

Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments.

Katherine F Mossop and Christine M Davidson*

Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, GLASGOW G1 1XL, UK

Abstract

This article describes a detailed comparison between the original BCR sequential extraction procedure, step 2 of which involves treatment with 0.1 mol L⁻¹ hydroxylammonium chloride at pH 2, and the revised BCR procedure (step 2 : 0.5 mol L⁻¹ hydroxylammonium chloride at pH 1.2). An intermediate protocol was also evaluated in which 0.5 mol L⁻¹ hydroxylammonium chloride at pH 2 was used. The procedures were applied to five soil and sediment substrates: a sewage sludge-amended soil, two different industrially contaminated soils, a river sediment and an inter-tidal sediment. Extractable iron and manganese concentrations were measured to assess the effects of the procedural modifications on dissolution of the reducible matrix components. Trace elements copper, lead and zinc were also determined. Statistical analysis (two-tailed t-tests at 95 % confidence interval) indicated that recovery of iron in step 2 was not markedly enhanced when the intermediate protocol was used. However, significantly greater amounts were isolated with the revised BCR scheme than with the original procedure. Copper behaved similarly to iron. Lead recoveries were increased by use of both modified protocols, with the greatest effect occurring for the revised BCR extraction. In contrast, manganese and zinc extraction did not vary markedly between procedures. The work indicates that the revised BCR sequential extraction provides better attack on the iron-based components of the reducible matrix for a wide range of soils and sediments.

* corresponding author : c.m.davidson@strath.ac.uk

1. Introduction

Sequential extraction is now a well-established approach for the fractionation of trace metal content in soils, sediments, and related materials [1]. The sample is treated with a series of reagents selected for their ability to react with different, major, components of the matrix and release associated trace metals. Difficulties have been reported with non-specificity of reagents [2] and re-adsorption of metals before they can be isolated for analysis [3].

Nevertheless, the lack of alternative approaches for the estimation of potential metal mobility has meant that a wide variety of sequential extraction schemes have been developed and used.

The operational nature of sequential extraction means that it is very hard to compare results obtained by different laboratories using different protocols. As a result, in the 1990's, a group of experts, working under the auspices of the Commission of the European Communities, Community Bureau of Reference (BCR, now superseded by the Standards, Measurement and Testing Programme) met with the aim of harmonising extraction methodology. The main outcomes were :

- (a) a simple, three-step sequential extraction protocol developed for fractionation of trace elements in sediment (Table 1) [4] and
- (b) a lake sediment reference material (BCR CRM 601) certified for metals extractable by the protocol [5].

The original BCR procedure was also applied to soil [6] and, later, to a variety of matrices including sewage sludge [7], marine sediment [8], industrial "made ground" [9], ash [10] and compost [11].

However, during certification of BCR CRM 601, significant inter-laboratory variability was apparent, in particular in step 2 of the extraction. This limited the number of elements for which certified values could be agreed and, eventually, led to a thorough re-evaluation of this step of the protocol [12]. The study, performed using CRM 601 as the test substrate, led to the development of a modified BCR sequential extraction procedure [13, 14]. The revised protocol involves use of an increased concentration of $\text{NH}_2\text{OH}\cdot\text{HCl}$ and lower pH. It improves reproducibility due, it is thought, to a more efficient dissolution of the reducible fraction of the soil matrix, most probably the iron oxyhydroxide phase.

The purpose of the present investigation was to compare the performance of original and modified BCR protocols when applied to a variety of soil and sediment substrates. The samples studied were: a sewage-sludge amended soil, a freshwater sediment, an inter-tidal sediment and two layers of “made- ground” obtained from a recently derelict industrial site. Iron and manganese were studied to assess the effects of the modification on reducible matrix components, which constitute the main target phases in step 2. The trace elements copper, lead and zinc were also determined. Analysis was performed by ICPAES.

