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Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates

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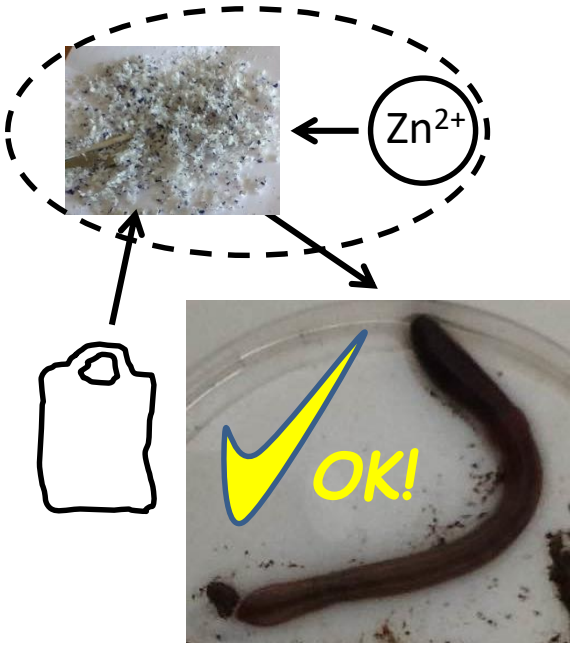
Table of Contents Graphic



Abstract

Microplastics are widespread contaminants in terrestrial environments but comparatively little is known about interactions between microplastics and common terrestrial contaminants such as zinc (Zn). In adsorption experiments fragmented HDPE bags c. 1 mm^2 in size showed similar sorption characteristics to soil. However, when present in combination with soil, concentrations of adsorbed Zn on a per mass basis were over an order of magnitude lower on microplastics . Desorption of the Zn was minimal from both microplastics and soil in synthetic soil solution (0.01 M $CaCl_2$), but in synthetic earthworm guts desorption was higher from microplastics (40 – 60%) than soil (2 – 15 %), suggesting microplastics could increase Zn bioavailability. Individual *Lumbricus terrestris* earthworms exposed for 28 days in mesocosms of 260 g moist soil containing 0.35 wt% of Zn-bearing microplastic (236-4505 mg kg^{-1}) ingested the microplastics, but there was no evidence of Zn accumulation, mortality or weight change. Digestion of the earthworms showed that they did not retain microplastics in their gut. These findings indicate that microplastics could act as vectors to increase metal exposure in earthworms, but that the associated risk is unlikely to be significant for essential metals such as Zn that are well regulated by metabolic processes.

Keywords: metals, microplastic, zinc, earthworm, uptake, toxicity

Introduction

Plastics are currently estimated to constitute up to 54 % (by mass) of anthropogenic waste materials released to the environment[1] and plastic debris is reported to be a prevalent pollutant in aquatic environments throughout the world.[1-9] Although, less widely reported, plastics may also accumulate in terrestrial environments from a range of sources, such as laundry dust, paint flakes, car tyre debris, sewage sludge, wind-blown dust from landfills, and agricultural plastic sheeting used to cover soil.[10-14] Nizetto et al. [14] estimate average and maximum annual additions of plastic particles to soil via sewage sludge in Europe at 0.2 and 8 mg ha^{-1} yr^{-1} per person but do not estimate absolute loadings. Huerta Lwanga et al.[15] state that bioturbation of soil with up to 40 % deliberate surface coverage of plastic bags results in plastic contents of 0.2 -1.2 % in the soil. Conventional high-density polyethylene (HDPE) bags are the lightweight single-use carrier bags used in almost all UK supermarkets; in 2014 it was estimated that 7.6 billion single-use plastic bags were given to customers by major supermarkets in England.[16] Most single-use bags ultimately end up in landfill, but they are also common items of litter in urban and rural environments, especially along roadsides, where they can come into contact with other common roadside contaminants such as metals.[17, 18]

Once in the environment, UV radiation and high temperatures can lead to fragmentation of plastic debris and the formation of microscopic particles (microplastics [MPs]; ≤5 mm plastic particles)[19] which, due to their small size, can be ingested by a range of aquatic invertebrates[20-28] and vertebrates.[29-36] Laboratory exposures, using aquatic invertebrates and fish suggest that this ingestion can lead to blockages in the digestive tract, inflammatory responses and reduced feeding due to plastic particles replacing digestible food.[26, 34, 37-40] Comparable studies on the impacts of microplastics on soil organisms are remarkably scarce, [41] however, investigations by Huerta Lwanga et al. [15,42] detected a significant decrease in weight and an increase in mortality for *L. terrestris* exposed to microplastic (< 150 μm low density polyethylene) loadings of ≥ 28 % by mass in the litter layer of soils. Huerta Lwanga et al.[42] also observed a decrease in particle size of microplastics between the microplastic-loaded litter and earthworm casts which suggests either preferential ingestion of the smaller particles, retention of larger particles in the earthworm gut subsequent to ingestion or breakdown of the larger particles into smaller sizes during transit through the earthworm gut. Given

the essential role earthworms play in soil processes[43] further studies on the impacts of microplastics on soil fauna are warranted.

