Environ Geochem Health (2009) 31:273–282
DOI 10.1007/s10653-008-9208-3

ORIGINAL PAPER

Earthworms and in vitro physiologically-based extraction tests: complementary tools for a holistic approach towards understanding risk at arsenic-contaminated sites

Mark Button · Michael J. Watts · Mark R. Cave · Chris F. Harrington · Gawen T. Jenkin

Received: 7 March 2008 / Accepted: 7 July 2008 / Published online: 29 October 2008

Abstract The relationship of the total arsenic content of a soil and its bioaccumulation by earthworms (*Lumbricus rubellus* and *Dendrodrilus rubidus*) to the arsenic fraction bioaccessible to humans, measured using an in vitro physiologically-based extraction test (PBET), was investigated. Soil and earthworm samples were collected at 24 sites at the former arsenic mine at the Devon Great Consols (DGC) in southwest England (UK), along with an uncontaminated site in Nottingham, UK, for comparison. Analysis of soil and earthworm total arsenic via inductively coupled plasma mass spectrometry (ICP-MS) was performed following a mixed acid digestion. Arsenic concentrations in the soil were elevated (204–9,025 mg kg^{-1}) at DGC. The arsenic bioaccumulation factor (BAF) for both earthworm species was found to correlate positively with the human bioaccessible fraction (HBF), although the correlation was only significant ($P \leq 0.05$) for *L. rubellus*. The potential use of both in vitro PBETs and earthworms as complementary tools is explored as a holistic and multidisciplinary approach towards understanding risk at contaminated sites. Arsenic resistant earthworm species such as the *L. rubellus* populations at DGC are presented as a valuable tool for understanding risk at highly contaminated sites.

Keywords Arsenic · Bioaccessibility · Bioaccumulation · Exposure · Risk

M. Button · M. J. Watts (✉) · M. R. Cave
British Geological Survey, Keyworth, Nottingham NG12 5GG, UK
e-mail: mb274@le.ac.uk; mwatts@bgs.ac.uk

M. Button · G. T. Jenkin
Department of Geology, University of Leicester, Leicester, UK

C. F. Harrington
School of Science and Technology, Nottingham Trent University, Nottingham, UK

Introduction

Arsenic-contaminated land is demanding increasing attention from environmental scientists due to its potential toxicity to humans, flora and fauna (Camm et al. 2004). A widely employed method for the assessment of risk to human health from contaminated land in the UK, the Contaminated Land Exposure Assessment (CLEA) model (Defra 2002), is arguably preoccupied with the derivation of a single universal guideline value, presumably to facilitate practicality and ease of application (Hamilton 2000). The current Soil Guideline Value (SGV) in the UK (implemented in 2002) for residential gardens and allotments is specified at 20 mg kg^{-1} dry weight (Defra 2002). In parts of the UK, such as the southwest, where arsenic contamination is widespread due to historic mining and calcination of polymetalic ores (Camm et al.

2004; Elteren et al. 2005; Hutton et al. 2005), the current SGV is unrealistic. One major criticism of the CLEA model is that contaminants are assumed to be completely available to a receptor following exposure (Hutton et al. 2005), leading to a potential overestimation of exposure. The primary pathways of human exposure to arsenic in soil that result in significant health effects are inhalation and oral ingestion, leading to both carcinogenic and non-carcinogenic responses, whilst dermal adsorption is not thought to be significant (Schultz and Bicksey 2003). Consideration of a contaminant's oral bioaccessibility is important in understanding exposure associated risk (Intawongse and Dean 2006). Numerous in vitro physiologically-based extraction tests (PBETs) have been developed as simple, inexpensive tools to investigate the bioaccessibility of soil contaminants (Oomen et al. 2002). Uncertainties as to whether these models produce similar estimations of bioaccessibility has hindered their incorporation into the contaminated land risk assessment process. The Bioaccessibility Research Group of Europe (BARGE 2008) undertook an international collaborative initiative to establish a unified PBET method (the Unified Barge Method; UBM) for estimating human bioaccessibility capable of providing reproducible, robust and defensible bioaccessibility data (Cave et al. 2006). Such efforts are likely to hasten the adoption of bioaccessibility testing in risk assessment, reinforced by the fact that the Scottish and Northern Ireland Forum For Environmental Research (SNIFFER) already propose a method for deriving site-specific human health assessment criteria for contaminants in soil that incorporates bioaccessibility testing (Fergusen et al. 2003).

Ecosystem indicator species such as earthworms have proven a useful tool in assessing soil contamination, particularly the accumulation of a contaminant by earthworm populations, as a guide to bioavailability (Langdon et al. 2001, 2003; Mariño and Morgan 1998; Morgan and Morgan 1999). The earthworm species *Lumbricus rubellus* and *Dendrodrilus rubidus* are known to inhabit soils and mine wastes in southwest England highly contaminated with arsenic (Morgan et al. 1994). They are thought to have developed a resistance to arsenic toxicity (Langdon et al. 1999), although not through avoidance of the contamination, since arsenic body burdens have been demonstrated up to 566 mg kg^{-1} (Langdon et al. 2002). This ability to accumulate high levels of arsenic makes these two earthworm species particularly useful tools in assessing arsenic bioavailability to indicator species in highly contaminated soils. Both earthworm species are epigeic (surface-living) and therefore ideal in assessing the soil surface, the soil fraction of most concern in assessing human exposure. Whilst many studies have investigated the impact of soil contamination on soil biota, in particular earthworms (Cotter-Howells et al. 2005; Piearce et al. 2002; Van Vliet et al. 2006), ecological input into contaminated land risk assessment is poor. A holistic approach, whereby the geochemical, human and ecological aspects of contaminated land are employed as multiple lines of evidence in understanding risk, requires investigation.

