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## Linking selective chemical extraction of iron oxyhydroxides to arsenic bioaccessibility in soil



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### ABSTRACT

The relationship between As bioaccessibility using the physiologically based extraction test (PBET) and As extracted by hydroxylamine hydrochloride (HH), targeting the dissolution of amorphous Fe oxyhydroxides, is established in soils from the British Geological Survey Geochemical Baseline Survey of SW England, UK, to represent low As background and high As mineralised/mined soils. The HH-extracted As was of the same order of magnitude as the As extracted in the bioaccessibility test and proved to be a better estimate of bioaccessible As than total As (bioaccessible As – total As:  $r = 0.955$ ; bioaccessible As – HH-extracted As:  $r = 0.974$ ;  $p$ -values = 0.000). These results provide a means of estimating soil As bioaccessibility on the basis of the HH extraction. Further selective extraction data, using hydrochloride acid that seeks to dissolve both amorphous and crystalline Fe oxyhydroxides, indicates a decrease in the As bioaccessible fraction with the increase of the soil Fe oxyhydroxide crystallinity.

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### 1. Introduction

Soil ingestion is typically the primary human health exposure pathway to consider for arsenic (As) contaminated soils. The health risk is related to the relative bioavailability (RBA) of the contaminant that is absorbed into systemic circulation (USEPA, 2007), which in soils is typically lower than soluble metal(loid) salts, from which oral metal toxicity values are derived. In the last decades, as a surrogate for RBA, several *in vitro* methods (IVG, PBET, Rel SBRC-I, RBALP, SBRC-G, UMB) have been developed for the determination of bioaccessibility of soil As, where bioaccessibility is considered to be the fraction of a soil contaminant that is soluble in the gastrointestinal (GI) tract and available for absorption (Ruby et al., 1996, 1999). The relationship between *in vivo* RBA and *in vitro* bioaccessibility for As documented in the studies of Basta et al., 2007; Bradham et al., 2011, 2015; Denys et al., 2012; Juhasz et al., 2007, 2009; Li et al., 2014; Rodriguez et al., 1999, has demonstrated the potential of *in vitro* assays to predict As RBA using swine, primates or rodents (Juhasz et al., 2014).

While it is well recognised that only a fraction of total As in soil is bioaccessible, understanding of which soil As pools are measured in *in vitro* tests is crucial in order to reach a broader acceptability of

*in vitro* tests in human health risk assessment. Moreover, knowledge of the solid phases hosting the bioaccessible As can help envisage bioaccessibility changes with environmental conditions affecting the stability of the As hosting solid phases (e.g. the mobilisation of As associated to iron (Fe) redox cycling in flooded or paddy soils (Liu et al., 2015)).

Numerous studies have successfully carried out predictive modelling of As bioaccessibility using multiple regression analysis based on soil physicochemical properties, such as the elemental composition of the soil and soil pH (Appleton et al., 2012 and references within; Cave et al., 2013; Mikutta et al., 2014). These model predictor variables can provide an insight into the phases and processes governing the bioaccessibility of As in the soils. Unsurprisingly, considering the well-known role of Fe oxyhydroxides phases in regulating As geochemical cycle, many of these studies converge in identifying Fe oxyhydroxides as key factors influencing As bioaccessibility where As is naturally enriched in soil (e.g. Appleton et al., 2012; Wragg et al., 2007). Yang et al. (2002, 2005) also indicate that soils with high Fe oxyhydroxide contents and low pH are able to sequester As in the lowest bioaccessible form in As (V) spiked soils.

Besides multiple regression analysis, selective/sequential chemical extractions and mineralogical analysis can help to understand As solid phase speciation and to gain a better understanding of the sources of bioaccessible As in the *in vitro* tests. Rodriguez et al., 2003 first evaluated the ability of chemical

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extractions to measure bioavailable As from soil ingestion for 15 contaminated soils and found the strongest relationship between *in vivo* bioavailable As and As determined by soil chemical extractions for hydroxylamine hydrochloride (HH) extractant, selected to dissolve As in Fe and Mn oxyhydroxides. Smith et al. (2008), by comparing sequential extraction results pre and post As bioaccessibility assessment (using the simplified bioaccessibility extraction test (SBET)), attributed the As bioaccessible fraction to the amorphous and poorly-crystalline oxyhydroxide Fe/Al fractions in 12 long-term contaminated soils. Yet, from these and other studies (Li et al., 2015; Mikutta et al., 2014; Whitacre et al., 2013) it is highlighted that various soil fractions are found to contribute to As bioaccessibility depending on the As contamination source, but also on the type of As solid speciation analysis/chemical extractant and As bioaccessibility *in vitro* methods in use. Most of these studies also analysed a small number of samples. Clearly, further investigation is warranted in order to consolidate the above studies, especially with large datasets.

In this study we aimed to investigate how As bioaccessibility using the PBET assay is related to the Fe oxyhydroxide phase pool based on chemical extraction assessments. The investigation is carried out on a robust dataset of 94 soils selected from the British Geological Survey Geochemical Baseline Survey (G-BASE) of the Tamar catchment, SW England, UK, including both low As background and high As mineralised/mined soils.

Previous work indicates the different importance of amorphous and crystalline Fe oxyhydroxides in affecting As bioaccessibility; higher bioaccessibility of As was observed for As bound to amorphous Fe oxyhydroxides than for As associated with crystalline Fe oxyhydroxides (Kim et al., 2014). Since the transformation of amorphous Fe oxyhydroxides like ferrihydrite ( $\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$ ) to more crystalline forms such as goethite ( $\alpha\text{-FeOOH}$ ) or hematite ( $\text{Fe}_2\text{O}_3$ ) is a major process of soil genesis (Blume and Schwertmann, 1969), the relationship between As bioaccessibility and degree of crystallinity of Fe oxyhydroxides can be important to predict bioaccessibility changes with soil development. In this study our further objective was to discern the relationship between bioaccessible As and the ratio “amorphous Fe oxyhydroxides/amorphous and crystalline Fe oxyhydroxides” in order to obtain evidence of the effect of soil Fe oxyhydroxide degree of crystallinity on As bioaccessibility in natural soils.