In addition to the direct effects of microplastics on organisms, there is evidence that microplastics can act as vectors to transport other pollutants into organisms.[44-47] These pollutants can adsorb to the microplastics in the environment and desorb post-ingestion with potential for toxicity and / or accumulation in the food chain. The majority of studies consider organic contaminants (see recent review[44]) but a few consider metals [45-47] and metal sorption to plastic has been reported in the literature in the context of sample storage and choice of vessels in adsorption experiments.[48] The impact of metal-contaminated microplastics on soil organisms has not previously been investigated. However, metals can be prevalent contaminants in urban and agricultural soils[49] and are likely to co-occur with plastic pollutants in these environments. Therefore it is important to consider the possible interaction between metals and microplastics when evaluating the risk that both pose to terrestrial organisms. If microplastics can act as vectors to increase body burdens of metals then this could act to reduce the soil concentrations at which metals have a negative impact on the soil organism health. Zinc occurs ubiquitously in soils; typical background concentrations lie in the range 10 – 100 mg kg^{-1} and concentrations are elevated by Zn-rich soil parent materials, atmospheric deposition, and applications of fertilisers, pesticides, animal manures and sewage sludge.[49, 50] Zn is an essential element for earthworms but at elevated concentrations ($\geq$ 200 mg kg^{-1}) can have a toxic effect.[51-54]

The aims of the experiments reported here were therefore to determine: 1) the potential for microplastics generated from HDPE plastic carrier bags to adsorb Zn; 2) whether this sorption was reversible; 3) whether earthworms ingest or avoid Zn-laden microplastics; and 4) whether ingestion of Zn-laden microplastics has a measurable toxic impact.

Experimental

Materials

An arable and woodland soil, (UK grid references SE 629 497 and SE 623 508 respectively), were selected for use in these experiments to provide soils with contrasting organic matter contents. The soils were air-dried, sieved to < 2 mm and characterised prior to use. The arable soil was a loam soil (1 % clay, 48 % silt, 52 % sand) with a pH of 6.66 ± 0.04, organic matter content of 5.87 ± 0.23 %, and contained 60.9 ± 1.24 mg kg^{-1} background Zn (n = 3, ± standard deviation). The woodland soil was a loam soil (1 % clay, 33 % silt, 67 % sand) with a pH of 6.70 ± 0.04, organic matter content of 10.86 ± 0.16 %, and contained 61.1 ± 3.14 mg kg^{-1} background Zn (n = 3, ± standard error). Characterisation methods are detailed in the Supporting information. Microplastics were obtained by manually cutting up white (with a red and blue pattern) HDPE single-use plastic carrier bags obtained from a UK national supermarket chain into small, irregularly shaped pieces. Average area of the pieces was 0.92 ± 1.09 mm^2 (n = 314, ± std dev), the average primary and secondary axis of the best fitting ellipse for each particle was 1.32 ± 0.72 mm and 0.71 ± 0.43 mm respectively (see Supporting Information, Table S1). The size falls below the 5mm^2 maximum size for plastics to be considered microplastics[19], is such that the material could potentially be ingested by earthworms and was large enough for ease of use in the laboratory and separation from soils during experiments. The plastic was confirmed to be HDPE using Fourier transform infra-red spectroscopy (FTIR) (Fig. S1) and contained 143 ± 4.37 mg kg^{-1} background Zn (n = 3, ± standard deviation). Characterisation methods are given in the Supporting information.

Adsorption experiments

Adsorption experiments were carried out to determine the potential for microplastics to adsorb Zn and to compare the sorption capacity of microplastics to that of soils. Initial adsorption experiments were carried out using 0.2 g of soil / microplastic in 8.7 – 10.0 mL of 5.67 mg L^{-1} Zn solution obtained by dissolving $Zn(NO_3)_2$ in a background electrolyte of 0.1 M $NaNO_3$. Samples were shaken at 220 rpm on a flatbed shaker. After 1, 3, 6, 12, 24 and 48 hours triplicate sacrificial replicates were filtered through Whatman 42 filter paper and the solutions analysed for Zn. Data (see Supporting Information, Fig. S2) indicate that adsorption had reached equilibrium within 24 hours and therefore this experimental duration was used for further adsorption experiments.

