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

Tom Sizmur[a]*, Barbara Palumbo-Roe[b], Michael J. Watts[b] and Mark E. Hodson[a]

[a]Soil Research Centre, Dept. Geography and Environmental Science, School of Human and Environmental Sciences, University of Reading, Whiteknights, Reading, RG6 6DW, U.K.

[b]British Geological Survey, Kingsley Dunham Centre, Keyworth, Nottingham, NG12 5GG, U.K.

*Corresponding author e-mail: t.p.sizmur@reading.ac.uk

Tel: +44(0) 118 378 8911 Fax: +44(0) 118 378 6666

Abstract

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

Keywords: earthworm, metal, mobility, availability, cast

Capsule

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

Introduction

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

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

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

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

Materials and methods

Soil and Earthworms

L. terrestris were sourced from Worms Direct, Ulting, UK. Three contaminated soils were used in this study (Table 1). Rookhope (54.780947 -2.121240; WGS84) and Devon Great Consols (DGC) (50.540851 -4.226920; WGS84) soils were collected from a former lead and fluorspar mine and a former copper and arsenic mine, respectively. Wisley soil (51.312975 -0.474771; WGS84) was amended with Pb nitrate and Cu and Zn sulphate salts 15 years ago (Alexander et al., 2006). Soil was collected from the top 30 cm of the soil profile and on return to the laboratory dried (40°C), sieved (<2mm), homogenised and stored until the start of the experiment. Soil pH was measured in a soil-water suspension (based on BS7755-3.2), percentage organic matter by loss on ignition (500°C) and soil texture by laser granulometry (Coulter LS 230 Particle Size Analyzer). Sand was classified as particles (2000-63µm, silt as 63-2µm and clay as < 2µm in diameter. Pseudototal elemental composition was determined by digestion in aqua regia (based on BS7755-3.9, 1995) and cation exchange capacity was measured at pH 7 using the ammonium acetate method (Rowell, 1994).

Experimental procedure

Single specimens of 48-hour depurated (Arnold and Hodson, 2007) *L. terrestris* (4.8g, SD = 0.79, n = 75) were incubated in bags (one earthworm per bag) containing 300g (dry wt.) of soil moistened to 80% of the water holding capacity (38%, 42% and 65% moisture content of Rookhope, Wisley and DGC soils respectively) at 20°C in darkness for 28 days alongside earthworm-free bags of moist soil. Bags were kept in vertical plastic cylinders made from disposable drinking cups in order to produce columns of soil at least 10 cm in depth. 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). No food was added to bags to ensure that observations made were due to the activity of the earthworms rather than the incorporation of organic matter. After 28 days 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. There were 25 bags for each treatment. Randomly selected bags were pooled in groups of five to give five samples for each replicate and a total of five replicates for each treatment. Earthworms were removed from the soil and their guts voided on moist filter paper for 48 hours, changing the paper every 12 hours (Arnold and Hodson, 2007) . The casts produced were air-dried and pooled to correspond with the same replicates as the bulk earthworm-inhabited and earthworm-free soil. Earthworms were re-weighed and frozen.

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

Pore water was extracted from moist bulk soil from each pooled sample by centrifuging at 6000rpm for 60 min. This extracted 51 % (SD = 0.9, n = 2), 56% (SD = 3.3, n = 2) and 65% (SD = 0.7, n = 2) of the soil moisture from the Rookhope, Wisley and DGC soils respectively. Pore water samples were analysed for pH (Jenway 3310 pH meter), elements (ICP-OES), major anions (Dionex DX-500 ion chromatograph), and Total Organic Carbon (TOC) (Shimadzu TOC 5000). Speciation of Cu, Pb and Zn in pore water samples was modelled using WHAM VI (Tipping, 1998). In the absence of characterisation of the TOC fractions, we assumed that 50% of TOC was fulvic in origin and that the fulvic acid contained 50% C (Tipping, 1996; Pribyl, 2010).

