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1 **Impact of earthworms on trace element solubility in contaminated mine soils amended**
2 **with green waste compost**

3

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5

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14

15

16 **Abstract**

17 The common practice of remediating metal contaminated mine soils with compost can reduce
18 metal mobility and promote re-vegetation, but the effect of introduced or colonising
19 earthworms on metal solubility is largely unknown. We amended two UK mine soils: As/Cu
20 (1150 mgAs kg⁻¹ and 362 mgCu kg⁻¹) and Pb/Zn (4550 mgPb kg⁻¹ and 908 mgZn kg⁻¹) with
21 0, 5, 10, 15 and 20 % compost and then introduced *Lumbricus terrestris*. Porewater was
22 sampled and soil extracted with water to determine trace element solubility, pH and soluble
23 organic carbon. Compost reduced Cu, Pb and Zn, but increased As solubility. Earthworms
24 decreased water soluble Cu and As but increased Pb and Zn in porewater. The effect of the
25 earthworms decreased with increasing compost amendment. The impact of the compost and
26 the earthworms on metal solubility is explained by their effect on pH and soluble organic
27 carbon and the environmental chemistry of each element.

28

29 **Keywords:** metal, arsenic, *Lumbricus terrestris*, remediation

30

31 **Capsule** The effect of earthworms on metal solubility was due to changes in dissolved
32 organic carbon and pH but was reduced with increasing compost amendments.

33

34 **Introduction**

35 The combination of large areas of trace element contaminated soils associated with former
36 mining and smelting activities and the generation of green waste from domestic, agricultural
37 and silvicultural management of vegetation has resulted in the practice of remediating mine
38 contaminated soils with green waste composts and other organic wastes (van Herwijnen et
39 al., 2007b; Pichtel and Bradway, 2008; Farrell et al., 2010). Composts generally increase
40 plant growth which can prevent wind and water erosion of contaminated soils (Tordoff et al.,
41 2000). In addition, cationic metals bind to exchange sites on the surface of organic matter
42 which reduces metal leaching from soils (Soler-Rovira et al., 2010).

43

44 Earthworms represent a significant proportion of the soil fauna and are considered ecosystem
45 engineers owing to the role that they play in organic matter degradation, nutrient cycling and
46 hydrology (Jones et al., 1994). For these reasons they have been the subject of inoculation
47 programmes during the reclamation of degraded soils (Butt, 1999). Earthworm inoculation
48 therefore has the potential to become a commonly used practice during remediation and
49 revegetation of metal contaminated mine soils. Earthworms are also able to colonise
50 contaminated land if climatic and material (organic matter, texture, pH, contaminant)
51 conditions are suitable (Eijsackers, 2010) and so, when organic amendments are incorporated
52 into contaminated soils, it is likely that earthworms will colonise leading to changes in the
53 chemical, biological and physical properties of the soil.

54

55 We reviewed the impact of earthworms on the mobility and availability of metals and found
56 that in the majority of studies earthworms increase the mobility of metals (Sizmur and
57 Hodson, 2009). Recent experiments have identified that this may be due to the impact of
58 earthworms on the degradation of organic matter and subsequent release of organically bound

59 elements and dissolved organic acids that lower the soil pH and lead to further mobilisation
60 of potentially toxic elements (Gomez-Eyles et al., 2011; Sizmur et al., 2011b). In contrast,
61 Beesley and Dickinson (2011) showed in an experiment with an urban soil contaminated with
62 As, Cd, Cu, Pb and Zn, that *Lumbricus terrestris* reduced dissolved organic carbon in
63 porewater and thereby reduced the solubility of As, Cu and Pb in a compost amended soil.

64

65 Different trace elements bind with organic compounds to varying degrees and react
66 differently to changes in soil pH. Therefore, the impact of compost or earthworm additions
67 on the solubility of trace elements depends not only on the changes in soluble organic carbon
68 and pH but also on the chemistry of the element in question. Copper and Pb both bind
69 strongly with organic carbon and therefore their solubility is much affected by changes in
70 soluble organic compounds (McBride et al., 1997). Zinc however, does not bind so strongly
71 with organic carbon and so solubility is relatively more affected by changes in pH (McBride,
72 1994). The solubility of Cu, Pb and Zn is increased with decreasing pH because these
73 elements are cationic (McBride et al., 1997) but As solubility is decreased with decreasing
74 pH because As forms an oxy-anion in solution and binds to positively charged soil surfaces
75 such as iron oxyhydroxides (Masscheleyn et al., 1991).

76

77 Lukkari et al. (2006) gave evidence that earthworms increase the extractability of Cu and Zn
78 in their faeces, but decrease the overall extractability of metals in the bulk earthworm-
79 inhabited soil. This indicates that there are probably at least two separate conflicting
80 mechanisms by which earthworms impact metal mobility. Earthworms burrow and create
81 casts that have elevated concentrations of soluble trace elements (Sizmur et al., 2011a). In
82 addition, they also release mucus into the soil solution which may decrease the solubility of
83 metals (Sizmur et al., 2010). Mucus is produced in greater quantities during copulation

84 (Edwards and Bohlen, 1996) and so this effect would be observed to a greater extent in
85 experiments where two or more earthworms are incubated in each test vessel.

86

87 In the current study we used anecic *L. terrestris* to determine the impact of earthworms on the
88 remediation of contaminated soils with green waste compost. Treatments of either one or two
89 earthworms, with a constant earthworm:soil mass ratio, were applied to determine the impact
90 of earthworm interactions on the solubility, extractability and speciation of As, Cu, Pb and Zn
91 in two highly contaminated mine soils.

92

93 **Methods**

94 Soils and Earthworms

95 *Lumbricus terrestris* (5.1 g, SD = 0.70, n = 150) were sourced from Worms Direct, Ulting,
96 UK. All earthworms were adult, fully clitellate and depurated for 48 hours (Arnold and
97 Hodson, 2007) prior to inoculation into the test medium. Rookhope (Pb/Zn) (54.780947 -
98 2.121240; WGS84) and Devon Great Consols (As/Cu) (50.540851 -4.226920; WGS84) soils
99 were collected from a former lead and fluorspar mine (contaminated with Pb and Zn) and a
100 former copper and arsenic mine (contaminated with As and Cu), respectively. Soil was
101 collected from the top 30 cm of the soil profile and on return to the laboratory dried (40 °C),
102 sieved (<2 mm), homogenised and stored until the start of the experiment. Green waste
103 compost was obtained commercially from B&Q (B&Q Organic living, Peat free vegetable
104 compost) and was not dried or sieved prior to use. Chemical properties of the soils and
105 compost are given in Table 1.

