

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

3 **Mark R. Cave^a, María Rosende^b, Ian Mounteney^a, Amanda Gardner^a, Manuel Miró^b**

4 *a) British Geological Survey, Keyworth, Nottingham NG12 5GG, United Kingdom.*

5 *b)FI-TRACE group, Department of Chemistry, University of the Balearic Islands, Carretera*
6 *de Valldemossa km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain*

7

8

9

10 **Abstract**

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

26

27 **Introduction**

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

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

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

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

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

60

61 **EXPERIMENTAL**

62 *Batch mode UBM bioaccessibility data*

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

67

68 *Sequential extraction-based CISED method*

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

73 All reagents were of analytical grade obtained from the ROMIL company and Milli-Q water
74 (Millipore Synthesis A10, Millipore Corporation, Billerica, MA, USA) was used throughout.
75 The Oakridge tubes were previously soaked in 10% (v/v) HNO₃ and rinsed three times
76 with deionized water.

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

- 79 i) 5 M aqua regia made up as 110 ml 37% HCl and 47.5 ml 70 % HNO₃ made up to 500 ml
80 volumetric flask with deionised water;
- 81 ii) 1.0 M aqua regia made up as 22 ml 37% HCl plus 9.5 ml 70% HNO₃ made up to 500 ml
82 in volumetric flask with deionised water;
- 83 iii) 0.5 M prepared as 50 ml of 5 M aqua regia made up to 500 ml in volumetric flask with
84 deionised water;
- 85 iv) 0.1 M prepared as 50 ml of 1 M aqua regia made up to 500 ml in volumetric flask with
86 deionised water;
- 87 v) 0.05 M prepared as 5 ml of 5 M aqua regia made up to 500 ml in volumetric flask with
88 deionised water; and

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

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

93 The extraction procedure is as follows:

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

100 For the extractions with 0.1, 0.5, 1.0 and 5 M acid extracts, carry out steps 2-5 with the
101 addition 0.25, 0.50, 0.75 and 1 ml, respectively, of 9% (v/v) H₂O₂ prior to making up the final
102 acid volume to 10 ml (as shown in Table 2).

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

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

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

116

117 ***Sequential extraction data processing***

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

127

128 ***Reagents and solutions for the dynamic extraction***

129 All reagents were of analytical grade and Milli-Q water (Millipore Synthesis A10, Millipore
130 Corporation, Billerica, MA, USA) was used throughout. All glassware and polyethylene
131 containers were previously soaked in 10% (v/v) HNO₃ and rinsed three times with deionized
132 water.

133 The inorganic salts, the organic reagents and the distinct enzymes for the preparation of the
134 synthetic gastric biofluid were specified by BARGE [20]. The chemical composition of the
135 gastric juice used in the dynamic method is as follows: 824 mg L⁻¹ KCl, 266 mg L⁻¹
136 NaH₂PO₄, 2752 mg L⁻¹ NaCl, 400 mg L⁻¹ CaCl₂, 306 mg L⁻¹ NH₄Cl, 3.6 g L⁻¹ HCl, 85 mg L⁻¹
137 urea, 650 mg L⁻¹ glucose, 20 mg L⁻¹ glucuronic acid, 330 mg L⁻¹ glucosamine hydrochloride,
138 1000 mg L⁻¹ pepsin from porcine gastric mucosa (0.7 FIP-U/mg, Merck, Darmstadt, Germany,
139 1000 mg L⁻¹ bovine serum albumin with a final pH of 1.1 ± 0.1. Previous studies indicate the
140 lack of digestive action in UBM by mucin, which merely acts as a lubricant in the mouth and
141 stomach [13]. Therefore, gastric fluid without mucin was used throughout (see further
142 explanations below).

143

144 ***Instrumentation for automatic on-line UBM extraction***

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

150 4S, Crison Instruments, Barcelona, Spain) was used for automatic handling of the extractant
151 and extracts. For quantitative injection of a metered digestive juice volume into the detection
152 system, a six-port rotary injection valve (IV) was furnished with a 500 μL injection loop. The
153 SV and the IV were connected via a 100 μL transfer line (0.8 mm i.d. PTFE).

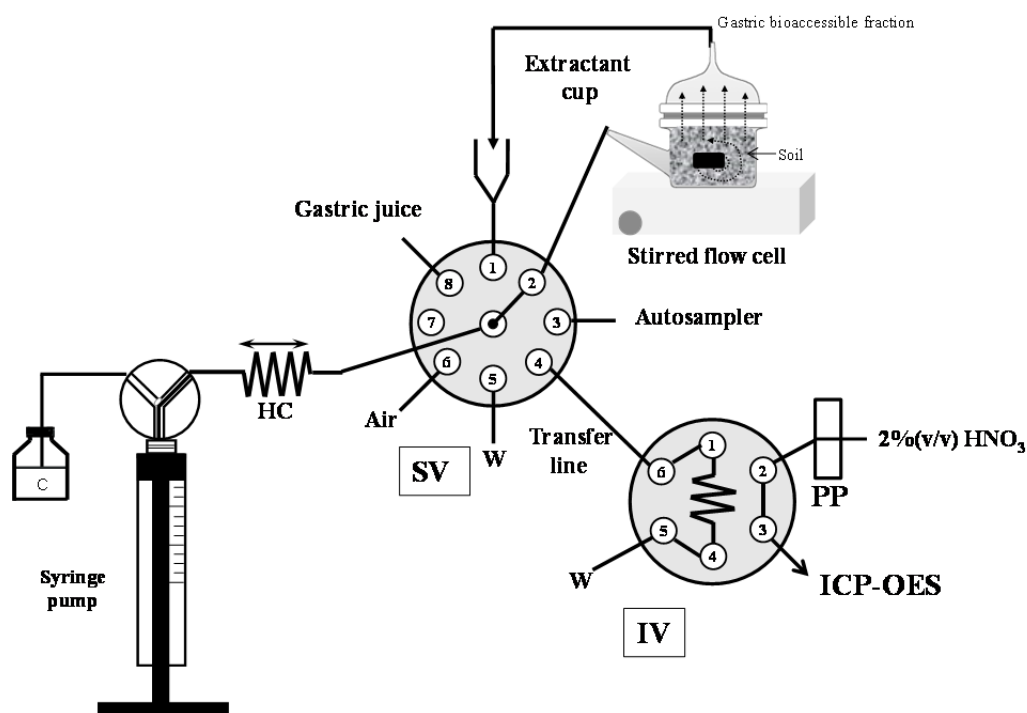
154 The syringe pump was furnished with a 5-mL gas-tight glass syringe (Hamilton, Switzerland)
155 and a three-way valve at its head, which allowed connection with either the manifold or the
156 carrier (water) reservoir. The central port of the SV was connected to the pump via a holding
157 coil (HC),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