Arsenic speciation in pore waters extracted from the DGC soil was determined in a separate experiment. This was carried out at the Analytical Geochemistry Laboratory at British Geological Survey, Keyworth separately to the previous experiment to ensure that freshly produced pore waters were analysed within 24 hours of extraction. Therefore experimental and analytical procedures differed in order to match instrument availability and adhere to local standard operating procedures. Five bags of DGC soil containing single specimens of *L. terrestris* and five earthworm-free bags were incubated for 26 days and the pore water extracted by centrifuging at 14 000rpm for 40 min. Arsenate (AsV), arsenite (AsIII), arsenobetaine (AB), methylarsonate (MA) and dimethylarsinate (DMA) species of As were then quantitatively determined in the pore water within 24 hours of extraction by HPLC-ICP-MS using the method described by Watts et al (2008). Spiked recoveries in pore waters were used to ensure transformation between species did not occur due to the procedure.

Statistical analysis

Minitab version 15 was used for all statistical analysis. Normality of data and equal variance between treatments was tested using the Kolmogorov-Smirnov test ($p>0.05$) and Bartlett's test ($p>0.05$) respectively. Data that was found not to be normal was Log transformed or outliers (> 2 standard deviations away from the mean) were removed. Where comparisons between treatments were made (e.g. between casts, bulk or control soil or between earthworm inhabited and earthworm free soil for one variable in one soil type), one-way ANOVA was carried out and Fisher's Least Significant Difference test ($p<0.05$ and $p<0.01$) used to identify significant differences between individual means.

Quality control

The aqua regia digestion of soil samples was carried out alongside an in-house reference material traceable to a certified reference material (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 (<14 mg kg^{-1}). During the ICP-OES analysis of all samples, calibration standards were analysed as samples at the end of each run to ensure that drift did not occur. Deviation was no greater than ± 4% for As, Cu, Pb and Zn.

Results

Earthworm mortality was low in the contaminated soils with 100% survival in Rookhope and Wisley soils and12% mortality in the DGC soil. Earthworms in all three soils lost weight during the test period. *L. terrestris* fresh weight decreased by 21% (1.1g, SD = 0.88, n = 25) in Rookhope soil, 11% (0.5g, SD = 0.48, n = 25) in the Wisley soil and 27% (1.3g, SD = 1.01, n = 22) in the DGC soil.

Mobility and partitioning of metals and metalloids in soil and casts

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

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

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

Mobility and speciation of metals and metalloids in pore water

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

Speciation modelling indicates that organic complexes and free ions of Cu, Pb and Zn were the major species present in the pore waters from all three soils (67 – 100% and 0 – 32 %; 27 – 99 % and 1 – 72% and, 3 – 14 % and 81 – 96 % respectively, Table 3). There was a greater modelled abundance of Cu ions and lower abundance of organically bound Cu in earthworm inhabited Wisley soil compared to earthworm free control soil. In the DGC earthworm-inhabited soil pore waters there were modelled decreases in free ions of Pb^{2+} and Zn^{2+} and increases in organo-Pb and -Zn relative to the controls, whereas for Wisley and Rookhope (Zn only) there were modelled increases in free Pb^{2+} and Zn^{2+} and decreases in organo-Pb and -Zn. Thus for Wisley and Rookhope not only are there greater concentrations of Pb and Zn in pore water due to the activity of *L. terrestris* (Fig. 3), but also a greater proportion are in a chemical form (free ions) that is potentially more available to organisms than in the earthworm-free soil (Di Toro et al., 2001; Thakali et al., 2006). In the DGC soil pore waters the majority (>90%) of the As was present as As(V). There was a significantly ($p< 0.01$) greater concentration of arsenobetaine (AB) in the pore water from earthworm-inhabited soil pore water relative to the control (Fig. 4) but when expressed as a percentage of total As, this difference is not significant.

Discussion

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

Other studies have reported increased mobility of metals in earthworm casts (Kizilkaya, 2004; Udovic and Lestan, 2007; Udovic et al., 2007), but the impact of earthworms on pore water concentrations has not previously been studied to our knowledge. The use of water as a metal extracting agent is likely to yield lower metal concentrations than weak salt, acid or chelating extractions. The water extractable fraction represents the most available portion of the total metal concentration in soil and it can be stated with a degree of certainty that this fraction is mobile. Weak salt solutions mimic soil solutions but do not effectively represent the complex mix of organic and inorganic components present in soil pore water. Therefore direct measurement of pore water was employed to determine metal mobility in the soil solution. Many of the impacts of earthworms on metal and metalloid mobility observed can be explained by earthworm induced changes in soil pH (Masscheleyn et al., 1991; Temminghoff et al., 1997; Martínez and Motto, 2000) or WEOC (Jordan et al., 1997; Temminghoff et al., 1997; Bauer and Blodau, 2009).