## 2. Study area

A recent study published by the UK Department for Environment, Food and Rural Affairs (Defra) on soil “normal” background concentrations (NBCs), which include contribution from both natural and diffuse anthropogenic sources, recognises different NBCs for different regions of England called domains (Ander et al., 2013). These domains are defined as distinct areas where a distinguishable factor is recognised as controlling the concentration of an element. Arsenic has three different NBC values for the “principal” (As  $32 \text{ mg kg}^{-1}$ ), “ironstone” (As  $220 \text{ mg kg}^{-1}$ ) and “mineralisation” (As  $290 \text{ mg kg}^{-1}$ ) UK domains, the last two domains largely exceeding the UK screening levels ( $32 \text{ mg kg}^{-1}$ ) for the assessment of contaminated land (DEFRA, 2014). Our study area includes part of the UK “mineralisation” domain, where the geogenic (geology and mineralisation, see Supporting information for description of the geology) and anthropogenic (associated mining activities, Dines, 1956) inputs have resulted in elevated NBC of As.

The Tamar catchment is situated in SW England (UK) and covers an area of  $976 \text{ km}^2$  (Fig. 1). As a result of intense exploitation of metalliferous ore deposits, combined with the natural geochemical dispersion from the copper-tin-arsenic mineralisation, SW England has significant areas of As rich soils and sediments (Abrahams and

Thornton, 1987; Aston et al., 1975; Colbourn et al., 1975; Rawlins et al., 2003; Rieuwerts et al., 2014). In particular, within the catchment Devon Great Consols mine (grid reference SX 426 733) was one of the major As producers in the world in the late 1800s (Dines, 1956).

## 3. Material and methods

The soil samples for this study were from a comprehensive geochemical survey of soils, sediments and water in the Tamar catchment, carried out as part of the British Geological Survey G-BASE project (Johnson et al., 2005). Sites for the soil samples were selected from every second kilometre square of the British National Grid by random location within each square, subject to the avoidance of roads, tracks, railways, domestic and public gardens, and other seriously disturbed ground.

### 3.1. Geochemical hierarchical clustering of the tamar soils and sample selection

The 468 soil samples, part of the G-BASE survey of the Tamar catchment, were grouped using k-mean clustering of the soil geochemical variables to derive more homogeneous data subsets (clusters) for further selecting the samples for bioaccessibility testing and chemical extractions. The soil geochemical dataset from Rawlins et al. (2003) consisted of 43 major and trace elements, pH, organic carbon and available phosphorus. The data were mean centred and scaled with Euclidean distance linkage using Ward's method (Ward, 1963). The spatial distribution of the resulting distinct 5 clusters was compared against the solid geology of the area (DiGMapGB-50, Smith, 2013) to identify possible relationships between regional soil geochemical data and geology (Fig. 1). The geochemical clusters largely reflected the distribution of the principal geological formations over which soils developed and therefore also referred as geo-domains (Supporting information).

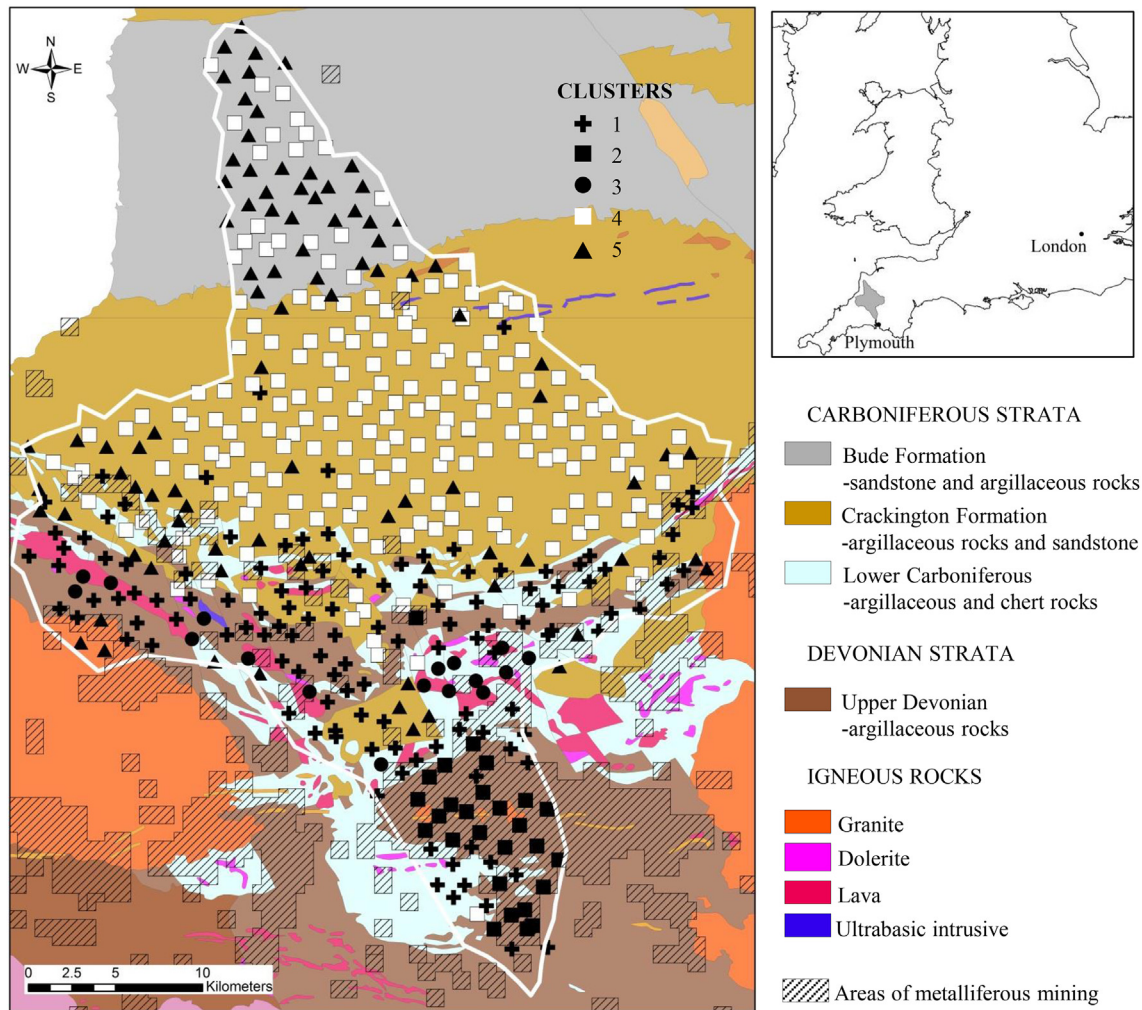
94 samples were chosen for bioaccessibility testing from the 5 clusters suggested by the k-mean clustering dendrogram. The clustering allowed the production of a reduced number of samples for further investigation, whilst ensuring that the selection of samples used for bioaccessibility testing were representative of the region under study.

