New insights into the reliability of automatic dynamic methods for oral bioaccessibility testing: A case study for BGS102 soil

Mark R. Cave[a], María Rosende[b], Ian Mounteney[a], Amanda Gardner[a], Manuel Miró[b]

a) British Geological Survey, Keyworth, Nottingham NG12 5GG, United Kingdom.
b)FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain

Abstract

Dynamic flow-through extraction is attracting a great deal of attention for real-time monitoring of the bioaccessible fraction of metal species in environmental solid substrates compared to its batchwise manual counterparts. There is however a lack of studies on the harmonization and validation of *in-vitro* dynamic methods for physiologically-based extraction tests against *in-vivo* bioavailability methods. This work is aimed at evaluating the reliability of dynamic flow-through extraction methods for estimation of oral bioaccessible fractions of Cu, Zn, Pb, Ni, Cr and As under worst-case extraction conditions in the gastric compartment based on the BGS102 guidance soil using the validated batchwise Unified BARGE test assay. Good overall agreement between batch and dynamic results was obtained for the tested elements, except for Pb, as a consequence of the slow leaching kinetics identified with the dynamic method and the contribution of readsorption phenomena in the course of the gastric digestion. Metal-soil phase associations and their relationship with gastric bioaccessible pools were elucidated using the so-called Chemometric Identification of Substrates and Element Distributions method based on sequential extraction with a variety of chemicals of increasing acidity as applied to both static and dynamic bioaccessibility data.

Introduction

Recent studies [1,2] have shown how batch mode in-vitro ingestion/digestion bioaccessibility testing is a good analogue for in-vivo bioavailability measurements for potentially harmful elements in soils. The in-vitro bioaccessibility tests are now increasingly being used by commercial testing laboratories (e.g. ALcontrol Laboratories UK) to provide data for human health risk assessment at contaminated land sites. Whilst batch testing is far more ethical, cheaper and rapid compared to animal testing it is still relatively time consuming and requires a large number of manual operation steps. Dynamic leaching methods are proven more suitable for rapid measurements at real time by automation and minimum extract manipulation [3-9] but their equivalence to validated batch tests for oral bioaccessibility data has not been established.

In dynamic leaching methods, in contrast to steady-state extraction methods, fresh portions of leaching reagents are continuously provided to the solid samples that are contained in flow-through micro-columns or chambers [3,10]. The dissolution equilibrium is thus driven to the liquid (extractant) phase so as to afford relevant insight into the maximum amount (worst-case extraction) of bioaccessible pools of target species, e.g., potentially harmful trace elements (TE) [11,12]. Entirely enclosed and (semi)automatic flow-based extraction methods also simplify operationally defined bioaccessibility tests, minimize accidental errors (e.g., sample contamination and analyte losses) and foster time-resolved (kinetic) data of the ongoing extraction. Further, re-adsorption phenomena of TE onto the remaining or freshly generated sorptive soil surfaces are circumvented [3,10].

In this work, the analytical setup described and validated by Rosende *et al.* [13] was applied to evaluate the Unified Barge Method (UBM) [14] gastric bioaccessible pools of TE (Cu, Zn, Pb, Ni, Cr and As) in the bioaccessibility guidance ironstone soil BGS 102. The flow manifold capitalizing upon the hyphenation of a sequential injection manifold accommodating a stirred flow-cell reaction to inductively coupled plasma-atomic emission spectrometry (ICP-AES) is devised for quantification of the UBM gastric bioaccessible TE in a conservative assessment while getting insight into the leaching kinetics as well.

The results from the dynamic test will be compared to data obtained from long term analysis of the BGS102 guidance soil using the UBM test assay [15]. The UBM batch and dynamic data will also be interpreted with respect to the fractionation data obtained from applying the

Chemometric Identification of Substrates and Element Distributions (CISED) sequential extraction method [16,17].

EXPERIMENTAL

Batch mode UBM bioaccessibility data

The batch mode gastric phase bioaccessibility data from UBM testing of BGS 102 comes from a recent study [15] where data had been collected over an extended period of time and compiled for 57 elements using Inductively Coupled Plasma Mass Spectrometry (ICP MS). The mean values for the elements under study in this work are given in Table 1.

Sequential extraction-based CISED method

The Chemometric Identification of Substrates and Element Distribution (CISED) method was used for elucidation of components in a sequential extraction procedure with increasing concentrations of aqua regia. The method was similar to previous work [18] but with a few modifications.

All reagents were of analytical grade obtained from the ROMIL company and Milli-Q water (Millipore Synthesis A10, Millipore Corporation, Billerica, MA, USA) was used throughout. The Oakridge tubes were were previously soaked in 10% (v/v) HNO_3 and rinsed three times with deionized water.

The 7 extraction solutions (1 of deionised water and 6 of acid) consist of mixtures of analytical reagent grade nitric and hydrochloric acid prepared as follows:

i) 5 M aqua regia made up as 110 ml 37% HCl and 47.5 ml 70 % HNO_3 made up to 500 ml volumetric flask with deionised water;
ii) 1.0 M aqua regia made up as 22 ml 37% HCl plus 9.5 ml 70% HNO_3 made up to 500 ml in volumetric flask with deionised water;
iii) 0.5 M prepared as 50 ml of 5 M aqua regia made up to 500 ml in volumetric flask with deionised water;
iv) 0.1 M prepared as 50 ml of 1 M aqua regia made up to 500 ml in volumetric flask with deionised water;
v) 0.05 M prepared as 5 ml of 5 M aqua regia made up to 500 ml in volumetric flask with deionised water; and

vi) 0.01 M prepared as 5 ml of 1 M aqua regia made up to 500 ml in volumetric flask with deionised water.

Each extractant is applied three times to the soil sample as outlined in Table 2 resulting 21 extracts per soil sample.

The extraction procedure is as follows:

1. Weigh approximately 2 g of each test sample into a clean 30 ml Oakridge tube.
2. Add a 10 ml aliquot of the required extractant, by pipette, to each of the tubes.
3. Rotate each tube on an end over end shaker for 10 minutes at 30 rpm.
4. Centrifuge the tubes at 4350 rpm for 5 minutes.
5. Pipette off the supernatant into a graduated sample vial and record the volume.
6. Add the next extracting solution in the sequence (see table 2) and repeat steps 2 to 5.

For the extractions with 0.1, 0.5, 1.0 and 5 M acid extracts, carry out steps 2-5 with the addition 0.25, 0.50, 0.75 and 1 ml, respectively, of 9% (v/v) H_2O_2 prior to making up the final acid volume to 10 ml (as shown in Table 2).

The hydrogen peroxide is added to aid the digestion of the organic material and Mn oxides as the sequential extraction proceeds.

The tubes are weighed before and after removal of the supernatant solution to determine the volume of solution withdrawn so that the volume of solution in contact with the soil during shaking is known. A small amount of solution is left behind (ca. 0.1-0.3ml) but this is taken into account by the self-modelling mixture resolution data processing which is carried out after the analysis of extracts.

The extracted solutions were analysed for major and trace elements required for the CISED data processing using a Perkin Elmer Optima 7300DV ICP-AES. The sample introduction system was a Conikal U-Series concentric glass nebulizer with a glass cyclonic spray chamber. The ICP-AES operating conditions and wavelengths used are given in tables S1 and S2 in the supplementary information. The wavelengths were chosen to give suitable detection limits, linear ranges and freedom from spectral interference in the CISED extraction matrix.

Sequential extraction data processing

The major and trace element data obtained from the sequential extracts for BGS102 soil were assembled into a data matrix consisting of 21 rows (the extracts) and 26 columns (the elements). The data was subjected to a previously described Self Modelling Mixture Resolution (SMMR) algorithm [16,19]. This procedure separates the data into geochemically distinct components which includes the chemical composition of each component, the amount of each component in each extract and the fractionation of each element between each of the identified components. The algorithm is programmed in the MatLab programming language and uses a bootstrap re-sampling approach to provide median and 95th percentile confidence intervals on all the outputs [19].

