

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**

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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 ( $\Sigma$ 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.  
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29       matter; inductively coupled plasma mass spectrometry.  
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## 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<sub>2.5</sub>) 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<sub>2.5</sub> and  
48 PM<sub>10</sub>) 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.

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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) HNO<sub>3</sub> 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:  $^{27}\text{Al}$ ,  $^{137}\text{Ba}$ ,  $^{40}\text{Ca}$ ,  $^{52}\text{Cr}$ ,  
183  $^{53}\text{Cr}$ ,  $^{63}\text{Cu}$ ,  $^{65}\text{Cu}$ ,  $^{56}\text{Fe}$ ,  $^{57}\text{Fe}$ ,  $^{39}\text{K}$ ,  $^{24}\text{Mg}$ ,  $^{55}\text{Mn}$ ,  $^{23}\text{Na}$ ,  $^{60}\text{Ni}$ ,  $^{31}\text{P}$ ,  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ ,  $^{28}\text{Si}$ ,  $^{29}\text{Si}$ ,  
184  $^{88}\text{Sr}$ ,  $^{45}\text{Ti}$ ,  $^{51}\text{V}$ ,  $^{64}\text{Zn}$ , and  $^{66}\text{Zn}$ . The internal standard was  $^{115}\text{In}$ . 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.

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415

416 **References**

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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 <sub>2</sub> O <sub>2</sub>
9, 10	95% aqua regia diluted 10 fold + 5% H <sub>2</sub> O <sub>2</sub>
11, 12	92.5% aqua regia diluted 5 fold + 7.5% H <sub>2</sub> O <sub>2</sub>
13, 14	90% aqua regia + 10% H <sub>2</sub> O <sub>2</sub>

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.

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618 List of Figures

619

620 Figure 1 Schematic diagrams of (a) centrifuge tube extraction arrangement used in  
621 Method 1 (after [37]) and (b) in-line polycarbonate filter holder extraction  
622 arrangement used in Method 2.

623

624 Figure 2 Extraction profile plots for the blank filters showing the chemical components  
625 derived from the filter used in the flow through method (F-flow through  
626 method, N-normal centrifuge method)

627

628 Figure 3 Extraction profile plots for the centrifugation method (S1 and S2 are the two  
629 replicate extractions and Bl is the blank extraction)

630

631 Figure 4 Extraction profile plots for the flow through method (S1 and S2 are the two  
632 replicate extractions and Bl is the blank extraction)

633

634 Figure 5 Chemical composition of each of the seven components (with elements that  
635 make up more than 1% of the total)

636

637 Figure 6 Comparison of analyte fractionation by the CISED and BCR extractions: (a)  
638 Cr (b) Cu (c) Ni (d) Pb (e) Zn