### 3.2. Sample preparation, total elemental digestion and As bioaccessibility extraction

The 94 selected topsoils (collected from 0 to 15 cm soil depth) were sub-sampled from the archived G-BASE soil samples and sieved to  $< 250 \mu\text{m}$ . This fraction was chosen for bioaccessibility and associated testing, as this is the upper bound of particle size that is likely to remain on the hands of children (the “at risk” receptor) and be incidentally ingested (Duggan et al., 1985).

A mixed acid ( $\text{HF}/\text{HClO}_4/\text{HNO}_3$ ) digest with Inductively Couple Plasma-Atomic Emission Spectrometry (ICP-AES) was used to determine the major and trace elements in the  $< 250 \mu\text{m}$  size fraction sub-samples.

The bioaccessible As concentration was determined using a modified physiologically based extraction test (PBET), based on the method first described by Ruby et al. (1993, 1996). The PBET method was a simple sequential extraction used to simulate a number of physical and chemical conditions in the human GI tract, such as transit time, pH and chemical conditions, in order to estimate the amount of As that may be absorbed after the accidental soil ingestion of  $100 \text{ mg day}^{-1}$  by a child. The methodology and the modifications have been previously described in full by Cave et al.



**Fig. 1.** —Hierarchical clustering of the 468 topsoils from Rawlins et al. (2003) against the solid geology of the Tamar catchment study area (DiGMapGB-50, Smith, 2013); top right: location of the study area in SW England, UK.

(2003). Each of the test samples was digested with simulated gastric and small intestinal solutions under laboratory conditions intended to recreate those occurring in the stomach (pH 2.5) and small intestine (pH 7.0). A temperature of 37 °C was used to represent body temperature, end-over-end mixing to simulate peristalsis, and 1 h and 4 h periods for stomach and intestinal extraction times. Sampling of the bioaccessibility extracts occurred at the end of both the stomach and intestinal phases and all sample solutions were filtered using Pall™ 0.45 µm cellulose acetate syringe filters and stored at 4.0 °C prior to the ICP-AES analysis of the bioaccessible As content. The highest bioaccessible As content observed for the stomach or intestine phases was used in all data interpretation as a conservative estimate of bioaccessibility.

Bioaccessibility results were also reported as percent value (percent As bioaccessibility), by dividing the bioaccessible As concentration by the total As concentration in the < 250µm size fraction and multiplying this quotient by 100%.

### 3.3. Soil As pools

In addition to the bioaccessibility extraction, chemical extraction methods and linear regression analysis were carried out to help elucidate the principal forms of As in the soil and to understand which of these forms are responsible for the bioaccessible

fraction. The < 250µm soil size fraction was used. The fractions dissolved during selective chemical extractions are operationally defined, and give information on a reactive pool targeted in the extraction rather than a specific mineralogical phase.

#### 3.3.1. Iron oxyhydroxide selective extractions

Amorphous Fe oxyhydroxides were extracted using the method from Chao and Zhou (1983) with 0.4 g of soil leached using 20 ml of a 0.25 M hydroxylamine hydrochloride (NH<sub>2</sub>OH·HCl) – 0.25 M hydrochloric acid mixture at 50 °C for 30 min, denoted as HH in further interpretation/discussion. After cooling rapidly to room temperature, the samples were centrifuged and the supernatant collected and acidified prior to analysis of major and trace elements by ICP-AES.

Both amorphous and crystalline Fe oxyhydroxides were extracted from 0.4 g of soils leached by 20 ml of 2 M hydrochloric acid (HCl) for 30 min at 96 °C in 15 samples chosen from the 5 soil clusters. The extracted solutions were analysed for their chemical composition by ICP-AES.

The above methods were chosen for the lack of re-adsorption effects during extraction (Van der Hoek and Comans, 1996).

The activity ratio “amorphous Fe oxyhydroxides/amorphous and crystalline Fe oxyhydroxides” (Fe<sub>HH</sub>/Fe<sub>HCl</sub>) was determined, modified from Blume and Schwertmann (1969).

### 3.4. Sequential extraction

Nine test soils representing the five identified geo-domains were subjected to the non-specific sequential extraction methodology described by Cave et al. (2004), called the Chemometric Identification of Substrates and Element Distributions (CISED). An aliquot (approximately 2 g) of each of the identified test soils was accurately weighed into the tube insert of a Schleicher and Schuell 'Centrex MF-25®' polypropylene centrifuge tubes with 0.45 µm regenerated cellulose acetate membrane filter inserts, and a 10 ml volume of extractant added. The vessels were centrifuged at 1034 G for 10 min and the resulting solution stored at < 8 °C. Each extraction fluid was sequentially passed through the original aliquot, of each test soil, in duplicate to produce a total of 14 extraction solutions per test soil. The extractants used were deionised water (DI) and HNO<sub>3</sub> (0.01 M, 0.05 M, 0.1 M, 0.5 M, 1.0 M and 5.0 M). To aid the dissolution of precipitated organic acids and manganese (Mn)/aluminium (Al) oxides, H<sub>2</sub>O<sub>2</sub> was added to the 0.5–5.0 M extracts (Wragg and Cave, 2012). All solutions were analysed for their major and trace element content by ICP-AES. The method assumes that the chemical composition data for each extract is made up from different proportions of the physico-chemical components in the soil. More easily extractable phases such as carbonates are dissolved in the low acid concentration extracts and less soluble components, such as Fe oxyhydroxides, are dissolved in the higher strength acid extracts. Since the acid used was nitric acid, the aluminosilicate component of the soil will only be attacked to a limited extent. Data processing of the sequential extraction data was carried out on a single data matrix in order to identify the main soil components across the whole area under study, rather than on a sample by sample basis (Wragg et al., 2014). This allows for a direct comparison of differences between the composition of the components between all samples and the As distribution between these components, for further comparison across the identified clusters. The data matrix for processing comprised of the elemental extraction data (25 elements) for the 14 extracts for each of the 9 test soils (126 rows of data). The data was processed using a modification (Cave, 2009) of the self-modelling mixture resolution (SMMR) algorithm previously described by Cave and co-workers (Cave et al., 2004; Wragg and Cave, 2012). Modelled soil component and element distribution data have been summarised by cluster (1–5) for ease of comparison.