Reagents and solutions for the dynamic extraction

All reagents were of analytical grade and Milli-Q water (Millipore Synthesis A10, Millipore Corporation, Billerica, MA, USA) was used throughout. All glassware and polyethylene containers were previously soaked in 10% (v/v) HNO_3 and rinsed three times with deionized water.

The inorganic salts, the organic reagents and the distinct enzymes for the preparation of the synthetic gastric biofluid were specified by BARGE [20]. The chemical composition of the gastric juice used in the dynamic method is as follows: 824 mg L^{-1} KCl, 266 mg L^{-1} NaH_2PO_4, 2752 mg L^{-1} NaCl, 400 mg L^{-1} $CaCl_2$, 306 mg L^{-1} NH_4Cl, 3.6 g L^{-1} HCl, 85 mg L^{-1} urea, 650 mg L^{-1} glucose, 20 mg L^{-1} glucuronic acid, 330 mg L^{-1} glucosamine hydrochloride, 1000 mg L^{-1} pepsin from porcine gastric mucosa (0.7 FIP-U/mg, Merck, Darmstadt, Germany, 1000 mg L^{-1} bovine serum albumin with a final pH of 1.1 ± 0.1. Previous studies indicate the lack of digestive action in UBM by mucin, which merely acts as a lubricant in the mouth and stomach [13]. Therefore, gastric fluid without mucin was used throughout (see further explanations below).

Instrumentation for automatic on-line UBM extraction

The automated flow system for assessment of oral bioaccessibility of TE in soils is schematically illustrated in Fig. 1. It comprises a 3,000-step bidirectional syringe pump (CAVRO XP3000, Tecan group, Männedorf, Switzerland) for automatic handling of the gastric fluid and delivery of well-controlled volumes to the solid sample as contained in a flow-through stirred chamber. An eight-port multiposition selection valve (SV; Multiburette

4S, Crison Instruments, Barcelona, Spain) was used for automatic handling of the extractant and extracts. For quantitative injection of a metered digestive juice volume into the detection system, a six-port rotary injection valve (IV) was furnished with a 500 μL injection loop. The SV and the IV were connected via a 100 μL transfer line (0.8 mm i.d. PTFE).

The syringe pump was furnished with a 5-mL gas-tight glass syringe (Hamilton, Switzerland) and a three-way valve at its head, which allowed connection with either the manifold or the carrier (water) reservoir. The central port of the SV was connected to the pump via a holding coil (HC), which consisted of a 3.0-m-long polytetrafluoroethylene (PTFE) tubing (1.5 mm i.d.), with an approximate internal volume of 5.3 mL. The outlets of SV were connected to the gastric fluid reservoir, the soil container, the extract cup consisting of a 5-mL polypropylene pipette tip, or the waste, through PTFE tubing (1.5 mm i.d.) using polyetheretherketone (PEEK) fittings.

The flow-through chamber for containing the soil was constructed from borosilicate glass as described elsewhere [21] with an inner volume capacity of ca. 15 mL (see Fig. 1). A rubber gasket was placed on top of the chamber followed by a nylon filter (GE Osmonics Labstore, MN, USA) of 0.45 μm pore size and 47 mm diameter to allow dissolved matter to flow through but retaining soil particles. The setup was completed with a second rubber gasket and the cover on top of the flow chamber. The inlet of the chamber was connected to SV, while the outlet to the extract cup (see Fig. 1) using small pieces of Tygon tube and PTFE tubing of 1.5 mm i.d.. A weighed soil sample (400 mg) was transferred to the flow chamber together with a small magnetic bar (1 cm long), and the overall components of the container were securely clamped. A magnetic stirring device (actuated at 480 rpm to ensure a stable soil-gastric fluid dispersion) was employed. All the programmable flow sequences were executed by a personal computer running the lab-made Cocosoft 4.3 software written in Phyton [22]. The software permits the control of syringe pump motion and speed through an RS232 interface, the selection of the distinct ports of the SV and IV as well as the relay activation of the detection instrument (ICP-AES) via the 6-pin barrier strip connector of the digital output of the SP.

The gastric juice leachates containing bioaccessible TE were analyzed using an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Optima 5330DV) furnished with a cross-flow pneumatic nebulizer. The operating conditions for ICP-AES detection are given as follows: RF power, 1300 W; plasma Ar flow rate, 15 L min^{-1}; auxiliary Ar flow rate, 0.2 L min^{-1}; nebulizer flow rate, 0.5 L min^{-1}; rinse time, 60 s; sample flush time, 0 s; sample uptake delay, 0 s; read time, 1 s; view mode, axial; and analytical wavelengths of 267.716 nm for Cr, 324.752 nm for Cu, 231.604 nm for Ni, 220.353 nm for Pb, 188.979 nm for As and 213.857 nm for Zn.

The instrument readouts were recorded on-line in a continuous mode at 1 Hz for the measurement of the overall leachate (or alternatively standard) content of the injection loop. The area of the transient peak in each leachate subfraction was used for plotting the oral bioaccessibility leaching profile or cumulative extraction profile for the suite of analyzed TE.

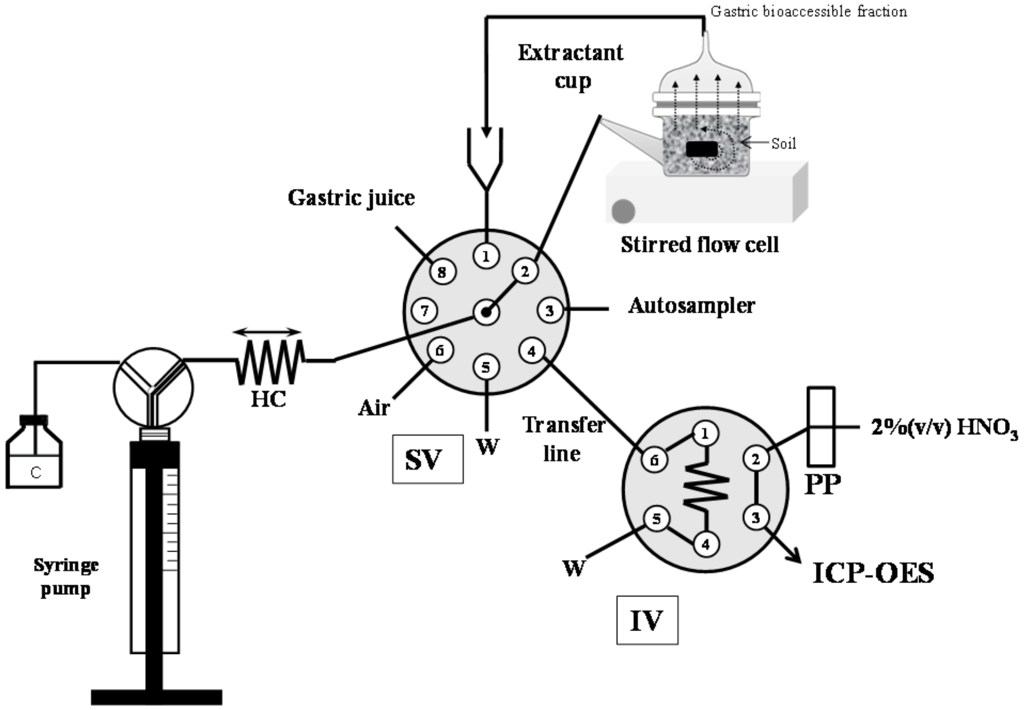

Fig. 1. Diagrammatic description of the hybrid flow setup hyphenated to ICP-AES for automated bioaccessibility tests of trace elements in BGS102 guidance soil using UBM gastric fluid. SV: Selection Valve; IV: Injection Valve; HC: Holding Coil; W: Waste; C: Carrier (H_2O); ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometer; PP: Peristaltic Pump.

Analytical procedure for the dynamic UBM extraction

The stirred-flow chamber was initially loaded with 400 mg of the BGS 102 soil. The peristaltic pump of the ICP-AES instrument was activated so as to provide the spectrometer with a constant flow of 2% HNO_3 (v/v) throughout via the IV in the load position.