106

107 The aqua regia digestion of soil samples was carried out alongside an in-house reference
108 material traceable to BCR-143R - trace elements in a sewage sludge amended soil

109 (Commission of the European Communities, Community Bureau of Reference) certified for
110 Pb and Zn and with an indicative value for Cu. Recoveries of these elements were 103 % (SD
111 = 2.4, n = 2) for Cu, 93 % (SD = 4.2, n = 2) for Pb and 90 % (SD = 0.81, n = 2) for Zn.
112 Arsenic was below detection limits in the in-house reference material (detection limit = 14
113 mg kg⁻¹). The nitric acid digestion of compost was carried out alongside an in-house plant
114 reference material traceable to CRM GBW 07603 - bush branches and leaves, (State Bureau
115 of Technical Supervision, The People's Republic of China, Institute of Geophysical and
116 Geochemical Exploration, Langfang, China) certified for Cu, Pb, and Zn. Recoveries were
117 101 % (SD = 2.1, n = 2) for Cu, 106 % (SD = 0.5, n = 2) for Pb and 104 % (SD = 4.0, n = 2)
118 for Zn. Arsenic was below detection limits in the in-house reference material (detection limit
119 = 17 mg kg⁻¹).

120

121 Experimental procedure

122 Both Pb/Zn and As/Cu soils were moistened to 80 % of their respective Water Holding
123 Capacities (WHCs). Fresh, moist (moisture content = 61.7%) compost was thoroughly mixed
124 with bulk soil samples from both sites at rates of 0, 5, 10, 15, and 20 % by dry weight (i.e. 6
125 kg of soil + 0, 0.3, 0.6, 0.9 or 1.2 kg of compost). These amended soils were left in buckets at
126 16 °C for 4 weeks to equilibrate, after which the moisture and WHC was determined (this
127 time in the compost/soil mixtures) and amended soils were re-wetted to 80 % of their WHC.
128 Amended soils were weighed out into perforated plastic bags kept in vertical plastic cylinders
129 made from disposable drinking cups in order to produce columns of soil at least 10 cm in
130 depth, as recommended by Lowe and Butt (2005). The surface area of the cups was 0.005 m²
131 so the earthworm density (500 m⁻²) was in the range (300-1000 m⁻²) found in temperate
132 pasture soils (Coleman et al., 2004). For each compost/soil treatment there were ten bags
133 containing 200 g and five bags containing 400 g of material (dry wt.). A single *L. terrestris*

134 was introduced into five of the bags containing 200 g of soil (leaving five bags earthworm-
135 free) and two *L. terrestris* per bag were introduced into the five bags containing 400 g. This
136 resulted in two soil treatments (As/Cu and Pb/Zn), five compost treatments (0, 5, 10, 15, and
137 20 %) and three earthworm treatments (0 earthworms, 1 earthworm and 2 earthworms).
138 Earthworms were incubated in these test media for 28 days at 16 °C in darkness.

139

140 At the end of the incubation the bags were emptied and the soil homogenised. Any bags
141 containing dead earthworms were disposed of and the soil was not used for further analysis.
142 A small sub-sample of the soil (c. 20 g) was air-dried (40 °C), ground and sieved to <2 mm,
143 while the remainder was frozen at -20 °C. Earthworms were removed from the soil, their guts
144 voided on moist filter paper for 48 hours (Arnold and Hodson, 2007) and frozen at -20 °C
145 until digestion in nitric acid to determine metal loadings by ICP-OES (Perkin Elmer Optima
146 7300 DV Inductively Coupled Plasma-Optical Emission Spectrometer) following the method
147 of Langdon et al. (2005). The digestion of earthworm tissue in nitric acid was run alongside
148 ERM CE278 – mussel tissue (European Commission, Institute for Reference Materials and
149 Measurements) certified for As, Cu, Pb and Zn. Recoveries were 106 % (SD = 3.1, n = 4) and
150 97 % (SD = 2.3, n = 4) for Cu and Zn, respectively. Arsenic and Pb were below the limit of
151 detection in the mussel tissue (detection limit = 15.5 mg kg⁻¹ and 4.5 mg kg⁻¹, respectively).

152

153 Five grams of air-dried (40 °C) soil from each experimental vessel was extracted with 20 ml
154 of >18.2 MΩ cm ultra pure water by mixing on a rotary shaker for 24 hours at 30 rpm at 20
155 °C. The soil pH was measured (Jenway 3310 pH meter) followed by centrifuging at 3000 g
156 for 20 min at 20 °C. The supernatants were analysed for water soluble organic carbon (WSC)
157 (Shimadzu TOC 5000) and water soluble As and Cu (As/Cu soil) or Pb and Zn (Pb/Zn soil)
158 by ICP-OES.

159

160 Pore water was extracted from defrosted soil from each experimental vessel by centrifuging
161 at 5000 g for 60 min. Pore water samples were analysed for pH (Jenway 3310 pH meter),
162 elements (ICP-OES), major anions (Dionex DX-500 ion chromatograph), and Dissolved
163 Organic Carbon (DOC) (Shimadzu TOC 5000). Please note the distinction between DOC
164 measured in the pore water and WSC measured in the soil-water extractions. Speciation of
165 Cu (As/Cu soil) or Pb and Zn (Pb/Zn soil) in pore water samples was modelled using WHAM
166 VI (Tipping, 1998). In the absence of characterisation of the DOC fractions, we assumed that
167 50 % of DOC was fulvic in origin and that the fulvic acid contained 50 % C (Tipping, 1996;
168 Pribyl, 2010). In all pore waters >98% of the Cu, Pb and Zn was modelled to be present as
169 either free ions or bound to fulvic acids so other species are not presented here.

170

171 Bioaccumulation factors were calculated as the ratio of metal loadings in the tissues of
172 earthworms to either pseudo-total soil metal concentrations corrected for dilution with
173 compost (BAF_{tot}), porewater metal concentrations (BAF_{pw}), or concentrations of modelled
174 free ions in porewater (BAF_{fi}).

175

176 Statistical analysis

177 Genstat version 11 was used for all statistical analysis. Normality of data and equal variance
178 between treatments was confirmed using the Shapiro-Wilk test ($p>0.01$) and Bartlett's test
179 ($p>0.01$), respectively. Where comparisons between treatments (e.g. compost or earthworm)
180 were made, two-way Analysis of Variance (ANOVA) was carried out. Where comparisons
181 between individual means were required, Fisher's Least Significant Difference test ($p<0.05$
182 and $p<0.01$) was used to identify significant differences. Pearson's correlation coefficient was
183 used to quantify relationships between water soluble metals and pH or WSC.

184

185 **Results**

186 Mortality, weight and trace element bioaccumulation in earthworms

187 Generally, mortality of the earthworms over the test duration was low and the majority of
188 treatments resulted in 0 % mortality (Table 2). In treatments containing two earthworms
189 where one earthworm died, the other also died in all cases. The As/Cu soil amended with 20
190 % compost treatment caused the greatest mortality. Earthworms in all treatments lost weight
191 over the test duration, but in both As/Cu and Pb/Zn soils, compost addition significantly
192 ($p < 0.05$) reduced the weight loss (Table 2).

193

194 Compost amendments also significantly ($p < 0.001$) reduced the loadings of Pb in earthworms
195 inhabiting the Pb/Zn soil (Table 2). There were significantly greater ($p < 0.05$) As loadings in
196 earthworms from treatments containing two specimens compared to treatments with one
197 earthworm. For Cu and Zn there was less variation in the pseudo-total soil metal
198 concentration bioaccumulation factors (BAF_{tot}) than the porewater (BAF_{pw}) or the free ion
199 (BAF_{fi}) bioaccumulation factors (Table SI-1).

