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

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Abstract

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

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

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

Introduction

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

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

We reviewed the impact of earthworms on the mobility and availability of metals and found that in the majority of studies earthworms increase the mobility of metals (Sizmur and Hodson, 2009). Recent experiments have identified that this may be due to the impact of earthworms on the degradation of organic matter and subsequent release of organically bound elements and dissolved organic acids that lower the soil pH and lead to further mobilisation of potentially toxic elements (Gomez-Eyles et al., 2011; Sizmur et al., 2011b). In contrast, Beesley and Dickinson (2011) showed in an experiment with an urban soil contaminated with As, Cd, Cu, Pb and Zn, that *Lumbricus terrestris* reduced dissolved organic carbon in porewater and thereby reduced the solubility of As, Cu and Pb in a compost amended soil.

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

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

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

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

Methods

Soils and Earthworms

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

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

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

Experimental procedure

Both Pb/Zn and As/Cu soils were moistened to 80 % of their respective water holding capacities (WHCs). Fresh, moist (moisture content = 61.7%) compost was throughly mixed with bulk soil samples from both sites at rates of 0, 5, 10, 15, and 20 % by dry weight (i.e. 6 kg of soil + 0, 0.3, 0.6, 0.9 or 1.2 kg of compost). These amended soils were left in buckets at 16 ºC for 4 weeks to equilibrate, after which the moisture and WHC was determined (this time in the compost/soil mixtures) and amended soils were re-wetted to 80 % of their WHC. Amended soils were weighed out into perforated plastic bags kept in vertical plastic cylinders made from disposable drinking cups in order to produce columns of soil at least 10 cm in depth, as recommended by Lowe and Butt (2005). The surface area of the cups was 0.005 m^2 so the earthworm density (500 m^{-2}) was in the range (300-1000 m^{-2}) found in temperate pasture soils (Coleman et al., 2004). For each compost/soil treatment there were ten bags containing 200 g and five bags containing 400 g of material (dry wt.). A single *L. terrestris* was introduced into five of the bags containing 200 g of soil (leaving five bags earthworm-free) and two *L. terrestris* per bag were introduced into the five bags containing 400 g. This resulted in two soil treatments (As/Cu and Pb/Zn), five compost treatments (0, 5, 10, 15, and 20 %) and three earthworm treatments (0 earthworms, 1 earthworm and 2 earthworms). Earthworms were incubated in these test media for 28 days at 16 ºC in darkness.

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

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

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

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

Statistical analysis

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

Results

Mortality, weight and trace element bioaccumulation in earthworms

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

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

Water soluble trace elements

In the As/Cu soil the concentration of water soluble As significantly ($p<0.001$) increased and the concentration of water soluble Cu significantly ($p<0.001$) decreased with increasing compost amendment (Figure 1 and Table 3). This was observed along with significantly greater ($p<0.001$) soil pH and water soluble organic carbon due to compost amendment (Figure 2 and Table 3). There were significant ($p<0.001$) positive correlations between water soluble As and both pH and water soluble organic carbon and a significant ($p<0.001$) negative correlation between water soluble Cu and soil pH (Figure 1). There were significantly ($p<0.05$) lower concentrations of water soluble As and Cu in As/Cu soil from the two earthworm treatments compared to the one earthworm or no earthworm treatments (Figure 1).

In the Pb/Zn soil the concentration of water soluble Pb significantly ($p<0.001$) increased and Zn significantly ($p<0.001$) decreased due to the compost addition (Figure 1 and Table 3). Lead was significantly ($p<0.001$) positively correlated to pH and water soluble organic carbon, while Zn was significantly ($p<0.001$) negatively correlated to pH and water soluble organic carbon (Figure 1). Water soluble Pb was significantly ($p<0.01$) lower in all compost treatments containing earthworms compared to the earthworm-free treatments, but water soluble Zn was significantly ($p<0.05$) greater in treatments containing earthworms compared to the earthworm-free treatments in Pb/Zn soil amended with 0, 5 and 10 % compost, but not in the 15 or 20 % amendments (Figure 1). This resulted in a significant ($p<0.001$) interaction between earthworms and compost affecting water soluble Zn in Pb/Zn soil (Table 3).

