

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

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15 **Table of Contents Graphic**



16

17 **Abstract**

18 Microplastics are widespread contaminants in terrestrial environments but comparatively little is
19 known about interactions between microplastics and common terrestrial contaminants such as zinc
20 (Zn). In adsorption experiments fragmented HDPE bags c. 1 mm² in size showed similar sorption
21 characteristics to soil. However, when present in combination with soil, concentrations of adsorbed Zn
22 on a per mass basis were over an order of magnitude lower on microplastics . Desorption of the Zn
23 was minimal from both microplastics and soil in synthetic soil solution (0.01 M CaCl₂), but in synthetic
24 earthworm guts desorption was higher from microplastics (40 – 60%) than soil (2 – 15 %), suggesting
25 microplastics could increase Zn bioavailability. Individual *Lumbricus terrestris* earthworms exposed for
26 28 days in mesocosms of 260 g moist soil containing 0.35 wt% of Zn-bearing microplastic (236-4505
27 mg kg⁻¹) ingested the microplastics, but there was no evidence of Zn accumulation, mortality or weight
28 change. Digestion of the earthworms showed that they did not retain microplastics in their gut. These
29 findings indicate that microplastics could act as vectors to increase metal exposure in earthworms, but
30 that the associated risk is unlikely to be significant for essential metals such as Zn that are well
31 regulated by metabolic processes.

32

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

34

35 **Introduction**

36 Plastics are currently estimated to constitute up to 54 % (by mass) of anthropogenic waste materials
37 released to the environment¹ and plastic debris is reported to be a prevalent pollutant in aquatic
38 environments throughout the world.¹⁻⁹ Although, less widely reported, plastics may also accumulate in
39 terrestrial environments from a range of sources, such as laundry dust, paint flakes, car tyre debris,
40 sewage sludge, wind-blown dust from landfills, and agricultural plastic sheeting used to cover soil.¹⁰⁻¹⁴
41 Nizetto et al.¹⁴ estimate average and maximum annual additions of plastic particles to soil via sewage
42 sludge in Europe at 0.2 and 8 mg ha⁻¹ yr⁻¹ per person but do not estimate absolute loadings. Huerta
43 Lwanga et al.¹⁵ state that bioturbation of soil with up to 40 % deliberate surface coverage of plastic
44 bags results in plastic contents of 0.2 -1.2 % in the soil. Conventional high-density polyethylene
45 (HDPE) bags are the lightweight single-use carrier bags used in almost all UK supermarkets; in 2014
46 it was estimated that 7.6 billion single-use plastic bags were given to customers by major
47 supermarkets in England.¹⁶ Most single-use bags ultimately end up in landfill, but they are also
48 common items of litter in urban and rural environments, especially along roadsides, where they can
49 come into contact with other common roadside contaminants such as metals.^{17, 18}

50 Once in the environment, UV radiation and high temperatures can lead to fragmentation of plastic
51 debris and the formation of microscopic particles (microplastics [MPs]; ≤5 mm plastic particles)¹⁹
52 which, due to their small size, can be ingested by a range of aquatic invertebrates²⁰⁻²⁸ and
53 vertebrates.²⁹⁻³⁶ Laboratory exposures, using aquatic invertebrates and fish suggest that this ingestion
54 can lead to blockages in the digestive tract, inflammatory responses and reduced feeding due to
55 plastic particles replacing digestible food.^{26, 34, 37-40} Comparable studies on the impacts of microplastics
56 on soil organisms are remarkably scarce,⁴¹ however, investigations by Huerta Lwanga et al.^{15,42}
57 detected a significant decrease in weight and an increase in mortality for *L. terrestris* exposed to
58 microplastic (< 150 µm low density polyethylene) loadings of ≥ 28 % by mass in the litter layer of soils.
59 Huerta Lwanga et al.⁴² also observed a decrease in particle size of microplastics between the
60 microplastic-loaded litter and earthworm casts which suggests either preferential ingestion of the
61 smaller particles, retention of larger particles in the earthworm gut subsequent to ingestion or
62 breakdown of the larger particles into smaller sizes during transit through the earthworm gut. Given

63 the essential role earthworms play in soil processes⁴³ further studies on the impacts of microplastics
64 on soil fauna are warranted.

