

1 **Comparison of two non-specific flow-through sequential extraction**
2 **approaches to identify the physico-chemical partitioning of potentially**
3 **harmful elements in a certified reference material**

4 Balarabe S. Sagagi^{a,1}, Christine M Davidson^{a*}, Mark R. Cave^b and Joanna Wragg^b
5 ^aWestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde,
6 295 Cathedral Street, GLASGOW, G1 1XL, UK
7 ^bBritish Geological Survey, KEYWORTH, NG12 5GG, UK
8
9

10 **Abstract**

11 Two non-specific sequential extraction methods utilising chemometric data processing
12 (chemometric identification of substrates and element distribution, CISED) have been
13 developed and used to determine the physico-chemical partitioning of potentially harmful
14 elements (PHE) in certified reference material BCR CRM 701 (lake sediment). A miniaturised
15 centrifugation method in which the sample was extracted on a filter insert in a polypropylene
16 centrifuging tube, and a quasi-flow through method in which the sample was supported on a
17 TX40 filter in a 47 mm in-line polycarbonate filter holder, gave similar results. The CISED
18 data processing identified nine components. Seven of these were of geochemical origin – two
19 carbonate components, an Al/Fe oxide/hydroxide component, three Fe-dominated components
20 and one Si-dominated component – while the others represented the TX40 filter blank. The
21 overall extraction capabilities of the methods were similar to that of the well-established BCR
22 sequential extraction (Σ steps 1-3). However, whilst the BCR extraction is operationally
23 defined, the CISED provides information on associations between PHE and the geochemical
24 components identified. The flow through CISED procedure has potential applications in
25 investigating the chemical speciation of PHE associated with urban airborne particulate matter.
26
27

28 *Keywords:* sequential extraction; CISED; chemometrics; BCR CRM 701; airborne particulate
29 matter; inductively coupled plasma mass spectrometry.
30

31 * Corresponding author: tel: +44 141 548 2134; email address: c.m.davidson@strath.ac.uk

32 ¹ Permanent address: Department of Chemistry, Kano University of Science and Technology,
33 Wudil, Nigeria

34 **1. Introduction**

35 The United Nations Department of Economic and Social Affairs predicts that global population
36 will rise from 7.63 billion in 2018 to 9.77 billion in 2050 [1] with the proportion of citizens
37 living in urban environments increasing from 55 to 68%. The intensity of anthropogenic
38 activity in rapidly urbanising areas has led to various environmental problems of which
39 airborne pollution by particulate matter is one major concern [2]. Airborne pollution falls
40 within the scope of (among others) EU Directive 2008/50/EC on ambient air quality and
41 cleaner air for Europe, and the WHO air quality guidelines, because of the risk of potential
42 harm to health. For example, long-term exposure to particulate matter of aerodynamic diameter
43 $< 2.5 \mu\text{m}$ (PM_{2.5}) was found to be strongly associated with mortality attributable to ischemic
44 heart disease, dysrhythmias, heart failure and cardiac arrest [3]. Many countries and urban
45 centres have established PM monitoring programmes and/or strategies (e.g. the Indian National
46 Air Monitoring Programme; USEPA National Air Monitoring Strategy; UK Automatic Urban
47 and Rural Network). Particulate matter of specific aerodynamic diameter (typically PM_{2.5} and
48 PM₁₀) is collected on filter material using various types of air samplers over different time
49 periods [4-6]. In the UK, the tapered element oscillating microbalance filter dynamics
50 measurement system (TEOM FDMS) has been widely used in ambient air-quality monitoring
51 stations.

52

53 Urban particulates contain a variety of potentially harmful elements (PHE) related to their
54 sources. These can be biologically essential for living organisms (including humans) [7-9] but
55 are toxic when present at higher concentrations [10]. Owing to the growing dominance of urban
56 living and the potential dangers posed, there is a pressing need to investigate the forms in which
57 these PHE exist in urban environments since these govern their mobility, transport, fate,
58 bioavailability and ultimately toxicity to humans [11].

59

60 One approach is to fractionate the total PHE content on the basis of lability by use of sequential
61 extraction. This employs a series of reagents to leach out elements associated with different
62 phases in a solid sample, these phases being operationally-defined. The reagents are applied in
63 order of increasing 'harshness' such that successive fractions correspond to element reservoirs
64 with lesser mobility. A variety of procedures and reagents have been reported [12-17].
65 However, a typical protocol begins with application of a mild extractant such as water or dilute
66 salt solution, progresses through various steps involving different combinations of reductants

67 and oxidants (often at elevated temperature), and concludes with digestion of residual material
68 in hot mineral acid.

69

70 Sequential extraction can provide valuable information when used appropriately [16]. The
71 approach has been applied successfully to urban soils [12, 13, 18-21]; street dusts [22-26]; and
72 airborne particle matter [26-31]. However, it has limitations. Numerous researchers have
73 reported that the reagents used do not target specific minerals [16, 32, 33], while potential re-
74 adsorption of released elements on residual solid during the process of leaching can
75 compromise the interpretation of analyte distribution in the sample [34-36]. A number of
76 workers [16, 33, 37, 38] have also pointed out the difficulties associated with the analysis of
77 the extractants and sample extracts because of, for example, their high salt content and complex
78 matrices.

79

80 In an attempt to address these problems, *Cave et al.* [37] proposed an extraction method which
81 uses a non-specific reagent, called “chemometric identification of substrates and element
82 distribution” (CISED). This methodology was based on earlier work [32, 33] where various
83 concentrations of nitric acid, sample to extractant ratios, and reaction times were studied, with
84 analysis of the resulting solutions for both major and trace elements. A chemometric self-
85 modelling mixture resolution (SMMR) procedure was then applied to the data generated to
86 determine the number of components present in the sample. The approach is based on the
87 assumption that environmental solids such as soil and dust consist of discrete physico-chemical
88 components, each having its own composition, and that extracting a sample using increasing
89 concentrations of acid will release into solution different proportions of these components. The
90 CISED procedure has the following merits:

- 91 i. The extracts have a simple matrix readily compatible with commonly-used analytical
92 techniques such as inductively coupled plasma mass spectrometry (ICP-MS)
- 93 ii. It is simple and rapid to carry out
- 94 iii. Re-absorption of the leached analytes is minimized due to the short contact time
95 between the sample and the reagent
- 96 iv. The results are not operationally defined and reflect the true physico-chemical
97 composition of the solid under investigation
- 98 v. The data processing helps to remove between-laboratory variability that may occur with
99 sequential extraction due to minor procedural differences.

100 It was more recently shown [38] that the use of aqua regia as the CISED extractant is
101 preferred to nitric acid because it dissolves Fe-bearing components more efficiently.

102
103 The CISED procedure has been applied to mining soils [39], soils enriched with As [40],
104 moderately contaminated agricultural soils [41] and river sediments [41, 42]. Despite interest
105 in developing methods to assess the mobility of PHE bound to respirable airborne particles [43-
106 45] little work has been carried out to apply the CISED approach in this context. The limited
107 mass of airborne particulate matter samples available – typically just a few tens of mg, usually
108 collected on a filter – is a key driver for miniaturization of sequential extraction techniques
109 and/or the development of flow through extraction methods based on sample extraction directly
110 from the collection filter [31].

111
112 The aim of this study was therefore to compare the physico-chemical partitioning of PHE in a
113 widely available and well-characterised certified reference material (BCR CRM 701) obtained
114 using a miniaturized version of the standard CISED method and a method in which the test
115 sample was supported on a TX40 filter (as used in FDMS continuous air monitoring systems)
116 mounted in a polycarbonate filter holder.

117
118

119 **2. Experimental**

120

121 *2.1 Apparatus*

122 Method 1: Whatman® “Vectaspin 20” polypropylene centrifuging tubes fitted with pigment-
123 free 0.2 µm pore size polypropylene filter inserts (Fisher Scientific, Leicestershire, UK) were
124 used to support the sample (Figure 1a). Method 2: 47 mm diameter TX40 filters (Air Monitors,
125 Gloucestershire, UK) loaded with sample were held firmly in a 47 mm in-line polycarbonate
126 filter holder (Pall Life Sciences, Portsmouth, UK) (Figure 1b). In Method 1, the centrifugation
127 of the extracts was performed using a ACL 4237 centrifuge (CAMLAB Ltd., Cambridge, UK).
128 In method 2 the extraction fluids were recovered using a 60 mL plastic hypodermic syringe
129 (Fisher Scientific, Leicestershire, UK). All extracts were analysed for Al, Ba, Ca, Cr, Cu, Fe,
130 K, Mg, Mn, Na, Ni, P, Pb, Si, Sr, Ti, V and Zn content using ICP-MS (Agilent 7700x ICP-MS
131 instrument fitted with an ASX-500 series autosampler, Agilent Technologies Ltd., Cheshire,
132 UK). All glassware and apparatus was soaked overnight in 5% (v/v) HNO₃ and washed
133 thoroughly with high purity water (HPW) before use.