The automatic analytical procedure for on-line gastric bioaccessibility measurements started with the aspiration of 100 µL of air (port 6 of the SV) into the HC so as to prevent dispersion of the surrogate gastric fluid into the carrier solution. A metered volume of 4900 µL of the gastric biofluid was aspirated (from port 8 in Fig 1) into the HC at 10 mL min^{-1}. Thereafter, the flow was reversed and the extractant plug perfused the soil sample contained in the stirred chamber at 1.5 mL min^{-1}, while retaining the air segment within the HC. The gastric leachate (after filling up of the chamber and connecting tubes) was collected into the extractant cup nested to port 1 of the SV. For physicochemical homogenization of the content of the cup, a 2.5 mL air zone was pumped up-flow into the extract solution at 5 mL min^{-1}. The extract cup was next emptied by aspiration of the overall content (leachate plus a 100 µL air) into HC. The syringe pump was then programmed to dispense a 2 mL of leachate volume toward the IV so as to fill the injection loop. The ICP-AES instrument was then triggered via the relay and the IV activated to the injection position whereupon the transient readout was recorded. The dynamic extraction method lasted 6.3 min per subfraction. The ICP-AES detection was synchronized with the collection of the next leachate subfraction.

The above-mentioned automatic procedure was repeated forty-fold to reach a baseline level, which was set to the extractant volume for which the increase of metal leached in five consecutive subfractions (ca. 25 mL) was less than 10 % of the cumulative extracted amount, thus indicating exhaustive extraction of the targeted TE.

A ten point matrix-match external calibration (in the UBM gastric medium) was selected for determination of oral bioaccessible TE in the certified soil.

RESULTS AND DISCUSSION

Gastric bioaccessibility of TE in BGS 102 under worst case conditions

The synthetic digestive fluids recommended by BARGE are composed of large amounts of salts, organic compounds and digestive enzymes that give rise to heterogeneous mixtures. As discussed previously by Rosende *et al.* [13], the handling of the UBM surrogate biofluids in flow systems is troublesome because of the progressive clogging of the tubing and membrane filters by suspended matter. Because turbid and viscous solutions were generated with the addition of mucin to the gastric media, and a good agreement was encountered at the 0.05 significance level between TE bioaccessibility in the presence and absence mucin [13] gastric fluid without mucin was thus selected for the ensuing studies. In order to assess the gastric bioaccessible fractions of Ni, Cu, Zn, Pb, As and Cr in the BGS 102 soil under worst-case scenarios as obtained by dynamic flow-through extraction, the critical parameters obtained previously by a factorial design [13], that is, extraction temperature, flow rate, and extraction flow rate were fixed to 400 mg, room temperature (27 ºC), and 1.5 mL min^{-1}, respectively.

Leaching profiles (so-called extractograms) were obtained by the graphical plot of the amount or concentration of gastric bioaccessible TE against time or cumulative extractant volume [23,24]. Fig. 2 depicts the average extractograms of Ni, Cu, Zn, Pb, As and Cr in the certified soil using dynamic gastric extraction as a front-end to ICP-AES. Fresh gastric phase was delivered to the soil containing extraction chamber until the bioaccessible TEs are completely leached out as seen from the signal gradually tailing off to baseline level, thereby simulating worst-case scenarios that cope with ISO/TS 17924:2007 specifications [25].

Similar trends in leaching patterns were recorded for the suite of analytes. Usually 12 (ca. 59 mL) subfractions were necessary for the extraction of more than 80% of the pools of gastric bioaccessible TE in BGS 102, except for Pb, which displayed a much slower leaching kinetics with the subsequent increase of the extraction volume to 103 mL for leaching of about 80% of the total bioaccessible fraction under dynamic conditions.

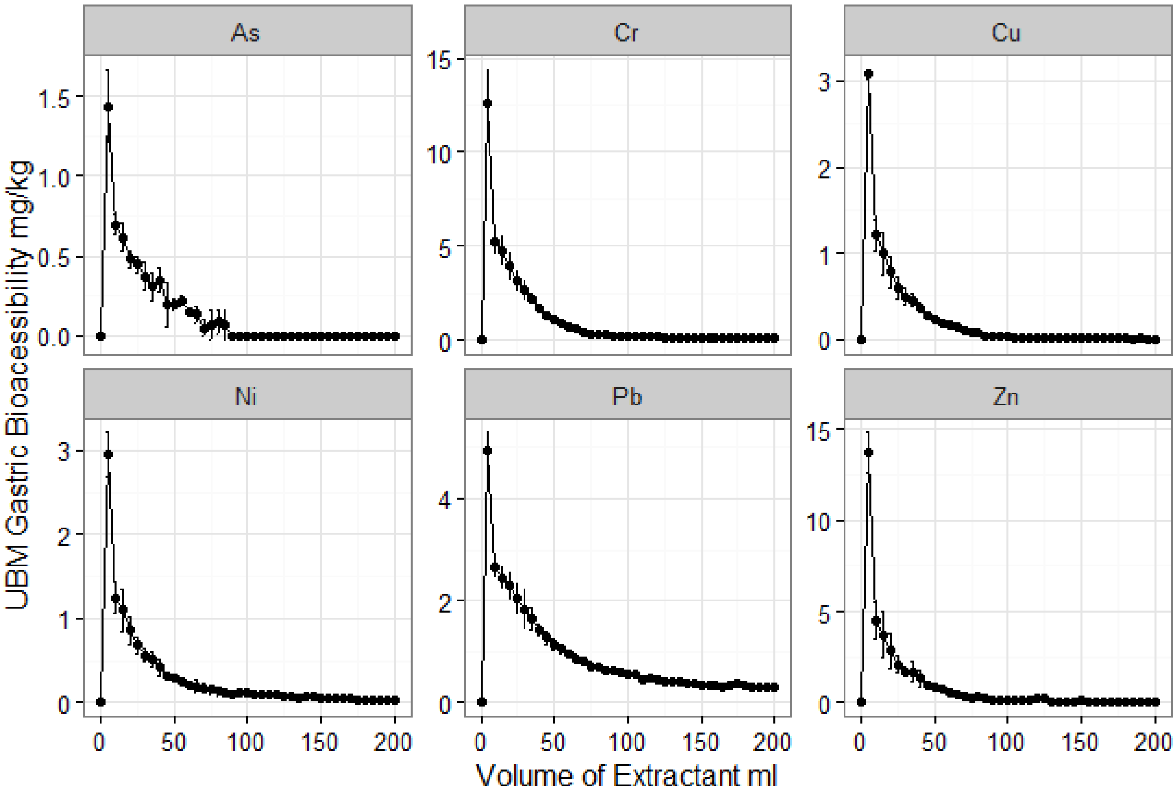

Fig. 2. Average extractograms of Cr, Cu, Ni, Pb, As and Zn in soils for evaluation of leaching kinetics and pools of bioaccessible elements under worst-case dynamic UBM gastric digestion scenarios (n = 3). Error bars indicate the standard deviation.

It should be noted that the final pH of the gastric phase in UBM bioaccessibility tests [14] has to be < 1.5 otherwise the procedure should be restarted from the beginning with the steady control of pH throughout. This was not an issue for the BSG 102 soil in the dynamic system, because the pH of the overall subfractions were < 1.5 in all cases and the nominal gastric fluid pH, that is, 1.1 ± 0.1, was attained after 5-10 subfractions (25-50 mL) as shown in Figure S1 (supplementary information).

Comparison of UBM batch mode against dynamic extraction mode

A direct comparison of the elements under study extracted by the two methods is shown in Fig. 3. All of the elements apart from Pb, showing slow leaching kinetics (see Fig. 2), are equivalent within the 95th percentile confidence limits on the measurements. The mean values for As, Cr, Cu and Pb are slightly higher for the dynamic mode test (in the case of Pb a factor ca. 2 higher) and slightly lower for Ni and Zn. The reasons for the significant difference for Pb are discussed later with respect to the BGS102 fractionation data. It is interesting to note, however, that for most of the elements studied the dynamic test gives statistically equivalent results to the batch method which suggests automation of the UBM method using a dynamic approach gives rise to reliable results.