2. Experimental

2.1 Apparatus

Metals were determined in digests and extracts by ICPAES using either a Plasma II (Perkin Elmer, Norwalk, Connecticut, USA) or a Liberty 220 (Varian Techtron Pty. Ltd., Mulgrave, Victoria, Australia) system. Quantification was with respect to reagent-matched multielement standards prepared by serial dilution of 1000 $\mu\text{g ml}^{-1}$ commercial standard solutions (Spectrosol, from Merck, Dorset, UK, for the Plasma II and Romil PrimAg, from Romil, Cambridge, UK for the Liberty 220). A single point standard addition was performed, for each analyte and reagent, in each batch of analyses, to check for matrix interference in digests and extracts. Few interferences were found but, where necessary, results were adjusted based on the recoveries of the added standards.

2.2 Reagents

Hydroxylammonium chloride, AnalaR grade, was provided by Merck, Poole, UK, as were AnalaR nitric (69 %, sp. gr. 1.42), hydrochloric (sp. gr. 1.18) and acetic (glacial, 100 %) acids. Hydrogen peroxide (30 %) was obtained from Fluka, Dorset, UK and ammonium acetate from Prolabo, Manchester, UK.

Soil and sediment samples were chosen to represent a variety of types of substrate. Details are given in Table 2.

2.3 Procedures

2.3.1 Digestion

Pseudototal (i.e. *aqua regia*-extractable) metal concentrations were determined by microwave-assisted digestion using a CEM-MDS-2000 system (CEM Corporation, NC, USA). 1 g of air-dried sediment was digested with 5 ml HNO₃ and 15 ml HCl in an advanced composite vessel. After cooling, the digest was filtered (Whatman type 50 filter paper) into a 100 ml volumetric flask and diluted to the mark with distilled water.

2.3.2 Sequential extraction

Extractions were performed using the reagents given in Table 1. Details of the experimental protocol are available elsewhere [9,14]. Briefly:

Step One 40 ml of acetic acid was added to 1 g air-dried sediment and shaken overnight. The mixture was centrifuged to separate the extract from the residue.

Step Two 40 ml of hydroxylammonium chloride, adjusted with nitric acid to the pH given in Table 1, was added to the residue from step one and the extraction performed as above.

Step Three The residue from step two was treated twice with 8.8 mol l⁻¹ hydrogen peroxide, evaporated to near dryness, then 50 ml of ammonium acetate, adjusted to pH 2 with nitric acid, was added and the extraction performed as above.

Residual fraction The material remaining at the end of the BCR procedure was digested in 20 ml *aqua regia*, with microwave assistance, as described above.

3. Results and discussion

3.1 Pseudo-total (*aqua-regia* soluble) metal contents

To provide data against which the results of sequential extraction could be compared, each substrate was digested in duplicate (Table 3). Copper, lead and zinc concentrations in the soils (GTB, I18 and I47) were higher than typical soil ranges [15]. They also generally exceeded the UK ICRCCL guideline values for redevelopment of land as gardens, allotments or for other uses where plants grow [16]. Iron in the industrial soils was present at a little above typical levels. Manganese concentrations were unremarkable.

3.2 *Effects of modification of sequential extraction protocol*

Iron and manganese

Five separate specimens each of sediment (WCR), soil (GTB) and industrial soil (I18) were sequentially extracted using procedures A, B, and C. Unfortunately, it was not possible to perform all extractions simultaneously due to limited capacity on the end-over-end shaker. Instead, extractions were performed in two batches in separate weeks. Procedures A and B were compared in the first batch of extractions, and procedures A and C in the second. It was thus also possible to investigate the reproducibility of procedure A.

Initial inspection of results presented in Table 4 suggests that, for iron, increasing the reductant concentration alone in step 2 has a small effect but the influence of extractant pH is more important. Procedure C releases much more of this element than either procedures A or B. In contrast, broadly similar amounts of manganese are released in all cases. Agreement between sum and pseudototal values are generally acceptable ($100 \pm 10\%$) for the first batch of sequential extractions (procedures A1 and B), but the second batch (procedures A2 and C) often appeared to give overall recoveries higher than aqua regia digestion.

