

1 **Impact of the earthworm *Lumbricus terrestris* (L.) on As, Cu, Pb and Zn mobility**
2 **and speciation in contaminated soils**

3

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16

17 **Abstract**

18 To assess the risks that contaminated soils pose to the environment properly a greater
19 understanding of how soil biota influence the mobility of metal(loid)s in soils is
20 required. *Lumbricus terrestris* L. were incubated in three soils contaminated with As,
21 Cu, Pb and Zn. The concentration and speciation of metal(loid)s in pore waters and the
22 mobility and partitioning in casts were compared with earthworm-free soil. Generally
23 the concentrations of water extractable metal(loid)s in earthworm casts were greater
24 than in earthworm-free soil. The impact of the earthworms on concentration and
25 speciation in pore waters was soil and metal specific and could be explained either by
26 earthworm induced changes in soil pH or soluble organic carbon. The mobilisation of
27 metal(loid)s in the environment by earthworm activity may allow for leaching or uptake
28 into biota.

29

30 **Keywords:** earthworm, metal, mobility, availability, cast

31

32 **Capsule**

33 *Lumbricus terrestris* change the partitioning of metal(loid)s between soil constituents
34 and increase the mobility of metal(loid)s in casts and and pore water.

35

36 **Introduction**

37 Human activities have resulted in an increase in the concentrations of metals and
38 metalloids in urban and rural soils due to diffuse and point source pollution. These
39 disruptions to the natural biogeochemical cycle of metals and metalloids can lead to
40 toxic effects on flora and fauna. Earthworms are found in soils containing elevated
41 levels of metals and metalloids (Spurgeon and Hopkin, 1996; Langdon et al., 2001;
42 Vijver et al., 2007) and represent a major constituent of soil fauna. Bioavailable- rather
43 than total- concentrations determine metal toxicity in soils (Harmsen, 2007) and this is
44 dependent on mobility and speciation in the living soil environment (Di Toro et al.,
45 2001; Thakali et al., 2006; Arnold et al., 2007). In order to assess properly the risks that
46 metal contamination of soil poses to the environment, a greater understanding of how
47 soil biota influence the mobility, partitioning and speciation of metals and metalloids in
48 contaminated soils is required.

49

50 Generally earthworms increase the mobility and availability of metals and metalloids in
51 soils (Sizmur and Hodson, 2009). This can result in greater concentrations of metals
52 leaching out of the soil into ground water (Tomlin et al., 1993) or greater uptake into
53 plants (Ma et al., 2003; Yu et al., 2005; Wang et al., 2006) and soil animals (Currie et
54 al., 2005; Coeurdassier et al., 2007). In addition to this, earthworms may reduce the
55 efficiency of soil remediation by mobilising recalcitrant metals (Udovic et al., 2007).
56 The mechanisms for earthworms increasing metal mobility and availability are unclear,
57 but may involve changes in microbial populations, pH, dissolved organic carbon or
58 metal speciation (Sizmur and Hodson, 2009).

59

60 Earthworms burrow in the soil and create casts that are chemically, biologically and
61 physically different from the surrounding soil (Edwards, 2004). Earthworm casts have
62 more active microbial communities than surrounding soil (Scheu, 1987); there is
63 evidence that they have a humifying capacity (Businelli et al., 1984) and contain a
64 higher concentration of soluble organic carbon compared to bulk soil (Daniel and
65 Anderson, 1992). Ireland (1975) extracted more water extractable Zn from earthworm
66 faeces compared with bulk contaminated soil and Devliegher and Verstraete (1996) give
67 evidence that gut associated processes in *Lumbricus terrestris* (L.) are responsible for
68 increases in metal availability in uncontaminated soils.

69

70 Anecic earthworms produce casts on the soil surface (Edwards and Bohlen, 1996) and
71 line their burrows with their own faeces (Binet and Curmi, 1992) leading to a potential
72 for metals and metalloids to be leached out of soils into surface waters or ground
73 waters. Therefore we carried out an experiment with a UK native anecic species (*L.*
74 *terrestris*) to determine the impact of soil passage through the earthworm gut on the
75 mobility and partitioning of metals and metalloids in casts and how this impacts on the
76 concentrations and speciation of metals and metalloids in the pore waters of earthworm-
77 inhabited soils compared to earthworm-free soils.

78

79 **Materials and methods**

80 Soil and Earthworms

81 *L. terrestris* were sourced from Worms Direct, Ulting, UK. Three contaminated soils
82 were used in this study (Table 1). Rookhope (54.780947 -2.121240; WGS84) and
83 Devon Great Consols (DGC) (50.540851 -4.226920; WGS84) soils were collected from

84 a former lead and fluorspar mine and a former copper and arsenic mine, respectively.
85 Wisley soil (51.312975 -0.474771; WGS84) was amended with Pb nitrate and Cu and
86 Zn sulphate salts 15 years ago (Alexander et al., 2006). Soil was collected from the top
87 30 cm of the soil profile and on return to the laboratory dried (40°C), sieved (<2mm),
88 homogenised and stored until the start of the experiment. Soil pH was measured in a
89 soil-water suspension (based on BS7755-3.2), percentage organic matter by loss on
90 ignition (500°C) and soil texture by laser granulometry (Coulter LS 230 Particle Size
91 Analyzer). Sand was classified as particles (2000-63µm, silt as 63-2µm and clay as <
92 2µm in diameter. Pseudototal elemental composition was determined by digestion in
93 aqua regia (based on BS7755-3.9, 1995) and cation exchange capacity was measured at
94 pH 7 using the ammonium acetate method (Rowell, 1994).