162 The flow-through chamber for containing the soil was constructed from borosilicate glass as
163 described elsewhere [21] with an inner volume capacity of ca. 15 mL (see Fig. 1). A rubber
164 gasket was placed on top of the chamber followed by a nylon filter (GE Osmonics Labstore,
165 MN, USA) of 0.45 μm pore size and 47 mm diameter to allow dissolved matter to flow
166 through but retaining soil particles. The setup was completed with a second rubber gasket and
167 the cover on top of the flow chamber. The inlet of the chamber was connected to SV, while
168 the outlet to the extract cup (see Fig. 1) using small pieces of Tygon tube and PTFE tubing of
169 1.5 mm i.d.. A weighed soil sample (400 mg) was transferred to the flow chamber together
170 with a small magnetic bar (1 cm long), and the overall components of the container were
171 securely clamped. A magnetic stirring device (actuated at 480 rpm to ensure a stable soil-
172 gastric fluid dispersion) was employed. All the programmable flow sequences were executed
173 by a personal computer running the lab-made Cocosoft 4.3 software written in Phyton [22].
174 The software permits the control of syringe pump motion and speed through an RS232
175 interface, the selection of the distinct ports of the SV and IV as well as the relay activation of
176 the detection instrument (ICP-AES) via the 6-pin barrier strip connector of the digital output
177 of the SP.

178 The gastric juice leachates containing bioaccessible TE were analyzed using an Inductively
179 Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Optima 5330DV)
180 furnished with a cross-flow pneumatic nebulizer. The operating conditions for ICP-AES
181 detection are given as follows: RF power, 1300 W; plasma Ar flow rate, 15 L min^{-1} ; auxiliary
182 Ar flow rate, 0.2 L min^{-1} ; nebulizer flow rate, 0.5 L min^{-1} ; rinse time, 60 s; sample flush

183 time, 0 s; sample uptake delay, 0 s; read time, 1 s; view mode, axial; and analytical
184 wavelengths of 267.716 nm for Cr, 324.752 nm for Cu, 231.604 nm for Ni, 220.353 nm for
185 Pb, 188.979 nm for As and 213.857 nm for Zn.

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



190

191

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

197

198 *Analytical procedure for the dynamic UBM extraction*

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

202 The automatic analytical procedure for on-line gastric bioaccessibility measurements started
203 with the aspiration of 100 μL of air (port 6 of the SV) into the HC so as to prevent dispersion
204 of the surrogate gastric fluid into the carrier solution. A metered volume of 4900 μL of the
205 gastric biofluid was aspirated (from port 8 in Fig 1) into the HC at 10 mL min^{-1} . Thereafter,
206 the flow was reversed and the extractant plug perfused the soil sample contained in the stirred
207 chamber at 1.5 mL min^{-1} , while retaining the air segment within the HC. The gastric leachate
208 (after filling up of the chamber and connecting tubes) was collected into the extractant cup
209 nested to port 1 of the SV. For physicochemical homogenization of the content of the cup, a
210 2.5 mL air zone was pumped up-flow into the extract solution at 5 mL min^{-1} . The extract cup
211 was next emptied by aspiration of the overall content (leachate plus a 100 μL air) into HC.
212 The syringe pump was then programmed to dispense a 2 mL of leachate volume toward the
213 IV so as to fill the injection loop. The ICP-AES instrument was then triggered via the relay
214 and the IV activated to the injection position whereupon the transient readout was recorded.
215 The dynamic extraction method lasted 6.3 min per subfraction. The ICP-AES detection was
216 synchronized with the collection of the next leachate subfraction.

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

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

223

224 **RESULTS AND DISCUSSION**

225 *Gastric bioaccessibility of TE in BGS 102 under worst case conditions*

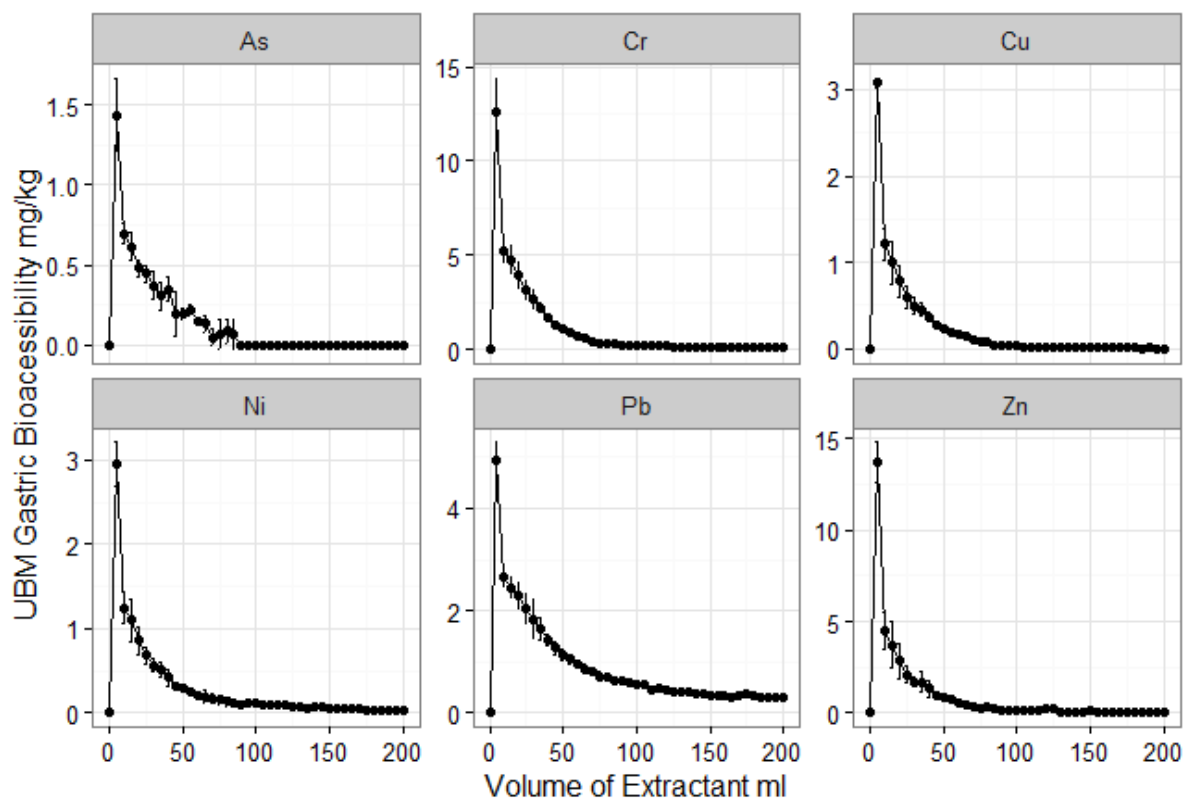
226 The synthetic digestive fluids recommended by BARGE are composed of large amounts of
227 salts, organic compounds and digestive enzymes that give rise to heterogeneous mixtures. As
228 discussed previously by Rosende *et al.* [13], the handling of the UBM surrogate biofluids in
229 flow systems is troublesome because of the progressive clogging of the tubing and membrane
230 filters by suspended matter. Because turbid and viscous solutions were generated with the
231 addition of mucin to the gastric media, and a good agreement was encountered at the 0.05
232 significance level between TE bioaccessibility in the presence and absence mucin [13] gastric
233 fluid without mucin was thus selected for the ensuing studies. In order to assess the gastric