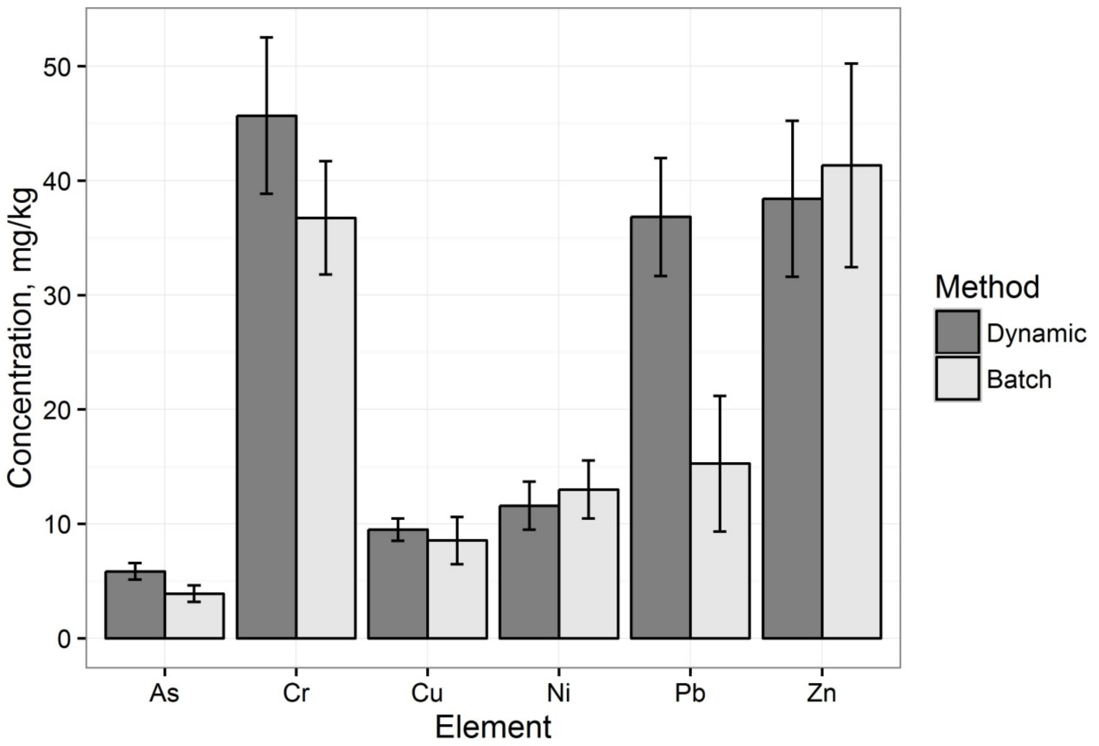

Figure 3. Comparison of the stomach phase bioaccessibility measurements for the batch and dynamic UBM bioaccessibility test on the BGS102 soil. Error bars represent 95 percentile confidence limits

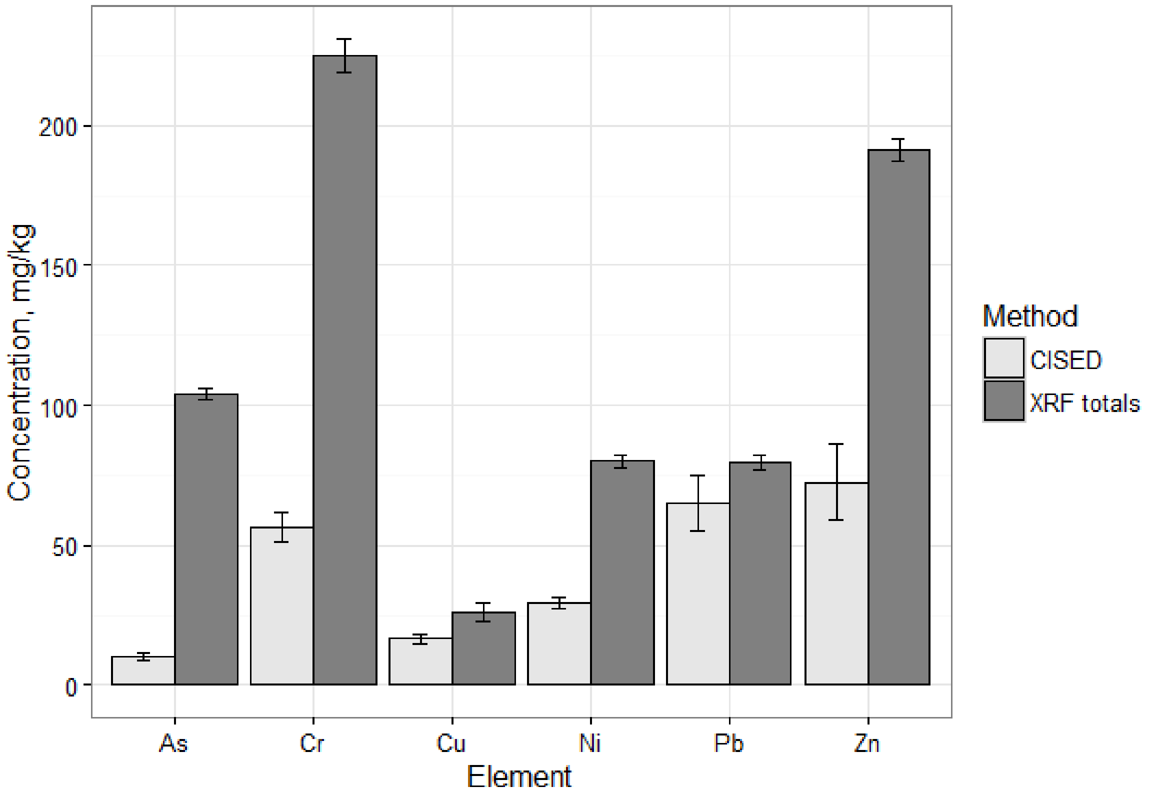

Figure 4. Total element concentrations in BGS 102 compared to the CISED extractable concentrations in BGS102. Error bars represent 95 percentile confidence limits

Figure 4 compares the total element concentrations in BGS 102, obtained by X-ray Fluorescence (XRF) analysis [26], to those extracted by summing the element concentrations in the CISED sequential extractions. Whilst the CISED method uses mineral acid extractants Fig. 5 clearly shows that it only extracts a proportion of the total elements under study (9.8% for As, 25.0% for Cr, 64.0% for Cu, 36.4% for Ni, 81.6% for Pb and 37.9% for Zn). The CISED method was designed to extract the more mobile physico-chemical phases of the soil, i.e. fine grained particulates and surface coatings of the silicate and aluminosilicates. Comparing the data from Fig. 3 with that of Fig. 4 shows that the CISED method extracts a larger proportion of the total element concentration than the oral bioaccessible fraction but is more comparable to the batch and dynamic UBM bioaccessibility data than the total element concentration, *viz*., the batch UBM extracts the following proportion of the total element concentration: 3.8% for As, 16.3% for Cr, 32.8% for Cu, 16.2% for Ni, 19.2% for Pb and 21.6% for Zn. The reason that the CISED method extracts a larger proportion of the metals than the gatric biofluid is that the CISED method uses a more aggressive extraction medium made up of mixed mineral acids at higher concentrations and lower pH than the gastric biofluid.

Fractionation of elements in BGS102 using the CISED sequential extraction

The CISED data processing identified 9 geochemically distinct physico-chemical components in the soil. Figure 5 illustrates the median extraction profiles for the 9 components along with 95th percentile confidence limits. The extraction number refers to the 21 solutions given in Table 2. The components are ordered by their ease of extraction (from K.S through to Fe). The names of the component are made up from the elements that make up more than 10% of the component composition. Figure 6 shows the chemical composition of each of the physico-chemical components with 95th percentile error bars, again ordered in by ease of extraction, from which tentative assignment of the components can be made as described below.