The aim of this work is to examine the interrelatedness of available tools in understanding the risk to human health and the ecosystem at arsenic contaminated sites. Comparison of the soil total arsenic, human bioaccessible fraction and bioaccumulation by earthworms will provide insight into whether or not these complementary tools can be used in parallel for a more holistic approach towards understanding risk at contaminated sites.

Materials and methods

Study site

The Devon Great Consols (DGC) is situated by the River Tamar in the Tavistock district of Devon (rgid reference: SX426735) and is one of many former mining sites in southwest England (Fig. 1). Soil arsenic concentrations found in and around the mine vary significantly depending on their proximity to the main tailings and range from 249 to 34,000 mg kg^{-1} (Klinck et al. 2002; Langdon et al. 2001). Klinck et al. (2002) demonstrated the high potential for the release of arsenic from sulphide ore and other wastes by carrying out leaching experiments. Arsenic bioaccessibility in soils in the mine area and mine tailings have previously been shown to be well above the 20 mg kg^{-1} SGV (Cave et al. 2002) for gardens and allotments. Notably, concentrations up to 624 mg kg^{-1} of bioaccessible arsenic in residential areas around the mine site were reported as giving cause for concern in terms of potential human exposure.

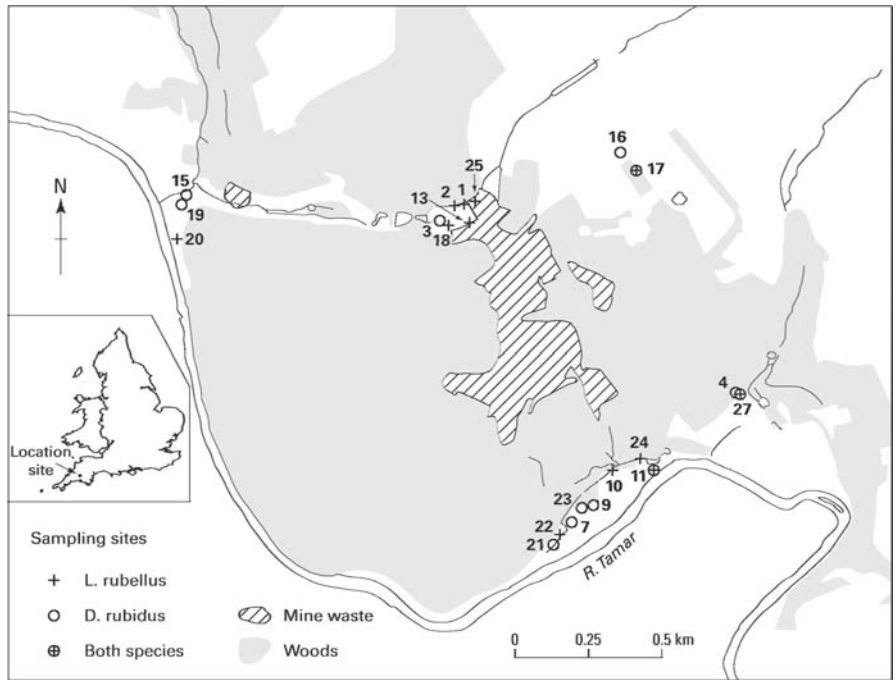

Fig. 1 Geographical location of study area and positioning and identification number of sampling sites

Sample collection and preparation

The following sample collection and preparation methods were employed at the contaminated study site (DGC) and at an uncontaminated Nottingham garden. The soil surface (0–20 cm) with an area of $\sim 1\ m^2$ was overturned with a spade allowing individual earthworms to be handpicked. Specimens were promptly separated according to species using a dichotomous earthworm key (WWC 2008), thoroughly rinsed with deionised water and placed in ventilated plastic tubes with moist filter paper to begin depuration of the gut contents. Filter papers were changed daily to prevent coprophagy. Earthworms were depurated for a minimum of 48 h since shorter times were unlikely to remove all soil particles in larger species such as *L. rubellus* (Langdon et al. 2003). Approximately 10–25 mature earthworms (clitellum present) were collected at each sampling point. Depurated earthworms were thoroughly rinsed with deionised water and dried in a low temperature oven (50°C) before homogenisation in a ceramic pestle and mortar. A composite soil sample from the overturned surface (~ 1 kg) was collected at each site, placed in a sealed paper bag and dried at room temperature. Soils were disaggregated in a ceramic pestle and mortar, sieved to <250 µm, homogenised by shaking, then stored in airtight containers prior to analysis.