200

201 Water soluble trace elements (WSTE)

202 In the As/Cu soil the concentration of water soluble As significantly ($p < 0.001$) increased and
203 the concentration of water soluble Cu significantly ($p < 0.001$) decreased with increasing
204 compost amendment (Figure 1 and Table 3). This was observed along with significantly
205 greater ($p < 0.001$) soil pH and WSC due to compost amendment (Figure 2 and Table 3).
206 There were significant ($p < 0.001$) positive correlations between water soluble As and both pH
207 and WSC and a significant ($p < 0.001$) negative correlation between water soluble Cu and soil
208 pH (Figure 1). There were significantly ($p < 0.05$) lower concentrations of water soluble As

209 and Cu in As/Cu soil from the two earthworm treatments compared to the one earthworm or
210 no earthworm treatments (Figure 1).

211

212 In the Pb/Zn soil the concentration of water soluble Pb significantly ($p<0.001$) increased and
213 Zn significantly ($p<0.001$) decreased due to the compost addition (Figure 1 and Table 3).

214 Lead was significantly ($p<0.001$) positively correlated to pH and WSC, while Zn was

215 significantly ($p<0.001$) negatively correlated to pH and WSC (Figure 1). Water soluble Pb

216 was significantly ($p<0.01$) lower in all compost treatments containing earthworms compared

217 to the earthworm-free treatments, but water soluble Zn was significantly ($p<0.05$) greater in

218 treatments containing earthworms compared to the earthworm-free treatments in Pb/Zn soil

219 amended with 0, 5 and 10 % compost, but not in the 15 or 20 % amendments (Figure 1). This

220 resulted in a significant ($p<0.001$) interaction between earthworms and compost affecting

221 water soluble Zn in Pb/Zn soil (Table 3).

222

223 Porewaters

224 The addition of compost to the As/Cu soil significantly ($p<0.001$) increased the concentration
225 of As and decreased the concentration of Cu in porewater (Figure 3 and Table 4) while pH

226 was significantly ($p<0.001$) increased and DOC significantly ($p<0.001$) decreased (Figure 4

227 and Table 4). The addition of compost also decreased the concentration of Cu present as the

228 Cu^{2+} ion and increased the relative proportion of Cu bound to fulvic acids.

229

230 There was a significant ($p<0.01$) interaction (Table 4) between compost and earthworms for

231 both Cu and As. This is because there were lower concentrations in porewaters from soil

232 containing two earthworms than earthworm-free soil in the unamended soils but not in the

233 compost amended soils. In the 10, 15 and 20 % compost treatments, As concentrations in

234 porewaters from the treatments containing two earthworms were greater than the earthworm
235 free treatments (Figure 3). A similar significant interaction ($p < 0.01$) can be seen with DOC,
236 as there is a significantly ($p < 0.05$) lower concentration of porewater DOC in the unamended,
237 two earthworm treatment compared to the earthworm-free soil, but significantly ($p < 0.01$)
238 greater DOC in the two earthworm treatment in As/Cu soil amended with 10 % compost
239 (Figure 4 and Table 4).

240

241 The concentration of Pb and Zn in porewaters from Pb/Zn soil significantly ($p < 0.001$)
242 decreased with increasing compost amendment and there were significantly ($p < 0.001$) lower
243 concentrations of Pb^{2+} and Zn^{2+} ions and a higher relative proportion of Pb and Zn complexed
244 with fulvic acids (Figure 3 and Table 4). This was observed alongside significant ($p < 0.001$)
245 increases in DOC and porewater pH with increasing compost amendment (Figure 4 and Table
246 4). The addition of earthworms significantly ($p < 0.001$) increased the concentration of both Pb
247 and Zn in porewater and significantly ($p < 0.001$) decreased porewater pH (Figure 3 and 4 and
248 Table 4). The inoculation of either one or two earthworms also significantly ($p < 0.001$)
249 increased the concentrations of free Pb^{2+} and Zn^{2+} ions in porewater (Figure 3 and Table 4).
250 The porewaters extracted from soils inoculated with one earthworm contained higher
251 concentrations of Pb and Zn than the two earthworm treatments in the unamended Pb/Zn soil,
252 but in the soils amended with 20 % compost the opposite was the case (Figure 3).

253

254 **Discussion**

255 Arsenic

256 The addition of compost increased the porewater and water soluble concentrations of As in
257 the As/Cu soil (Figure 1 and 3), as has been previously observed (Beesley et al., 2010). This
258 is due to the increase in soil and porewater pH brought about from the addition of compost

259 with pH 6.8 to a soil with a pH of 4.1 (Table 1). As the pH increases, soil Fe and Mn oxide
260 and oxyhydroxide surfaces become increasingly negatively charged and favour the desorption
261 of arsenic oxyanions (Masscheleyn et al., 1991). This is an important observation concerning
262 the use of compost to remediate soils contaminated with As.

263

264 Whilst both water soluble As and porewater As concentrations were increased in the As/Cu
265 soil with increasing compost amendment, there was a decrease in soil pH in the As/Cu soil
266 brought about by earthworm activity, and this resulted in a decrease in the concentration of
267 water soluble As (Figure 1 and 2). The two earthworm treatment resulted in significantly
268 ($p < 0.01$) lower water soluble As compared to the one earthworm treatment (Figure 1), but
269 this is not seen in the porewater data (Figure 3). The reason for the lower water soluble As in
270 the two earthworm treatment compared to the one earthworm treatment was due to the
271 significantly ($p < 0.05$) lower WSC (Bauer and Blodau, 2006) in the two earthworm treatment
272 (Figure 2), a change not reflected in the DOC data (Figure 4).

273

274 In the As/Cu porewaters there was a significant ($p < 0.01$) interaction between earthworms and
275 compost on DOC and As concentration (Table 4). The addition of two earthworms decreased
276 both the DOC and As concentration in the unamended and 5 % amended soils, but increased
277 the DOC and As concentration in the 10, 15, and 20 % amended soils (Figure 3 and 4). This
278 relationship between As and DOC was due to competition between As and DOC for binding
279 surfaces on positively charged soil constituents such as Fe and Mn oxide oxyhydroxide
280 surfaces (Bauer and Blodau, 2006).

281

282 Copper

283 The addition of green waste compost reduced the porewater and water soluble concentrations
284 of Cu in As/Cu soil (Figure 1 and 3). This may be due to two mechanisms; the first being the
285 binding of metals to an increasing number of organic ligands on the surface of the compost
286 (McBride, 1994; McBride et al., 1997) due to the much greater CEC of the compost
287 compared to the soils (Table 1). The second being an increase in pH leading to less
288 competition with hydrogen ions for pH-dependent cation exchange sites on the compost or
289 soil constituents (Martínez and Motto, 2000). There is also a decrease in the modelled
290 concentration of free Cu^{2+} ions in the porewaters due to the addition of compost (Figure 3).
291 This is because of the reduction of total porewater Cu and the increase in porewater pH with
292 increasing compost.