134

135 2.2 *Reagents*

136 All solutions were prepared using HPW (18 MΩ cm) supplied from a Direct-Q 3 UV system
137 (Fisher Scientific, Loughborough, UK). Aqua regia was prepared by mixing extra-pure
138 hydrochloric and nitric acids (Sigma-Aldrich, Gillingham, UK), in a ratio 3:1 (v/v). Analytical
139 grade hydrogen peroxide (30%) solution (VWR, Leicestershire, England, UK) was used as
140 supplied.

141

142 2.3 *Test material*

143 A certified reference material (CRM), BCR CRM 701, was used as the test sample in this study.
144 This sediment from Lake Orta (Piemonte, Italy) was preferred to a CRM of urban particulates
145 such as NIST SRM 1648a for use in method development because of the known information
146 on the relative labilities of elements and possible inter-element associations. The material is
147 certified for the extractable contents of Cd, Cr, Cu, Ni, Pb and Zn using the modified BCR
148 sequential extraction procedure, and has indicative values for the pseudo-total contents of these
149 PHE. Their presence in the CRM is a consequence of Cu and ammonium sulfate discharge to
150 the lake over several decades, augmented by Cu, Cr, Ni and Zn release from nearby plating
151 factories in the 1960s and 1970s [46].

152

153 2.4 *CISED extraction*

154 *Method 1*

155 The reagents used for the CISED extraction are shown in Table 1. About 0.1 g of the test sample
156 was accurately weighed and placed into a clean Whatman® “Vectaspin 20” tube. The
157 extraction was performed by pipetting a 3 mL aliquot of extractant into the vessel,
158 centrifugation for 10 minutes at 1034g and collection of the extracts for analysis (the sample
159 mass and extractant volume used are smaller than the conventional CISED method, which uses
160 10 mL aliquots of extractant and 2.0 g test portions). This was repeated until a total of 14
161 extracts had been created, as extraction with each reagent shown in Table 1 was carried out
162 twice. The procedure was performed in duplicate together with a blank extraction.

163

164 *Method 2*

165 In this method the CISED extraction was performed on test sample supported on a 47 mm
166 TX40 FDMS filter. About 0.1 g of the test sample was weighed accurately and smeared as
167 evenly as possible using a plastic spatula onto the filter. It was then placed in a 47 mm in-line

168 polycarbonate filter holder, taking care to avoid any loss of sample. The extractants used and
169 the sequence were the same as in Method 1. The extraction was performed by pipetting 3 mL
170 of the extractant onto the sample through the inlet vent on the top of the holder. The vent was
171 stoppered and the filter holder held flat and shaken gently in a circular motion for 10 minutes.
172 The solution was then forced through the filter using a 60 mL plastic hypodermic syringe and
173 the filtrate collected for analysis. The procedure was performed in duplicate together with a
174 blank extraction.

175

176 2.5 ICP-MS analysis

177 The ICP-MS was operated at 1550 W and 27 MHz in collision cell mode using He as the cell
178 gas (flow rate 4.3 mL/ min) to give a $^{140}\text{Ce}^{16}\text{O}:^{140}\text{Ce}$ ratio of $\leq 0.07\%$. The plasma, auxiliary
179 and nebuliser (carrier) gas flows were 15, 0.9 and 1.0 L/min, respectively. Daily performance
180 checks were carried out using a 10 $\mu\text{g/L}$ solution of Ba, Ce, Co, In, Li, Ni and Pb. Calibration
181 was with respect to solutions freshly-prepared by serial dilution of Agilent Technologies
182 multi-element standard solution 2A. The nuclides quantified were: ^{27}Al , ^{137}Ba , ^{40}Ca , ^{52}Cr ,
183 ^{53}Cr , ^{63}Cu , ^{65}Cu , ^{56}Fe , ^{57}Fe , ^{39}K , ^{24}Mg , ^{55}Mn , ^{23}Na , ^{60}Ni , ^{31}P , ^{206}Pb , ^{207}Pb , ^{208}Pb , ^{28}Si , ^{29}Si ,
184 ^{88}Sr , ^{45}Ti , ^{51}V , ^{64}Zn , and ^{66}Zn . The internal standard was ^{115}In . One of the calibration
185 standards was re-analysed every 8 samples, and again at the end of the sample run, to check
186 for instrumental drift.

187

188 2.6 Chemometric processing of the CISED extraction data

189 The 14 extracts obtained from each replicate in both methods were analysed by ICP-MS. The
190 extraction data obtained from the two methods (including the blank extractions with no sample
191 present) were combined to give one large data set for further data processing. Previous work
192 [36] comparing different extractants generated different numbers of components of similar
193 composition. The outputs required further data manipulation to understand the importance of
194 the acid extractant used and the distribution of PHE. In the current work, the data sets from
195 the two methods were combined so that a consistent set of components was generated without
196 the need for further data manipulation.

197

198 The combined data set was processed using a chemometric data processing method that has
199 been described in detail by Cave et al. [31, 35]. This resolves chemical extraction data into

200 components, defining the number of components and their composition. The data processing
201 generates:

- 202 i. a series of profile plots or an ‘extractogram’ of each of the identified components
203 showing where within the extraction timescale the component was removed;
- 204 ii. a bar chart showing the percentage elemental composition of each component; the
205 components are named using the elements with a percentage composition > 10%
206 therein (in decreasing order of their contribution).

207 The modelling algorithm also incorporates a bootstrap resampling routine. Briefly, this
208 involves calculating the difference between the SMMR modelled data and the original data
209 from the analysis of the extracts (the residuals). The residuals are resampled with replacement
210 and added to the original data set producing a new original data set which is taken through the
211 modelling process. This resampling of the residuals and adding to the original data is repeated
212 1000 times which produces 1000 modelling outputs of the extractograms and component
213 element compositions. Taking the median value and the 2.5th and 97.5th percentile produces a
214 median value with a 95th percentile uncertainty limits. These uncertainties have been used in
215 the plots shown in Figures 5-7.

216

217

218 **3. Results and discussion**

219

220 *3.1 Identification of the components*

221 When the CISED SMMR algorithm was applied to the combined data set from the centrifuge
222 method and the flow through method (including the blank extractions) nine distinct
223 components were identified. Figures 2-4 show the ‘extractograms’ obtained.

224

225 Two of the components originated from the filter media used in the flow through method. This
226 is shown Figure 2, which highlights the marked difference between the blank extraction
227 profiles obtained using the normal centrifuge method (N) and the flow through method (F).
228 The presence of a substantial PHE blank in TX40 FDMS filters has previously been reported
229 [47, 31]. As these “blank” components can be identified separately, they were able to be
230 excluded from further data processing. This is an advantage of the SMMR data processing
231 procedure as it has identified the blank contribution from the filter within the sample being
232 analysed and does not require the subtraction of the data from a blank filter extraction run. This

233 is particularly important when the blank content of the filter is variable and a separate blank
234 run may not be an accurate reflection of the filter contribution to the sample run. Figures 3 and
235 4 show the extraction profiles for the seven remaining geochemical components identified in
236 the BCR CRM 701 sample using the centrifugation (Figure 3) and flow through (Figure 4)
237 methods. In each case S1 and S2 are the two replicate extractions and B1 is the blank extraction.
238 Visual inspection indicates that the repeatability of the centrifuge method was better than the
239 flow through method (as shown by replicate extraction profiles S1 and S2) whilst the shapes
240 of the extraction profiles were broadly similar for the two methods.

241

242 Figure 5 shows the chemical composition of each of the seven geochemical components (with
243 elements that make up more than 1% of the total). The components are: Al.Fe.P; Ca.P.Mg; Fe;
244 Fe.Al; Fe.K.Si; Mg.Ca and Si.Na. As discussed below, they can be categorised into four broad
245 categories: carbonate, Al/Fe oxides/hydroxide, Fe dominated and Si dominated.

246

247 *Carbonates*

248 There are two components dominated by Ca and Mg: Ca.P.Mg (ca. Ca 30%, Mg 20%) and
249 Mg.Ca (ca. Mg 50%, Ca 25%). Both of these were extracted at low acid concentration
250 (Ca.P.Mg in extraction steps 1-5 and Mg.Ca in extraction steps 4-7). The composition of these
251 components, together with their extraction in the early stages of the methods indicates that they
252 originate from dissolution of calcareous material. This may be indigenous to the watershed of
253 Lake Orta [48, 49] or perhaps reflect liming that took place in 1989-1990 and involved the
254 addition of >10,000 tons of finely powdered natural limestone to restore the lake's alkalinity
255 [46, 50, 51].

256

257 *Al/Fe oxides/hydroxides*

258 The Al.Fe.P component was extracted over a wide range of acid concentrations (extracts 2 to
259 12) and is probably a mixed Al/Fe oxide/hydroxide [48, 52]. These are known to be an
260 important sink for phosphates in sediments [53] and this would account for the relatively high
261 concentration of P present (ca. 20%).