Standards and reagents

All reagents used were analytical grade or better quality. All aqueous solutions were prepared using deionised water (18.2 MΩ; Millipore, UK). Concentrated HNO_3, HF, 30% v/v H_2O_2 and $HClO_4$ (BDH; Aristar, UK) were used for the dissolution of earthworms and soil samples. $CaCl_2$ (Fisher Scientific, UK) was used for the measurement of soil pH. NaCl, KSCN, Anhydrous Na_2SO_4, KCl, $CaCl_2$ $2H_2O$, NH_4Cl, $NaHCO_3$, KH_2PO_4, $MgCl_2$ $6H_2O$, NaOH, HCl, urea, anhydrous D+ glucose, D-glucosaminehydrochloride, pepsin (pig), bovine serum albumin (BSA), pancreatin (pig), 69% HNO_3 (Merck, UK), α-amylase (bacillus species), lipase (pig), bile salts (bovine) (Sigma, UK), NaH_2PO_4 (Baker, UK), Mucin (pig) (Carl Roth, Germany), D-glucuronic acid (Fluka, Germany) and uric acid (Merck-Prolabo, UK) were used in the in vitro UBM PBET for estimating human bioaccessibility (Cave et al. 2006).

Total digestion of earthworm

Microwave-assisted (CEM MARS5; CEM, UK) dissolution of the earthworms using a closed vessel system was performed on 0.1 g of earthworm homogenate (dry weight). Concentrated nitric acid (10 ml) and hydrofluoric acid (100 µl) was added, allowed to stand for 30 min and then microwaved. Following an initial heating program (ramp to 100°C over 5 min, then hold for 5 min, ramp to 200°C over 5 min, then hold for 5 min) the vessels were allowed to cool (<50°C) and then 1 ml of 30% H_2O_2 was added. The vessels were sealed and the microwave cycle repeated. After cooling, the sample solutions were transferred to PTFA Savillex containers and evaporated to dryness on a hotplate (100°C) to reduce the presence of organic compounds that could form possible polyatomic interferences on analysis by ICP-MS. Samples were reconstituted by the addition of 2 ml of 50% v/v nitric acid, heated at 50°C for 30 min and then made up to 10 ml with deionised water. This final stage reduced the dilution of the acid to that required for ICP-MS measurement (<2.5% v/v). The procedure was monitored using a certified reference material, CRM 627 tuna fish (BCR, Brussels). Mean total arsenic recoveries of 96 ± 7% ($n = 6$) were obtained, compared to the certified value. The method precision, expressed as the mean % difference (±1 SD), between duplicate earthworm samples was 1.7 ± 0.9% ($n = 4$ duplicates).

Soil chemistry

Soil pH was determined by adding 0.01 M aqueous $CaCl_2$ (6.25 ml) to 0.25 g of homogenised soil. Each soil slurry was mixed for 5 min and left to stand for 15 min prior to analysis using a pH meter (SA720; Orion, UK). Readings were checked at the start and end of the run using a pH 7 buffer solution and an in-house QC standard (pH 7.3). Loss on ignition (LOI) was also determined for each soil sample to provide an estimation of the organic matter content. One gram (dry weight) of each soil was weighed into a glass crucible before heating to 450°C for 4 h. The percentage weight reduction after heating was recorded as the estimated organic matter content.

Soil dissolution

Homogenised soils (0.25 g) were prepared for total elemental measurements by ICP-MS based on a mixed acid digestion approach ($HF/HNO_3/HClO_4$) (Green et al. 2006). Samples were weighed directly into PFA vials, acids added and heated on a temperature programmable graphite hot-block (80°C for 8 h, 100°C for 2 h, 120°C for 1 h, 140°C for 3 h, 160°C for 4 h). This mixture was used, rather than the more widely used aqua regia, as the hydrofluoric acid breaks down the silicate structure, except for a few accessory minerals to give an almost total digest and hence total concentrations can be determined. $HClO_4$ was used to breakdown more resistant minerals and ensure complete evaporation of the hydrofluoric acid. Once digested and evaporated, the sample was taken up in 2.5 ml of concentrated nitric acid, heated at 50°C for 30 min and then treated with 30% v/v H_2O_2 to avoid precipitation of meta-stable hydroxyl-fluorides, before being made up to volume (25 ml) with deionised water to give a final solution of 5% HNO_3 for analysis by ICP-MS. Certified reference materials were included with each batch of soil digestions as a measure of quality control. These were NIST CRM 2710 Montana Soil I and NIST CRM 2711 Montana Soil II. Recoveries of 98 ± 4% ($n = 6$) and 91 ± 3% ($n = 3$), respectively, were achieved during the course of the study. The repeatability precision for the method was additionally assessed using the Thompson Howarth precision control method (RSC 2002). Thompson Howarth Precision Control Charts are a simple graphical method for assessing and controlling repeatability precision from a moderate number of duplicated analytical results, in this case $n = 21$ duplicate analyses. The repeatability precision was found to exceed the specified fitness for purpose (FFP) criteria of 5% RSD on the duplicate analyses.

