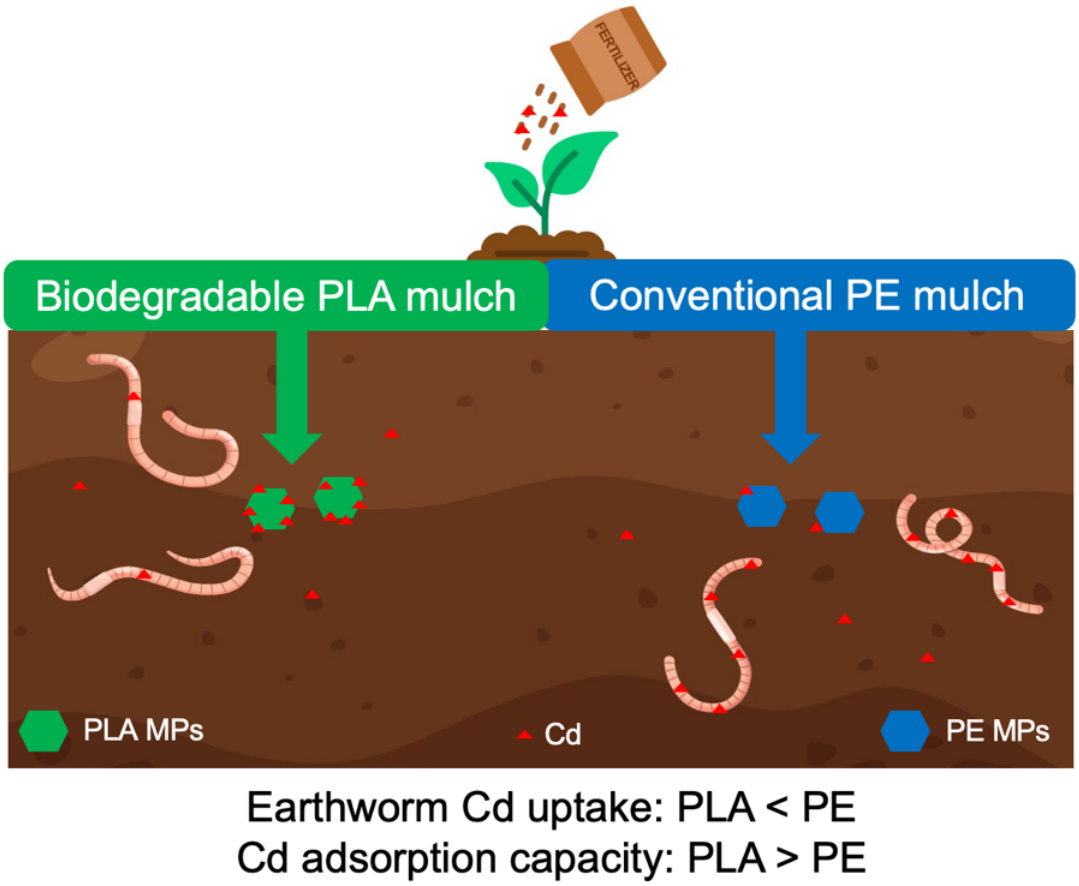
measured. Earthworm mass change and mortality were also assessed. In addition, an initial adsorption experiment was carried out to aid interpretation of the earthworm exposure experiments. Based on previous research, we hypothesised that (1) Cd concentrations in soil pore water would decrease, (2) as Cd concentrations in the pore water decreases, less Cd would bioaccumulate in earthworm body and (3) reduction in Cd bioavailability with the presence of MPs probably due to Cd would be adsorbed on MPs.

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

Highlights

- Realistic levels of MP in soils did not impact earthworm mortality or weight change
- The presence of MPs decreased Cd concentrations in both earthworms and pore water
- PLA MPs decreased Cd accumulation in earthworm relative to PE MPs
- PLA MPs adsorbed significantly more Cd than PE MPs

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 this 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.



Graphical abstract

Key words: *Lumbricus terrestris*, soil pore water, adsorption, bioavailability

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 et al., 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 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 damages 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, Quezada-Hinojosa et al., 2009; Korea, Park et al., 2010; USA, 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 the common European earthworm *L. terrestris* was exposed 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 the earthworm exposure experiments. 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.

Materials and methods

Soils

Because this study was designed to investigate mechanisms and to avoid biosecurity issues associated with overseas soil use, commercially available uncontaminated natural topsoil was used. The source and characteristics of the purchased topsoil are provided in Section 3.2.

Microplastics

The source of two types of plastic mulches, non-biodegradable PE and biodegradable PLA, is presented in Section 3.4.1. The MPs were generated from these two plastic mulches (Section 3.4.2) and their characterisation are given in Section 3.4.3.

Earthworms

In these exposure experiments, the common UK earthworm *Lumbricus terrestris* was used. The reason for using this species and the treatments of the earthworms prior to use are presented in Sections 1.4 and 3.3.

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. S5a.1). 1200g 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 $Cd(NO_3)_2 \cdot 4H_2O$ 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 Davis et al., (2002); details are given in Text S5a.1. Pore waters were extracted from the moist soils by centrifugation following the method of Carter et al., (2014); details are given in Text S5a.2. 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 S5a.1. 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.

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 $Cd(NO_3)_2 \cdot 4H_2O$ 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.

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 Section 3.6.2.

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.

Given the operability of these exposure experiments, PE and PLA treatments were separated carried out. There were therefore two sets of controls (8 controls), which are the exactly same treatments using same soils, however other treatments were all 4 replicates. It was the case that asymmetry was brought into this statistical analysis affected by the control bias. Sigmaplot and SPSS can handle the unequal and unbalanced designs when calculating statistics and their statistical power is the same as if there would be 4 or 8 replicates in the control group. Even though, the statistical analyses were run again using symmetrical datasets. The values of random 2 controls from the 8 controls were averaged. Total 4 values were got which were

represented as values for the 4 controls for symmetrical statistical analysis. All the datasets were tried to analyse again using the datasets including the recalculated values of 4 controls. Although statistical values (parametric ANOVA test: SS, MS and F; non-parametric Scheirer Ray Hare test: SS, MS and H) were slightly different between the datasets including 4 controls and 8 controls, degrees of freedom and the results were not substantially different. Furthermore, when carrying out the Games-Howell post toc tests, the statistical tools (SPSS) used can cope with the asymmetry and controls were not included in the 3 way analysis of the pore water concentrations, $K_{d\text{-}exposure}$ and BAF_{pw}. Therefore, the control bias did not affect the whole statistical results in this particular experiment.

Results

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 S5a.1). 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. 5.1, Table S5a.2), increasing significantly with increasing concentrations of Cd in the soil (Fig. 5.1) ($H_{7,209}$ = 94.623, P ≤ 0.001, 2 way Scheirer Ray Hare Test; Games - Howell post hoc test; Tables S5b.1, S5b.3). Cd pore water concentrations increased with increasing measured bulk soil Cd concentrations for the different MP treatments (R^2 = 0.88 - 0.95, P ≤ 0.001, linear regressions) (Table S5a.3). For a given nominal soil Cd concentration, pore water concentrations were significantly greater in the absence of MPs than in their presence ($H_{1,209}$ = 17.356, P ≤ 0.001, 2 way Scheirer Ray Hare Test; Table S5b.1) and were lower in the presence of PLA than in the presence of PE although this difference was not significant ($H_{1,141}$ = 0.644, P = 0.422, 3 way Scheirer Ray Hare Test; Table S5b.2). Increasing

concentrations of MP at a given nominal soil Cd concentration resulted in lower pore water concentrations ($P \leq 0.05$, Games - Howell post hoc test; Table S5b.3).

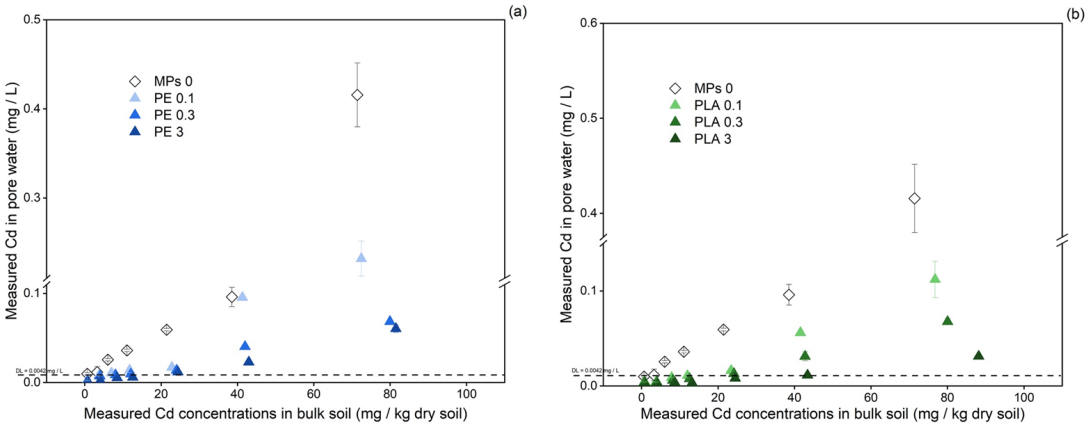


Fig. 5.1. Mean Cd concentrations in pore water following exposure to Cd with and without MPs over 28 days. (a) PE treatments, (b) PLA treatments. Results are mean ± standard deviation, n = 3-4 (for some replicates no pore water was extracted). Values that were below detection are expressed as the detection limit divided by the square root of two (Croghan and Egeghy, 2003). Dashed horizontal line on the figures indicates the detection limit.

$K_{d\text{-exposure}}$ values were determined for all treatments for which Cd was detected in both the bulk soil and soil pore water (Fig. 5.1, Table S5a.1 and S5a.2); values ranged from 68.70 ± 9.3 to 4154 ± 808.5 L / kg (n =3 - 4, ± standard deviation) (Fig. 5.2, Table S5a.4). 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 ($H_{1,179} = 55.628$, $P \leq 0.001$, 2 way Scheirer Ray Hare Test; Table S5b.4) and were significantly greater in the presence of PLA than in the presence of PE ($H_{1,123} = 6.450$, $P \leq 0.05$, 3 way Scheirer Ray Hare Test; Table S5b.5). Increasing concentrations of MP at a given nominal bulk soil Cd concentration resulted in greater $K_{d\text{-exposure}}$ values (Fig. 5.2, Table S5a.4) 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 S5a.4, S5b.6). Although there were some significant differences between $K_{d\text{-exposure}}$ values for different Cd concentrations at a given MP treatment ($P \leq 0.05$, Game - Howell post hoc test;

Table S5b.6) there was no systematic variation in these values (Fig. 5.2, Table S5a.4), consistent with the linear relationship between bulk soil and pore water Cd concentrations (Table S5a.3).

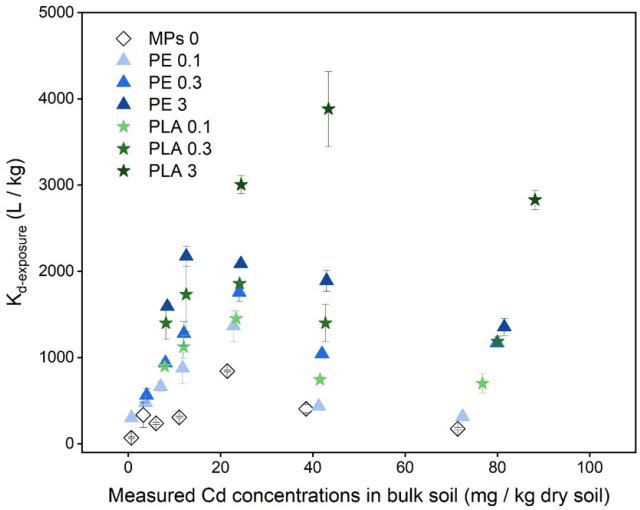


Fig. 5.2. Mean soil-pore water distribution coefficients ($K_{d\text{-}exposure}$) as a function of soil Cd concentration for the different treatments at the end of the 28 day experiment (n = 3-4, for some replicates no pore water was extracted). Error bars represent standard deviation; where not visible values are smaller than the symbols.

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 (plastic: $H_{1,212} = 0.524$, $P \geq 0.05$; Cd: $H_{7,212} = 4.516$, $P \geq 0.05$; their interaction: $H_{7,212} = 5.499$, $P \geq 0.05$; 2 way Scheirer Ray Hare Test; Table S5b.7). Weight change of the surviving earthworms was 0.13 ± 0.40 g (n = 215, ± standard deviation) and showed no systematic

variation between treatments (plastic: $H_{1,199} = 0.272$, $P \geq 0.05$; Cd: $H_{7,199} = 1.459$, $P \geq 0.05$; their interaction: $H_{7,199} = 1.210$, $P \geq 0.05$; 2 way Scheirer Ray Hare Test; Table S5b.8) (Fig. S5a.2). 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).

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. 5.3, Table S5a.2) increasing with soil concentrations ($H_{7,140} = 303.4$, $P \leq 0.001$, 2 way ANOVA; Games - Howell post hoc test; Tables S5b.9). Earthworm Cd concentration was greater in the MP-free treatments and, in the MP treatments, decreased with increasing MP concentration, but none of these differences were significant ($P \geq 0.05$, Holm-Sidak post hoc tests; Table S5b.9). Cd concentrations in earthworms exposed to Cd were significantly lower in the 3% PLA treatments compared with the equivalent PE treatments ($P \leq 0.05$, Holm-Sidak post hoc test; Table S5b.9); at other plastic concentrations although the Cd concentrations were lower for the PLA, the differences were not significant ($P \geq 0.05$, Holm-Sidak post hoc tests; Table S5b.9).

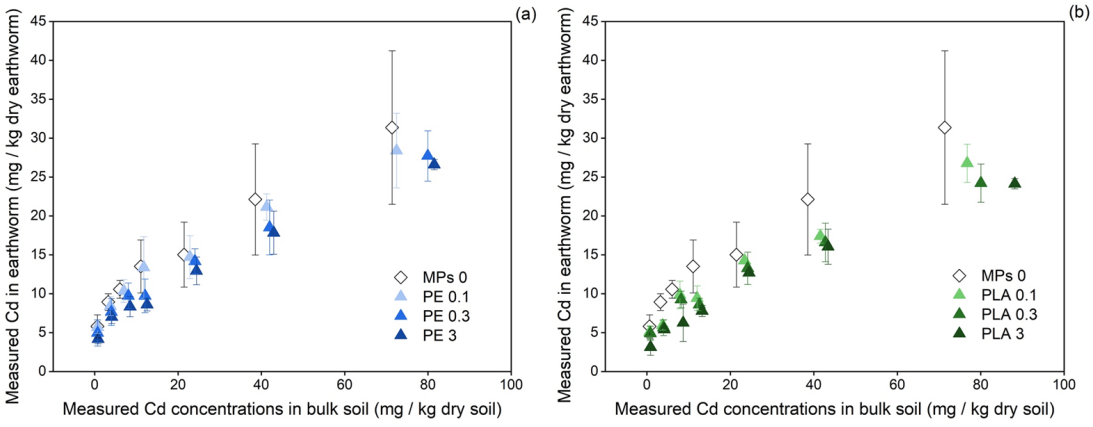


Fig. 5.3. Mean of measured Cd concentrations in earthworm bodies following exposure to Cd with and without MP over 28 days, (a) PE treatments, (b) PLA treatments. Results are mean ± standard deviation, n = 2-4 (earthworm mortality in some treatments reduced the number of replicates).

Bioaccumulation factors (BAF) were calculated for treatments where concentrations of Cd in earthworms and bulk soil / pore water were above detection limits (Figs. 5.1 and 5.3, Tables S5a.1 and S5a.2). 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) (Figs. 5.4, S5a.3). The BAF values decreased with increasing MP concentrations (BAF_s: $F_{6,121} = 33.45$, $P \leq 0.001$, 2 way ANOVA; Holm-Sidak post hoc test; Table S5b.10; BAF_{pw}: $H_{2,98} = 20.81$, $P \leq 0.001$, 3 way Scheirer Ray Hare Test; Game-Howell post hoc test; Tables S5b.12, S5b.13) and were significantly higher in PE treatments relative to PLA treatments with the same MP concentrations (BAF_s: $P \leq 0.05$, Holm-Sidak post hoc test; Table S5b.10; BAF_{pw}: $P \leq 0.05$, Game-Howell post hoc test; Table S5b.13) (Fig. 5.4).

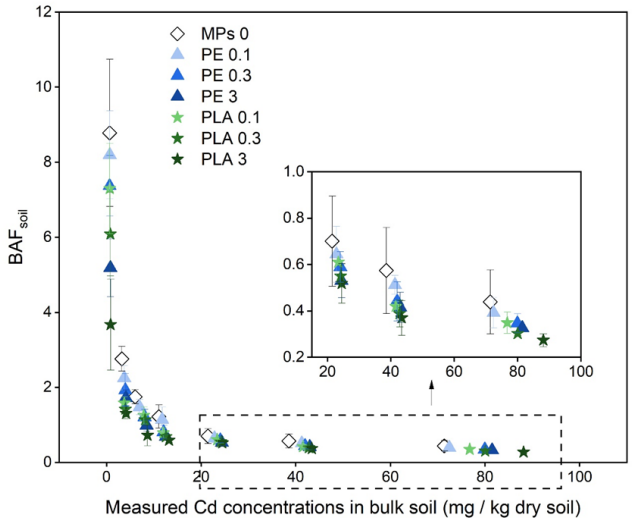


Fig. 5.4. Earthworm-soil bioaccumulation factors (BAF_s) of the Cd following exposure to Cd with and without MP over 28 days (n = 2-4, earthworm mortality in some treatments reduced the number of replicates). Error bars represent standard deviation.

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 S5a.6). There was no significant difference between the mass of depurate recovered between Cd treatments ($F_{7,159}$ = 0.801, P ≥ 0.05, 2 way ANOVA; Table S5b.14). 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. 5.5, Table S5a.6) (P ≤ 0.05, Holm-Sidak post hoc test; Table S5b.14). Mass of earthworm depurate decreased with increasing MP concentration (Fig. 5, Table S5a.6); significant differences were found between the 0.1 % and 3 %, and the 0.3 % and 3 % MP treatments (P ≤ 0.05, Holm-Sidak post hoc test; Table S5b.14). For a given MP concentration, mass of earthworm depurate was greater in the presence of PLA than in the presence of PE (Fig. 5.5, Table S5a.6), however, there were no significant differences between PLA and equivalent PE treatments (P ≥ 0.05, Holm-Sidak post hoc test; Table S5b.14).

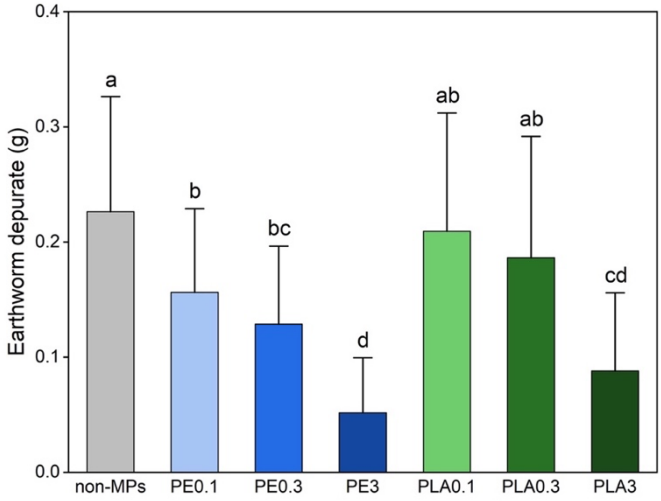


Fig. 5.5. Mean of earthworm depurates recovered after 28 days exposure between different MP treatments. Error bars represent standard deviation. Different letters above the bars indicate significant differences at P ≤ 0.05 (2 way ANOVA followed by Holm Sidak post hoc tests).

Adsorption experiment

In the adsorption experiments, the amount of Cd adsorbed on MPs ranged from 4.67 to 875 mg / kg (Fig. 5.6a). 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 5.1 and S5a.7). 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. 5.6, Table 5.1). Isotherm fit parameters indicate greater adsorption to PLA than PE.

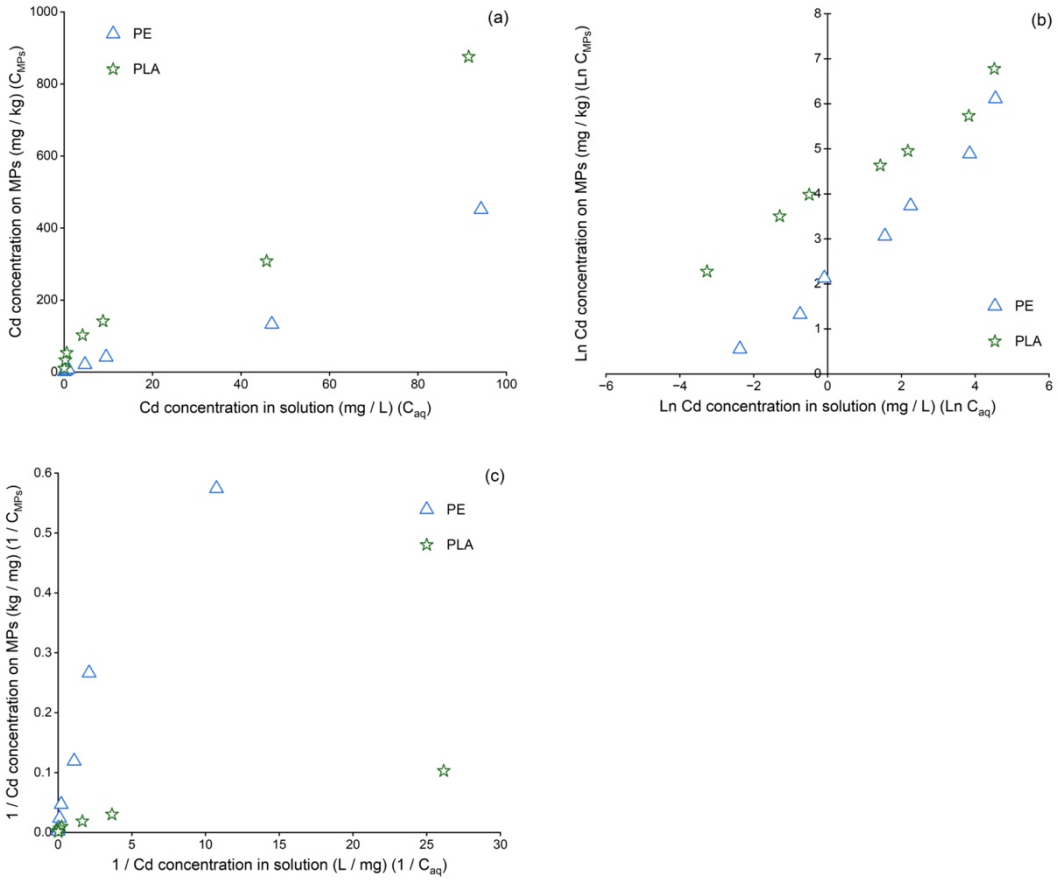


Fig. 5.6. (a) Adsorption data for PE and PLA MPs. (b) Freundlich isotherms for PE and PLA MPs. (c) Langmuir isotherms for PE and PLA MPs.

Table 5.1. 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)	0.82	< 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)	0.98	< 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

Discussion

The 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 the 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 S5a.1) 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 $Cd(NO_3)_2.4H_2O$ used to make the Cd solutions contained additional water less Cd would have been added 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 (Tables S5a.8, S5a.9) ($P \geq 0.05$, t test; S5b.15). However, because we measured Cd concentrations rather than assuming them, the mismatch between the nominal and measured concentrations does not affect this 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 S5a.10). Reductions in pore water concentration in the presence of the MPs (Fig. 5.1) and the adsorption experiments (Fig. 5.5) suggest that the MPs increased the amount of adsorption. The $K_{d\text{-exposure}}$ values calculated from the exposure experiment (Fig. 5.2) and the calculated adsorption parameters (Table 5.1) are similar to those found in other studies (Tables S5a.11 and S5a.12 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 $K_{d\text{-adsorption}}$, 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. 3.5 in Chapter 3). 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 1.82 and 0.325 m^2 / g, respectively (Section 3.4.3.2). 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 ether functional groups (Fig. 3.2, Table 3.2 in Chapter 3) and the Cd^{2+} could bind to these by surface complexation (Fan et al., 2018, Wu et al., 2019). Thus, consistent with the 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 S5a.13, 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 this study had an organic matter content of 12.67 ± 0.17 % (n = 5, ± standard deviation), much greater than those from the above reported studies (1 - 5.3 %, Table S5a.13), 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. 5.5) and pore water (Fig. S5a.3). 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., 2018, Richardson et al., 2020). The data are well described by Log10-Log10 relationships (Fig. S5a.5; Tables S5a.5, S5a.14) 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 this 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. 5.5). 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). The 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 bioavailability due to adsorption on PLA will be reversed as the PLA degrades.

Although the depurate data suggest that the presence of MPs reduced soil ingestion, no systematic earthworm mortality or weight change was detected. 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 S5a.15) than this study. At the end of the 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. S5a.6) 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. The 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.

As mentioned above, for a given MP concentration no significant differences in earthworm mortality or weight was found between PE piece and PLA fibre treatments, indicating that the differences in particle shape of MPs did not have an impact on earthworm, in terms of weight or mortality. However, Qiao et al., (2019) found that PP MPs fibres were more toxic to zebrafish, in terms of intestinal damage than PS MPs films, probably because MPs fibres with a relatively higher ratio of the length and diameter may be more able to embed in tissue relative to MPs films (Hurley et al., 2017). As well as being a different polymer type to that used in these experiments, more than 70 % of the fibres used in Qian et al., (2019) study were in the range

from 20 to 100 μm in length, while the fibres used in this study were 2.170 ± 0.749 mm in length (n = 50; Section 3.4.3.1). Order of magnitude longer fibres indicate larger MPs used in current study than Qian et al., (2019) study. Larger MPs may be harder to embed into organisms tissues relative to smaller MPs, which may contribute to less accumulation in tissues so that less toxicity or even no toxicity to earthworm.

Conclusion

Perhaps counter intuitively these 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 warrants further study.

Acknowledgements

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6 An investigation into the adsorption of Cd on pristine and naturally weathered MPs

6.1 Introduction

The exposure and adsorption experiments in Chapter 5 indicated plastic mulches-derived MPs present in soil can decrease earthworm exposure to Cd by adsorption which removes the Cd from pore water therefore reducing Cd bioavailability. MP mulch residues could be weathered and their surface characteristics may change during mulch application in farmland and exposure to nature, which may lead to different adsorption of Cd on MPs. Over time weathering of MPs might have different impacts on mobility and distribution of Cd in agricultural soil to the one found in Chapter 5. This chapter therefore concerns an adsorption experiment that investigated the adsorption of Cd on pristine and naturally weathered MPs. This study has been published in Chemosphere (Xiao et al., 2024). A front introduction has been added before the published paper to introduce this work. As this adsorption chapter has been published, it has been presented in much the same form here. However, repetition in the contents of Materials and Methods has been removed because those contents are presented in Chapter 3 Materials and Methods. Furthermore, the numbers of the figures and tables in the published manuscript have been updated, such as Fig. 1 was changed into Fig. 6.1 and Table 1 into Table 6.1 to keep the format of figures and tables numbers consistent in the whole thesis. Additional details of statistical parameters e.g. N, degrees of freedom and F or H values that were not required for publication have also been added throughout the text. Finally, a paragraph discussing the adsorption isotherm fitting in this particular experiment has been added in the Materials and methods - Adsorption experiments. The supplementary information includes two sections, SIa supporting figures and tables and SIb statistical results, which have also been published in the Chemosphere (Xiao et al., 2024). This introduction summarises the existing research into the topics of adsorption of Cd on MPs and increase in adsorption capacity of Cd on MPs after weathering, to contextualise this study.

6.1.1 Adsorption of Cd on MPs

Once MPs and non-essential metal Cd are in the environment, interaction between MPs and Cd occur by adsorption (Sections 1.6 and 2.4.2). MPs with different properties (such as chemical

structure and specific surface area) could have impacts on MPs-Cd adsorption (Sections 1.7 and 2.4.3). So far a growing number of studies have investigated on Cd adsorption on various polymers of MPs and their mechanisms (Guo et al., 2020; Zhou et al., 2020; Zou et al., 2020; Li et al., 2022). Most of the previous research focused on conventional MPs, such as PE, PS and PP MPs. Adsorption between Cd and these conventional MPs could be highly affected by the physical characteristics of these MPs. Furthermore, specific surface area of MPs is an important factor and it determines how much surface area is available for sorption per mass of MPs (Gao et al., 2021).

Given the growing concerns about plastic pollution, biodegradable plastics (PLA, the largest production of global biodegradable plastics) have been developed to replace conventional plastics (Sections 1.2.6 and 2.3.6). Biodegradable PLA is derived from lactic acid made from renewable resources, such as corn starch or sugar-cane (Jem et al., 2010; Pang et al., 2010). PLA as aliphatic polyester contains oxygen-containing functional groups (Dreier et al., 2021; Li et al., 2023). The different chemical structures between polar biodegradable PLA and non-polar conventional plastics may lead to different adsorption capacity of Cd, the research of which is still lacking. Previous research indicated that PLA MPs adsorbed more Cd than conventional MPs (PE, PP and PVC), probably because of the oxygen-containing functional groups on PLA surface that can dissociate and provide adsorption sites for Cd cations (Fei et al., 2023; Jiang et al., 2024; Zhang et al., 2024). These above mentioned studies focused on purchased MPs, which may not represent well naturally generated MPs such as plastic mulch-derived MPs in agricultural system (Sections 1.2.6 and 2.3.5). However, it is not known how adsorption of Cd on plastic mulch-derived MPs behaves, especially on most commonly used mulching plastics conventional PE and biodegradable PLA (Sections 1.2.6, 2.3.5 and 2.3.6).

6.1.2 Increase in adsorption capacity of MPs after weathering

When exposed to the natural environment MPs can undergo various weathering processes, including physical abrasion, ultraviolet radiation, chemical oxidation and biodegradation (Andrady, 2011; Jahnke et al., 2017; Luo et al., 2022), which may impact their adsorption capacity due to changes in their physical and chemical properties (Sections 1.8 and 2.4.3).

Generally, the surface morphology, roughness, hydrophilicity and chemical composition of MPs could change after weathering (Liu et al., 2020). Furthermore, weathering of MPs could increase the adsorption capacity of MPs because of the roughening of the MPs surface, creating additional surface area, and the generated oxygen-containing groups on the surface of the MPs (Holmes et al., 2012; Mao et al., 2020; Wang et al., 2020). Previous reviews (Liu et al., 2020; Sun et al., 2020; Luo et al., 2022) summarised that the majority of studies used laboratory simulation weathering methods, such as UV light, photo-Fenton reaction, physical abrasion (mechanical fragmentation), chemically or thermally induced oxidation, to investigate the impacts of weathering on MPs adsorption. Additionally, MPs weathering process not only can be affected by the above mentioned physical and chemical factors but also affected by microbial attachment and colonisation (biological weathering) (He et al., 2023; Wang et al., 2023), which has been neglected. Generally, microorganisms colonise on MPs and promote biodegradation and biotransformation by adhering to the surface of the MPs and forming biofilms (Luo et al., 2022; Wang et al., 2021). The presence of biofilms could also affect the sorption behavior of the MPs, and previous studies suggested that greater adsorption of the weathered MPs due to the development of biofilms compared to the pristine MPs (Qi et al., 2021; Qiongjie et al., 2022).

6.1.3 Rational, aim and hypotheses

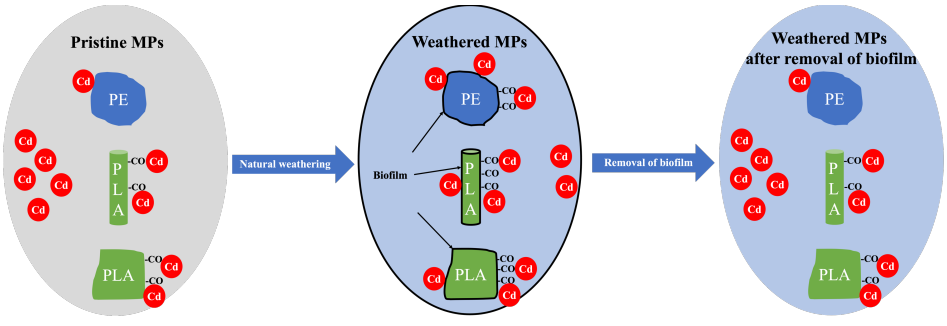
Previous studies mainly focused on the purchased MPs after artificial weathering which may not fairly represent naturally weathered MPs. Therefore, adsorption experiments in this chapter to investigate the adsorption of Cd on pristine and naturally weathered plastic mulch-derived MPs are warranted. The adsorption experiment documented in this chapter aimed to compare the adsorption of Cd on MPs of the most commonly used conventional and biodegradable mulching plastics, PE and PLA (Sections 1.2.6 and 2.3.6) respectively, and their adsorption capacity before and after natural weathering. Based on previous research, we hypothesised that (1) the different chemical structures of PE and PLA MPs would be the main factor causing their different adsorption capacity (greater adsorption capacity of PLA MPs relative to PE MPs), (2) natural weathering would increase the adsorption of Cd on MPs, and (3) biofilms would be the main reason impacting the adsorption after natural weathering.

Biodegradable PLA microplastics adsorb more Cd than conventional PE microplastic and biofilms enhance adsorption

Highlights

- PLA MPs adsorbed significantly more Cd than PE MPs
- Weathering led to the appearance of oxygen-containing functional groups in PE MPs
- Weathering increased abundance of oxygen-containing functional groups in PLA MPs
- Biofilms developed on MPs during weathering
- Increased adsorption of Cd after weathering was due to biofilms

Abstract: Biodegradable polylactic acid (PLA) mulch has been developed to replace conventional polyethylene (PE) mulch in agriculture to reduce plastic pollution and the accumulation of microplastics (MPs) in soil. Cadmium (Cd) is a significant soil contaminant, and can be adsorbed by MPs. It is increasingly recognised that in the natural environment biofilms can develop on MPs and that this can affect their adsorption properties. PLA and PE mulches were exposed outdoors for 16 months. MPs were then generated from pristine and weathered mulches. Biofilms developed on the weathered plastics. Oxygen-containing functional groups were detected on the weathered, but not the pristine PE, abundance of these groups increased for the weathered PLA. After removal of the biofilm the observed increases in oxygen-containing functional groups relative to the pristine plastics remained. In adsorption experiments pristine PLA MPs had a greater maximum adsorption capacity than pristine PE MPs (106 - 126 vs 23.2 mg / kg) despite having a lower specific surface area (0.325 m^2 / g vs 1.82 m^2 / g) suggesting that the greater levels of adsorption were due to MP chemistry. The weathered plastics adsorbed more Cd than the pristine plastics (e.g. maximum adsorption capacities of 153 - 185 and 152 mg / kg for the weathered PLA and PE respectively). However, after removal of the biofilm, adsorption of Cd to the weathered MPs was no greater than for the pristine plastics. This suggests that the increased adsorption of Cd due to weathering was caused primarily by adsorption onto the biofilm.



Graphical abstract

Key words: pristine MPs, natural weathering, biofilm, oxygen-containing functional groups, shape

Introduction

Agricultural plastic mulches have been developed to increase yields and improve crop quality (Deng et al., 2006, Kasirajan and Ngouajio, 2012). The most commonly used type is polyethylene (PE) (Khalid et al., 2023, Qiang et al., 2023). It is estimated that over 8300 million tonnes of plastics have been produced globally, with PE accounting for 36.3 % of that figure, in addition, by 2023 annual plastic production rates were over 400 million tonnes (Singh et al., 2024, Plastics Europe, 2024). Biodegradable plastic mulches have been developed to replace conventional PE mulch as a response to growing concerns about plastic pollution (Sintim and Flury, 2017). In 2028, global bioplastics production capacity is set to increase significantly to approximately 7.43 million tonnes (Plastics Europe, 2024). Polylactic acid (PLA) accounts for the largest share (24 %) of total global biodegradable plastics manufacturing (Haider et al., 2019) and it has wider application potential in the future (European Bioplastics, 2023).

Under some management practices, plastic mulches are ploughed into the soil at the end of their use which could generate plastic debris with a range of sizes including microplastics (MPs), i.e. plastic material less than 5 mm in size (Thompson et al., 2004). Microplastic mulch residues could also form when mulch degrades during exposure to ultraviolet (UV) radiation and weather (Huang et al., 2020, Luo et al., 2022). Once MPs enter the environment, they can interact with contaminants, such as metals (Alimi et al., 2018, Gao et al., 2021a; Ricardo et al., 2021, Liu et al., 2022a, Luo et al., 2022, Li and Wang, 2023), potentially reducing the availability of metals to soil organisms (Cao et al., 2021, Hodson et al., 2017, Khalid et al., 2021, Xiao et al., 2024). Cadmium (Cd) contamination of agricultural soils is an ongoing challenge around the world (Burau, 1981, Quezada-Hinojosa et al., 2009, Park et al., 2010, Zhao et al., 2015, Niño-Savala et al., 2019, Gray and Cavanagh, 2023) often due to the use of Cd-bearing phosphate fertilisers (Niño-Savala et al., 2019) with Cd concentrations in soils being reported up to 47.7 mg / kg (Huang et al., 2024a). It is already known that MPs can adsorb Cd (Alimi et al., 2018, Wen et al., 2018, Zhou et al., 2020). Fei et al., (2023) and Jiang et al., (2024) found that pristine biodegradable PLA MPs adsorbed more Cd than conventional polyvinyl chloride (PVC) and polypropylene (PP) MPs. Most (> 99 %) conventional MPs found in arable land are PE MPs (Liu et al., 2022b) and PE MPs have been found to adsorb Cd (Wang et al., 2019, Zhang et al., 2020). However, it is not known how adsorption of Cd compares between PE MPs and biodegradable PLA MPs.

Weathering of plastic mulches may impact their adsorption of contaminants due to changes in their physical and chemical properties (Zhang et al., 2018, Duan et al., 2021; Ventura et al., 2024). The mechanisms of plastic ageing are complex and diverse and can be affected by environmental factors (Ding et al., 2020, Ge et al., 2023). The mechanisms include physical ageing (abrasion), chemical ageing (advanced oxidation and photo-oxidation) and biological ageing (microbial attachment and colonization) (He et al., 2023, Wang et al., 2023a). However, the majority of studies on ageing of MPs consider UV ageing and biological ageing has been neglected (Ge et al., 2023, Wang et al., 2023a). When exposed to the natural environment plastic mulches are readily colonised by microorganisms (Bhagwat et al., 2021). Microorganisms attach to the surface of plastics, reproduce and secrete polysaccharide substrates that encapsulate the bacterial colonies and lead to the formation of biofilms (Luo et al., 2022, Tu et al., 2020, Wang et al., 2021, Moyal et al., 2023). These biofilms can increase the size, density and surface hydrophobicity of MPs, which may promote the transport and sedimentation of MPs in the environment (Ding et al., 2015, Chen et al., 2019). Furthermore, these biofilms can act as reservoirs for pathogenic microorganisms which can spread through the food chain (Keswani et al., 2016, Imran et al., 2019, Hirt and Body-Malapel, 2020). The biofilms may also modify the sorption behavior of the MPs. The adsorption capacity of conventional MPs (including PE, PS, PET, PA, PVC and PP) for divalent metals generally increases with artificial ageing (Chen et al., 2021, Gao et al., 2021b, Wang et al., 2023b) due to the roughening of the MPs surface, creating additional surface area, and the appearance of oxygen-containing groups on the surface of the MPs (Mao et al., 2020, Liu et al., 2022c). Huang et al., (2023, 2024b) found that biodegradable PLA had a greater adsorption capacity for divalent metal ions after artificial ageing, mainly due to an increase in oxygen-containing functional groups. However, the above studies (Chen et al., 2021, Gao et al., 2021b, Huang et al., 2023, Wang et al., 2023b, Huang et al., 2024b) only compared pristine with artificially aged (UV-aged and H_2O_2-aged) conventional and biodegradable MPs which may not be good representatives of naturally weathered MPs, particularly in terms of the development of biofilms (Hanun et al., 2021, Alimi et al., 2022, Lozano et al., 2023).