#### 3.4.1. Linear regression modelling

The Pearson correlation coefficient was used. The correlation coefficients between both total As and bioaccessible As and other parameters were used to complement the assessment of controls on the total and bioaccessible As content of the soils.

### 3.5. Chemical analysis

Analysis of all extraction solutions (total digestion, bioaccessibility, Fe oxyhydroxides and solutions from the CISED sequential extraction) were carried out using a Varian Vista AX CCD simultaneous ICP-AES with dedicated Varian SPS-5 Autosampler, using operating conditions previously described by Palumbo-Roe et al., 2005. The instrument was calibrated using a minimum of four mixed element standards (in 1% v/v HNO<sub>3</sub>), covering a range of concentrations for the analysis of the elements of interest in each of the extraction solutions.

### 3.6. Quality control

For each extraction type (total digestion, bioaccessibility, Fe oxyhydroxide) a representative number (1 per batch of 10

unknown samples) of blank and extraction duplicates were carried out, in addition to the extraction of standard reference or guidance materials to check the accuracy of the extraction, where applicable. Matrix matched QC solutions and blanks were run as part of the analytical runs to check for instrument drift, accuracy and precision. Extraction solutions were matrix matched to the calibration standards in order to ensure data quality. The NIST Standard Reference Material (SRM) 2710 was included in the total digestion procedure and the results obtained for As are comparable with the certified values; the recovery obtained in this study was 97.7%. The average repeatability of the digestion of duplicate samples for their total As content was 10.4% (n = 7). At the time of extraction development of standard reference materials for bioaccessibility assessment in soils was in its infancy, with no certified reference materials to assess the quality of bioaccessibility extractions. However a guidance material, a natural ironstone soil, with bioaccessibility data for As and Pb, called BGS 102 (Wragg, 2009) was available and utilised in this study. Bioaccessibility/availability information has since become available for the NIST SRM's 2710 and 2711 and these reference materials are recommended for use with the USEPA IVBA methodology for Pb (<http://www.epa.gov/superfund/bioavailability/faqs.htm#ivbaref>). BGS 102 was used for QC checks in this study and has been the subject of an international inter-laboratory trial (Wragg et al., 2011), which has generated the As reference values used in this study. Results for the extraction of BGS 102 have been assessed against, and are in good agreement with previously reported consensus values for As. The As bioaccessibility values for the stomach and the intestine phases were  $3.6 \pm 0.2 \text{ mg kg}^{-1}$  and  $3.3 \pm 0.3 \text{ mg kg}^{-1}$ , respectively, compared to guidance values of  $4.5 \pm 1.3 \text{ mg kg}^{-1}$  and  $5.4 \pm 2.4 \text{ mg kg}^{-1}$ . The As repeatability in BGS 102 (n = 9) for the stomach and intestinal compartments was 5.4% and 8.0%, respectively. The average repeatability of bioaccessible As extracted from duplicate samples was 13.8% and 8.43% for the stomach and the intestinal phases. All blank extractions for all digestion types returned values below the method detection limits.

