

Improvement of the sequential extraction procedure based on supercritical CO₂ and subcritical H₂O solvents for the estimation of the environmentally mobile potentially toxic element fractions of sediments and soils

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Abstract

The estimation of environmental risk caused by pollution with potentially toxic elements (PTE) is usually carried out using the (3+1) step sequential extraction procedure suggested in 1993 by the Community Bureau of Reference (BCR). In the 1st step the water-soluble, exchangeable and carbonate-bound element content is extracted with acetic acid. In 2002 a fractionation procedure based on the application of supercritical CO₂, subcritical H₂O and of a mixture of subcritical H₂O/CO₂ was proposed, which allowed the water-soluble and carbonate-bound element contents to be extracted separately from sediment or soil samples weighed into the preparative column of a supercritical fluid extractor and diluted with quartz sand in a mass ratio of 1:20. The aim of the present study was to develop a new reduced-size column construction with which this dilution rate could be decreased to 1:2. A kinetic study was performed to determine the extraction time necessary for samples with different carbonate contents and the extracted element contents were compared to the results of the BCR sequential procedure on the same samples. It was established that fractionation using the reduced-size column may be a rapid way to obtain more reliable information on the easily mobilizable (water-soluble and carbonate-bound) PTE content of soils and sediments than was previously available to supplement BCR fractionation.

Keywords: fractionation, kinetics, mobilization, sequential extraction, supercritical, potentially toxic elements (PTE)

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Introduction

Since the 1990s the need to improve elemental speciation and fractionation methods has continuously increased due to the growing risks of pollution by potentially toxic elements (PTE). In addition, the terms chemical speciation, operational speciation, etc. have been used with various meanings in many scientific papers. Therefore, in 2000 the IUPAC committees proposed a clear nomenclature for elemental speciation, speciation analysis and fractionation (TEMPLETON et al., 2000), which will be used in the present paper.

The use of total speciation analysis to estimate the environmental risk represented by the PTEs detectable in soils and sediments would be an unrealistic analytical task, so up to now fractionation by multistep extraction procedures has been used for this purpose. One strategy frequently used in agrochemistry is the individual application of single-step extractions with different extractants on the original solid sample, e.g. distilled water (MSZ 21470-50:2006), KCl-EDTA (MSZ 20135:1999), CaCl₂-DTPA (SAHUQUILLO et al., 2003; BAKIRCIOGLU et al., 2011), Lakanen-Erviö (MSZ 20135:1999; LAKANEN & ERVIÖ, 1971), aqua regia (MSZ 21470-50:2006) or HNO₃/H₂O₂ digestion. In environmental risk assessment the sequential extraction strategy has become the accepted approach, in which extractants with increasing aggressiveness are applied successively after phase separation on the same solid sample (TESSIER et al., 1979; BOWARDT & HAWTHORNE, 1995; KAASALAINEN & YLI-HALLA, 2003). Sequential extraction procedures proved to be useful for estimating the mobility and toxicity of PTEs and their temporal changes in various environmental matrices (FIEDLER et al., 1994). The (3+1) step sequential extraction procedure suggested by the Community Bureau of Reference (BCR) in 1993 (URE et al., 1993; LÓPEZ-SÁNCHEZ et al., 1998; RAURET et al., 1999; ONDOÑO et al., 2017) has been used by the authors for two decades (HELTAI et al., 1998). Simultaneously, however, a supplementary alternative method was developed by which the species can be preserved intact (PUEYO et al., 2008). For this purpose HELTAI et al., (2000; 2002) elaborated a new three-step continuous flow procedure performed in a supercritical extractor using a sequence of solvents (1. supercritical CO₂, 2. subcritical H₂O, and 3. a subcritical mixture of H₂O and CO₂) to supplement the BCR procedure. Soil or sediment samples of 0.500-1.000 g are weighed into a supercritical fluid extractor column and diluted with high purity quartz up to a mass ratio of 1:20. In the 3rd step of this procedure, carbonates were dissolved by hydrocarbonate formation and the necessary extraction time was adjusted according to the carbonate content of the samples (HELTAI et al., 2005; HALÁSZ et al., 2008). The information supplied by this supercritical fluid extraction (SFE) method differs from that provided by BCR extraction, which uses acetic acid in the first step to characterize the sum of easily mobilizable element fractions (HORVÁTH et al., 2010). The SFE procedure divides this into two (water-soluble and carbonate-bound) fractions. The advantage of this method is that it needs much less time than the BCR procedure (SFE: 7-8 hours, BCR: 5 days); moreover it is possible to search for and identify the original species

in the aqueous and CO₂-soluble fractions using appropriate analytical techniques (ICP-OES, ICP-MS) (HELTAI et al., 2005; HALÁSZ et al., 2008).