Physiologically-based extraction technique

The UBM PBET (Cave et al. 2006) was employed in this study with the permission and assistance of BARGE members. 0.6 g of <250 µm dried and homogenised soil was mixed with 9 ml of simulated saliva at pH 6.5 for 5 min. About 13.5 ml of simulated gastric solution was then added at pH 0.9–1.0 to give a final pH of 1.2 and shaken

end-over-end at 37°C for 1 h. This first stage constituted the stomach-only phase of the extraction technique. In order to simulate the stomach and intestinal phase together, a duplicate stomach phase solution was produced and to this 27 ml of simulated duodenal fluid and 9 ml of simulated bile fluid at pH 6.3 were added and shaken end-over-end at 37°C for 4 h. The phase giving the highest value was taken as the estimation of arsenic bioaccessibility. Certified reference material NIST 2710 (Montana soil) was included in each batch of samples ($n = 5$) along with duplicates and reagent blanks. CRM 2710 was also employed by BARGE in an inter-laboratory study (Cave et al. 2006) facilitating comparison of the results obtained for NIST 2710 in this study with those of BARGE. The results for arsenic (in mg kg^{-1}, errors expressed as ±1 SD) were highly comparable. The BARGE inter-laboratory study obtained 323 ± 45 mg kg^{-1} ($n = 4$) for the stomach-only phase and 264 ± 18 mg kg^{-1} ($n = 3$) for the stomach and intestine phase. In the present study, 310 ± 8 mg kg^{-1} ($n = 5$) was obtained for the stomach-only phase and 249 ± 2 mg kg^{-1} ($n = 5$) for the stomach and intestine. These recoveries were well within error on the BARGE inter-laboratory values suggesting good reproducibility and accuracy of results using the UBM PBET method. The method precision expressed as the mean percentage difference (±1 SD) between duplicate samples was 3.8 ± 3.5% ($n = 12$ duplicates).

Instrumentation

Earthworm and soil digests were analysed for trace metal contents using a Thermoelemental PQ ExCell ICP-MS. The standard operating conditions were as follows: RF power 1,350 W; gas flow rates: coolant 13 l min^{-1}, auxiliary 0.9 l min^{-1}, nebuliser 0.93 l min^{-1}; spraychamber temperature 3°C; Meinhardt nebuliser. The instrument was tuned using a 1 μg l^{-1} dilution of Claritas PPT multielement tune solution 1 (GlenSpectra Reference Materials, UK). Data was acquired in peak jump mode with an acquisition of 3 × 30 s. Indium at a concentration of 10 μg l^{-1} was used as an internal standard and was added to the sample stream via a T-piece. UBM PBET solutions were analysed using a Fisons ARL ICP-AES, with a low flow torch, Babington nebuliser and impact bead spray chamber. Simultaneous detection of analytes was employed with radial viewing of plasma at 650 W forward power. All samples were analysed at maximum dilution to minimise the occurrence of matrix effects.

Results

Total arsenic concentrations

Arsenic concentrations in soils were highly variable depending on their proximity to the mine tailings. Sampling sites 1–3, 13, 18 and 25 to the north of the study area (Fig. 1) and closest to the mine tailings demonstrated the highest soil arsenic concentrations in the range 1,005–9,025 mg kg^{-1}. Sampling sites 4, 7, 9–11, 21–24 and 27 to the south of the study area, closest to the River Tamar and further from mine tailings demonstrated lower soil arsenic concentrations in the range 204–1,306 mg kg^{-1}. Sites 15, 19–20 and 16–17 to the northwest and northeast, respectively, of the study area displayed soil arsenic concentrations in the range 622–6,308 mg kg^{-1}. The soil arsenic concentration at the uncontaminated Nottingham comparison site was 16 mg kg^{-1}, below the current SGV of 20 mg kg^{-1} (Defra 2002).

L. rubellus were found inhabiting soils covering a wide arsenic concentration range from 204 to 9,025 mg kg^{-1}, with a mean of 2,301 mg kg^{-1} ($n = 12$) (Table 1). *D. rubidus* were only found in soils up to an arsenic concentration of 3,995 mg kg^{-1} with a mean of 837 mg kg^{-1} ($n = 12$). Both earthworm species were found cohabiting at three sites (11, 17 and 27) where arsenic concentrations were comparatively low, 289–622 mg kg^{-1}. The high mean soil arsenic concentrations at *L. rubellus* sites were reflected by high mean arsenic body burdens for this species (mean 287 mg kg^{-1}, $n = 12$). The mean arsenic body burden for *D. rubidus* was 134 mg kg^{-1} ($n = 12$). The arsenic body burden ranges for both earthworm species were similar (Table 1), and the difference between the mean values was not significant (Table 2). At the uncontaminated comparison site where both species of earthworm were also found residing together, the arsenic body burdens were similar (Table 1). A positive linear correlation was observed between the arsenic concentration in the soil and arsenic body burdens for both earthworm

species (Fig. 2) with R^2 values of 0.73 and 0.93 for *L. rubellus* and *D. rubidus* respectively.

Bioaccumulation

Earthworm bioaccumulation factors (BAFs) were calculated as the earthworm total arsenic (mg kg^{-1}) divided by soil total arsenic (mg kg^{-1}). BAFs of <1.00 were observed at all sites indicating no enrichment above soil concentration was occurring. The mean BAF for *L. rubellus* of 0.15 ($n = 12$) was slightly higher than for *D. rubidus* at 0.12 ($n = 12$), although the BAF range for both earthworm species were similar at around 0.04–0.30 (Table 1), while the difference between the mean values was not statistically significant (Table 2).