Porewaters

The addition of compost to the As/Cu soil significantly ($p<0.001$) increased the concentration of As and decreased the concentration of Cu in porewater (Figure 3 and Table 4) while pH was significantly ($p<0.001$) increased and dissolved organic carbon significantly ($p<0.001$) decreased (Figure 4 and Table 4). The addition of compost also decreased the concentration of Cu present as the Cu^{2+} ion and increased the relative proportion of Cu bound to fulvic acids.

There was a significant ($p<0.01$) interaction (Table 4) between compost and earthworms for both Cu and As. This is because there were lower concentrations in porewaters from soil containing two earthworms than earthworm-free soil in the unamended soils but not in the compost amended soils. In the 10, 15 and 20 % compost treatments, As concentrations in porewaters from the treatments containing two earthworms were greater than the earthworm free treatments (Figure 3). A similar significant interaction ($p<0.01$) can be seen with dissolved organic carbon, as there is a significantly ($p<0.05$) lower concentration of porewater dissolved organic carbon in the unamended, two earthworm treatment compared to the earthworm-free soil, but significantly ($p<0.01$) greater dissolved organic carbon in the two earthworm treatment in As/Cu soil amended with 10 % compost (Figure 4 and Table 4).

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

Discussion

Arsenic

The addition of compost increased the porewater and water soluble concentrations of As in the As/Cu soil (Figure 1 and 3), as has been previously observed (Beesley et al., 2010). This is due to the increase in soil and porewater pH brought about from the addition of compost with pH 6.8 to a soil with a pH of 4.1 (Table 1). As the pH increases, soil Fe and Mn oxide and oxyhydroxide surfaces become increasingly negatively charged and favour the desorption of arsenic oxyanions (Masscheleyn et al., 1991). This is an impotant observation concerning the use of compost to remediate soils contaminated with As.

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

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

Copper

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

The addition of two earthworms reduced the water soluble concentrations of Cu in the As/Cu soil (Figure 1). Although there is a significant negative correlation between soil pH and water soluble Cu, the majority of the variation in water soluble Cu that is explained by changes in pH is due to the effect of the compost. The earthworms significantly ($p<0.01$) decreased the water soluble organic carbon in the two earthworm treatments compared to the earthworm-free treatments (Figure 2). Therefore the lower solubility of organic carbon in the soils innoculated with two earthworms may have reduced the binding between Cu^{2+} ions and organic acids in solution, allowing for less Cu to become soluble in the soil solution

(Temminghoff et al., 1997). Beesley and Dickinson (2011) also found that *L. terrestris* earthworms reduced dissolved organic carbon (measured in porewater extracted with rhizon samplers) and therefore reduced Cu mobilisation in a compost-amended, contaminated soil.

Lead

Water soluble Pb was increased due to compost amendments and decreased due to the innoculation of earthworms (Figure 1), while porewater Pb concentrations were decreased by compost amendments and increased by the innoculation of earthworms (Figure 3). There was a significant ($p<0.001$) positive correlation between both water soluble organic carbon and soil pH and water soluble Pb in the Pb/Zn soil, but it is known that increases in soil pH reduce the solubility of Pb in soils (Martínez and Motto, 2000). Therefore it appears that, in the water extractions, water soluble organic carbon was responsible for the increase in solubility of Pb rather than pH. This is confirmed by the significantly ($p<0.001$) lower water soluble organic carbon due to earthworm addition resulting in significantly ($p<0.001$) lower soluble Pb (Figure 1 and 2 and Table 3). In the porewater data, changes in pH, rather than dissolved organic carbon were responsible for the changes in the concentration of Pb. A significant ($p<0.001$) increase in porewater pH due to the addition of compost led to a significant ($p<0.001$) reduction in the porewater Pb concentration. A significant ($p<0.001$) decrease in porewater pH due to earthworm addition led to a significant ($p<0.001$) increase in porewater Pb concentrations (Figures 3 and 4 and Table 4).