Adsorption isotherms for the arable and woodland soil and the microplastics were constructed using data from experiments in which c. 0.2 g soil or plastic or a mixture of 0.1 g soil and 0.1 g plastic were shaken at 220 rpm in glass vials for 24 hours on a flatbed shaker in 20 mL Zn solution (from $Zn(NO_3)_2$) in a background electrolyte of 0.1M $NaNO_3$ to give a constant solution ionic strength. Initial target Zn concentrations were in the range 0.1 to 100 mg L^{-1} with triplicate soil/plastic and solid-free control treatments at each concentration. Suspensions were filtered through Whatman 42 filter paper and analysed for Zn. Solid-free controls were used to measure true values of initial solution concentrations (Supporting information, Table S2) and concentrations adsorbed were calculated by difference between the Zn concentrations in the filtered solutions of the solid-free control and soil/plastic treatments at the end of the adsorption period. Langmuir and Freundlich isotherms were fitted to the adsorption data. For the mixed soil / microplastic adsorption experiments, after filtering, the microplastics were separated from the soil by flotation in water, washed in deionised water in an ultrasonic bath to remove adhering soil particles, air dried, weighed and then digested in concentrated HNO_3 to determine their adsorbed Zn content. The adsorbed Zn content of the soil was determined by mass balance considering total Zn adsorbed and the measured Zn adsorption on the microplastics.

Desorption experiments

Desorption experiments were performed to determine whether microplastics could release previously sorbed Zn and to compare release rates with those from soils. To produce Zn-laden solids of three increasing Zn contents (C1, C2, C3) 5 g of soil or plastic were shaken in 500 mL of 1, 10 or 100 mg L^{-1} Zn solution (from $Zn(NO_3)_2$) for 24 hours. Suspensions were filtered through Whatman 42 filter paper and solutions analysed for Zn. Sorbed concentrations of 236 – 7171 mg kg^{-1} Zn were determined from the difference in concentrations between solid-free and solid-bearing solutions (Supporting information, Table S3). The metal-loaded solids were washed in deionised water to remove residual Zn solution, air-dried and stored for use in desorption experiments. At the highest Zn concentration the Woodland soil adsorbed significantly more Zn than the arable soil or plastic and was therefore not used in the desorption experiments as Zn loadings were not equivalent.

Potential desorption in soil solution was investigated using 0.01M $CaCl_2$ solution.[55] 0.8 g solids were shaken for 2 hours in 8 mL 0.01 M $CaCl_2$ solution at 220 rpm in glass vials on a flatbed shaker. The suspensions were filtered through Whatman 42 filter paper which was then washed through with an additional 8 mL $CaCl_2$ solution that was added to the filtrate prior to analysis for Zn. Potential desorption in the earthworm gut was investigated using a synthetic oxic earthworm gut with the following enzyme activity per mL: amylase 675 U; cellulase 186 U, phosphatase 37 U, trypsin 250000 U.[56] Smith et al.[56] used a 2:1 (v:w) liquid to soil ratio based on the moisture content of earthworm guts. We used 1.2 mL of solution and 0.6 g soil. However, the low density of plastic relative to soil meant that this mass of microplastic had a high volume compared to soil. Therefore we decided to use a volume of microplastic (0.5 mL) equivalent to the volume of 0.6 mg of soil. This gave a mass of 0.05 g microplastic in our desorption experiments. As desorption in the extract might be a function of mass rather than volume of solid in contact with solution, we also performed experiments using 0.05 g of soil for comparison with the microplastic extractions. The soil-synthetic gut mixes were shaken for 3.5 hours at 220 rpm in glass vials on a flatbed shaker and then centrifuged for 15 minutes at 16500 rcf. One millilitre of solution was pipetted out and filtered through a Whatman 42 filter paper which was then washed through with 19 mL of 5% HNO_3 which was added to the initial filtrate. Solutions were analysed for Zn.

Earthworm exposure experiments

In order to assess earthworm exposure to metal-bearing microplastics, *Lumbricus terrestris* earthworms were individually exposed to Zn-adsorbed microplastics. The exposure experiments were carried out using only arable soil to minimise the number of live earthworms used in our experiments. The arable soil was selected for the experiments on the basis of results from the adsorption / desorption experiments; it represents the worst case scenario for exposure to microplastic-adsorbed Zn as in the soil-microplastic adsorption experiments there was less preferential adsorption of Zn to the soil relative to the microplastic for the arable soil compared to the woodland soil. Clitellate, i.e. sexually mature individuals with a clitellum, *L. terrestris* weighing 5.40 $\pm$ 1.16 g (n = 24, $\pm$ standard deviation) were obtained from Worms Direct (Drylands, Ulting, Nr Maldon, Essex, CM9 6QS, United