Therefore, the aim of this study was to compare the adsorption of Cd on MPs of the most commonly used conventional and biodegradable mulching plastics, PE and PLA respectively, and also to investigate their adsorption capacity before and after natural weathering. Previous studies mainly focused on MPs after artificial weathering which may not fairly represent naturally weathered MPs. As far as the authors are aware, this is the first study to directly compare adsorption of Cd on pristine and naturally weathered PE and PLA MPs generated from plastic mulches. This study will provide a better understanding of the mobility and distribution of Cd in agricultural environments where plastic mulches are used.

Materials and methods

Plastic mulch selection

Two types of plastic mulches were selected, non-biodegradable PE and biodegradable PLA (Section 3.4.1). They were confirmed to be PE and PLA using Fourier transform infrared (FTIR) spectroscopy (Section 3.4.1.1). The details of PE and PLA mulches naturally age and the generation of their MPs are in Section 3.4.2. Details of their characterisation are given in the Chapter 3 Materials and methods (Sections 3.4.1.1, 3.4.1.2, 3.4.1.3 and 3.4.3).

Adsorption experiments

A series of batch adsorption experiments were conducted to investigate the potential for the different types of MPs to adsorb Cd. Approximately 0.2 g of MPs were accurately weighed and added to 30 mL of a Cd solution obtained by dissolving $Cd(NO_3)_2 \cdot 4H_2O$ in a background electrolyte of 0.01 mol / L $NaNO_3$ at a pH of 6.0 to give a constant ionic strength (Hodson et al., 2017). Initial Cd target concentrations were 0.1, 0.5, 1, 5, 10, 50 and 100 mg / L with triplicate control and MP treatments at each concentration. These concentrations were chosen due to the high levels of Cd found in contaminated soils (e.g. Huang et al., 2024a; Section 3.5) and to provide a range in concentrations of several orders of magnitude to generate adsorption isotherms (Kettum et al., 2018, Shahrokhi-Shahraki et al., 2021, Qu et al., 2022). Samples were shaken in 50 mL centrifuge tubes at 220 rpm on a flatbed shaker in a 12 °C temperature controlled room for 24 h. This temperature was used for consistency with the previously experiment conducted by the authors in which earthworms were exposed to both MPs and Cd (Xiao et al., 2024; Chapter 5). 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, Khan and Hodson, 2024). After 24 h, suspensions were centrifuged at 4000 RPM for 15 minutes and filtered through Whatman no. 540 12.5 cm diameter filter paper. All filtered solutions were analysed for Cd using a ICP-OES and their quality control was calculated (Section 3.6.2). Controls were used to measure actual concentrations of initial Cd solution. Cd adsorption by MPs was calculated by difference between the average Cd concentrations in the controls and MP treatments at the end of the adsorption period.

As adsorption is a surface phenomenon, adsorption isotherms are used to predict the amount of sorbate that can sorb to a solid surface. Linear, Langmuir and Freundlich isotherms are the most common adsorption isotherms for explaining the adsorption process (Fred-Ahmadu et al., 2022; Biswas et al., 2024), which were used to fit the solution-solid (Cd-MPs) adsorption data in this particular study. Linear isotherm (one-parameter model) as the most fundamental isotherm model can be used to describe the partitioning of adsorbates between liquid (Cd solution) and

solid phases (MPs) (Ayawei et al., 2017). Langmuir and Freundlich isotherms (two-parameter models) also as the most extensively used models can be used to determine the adsorption affinity and maximum sorption capacity (Teuten et al., 2007; Ayawei et al., 2017). Furthermore, Langmuir model is a monolayer phenomenon, while Freundlich model is a multilayer phenomenon (Biswas et al., 2024). Godoy et al,. (2019) indicated Langmuir model was better fitted for the adsorption of bivalent metals on MPs (PE, PET, PS, PP and PVC), whereas Zou et al., (2020) indicated Freundlich model was better described the data on the adsorption of bivalent metals on MPs (PE and PVC). Additionally, Liu et al., (2019a) suggested adsorption on aged MPs matched well with Freundlich model because multilayer adsorption occurred on the heterogeneous surface of the aged MPs with pores or cracks. These three isotherms, Linear, Langmuir and Freundlich isotherms, therefore were all used in this study to describe the adsorption of Cd on pristine and weathered MPs. For fitting the linear isotherm the regression line was constrained to pass through the origin because there is no Cd adsorbed on MPs if the there is no Cd in the solution (0 mg / L in control treatments) and measured Cd of MPs is negligible (Section 3.4.3.4).

Data analysis

Statistical analysis was carried out using SigmaPlot version 15.0 software, SPSS version 28.0.1.1 (15) and Excel version 16.72. The variation of adsorption data (the Cd concentration adsorbed to MPs at equilibrium C_{MPs}, mg / kg) was analysed using a non-parametric 3 way Scheirer Ray Hare test (Holmes et al., 2017) as it proved not possible to transform the adsorption data to obtain a normal distribution and equal variance. Initial target Cd concentration (0.1, 0.5, 1, 5, 10, 50, 100 mg / L), plastic type (PE, PLAF, PLAP) and plastic treatment (pristine, weathered, weathered-washed) were used as factors, Games-Howell post hoc tests were then carried out (Field, 2013). Variation in relative intensity of FTIR peaks and amount of biofilm (OD_{595}, OD_{595} / mass and OD_{595} / surface area) was analysed with plastic type (PE, PLAF, PLAP) and plastic treatment (pristine, weathered, weathered-washed) as factors. Normality and equal variance were assessed using the Shapiro-Wilks and Brown-Forsythe tests respectively. The relative intensity of FTIR peaks data set did not have equal variance ($P \leq 0.05$) and the OD_{595} / mass data set was not normally distributed ($P \leq 0.05$). It was not possible to transform the relative intensity of FTIR peaks data set to both a normal distribution and equal variance so this data was analysed using the non-parametric 2 way Scheirer Ray Hare test (Holmes et al., 2017) followed by Games-Howell post hoc tests (Field, 2013). A log10 transformation was used to convert the OD_{595} / mass data set to obtain normal distributions and equal variance. Data sets were then analysed using a two way ANOVA analysis followed by Holm-sidak post doc tests (Underwood, 1997).

Results

Adsorption of Cd on different types of MP

Cadmium adsorption isotherms of pristine MPs, weathered MPs and weathered then washed MPs are shown in Fig. 6.1 with the fitting parameters listed in Table 6.1. In these adsorption experiments, the amount of Cd adsorbed on MPs ranged from 1.742 to 993.1 mg / kg (Fig. 6.1a). The adsorption data obtained at an initial Cd concentration of 100 mg / L appeared to deviate slightly from the trend of the other data. However, based on 95 % confidence intervals from fitted parameters of the isotherms, there were no significant differences between when the 100 mg / L data were included and excluded (Table 6.1 and S6a.1). Thus, the Cd data at 100 mg / L were included in this analysis.

The results of the 3 way Scheirer Ray Hare Test showed that initial Cd concentration, plastic type and plastic treatment had a significant impact on adsorption (initial Cd concentration: $H_{6,126}$ = 66.5, $P \leq 0.05$; plastic type: $H_{2,126}$ = 13.2, $P \leq 0.05$; plastic treatment: $H_{2,126}$ = 82.8, $P \leq 0.05$; Table S6b.1); there were no significant interactions (initial Cd concentration x plastic type: $H_{12,126}$ = 2.29, $P \geq 0.05$; initial Cd concentration x plastic treatment: $H_{12,126}$ = 17.0, $P \geq 0.05$; plastic type x plastic treatment: $H_{4,126}$ = 4.06, $P \leq 0.05$; initial Cd concentration x plastic type x plastic treatment: $H_{24,126}$ = 0.839, $P \geq 0.05$; Table S6b.1). Adsorption increased with initial Cd concentrations (from below detection to almost 1000 mg / kg; Fig. 6.1a). PLA adsorbed more Cd than PE (106 - 126 vs 23.2 mg / kg; Table 6.1). Adsorption onto the weathered MPs (maximum adsorption capacities of 153 - 185 and 152 mg / kg for the weathered PLA and PE respectively; Table 6.1) was significantly greater ($P \leq 0.05$, Games - Howell post hoc test; Table S6b.2) than that onto either the pristine (maximum adsorption capacities of 106 - 126 and 23.2 mg / kg for the pristine PLA and PE respectively; Table 6.1) or weathered then washed MPs (maximum adsorption capacities of 108 - 153 and 108 mg / kg for the weathered then washed PLA and PE respectively; Table 6.1) but there were no significant differences ($P \geq 0.05$, Games - Howell post hoc test; Table S6b.2) between adsorption to the pristine and weathered then washed MPs.

The linear, Freundlich and Langmuir isotherms all described the data well though fits to the Langmuir and Freundlich isotherms were better than those to the linear isotherm (Table 6.1). Pristine PLA adsorbed more Cd than pristine PE, regardless of its shape (Fig. 6.1a). The maximum adsorption capacity (C_m) of the pristine PLA MPs was significantly greater than that of the pristine PE MPs when 95 % confidence intervals were considered (Table 6.1). Isotherm fit parameters (K_d, K_F, and C_m) indicated no significant difference of adsorption capacity between the PLA fibres and PLA pieces given the over lapping 95 % confidence intervals (Table 6.1). Consistent with the results of the 3 way Scheirer Ray Hare Test both the weathered

and the weathered then washed PE showed significantly greater adsorption of Cd relative to the pristine PE (Fig. 6.1a) but this was not the case for the pristine and weathered or weathered then washed PLA (Table 6.1).

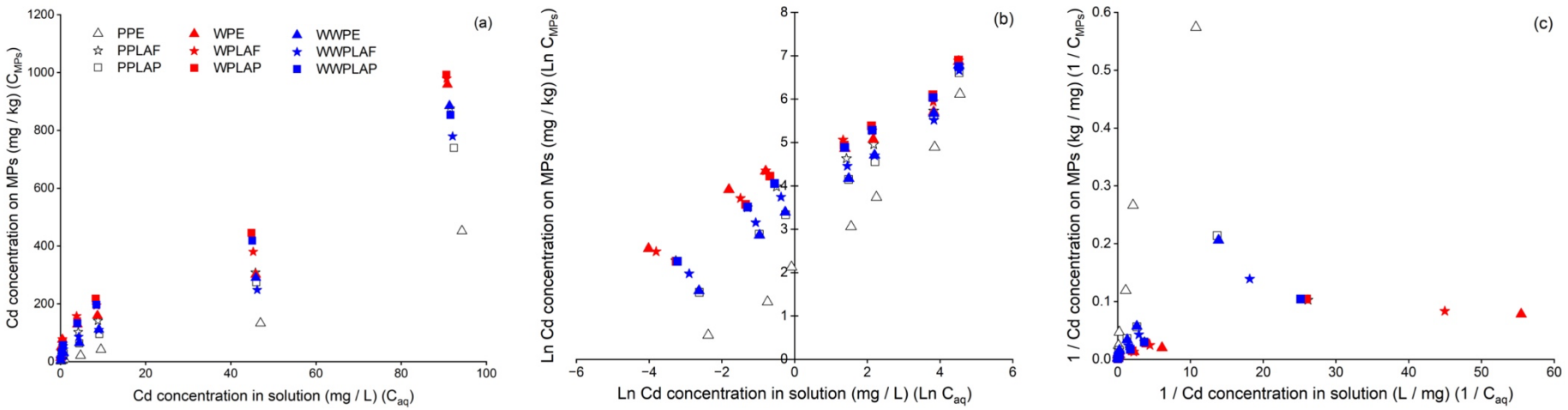


Fig. 6.1. (a) Adsorption data for different types of MPs expressed per unit mass. (b) Freundlich isotherms for different types of MPs (per unit mass). (c) Langmuir isotherms for different types of MPs (per unit mass). White symbols are pristine MPs, red are weathered MPs and blue are weathered and washed MPs. Triangles are PE, stars are PLAF and squares are PLAP. Individual plots are presented in the SI (Figs. S6a.2-S6a.4).

Table 6.1. Linear, Freundlich and Langmuir isotherm parameters for Cd adsorption to different types of MPs with concentrations adsorbed expressed on a per unit mass basis, 95 % confidence intervals are given in brackets. Initial Cd concentrations range from 0.1 to 100 mg / L. C_{MPs} is concentration adsorbed to MPs at equilibrium, mg / kg, C_{aq} is concentration in solution at equilibrium, mg / L. The linear adsorption model is expressed as $C_{MPs} = K_d C_{aq}$ where K_d 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, $(L / mg)^{1/n}$. 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 constant, mg / L and C_m is the maximum concentration adsorbed to MPs, mg / kg.

	Linear			**Freundlich**				**Langmuir**			
MPs type	**K_d**	**R^2**	**P**	**LnK_F**	**1/n**	**R^2**	**P**	**K_L**	**C_m**	**R^2**	**P**
pristine PE / PPE	4.405 (3.625-5.184)	0.82	< 0.001	2.115 (1.771-2.460)	0.7785 (0.6485-0.9085)	0.98	< 0.001	0.8357 (0.6567-1.622)	23.2 (9.115-42.72)	0.92	< 0.001
pristine PLA fibre / PPLAF	9.082 (7.550-10.62)	0.81	< 0.001	4.041 (3.758-4.324)	0.5172 (0.4156-0.6187)	0.97	< 0.001	2.158 (0.4991-3.324)	126 (69.66-660.1)	0.97	< 0.001
pristine PLA piece / PPLAP	7.634 (6.711-8.557)	0.8	< 0.001	3.332 (3.074-3.591)	0.6507 (0.5541-0.7474)	0.98	< 0.001	0.6238 (0.1552-1.014)	106.2 (59.87-469.7)	0.99	< 0.001
weathered PE / WPE	9.841 (7.703-11.98)	0.79	< 0.001	4.435 (4.075-4.796)	0.4284 (0.3068-0.5500)	0.93	< 0.001	5.032 (1.912-7.279)	152.4 (90.62-479.0)	0.98	< 0.001
weathered PLA fibre / WPLAF	10.45 (8.449-12.46)	0.8	< 0.001	4.425 (4.166-4.683)	0.4791 (0.3898-0.5684)	0.97	< 0.001	3.767 (0.2845-6.055)	153.2 (78.98-2560)	0.96	< 0.001

weathered PLA pieces / WPLAP	10.9 (9.123-12.68)	0.81	< 0.001	4.258 (4.024-4.492)	0.5497 (0.4656-0.6338)	0.98	< 0.001	1.399 (0.1617-2.359)	185.2 (97.52-1834)	0.99	< 0.001
washed, weathered PE / WWPE	9.045 (7.627-10.46)	0.81	< 0.001	3.386 (3.107-3.666)	0.6684 (0.5637-0.7731)	0.98	< 0.001	0.646 (0.09841-1.090)	107.7 (57.81-789.4)	0.99	< 0.001
washed, weathered PLA fibre / WWPLAF	7.902 (6.460-9.344)	0.8	< 0.001	3.695 (3.386-4.005)	0.5647 (0.4509-0.6785)	0.96	< 0.001	1.278 (0.2034-2.059)	107.6 (57.67-804.2)	0.98	< 0.001
washed, weathered PLA pieces / WWPLAP	9.449 (7.854-11.04)	0.81	< 0.001	4.156 (3.997-4.316)	0.5452 (0.4877-0.6028)	0.99	< 0.001	1.643 (0.1599-2.725)	153.9 (80.26-1876)	0.98	< 0.001

MP characteristics

The 16 months of weathering outside had no obvious optically-visible changes in the appearance of the plastic mulch-derived MPs before and after weathering or washing in ethanol (Fig. 3.5 in Chapter 3). However, SEM images and FTIR spectra suggested that biofilms had formed on the surfaces of the weathered MPs and that the biofilms were removed by the ethanol-wash (Figs. 3.6-3.8 in Chapter 3). The surfaces of the weathered and the weathered then washed MPs were rougher and had creases (PE) or were more uneven (PLA) compared with those of the pristine MPs (Figs. 3.6-3.8 in Chapter 3). Furthermore, the FTIR spectra of both the weathered PE (WPE) and weathered PE after ethanol washing (WWPE) showed a new peak at around 1100 cm^{-1} compared to the pristine PE (PPE). This peak corresponds to the oxygen-containing hydroxyl functional group C-O-H (Fig. 6.2a). The intensity of this peak, relative to that of the C-H peak (around wave numbers 3200 - 2600 cm^{-1}) decreased significantly after the WPE was washed with ethanol ($H_{2,18} = 24.4$, $P \leq 0.001$, 2 way Scheirer Ray Hare Test; Games - Howell post hoc test; Tables S6b.3, S6b.4) (Table 3.3 in Chapter 3). There was no obvious change in the peaks present in the FTIR spectra of the PLA across different treatments (Fig. 6.2b). However, the relative peak intensity of the C-O peak (1300 - 800 cm^{-1}) to the C-H peak (3200 - 2600 cm^{-1}) increased significantly in the order pristine PLA (PPLAF and PPLAP) < weathered then washed PLA (WWPLAF and WWPLAP) < weathered PLA (WPLAF and WPLAP) ($H_{2,18} = 24.4$, $P \leq 0.001$, 2 way Scheirer Ray Hare Test; Games - Howell post hoc test; Tables S6b.3, S6b.4) (Table 3.3 in Chapter 3).

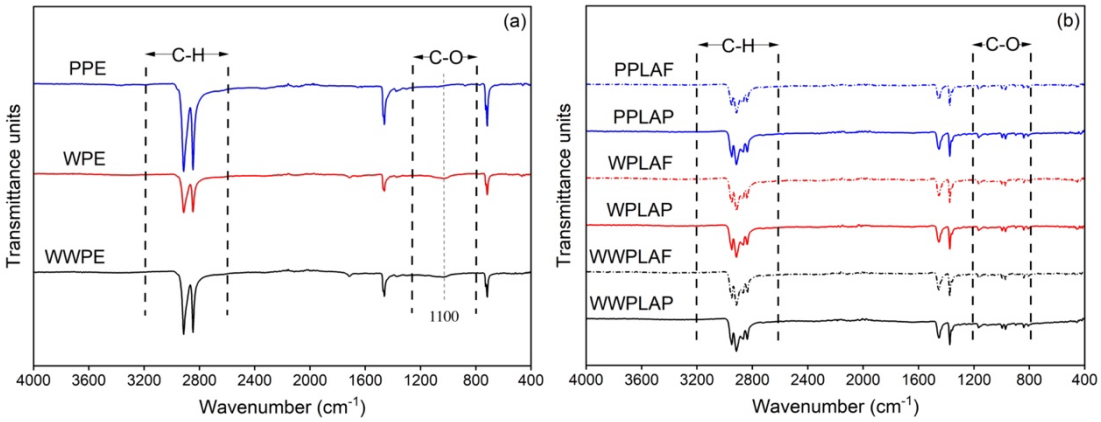


Fig. 6.2. FTIR spectra of different types of MPs: (a) pristine (PPE), weathered (WPE) and ethanol-washed weathered PE (WWPE) and (b) pristine (PPLAF, PPLAP), weathered (WPLAF, WPLAP) and ethanol-washed weathered PLA fibres and pieces (WWPLAF, WWPLAP). The grey vertical dotted line in (a) indicates the position of the new peak at 1100 cm^{-1} in the WPE and WWPE samples.

The amount of biofilm on the MPs was determined using crystal violet staining and absorption at 595 nm (OD_{595}, Fig. 6.3). There was no significant difference in the OD_{595} values between the different pristine MPs ($P \geq 0.05$, Holm-Sidak post hoc test; Table S6b.5). After being weathered or weathered and then washed, PE and PLA showed significant differences in the amount of biofilm present; significantly less biofilm was associated with the PE ($F_{2,18} = 220$, $P \leq 0.001$, two way ANOVA; Holm-Sidak post hoc test; Table S6b.5) (Fig. 6.3). Biomass per unit mass was greater for the PE than the PLA ($F_{2,18} = 1605$, $P \leq 0.001$, two way ANOVA; Holm-Sidak post hoc test; Table S6b.6) (Fig. 6.3b), whereas biomass per unit area was greater for the PLA ($F_{2,18} = 454$, $P \leq 0.001$, two way ANOVA; Holm-Sidak post hoc test; Table S6b.7) (Fig. 6.3c). Regardless of the normalisation of biofilm biomass (biomass: Fig. 6.3a; biomass per unit mass: Fig. 6.3b; biomass per surface area: Fig. 6.3c), the amount of biofilm decreased significantly in the order weathered MPs > weathered then washed MPs > pristine MPs for both PE and PLA (OD_{595}: $F_{2,18} = 10134$; OD_{595} / MP mass: $F_{2,18} = 6550$; OD_{595} / MP surface area: $F_{2,18} = 5115$; $P \leq 0.001$, two way ANOVA; Holm-Sidak post hoc test; Tables S6b.5, S6b.6, S6b.7) (Fig. 6.3). The pristine PLA fibres and particles did not have significantly different amounts of biofilm associated with them regardless of normalization to mass or surface area ($P \geq 0.05$, Holm-Sidak post hoc test; Tables S6b.6, S6b.7) (Fig. 6.3b, 6.3c). However, the weathered and weathered then washed mass normalised biofilm concentrations were greater for the PLAP than the PLAF ($P \leq 0.001$, Holm-Sidak post hoc test; Table S6b.6) (Fig. 6.3b), whereas the weathered and weathered then washed surface area normalised biofilm concentrations were greater for the PLAF than the PLAP (Fig. 6.3c) ($P \leq 0.001$, Holm-Sidak post hoc test; Table S6b.7) (Fig. 6.3c).

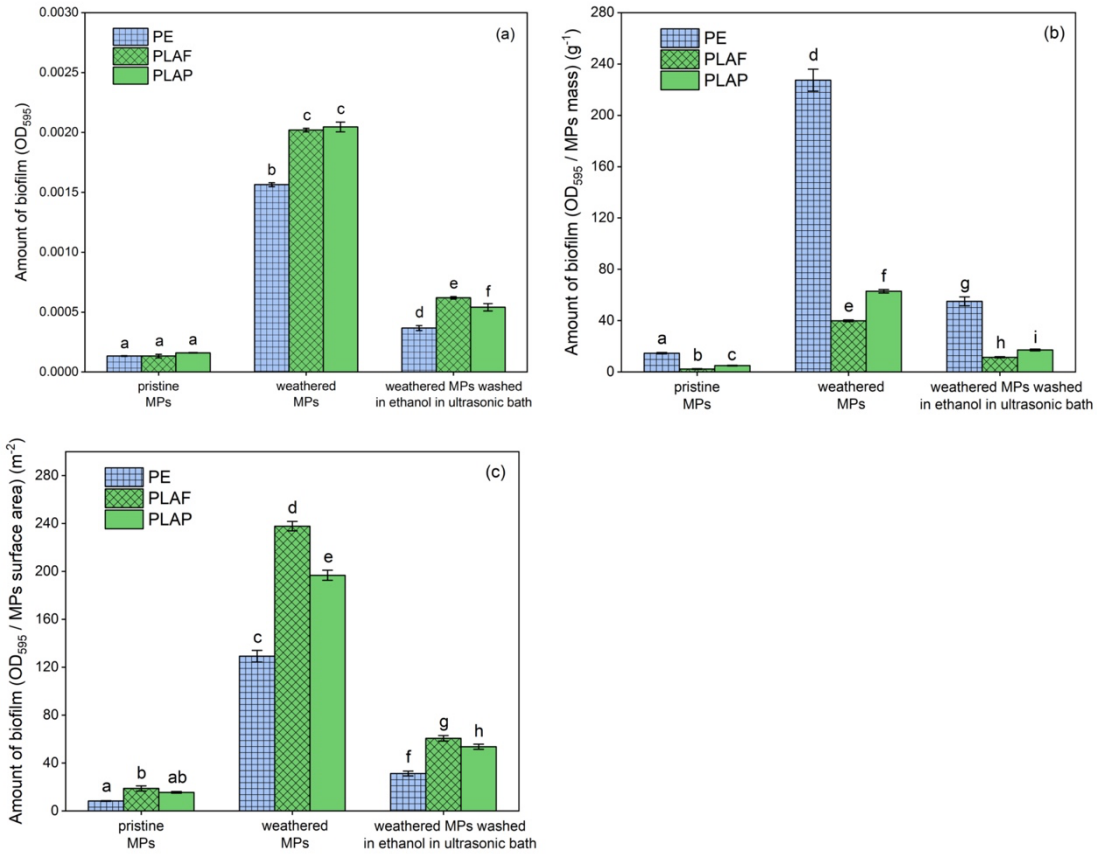


Fig. 6.3. Amount of biofilm on the PE, PLAF and PLAP samples expressed as (a) OD_{595}, (b) OD_{595} / MP mass, and (c) OD_{595} / MPs surface area. Values are averages ± standard deviations (n = 3). Different letters above the bars indicate a significant difference at $P \leq 0.001$ (2 way ANOVA followed by Holm-Sidak post hoc test).

Discussion

Cd adsorbed to all the MPs tested and was better described by Freundlich and Langmuir than by linear isotherms (Table 6.1). Adsorption was greater for the biodegradable PLA than the conventional PE MPs and significantly greater for the weathered than either the weathered then washed or pristine MPs (Fig. 6.1, Table 6.1). The amount of biofilm extracted from the MPs decreased in the order weathered > weathered then washed > pristine (Fig. 6.3) as did the relative intensity of the oxygen-bearing functional groups present in the FTIR spectra.

Impacts of MPs shape and chemistry on adsorption of Cd

Adsorption is a surface phenomenon; as SSA increases more surface is available for sorption per mass of material (Wang et al., 2019, Zhang et al., 2020, Zhou et al., 2020, Li et al., 2022, Chang et al., 2024). The PE MPs used in this study were platy particles, while PLA MPs were either platy or fibrous particles (Fig. 3.5 in Chapter 3). These calculations suggest that the mean SSAs of the platy PE MPs, platy PLA MPs and fibrous PLA MPs were 1.82, 0.217 and 0.433

m^2 / g respectively (Section 3.4.3.2). Although the SSA calculations do not take into account surface roughness and are therefore approximations, visual analysis of the MP surfaces by SEM suggests that the pristine PE and PLA show no significant difference in terms of their roughness (Figs. 3.6-3.8 in Chapter 3). Surface roughness is a scaling factor between BET and geometric SSA (Brantley and Mellott, 2000) and thus whilst the absolute values of SSA may vary from the calculations in this study, these calculations do indicate that there was substantially more surface area present in the pristine PE adsorption experiments than in those that used the pristine PLA, whilst the platy and fibrous PLA had very similar SSA. Therefore, the lack of a significant difference in adsorption between the platy and the fibrous PLA (Table 6.1) was not unexpected; for a given mass of each shape of particle a similar amount of surface should have been available for adsorption. However, there would have been more PE than PLA surface area for a given mass of particles in our adsorption experiments. Despite this, the PLA MPs showed greater adsorption of Cd than the PE MPs (Fig. 6.1, Fig. S6a.1, Table 6.1) suggesting that this was due to differences in the chemistry of the MPs rather than available surface area for adsorption (Gao et al., 2021a, Khalid et al., 2021). Similarly, previous studies also found that biodegradable MPs were able to adsorb more metal ions than conventional MPs (Li et al., 2018, Huang et al., 2023, Shi et al., 2023, Huang et al., 2024b, Jiang et al., 2024), however, they did not take surface area into account. Therefore, they were unable to unambiguously attribute this to compositional differences between the plastics rather than their size and shape. The PLA contained polar, oxygen-containing ether functional groups whereas PE contained no polar functional groups (Fig. 3.2, Tables 3.2 and 3.3 in Chapter 3). Adsorption between non-polar PE and metal ions is likely to be a non-specific interaction such as by weak van der waal forces (Gao et al., 2021a, Xu et al., 2021). In contrast, metal ions can adsorb by surface complexation with the polar functional groups of PLA (Fan et al., 2018, Wu et al., 2019) leading to an increase in adsorption (Brennecke et al., 2016).

Impacts of natural weathering on adsorption of Cd on MPs

The adsorption of Cd on the weathered MPs was significantly higher than that on the pristine or weathered then washed MPs (Fig. 6.1 and Table 6.1). A biofilm was associated with the weathered MPs and was significantly reduced by the ethanol washing (Fig. 6.3). Previous studies have demonstrated an increase in adsorption by artificially aged MPs (Bhagat et al., 2022, Huang et al., 2023, Huang et al., 2024b) and the adsorption of metals by biofilms (Qi et al., 2021, Qiongjie et al., 2022). Ageing can alter the surface morphology (Liu et al., 2019b, Mao et al., 2020, Gao et al., 2024) and physicochemical properties of MPs (Guo and Wang, 2019, Wei et al., 2021, Deng et al., 2023). In this study, it was not able to quantify the additional surface area generated by weathering of the MPs (either from the biofilm or changes in the roughness of the MP itself) but SEM observation suggests that the biofilm associated with the

weathered MPs will have increased particle surface roughness and thus SSA to a greater extent than the physical affects of weathering but that the physical weathering of the MPs also increased the roughness of the particles, and thus SSA, relative to the pristine MPs (Figs. 3.6-3.8 in Chapter 3). Furthermore, the FTIR spectra of the weathered PE included a new peak at around 1100 cm^{-1} which corresponds to polar, oxygen-containing hydroxyl functional groups. Although their relative intensity decreased, the peaks remained present after the MPs were washed to remove the biofilm. In contrast to the PE, no new peaks were found in the PLA spectra after weathering. However, there was a significant increase in the relative intensity of peaks representing oxygen-containing ether and hydroxyl functional group (Fig. 6.2b, Table 3.3 in Chapter 3) on weathering of the PLA and a subsequent decrease after washing in ethanol. The relative intensities after washing were still greater than those for the pristine material. This suggests that some of the oxygen-containing functional groups were associated with the biofilm but that some were associated with alteration of the MP chemical structure. The new functional groups would provide new adsorption sites (Li et al. 2019a, Li et al. 2019b, Guan et al. 2020). However, once the biofilm was removed by washing in ethanol there was no significant difference in the adsorption of Cd between the weathered then washed compared to the pristine MPs. This suggests that the increased adsorption of Cd after weathering of the MPs was mainly due to adsorption by the biofilm rather than increased adsorption directly onto the surface of the MPs. However, it was unable to determine how much of the additional adsorption on the weathered MPs was due to the chemical groups in the biofilm as opposed to the additional surface area associated with the biofilm in this study. In slight contrast to the statistical analysis of the adsorption data, the differences in modelled K_d and C_m perhaps suggest some increased adsorption due to chemical changes in response to weathering, at least for the PE where the introduction of new functional groups might be considered a more significant chemical change than a slight increase in their abundance as was the case for the PLA. The majority of studies that have demonstrated an increase in adsorption to MPs after ageing have used artificial ageing methods such as UV light (Han et al., 2021, Bhagat et al., 2022, Qiongjie et al., 2022, Huang et al., 2023, Huang et al., 2024b), the photo-Fenton reaction (Lang et al., 2020, Liu et al., 2020, Jiang et al., 2022), or heat activated potassium persulfate oxidation (Wu et al., 2020) or reducing agents $NaBH_4$ (Xiao et al., 2023) which alter the physical (increased surface roughness, cracks and holes formation, decreased particle size) (Bhagat et al., 2022, Qiongjie et al., 2022, Huang et al., 2023) and chemical (changed functional groups on MPs and chain scission) (Han et al., 2021, Xiao et al., 2023, Huang et al., 2024b) properties of MPs rather than leading to the development of biofilms which potentially raises questions about the relevance of those studies to naturally weathering processes and environmental MPs. The results in this particular study confirm that natural weathering leads to the incorporation of oxygen-bearing functional groups into the weathered plastics, which could ultimately lead to increased adsorption of Cd. However,

the above mentioned studies and this study also suggest that whilst natural and artificial ageing can both result in increased adsorption, at least in the short (16 month of natural weathering) term this is likely due to different mechanisms with the changes in adsorption by the naturally weathered MPs being more closely linked to biofilm formation than structural / compositional changes.

Differences in biofilm formation between biodegradable PLA and conventional PE

The PE particles had a higher SSA than that of the PLA and would therefore have a higher surface area for a given mass of particles. As biofilms form on surfaces, this is consistent with the amount of biofilm being greater in the PE than PLA particles when normalized to mass as that mass of material represents a greater amount of surface. However, when normalized to surface area the opposite is seen (Fig. 6.3). PLA is, by design, biodegradable (Kasirajan et al., 2012, Haider et al., 2019) and the results of this study suggests that it is perhaps easier for biofilm to colonise PLA surfaces, most likely because the biodegradable PLA could act as a food source resulting in more biofilm being present on the particles per unit area.

Conclusions

The ecological hazards of Cd depend on its mobility, which can be influenced by adsorption. This study showed that polymer type, weathering and biofilms had a significant impact on adsorption of Cd by the MPs ($P \leq 0.05$). Pristine PLA was significantly more sorptive to Cd than pristine PE (e.g. maximum adsorption capacities of 106 - 126 vs 23.2 mg / kg). Thus, initially, reductions in Cd bioavailability in Cd-bearing soils may occur if PE mulches are replaced by PLA mulches which could lead to increased accumulation of Cd in soils but a reduced risk of this Cd entering the food chain by biomagnification. Weathering of the plastics resulted in the appearance (PE) or increased abundance (PLA) of oxygen-containing functional groups in their chemical structure, together with the development of biofilms leading to an increase in adsorption of Cd (e.g. increases in maximum adsorption capacity to 153 - 185 and 152 mg / kg for the PLA and PE respectively) which could decrease Cd mobility further. Artificial weathering of MPs which does not result in biofilm formation may lead to differing results between studies of naturally and artificially weathered MPs or mean that conclusions drawn from artificial weathering studies are not applicable to short term (months long duration) natural weathering. Although PLA is designed to biodegrade it showed little signs of degradation after 16 months exposure to natural conditions. In the short term use of PLA could therefore lead to increased retention of Cd in soil but reduced Cd bioavailability. If Cd has been retained in soil by adsorption to biodegradable MPs this might ultimately lead to a larger pool of bioavailable Cd on degradation of the MPs.

Limitations and future research recommendations

This study produced naturally weathered MPs by hanging up plastic mulch sheets to expose them to ambient weather conditions. However, when mulches are used in practice they are laid on the soil surface. Therefore only one side of the mulch would be exposed to sunlight, the other would be in contact with the soil which could result in different weathering mechanisms and / or different rates of weathering which could in turn result in different adsorption properties. Thus whilst authors believe that using naturally weathered MPs is an advance on using artificially weathered MPs in terms of environmental realism, further consideration to the exposure scenarios of pristine plastics is warranted when generating weathered MPs.

Whilst the amount of biofilm present on the MPs was quantified, further research on how biofilms form on MPs and adsorb metals (and other contaminants) at a molecular level is warranted to better understand the potential risk that this poses in the environment. Furthermore, genetic characterization of the biofilms would support understanding of their origins and properties. The use of isotopically labelled MPs in experiments could be investigated to determine whether the micro-organisms present in biofilms are using the MPs as a carbon source and actively metabolizing them.

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7 A field-based investigation into the impacts of conventional and biodegradable plastic mulch film on earthworm abundance, biomass and diversity

7.1 Introduction

The previous avoidance chapter (Chapter 4) compare the differences in the impacts of conventional PE and biodegradable PLA plastic mulch films and interaction between them and Cd on earthworm avoidance at a laboratory scale. An existing field experiment was carried out by the colleagues in the university of Bangor. Conventional and biodegradable plastic mulch films were used in their experimental site, which is matched with the Cd-free treatments of the laboratory experiments of this thesis. During the course of the PhD, this opportunity of sampling earthworms and soils at the Bangor field trial was offered. Although it is not a major part of the work carried out during the PhD, it has been reported here as the field sampling experience.

The aim of the field sampling was to compare the differences in the impacts of conventional and biodegradable mulch films on earthworm populations in natural farmland, which complements the Cd-free parts of the previous laboratory-scale avoidance experiments. To achieve this aim, both soils and earthworms were sampled from each plots and then transferred back to York. Afterwards, soil properties were measured and earthworm abundance and biomass were identified. Based on the results of previous Cd-free avoidance tests (Chapter 4) that earthworm had slightly preference to PLA relative to PE, this field sampling therefore tested the hypotheses that greater earthworm population would be found in PLA treatments.

7.2 Materials and methods

7.2.1 Sampling site

The sampling was conducted on an existing field experiment located at the Henfaes Agricultural Research Station, Abergwyngregyn, North Wales (53º14’29”N, 4º01’15”W) carried out by colleagues at the University of Bangor. More information of this field experiment was showed in Graf et al., (2024). The soil of this arable field is a freely draining Eutric Cambisol with crumb structure and there is no known history of previous plastic mulching or

application of known plastic contaminants, e.g., biosolids, compost, or coated fertilisers in this field. The field was ploughed one month before (May 2021) the start of this multi-year field experiment (14th June 2021). Prior to the experimental setup, each plots were prepared by manually weeding and rotavating the soil to a depth of 15 cm, which have also been repeated annually before each seeding. Forage maize seed were germinated in a damp tissue 4 days prior to sowing. The maize was sown by hand in two parallel rows per plot with 20 cm distance to the plot edges and 12 cm distance between seeds within the same row, resulting in an average of 14 seeds / m^2. The seeds were placed at a depth of 3 cm. This study site was covered by conventional PE or biodegradable PLA / PBAT (ratio 85 % PBAT; 15 % PLA) plastic mulch films and small holes were cut in the films by hand to plant the seeds directly into the soil and aid emergence.

This experiment has been repeated annually, using the same plots as the previous year. The maize was grown to maturity and harvested after 4 months (October of each year). After each year harvest, the remaining mulch film has been collected, cut into hand-sized pieces, and mixed back into the soil when rotavating the soil before the next sowing season (June of each year) to simulate the ploughing of mulch film into the soil. No agricultural activities occur on this site during the period of time between harvest and seeding for the next year.

7.2.2 Sampling details

This sampling took place on 24th April 2023, which was between the last harvest season (October in 2022) and the next sowing season (June in 2023). The field site layout and the sampling details are showed in Fig. 7.1. This sampling was for both soils and earthworms. There were 4 plots per treatment and one sample was taken in the middle of each plot (solid black square in Fig. 7.1). The experimental site is shallow and has a lot of rocks in the soil, so only a maximum depth of 10 cm could be dug into.