The parameters that affect the solubility of an element in soils are the concentration of an element in the soil solution and the ability for the solid phase to replenish the soil solution. The main difference between the water and pore water extractions was the soil to liquid ratio. The solid to liquid ratio of the water extraction was greater than the porewater extraction. The concentration of dissolved organic carbon was much greater in the porewaters (ranging from approximately 55 to 200 µg L^{-1}) compared to water soluble organic carbon (converted to µg L^{-1}) in the water extraction (ranging from approximately 25 to 85 µg L^{-1}). As the concentration of dissolved organic carbon increases, its influence on Pb solubility decreases as the pool of Pb in the solid phase that can replenish the soil solution is increasingly diminished with increasing dissolved organic carbon concentration. In the water extraction modest decreases in the relatively low water soluble organic carbon in earthworm treatments resulted in large decreases in Pb (and As) solubility. This particularly affected Pb in the Pb/Zn soil, because Pb binds very strongly with organic carbon, while Zn does not (McBride, 1994). In the porewater extraction, dissolved organic carbon had less of an effect on Pb solubility because the capacity for the solid phase to replace elements, becoming organically complexed in the liquid phase, had become more diminished, due to greater dissolved organic carbon concentrations, and so changes in pH, rather than dissolved organic carbon, had a greater impact on the dissolution of Pb. In this instance, relatively modest decreases in porewater pH from earthworm inhabited soils resulted in large increases in porewater Pb concentrations.

Zinc

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

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

Impact of compost and earthworms on trace element solubility

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

A number of studies have reported increases in metal solubility and availability due to the activities of earthworms (Ma et al., 2000; Kizilkaya, 2004; Wen et al., 2004; Zorn et al., 2005; Wang et al., 2006; Wen et al., 2006). This is due to the degradation of organic matter and release of organically bound metals into solution and the effect of passage through the gut of the earthworms on the soil pH and solubility of organic carbon (Sizmur et al., 2011a; Sizmur et al., 2011b). In this study earthworms decreased the water soluble As and Cu in the As/Cu soil, but increased the water soluble and porewater Pb and Zn concentrations in the Pb/Zn soil and, while results appear contradictory, they could be easily explained by the impact of the earthworms on pH and mobile organic carbon.

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

Environmental relevance

When compost is added to contaminated soils to imobilise metals or to promote vegetation establishment, earthworms may be innoculated or colonise the soil. This results in a number of 'ecosystem services' that are beneficial to pedogenesis, revegetation, and bio-stabilisation of organic amendments (Boyer and Wratten, 2010). It is therefore important to understand the effect that such soil biota may have on the solubility of metals that are sequestered by these amendments. Most laboratory experiments performed to test the performance of various soil amendments on the solubility of metals in soils do not take into consideration the influence of soil biota on metal solubility or soil properties that influence metal chemistry. This experiment has shown that earthworms effect the solubility of trace elements in soils, but this effect was reduced in soils with increasing compost additions. However, As in porewaters was increased by earthworm and compost addition. Therefore care must be taken when innoculating earthworms and adding organic amendments to contaminated soils that contain anionic metalloids such as As as increases in pH and dissolved organic carbon may mobilise these elements and cause toxic effects.

Conclusions

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

Supporting Information

There is one table provided as supporting information

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

	pH[1] (H_2O)	**%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	15.9	1150	362	109	88.6	21.0	87.0
	±0.0	±0.0	±14	±2.9	±2.4	±1.2	±0.30	±0.91
Pb/Zn soil	5.9	7.60	<14	38.5	4550	908	13.6	55.9
	±0.0	±0.1		±3.7	±270	±77	±0.14	±0.37
Green waste compost	6.8	69.2	<16	25.8	45.5	127	67.4	N/A
	±0.0	±7.2		±0.8	±0.8	±5.2	±2.5	

[1] Based on BS7755-3.2, 1995. [2] Loss on ignition [3] For soil these are aqua regia extractable concentrations based on BS7755-3.9, 1995. For compost these are nitric acid extractable concentrations based on Alexander et al., (2006). [4] Cation exchange capacity based on (Rowell, 1994). [5] Water holding capacity based on (OECD, 2004)

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 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 Analysis of variance (ANOVA) show the significance of earthworm inoculation, compost amendments and their interaction on the weight loss and metal loadings of the earthworms.

		% Mortality		**% Weight loss**		**As/Cu soil (mg kg^{-1})**		**Pb/Zn soil (mg kg^{-1})**	
Compost	**Earthworms**	**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

ns = Not significant (p>0.05)

Table 3. P values to describe the significance of earthworm inoculation, compost amendments and their interaction on soil pH, Water soluble organic carbon (WSC) and 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.

