# Earthworms and *in vitro* Physiologically Based Extraction Tests: Complementary Tools for a Holistic Approach towards Understanding Risk at Arsenic Contaminated Sites

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**Abstract**

The relationship of the total arsenic content of a soil and its bioaccumulation by earthworms (*L. rubellus* and *D. 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 South West 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 – 9025 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 ($P = <0.05$) for *L. rubellus*. The potential use of both *in vitro* PBETs and earthworms as complementary tools is explored as an holistic and multidisciplinary approach to understanding risk at contaminated sites.

**Keywords:** Arsenic**,** Exposure, Bioaccessibility, Bioaccumulation, Risk

## 1. 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 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 *et al.* 2003). Consideration of a contaminants oral bioaccessibility is important in understanding exposure associated risk (Intawongse *et al.* 2006). Numerous *in vitro* Physiologically Based Extraction Test's (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, Langdon *et al.* 2003, Mariño *et al.* 1998, Morgan *et al.* 1999). The earthworm species *L. rubellus* and *D. rubidus* are known to inhabit soils and mine wastes in South West England highly contaminated with arsenic (Morgan 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. An 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 inter-relatedness of available tools in understanding the risk to human health and the ecosystem at arsenic contaminated sites. Comparison of the 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.

## 2. Materials and Methods

### 2.1 Study Site

The Devon Great Consols (DGC) is situated by the River Tamar in the Tavistock district of Devon (SX 426 735) and is one of many former mining sites in South West England (Figure 1). Soil arsenic concentrations found in and around the mine vary significantly depending on their proximity to the main tailings ranging 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 giving cause for concern in terms of potential human exposure.

### 2. 2 Sample Collection

The soil surface (0 – 20 cm) with an area of approximately 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. Earthworms were depurated for a minimum of 48 hours, since shorter times were unlikely to remove all soil particles in larger species such as *L. rubellus* (Langdon, 2003). Approximately 10 to 25 earthworms 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 (approximately 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.

**2. 3 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 and 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) and 69 % $HNO_3$ (Merck, UK). α-amylase (bacillus species), lipase (pig) and 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.

**2. 4 Total Digestion of Earthworm**

Microwave assisted (CEM MARS5, CEM Corporation, 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 minutes and then microwaved. Following an initial heating program (ramp to 100 ºC over 5 minutes then hold for 5 minutes, ramp to 200 ºC over 5 minutes then hold for 5 minutes) the vessels were allowed to cool (<50 ºC) 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 minutes 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).

**2. 5 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 minutes and left to stand for 15 minutes prior to analysis using a pH meter (Orion SA720, 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. 1 g (dry weight) of each soil was weighed into a glass crucible before heating to 450 ºC for 4 hours. The percentage weight reduction after heating was recorded as the estimated organic matter content.

**2. 6 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 ºC for 8 hrs, 100 ºC for 2 hrs, 120 ºC for 1 hr, 140 ºC for 3 hrs, 160 ºC for 4 hrs). 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 ºC for 30 minutes and then treated with 30 % $H_2O_2$ (v/v) 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.

**2. 7 Physiologically Based Extraction Technique (PBET)**

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 minutes. 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 ºC for 1 hour. 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 was added to the mixture and shaken end over end at 37 ºC for 4 hours. 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 interlaboratory 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 interlaboratory 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).

### 2. 8 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 1350 W; gas flow rates, coolant 13 L $min^{-1}$, auxiliary 0.9 L $min^{-1}$, nebuliser 0.93 l $min^{-1}$; spraychamber temperature 3 ºC; 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 x 30 seconds. 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.

## 3. Results

### 3. 1 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 (Figure 1) and closest to the mine tailings demonstrated the highest soil arsenic concentrations

in the range 1005 – 9025 mg $kg^{-1}$. Sampling sites 4, 7, 9 - 11, 21 - 24 and 27 to the south of the of the study area, closest to the river Tamar and further from mine tailings demonstrated lower soil arsenic concentrations in the range 204 – 1306 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 - 6308 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 – 9025 mg $kg^{-1}$, with a mean of 2301 mg $kg^{-1}$ (n = 12) (Table 1). *D. rubidus* were only found present in soils up to an arsenic concentration of 3995 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 (Figure 2) with $r^2$ values of 0.73 and 0.93 for *L. rubellus* and *D. rubidus* respectively.