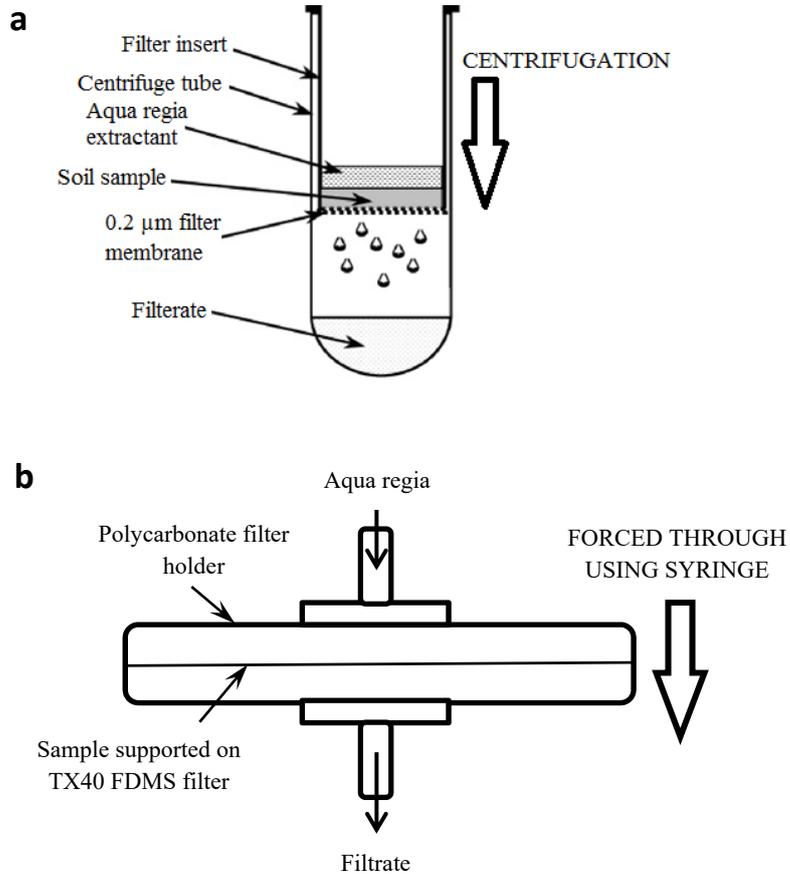
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640 Figure 7 Comparison between the total amounts of analytes extracted by the CISED  
641 and BCR methods (a) with and (b) without inclusion of the aqua-regia pseudo-  
642 total (step 4) in the BCR method.