262

263 *Fe dominated*

264 Three Fe dominated components were observed and likely to be related to sedimentary material
265 enriched in Fe and Al [48].

266

267 The Fe component has a composition of 75% Fe and is probably a relatively pure iron oxide.
268 It has two windows of extraction (3-7 and 7-14). The earlier window of extraction occurs in
269 the same region as the Ca.P.Mg carbonate extraction peak suggesting that some Fe oxides are
270 trapped within the carbonate matrix and released as the carbonate is dissolved. The extraction
271 peaks occurring later in the extraction (extracts 9-14) are associated with the clay and Fe/Al
272 silicates, released at high acid concentrations.

273

274 The Fe.Al component is ca 60% Fe, 15% Al and 10% Si with smaller amounts of Mg, P Ti and
275 Cr and is extracted at high acid concentrations (extracts 5-14). This probably represents the
276 alumino-silicate matrix of the sediment.

277

278 In the Fe.K.Si component, although the median Fe value is ca. 40% it has a large uncertainty
279 showing that the Fe concentration is not well defined. The K content is more clearly defined at
280 ca. 20%. A high K content and extraction at higher acid concentrations suggests that this could
281 represent the clay fraction within the sediment.

282

283 *Si dominated*

284 Figure 5 shows the Si.Na component to be ca. 50 % Si with a less well defined Na content. It
285 has a relatively wide extraction window (ca. extracts 2-11). In lakes, biogenic Si often
286 constitutes a significant part of the sediment [54]. Suggesting that this component is biogenic
287 Si that mainly comes from diatom frustules.

288

289 3.2 *Fractionation of the metals*

290 Figure 6 compares the fractionation of Cr, Cu, Ni, Pb and Zn obtained with the CISED method
291 and with the BCR method. The BCR data were obtained from the BCR CRM 701 certificate.
292 Tentative assignments of the geochemical source of the CISED components are given above;
293 the reagents used and nominal geochemical targets [55] for the steps of the BCR extraction are
294 below:

- 295 • Step 1 (exchangeable/acid-extractable fraction) - Acetic acid extraction to recover the
296 fraction that is mobile, soluble in water or weak acids, or adsorbed to carbonates;
- 297 • Step 2 (reducible fraction) - Hydroxylammonium chloride extraction to recover the
298 fraction bound to iron and manganese oxides/hydroxides.

- 299 • Step 3 (oxidisable fraction) - Hydrogen peroxide followed by ammonium acetate to
300 recover the fraction bound to organic matter and sulfides.
- 301 • Step 4 (residual fraction) - Aqua regia pseudo-total digestion to provide information on
302 how much of the analyte is bound to the more resistant components of the mineral
303 matrix (also used for quality control purposes to allow mass balance with respect to
304 results of pseudo-total digestion of a separate test portion of the same material [56]).

305

306 Whilst the BCR order of extraction is defined by the method, the CISED order of extraction of
307 the different components is derived from the extraction profiles (Figures 3 and 4). These have
308 been ordered by the extraction step at which 75% of the total mass was extracted, giving an
309 approximate order of their relative solubility in the increasing acid concentration in the
310 extraction solutions.

311

312 *Chromium*

313 Figure 6a shows Cr is almost exclusively associated with the Fe oxide component and the Fe
314 aluminosilicate components identified by the CISED, which are only dissolved at higher acid
315 concentration. In contrast, the BCR extraction suggests most of the Cr is associated with
316 organic matter and sulfides (the nominal target of step 3) with smaller amounts associated with
317 more refractory components (step 4) and iron and manganese oxyhydroxides (step 2). This
318 difference probably arises from a combination of two factors. First, as already emphasised, the
319 BCR extraction is operationally-defined and was not originally proposed with the intention that
320 it be used to determine geochemical phase associations [16, 55]. Second, the CISED has
321 difficulty distinguishing PHE bound to organic matter (since C cannot be quantified by ICP-
322 MS it is not amongst the analytes whose concentration is measured and fed into the algorithm).

323

324 *Copper*

325 According to the CISED, Cu is mainly associated with the mixed Al/Fe oxide/hydroxide
326 (Al.Fe.P) component and, to a lesser extent, with the carbonate (Ca.P.Mg) and the Fe oxide
327 components (Figure 6b). This is in reasonable agreement with the BCR method where most of
328 the Cu was released in step 2, which nominally targets PHE bound to iron and manganese
329 oxyhydroxides, with a smaller amount associated with the carbonate fraction released in step
330 1. However, the BCR also shows a significant portion of Cu associated with step 3 and 4, the
331 oxidisable and residual fractions, which is not indicated by the CISED data interpretation.

332 Again, this may reflect the non-specific nature of the BCR procedure and that the CISED has
333 difficulty distinguishing PHE bound to organic matter.

334

335 *Nickel*

336 Of the five PHE investigated, Ni (Figure 6c) is the analyte most widely distributed across the
337 identified components of the CISED. It is found mainly in association with the Ca.P.Mg,
338 Al.Fe.P, Si.Na, Fe.K.Si and Fe components. This is also reflected in the results of the BCR
339 extraction where considerable amounts of Ni were released in all four steps of the procedure.

340

341 *Lead*

342 The CISED shows that Pb is predominantly associated with the mixed Al/Fe oxyhydroxide
343 component (Figure 6d). This agrees well with the BCR extraction method where Pb is
344 predominantly associated with step 2, the nominal target of which is iron and manganese
345 oxyhydroxides.

346

347 *Zinc*

348 The CISED shows Zn to be predominantly associated with the Ca.P.Mg carbonate component
349 with smaller amounts associated with the Si.Na and the Fe components. Similarly, the BCR
350 extraction released a large proportion of Zn in step 1, which targets species that are mobile,
351 soluble in water or weak acids, or adsorbed to carbonates. However, the BCR method also
352 found substantial amounts of Zn in steps 2-4, which is not in agreement with the CISED. This
353 may indicate re-distribution of the Zn released in step 1 to other components in the sediment
354 during the BCR extraction process.

355

356 Figure 7 compares the total amounts of the five elements extracted by the CISED and BCR
357 methods, either including (Figure 7a) or excluding (Figure 7b) the aqua-regia pseudo-total
358 digestion (step 4) in the BCR method. With step 4 included, the BCR procedure extracts more
359 than the CISED method, except for Pb. When, however, the aqua-regia step is not included
360 there is agreement in the amount extracted for Cu, Pb and Zn. This suggests that the CISED
361 method has very similar overall extraction capability to the BCR method except for elements
362 more strongly associated with refractory minerals that can only be solubilised by hot mineral
363 acid digestion (Cr and Ni in this case).

364

365 **4. Conclusions**

366 The centrifugation and the quasi-flow through methods gave comparable results despite quite
367 different approaches and suggest that use of a flow through method, as required for application
368 to samples of urban airborne PM collected during routine air quality monitoring, would be
369 feasible. The CISED data processing identified seven geochemical components in BCR CRM
370 701: two carbonate components; an Al/Fe oxides/hydroxide component; three Fe dominated
371 components and one Si dominated component. Two additional components were identified as
372 originating from the TX40 FDMS filter used to support the sample in the flow through method.
373 The CISED thus provides an efficient way of dealing with blank correction.

374

375 The overall extraction capability of the CISED and BCR methods were similar provided step
376 4 of the BCR sequential extraction (aqua regia digestion to obtain the residual fraction) was
377 excluded. However, whilst the BCR method is operationally defined, the CISED provides
378 information about the geochemical source(s) of the analytes in the sample (although PHE
379 bound to organic matter can be difficult to distinguish). In terms of fractionation, agreement
380 between the CISED method and the BCR sequential extraction was good for some analytes
381 (e.g. Ni and Pb) but less good for others (e.g. Cr and Cu). Marked contrast between the
382 extraction of Zn predominantly at low acid concentration in the early stages of the CISED, but
383 across all steps of the BCR method, highlights the re-adsorption effects that can occur with use
384 of sequential extraction and confirms that such procedures are not well-optimised to determine
385 PHE geochemical associations [16].

386

387 In terms of practicalities, the CISED is simpler to carry out than the BCR method. It is more
388 rapid (can be completed in ca. three hours in contrast to the three 16-hour shaking periods
389 required in the BCR protocol) and produces solutions with simpler matrix composition more
390 compatible with ICP-MS.

391

392 Although the CISED procedure does not incorporate simulated biological fluids, and so does
393 not accurately mimic dissolution processes within the human body, it does provide an
394 indication of the physico-chemical components in the test material and how PHE are distributed
395 between them, from which information on relative lability, mobility and bioavailability can be
396 inferred. There are also indications that the output of the CISED can be related to results of the
397 physiologically relevant Unified BARGE Method [57]. Application of the flow through CISED
398 procedure to samples collected during air quality monitoring programmes worldwide could
399 thus provide valuable information on the binding and potential mobility of PHE associated with

400 respirable PM, and hence contribute towards greater understanding of the exposure of urban
401 populations to airborne pollutants.