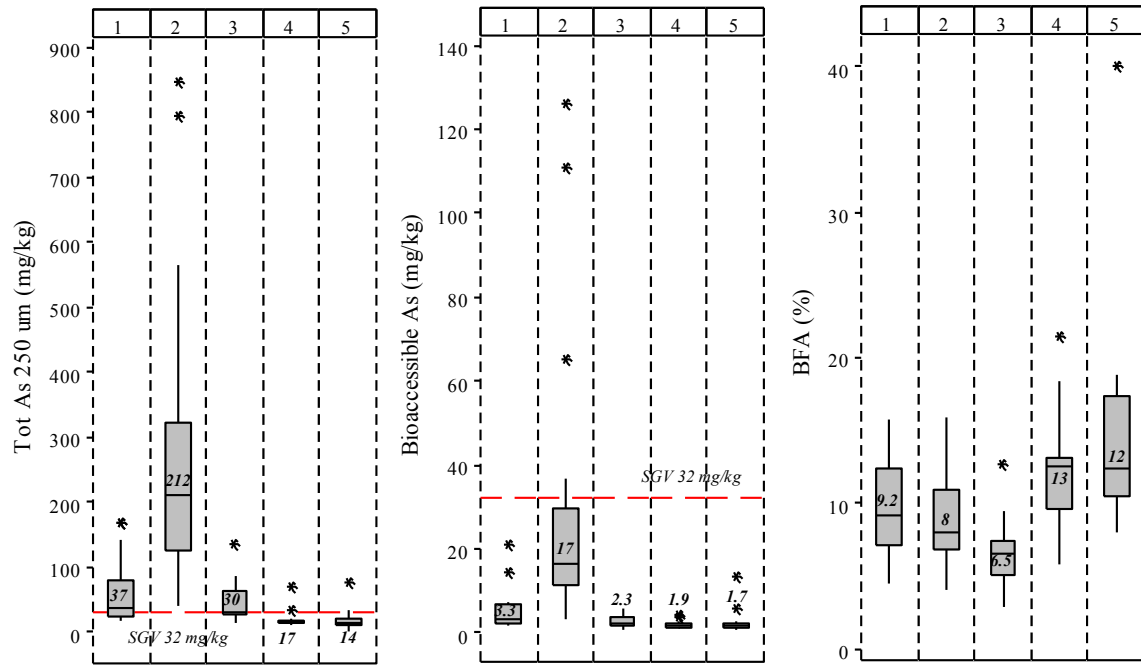
## 4. Results and discussion

### 4.1. Total As and bioaccessible As distribution

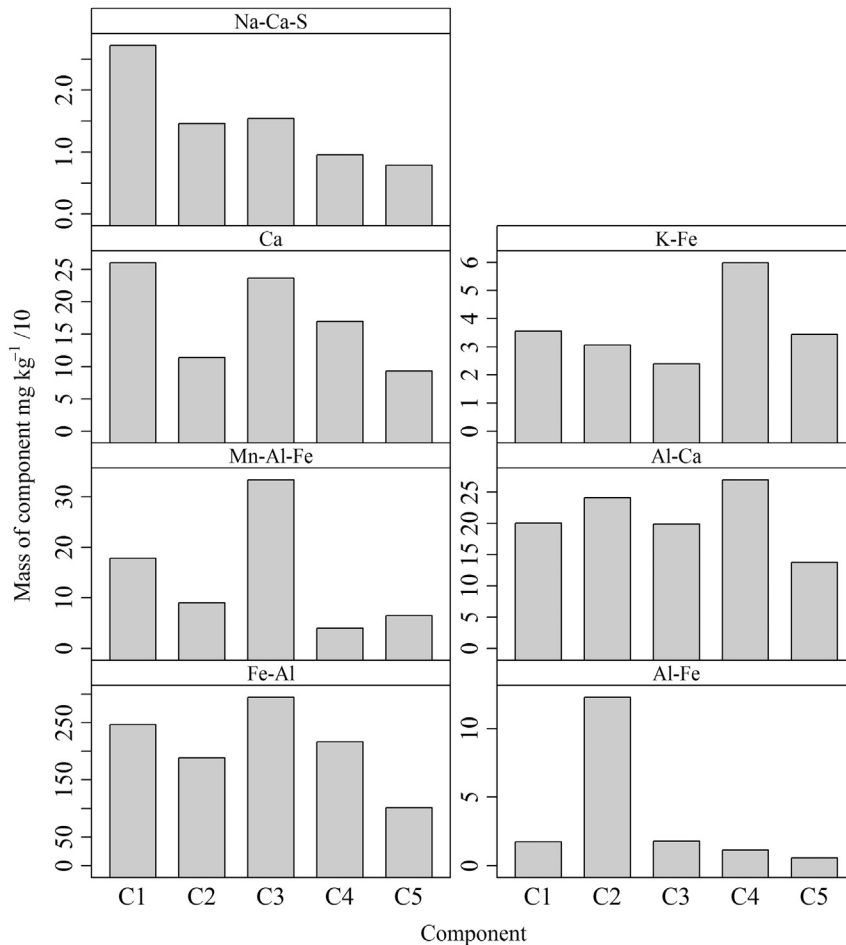
Total As concentrations in the < 250µm fraction (total As<sub>250µm</sub>) of the Tamar soils ranged from a minimum of 3.8 mg kg<sup>-1</sup> to a maximum of 848 mg kg<sup>-1</sup>, a mean of 89 mg kg<sup>-1</sup> and a median of 30 mg kg<sup>-1</sup> (N = 94). These values compared with the As mean and median of the less than 2 mm fraction (from Rawlins et al., 2003) indicated only small differences in the As concentrations between the two size fractions. 44 out of 94 soils had total As<sub>250µm</sub> > 32 mg kg<sup>-1</sup> UK screening levels for the assessment of contaminated land (DEFRA, 2014).

Bioaccessible As concentrations varied from 1 mg kg<sup>-1</sup> to 126 mg kg<sup>-1</sup> with a mean of 8.74 mg kg<sup>-1</sup> and a median value of 2.49 mg kg<sup>-1</sup> (N = 94). Only 4 out of 94 soils had bioaccessible As > 32 mg kg<sup>-1</sup>. These were soils with a total As<sub>250µm</sub> > 465 mg kg<sup>-1</sup>. The percent bioaccessible As ranged from 2.94% to 21.47%, with a mean of 10% and a median of 9.5%.

The distribution of total As<sub>250µm</sub>, bioaccessible As and percent As bioaccessibility, varied through the five geo-domains identified by the clusters (Fig. 2). Three (C1–C2–C3) of the five domains had total As concentrations above the 32 mg kg<sup>-1</sup> UK soil screening values for assessment of contaminated land in the southern catchment area, Fig. 1. Within these soils relatively enriched in As, the highest As concentrations associated with C2 are likely to reflect the influence of both mineralisation and diffuse pollution from the mining legacy in the Tavistock mining district, as reported



**Fig. 2.** Box and whisker plots for total As < 250µm fraction, bioaccessible As and percent As bioaccessibility among the 5 soil clusters C1 to C5 (N<sub>C1</sub> = 20; N<sub>C2</sub> = 20; N<sub>C3</sub> = 17; N<sub>C4</sub> = 20; N<sub>C5</sub> = 17). The bars show the median values (in italics). SGV reference line is the UK regulatory soil threshold value for residential land use (32 mg kg<sup>-1</sup>).



**Fig. 3.** Average mass (sum of the total elements measured) of each soil component (mg kg<sup>-1</sup>) defined by the CISED extraction associated with each soil cluster (C1–C5).

by Abrahams and Thornton (1987). For this domain (C2) As bioaccessibility was the highest with a median value of  $16.6 \text{ mg kg}^{-1}$  and the percent As bioaccessibility median of 8.01%. These data consolidate low percent As bioaccessibility median values in soils affected by mining and ore processing reported worldwide (e.g.: 9% in the mining Tavistock district, UK (Palumbo-Roe and Klinck, 2007), 8.4% in Gold Mine Districts of Nova Scotia, Canada (Meunier et al., 2010), 3.7% in Murcia, S.E. Spain (Martínez-Sánchez et al., 2013), 7–19% (Mikutta et al., 2014).

The pattern of distribution of percent As bioaccessibility across the domains did not match the respective patterns shown by the total  $\text{As}_{250\mu\text{m}}$  and bioaccessible As (Fig. 2). It was observed that the highest percent As bioaccessibility median values were in the As poor soils over the sandstone and argillaceous rock types in the north of the catchment (C4 and C5) and the lowest percent As bioaccessibility in the soils on basic igneous rocks (C3) with medium to high As concentrations. This variability across soils from different parent material emphasizes the importance of As-bearing minerals and soil characteristics in controlling the percent As bioaccessibility (Ruby et al., 1999; Meunier et al., 2010).