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

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

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

The greater solubility of Cu in casts compared with control or bulk soil (Fig. 1) can be explained by the higher concentration of WEOC in the casts compared with the control and bulk soil (Table 2) as Cu binds strongly to organic complexes in solution (Temminghoff et al., 1997). The lower concentrations of Cu in the exchangeable fraction of the casts of DGC soil compared with control may indicate a movement of Cu from the exchangeable fraction to the water extractable fraction (Table S1 and Fig. 1). Li et al. (2009) explain an increase in Cu mobility after transit through the gut of *Eisenia fetida* by the formation of ‘mini-molecule organic acids’, due to the breakdown of organic matter, that have a high capacity for Cu^{2+} complexation. This process may also be occurring in these soils as the earthworms degrade the organic matter and release organic compounds into solution. It therefore seems that *L. terrestris* are mobilising Cu that is exchangeable into solution by organic complexation rather than mobilising organically bound Cu. However, there is a lower concentration of Cu in pore waters from Wisley and DGC earthworm-inhabited soils (Fig. 3) despite greater TOC in the DGC earthworm-inhabited pore waters and lower pH in Wisley earthworm-inhabited pore waters (Table 2).

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

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

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

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

Conclusions

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

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Supporting information

One table is included in the Supporting Information.

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

	pH[1] (H_2O)	%OM (LOI)[2]	Pseudo-total elements[3] (mg kg^{-1}) As	Cu	Pb	Zn	CEC[4] ($\text{cmol}_c\text{kg}^{-1}$)	Texture[5] % Sand	% Silt	% Clay	Classification[6]
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

[1]Based on BS7755-3.2, 1995. [2]Loss on ignition [3]Aqua regia extractable concentrations based on BS7755-3.9, 1995. [4]Based on (Rowell, 1994). [5]Laser granulometry. [6]Using 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 contaminted soils (n = 5, ± standard error).

		Soil pH (H_2O)	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 contaminted soils (n = 5, ± standard error).

		% Cu species			% Pb species			% Zn species		
		Cu^{2+}	Cu-Org	Cu-Inorg	Pb^{2+}	Pb- Org	Pb-Inorg	Zn^{2+}	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.