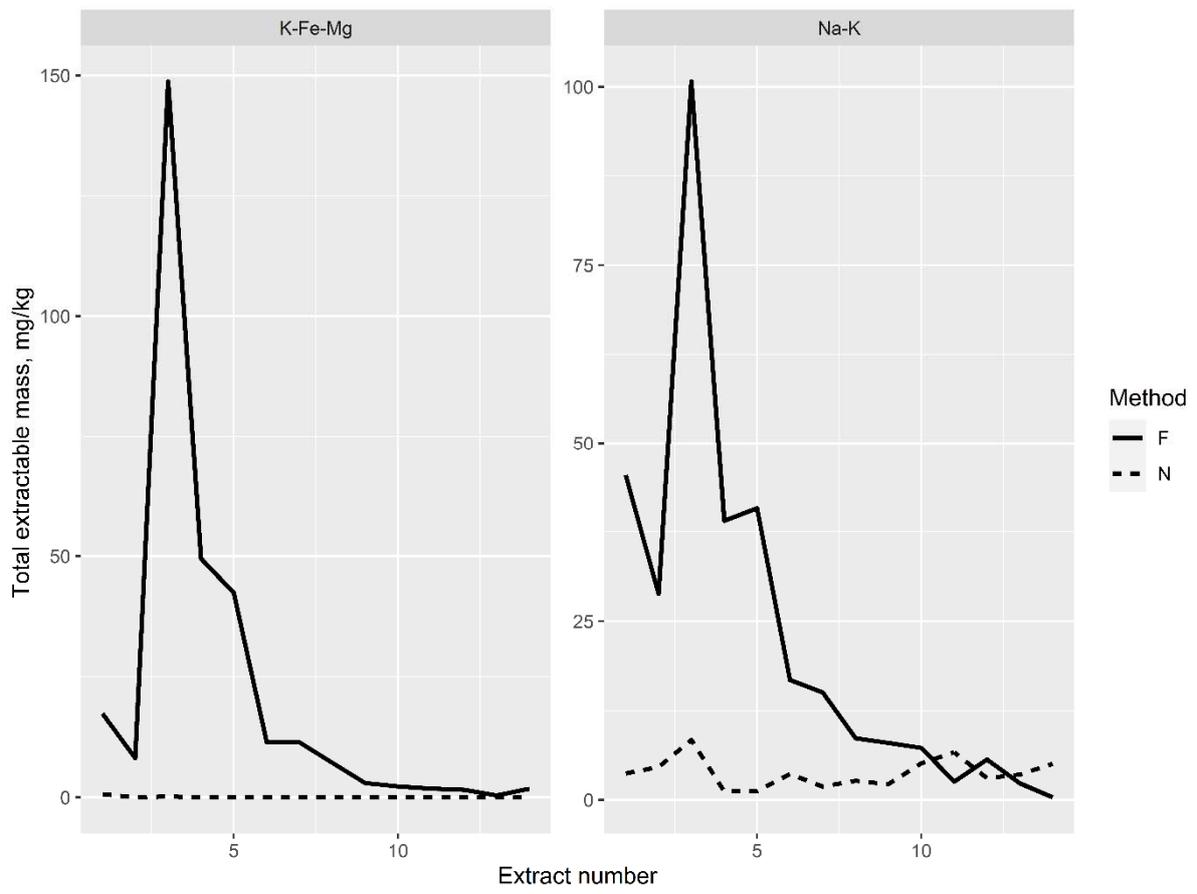
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