234 bioaccessible fractions of Ni, Cu, Zn, Pb, As and Cr in the BGS 102 soil under worst-case
235 scenarios as obtained by dynamic flow-through extraction, the critical parameters obtained
236 previously by a factorial design [13], that is, extraction temperature, flow rate, and extraction
237 flow rate were fixed to 400 mg, room temperature (27 °C), and 1.5 mL min⁻¹, respectively.

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

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

250



251

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

255

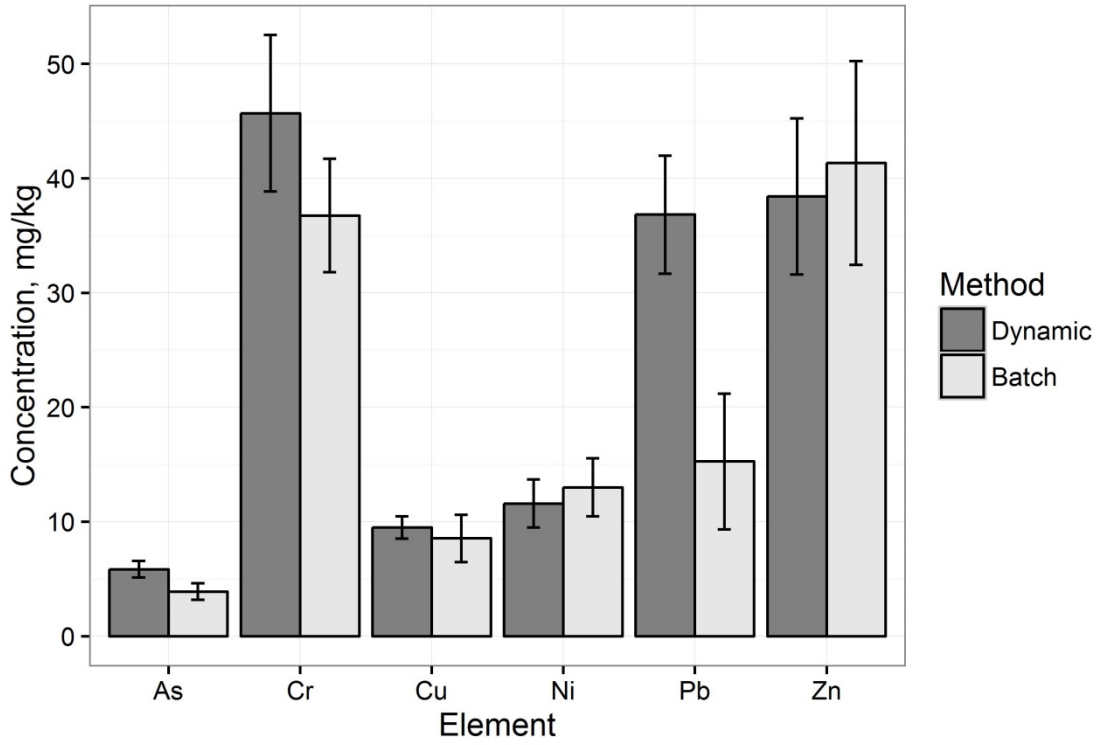
256

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

263

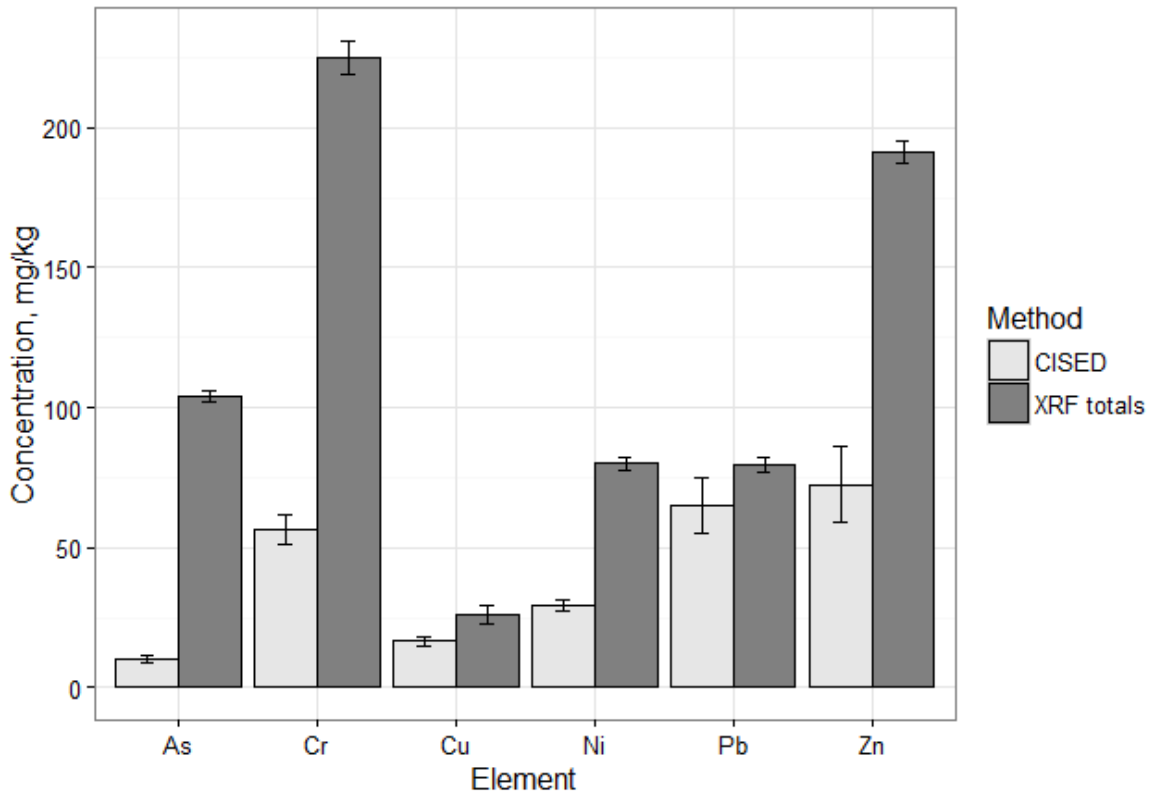
264 ***Comparison of UBM batch mode against dynamic extraction mode***