At each sampling location, the soil temperature at the depth of 10 cm were measured using a thermometer. In the meantime, a 25 cm x 25 cm x 10 cm deep volume of soil was dug up using a spade and earthworms were gently removed by hand. A density soil sample was collected by

horizontally inserting a density ring of fixed volume into the wall of the soil pit (Walter et al., 2016). Afterwards, 1 L mustard solution (1 tablespoon of dry Colman's original mustard powder per 1 L tap water) was poured into the hole and for up to 15 minutes after the solution draining, any earthworms which emerged from the base of the soil pit were collected (Gunn, 1992; Lawrence and Bowers, 2002). All found earthworms and 1 kg soil were sampled on each plot and transported back to York for the further measurement. Collected earthworms were separated in those sampled in the 25 cm x 25 cm x 10 cm deep volume of soil, and those emerged from the bottom of the soil after pouring mustard solution.

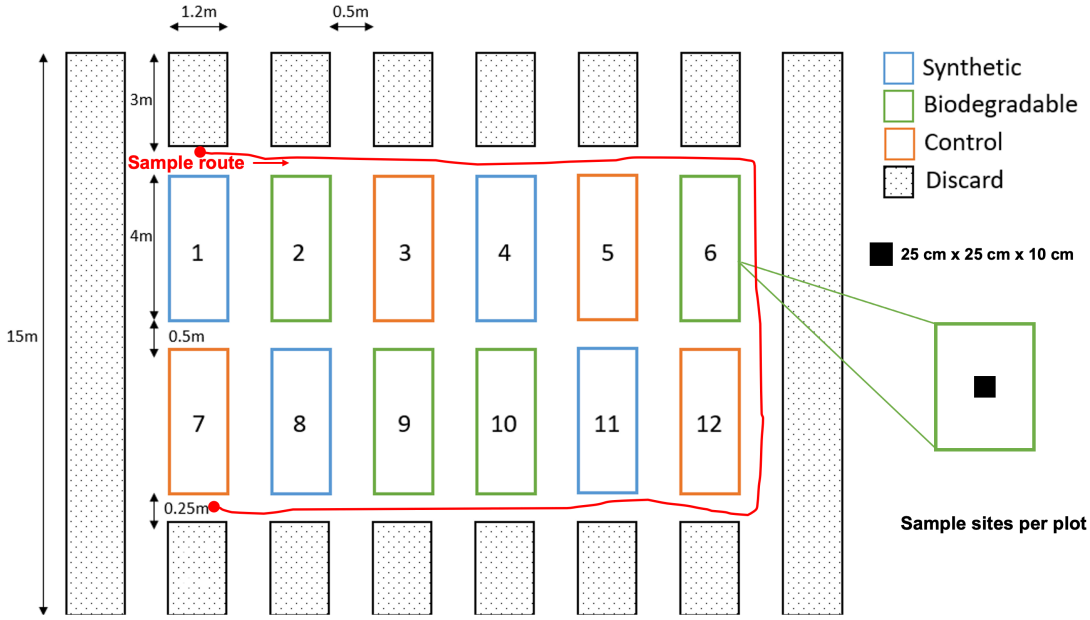

Fig. 7.1. Sampling diagram of Bangor existing field experiment treated with control, synthetic LDPE and biodegradable PLA / PBAT with sampling details. Solid black square represents our sampling location which is in the middle of each plot.

7.2.3 Soil properties measurement

The soil samples were air-dried to constant weight, sieved to < 2 mm and characterised. Physicochemical properties, soil pH, moisture (i.e. water holding capacity, WHC), organic matter and texture were measured for soil. The detailed methods were described in Section 3.2.

7.2.4 Earthworm identification

Earthworms were identified in accordance with the Earthworm Identification Guide (Jones, Imperial College London, 2015) and weighted in the laboratory. Total number of earthworms in each pot was recorded. Total mass was calculated by summing up all the earthworm biomass in each plot, and individual biomass was presented as their average weight (total biomass divided by total number in each plot).

The number of earthworm in this study is expressed as number per m^2 calculated by dividing the above recorded number of earthworms by the area of the pit. Earthworm total biomass is presented as gram per m^2 and individual biomass is presented as gram per individual.

7.2.5 Statistical analysis

A one way analysis of variance (ANOVA) followed by Holm-Sidak post hoc test was run using SigmaPlot version 15.0 software, with plastic type (non-MPs, PE and PLA) as the factor and measured soil pH, moisture, organic matter, earthworm number and biomass as the variables. Normality and equal variance were assessed using the Shapiro-Wilks and Brown-Forsythe tests respectively. Of the data investigated, the soil temperature, moisture, organic matter and biomass per earthworm data sets were normally distributed and had equal variance ($P \geqslant 0.05$), and soil pH, earthworm number and earthworm total biomass did not have equal variance ($P \leq 0.05$). Where possible data were transformed using a square root (earthworm total biomass) transform to obtain normal distributions and equal variance. Data were then analysed using one way ANOVA followed by Holm-Sidak post hoc tests. However, it was not possible to transform the soil pH data using log 10 , square, square root and 1 / X transformation to have equal variance. Therefore, soil pH data was analysed using the non-parametric Kruskal-Wallis test (i.e. one-way ANOVA on ranks). Pearson correlation was used to analyse the relationship between soil properties and earthworm abundance and biomass run by SPSS version 28.0.1.1 (15).

7.3 Results

7.3.1 Soil properties

Soil characteristics in the three different treatments (control, PE and PLA) are presented in Table 7.1. The soil at the field site was a sandy loam soil had a temperature of 11.0 ± 0.6 ^{o}C (mean ± standard deviation, n = 12), a pH of 6.4 ± 0.1 (mean ± standard deviation, n = 12), 27.99 ± 1.96 g / 100 g oven-dry soil (mean ± standard deviation, n = 12) moisture and 4.65 ± 0.10 g / 100 g oven-dry soil (mean ± standard deviation, n = 12) organic matter. There were no significant differences in these four characteristics between treatments (temperature: $F_{2,9}$ = 0.105, P = 0.901, one way ANOVA; pH: $H_{2,9}$ = 3.975, P = 0.145, Kruskal-Wallis test; moisture: $F_{2,9}$ = 2.121, P = 0.176, one way ANOVA; organic matter: $F_{2,9}$ = 0.813, P = 0.474, one way ANOVA; Tables S7.1-S7.4).

Table 7.1. Soil temperature, pH, moisture and organic matter of different microplastic plots, non MPs control treatment, conventional PE treatment and biodegradable PLA treatment. Results are mean ± standard deviation, n = 4.

Treatment	Soil temperature (^{o}C)	pH	Moisture (g H_2O / 100 g oven-dry soil)	Organic matter (g / 100 g oven-dry soil)
Control	11.10 ± 0.60	6.33 ± 0.05	26.71 ± 3.13	4.64 ± 0.07
PE	10.90 ± 0.80	6.39 ± 0.12	27.95 ± 1.64	4.61 ± 0.08
PLA	10.90 ± 0.60	6.43 ± 0.10	29.31 ± 1.12	4.71 ± 0.08

7.3.2 Earthworm abundance and biomass

Earthworms were found except in four plots, a replicate of PE treatments and three replicates of control treatments (Table S7.5). Only one earthworm was collected from a replicate of control treatments, the recorded biomass of that was 0.0651 g (Table S7.5). The earthworm abundance was calculated in all the treatments, was in the range 0 - 80 (individuals / m^2) with an average value was 22 ± 26 (number / m^2; mean ± standard deviation, n = 12), based on the samples earthworm number (Table S7.5) and the area of the dug sampling pit (Fig. 7.1). The range of total earthworm biomass was from 0.0768 to 8.07 g / m^2 calculated using the recorded earthworm total biomass (Table S7.5) and the area of the dug sampling pit (Fig. 7.1).

Earthworm number decreased in the order PLA > PE > control treatments (Table 7.2), however these differences were not significant ($F_{1,5}$ = 2.191, P = 0.199, one way ANOVA; Table S7.6). There were significant differences in total earthworm biomass and biomass per earthworm between treatments (total earthworm biomass: $F_{2,9}$ = 5.681, P = 0.025, one way ANOVA; biomass per earthworm: $F_{2,9}$ = 7.071, P = 0.014, one way ANOVA; Tables S7.7, S7.8). Total biomass of earthworms decreased in the order PLA < PE < control treatments (Table 7.2), the significant differences were only found between PLA and control treatments (P = 0.035, Holm-Sidak post toc test). Furthermore, a significantly greater biomass per earthworms were found in the PLA treatments than PE and control treatments (PLA vs control: P = 0.025, Holm-Sidak post toc test; PLA vs PE: P = 0.025, Holm-Sidak post toc test; Tables 7.2, S7.8).

Table 7.2. Summary of calculated earthworm number and biomass in control, PLA and PE plots. Results are mean ± standard deviation, n = 4.

Treatment	Earthworm number (individuals / m^2)	Earthworm total biomass (g / m^2)	Biomass per earthworm (g / individuals)
Control	4 ± 8	0.260 ± 0.521	0.016 ± 0.033
PE	16 ± 13	0.669 ± 1.17	0.023 ± 0.035
PLA	46 ± 31	5.860 ± 5.310	0.119 ± 0.057

7.3.3 Earthworm diversity

Two types of earthworms were found from the Bangor field site, *Aporrectodea caliginosa* and *Aporrectodea rosea*. These two types belong to the same species category, endogeic (Table 7.3).

Table 7.3. Calculated numbers of earthworm species (individuals / m^2) found at the different plots of the Bangor field site. Results are mean ± standard deviation, n = 4.

Earthworm species	Ecological category	Treatment

		PE	PLA	Control
Aporrectodea caliginosa	Endogeic	4 ± 8	18 ± 16	0
Aporrectodea rosea	Endogeic	12 ± 19	28 ± 15	4 ± 8

7.4 Discussion

Soil characteristics, in terms of temperature, pH, moisture and organic matter, at this experimental site were not significantly different between PLA, PE and control treatments. Total number and biomass of earthworm were significantly greater in PLA treatments than in the control treatments. Only endogeic species of earthworms *A. caliginosa* and *A. rosea* were found in the soil.

7.4.1 Impacts of plastic mulch films on soil properties

Nieminen et al., (2011) showed that earthworms remain active at the temperature, pH, moisture and organic matter similar to the ranges measured in this study (Table 7.1). Therefore, the soils should be valid in terms of supporting earthworm populations. There was no significant difference in soil characteristics between control, PE and PLA treatments, indicating the low rate of PE or PLA mulching did not have significant impacts on the measured soil properties (Jordán et al., 2010).

7.4.2 Impacts of agricultural activities and environmental condition on earthworm abundance and diversity

The earthworm abundance at this experimental site was low (0 - 80 number / m^2) compared to typical values range of 137 - 1017 number / m^2 from the global non-tillage arable sites and pasture systems as summarised by Chan (2001). The field was ploughed in May 2021 before the start of this experiment (June 2021), which could negatively affected earthworm abundances (Chan, 2001; Nieminen et al., 2011; Crittenden et al., 2014). Before first sowing (June 2021), the number of earthworms was already low due to the initial ploughing. Afterwards, the soils in each plots have been annually rotavated to a depth of 15 cm, which completely broke up the surface layers probably leading to a further decrease in earthworm

populations (van Eekeren et al., 2008). Furthermore, this damage from rotary tillage has been repeated annually. During periods when there have been no agricultural activities, the replenishment of earthworms may be due to two ways: 1) hatching of cocoons and 2) migration of earthworms from the surrounding area. Earthworm cocoons would typically start to hatch when the soil warms up in the spring (Nouri-Aiin and Görres, 2019; Edwards and Arancon, 2022). Although cocoons may survive after soil being annually rotavated in June, this annual rotavating could disturb their normal hatching in this case so that there may not have been sufficient time for cocoons to hatch and then develop to adults until next disturbance (Gerard, 1967). Furthermore, earthworms can make burrows to move around and to feed (Le Couteulx et al., 2015), however, they reuse these burrows and they advance slowly (Bastardie et al., 2005), typically up to a few meters per years (Bastardie et al., 2002; Caro et al., 2012). Therefore, slow movement rates of earthworms leads to difficulties in the rapid recovery (in years) of earthworm population in this experimental trail (15 m x 13 m; Fig. 7.1) by the migration of earthworms in the soil which is not disturbed (Hale et al., 2005; Madarász and Benke, 2023). Also, the migration of earthworms between soil habitats is due to a lowering suitability of their local habitat resulting from environmental pressures (such as overpopulation, predation and natural / anthropogenic habitat disturbance) (Mather and Christensen, 1988). Therefore it might be that earthworms in other soil habitats do not migrate because they are fine where they are and there are no population pressures driving migration.

Earthworm abundances can inevitably decrease in frosty and dry soils (Eggleton et al., 2009), however, when conducting the sampling the soils properties were fine in terms of supporting earthworm populations (Section 7.4.1), indicating environmental temperature and moisture would not be expected to have impacts on earthworm populations in this case. Furthermore, soil type can affect earthworm populations (Guild, 1948). This experimental site was shallow and rocky so that only a maximum depth of 10 cm could be dug instead of the normal sampling depth of 20 cm (Bartz et al., 2014). More density of earthworms has been reported in deeper soils (20-30 cm) (Kooch et al., 2008), thus less digging depth may also be the reason for the low abundance of earthworms in this site especially for endogeic species (which typically live at depths of 10 - 30 cm in the soil; Palm et al., 2013; Fierer, 2019) collected in this study.

Furthermore, Nieminen et al., (2011) also found that cultivation simplified earthworm communities in terms of inducing dominance by endogeic species, which is in accordance with the results in this study that only endogeic species was collected (Table 7.3). Other studies also confirmed the small bodies species (endogeic species, such as *A. rosea* and *A. caliginosa*) were able to survive better in the ploughed soil than the large bodies (anecic species, such as *L. terrestris* and *A. longa*) (Gerard and Hay,1979; Edwards and Lofty, 1982; Wyss and Glasstetter, 1992).

7.4.3 Impacts of plastic mulch films on earthworm biomass

The total earthworm biomass and biomass per earthworm in the PLA treatments were significantly greater than control treatments, and there was no significant difference between PE and control treatments (Tables 7.2, S7.7, S7.8). One mulch being PE and the other PLA could be the main reason for the differences in earthworm biomass. Earthworm could prefer PLA compared to PE, probably because PLA, by design, is readily biodegradable and acts as a food source to microorganisms (such as fungi and bacteria) during the degradation of PLA (Darie et al., 2012; Wang et al., 2019; Swetha et al., 2023). These microorganisms can provide food for earthworms, and the microbial-earthworm interactions promote the degradation of organic matter from which earthworm can also extract food (Edwards and Fletcher, 1988; Jager et al., 2003; Li et al., 2014; Huang et al., 2020). Furthermore, Ding et al., (2021) and Ju et al., (2023) both found that exposure to only PLA led to increase more earthworm weight than exposure to only PE. Although the results of Cd-free avoidance test (Chapter 4) showed that there was no significant avoidance behaviour of the earthworms for either PE and PLA, earthworms had a slight tendency to avoid PE treated soils compared to PLA treated soils, which is somewhat consistent with the results of more earthworm in PLA plots.

7.5 Conclusions

Significantly greater earthworm biomass were found in the PLA treatments than control treatments and no significant difference between PE and control treatments, which is somewhat

consistent with the laboratory results that earthworms had a preference to PLA over PE in the Avoidance test (Chapter 4). However, due to tillage and sampling depth, the numbers of collected earthworms from this Bangor existing field site were so low that it was hard to draw any definitive conclusions. This field sampling chapter is a small part of the thesis, which acts to compliment the previous major laboratory work.

The low number of earthworms found in this study suggests that a preliminary survey of the earthworm populations may be required prior to long-term field trials to determine if the site is suitable for experimentation to avoid wasting time and resources. Otherwise, it would be better to conduct field experiments in a more suitable place. Also, it is worth to investigate the impacts of MPs on earthworm in the field. Also, the change in earthworm population before and after rotation is also worth measuring by the people who set up the experiment.

8 Conclusions

This study aimed to investigate the interactions between mulch-derived MPs, Cd and earthworms. The following three questions identified in Chapter 2 (Literature review) have been addressed via three laboratory experiments and to a limited extent, some field work. The general conclusions, environmental implications and limitations of the work carried out are discussed and future research recommendations resulting from our findings are presented below.

(1) Do mulch-derived MPs interact with Cd and further alter the responses of earthworms to Cd? If so, why does this altered response occur?

(2) Do interactions between biodegradable mulch-derived MPs (PLA) and Cd have different impacts on earthworms in the soil compared with interactions between conventional MPs (PE) and Cd? If so, what are the driving factors of this difference?

(3) Does naturally weathering lead to the alteration of the surface and physiochemical properties of mulch-derived MPs? If so, how do these changes have further impacts on the interaction between mulch-derived MPs and Cd?

8.1 General conclusions

The laboratory experiments found that MPs and Cd at field-relevant concentrations (0.3 % w / w and up to 10 mg / kg respectively) did not impact earthworm mortality, avoidance or weight change (Chapters 4 and 5). Earthworm mortality and avoidance were detected in the 100 mg / kg Cd-only treatments (Chapter 4). Furthermore, earthworm mortality and avoidance decreased with the presence of MPs (Chapter 4) probably due to decrease in Cd concentrations in both earthworms and pore water (Chapter 5) via MP adsorption (Chapter 6). PLA MPs reduced Cd concentrations in earthworms and pore water more than PE MPs (Chapter 5) because PLA MPs (polar polymers with oxygen-containing functional groups) adsorbed significantly more Cd than PE MPs (non-polar polymers) (Chapter 6). Additionally, natural weathering of the plastics mulches resulted in the appearance (PE) or increased abundance (PLA) of oxygen-containing functional groups and biofilms also developed on the plastics leading to increased adsorption

of Cd by them (Chapter 6). The final small part, field work (Chapter 7) showed significantly greater earthworm biomass were found in the PLA treatments than control treatments and no significant difference between PE and control treatments, which is somewhat consistent with the laboratory results that PLA had less impacts on earthworms relative to PE (Chapter 4 and 5).

8.2 Environmental implications

This study used environmentally relevant levels of plastic mulch-derived MP and / or Cd in soil and found that they did not cause avoidance, mortality or weight change in earthworm *L. terrestris*, a common UK earthworm which is widely distributed globally. Thus this study shows that, at least for these measures, the concentrations of MP and Cd used do not necessarily cause environment harm. This study also suggested the use of plastic mulches on Cd-bearing farmland could reduce the ecological hazards of Cd, by MP residues adsorbing Cd on their surfaces and removing Cd from soil solution (pore water) and thus reducing Cd bioavailability. These MP residues therefore could reduce the risk of Cd bioaccumulation in earthworm and further up the food chain by biomagnification in the Cd-bearing agricultural soils. Also, if Cd is removed from pore water, it is likely that bioavailability to other potential receptors, such as plants, could also be reduced. Furthermore, when plastic mulches are used in practice, they are exposed to nature and weathered leading to a change in their surface characteristics; biofilms also form on their surfaces. Both these changes can increase their adsorption capacities of Cd. Therefore, over time (16 months long duration in this particular study) the use of plastic mulches could further increase retention of Cd in soil but reduce Cd bioavailability. Additionally, the current replacement of conventional mulches (PE) by biodegradable mulches (PLA) could lead to greater reductions in the risk of Cd, because PLA MPs are able to adsorb more Cd than PE.

However, PLA is designed to incorporate into the soil after use, which could then be completely decomposed by soil organisms into microbial biomass, carbon dioxide (CO_2) and water (Taib et al., 2023). Thus, any Cd retained in soil by adsorption to PLA MPs might ultimately release

back into the soil pore water and lead to a larger pool of bioavailable Cd once PLA totally degrades, thereby increasing the risk of Cd uptake in the longer term.

8.3 Limitations of this PhD project and future research recommendations

8.3.1 Environmental relevance of the soil testing system

This laboratory study focused on a single earthworm species, native *L. terrestris* (Sections 1.4 and 3.3), however, there are other species (such as *A. caliginosa* and *A. rosea*) which can be found in UK soils (Chapter 7). Previous studies showed that difference earthworm species had different sensitivities to metals (Langdon et al., 2005; Lukkari and Haimi, 2005; Fourie et al., 2007). That indicates the results of avoidance and exposure experiments might not be applicable to all earthworms found in UK soils and even in global soils. Therefore, future work to compare whether there may be different impacts of MPs and / or Cd on different species of earthworms such as avoidance, mortality and weight change should be considered.

This study found there were differences between most commonly used conventional PE and biodegradable PLA (Sections 1.2.6 and 2.3.6) due to their different adsorption capacities caused by their surface and physicochemical properties. However, there are other widely used plastics, such as polar PVC and non-polar PS. PVC chemical formula $(C_2H_3Cl)_n$, consists of polar molecules, and PS chemical formula $(C_8H_8)_n$ is an aromatic polymer. Previous studies reported differences in sorption behaviour between different plastic polymers (Zou et al., 2020; Li et al,. 2022; Zhu et al., 2023). These conventional plastics such as PS and PVC have different chemical structures from the new type of PLA might lead to different adsorption capacities via different mechanisms, suggesting the findings from this study might not be suitable for all types of plastics. Therefore, future research on the adsorption of different types of plastics are warranted. Also, based on existing research that takes into account the functional groups / polar vs non polar nature of the plastics, it could be worthwhile to build a predictive framework / model so that each newly produced plastic may not need to undergo repeated testing. Some modelling approaches could be considered to propose a conceptual integration of how the structures of plastics and functional groups present impacts on adsorption

Furthermore, this study showed that natural weathering led to the biofilm generation and physiochemical properties change of plastic mulch-derived MP (Sections 1.2.6 and 2.3.5) by adsorption experiments (Chapter 6), and the additives associated with MPs (Marturano et al., 2017) are more likely able to be released during this process (Sections 1.2.4 and 1.7). Future studies to identify the chemicals leaching from MPs into soil pore water using non-target or target analysis after setting up long-term exposure experiment are warranted. If any chemicals are detected, it should be considered that the toxicity experiments with the native forms of these chemicals to confirm whether it is the chemicals or the MPs that are producing any of the observed effects.

Although Cd-amended natural uncontaminated soils used in this study have the advantage of being able to control the concentration of addition, they may not be a good representation of real Cd-contaminated soils in farmland. Previous review summarised that metals that are artificially added may be more available than metals present in contaminated soils (metals concentrations built up over time) (Sizmur and Hodson, 2009). Furthermore, most contaminated soils are contaminated with more than one metal, and there may be synergistic effects between these contaminants in the field (Nahmani et al., 2007; Redha et al., 2021), which might affect the availability of the target metal. Thus, laboratory-based research on ecotoxicology using Cd-contaminated agricultural soils with a variety of properties should be considered. Furthermore, soil pH can vary from 4.5 to 8.5 (McCauley et al., 2009). Soluble Cd increases with decreasing pH in the soil which typically move from neutral to somewhat acidic (Kiewiet and Ma, 1991). Soil organic matters are essential component of soil, which are an important metal sorbent in soils (Gustafsson et al., 2003; Zhang et al., 2022), so that soluble Cd decreases with increasing organic matter. Previous review summarised that Cd solubility varies with soil pH and organic matter (Sauvé et al., 2000). However, studies on modelling the relationship between soils with different pH and organic matters and soluble Cd contents under actual field conditions are warranted. Altogether, these recommended research could develop a more realistic understanding of Cd solubility in field.

8.3.2 Possible alteration of soil pH

Soil pH is an important driver for Cd solubility in soil, Cd solubility increase with decreasing pH (Kiewiet and Ma, 1991; Peijnenburg et al., 1999), which could further affect Cd bioaccumulation in earthworms (Section 5.1.2). However, pH was not controlled in the 28-days exposure experiments (Chapter 5) and therefore pH might have impact on the results. To address this issue some post experiment measurements were made. There were two groups of treatments, pre-exposure soils (initial uncontaminated soils after drying and sieving, Section 3.2) and post-exposure soils (stored soils after the end of the exposure experiments, Chapter 5). Pre-exposure soils were mixed with the 0.6006 g / L stock solution of $Cd(NO_3)_2$ or diluted $Cd(NO_3)_2$ stock solution or deionised water to generate a series of soils with selected nominal soil Cd concentrations of 0, 1.0, 10, 50 and 100 mg / kg in triplicate. pH was measured (Section 3.2.1) on these 15 soils together with post-exposure soils with the same Cd concentrations from the exposure experiments (Chapter 5) to see whether pH in this particular study system significantly alters between the start and end of the experiment and whether the different levels of Cd addition significantly affected pH. Statistical analyses were run using SigmaPlot version 15.0. Variation in pH was analysed with soil group (pre-exposure, post-exposure) and Cd concentration (0, 1.0, 10, 50, 100 mg / kg) as factors. pH data set was normally distributed and had equal variance using the Shapiro-Wilks and Brown-Forsythe tests respectively. Data set was then analysed using a two way ANOVA analysis followed by Holm-sidak post doc tests. pH of pre-exposure soils was 7.50 ± 0.01 (n = 15, ± standard deviation) and pH of chosen post-exposure soils was 7.49 ± 0.02 (n = 15, ± standard deviation). Soil pH did not significantly alter ($F_{1,20}$ = 3.462, P = 0.078, two way ANOVA) before and after short-term (28 days) exposure and the different levels of Cd addition did not significantly affect pH ($F_{4,20}$ = 1.438, P = 0.258, two way ANOVA), and not significant interaction between two ($F_{4,20}$ = 0.654, P = 0.631, two way ANOVA) in this particular experiments. Therefore it seems unlikely that pH was a confounding factor in the exposure experiments. However, it is also worth to carry out laboratory experiments with soils of different pH. Additionally, investigations on changes in pore water Cd concentrations over time, as well as pH of the soil for long-term field experiments are warranted to determine the relationship between pH and Cd bioavailability.

pH was also not controlled in the adsorption experiments (Chapter 6), and therefore adsorption might vary with pH. Although pH was not controlled during the adsorption experiments, experiments were carried out in a background electrolyte of 0.01 mol / L $NaNO_3$. $NaNO_3$ solution was 23.809 - 23809 orders of magnitude more concentrated than the Cd concentrations (0.1 - 100 mg / L equivalent to 4.2×10^{-7} - 4.2×10^{-4} mol / L) and could have had a far bigger influence on pH than the Cd and therefore although it was not measured, pH variation was unlikely to be that significant.

8.3.3 Simulation of natural weathering of plastic mulches

This study produced naturally weathered MPs by hanging up large metre scale sheets of plastic mulch (PE and PLA) to expose them to ambient weather conditions (Section 3.4.2). However, when mulches are used in agricultural system they are laid on the soil surface as row cover. Therefore the upside of the mulch would be directly exposed to sunlight and rain etc., whilst the underside would be in direct contact with the soil and not exposed to sunlight, which may lead to different weathering mechanisms of mulches, such as different degree of UV photooxidation, different environmental parameters (e.g., temperature, pH and moisture), and different types of microorganisms involved. In terms of environmental relevance, this study using naturally weathered MPs is more relevant than artificially weathered MPs, however it is also necessary to further consider the exposure scenarios of the pristine plastic mulches when generating weathered MPs. Weathering process leads to fragmentation of MPs, therefore, future long-term field experiments should be considered to bury larger, retrievable plastic fragments then recover them and break them up to be smaller fragments and then release back to the soil. In the mean time, the changes of their physiochemical properties and the formation of biofilms, as well as possible chemicals (additives and low-molecular products) release are also worthy to monitor.

8.3.4 Analysis method limitations

Although this study detected increase in the roughness of MP particles and biofilm formation on MP surfaces by SEM observation (Figs. 3.6 - 3.8 in Chapter 3), quantification on the additional surface area generated by weathering of the MPs (either from changes in the

roughness of the MP itself or the biofilm) was unable to be carried out. Therefore, to explore the changes in MP surface area during the MP weathering process, more advanced technologies such as atomic force microscopy (Demir-Yilmaz et al., 2022; Kang et al., 2025), which can not be used in this study due to budget constraints, could be considered.

Although the amount of biofilm present on the MPs was quantified in this study, further research on mechanisms of biofilms formation on MPs and how they adsorb metals (and other contaminants) at a molecular level (such as using X-ray spectroscopy) is warranted to better understand the potential risk that this poses in the environment. Furthermore, genetic characterisation of the biofilms on MPs, such as the microorganisms measurement and the composition identification of extracellular polymeric substances produced by the microorganisms, would provide a better understanding that whether the biofilms are likely to be degrading the MP and how the biofilms are affected by the Cd.

8.3.5 MP degradation

This study found biodegradable PLA MPs slightly degraded after 16 months exposure to natural conditions. The Cd sorbed on PLA MPs may gradually release back into the soil pore water and thus become bioavailable again with the further degradation of PLA MPs over time. Therefore future studies that are designed to quantify the lifetime of PLA fragments in soils as well as degradation mechanisms and products and the release of any adsorbed chemicals, warrant to test this hypothesis.

8.3.6 Feasibility of field trials

The low number of earthworms found in the field chapter of this study suggests that an initial survey on the earthworm population may be required to determine if the selected site is suitable for experimentation to avoid wasting time and resources, before conducting this long-term field trials. Furthermore, investigations on the impacts of MPs on earthworm (such as biomass, number and behaviour) in this field and the impacts of agricultural activities (such as rotation) on earthworm population are worth carrying out by the university of Bangor.

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Supporting information

1 Chapter 2 Literature review supporting information

Number of table: 7

List of contents:

Table S2.1 - S2.7:
(S2.1) A detailed summary (sampling location classified by continent, depth, MPs abundance) about MPs sample collected from agricultural soil based on previous studies;
(S2.2) Previous studies showing possible sources for MPs, their concentration and found location in agricultural soil;
(S2.3) Impact of MPs on agricultural soil health in terms of soil properties, soil organisms and plants;
(S2.4) Previous academic studies about potential mechanisms of MPs-metals interaction;
(S2.5) Previous academic studies summarised about different isotherm models of the adsorption of metals on MPs;
(S2.6) Summarised factors that influence the MPs-metals adsorption from previous studies including characteristics of MPs and environmental factors;
(S2.7) Impact of MPs-metals interactions on earthworms from previous studies.

Tables

Table S2.1. A detailed summary (sampling location classified by continent, depth, MPs abundance) about MPs sample collected from agricultural soil based on previous studies.

Continent	Sampling location	Sampling Depth (cm)	Abundance	Unit	Reference
Asia	Hokuriku, Japan	15	6 - 369	mg / kg	Katsumi et al., 2021
	Gyeonggi-do, Korea	20	195 - 306	particles / kg	Park and Kim, 2022
	Yeoju, Korea	/	664 ± 83	piece / kg	Choi et al.,2021
	Yong-In, Korea	/	20 - 325	items / kg	Kim et al.,2021
	Hainan, China	0 - 30	2800 - 82500	particles / kg	Khan et al., 2023
	Shenyang, China	0 - 10	7183 - 10586	particles / kg	Li et al., 2022
	Tainan, China	0 - 5 5 - 20	26 - 117 12 - 67	items / m^2	Fakour et al.,2021
	Jiangxi, China	/	43.8 ± 16.2	particle / kg	Yang et al.,2021
	Qinghai-Tibet plateau, China	0 - 3 (Shallow) 3 - 6 (Deep)	53.2 ± 29.7 43.9 ± 22.3	items / kg	Feng et al.,2021
	Shandong, China	0 - 5 (Shallow) 5 - 10 (Middle) 10 - 25 (Deep)	454 - 5411 (1860 ± 1212) 412 - 7175 (1726 ± 1596) 307 - 4507 (1065 ± 942)	items / kg	Yu et al.,2021
	Jilin, China Hubei, China Shanxi, China Hebei, China Shandong, China	/	2783 - 6366 (4496 ± 1082)	items / kg	Wang et al.,2021
	Guilin, China	0 - 5 (Shallow) 5 - 15 (Middle)	35.5 ± 5.3 330.4 ± 21.5	items / kg	Zhang et al.,2020

		15 - 25 (Deep)	180.0 ± 28.3		
	Hebei, China	/	/	/	Du et al.,2020a
	Hebei, China	10	/	/	Du et al.,2020b
	Xinjiang, China	/	10.10 - 61.05	mg / kg	Li et al.,2020a
	Xinjiang, China		230.9		
	Inner Mongolia, China	/	77.6	kg / ha	Huang et al.,2020
	Gansu, China		65.9		
	Shanxi, China	10	1430 - 3410	items / kg	Ding et al.,2020
	Wuhan, China	5	320 - 12560 (2020)	items / kg_{dw}	Chen et al.,2020
	Wuhan, China	5	2.2×10^4 - 6.9×10^5 (2.2×10^5)	particle / kg	Zhou et al.,2019a
	Hangzhou, China	10	503.3 - 2760	piece / kg	Zhou et al.,2019b
	Shanghai, China	10	10.3 ± 2.2	items / kg	Lv et al.,2019
	Shanghai, China	6	136.7 ± 41.7	items / kg	Liu et al.,2019
	Shanghai, China	0 - 3 (Shallow) 3 - 6 (Deep)	78 ± 12.9 62.5 ± 13	items / kg	Liu et al.,2018
	Loess plateau, China	30	40 - 100	particle / kg	Zhang et al.,2018
	Yunnan, China	10	7100 - 42960 (18760)	particle / kg	Zhang and Liu, 2018
	Nanjing and Wuxi, China	/	420 - 1290	items / kg	Li et al.,2018
Europe	UK	0 - 5	1320 - 8190	particle / kg	Cusworth et al., 2023
	North Rhine-Westphalia, Germany	0 - 10 10 - 40	2.3 2.3	items / kg	Steinmetz and Schröder, 2022
	Schleswig-Holstein, Germany	0 - 10 (Shallow) 10 - 20 (Middle) 20 - 30 (Deep)	5.8 ± 8 3.3 ± 3.1 1.9 ± 1.6	particle / kg	Harms et al.,2021

	Valencia, Spain	0 - 10 (Shallow) 10 - 30 (Deep)	1730 ± 920 (Light density) 3410 ± 2330 (Heavy density) 1730 ± 920 (Light density) 3180 ± 2430 (Heavy density)	particle / kg	van den Berg et al.,2020
	Franconia, Germany	5	0.34 ± 0.36 (0 - 1.25)	particle / kg	Piehl et al.,2018
	San Juan Cotzocón Municipality, Mexico	20	1.49 - 1.53	particle / g	Álvarez-Lopeztello et al.,2021
North America	USA Ontario, Canada	15	9 - 63 24.3 - 358.4	particle / kg	Crossman et al.,2020
	Campeche, Mexico	20	0.87 - 1.9	particle / g	Huerta Lwanga et al.,2017a
	Buenos Aires, Argentina	10	2383 - 3815	items / m^2	Berenstein et al., 2024
Latin America	Región Metropolitana, Chile	25	306 ± 360	particles / kg	Corradini et al.,2021
	Mellipilla, Chile	25	2.01 (1.1 - 3.5)	items / kg	Corradini et al.,2019
Oceania	Australia	/	2.3 - 15.8	t / ha	Ng et al.,2018
Africa	Arusha, Tanzania,	30	0.21 - 1.50	items / g	Kundu et al., 2022
Antarctica	/	/	/	/	/

Table S2.2. Previous studies showing possible sources for MPs, their concentration and found location in agricultural soil.

Possible source	Concentration	Unit	Location	Reference
Plastic mulches	0.34 ± 0.36	particles / kg	Southeast Germany	Piehl et al.,2018
	10.10 - 61.05	mg / kg	Xinjiang, China	Li et al.,2020a
	0.1 - 324.5	kg / ha	19 provinces across China	Huang et al.,2020
	900	particles / kg	Gansu, China	Meng et al., 2020
	2283 - 6366	items / kg	5 provinces in China	Wang et al.,2021
	5.8 ± 8	particles / kg	Schleswig-Holstein, Germany	Harms et al., 2021
	10 - 7630	items / kg	Yong-In, Korea	Kim et al., 2021
	195 - 306	particles / kg	Gyeonggi-do, Korea	Park and Kim, 2022
	7183 - 10586	particles / kg	Shenyang, China	Li et al., 2022
	1320 - 8190	particles / kg	108 sites across the UK	Cusworth et al., 2023
	2838 - 3815	items / m^2	Buenos Aires, Argentina	Berenstein et al., 2024
Plastic mulches / sewage sludge	306 ± 360	particles / kg	Región Metropolitana, Chile	Corradini et al.,2021
Plastic mulches + sewage sludge	78.00 ± 12.91 (shallow soil) 62.50 ± 12.97 (deep soil)	items / kg	Shanghai, China	Liu et al.,2018
	0 - 217.8	particles / kg	Schleswig - Holstein of Northern Germany	Harms et al.,2021
Wastewater irrigation Plastic mulches + wastewater irrigation	263 571	pieces / kg	Hangzhou Bay of China	Zhou et al.,2019b
Sewage sludge	10.3 ± 2.2	particles / kg	Shanghai City in China	Lv et al.,2019
	4196 - 15385	particles / kg	Ireland	Mahon et al.,2017
	2.3 - 15.8	t / ha	Australia	Ng et al.,2018
	0.6 - 10.4	particles / g	Chile	Corradini et al.,2019

	5190	particles / kg	Spain	van den Berg et al.,2020
	420 - 1290	items / kg	Nanjing, Wuxi, Jiangsu Province of China	Li et al.,2019
Sewage sludge + wastewater irrigation	7100 - 42960	particles / kg	Dian Lake of southwestern China	Zhang and Liu, 2018
Wastewater irrigation + street runoff + littering	$1.6x10^5$	particles / kg	Central China	Zhou et al.,2019a
Plastic waste	0.87 - 1.9	particles / g	Yucatan Peninsula, Mexico	Huerta Lwanga et al.,2017a
Plastic waste + wastewater	320 - 12560	items / kg	Wuhan, China	Chen et al.,2020
Littering (open-air dump site)	1.53	particles / g	Gulf, Mexico	Álvarez-Lopeztello et al.,2021
Atmospheric input	29 - 280	Items / (m^2·day)	Paris, France	Dris et al.,2015
Atmospheric input + flooding	55.5 - 593	mg / kg - particles / kg	Swiss	Scheurer and Bigalk, 2018

Table S2.3. Impact of MPs on agricultural soil health in terms of soil properties, soil organisms and plants.