65 In addition to the direct effects of microplastics on organisms, there is evidence that microplastics can
66 act as vectors to transport other pollutants into organisms.⁴⁴⁻⁴⁷ These pollutants can adsorb to the
67 microplastics in the environment and desorb post-ingestion with potential for toxicity and / or
68 accumulation in the food chain. The majority of studies consider organic contaminants (see recent
69 review⁴⁴) but a few consider metals ⁴⁵⁻⁴⁷ and metal sorption to plastic has been reported in the
70 literature in the context of sample storage and choice of vessels in adsorption experiments.⁴⁸ The
71 impact of metal-contaminated microplastics on soil organisms has not previously been investigated.
72 However, metals can be prevalent contaminants in urban and agricultural soils⁴⁹ and are likely to co-
73 occur with plastic pollutants in these environments. Therefore it is important to consider the possible
74 interaction between metals and microplastics when evaluating the risk that both pose to terrestrial
75 organisms. If microplastics can act as vectors to increase body burdens of metals then this could act
76 to reduce the soil concentrations at which metals have a negative impact on the soil organism health.
77 Zinc occurs ubiquitously in soils; typical background concentrations lie in the range 10 – 100 mg kg⁻¹
78 and concentrations are elevated by Zn-rich soil parent materials, atmospheric deposition, and
79 applications of fertilisers, pesticides, animal manures and sewage sludge.^{49, 50} Zn is an essential
80 element for earthworms but at elevated concentrations (≥ 200 mg kg⁻¹) can have a toxic effect.⁵¹⁻⁵⁴

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

86

87 **Experimental**

88 *Materials*

89 An arable and woodland soil, (UK grid references SE 629 497 and SE 623 508 respectively), were
90 selected for use in these experiments to provide soils with contrasting organic matter contents. The
91 soils were air-dried, sieved to < 2 mm and characterised prior to use. The arable soil was a loam soil
92 (1 % clay, 48 % silt, 52 % sand) with a pH of 6.66 ± 0.04 , organic matter content of 5.87 ± 0.23 %,
93 and contained 60.9 ± 1.24 mg kg⁻¹ background Zn (n = 3, \pm standard deviation). The woodland soil
94 was a loam soil (1 % clay, 33 % silt, 67 % sand) with a pH of 6.70 ± 0.04 , organic matter content of
95 10.86 ± 0.16 %, and contained 61.1 ± 3.14 mg kg⁻¹ background Zn (n = 3, \pm standard error).
96 Characterisation methods are detailed in the Supporting information. Microplastics were obtained by
97 manually cutting up white (with a red and blue pattern) HDPE single-use plastic carrier bags obtained
98 from a UK national supermarket chain into small, irregularly shaped pieces. Average area of the
99 pieces was 0.92 ± 1.09 mm² (n = 314, \pm std dev), the average primary and secondary axis of the best
100 fitting ellipse for each particle was 1.32 ± 0.72 mm and 0.71 ± 0.43 mm respectively (see Supporting
101 Information, Table S1). The size falls below the 5mm² maximum size for plastics to be considered
102 microplastics¹⁹, is such that the material could potentially be ingested by earthworms and was large
103 enough for ease of use in the laboratory and separation from soils during experiments. The plastic
104 was confirmed to be HDPE using Fourier transform infra-red spectroscopy (FTIR) (Fig. S1) and
105 contained 143 ± 4.37 mg kg⁻¹ background Zn (n = 3, \pm standard deviation). Characterisation methods
106 are given in the Supporting information.

107

108 *Adsorption experiments*

109 Adsorption experiments were carried out to determine the potential for microplastics to adsorb Zn and
110 to compare the sorption capacity of microplastics to that of soils. Initial adsorption experiments were
111 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
112 dissolving Zn(NO₃)₂ in a background electrolyte of 0.1 M NaNO₃. Samples were shaken at 220 rpm on
113 a flatbed shaker. After 1, 3, 6, 12, 24 and 48 hours triplicate sacrificial replicates were filtered through
114 Whatman 42 filter paper and the solutions analysed for Zn. Data (see Supporting Information, Fig. S2)
115 indicate that adsorption had reached equilibrium within 24 hours and therefore this experimental
116 duration was used for further adsorption experiments.