#### 4.2. As solid phase associations and bioaccessibility

Seven distinct components were identified using the CISED sequential extraction and data processing. The components were named and summarised according to their elemental composition, where elements were present at > 10%. The amount of each component in each soil cluster is provided in Fig. 3 (summary data in Tables S1, S2 and S3, Figure S1 in the Supporting information). Despite small differences across the soil clusters, the dominant component was a Fe-dominated component (Fe–Al) extracted at high acid concentrations, likely derived from the dissolution of a Fe oxyhydroxide phase.

Arsenic occurs as a major constituent in ore minerals such as arsenian pyrite, arsenopyrite, realgar, orpiment, cobaltite, niccolite and scorodite. As weathering processes occur over time the As from primary ore minerals is mainly sequestered by Fe oxyhydroxide phases, due to the specific high affinity of As for these minerals. Previous work (Palumbo et al., 2007; Rieuwerts et al., 2014) has shown Fe oxyhydroxide-dominated soil components to be the most important hosts of As in mineralised and mined soils from the area. Application of the CISED extraction to the study soils shows the majority of the As (>60%) to be associated with Fe oxyhydroxides

(the Fe–Al component), regardless of the soil cluster. Up to 30% of the As was further associated with the Al–Fe component, with smaller amounts distributed across the other five identified CISED components (Fig. 4). Given the above indications of the strong Fe–As association, hydroxylamine hydrochloride-hydrochloric acid (HH) extraction was aimed at targeting the pools of As bound to amorphous Fe oxyhydroxides to evaluate their relation to total and bioaccessible As.

Fig. 5 shows the mean, median, minimum and maximum values ( $N = 81$ ) of As extracted by the HH mixture ( $\text{As}_{\text{HH}}$ ). On average, 18% of total As was extracted by the HH extraction, with a minimum of 5% and a max of 56%. While total  $\text{As}_{250\mu\text{m}}$  and total  $\text{Fe}_{250\mu\text{m}}$  were not significantly correlated, the HH-extraction of the soil, by which amorphous and poor crystalline Fe oxyhydroxides dissolve, show a significant correlation ( $r = 0.407$ ,  $p = 0.000$ ) between HH-extracted As as percent of total  $\text{As}_{250\mu\text{m}}$  ( $\% \text{As}_{\text{HH}}/\text{total As}$ ) and HH-extracted Fe as percent of total  $\text{Fe}_{250\mu\text{m}}$  ( $\% \text{Fe}_{\text{HH}}/\text{total Fe}$ ), which indicates the amorphous Fe oxyhydroxides as a plausible soil As pool.

Selected samples ( $N = 15$ ) to represent the 5 geo-domains were further analysed for the content of both amorphous and crystalline Fe oxyhydroxides to test the correlation between Fe oxyhydroxides degree of crystallinity and As bioaccessibility. The mean, median, minimum and maximum values ( $N = 15$ ) of As extracted by HCl were  $22.62 \text{ mg kg}^{-1}$ ,  $8.92 \text{ mg kg}^{-1}$ ,  $1.48 \text{ mg kg}^{-1}$  and  $71.39 \text{ mg kg}^{-1}$ , respectively. On average more As was extracted in the HCl extraction than in the HH extraction, as would be expected from a strong mineral acid extraction.

#### 4.3. Correlation between As bioaccessibility and As solid phase associations

The relationship between As bioaccessibility and various chemical extraction results and soil physico-chemical properties has been investigated by calculating the Pearson correlation coefficients (Table 1).

Bioaccessible As was very strongly correlated with total  $\text{As}_{250\mu\text{m}}$  ( $N = 94$ ) (Table 1), therefore as the total concentrations of As increased, so did the bioaccessible As. This relationship held true for each soil cluster group (C1:  $r = 0.816$ ,  $p = 0.000$ ; C2:  $r = 0.961$ ,  $p = 0.000$ ; C3:  $r = 0.68$ ,  $p = 0.003$ ; C4:  $r = 0.589$ ,  $p = 0.006$ , C5:  $r = 0.961$ ,  $p = 0.000$ ). Bioaccessible As did not correlate significantly to any other element, neither to soil properties such as pH and organic matter.

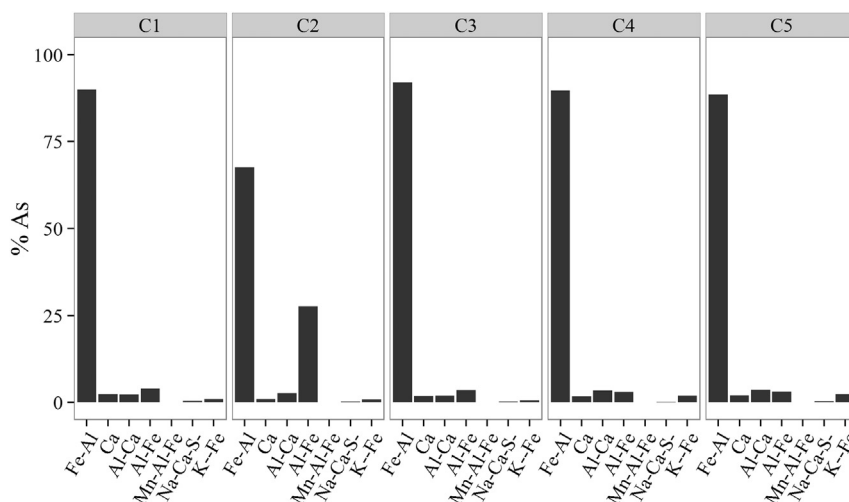


Fig. 4. Distribution of As among the different CISED components in each soil cluster (expressed as % of the total As extracted in the soils from that cluster).