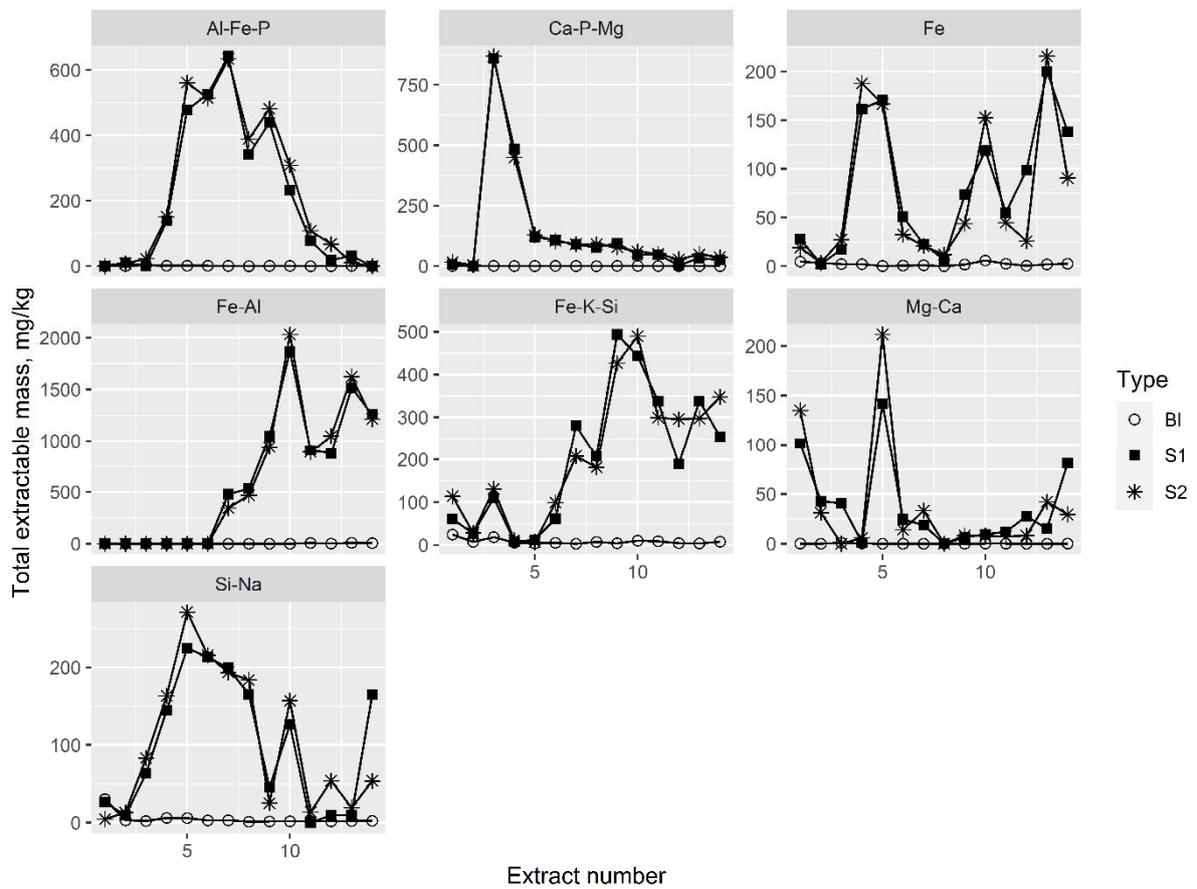
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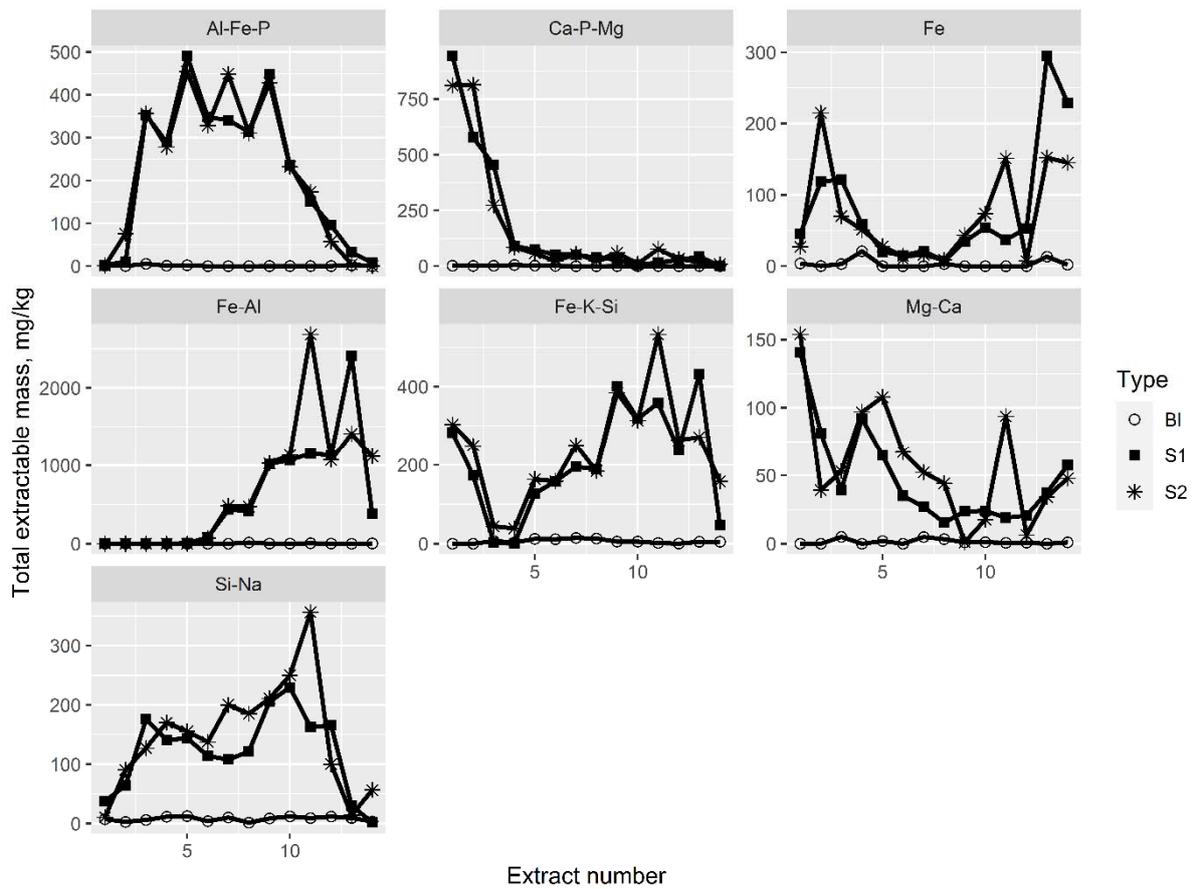