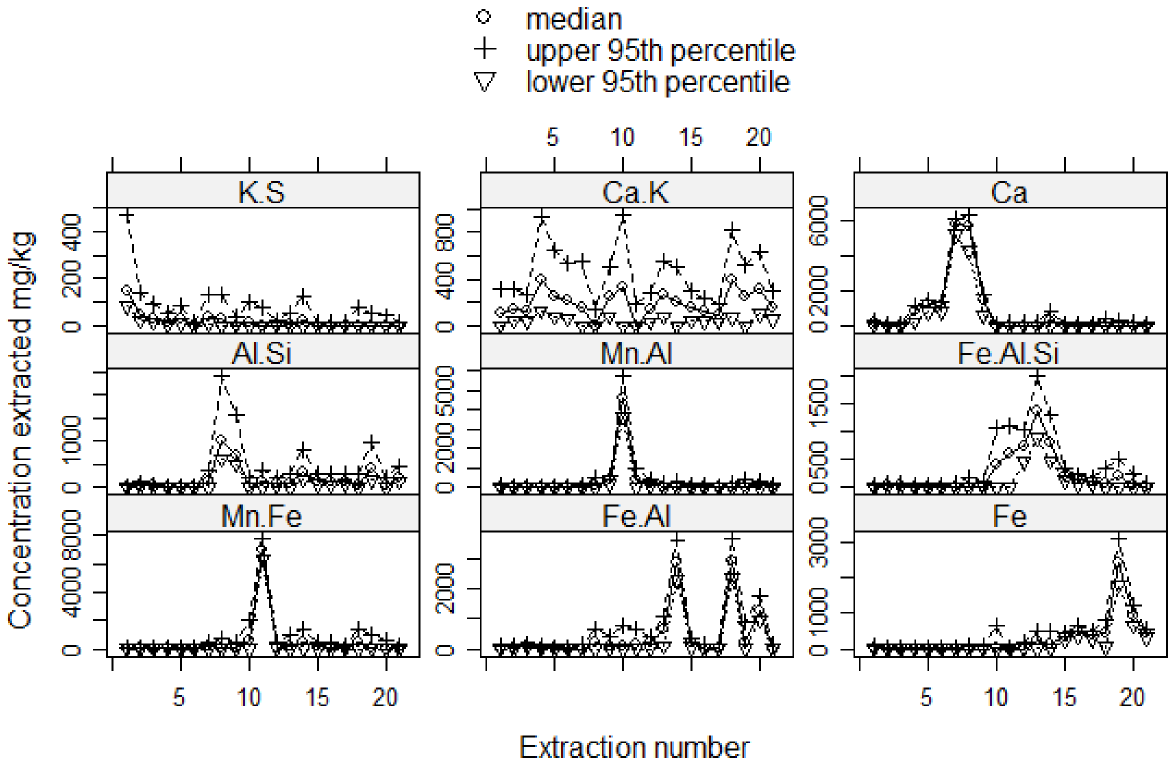

Figure 5. Extraction profiles of the 9 physico-chemical components identified in BGS102 by the CISED sequential extraction. ○ is the median value, ∇ upper 95th percentile confidence limit, + lower 95th percentile confidence limit

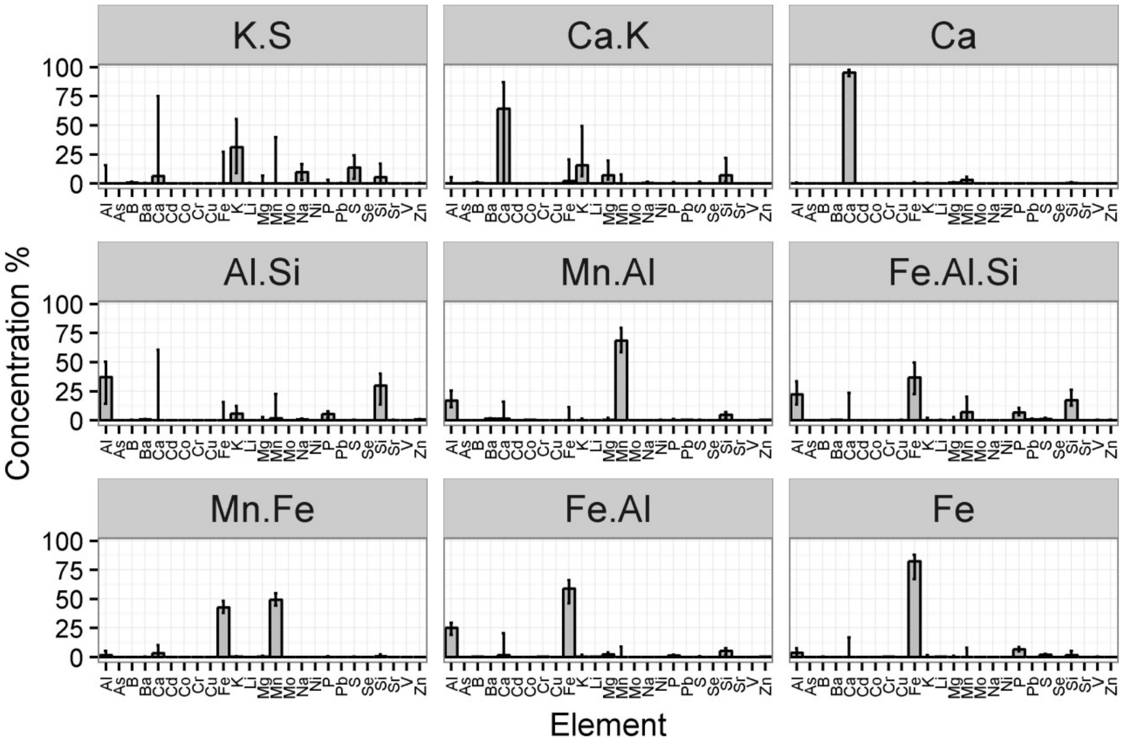

Figure 6. Chemical compositions of the 9 physico-chemical components identified in BGS102 by the CISED sequential extraction. Error bars represent the 95th percentile confidence limit

Tentative assignment of the physico-chemical components

The elucidation of the origin of the 9 components identified in the BGS102 soil is based on the combination of the chemical composition of the component as outlined in Fig. 6, the extraction profiles (Figure 5) which indicate the solubility of the component in increasing strength of acid extractants (see Table 2), the mass and concentration of TE extracted and knowledge of geochemical history of the soil. Insights are provided below:

K.S – This component consists mainly of K, Na, and S and is extracted with the pure water extracts suggesting that this is derived from residual salts from porewater in the soil.

Ca.K - Composed of K, Mg and Si with a poorly defined Ca content; the extraction profile is spread out over the whole range of extractants; and the mass extracted is relatively low compared to other components. This is possibly an organic component which is being released as its inorganic hosts are being dissolved.

Ca – Made up of over 90% Ca with a very clearly defined extraction window at lower acid strengths. This is clearly a calcium carbonate component.

Al.Si - Made up principally of Al, Si with some K and P this component has a similar extraction window to the calcium carbonate component; it has a relatively low extractable mass compared to other components. This is probably an organic material which is associated with the calcium carbonate in the soil.

Mn.Al – Made up of more than ca. 70% Mn by mass this component has a very tightly defined extraction window which coincides with the first addition of H_2O_2 to the extracts. As Mn oxides are known to be highly soluble in this reagent [27] this component is clearly Mn oxide.

The next three components all have ca.50% or greater Fe content and, given that the soil is a ferritic brown earth from North Lincolnshire in the UK which is developed over underlying ironstone geology [26], are likely to be derived from iron oxide sources.

Fe.Al.Si –The major contributors to this component's composition are Fe (ca. 30%), Al(ca. 25%), Si (ca. 20%) and P(ca. 5%). As it has a reasonably well defined extraction window at medium to high acid strength this component is probably a fine grained Fe oxy-hydroxide which are known to have variable composition and are contaminated by a variety of elements [28].