402

403 **CRedit authorship contribution statement**

404 **Balarabe S Sagagi:** conceptualisation, investigation, funding acquisition, writing – review &
405 editing. **Christine M Davidson:** supervision, resources, writing – original draft. **Mark R**
406 **Cave:** formal analysis, visualisation, writing – review & editing. **Joanna Wragg:**
407 investigation. verification, writing – review & editing

408

409 **Declaration of competing interest**

410 The authors declare no conflicts of interest.

411

412 **Acknowledgements**

413 BSS wishes to thank the Tertiary Education Trust Fund, Nigeria, for award of a PhD
414 scholarship to allow him to study in the UK.

415

416 **References**

417

- 418 1. World Urbanisation Prospects: the 2018 Revision, United Nations Department of Economic
419 and Social Affairs, New York, 2019.
420 <https://population.un.org/wup/Publications/Files/WUP2018-Report.pdf>
- 421 2. B. Grobéty, R. Gleré, V. Dietze, P. Stille, Airborne particles in the urban environment,
422 *Elements*. 6 (2010) 229-234. **DOI:** 10.2113/gselements.6.4.229
- 423 3. C.A. Pope, R.T. Burnett, G.D. Thurston, M.J. Thun, E.E. Calle, D. Krewski, J.J. Godleski,
424 Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological
425 evidence of general pathophysiological pathways of disease, *Circulation*. 109 (2004) 71-77.
426 **DOI:** 10.1161/01.CIR.0000108927.80044.7F
- 427 4. Y.I. Chirino, Y. Sanchez-Perez, A.R. Orsonio, I. Rosas, C.M. Garcia-Ceuellar, Sampling and
428 compositions of airborne particulate matter (PM₁₀) from two locations of Mexico City, *Data in*
429 *Brief*. 4 (2015) 353-356. **DOI:** 10.1016/j.dib.2015.06.017
- 430 5. J. Zheng, M.G. Tan, Y. Shibata, A. Tanaka, Y. Li, G.L Zhang, Y.M. Zhang, Z. Shan,
431 Characteristics of lead isotope ratios and elemental concentrations in PM₁₀ fraction of airborne
432 particulate matter in Shanghai after the phase-out of leaded gasoline, *Atmospheric*
433 *International*. 38 (2004) 1191-1200. **DOI:** 10.1016/j.atmosenv.2003.11.004
- 434 6. J.J. Schauer, W.F. Rogge, L.M. Hildemann, M.A. Mazurek, G.R. Cass, B.R.T. Simoneit,
435 Source apportionment of airborne particulate matter using organic compounds as tracers,
436 *Atmos. Environ*. 30 (1996) 3837-3855. **DOI:** 10.1016/1352-2310(96)00085-4
- 437 7. B.J. Alloway, *Heavy Metals in Soils*, second ed., Blackie Academic and Professional, London,
438 1995.
- 439 8. A. Kabata-Pendias and H. Pendias, *Trace Elements in Soils and Plants*, third ed., CRC Press,
440 London, 2001.
- 441 9. C.S.C. Wong, X.D. Li, I. Thornton, Urban environmental geochemistry of trace metals,
442 *Environ. Pollut*. 142 (2006) 1-16. **DOI:** 10.1016/j.envpol.2005.09.004
- 443 10. S. Davydova, Heavy metals as toxicants in big cities, *Microchem. J*. 79 (2005) 133-136.
444 **DOI:** 10.1016/j.microc.2004.06.010
- 445 11. A.M. Ure and C.M. Davidson, eds. *Chemical Speciation in the Environment.*, second ed.
446 Blackwell Science, London, 2002.
- 447 12. C.M. Davidson, G.J. Urquhart, F. Ajmone-Marsan, M. Biasioli, A.D. Duarte, E. Diaz-
448 Barrientos, H. Grcman, L. Hossack, A.S. Hursthouse, L. Madrid, S. Rodrigues, M. Zupan,
449 Fractionation of potentially toxic elements in urban soils from five European cities by means
450 of a harmonised sequential extraction procedure, *Anal. Chim. Acta*. 565 (2006) 63-72.
451 **DOI:** 10.1016/j.aca.2006.02.014

- 452 13. M. Imperato, P Adamo, D. Naimo, M. Arienzo, D. Stanzione, P. Violante, Spatial distribution
453 of heavy metals in urban soils of Naples city (Italy), *Environ. Pollut.* 124 (2003) 247-256.
454 **DOI:** 10.1016/S0269-7491(02)00478-5
- 455 14. P. Adamo, D. Agrellie, M. Zampella, Chemical speciation to assess bioavailability,
456 bioaccessibility and geochemical forms of potentially toxic metals (PTMs) in polluted soils in:
457 B. de Vivo, H.E. Belkin, A. Lima (eds) *Environmental Geochemistry: Site characterisation,*
458 *data analysis and case histories.* Elsevier, Amsterdam, 2008, pp. 175-212.
- 459 15. C.R.M. Rao, A. Sahuquillo, J.F. Lopez-Sanchez, A review of the different methods applied
460 in environmental geochemistry for single and sequential extraction of trace elements in soils
461 and related materials, *Water Air Soil Poll.* 189 (2008) 291-333. **DOI:** 10.1007/s11270-007-
462 9564-0
- 463 16. J.R. Bacon, C.M. Davidson, Is there a future for sequential chemical extraction? *Analyst.* 133
464 (2008) 25-46. **DOI:** 10.1039/b711896a
- 465 17. M. Horváth, V. Boková, G. Heltai, K. Flórián, I. Fekete, Study of application of BCR sequential
466 extraction procedure for fractionation of heavy metal content of soils, sediments, and
467 gravitation dusts, *Toxicol. Environ. Chem.* 92 (2010) 429-441. **DOI:**
468 10.1080/02772240903036147
- 469 18. X.P. Li, L.N. Feng, Geostatistical analyses and fractionation of heavy metals in urban soil from
470 industrial district in Weinan, NW China, *Environ. Earth Sci.* 67 (2012) 2129-2140.
471 **DOI:** 10.1007/s12665-012-1653-8
- 472 19. Y. Lu, F. Zhu, J. Chen, H.H. Gan, Y.B. Guo, Chemical fractionation of heavy metals in urban
473 soils of Guangzhou, China, *Environ. Monit. Assess.* 134 (2007) 429-439.
474 **DOI:** 10.1007/s10661-007-9634-1
- 475 20. C. Waterlot, G. Bidar, A. Pelfrène, H. Roussel, H. Fourier, F. Douay, Contamination,
476 fractionation and availability of metals in urban soils in the vicinity of former lead and zinc
477 smelters, France, *Pedosphere.* 23 (2013) 143-159. **DOI:** 10.1016/S1002-0160(13)60002-8
- 478 21. X.S. Luo, S. Yu, X.D. Li, The mobility, bioavailability, and human bioaccessibility of trace
479 metals in urban soils of Hong Kong, *Appl. Geochem.* 27 (2012) 995-1004.
480 **DOI:** 10.1016/j.apgeochem.2011.07.001
- 481 22. H.M. Li, X. Qian, W. Hu, Y.L. Wang, H.L. Gao, Chemical speciation and human health risk of
482 trace metals in urban street dusts from a metropolitan city, Nanjing, SE China, *Sci. Total*
483 *Environ.* 456 (2013) 212-221. **DOI:** 10.1016/j.scitotenv.2013.03.094
- 484 23. N. Ozcan, H. Altundag, Speciation of heavy metals in street dust samples from Sakarya I.
485 organized industrial district using the BCR sequential extraction procedure by ICP-OES, *B.*
486 *Chem. Soc. Ethiopia.* 27 (2013) 205-212. **DOI:** 10.4314/bcse.v27i2.5