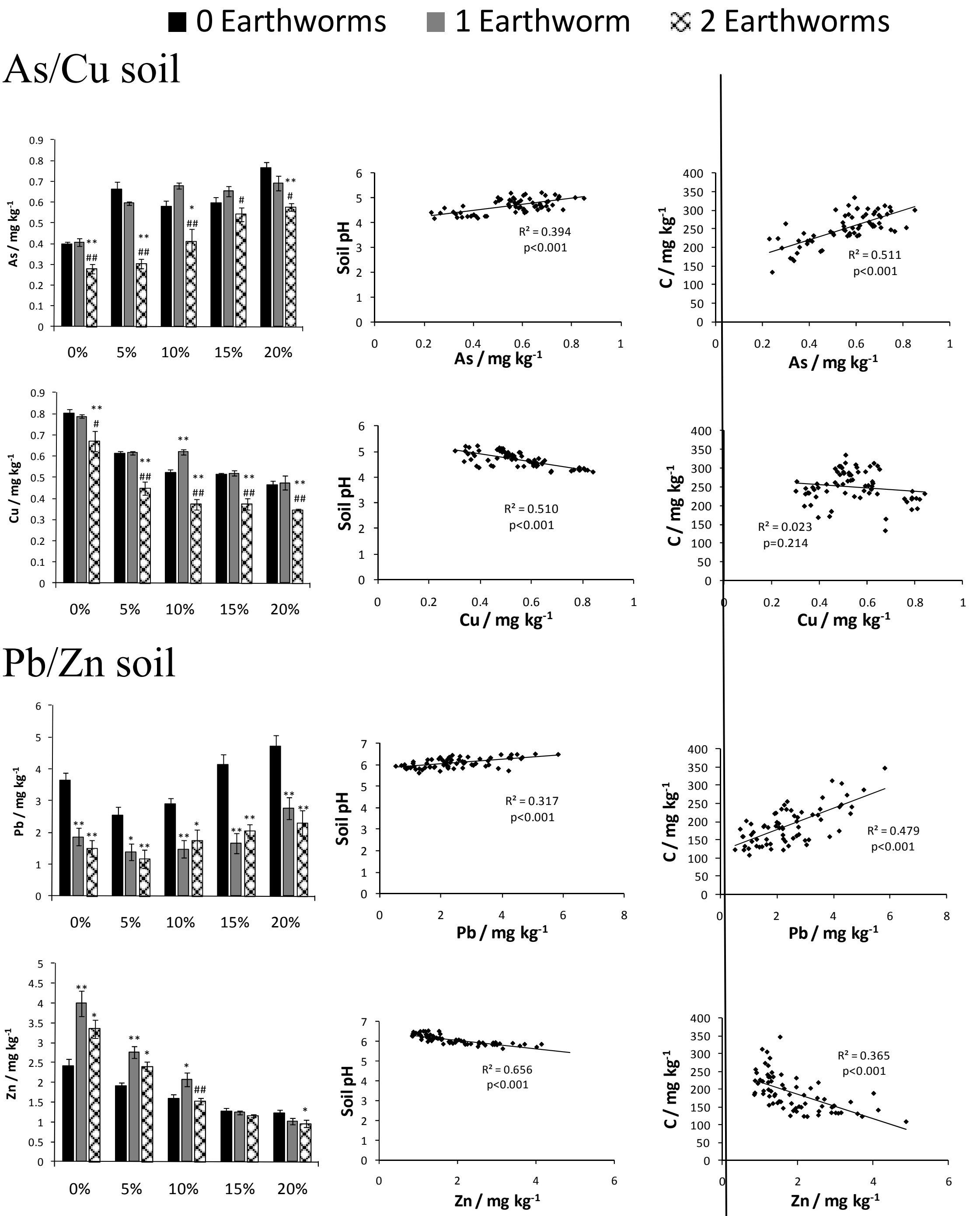
	Earthworm	Compost	Earthworm*Compost
As/Cu soil			
As	<0.001	<0.001	<0.001
Cu	<0.001	<0.001	ns
pH	ns	<0.001	0.002
WSC	<0.001	<0.001	ns
Pb/Zn soil			
Pb	<0.001	<0.001	ns
Zn	<0.001	<0.001	<0.001
pH	<0.001	<0.001	ns
WSC	<0.001	<0.001	0.035

ns = Not significant ($p>0.05$)

Table 4. P values to describe the significance of earthworm inoculation, compost amendments and their interaction on pH, dissolved organic carbon (DOC), As, Cu, Pb and Zn and the WHAM-modelled concentrations of free ions of Cu, Pb and Zn in solution or bound to fulvic acids (FA) in porewaters extracted from As/Cu (As and Cu) and Pb/Zn (Pb and Zn) soil.