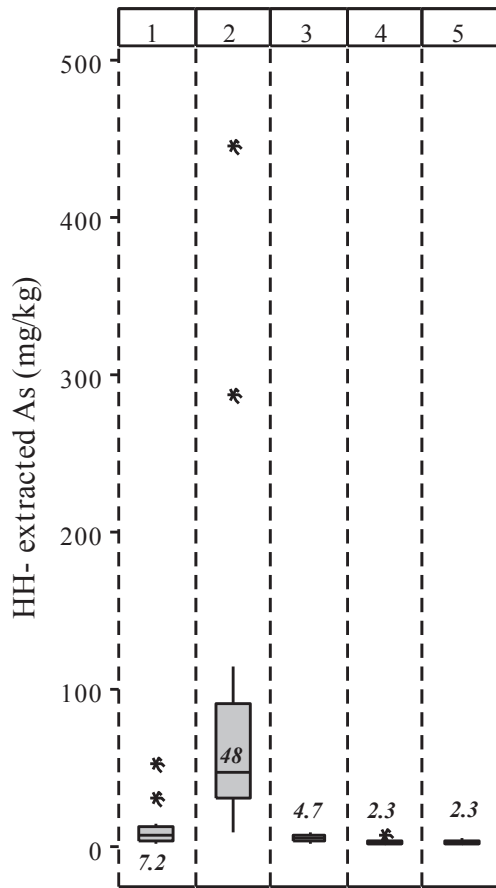


Fig. 5. Box and whisker plots for HH-extracted As among the 5 soil clusters ( $N_{C1} = 18$ ;  $N_{C2} = 18$ ;  $N_{C3} = 14$ ;  $N_{C4} = 17$ ;  $N_{C5} = 14$ ). The bars show the median values (in italics).

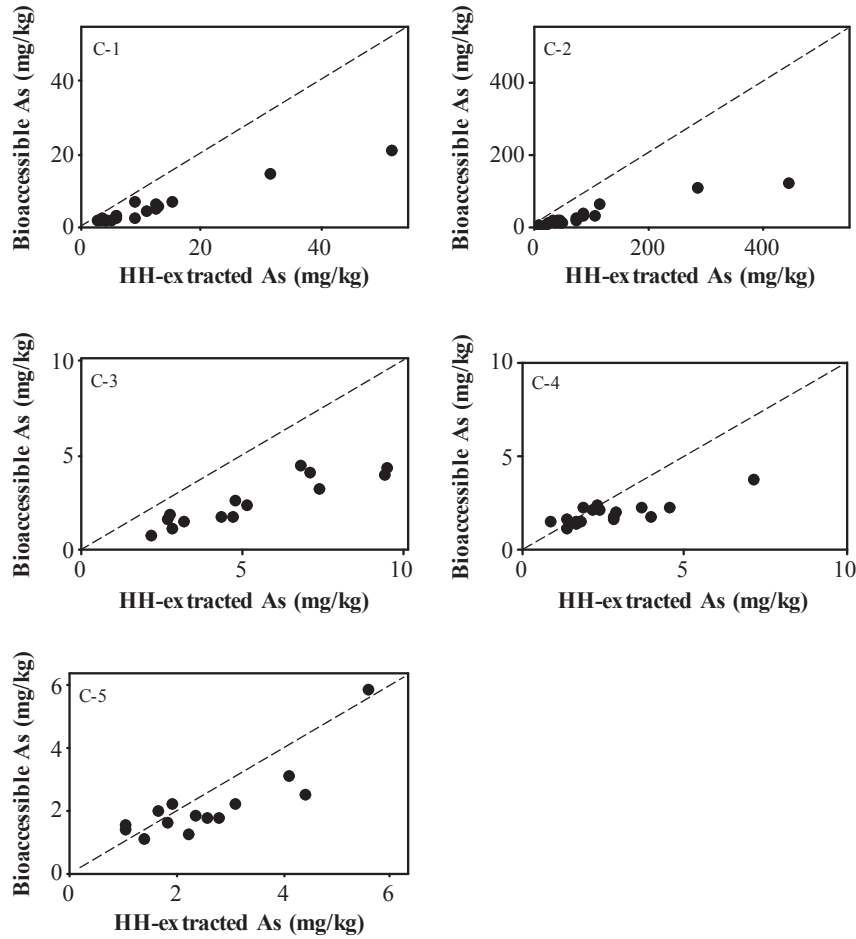
However, the  $As_{HH}$  proved to be a better estimate of bio-accessible As (improved Pearson correlation coefficient) than total  $As_{250\mu m}$  ( $N = 81$ ) (Table 1). The amount of As in the HH extraction

was of the same order of magnitude as the As extracted in the bioaccessibility test, although almost consistently overestimating the bioaccessible As concentration. The results suggest that this reactive Fe phase is the pool of As that is bioaccessible, thus consolidating the findings of Rodriguez et al. (2003). The strong correlation between bioaccessible As and  $As_{HH}$  was observed across all of the soil cluster types (Fig. 6). In particular for the sandstone and argillaceous geo-domain (C5) low in As, soil bioaccessible As versus  $As_{HH}$ , closely conforming to the 1:1 line (slope = 1), suggests that  $As_{HH}$  was completely bioaccessible.

Fig. 7 shows the percent As bioaccessibility significantly ( $p < 0.001$ ) negatively correlated to total  $Fe_{250\mu m}$ , while significantly ( $p < 0.05$ ) positively correlated to the  $Fe_{HH}/Fe_{HCl}$  ratio ( $N = 15$ ). The activity ratio  $Fe_{HH}/Fe_{HCl}$ , representing the “amorphous Fe oxyhydroxides” versus “amorphous and crystalline Fe oxyhydroxides”, can serve as a relative measure of the degree of crystallinity of Fe oxyhydroxides. Our results indicate that the decrease in As bioaccessibility (%) corresponds to a decrease in amorphous Fe oxyhydroxides in favour of the more crystalline Fe oxyhydroxides, in accordance with the findings of Kim et al. (2014). The difference in As bioaccessibility with varying Fe oxide mineralogy has major implications when carrying out risk assessments on the basis of total element concentrations. Our data provides more evidence of the importance of Fe speciation (i.e. Fe oxide mineralogy) rather than the total Fe content in soils when considering As bioaccessibility. The relatively lower percent As bioaccessibility of more crystalline Fe oxyhydroxides than amorphous Fe oxyhydroxides corresponds to changes in the As bonding with Fe oxide mineralogy, previously shown by Dixit and Hering (2003); Ford (2002); and Pedersen et al. (2006). For ferrihydrite most As remains associated with the surface, but in goethite As is, to a larger extent, incorporated in the crystalline structure (Pedersen et al., 2006). However, it has been highlighted by Dixit and Hering (2003) and Kumpiene et al. (2012) that the decrease in specific surface area that accompanies transformation of amorphous Fe oxyhydroxides to more crystalline phases could increase the mobility of As in the soil. Ultimately, the likelihood of As mobilisation would depend on the As concentrations, As