- 487 24. T.T.T. Duong, B.K. Lee, Partitioning and mobility behavior of metals in road dusts from
488 national-scale industrial areas in Korea, *Atmos. Environ.* 43 (2009) 3502-3509.
489 **DOI:** 10.1016/j.atmosenv.2009.04.036
- 490 25. A.D.K. Banerjee, Heavy metal levels and solid phase speciation in street dusts of Delhi, India,
491 *Environ. Pollut.* 123 (2003) 95-105. **DOI:** 10.1016/S0269-7491(02)00337-8
- 492 26. J. Sysalová, J. Száková, Mobility of important toxic analytes in urban dust and simulated air
493 filters determined by sequential extraction and GFAAS/ICP-OES methods, *Chem. Pap.* 61
494 (2007) 271-275. **DOI:** 10.2478/s11696-007-0032-5
- 495 27. E. Dabek-Zlotorzynska, M. Kelly, H.D. Chen, C.L. Chakrabarti, Application of capillary
496 electrophoresis combined with a modified BCR sequential extraction for estimating of
497 distribution of selected trace metals in PM_{2.5} fractions of urban airborne particulate matter,
498 *Chemosphere.* 58 (2005) 1365-1376. **DOI:** 10.1016/j.chemosphere.2004.09.082
- 499 28. Y.B. Zhai, X.T. Liu, H.M. Chen, B.B. Xu, L. Zhu, C.T. Li and G.M. Zeng, Source identification
500 and potential ecological risk assessment of heavy metals in PM_{2.5} from Changsha, *Sci. Total*
501 *Environ.* 493, (2014) 109-115. **DOI:** 10.1016/j.scitotenv.2014.05.106
- 502 29. Y.Y. Sun, X. Hu, J.C. Wu, H.Z. Lian and Y.J. Chen, Fractionation and health risks of
503 atmospheric particle-bound As and heavy metals in summer and winter, *Sci. Total Environ.*
504 493, (2014) 487-494. **DOI:** 10.1016/j.scitotenv.2014.06.017
- 505 30. P. Sipos, C. Choi and Z. May, Combination of single and sequential chemical extractions to
506 study the mobility and host phases of potentially toxic elements in airborne particulate matter,
507 *Chem. Erde–Geochem.* 76 (2016) 481-489. **DOI:** 10.1016/j.chemer.2016.08.005
- 508 31. B. S. Sagagi, C.M. Davidson, A. S. Hursthouse, Adaptation of the BCR sequential extraction
509 procedure for fractionation of potentially toxic elements in airborne particulate matter
510 collected during routine air quality monitoring, *Int. J. Environ. An. Ch.* Early access
511 **DOI:** 10.1080/03067319.2019.1674847
- 512 32. M.R. Cave, K. Harmon, Determination of trace metal distributions in the iron oxide phases of
513 red bed sandstones by chemometric analysis of whole rock and selective leachate data, *Analyst.*
514 122 (1997) 501-512. **DOI:** 10.1039/a607953i
- 515 33. M.R. Cave, J. Wragg, Measurement of trace element distributions in soils and sediments using
516 sequential leach data and a non-specific extraction system with chemometric data processing,
517 *Analyst.* 122 (1997) 1211-1221. **DOI:** 10.1039/a705163h
- 518 34. J.L.G. Ariza, I. Giraldez, D. Sanchez-Rodas, E. Morales, Metal sequential extraction procedure
519 optimized for heavily polluted and iron oxide rich sediments, *Anal. Chim. Acta.* 414 (2000)
520 151-164. **DOI:** 10.1016/S0003-2670(00)00804-7
- 521 35. J.L. Gomez-Ariza, I. Giraldez, D. Sanchez-Rodas, E. Morales, Metal readsorption and
522 redistribution during the analytical fractionation of trace elements in oxic estuarine sediments,
523 *Anal. Chim. Acta.* 399 (1999) 295-307. **DOI:** 10.1016/S0003-2670(99)00460-2

- 524 36. M.D. Ho, G.J. Evans, Sequential extraction of metal contaminated soils with radiochemical
525 assessment of readsorption effects, *Environ. Sci. Technol.* 34 (2000) 1030-1035.
526 **DOI:** 10.1021/es981251z
- 527 37. M.R. Cave, A.E. Milodowski, E.N. Friel, Evaluation of a method for identification of host
528 physico-chemical phases for trace metals and measurement of their solid-phase partitioning in
529 soil samples by nitric acid extraction and chemometric mixture resolution, *Geochem-Explor.*
530 *Env. A.* 4 (2004) 71-86. **DOI:** 10.1144/1467-7873/03-025
- 531 38. J. Wragg, M. Cave, Assessment of a geochemical extraction procedure to determine the solid
532 phase fractionation and bioaccessibility of potentially harmful elements in soils: A case study
533 using the NIST 2710 reference soil, *Anal. Chim. Acta.* 722 (2012) 43-54.
534 **DOI:** 10.1016/j.aca.2012.02.008
- 535 39. B. Palumbo-Roe, B. Klinck, Bioaccessibility of arsenic in mine waste-contaminated soils: A
536 case study from an abandoned arsenic mine in SW England (UK), *J. Environ. Sci. Heal. A.* 42
537 (2007) 1251-1261. **DOI:** 10.1080/10934520701435692
- 538 40. B. Palumbo-Roe, M.R. Cave, B.A. Klinck, J. Wragg, H. Taylor, K. O'Donnell, R.A. Shaw,
539 Bioaccessibility of arsenic in soils developed over Jurassic ironstones in eastern England,
540 *Environ. Geochem. Hlth.* 27 (2005) 121-130. **DOI:** 10.1007/s10653-005-0128-1
- 541 41. R. Santamaria-Fernandez, M.R. Cave, S.J. Hill, The effect of humic acids on the sequential
542 extraction of metals in soils and sediments using ICP-AES and chemometric analysis, *J.*
543 *Environ. Monit.* 5 (2003) 929-934. **DOI:** 10.1039/b306865j
- 544 42. R. Santamaria-Fernandez, M.R. Cave, S.J. Hill, Trace metal distribution in the Arosa estuary
545 (NW Spain): The application of a recently developed sequential extraction procedure for metal
546 partitioning, *Anal. Chim. Acta.* 557 (2006) 344-352. **DOI:** 10.1016/j.aca.2005.10.029
- 547 43. A. Mukhtar, A. Limbeck, Recent developments in assessment of bio-accessible trace metal
548 fractions in airborne particulate matter: a review, *Anal. Chim. Acta.* 774 (2013) 11-25.
549 **DOI:** 10.1016/j.aca.2013.02.008
- 550 44. M. Guney, R.P. Chapuis, G.J. Zagury, Lung bioaccessibility of contaminants in particulate
551 matter of geological origin, *Environ. Sci. Pollut. R.* 23 (2016) 24422-24434.
552 **DOI:** 10.1007/s11356-016-6623-3
- 553 45. F. Kastury, E. Smith, A.L. Juhasz, A critical review of approaches and limitations of inhalation
554 bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust, *Sci.*
555 *Total Environ.* 574 (2017) 1054-1074. **DOI:** 10.1016/j.scitotenv.2016.09.056
- 556 46. M. Rogora, L. Kamburska, R. Mosello, G. Tartari, Lake Orta chemical status 25 years after
557 liming: problems solved and emerging critical issues. *J. Limnol.* 75 (2016) 93-106.
558 **DOI:** 10.4081/jlimnol.2016.1320
- 559

- 560 47. J.A.H. Alpofoead, C.M. Davidson, D. Littlejohn, Oral bioaccessibility tests to measure
561 potentially toxic elements in inhalable particulate matter collected during routine air quality
562 monitoring, *Anal Methods-UK*. 8 (2016) 5466-5474. **DOI:** 10.1039/c6ay01403h
- 563 48. R. Baudo, L. Amantini, F. Bo, R. Cenci, P. Hannaert, A. Lattanzio, G. Marrengo, H. Muntau,
564 Spatial distribution patterns of metals in the surface sediments of Lake Orta (Italy), *Sci. Total*
565 *Environ.* 87/88 (1989) 117-128. **DOI:** 10.1016/0048-9697(89)90229-5
- 566 49. R. Baudo, M. Beltrami, Chemical composition of Lake Orta sediments, *J. Limnol.* 60 (2001)
567 213-236. **DOI:** 10.4081/jlimnol.2001.1.213
- 568 50. A. Calderoni, R. Mosello, A. Quirci, Chemical response of Lake Orta (Northern Italy) to liming,
569 *Arch. Hydrobiol.* 122 (1991) 421-439.
- 570 51. D. A. L. Vignati, R. Bettinetti, A. Marchetto, Long-term persistence of sedimentary copper
571 contamination in Lake Orta: potential environmental risks 20 years after liming, *J. Limnol.* 75
572 (2016) 107-119. **DOI:** 10.4081/jlimnol.2016.1232
- 573 52. B.P. von der Heyden, M.G. Frith, S. Bernasek, T. Tylizszak, A.N. Roychoudhury, S.C.B.
574 Myneni, Geochemistry of Al and Fe in freshwater and coastal water colloids from the west
575 coast of Southern Africa, *Geochim. Cosmochim. Ac.* 241 (2018) 56-68.
576 **DOI:** 10.1016/j.gca.2018.08.043
- 577 53. K.C. Ruttenberg, Development of a sequential extraction method for different forms of
578 phosphorus in marine sediments, *Limnol. Oceanogr.* 37 (1992) 1460-1482.
579 **DOI:** 10.4319/lo.1992.37.7.1460
- 580 54. E.K. Peinerud, Interpretation of Si concentrations in lake sediments: three case studies,
581 *Environ. Geol.* 40 (2000) 64-72. **DOI:** 10.1007/PL00013330
- 582 55. A. Sahuquillo, J.F. Lopez-Sanchez, R. Rubio, G. Rauret, R.P. Thomas, C.M. Davidson and
583 A.M Ure, Use of a certified reference material for extractable trace metals to assess sources of
584 uncertainty in the BCR three-stage sequential extraction procedure, *Anal. Chim. Acta.* 382
585 (1999) 317-327. **DOI:** 10.1016/S0003-2670(98)00754-5
- 586 56. G.Rauret, J.F. Lopez-Sanchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure and P.
587 Quevauviller, Improvement of the BCR three step sequential extraction procedure prior to the
588 certification of new sediment and soil reference materials, *J. Environ. Monit.* 1 (1999) 57-61.
589 **DOI:** 10.1039/a807854h
- 590 57. J. Wragg, A. Broadway, M.R. Cave, F.M. Fordyce, B. Palumbo-Roe, D.J. Beriro, J.G.
591 Farmer, M.C. Graham, B.T. Ngwenya and R.J. Bewley, Linkage between solid-phase
592 apportionment and bioaccessible arsenic, chromium and lead in soil from Glasgow, Scotland,
593 UK, *Earth Env. Sci. T. R. So.* 108 (2019) 217-230. **DOI:** 10.1017/S1755691018000762