A more rigorous statistical analysis of the data was performed. Pairs of results (procedures A2 and A1; procedures B and A1; procedures C and A2) were compared. For each step, an F-test was performed to determine whether the variances of the two sets of results were equal, then an appropriate form of the two-tailed t-test was applied at 95 % confidence interval [17].

Results indicated that similar amounts of iron were released in step 1 of all sequential extractions, for all substrates, which is expected since the extraction procedures are identical at this stage. Significant differences were observed in step 2. The between batch variation (A1 vs. A2) was similar to the variation between procedures A1 and B for this step. It is thus unclear whether the increased reductant concentration alone had any effect. However, the difference between procedures A2 and C was much more pronounced. Some pairs of similar results were obtained in step 3 (procedures C and A2 for WCR and GTB, and procedures B and A1 for I18) and for step 4 (procedures B and A1 for WCR, and all procedures for I18), but most comparisons revealed significant differences between the procedures. Presumably

increasing the amount of analyte recovered in step 2 also affects recoveries at subsequent stages of the procedure since less analyte remains in association with the solid phase.

For manganese, some differences between step 1 results were observed especially, surprisingly, for the replicates of procedure A. In step 2, results for all procedures were similar for sediment WCR and soil GTB, except for WCR procedure C. Some significantly different results were obtained, for some substrates, in steps 3 and 4. However, in all cases, these differences were less than typical, between-batch variation.

Overall, significantly more iron is released by the modified than by the original BCR procedure. Effects on manganese are less marked. This is consistent with previous work on CRM 601, which suggested that the revised BCR protocol is able to more effectively target resistant components of the iron oxyhydroxide phase than the original protocol, whereas the original reagent dissolves manganese oxyhydroxides effectively [12]. It is also clear that increasing the $\text{NH}_2\text{OH}\cdot\text{HCl}$ concentration alone does not markedly enhance the recovery of iron unless accompanied by a decrease in the extractant pH. This may indicate that the ability to prevent re-adsorption is, perhaps, more important than the greater amount of reductant present.

Copper, lead and zinc

The effect of procedures A, B and C on the release of selected trace elements is shown in figure 1. Copper extraction in step 2 was only marginally increased, relative to the original BCR protocol, when procedure B was applied. However, significant additional analyte was isolated in this step using procedure C. Procedure B recovered more lead in step 2 than procedure A, and procedure C more than procedure B, indicating that both the increased reductant concentration and pH have an effect for this element. There was a concomitant decrease in the amount of lead isolated in step 3. More unexpectedly, for the soils, a portion of the lead that was not released in the original BCR procedure until the residual (aqua regia) stage was isolated in step 2 when modified procedure C was applied. In contrast, zinc partitioning was relatively unaffected by the changes in the sequential extraction. Total amounts of analytes recovered (Σ steps 1-4) were generally within 15 % of aqua regia pseudototals, except for zinc in WCR (recoveries of 129 % and 150 % for procedures A and C, respectively).

Application to further samples

Procedures A and C (original and modified BCR protocols) were applied to two further substrates, industrial soil I47 and marine sediment WHN. The modified protocol extracted larger proportions of all analytes from the soil in step 2 than did the original procedure (Figures 2). As before, the effect was more marked for copper, iron and lead, and less pronounced for manganese and zinc. The total amounts of analytes recovered were within 10 % of pseudototal values, except for iron, whose recovery was only ~ 75 %. Broadly similar trends were obtained for the sediment sample.

Comparison with indicative values

Reference samples were extracted to assess the quality of the data obtained. Lake sediment reference material BCR CRM 601 was used. Results obtained for GTB may also be compared with literature values for BCR CRM 483, since the soil originated from the same source as the reference material. Unfortunately, neither CRM is certified for extractable iron or manganese, the main focus of the present work. Results for trace elements (Table 5) indicate that, with a few exceptions (e.g. copper in step 2 for CRM 601 and lead in steps 3 and 4 for GTB) agreement with literature values was generally acceptable.