293

294 The addition of two earthworms reduced the water soluble concentrations of Cu in the As/Cu
295 soil (Figure 1). Although there is a significant negative correlation between soil pH and water
296 soluble Cu, the majority of the variation in water soluble Cu that is explained by changes in
297 pH is due to the effect of the compost. The earthworms significantly ($p < 0.01$) decreased the
298 WSC in the two earthworm treatments compared to the earthworm-free treatments (Figure 2).
299 Therefore the lower solubility of organic carbon in the soils inoculated with two earthworms
300 may have reduced the binding between Cu^{2+} ions and organic acids in solution, allowing for
301 less Cu to become soluble in the soil solution (Temminghoff et al., 1997). Beesley and
302 Dickinson (2011) also found that *L. terrestris* earthworms reduced DOC and therefore
303 reduced Cu mobilisation in a compost-amended, contaminated soil.

304

305 Lead

306 Water soluble Pb was increased due to compost amendments and decreased due to the
307 inoculation of earthworms (Figure 1), while porewater Pb concentrations were decreased by

308 compost amendments and increased by the inoculation of earthworms (Figure 3). There was
309 a significant ($p < 0.001$) positive correlation between both WSC and soil pH and water soluble
310 Pb in the Pb/Zn soil, but it is known that increases in soil pH reduce the solubility of Pb in
311 soils (Martínez and Motto, 2000). Therefore it appears that, in the WSM extraction, WSC
312 was responsible for the increase in solubility of Pb rather than pH. This is confirmed by the
313 significantly ($p < 0.001$) lower WSC due to earthworm addition resulting in significantly
314 ($p < 0.001$) lower soluble Pb (Figure 1 and 2 and Table 3). In the porewater data, changes in
315 pH, rather than DOC were responsible for the changes in the concentration of Pb. A
316 significant ($p < 0.001$) increase in porewater pH due to the addition of compost led to a
317 significant ($p < 0.001$) reduction in the porewater Pb concentration. A significant ($p < 0.001$)
318 decrease in porewater pH due to earthworm addition led to a significant ($p < 0.001$) increase in
319 porewater Pb concentrations (Figure 3 and 4 and Table 4).

320

321 The parameters that affect the solubility of an element in soils are the concentration of an
322 element in the soil solution and the ability for the solid phase to replenish the soil solution.
323 The main difference between the WSTE and pore water extractions was the soil to liquid
324 ratio. The solid to liquid ratio of the WSTE extraction was greater than the porewater
325 extraction. The concentration of DOC was much greater in the porewaters (ranging from
326 approximately 55 to 200 $\mu\text{g L}^{-1}$) compared to DOC (converted to $\mu\text{g L}^{-1}$) in the WSTE
327 extraction (ranging from approximately 25 to 85 $\mu\text{g L}^{-1}$). As the concentration of DOC
328 increases, its influence on Pb solubility decreases as the pool of Pb in the solid phase that can
329 replenish the soil solution is increasingly diminished with increasing DOC concentration. In
330 the WSTE extraction modest decreases in WSC in earthworm treatments resulted in large
331 decreases in Pb (and As) solubility. This particularly affected Pb in the Pb/Zn soil, because
332 Pb binds very strongly with organic carbon, while Zn does not (McBride, 1994). In the

333 porewater extraction, DOC had less of an effect on Pb solubility because the capacity for the
334 solid phase to replace elements, becoming organically complexed in the liquid phase, had
335 become more diminished, due to greater DOC concentrations, and so changes in pH, rather
336 than DOC, had a greater impact on the dissolution of Pb. In this instance, relatively modest
337 decreases in porewater pH from earthworm inhabited soils resulted in large increases in
338 porewater Pb concentrations.

339

340 Zinc

341 Compost amendments reduced the porewater and water soluble concentrations of Zn in Pb/Zn
342 soil (Figure 1 and 3). This is probably due to an increase in pH leading to less competition
343 with hydrogen ions for pH-dependent cation exchange sites on the compost or soil
344 constituents (Martínez and Motto, 2000). There is also a decrease in the modelled
345 concentration of free and Zn^{2+} (and Pb^{2+}) ions in the porewaters due to the addition of
346 compost (Figure 3). This is presumably due to an increase in pH and the concentration of
347 DOC in porewater from the Pb/Zn soil, resulting in an increase in the relative proportion of
348 the Zn that is complexed with organic and inorganic ligands (Figure 3 and 4 and Table 4). It
349 has been suggested in the literature that free ions in solution represent the most
350 toxicologically relevant parameter of metal contaminated soils and solutions (Di Toro et al.,
351 2001; Thakali et al., 2006) and that complexation with organic ligands reduces metal uptake
352 by earthworms (Steenbergen et al., 2005; Arnold et al., 2007). However, the bioaccumulation
353 factors (Table SI-1) suggest that pseudo-total concentrations of Zn (and Cu) in soils are a
354 better predictor of metal bioavailability to earthworms. This is presumably because after the
355 uptake of free ions from porewater, the ions complexed in solution and sorbed to the soil
356 constituents may have re-equilibrated and provided more free ions for uptake.

357

358 The addition of earthworms to Pb/Zn soil had a larger effect on the solubility of Zn than
359 compost. The earthworms increased the solubility of Zn and the addition of one earthworm
360 had a greater effect than two. This is due to the significantly ($p < 0.001$) lower soil and
361 porewater pH in the earthworm inhabited soils (Table 3 and 4). The lower pH increased the
362 competition between the or Zn^{2+} ions and H^+ ions for negatively charged binding sites on the
363 surface of soil constituents such as clays or organic matter and therefore increased the
364 concentrations of Zn in solution (Jordan et al., 1997).

365

366 Impact of compost and earthworms on trace element solubility

367 Compost has been used to remediate and revegetate metal contaminated soil in a number of
368 experiments (Gadepalle et al., 2007; Clemente et al., 2010; Farrell et al., 2010). Often it is
369 found that this reduces the solubility of metals, especially when combined with other
370 amendments (Pérez-de-Mora et al., 2007; van Herwijnen et al., 2007a; Gadepalle et al., 2008;
371 Gadepalle et al., 2009), but other studies have shown that a resulting increase in DOC leads
372 to greater solubility of metals (Hartley et al., 2009; Beesley and Dickinson, 2010; Farrell et
373 al., 2010) and elevated pH may mobilise oxy-anions such as arsenic (Beesley et al., 2010). In
374 the current study the addition of green waste compost reduced the porewater and water
375 soluble concentrations of Cu in As/Cu soil and Zn in Pb/Zn soil (Figure 1 and 3). However
376 porewater and water soluble concentrations of As in the As/Cu soil were increased and water
377 soluble (but not porewater) Pb was increased by compost addition.