Kingdom). Determining appropriate realistic exposure levels is difficult as there are no standard methods for quantifying microplastic levels in soils. In our experiments we used a soil microplastic content of 0.35 % by mass, similar to those reported in Huerta Lwanga et al.[15] for soils with a c. 40% surface coverage of plastic bags. 0.7 g of either control or Zn adsorbed microplastics (236, 1261 and 4505 mg kg^{-1} as prepared for the desorption experiments) were mixed into 200 g air dried arable soil which was then moistened with 60 g deionised water. The soils were placed in ziplock bags which were placed inside 480 cm^3 plastic drinking cups. Adding the Zn adsorbed microplastics leads to a slight increase in the total Zn concentration in the soil. To control for this affect, a further set of treatments, in which $Zn(NO_3)_2$ solution was added to the arable soil were prepared. These treatments increased the total Zn content of the soil to the same extent as the additions of the Zn-adsorbed microplastics (i.e. by 0, 0.6 (CS1), 3.4 (CS2) and 12 (CS3) mg kg^{-1}) (Table S4). Concentrations were increased to a greater extent than our target but in statistical analysis of our results where the increased concentration in Zn due to either the Zn-adsorbed plastics (C1 – C3) or Zn amendments to soil (CS1 – CS3) is treated as a factor in Analysis of Variance (ANOVA) we assume that the increases are the same (i.e. C1 = CS1, C2 = CS2 and C3 = CS3). Treatments were prepared in triplicate. Individual *L. terrestris* were depurated for 2 days,[57] weighed and then added to each replicate. We chose not to feed the earthworms during the experiment as this may have reduced rates of soil ingestion and / or complicated data interpretation. The earthworms were exposed for 28 days at 10 °C; the mass of each replicate was measured daily for the first week and then once weekly for the remainder of the experiment. No significant mass loss occurred so no additional water was added to the treatments. After 28 days earthworms were removed, depurated for two days and weighed. Toxicity was assessed by mortality and weight change. Earthworms were then killed by freezing, defrosted and the posterior dissected to separate out the gut plus chloragog section from other body tissues and skin. The chloragog is a diffuse tissue that surrounds the gut and which containts chloragosome granules which concentrate potentially toxic metals.[58-62] The two fractions (i.e. gut plus chloragog; body tissues) were digested and analysed for Zn (c. 1 g of air dried tissue was digested overnight in 10 mL concentrated nitric acid and then further digested fo 6 – 8 hours at 90 °C, see Supporting Information for details). The microplastics do not dissolve in nitric acid and therefore the digestate was examined to determine whether earthworms retained microplastics in their gut. The depurate is the soil that earthworms excrete and which forms casts. The mass of depurate produced

over two days was weighed. Microplastics in the depurate and also the bulk soil were picked out from the soil by hand, washed in deionised water in an ultra-sonic bath for 5 minutes, air-dried, weighed and digested for Zn analysis. However, due to the low mass of microplastic recovered, there was insufficient Zn after digestion for detection by ICP-OES (See Supporting Information).

Analysis and Quality control

All solutions were analysed for Zn using a Thermo Scientific iCAP 7000 inductively coupled plasma-optical emission spectrometer (ICP-OES). Quality control data for chemical analysis associated with each set of experiments are provided in the Supporting information (Table S8). Statistical analysis was carried out using SigmaPlot for Windows 12.0.