95

96 Experimental procedure

97 Single specimens of 48-hour depurated (Arnold and Hodson, 2007) *L. terrestris* (4.8g,
98 SD = 0.79, n = 75) were incubated in bags (one earthworm per bag) containing 300g
99 (dry wt.) of soil moistened to 80% of the water holding capacity (38%, 42% and 65%
100 moisture content of Rookhope, Wisley and DGC soils respectively) at 20°C in darkness
101 for 28 days alongside earthworm-free bags of moist soil. Bags were kept in vertical
102 plastic cylinders made from disposable drinking cups in order to produce columns of
103 soil at least 10 cm in depth. The surface area of the cups was 0.005 m² so the
104 earthworm density (500 m⁻²) was in the range (300-1000 m⁻²) found in temperate
105 pasture soils (Coleman et al., 2004). No food was added to bags to ensure that
106 observations made were due to the activity of the earthworms rather than the
107 incorporation of organic matter. After 28 days the bags were emptied and the soil

108 homogenised. Any bags containing dead earthworms were disposed of and the soil was
109 not used for further analysis. There were 25 bags for each treatment. Randomly selected
110 bags were pooled in groups of five to give five samples for each replicate and a total of
111 five replicates for each treatment. Earthworms were removed from the soil and their
112 guts voided on moist filter paper for 48 hours, changing the paper every 12 hours
113 (Arnold and Hodson, 2007) . The casts produced were air-dried and pooled to
114 correspond with the same replicates as the bulk earthworm-inhabited and earthworm-
115 free soil. Earthworms were re-weighed and frozen.

116

117 One gram of air dried casts, bulk soil and earthworm-free control soil of each soil type
118 was extracted with 10ml of $>18.2 \text{ M}\Omega \text{ cm}$ ultra pure water by mixing on a rotary shaker
119 for 24 hours at 30rpm at 20°C. The soil pH was measured (Jenway 3310 pH meter)
120 followed by centrifuging at 3600rpm for 10 min at 20°C. The supernatants were
121 analysed for water extractable organic carbon (WEOC) (Shimadzu TOC 5000) and
122 water extractable As, Cu, Pb and Zn by ICP-OES. The binding of metals and metalloids
123 to different soil constituents was then determined on this 1g of soil by a sequential
124 extraction following the method described by Rauret et al. (1999) to obtain the
125 partitioning between the exchangeable, reducible, oxidisable and residual fractions of
126 As, Cu, Pb and Zn by ICP-OES.

127

128 Pore water was extracted from moist bulk soil from each pooled sample by centrifuging
129 at 6000rpm for 60 min. This extracted 51 % (SD = 0.9, n = 2), 56% (SD = 3.3, n = 2)
130 and 65% (SD = 0.7, n = 2) of the soil moisture from the Rookhope, Wisley and DGC
131 soils respectively. Pore water samples were analysed for pH (Jenway 3310 pH meter),

132 elements (ICP-OES), major anions (Dionex DX-500 ion chromatograph), and Total
133 Organic Carbon (TOC) (Shimadzu TOC 5000). Speciation of Cu, Pb and Zn in pore
134 water samples was modelled using WHAM VI (Tipping, 1998). In the absence of
135 characterisation of the TOC fractions, we assumed that 50% of TOC was fulvic in
136 origin and that the fulvic acid contained 50% C (Tipping, 1996; Pribyl, 2010).

137

138 Arsenic speciation in pore waters extracted from the DGC soil was determined in a
139 separate experiment. This was carried out at the Analytical Geochemistry Laboratory at
140 British Geological Survey, Keyworth separately to the previous experiment to ensure
141 that freshly produced pore waters were analysed within 24 hours of extraction.

142 Therefore experimental and analytical procedures differed in order to match instrument
143 availability and adhere to local standard operating procedures. Five bags of DGC soil
144 containing single specimens of *L. terrestris* and five earthworm-free bags were
145 incubated for 26 days and the pore water extracted by centrifuging at 14 000rpm for 40
146 min. Arsenate (AsV), arsenite (AsIII), arsenobetaine (AB), methylarsonate (MA) and
147 dimethylarsinate (DMA) species of As were then quantitatively determined in the pore
148 water within 24 hours of extraction by HPLC-ICP-MS using the method described by
149 Watts et al (2008). Spiked recoveries in pore waters were used to ensure transformation
150 between species did not occur due to the procedure.

151

152 Statistical analysis

153 Minitab version 15 was used for all statistical analysis. Normality of data and equal
154 variance between treatments was tested using the Kolmogorov-Smirnov test ($p > 0.05$)
155 and Bartlett's test ($p > 0.05$) respectively. Data that was found not to be normal was Log

156 transformed or outliers (> 2 standard deviations away from the mean) were removed.
157 Where comparisons between treatments were made (e.g. between casts, bulk or control
158 soil or between earthworm inhabited and earthworm free soil for one variable in one soil
159 type), one-way ANOVA was carried out and Fisher's Least Significant Difference test
160 ($p < 0.05$ and $p < 0.01$) used to identify significant differences between individual means.

161

162 Quality control

163 The aqua regia digestion of soil samples was carried out alongside an in-house reference
164 material traceable to a certified reference material (BCR-143R - trace elements in a
165 sewage sludge amended soil; Commission of the European Communities, Community
166 Bureau of Reference) certified for Pb and Zn and with an indicative value for Cu.
167 Recoveries of these elements were 103%, SD = 2.4, n = 2 for Cu, 93%, SD = 4.2, n = 2
168 for Pb and 90%, SD = 0.81, n = 2 for Zn. Arsenic was below detection limits in the in-
169 house reference material ($< 14 \text{ mg kg}^{-1}$). During the ICP-OES analysis of all samples,
170 calibration standards were analysed as samples at the end of each run to ensure that drift
171 did not occur. Deviation was no greater than $\pm 4\%$ for As, Cu, Pb and Zn.