594

595

596 Table 1. Reagents used in the CISED method

Extraction number	Reagent(s)
1, 2	HPW
3, 4	aqua regia diluted 500 fold
5, 6	aqua regia diluted 100 fold
7, 8	97.5% aqua regia diluted 50 fold + 2.5% H ₂ O ₂
9, 10	95% aqua regia diluted 10 fold + 5% H ₂ O ₂
11, 12	92.5% aqua regia diluted 5 fold + 7.5% H ₂ O ₂
13, 14	90% aqua regia + 10% H ₂ O ₂

597 Concentrated aqua regia was used for extracts 13 and 14. This was diluted 5-fold for use in extracts
 598 11 and 12; 10-fold for extracts 9 and 10; 50-fold for extracts 7 and 8; 100-fold for extracts 5 and 6; and
 599 500-fold for extracts 3 and 4.

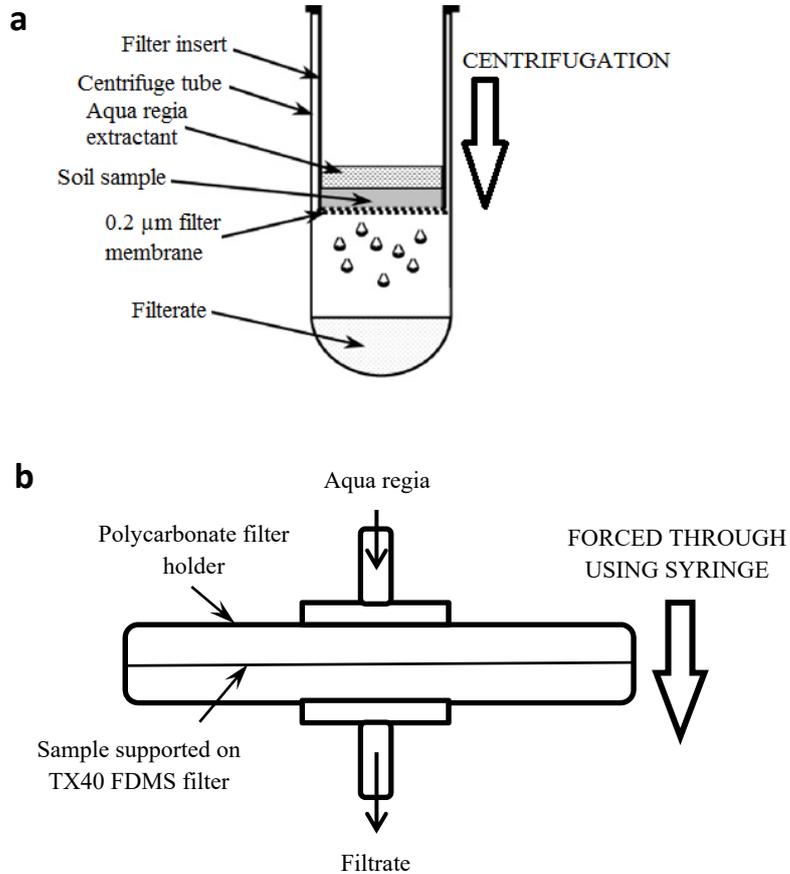
600
 601
 602
 603
 604
 605
 606
 607
 608
 609
 610
 611
 612
 613
 614
 615
 616
 617

618 List of Figures

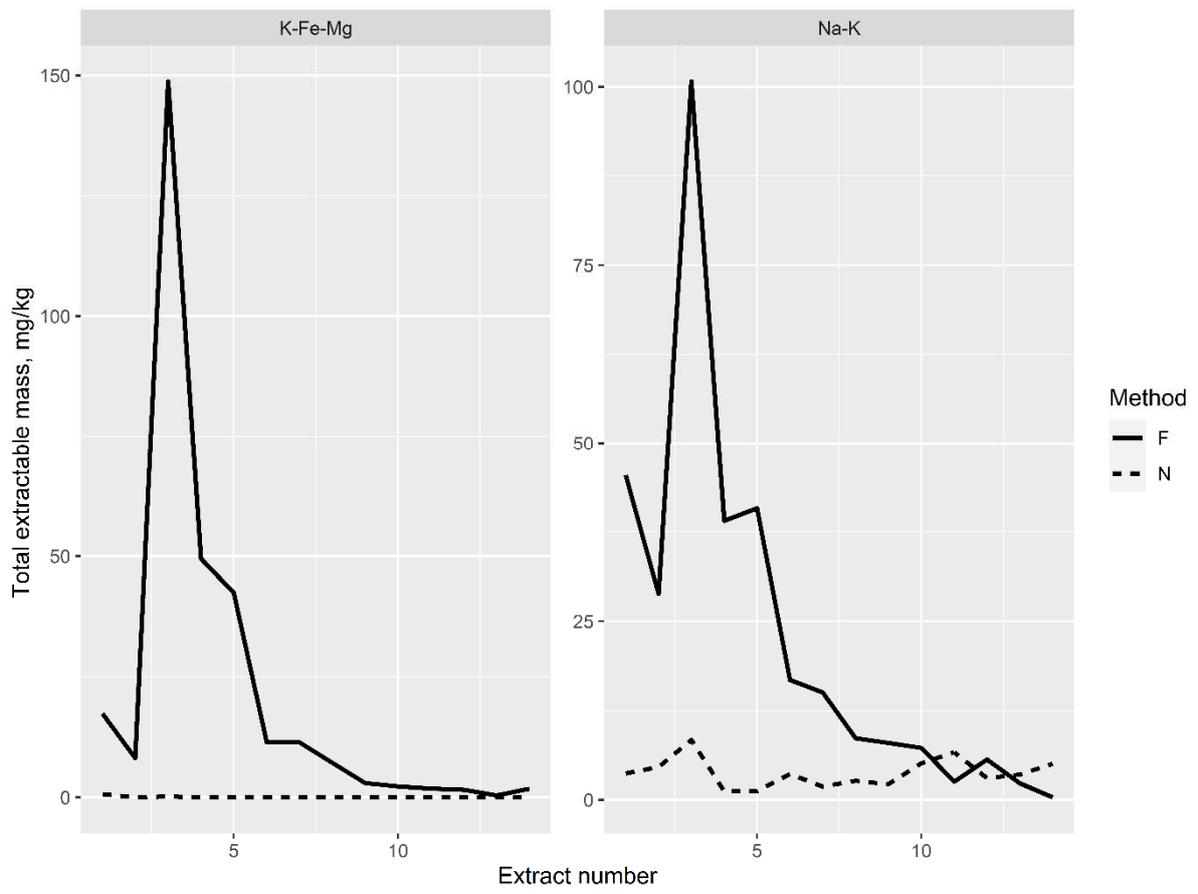
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644

- Figure 1 Schematic diagrams of (a) centrifuge tube extraction arrangement used in Method 1 (after [37]) and (b) in-line polycarbonate filter holder extraction arrangement used in Method 2.
- Figure 2 Extraction profile plots for the blank filters showing the chemical components derived from the filter used in the flow through method (F-flow through method, N-normal centrifuge method)
- Figure 3 Extraction profile plots for the centrifugation method (S1 and S2 are the two replicate extractions and Bl is the blank extraction)
- Figure 4 Extraction profile plots for the flow through method (S1 and S2 are the two replicate extractions and Bl is the blank extraction)
- Figure 5 Chemical composition of each of the seven components (with elements that make up more than 1% of the total)
- Figure 6 Comparison of analyte fractionation by the CISED and BCR extractions: (a) Cr (b) Cu (c) Ni (d) Pb (e) Zn
- Figure 7 Comparison between the total amounts of analytes extracted by the CISED and BCR methods (a) with and (b) without inclusion of the aqua-regia pseudo-total (step 4) in the BCR method.