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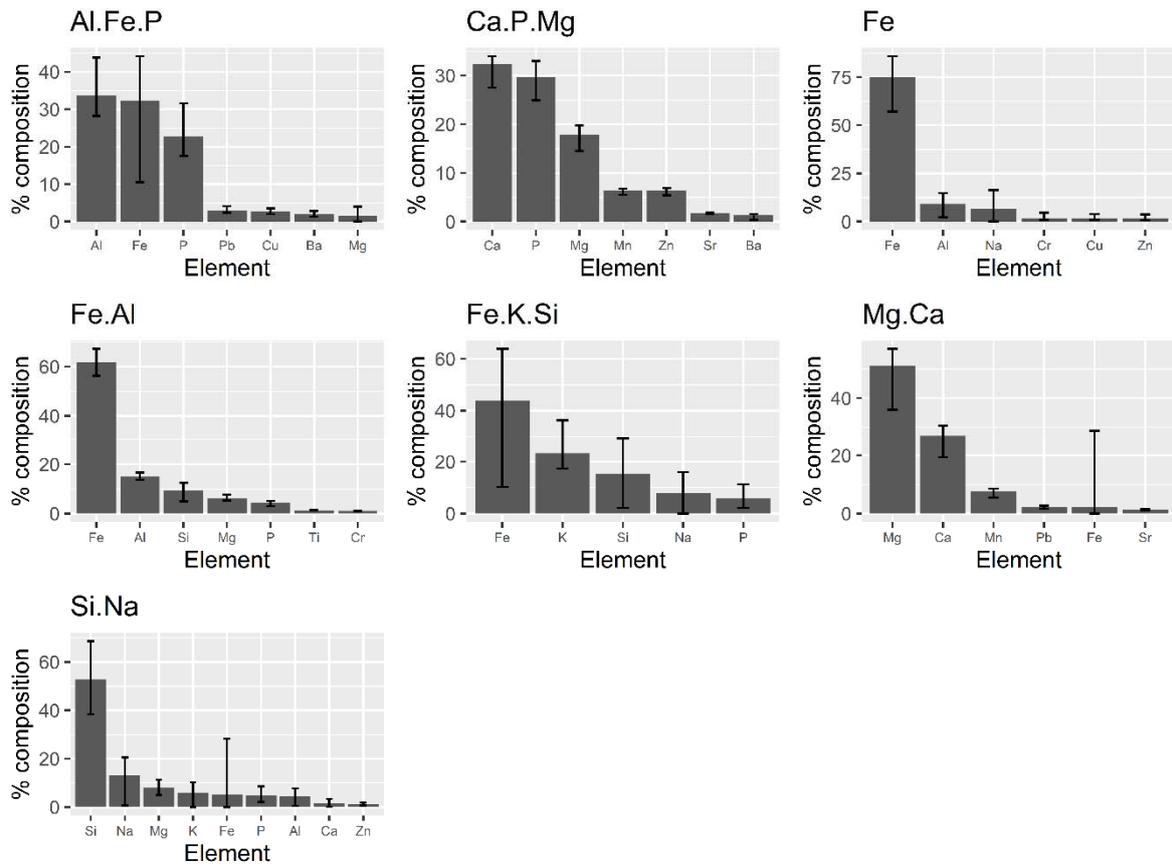