Mn.Fe – Made up of equal amounts of Fe and Mn (ca. 50:50) this component has a very tightly defined extraction window which coincides with the first addition of H_2O_2 to the extracts. This component is likely to be a mixed Fe/Mn oxide.

Fe.Al - Made up of ca. 50% Fe and 25% Al with smaller amounts of Si this component is extracted at medium to high acid strength. The extraction profile shows two main extraction peaks. The first appears in the same extraction window as the Fe.Al.Si component and the second as the Fe component window. This suggests that this component is closely associated with the dissolution of both these components suggesting that it could be a more recently formed component that overlays the older components. Like the Fe.Al.Si this component is probably a fine grained Fe oxy-hydroxide.

Fe – This component is over 80% Fe with a small amount of P and is extracted at the highest acid strength. It has a relatively high purity compared to the other Fe dominated components and its low mobility suggests it is a crystalline iron oxide e.g. hematite or goethite which are known to be present in these soils [29].

Figures 7 and S2 to S6 (supplementary information) show the cumulative concentration of each of the elements under study extracted for each of the 9 physico-chemical CISED components with increasing difficulty of extraction. This shows the fractionation of the elements between the identified physico-chemical components and their relative mobility. In addition, stomach phase bioaccessible fractions measured by the batch and dynamic UBM tests are plotted for each element. Where these lines cross cumulative extraction curve provides information on what fraction of the BGS102 soil is being accessed by the bioaccessibility extraction. Some detailed explanations are given below for the suite of target TE:

Lead (see Fig. 7) – The majority of the lead is held in the Mn.Al, Fe.Al.Si and Mn.Fe components in the soil and the bioaccessible fraction is mostly coming from the Mn.Al component. The higher value for the dynamic extraction suggests that some is also being dissolved out of the Fe.Al.Si and Mn.Fe fractions but in the batch mode process some of this is being reabsorbed back onto some of the fine grained material in the soil.

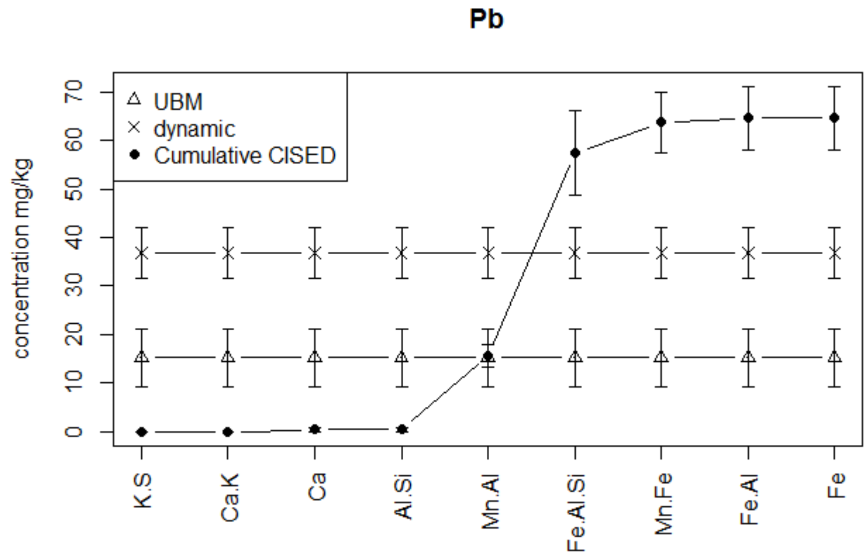

Figure 8. Cumulative Extraction Curve for Pb from the CISED identified physico-chemical fractions

Arsenic (see Fig. S2, supplementary information) – The extractable As is mostly found in the crystalline Fe component although small but significant amounts are found in the components Ca.K through to Fe.Al. The dynamic bioaccessible (worst-case) fraction appears to come from the sum of the Ca.K through to Fe.Al components. The extraction solutions (Table 2) are not very efficient at extracting As from the soil matrix as only 8 mg/kg of the total As concentration of 104 mg/kg is accessed during CISED extractions. These results are in agreement with previous work on the fractionation of ironstone derived soils from Lincolnshire [29].

Chromium (Fig. S3, supplementary information) – Is only associated with the two physico – chemical components with the lowest mobility (Fe.Al and Fe). The two bioaccessibility methods agree and show they are probably accessing the same components as the CISED test.

Copper (Fig. S4, supplementary information) – Like Pb, the majority of the Cu is held in the Mn.Al, Fe.Al.Si and Mn.Fe components in the soil. The two bioaccessibility methods agree and the bioaccessible fraction is mostly coming from the Mn.Al component with some addition dissolution of the Fe.Al.Si component.

Nickel (Fig. S5, supplementary information) - The majority of the Ni is associated with the Mn.Al and the Fe.Al components. The two bioaccessibility methods agree and the bioaccessible fraction is mostly coming from the Mn.Al component.

Zinc (Fig. S6, supplementary information) – The majority of the Zn is associated with the Al.Si, Mn.Al and the Fe.Al components. The two bioaccessibility methods agree and the bioaccessible fraction is mostly coming from the Al.Si and Mn.Al components.

In general, the UBM gastric bioaccessible fraction for most of the metals studied comes from the more mobile, probably fine grained, Mn and Fe oxide fractions in the BGS102 soil with reasonable agreement between the bioaccessible fraction and the sum of the CISED fractions associated with these mid-range mobility fractions.

In the quest of validating new physiologically-based extraction tests, a bioaccessibility guidance soil (BGS 102) has been used in this work to demonstrate that a dynamic UBM extraction procedure for in-vitro gastric phase bioaccessibility gives comparable results to a batch mode bioaccessibility test that has been validated against an animal model. In addition the CISED sequential extraction test provides information on the fractionation of metals in the soil and how this relates to the oral bioaccessible fraction.

The results for the dynamic extraction suggest that it could be used to produce an automated UBM testing standard procedure that would be more efficient and expedite than the batch mode test, and offer relevant insights into the leaching kinetics and the occurrence of potential metal re-adsorption phenomenon.

The additional information on the fractionation of the elements in BGS 102 soil should provide useful data for researchers who wish to use the soil as a reference in contaminated land research.

Note

The authors declare no competing financial interest.

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Table 1. Mean values for study elements obtained from the UBM batch mode bioaccessibility test in the gastric compartment on the BGS 102 bioaccessibility guidance soil

Element	Number of Measurements (n)	Mean (mg kg^{-1})	S.D.	%RSD
As	89	3.9	0.4	9
Cd	72	0.24	0.03	11
Cu	68	8.6	1.0	12
Cr	74	36.7	2.5	7
Ni	72	13.0	1.3	10
Pb	75	15.3	3.0	19
Zn	71	41.3	4.4	11

Table 2. Extraction reagents for the CISED extraction test

Extraction order	Extractant concentration	Volume of extractant (ml)	No of repeat extractions	Volume of 30vol H_2O_2 (ml)
1-3	Deionized water	10	3	0
4-6	0.01M	10	3	0
5-9	0.05M	10	3	0
10-12	0.1M	9.75	3	0.25
13-15	0.5M	9.50	3	0.50
16-18	1.0M	9.25	3	0.75
19-21	5.0M	9.00	3	1.00

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New insights into the reliability of automatic dynamic methods for oral bioaccessibility testing: A case study for BGS102 soil

Mark R. Cave[a], María Rosende[b], Ian Mounteney[a], Amanda Gardner[a], Manuel Miró[b]

a) British Geological Survey, Keyworth, Nottingham NG12 5GG, United Kingdom.
b)FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera de Valldemossa km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain

Supplementary Information

No of figures 6

No. of tables 2

Table S1 ICP operating conditions for the Perkin Elmer Optima 7300DV used for the sequential extraction analysis