645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665



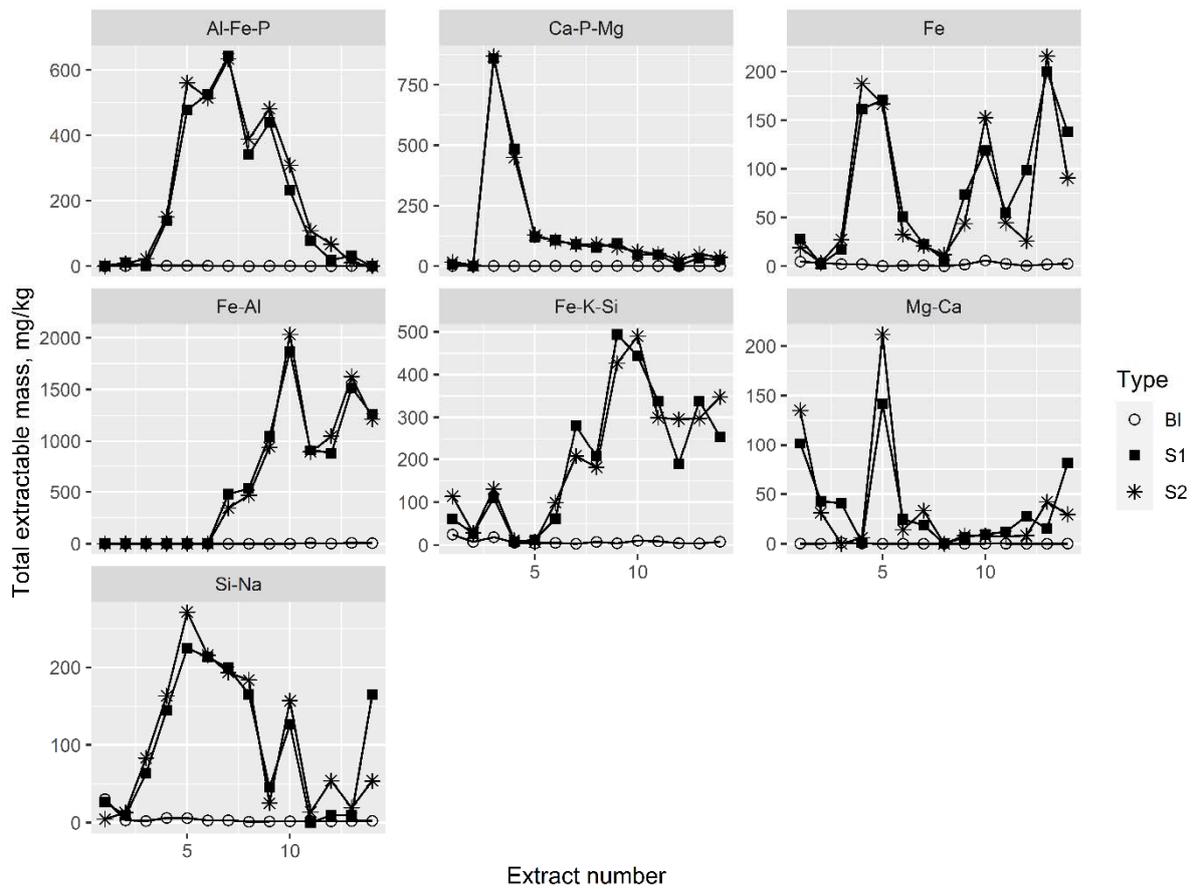
666



667

668

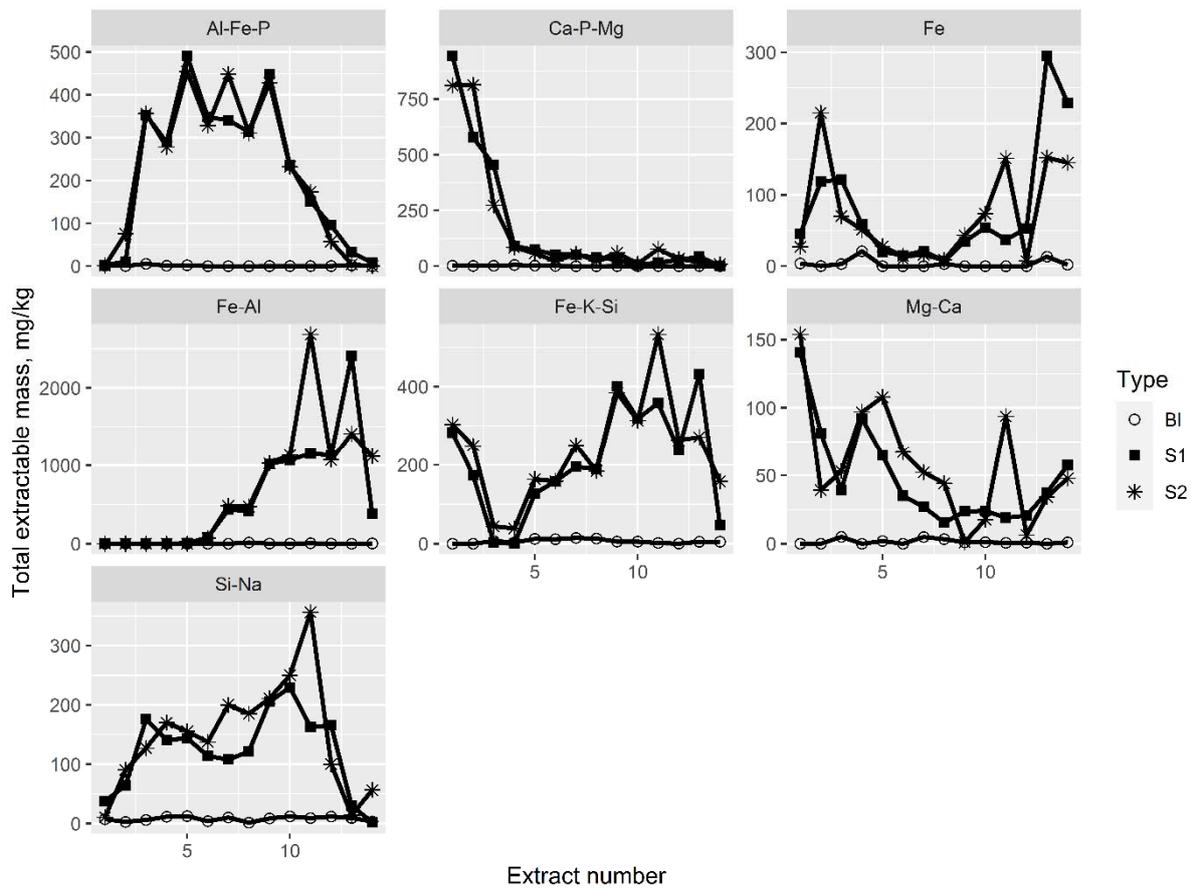
669



670

671

672

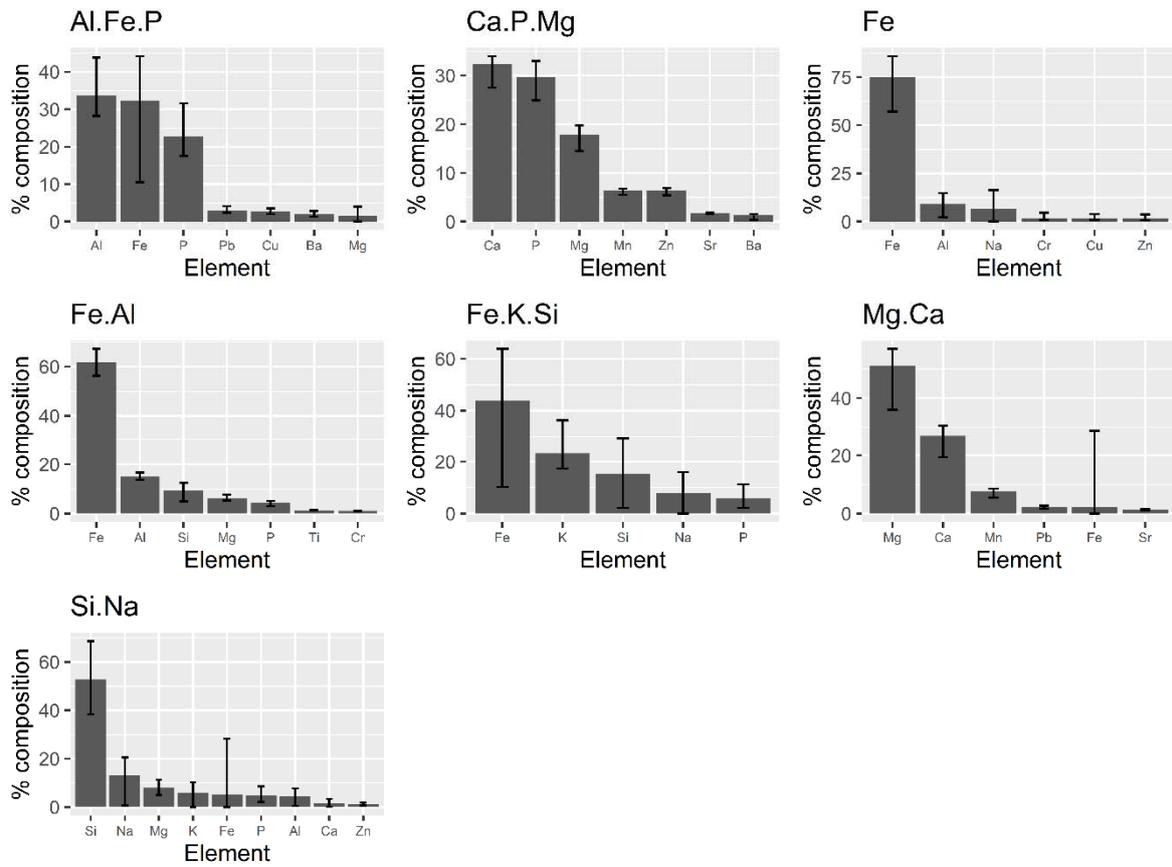


673

674

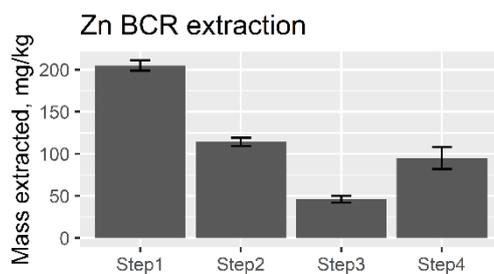
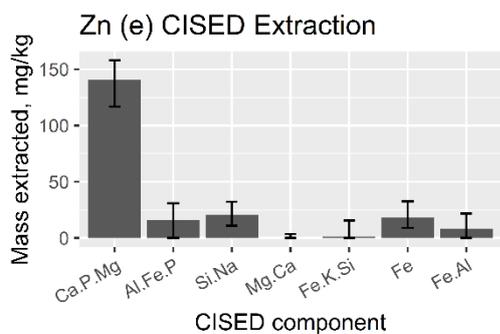
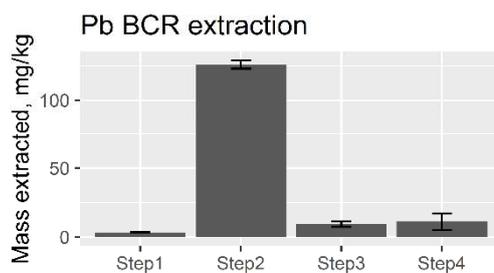
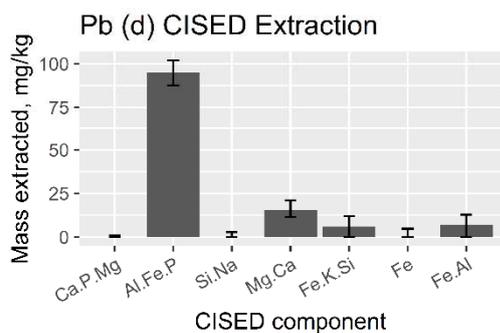