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



274

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



278

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

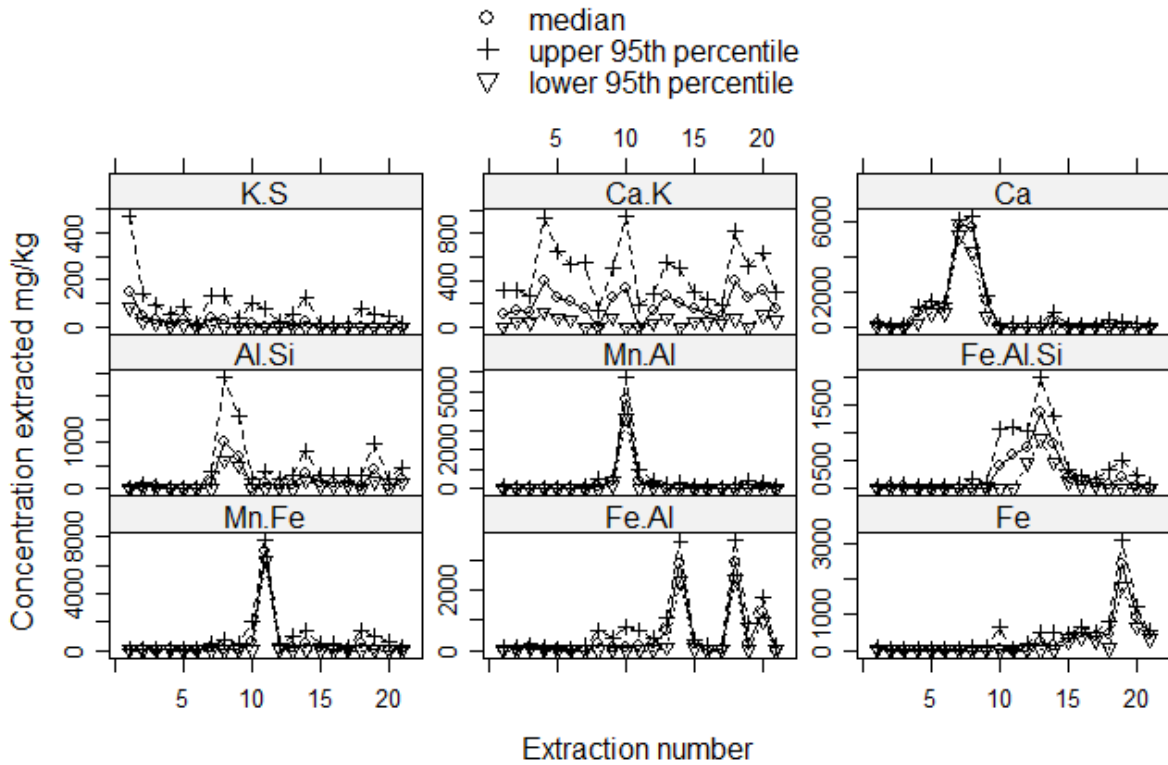
281

282 Figure 4 compares the total element concentrations in BGS 102, obtained by X-ray
283 Fluorescence (XRF) analysis [26], to those extracted by summing the element concentrations
284 in the CISED sequential extractions. Whilst the CISED method uses mineral acid extractants
285 Fig. 5 clearly shows that it only extracts a proportion of the total elements under study (9.8%
286 for As, 25.0% for Cr, 64.0% for Cu, 36.4% for Ni, 81.6% for Pb and 37.9% for Zn). The
287 CISED method was designed to extract the more mobile physico-chemical phases of the soil,
288 i.e. fine grained particulates and surface coatings of the silicate and aluminosilicates.
289 Comparing the data from Fig. 3 with that of Fig. 4 shows that the CISED method extracts a
290 larger proportion of the total element concentration than the oral bioaccessible fraction but is
291 more comparable to the batch and dynamic UBM bioaccessibility data than the total element
292 concentration, *viz.*, the batch UBM extracts the following proportion of the total element
293 concentration: 3.8% for As, 16.3% for Cr, 32.8% for Cu, 16.2% for Ni, 19.2% for Pb and
294 21.6% for Zn. The reason that the CISED method extracts a larger proportion of the metals
295 than the gastric biofluid is that the CISED method uses a more aggressive extraction medium
296 made up of mixed mineral acids at higher concentrations and lower pH than the gastric
297 biofluid.

298

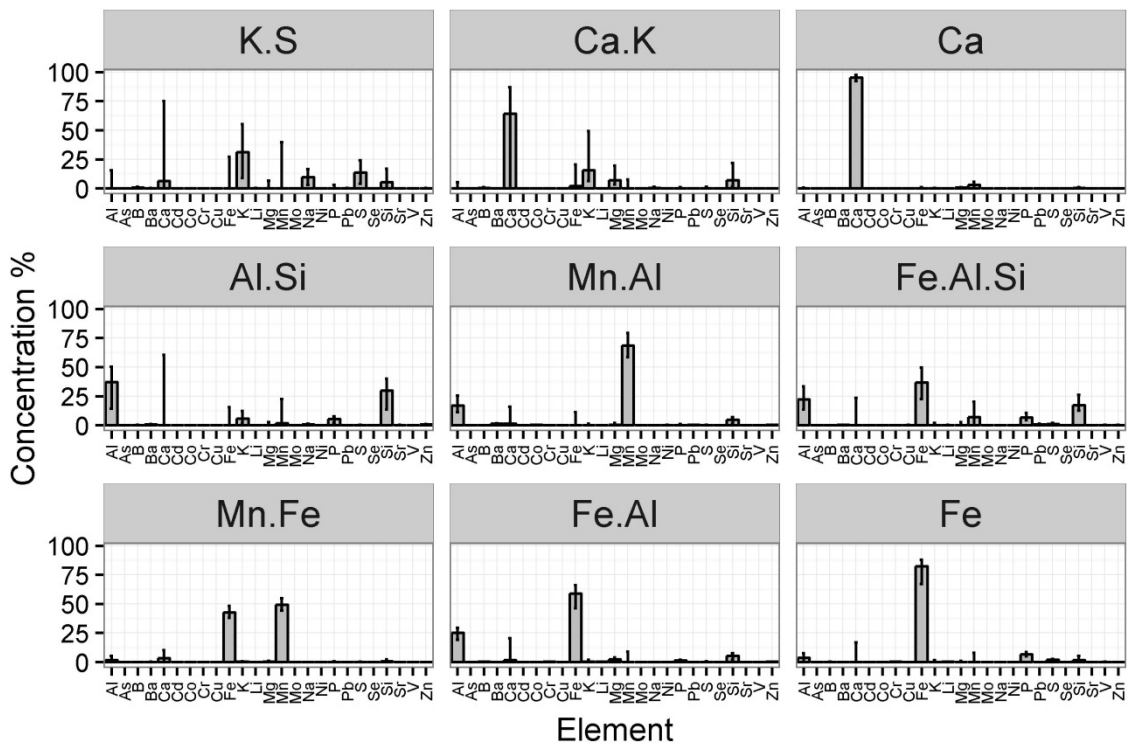
299 ***Fractionation of elements in BGS102 using the CISED sequential extraction***