	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

ns = Not significant ($p>0.05$)



As/Cu soil

Pb/Zn soil

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 trace element data and soil pH or water soluble organic carbon. Error bars are standard errors, n = 5.

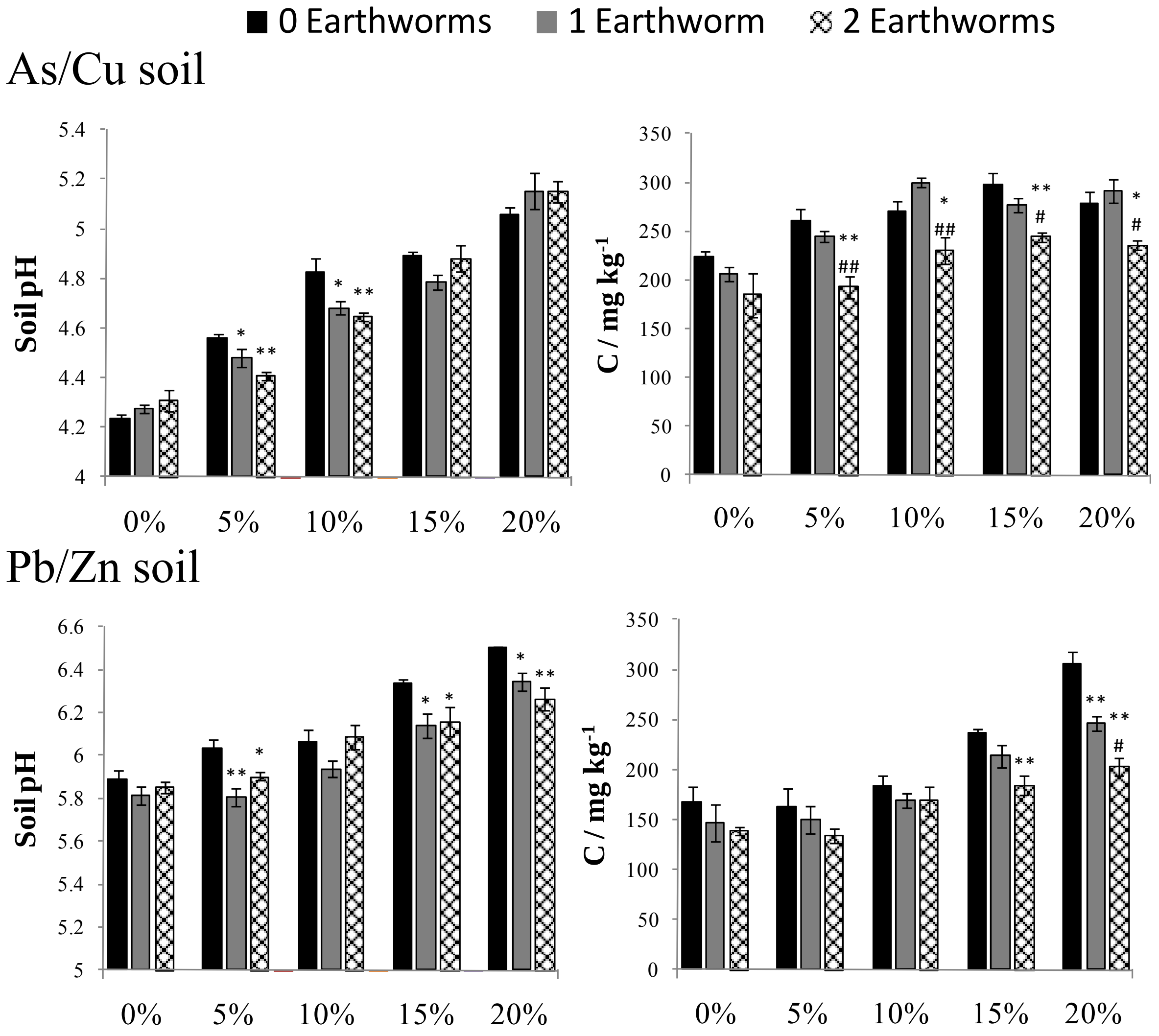
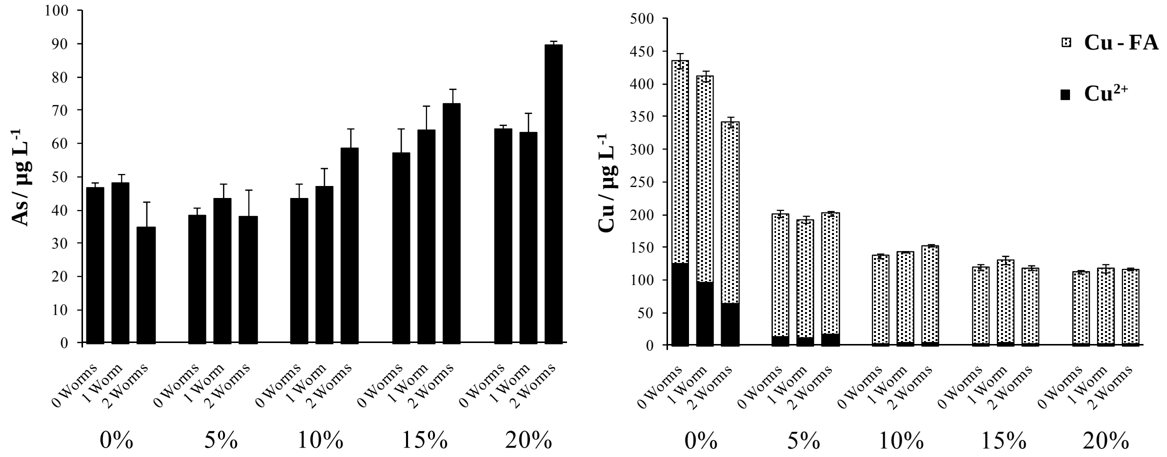