4. Conclusions

Step 2 of the revised BCR sequential extraction has been shown to extract more iron than the equivalent step of the original BCR extraction, for several soil and sediment substrates. This suggests that, although developed on the basis of a single type of sediment (lake sediment reference material BCR CRM 601) the modified procedure should afford better attack on the iron-based components of the reducible matrix for a range of soils and sediments. No clear improvement in precision was obtained with use of the modified protocol. However, it is possible that this would be more evident in inter-laboratory trials where potential sources of variability are greater than in the present work.

5. Acknowledgements

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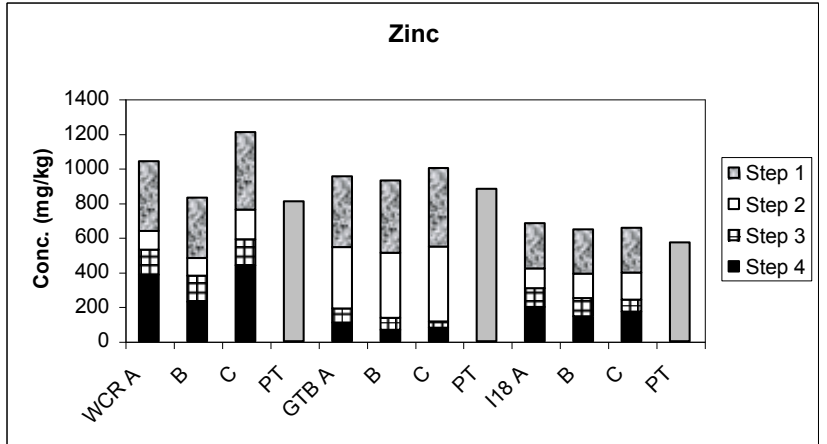
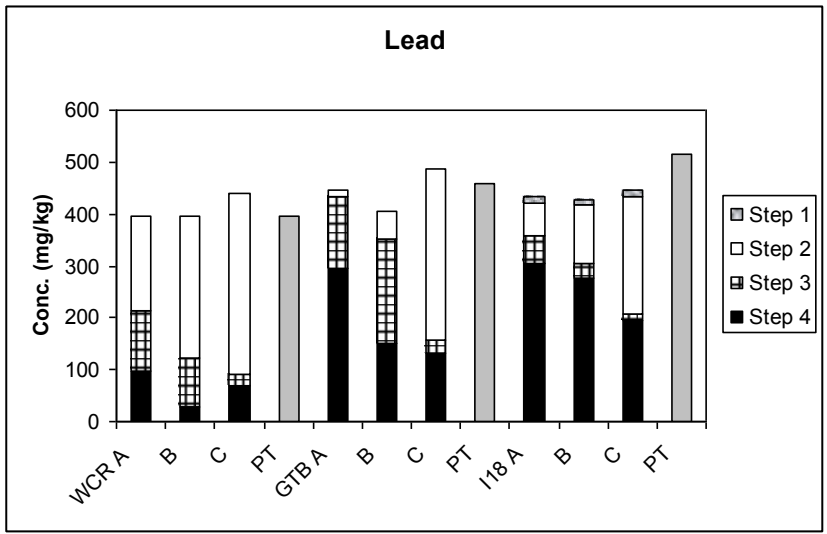
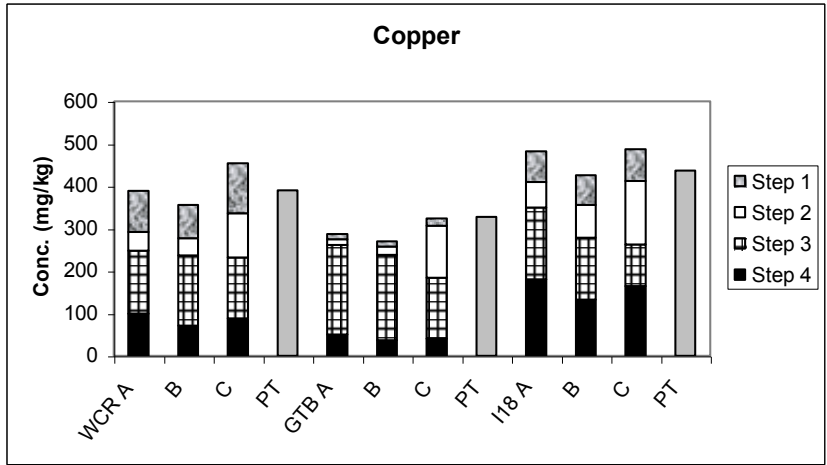
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Legends for figures