Results and Discussion

Adsorption experiments

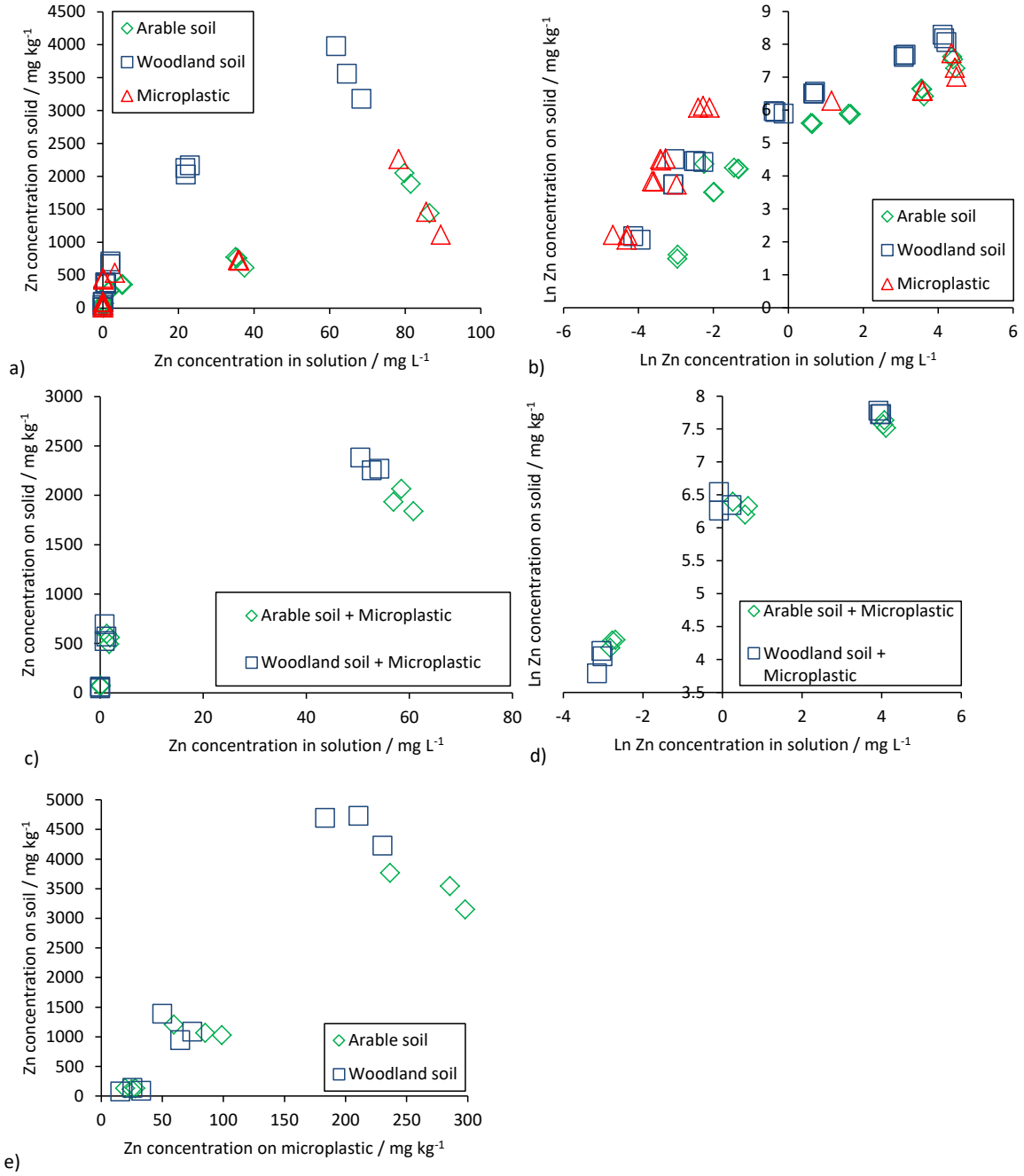
When present as the only solid in the adsorption experiment both the soils and the microplastics adsorbed similar amounts of Zn (Fig. 1, Table 1). These data support the relatively few other studies that show that microplastics have the potential to adsorb metals.[45-48] Although both Langmuir and Freundlich isotherms described the data well, the fits to the Langmuir isotherm gave a negative value for the maximum adsorption capacity and are therefore not reported here. Values of 1/*n* for the plastic are similar to those reported for polyethylene pellets and a range of other metals[45, 46] but values of K_f are far higher, possibly reflecting differences in the surface chemistry of the plastics, particle size or surface area of the experimental material or the higher ionic strength of the filtered seawater used by Holmes et al. [45, 46] The higher level of adsorption exhibited by the woodland soil is most likely due to its higher organic matter content relative to the arable soil.[50] The adsorption experiments demonstrate that there is the potential for microplastics to accumulate metals and therefore be a source of exposure to metals for soil fauna.

When both soil and microplastic was present in the sorption experiment, overall adsorption and the Freundlich parameters were similar to the adsorption experiments when only soil or plastic was present. However, the digestion data indicate that the concentration of Zn adsorbed to the soil is far greater than that sorbed to the plastic. On average adsorbed Zn concentrations are 13.0 $\pm$ 7.6 (n = 18, $\pm$ standard deviation) times greater on the soil than on the plastic. Although the mean ratio of Zn concentration on soil to plastic is 15.1 $\pm$ 9.3 for the woodland soil and 10.9 $\pm$ 5.2 for the arable soil these are not significantly different (t test, $p = 0.12$). The data suggest that at higher concentrations of adsorbed Zn the extent of preferential concentration of adsorbed Zn on soils increases. There are no significant differences in the ratio of concentration of adsorbed on the soil to plastic between the arable and woodland soils at plastic concentrations of < c. 50 mg kg^{-1} and > c. 50 mg kg^{-1} (t tests, $p \geq 0.08$). Combining the data for both soils, the soil to plastic ratio is greater at plastic Zn concentrations above c. 50 mg kg^{-1} (17.1 $\pm$ 5.8, n = 12) compared to the ratio at plastic Zn concentrations below c. 50 mg kg^{-1} (4.7 $\pm$ 1.3, n = 6) (Mann-Whitney test, $p \leq 0.01$). These data suggest that although microplastics exposed to Zn in soils have the potential to sorb the metal, competitive adsorption with soil particles will result in relatively low levels of metal sorption to the microplastics, particularly at high Zn loadings, thereby reducing the extent of possible exposure to Zn via microplastics for soil organisms.