Bioaccessibility

The estimated human bioaccessible fraction (HBF) of arsenic, calculated as the bioaccessible arsenic (mg kg^{-1}) divided by total arsenic in the soil (mg kg^{-1}), varied substantially across sites from 0.10 to 0.34. The HBF at the uncontaminated comparison site was higher at 0.42. A positive linear correlation was observed between the bioaccessible arsenic and total arsenic in the soil ($R^2 = 0.93$) (Fig. 2) when all sites were combined. This trend did not differ when the sites were split into groups for *L. rubellus* and *D. rubidus* (Fig. 3). The trend was similar to that of total arsenic in both earthworm species suggesting colinearity between earthworm BAFs and the HBF at the investigated sites. Table 1 shows the mean arsenic bioaccessibility was higher for *L. rubellus* sites (413 mg kg^{-1}) than for *D. rubidus* (177 mg kg^{-1}), although the ranges were similar (Table 1) and differences between the two earthworm species were not significant (Table 2).

Table 2 Results of the Wilcoxon signed-rank test for significance of difference between paired groups (*L. rubellus* and *D. rubidus* sites)

Group variable	*P* value
Soil total As (mg kg^{-1})	0.13
Worm total As (mg kg^{-1})	0.13
Bioaccessible As (mg kg^{-1})	0.53
Soil pH	0.82
Soil LOI (%)	0.29
HBF	0.56
BAF	0.40

Difference between group variables significant at $P < 0.05$

HBF Human bioaccessible fraction, *BAF* earthworm bioaccumulation factor, *LOI* loss on ignition

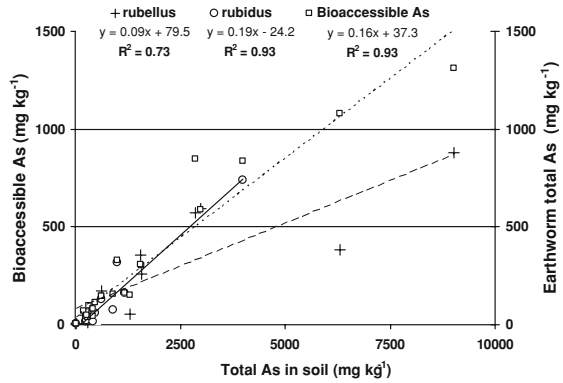

Fig. 2 Correlations between bioaccessible As and earthworm total As to soil total arsenic for *Lumbricus rubellus* and *Dendrodrilus rubidus* (includes DGC and control site)

Table 1 Mean As data presented with the range encountered across *Lumbricus rubellus* and *Dendrodrilus rubidus* sampling sites

	Mean*rubellus* ($n = 12$)	Min	Max	Control *rubellus*	Mean*rubidus* ($n = 12$)	Min	Max	Control *rubidus*
Soil total (mg kg^{-1})	2,301	204	9,025	16	837	255	3,995	16
Worm total (mg kg^{-1})	287	11	877	6.5	134	15	737	7.1
Bioaccessible (mg kg^{-1})	413	36	1,312	6.7	177	36	837	6.7
HBF	0.19	0.10	0.34	0.42	0.21	0.13	0.33	0.42
BAF	0.15	0.04	0.28	0.41	0.12	0.04	0.32	0.44
Soil pH	4.6	3.5	6.1	6.7	4.7	4.0	6.8	6.7
Soil LOI (%)	5.7	1.9	12	3.8	4.6	1.9	12	3.8

HBF Human bioaccessible fraction, *BAF* earthworm bioaccumulation factor, *LOI* loss on ignition

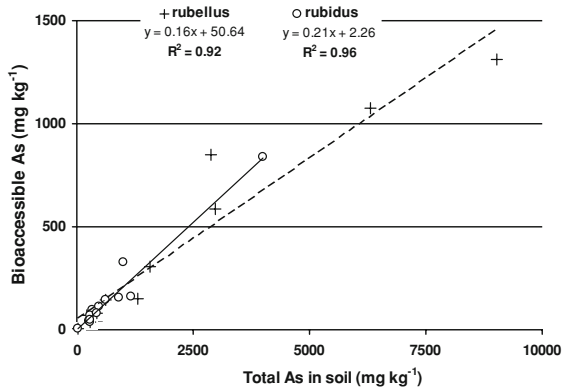

Fig. 3 Correlations between bioaccessible As and soil total arsenic at *L. rubellus* and *D. rubidus* sites

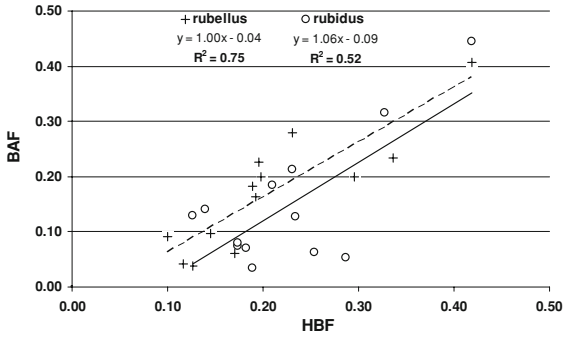

Fig. 4 Correlations between the human bioaccessible fraction (*HBF*) and earthworm bioaccumulation factors (*BAF*) at *L. rubellus* and *D. rubidus* sites