300 The CISED data processing identified 9 geochemically distinct physico-chemical
301 components in the soil. Figure 5 illustrates the median extraction profiles for the 9
302 components along with 95th percentile confidence limits. The extraction number refers to the
303 21 solutions given in Table 2. The components are ordered by their ease of extraction (from
304 K.S through to Fe). The names of the component are made up from the elements that make
305 up more than 10% of the component composition. Figure 6 shows the chemical composition
306 of each of the physico-chemical components with 95th percentile error bars, again ordered in
307 by ease of extraction, from which tentative assignment of the components can be made as
308 described below.



309

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



313

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

317

318 *Tentative assignment of the physico-chemical components*

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

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

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

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

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

336 Mn.Al – Made up of more than ca. 70% Mn by mass this component has a very tightly
337 defined extraction window which coincides with the first addition of H₂O₂ to the extracts. As
338 Mn oxides are known to be highly soluble in this reagent [27] this component is clearly Mn
339 oxide.

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

343 Fe.Al.Si –The major contributors to this component's composition are Fe (ca. 30%), Al(ca.
344 25%), Si (ca. 20%) and P(ca. 5%). As it has a reasonably well defined extraction window at

345 medium to high acid strength this component is probably a fine grained Fe oxy-hydroxide
346 which are known to have variable composition and are contaminated by a variety of elements
347 [28].

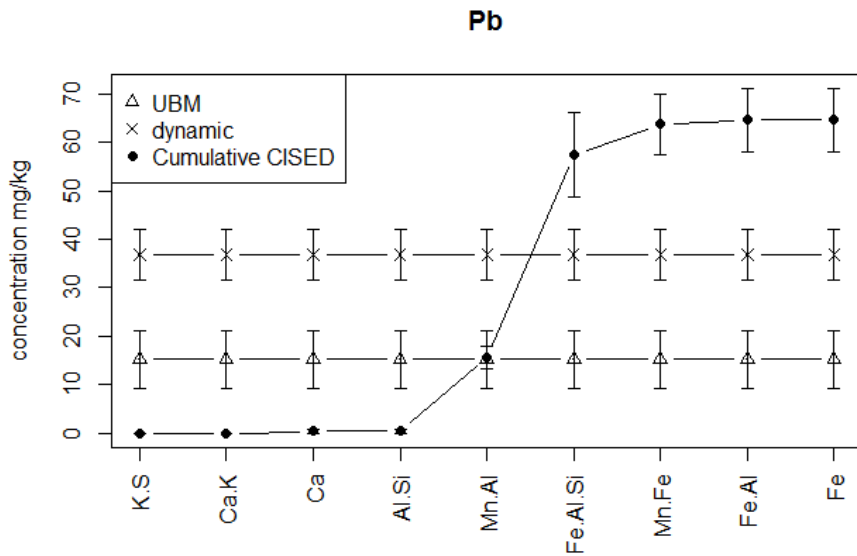
348 Mn.Fe – Made up of equal amounts of Fe and Mn (ca. 50:50) this component has a very
349 tightly defined extraction window which coincides with the first addition of H₂O₂ to the
350 extracts. This component is likely to be a mixed Fe/Mn oxide.

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

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

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

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



376

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

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

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

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

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

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

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

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

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

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

418

419 **Note**

420 The authors declare no competing financial interest.

421

422 **Acknowledgement**

423 Manuel Miró and María Rosende acknowledges financial support from the Spanish Ministry of
424 Economy and Competitiveness (MINECO) through projects CTM2014-56628-C3-3-R and CTM2014
425 -61553-EXP.

426

427

428

429 **Table 1. Mean values for study elements obtained from the UBM batch mode**
430 **bioaccessibility test in the gastric compartment on the BGS 102 bioaccessibility**
431 **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

432

433

434

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 ₂ O 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

435

436

- (1) Denys, S.; Caboche, J.; Tack, K.; Rychen, G.; Wragg, J.; Cave, M.; Jondreville, C.; Feidt, C. In Vivo Validation of the Unified BARGE Method to Assess the Bioaccessibility of Arsenic, Antimony, Cadmium, and Lead in Soils. *Environ. Sci. Technol.* **2012**, *46*, 6252-6260.
- (2) Li, J.; Li, K.; Cave, M.; Li, H.-B.; Ma, L.-Q. Lead bioaccessibility in 12 contaminated soils from China: Correlation to lead relative bioavailability and lead in different fractions. *Journal of Hazardous Materials* **2015**, *295*, 55-62.
- (3) Rosende, M.; Miró, M. Recent trends in automatic dynamic leaching tests for assessment of bioaccessible forms of trace elements in solid substrates. *TrAC-Trends Anal. Chem.* **2013**, *45*, 67-78.
- (4) Zhang, Y.-L.; Miró, M.; Kolev, S.D. Hybrid Flow System for Automatic Dynamic Fractionation and Speciation of Inorganic Arsenic in Environmental Solids. *Environ. Sci. Technol.* **2015**, *49*, 2733-2740.
- (5) Boonjob, W.; Zevenhoven, M.; Ek, P.; Hupa, M.; Ivaska, A.; Miró, M. Automatic dynamic chemical fractionation method with detection by plasma spectrometry for advanced characterization of solid biofuels. *J. Anal. At. Spectrom.* **2012**, *27*, 841-849.
- (6) Fedotov, P.S.; Savonina, E.Y.; Wennrich, R.; Spivakov, B.Ya. A hyphenated flow-through analytical system for the study of the mobility and fractionation of trace and major elements in environmental solid samples. *Analyst* **2006**, *131*, 509-515.
- (7) Limbeck, A.; Wagner, C.; Lendl, B.; Mukhtar, A. Determination of water soluble trace metals in airborne particulate matter using a dynamic extraction procedure with on-line inductively coupled plasma optical emission spectrometric detection. *Anal. Chim. Acta* **2012**, *750*, 111-119.
- (8) Boonjob, W.; Miró, M.; Cerdà, V. Multiple stirred-flow chamber assembly for simultaneous automatic fractionation of trace elements in fly ash samples using a multisyringe-based flow system. *Anal. Chem.* **2008**, *80*, 7319-7326.
- (9) Leufroy, A.; Noël, L.; Beauchemin, D.; Guérin, T. Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. *Anal. Bioanal. Chem.* **2012**, *402*, 2849-2859.
- (10) Miró, M.; Hansen, E.H.; Chomchoei, R.; Frenzel, W. Dynamic flow-through approaches for metal fractionation in environmentally relevant solid samples. *TrAC-Trends Anal. Chem.* **2005**, *24*, 759-771.