- Figure 1 Fractionation of copper, lead and zinc in sediment WCR and soils GTB and I18, by means of sequential extraction procedures A, B and C. Pseudototal (PT) concentrations are shown for comparison. Results plotted for WCR are 10 x actual values, for all analytes.
- Figure 2 Comparison of fractionation obtained by means of original and modified BCR procedures (procedures A and C). (a) industrial soil I47 (results plotted for iron are 1/100th actual values and for zinc are 1/2 actual values), (b) inter-tidal sediment WHN (results plotted for copper are 10 x actual values and for iron are 1/100th actual values).



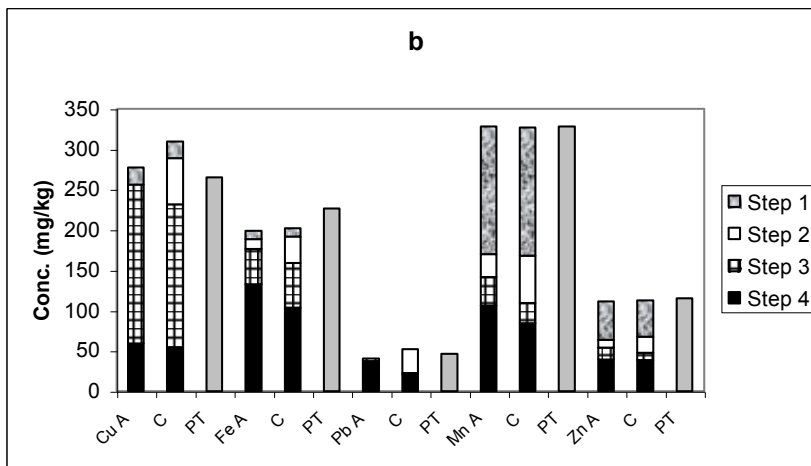
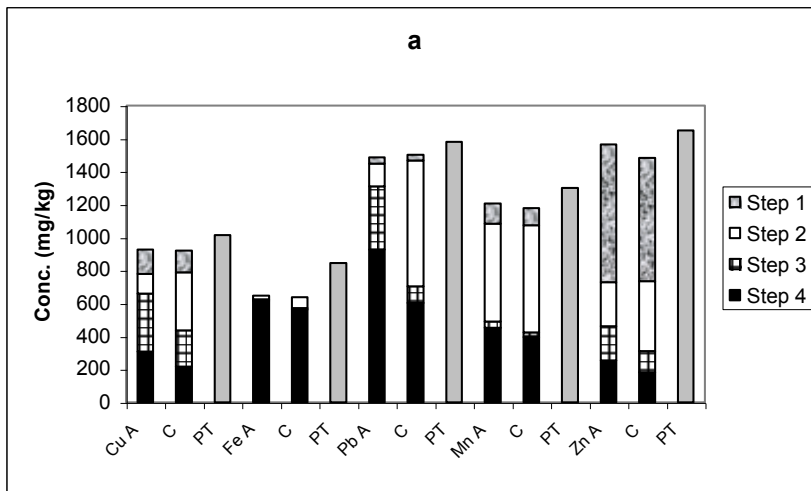