Figure 2. Soil pH and water soluble organic carbon in As/Cu and Pb/Zn soil amended with 0, 5, 10, 15 or 20 % compost and inoculated with 0, 1 or 2 earthworms for 28 days. * = statistically significantly different from the 0 earthworm treatment at the 95 % (*) and 99 % (**) level. # = statistically significantly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

As/Cu soil



Pb/Zn soil

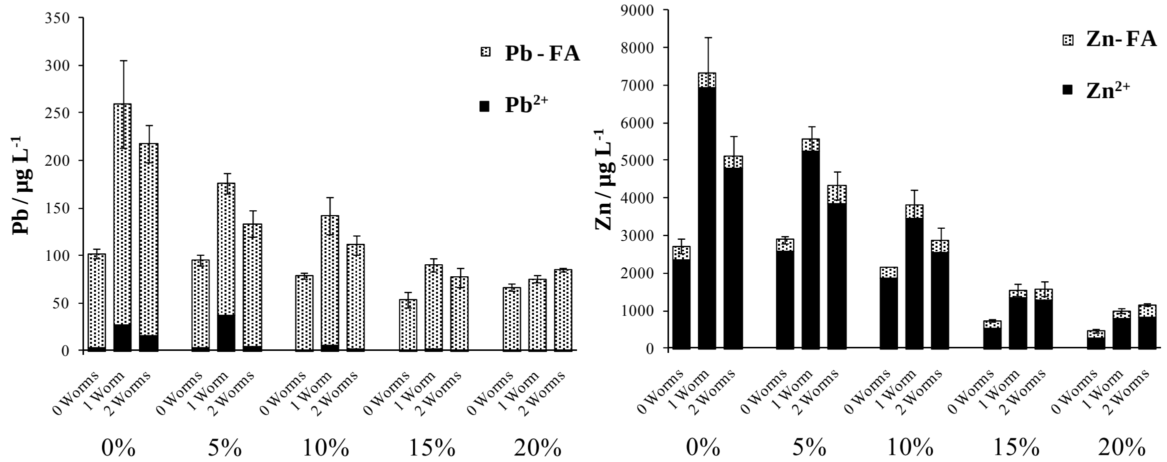


Figure 3. Concentration of As and Cu and Pb and Zn in porewaters of As/Cu and Pb/Zn soils respectively after earthworms were inoculated for 28 days in soils amended with 0, 5, 10, 15 or 20 % compost. Cu, Pb and Zn concentrations are split into free ions or complexed with fulvic acids (FA), modelled using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