- (11) Fedotov, P.S.; Miró, M. Fractionation and mobility of trace elements in soils and sediments, In: Violante, A.; Huang, P.M.; Gadd, G.M. (Eds), *Biophysico-Chemical Processes of Heavy Metals and Metalloids in Soil Environments*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2008, pp. 467–520.
- (12) Lamsal, R.P.; Beauchemin, D. Estimation of the bio-accessible fraction of Cr, As, Cd and Pb in locally available bread using on-line continuous leaching method coupled to inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* **2015**, *867*, 9-17.
- (13) Rosende, M.; Magalhaes, L.M.; Segundo, M.A.; Miró, M. Assessing oral bioaccessibility of trace elements in soils under worst-case scenarios by automated in-line dynamic extraction as a front end to inductively coupled plasma atomic emission spectrometry. *Anal. Chim. Acta* **2014**, *842*, 1-10.
- (14) Wragg, J.; Cave, M.; Taylor, H.; Basta, N.; Brandon, E.; Casteel, S.; Denys, S.; Gron, C.; Oomen, A.; Reimer, K.; Tack, K.; Van de Wiele, T. Interlaboratory Trial of a Unified Bioaccessibility Procedure. British Geological Survey, OR/07/027, 2009. <http://www.bgs.ac.uk/barge/home.html>. (last accessed May 1, 2016).
- (15) Hamilton, E. M.; Barlow, T. S.; Gowing, C. J. B.; Watts, M. J. Bioaccessibility performance data for fifty-seven elements in guidance material BGS 102. *Microchem J.* **2015**, *123*, 131-138.
- (16) Cave, M. R.; Milodowski, A. E.; Friel, E. N. Evaluation of a method for Identification of Host Physico-chemical Phases for Trace Metals and Measurement of their Solid-Phase Partitioning in Soil Samples by Nitric Acid Extraction and Chemometric Mixture Resolution. *Geochemistry: Exploration, Environment, Analysis* **2004**, *4*, 71-86.
- (17) Wragg, J.; Cave, M. Assessment of a geochemical extraction procedure to determine the solid phase fractionation and bioaccessibility of potentially harmful elements in soils: A case study using the NIST 2710 reference soil. *Anal. Chim. Acta* **2012**, *722*, 43-54.
- (18) Cave, M.; Wragg, J.; Gowing, C.; Gardner, A. Measuring the solid-phase fractionation of lead in urban and rural soils using a combination of geochemical survey data and chemical extractions. *Environ. Geochem. Health* **2015**, *37*, 779-790.
- (19) Cave, M. *The use of self modelling mixture resolution methods for the interpretation of geochemical data sets* IR/08/035; British Geological Survey: UK, 2008.
- (20) BARGE Bioaccessibility Research Group of Europe. <http://www.bgs.ac.uk/barge/home.html> (last accessed date, May 1, 2016)

- (21) Shiowatana, J.; Tantidanai, N.; Nookabkaew, S.; Nacapricha, D. A flow system for the determination of metal speciation in soil by sequential extraction. *Environ. Int.* **2001**, *26*, 381–387.
- (22) Cocovi-Solberg, D.J.; Miró, M. CocoSoft: educational software for automation in the analytical chemistry laboratory. *Anal Bioanal. Chem.* **2015**, *407*, 6227–6233.
- (23) Rosende, M.; Miró, M.; Segundo, M.A.; Lima, J.L.F.C.; Cerdà, V. Highly integrated flow assembly for automated dynamic extraction and determination of readily bioaccessible chromium (VI) in soils exploiting carbon nanoparticle-based solid-phase extraction. *Anal. Bioanal. Chem.* **2011**, *400*, 2217–2227.
- (24) Rosende, M.; Miró, M.; Cerdà, V. Fluidized-bed column method for automatic dynamic extraction and determination of trace element bioaccessibility in highly heterogeneous solid wastes. *Anal. Chim. Acta* **2010**, *658*, 41–48.
- (25) ISO/TS 17924:2007, *Soil quality-Assessment of human exposure from ingestion of soil and soil material- Guidance on the application and selection of physiologically based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil*. Geneva: Switzerland, 2007.
- (26) Survey, B. G. *Certificate of Analysis BGS Guidance Material 102 Ironstone Soil*; IR/09/006; British Geological Survey: UK, 2009.
- (27) Clark, J. R., Enzyme-Induced Leaching of B-Horizon Soils for Mineral Exploration in Areas of Glacial Overburden. *Tran. Inst. Min. Metall. Sect. B-App. Earth Sci.* **1993**, *102*, B19-B29.
- (28) Cornell, R. M.; Schwertmann, U. *The Iron Oxides - Structure Properties, Reactions, occurrences and Uses*. VCH Publishers: Weinheim, 1996.
- (29) Palumbo-Roe, B.; Cave, M. R.; Klinck, B. A.; Wragg, J.; Taylor, H.; O'Donnell, K.; Shaw, R. A. Bioaccessibility of arsenic in soils developed over Jurassic ironstones in eastern England. *Environ. Geochem. Health* **2005**, *27*, 121-130.