172

173 **Results**

174 Earthworm mortality was low in the contaminated soils with 100% survival in
175 Rookhope and Wisley soils and 12% mortality in the DGC soil. Earthworms in all three
176 soils lost weight during the test period. *L. terrestris* fresh weight decreased by 21%
177 (1.1g, SD = 0.88, n = 25) in Rookhope soil, 11% (0.5g, SD = 0.48, n = 25) in the Wisley
178 soil and 27% (1.3g, SD = 1.01, n = 22) in the DGC soil.

179

180 Mobility and partitioning of metals and metalloids in soil and casts

181 Generally the concentrations of water extractable metals and metalloids in earthworm
182 casts were greater than in bulk earthworm-inhabited or earthworm-free control soil (Fig.
183 1). The exception to this was the water extractable Zn which was significantly ($p < 0.01$)
184 lower than bulk and control DGC soil and significantly lower ($p < 0.01$) than bulk (but
185 not control) Rookhope soil.

186

187 Water extractable carbon and pH in the casts of all three soil types was significantly
188 ($p < 0.01$) greater than the bulk or control soil (Table 2). There were also significant
189 ($p < 0.05$) decreases in the pH of the bulk earthworm-inhabited soil compared to the
190 control Rookhope ($p < 0.05$) and Wisley ($p < 0.01$) soils and a significant ($p < 0.01$)
191 increase in the pH of the bulk DGC soil compared to the control (Table 2).

192

193 Generally, there was a shift in the partitioning of the metals and metalloids in the
194 earthworm casts from the less available fractions to the more available fractions (Table
195 S1), though the percentage changes are relatively small (Fig. 2). In the DGC casts there
196 were significantly ($p < 0.01$) greater concentrations of As in the exchangeable and
197 reducible fractions and significantly ($p < 0.01$) lower concentrations in the oxidisable
198 fraction compared with the bulk earthworm-inhabited and control soil. There were
199 greater concentrations of Pb and Zn extracted from the oxidisable fractions of the
200 Rookhope and Wisley casts compared with the control and bulk earthworm-inhabited
201 soils (Table S1). However none of the other fractions were consistently depleted to
202 compensate for this. There were no observed differences in the partitioning of the

203 metals and metalloids between the control and bulk earthworm-inhabited soils (Table
204 S1).

205

206 Mobility and speciation of metals and metalloids in pore water

207 While typically there was a greater concentration of water extractable metals and
208 metalloids in earthworm casts compared to earthworm-free control soil, this was not
209 always reflected in the pore waters. Generally Cu was lower and Pb and Zn (apart from
210 DGC Zn) was greater in earthworm-inhabited pore water compared to pore waters from
211 earthworm-free soils (Fig. 3).

212

213 Speciation modelling indicates that organic complexes and free ions of Cu, Pb and Zn
214 were the major species present in the pore waters from all three soils (67 – 100% and 0
215 – 32 %; 27 – 99 % and 1 – 72% and, 3 – 14 % and 81 – 96 % respectively, Table 3).

216 There was a greater modelled abundance of Cu ions and lower abundance of organically
217 bound Cu in earthworm inhabited Wisley soil compared to earthworm free control soil.

218 In the DGC earthworm-inhabited soil pore waters there were modelled decreases in free
219 ions of Pb^{2+} and Zn^{2+} and increases in organo-Pb and -Zn relative to the controls,

220 whereas for Wisley and Rookhope (Zn only) there were modelled increases in free Pb^{2+}
221 and Zn^{2+} and decreases in organo-Pb and -Zn. Thus for Wisley and Rookhope not only
222 are there greater concentrations of Pb and Zn in pore water due to the activity of *L.*

223 *terrestris* (Fig. 3), but also a greater proportion are in a chemical form (free ions) that is
224 potentially more available to organisms than in the earthworm-free soil (Di Toro et al.,
225 2001; Thakali et al., 2006). In the DGC soil pore waters the majority (>90%) of the As
226 was present as As(V). There was a significantly ($p < 0.01$) greater concentration of

227 arsenobetaine (AB) in the pore water from earthworm-inhabited soil pore water relative
228 to the control (Fig. 4) but when expressed as a percentage of total As, this difference is
229 not significant.

230

231 **Discussion**

232 Earthworms lost weight in all three soils used in this study. This weight loss is most
233 likely due to the absence of food supplied on the surface of the soil. Food was withheld
234 in order to ensure that any changes in metal chemistry that were observed were due to
235 the burrowing activity of the earthworms rather than the effect of mixing the food in
236 with the soil matrix. The greatest weight loss occurred in the DGC soil which was the
237 only soil which had a pH below the recommended range (4.5-7) for culturing *L.*
238 *terrestris* (Lowe and Butt, 2005). The soil organic matter content of the soils used in
239 this study were in the range of pasture soils within which *L. terrestris* reside in the UK.
240

241 Other studies have reported increased mobility of metals in earthworm casts (Kizilkaya,
242 2004; Udovic and Lestan, 2007; Udovic et al., 2007), but the impact of earthworms on
243 pore water concentrations has not previously been studied to our knowledge. The use of
244 water as a metal extracting agent is likely to yield lower metal concentrations than weak
245 salt, acid or chelating extractions. The water extractable fraction represents the most
246 available portion of the total metal concentration in soil and it can be stated with a
247 degree of certainty that this fraction is mobile. Weak salt solutions mimic soil solutions
248 but do not effectively represent the complex mix of organic and inorganic components
249 present in soil pore water. Therefore direct measurement of pore water was employed to
250 determine metal mobility in the soil solution. Many of the impacts of earthworms on

251 metal and metalloid mobility observed can be explained by earthworm induced changes
252 in soil pH (Masscheleyn et al., 1991; Temminghoff et al., 1997; Martínez and Motto,
253 2000) or WEOC (Jordan et al., 1997; Temminghoff et al., 1997; Bauer and Blodau,
254 2009).