378

379 A number of studies have reported increases in metal solubility and availability due to the
380 activities of earthworms (Ma et al., 2000; Kizilkaya, 2004; Wen et al., 2004; Zorn et al.,
381 2005; Wang et al., 2006; Wen et al., 2006). This is due to the degradation of organic matter
382 and release of organically bound metals into solution and the effect of passage through the

383 gut of the earthworms on the soil pH and solubility of organic carbon (Sizmur et al., 2011a;
384 Sizmur et al., 2011b). In this study earthworms decreased the water soluble As and Cu in the
385 As/Cu soil, but increased the water soluble and porewater Pb and Zn concentrations in the
386 Pb/Zn soil and, while results appear contradictory, they could be easily explained by the
387 impact of the earthworms on pH and mobile organic carbon.

388

389 The addition of two earthworms did not always have the same impact as one earthworm in
390 these experiments. This indicates that earthworms interact in the soil to affect soil chemistry.
391 In the As/Cu soil, the two earthworm treatments significantly ($p < 0.001$) decreased the WSC
392 (Figure 2, Table 3) more than the single earthworm treatments, leading to significantly
393 ($p < 0.001$) lower water soluble Cu and As (Figure 1, Table 3). This may be explained by the
394 ingestion of soil to produce casts with elevated WSC, caused by microbial stimulation and
395 mucus excretion (Brown et al., 2000), by one earthworm and then reingestion of casts (Curry
396 and Schmidt, 2007) by the other earthworm which then assimilates the mobile carbon.
397 Because the casts are also known to contain elevated concentrations of water soluble As
398 (Sizmur et al., 2011a), this may also explain the significantly ($p < 0.05$) greater As loadings in
399 earthworms from treatments containing two earthworms (Table 2).

400

401 Environmental relevance

402 When compost is added to contaminated soils to immobilise metals or to promote vegetation
403 establishment, earthworms may be inoculated or colonise the soil. This results in a number
404 of 'ecosystem services' that are beneficial to pedogenesis, revegetation, and bio-stabilisation
405 of organic amendments (Boyer and Wratten, 2010). It is therefore important to understand the
406 effect that such soil biota may have on the solubility of metals that are sequestered by these
407 amendments. Most laboratory experiments performed to test the performance of various soil

408 amendments on the solubility of metals in soils do not take into consideration the influence of
409 soil biota on metal solubility or soil properties that influence metal chemistry. This
410 experiment has shown that earthworms effect the solubility of trace elements in soils, but this
411 effect was reduced in soils with increasing compost additions. However, As in porewaters
412 was increased by earthworm and compost addition. Therefore care must be taken when
413 innoculating earthworms and adding organic amendments to contaminated soils that contain
414 anionic metalloids such as As as increases in pH and DOC may mobilise these elements and
415 cause toxic effects.

416

417 **Conclusions**

418 Generally, the effect of compost increased the solubility of As and decreased the solubility of
419 Cu in As/Cu soil and decreased the solubility of Pb and Zn in Pb/Zn soil. Earthworm addition
420 decreased the solubility of As and Cu in the As/Cu soil and increased the solubility of Pb and
421 Zn in the Pb/Zn soil, apart from when Pb solubility was determined by water soluble Pb and
422 As solubility was determined in porewater. These differences are probably due to the
423 difference in the soil to liquid ratio in porewater extractions compared to the water soluble
424 metals extraction. The addition of compost to contaminated soils buffered the metal solubility
425 and reduced the influence of earthworms on the solubility of metals. Whilst the effects of the
426 earthworms may have been buffered in the higher compost treatments, we do not know how
427 long this buffering is likely to last. The impact of earthworms on metal solubility needs to be
428 tested in a longer term experiment to determine if, after decomposition of compost,
429 earthworms will continue to mobilise trace elements from the soil constituents.

430

431 **Acknowledgements**

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433

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599

600

601 **Table 1. Chemical properties of soils and compost (n = 3, ± standard error).**

| | pH ¹ (H ₂ O) | %Organic mater ² | Pseudo-total elements ³ (mg kg ⁻¹) | | | | CEC ⁴ (cmol _c kg ⁻¹) | %WHC ⁵ |
|--------------------------------|---------------------------------------|--------------------------------|---|--------------|--------------|--------------|---|-------------------|
| | | | As | Cu | Pb | Zn | | |
| As/Cu soil | 4.1 ±0.0 | 15.9 ±0.0 | 1150 ±14 | 362 ±2.9 | 109 ±2.4 | 88.6 ±1.2 | 21.0 ±0.30 | 87.0 ±0.91 |
| Pb/Zn soil | 5.9 ±0.0 | 7.60 ±0.1 | <14 | 38.5 ±3.7 | 4550 ±270 | 908 ±77 | 13.6 ±0.14 | 55.9 ±0.37 |
| Green waste compost | 6.8 ±0.0 | 69.2 ±7.2 | <16 | 25.8 ±0.8 | 45.5 ±0.8 | 127 ±5.2 | 67.4 ±2.5 | N/A |

602 ¹Based on BS7755-3.2, 1995. ²Loss on ignition ³For soil these are aqua regia extractable concentrations based
603 on BS7755-3.9, 1995. For compost these are nitric acid extractable concentrations based on Alexander et al.,
604 (2006). ⁴Cation Exchange Capacity based on (Rowell, 1994). ⁵Water Holding Capacity based on (OECD, 2004)
605

606

607 **Table 2. Mortality, % weight loss and concentrations of As and Cu and Pb and Zn in the tissues of earthworms after incubation individually or in**
 608 **pairs for 28 days in As/Cu and Pb/Zn soils that were remediated with 0, 5, 10, 15 or 20 % compost. n = 5, ± standard errors. P values derived from**
 609 **Analysis of variance (ANOVA) show the significance of earthworm inoculation, compost amendments and their interaction on the weight loss and**
 610 **metal loadings of the earthworms.**

| Compost | Earthworms | % Mortality | | % Weight loss | | As/Cu soil (mg kg ⁻¹) | | Pb/Zn soil (mg kg ⁻¹) | |
|----------|--------------------|-------------|-------|---------------|------------|-----------------------------------|------------|-----------------------------------|-----------|
| | | As/Cu | Pb/Zn | As/Cu | Pb/Zn | As | Cu | Pb | Zn |
| 0 % | 1 | 0 | 0 | 22.0 ± 2.5 | 27.9 ± 6.3 | 64.4 ± 11. | 39.6 ± 3.1 | 1260 ± 110 | 535 ± 120 |
| | 2 | 20 | 0 | 20.6 ± 2.4 | 25.3 ± 2.0 | 86.2 ± 19 | 45.3 ± 6.0 | 995 ± 140 | 556 ± 20 |
| 5 % | 1 | 0 | 0 | 20.0 ± 3.6 | 24.4 ± 3.7 | 110 ± 26 | 50.4 ± 11 | 951 ± 150 | 602 ± 83 |
| | 2 | 0 | 20 | 15.4 ± 2.1 | 29.6 ± 4.4 | 116 ± 19 | 43.8 ± 6.1 | 802 ± 84 | 623 ± 48 |
| 10 % | 1 | 20 | 0 | 22.1 ± 1.7 | 27.9 ± 3.4 | 82.7 ± 28 | 38.6 ± 10 | 712 ± 150 | 546 ± 64 |
| | 2 | 0 | 0 | 22.7 ± 6.9 | 16.3 ± 4.2 | 160 ± 17 | 59.1 ± 4.2 | 698 ± 87 | 544 ± 45 |
| 15 % | 1 | 0 | 0 | 16.3 ± 2.7 | 17.0 ± 2.6 | 87.4 ± 7.1 | 33.7 ± 1.9 | 541 ± 70 | 479 ± 48 |
| | 2 | 0 | 20 | 21.5 ± 2.5 | 17.0 ± 4.5 | 99.0 ± 15 | 37.6 ± 3.4 | 612 ± 77 | 485 ± 44 |
| 20 % | 1 | 20 | 0 | 13.4 ± 2.2 | 11.4 ± 5.4 | 81.2 ± 15 | 32.9 ± 6.2 | 480 ± 97 | 542 ± 47 |
| | 2 | 40 | 20 | 7.04 ± 2.5 | 17.2 ± 7.2 | 99.3 ± 12 | 35.0 ± 5.8 | 571 ± 81 | 488 ± 64 |
| P values | Earthworms | | | ns | ns | 0.036 | ns | ns | ns |
| | Compost | | | 0.035 | 0.041 | ns | ns | <0.001 | ns |
| | Earthworms*Compost | | | ns | ns | s | ns | ns | ns |