117

118 Adsorption isotherms for the arable and woodland soil and the microplastics were constructed using
119 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
120 shaken at 220 rpm in glass vials for 24 hours on a flatbed shaker in 20 mL Zn solution (from
121 $\text{Zn}(\text{NO}_3)_2$) in a background electrolyte of 0.1M NaNO_3 to give a constant solution ionic strength. Initial
122 target Zn concentrations were in the range 0.1 to 100 mg L^{-1} with triplicate soil/plastic and solid-free
123 control treatments at each concentration. Suspensions were filtered through Whatman 42 filter paper
124 and analysed for Zn. Solid-free controls were used to measure true values of initial solution
125 concentrations (Supporting information, Table S2) and concentrations adsorbed were calculated by
126 difference between the Zn concentrations in the filtered solutions of the solid-free control and
127 soil/plastic treatments at the end of the adsorption period. Langmuir and Freundlich isotherms were
128 fitted to the adsorption data. For the mixed soil / microplastic adsorption experiments, after filtering,
129 the microplastics were separated from the soil by flotation in water, washed in deionised water in an
130 ultrasonic bath to remove adhering soil particles, air dried, weighed and then digested in concentrated
131 HNO_3 to determine their adsorbed Zn content. The adsorbed Zn content of the soil was determined by
132 mass balance considering total Zn adsorbed and the measured Zn adsorption on the microplastics.

133

134 *Desorption experiments*

135 Desorption experiments were performed to determine whether microplastics could release previously
136 sorbed Zn and to compare release rates with those from soils. To produce Zn-laden solids of three
137 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}
138 Zn solution (from $\text{Zn}(\text{NO}_3)_2$) for 24 hours. Suspensions were filtered through Whatman 42 filter paper
139 and solutions analysed for Zn. Sorbed concentrations of 236 – 7171 mg kg^{-1} Zn were determined from
140 the difference in concentrations between solid-free and solid-bearing solutions (Supporting
141 information, Table S3). The metal-loaded solids were washed in deionised water to remove residual
142 Zn solution, air-dried and stored for use in desorption experiments. At the highest Zn concentration
143 the Woodland soil adsorbed significantly more Zn than the arable soil or plastic and was therefore not
144 used in the desorption experiments as Zn loadings were not equivalent.

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Potential desorption in soil solution was investigated using 0.01M CaCl₂ solution.⁵⁵ 0.8 g solids were shaken for 2 hours in 8 mL 0.01 M CaCl₂ 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 CaCl₂ 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% HNO₃ which was added to the initial filtrate. Solutions were analysed for Zn.

164 *Earthworm exposure experiments*

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

174 Kingdom). Determining appropriate realistic exposure levels is difficult as there are no standard
175 methods for quantifying microplastic levels in soils. In our experiments we used a soil microplastic
176 content of 0.35 % by mass, similar to those reported in Huerta Lwanga et al.¹⁵ for soils with a c. 40%
177 surface coverage of plastic bags. 0.7 g of either control or Zn adsorbed microplastics (236, 1261 and
178 4505 mg kg⁻¹ as prepared for the desorption experiments) were mixed into 200 g air dried arable soil
179 which was then moistened with 60 g deionised water. The soils were placed in ziplock bags which
180 were placed inside 480 cm³ plastic drinking cups. Adding the Zn adsorbed microplastics leads to a
181 slight increase in the total Zn concentration in the soil. To control for this affect, a further set of
182 treatments, in which Zn(NO₃)₂ solution was added to the arable soil were prepared. These treatments
183 increased the total Zn content of the soil to the same extent as the additions of the Zn-adsorbed
184 microplastics (i.e. by 0, 0.6 (CS1), 3.4 (CS2) and 12 (CS3) mg kg⁻¹) (Table S4). Concentrations were
185 increased to a greater extent than our target but in statistical analysis of our results where the
186 increased concentration in Zn due to either the Zn-adsorbed plastics (C1 – C3) or Zn amendments to
187 soil (CS1 – CS3) is treated as a factor in Analysis of Variance (ANOVA) we assume that the
188 increases are the same (i.e. C1 ≡ CS1, C2 ≡ CS2 and C3 ≡ CS3). Treatments were prepared in
189 triplicate. Individual *L. terrestris* were depurated for 2 days,⁵⁷ weighed and then added to each
190 replicate. We chose not to feed the earthworms during the experiment as this may have reduced rates
191 of soil ingestion and / or complicated data interpretation. The earthworms were exposed for 28 days
192 at 10 °C; the mass of each replicate was measured daily for the first week and then once weekly for
193 the remainder of the experiment. No significant mass loss occurred so no additional water was added
194 to the treatments. After 28 days earthworms were removed, depurated for two days and weighed.
195 Toxicity was assessed by mortality and weight change. Earthworms were then killed by freezing,
196 defrosted and the posterior dissected to separate out the gut plus chloragog section from other body
197 tissues and skin. The chloragog is a diffuse tissue that surrounds the gut and which contains
198 chloragosome granules which concentrate potentially toxic metals.⁵⁸⁻⁶² The two fractions (i.e. gut plus
199 chloragog; body tissues) were digested and analysed for Zn (c. 1 g of air dried tissue was digested
200 overnight in 10 mL concentrated nitric acid and then further digested fo 6 – 8 hours at 90 °C, see
201 Supporting Information for details). The microplastics do not dissolve in nitric acid and therefore the
202 digestate was examined to determine whether earthworms retained microplastics in their gut. The
203 depurate is the soil that earthworms excrete and which forms casts. The mass of depurate produced