Parameter	Standard Operating Conditions
Power	1300 W
Plasma gas flow	15 min^{-1}
Auxiliary gas flow	0.2 l min^{-1}
Nebuliser gas flow	0.65 min^{-1}
Integration time	3 measurements of up to 10 s each
Sample uptake rate	1 ml min^{-1}
Rinse time	Minimum 30 s (sample type dependant)
Uptake delay	60 s
Stabilisation delay	10 s
View distance	15 mm

Table S2 ICP emission wavelengths used for the sequential extraction analysis using the Perkin Elmer Optima 7300DV instrument

Element	Wavelength nm	Atom line (I)/Ion line (II)	Viewing orientation
Al	396.153	I	Axial
As	188.979	I	Axial
B	249.772	I	Radial
Ba	455.403	II	Axial
Ca	315.887	II	Radial
Cd	228.802	I	Axial
Co	228.616	II	Axial
Cr	205.560	II	Axial
Cu	324.752	I	Axial
Fe	238.204	II	Radial
K	766.490	I	Radial
Li	460.286	I	Axial
Li	670.783	I	Axial
Mg	279.077	II	Radial
Mn	257.610	II	Radial
Mo	203.845	II	Axial
Na	330.237	I	Radial
Ni	231.604	II	Axial
P	177.434	I	Axial
Pb	220.353	II	Axial
S	181.975	I	Radial
Se	196.026	I	Axial
Si	251.611	I	Radial
Sr	421.552	II	Radial
V	292.402	II	Axial
Zn	213.857	II	Radial

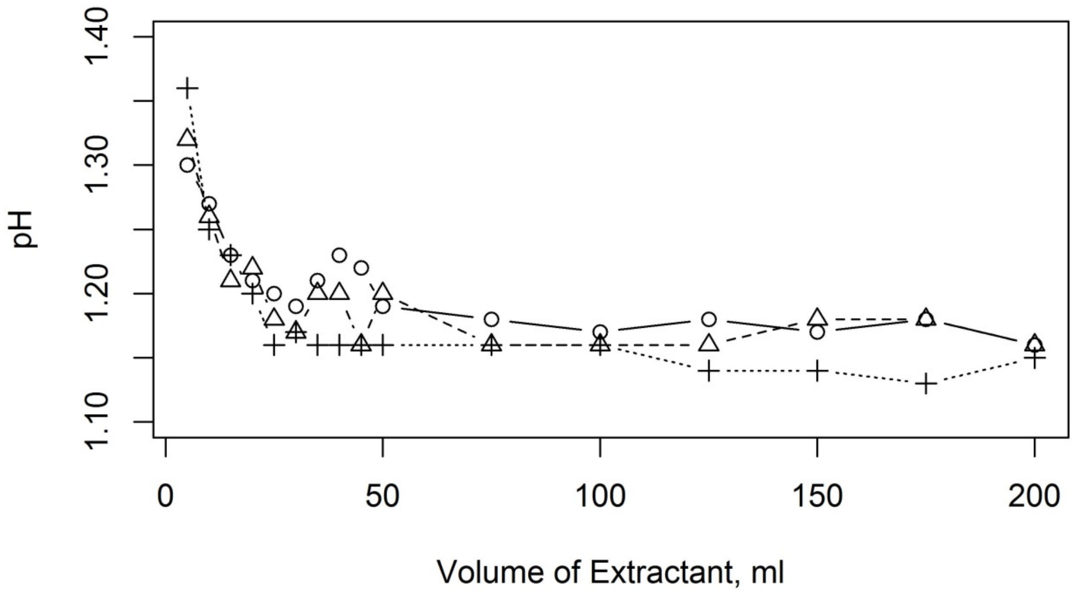

Figure S1 Leachate pH profiles of three replicate measurements of the BGS 102 soil obtained by exploiting in-line leaching of TE in the UBM gastric phase

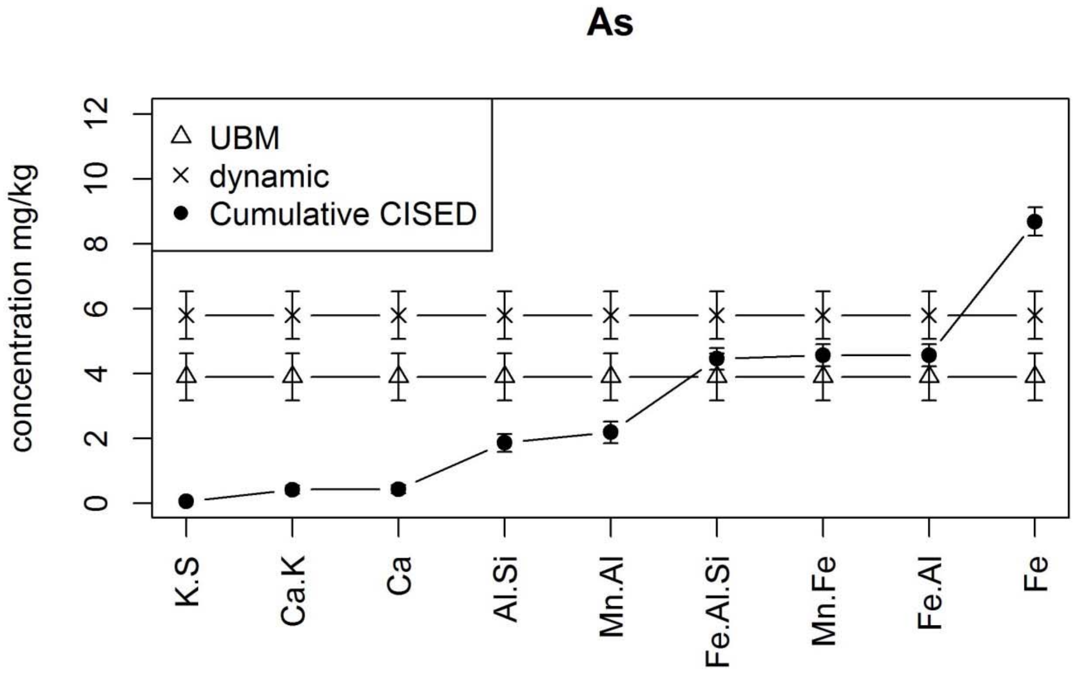

Figure S2 Cumulative Extraction Curve for As from the CISED identified physico-chemical fractions

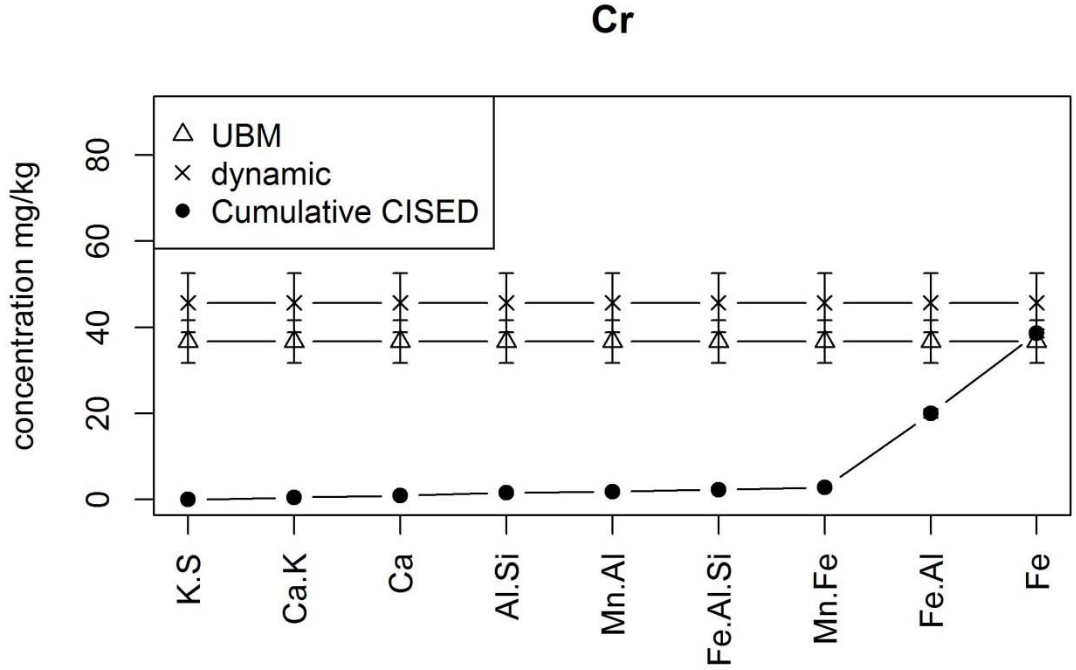

Figure S3 Cumulative Extraction Curve for Cr from the CISED identified physico-chemical fractions