Table 1  
Pearson’s correlation coefficients for As bioaccessibility and selected geochemical parameters.

	total $As_{250\mu m}$	Bioaccessible As	% As Bioaccessibility	$As_{HH}$	$As_{HH}/total As$ (%)	total $Fe_{250\mu m}$	$Fe_{HH}$	$Fe_{HH}/total Fe$ (%)	pH	% Carbon
total $As_{250\mu m}$	1.000									
Bioaccessible As	0.955 <i>0.000</i>	1.000								
% As Bioaccessibility	-0.066 <i>0.530</i>	0.078 <i>0.453</i>	1.000							
$As_{HH}$	0.910 <i>0.000</i>	0.974 <i>0.000</i>	0.085 <i>0.452</i>	1.000						
$As_{HH}/total As$ (%)	0.451 <i>0.000</i>	0.529 <i>0.000</i>	0.496 <i>0.000</i>	0.582 <i>0.000</i>	1.000					
total $Fe_{250\mu m}$	0.096 <i>0.356</i>	0.040 <i>0.703</i>	-0.576 <i>0.000</i>	0.081 <i>0.470</i>	-0.138 <i>0.220</i>	1.000				
$Fe_{HH}$	0.237 <i>0.033</i>	0.231 <i>0.038</i>	-0.197 <i>0.078</i>	0.272 <i>0.014</i>	0.341 <i>0.002</i>	0.437 <i>0.000</i>	1.000			
$Fe_{HH}/total Fe$ (%)	0.001 <i>0.990</i>	0.057 <i>0.615</i>	0.462 <i>0.000</i>	0.051 <i>0.649</i>	0.407 <i>0.000</i>	-0.651 <i>0.000</i>	0.293 <i>0.008</i>	1.000		
pH	-0.062 <i>0.555</i>	-0.059 <i>0.572</i>	-0.072 <i>0.491</i>	-0.023 <i>0.837</i>	0.139 <i>0.214</i>	0.014 <i>0.893</i>	-0.065 <i>0.564</i>	0.016 <i>0.887</i>	1.000	
% Carbon	0.008 <i>0.937</i>	0.042 <i>0.689</i>	-0.015 <i>0.889</i>	0.025 <i>0.823</i>	0.120 <i>0.286</i>	0.123 <i>0.239</i>	0.216 <i>0.053</i>	0.068 <i>0.546</i>	-0.243 <i>0.018</i>	1.000
Cell Contents:	Pearson correlation <i>P-Value</i>									



**Fig. 6.** Comparison of HH-extracted As with bioaccessible As for each soil cluster. The broken line shows the 1:1 line. (C1:  $r = 0.982$ ,  $N = 18$ ; C2:  $r = 0.962$ ,  $N = 18$ ; C3:  $r = 0.915$ ,  $N = 14$ ; C4:  $r = 0.824$ ,  $N = 17$ , C5:  $r = 0.848$ ,  $N = 14$ ; all P-values = 0.000).

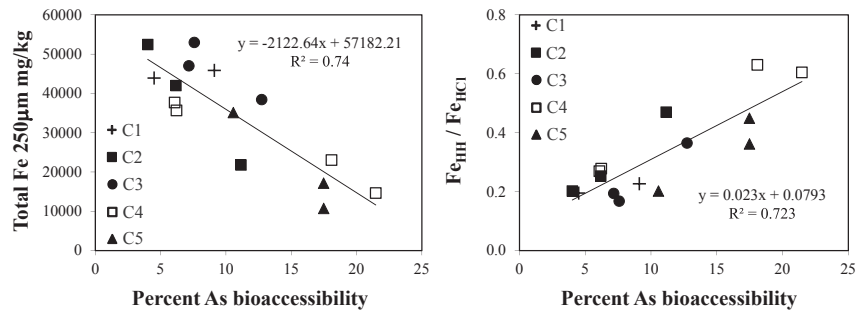
adsorption maximum, other factors such as pH, redox, organic acids, presence of competing ions and the overall soil adsorptive properties.

Laboratory studies on the effect of soil ageing on bioaccessibility have indicated the decrease in the bioaccessible As fraction with incubation time (in the order of months) after As soil spiking (Fendorf et al., 2004; Juhasz et al., 2008; Tang et al., 2007; Yang et al., 2002). The decrease in the As bioaccessible fraction is assumed to be related to a shift from the rapidly formed outer-sphere complexes of As freshly adsorbed on soil material to inner-sphere complexes (Fendorf et al., 2004). These short incubation time experiments may well represent processes in freshly contaminated soils; however, the crystallisation of Fe oxides in

soils, often used as an indicator of the stage of soil development and age, is a common, but slow, pedogenic process. Our findings then extends the evidence collected for As-spiked soils on the influence of soil ageing on As bioaccessibility, to soils naturally enriched in As. The relationship between As bioaccessibility and Fe oxide crystallinity shown in this study can serve to model bioaccessibility changes with soil development, soil horizons and soil types.

**5. Conclusion**

The amount of As in the amorphous Fe oxyhydroxides was very strongly correlated with, and of the same order of magnitude as, the As extracted in the PBET bioaccessibility test, although almost



**Fig. 7.** Correlations between percent As bioaccessibility and total  $Fe_{250\ \mu m}$  (left) and percent As bioaccessibility and  $Fe_{HH}/Fe_{HCl}$  (right) ( $N = 15$ ).



consistently overestimating the bioaccessible As.

Allowing for the inherent limitations of the chemical fractionation procedures, these results hint to the source of bioaccessible As in the studied soils being attributable to the amorphous Fe oxyhydroxides. Considering the agreement shown between bioaccessible As and As present in the soil amorphous Fe oxyhydroxide pool, these results provide a means of estimating As bioaccessibility of soil-bound As on the basis of the HH extraction.

Interpretation of further selective extraction data, using hot HCl that seeks to dissolve both amorphous and crystalline Fe hydroxides, points to a decrease in the As bioaccessible fraction with the increase of the soil Fe oxyhydroxide crystallinity. Since the transformation of amorphous Fe oxyhydroxides to more crystalline forms is a major process of soil genesis and ageing, these findings can serve to envisage As bioaccessibility changes with soil development.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.09.026>.

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