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

208

209 *Analysis and Quality control*

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

214

215 **Results and Discussion**

216 *Adsorption experiments*

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

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230 When both soil and microplastic was present in the sorption experiment, overall adsorption and the
 231 Freundlich parameters were similar to the adsorption experiments when only soil or plastic was
 232 present. However, the digestion data indicate that the concentration of Zn adsorbed to the soil is far
 233 greater than that sorbed to the plastic. On average adsorbed Zn concentrations are 13.0 ± 7.6 ($n =$
 234 18 , \pm standard deviation) times greater on the soil than on the plastic. Although the mean ratio of Zn
 235 concentration on soil to plastic is 15.1 ± 9.3 for the woodland soil and 10.9 ± 5.2 for the arable soil
 236 these are not significantly different (t test, $p = 0.12$). The data suggest that at higher concentrations of
 237 adsorbed Zn the extent of preferential concentration of adsorbed Zn on soils increases. There are no
 238 significant differences in the ratio of concentration of adsorbed on the soil to plastic between the
 239 arable and woodland soils at plastic concentrations of $< c. 50 \text{ mg kg}^{-1}$ and $> c. 50 \text{ mg kg}^{-1}$ (t tests, $p \geq$
 240 0.08). Combining the data for both soils, the soil to plastic ratio is greater at plastic Zn concentrations
 241 above $c. 50 \text{ mg kg}^{-1}$ (17.1 ± 5.8 , $n = 12$) compared to the ratio at plastic Zn concentrations below $c. 50$
 242 mg kg^{-1} (4.7 ± 1.3 , $n = 6$) (Mann-Whitney test, $p \leq 0.01$). These data suggest that although
 243 microplastics exposed to Zn in soils have the potential to sorb the metal, competitive adsorption with
 244 soil particles will result in relatively low levels of metal sorption to the microplastics, particularly at high
 245 Zn loadings, thereby reducing the extent of possible exposure to Zn via microplastics for soil
 246 organisms.