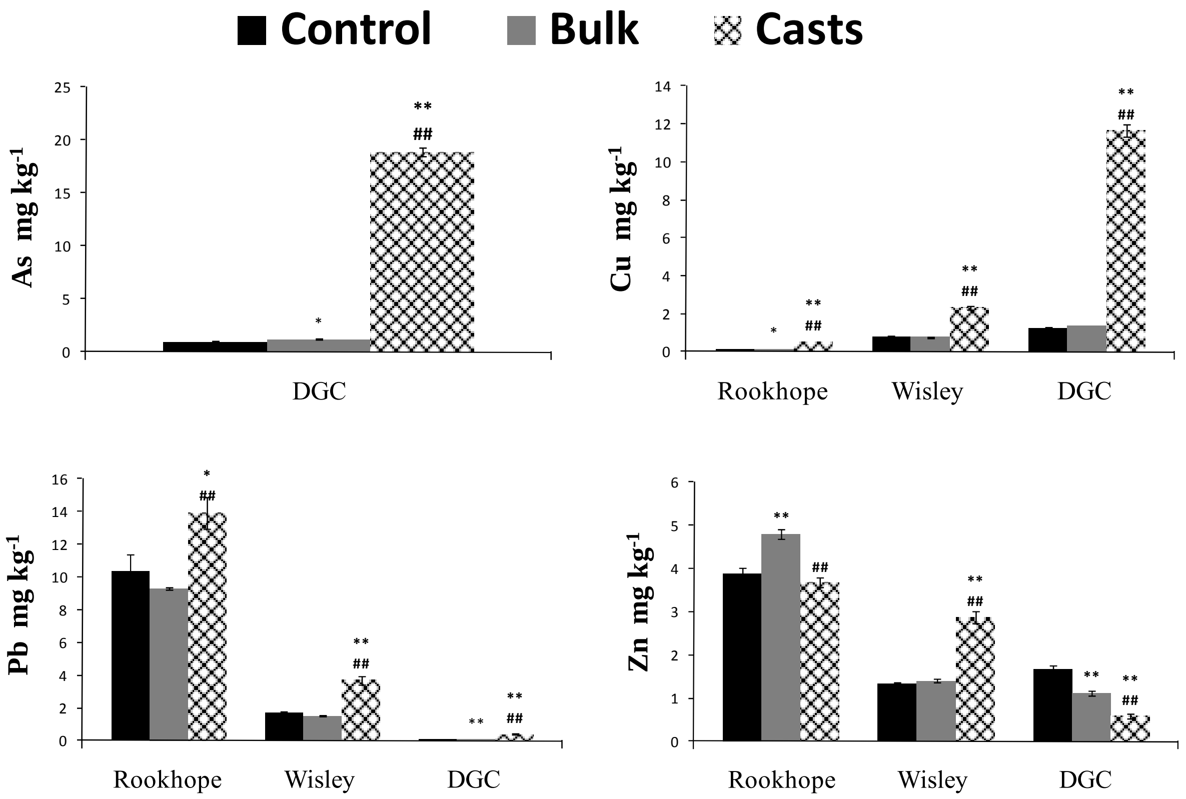

Figure 1. Water extractable As, Cu, Pb and Zn in the control soil, bulk earthworm-inhabited soil and casts of *Lumbricus terrestris* after incubation for 28 days in three contaminated soils. n = 5, error bars represent standard errors of the mean. * = significantly different from the control at the 95% 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.