Test aspect		Soil	MPs polymer types and size	Option and details	Exposure concentration	Exposure time	Characteristics	Reference
Soil properties	/	Loamy sandy soil collected at the experimental facilities of Freie Universität Berlin	PP, PS, PA, PES, PET, PEHD, 1mm	PA from Good Fellow- AM306010; Cambridge, U.K.; PES obtained by manually cutting 100% polyester wool "Dolphin Baby" (product number 80313, Himalaya Co.,Turkey); The other microplastics were fabricated by industrial pellets into microplastic fragments.	2 % of soil fresh weight	2 months	Soil bulk density, water stable aggregates decreased;	de Souza Machado et al., 2019
	/	Loamy sandy soil collected at the experimental facilities of Freie Universität Berlin	polyacrylic, polyester, polyamide and polyethylene, < 0.63 mm	The polyacrylic fibers were obtained manually cutting 100 % acrylic "Rozetti Puzzle" yarn (product	polyacrylic and polyester (0.05 %, 0.10 %, 0.20 %, and 0.40 %); polyamide and	5 weeks	Noticeable changes in soil bulk density, and water holding capacity; not significant change in hydraulic	de Souza Machado et al., 2018

			number 233-01, Himalaya Co. Turkey); Polyester fibers were manufactured by manually cutting 100 % polyester wool “Dolphin Baby” (product number 80313,Himalaya Co. Turkey); Polyamide beads of (product AM306010) were acquired from Good Fellow (Cambridge, United Kingdom); polyethylene were fabricated by cryo-milling pristine industrial pellets (Berlin, Germany).	polyethylene (0.25 %, 0. 50 %, 1.00 %, and 2.00 %)		conductivity; nonlinear microplastic-driven increased microbial activity	
/	Top soil sourced from Westland Garden Health	PLA, HDPE, fibers, 0.6 – 363 μm (PLA,	Fibers were collected from a	0.1 % PLA and HDPE (w / w);	30 days	Soil pH decreased with HDPE present; alter soil	Boots et al., 2019

		(Dungannon, Northern Ireland)	HDPE); 0.48 – 316 μm (fiber)	standard household washing machine	0.001 % fibers (w / w)		stability when microplastics were present. Microplastics may affect soil water content. Interaction between microplastic fibres and drought affected ecosystem functions and multifunctionality.	
/		Dry sandy loam soil from grasslands communities	Microplastic fibers, Length of 1.28 ± 0.03 mm and a diameter of 0.030 ± 0.0008 mm	Manually cut with scissors polyester fibres (Rope Paraloc Mamutec polyester white, item number, 8442172, Hornbach.de).	0.4 % (w / w)	14 days	Microplastics increased soil aggregation by 18%, soil pH by 4% and nutrient retention by up to 70 %. Microplastic fibres also impacted soil enzymes, respiration and ecosystem multifunctionality.	Lozano et al., 2021a

/	Dry sandy loam soil from grasslands communities	PES, PA, PP, LDPE, PET, PU, PS and PC, < 5 mm	Manually cut with scissors.	0.1 %, 0.2 %, 0.3 % and 0.4 % (w / w)	28 days	All shapes decreased soil aggregation by 25 %. MPs have potential negative effects on soil biota and decrease in microbial activity.	Lozano et al., 2021b
/	Dry sandy loam soil from grasslands communities	PET, PA, < 5 mm	Manually cutting them into fragments of approximately 5 mm in length.	0.3 % (w / w)	42 days	Effects of microplastic fibers on soil aggregation and enzyme activities are organic matter dependent.	Liang et al., 2021
/	Dry sandy loam soil from grasslands communities	PA, PC, PE, PES, PET, PP, PS and PU, The length for fibers was 1.26 ± 0.03 mm, and the size of films was 1.55 ± 0.03 mm x	Manually cut with scissors.	0.4 % (w / w)	7 days	Soil pH increased with foams and fragments and overall decreased in the first days of incubation and then increased. Soil respiration increased with PE foams and was	Zhao et al., 2021

			2.26 ± 0.04 mm				affected by the incubation time, declining over time. Enzymatic activities fluctuated during the incubation time	
Soil organism	Earthworm (*Eisenia fetida*)	Uncultivated land in the Shang zhuang Agricultural Experimental Field at China Agricultural University	LDPE, 1 - 2, 0.71 - 1, 0.425 - 0.71, 0.25 - 0.425, 0.15 - 0.25, and 0.05 - 0.15 mm	Purchased from Chenlin Plastic (Dongguan,China)	1 % (w / w)	28 days	PE microplastics with smaller size range can sorb and desorb more PCBs; soil with clean plastic were likely to extract PCBs from earthworm tissues than polluted plastic; Smallest particles (0.05 - 0.15 mm) showed the greatest accumulation.	Wang et al., 2020a
	Earthworm (*Eisenia fetida*)	A clean agricultural sandy loam soil	LDPE, PS, LDPE (< 300	Purchased from Goodfellow (Coraopolis,PA)	1 %, 5 %, 10 %, 20 % (w / w)	14 days	Microplastics were found in earthworm casts,	Wang et al., 2019a

	sample collected from Riverside in Southern California	μm) and PS (< 250 μm)				suggesting particle ingestion.; Presence of microplastics in PAH- and PCB-contaminated soils decreased the bioaccumulation of the environmental contaminants.	
Earthworm (*Eisenia fetida*)	Agricultural sandy loam were collected from agricultural regions in NSW (Kirby Sand, Kirby Clay and Warialda Loam Near Armidale, NSW. Vegetative cover was removed from the surface of soils and the top 20 cm of the	HDPE, PET, PVC, < 2 mm	HDPE was sourced from consumer shopping bags (QIS Packaging, Australia), PET was sourced from drink bottles (Synergy Packaging, Australia) and PVC was sourced from tablecloth (Spotlight, Australia).	HDPE (1.43 ± 0.55 mm), PET (1.51 ± 0.46 mm) and PVC (1.18 ± 0.36 mm)	56 days	Earthworms did not avoid the microplastic-spiked soils, irrespective of both microplastic type and soil type. Earthworm survival, body weight, and reproduction rate did not differ statistically between control test media	Judy et al., 2019

		soil profile collected.				(compost amended soils) and microplastic treatments. 0.5 % microplastics in compost-amended soils were not toxic to *E.fetida*.	
Earthworm (*Lumbricus terrestris*)	The top 20 cm of an arable loam soil the University of Leeds commercial farm	Polyester microfibres, < 2 mm	Textile laundering	0.1 % and 1.0 % (w / w)	35 days	Be not lethal to earthworms, nor did earthworms actively avoid MFs; in theMF1.0 % treatment showed a 1.5-fold lower cast production	Prendergast-Miller et al., 2019
Earthworm (*Lumbricus terrestris*)	An organic farm in thecity of Chilln	PS, PP, PET, LDPE, 0.25 mm	Commercial	2.5 %, 5 %, 7 % (w / w)	48 hours	Earthworms suffered physical damage causing by microplastics, such as losing surface mucous, resulting in burns and	Baeza et al., 2020

						lesions on their bodies.	
Earthworm (*Lumbricus terrestris*)	Field collected from a meadow in Berlin	PE, 710 - 850 µm, 1180 – 1400 µm, 1700 – 2000 µm and 2360 – 2800 µm	clear, approximately spherical and bought from Cospheric, SantaBarbara, CA, USA	750 mg of the differently sized PE-microplastic particles were added to the soil surface.	21 days	Microplastic transport and could potentially also reach groundwater by earthworms.	Rillig et al ., 2017
Earthworm (*Lumbricus terrestris*)	An arable and woodland soil	HDPE, 0.92 ± 1.09 mm^2	Single-use plastic carrier bags obtained from a U.K. national supermarket chain into small, irregularly shaped pieces.	0.7 ± 0.8 % (w / w)	28 days	No mortality and alteration of body weight in microplastic exposed earthworms with respect to controls.; Ingestion of Zn-bearing microplastics did not increase bioaccumulation of the metal.; Metal desorption from microplastics incubated in earthworm guts	Hodson et al., 2017

						imulated fluid suggested that Zn-bearing microplastics might facilitate metal uptake via the alimentary canal.	
Earthworm (*Lumbricus terrestris*)	Sandy soil (50 % sand, 50 % loamy silt, with 0.2 % organic matter).	LDPE, 200 - 300 µm	Purchased from Riblon, Ter Hell Plastic GmbH	7 %, 28 %, 45 % and 60 % (w / w)	14 days	Highest burrows and MP bioturbation efficiency ratio were found in 7 % LDPE.	Huerta Lwanga et al., 2017b
Earthworm (*Lumbricus terrestris*)	Sandy soil (26.6 % brown sand, 24 % silver sand, and 50 % loamy silt with0.2 % organic matter)	LDPE, 200 - 300 µm	Purchased from Riblon, Ter Hell Plastic GmbH	1 %, 5 %, 10 %, 15 % (w / w)	40 days	Growth rate was reduced and microplastics were concentrated in casts.	Huerta Lwanga et al., 2016
Earthworm (*Aporrectodea rosea)*	Topsoil sourced from Westland Garden Health (Dungannon,	PLA, HDPE, fibers, 0.6 - 363 µm (PLA,	Fibers were collected from a standard household washing machine	0.1 % PLA and HDPE (w / w); 0.001 % fibers (w / w)	30 days	Affect the health of earthworm	Boots et al., 2019

	Northern Ireland)	HDPE); 0.48 - 316 μm (fiber)					
Earthworm (*Metaphire californica*)	Framland soil (the background arsenic concentration is 8 mg / kg) were both obtained from a non-arsenic contaminated farmland in Yinzhou district, Ningbo,south-east China.	PVC	Purchased from Aladdin Industrial Corporation (Shanghai, China)	2000 mg / kg	28 days	PVC reduced arsenic accumulation in gut and body tissues. PVC alleviated the effect of arsenic on the gut microbiota.	Wang et al., 2019b
Enchytraeid (E*nchytraeus crypticus*)	Standard agricultural soil Lufa 2.2	PES, short (0.012 - 2.87 mm) and long (4 - 24 mm)	A pink polyester fleece blanket (Skogsklocka, IKEA)	0.02 - 1.5 % (w / w)	28 days	Survival was slightly decreased after 21 d exposure to long polyester fibers at 0.17 % and 0.5 % w / w. Reproduction rate significantly decreased in worms exposed to	Selonen et al., 2020

Enchytraeid (E*nchytraeus crypticus*)	Standard agricultural soil Lufa 2.2	PA, PVC, 0.013 - 0.018 mm, 0.063 - 0.09 and 0.09 - 0.150 mm (PA); 0.106 - 0.150 mm (PVC)	PA was generated in-house by grinding nylon-6 pellets (SigmaAldrich, United Kingdom); High molecular weight PVC was obtained fromSigma Aldrich, UK.	PA: 2 - 12 % (w / w); PVC: 9 % (w / w)	21 days	long-sized fibers at all concentrations except in the 0.06%treatment group. Short fibers did not affect both survival and reproduction rate at any concentration. Enchytraeids ingested all size fractions, although a higher number of particles were found in worms exposed to 0.0 - 0.018 mm particles. No clear evidence for PVC. After 21 d of exposure, no effect of nylon-6 microplastics on worm survival, but reproduction	Lahive et al., 2019

						(number of juveniles produced per worm) decreased. Short- and long-sized fibers did not affect the survival, biomass, and feeding activity of isopods. No significant variations were found in total concentration of proteins, carbohydrates, and lipids in fiber-exposed isopods.	
Isopods (*Porcellio scaber*)	Standard agricultural soil Lufa 2.2	PES, short (0.012 - 2.87 mm) and long (4 - 24 mm)	A pink polyester fleece blanket (Skogsklocka, IKEA)	0.02 - 1.5 % (w / w)	28 days		Selonen et al., 2020
Springtail (*Folsomia candida*)	Standard agricultural soil Lufa 2.2	PES, short (0.012 - 2.87 mm) and long (4 - 24 mm)	A pink polyester fleece blanket (Skogsklocka, IKEA)	0.02 - 1.5 % (w / w)	28 days	No significant effect of polyester-contaminated soil or food on springtail survival and reproduction.	Selonen et al., 2020

	Mites (*Oppia nitens*)	Standard agricultural soil Lufa 2.2	PES, 4 -24 mm	A pink polyester fleece blanket (Skogsklocka, IKEA)	0.5 % (w / w)	28 days	No significant effect of polyester-contaminated soil or food on mite survival and reproduction.	Selonen et al., 2020
	Snails (*Achatina fulica*)	Cultivation soils were collected from the campus of East China Normal University	PET, 1257.8 μm	Purchased from Asahi exhibition plastic wire drawing Co., LTD (Linhai, China).	0.014, 0.14 and 0.71 g / kg	28 days	Snails progressively accumulated microfibers along digestive tract. Significant fragmentation and deterioration in microfibers collected from both digestive tracts and excrements. No significant impact of microfibers on snail growth but 0.71 g / kg PET induced villi damages in walls	Song et al., 2019

							of gastrointestinal tract.	
Plants	Wheat (*Triticum aestivum*)	Sandy soil used obtained from the agricultural land in Wageningen, the Netherlands	LDPE; Based biodegradable plastic, 0.5 - 1 mm, 12.5 %; 0.25 - 0. 5 mm, 62.5 %; 0.05 - 0.25 mm, 25 %	Based biodegradable plastic: 37.1 % Pullulan, 44.6 % Polyethylene Terephthalate (PET) and 18.3 % Polybutylene Terephthalate (PBT)	1 % (w / w)	139 days	PP and biodegradable mulch films had a negative impact on both above- and below-ground parts of the wheat. Starch-based biodegradable fragments showed stronger negative effects on plant growth and development than those of PP-based fragments. Impact of the size of both mulch films on plant growth and development was insignificant.	Qi et al., 2018

Spring onion (*Allium fistulosum*)	Loamy sandy soil collected at the experimental facilities of Freie Universität Berlin	PP, PS, PA, PES, PET, PEHD, 1 mm	PA from Good Fellow- AM306010; Cambridge, U.K.; PES obtained by manually cutting 100 % polyester wool“Dolphin Baby” (product number 80313, Himalaya Co.,Turkey); The other microplastics were fabricated by industrial pellets into microplastic fragments.	2 % of soil fresh weight	2 months	Root and total biomass and length increased; root tissue density increased (PA, PES and PS); dry biomass decreased in PA-treated plants and nearly doubled after PES exposure; total biomass increased. Microplastic impact on A. fistulosum was due to indirect effects of microplastics on soil physicochemical properties (soil bulk density, soil aggregate, and water dynamics).	de Souza Machado et al., 2019
Perennial ryegrass	Top soil sourced from Westland	PLA, HDPE, fibers, 0.6 -	Fibers were collected from a	0.1 % PLA and HDPE (w / w);	30 days	When exposed to microplastics,	Boots et al., 2019

	(*Lolium perenne*)	Garden Health (Dungannon, Northern Ireland)	363 µm (PLA, HDPE); 0.48 - 316 µm (fiber)	standard household washing machine	0.001 % fibers (w / w)		fewer seeds germinated; average shoot length was suppressed for PLA; root biomass was greater for HDPE
	D. carota	Dry sandy loam soil from grasslands communities	PES, PA, PP, LDPE, PET, PU, PS and PC, < 5 mm	Manually cut with scissors.	0.1 %, 0.2 %, 0.3 % and 0.4 % (w / w)	28 days	All shapes increased plant biomass. Shoot mass increased by 27 % with fibers, 60 % with films,45 % with foams, and by 54 % with fragments.

Table S2.4. Previous academic studies about potential mechanisms of MPs-metals interaction.

MP type	Metal types	Non-specific interaction			Specific interaction		References
		Electrostatic interaction	Van der Waals Force	π-π interaction	Surface complexation	Hydrogen bonding	
PS	AgNPs	*		*			Li et al., 2020a
PSMPs	As(III)	*				*	Dong et al., 2020
PS	Pb, Cu, Cd, Ni, Zn	*					Mao et al., 2020
PA, PS, PP	Sr^{2+}	*					Guo et al., 2020a
PS				*			
PE	Pb(II)		*				Lin et al., 2021a
PVC							
CPE, PVC, PE	Pb^{2+}, Cd^{2+}, Cu^{2+}	*			*		Zuo et al., 2020
HDPE	Hg	*					Fernández et al., 2020
PTFE	As(III)	*				*	Dong et al., 2019
nylon MPs	Cu(II), Ni(II), Zn(II)				*		Tang et al., 2021
nylon MPs	Pb(II)	*			*		Tang et al., 2020
MPs (no special types)	Pb(II)	*					Fu et al., 2021

PS, Polystyrene; PE, Polyethylene; HDPE, High density polyethylene; PVC, Polyvinyl Chloride; PA, Polyamide; PP, Polypropylene; CPE, Chlorinated polyethylene; PSMPs, Polystyrene microplastic particles; PTFE, Polytetrafluoroethylene.

Table S2.5. Previous academic studies summarised about different isotherm models of the adsorption of metals on MPs.

MPa type	Concentration (g / L); Size	Metal type	Dosage (mg / L)	Allowed time to reach Equilibrium	Adsorption model		References
					Fitted model	Parameters	
PS	0.1, 0.2, 0.25, 0.4, 0.5, 1	AgNPs	10		Langmuir model	Qmax = 26.12 mg / g	Li et al., 2020b
HDPE	28.57	Cd	5		Langmuir model	Qmax = 0.0305 mg / g	Wang et al., 2019c
PSMPs (100 - 1000 nm)	0.4	As(III)	10, 20, 30, 40, 50	24 h	Langmuir and Freundlich model	Qmax = 1.12 μg / g; KF = 0.017 mL^3 / g	Dong et al., 2020
PSMPs (1000 - 10000 nm)						Qmax = 1.047 μg / g; KF = 0.0168 mL^3 / g	
PSMPs (> 10000 nm)						Qmax = 0.92 μg / g; KF = 0.0146 mL^3 / g	
PTFE (100 -1000 nm)	1	As(III)	50		Langmuir model	Qmax = 1.03 mg / g	Dong et al., 2019
PTFE (1000 - 10000 nm)						Qmax = 0.94 mg / g	
PTFE (> 10000 nm)						Qmax = 0.83 mg / g	

CPE	0.25 - 2; 0.28 mm	Pb^{2+}	0.1 50	24 h	Freundlich model	KF = 369 $mmol^{1-n}L^n$ / kg	Zuo et al., 2020
		Cd^{2+}				KF = 227 $mmol^{1-n}L^n$ / kg	
		Cu^{2+}				KF = 140 $mmol^{1-n}L^n$ / kg	
PVC		Pb^{2+}				KF = 17.8 $mmol^{1-n}L^n$ / kg	
		Cd^{2+}				KF = 55 $mmol^{1-n}L^n$ / kg	
		Cu^{2+}				KF = 13.4 $mmol^{1-n}L^n$ / kg	
		Pb^{2+}				KF = 18.3 $mmol^{1-n}L^n$ / kg	
HPE		Cd^{2+}				KF = 6.4 $mmol^{1-n}L^n$ / kg	
		Cu^{2+}				KF = 14.4 $mmol^{1-n}L^n$ / kg	
		Pb^{2+}				KF = 3.5 $mmol^{1-n}L^n$ / kg	
LPE		Cd^{2+}				KF = 14 $mmol^{1-n}L^n$ / kg	
		Cu^{2+}				KF = 1.55 $mmol^{1-n}L^n$ / kg	
Virgin PE pellets	10	Cr	0 - 0.02	48 h	Langmuir and Freundlich model	Qmax = 0.297 μg / g; KF = 0.038 $μg^{1-1/n}$ / g	Holmes et al., 2012
		Co				Qmax = 0.018 μg / g; KF = 0.0006$μg^{1-1/n}$ / g	
		Ni				Qmax = 0.008μg/g; KF=0.0005 $μg^{1-1/n}$ / g	
		Cu				Qmax=0.261 μg / g; KF = 0.036 $μg^{1-1/n}$ / g	
		Cd				Qmax = 0.0004 μg / g; KF = 0.0002 $μg^{1-1/n}$ / g	
		Pb				/	
Beached PE pellets		Cr				Qmax = 0.441 μg / g; KF = 0.131$μg^{1-1/n}$ / g	

	Co			Qmax = 0.038 μg / g; KF = 0.014 $\mu g^{1-1/n}$ / g	
	Ni			Qmax = 0.070 μg / g; KF = 0.057 $\mu g^{1-1/n}$ / g	
	Cu			/; KF = 0.069 $\mu g^{1-1/n}$ / g	
	Cd			Qmax = 0.01 μg / g; KF = 0.002 $\mu g^{1-1/n}$ / g	
	Pb			Qmax = 0.716 μg / g; KF = 0.214 $\mu g^{1-1/n}$ /g	
	Cd			Qmax = 0.0894 nmol / g; KF = 0.000173 $nmol^{1-1/n}g^{-1}L^{1/n}$	
	Co			Qmax = 1.17 nmol / g; KF = 0.000293 $nmol^{1-1/n}g^{-1}L^{1/n}$	
Virgin pellets-river water	Cr	0 - 0.02	Langmuir and Freundlich model	/; KF = 0.000243 $nmol^{1-1/n}g^{-1}L^{1/n}$	Holmes et al., 2014
	Cu			Qmax = 1.58 nmol / g; KF = 0.0162 $nmol^{1-1/n}g^{-1}L^{1/n}$	
	Ni			Qmax = 0.282 nmol / g; KF = 0.00124 $nmol^{1-1/n}g^{-1}L^{1/n}$	
	Pb			Qmax = 0.922 nmol / g; KF = 0.013 $nmol^{1-1/n}g^{-1}L^{1/n}$	
Virgin pellets-sea water	Cd			Qmax = 0.00383 nmol / g; KF = 0.000816 $nmol^{1-1/n}g^{-1}L^{1/n}$	
	Co			Qmax = 0.299 nmol / g; KF = 0.000982 $nmol^{1-1/n}g^{-1}L^{1/n}$	

	Cr	Qmax = 5.72 nmol / g; KF = 0.0987 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Cu	Qmax = 4.11 nmol / g; KF = 0.0992 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Ni	Qmax = 0.0129nmol/g; KF = 0.000959 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Pb	/; KF = 0.000140 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Cd	Qmax = 2.21nmol/g; KF = 0.193 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Co	Qmax = 1.35 nmol / g; KF = 0.507 $nmol^{1-1/n}g^{-1}L^{1/n}$
Beached pellets-river water	Cr	Qmax = 1.79 nmol / g; KF = 0.0452 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Cu	/; KF = 0.0508 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Ni	Qmax = 2.58 nmol / g; KF = 0.421 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Pb	Qmax = 13.2 nmol / g; KF = 0.373 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Cd	Qmax = 0.0904 nmol / g; KF = 0.00379 $nmol^{1-1/n}g^{-1}L^{1/n}$
Beached pellets-sea water	Co	Qmax = 0.717 nmol / g; KF = 0.0905 $nmol^{1-1/n}g^{-1}L^{1/n}$
	Cr	Qmax = 8.48 nmol / g; KF = 0.812 $nmol^{1-1/n}g^{-1}L^{1/n}$

		Cu				/	
		Ni				Qmax = 1.25 nmol / g; /	
		Pb				Qmax = 3.3 nmol / g; KF = 0.65 $nmol^{1-1/n}g^{-1}L^{1/n}$	
	0.25	Pb	0.1			Kf = 108.66 (mg / kg) / (mg / L)^n	
	0.5	Cu	0.3			Kf = 136.23 (mg / kg) / (mg / L)^n	
Pristine-PS	0.75	Cd	0.7			Kf = 142.51 (mg / kg) / (mg / L)^n	
	1.25	Ni	1			Kf = 154.46 (mg / kg) / (mg / L)^n	
	2	Zn	2			Kf = 70.297 (mg / kg) / (mg / L)^n	
	0.25	Pb	0.1	48 h	Freundlich model	Kf = 91.11 (mg / kg) / (mg / L)^n	Mao et al., 2020
UV pure	0.5	Cu	0.3			Kf = 94.08 (mg / kg) / (mg / L)^n	
water	0.75	Cd	0.7			Kf = 101.1 (mg / kg) / (mg / L)^n	
aged-PS	1.25	Ni	1			Kf = 49.12 (mg / kg) / (mg / L)^n	
	2	Zn	2			Kf = 72.63 (mg / kg) / (mg / L)^n	
	0.25	Pb	0.1			Kf = 75.66 (mg / kg) / (mg / L)^n	
UV	0.5	Cu	0.3			Kf = 82.56 (mg / kg) / (mg / L)^n	
seawater	0.75	Cd	0.7			Kf = 37.77 (mg / kg) / (mg / L)^n	
aged-PS	1.25	Ni	1			Kf = 63.24 (mg / kg) / (mg / L)^n	
	2	Zn	2			Kf = 66.20 (mg / kg) / (mg / L)^n	
	0.25	Pb	0.1			Kf = 70.97 (mg / kg) / (mg / L)^n	

	0.5	Cu	0.3			Kf = 26.41 (mg / kg) / (mg / L)^n	
UV air	0.75	Cd	0.7			Kf = 48.59 (mg / kg) / (mg / L)^n	
aged-PS	1.25	Ni	1			Kf = 52.12 (mg / kg) / (mg / L)^n	
	2	Zn	2			Kf = 56.52 (mg / kg) / (mg / L)^n	
PS-S	0.04; 0.1 μm	Cu	0.2 - 5	14 d	Freundlich model	Kf = 9.85 (mg / kg) / (mg / L)^n	Qiao et al., 2019
PS-L	0.04; 20 μm					Kf = 8.47 (mg / kg) / (mg / L)^n	
PE					BET model	QBet = 144 μg / g	
PS	20	Pb(II)	25		BET model	QBet = 114.5 μg / g	Lin et al., 2021
PVC					Langmuir and Freundlich model	Qmax = 794.8 μg / g; KF = 0.089 μg$^{1-1/n}$ / g	
MPs		Pb			Freundlich model	Kf = 275.677 L / mg	Purwiyanto et al., 2020
		Cu				Kf = 1867.67 L / mg	
PET						Kf = 0.065 mg / g	
PA		Pb(II)				Kf = 0.069 mg / g	
EVA						Kf = 0.047 mg / g	
PET					Freundlich model	Kf = 0.012 mg / g	Öz et al., 2019
PA		Al(III)				Kf = 0.01 mg / g	
EVA						/	
PA	4.5; 0.1 - 0.15 mm	Sr^{2+}	0 - 3.4	24 h	Temkin model	A = 0.00753 L / g	Guo et al., 2020a
PS						A = 0.0101 L / g	
PP						A = 0.0091 L / g	
Aged nylon MPs	1	Cu(II)	30.69 - 122.34 μmol / L	72 h	Langmuir and Freundlich model	Qmax = 16.712 μmol / g; KF = 1.7398 (μmol / g) / (μmol / L)$^{1/n}$	Tang et al., 2021
		Ni(II)				Qmax = 10.565 μmol / g; KF = 0.1743 (μmol / g) / (μmol / L)$^{1/n}$	

		Zn(II)				Qmax = 12.726 μmol / g; KF = 2.6319 (μmol / g) / (μmol / L)$^{1/n}$	
Aged nylon MPs	3	Pb(II)	2 - 14	36 h	Langmuir model	Qmax = 1.0315 mg / g	Tang et al., 2020
Natural-aged MPs	0.4	Pb(II)	10		Langmuir model	Qmax = 13.60 mg / g	Fu et al., 2021
PET	50	Pb Cd Zn		24 h	Freundlich model	KF = 0.0398 mg^{1-n}L^{n} / g KF = 0.170 mg^{1-n}L^{n} / g KF = 0.490 mg^{1-n}L^{n} / g	Abbasi et al., 2020
PE PP PVC PS	2	Cd	1				Guo et al., 2020b
HDPE	1	Hg	10	24 h	Langmuir model	Qmax = 0.03663 mg / g Qmax = 0.03610 mg / g Qmax = 0.05348 mg / g	Fernández et al., 2020
UV aged-PET	1 - 5	Cu^{2+} Zn^{2+}	2 - 10	144 h	Langmuir model	Qmax = 0.04049 mg / g Qmax = 0.35753 mg / g Qmax = 0.21103 mg / g	Wang et al., 2020b
PE	1	Cr Cu Pb Zn	0.5, 1, 2, 4, 8, 16, 32	14 d	Langmuir model	Qmax = 4.70 mg / g Qmax = 0.259 mg / g Qmax = 2.36 mg / g Qmax = 0.505 mg / g	Godoy et al., 2019
PET PP		Pb Cr				Qmax = 4.93 mg / g Qmax = 0.624 mg / g	

PS		Cu				Qmax = 2.95 mg / g	
		Pb				Qmax = 5.55 mg / g	
		Co				Qmax = 0.813 mg / g	
		Cr				Qmax = 0.473 mg / g	
		Cu				Qmax = 0.358 mg / g	
		Pb				Qmax = 2.94 mg / g	
		Cr				Qmax = 2.44 mg / g	
PVC		Pb				Qmax = 1.90 mg / g	
		Zn				Qmax = 0.634 mg / g	
PS-S	1; 0.01 mm	Pb^{2+}	10, 20, 30, 40,50	72 h	Langmuir model	Qmax = 1.3482 mg / g	Yuan et al., 2020
		Cu^{2+}				Qmax = 0.7584 mg / g	
		Cd^{2+}				Qmax = 0.6553 mg / g	
		Ni^{2+}				Qmax = 0.7663 mg / g	
PS-L	1; 0.05 mm	Pb^{2+}				Qmax = 1.0635 mg / g	
		Cu^{2+}				Qmax = 0.6922 mg / g	
		Cd^{2+}				Qmax = 0.6046 mg / g	
		Ni^{2+}				Qmax = 0.5898 mg / g	
PMB		Cu(II)			Langmuir model	Qmax = 0.0630 mg / g	Wijesekara et al., 2018
BPMB						Qmax = 0.1058 mg / g	
SEMB						Qmax = 1.3196 mg / g	
SMB						Qmax = 0.08507 mg / g	

PS, Polystyrene; PE, Polyethylene; HDPE, High density polyethylene; HPE, High crystallinity polyethylene; LPE, Low crystallinity polyethylene; PVC, Polyvinyl Chloride; PET, Polyethylene terephthalate; PA, Polyamide; PP, Polypropylene; CPE, Chlorinated polyethylene; PSMPs, Polystyrene microplastic particles; PTFE, Polytetrafluoroethylene; EVA, Ethylene vinyl acetate; PMB, Pure polyethylene microbeads; BPMB, Extracted microbeads from biosolids; SEMB, Extracted microbeads from sediment; SMB, Extracted microbeads from soil.

Table S2.6. Summarised factors that influence the MPs-metals adsorption from previous studies including characteristics of MPs and environmental factors.

MP type	Metal type	Influencing factor	Effect	Finding	References
HDPE	Cd	Contact time	+	The high adsorption rate at the initial stage, with a plateau value occurring at 60 min, then remained unchanged after 90 min.	Wang et al., 2019b
		Particle size	-	Microplastics with a smaller particle size showed higher adsorption capacity.	
		pH	+	Increasing the pH of Cd solution led to an increase in Cd adsorption.	
		Ionic strength	-	Addition of NaCl decreased Cd adsorption by microplastics.	
PSMPs	As(III)	Point of zero charge (PZC)	+	The higher the PZC is, the higher the adsorbed amount of As(III) is.	Dong et al., 2020
		Particle size	-	Increase in the PSMP particle size decreased the amount of As(III) adsorbed.	
		Temperature	-	Hydrogen bonds between As(III) and PSMPs were broken at high temperatures, resulting in a decrease in As(III) adsorption onto PSMP.	
		pH	-	Increases in the pH in the solution led to inhibited As(III) adsorption of PSMPs.	
		Ionic strength	-	Increases in concentrations of interfering nitrate and phosphate ions in the solution led to inhibited As(III) adsorption of PSMPs.	
PTFE	As(III)	Temperature	-	The amount of adsorption was decreased with the increase in temperature.	Dong et al., 2019
		pH	-	In the pH range of 3 - 7, as the pH value increased, the amount of As(III) adsorbed by PTFE gradually decreased.	
		Ionic strength	-	PTFE slightly decreased with an increase in NO^{3-} and PO_4^{3-} concentrations.	
CEP, PVC, HPE, LPE	Cu^{2+}, Cd^{2+}, Pb^{2+}	pH	+	The adsorption strengthened with increased pH.	Zuo et al., 2020

		Ionic strength	-	The increasing ionic strength in solutions only slightly inhibited the adsorption of Pb^{2+} to MPs and Cu^{2+} to CPE, while, significantly decreased for others.	
		Surface area and crystallinity of MPs		Surface area had a minimal effect and crystallinity seemed to have no effect on the adsorption of metals on MPs.	
Virgin and benched PE pellets	Cr, Co, Ni, Cu, Zn, Cd, Pb	Aging	+	Adsorption to beached pellets is greater than adsorption to virgin pellets.	Holmes et al., 2012
Pristine and aging PS	Pb, Cu, Cd, Ni, Zn	Aging	+	Adsorption capacity of PS increases with increasing aging degree.	Mao et al., 2020
		Aging under different condition		The order of aging degree of PS under three different conditions is: UV air > UV seawater > UV pure water.	
		Aging time	+	With increasing aging time, MPs surface generates pores and becomes rough and adsorbs more metals.	
PS	Cu	Particle size	-	Under the same initial concentration of Cu, SMPs have a higher adsorption capacity than that of LMPs.	Qiao et al., 2019
		pH	+	With increasing pH, the adsorption of Cu on SMPs and LMPs significantly increased from 2.19 to 18.04 mg / g and from 2.09 to 10.87mg / g.	

		NOM	+	Within the pH range of 6-8, Cu adsorption significantly increased by 5.42 - 5.87 mg / g and 8.12 - 9.39 mg / g for SMPs and LMPs in the presence of NOM compared with those in the absence of NOM.	
PS, PE, PVC	Pb(II)	pH	+	Pb(II) sorption onto different MPs were all increased with the increasing pH from 2.0 to 6.0.	Lin et al., 2021
		Ionic strength	-	Higher ionic strength inhibited the Pb(II) sorption onto the MPs.	
		Temperature	+	High temperature was beneficial to the Pb(II) sorption onto MPs.	
PET, PA, EVA	Pb(II), Al(III)	pH		The highest adsorption capacities were obtained in experiments conducted in optimum conditions with a pH value of 5.5 (2 - 10).	Öz et al., 2019
		Contact time		The highest adsorption capacities were obtained in experiments conducted in optimum conditions with a 60-min stirring time (5 - 90 min).	
		Initial concentration of metals		The highest adsorption capacities were obtained in experiments conducted in optimum conditions with an initial concentration of 4 ppm (0.5 - 7 ppm).	
		Temperature	+	Adsorption capacity increased for MPs with increasing temperatures but it was not affected much.	
Aged nylon MPs	Cu(II), Ni(II), Zn(II)	Temperature	+	The amounts adsorbed increased with the rising temperature.	Tang et al., 2021
		pH	+	The decrement of initial solution pH contribute to gradually decreased adsorption amounts of metals onto collected nylon MPs.	
		Ionic strength	-	The increment of NaCl concentration contribute to gradually decreased adsorption amounts of metals onto collected nylon MPs.	

		FA concentration	+ & -	The rising concentration of FA in solution slightly increases the amounts of Ni(II) adsorbed but inhibits the adsorption capacities of Cu(II) and Zn(II).	
Aged nylon MPs	Pb(II)	Temperature	+	The amounts adsorbed increased with the rising temperature.	Tang et al., 2020
		pH	+	The amounts of lead(II) ions adsorbed increases with an increase in solution pH.	
		Ionic strength	-	The presence of NaCl inhibited the Pb(II) sorption onto the MPs.	
		FA concentration	-	The adsorption of lead(II) was inhibited due to the increase of FA concentration.	
Pristine and natural-aged MPs	Pb(II)	Aging	+	Natural-aged MPs had a stronger adsorption efficiency than pristine MPs.	Fu et al., 2021
		Initial amount of MPs	+	When the used amount of MPs was reduced, the final adsorption capacity to Pb(II) was low.	
		Initial concentration of Pb(II)	+	Adsorption capacity of MPs enhanced with increasing initial Pb(II) concentration in the range of 2 - 15 mg / L.	
		Ionic strength	-	When the salinity increased, the adsorption efficiency and rate of MPs was decreased.	
		pH	+	The capture efficiency increased with the increase of pH in the solution.	
		Background electrolyte		The concentration of Pb(II) decreased rapidly forming the precipitation immediately in the present of HCO^{3-}; The presence of $SO4^{2-}$ and HA significantly reduced the concentration of Pb(II) in the solution and promoted the adsorption process between MPs and Pb(II).	

		Aqueous medium		Adsorption capacity of Milli-Q water and stormwater were basically same, while the adsorption efficiency in surface water and tap water was higher, and the adsorption process showed the best efficiency in tap water.	
MPs	Hg	MPs concentration		The decay of mercury in the water increased with MPs concentration.	Barboza et al., 2018
PE, PP, PVC, PS	Cd	pH	+	The sorption tendency increased as the pH increased.	Guo et al., 2020b
		Ionic strength	-	The sorption capacity of Cd^{2+} on polymers decreased as the salinity increased, becoming extraordinary under high salinity.	
		HA	+	In the presence of HA, the sorption increased with an increase in HA concentration.	
Origin and UV-aged-PET	Cu^{2+}, Zn^{2+}	Aging use UV radiation	+	Aged microplastics had higher adsorption capacity of heavy metals than original ones. When prolonging the time of radiation, the enhanced adsorption capacities of microplastics appeared for Cu^{2+} and Zn^{2+}.	Wang et al., 2020b
		Temperature	+	The adsorption capacities of metal ions also enhanced with increasing temperature.	
		pH	+	Higher the pH value would lead to an effective adsorption of positive charged metal ions.	
		Initail amount of MPs	+	The adsorption capacity of MPs increases sharply when the dosage of MPs increases from 1.0 to 2.0 g / L, and the trend is smoothly when the dosage is higher than 2.0 g / L.	
PE beads	Cr(VI)	SDBS	+	SDBS could increase the attraction between PE and Cr(VI) ions in the water environment. The adsorption capacity was increased from 0.18 mg / g 1 to 0.50 mg / g 1 when the SDBS concentration was increased from 0 to approximate 1 mM.	Zhang et al., 2020
		pH	-	The adsorption capability was decreased with increasing pH.	

PE, PP, PS, PVC, PET	Cd, Co, Cr, Cu, Ni, Pb, Zn	Different natural waters	+	In wastewater and irrigation water, the presence of organic matter increased the adsorption of lead and chromium on the plastic. The pH of these waters altered the adsorption of metals, as precipitation phenomena normally occur at pH values higher than 7. The presence of carbonates, sulphates or phosphates can induce the reaction of metals with them and consequently, precipitation occurs. An adsorption precipitation phenomenon occurred in the irrigation water and wastewater, especially for lead and chromium. Respect to seawater, the combined effects of salinity and solution chemistry sometimes increased the adsorption of metals (Cu and Cr on PP and PVC) and sometimes decreased it (Cr and Co on PE and PS).	Godoy et al., 2019
PS	Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}	Particle size	-	The smaller particle size of MPs was accompanied with the more favorable adsorption for heavy metals.	Yuan et al., 2020
PMB	Cu(II)	Types of surface-modified		The microbeads need to be supported with organic matter and minerals to adsorb metals in high contents.	Wijesekara et al., 2018

"+" indicates a positive correlation; "-" indicates a negative correlation.

PS, Polystyrene; PE, Polyethylene; HDPE, High density polyethylene; PVC, Polyvinyl Chloride; PET, Polyethylene terephthalate; PA, Polyamide; PP, Polypropylene; CPE, Chlorinated polyethylene; PSMPs, Polystyrene microplastic particles; PTFE, Polytetrafluoroethylene; EVA, Ethylene vinyl acetate; PMB, Pure polyethylene microbeads.

Table S2.7. Impact of MPs-metals interactions on earthworms from previous studies.