### 3. 2 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) and the difference between the mean values was not significant (Table 2).

### 3. 3 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 – 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) (Figure 2) when all sites were combined. This trend did not differ when the sites were split into groups for *L. rubellus* and *D rubidus* (Figure 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).

### 3. 4 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.

### 3. 5 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 x $10^4$ 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 to 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.

**4. 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 effected by mining (Palumbo-Roe *et al.* 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 (Figure 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 (figure 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 (Marino *et al.* 1999). The correlation between BAF and HBF was 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 4). This finding agrees with the suggestion by Morgan *et al* (1999) that no simple universal relationship exists between soil and earthworm arsenic concentrations. *L. rubellus* were reported to be less sensitive to mining derived contamination than other species (Spurgeon *et al.* 1996). The differences between the earthworm species reported here may be related, in part, to variation in sensitivity to the contamination, this may also explain differences in the distributions of earthworm species around the mine area (Figure 1).

The accumulation of arsenic by both earthworm species reinforces the observed trends in bioaccessibility at DGC (Figures 2 and 4). However, the intra-species differences in the relationship between BAF and HBF (Figure 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. 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.

## 5. 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 contaminants 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.

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**Figures and Tables:**

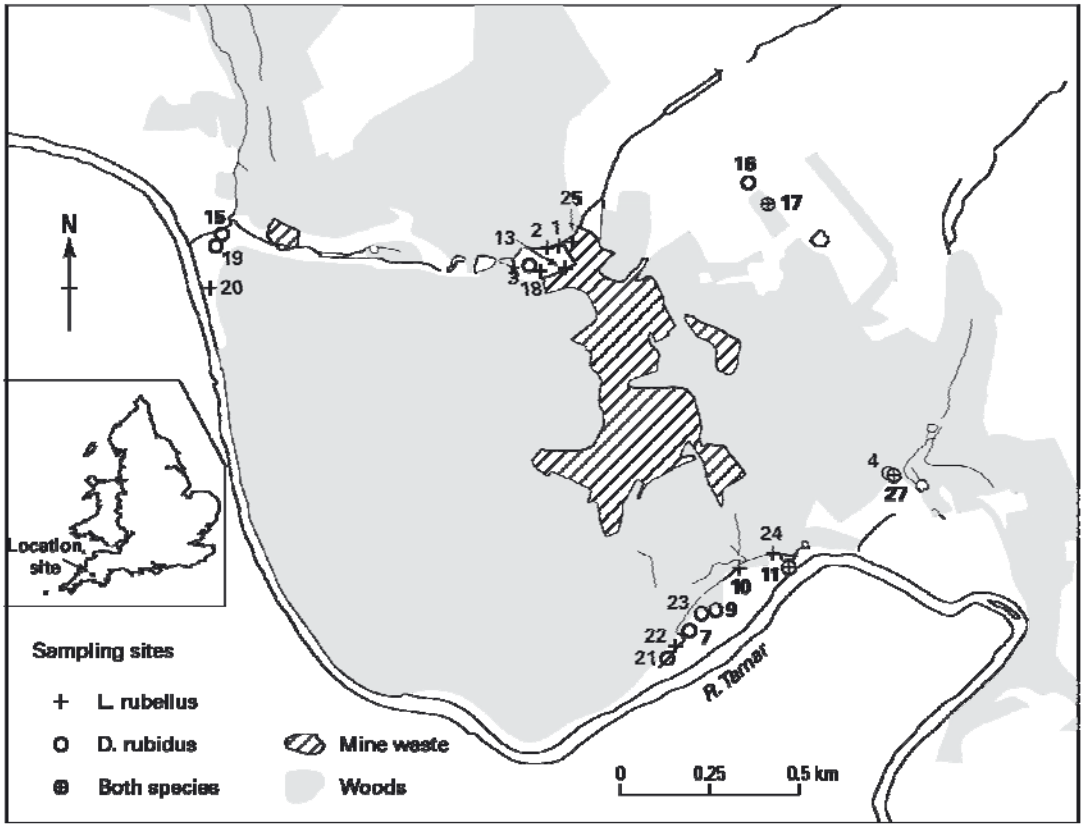



**Figure 1:** Geographical location of study area and positioning and identification number of sampling sites with site.

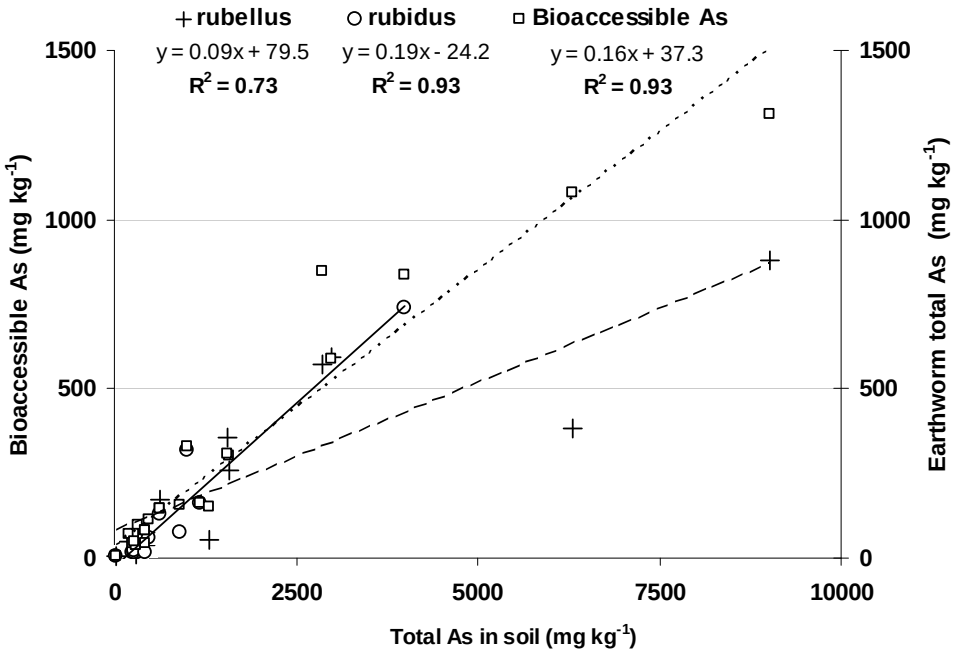



**Figure 2:** Correlations between bioaccessible As and earthworm total As to soil total arsenic for *L. rubellus* and *D. rubidus (includes DGC and control site)*.