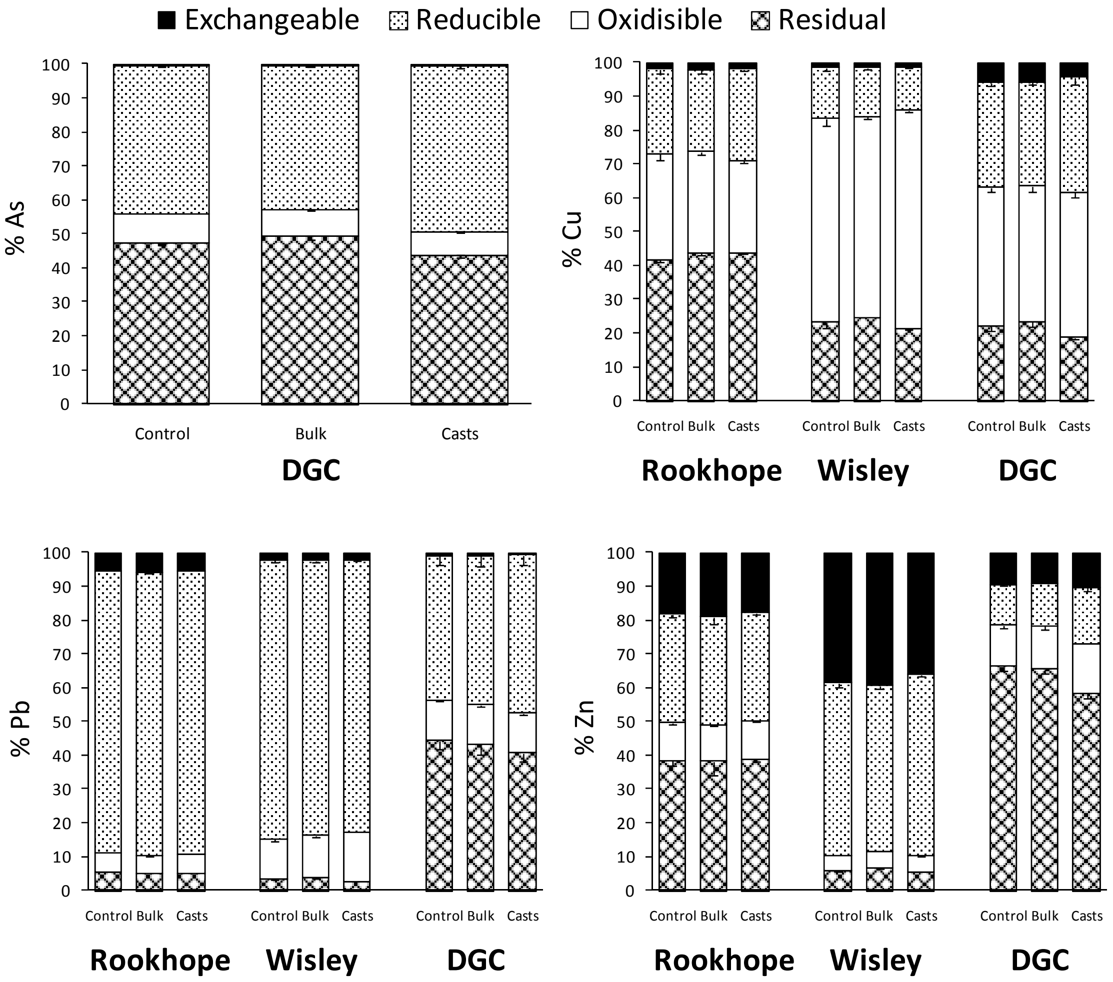

Figure 2 Percentage abundance of As, Cu, Pb and Zn in the exchangeable, reducible, oxidisable and residual fractions of earthworm-free control soil, bulk earthworm-inhabited soil and casts of *Lumbricus terrestris* after incubation for 28 days in three contaminated soils. n = 5, error bars represent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