Soil organism	Metal	MPs	Test condition	Exposure concentration	Exposure time	Evaluating indicators	Highlight results	Reference
Earthworm, *Eisenia fetida*	Cadmium (Cd)	PP (< 150 μm) particles	OECD artificial soil	MPs: 300, 3000, 6000, 9000 mg / kg; Cd: 8mg / kg	14, 28, 42 days	Growth; mortality; bioaccumulation; oxidative damage	Generally, decrease in growth, mortality. MPs particles can increase the accumulation of Cd in earthworm from 9.7 - 161.3 %. The presence of Cd accelerates the oxidative damage in *E. fetida* of MPs.	Zhou et al., 2020
Earthworm, *Eisenia fetida*	Cadmium (Cd)	PE (< 300 μm) particles	Agricultural experimental field soil	MPs: 0, 7, 15, 20, 30 % (w / w); Cd: 2, 10 mg / kg	28 days	Growth; reproduction; oxidative stress; histopathological damage; sperm DNA damages; bioaccumulation	Co-exposure to MPs and Cd inhibited growth and reproduction, induced the oxidative stress, histopathological and sperm DNA damages of *E. fetida*. Increase in	Huang et al., 2021

							the Cd bioaccumulation and availability in *E. fetida* and availability in soil. PE-MPs enhanced the accumulation of metals in earthworms.	
Earthworm, *Eisenia fetida*	Copper (Cu), Nickel (Ni)	PE (30, 100 μm) particles	Agricultural experiment station soil	MPs: 0.01, 0.05, 0.1 % (w / w); Cu: 100 mg / kg; Ni: 40 mg / kg	21 days	Bioaccumulation; enzyme activities; gene expression	Enzyme activities and gene expression showed combined two pollutants brought severer damage to earthworms.	Li et al., 2021
Earthworm, *Eisenia andrei*	Silver nanoparticles (AgNP), $AgNO_3$	Polyester MP (50 - 3000 μm) fibers	LUFA 2.2 soil	MPs: 0.01 % (w / w); AgNP (32, 100, 320, 1000, 3200 mg Ag / kg); $AgNO_3$ (12.8, 32, 80, 200, 500 mg Ag / kg)	28 days	Bioaccumulation; survival; reproduction	No effect on survival. The presence of MF increased the toxicity of AgNP to earthworm reproduction, did not alter the toxicity of $AgNO_3$.	Tourinho et al., 2021

Earthworm, *Lumbricus terrestris*	Zinc (Zn)	HDPE (< 400 mm) pieces	Woodland soil	0.35 % (w / w) of Zn-bearing MP (236 - 4505 mg / kg)	28 days	Growth; bioaccumulation; mortality	MF may lower the bioavailable Ag fraction at high concentrations due to sorption. Increased Zinc exposure to earthworm and no evidence of Zn accumulation, mortality or weight change.	Hodson et al., 2017
Earthworm, *Metaphire californica*	Arsenic (As)	PVC particles	Farmland soiil	MPs: 2000 mg / kg; As(V): 40 mg / kg	28 days	Bioaccumulation	Decrease in accumulation of total As and transformation of As(IV) to As(III).	Wang et al., 2019b

PP, Polypropylene; PE, Polyethylene; HDPE, High density polyethylene; PVC, Polyvinyl Chloride; PS, Polystyrene; PTFE, Polytetrafluoroethylene; PLA, polylactic acid.

2 Chapter 4 An investigation into the avoidance of Cd and MPs individually and in combination by the earthworm *Lumbricus terrestris* supporting information

Number of tables: 3

List of contents:

Table S4.1. 2-way ANOVA on ranked earthworm mortality after 72 h exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test					
Source of Variation	DF	SS	MS	H	P
plastic type	2	106.9		0.718346392	0.69825341
Cd	4	7244.167		48.67933797	6.8111E-10
plastic type x Cd	8	323.433		2.173404384	0.97522886
Residual	45	1105.5			
Total	59	8780	148.814		

Table S4.2. 2-way ANOVA results for earthworm avoidance after 72 h exposure.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.739)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.099)

Source of Variation	DF	SS	MS	F	P
plastic type	2	5775.525	2887.763	3.279	0.047
Cd	4	30013.45	7503.363	8.52	<0.001
plastic type x Cd	8	18198.98	2274.872	2.583	0.02
Residual	46	40512.61	880.709		
Total	60	104004.4	1733.406		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of plastic type depends on what level of Cd is present.
There is a statistically significant interaction between plastic type and Cd. (P = 0.020)

Power of performed test with alpha = 0.0500: for plastic type : 0.440
Power of performed test with alpha = 0.0500: for Cd : 0.994
Power of performed test with alpha = 0.0500: for plastic type x Cd : 0.627

Table S4.3. Holm-Sidak post hoc test results for earthworm avoidance after 72 h exposure.

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic type				
Comparison	Diff of Means	t	P	P<0.050
PLA vs. PE	21.7	2.312	0.074	No
None vs. PE	20.129	2.086	0.083	No
PLA vs. None	1.571	0.163	0.871	No

Comparisons for factor: Cd				
Comparison	Diff of Means	t	P	P<0.050
10.000 vs. 100.000	69.917	5.343	<0.001	Yes
0.000 vs. 100.000	59.048	4.657	<0.001	Yes
0.100 vs. 100.000	60	4.585	<0.001	Yes
1.000 vs. 100.000	57.5	4.394	<0.001	Yes
10.000 vs. 1.000	12.417	1.025	0.893	No
10.000 vs. 0.000	10.869	0.931	0.89	No
10.000 vs. 0.100	9.917	0.819	0.885	No
0.100 vs. 1.000	2.5	0.206	0.996	No
0.000 vs. 1.000	1.548	0.133	0.989	No
0.100 vs. 0.000	0.952	0.0816	0.935	No

Comparisons for factor: Cd within None				
Comparison	Diff of Means	t	P	P<0.050
10.000 vs. 0.000	41.357	2.223	0.271	No
0.100 vs. 0.000	40.107	2.156	0.283	No
10.000 vs. 100.000	35	1.362	0.795	No
0.100 vs. 100.000	33.75	1.313	0.782	No
10.000 vs. 1.000	22.5	1.072	0.871	No
1.000 vs. 0.000	18.857	1.014	0.85	No
0.100 vs. 1.000	21.25	1.013	0.782	No
1.000 vs. 100.000	12.5	0.486	0.949	No
100.000 vs. 0.000	6.357	0.267	0.956	No
10.000 vs. 0.100	1.25	0.0596	0.953	No

Comparisons for factor: Cd within PE				
Comparison	Diff of Means	t	P	P<0.050
0.000 vs. 100.000	109.75	5.23	<0.001	Yes
0.100 vs. 100.000	84.5	4.027	0.002	Yes
10.000 vs. 100.000	74.75	3.562	0.007	Yes
1.000 vs. 100.000	70.25	3.348	0.011	Yes

0.000 vs. 1.000	39.5	1.882	0.337	No
0.000 vs. 10.000	35	1.668	0.416	No
0.000 vs. 0.100	25.25	1.203	0.658	No
0.100 vs. 1.000	14.25	0.679	0.875	No
0.100 vs. 10.000	9.75	0.465	0.874	No
10.000 vs. 1.000	4.5	0.214	0.831	No

Comparisons for factor: Cd within PLA

Comparison	Diff of Means	t	P	P<0.050
10.000 vs. 100.000	100	4.765	<0.001	Yes
1.000 vs. 100.000	89.75	4.277	<0.001	Yes
0.000 vs. 100.000	73.75	3.514	0.008	Yes
0.100 vs. 100.000	61.75	2.943	0.035	Yes
10.000 vs. 0.100	38.25	1.823	0.373	No
1.000 vs. 0.100	28	1.334	0.648	No
10.000 vs. 0.000	26.25	1.251	0.625	No
1.000 vs. 0.000	16	0.762	0.833	No
0.000 vs. 0.100	12	0.572	0.815	No
10.000 vs. 1.000	10.25	0.488	0.628	No

Comparisons for factor: plastic type within 0

Comparison	Diff of Means	t	P	P<0.050
PE vs. None	43.107	2.317	0.073	No
PLA vs. None	31.607	1.699	0.183	No
PE vs. PLA	11.5	0.548	0.586	No

Comparisons for factor: plastic type within 0.1

Comparison	Diff of Means	t	P	P<0.050
None vs. PE	22.25	1.06	0.649	No
None vs. PLA	20.5	0.977	0.556	No
PLA vs. PE	1.75	0.0834	0.934	No

Comparisons for factor: plastic type within 1

Comparison	Diff of Means	t	P	P<0.050
PLA vs. PE	44	2.097	0.12	No
PLA vs. None	28.75	1.37	0.323	No
None vs. PE	15.25	0.727	0.471	No

Comparisons for factor: plastic type within 10

Comparison	Diff of Means	t	P	P<0.050
PLA vs. PE	49.75	2.371	0.065	No
None vs. PE	33.25	1.584	0.225	No
PLA vs. None	16.5	0.786	0.436	No

Comparisons for factor: plastic type within 100				
Comparison	Diff of Means	t	P	P<0.050
None vs. PE	73	2.84	0.02	Yes
None vs. PLA	48.5	1.887	0.127	No
PLA vs. PE	24.5	1.168	0.249	No

3 Chapter 5 An investigation into the impacts of MPs on the bioaccumulation of cadmium in earthworms *Lumbricus terrestris* supporting information a

Number of text: 2
Number of figures: 6
Number of table: 15

List of contents:

(S5a.7) 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 50 mg / L). The Linear regression model is expressed as $C_{MPs} = K_d C_{aq}$ where $K_{d\text{-adsorption}}$ is coefficient constant, L / kg. The Freundlich model is expressed as $C_{MPs} = K_F C_{aq}^{1/n}$ where K_F is 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 binding coefficient constant, mg / L and C_m is maximum concentration adsorbed to MPs, mg / kg. C_{MPs} is concentration adsorbed to MPs at equilibrium, mg / kg, C_{aq} is concentration in solution at equilibrium, mg / L;
(S5a.8) Measured Cd concentrations of the bulk soil and mean Cd concentrations accounting for both the bulk soil and earthworm body concentration after 28 days exposure;
(S5a.9) Independent t test results for difference between measured Cd concentration of the bulk soil and the average concentration accounting for both the bulk soil and earthworm tissue concentrations and mass;
(S5a.10) The total mass of Cd in the pore water and bulk soil of the MP-free treatments;
(S5a.11) Distribution coefficients ($K_{d\text{-exposure}}$) of Cd from previous studies;
(S5a.12) Isotherm parameters for Cd adsorption to MPs from previous studies;
(S5a.13) BAF_s values, earthworm species used and organic matter values from previous studies;
(S5a.14) Linear regression parameters of Log10 transformed measured Cd concentration in earthworm body (mg / kg dry weight) against Log10 transformed measured total Cd concentration in bulk soil (mg / kg dry soil). 95 % confidence intervals are given in brackets.;
(S5a.15) Cd LC_{50} values from previous studies.

Text:

Test S5a.1: Digestion methods

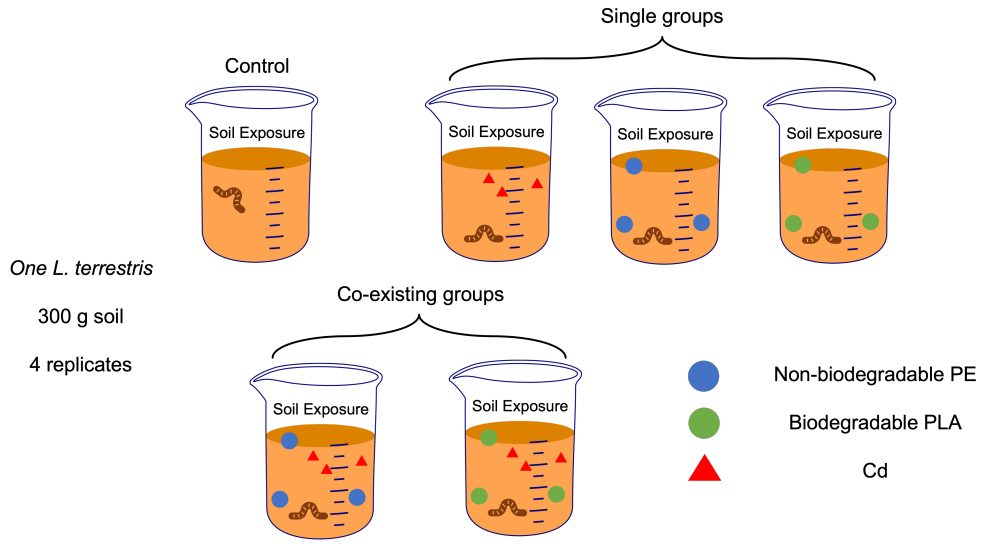
Soil digestions for pseudo-total Cd concentration followed the digestion method of British Standard BS 7755, (1995). At the end of the exposure experiment soils were air-dried. One replicate soil of each treatment was weighed to 1.5 g transferred to a 100 ml Kjeldahl digestion tube. When we set up the exposure experiment, we thoroughly mixed chemicals with soil of each treatment and then divided it into four replicates, so we assume that each replicate had the same Cd concentration. 10.5 ml of concentrated AnalaR hydrochloric acid was added to each tube followed by 3.5 ml concentrated AnalaR nitric acid (aqua regia, 3:1 HCl-HNO_3). A glass bubble was placed on top of each tube and the tubes were left to stand overnight in a fume cupboard. The next day, the tubes were heated to 50 °C, the temperature was then gradually increased to 140 °C at 1 °C per minute. The soils were digested for 2 - 2.5 hours. After cooling down, the digestions were filtered through a Whatman no. 540 12.5 cm diameter filter paper and diluted with 0.5M nitric acid into a 100 ml volumetric flask prior to analysis by ICP-OES. PE and PLA MPs were also digested following the above procedures.

Earthworm digestion followed the protocol of Davies et al. (2002). Frozen earthworms were defrosted for approximately 40 minutes. Each individual was placed on a Whatman no. 540 12.5 cm diameter filter paper, and then dried in an oven at 40 ^{o}C for approximately 12 hours. The dry weight of the earthworm was recored. Both filter papers and earthworms were transferred to a 100 ml Kjeldahl digestion tube, and a 10 ml of concentrated AnalaR nitric acid was added to each tube and then a glass bubble was placed on top of the tube. The sample was left to stand overnight in a fume cupboard. The next day, the tubes were heated cautiously to 90 °C and digested for 6-8 hours. After cooling down, the digestions were filtered through a Whatman no. 540 12.5 cm diameter filter paper and diluted with deionized water into a 100 ml volumetric flask prior to analysis by ICP-OES. PE and PLA MPs were also digested following the above procedures.

Text S5a.2: Pore water extraction from soil

Around 30 g soil was placed in a disposable syringe with a layer of 3 cm of glass wool inserted into the bottom, which was placed in a 50 ml centrifuge tube. After 15 minutes centrifugation at 4000 RPM, the separated solution was carefully collected from the tube and transferred to a 2 mL plastic microfuge tube (Carter et al., 2014). The pore water samples were diluted by mass using deionized water prior to further ICP analysis.

Figures



Note: CT = controlled temperature; Cadmium= Cd; Polylactic acid = PLA; Polyethylene = PE.
Fig. S5a.1. Exposure experiment for 28 days setup diagram.

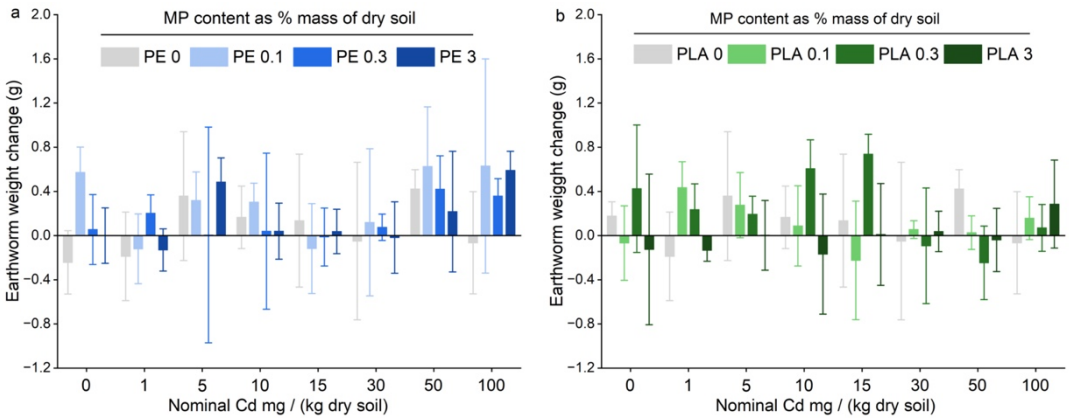


Fig. S5a.2. Mean of earthworms weight change in soil following exposure to Cd with and without MPs (a) PE and (b) PLA over 28 days. Results are mean ± standard deviation, n = 2-4 (dead earthworms were not counted). A positive weight change indicates an increase in earthworm weight and a negative weight change indicates a decrease in earthworm weight.

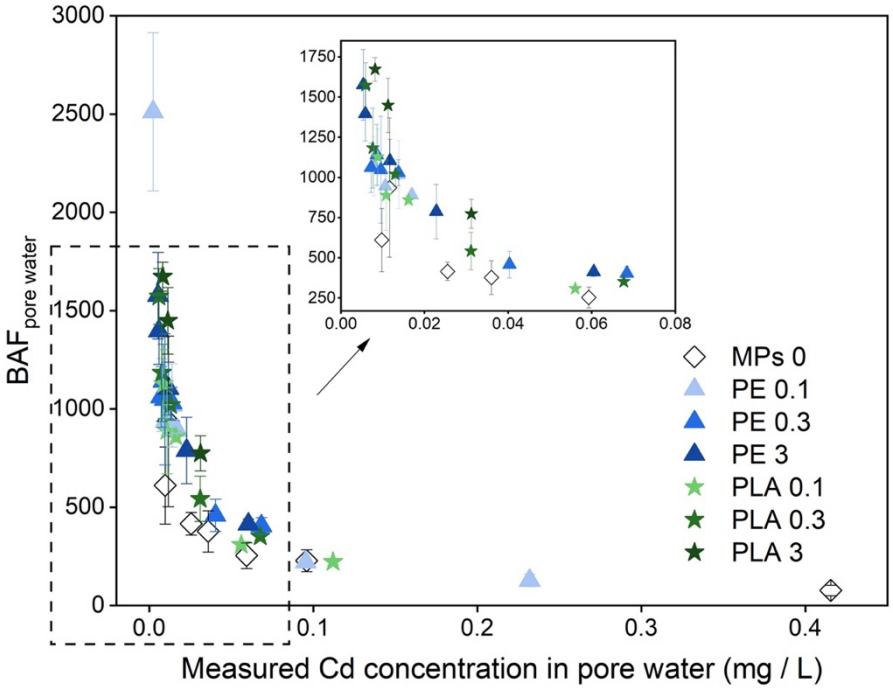


Fig. S5a.3. Earthworm-pore water bioaccumulation factors (BAF_{pw}) of the Cd following exposure to Cd with and without MPs over 28 days (n = 2-4, some replicates did not have pore water samples or did not have live earthworms to digest). Error bars represent standard deviation and some symbols don't show error bars because the values of standard deviation are low.

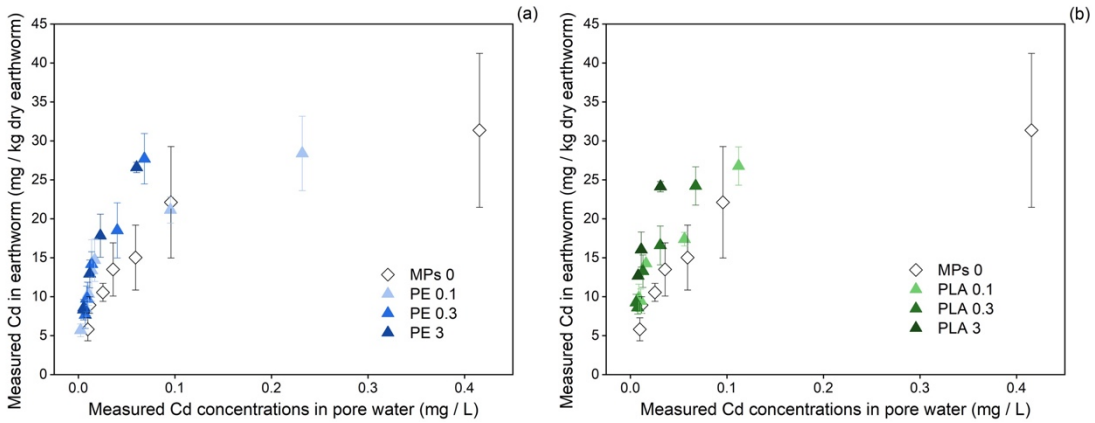


Fig. S5a.4. Mean of measured Cd concentrations in earthworm bodies following exposure to Cd with and without MPs over 28 days against measured Cd concentrations in pore water, (a) PE treatments, (b) PLA treatments. (n = 2-4, earthworm mortality in some treatments reduced the number of replicates). Error bars represent standard deviation.

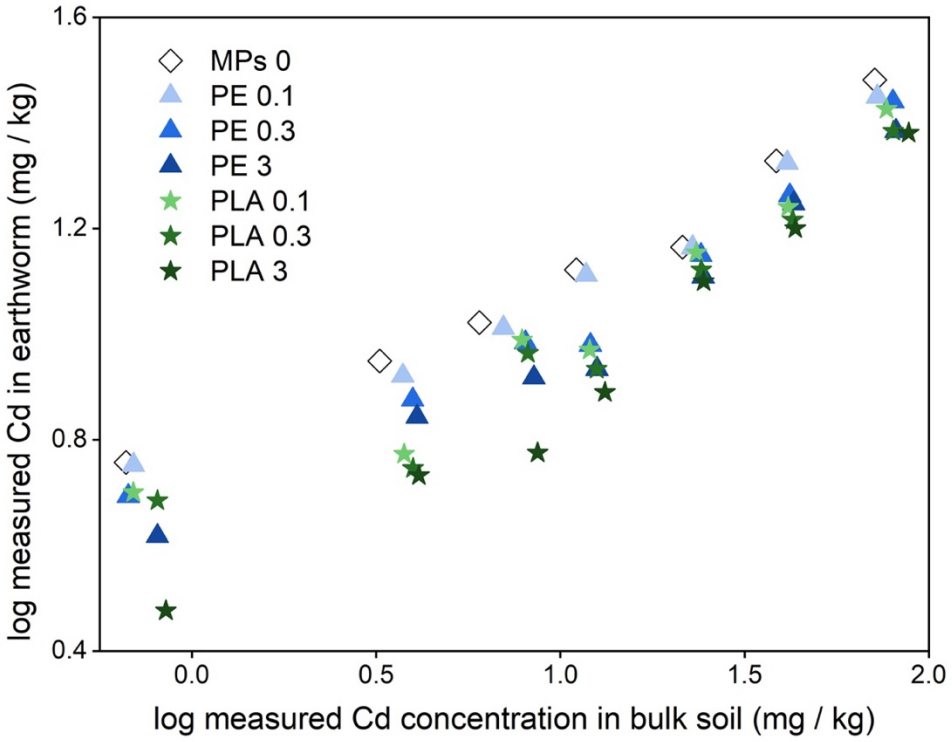


Fig. S5a.5. Log of mean measured Cd concentrations in earthworm (mg / kg dry weight) following exposure to Cd with and without MPs over 28 days against log of mean measured Cd concentrations in bulk soil (mg / kg dry soil).

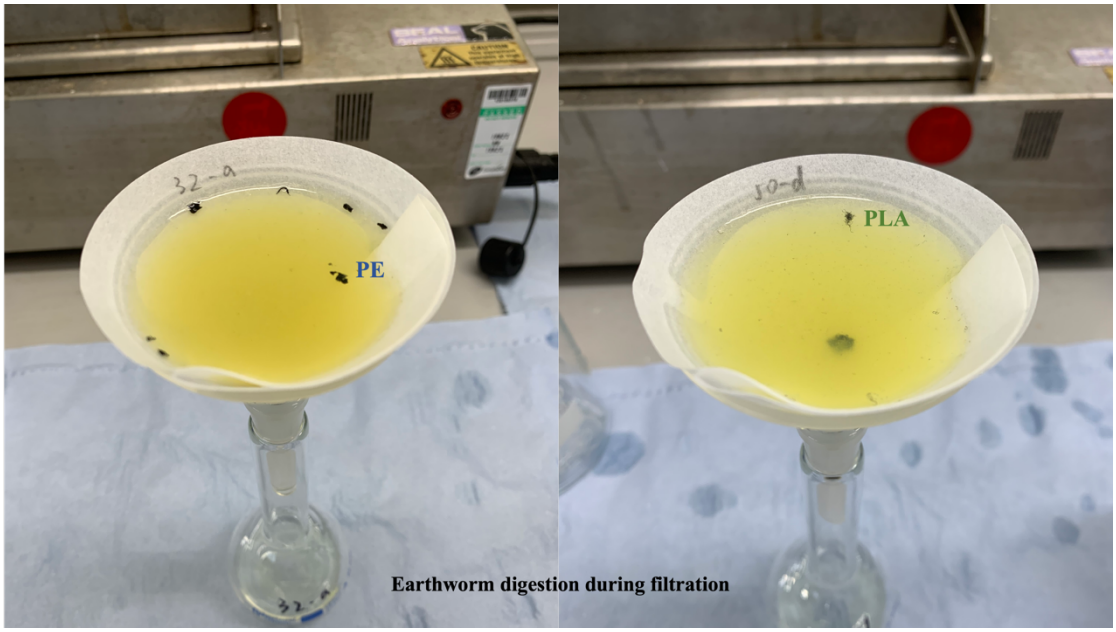


Fig. S5a.6. Residual PE and PLA MPs in earthworm digestate after nitric acid digestion.

Tables

Table S5a.1. Nominal and measured Cd concentrations (mg / kg dry soil) in soil of the exposure experiment and their accuracy (100 * measured Cd concentration / nominal Cd concentration). Soil, PE and PLA are the starting materials, PE control and PLA control are earthworm exposure soils to which no MPs or Cd had been added.

Treatment	Cd concentrations (mg / kg dry soil)		
	Nominal	Measured	Accuracy (%)
Soil	/	< DL	/
PE	/	< DL	/
PLA	/	< DL	/
PE control (no MPs)	0	< DL	/
PLA control (no MPs)	0	< DL	/
PE0.1	0	< DL	/
PE0.3	0	< DL	/
PE3	0	< DL	/
PLA0.1	0	< DL	/
PLA0.3	0	< DL	/
PLA3	0	< DL	/
Cd1	1	0.662	66.239
Cd5	5	3.237	64.732
Cd10	10	6.038	60.380
Cd15	15	11.064	73.758
Cd30	30	21.450	71.500
Cd50	50	38.549	77.097
Cd100	100	71.401	71.401
PE0.1Cd1	1	0.694	69.440
PE0.1Cd5	5	3.737	74.742
PE0.1Cd10	10	7.004	70.038
PE0.1Cd15	15	11.750	78.332
PE0.1Cd30	30	22.833	76.112
PE0.1Cd50	50	41.290	82.580
PE0.1Cd100	100	72.456	72.456
PE0.3Cd1	1	0.672	67.238
PE0.3Cd5	5	3.973	79.463
PE0.3Cd10	10	8.040	80.399
PE0.3Cd15	15	12.066	80.441
PE0.3Cd30	30	24.053	80.177
PE0.3Cd50	50	41.971	83.942
PE0.3Cd100	100	79.947	79.947
PE3Cd1	1	0.806	80.632
PE3Cd5	5	4.082	81.632
PE3Cd10	10	8.479	84.789
PE3Cd15	15	12.570	83.802

PE3Cd30	30	24.407	81.357
PE3Cd50	50	42.974	85.948
PE3Cd100	100	81.503	81.503
PLA0.1Cd1	1	0.694	69.363
PLA0.1Cd5	5	3.768	75.358
PLA0.1Cd10	10	7.878	78.782
PLA0.1Cd15	15	12.011	80.071
PLA0.1Cd30	30	23.399	77.998
PLA0.1Cd50	50	41.547	83.093
PLA0.1Cd100	100	76.769	76.769
PLA0.3Cd1	1	0.806	80.625
PLA0.3Cd5	5	3.981	79.626
PLA0.3Cd10	10	8.162	81.618
PLA0.3Cd15	15	12.547	83.645
PLA0.3Cd30	30	24.135	80.448
PLA0.3Cd50	50	42.744	85.487
PLA0.3Cd100	100	80.041	80.041
PLA3Cd1	1	0.851	85.056
PLA3Cd5	5	4.133	82.653
PLA3Cd10	10	8.675	86.749
PLA3Cd15	15	13.215	88.099
PLA3Cd30	30	24.476	81.586
PLA3Cd50	50	43.405	86.811
PLA3Cd100	100	88.165	88.165

Note: DL = detection limit.

Table S5a.2. Mean Cd concentrations in pore water and earthworm body after 28 days exposure. Results are mean ± standard deviation, n = 2-4 (no pore water extracted from soil or dead earthworms were not able to digest). PE control and PLA control are earthworm exposure soils to which no MPs or Cd had been added.

Treatments	**Cd concentration in pore water (mg / L)**	**Cd concentrations in earthworm body (mg / kg)**
PE control (no MPs)	0.003 ± 0.0005 (< DL)	3.786 ± 0.808
PLA control (no MPs)	0.003 ± 0.0005 (< DL)	3.858 ± 0.742
PE0.1	0.003 ± 0.0007 (< DL)	3.972 ± 0.496
PE0.3	0.003 ± 0.0005 (< DL)	4.712 ± 0.230
PE3	0.003 ± 0.0007 (< DL)	3.674 ± 0.645
PLA0.1	0.003 ± 0.0002 (< DL)	4.639 ± 1.100
PLA0.3	0.004 ± 0.0003 (< DL)	3.552 ± 1.331
PLA3	0.003 ± 0.0001 (< DL)	3.266 ± 0.474
Cd1	0.010 ± 0.0013	5.814 ± 1.474
Cd5	0.012 ± 0.0062	8.936 ± 1.067
Cd10	0.025 ± 0.0012	10.563 ± 1.151
Cd15	0.036 ± 0.0012	13.510 ± 3.411
Cd30	0.059 ± 0.0013	15.027 ± 4.167

Cd50	0.096 ± 0.0109	22.124 ± 7.150
Cd100	0.416 ± 0.0359	31.363 ± 9.872
PE0.1Cd1	0.002 ± 0.0001	5.691 ± 0.813
PE0.1Cd5	0.008 ± 0.0003	8.405 ± 1.208
PE0.1Cd10	0.011 ± 0.0009	10.339 ± 1.452
PE0.1Cd15	0.014 ± 0.0026	13.376 ± 3.943
PE0.1Cd30	0.017 ± 0.0021	14.710 ± 2.746
PE0.1Cd50	0.095 ± 0.0008	21.142 ± 1.693
PE0.1Cd100	0.232 ± 0.0198	28.395 ± 4.783
PE0.3Cd1	0.001 ± 0.0005 (< DL)	4.955 ± 1.683
PE0.3Cd5	0.007 ± 0.0010	7.652 ± 1.725
PE0.3Cd10	0.009 ± 0.0003	9.729 ± 1.643
PE0.3Cd15	0.010 ± 0.0011	9.728 ± 2.160
PE0.3Cd30	0.014 ± 0.0008	14.178 ± 1.600
PE0.3Cd50	0.040 ± 0.0007	18.517 ± 3.529
PE0.3Cd100	0.068 ± 0.0014	27.705 ± 3.238
PE3d1	0.001 ± 0.0004 (< DL)	4.179 ± 0.622
PE3Cd5	0.002 ± 0.0005 (< DL)	7.008 ± 0.849
PE3Cd10	0.005 ± 0.0001	8.342 ± 1.309
PE3Cd15	0.006 ± 0.0003	8.606 ± 0.821
PE3Cd30	0.012 ± 0.0002	12.938 ± 1.777
PE3Cd50	0.023 ± 0.0014	17.835 ± 2.771
PE3Cd100	0.060 ± 0.0043	26.602 ± 0.661
PLA0.1Cd1	0.004 ± 0.0007 (< DL)	5.061 ± 0.838
PLA0.1Cd5	0.004 ± 0.0006 (< DL)	5.947 ± 0.623
PLA0.1Cd10	0.009 ± 0.0003	9.856 ± 1.768
PLA0.1Cd15	0.011 ± 0.0012	9.442 ± 1.564
PLA0.1Cd30	0.016 ± 0.0010	14.266 ± 0.025
PLA0.1Cd50	0.056 ± 0.0029	17.397 ± 0.873
PLA0.1Cd100	0.112 ± 0.0191	26.770 ± 2.456
PLA0.3d1	0.004 ± 0.0002 (< DL)	4.905 ± 0.897
PLA0.3Cd5	0.004 ± 0.0002 (< DL)	5.639 ± 1.016
PLA0.3Cd10	0.006 ± 0.0008	9.254 ± 1.069
PLA0.3Cd15	0.008 ± 0.0019	8.606 ± 0.821
PLA0.3Cd30	0.013 ± 0.0001	13.272 ± 0.025
PLA0.3Cd50	0.031 ± 0.0047	16.595 ± 2.485
PLA0.3Cd100	0.068 ± 0.0031	24.217 ± 0.661
PLA3Cd1	0.002 ± 0.0003 (< DL)	3.124 ± 1.031
PLA3Cd5	0.002 ± 0.0002 (< DL)	5.407 ± 0.394
PLA3Cd10	0.004 ± 0.0006 (< DL)	6.257 ± 2.409
PLA3Cd15	0.003 ± 0.0008 (< DL)	7.783 ± 0.724
PLA3Cd30	0.008 ± 0.0003	12.689 ± 2.085
PLA3Cd50	0.011 ± 0.0013	16.047 ± 3.259

PLA3Cd100	0.031 ± 0.0012	24.126 ± 2.456

Note: Detection limit (DL) for pore water is 0.0010 – 0.0015 mg / L. Measured Cd concentrations of pore water that were below the DL were set to the DL divided by the square root of 2 and multiplied when used in further statistical analysis (Croghan and Egeghy, 2003).

Table S5a.3. R^2 and P values for linear regression of measure Cd in pore water (mg / L) against measure Cd in bulk soil (mg / kg) for the different plastic treatments. Regression lines were constrained to pass through the origin.

MP type	R^2	P
No-MPs	0.88	< 0.001
PE0.1	0.90	< 0.001
PE0.3	0.95	< 0.001
PE3	0.94	< 0.001
PLA0.1	0.92	< 0.001
PLA0.3	0.94	< 0.001
PLA3	0.94	< 0.001

Table S5a.4. Mean soil-pore water distribution coefficients, $K_{d\text{-}exposure}$ and earthworm bioaccumulation factors based on bulk soil BAF_s and based on pore water BAF_{pw}. Results are mean ± standard deviation, n = 2-4 (lower values due to no pore water being extracted or earthworm mortality).

$K_{d\text{-}exposure}$ (L / kg)	MPs 0	PE 0.1	PE 0.3	PE 3	PLA 0.1	PLA 0.3	PLA 3
Cd 1	68.70 ± 9.3^{aA}	302.0 ± 17.9^{aB}	631.4 ± 24.0*^{aC}	739.7 ± 176.7*^{aBC}	169.5 ± 27.6*^{aD}	198.3 ± 8.4*^{aD}	473.4 ± 61.5*^{aC}
Cd 5	334.6 ± 145.5^{abcdeA}	474.8 ± 20.8^{bB}	561.0 ± 82.1^{aB}	1941 ± 434.3*^{bcCD}	954.8 ± 122.7*^{bcdC}	1030 ± 53.9*^{bC}	2307 ± 190.7*^{bD}
Cd 10	237.3 ± 11.3^{bA}	663.5 ± 53.7^{cE}	938.9 ± 36.4^{bB}	1592 ± 38.0^{bdCD}	895.9 ± 34.5^{cB}	1399 ± 189.2^{bcBC}	2481 ± 376.2*^{bcD}
Cd 15	307.5 ± 10.4^{cA}	876.2 ± 180.7^{bcdB}	1278 ± 140.4^{bcB}	2173 ± 118.5^{cCD}	1124 ± 129.0^{dB}	1731 ± 428.9^{bcBC}	4154 ± 808.5*^{bcD}
Cd 30	843.0 ± 8.2^{dA}	1366 ± 182.9^{dBC}	1754 ± 107.3^{dBD}	2085 ± 33.9^{cE}	1451 ± 90.1^{dCD}	1858 ± 16.2^{cB}	3004 ± 104.1^{cF}
Cd 50	405.7 ± 49.5^{cdA}	432.9 ± 3.7^{bD}	1042 ± 18.0^{cB}	1889 ± 123.2^{cdC}	743.1 ± 39.4^{bcfE}	1399 ± 212.7^{bcBC}	3881 ± 435.5^{bcF}
Cd 100	172.7 ± 14.3^{eA}	314.2 ± 27.9^{aC}	1169 ± 24.9^{cB}	1354 ± 98.5^{bB}	699.1 ± 111.1^{bcD}	1185 ± 55.2^{bB}	2828 111.0^{cE}

BAF_s	MPs 0	PE 0.1	PE 0.3	PE 3	PLA 0.1	PLA 0.3	PLA 3
Cd 1	8.777 ± 1.961^{aA}	8.195 ± 1.171^{aA}	7.369 ± 0.807^{aAB}	5.182 ± 0.772^{aBCD}	7.296 ± 1.208^{aAC}	6.084 ± 1.113^{aAC}	3.673 ± 1.212^{aD}
Cd 5	2.761 ± 0.330^{bA}	2.249 ± 0.323^{bAB}	1.926 ± 0.434^{bAC}	1.717 ± 0.208^{bBC}	1.578 ± 0.165^{bBC}	1.416 ± 0.255^{bC}	1.308 ± 0.095^{bC}
Cd 10	1.749 ± 0.191^{cA}	1.476 ± 0.207^{cAB}	1.210 ± 0.204^{cAB}	0.9839 ± 0.154^{cBCD}	1.251 ± 0.224^{bcAC}	1.134 ± 0.131^{bcBC}	0.7213 ± 0.278^{cD}
Cd 15	1.221 ± 0.308^{dA}	1.138 ± 0.336^{cdAB}	0.8062 ± 0.179^{dBC}	0.6847 ± 0.065^{cdC}	0.7862 0.130^{dBC}	0.6859 ± 0.065^{dC}	0.5889 ± 0.055^{cdC}
Cd 30	0.7006 ± 0.194^{eA}	0.6442 ± 0.120^{eA}	0.5895 ± 0.066^{deA}	0.5301 ± 0.073^{deA}	0.6097 ± 0.001^{deA}	0.5499 ± 0.047^{deA}	0.5184 ± 0.085^{cdeA}
Cd 50	0.5739 ± 0.185^{efA}	0.5120 ± 0.041^{efA}	0.4412 ± 0.084^{efA}	0.4150 ± 0.064^{dfA}	0.4187 ± 0.021^{fA}	0.3882 ± 0.058^{fA}	0.3697 ± 0.075^{efA}
Cd 100	0.4393 ± 0.138^{fA}	0.3919 ± 0.066^{fAB}	0.3465 ± 0.040^{fAB}	0.2971 ± 0.008^{fAB}	0.3487 ± 0.045^{fAB}	0.3026 ± 0.008^{fAB}	0.2736 ± 0.028^{fB}

BAF_{pw} (L / kg)	MPs 0	PE 0.1	PE 0.3	PE 3	PLA 0.1	PLA 0.3	PLA 3
Cd 1	610.6 ± 196.5^{abcA}	2511 ± 401.8^{aB}	4658 ± 583.1*^{aC}	3820 ± 1099*^{abC}	1248 ± 358.3*^{abdeC}	1202 ± 195.5*^{abC}	1763 ± 697.8*^{acefBC}
Cd 5	937.0 ± 434.1^{abcA}	1071 ± 185.3^{aB}	1062 ± 154.0^{bB}	3353 ± 978.3*^{bC}	1582 ± 48.9*^{aBC}	1456 ± 245.2*^{abC}	2920 ± 236.0*^{aC}
Cd 10	416.0 ± 56.8^{aA}	945.5 ± 75.8^{aB}	1140 ± 189.4^{bcdBC}	1576 ± 220.4^{abBC}	1127 ± 174.0^{acdBC}	1572 ± 141.7^{aC}	1739 ± 869.6*^{adBC}
Cd 15	377.1 ± 105.2^{abA}	1018 ± 211.3^{abB}	1049 ± 333.3^{bdB}	1397 ± 169.9^{abcB}	888.9 ± 219.0^{cdB}	1183 ± 247.9^{abcB}	2438 ± 505.0*^{abgC}
Cd 30	253.8 ± 65.1^{abcA}	890.8 ± 15.8^{aBC}	1030 ± 80.7^{bcB}	1104 ± 134.5^{abB}	859.4 ± 27.3^{bcBC}	1020 ± 76.7^{bB}	167 ± 73.3^{begC}
Cd 50	228.1 ± 55.5^{bA}	221.0 ± 16.3^{bB}	458.9 ± 81.9^{dCD}	788.8 ± 169.4^{abcC}	309.1 ± 13.3^{deD}	542.5 ± 115.4^{cCD}	1448 ± 169.2^{bdfBCD}
Cd 100	76.51 ± 26.6^{cA}	127.1 ± 31.8^{bB}	404.7 ± 42.7^{dC}	413.0 ± 28.4^{cC}	222.4 ± 15.7^{eB}	351.5 ± 19.2^{cC}	774.5 ± 89.7^{cdD}

Note: * means pore water concentrations used to calculate $K_{d\text{-}exposure}$ and BAF_{pw} were below detection limit (Table S6); values used were detection limit divided by the square root of 2. Values with different superscript letters are significantly different at $P \leq 0.05$. For a given nominal Cd concentration, values between different MP treatments are compared using uppercase letters and for MP treatments, values between different Cd concentrations are compared using lowercase letters.