Comparability of estimated HBF and earthworm BAF

Figure 4 displays the HBF plotted against the BAFs of both earthworm species. The bioaccumulation of arsenic by *L. rubellus* correlates positively with the HBF at each site ($R^2 = 0.75$). This is reflected in similar mean values for *L. rubellus* sites of 0.19 ($n = 12$) for the HBF and 0.15 ($n = 12$) for the mean BAF (Table 1). The BAFs for *D. rubidus* also showed a positive correlation with the HBF at each site ($R^2 = 0.52$), although the correlation was not significant, as reflected by the greater difference between the means of each measure for this species (0.21 and 0.12, $n = 12$) for HBF and BAF, respectively.

Statistical analysis

Potential causes for the differing correlations between BAF and HBF for *L. rubellus* and *D. rubidus*, such as differing soil edaphic and geochemical factors were investigated. The non-parametric Wilcoxon signed-rank test for two related samples was applied (SPSS 14.0) to the groups (*L. rubellus* and *D. rubidus* sites) for each of the variables listed in Table 2. The hypothesis that the two groups differ is significant at P values <0.05. No significant difference was observed between the groups for any of the variables tested.

The significance of the positive correlation between BAF and HBF for both earthworm species was also investigated via a non-parametric significance test using bootstrap resampling (Efron et al. 1993) of the paired datasets. The datasets were resampled 1×10^4 times using a resampling statistics add-in package for Excel (Blank et al. 2001). For each resample, the slope of the BAF to HBF least square linear fit was recalculated. The upper and lower 95% significance limits were calculated from the resampled data (97.5 and 0.025 percentiles). The 95% confidence limits for *L. rubellus* were 0.66–1.53, showing that the slope was significantly different from zero and, therefore, a significant relationship exists. For the *D. rubidus* samples, however, the 95% confidence limits were −0.31 to 1.52, showing that the slope was not significantly different from zero and that there was not a significant relationship between BAF and HBF for this species of earthworm.

Discussion

Soil arsenic concentrations at the sites investigated at DGC were found to be elevated well above the current SGV (20 mg kg^{-1}). The values presented in this paper are in agreement with levels reported in previous studies (Kavanagh et al. 1997; Klinck et al. 2002; Langdon et al. 2002). Whilst the HBF of arsenic was never greater than 0.34 (Table 1) of the total arsenic in the soil, bioaccessible arsenic levels at all sites were well above the SGV. Soils at DGC are reported to show higher arsenic bioaccessibility than other mineralised soils not affected by mining (Palumbo-Roe and Klinck 2007). Anthropogenic sources of contamination such as mine wastes are

likely to give rise to higher bioaccessibility as the contaminant has relatively little time to bind to soil phases such as iron oxyhydroxides. This may also help to explain the linearity between bioaccessible and total arsenic in the soils at DGC (Fig. 2). The same linear trend was not observed in studies of arsenic bioaccessibility where the source of contamination was geogenic (Palumbo-Roe et al. 2005; Wragg et al. 2007). The higher bioaccessibility of arsenic at DGC is reflected in the arsenic body burdens in both *L. rubellus* and *D. rubidus* populations, which also demonstrate a degree of linearity with increasing soil concentrations (Fig. 2). These results differ from those in the literature where bioaccumulation of contaminants by earthworms is reported to decrease as soil concentrations increase (Neuhauser et al. 1995; Sample et al. 1999). The fact that arsenic accumulation in earthworms at DGC does not conform to models in the literature is likely due to their reported resistance to arsenic toxicity (Langdon et al. 1999).

Previous studies have failed to provide firm evidence about species differentiation in terms of contaminant uptake from soils by earthworms (Mariño and Morgan 1999). The correlation between BAF and HBF was statistically significant for *L. rubellus,* but not for *D. rubidus* in this study and could not be explained by any of the edaphic and geochemical soil characteristics investigated (Table 2). This finding agrees with the suggestion by Morgan and Morgan (1999) that no simple universal relationship exists between soil and earthworm arsenic concentrations. Further research on the ratio of earthworm body mass to the mass of ingested soil would be useful in elucidating causes for the observed difference between earthworm species. *L. rubellus* were reported to be less sensitive to mining derived contamination than other species (Spurgeon and Hopkin 1996). The differences between the earthworm species reported here may be related, in part, to variation in sensitivity to the contamination, which may also explain differences in the distributions of earthworm species around the mine area (Fig. 1).

The accumulation of arsenic by both earthworm species reinforces the observed trends in bioaccessibility at DGC (Figs. 2, 4). However, the intra-species differences in the relationship between BAF and HBF (Fig. 4) highlight the need for a degree of standardisation if biological receptors are to be used in conjunction with in vitro estimates of arsenic bioaccessibility. This need for standardisation also applies when using in vitro bioaccessibility models at contaminated sites if they are to be adopted in the risk assessment process. Small variations between bioaccessibility tests, such as the solid-solution, pH and residence times, have been shown to cause significant differences in bioaccessibilty estimates (Oomen et al. 2002). These differences may also impact upon the relationship of in vitro bioaccessibility tests to in vivo estimates of a contaminant's bioavailability.