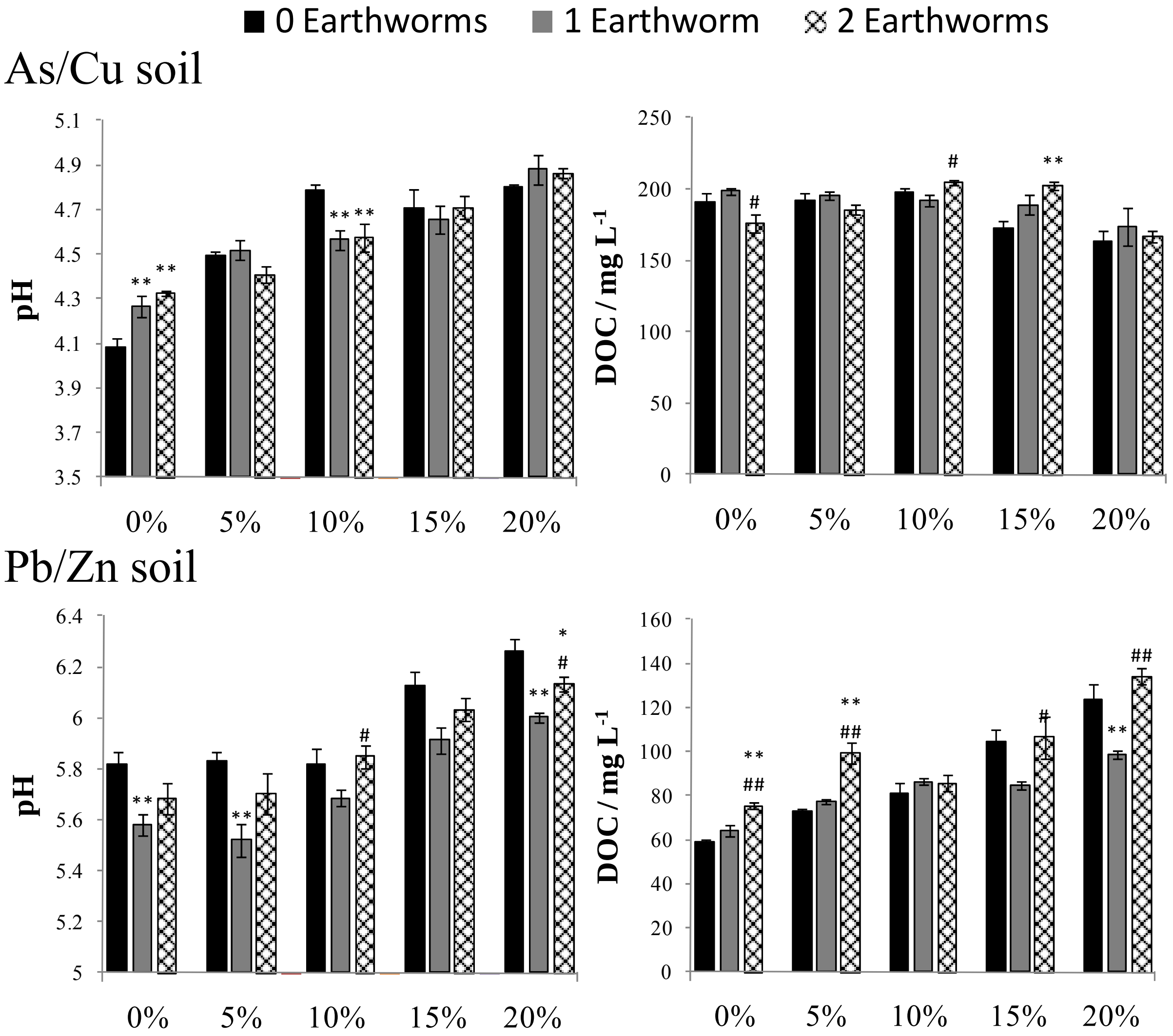


Figure 4. pH and dissolved organic carbon (DOC) in porewaters of As/Cu and Pb/Zn soils respectively after earthworms were inoculated for 28 days in soils amended with 0, 5, 10, 15 or 20 % compost. * = statistically significantly different from the 0 earthworm treatment at the 95 % (*) and 99 % (**) level.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