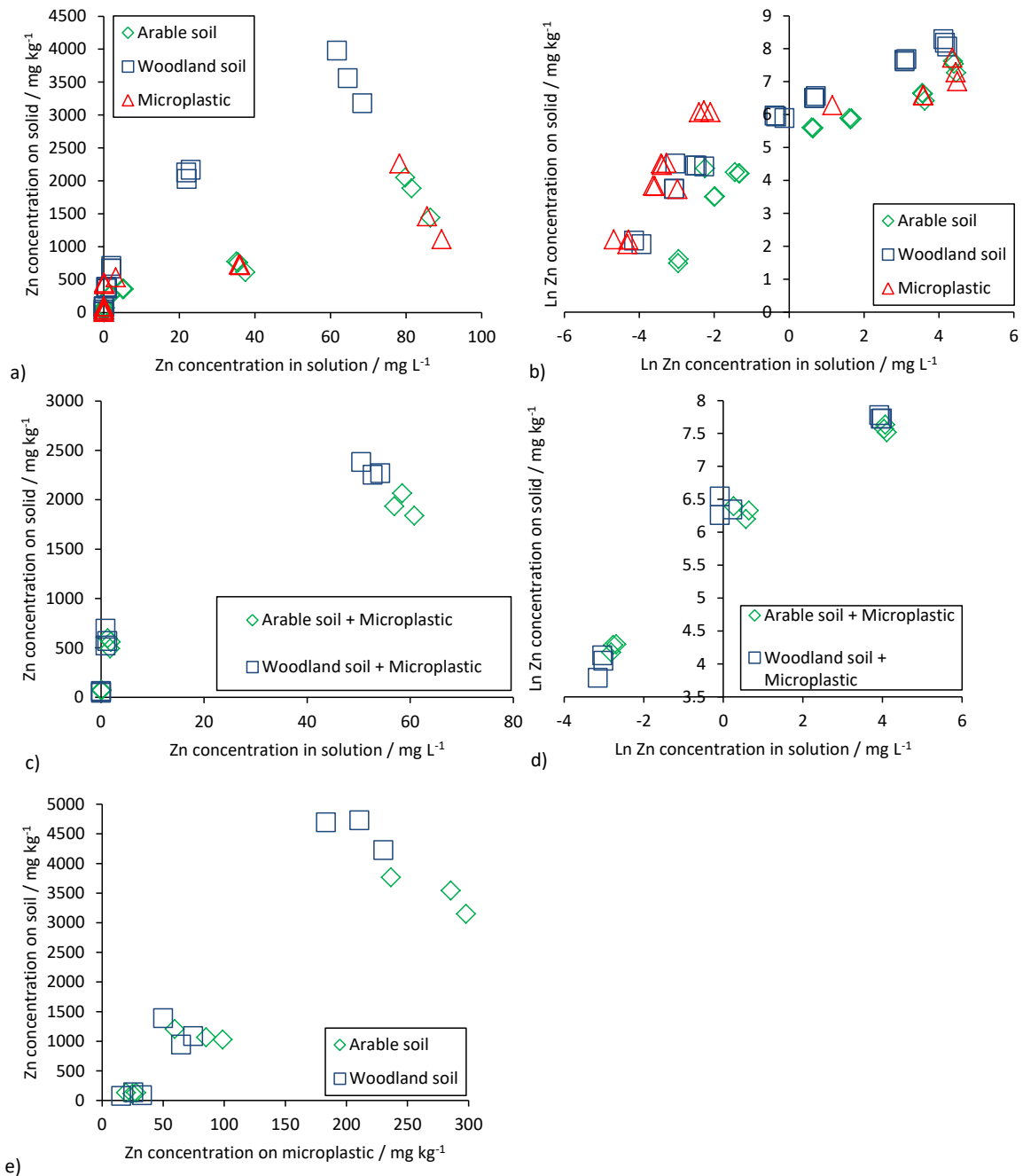
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248 Table 1. Freundlich isotherm parameters for Zn adsorption to the Arable and Woodland soils and the
 249 microplastics. 95% confidence intervals are given in brackets. The Freundlich equation is expressed
 250 as $C_s = K_f C_{aq}^{1/n}$ where C_s = concentration adsorbed to the solid at equilibrium, C_{aq} = concentration in
 251 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	≤ 0.001
Woodland soil	5.76 (5.54 – 5.98)	0.65 (0.57 – 0.73)	0.94	≤ 0.001
Microplastic	5.49 (5.01 – 5.96)	0.43 (0.30 – 0.56)	0.72	≤ 0.001
Arable soil + microplastic	5.75 (5.53 – 5.97)	0.48 (0.41 – 0.56)	0.96	≤ 0.001
Woodland soil + microplastic	5.88 (5.54 – 6.21)	0.53 (0.41 – 0.64)	0.94	≤ 0.01