Table 1: Sequential extraction procedures

step	Fraction	Nominal target phase(s)	procedure A (Original BCR) [4]	procedure B	procedure C (Revised BCR) [14]
1	Exchangeable, water and acid soluble	Soluble species, carbonates, cation exchange sites	0.11 mol l ⁻¹ acetic acid	0.11 mol l ⁻¹ acetic acid	0.11 mol l ⁻¹ acetic acid
2	Reducible	Iron and manganese oxyhydroxides	0.1 mol l ⁻¹ hydroxylammonium chloride at pH 2	0.5 mol l ⁻¹ hydroxylammonium chloride at pH 2	0.5 mol l ⁻¹ hydroxylammonium chloride at pH 1.5
3	Oxidisable	Organic matter and sulfides	hydrogen peroxide followed by 1.0 mol l ⁻¹ ammonium acetate at pH 2	hydrogen peroxide followed by 1.0 mol l ⁻¹ ammonium acetate at pH 2	hydrogen peroxide followed by 1.0 mol l ⁻¹ ammonium acetate at pH 2
4 ^a	Residual		<i>aqua regia</i>	<i>aqua regia</i>	<i>aqua regia</i>

^a although not part of the original procedure, the addition of a residual step is useful for quality control, since Σ (step 1-4) can be compared with results of a separate aqua regia digestion [14].

Table 2: Substrates studied

Substrate	Material	Source	Moisture content (%) ^a	Loss on ignition (%) ^b
WCR	Freshwater sediment	White Cart River, Paisley, UK	0.58	2.84
GTB	Sewage-sludge amended soil	Great Billing, Northampton, UK	3.26	19.7
I18	Industrial soil (depth 18 – 28 cm)	Derelict industrial site, NW England, UK	2.00	12.9
I47	Industrial soil (depth 47-50 cm)	Derelict industrial site, NW England, UK	4.28	18.8
WHN	Inter-tidal sediment	Whitehaven Harbour, Cumbria, UK	0.89	4.78

^a obtained by drying to constant mass at 105 °C, ^b obtained by combustion at 500 °C

Table 3: Pseudototal (aqua regia soluble) metal concentrations (mg kg⁻¹ dry weight)

Substrate	Cu	Fe	Pb	Mn	Zn
WCR	39.0	14300	39.5	109	80.7
GTB	327	22700	459	220	879
I18	437	46600	515	370	572
I47	1020	84500	1580	1300	3300
WHN	26.5	22600	46.2	328	115
typical soil range [15]	1 - 80	7000 - 42000	0.1 – 200	20 – 3000	3 – 300
ICRCL guideline [16]	130 ^a		500 ^b 2000 ^c		300 ^a

Results shown are mean values for duplicate digestion. ^a any uses where plants grow, ^b domestic gardens and allotments, ^c parks and open spaces

Table 4: Effect of sequential extraction procedure on release of iron and manganese.