611 ns = Not significant (p>0.05)

612

613 **Table 3. P values to describe the significance of earthworm inoculation, compost amendments**
614 **and their interaction on soil pH, Water Soluble organic Carbon (WSC) and water soluble As**
615 **and Cu in As/Cu soil and Pb and Zn in Pb/Zn soil amended with 0, 5, 10, 15 or 20 % compost**
616 **and inoculated with 0, 1 or 2 earthworms for 28 days.**
617

| | Earthworm | Compost | Earthworm*Compost |
|--------------|------------------|----------------|--------------------------|
| As/Cu | | | |
| soil | | | |
| As | <0.001 | <0.001 | <0.001 |
| Cu | <0.001 | <0.001 | ns |
| pH | ns | <0.001 | 0.002 |
| WSC | <0.001 | <0.001 | ns |
| Pb/Zn | | | |
| soil | | | |
| Pb | <0.001 | <0.001 | ns |
| Zn | <0.001 | <0.001 | <0.001 |
| pH | <0.001 | <0.001 | ns |
| WSC | <0.001 | <0.001 | 0.035 |

618 ns = Not significant (p>0.05)

619

621 **Table 4. P values to describe the significance of earthworm inoculation, compost amendments**
 622 **and their interaction on pH, Dissolved Organic Carbon (DOC), As, Cu, Pb and Zn and the**
 623 **WHAM-modelled concentrations of free ions of Cu, Pb and Zn in solution or bound to fulvic**
 624 **acids (FA) in porewaters extracted from As/Cu (As and Cu) and Pb/Zn (Pb and Zn) soil.**
 625

| | Earthworm | Compost | Earthworm*Compost |
|------------------|------------------|----------------|--------------------------|
| As/Cu | | | |
| soil | | | |
| As | 0.034 | <0.001 | 0.008 |
| Cu | <0.001 | <0.001 | <0.001 |
| Cu ²⁺ | <0.001 | <0.001 | <0.001 |
| Cu - FA | 0.049 | <0.001 | <0.001 |
| pH | ns | <0.001 | <0.001 |
| DOC | ns | <0.001 | 0.008 |
| Pb/Zn | | | |
| soil | | | |
| Pb | <0.001 | <0.001 | 0.001 |
| Pb ²⁺ | <0.001 | <0.001 | 0.004 |
| Pb - FA | <0.001 | <0.001 | 0.001 |
| Zn | <0.001 | <0.001 | <0.001 |
| Zn ²⁺ | <0.001 | <0.001 | <0.001 |
| Zn - FA | <0.001 | <0.001 | <0.001 |
| pH | <0.001 | <0.001 | ns |
| DOC | <0.001 | <0.001 | <0.001 |

626 ns = Not significant (p>0.05)

628

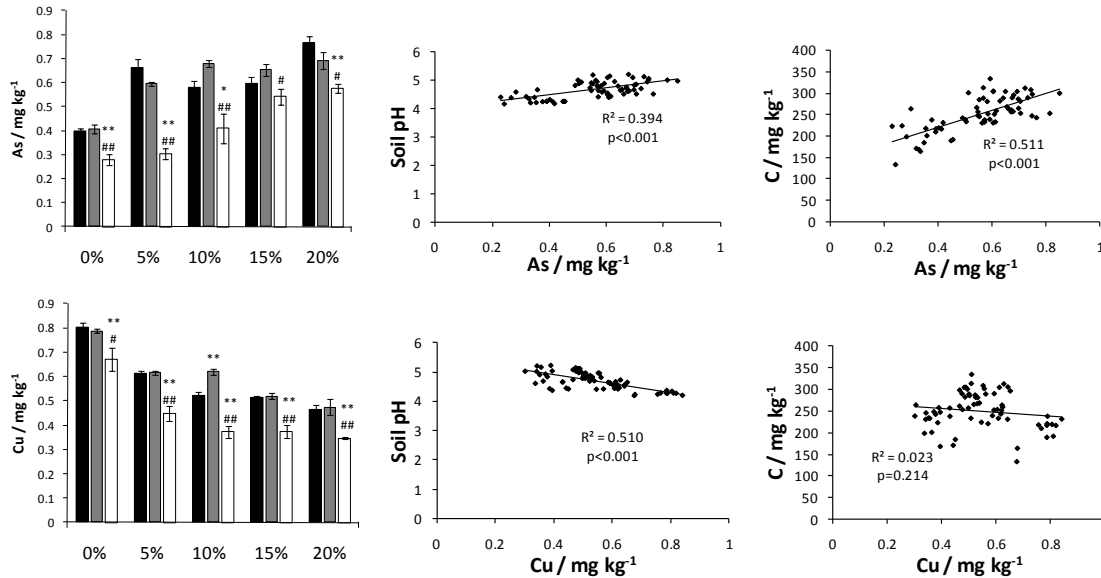
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631