255

256 All four elements studied here are sensitive to changes in both soil pH and WEOC.
257 However they have different affinities for binding to organic carbon. The relative
258 importance of their sensitivity to pH and WEOC determines which property governs
259 their mobility and therefore bioavailability and toxicity in soil. Cu and Pb bind more
260 readily with soluble organic carbon than Zn and therefore are more sensitive to changes
261 in WEOC. As a result Zn is more sensitive to changes in pH than Cu and Pb (McBride
262 et al., 1997). Unlike Cu, Pb and Zn, As is present in soil solutions as an oxy-anion and
263 therefore does not bind with negatively charged organic carbon. However increases in
264 WEOC increase the competition between As and dissolved organic matter for binding
265 surfaces on positively charged soil constituents such as iron and manganese oxides
266 (Bauer and Blodau, 2006). Little is known about the relative importance of an
267 increasing soil pH and increasing WEOC on the mobility of As in soils.

268

269 The greater concentration of As extracted from casts compared to both control and bulk
270 DGC soil (Fig. 1) may be due to greater pH (Masscheleyn et al., 1991) or greater
271 concentration of WEOC (Bauer and Blodau, 2009) in casts compared with both control
272 or bulk soil (Table 2). The greater concentrations of WEOC and As in the water
273 extractable, exchangeable and reducible fractions of the DGC casts and lower
274 concentrations of As in the oxidisable fraction compared with the control soil (Table

275 S1) indicates that *L. terrestris* mobilises previously sequestered As in DGC soil. It also
276 indicates that this occurs via the degradation (or oxidation) of organic matter in the soil
277 and the release of organically bound As into the pore water solution. Several other
278 authors have reported decreases in the concentrations of oxidisable metals in
279 earthworm-inhabited soils with concurrent increases in the more labile fractions (El-
280 Gharmali, 2002; Kizilkaya, 2004; Wen et al., 2004; Li et al., 2009). The significantly
281 greater concentration of arsenobetaine (AB) in the earthworm-inhabited soil pore water
282 relative to the control (Fig. 4) may be due to changes in the speciation of As in
283 earthworm tissue. AB has previously been detected in earthworm casts (Button et al.,
284 2009) and it has been suggested that it is synthesised in earthworm tissue as a
285 detoxification mechanism (Langdon et al., 2003).

286

287 The greater solubility of Cu in casts compared with control or bulk soil (Fig. 1) can be
288 explained by the higher concentration of WEOC in the casts compared with the control
289 and bulk soil (Table 2) as Cu binds strongly to organic complexes in solution
290 (Temminghoff et al., 1997). The lower concentrations of Cu in the exchangeable
291 fraction of the casts of DGC soil compared with control may indicate a movement of Cu
292 from the exchangeable fraction to the water extractable fraction (Table S1 and Fig. 1).
293 Li et al. (2009) explain an increase in Cu mobility after transit through the gut of
294 *Eisenia fetida* by the formation of ‘mini-molecule organic acids’, due to the breakdown
295 of organic matter, that have a high capacity for Cu²⁺ complexation. This process may
296 also be occurring in these soils as the earthworms degrade the organic matter and
297 release organic compounds into solution. It therefore seems that *L. terrestris* are
298 mobilising Cu that is exchangeable into solution by organic complexation rather than

299 mobilising organically bound Cu. However, there is a lower concentration of Cu in pore
300 waters from Wisley and DGC earthworm-inhabited soils (Fig. 3) despite greater TOC in
301 the DGC earthworm-inhabited pore waters and lower pH in Wisley earthworm-
302 inhabited pore waters (Table 2).

303

304 The greater solubility of Pb in the casts of all three soils compared to the control or bulk
305 soil (Fig. 1) can also be explained by the higher concentrations of WEOC (Jordan et al.,
306 1997) (Table 2). More of the Pb in the earthworm-inhabited Wisley pore water is
307 present as free ions and less in the earthworm-inhabited DGC pore water relative to
308 controls (Table 3). This is due to the lower pH in the pore waters from earthworm-
309 inhabited Wisley soils and the greater TOC in the earthworm-inhabited DGC soils
310 compared to earthworm-free soils (Table 2). The lower mobility of Zn in the casts and
311 bulk DGC soil compared with the control (Fig. 1) are probably due to increases in pH
312 (Martínez and Motto, 2000) in the casts and bulk soil compared to control (Table 2).
313 The changes in pore water pH and TOC also explain the differences in modelled Zn
314 speciation in pore waters.

315

316 Greater concentrations of Pb and Zn were extracted in the oxidisable fractions of the
317 Rookhope and Wisley casts compared with the control and bulk earthworm-inhabited
318 soils (Table S1). This is in contrast to the observations made in the DGC soil whereby
319 As moves from the oxidisable fraction to more mobile fractions in casts and to other
320 reports in the literature of oxidisable metals being mobilised due to earthworm activity
321 (El-Gharmali, 2002; Kizilkaya, 2004; Wen et al., 2004; Li et al., 2009).