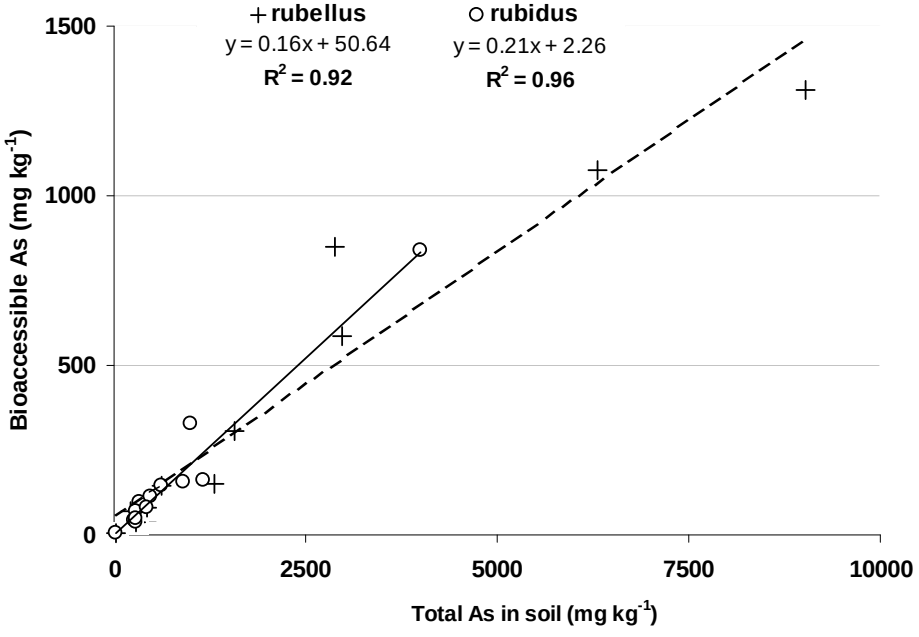
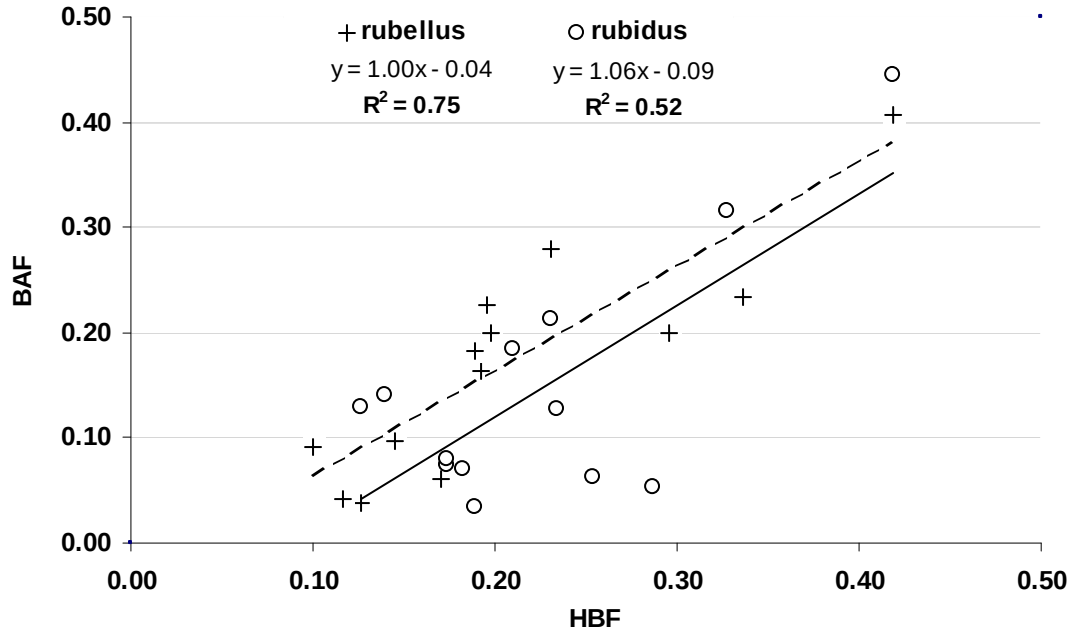



**Figure 3:** Correlations between bioaccessible As and soil total arsenic at *L. rubellus* and *D. rubidus* sites.




F**igure 4:** Correlations between the Human Bioaccessible Fraction (HBF) and earthworm Bioaccumulation Factors (BAF) at *L. rubellus* and *D. rubidus* sites.

**Table 1:** Mean As data (*mg $kg^{-1}$) presented with the range encountered across *L. rubellus* and *D. rubidus* sampling sites. HBF = Human Bioaccessible Fraction. BAF = earthworm Bioaccumulation Factor. OM = soil organic matter.

| | **Mean rubellus (n=12)** | **min** | **max** | **Control rubellus** | **Mean rubidus (n=12)** | **min** | **max** | **Control rubidus** |
|---|---|---|---|---|---|---|---|---|
| **Soil Total*** | 2301 | 204 | 9025 | 16 | 837 | 255 | 3995 | 16 |
| **Worm Total*** | 287 | 11 | 877 | 6.5 | 134 | 15 | 737 | 7.1 |
| **Bioaccessible*** | 413 | 36 | 1312 | 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 |
| **OM (%)** | 5.7 | 1.9 | 12 | 3.8 | 4.6 | 1.9 | 12 | 3.8 |

**Table 2:** Results of the Wilcoxon signed-rank test for significance of difference between paired groups (*L. rubellus* and *D. rubidus* sites). Difference between group variables significant at $P < 0.05$.

| 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 OM (%)** | 0.29 |
| **HBF** | 0.56 |
| **BAF** | 0.40 |