Table 1. Freundlich isotherm parameters for Zn adsorption to the Arable and Woodland soils and the microplastics. 95% confidence intervals are given in brackets. The Freundlich equation is expressed as $C_s = K_f C_{aq}^{1/n}$ where C_s = concentration adsorbed to the solid at equilibrium, C_{aq} = concentration in solution at equilibrium; K_f and n are constants.

	Ln K_f	1 / n	R^2	P
Arable soil	4.72 (4.43 – 5.01)	0.63 (0.52 – 0.74)	0.89	$\leq$ 0.001
Woodland soil	5.76 (5.54 – 5.98)	0.65 (0.57 – 0.73)	0.94	$\leq$ 0.001
Microplastic	5.49 (5.01 – 5.96)	0.43 (0.30 – 0.56)	0.72	$\leq$ 0.001
Arable soil + microplastic	5.75 (5.53 – 5.97)	0.48 (0.41 – 0.56)	0.96	$\leq$ 0.001
Woodland soil + microplastic	5.88 (5.54 – 6.21)	0.53 (0.41 – 0.64)	0.94	$\leq$ 0.01

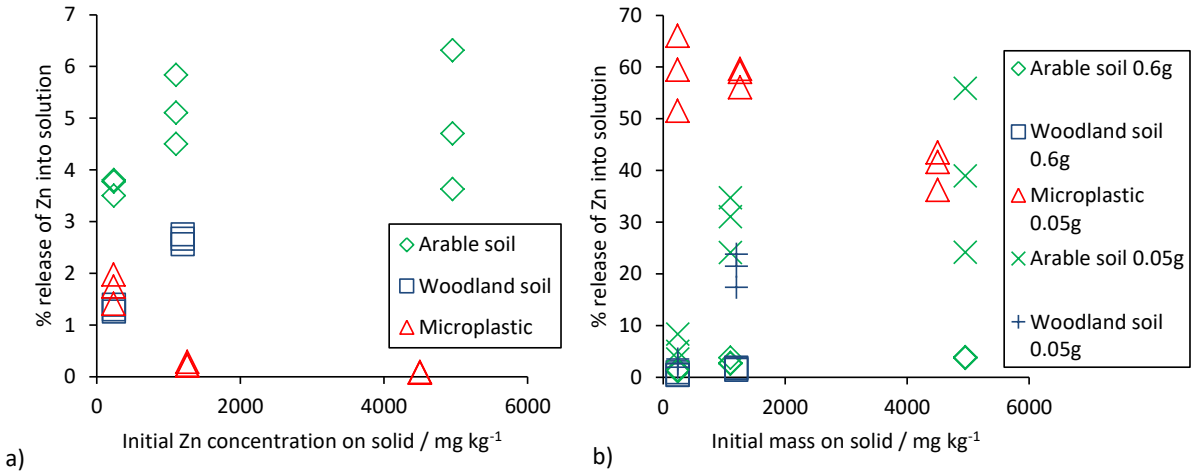
Fig 1. (a) Adsorption data for Arable soil, Woodland soil and microplastics. (b) Freundlich isotherms for Arable soil, Woodland soil and microplastics, (c) adsorption data for mixed Arable soil + microplastics and Woodland soil + microplastics, (d) Freundlich isotherms for mixed Arable soil + microplastics and Woodland soil + microplastics, (e) concentrations of Zn adsorbed to Arable soil and microplastic and Woodland soil and microplastic in mixed adsorption experiments.



Desorption experiments

Very little desorption was measured for the 0.01M $CaCl_2$ solution experiments (Fig. 2), although the soils did desorb more Zn (3.5 ±1.6%, n = 15, ± standard deviation) than the microplastics (0.7±0.8%, n = 9, ± standard deviation) (t test, $p \leq 0.001$). The low % desorption values suggest that once adsorbed, Zn would remain associated with microplastics in soil systems. In contrast, more desorption, particularly from the microplastics was measured in the synthetic earthworm gut. For samples of the same volume (0.6 g soil, 0.05 g microplastic) at < 2000 mg Zn kg^{-1} metal loadings, 30 times more Zn was found to desorb from the plastic (59 ± 5 %, n = 6, ± standard deviation) than soil (2 ± 1 %, n = 12, ± standard deviation) (Mann Whitney test, $p \leq 0.01$). This was also observed at c. 5000 mg Zn kg^{-1} metal loadings; 10 times more Zn was found to desorb from the plastic (40 ± 4 %, n = 3, ± standard deviation) than soil (4 ± 0.1 %, n = 3, ± standard deviation) (t-test, $p \leq 0.01$). Desorption was more comparable for samples of the same mass (0.05 g soil or plastic); at < 2000 mg Zn kg^{-1} metal loadings, 4 times more Zn was found to desorb from the plastic (59 ± 5 %, n = 6, ± standard deviation) than soil; 15 ± 12 % (n = 12, ± standard deviation) (Mann Whitney test, $p \leq 0.01$) but above 2000 mg kg^{-1} there was no significant difference in the amount of Zn that desorbed (Mann Whitney test, p = 1.00) (40 ± 4 %, for plastic; 40 ± 16% for Arable soil; n = 3, ± standard deviation for each). Collectively, these results suggest that, at least for Zn loadings up to 2000 mg kg^{-1}, the Zn adsorbed to microplastics may be more available to earthworms than that adsorbed to soil particles. Therefore if microplastics do become loaded with Zn this has the potential to be a significant exposure pathway for earthworms.