322

323 The binding of metals and metalloids to different soil constituents (partitioning), affects
324 their mobility in the environment. The lack of observed differences in the partitioning of
325 the metals and metalloids between the control and bulk earthworm-inhabited soils (Fig.
326 2) may indicate that although passage through the earthworm gut has an impact on
327 metal mobility, this is a temporary effect (Lukkari et al., 2006). However, if *L. terrestris*
328 ingest 0.2 g of soil per day (Arnold and Hodson, 2007), then during the 28 day test
329 period only about 5.6 g of soil would have passed through their gut, less than 2% of the
330 soil they inhabited. Therefore a dilution effect could be occurring in these experiments
331 due to the relatively low proportion of the soil that passed through the earthworm gut in
332 relation to the total bulk soil. If one extrapolates this effect over a longer period of time,
333 earthworms may have a major impact on the partitioning and mobility of metals and
334 metalloids at contaminated sites where these effects could occur over years and decades.

335

336 The measurement and use of ‘mobile’ and ‘mobilisable’ metal concentrations has been
337 suggested and considered for use in the risk assessment of metals and metalloids in soil
338 for a reasonably long time (Gupta et al., 1996) and bioavailability now forms part of
339 many higher tier risk assessment guidelines (e.g. Fairbrother et al., 2007). However,
340 within these risk assessments no provision is made for considering the effect of soil
341 inhabitants on the mobility and therefore bioavailability of contaminants to receptors
342 and water courses. This study clearly demonstrates that soil biota impacts metal
343 mobility and speciation in soils. Therefore we recommend against the use of ‘mobile’
344 concentrations in risk assessment and instead suggest that ‘potentially mobile’ or
345 ‘potentially mobilisable’ concentrations are instead adopted to allow for the complex
346 biological interactions that take place in the living soil environment.

347

348 Conclusions

349 The impact of earthworms on metal mobility, partitioning and speciation in soils and
350 solution is both soil and metal specific and depends on whether earthworm activity
351 increases or decreases pH and the solubility of organic carbon. The speciation (and
352 therefore bioavailability) of metals leached out of contaminated soils to water courses is
353 an important consideration for risk assessment and it is clear that earthworms influence
354 this. In the soil environment the mobilisation of previously sequestered metals and
355 metalloids, even temporarily, allows for their transport from the soil into surface or
356 ground waters or to soil flora and fauna. This should be considered when risk assessing
357 metal contaminated soils. Soils should be considered in risk assessments as dynamic
358 living systems whereby the soil biota can influence the distribution, mobility and
359 therefore the bioavailability of metals and metalloids.

360

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365

366 Supporting information

367 One table is included in the Supporting Information.

368

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Table 1 Mean chemical properties of soils used for earthworm experiments (n = 3, ± standard error)

	pH ¹ (H ₂ O)	%OM (LOI) ²	Pseudo-total elements ³ (mg kg ⁻¹)				CEC ⁴ (cmol _c kg ⁻¹)	% Sand	Texture ⁵		Classification ⁶
			As	Cu	Pb	Zn			% Silt	% Clay	
Rookhope	5.9 ±0.02	7.7 ±0.20	< 14	38 ±4	4550 ±271	908 ±77	13.6 ±0.14	65.7 ±0.78	31.3 ±0.67	2.98 ±0.10	Sandy loam
Wisley	6.6 ±0.01	10.4 ±0.03	< 14	120 ±11	988 ±74	241 ±21	18.4 ±0.09	67.7 ±1.16	29.6 ±1.16	2.72 ±0.07	Sandy loam
DGC	4.1 ±0.00	15.9 ±0.03	1150 ±14	362 ±3	109 ±2	89 ±1	21.0 ±0.30	41.5 ±1.12	54.9 ±1.13	3.63 ±0.12	Silt loam

¹Based on BS7755-3.2, 1995. ²Loss on ignition ³Aqua regia extractable concentrations based on BS7755-3.9, 1995. ⁴Based on (Rowell, 1994). ⁵Laser granulometry. ⁶Using the United States Department of Agriculture soil texture triangle.

Table 2 Soil pH and water extractable organic carbon (WEOC) of control, bulk earthworm-inhabited soil and casts and pH and total organic carbon (TOC) in pore water solutions from earthworm-free control and bulk earthworm-inhabited soils after 28 days of incubation with single specimens of *Lumbricus terrestris* in three contaminated soils (n = 5, ± standard error).

		Soil pH (H ₂ O)	WEOC (mg kg ⁻¹)	Pore water pH	TOC (mg L ⁻¹)
Rookhope	Control	6.0±0.05	237±13.6	5.1±0.03	53.2±1.76
	Bulk	5.9±0.02 *	225±11.6	5.0±0.05	57.9±4.16
	Casts	6.7±0.02 ** ##	649±24.1 ** ##		
Wisley	Control	6.8±0.04	309±13.3	6.2±0.05	63.9±1.32
	Bulk	6.6±0.03 **	294±9.1	5.9±0.21	63.3±2.67
	Casts	7.1±0.02 ** ##	738±23.2 ** ##		
DGC	Control	4.6±0.01	279±20.9	4.1±0.01	168±3.64
	Bulk	4.7±0.02 **	327±6.5	4.1±0.02	175±3.90
	Casts	6.8±0.04 ** ##	1970±157 ** ##		

* = significantly different from the control at the 95% level (*) or 99% level (**) and # = significantly different from the bulk soil at the 95% level (#) or 99% level (##).