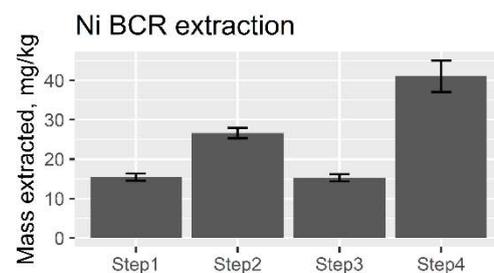
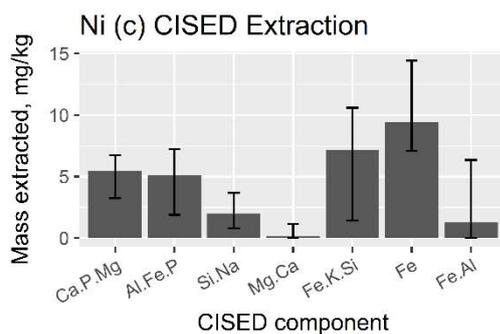
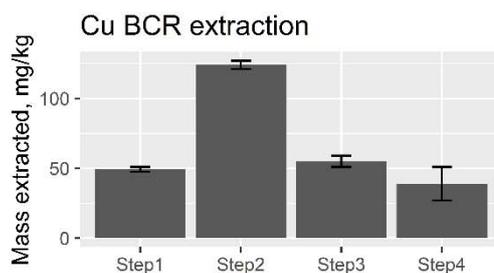
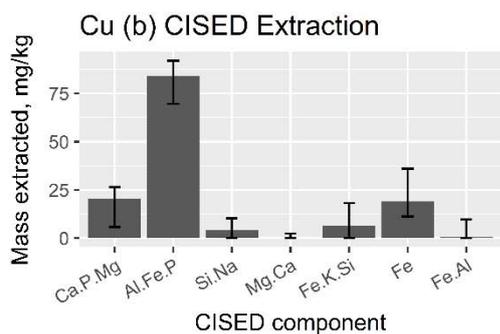
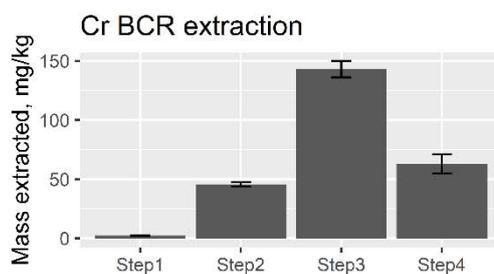
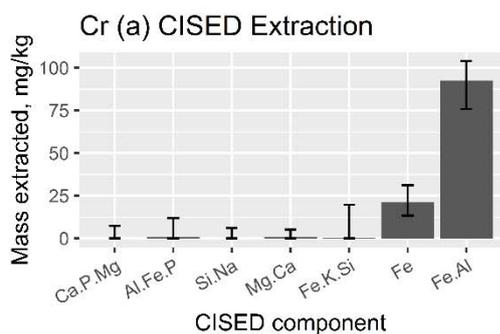
675

676



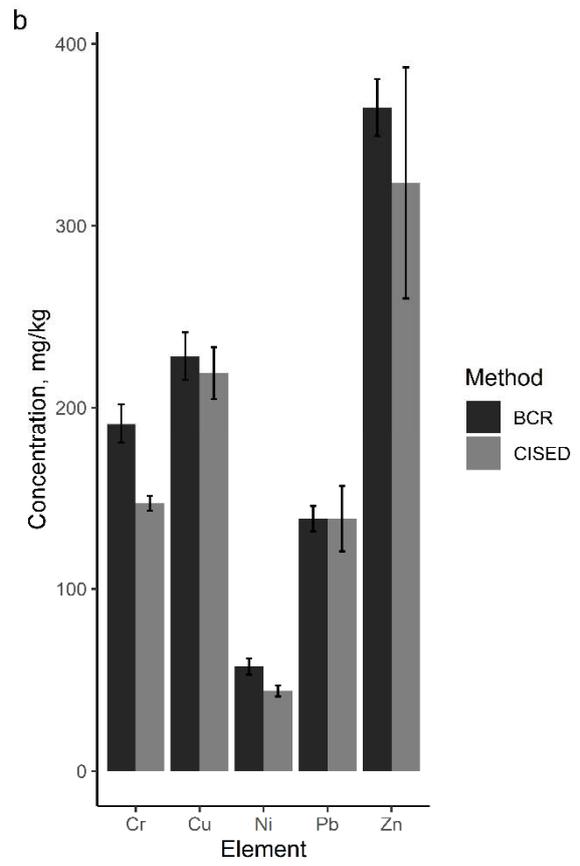
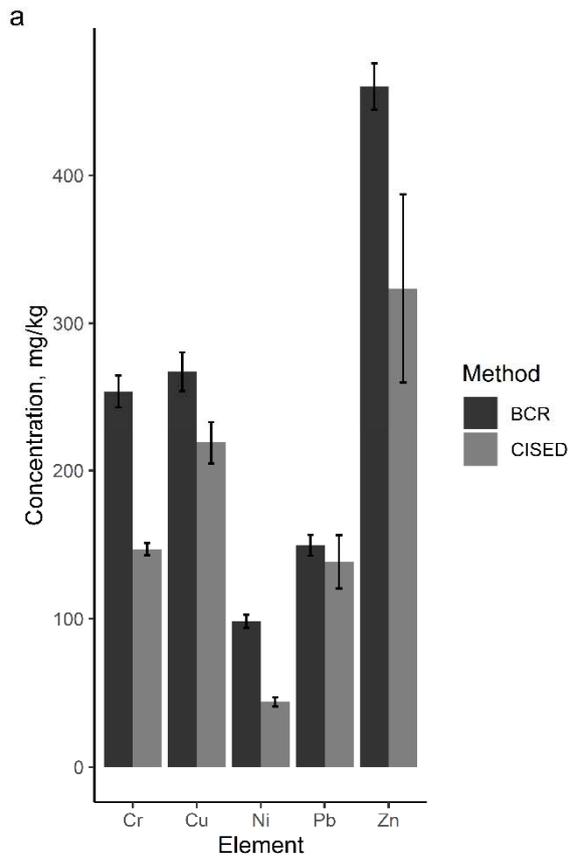
677

678



682

683



684

685