Fig. 2. Desorption of Zn from soils and plastic in a) 0.01M $CaCl_2$ and b) a synthetic oxic earthworm gut.



Earthworm exposure experiments

Concentrations of Zn adsorbed to the plastic and the increases in soil Zn content due to addition of $Zn(NO_3)_2$ to the soil used in the exposure experiments are reported in the Supporting information (Tables S3 and S4 respectively). Two earthworms either died or escaped from the exposure experiments, one from a replicate for the arable soil with no plastic or Zn amendment treatment and one from a replicate for the highest concentration $Zn(NO_3)_2$-amended treatment. Earthworms lost weight over the duration of the experiment in all treatments (19 ± 7.7 %, n = 22, ± standard deviation; Table S5), most likely because they were not fed during the experiment but there were no significant differences in weight loss experienced by earthworms between the different treatments ($p \geq 0.05$, Two way analysis of variance with increased Zn concentration in the soil (Control, C1 / CS1, C2 / CS2 and C3 / CS3) and source of metal (microplastic or $Zn(NO_3)_2$) as factors). The lack of any survival or weight loss effect on the earthworms at the bulk Zn concentrations used was not unexpected given that the bulk concentration of Zn used in these experiments were below those reported to be toxic to earthworms.[54, 63] Similarly, Huerta Lwanga et al.[42] recorded no toxic effects at higher microplastic concentrations than those used here (7% plastic). Analysis of the earthworm tissues indicated the presence of Zn (Table S6). Zn concentration in the gut plus chloragog fraction was higher compared to the "other tissues" fraction (1698 ± 808 mg kg^{-1} vs 362 ± 232 mg kg^{-1}, n = 21 for each fraction, ±

304 standard deviation, $p \leq 0.01$, Holm-Sidak pair wise comparison) but there were no significant
305 differences in Zn load associated with either the source of the Zn (microplastic or $Zn(NO_3)_2$) or the
306 increased Zn concentration in the soil (Control, C1/CS1, C2/CS3 or C3/CS3) ($p > 0.05$, three way
307 analysis of variance). Zn is a highly regulated essential element in earthworms that is concentrated in
308 the chloragog[58-62] and the tissue concentrations recorded in our study are similar to those reported for
309 earthworms collected from uncontaminated and Zn-enriched soils.[62, 64] In addition the high
310 concentration in the gut plus chloragog fraction compared to the "other tissues" fraction was seen in
311 both the control and Zn-exposed earthworms and, following digestion, no microplastic fragments were
312 observed in the digestate. Thus the high Zn concentrations in the gut plus chloragog fraction appear
313 to be due to "normal" earthworm metabolic processes and not the accumulation of Zn-laden
314 microplastics in the earthworm gut.

315

316 The average mass of depurate recovered from the treatments was 0.28 ± 0.12 g ($n = 21$, $\pm$ standard
317 deviation, Table S7). There was no significant difference between the mass of depurate recovered
318 between treatments ($p \geq 0.05$, Two way analysis of variance with increased Zn concentration (Control,
319 C1/CS1, C2/CS2, C3/CS3) and source of metal (microplastic or $Zn(NO_3)_2$) as factors). This is
320 consistent with Huerta Lwanga et al.[42] who observed no impact on soil ingestion rate between soils
321 with a control leaf litter covering and soils with leaf litter containing 7% microplastic. The average
322 mass of microplastic recovered from the bulk soil and depurate was 0.0056 ± 0.0028 g of microplastic
323 per gram of soil ($n = 22$, $\pm$ standard deviation) and there were no significant differences in mass of
324 microplastic recovered either between bulk soil and depurate or between the different metal loads ($p \geq$
325 0.05, Two way analysis of variance). This suggests that there was no preferential ingestion or
326 avoidance of the microplastics as earthworms ingested soil. This is perhaps surprising: firstly,
327 assuming that Zn did not desorb from the plastic when added to the soil (we were unable to confirm
328 this by measurement due to low masses of microplastic recovered from the soil but this assumption is
329 supported by our 0.01M $CaCl_2$ extraction data) the higher concentrations of Zn on the microplastics
330 (1261 and 4505 mg kg^{-1}) are within the range of Zn soil concentrations reported to have a toxic effect
331 on earthworms[54] and higher than soil concentrations in which avoidance of Zn-amended[51, 52] and field-
332 contaminated[53, 65] soils by earthworms have been observed; and secondly, several studies suggest

that earthworms are able to express preferences both for the particle size of ingested material and the actual material ingested.[66-70]