**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

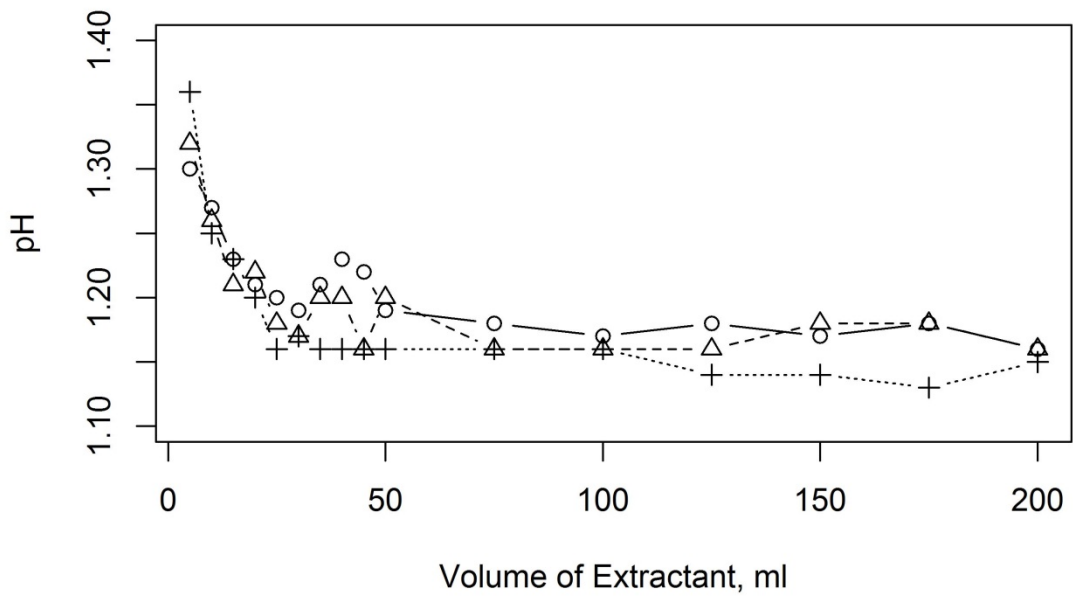


Figure S1 Leachate pH profiles of three replicate measurements of the BGS 102 soil obtained by exploiting in-line leaching of TE in the UBM gastric phase

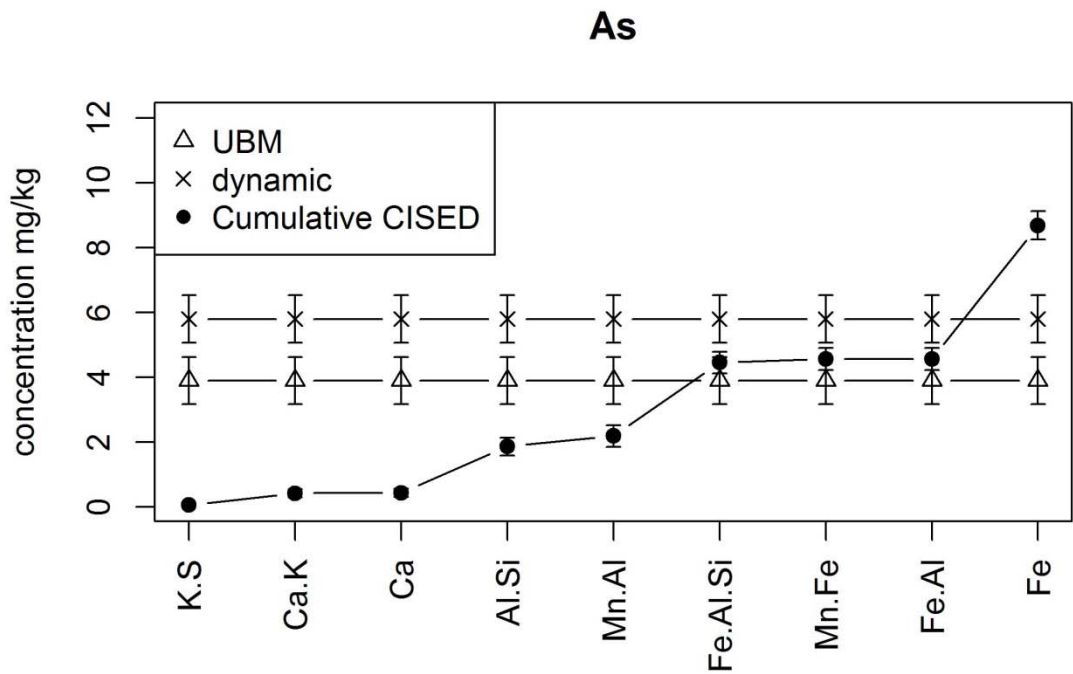


Figure S2 Cumulative Extraction Curve for As from the CISED identified physico-chemical fractions

Cr

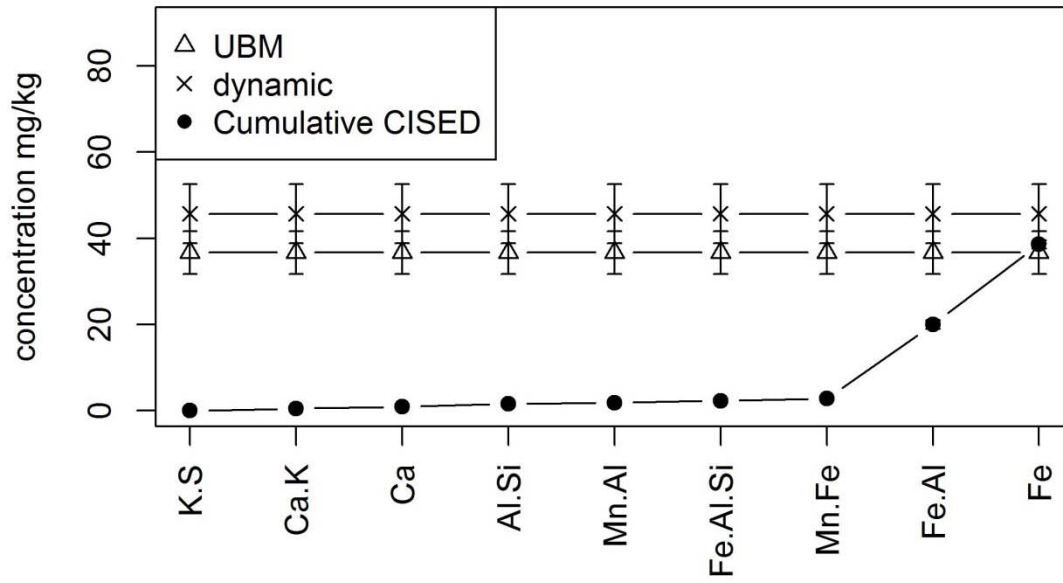


Figure S3 Cumulative Extraction Curve for Cr from the CISED identified physico-chemical fractions