■ 0 Earthworms ■ 1 Earthworm ▨ 2 Earthworms

As/Cu soil

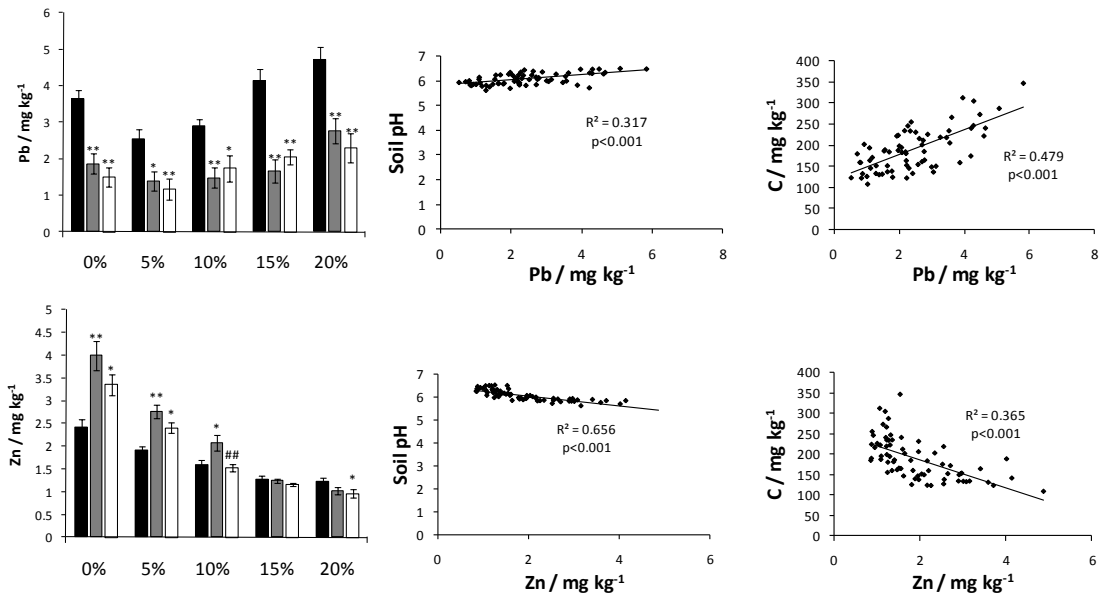


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Pb/Zn soil



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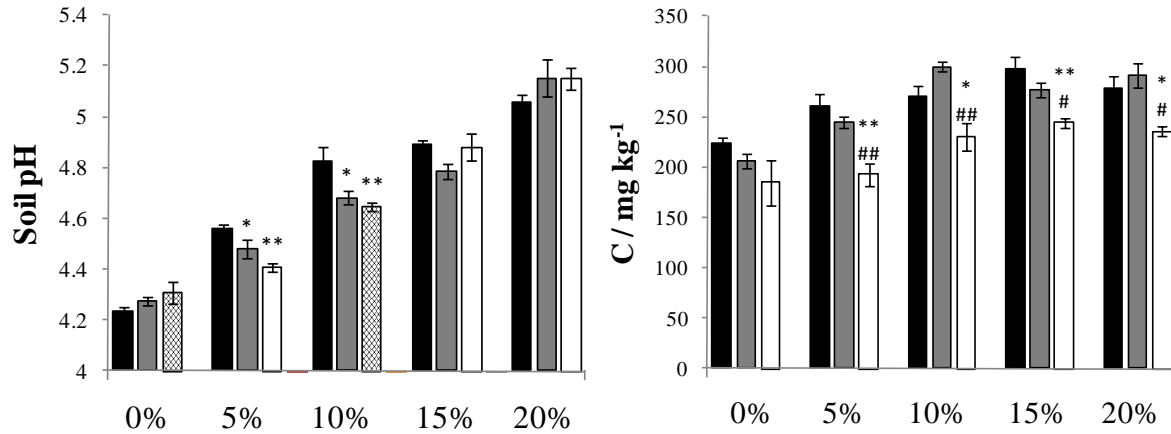
Figure 1. Water soluble As and Cu in As/Cu soil and Pb and Zn in Pb/Zn soil amended with 0, 5, 10, 15 or 20 % compost and inoculated with 0, 1 or 2 earthworms for 28 days. * = statistically significantly different from the 0 earthworm treatment at the 95 % (*) and 99 % () level. # = statistically significantly different from the 1 earthworm treatment at the 95 % (#) and 99 % (##) level. Scatter plots show the strength and significance of correlations between water soluble metals data and soil pH or water soluble organic carbon. Error bars are standard errors, n = 5.**

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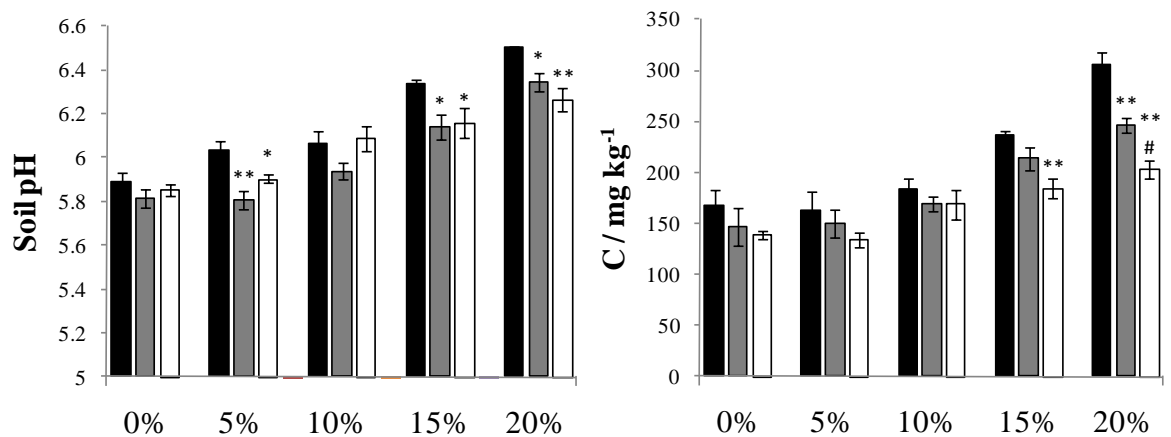
As/Cu soil



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Pb/Zn soil



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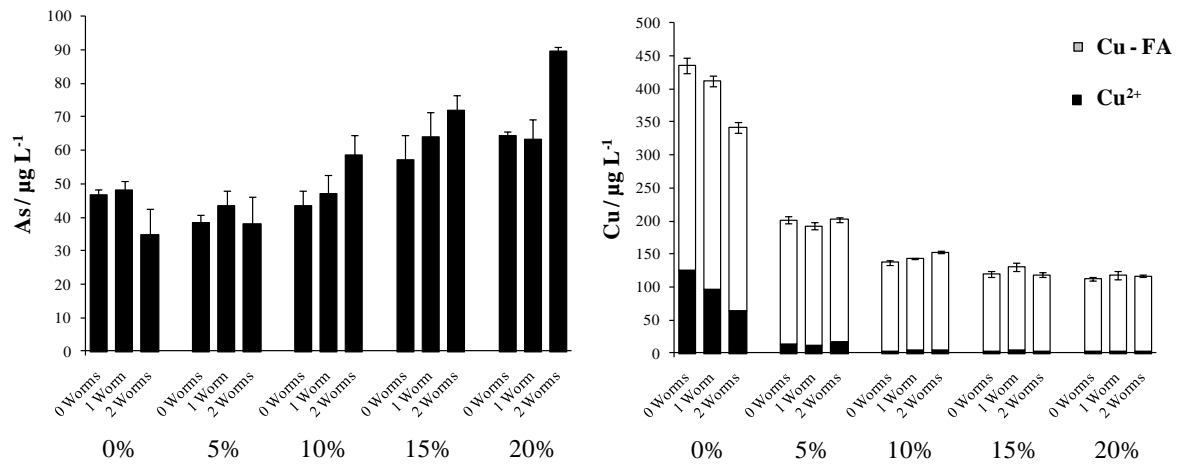
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Figure 2. Soil pH and Water Soluble organic Carbon (WSC) in As/Cu and Pb/Zn soil amended with 0, 5, 10, 15 or 20 % compost and inoculated with 0, 1 or 2 earthworms for 28 days. * = statistically significantly different from the 0 earthworm treatment at the 95 % (*) and 99 % () level. # = statistically significantly different from the 1 earthworm treatment at the 95 % (#) and 99 % (##) level. Error bars are standard errors, n = 5.**