Thus in our experiments there was no evidence for either preferential ingestion or avoidance of microplastics by *L. terrestris* at Zn loadings up to 4505 mg kg^{-1} suggesting that earthworms could be exposed to metals by ingesting Zn-bearing plastics. However, ingestion of Zn-laden microplastics at these concentrations had no significant effect on survival or body weight though we note that any subtle effects due to microplastic ingestion may have been masked by the weight loss experienced by all the earthworms due to our not feeding them over the duration of the experiment.

Another question of environmental relevance is whether Zn was concentrated in cast material due to the presence of Zn-laden microplastics. A conceptual mass balance calculation using our data suggests that this was not the case. In our experimental design, for each Zn-loaded, microplastic-amended treatment there was a $Zn(NO_3)_2$-amended treatment to give an equivalent bulk Zn concentration. The lack of a significant difference between treatments for the mass of depurate recovered from earthworms indicates the same rate of soil ingestion and excretion between treatments. The lack of a significant difference in the concentration of microplastics in the depurate and microplastic-amended bulk soil indicates no avoidance or preferential ingestion of the microplastics. As the earthworms did not accumulate Zn over the course of the experiment, mass balance indicates that the earthworms must have excreted the same mass of Zn as they ingested and that this was the same between the equivalent microplastic-amended and $Zn(NO_3)_2$-amended treatments that had the same bulk Zn concentration. Thus the bulk Zn concentration in the depurate (and casts) from the microplastic-amended and $Zn(NO_3)_2$-amended treatments would have been the same. Given that earthworm activity typically increases metal availability[71] it would be interesting to determine whether passage through the gut changes the partitioning of Zn between the soil and microplastic and / or the availability in the casts. However, although we extracted microplastics from the depurate, there was insufficient mass for Zn analysis so we are unable to answer this question.

Environmental implications

Our co-adsorption data show that soil concentrates Zn relative to microplastics by a factor of 5 – 20. Once adsorbed by the microplastics or soil the Zn is unlikely to desorb into the soil solution (0.01 M $CaCl_2$ extractions). In contrast, the synthetic earthworm gut extractions performed on equal volumes of soil and microplastics suggest Zn is 10-30 times more likely to desorb from microplastics than soil implying microplastics could increase the bioavailability of metals. However, when the adsorption and desorption characteristics are compared they approximately balance each other, suggesting that, as with the exposure of aquatic organisms to organic chemicals due to microplastic ingestion,exposure of earthworms to Zn would be relatively unaffected by microplastics. [44]When the synthetic earthworm gut extractions were carried out on the same mass of material, Zn adsorbed to the plastic was determined as only being up to 4 times more available than that adsorbed to the soil suggesting that the presence of microplastics could reduce the exposure of earthworms to Zn..

Our exposure experiment and analysis of microplastic content of depurate suggests that earthworms do not avoid the ingestion of Zn-laden microplastics but also that there is no preferential ingestion of microplastics. Thus scenarios of ingestion of equal amounts of soil and microplastics appear to be plausible since Huerta Lwanga et al.[15] report microplastic contents of litter layer, where *L. terrestris* feed, of up to 40%. Under these scenarios our data of relative greater adsorption of Zn to soils than microplastic and relatively greater bioavailability of adsorbed Zn to earthworms from microplastics than soils suggests that ingestion of microplastics is unlikely to increase earthworm exposure to Zn.

Our study highlights the potential for plastics to act as vectors for increasing uptake of metals in terrestrial environments highlighting the need for a wider range of metals, particularly non-essential metals that earthworms (and other organisms) are less able to regulate, together with a wider range of plastic feedstocks with potentially different surface chemistries and sorption characteristics, to be investigated. Further, smaller particles than those investigated here will have a higher surface area to mass ratio which may impact on relative adsorption between soil and microplastics such that the impact of particle size also warrants further investigation. Finally, changes in metal partitioning between microplastics and soil and consequent changes in metal bioavailability in casts relative to bulk soil due to passage through the earthworm gut may also warrant investigation.

Supporting information

Material characterisation methods; Earthworm synthetic gut method; Results from temporal adsorption experiments; Solution Zn concentrations used in adsorption experiments; Solid Zn concentrations used in desorption experiments; Zn concentration increases in earthworm exposure experiments; Earthworm weight changes in earthworm exposure experiments; Earthworm Zn concentrations in earthworm exposure experiments; Mass of depurate and microplastic content in earthworm exposure experiments;Quality control data

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