

This is a peer-reviewed, accepted author manuscript of the following article: Sagagi, B. S., Davidson, C. M., Cave, M. R., & Wragg, J. (2021). Comparison of two non-specific flow-through sequential extraction approaches to identify the physico-chemical partitioning of potentially harmful elements in a certified reference material. *Talanta*, 223(Pt. 1), Article 121685.

<https://doi.org/10.1016/j.talanta.2020.121685>

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

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

Abstract

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

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

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

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

1. Introduction

The United Nations Department of Economic and Social Affairs predicts that global population will rise from 7.63 billion in 2018 to 9.77 billion in 2050 [1] with the proportion of citizens living in urban environments increasing from 55 to 68%. The intensity of anthropogenic activity in rapidly urbanising areas has led to various environmental problems of which airborne pollution by particulate matter is one major concern [2]. Airborne pollution falls within the scope of (among others) EU Directive 2008/50/EC on ambient air quality and cleaner air for Europe, and the WHO air quality guidelines, because of the risk of potential harm to health. For example, long-term exposure to particulate matter of aerodynamic diameter $< 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) was found to be strongly associated with mortality attributable to ischemic heart disease, dysrhythmias, heart failure and cardiac arrest [3]. Many countries and urban centres have established PM monitoring programmes and/or strategies (e.g. the Indian National Air Monitoring Programme; USEPA National Air Monitoring Strategy; UK Automatic Urban and Rural Network). Particulate matter of specific aerodynamic diameter (typically $\text{PM}_{2.5}$ and PM_{10}) is collected on filter material using various types of air samplers over different time periods [4-6]. In the UK, the tapered element oscillating microbalance filter dynamics measurement system (TEOM FDMS) has been widely used in ambient air-quality monitoring stations.

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

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

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

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

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

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

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

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

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

2. Experimental

2.1 Apparatus

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

2.2 Reagents

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

2.3 Test material

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

2.4 CISED extraction

Method 1

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

Method 2

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

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

2.5 ICP-MS analysis

The ICP-MS was operated at 1550 W and 27 MHz in collision cell mode using He as the cell 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 and nebuliser (carrier) gas flows were 15, 0.9 and 1.0 L/min, respectively. Daily performance checks were carried out using a 10 $\mu\text{g/L}$ solution of Ba, Ce, Co, In, Li, Ni and Pb. Calibration was with respect to solutions freshly-prepared by serial dilution of Agilent Technologies multi-element standard solution 2A. The nuclides quantified were: ^{27}Al , ^{137}Ba , ^{40}Ca , ^{52}Cr , ^{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 , ^{88}Sr , ^{45}Ti , ^{51}V , ^{64}Zn , and ^{66}Zn . The internal standard was ^{115}In . One of the calibration standards was re-analysed every 8 samples, and again at the end of the sample run, to check for instrumental drift.

2.6 Chemometric processing of the CISED extraction data

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

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

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

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

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

3. Results and discussion

3.1 Identification of the components

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

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

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

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

Carbonates

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

Al/Fe oxides/hydroxides

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

Fe dominated

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

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

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

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

Si dominated

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

3.2 Fractionation of the metals

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

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

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

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

Chromium

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

Copper

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

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

Nickel

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

Lead

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

Zinc

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

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

4. Conclusions

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

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

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

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

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

CRedit authorship contribution statement

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

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

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

References

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

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

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

36. M.D. Ho, G.J. Evans, Sequential extraction of metal contaminated soils with radiochemical assessment of readsorption effects, *Environ. Sci. Technol.* 34 (2000) 1030-1035. **DOI:** 10.1021/es981251z
37. M.R. Cave, A.E. Milodowski, E.N. Friel, 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, *Geochem-Explor. Env. A.* 4 (2004) 71-86. **DOI:** 10.1144/1467-7873/03-025
38. J. Wragg, M. Cave, 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.* 722 (2012) 43-54. **DOI:** 10.1016/j.aca.2012.02.008
39. B. Palumbo-Roe, B. Klinck, Bioaccessibility of arsenic in mine waste-contaminated soils: A case study from an abandoned arsenic mine in SW England (UK), *J. Environ. Sci. Heal. A.* 42 (2007) 1251-1261. **DOI:** 10.1080/10934520701435692
40. B. Palumbo-Roe, M.R. Cave, B.A. Klinck, J. Wragg, H. Taylor, K. O'Donnell, R.A. Shaw, Bioaccessibility of arsenic in soils developed over Jurassic ironstones in eastern England, *Environ. Geochem. Hlth.* 27 (2005) 121-130. **DOI:** 10.1007/s10653-005-0128-1
41. R. Santamaria-Fernandez, M.R. Cave, S.J. Hill, The effect of humic acids on the sequential extraction of metals in soils and sediments using ICP-AES and chemometric analysis, *J. Environ. Monit.* 5 (2003) 929-934. **DOI:** 10.1039/b306865j
42. R. Santamaria-Fernandez, M.R. Cave, S.J. Hill, Trace metal distribution in the Arosa estuary (NW Spain): The application of a recently developed sequential extraction procedure for metal partitioning, *Anal. Chim. Acta.* 557 (2006) 344-352. **DOI:** 10.1016/j.aca.2005.10.029
43. A. Mukhtar, A. Limbeck, Recent developments in assessment of bio-accessible trace metal fractions in airborne particulate matter: a review, *Anal. Chim. Acta.* 774 (2013) 11-25. **DOI:** 10.1016/j.aca.2013.02.008
44. M. Guney, R.P. Chapuis, G.J. Zagury, Lung bioaccessibility of contaminants in particulate matter of geological origin, *Environ. Sci. Pollut. R.* 23 (2016) 24422-24434. **DOI:** 10.1007/s11356-016-6623-3
45. F. Kastury, E. Smith, A.L. Juhasz, A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust, *Sci. Total Environ.* 574 (2017) 1054-1074. **DOI:** 10.1016/j.scitotenv.2016.09.056
46. M. Rogora, L. Kamburska, R. Mosello, G. Tartari, Lake Orta chemical status 25 years after liming: problems solved and emerging critical issues. *J. Limnol.* 75 (2016) 93-106. **DOI:** 10.4081/jlimnol.2016.1320

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

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

Concentrated aqua regia was used for extracts 13 and 14. This was diluted 5-fold for use in extracts 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 500-fold for extracts 3 and 4.

- 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.