Table S5a.5. Linear regression parameters for Log10 transformed BAF_s against Log10 transformed measured bulk soil and for Log10 transformed BAF_{pw} against pore water Cd concentrations respectively.

MP type	BAF_s - measured soil Cd		BAF_{pw} - pore water Cd	
	R^2	P	R^2	P
No MPs	0.95	< 0.001	0.89	< 0.001
PE0.1	0.97	< 0.001	0.97	< 0.001
PE0.3	0.96	< 0.001	0.93	< 0.001
PE3	0.97	< 0.001	0.96	< 0.001
PLA0.1	0.96	< 0.001	0.91	< 0.001
PLA0.3	0.96	< 0.001	0.87	< 0.001
PLA3	0.93	< 0.001	0.57	< 0.001

Table S5a.6. Mass of earthworm depurate recovered from each replicate at the end of earthworm exposure experiment. PE control and PLA control are earthworm exposure soils to which no MPs or Cd had been added.

	Mass of depurate (g)			
Treatments	Replicate a	Replicate b	Replicate c	Replicate d
PE control (no MPs)	0.27	0.24	0.08	0.23
PLA control (no MPs)	0.36	0.29	0.10	0.12
PE0.1	0.13	0.22	0.18	0.24
PE0.3	0.26	0.13	0.06	0.10
PE3	0.02	0.05	0.04	0.03
PLA0.1	0.32	0.19	0.12	0.20
PLA0.3	0.16	0.18	0.19	0.17
PLA3	0.13	0.15	0.07	0.31
Cd1	0.12	0.16	0.26	0.21
Cd5	0.21	0.13	0.18	0.15
Cd10	0.13	0.28	0.25	0.12
Cd15	0.16	0.18	0.31	0.28
Cd30	0.16	0.23	0.22	Dead
Cd50	0.15	0.28	0.18	0.33
Cd100	0.38	0.58	0.28	0.34
PE0.1Cd1	0.15	0.27	0.11	0.13
PE0.1Cd5	0.18	0.07	0.13	0.02
PE0.1Cd10	0.21	0.11	0.22	0.13
PE0.1Cd15	0.16	0.11	0.10	0.06
PE0.1Cd30	0.19	0.14	Dead	0.17
PE0.1Cd50	0.22	0.06	0.09	0.16
PE0.1Cd100	0.14	0.39	Dead	0.13
PE0.3d1	0.18	0.09	0.07	0.06
PE0.3Cd5	0.09	0.18	0.00	0.11
PE0.3Cd10	0.06	0.03	0.28	Dead
PE0.3Cd15	0.13	0.32	0.10	0.07

PE0.3Cd30	0.18	0.06	0.17	0.17
PE0.3Cd50	0.21	0.12	0.12	0.19
PE0.3Cd100	0.09	0.16	0.14	0.11
PE3d1	0.06	0.11	0.05	Dead
PE3Cd5	Dead	Dead	0.04	0.06
PE3Cd10	0.09	0.01	0.04	0.10
PE3Cd15	0.03	0.02	0.11	0.03
PE3Cd30	0.07	0.02	0.01	0.04
PE3Cd50	0.04	0.00	0.14	0.21
PE3Cd100	Dead	0.02	0.01	0.01
PLA0.1Cd1	0.21	0.02	0.32	0.04
PLA0.1Cd5	0.13	0.36	0.29	0.17
PLA0.1Cd10	0.15	0.31	0.10	0.12
PLA0.1Cd15	0.16	0.11	0.21	0.19
PLA0.1Cd30	0.04	0.29	0.28	0.20
PLA0.1Cd50	0.26	0.35	0.43	Dead
PLA0.1Cd100	0.24	0.07	0.28	0.19
PLA0.3d1	0.26	0.10	0.17	0.12
PLA0.3Cd5	0.02	0.27	0.31	0.33
PLA0.3Cd10	0.11	0.02	Dead	0.52
PLA0.3Cd15	0.18	0.19	0.19	0.13
PLA0.3Cd30	0.18	0.32	0.24	0.14
PLA0.3Cd50	0.01	0.27	0.19	Dead
PLA0.3Cd100	0.16	Dead	Dead	0.15
PLA3d1	0.04	0.01	0.18	0.10
PLA3Cd5	0.02	0.07	0.02	0.13
PLA3Cd10	0.04	0.07	0.13	0.00
PLA3Cd15	0.07	0.13	0.04	0.13
PLA3Cd30	0.04	0.11	0.04	0.14
PLA3Cd50	0.07	0.01	0.08	0.15
PLA3Cd100	0.21	0.07	0.05	0.01

Table S5a.7. 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 50 mg / L). The Linear regression model is expressed as $C_{MPs} = K_{d\text{-}exposure}C_{aq}$ where $K_{d\text{-}adsorption}$ is coefficient constant, L / kg. The Freundlich model is expressed as $C_{MPs} = K_F C_{aq}^{1/n}$ where K_F is 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 binding coefficient constant, mg / L and C_m is maximum concentration adsorbed to MPs, mg / kg. C_{MPs} is concentration adsorbed to MPs at equilibrium, mg / kg, C_{aq} is concentration in solution at equilibrium, mg / L.

MP type	Linear		
	$K_{d\text{-}adsorption}$	R^2	P
PE	2.913 (2.489-3.338)	0.78	< 0.001
PLA	7.221 (4.185-10.26)	0.68	< 0.001

MP type	Freundlich			
	LnK_F	1/n	R^2	P
PE	2.090 (1.887-2.293)	0.7091 (0.6159-0.8024)	0.99	< 0.001
PLA	3.994 (3.808-4.180)	0.4675 (0.3894-0.5455)	0.98	< 0.001

MP type	Langmuir			
	K_L	C_m	R^2	P
PE	1.044 (-0.9708-1.974)	18.96 (-32.31-7.33)	0.92	< 0.001
PLA	2.615 (0.8181-3.863)	106.0 (60.79-413.0)	0.98	< 0.001

Table S5a.8. Measured Cd concentrations of the bulk soil and mean Cd concentrations accounting for both the bulk soil and earthworm body concentration after 28 days exposure.

Treatments	Cd concentrations (mg / kg)	
	Bulk soil after exposure	Bulk soil + earthworm after exposure
Cd1	0.662	0.675
Cd5	3.237	3.258
Cd10	6.038	6.066
Cd15	11.06	11.11
Cd30	21.45	21.47
Cd50	38.55	38.60
Cd100	71.40	71.46
PE0.1Cd1	0.694	0.704
PE0.1Cd5	3.737	3.755
PE0.1Cd10	7.004	7.028
PE0.1Cd15	11.75	11.79
PE0.1Cd30	22.83	22.88
PE0.1Cd50	41.29	41.33
PE0.1Cd100	72.46	72.51
PE0.3Cd1	0.672	0.684
PE0.3Cd5	3.973	3.993
PE0.3Cd10	8.040	8.075
PE0.3Cd15	12.07	12.08
PE0.3Cd30	24.05	24.09
PE0.3Cd50	41.97	42.02
PE0.3Cd100	79.95	80.02
PE3Cd1	0.806	0.813
PE3Cd5	4.082	4.097
PE3Cd10	8.479	8.503
PE3Cd15	12.57	12.59
PE3Cd30	24.41	24.43
PE3Cd50	42.97	43.01
PE3Cd100	81.50	81.55
PLA0.1Cd1	0.694	0.705
PLA0.1Cd5	3.768	3.784
PLA0.1Cd10	7.878	7.905
PLA0.1Cd15	12.01	12.03
PLA0.1Cd30	23.40	23.43
PLA0.1Cd50	41.55	41.59
PLA0.1Cd100	76.77	76.82
PLA0.3Cd1	0.806	0.815
PLA0.3Cd5	3.981	3.993
PLA0.3Cd10	8.162	8.183
PLA0.3Cd15	12.55	12.57

PLA0.3Cd30	24.13	24.16
PLA0.3Cd50	42.74	42.77
PLA0.3Cd100	80.04	80.10
PLA3Cd1	0.851	0.858
PLA3Cd5	4.133	4.148
PLA3Cd10	8.675	8.688
PLA3Cd15	13.21	13.23
PLA3Cd30	24.48	24.51
PLA3Cd50	43.40	43.45
PLA3Cd100	88.16	88.21

Table S5a.9. Independent t test results for difference between measured Cd concentration of the bulk soil and the average concentration accounting for both the bulk soil and earthworm tissue concentrations and mass.

	Measured Cd concentrations in bulk soil (mg / kg)				Measured Cd concentrations accounting for the bulk soil and earthworm tissues(mg / kg)				t-value	P- value
	mean	standard deviation	variance	number	mean	standard deviation	variance	number		
Cd 1	0.741	0.077	0.006	7	0.751	0.075	0.006	7	0.241	> 0.05
Cd 5	3.844	0.306	0.094	7	3.861	0.304	0.092	7	0.103	> 0.05
Cd 10	7.754	0.926	0.857	7	7.778	0.924	0.853	7	0.050	> 0.05
Cd 15	12.175	0.687	0.472	7	12.201	0.678	0.459	7	0.071	> 0.05
Cd 30	23.536	1.090	1.189	7	23.565	1.089	1.186	7	0.050	> 0.05
Cd 50	41.783	1.621	2.628	7	41.823	1.617	2.615	7	0.047	> 0.05
Cd 100	78.612	5.726	32.788	7	78.667	5.724	32.769	7	0.018	> 0.05

Table S5a.10. The total mass of Cd in the pore water and bulk soil of the MP-free treatments.

Nominal Cd concentration in soil (mg / kg)	Cd concentration in pore water (mg / L)	Cd mass in pore water (mg)	Cd concentration in bulk soil (mg / kg)	Cd mass in bulk soil (mg)
1	0.010	0.001	0.662	0.199
5	0.012	0.001	3.237	0.971
10	0.025	0.003	6.038	1.811
15	0.036	0.004	11.064	3.319
30	0.059	0.007	21.450	6.435
50	0.096	0.011	38.549	11.565
100	0.416	0.047	71.401	21.420

Table S5a.11. Distribution coefficients ($K_{d\text{-}exposure}$) of Cd from previous studies.

$K_{d\text{-}exposure}$ (L / kg)	References
0.44 - 192000	Sauvé et al., 2000
116.3	Yan et al., 2008
375	Karapinar and Donat, 2009
402	Lukman, et. al., 2013
190 - 4590	Coulombe et al., 2023
59.55 - 4763	This study

Table S5a.12. Isotherm parameters for Cd adsorption to MPs from previous studies.

Langmuir - C_m (mg / kg)	Freundlich - LnK_F (L / mg)$^{1/n}$	MP type	References
30.5	4.459	HDPE	Wang, et al., 2019
247	8.9	PET	Zhou et al., 2020
323, 314	4.762, 4.234	PP, PS	Li et al., 2022
192, 254	3.569, 3.239	PS, PET	Wang et al., 2022
151.8	4.487	PLA	Sun et al., 2022
23.2, 126.0	2.115, 4.041	PE, PLA	This study

Table S5a.13. BAF_s values, earthworm species used and organic matter values from previous studies.

BAF_s value	Earthworm species	Organic matter	References
3.60 - 28.94	*E. fetida*	2 %	Ge et al., 2023
4.38 - 11.5	*A. robustu, A. corticis*	16.3 ± 0.32 g / kg	Xiao et al., 2020
10.6 - 18.8	*M. californica*, *A. homochaetus*, *A. pecteniferus*, and *A. heterochaetus*.	15.78 - 25.17 g / kg	Wang et al., 2018
7.1 - 59.7	*N. caliginosus, A. rosea*	1 - 5.3 %	Nannoni et al., 2011
0.2444 - 11.71	*L. terrestris*	12.67 ± 0.17 %	This study

Table S5a.14. Linear regression parameters of Log10 transformed measured Cd concentration in earthworm body (mg / kg dry weight) against Log10 transformed measured total Cd concentration in bulk soil (mg / kg dry soil). 95 % confidence intervals are given in brackets.

MP type	a	b	R^2	P
No MPs	0.342 (0.267-0.416)	0.779 (0.692-0.867)	0.96	< 0.001
PE0.1	0.341 (0.267-0.415)	0.756 (0.667-0.844)	0.96	< 0.001
PE0.3	0.350 (0.253-0.448)	0.689 (0.569-0.808)	0.93	< 0.001
PE3	0.378 (0.290-0.465)	0.604 (0.496-0.712)	0.95	< 0.001
PLA0.1	0.359 (0.236-0.481)	0.664 (0.515-0.813)	0.90	< 0.001
PLA0.3	0.358 (0.235-0.481)	0.627 (0.476-0.778)	0.90	< 0.001
PLA3	0.452 (0.351-0.552)	0.447 (0.322-0.573)	0.96	< 0.001

Table S5a.15. Cd LC_{50} values from previous studies.

Earthworm species	LC_{50} (mg / kg)	Nominal Cd concentration (mg / kg)	Soil organic matter (%)	Exposure time	References
E. fetida	1843	/	Artificial soil	14 days	Neuhauser et al., 1985
E. andrei	> 1000	0 - 1000	1.7	14 days	Van Gestel and van Dis, 1988
A. caliginosa	540	/	/	56 days	Khalil et al., 1996
A. caliginosa	344	0 - 1000	5.7	28 days	Qiu et al., 2011

3 Chapter 5 An investigation into the impacts of MPs on the bioaccumulation of cadmium in earthworms *Lumbricus terrestris* supporting information b – statistical results

Table S5b.1 - S5b.15:

List of contnets:

Table S5b.1. 2-way ANOVA on ranked measured Cd in pore water after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
plastic N/Y	1	73545.3	73545.3	17.356	0.000
Cd	7	400960.2	57280.03	94.623	0.000
plastic N/Y x Cd	7	27806.93	3972.418	6.562	0.476
Residual	209	124460.6	595.505		
Total	224	949193	4237.469		

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic

Comparison	Diff of Means	t	P	P<0.050
Y vs. N	50.438	11.113	<0.001	Yes

Power of performed test with alpha = 0.0500: for plastic N/Y : 1.000

Table S5b.2. 3-way ANOVA on ranked measured Cd in pore water after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
MP type	1	1927.628	1927.628	0.644	0.422
MP concentration	2	45015.85	22507.92	15.043	0.001
Cd	7	464059	66294.15	155.075	0.000
MP type x MP concentration	2	1973.216	986.608	0.659	0.719
MP type x Cd	7	17730.59	2532.941	5.925	0.549
MP concentration x Cd	14	13549.38	967.812	4.528	0.991
MP type x MP concentration x C	14	3888.489	277.749	1.299	1.000
Residual	141	7252.292	51.435		
Total	188	562586.5	2992.481		

Table S5b.3. Game-Howell post hoc test results for measured Cd in pore water after 28 days exposure.

*. The mean difference is significant at the 0.05 level.

Comparisons for factor: plastic within 1

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	.0012393*	0.000	0.001
	PE3	.0011518*	0.000	0.028
	PLA0.1	(0.002)	0.000	0.059
	PLA0.3	-.0017650*	0.000	0.000

	PLA3	0.000	0.000	0.182
PE0.3	PE0.1	-.0012393*	0.000	0.001
	PE3	(0.000)	0.000	0.997
	PLA0.1	-.0031109*	0.000	0.015
	PLA0.3	-.0030043*	0.000	0.000
	PLA3	(0.001)	0.000	0.058
PE3	PE0.1	-.0011518*	0.000	0.028
	PE0.3	0.000	0.000	0.997
	PLA0.1	-.0030234*	0.000	0.007
	PLA0.3	-.0029168*	0.000	0.001
	PLA3	(0.001)	0.000	0.184
PLA0.1	PE0.1	0.002	0.000	0.059
	PE0.3	.0031109*	0.000	0.015
	PE3	.0030234*	0.000	0.007
	PLA0.3	0.000	0.000	1.000
	PLA3	.0023537*	0.000	0.022
PLA0.3	PE0.1	.0017650*	0.000	0.000
	PE0.3	.0030043*	0.000	0.000
	PE3	.0029168*	0.000	0.001
	PLA0.1	(0.000)	0.000	1.000
	PLA3	.0022471*	0.000	0.000
PLA3	PE0.1	(0.000)	0.000	0.182
	PE0.3	0.001	0.000	0.058
	PE3	0.001	0.000	0.184
	PLA0.1	-.0023537*	0.000	0.022
	PLA0.3	-.0022471*	0.000	0.000

Comparisons for factor: plastic within 5

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	0.001	0.001	0.838
	PE3	.0056976*	0.000	0.000
	PLA0.1	.0038817*	0.000	0.001
	PLA0.3	.0040109*	0.000	0.000
	PLA3	.0060820*	0.000	0.000
PE0.3	PE0.1	(0.001)	0.001	0.838
	PE3	.0050100*	0.001	0.005
	PLA0.1	.0031941*	0.001	0.027
	PLA0.3	.0033233*	0.001	0.037
	PLA3	.0053944*	0.001	0.009
PE3	PE0.1	-.0056976*	0.000	0.000

	PE0.3	-.0050100*	0.001	0.005
	PLA0.1	-.0018159*	0.000	0.027
	PLA0.3	-.0016866*	0.000	0.022
	PLA3	0.000	0.000	0.743
PLA0.1	PE0.1	-.0038817*	0.000	0.001
	PE0.3	-.0031941*	0.001	0.027
	PE3	.0018159*	0.000	0.027
	PLA0.3	0.000	0.000	0.999
	PLA3	.0022003*	0.000	0.019
PLA0.3	PE0.1	-.0040109*	0.000	0.000
	PE0.3	-.0033233*	0.001	0.037
	PE3	.0016866*	0.000	0.022
	PLA0.1	(0.000)	0.000	0.999
	PLA3	.0020711*	0.000	0.000
PLA3	PE0.1	-.0060820*	0.000	0.000
	PE0.3	-.0053944*	0.001	0.009
	PE3	(0.000)	0.000	0.743
	PLA0.1	-.0022003*	0.000	0.019
	PLA0.3	-.0020711*	0.000	0.000

Comparisons for factor: plastic within 10

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	0.002	0.000	0.087
	PE3	.0052831*	0.000	0.006
	PLA0.1	0.002	0.000	0.124
	PLA0.3	.0046948*	0.001	0.002
	PLA3	.0070504*	0.001	0.000
PE0.3	PE0.1	(0.002)	0.000	0.087
	PE3	.0032425*	0.000	0.001
	PLA0.1	(0.000)	0.000	0.944
	PLA0.3	.0026543*	0.000	0.024
	PLA3	.0050099*	0.000	0.000
PE3	PE0.1	-.0052831*	0.000	0.006
	PE0.3	-.0032425*	0.000	0.001
	PLA0.1	-.0034734*	0.000	0.000
	PLA0.3	(0.001)	0.000	0.759
	PLA3	.0017674*	0.000	0.038
PLA0.1	PE0.1	(0.002)	0.000	0.124
	PE0.3	0.000	0.000	0.944

	PE3	.0034734*	0.000	0.000
	PLA0.3	.0028851*	0.000	0.018
	PLA3	.0052407*	0.000	0.000
PLA0.3	PE0.1	-.0046948*	0.001	0.002
	PE0.3	-.0026543*	0.000	0.024
	PE3	0.001	0.000	0.759
	PLA0.1	-.0028851*	0.000	0.018
	PLA3	.0023556*	0.000	0.032
PLA3	PE0.1	-.0070504*	0.001	0.000
	PE0.3	-.0050099*	0.000	0.000
	PE3	-.0017674*	0.000	0.038
	PLA0.1	-.0052407*	0.000	0.000
	PLA0.3	-.0023556*	0.000	0.032

Comparisons for factor: plastic within 15

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	0.004	0.001	0.213
	PE3	.0080099*	0.001	0.043
	PLA0.1	0.003	0.001	0.467
	PLA0.3	0.006	0.002	0.077
	PLA3	.0105116*	0.001	0.015
PE0.3	PE0.1	(0.004)	0.001	0.213
	PE3	.0037286*	0.001	0.022
	PLA0.1	(0.001)	0.001	0.708
	PLA0.3	0.002	0.001	0.629
	PLA3	.0062303*	0.001	0.001
PE3	PE0.1	-.0080099*	0.001	0.043
	PE0.3	-.0037286*	0.001	0.022
	PLA0.1	-.0049908*	0.001	0.015
	PLA0.3	(0.002)	0.001	0.606
	PLA3	.0025017*	0.000	0.027
PLA0.1	PE0.1	(0.003)	0.001	0.467
	PE0.3	0.001	0.001	0.708
	PE3	.0049908*	0.001	0.015
	PLA0.3	0.003	0.001	0.245
	PLA3	.0074925*	0.001	0.001
PLA0.3	PE0.1	(0.006)	0.002	0.077
	PE0.3	(0.002)	0.001	0.629
	PE3	0.002	0.001	0.606
	PLA0.1	(0.003)	0.001	0.245

	PLA3	0.004	0.001	0.095
PLA3	PE0.1	-.0105116*	0.001	0.015
	PE0.3	-.0062303*	0.001	0.001
	PE3	-.0025017*	0.000	0.027
	PLA0.1	-.0074925*	0.001	0.001
	PLA0.3	(0.004)	0.001	0.095

Comparisons for factor: plastic within 30

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	0.003	0.001	0.275
	PE3	0.005	0.001	0.085
	PLA0.1	0.001	0.001	0.990
	PLA0.3	0.004	0.001	0.172
	PLA3	.0087768*	0.001	0.019
PE0.3	PE0.1	(0.003)	0.001	0.275
	PE3	0.002	0.000	0.074
	PLA0.1	(0.002)	0.001	0.074
	PLA0.3	0.001	0.000	0.611
	PLA3	.0055846*	0.000	0.002
PE3	PE0.1	(0.005)	0.001	0.085
	PE0.3	(0.002)	0.000	0.074
	PLA0.1	-.0044628*	0.000	0.013
	PLA0.3	-.0012860*	0.000	0.001
	PLA3	.0035542*	0.000	0.001
PLA0.1	PE0.1	(0.001)	0.001	0.990
	PE0.3	0.002	0.001	0.074
	PE3	.0044628*	0.000	0.013
	PLA0.3	.0031769*	0.000	0.038
	PLA3	.0080170*	0.001	0.001
PLA0.3	PE0.1	(0.004)	0.001	0.172
	PE0.3	(0.001)	0.000	0.611
	PE3	.0012860*	0.000	0.001
	PLA0.1	-.0031769*	0.000	0.038
	PLA3	.0048401*	0.000	0.002
PLA3	PE0.1	-.0087768*	0.001	0.019
	PE0.3	-.0055846*	0.000	0.002
	PE3	-.0035542*	0.000	0.001
	PLA0.1	-.0080170*	0.001	0.001
	PLA0.3	-.0048401*	0.000	0.002

Comparisons for factor: plastic within 50

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	.0550921*	0.001	0.000
	PE3	-.1327469*	0.007	0.002
	PLA0.1	.0393648*	0.002	0.000
	PLA0.3	.0643151*	0.002	0.000
	PLA3	.0841116*	0.001	0.000
PE0.3	PE0.1	-.0550921*	0.001	0.000
	PE3	-.1878390*	0.007	0.001
	PLA0.1	-.0157273*	0.002	0.007
	PLA0.3	0.009	0.002	0.142
	PLA3	.0290196*	0.001	0.000
PE3	PE0.1	.1327469*	0.007	0.002
	PE0.3	.1878390*	0.007	0.001
	PLA0.1	.1721117*	0.007	0.001
	PLA0.3	.1970620*	0.007	0.000
	PLA3	.2168586*	0.007	0.000
PLA0.1	PE0.1	-.0393648*	0.002	0.000
	PE0.3	.0157273*	0.002	0.007
	PE3	-.1721117*	0.007	0.001
	PLA0.3	.0249503*	0.003	0.002
	PLA3	.0447469*	0.002	0.000
PLA0.3	PE0.1	-.0643151*	0.002	0.000
	PE0.3	(0.009)	0.002	0.142
	PE3	-.1970620*	0.007	0.000
	PLA0.1	-.0249503*	0.003	0.002
	PLA3	.0197965*	0.002	0.013
PLA3	PE0.1	-.0841116*	0.001	0.000
	PE0.3	-.0290196*	0.001	0.000
	PE3	-.2168586*	0.007	0.000
	PLA0.1	-.0447469*	0.002	0.000
	PLA0.3	-.0197965*	0.002	0.013

Comparisons for factor: plastic within 100

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	.1635327*	0.010	0.003
	PE3	.1714884*	0.010	0.002
	PLA0.1	.1198649*	0.014	0.001
	PLA0.3	-.4446034*	0.018	0.000

	PLA3	.2007226*	0.010	0.001
PE0.3	PE0.1	-.1635327*	0.010	0.003
	PE3	0.008	0.002	0.164
	PLA0.1	(0.044)	0.010	0.101
	PLA0.3	-.6081361*	0.016	0.000
	PLA3	.0371899*	0.001	0.000
PE3	PE0.1	-.1714884*	0.010	0.002
	PE0.3	(0.008)	0.002	0.164
	PLA0.1	(0.052)	0.010	0.059
	PLA0.3	-.6160918*	0.016	0.000
	PLA3	.0292342*	0.002	0.003
PLA0.1	PE0.1	-.1198649*	0.014	0.001
	PE0.3	0.044	0.010	0.101
	PE3	0.052	0.010	0.059
	PLA0.3	-.5644684*	0.018	0.000
	PLA3	.0808577*	0.010	0.019
PLA0.3	PE0.1	.4446034*	0.018	0.000
	PE0.3	.6081361*	0.016	0.000
	PE3	.6160918*	0.016	0.000
	PLA0.1	.5644684*	0.018	0.000
	PLA3	.6453260*	0.016	0.000
PLA3	PE0.1	-.2007226*	0.010	0.001
	PE0.3	-.0371899*	0.001	0.000
	PE3	-.0292342*	0.002	0.003
	PLA0.1	-.0808577*	0.010	0.019
	PLA0.3	-.6453260*	0.016	0.000

Table S5b.4. 2-way ANOVA on ranked K_d after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
plastic N/Y	1	173568.917	173568.917	55.628	0.000
Cd	6	44772.479	7462.08	14.349	0.026
plastic N/Y x Cd	6	14527.595	2421.266	4.656	0.589
Residual	179	247919.716	1385.026		
Total	192	599072	3120.167		

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic

Comparison	Diff of Means	t	P	P<0.050
N vs. Y	85.155	11.195	<0.001	Yes

Power of performed test with alpha = 0.0500: for plastic N/Y : 1.000

Table S5b.5. 3-way ANOVA on ranked K_d after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
MP type	1	14722.917	14722.917	6.450	0.011
MP concentration	2	160763.481	80381.741	70.433	0.000
Cd	6	149349.42	24891.57	65.432	0.000
MP type x MP concentration	2	242.623	121.311	0.106	0.948
MP type x Cd	6	12562.339	2093.723	5.504	0.481
MP concentration x Cd	12	28174.69	2347.891	12.344	0.418
MP type x MP concentration x C	12	4448.635	370.72	1.949	0.999
Residual	123	8211.167	66.757		
Total	164	374330	2282.5		

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: MP type

Comparison	Diff of Means	t	P	P<0.050
PE vs. PLA	18.944	14.851	<0.001	Yes

Power of performed test with alpha = 0.0500: for MP type : 1.000

Table S5b.6. Game-Howell post hoc test results for K_d after 28 days exposure.

*. The mean difference is significant at the 0.05 level.

Comparisons for factor: plastic within 1

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-329.3495750*	14.976	0.000
	PE3	(437.742)	88.802	0.071
	PLA0.1	132.5505972*	16.457	0.003
	PLA0.3	103.6554962*	9.903	0.002
	PLA3	-171.3562891*	32.053	0.044
PE0.3	PE0.1	329.3495750*	14.976	0.000
	PE3	(108.392)	89.158	0.812
	PLA0.1	461.9001722*	18.279	0.000
	PLA0.3	433.0050712*	12.702	0.000
	PLA3	157.993	33.025	0.051
PE3	PE0.1	437.742	88.802	0.071
	PE0.3	108.392	89.158	0.812
	PLA0.1	570.2921101*	89.419	0.033
	PLA0.3	541.3970091*	88.447	0.040
	PLA3	266.385	93.554	0.233
PLA0.1	PE0.1	-132.5505972*	16.457	0.003
	PE0.3	-461.9001722*	18.279	0.000
	PE3	-570.2921101*	89.419	0.033
	PLA0.3	(28.895)	14.419	0.473
	PLA3	-303.9068864*	33.723	0.004
PLA0.3	PE0.1	-103.6554962*	9.903	0.002
	PE0.3	-433.0050712*	12.702	0.000
	PE3	-541.3970091*	88.447	0.040
	PLA0.1	28.895	14.419	0.473
	PLA3	-275.0117854*	31.056	0.013
PLA3	PE0.1	171.3562891*	32.053	0.044
	PE0.3	(157.993)	33.025	0.051
	PE3	(266.385)	93.554	0.233
	PLA0.1	303.9068864*	33.723	0.004
	PLA0.3	275.0117854*	31.056	0.013

Comparisons for factor: plastic within 5

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	(86.193)	42.344	0.466
	PE3	-1465.8869313*	217.423	0.031
	PLA0.1	-479.9930175*	62.227	0.018
	PLA0.3	-555.6517784*	28.891	0.000
	PLA3	-1832.4428860*	95.904	0.001
PE0.3	PE0.1	86.193	42.344	0.466
	PE3	-1379.6934773*	221.020	0.033
	PLA0.1	-393.7995635*	73.820	0.018

	PLA0.3	-469.4583244*	49.110	0.001
	PLA3	-1746.2494321*	103.801	0.000
PE3	PE0.1	1465.8869313*	217.423	0.031
	PE0.3	1379.6934773*	221.020	0.033
	PLA0.1	985.894	225.674	0.081
	PLA0.3	910.235	218.841	0.108
	PLA3	(366.556)	237.180	0.662
PLA0.1	PE0.1	479.9930175*	62.227	0.018
	PE0.3	393.7995635*	73.820	0.018
	PE3	(985.894)	225.674	0.081
	PLA0.3	(75.659)	67.015	0.850
	PLA3	-1352.4498685*	113.375	0.000
PLA0.3	PE0.1	555.6517784*	28.891	0.000
	PE0.3	469.4583245*	49.110	0.001
	PE3	(910.235)	218.841	0.108
	PLA0.1	75.659	67.015	0.850
	PLA3	-1276.7911076*	99.078	0.003
PLA3	PE0.1	1832.4428860*	95.904	0.001
	PE0.3	1746.2494321*	103.801	0.000
	PE3	366.556	237.180	0.662
	PLA0.1	1352.4498685*	113.375	0.000
	PLA0.3	1276.7911076*	99.078	0.003

Comparisons for factor: plastic within 10

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-275.4628030*	32.432	0.002
	PE3	-928.3585045*	32.887	0.000
	PLA0.1	-232.4673749*	31.902	0.005
	PLA0.3	-735.9345663*	98.352	0.015
	PLA3	-1817.3013895*	189.994	0.010
PE0.3	PE0.1	275.4628030*	32.432	0.002
	PE3	-652.8957015*	26.339	0.000
	PLA0.1	42.995	25.098	0.569
	PLA0.3	(460.472)	96.360	0.071
	PLA3	-1541.8385865*	188.971	0.017
PE3	PE0.1	928.3585045*	32.887	0.000
	PE0.3	652.8957015*	26.339	0.000
	PLA0.1	695.8911296*	25.683	0.000
	PLA0.3	192.424	96.514	0.485
	PLA3	(888.943)	189.050	0.080
PLA0.1	PE0.1	232.4673750*	31.902	0.005
	PE0.3	(42.995)	25.098	0.569
	PE3	-695.8911296*	25.683	0.000

	PLA0.3	(503.467)	96.182	0.055
	PLA3	-1584.8340146*	188.881	0.016
PLA0.3	PE0.1	735.9345663*	98.352	0.015
	PE0.3	460.472	96.360	0.071
	PE3	(192.424)	96.514	0.485
	PLA0.1	503.467	96.182	0.055
	PLA3	-1081.3668232*	210.550	0.031
PLA3	PE0.1	1817.3013895*	189.994	0.010
	PE0.3	1541.8385865*	188.971	0.017
	PE3	888.943	189.050	0.080
	PLA0.1	1584.8340146*	188.881	0.016
	PLA0.3	1081.3668233*	210.550	0.031

Comparisons for factor: plastic within 15

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	(402.056)	114.448	0.088
	PE3	-1296.7046994*	113.337	0.001
	PLA0.1	(248.051)	111.023	0.350
	PLA0.3	(854.754)	232.705	0.111
	PLA3	-3277.8214293*	414.238	0.015
PE0.3	PE0.1	402.056	114.448	0.088
	PE3	-894.6489011*	98.028	0.002
	PLA0.1	154.005	95.343	0.618
	PLA0.3	(452.698)	225.646	0.471
	PLA3	-2875.7656310*	410.314	0.024
PE3	PE0.1	1296.7046994*	113.337	0.001
	PE0.3	894.6489011*	98.028	0.002
	PLA0.1	1048.6538076*	94.007	0.001
	PLA0.3	441.951	225.084	0.488
	PLA3	(1981.117)	410.006	0.070
PLA0.1	PE0.1	248.051	111.023	0.350
	PE0.3	(154.005)	95.343	0.618
	PE3	-1048.6538076*	94.007	0.001
	PLA0.3	(606.703)	223.928	0.268
	PLA3	-3029.7705375*	409.372	0.021
PLA0.3	PE0.1	854.754	232.705	0.111
	PE0.3	452.698	225.646	0.471
	PE3	(441.951)	225.084	0.488
	PLA0.1	606.703	223.928	0.268
	PLA3	-2423.0677362*	457.615	0.026
PLA3	PE0.1	3277.8214293*	414.238	0.015
	PE0.3	2875.7656310*	410.314	0.024
	PE3	1981.117	410.006	0.070

	PLA0.1	3029.7705375*	409.372	0.021
	PLA0.3	2423.0677363*	457.615	0.026

Comparisons for factor: plastic within 30

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	(388.319)	106.038	0.091
	PE3	-719.2061222*	93.034	0.018
	PLA0.1	(85.162)	101.977	0.946
	PLA0.3	(491.593)	91.831	0.057
	PLA3	-1638.1875664*	109.465	0.000
PE0.3	PE0.1	388.319	106.038	0.091
	PE3	-330.8874627*	56.253	0.031
	PLA0.1	303.1563542*	70.060	0.037
	PLA0.3	(103.274)	54.242	0.521
	PLA3	-1249.8689069*	80.571	0.000
PE3	PE0.1	719.2061222*	93.034	0.018
	PE0.3	330.8874627*	56.253	0.031
	PLA0.1	634.0438170*	48.163	0.001
	PLA0.3	227.6130577*	18.797	0.001
	PLA3	-918.9814442*	62.473	0.010
PLA0.1	PE0.1	85.162	101.977	0.946
	PE0.3	-303.1563542*	70.060	0.037
	PE3	-634.0438170*	48.163	0.001
	PLA0.3	-406.4307592*	45.797	0.012
	PLA3	-1553.0252612*	75.146	0.000
PLA0.3	PE0.1	491.593	91.831	0.057
	PE0.3	103.274	54.242	0.521
	PE3	-227.6130577*	18.797	0.001
	PLA0.1	406.4307592*	45.797	0.012
	PLA3	-1146.5945019*	60.669	0.009
PLA3	PE0.1	1638.1875664*	109.465	0.000
	PE0.3	1249.8689069*	80.571	0.000
	PE3	918.9814442*	62.473	0.010
	PLA0.1	1553.0252612*	75.146	0.000
	PLA0.3	1146.5945019*	60.669	0.009

Comparisons for factor: plastic within 50

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-608.9100006*	9.207	0.000
	PE3	-1456.5869809*	61.633	0.001
	PLA0.1	-310.2465184*	19.790	0.003
	PLA0.3	-966.4060122*	106.361	0.013
	PLA3	-3448.5164396*	251.446	0.019
PE0.3	PE0.1	608.9100007*	9.207	0.000

	PE3	-847.6769802*	62.263	0.003
	PLA0.1	298.6634823*	21.671	0.001
	PLA0.3	(357.496)	106.727	0.185
	PLA3	-2839.6064389*	251.601	0.027
PE3	PE0.1	1456.5869809*	61.633	0.001
	PE0.3	847.6769802*	62.263	0.003
	PLA0.1	1146.3404625*	64.680	0.001
	PLA0.3	490.181	122.901	0.068
	PLA3	-1991.9294587*	258.877	0.045
PLA0.1	PE0.1	310.2465184*	19.790	0.003
	PE0.3	-298.6634823*	21.671	0.001
	PE3	-1146.3404625*	64.680	0.001
	PLA0.3	-656.1594938*	108.155	0.036
	PLA3	-3138.2699212*	252.210	0.022
PLA0.3	PE0.1	966.4060122*	106.361	0.013
	PE0.3	357.496	106.727	0.185
	PE3	(490.181)	122.901	0.068
	PLA0.1	656.1594938*	108.155	0.036
	PLA3	-2482.1104274*	273.004	0.018
PLA3	PE0.1	3448.5164396*	251.446	0.019
	PE0.3	2839.6064389*	251.601	0.027
	PE3	1991.9294587*	258.877	0.045
	PLA0.1	3138.2699212*	252.210	0.022
	PLA0.3	2482.1104274*	273.004	0.018

Comparisons for factor: plastic within 100

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-855.0570783*	18.695	0.000
	PE3	-1039.5395132*	51.198	0.001
	PLA0.1	-384.9489874*	57.261	0.023
	PLA0.3	-870.8203485*	30.930	0.000
	PLA3	-2514.0864077*	57.208	0.000
PE0.3	PE0.1	855.0570783*	18.695	0.000
	PE3	(184.482)	50.806	0.138
	PLA0.1	470.1080908*	56.910	0.013
	PLA0.3	(15.763)	30.277	0.992
	PLA3	-1659.0293295*	56.857	0.000
PE3	PE0.1	1039.5395133*	51.198	0.001
	PE0.3	184.482	50.806	0.138
	PLA0.1	654.5905258*	74.233	0.001
	PLA0.3	168.719	56.466	0.176
	PLA3	-1474.5468945*	74.192	0.000
PLA0.1	PE0.1	384.9489875*	57.261	0.023
	PE0.3	-470.1080908*	56.910	0.013

	PE3	-654.5905258*	74.233	0.001
	PLA0.3	-485.8713610*	62.016	0.006
	PLA3	-2129.1374203*	78.498	0.000
PLA0.3	PE0.1	870.8203485*	30.930	0.000
	PE0.3	15.763	30.277	0.992
	PE3	(168.719)	56.466	0.176
	PLA0.1	485.8713610*	62.016	0.006
	PLA3	-1643.2660593*	61.967	0.000
PLA3	PE0.1	2514.0864078*	57.208	0.000
	PE0.3	1659.0293295*	56.857	0.000
	PE3	1474.5468945*	74.192	0.000
	PLA0.1	2129.1374203*	78.498	0.000
	PLA0.3	1643.2660593*	61.967	0.000

Table S5b.7. 2-way ANOVA on ranked earthworm mortality after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test					
Source of Variation	DF	SS	MS	H	P
plastic	1	367.811	367.811	0.524	0.469
Cd	7	3169.433	452.776	4.516	0.719
plastic N Y x Cd	7	3859.016	551.288	5.499	0.599
Residual	212	149454	704.972		
Total	227	159315	701.828		

Table S5b.8. 2-way ANOVA on ranked earthworm weight change before and after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test					
Source of Variation	DF	SS	MS	H	P
plastic	1	1045.117	1045.117	0.272	0.602
Cd	7	39200.21	5600.03	1.459	0.984
plastic x Cd	7	32503.74	4643.391	1.21	0.991
Residual	199	763835.1	3838.367		
Total	214	828113	3869.687		

Table S5b.9. 2-way ANOVA results for log transformed measured Cd in earthworm body after 28 days exposure.

