Northumbria Research Link

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

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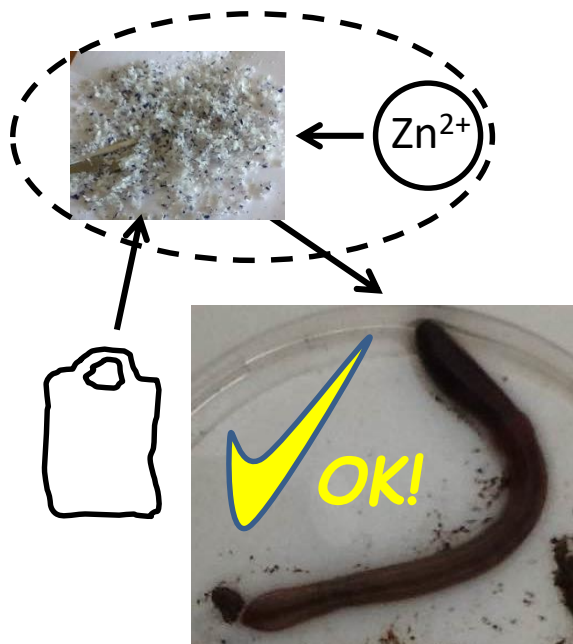
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15   **Table of Contents Graphic**

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 mm^2 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 CaCl$_2$), 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

2

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 $\geq$ 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

63　the essential role earthworms play in soil processes[43] 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　review[44]) 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 soils[49] 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]

81

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*

4

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 mm^2 (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 5mm^2 maximum size for plastics to be considered

102 microplastics[19], 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(NO$_3$)$_2$ in a background electrolyte of 0.1 M NaNO$_3$. 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 $Zn(NO_3)_2$) 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^-

138 ^{1} Zn solution (from $Zn(NO_3)_2$) 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.

145

146 Potential desorption in soil solution was investigated using 0.01M $CaCl_2$ solution.[55] 0.8 g solids were

147 shaken for 2 hours in 8 mL 0.01 M $CaCl_2$ solution at 220 rpm in glass vials on a flatbed shaker. The

148 suspensions were filtered through Whatman 42 filter paper which was then washed through with an

149 additional 8 mL $CaCl_2$ solution that was added to the filtrate prior to analysis for Zn. Potential

150 desorption in the earthworm gut was investigated using a synthetic oxic earthworm gut with the

151 following enzyme activity per mL: amylase 675 U; cellulase 186 U, phosphatase 37 U, trypsin 250000

152 U.[56] Smith et al.[56] used a 2:1 (v:w) liquid to soil ratio based on the moisture content of earthworm

153 guts. We used 1.2 mL of solution and 0.6 g soil. However, the low density of plastic relative to soil

154 meant that this mass of microplastic had a high volume compared to soil. Therefore we decided to

155 use a volume of microplastic (0.5 mL) equivalent to the volume of 0.6 mg of soil. This gave a mass of

156 0.05 g microplastic in our desorption experiments. As desorption in the extract might be a function of

157 mass rather than volume of solid in contact with solution, we also performed experiments using 0.05 g

158 of soil for comparison with the microplastic extractions. The soil-synthetic gut mixes were shaken for

159 3.5 hours at 220 rpm in glass vials on a flatbed shaker and then centrifuged for 15 minutes at 16500

160 rcf. One millilitre of solution was pipetted out and filtered through a Whatman 42 filter paper which

161 was then washed through with 19 mL of 5% HNO_3 which was added to the initial filtrate. Solutions

162 were analysed for Zn.

163

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 cm^3 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 $Zn(NO_3)_2$ 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 $\equiv$ CS1, C2 $\equiv$ CS2 and C3 $\equiv$ 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 containts

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 depurate 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.

229

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 mg kg^{-1} and > c. 50 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 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.

247

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	$\leq$ 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