252

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



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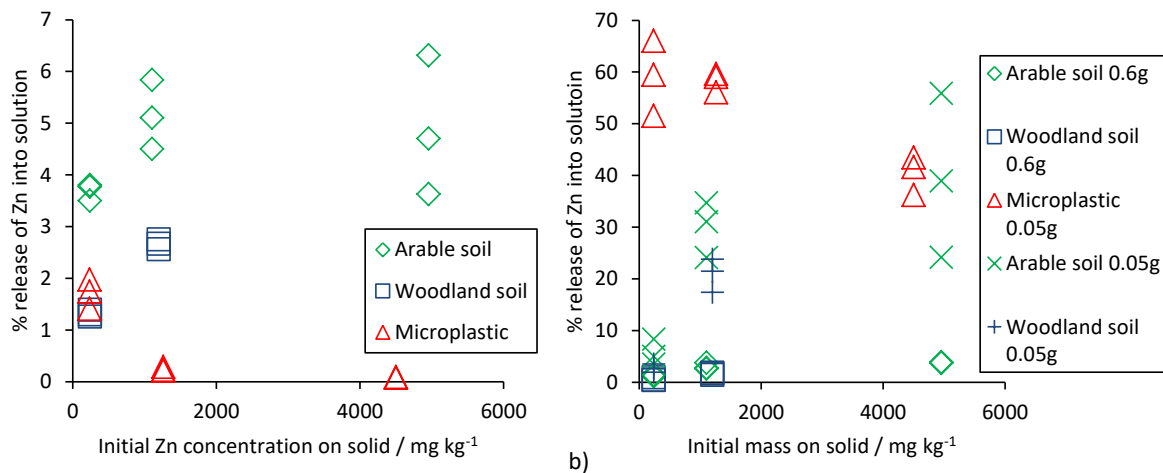
261 *Desorption experiments*

262 Very little desorption was measured for the 0.01M CaCl₂ solution experiments (Fig. 2), although the
263 soils did desorb more Zn ($3.5 \pm 1.6\%$, $n = 15$, \pm standard deviation) than the microplastics ($0.7 \pm 0.8\%$,
264 $n = 9$, \pm standard deviation) (t test, $p \leq 0.001$). The low % desorption values suggest that once
265 adsorbed, Zn would remain associated with microplastics in soil systems. In contrast, more
266 desorption, particularly from the microplastics was measured in the synthetic earthworm gut. For
267 samples of the same volume (0.6 g soil, 0.05 g microplastic) at < 2000 mg Zn kg⁻¹ metal loadings, 30
268 times more Zn was found to desorb from the plastic ($59 \pm 5 \%$, $n = 6$, \pm standard deviation) than soil
269 ($2 \pm 1 \%$, $n = 12$, \pm standard deviation) (Mann Whitney test, $p \leq 0.01$). This was also observed at c.
270 5000 mg Zn kg⁻¹ metal loadings; 10 times more Zn was found to desorb from the plastic ($40 \pm 4 \%$, n
271 $= 3$, \pm standard deviation) than soil ($4 \pm 0.1 \%$, $n = 3$, \pm standard deviation) (t-test, $p \leq 0.01$).
272 Desorption was more comparable for samples of the same mass (0.05 g soil or plastic); at < 2000 mg
273 Zn kg⁻¹ metal loadings, 4 times more Zn was found to desorb from the plastic ($59 \pm 5 \%$, $n = 6$, \pm
274 standard deviation) than soil; $15 \pm 12 \%$ ($n = 12$, \pm standard deviation) (Mann Whitney test, $p \leq 0.01$)
275 but above 2000 mg kg⁻¹ there was no significant difference in the amount of Zn that desorbed (Mann
276 Whitney test, $p = 1.00$) ($40 \pm 4 \%$, for plastic; $40 \pm 16\%$ for Arable soil; $n = 3$, \pm standard deviation for
277 each). Collectively, these results suggest that, at least for Zn loadings up to 2000 mg kg⁻¹, the Zn
278 adsorbed to microplastics may be more available to earthworms than that adsorbed to soil particles.
279 Therefore if microplastics do become loaded with Zn this has the potential to be a significant exposure
280 pathway for earthworms.

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283 Fig. 2. Desorption of Zn from soils and plastic in a) 0.01M CaCl₂ and b) a synthetic oxic earthworm
 284 gut.