Normality Test (Shapiro-Wilk): Passed (P = 0.500)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.109)

Source of Variation	DF	SS	MS	F	P
MP	6	0.579	0.0965	15.378	<0.001
Cd	7	13.327	1.904	303.428	<0.001
MP x Cd	42	0.26	0.00619	0.987	0.503
Residual	140	0.878	0.00627		
Total	195	15.899	0.0815		

The difference in the mean values among the different levels of MP is greater than would be expected by chance after allowing for effects of differences in Cd. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Cd is greater than would be expected by chance after allowing for effects of differences in MP. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of MP does not depend on what level of Cd is present. There is not a statistically significant interaction between MP and Cd. (P = 0.503)

Power of performed test with alpha = 0.0500: for MP : 1.000

Power of performed test with alpha = 0.0500: for Cd : 1.000

Power of performed test with alpha = 0.0500: for MP x Cd : 0.0500

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):

Overall significance level = 0.05

Comparisons for factor: MP

Comparison	Diff of Means	t	P	P<0.050
NONE vs. PLA3	0.168	8.077	<0.001	Yes
PE0.1 vs. PLA3	0.168	7.458	<0.001	Yes
PE0.3 vs. PLA3	0.11	5.233	<0.001	Yes
NONE vs. PLA0.3	0.102	4.893	<0.001	Yes
NONE vs. PE3	0.0967	4.751	<0.001	Yes
PLA0.1 vs. PLA3	0.106	4.667	<0.001	Yes
PE0.1 vs. PLA0.3	0.103	4.529	<0.001	Yes
PE0.1 vs. PE3	0.097	4.379	<0.001	Yes
PE3 vs. PLA3	0.0709	3.315	0.015	Yes

PLA0.3 vs. PLA3	0.0651	2.966	0.042	Yes
NONE vs. PE0.3	0.0577	2.892	0.048	Yes
NONE vs. PLA0.1	0.0617	2.843	0.05	No
PE0.1 vs. PE0.3	0.0579	2.662	0.075	No
PE0.1 vs. PLA0.1	0.062	2.649	0.075	No
PE0.3 vs. PLA0.3	0.0448	2.113	0.228	No
PE0.3 vs. PE3	0.039	1.892	0.312	No
PLA0.1 vs. PLA0.3	0.0408	1.783	0.329	No
PLA0.1 vs. PE3	0.035	1.566	0.399	No
PE3 vs. PLA0.3	0.0058	0.269	0.991	No
PE0.3 vs. PLA0.1	0.00403	0.184	0.991	No
PE0.1 vs. NONE	0.000248	0.0115	0.991	No

Comparisons for factor: Cd

Comparison	Diff of Means	t	P	P<0.050
100.000 vs. 0.000	0.835	35.219	<0.001	Yes
100.000 vs. 1.000	0.752	32.887	<0.001	Yes
50.000 vs. 0.000	0.674	29.272	<0.001	Yes
50.000 vs. 1.000	0.591	26.672	<0.001	Yes
100.000 vs. 5.000	0.587	24.899	<0.001	Yes
30.000 vs. 0.000	0.552	23.055	<0.001	Yes
30.000 vs. 1.000	0.469	20.291	<0.001	Yes
100.000 vs. 10.000	0.469	19.715	<0.001	Yes
50.000 vs. 5.000	0.425	18.605	<0.001	Yes
100.000 vs. 15.000	0.429	18.405	<0.001	Yes
15.000 vs. 0.000	0.406	17.624	<0.001	Yes
10.000 vs. 0.000	0.366	15.599	<0.001	Yes
15.000 vs. 1.000	0.323	14.563	<0.001	Yes
50.000 vs. 10.000	0.308	13.324	<0.001	Yes
30.000 vs. 5.000	0.303	12.749	<0.001	Yes
10.000 vs. 1.000	0.283	12.512	<0.001	Yes
50.000 vs. 15.000	0.268	11.854	<0.001	Yes
100.000 vs. 30.000	0.283	11.695	<0.001	Yes
5.000 vs. 0.000	0.249	10.694	<0.001	Yes
30.000 vs. 10.000	0.186	7.728	<0.001	Yes
5.000 vs. 1.000	0.166	7.39	<0.001	Yes
100.000 vs. 50.000	0.161	6.905	<0.001	Yes
15.000 vs. 5.000	0.157	6.872	<0.001	Yes
30.000 vs. 15.000	0.146	6.199	<0.001	Yes
50.000 vs. 30.000	0.122	5.19	<0.001	Yes
10.000 vs. 5.000	0.118	5.044	<0.001	Yes
1.000 vs. 0.000	0.0833	3.69	<0.001	Yes
15.000 vs. 10.000	0.0395	1.71	0.09	No

Table S5b.10. 2-way ANOVA results for log transformed BAF_s after 28 days exposure.

Normality Test (Shapiro-Wilk): Passed (P = 0.411)

Equal Variance Test (Brown-Forsythe): Passed (P = 0.081)

Source of Variation	DF	SS	MS	F	P
MP	6	1.235	0.206	33.454	<0.001
Cd	6	28.131	4.689	761.742	<0.001
MP x Cd	36	0.257	0.00714	1.161	0.271
Residual	121	0.745	0.00615		
Total	169	31.088	0.184		

The difference in the mean values among the different levels of MP is greater than would be expected by chance after allowing for effects of differences in Cd. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Cd is greater than would be expected by chance after allowing for effects of differences in MP. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of MP does not depend on what level of Cd is present. There is not a statistically significant interaction between MP and Cd. (P = 0.271)

Power of performed test with alpha = 0.0500: for MP : 1.000
Power of performed test with alpha = 0.0500: for Cd : 1.000
Power of performed test with alpha = 0.0500: for MP x Cd : 0.143

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: MP

Comparison	Diff of Means	t	P	P<0.050
NONE vs. PLA3	0.274	12.363	<0.001	Yes
PE0.1 vs. PLA3	0.228	9.576	<0.001	Yes
NONE vs. PE3	0.189	8.602	<0.001	Yes
NONE vs. PLA0.3	0.183	8.328	<0.001	Yes
PE0.3 vs. PLA3	0.155	7.001	<0.001	Yes
PE0.1 vs. PE3	0.142	6.038	<0.001	Yes
PLA0.1 vs. PLA3	0.144	6.013	<0.001	Yes
PE0.1 vs. PLA0.3	0.136	5.783	<0.001	Yes
NONE vs. PLA0.1	0.13	5.569	<0.001	Yes
NONE vs. PE0.3	0.119	5.541	<0.001	Yes

PLA0.3 vs. PLA3	0.0915	4.039	0.001	Yes
PE3 vs. PLA3	0.0855	3.773	0.003	Yes
PE0.1 vs. PLA0.1	0.0834	3.356	0.009	Yes
PE0.3 vs. PE3	0.0698	3.182	0.015	Yes
PE0.1 vs. PE0.3	0.0724	3.133	0.015	Yes
PE0.3 vs. PLA0.3	0.0638	2.908	0.026	Yes
PLA0.1 vs. PE3	0.0588	2.474	0.072	No
PLA0.1 vs. PLA0.3	0.0528	2.221	0.108	No
NONE vs. PE0.1	0.0465	2.014	0.132	No
PE0.3 vs. PLA0.1	0.011	0.472	0.869	No
PLA0.3 vs. PE3	0.00601	0.268	0.869	No

Comparisons for factor: Cd

Comparison	Diff of Means	t	P	P<0.050
1.000 vs. 100.000	1.275	56.286	<0.001	Yes
1.000 vs. 50.000	1.162	52.956	<0.001	Yes
1.000 vs. 30.000	1.035	45.236	<0.001	Yes
1.000 vs. 15.000	0.894	40.763	<0.001	Yes
1.000 vs. 10.000	0.736	32.822	<0.001	Yes
5.000 vs. 100.000	0.724	31.05	<0.001	Yes
5.000 vs. 50.000	0.612	27.01	<0.001	Yes
1.000 vs. 5.000	0.55	24.811	<0.001	Yes
10.000 vs. 100.000	0.539	22.885	<0.001	Yes
5.000 vs. 30.000	0.485	20.573	<0.001	Yes
10.000 vs. 50.000	0.426	18.635	<0.001	Yes
15.000 vs. 100.000	0.38	16.458	<0.001	Yes
5.000 vs. 15.000	0.344	15.196	<0.001	Yes
10.000 vs. 30.000	0.299	12.584	<0.001	Yes
15.000 vs. 50.000	0.268	11.936	<0.001	Yes
30.000 vs. 100.000	0.24	9.996	<0.001	Yes
5.000 vs. 10.000	0.185	8.024	<0.001	Yes
10.000 vs. 15.000	0.159	6.939	<0.001	Yes
15.000 vs. 30.000	0.14	6.019	<0.001	Yes
30.000 vs. 50.000	0.127	5.449	<0.001	Yes
50.000 vs. 100.000	0.113	4.878	<0.001	Yes

Table S5b.11. 2-way ANOVA on ranked BAF_{pw} after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
Plastic N/Y	1	77683.03	77683.03	33.226	0.000
Cd	6	77344.162	12890.694	33.081	0.000
plastic N/Y x Cd	6	9600.432	1600.072	4.106	0.662
Residual	153	111660.052	729.804		
Total	166	388108	2338		

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic N/Y

Comparison	Diff of Means	t	P	P<0.050
N vs. Y	58.859	10.317	<0.001	Yes

Power of performed test with alpha = 0.0500: for plastic N/Y : 1.000

Table S5b.12. 3-way ANOVA on ranked BAF_{pw} after 28 days exposure and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test

Source of Variation	DF	SS	MS	H	P
MP type	1	1080.75	1080.75	0.657	0.418
MP concentration	2	34260.58	17130.29	20.814	0.000
Cd	6	132588.5	22098.08	80.552	0.000
MP type x MP concentration	2	1152.401	576.201	0.700	0.705
MP type x Cd	6	16897.91	2816.319	10.266	0.114
MP concentration x Cd	12	8597.15	716.429	5.223	0.950
MP type x MP concentration x C	12	8230.23	685.853	5.000	0.958
Residual	98	21546.85	219.866		
Total	139	228794.5	1646.004		

Table S5b.13. Game-Howell post hoc test results for BAF_{pw} after 28 days exposure.

*. The mean difference is significant at the 0.05 level.

Comparisons for factor: plastic within 1

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-3410.1169154*	398.626	0.003
	PE3	-2482.5824449*	438.272	0.018
	PLA0.1	-3536.8987037*	551.803	0.016
	PLA0.3	-3350.5839144*	584.355	0.026
	PLA3	(1222.186)	658.356	0.526
PE0.3	PE0.1	3410.1169154*	398.626	0.003

	PE3	927.534	493.302	0.489
	PLA0.1	(126.782)	596.449	1.000
	PLA0.3	59.533	626.686	1.000
	PLA3	2187.931	696.202	0.156
PE3	PE0.1	2482.5824449*	438.272	0.018
	PE0.3	(927.534)	493.302	0.489
	PLA0.1	(1054.316)	623.643	0.583
	PLA0.3	(868.001)	652.622	0.763
	PLA3	1260.396	719.637	0.558
PLA0.1	PE0.1	3536.8987037*	551.803	0.016
	PE0.3	126.782	596.449	1.000
	PE3	1054.316	623.643	0.583
	PLA0.3	186.315	733.703	1.000
	PLA3	2314.713	793.903	0.166
PLA0.3	PE0.1	3350.5839144*	584.355	0.026
	PE0.3	(59.533)	626.686	1.000
	PE3	868.001	652.622	0.763
	PLA0.1	(186.315)	733.703	1.000
	PLA3	2128.398	816.864	0.230
PLA3	PE0.1	1222.186	658.356	0.526
	PE0.3	(2187.931)	696.202	0.156
	PE3	(1260.396)	719.637	0.558
	PLA0.1	(2314.713)	793.903	0.166
	PLA0.3	(2128.398)	816.864	0.230

Comparisons for factor: plastic within 5

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	8.976	120.472	1.000
	PE3	-7305.2110461*	515.650	0.003
	PLA0.1	(6036.569)	534.299	0.113
	PLA0.3	-5669.2157717*	614.073	0.011
	PLA3	-5390.8763385*	287.232	0.004
PE0.3	PE0.1	(8.976)	120.472	1.000
	PE3	-7314.1874558*	513.069	0.003
	PLA0.1	(6045.545)	531.808	0.116
	PLA0.3	-5678.1921813*	611.907	0.011
	PLA3	-5399.8527481*	282.572	0.005
PE3	PE0.1	7305.2110461*	515.650	0.003
	PE0.3	7314.1874558*	513.069	0.003
	PLA0.1	1268.642	730.891	0.593
	PLA0.3	1635.995	791.082	0.408
	PLA3	1914.335	575.524	0.135
PLA0.1	PE0.1	6036.569	534.299	0.113
	PE0.3	6045.545	531.808	0.116
	PE3	(1268.642)	730.891	0.593

	PLA0.3	367.353	803.363	0.995
	PLA3	645.692	592.291	0.854
PLA0.3	PE0.1	5669.2157717*	614.073	0.011
	PE0.3	5678.1921813*	611.907	0.011
	PE3	(1635.995)	791.082	0.408
	PLA0.1	(367.353)	803.363	0.995
	PLA3	278.339	665.145	0.997
PLA3	PE0.1	5390.8763385*	287.232	0.004
	PE0.3	5399.8527481*	282.572	0.005
	PE3	(1914.335)	575.524	0.135
	PLA0.1	(645.692)	592.291	0.854
	PLA0.3	(278.339)	665.145	0.997

Comparisons for factor: plastic within 10

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	(194.459)	117.797	0.632
	PE3	(630.626)	134.554	0.110
	PLA0.1	(181.668)	109.570	0.627
	PLA0.3	-626.8258152*	83.290	0.006
	PLA3	(6532.782)	1663.130	0.198
PE0.3	PE0.1	194.459	117.797	0.632
	PE3	(436.168)	167.788	0.280
	PLA0.1	12.791	148.504	1.000
	PLA0.3	(432.367)	130.327	0.163
	PLA3	(6338.323)	1666.148	0.207
PE3	PE0.1	630.626	134.554	0.110
	PE0.3	436.168	167.788	0.280
	PLA0.1	448.959	162.117	0.246
	PLA0.3	3.801	145.649	1.000
	PLA3	(5902.155)	1667.417	0.233
PLA0.1	PE0.1	181.668	109.570	0.627
	PE0.3	(12.791)	148.504	1.000
	PE3	(448.959)	162.117	0.246
	PLA0.3	(445.158)	122.941	0.121
	PLA3	(6351.114)	1665.587	0.207
PLA0.3	PE0.1	626.8258152*	83.290	0.006
	PE0.3	432.367	130.327	0.163
	PE3	(3.801)	145.649	1.000
	PLA0.1	445.158	122.941	0.121
	PLA3	(5905.956)	1664.065	0.234
PLA3	PE0.1	6532.782	1663.130	0.198
	PE0.3	6338.323	1666.148	0.207
	PE3	5902.155	1667.417	0.233
	PLA0.1	6351.114	1665.587	0.207

	PLA0.3	5905.956	1664.065	0.234

Comparisons for factor: plastic within 15

Comparison		Mean Differenc	Std. Error	Sig.
PE0.1	PE0.3	(30.573)	206.536	1.000
	PE3	(378.816)	171.205	0.432
	PLA0.1	129.160	163.939	0.957
	PLA0.3	(165.209)	188.061	0.934
	PLA3	-8282.9208065*	449.277	0.001
PE0.3	PE0.1	30.573	206.536	1.000
	PE3	(348.243)	205.454	0.596
	PLA0.1	159.732	199.440	0.956
	PLA0.3	(134.636)	219.697	0.985
	PLA3	-8252.3481756*	463.411	0.000
PE3	PE0.1	378.816	171.205	0.432
	PE0.3	348.243	205.454	0.596
	PLA0.1	507.976	162.573	0.233
	PLA0.3	213.607	186.871	0.840
	PLA3	-7904.1050717*	448.780	0.001
PLA0.1	PE0.1	(129.160)	163.939	0.957
	PE0.3	(159.732)	199.440	0.956
	PE3	(507.976)	162.573	0.233
	PLA0.3	(294.368)	180.238	0.621
	PLA3	-8412.0806492*	446.059	0.001
PLA0.3	PE0.1	165.209	188.061	0.934
	PE0.3	134.636	219.697	0.985
	PE3	(213.607)	186.871	0.840
	PLA0.1	294.368	180.238	0.621
	PLA3	-8117.7121790*	455.477	0.001
PLA3	PE0.1	8282.9208065*	449.277	0.001
	PE0.3	8252.3481757*	463.411	0.000
	PE3	7904.1050718*	448.780	0.001
	PLA0.1	8412.0806492*	446.059	0.001
	PLA0.3	8117.7121791*	455.477	0.001

Comparisons for factor: plastic within 30

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	(139.243)	41.849	0.170
	PE3	(212.710)	68.174	0.211
	PLA0.1	31.391	19.294	0.636
	PLA0.3	(129.106)	45.695	0.316
	PLA3	(782.049)	53.030	0.081
PE0.3	PE0.1	139.243	41.849	0.170
	PE3	(73.467)	78.425	0.920

	PLA0.1	170.634	43.302	0.095
	PLA0.3	10.137	59.923	1.000
	PLA3	-642.8058560*	65.689	0.025
PE3	PE0.1	212.710	68.174	0.211
	PE0.3	73.467	78.425	0.920
	PLA0.1	244.100	69.075	0.150
	PLA0.3	83.603	80.542	0.886
	PLA3	-569.3392799*	84.920	0.019
PLA0.1	PE0.1	(31.391)	19.294	0.636
	PE0.3	(170.634)	43.302	0.095
	PE3	(244.100)	69.075	0.150
	PLA0.3	(160.497)	47.029	0.210
	PLA3	(813.439)	54.185	0.066
PLA0.3	PE0.1	129.106	45.695	0.316
	PE0.3	(10.137)	59.923	1.000
	PE3	(83.603)	80.542	0.886
	PLA0.1	160.497	47.029	0.210
	PLA3	-652.9426413*	68.203	0.024
PLA3	PE0.1	782.049	53.030	0.081
	PE0.3	642.8058560*	65.689	0.025
	PE3	569.3392799*	84.920	0.019
	PLA0.1	813.439	54.185	0.066
	PLA0.3	652.9426413*	68.203	0.024

Comparisons for factor: plastic within 50

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-237.9379249*	42.028	0.041
	PE3	-567.8830158*	85.247	0.031
	PLA0.1	-88.1375622*	12.127	0.013
	PLA0.3	-321.5170669*	58.475	0.049
	PLA3	(1227.447)	119.996	0.136
PE0.3	PE0.1	237.9379249*	42.028	0.041
	PE3	(329.945)	94.109	0.118
	PLA0.1	149.800	41.672	0.149
	PLA0.3	(83.579)	70.773	0.831
	PLA3	(989.509)	126.446	0.133
PE3	PE0.1	567.8830158*	85.247	0.031
	PE0.3	329.945	94.109	0.118
	PLA0.1	479.7454536*	85.072	0.049
	PLA0.3	246.366	102.516	0.297
	PLA3	(659.564)	146.592	0.149
PLA0.1	PE0.1	88.1375622*	12.127	0.013
	PE0.3	(149.800)	41.672	0.149
	PE3	-479.7454536*	85.072	0.049

	PLA0.3	(233.380)	58.220	0.118
	PLA3	(1139.309)	119.872	0.147
PLA0.3	PE0.1	321.5170669*	58.475	0.049
	PE0.3	83.579	70.773	0.831
	PE3	(246.366)	102.516	0.297
	PLA0.1	233.380	58.220	0.118
	PLA3	(905.929)	132.822	0.120
PLA3	PE0.1	1227.447	119.996	0.136
	PE0.3	989.509	126.446	0.133
	PE3	659.564	146.592	0.149
	PLA0.1	1139.309	119.872	0.147
	PLA0.3	905.929	132.822	0.120

Comparisons for factor: plastic within 100

Comparison		Mean Difference	Std. Error	Sig.
PE0.1	PE0.3	-277.5557493*	28.172	0.001
	PE3	-285.9088807*	24.639	0.002
	PLA0.1	(95.241)	21.488	0.098
	PLA0.3	-224.4223876*	21.476	0.006
	PLA3	-647.3584177*	48.478	0.001
PE0.3	PE0.1	277.5557493*	28.172	0.001
	PE3	(8.353)	26.923	0.999
	PLA0.1	182.3144159*	24.072	0.010
	PLA0.3	53.133	24.062	0.381
	PLA3	-369.8026685*	49.678	0.008
PE3	PE0.1	285.9088807*	24.639	0.002
	PE0.3	8.353	26.923	0.999
	PLA0.1	190.6675473*	19.822	0.011
	PLA0.3	61.486	19.810	0.197
	PLA3	-361.4495370*	47.763	0.012
PLA0.1	PE0.1	95.241	21.488	0.098
	PE0.3	-182.3144159*	24.072	0.010
	PE3	-190.6675473*	19.822	0.011
	PLA0.3	-129.1810542*	15.717	0.025
	PLA3	-552.1170843*	46.216	0.004
PLA0.3	PE0.1	224.4223876*	21.476	0.006
	PE0.3	(53.133)	24.062	0.381
	PE3	(61.486)	19.810	0.197
	PLA0.1	129.1810542*	15.717	0.025
	PLA3	-422.9360301*	46.211	0.009
PLA3	PE0.1	647.3584177*	48.478	0.001
	PE0.3	369.8026685*	49.678	0.008
	PE3	361.4495370*	47.763	0.012
	PLA0.1	552.1170843*	46.216	0.004

	PLA0.3	422.9360301*	46.211	0.009

Comparisons for factor: Cd within non-MPs

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	(326.364)	238.246	0.797
	Cd10	194.583	102.250	0.567
	Cd15	233.443	111.421	0.470
	Cd30	356.826	105.184	0.170
	Cd50	382.478	102.069	0.143
	Cd100	534.076	99.125	0.062
Cd5	Cd1	326.364	238.246	0.797
	Cd10	520.948	218.903	0.410
	Cd15	559.807	223.334	0.365
	Cd30	683.190	220.289	0.242
	Cd50	708.842	218.818	0.224
	Cd100	860.440	217.461	0.144
Cd10	Cd1	(194.583)	102.250	0.567
	Cd5	(520.948)	218.903	0.410
	Cd15	38.860	59.767	0.990
	Cd30	162.242	47.128	0.155
	Cd50	187.8946411*	39.693	0.028
	Cd100	339.4921728*	31.356	0.002
Cd15	Cd1	(233.443)	111.421	0.470
	Cd5	(559.807)	223.334	0.365
	Cd10	(38.860)	59.767	0.990
	Cd30	123.383	64.658	0.546
	Cd50	149.035	59.456	0.327
	Cd100	300.6325678*	54.247	0.049
Cd30	Cd1	(356.826)	105.184	0.170
	Cd5	(683.190)	220.289	0.242
	Cd10	(162.242)	47.128	0.155
	Cd15	(123.383)	64.658	0.546
	Cd50	25.652	46.734	0.996
	Cd100	177.250	39.897	0.137
Cd50	Cd1	(382.478)	102.069	0.143
	Cd5	(708.842)	218.818	0.224
	Cd10	-187.8946411*	39.693	0.028
	Cd15	(149.035)	59.456	0.327
	Cd30	(25.652)	46.734	0.996
	Cd100	151.5975317*	30.760	0.045
Cd100	Cd1	(534.076)	99.125	0.062
	Cd5	(860.440)	217.461	0.144
	Cd10	-339.4921728*	31.356	0.002
	Cd15	-300.6325678*	54.247	0.049

	Cd30	(177.250)	39.897	0.137
	Cd50	-151.5975317*	30.760	0.045

Comparisons for factor: Cd within PE0.1

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	1440.828	249.807	0.070
	Cd10	1565.882	236.078	0.076
	Cd15	1493.335	262.102	0.056
	Cd30	1620.545	232.257	0.077
	Cd50	2290.4017587*	232.180	0.040
	Cd100	2384.2400306*	232.716	0.036
Cd5	Cd1	(1440.828)	249.807	0.070
	Cd10	125.054	102.463	0.858
	Cd15	52.507	153.177	1.000
	Cd30	179.716	93.321	0.566
	Cd50	849.5734972*	93.129	0.014
	Cd100	943.4117691*	94.459	0.009
Cd10	Cd1	(1565.882)	236.078	0.076
	Cd5	(125.054)	102.463	0.858
	Cd15	(72.547)	129.586	0.993
	Cd30	54.663	45.148	0.852
	Cd50	724.5196129*	44.750	0.011
	Cd100	818.3578848*	47.456	0.004
Cd15	Cd1	(1493.335)	262.102	0.056
	Cd5	(52.507)	153.177	1.000
	Cd10	72.547	129.586	0.993
	Cd30	127.209	122.486	0.906
	Cd50	797.066	122.340	0.087
	Cd100	890.905	123.355	0.067
Cd30	Cd1	(1620.545)	232.257	0.077
	Cd5	(179.716)	93.321	0.566
	Cd10	(54.663)	45.148	0.852
	Cd15	(127.209)	122.486	0.906
	Cd50	669.8570232*	14.584	0.001
	Cd100	763.6952951*	21.499	0.000
Cd50	Cd1	-2290.4017587*	232.180	0.040
	Cd5	-849.5734972*	93.129	0.014
	Cd10	-724.5196129*	44.750	0.011
	Cd15	(797.066)	122.340	0.087
	Cd30	-669.8570232*	14.584	0.001
	Cd100	93.838	20.649	0.104
Cd100	Cd1	-2384.2400306*	232.716	0.036
	Cd5	-943.4117691*	94.459	0.009
	Cd10	-818.3578848*	47.456	0.004

	Cd15	(890.905)	123.355	0.067
	Cd30	-763.6952951*	21.499	0.000
	Cd50	(93.838)	20.649	0.104

Comparisons for factor: Cd within PE0.3

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	3596.1661960*	301.530	0.004
	Cd10	3517.7850092*	311.373	0.003
	Cd15	3609.1243836*	335.812	0.001
	Cd30	3627.6631987*	294.310	0.006
	Cd50	4198.8253587*	294.396	0.004
	Cd100	4253.0458062*	292.313	0.004
Cd5	Cd1	-3596.1661960*	301.530	0.004
	Cd10	(78.381)	133.757	0.994
	Cd15	12.958	183.596	1.000
	Cd30	31.497	86.925	1.000
	Cd50	602.6591627*	87.217	0.011
	Cd100	656.8796102*	79.904	0.014
Cd10	Cd1	-3517.7850092*	311.373	0.003
	Cd5	78.381	133.757	0.994
	Cd15	91.339	199.351	0.998
	Cd30	109.878	116.573	0.937
	Cd50	681.040	116.791	0.072
	Cd100	735.261	111.435	0.076
Cd15	Cd1	-3609.1243836*	335.812	0.001
	Cd5	(12.958)	183.596	1.000
	Cd10	(91.339)	199.351	0.998
	Cd30	18.539	171.481	1.000
	Cd50	589.701	171.629	0.183
	Cd100	643.921	168.031	0.152
Cd30	Cd1	-3627.6631987*	294.310	0.006
	Cd5	(31.497)	86.925	1.000
	Cd10	(109.878)	116.573	0.937
	Cd15	(18.539)	171.481	1.000
	Cd50	571.1621600*	57.489	0.001
	Cd100	625.3826075*	45.637	0.001
Cd50	Cd1	-4198.8253587*	294.396	0.004
	Cd5	-602.6591627*	87.217	0.011
	Cd10	(681.040)	116.791	0.072
	Cd15	(589.701)	171.629	0.183
	Cd30	-571.1621600*	57.489	0.001
	Cd100	54.220	46.191	0.877
Cd100	Cd1	-4253.0458062*	292.313	0.004
	Cd5	-656.8796102*	79.904	0.014

	Cd10	(735.261)	111.435	0.076
	Cd15	(643.921)	168.031	0.152
	Cd30	-625.3826075*	45.637	0.001
	Cd50	(54.220)	46.191	0.877

Comparisons for factor: Cd within PE3

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	467.384	735.525	0.992
	Cd10	2244.345	563.829	0.128
	Cd15	2423.609	562.268	0.106
	Cd30	2716.924	553.386	0.081
	Cd50	3031.608	555.780	0.059
	Cd100	3407.4200893*	549.529	0.045
Cd5	Cd1	(467.384)	735.525	0.992
	Cd10	1776.961	505.446	0.172
	Cd15	1956.225	503.704	0.136
	Cd30	2249.540	493.769	0.098
	Cd50	2564.224	496.451	0.067
	Cd100	2940.0363251*	489.442	0.050
Cd10	Cd1	(2244.345)	563.829	0.128
	Cd5	(1776.961)	505.446	0.172
	Cd15	179.264	174.995	0.916
	Cd30	472.579	143.924	0.216
	Cd50	787.263	152.870	0.052
	Cd100	1163.0753226*	128.296	0.044
Cd15	Cd1	(2423.609)	562.268	0.106
	Cd5	(1956.225)	503.704	0.136
	Cd10	(179.264)	174.995	0.916
	Cd30	293.315	137.680	0.554
	Cd50	607.999	147.007	0.194
	Cd100	983.811	121.250	0.177
Cd30	Cd1	(2716.924)	553.386	0.081
	Cd5	(2249.540)	493.769	0.098
	Cd10	(472.579)	143.924	0.216
	Cd15	(293.315)	137.680	0.554
	Cd50	314.684	108.176	0.198
	Cd100	690.4960522*	69.228	0.008
Cd50	Cd1	(3031.608)	555.780	0.059
	Cd5	(2564.224)	496.451	0.067
	Cd10	(787.263)	152.870	0.052
	Cd15	(607.999)	147.007	0.194
	Cd30	(314.684)	108.176	0.198
	Cd100	375.812	86.300	0.105
Cd100	Cd1	-3407.4200893*	549.529	0.045

	Cd5	-2940.0363251*	489.442	0.050
	Cd10	-1163.0753226*	128.296	0.044
	Cd15	(983.811)	121.250	0.177
	Cd30	-690.4960522*	69.228	0.008
	Cd50	(375.812)	86.300	0.105

Comparisons for factor: Cd within PLA0.1

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	(333.896)	182.467	0.602
	Cd10	121.015	205.403	0.994
	Cd15	359.296	209.992	0.638
	Cd30	388.736	179.853	0.482
	Cd50	939.065	179.326	0.071
	Cd100	1025.800	179.507	0.056
Cd5	Cd1	333.896	182.467	0.602
	Cd10	454.911	106.237	0.153
	Cd15	693.1923345*	114.859	0.035
	Cd30	722.6322430*	37.987	0.033
	Cd50	1272.9611986*	35.406	0.032
	Cd100	1359.6956993*	36.314	0.023
Cd10	Cd1	(121.015)	205.403	0.994
	Cd5	(454.911)	106.237	0.153
	Cd15	238.281	148.623	0.690
	Cd30	267.721	101.683	0.400
	Cd50	818.050	100.748	0.057
	Cd100	904.7843456*	101.070	0.046
Cd15	Cd1	(359.296)	209.992	0.638
	Cd5	-693.1923344*	114.859	0.035
	Cd10	(238.281)	148.623	0.690
	Cd30	29.440	110.659	1.000
	Cd50	579.769	109.800	0.069
	Cd100	666.5033649*	110.097	0.047
Cd30	Cd1	(388.736)	179.853	0.482
	Cd5	-722.6322430*	37.987	0.033
	Cd10	(267.721)	101.683	0.400
	Cd15	(29.440)	110.659	1.000
	Cd50	550.3289557*	17.511	0.001
	Cd100	637.0634564*	19.281	0.000
Cd50	Cd1	(939.065)	179.326	0.071
	Cd5	-1272.9611986*	35.406	0.032
	Cd10	(818.050)	100.748	0.057
	Cd15	(579.769)	109.800	0.069
	Cd30	-550.3289557*	17.511	0.001
	Cd100	86.735	13.505	0.095

Cd100	Cd1	(1025.800)	179.507	0.056
	Cd5	-1359.6956993*	36.314	0.023
	Cd10	-904.7843456*	101.070	0.046
	Cd15	-666.5033649*	110.097	0.047
	Cd30	-637.0634564*	19.281	0.000
	Cd50	(86.735)	13.505	0.095

Comparisons for factor: Cd within PLA0.3

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	(253.388)	156.824	0.682
	Cd10	(369.949)	120.752	0.173
	Cd15	19.121	173.338	1.000
	Cd30	182.433	107.339	0.648
	Cd50	659.8792613*	113.529	0.019
	Cd100	850.8122125*	98.393	0.017
Cd5	Cd1	253.388	156.824	0.682
	Cd10	(116.561)	141.629	0.971
	Cd15	272.509	188.478	0.764
	Cd30	435.821	130.382	0.180
	Cd50	913.2672535*	135.524	0.015
	Cd100	1104.2002046*	123.122	0.015
Cd10	Cd1	369.949	120.752	0.173
	Cd5	116.561	141.629	0.971
	Cd15	389.071	159.723	0.399
	Cd30	552.3821285*	83.587	0.012
	Cd50	1029.8283612*	91.400	0.000
	Cd100	1220.7613124*	71.738	0.002
Cd15	Cd1	(19.121)	173.338	1.000
	Cd5	(272.509)	188.478	0.764
	Cd10	(389.071)	159.723	0.399
	Cd30	163.312	149.840	0.893
	Cd50	640.758	154.335	0.149
	Cd100	831.691	143.567	0.108
Cd30	Cd1	(182.433)	107.339	0.648
	Cd5	(435.821)	130.382	0.180
	Cd10	-552.3821285*	83.587	0.012
	Cd15	(163.312)	149.840	0.893
	Cd50	477.4462326*	72.764	0.010
	Cd100	668.3791838*	45.684	0.012
Cd50	Cd1	-659.8792613*	113.529	0.019
	Cd5	-913.2672534*	135.524	0.015
	Cd10	-1029.8283612*	91.400	0.000
	Cd15	(640.758)	154.335	0.149
	Cd30	-477.4462326*	72.764	0.010

	Cd100	190.933	58.773	0.216
Cd100	Cd1	-850.8122125*	98.393	0.017
	Cd5	-1104.2002046*	123.122	0.015
	Cd10	-1220.7613124*	71.738	0.002
	Cd15	(831.691)	143.567	0.108
	Cd30	-668.3791838*	45.684	0.012
	Cd50	(190.933)	58.773	0.216

Comparisons for factor: Cd within PLA3

Comparison		Mean Difference	Std. Error	Sig.
Cd1	Cd5	(1157.199)	374.573	0.215
	Cd10	23.561	611.394	1.000
	Cd15	(675.569)	430.710	0.706
	Cd30	89.668	352.747	1.000
	Cd50	314.128	368.854	0.962
	Cd100	988.054	351.788	0.302
Cd5	Cd1	1157.199	374.573	0.215
	Cd10	1180.760	520.214	0.479
	Cd15	481.630	286.933	0.656
	Cd30	1246.8668975*	145.774	0.030
	Cd50	1471.3262935*	181.309	0.024
	Cd100	2145.2526925*	143.438	0.009
Cd10	Cd1	(23.561)	611.394	1.000
	Cd5	(1180.760)	520.214	0.479
	Cd15	(699.130)	561.987	0.844
	Cd30	66.107	504.726	1.000
	Cd50	290.566	516.111	0.993
	Cd100	964.493	504.056	0.600
Cd15	Cd1	675.569	430.710	0.706
	Cd5	(481.630)	286.933	0.656
	Cd10	699.130	561.987	0.844
	Cd30	765.237	257.792	0.263
	Cd50	989.696	279.427	0.148
	Cd100	1663.6226253*	256.477	0.035
Cd30	Cd1	(89.668)	352.747	1.000
	Cd5	-1246.8668975*	145.774	0.030
	Cd10	(66.107)	504.726	1.000
	Cd15	(765.237)	257.792	0.263
	Cd50	224.459	130.379	0.687
	Cd100	898.3857950*	68.557	0.010
Cd50	Cd1	(314.128)	368.854	0.962
	Cd5	-1471.3262935*	181.309	0.024
	Cd10	(290.566)	516.111	0.993
	Cd15	(989.696)	279.427	0.148

	Cd30	(224.459)	130.379	0.687
	Cd100	673.926	127.761	0.220
Cd100	Cd1	(988.054)	351.788	0.302
	Cd5	-2145.2526925*	143.438	0.009
	Cd10	(964.493)	504.056	0.600
	Cd15	-1663.6226253*	256.477	0.035
	Cd30	-898.3857950*	68.557	0.010
	Cd50	(673.926)	127.761	0.220

Table S5b.14. 2-way ANOVA results for square root transformed mass of earthworm depurate after 28 days exposure.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.451)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.094)

Source of Variation	DF	SS	MS	F	P
MP	6	1.521	0.254	19.658	<0.001
Cd	7	0.0723	0.0103	0.801	0.588
MP x Cd	42	0.479	0.0114	0.884	0.672
Residual	159	2.051	0.0129		
Total	214	4.159	0.0194		

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: MP

Comparison	Diff of Means	t	P	P<0.050
NONE vs. PE3	0.259	8.72	<0.001	Yes
PLA0.1 vs. PE3	0.231	7.67	<0.001	Yes
NONE vs. PLA3	0.196	6.927	<0.001	Yes
PLA0.3 vs. PE3	0.201	6.452	<0.001	Yes
PLA0.1 vs. PLA3	0.168	5.846	<0.001	Yes
PE0.1 vs. PE3	0.175	5.764	<0.001	Yes
PLA0.3 vs. PLA3	0.138	4.629	<0.001	Yes
NONE vs. PE0.3	0.127	4.459	<0.001	Yes
PE0.3 vs. PE3	0.132	4.373	<0.001	Yes
PE0.1 vs. PLA3	0.112	3.861	0.002	Yes
PLA0.1 vs. PE0.3	0.0993	3.427	0.009	Yes
NONE vs. PE0.1	0.0838	2.905	0.041	Yes
PE0.3 vs. PLA3	0.0684	2.385	0.153	No
PLA0.3 vs. PE0.3	0.0697	2.314	0.163	No
PLA3 vs. PE3	0.0633	2.121	0.223	No
NONE vs. PLA0.3	0.0576	1.939	0.285	No
PLA0.1 vs. PE0.1	0.0558	1.907	0.285	No
PE0.1 vs. PE0.3	0.0435	1.486	0.451	No
PLA0.1 vs. PLA0.3	0.0296	0.983	0.695	No
NONE vs. PLA0.1	0.028	0.98	0.695	No
PLA0.3 vs. PE0.1	0.0262	0.862	0.695	No

Power of performed test with alpha = 0.0500: for MP : 1.000
Power of performed test with alpha = 0.0500: for Cd : 0.0500
Power of performed test with alpha = 0.0500: for MP x Cd : 0.0500

Table S5b.15. Independent t tests result for comparing the mass of Cd in the soil and in the soil plus earthworms.