Table 3 Percentage abundance of Cu, Pb and Zn modelled using WHAM VI (Tipping, 1994) modelled to be present as free ions, organic or inorganic complexes in pore water extracted from earthworm-free control and earthworm-inhabited soils after 28 days of incubation with single specimens of *Lumbricus terrestris* in three contaminated soils (n = 5, ± standard error).

		% Cu species			Pb ²⁺	% Pb species		Zn ²⁺	% Zn species	
		Cu ²⁺	Cu-Org	Cu-Inorg		Pb- Org	Pb-Inorg		Zn- Org	Zn-Inorg
Rookhope	Control	4.15±1.03	95.8±1.04	0.07±0.02	20.4±3.26	79.2±3.36	0.42±0.10	95.25±0.07	4.17±0.10	0.74±0.06
	Earthworm	3.25±0.35	96.8±0.35	0.04±0.00	22.6±1.89	77.0±1.94	0.41±0.05	95.8±0.26**	3.55±0.29**	0.69±0.04
Wisley	Control	0.38±0.03	99.6±0.04	0.07±0.01	1.20±0.12	98.6±0.14	0.21±0.02	80.9±0.46	13.63±0.49	5.57±0.11
	Earthworm	1.10±0.53*	98.8±0.55*	0.11±0.02	2.20±0.31*	97.5±0.36*	0.35±0.08*	85.8±1.22**	9.10±1.16**	5.19±0.06*
DGC	Control	32.1±1.19	67.5±1.20	0.45±0.04	71.5±1.03	26.5±1.01	2.03±0.04	95.8±0.15	3.15±0.13	1.12±0.03
	Earthworm	26.2±2.71	73.4±2.76	0.43±0.05	65.9±1.77*	31.6±1.82*	2.66±0.08**	94.6±0.28**	3.94±0.27*	1.56±0.04**

* = abundance of free ions, organic or inorganic complexes are significantly different from the earthworm-free control soil at the 95% (*) or the 99% (**) level.

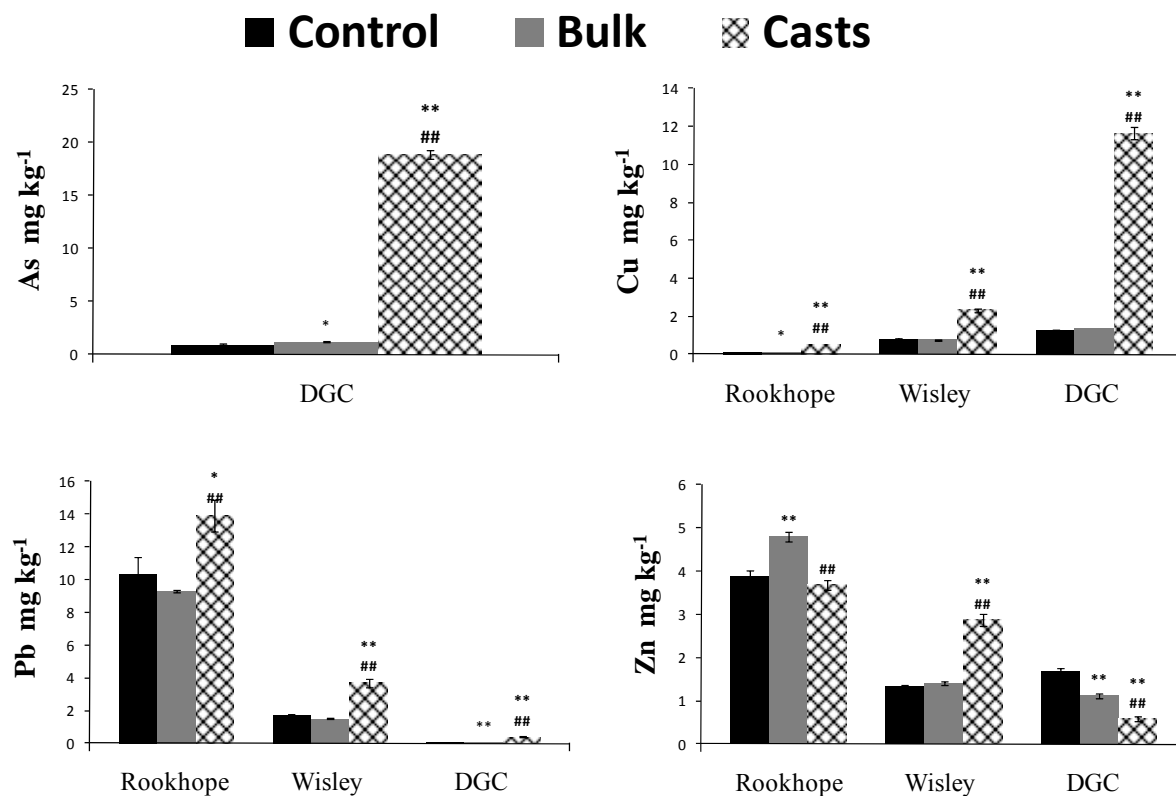


Figure 1. Water extractable As, Cu, Pb and Zn in the control soil, bulk earthworm-inhabited soil and casts of *Lumbricus terrestris* after incubation for 28 days in three contaminated soils. $n = 5$, error bars represent standard errors of the mean. * = significantly different from the control at the 95% level (*) or 99% level (**) and # = significantly different from the bulk soil at the 95% level (#) or 99% level (##). As was below detection (0.05 mg kg^{-1}) in Rookhope and Wisley soils.