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286

287 *Earthworm exposure experiments*

288 Concentrations of Zn adsorbed to the plastic and the increases in soil Zn content due to addition of
 289 Zn(NO₃)₂ to the soil used in the exposure experiments are reported in the Supporting information
 290 (Tables S3 and S4 respectively). Two earthworms either died or escaped from the exposure
 291 experiments, one from a replicate for the arable soil with no plastic or Zn amendment treatment and
 292 one from a replicate for the highest concentration Zn(NO₃)₂-amended treatment. Earthworms lost
 293 weight over the duration of the experiment in all treatments (19 ± 7.7 %, n = 22, ± standard deviation;
 294 Table S5), most likely because they were not fed during the experiment but there were no significant
 295 differences in weight loss experienced by earthworms between the different treatments (p ≥ 0.05, Two
 296 way analysis of variance with increased Zn concentration in the soil (Control, C1 / CS1, C2 / CS2 and
 297 C3 / CS3) and source of metal (microplastic or Zn(NO₃)₂) as factors). The lack of any survival or
 298 weight loss effect on the earthworms at the bulk Zn concentrations used was not unexpected given
 299 that the bulk concentration of Zn used in these experiments were below those reported to be toxic to
 300 earthworms.^{54, 63} Similarly, Huerta Lwanga et al.⁴² recorded no toxic effects at higher microplastic
 301 concentrations than those used here (7% plastic). Analysis of the earthworm tissues indicated the
 302 presence of Zn (Table S6). Zn concentration in the gut plus chloragoc fraction was higher compared
 303 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 $\text{Zn}(\text{NO}_3)_2$) 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 chloragoc⁵⁸⁻⁶² 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 chloragoc 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 chloragoc 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 $\text{Zn}(\text{NO}_3)_2$) 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 earthworms⁵⁴ 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

333 that earthworms are able to express preferences both for the particle size of ingested material and the
334 actual material ingested.⁶⁶⁻⁷⁰

335

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

342

343 Another question of environmental relevance is whether Zn was concentrated in cast material due to
344 the presence of Zn-laden microplastics. A conceptual mass balance calculation using our data
345 suggests that this was not the case. In our experimental design, for each Zn-loaded, microplastic-
346 amended treatment there was a Zn(NO₃)₂-amended treatment to give an equivalent bulk Zn
347 concentration. The lack of a significant difference between treatments for the mass of depurate
348 recovered from earthworms indicates the same rate of soil ingestion and excretion between
349 treatments. The lack of a significant difference in the concentration of microplastics in the depurate
350 and microplastic-amended bulk soil indicates no avoidance or preferential ingestion of the
351 microplastics. As the earthworms did not accumulate Zn over the course of the experiment, mass
352 balance indicates that the earthworms must have excreted the same mass of Zn as they ingested and
353 that this was the same between the equivalent microplastic-amended and Zn(NO₃)₂-amended
354 treatments that had the same bulk Zn concentration. Thus the bulk Zn concentration in the depurate
355 (and casts) from the microplastic-amended and Zn(NO₃)₂-amended treatments would have been the
356 same. Given that earthworm activity typically increases metal availability⁷¹ it would be interesting to
357 determine whether passage through the gut changes the partitioning of Zn between the soil and
358 microplastic and / or the availability in the casts. However, although we extracted microplastics from
359 the depurate, there was insufficient mass for Zn analysis so we are unable to answer this question.

360

361 *Environmental implications*

362 Our co-adsorption data show that soil concentrates Zn relative to microplastics by a factor of 5 – 20.
363 Once adsorbed by the microplastics or soil the Zn is unlikely to desorb into the soil solution (0.01 M
364 CaCl₂ extractions). In contrast, the synthetic earthworm gut extractions performed on equal volumes
365 of soil and microplastics suggest Zn is 10-30 times more likely to desorb from microplastics than soil
366 implying microplastics could increase the bioavailability of metals. However, when the adsorption and
367 desorption characteristics are compared they approximately balance each other, suggesting that, as
368 with the exposure of aquatic organisms to organic chemicals due to microplastic ingestion, exposure
369 of earthworms to Zn would be relatively unaffected by microplastics. ⁴⁴When the synthetic earthworm
370 gut extractions were carried out on the same mass of material, Zn adsorbed to the plastic was
371 determined as only being up to 4 times more available than that adsorbed to the soil suggesting that
372 the presence of microplastics could reduce the exposure of earthworms to Zn..

373

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

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

390 **Supporting information**

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

397

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404

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451 [for-2015-to-2016/single-use-plastic-carrier-bags-charge-data-in-england-for-2015-to-2016](https://www.gov.uk/government/publications/carrier-bag-charge-summary-of-data-in-england-for-2015-to-2016/single-use-plastic-carrier-bags-charge-data-in-england-for-2015-to-2016)
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