		Fe concentration (mg kg ⁻¹ dry weight)						Mn concentration (mg kg ⁻¹ dry weight)					
	Method	step 1	step 2	step 3	step 4	Sum	Recovery	step 1	step 2	step 3	step 4	Sum	Recovery
WCR	A1	217 (2.30) ^a	1070 (3.08) ^a	1460 (2.47) ^a	11300 (8.22) ^a	14000 (6.62)	97.9	21.0 (2.38) ^a	8.45 (12.7) ^b	10.2 (13.7) ^a	64.6 (13.3) ^a	104 (8.65)	95.4
	A2	208 (6.25)	1430 (4.06)	796 (2.14)	15900 (5.45) ^a	18300 (4.74)	128	27.6 (1.45)	9.13 (3.40)	9.88 (2.83)	105 (3.81)	152 (2.63)	139
	B	213 (2.82)	1290 (1.55)	1360 (4.94)	11800 (3.99) ^a	14700 (3.25)	103	21.3 (2.82)	9.54 (10.1)	9.61 (4.27)	63.1 (5.55)	104 (3.85)	95.4
	C	209 (30.1)	2320 (2.68)	676 (14.6)	12800 (15.2) ^b	16000 (12.2)	112	28.5 (2.81)	15.4 (3.25)	9.13 (8.76)	98.3 (13.7)	151 (9.27)	138
GTB	A1	< DL	767 (24.8)	3950 (7.08)	18000 (7.79)	22700 (6.35)	100	85.4 (5.85)	92.7 (7.67)	14.4 (16.0)	50.2 (7.37)	243 (4.12)	110
	A2	31.6 (5.38)	1900 (4.43)	686 (9.33)	22200 (9.16)	24800 (8.20)	109	119 (8.52)	85.6 (9.58)	8.94 (7.16)	54.9 (7.83)	268 (3.73)	122
	B	< DL	2120 (9.44)	3314 (9.99)	15696 (7.45)	21100 (5.83)	93.0	92.8 (3.45)	84.7 (6.49)	10.4 (11.5)	41.8 (11.5)	230 (3.48)	104
	C	32.8 (3.35)	7000 (2.63) ^b	724 (19.5)	15340 (3.55) ^a	23100 (2.56)	102	120 (2.50)	95.7 (8.88)	7.90 (7.34)	41.9 (8.83)	266 (3.76)	121
I18	A1	37.2 (28) ^a	1360 (5.57)	1010 (10.6)	53400 (4.88)	55800 (4.68)	120	38.3 ^a (4.18)	85.7 (6.30)	19.2 (5.21)	262 (6.49)	405 (4.43)	110
	A2	37.5 (29.3)	1670 (2.64)	666 (7.21)	66500 (14.1) ^b	68900 (13.6)	148	44.0 (3.41)	72.8 (3.30) ^a	17.4 (14.9)	439 (8.88) ^b	573 (6.84)	155
	B	36.6 (43.2)	1900 (5.35)	1060 (12.7)	48800 (7.78)	51800 (7.34)	111	38.0 (5.00)	97.1 (6.08)	22.1 (13.6)	245 (7.76)	402 (5.03)	109
	C	25.6 (12.5)	4280 (2.71)	1060 (18.2)	60800 (15.8) ^b	66200 (14.5)	142	45.8 (14.2)	96.9 (9.60)	23.6 (17.2)	392 (15.0) ^b	558 (10.8)	151

Results presented are mean values for n = 5 unless otherwise stated. ^a denotes n = 4. ^b denotes n = 3. RSD values are given in brackets. Recovery is expressed as a percentage relative to pseudototal value (Table 3). Extraction A1 was performed at the same time as extraction B, and extraction A2 with extraction C.

Table 5: Comparison between current and literature values for extractable trace elements (revised BCR procedure).

	Cu concentration (mg kg ⁻¹ dry weight)		Pb concentration (mg kg ⁻¹ dry weight)		Zn concentration (mg kg ⁻¹ dry weight)	
	indicative [18]	found	indicative [18]	found	indicative [18]	found
CRM 601						
step 1	10.5 ± 0.8	9.6 ± 0.4	2.28 ± 0.44	2.42 ± 1.70	261 ± 13	211 ± 9.0
step 2	72.8 ± 4.9	48 ± 6.0	205 ± 11	182 ± 11	266 ± 17	240 ± 10
step 3	78.6 ± 8.9	93 ± 4.0	19.7 ± 5.8	23.1 ± 2.0	106 ± 11	135 ± 8.0
step 4	60.4 ± 4.9	57 ± 8.0	38.0 ± 8.7	53.2 ± 7.5	161 ± 14	167 ± 19
CRM 483 / GTB	indicative [19]		indicative [19]		indicative [19]	
step 1	16.8 ± 1.5	17.6 ± 0.3	0.76 ± 0.70	< 0.3	441 ± 39	453 ± 9.0
step 2	141 ± 20	123 ± 3.7	379 ± 21	329 ± 6.0	438 ± 56	433 ± 22
step 3	132 ± 29	143 ± 8.0	66.5 ± 22	27.1 ± 4.6	37.1 ± 9.9	36.3 ± 5.6
step 4	43.3 ± 3.8	40.6 ± 2.3	76.9 ± 17	131 ± 5.0	82.1 ± 9.6	78.1 ± 9.6

Results presented are mean values ± one standard deviation. Indicative values for CRM 601 are n = 7 (steps 1-3) and n = 6 (step 4). Indicative values for CRM 483 are n = 6. Results from the present study are n = 7 (CRM 601) and n = 5 (GTB).