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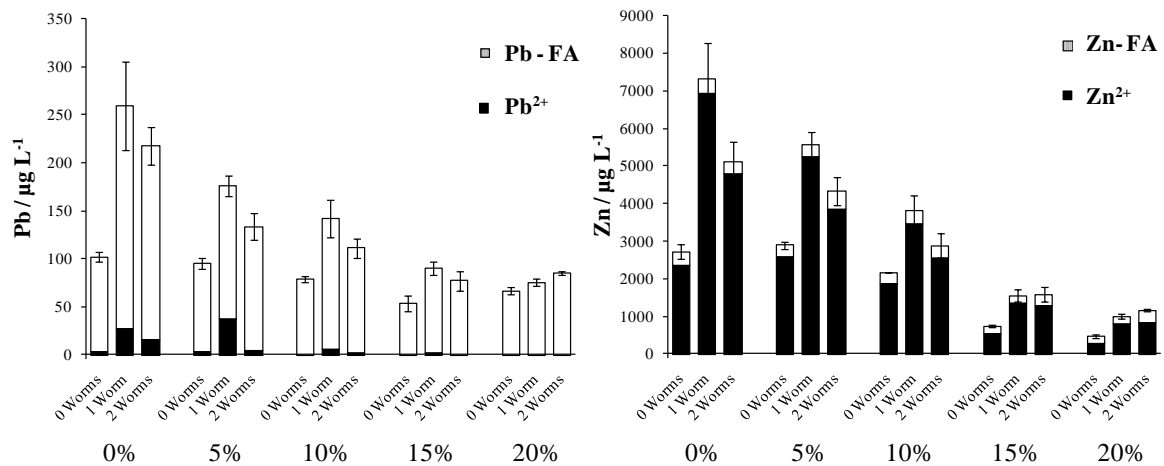
As/Cu soil



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Pb/Zn soil



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Figure 3. Concentration of As and Cu and Pb and Zn in porewaters of As/Cu and Pb/Zn soils respectively after earthworms were inoculated for 28 days in soils amended with 0, 5, 10, 15 or 20 % compost. Cu, Pb and Zn concentrations are split into free ions or complexed with fulvic acids (FA), modelled using WHAM. Error bars are standard errors of total concentrations, n = 5.

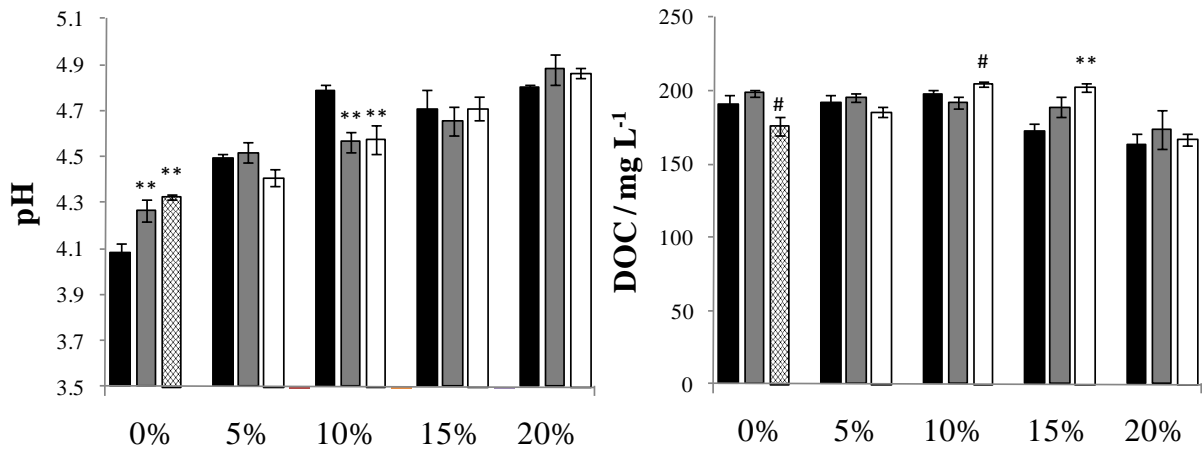
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669

0 Earthworms
 1 Earthworm
 2 Earthworms

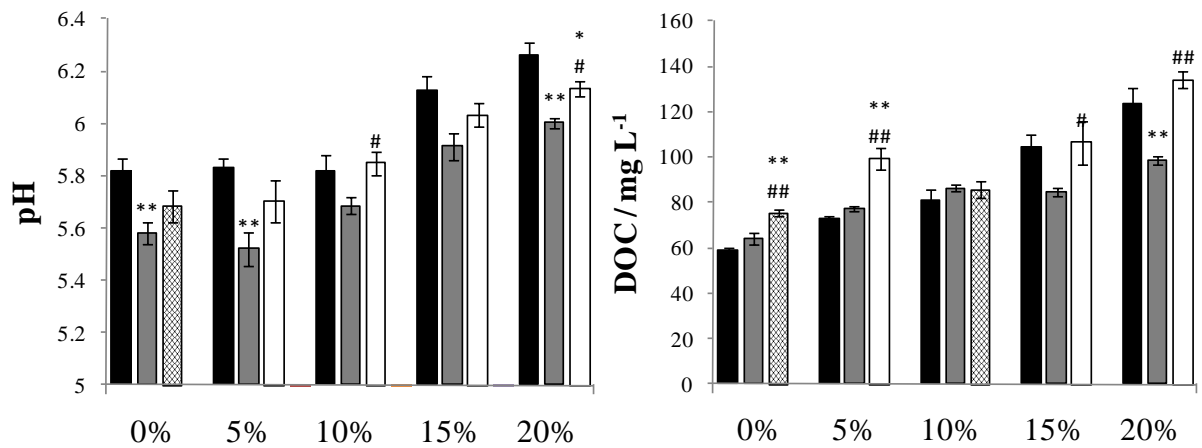
As/Cu soil



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Pb/Zn soil



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Figure 4. pH and Dissolved Organic Carbon (DOC) in porewaters of As/Cu and Pb/Zn soils respectively after earthworms were inoculated for 28 days in soils amended with 0, 5, 10, 15 or 20 % compost. * = statistically significantly different from the 0 earthworm treatment at the 95 % (*) and 99 % (**) level. # = statistically significantly different from the 1 earthworm treatment at the 95 % (#) and 99 % (##) level. Error bars are standard errors, n = 5.