For obvious reasons, earthworm species with a developed resistance to arsenic contamination are unsuitable for determining the contaminants toxicity. However, the results presented here suggest resistant earthworm species may be more useful in the indirect assessment of bioavailability at sites with highly elevated levels of arsenic. The incorporation of earthworm BAFs alongside bioaccessibility testing at contaminated sites would provide complementary lines of evidence in support of existing methods for assessing risk such as the CLEA (Defra 2002) and SNIFFER (Fergusen et al. 2003) models.

Conclusions

This study is in no way presented as an alternative to existing methods for understanding risk at contaminated sites. These results represent a focal point for discussion on more holistic, multidisciplinary approaches towards understanding risk at contaminated sites. Indirect measures of a contaminant's bioavailability, such as its accumulation by earthworms, can be used as complementary lines of evidence to reinforce site-wide trends using in vitro bioaccessibility when estimating the potential for human exposure to a contaminant. Further research into the inter-relatedness of earthworm BAFs and in vitro PBETs at sites with differing contamination characteristics would be of benefit. This should include the study of a wider range of earthworm species to qualify the applicability of earthworms for a holistic approach towards understanding risk at contaminated sites.

Acknowledgements The authors wish to thank the British Geological Survey University Funding Initiative (BUFI) for funding this research as part of a PhD studentship. We are also grateful to Joanna Wragg of the British Geological Survey for reviewing the manuscript and the late Tim Brewer for his

guidance early on in the studentship. We would also like to thank the Tavistock estate for granting permission to access the Devon Great Consols site.

References

BARGE (the Bioaccessibility Research Group of Europe). (2008). Retrieved on 22 February 2008, from http://www.bgs.ac.uk/barge/home.html.

Blank, S., Seiter, C., & Bruce, P. (2001). Resampling stats in excel version 2. Arlington.

Camm, G. S., Glass, H. J., Bryce, D. W., & Butcher, A. R. (2004). Characterisation of a mining-related arsenic-contaminated site, Cornwall, UK. *Journal of Geochemical Exploration, 82*, 1–15.

Cave, M. R., Wragg, J., Palumbo, B., & Klinck, B. A. (2002). *Measurement of the bioaccessibility of arsenic in UK soils*. Environment Agency R&D Technical Report, P5-062/TR002.

Cave, M., Wragg, J., Klinck, B., Gron, C., Oomen, A., van de Wiele, T., et al. (2006). Preliminary assessment of a unified bioaccessibility method for potentially harmful elements in soils. *Epidemiology, 17*, 39.

Cotter-Howells, J., Charnock, J. M., Winters, C., Kille, P., Fry, J. C., & Morgan, A. J. (2005). Metal compartmentation and speciation in a soil sentinel: The earthworm, *Dendrodrilus rubidus*. *Environmental Science and Technology, 39*, 7731–7740.

Defra. (2002). *The contaminated land exposure assessment model (CLEA): Technical basis and algorithms CLR10*. Environment Agency.

Efron, B., & Tibshirani, R. J. (1993). *An introduction to the bootstrap*. Chapman & Hall.

Elteren, J., Zdenka, S., Iztok, A., & Hylke-Jan, G. (2005). An interdisciplinary physical-chemical approach for characterization of arsenic in a calciner residue dump in Cornwall (UK). *Environmental Pollution, 139*, 477–488.

Fergusen, C., Nathanail, P., McCaffrey, C., Earl, N., Foster, N., Gillet, A., & Ogden, R. (2003). Method for deriving site-specific human health assessment criteria for contaminants in soil. Retrieved on 22 February 2000, from Scottish and Northern Ireland Forum for Environmental Research website. http://www.sniffer.org.uk/results.asp.

Green, K. A., Chenery, S. R., Barlow, T. S., Taylor, H., & Cook, J. M. (2006). A high productivity sample digestion and analysis methodology for the determination of major and trace elements by ICP, poster presentation. In *13th Biennial National Atomic Spectroscopy Symposium*, Glasgow, UK.

Hamilton, E. I. (2000). Environmental variables in a holistic evaluation of land contaminated by historic mine wastes: A study of multi-element mine wastes in West Devon, England using arsenic as an element of potential concern to human health. *Science of the Total Environment, 249*, 171–221.

Hutton, C., Bryce, D. W., Russeau, W., Glass, H. J., Jenkin, L. E. T., Corns, W. T., et al. (2005). Aqueous and solid-phase speciation of arsenic in Cornish soils. *Mineralogical Magazine, 69*, 577–589.

Intawongse, M., & Dean, J. R. (2006). In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples. *TrAC—Trends in Analytical Chemistry, 25*, 876–886.

Kavanagh, P. J., Farago, M. E., Thornton, I., & Braman, R. S. (1997). Bioavailability of arsenic in soil and mine wastes of the Tamar valley, SW England. *Chemical Speciation and Bioavailability, 9*, 77–81.