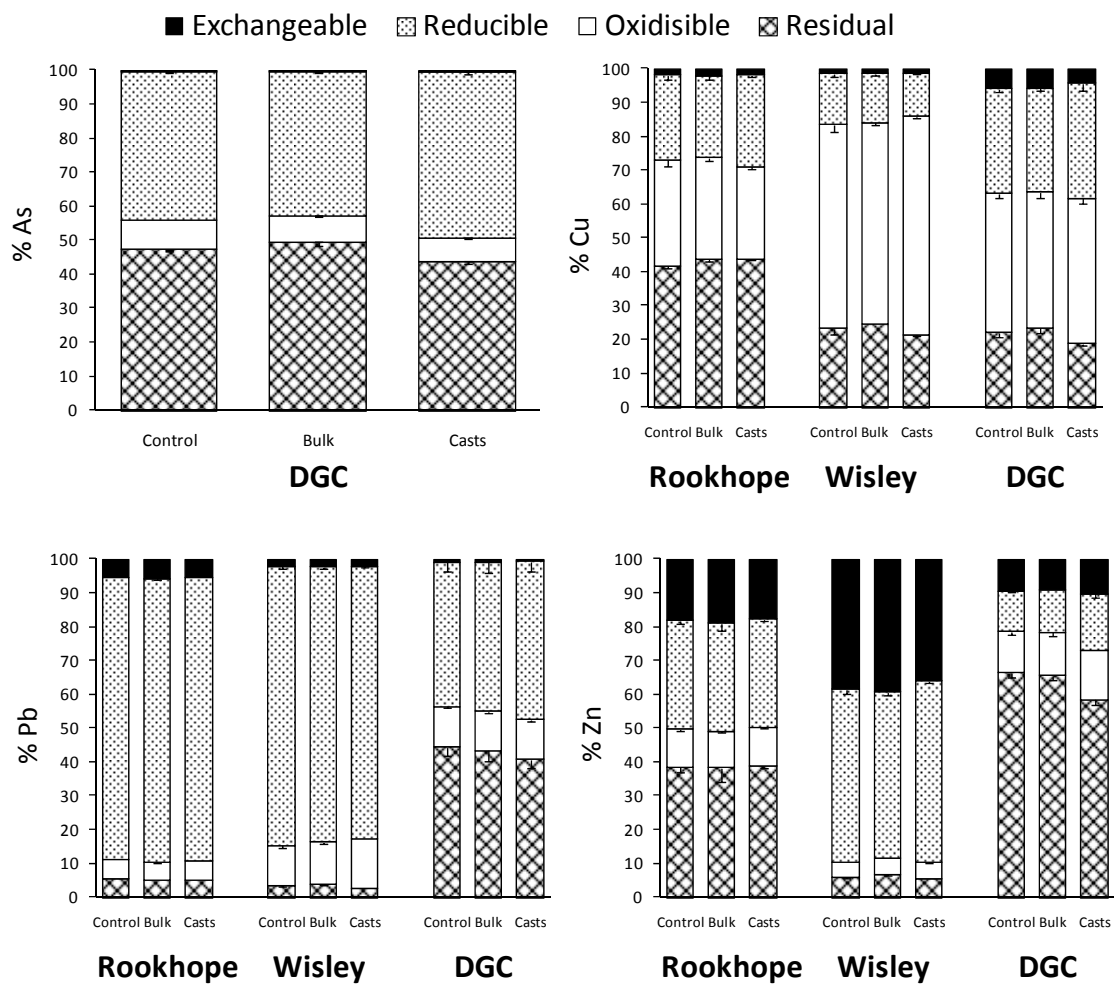


Figure 2 Percentage abundance of As, Cu, Pb and Zn in the exchangeable, reducible, oxidisable and residual fractions of earthworm-free control soil, bulk earthworm-inhabited soil and casts of *Lumbricus terrestris* after incubation for 28 days in three contaminated soils. n = 5, error bars represent standard errors of the mean. As was not determined in extractions of Rookhope and Wisley soil.