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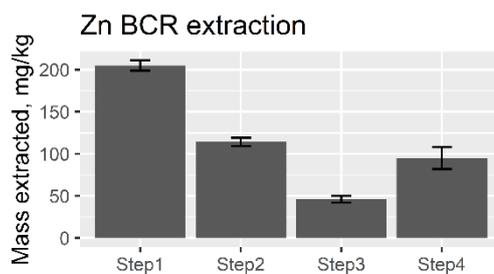
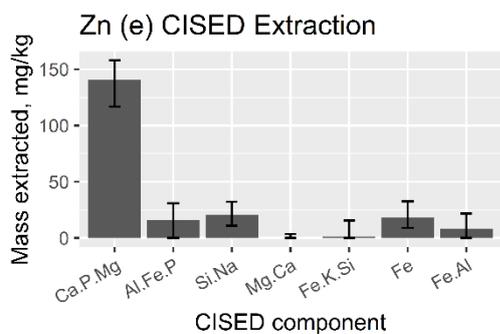
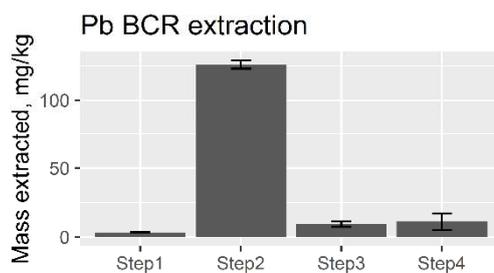
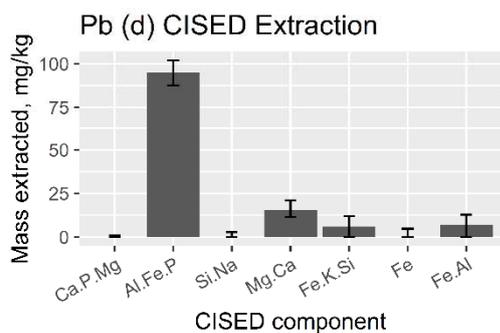
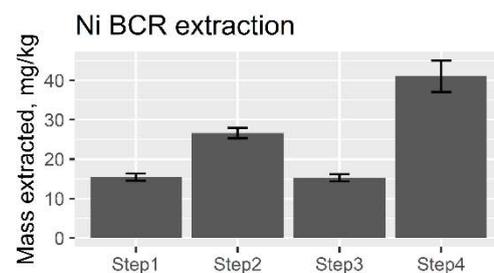
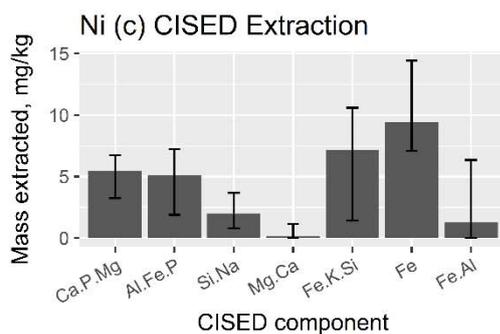
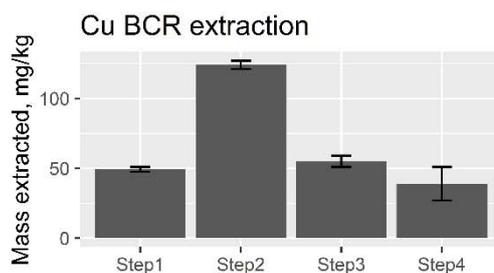