252

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.
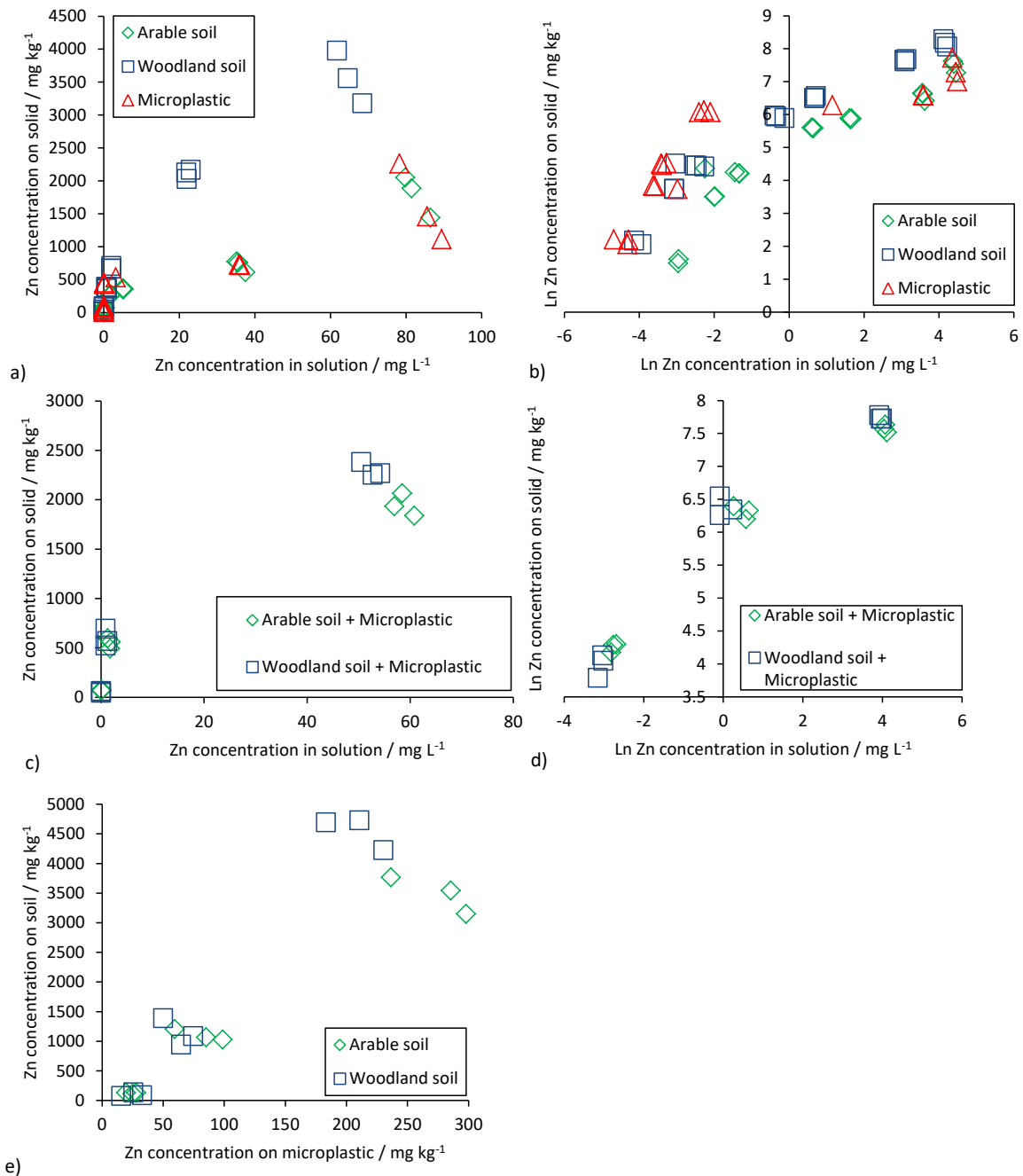
Desorption experiments

262 Very little desorption was measured for the 0.01M CaCl$_2$ 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.

281

282

283 Fig. 2. Desorption of Zn from soils and plastic in a) 0.01M CaCl$_2$ and b) a synthetic oxic earthworm

284 gut.

285 a)   b)
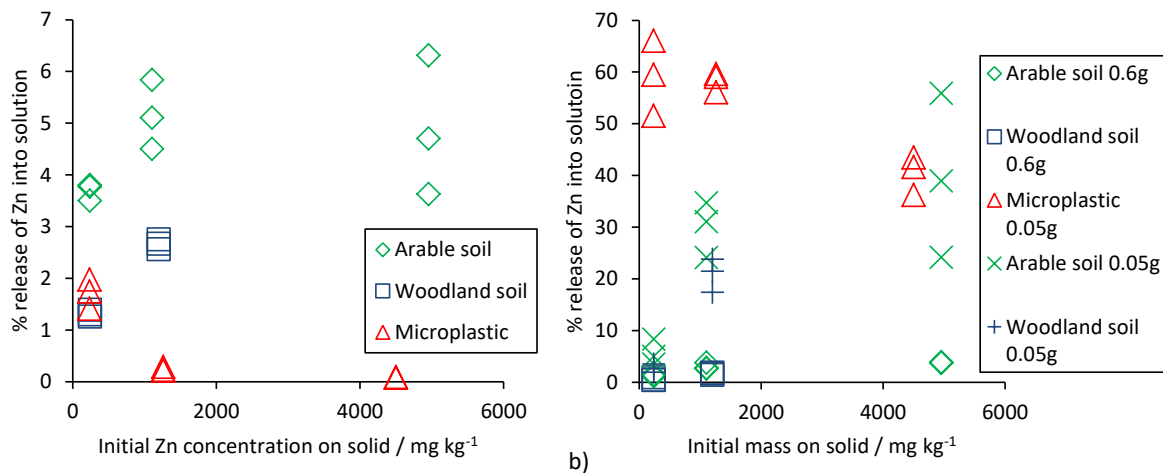
286

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(NO$_3$)$_2$ 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(NO$_3$)$_2$-amended treatment. Earthworms lost

293 weight over the duration of the experiment in all treatments (19 $\pm$ 7.7 %, n = 22, $\pm$ 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 $\geq$ 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(NO$_3$)$_2$) 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 chloragog fraction was higher compared

303 to the "other tissues" fraction (1698 $\pm$ 808 mg kg^{-1} vs 362 $\pm$ 232 mg kg^{-1}, n = 21 for each fraction, $\pm$

13

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

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 $Zn(NO_3)_2$-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 $Zn(NO_3)_2$-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 $Zn(NO_3)_2$-amended treatments would have been the

356 same. Given that earthworm activity typically increases metal availability[71] 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

15

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 CaCl$_2$ 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. [44]When 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

404

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