Cu

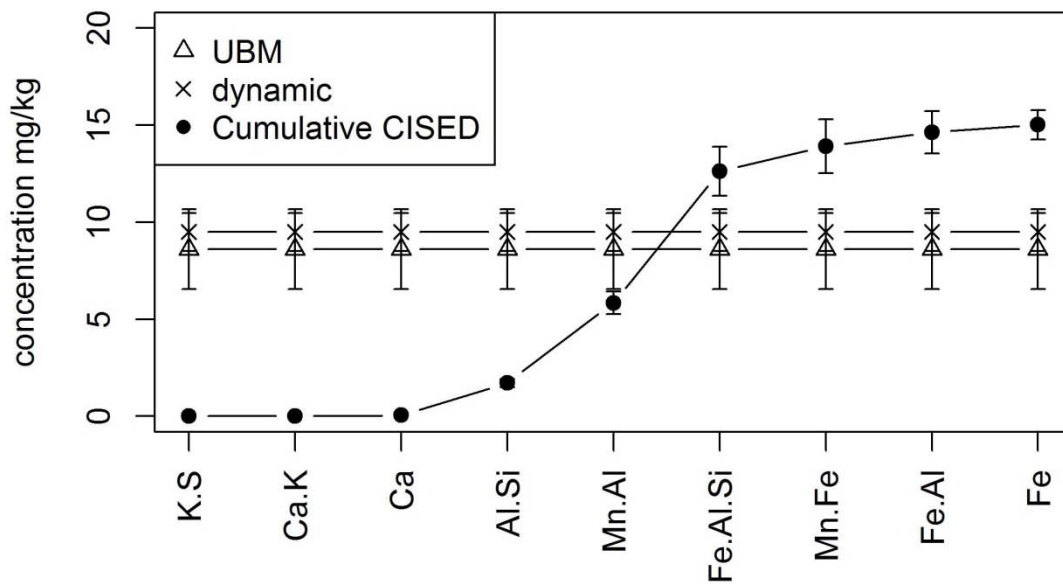


Figure S4 Cumulative Extraction Curve for Cu from the CISED identified physico-chemical fractions

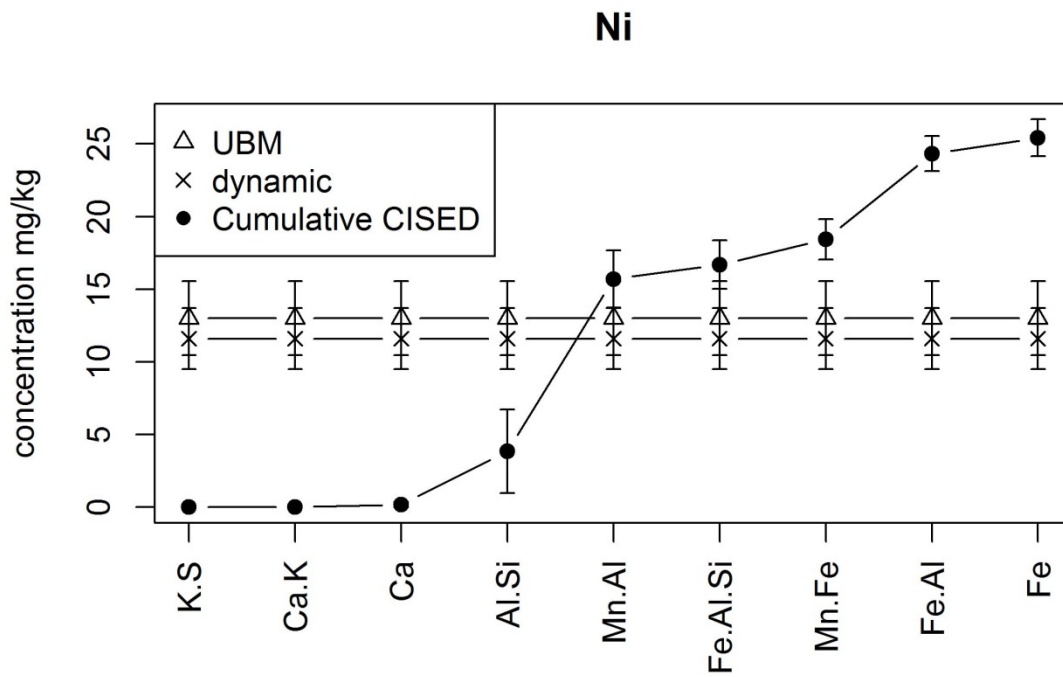


Figure S5 Cumulative Extraction Curve for Ni from the CISED identified physico-chemical fractions

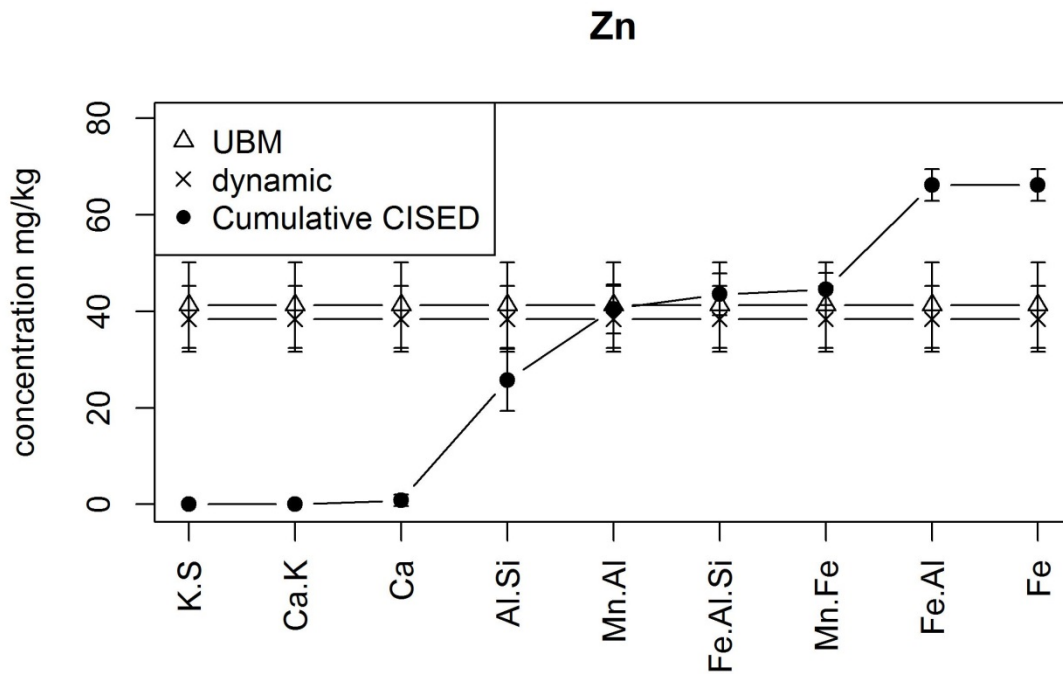


Figure S6 Cumulative Extraction Curve for Zn from the CISED identified physico-chemical fractions