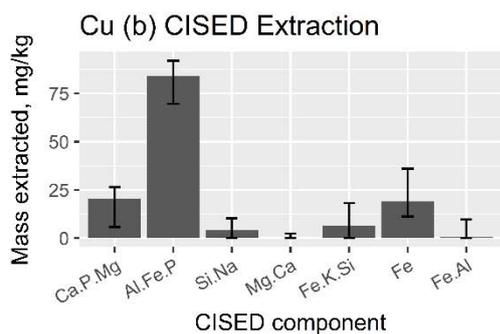
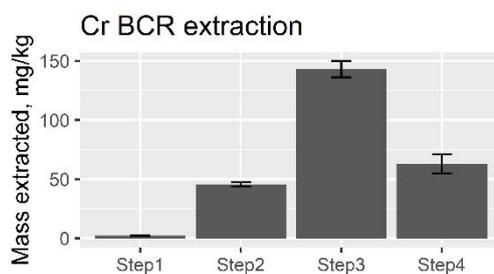
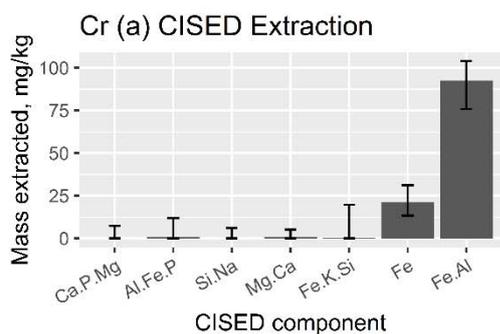
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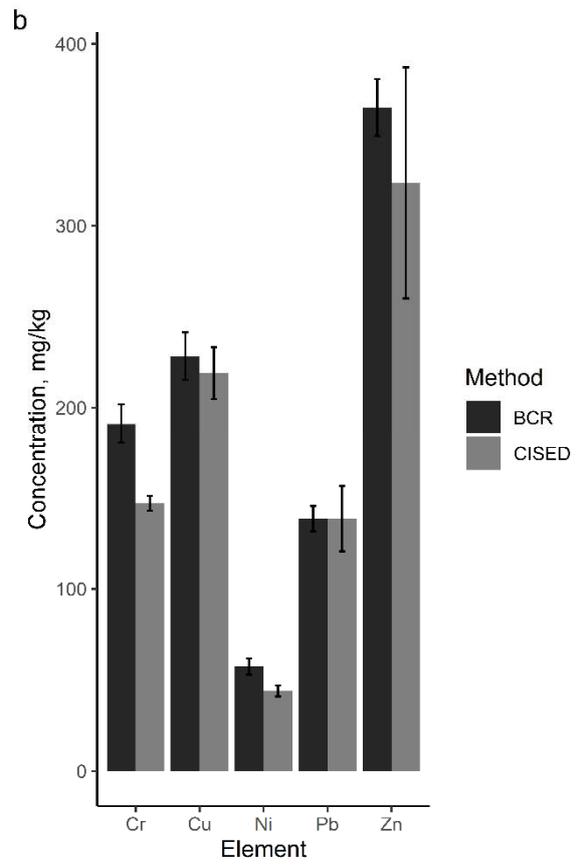
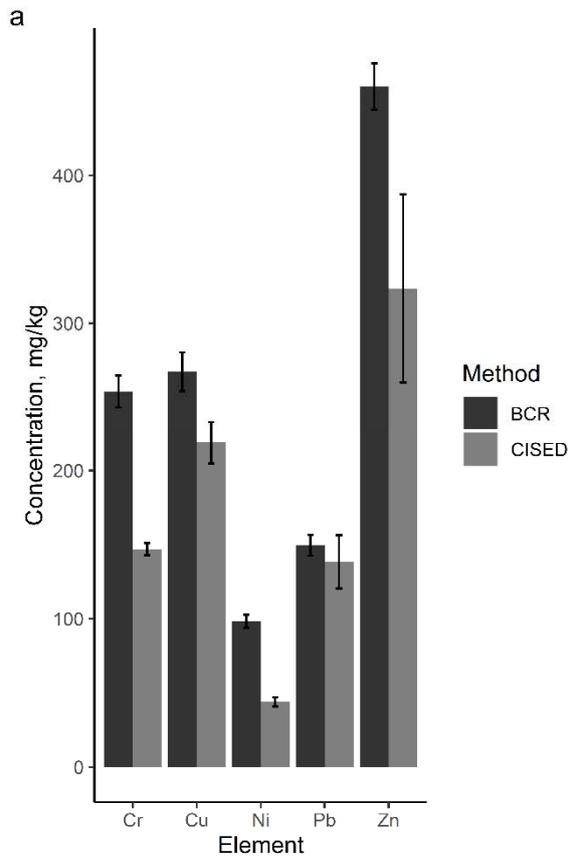
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