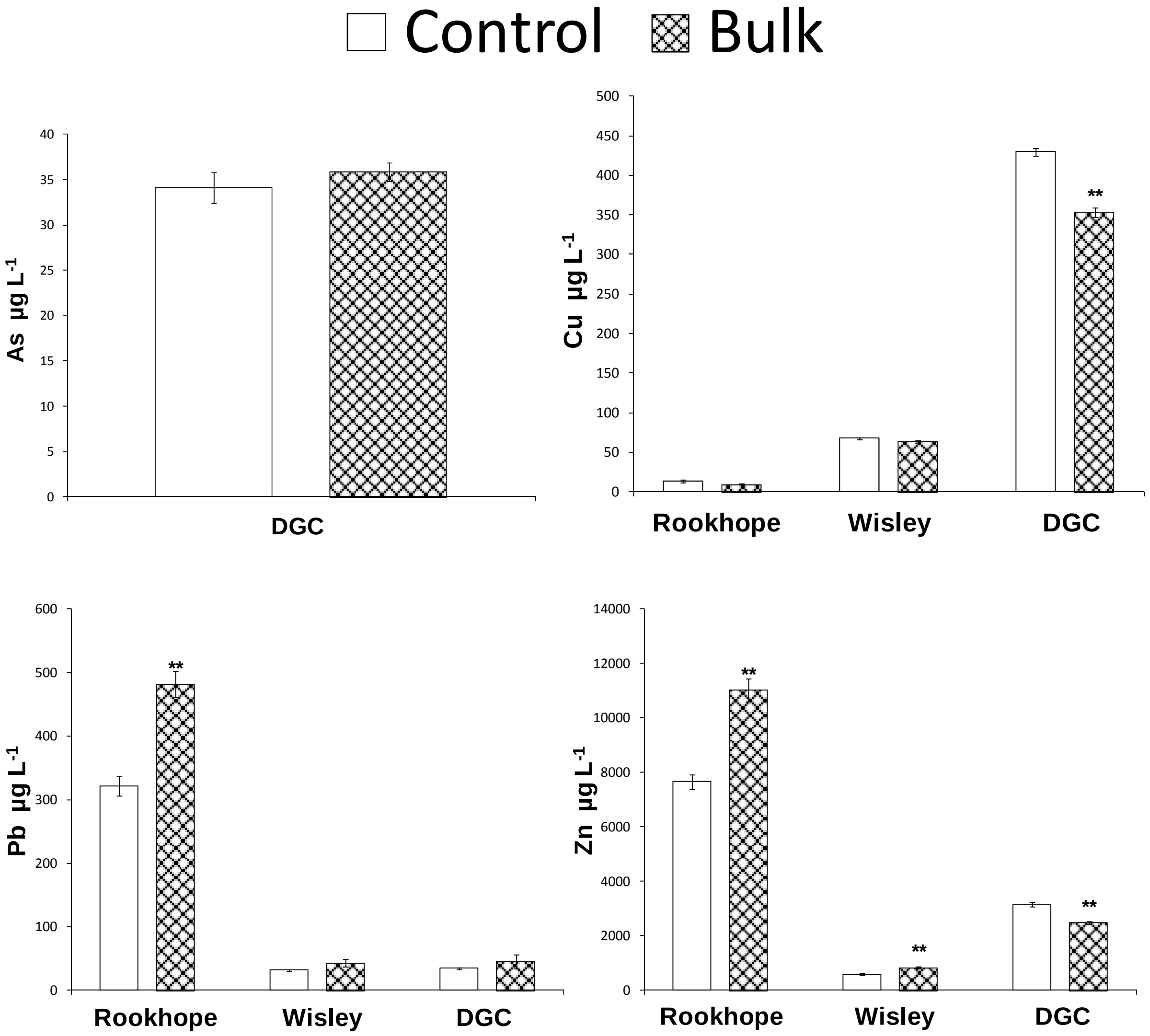

Figure 3 Mean concentration of metals and metalloids in pore water extracted from three contaminated earthworm-free control and bulk earthworm-inhabited soils by centrifuging. *Lumbricus terrestris* were incubated in soils for 28 days. n = 5, error bars represent standard errors of the mean. ** = significantly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

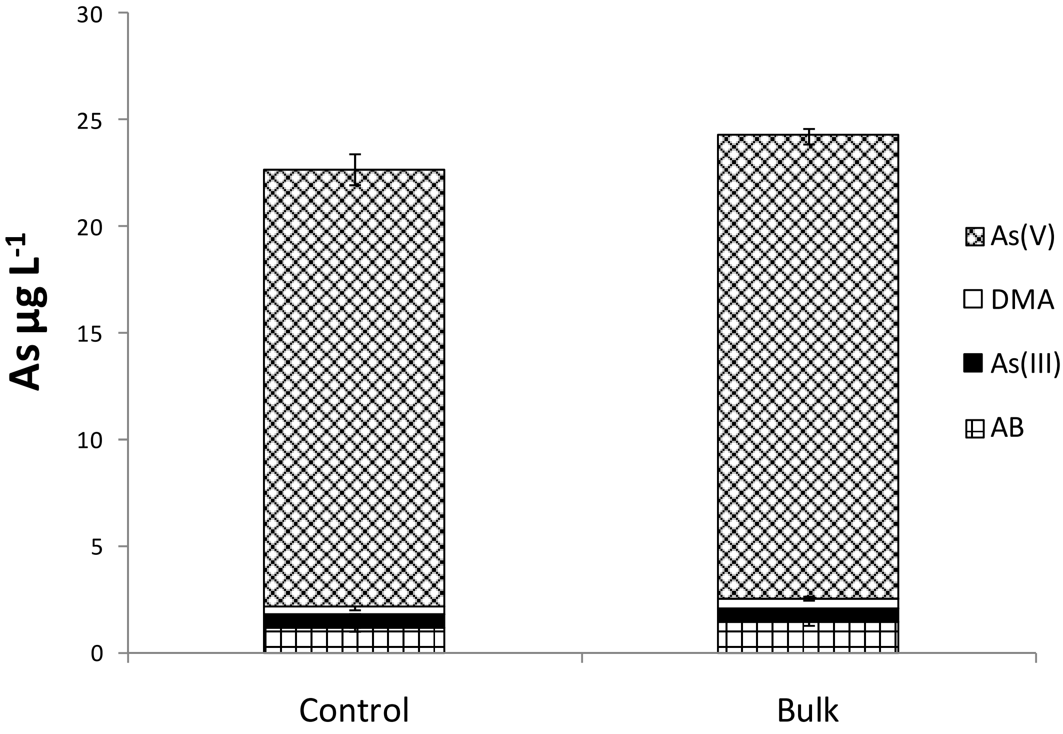

Figure 4 Mean concentration of As species in pore water extracted from earthworm-free control and bulk earthworm-inhabited DGC soil by centrifuging. *Lumbricus terrestris* were incubated in soils for 26 days. n = 5, error bars represent standard errors of the mean.
Recovery was 93% of total As in pore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