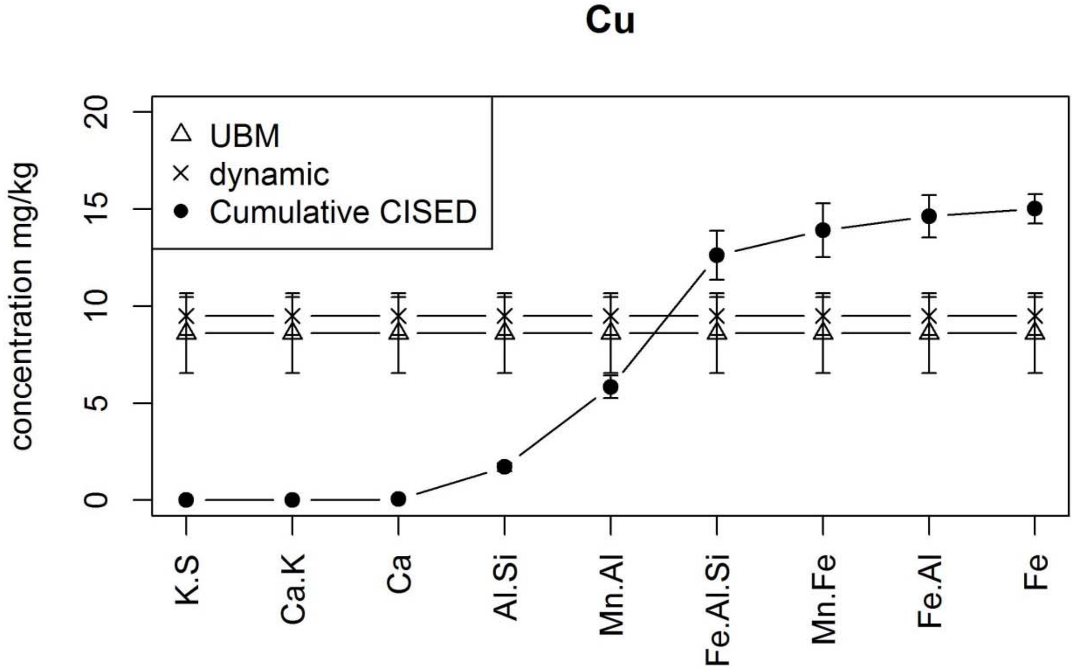

Figure S4 Cumulative Extraction Curve for Cu from the CISED identified physico-chemical fractions

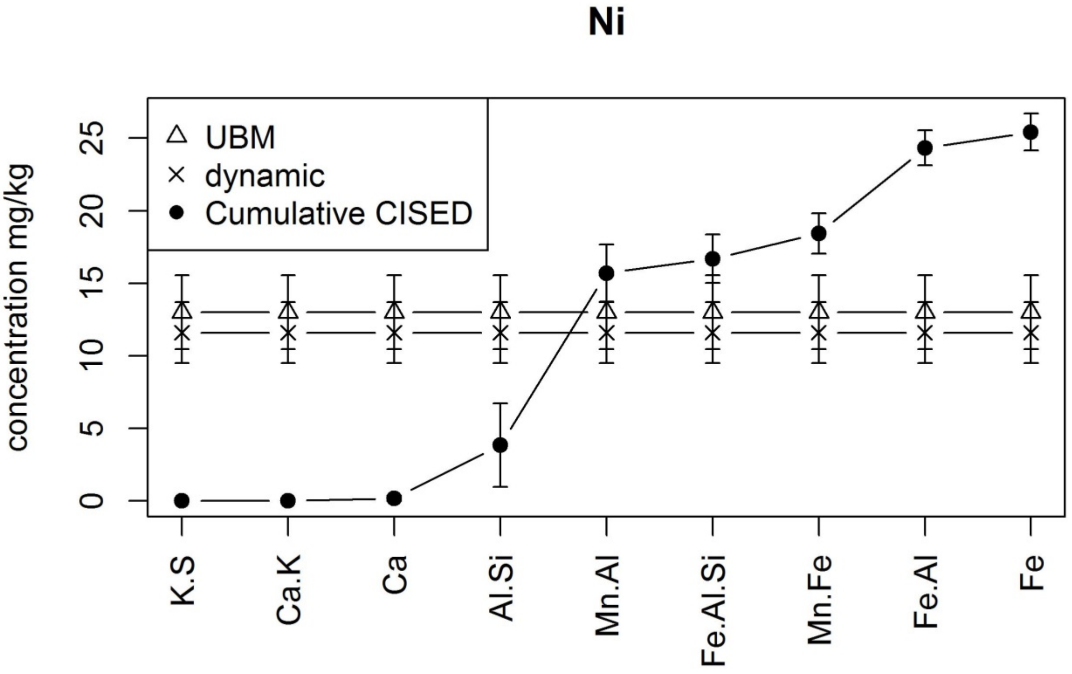

Figure S5 Cumulative Extraction Curve for Ni from the CISED identified physico-chemical fractions

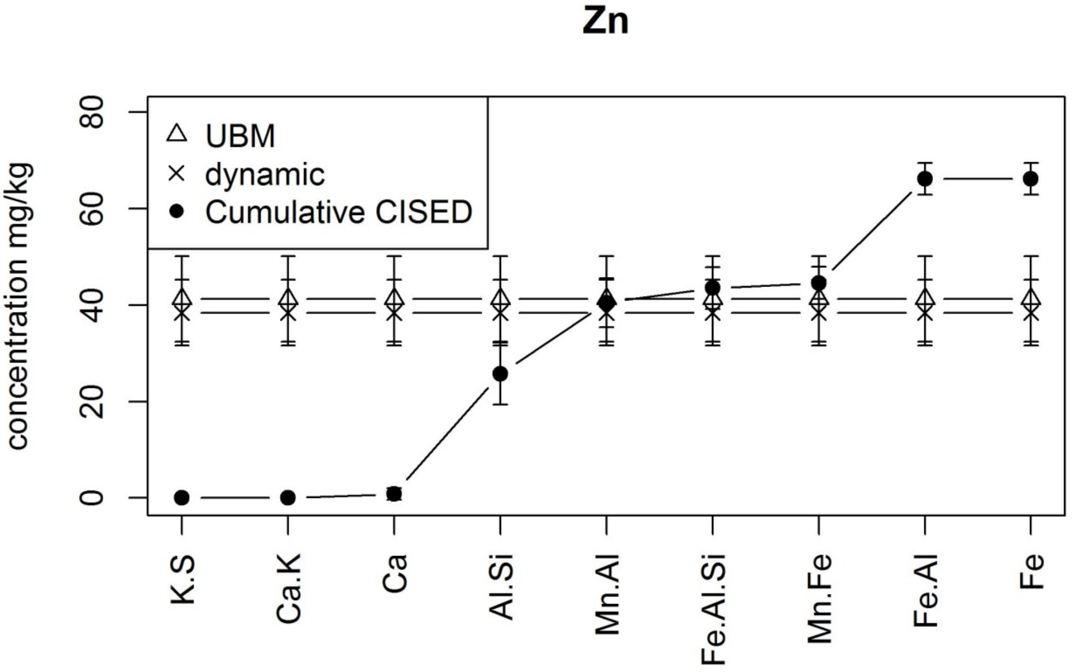

Figure S6 Cumulative Extraction Curve for Zn from the CISED identified physico-chemical fractions