Klinck, B. A., Palumbo, B., Cave, M. R., & Wragg, J. (2002). Arsenic dispersal and bioaccessibility in mine contaminated soils: A case study from an abandoned arsenic mine in Devon, UK. British Geological Survey, Research Report RR/04/003.

Langdon, C. J., Piearce, T. G., Black, S., & Semple, K. T. (1999). Resistance to arsenic-toxicity in a population of the earthworm *Lumbricus rubellus*. *Soil Biology and Biochemistry, 31*, 1963–1967.

Langdon, C. J., Piearce, T. G., Meharg, A. A., & Semple, K. T. (2001). Survival and behaviour of the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus* from arsenate-contaminated and non-contaminated sites. *Soil Biology and Biochemistry, 33*, 1239–1244.

Langdon, C. J., Meharg, A. A., Feldmann, J., Balgar, T., Charnock, J., Farquhar, M., et al. (2002). Arsenic-speciation in arsenate-resistant and non-resistant populations of the earthworm, *Lumbricus rubellus*. *Journal of Environmental Monitoring, 4*, 603–608.

Langdon, C. J., Piearce, T. G., Meharg, A. A., & Semple, K. T. (2003). Interactions between earthworms and arsenic in the soil environment: A review. *Environmental Pollution, 124*, 361–373.

Mariño, F., & Morgan, A. J. (1998). Equilibrated body metal concentrations in laboratory exposed earthworms: Can they be used to screen candidate metal-adapted populations? *Applied Soil Ecology, 12*, 179–189.

Mariño, F., & Morgan, A. J. (1999). The time-course of metal (Ca, Cd, Cu, Pb, Zn) accumulation from a contaminated soil by three populations of the earthworm, *Lumbricus rubellus*. *Applied Soil Ecology, 12*, 169–177.

Morgan, J. E., & Morgan, A. J. (1999). The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two ecologically contrasting earthworm species (*Lumbricus rubellus* and *Aporrectodea caliginosa*): Implications for ecotoxicological testing. *Applied Soil Ecology, 13*, 9–20.

Morgan, A. J., Winters, C., & Yarwood, A. (1994). Speed-mapping of arsenic distribution in the tissues of earthworms inhabiting arsenious soil. *Cell Biology International, 18*, 911–914.

Neuhauser, E. F., Cukic, Z. V., Malecki, M. R., Loehr, R. C., & Durkin, P. R. (1995). Bioconcentration and biokinetics of heavy metals in the earthworm. *Environmental Pollution, 89*, 293–301.

Oomen, A. G., Hack, A., Minekus, M., Zeijdner, E., Cornelis, C., Schoeters, G., et al. (2002). Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental Science and Technology, 36*, 3326–3334.

Palumbo-Roe, B., & Klinck, B. (2007). Bioaccessibility of arsenic in mine waste-contaminated soils: A case study from an abandoned arsenic mine in SW England (UK). *Journal of Environmental Science and Health—Part A*

Toxic/Hazardous Substances and Environmental Engineering, 42, 1251–1261.

Palumbo-Roe, B., Cave, M. R., Klinck, B. A., Wragg, J., Taylor, H., O'Donnell, K. E., et al. (2005). Bioaccessibility of arsenic in soils developed over Jurassic ironstones in eastern England. *Environmental Geochemistry and Health, 27*, 121–130.

Piearce, T. G., Langdon, C. J., Meharg, A. A., & Semple, K. T. (2002). Yellow earthworms: Distinctive pigmentation associated with arsenic- and copper-tolerance in *Lumbricus rubellus*. *Soil Biology and Biochemistry, 34*, 1833–1838.

Royal Society of Chemistry: A simple fitness-for-purpose control chart based on duplicate results obtained from routine test materials: Analytical Methods Committee Technical brief number 9. (2002). Retrieved on 22 February 2008 from http://www.rsc.org/images/brief9_tcm18-25951.pdf.

Sample, B. E., Suter Ii, G. W., Beauchamp, J. J., & Efroymson, R. A. (1999). Literature-derived bioaccumulation models for earthworms: Development and validation. *Environmental Toxicology and Chemistry, 18*, 2110–2120.

Schultz, A. C., & Biksey, T. M. (2003). Arsenic speciation and its effect on soil cleanup standards. *Environmental Claims Journal, 15*, 107–118.

Spurgeon, D. J., & Hopkin, S. P. (1996). The effects of metal contamination on earthworm populations around a smelting works: Quantifying species effects. *Applied Soil Ecology, 4*, 147–160.

Van Vliet, P. C. J., Didden, W. A. M., Van der Zee, S. E. A. T. M., & Peijnenburg, W. J. G. M. (2006). Accumulation of heavy metals by enchytraeids and earthworms in a floodplain. *European Journal of Soil Biology, 42*, S117–S126.

Wragg, J., Cave, M., & Nathanail, P. (2007). A study of the relationship between arsenic bioaccessibility and its solid-phase distribution in soils from Wellingborough, UK. *Journal of Environmental Science and Health—Part A Toxic/Hazardous Substances and Environmental Engineering, 42*, 1303–1315.

Worm Watch Canada: Key to reproductively mature earthworms. (2008). Retrieved on 22 February 2008 from http://www.naturewatch.ca/english/wormwatch/resources/key/taxonomic.html.