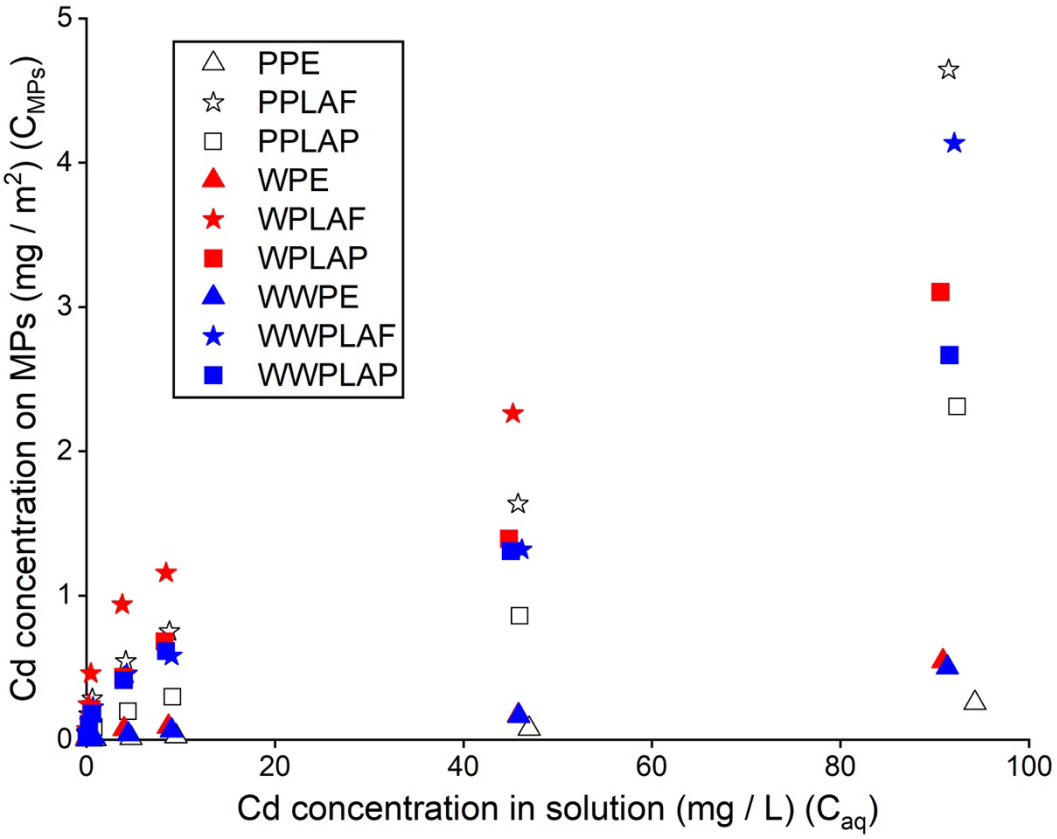
	Measured Cd concentrations in bulk soil (mg / kg)				Measured Cd concentrations in bulk soil and earthworm body (mg / kg)					
	mean	standard deviation	variance	number	mean	standard deviation	variance	number	t-value	P-value
Cd 1	0.741	0.077	0.006	7	0.751	0.075	0.006	7	0.241	> 0.05
Cd 5	3.844	0.306	0.094	7	3.861	0.304	0.092	7	0.103	> 0.05
Cd 10	7.754	0.926	0.857	7	7.778	0.924	0.853	7	0.050	> 0.05
Cd 15	12.175	0.687	0.472	7	12.201	0.678	0.459	7	0.071	> 0.05
Cd 30	23.536	1.090	1.189	7	23.565	1.089	1.186	7	0.050	> 0.05
Cd 50	41.783	1.621	2.628	7	41.823	1.617	2.615	7	0.047	> 0.05
Cd 100	78.612	5.726	32.788	7	78.667	5.724	32.769	7	0.018	> 0.05

4 Chapter 6 An investigation into the adsorption of Cd on pristine and naturally weathered MPs supporting information a

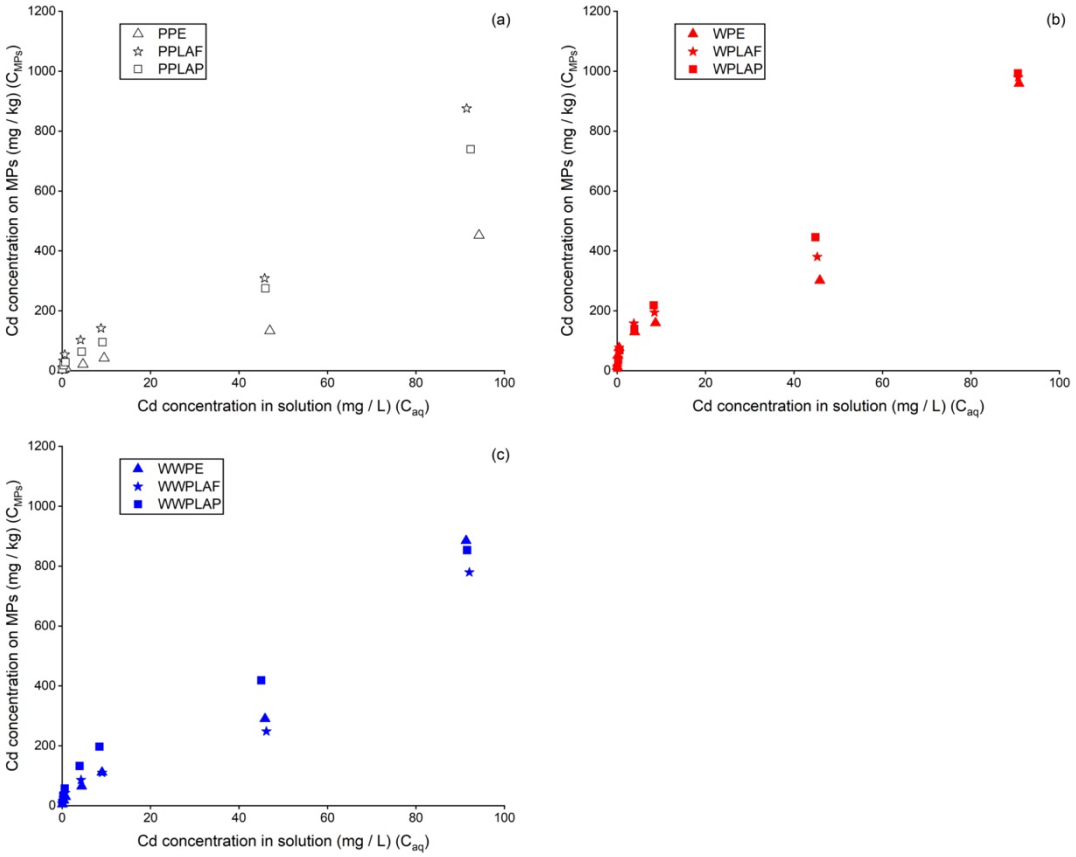
Number of pages: 8
Number of figures: 4
Number of table: 1

List of contents:

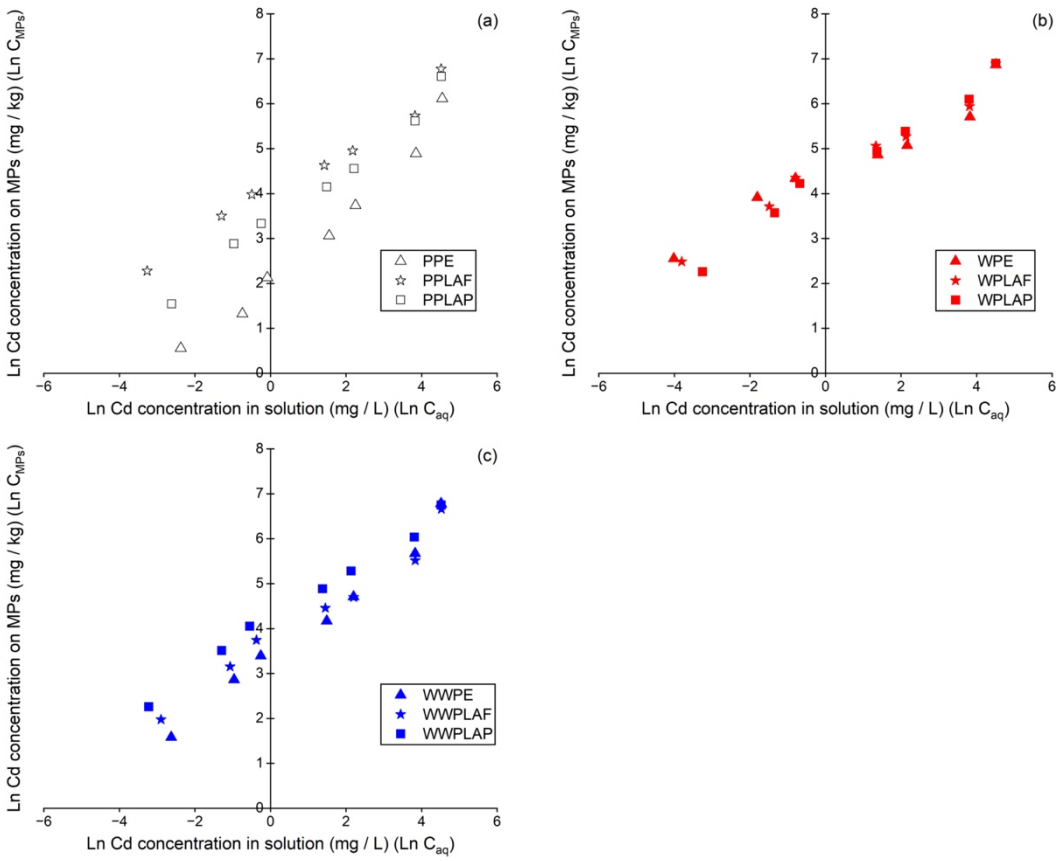
Figures



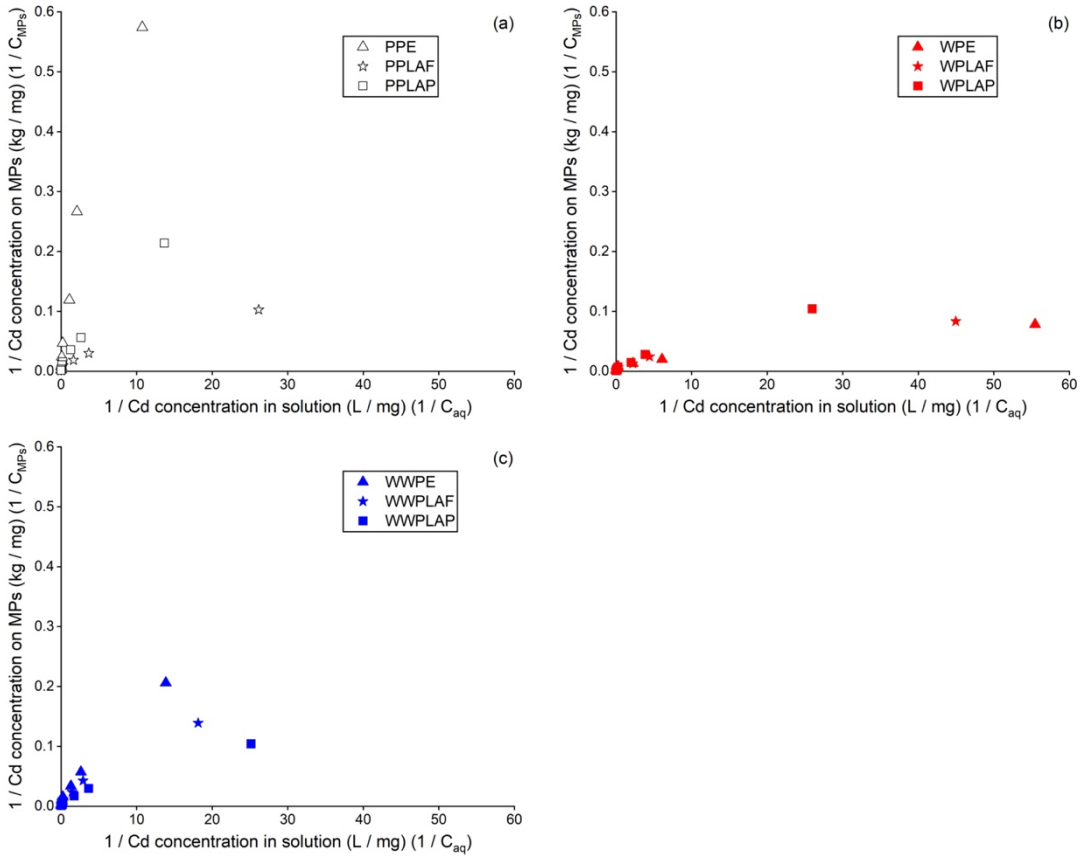
(S6a.1) Adsorption data for different types of MPs expressed per unit surface area. (White symbols are pristine MPs, red are weathered MPs and blue are weathered and washed MPs.



(S6a.2) Adsorption data for different types of MPs expressed per unit mass. (a) White symbols are pristine MPs, (b) red are weathered MPs and (c) blue are weathered and washed MPs. Triangles are PE, stars are PLAF and squares are PLAP.



(S6a.3) Freundlich isotherms for different types of MPs (per unit mass). (a) White symbols are pristine MPs, (b) red are weathered MPs and (c) blue are weathered and washed MPs. Triangles are PE, stars are PLAF and squares are PLAP.



(S6a.4) Langmuir isotherms for different types of MPs (per unit mass). (a) White symbols are pristine MPs, (b) red are weathered MPs and (c) blue are weathered and washed MPs. Triangles are PE, stars are PLAF and squares are PLAP.

Tables

Table S6a.1. Linear, Freundlich and Langmuir isotherm parameters for Cd adsorption to different types of MPs with concentrations adsorbed expressed on a per unit mass basis, 95 % confidence intervals are given in brackets. Initial Cd concentrations range from 0.1 to 50 mg / L. C_{MPs} is concentration adsorbed to MPs at equilibrium, mg / kg, C_{aq} is concentration in solution at equilibrium, mg / L. The linear adsorption model is expressed as $C_{MPs} = K_d C_{aq}$ where K_d is coefficient constant, L / kg. The Freundlich model is expressed as $C_{MPs} = K_F C_{aq}^{1/n}$ where K_F is 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 binding coefficient constant, mg / L and C_m is maximum concentration adsorbed to MPs, mg / kg.

	Linear			**Freundlich**				**Langmuir**			
MPs type	**K_d**	**R^2**	**P**	**LnK_F**	**1/n**	**R^2**	**P**	**K_L**	**Q_m**	**R^2**	**P**
pristine PE / PPE	2.913 (2.489-3.338)	0.78	< 0.001	2.090 (1.887-2.293)	0.7091 (0.6159-0.8024)	0.99	< 0.001	1.044 (-0.9708-1.974)	18.96 (-32.31-7.33)	0.92	< 0.001
pristine PLA fibre / PPLAF	7.221 (4.185-10.26)	0.68	< 0.001	3.994 (3.808-4.180)	0.4675 (0.3894-0.5455)	0.98	< 0.001	2.614 (0.8180-3.863)	106.0 (60.79-413.0)	0.98	< 0.001
pristine PLA piece / PPLAP	6.229 (4.751-7.707)	0.76	< 0.001	3.307 (3.120-3.494)	0.6039 (0.5197-0.6880)	0.99	< 0.001	0.7575 (0.2697-1.165)	88.47 (52.79-273.0)	0.99	< 0.001
weathered PE / WPE	7.175 (3.056-11.29)	0.60	< 0.001	4.359 (4.082-4.636)	0.3753 (0.2695-0.4811)	0.95	< 0.001	6.051 (3.008-8.282)	129.1 (81.77-306.9)	0.98	< 0.001
weathered PLA fibre / WPLAF	9.132 (4.343-13.92)	0.63	< 0.001	4.381 (4.144-4.618)	0.4447 (0.3508-0.5386)	0.97	< 0.001	4.527 (0.4106-7.115)	129.8 (67.24-1853)	0.97	< 0.001
weathered PLA pieces / WPLAP	10.69 (6.150-15.22)	0.68	< 0.001	4.238 (3.975-4.500)	0.5288 (0.4179-0.6397)	0.97	< 0.001	1.674 (0.2290-2.770)	156.6 (83.24-1328)	0.99	< 0.001
washed, weathered PE / WWPE	6.626 (4.909-8.342)	0.75	< 0.001	3.356 (3.184-3.528)	0.6140 (0.5366-0.6915)	0.99	< 0.001	0.7879 (0.1874-1.272)	89.40 (50.01-421.1)	0.99	< 0.001

washed, weathered PLA fibre / WWPLAF	5.762 (3.406-8.119)	0.69	< 0.001	3.656 (3.439-3.873)	0.5096 (0.4145-0.6046)	0.98	< 0.001	1.554 (0.3534-2.410)	90.06 (49.74-475.5)	0.98	< 0.001
washed, weathered PLA pieces / WWPLAP	9.969 (5.934-14.00)	0.69	< 0.001	4.141 (3.969-4.313)	0.5286 (0.4557-0.6015)	0.99	< 0.001	1.972 (0.226-3.206)	130.2 (68.34-130.2)	0.98	< 0.001

4 Chapter 6 An investigation into the adsorption of Cd on pristine and naturally weathered MPs supporting information b - statistical results

Table S6b.1 - S6b.7:

List of contents:

Table S6b.1. Three way ANOVA on ranked adsorption data (the Cd concentration adsorbed to MPs at equilibrium C_{MPs}, mg / kg) and Scheirer Ray Hare test H values and significance calculated from them.

Source of Variation	DF	SS	MS	H	P
initial Cd concentration	6	160709.46	26784.91	66.55303	2.07743E-12
plastic type	2	31840.222	15920.11	13.18568	0.001370144
plastic treatment	2	199988.98	99994.49	82.81947	1.03748E-18
initial Cd co x plastic type	12	5521.778	460.148	2.28668	0.998823131
initial Cd co x plastic treat	12	41013.016	3417.751	16.98432	0.150188195
plastic type x plastic treat	4	9810.794	2452.698	4.062848	0.397567151
initial Cd concentration x pla	24	2025.651	84.402	0.838863	1
Residual	126	3064.667	24.323		
Total	188	453974.57	2414.758		

Table S6b.2. Game-Howell post hoc test results for adsorption data (the Cd concentration adsorbed to MPs at equilibrium C_{MPs}, mg / kg).

*. The mean difference is significant at the 0.05 level.

Comparisons for factor: plastic within all Cd

(I) plastic type for all Cd	Mean Difference (I-J)	Std. Error	Sig.	
PE	PLAF	-43.4749	49.99108	0.660
	PLAP	-51.5501	50.18053	0.561
PLAF	PE	43.4749	49.99108	0.660
	PLAP	-8.0752	51.94843	0.987
PLAP	PE	51.5501	50.18053	0.561
	PLAF	8.0752	51.94843	0.987

Comparisons for factor: plastic within 0.1

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-6.0804*	1.29362	0.002
	weathered-washed	-1.8270	1.35769	0.396
weathered	pristine	6.0804*	1.29362	0.002
	weathered-washed	4.2535*	0.88268	0.001
weathered-washed	pristine	1.8270	1.35769	0.396
	weathered	-4.2535*	0.88268	0.001

Comparisons for factor: plastic within 0.5

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-23.9625*	4.86754	0.001
	weathered-washed	-6.5435	4.87424	0.399
weathered	pristine	23.9625*	4.86754	0.001
	weathered-washed	17.4190*	3.34919	0.000
weathered-washed	pristine	6.5435	4.87424	0.399
	weathered	-17.4190*	3.34919	0.000

Comparisons for factor: plastic within 1

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-44.0294*	6.96623	0.000
	weathered-washed	-13.2198	7.80491	0.243
weathered	pristine	44.0294*	6.96623	0.000
	weathered-washed	30.8096*	4.82681	0.000
weathered-washed	pristine	13.2198	7.80491	0.243
	weathered	-30.8096*	4.82681	0.000

Comparisons for factor: plastic within 5

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-79.7109*	14.13922	0.000
	weathered-washed	-32.1058	15.53681	0.129
weathered	pristine	79.7109*	14.13922	0.000
	weathered-washed	47.6050*	12.74019	0.005
weathered-washed	pristine	32.1058	15.53681	0.129
	weathered	-47.6050*	12.74019	0.005

Comparisons for factor: plastic within 10

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-97.8976*	18.52328	0.000
	weathered-washed	-46.2437	20.98481	0.101
weathered	pristine	97.8976*	18.52328	0.000
	weathered-washed	51.6539*	18.71833	0.037
weathered-washed	pristine	46.2437	20.98481	0.101
	weathered	-51.6539*	18.71833	0.037

Comparisons for factor: plastic within 50

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.

pristine	weathered	-137.0395*	39.51530	0.009
	weathered-washed	-80.3890	44.10136	0.194
weathered	pristine	137.0395*	39.51530	0.009
	weathered-washed	56.6505	38.16112	0.326
weathered-washed	pristine	80.3890	44.10136	0.194
	weathered	-56.6505	38.16112	0.326

Comparisons for factor: plastic within 100

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-288.5022*	72.02997	0.005
	weathered-washed	-150.6020	66.62122	0.111
weathered	pristine	288.5022*	72.02997	0.005
	weathered-washed	137.9002*	38.80949	0.009
weathered-washed	pristine	150.6020	66.62122	0.111
	weathered	-137.9002*	38.80949	0.009

Table S6b.3. Two way ANOVA on ranked the relative peak intensity of the C-O peak (1300 - 800 cm^{-1}) to the C-H peak (3200 - 2600 cm^{-1}) and Scheirer Ray Hare test H values and significance calculated from them.

Scheirer Ray Hare Test					
Source of Variation	DF	SS	MS	H	P
plastic type	2	36.5	18.25	0.43270542	0.805
plastic treatment	2	2059.056	1029.528	24.4099913	0.000
plastic type x plastic treat	4	87.778	21.944	1.04060318	0.904
Residual	18	9.833	0.546		
Total	26	2193.167	84.353		

Table S6b.4. Game-Howell post hoc test results for the relative peak intensity of the C-O peak (1300 - 800 cm^{-1}) to the C-H peak (3200 - 2600 cm^{-1}).

*. The mean difference is significant at the 0.05 level.

Comparisons for factor: PE

(I) plastic treatment		Mean Difference (I-J)	Std. Error	Sig.
pristine	weathered	-.2930*	0.00017	0.000
	weathered-washed	-.2074*	0.00023	0.000
weathered	pristine	.2930*	0.00017	0.000
	weathered-washed	.0856*	0.00029	0.000
weathered-washed	pristine	.2074*	0.00023	0.000
	weathered	-.0856*	0.00029	0.000

Comparisons for factor: PLAF

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-.0455*	0.00024	0.000
	weathered-washed	-.0132*	0.00031	0.000
weathered	pristine	.0455*	0.00024	0.000
	weathered-washed	.0323*	0.00021	0.000
weathered-washed	pristine	.0132*	0.00031	0.000
	weathered	-.0323*	0.00021	0.000

Comparisons for factor: PLAP

(I) plastic treatment	Mean Difference (I-J)	Std. Error	Sig.	
pristine	weathered	-.0463*	0.00013	0.000
	weathered-washed	-.0062*	0.00018	0.000

weathered	pristine	.0463*	0.00013	0.000
	weathered-washed	.0401*	0.00021	0.000
weathered-washed	pristine	.0062*	0.00018	0.000
	weathered	-.0401*	0.00021	0.000

Table S6b.5. Two way ANOVA followed by Holm-Sidak post hoc test results for amount of biofilm OD_{595}.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.262)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.663)

Source of Variation	DF	SS	MS	F	P
plastic type	2	2.96E-07	1.48E-07	220.065	< 0.001
plastic treatment	2	1.36E-05	6.82E-06	10134.14	< 0.001
plastic type x plastic treat	4	2.64E-07	6.6E-08	98.108	< 0.001
Residual	18	1.21E-08	6.73E-10		
Total	26	1.42E-05	5.46E-07		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of plastic type depends on what level of plastic treatment is present. There is a statistically significant interaction between plastic type and plastic treatment. (P = <0.001)

Power of performed test with alpha = 0.0500: for plastic type : 1.000
Power of performed test with alpha = 0.0500: for plastic treatment : 1.000
Power of performed test with alpha = 0.0500: for plastic type x plastic treat : 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic type

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	0.000242	19.829	<0.001	Yes
PLAP vs. PE	0.000194	15.848	<0.001	Yes
PLAF vs. PLAP	4.87E-05	3.98	<0.001	Yes

Comparisons for factor: plastic treatment

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	0.00173	141.854	<0.001	Yes
weathered vs. washed weathered	0.000995	81.378	<0.001	Yes
washed weathered vs. pristine	0.000739	60.477	<0.001	Yes

Comparisons for factor: plastic treatment within PE

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	0.00143	67.555	<0.001	Yes
weathered vs. washed weathered	0.000798	37.665	<0.001	Yes
washed weathered vs. pristine	0.000633	29.89	<0.001	Yes

Comparisons for factor: plastic treatment within PLAF

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	0.00189	89.103	<0.001	Yes
weathered vs. washed weathered	0.000982	46.37	<0.001	Yes
washed weathered vs. pristine	0.000905	42.734	<0.001	Yes

Comparisons for factor: plastic treatment within PLAP

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	0.00189	89.04	<0.001	Yes
weathered vs. washed weathered	0.00121	56.915	<0.001	Yes
washed weathered vs. pristine	0.00068	32.125	<0.001	Yes

Comparisons for factor: plastic type within pristine

Comparison	Diff of Means	t	P	P<0.050
PLAP vs. PLAF	2.67E-05	1.259	0.533	No
PLAP vs. PE	2.63E-05	1.243	0.533	No
PE vs. PLAF	3.33E-07	0.0157	0.988	No

Comparisons for factor: plastic type within weathered

Comparison	Diff of Means	t	P	P<0.050
PLAP vs. PE	0.000481	22.728	<0.001	Yes
PLAF vs. PE	0.000456	21.532	<0.001	Yes
PLAP vs. PLAF	2.53E-05	1.196	0.247	No

Comparisons for factor: plastic type within washed weathered

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	0.000272	12.828	<0.001	Yes
PLAF vs. PLAP	0.000198	9.349	<0.001	Yes
PLAP vs. PE	7.37E-05	3.479	0.003	Yes

Table S6b.6. Two way ANOVA followed by Holm-Sidak post hoc test results for log transformed OD_{595} / MP mass.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.155)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.306)

Source of Variation	DF	SS	MS	F	P
plastic type	2	1.626	0.813	1605.968	< 0.001
plastic treatment	2	6.63	3.315	6550.109	< 0.001
plastic type x plastic treat	4	0.0336	0.0084	16.59	< 0.001
Residual	18	0.00911	0.000506		
Total	26	8.298	0.319		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of plastic type depends on what level of plastic treatment is present. There is a statistically significant interaction between plastic type and plastic treatment. (P = <0.001)

Power of performed test with alpha = 0.0500: for plastic type : 1.000
Power of performed test with alpha = 0.0500: for plastic treatment : 1.000
Power of performed test with alpha = 0.0500: for plastic type x plastic treat : 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic type

Comparison	Diff of Means	t	P	P<0.050
PE vs. PLAP	0.555	52.297	<0.001	Yes
PE vs. PLAF	0.478	45.062	<0.001	Yes
PLAF vs. PLAP	0.0767	7.234	<0.001	Yes

Comparisons for factor: plastic treatment

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	1.176	110.919	<0.001	Yes

washed weathered vs. pristine	0.847	79.912	<0.001	Yes
weathered vs. washed weathered	0.329	31.007	<0.001	Yes

Comparisons for factor: plastic treatment within PE

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	1.19	64.807	<0.001	Yes
washed weathered vs. pristine	0.895	48.71	<0.001	Yes
weathered vs. washed weathered	0.296	16.098	<0.001	Yes

Comparisons for factor: plastic treatment within PLAF

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	1.236	67.313	<0.001	Yes
washed weathered vs. pristine	0.918	49.985	<0.001	Yes
weathered vs. washed weathered	0.318	17.328	<0.001	Yes

Comparisons for factor: plastic treatment within PLAP

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	1.102	59.997	<0.001	Yes
washed weathered vs. pristine	0.73	39.716	<0.001	Yes
weathered vs. washed weathered	0.373	20.28	<0.001	Yes

Comparisons for factor: plastic type within pristine

Comparison	Diff of Means	t	P	P<0.050
PE vs. PLAF	0.501	27.277	<0.001	Yes
PE vs. PLAP	0.47	25.592	<0.001	Yes
PLAP vs. PLAF	0.0309	1.685	0.109	No

Comparisons for factor: plastic type within weathered

Comparison	Diff of Means	t	P	P<0.050
PE vs. PLAP	0.558	30.403	<0.001	Yes
PE vs. PLAF	0.455	24.771	<0.001	Yes
PLAF vs. PLAP	0.103	5.631	<0.001	Yes

Comparisons for factor: plastic type within washed weathered

Comparison	Diff of Means	t	P	P<0.050
PE vs. PLAP	0.635	34.586	<0.001	Yes
PE vs. PLAF	0.478	26.002	<0.001	Yes
PLAF vs. PLAP	0.158	8.584	<0.001	Yes

Table S6b.7. Two way ANOVA followed by Holm-Sidak post hoc test results for OD_{595} / MP surface area.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.102)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.566)

Source of Variation	DF	SS	MS	F	P
plastic type	2	12225.45	6112.727	454.401	< 0.001
plastic treatment	2	137622.3	68811.17	5115.209	< 0.001
plastic type x plastic treat	4	7973.691	1993.423	148.185	< 0.001
Residual	18	242.141	13.452		
Total	26	158063.6	6079.37		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of plastic type depends on what level of plastic treatment is present. There is a statistically significant interaction between plastic type and plastic treatment. (P = <0.001)

Power of performed test with alpha = 0.0500: for plastic type : 1.000
Power of performed test with alpha = 0.0500: for plastic treatment : 1.000
Power of performed test with alpha = 0.0500: for plastic type x plastic treat : 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: plastic type

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	51.792	29.955	<0.001	Yes
PLAP vs. PE	30.969	17.912	<0.001	Yes
PLAF vs. PLAP	20.824	12.044	<0.001	Yes

Comparisons for factor: plastic treatment

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	173.701	100.464	<0.001	Yes

weathered vs. washed weathered	104.4	60.382	<0.001	Yes
washed weathered vs. pristine	69.301	40.082	<0.001	Yes

Comparisons for factor: plastic treatment within PE

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	120.855	40.356	<0.001	Yes
weathered vs. washed weathered	63.8	21.304	<0.001	Yes
washed weathered vs. pristine	57.055	19.052	<0.001	Yes

Comparisons for factor: plastic treatment within PLAF

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	219.03	73.139	<0.001	Yes
weathered vs. washed weathered	136.084	45.442	<0.001	Yes
washed weathered vs. pristine	82.945	27.697	<0.001	Yes

Comparisons for factor: plastic treatment within PLAP

Comparison	Diff of Means	t	P	P<0.050
weathered vs. pristine	181.219	60.513	<0.001	Yes
weathered vs. washed weathered	113.315	37.839	<0.001	Yes
washed weathered vs. pristine	67.903	22.675	<0.001	Yes

Comparisons for factor: plastic type within pristine

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	10.437	3.485	0.008	Yes
PLAP vs. PE	7.231	2.415	0.053	No
PLAF vs. PLAP	3.206	1.071	0.299	No

Comparisons for factor: plastic type within weathered

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	108.612	36.268	<0.001	Yes
PLAP vs. PE	67.595	22.572	<0.001	Yes
PLAF vs. PLAP	41.017	13.697	<0.001	Yes

Comparisons for factor: plastic type within washed weathered

Comparison	Diff of Means	t	P	P<0.050
PLAF vs. PE	36.328	12.131	<0.001	Yes
PLAF vs. PLAP	18.248	6.093	<0.001	Yes
PLAP vs. PE	18.08	6.037	<0.001	Yes

5 Chapter 7 A field-based investigation into the impacts of conventional and biodegradable plastic mulch film on earthworm abundance, biomass and diversity supporting information

Table S7.1 - S7.8:

List of contents:

Table S7.1. One way ANOVA results for measured soil temperature.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.256)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.741)

Group Name	N	Missing	Mean	Std Dev	SEM
Non-MPs	4	0	11.075	0.574	0.287
PE	4	0	10.875	0.763	0.382
PLA	4	0	10.925	0.568	0.284

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0867	0.0433	0.105	0.901
Residual	9	3.702	0.411		
Total	11	3.789			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.901).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Table S7.2. One way ANOVA on ranks (Kruskal-Wallis test) results for measured soil pH.

Normality Test (Shapiro-Wilk): Passed (P = 0.147)

Equal Variance Test (Brown-Forsythe): Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks

Dependent Variable: pH

Group	N	Missing	Median	25%	75%
PE	4	0	6.38	6.29	6.507
PLA	4	0	6.465	6.377	6.568
Non-MPs	4	0	6.335	6.275	6.373

H = 3.975 with 2 degrees of freedom. P(est.)= 0.137 P(exact)= 0.145

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.145)

Table S7.3. One way ANOVA results for measured soil moisture.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.985)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.468)

Group Name	N	Missing	Mean	Std Dev	SEM
PE	4	0	27.946	1.366	0.683
PLA	4	0	29.306	1.009	0.504
Non-MPs	4	0	26.707	2.583	1.292

Source of Variation	DF	SS	MS	F	P
Between Groups	2	13.514	6.757	2.121	0.176
Residual	9	28.671	3.186		
Total	11	42.185			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.176).

Power of performed test with alpha = 0.050: 0.189

The power of the performed test (0.189) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Table S7.4. One way ANOVA results for measured soil organic matter.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.786)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.518)

Group Name	N	Missing	Mean	Std Dev	SEM
PE	4	0	4.615	0.0714	0.0357
PLA	4	0	4.707	0.133	0.0663
Non-MPs	4	0	4.638	0.106	0.0531

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0184	0.0092	0.813	0.474
Residual	9	0.102	0.0113		
Total	11	0.12			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.474).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Table S7.5. Sampled earthworm abundance and biomass in control, PLA and PE plots.

Plot	earthworm number	earthworm total biomass (g)	biomass per earthworm (g)
01 PE	2	0.1509	0.07545
02 PLA/PBAT	1	0.1595	0.1595
03 Control	1	0.0651	0.0651
04 PE	0	0	0
05 Control	0	0	0
06 PLA/PBAT	5	0.765	0.153
07 Control	0	0	0
08 PE	1	0.0116	0.0116
09 PLA/ PBAT	4	0.5047	0.126175
10 PLA/PBAT	1.5	0.0358	0.0358
11 PE	1	0.0048	0.0048
12 Control	0	0	0

Table S7.6. One way ANOVA results for 1 / X transformed measured earthworm number.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.266)
Equal Variance Test (Brown-Forsythe):	Passed	(P = 1.000)

Group Name	N	Missing	Mean	Std Dev	SEM
PE	4	1	0.833	0.289	0.167
PLA	4	0	0.529	0.377	0.189
Non-MPs	4	3	1	0	0

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.26	0.26	2.191	0.199
Residual	5	0.594	0.119		
Total	6	0.854			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.199).

Power of performed test with alpha = 0.050: 0.145

The power of the performed test (0.145) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Table S7.7. One way ANOVA results for square root transformed measured earthworm total biomass.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.354)			
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.141)			
Group Name	N	Missing	Mean	Std Dev	SEM
PE	4	0	0.141	0.171	0.0853
PLA	4	0	0.543	0.308	0.154
Non-MPs	4	0	0.0638	0.128	0.0638

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.53	0.265	5.681	0.025
Residual	9	0.42	0.0467		
Total	11	0.95			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.025).

Power of performed test with alpha = 0.050: 0.630

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: Col 1

Comparison	Diff of Means	t	P	P<0.050
PLA vs. non-MPs	0.48	3.14	0.035	Yes
PLA vs. PE	0.402	2.632	0.054	No
PE vs. non-MPs	0.0776	0.508	0.624	No

Table S7.8. One way ANOVA results for measured biomass per earthworm.

Normality Test (Shapiro-Wilk):	Passed	(P = 0.135)			
Equal Variance Test (Brown-Forsythe):	Passed	(P = 0.692)			
Group Name	N	Missing	Mean	Std Dev	SEM
PE	4	0	0.023	0.0353	0.0177
PLA	4	0	0.119	0.0571	0.0285
Non-MPs	4	0	0.0163	0.0326	0.0163

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0262	0.0131	7.071	0.014
Residual	9	0.0167	0.00185		
Total	11	0.0429			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.014).

Power of performed test with alpha = 0.050: 0.748

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: Col 1

Comparison	Diff of Means	t	P	P<0.050
PLA vs. non-MPs	0.102	3.361	0.025	Yes
PLA vs. PE	0.0957	3.141	0.025	Yes
PE vs. non-MPs	0.00669	0.22	0.831	No