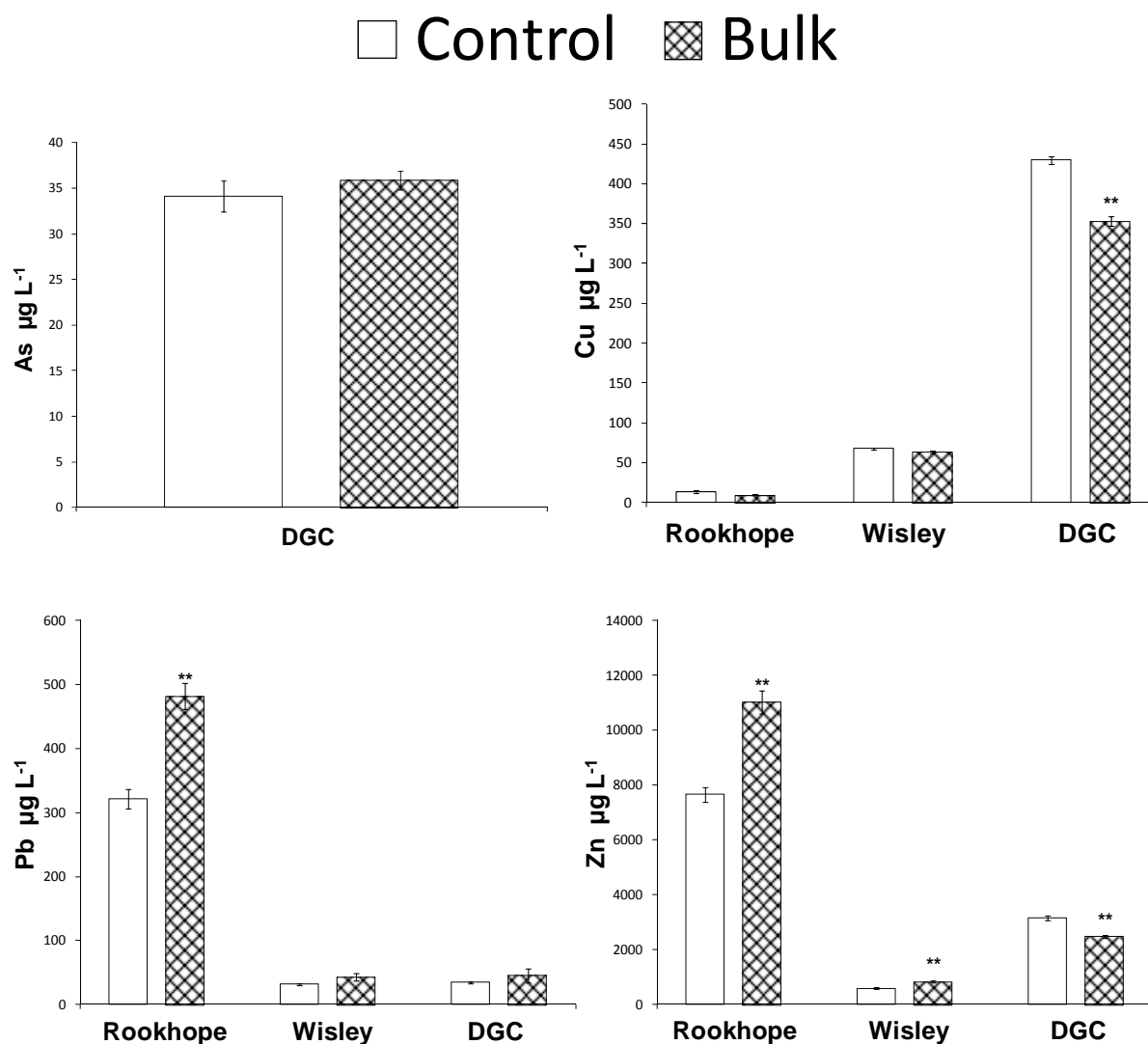


Figure 3 Mean concentration of metals and metalloids in pore water extracted from three contaminated earthworm-free control and bulk earthworm-inhabited soils by centrifuging. *Lumbricus terrestris* were incubated in soils for 28 days. $n = 5$, error bars represent standard errors of the mean. ** = significantly different from the control at the 99% level. As was below detection (2.3 mg L^{-1}) in Rookhope and Wisley pore waters.

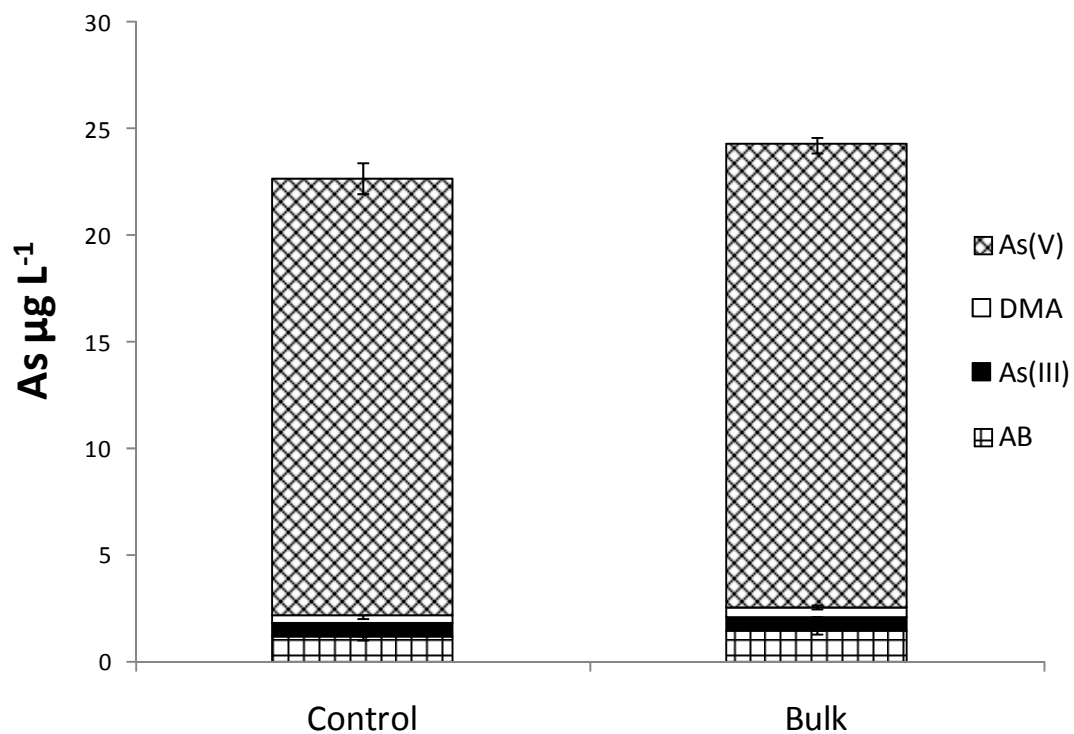


Figure 4 Mean concentration of As species in pore water extracted from earthworm-free control and bulk earthworm-inhabited DGC soil by centrifuging. *Lumbricus terrestris* were incubated in soils for 26 days. $n = 5$, error bars represent standard errors of the mean. Recovery was 93% of total As in pore water.