The special aim of the present study was to decrease the uncertainty caused in the SFE procedure by the 1:20 solid dilution of the samples with high purity quartz by constructing a new reduced-size SFE column (MCHUGH & KRUKONIS, 1986; MCHARDY & SAWAN, 1998; BAIG et al., 2011). In the first step the length of the column was decreased from 12.86 cm to 7.25 cm, which resulted in a 43.61% decrease in the volume. The extraction time required for samples with different carbonate contents was determined on the basis of kinetic studies. The results of the SFE procedure were compared with those of the BCR sequential procedure on the same samples. It was hypothesized that the easily extractable element content (water-soluble, carbonate-bound) could be characterized more reliably with the reduced size SFE-column than with the earlier version of the procedure.

Materials and methods

Samples

SFE kinetic studies were carried out using soil samples from the soil bank of the Department of Soil Science and Agrochemistry of Szent István University, having the following characteristics:

- Mosonmagyaróvár soil (alluvial loam from the Danube); 22.1% CaCO₃, 2-3% humus;
- Nagyhörcsök soil (loam on loess); 4.8% CaCO₃, 3-3.5% humus;
- Kecskemét soil (alluvial sand); 3.8% CaCO₃, 1% humus.

The following samples were used to compare the information content of BCR and SFE fractionation:

- The BCR CRM 701 lake sediment reference sample (RAURET et al., 2001) and
- A sediment sample from a fish pond chain constructed on the Rákos Creek between Gödöllő and Isaszeg (HELTAI et al., 1998).

Modified Supercritical Fluid Extraction

The SFE sequential extraction procedure was carried out in supercritical extraction equipment constructed from Jasco type modules, which allows the continuous flow extraction of the sample with supercritical CO₂, subcritical H₂O and a CO₂/H₂O mixture (Table 1). The extractor (Figure 1) consisted of two Jasco PU 980 HPLC pumps (a), one of them equipped with a cooler (b) to keep the flow of carbon dioxide at a temperature of between -6 and -8°C using a Neslab RTE-100 thermostat, a Jasco CO 980 column oven (d), and a Jasco 880-81 back pressure regulator (e). The cooled pump was connected directly to the carbon dioxide bottle

(c) by means of a conical tube (HELTAI et al., 2002; HELTAI et al., 2005; HELTAI, 2005; HORVÁTH et al., 2013).

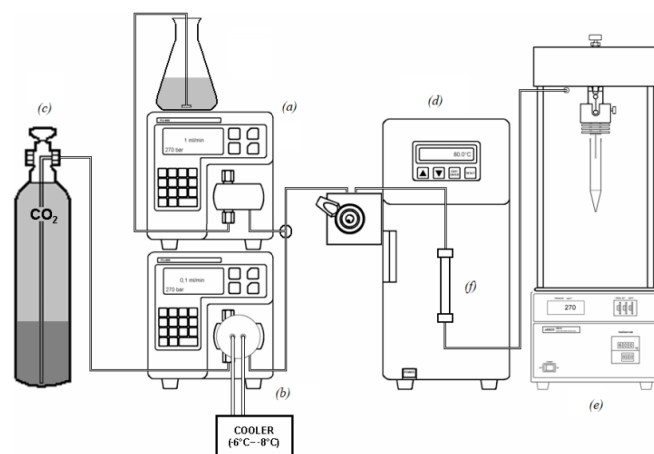


Figure 1
Schematic illustration of the supercritical fluid extractor (HALÁSZ et al. 2008)

The reduced-size column (*f*) was filled with a mixture of 0.500 g sample, with different carbonate contents, and 1.000 g analytical grade quartz sand (VWR, Radnor) (that also served as a blank). The duration of extraction was determined according to the CaCO_3 content ($m/m\%$) of the samples (*Table 1*). The flow rates of the pumps are summarized in *Table 2*. During the SFE sequential extraction the aqueous phase extracts were collected for 5 min intervals and 1 mL 0.5 mol dm^{-3} HNO_3 was added to each fraction.

Table 1
Basic scheme of the modified 3-step $\text{H}_2\text{O}/\text{CO}_2$ sequential extraction

Step	Extractant	Temperature (°C)	Pressure (MPa)	Duration (min)	Chemical information (fraction)
1.	Supercritical CO_2	80	27	60	CO_2 -soluble organic fraction and sulphides
2.	Subcritical H_2O	80	27	90	H_2O -soluble fraction
3.	Subcritical $\text{H}_2\text{O}/\text{CO}_2$ ($\approx 10 \text{ (V/V)\%}$)	80	27	90-240	Fraction bound to carbonates

Table 2
Flow parameters of the SFE pumps

Step	Extractant	Pump (H ₂ O) (mL/min)	Pump (CO ₂) (mL/min)
1.	Supercritical CO ₂	0	1
2.	Subcritical H ₂ O	1	0
3.	Subcritical H ₂ O/CO ₂ (≈ 10 (V/V)%)	1	0.1

BCR sequential extraction

The reagents applied in the BCR (3+1) step sequential extraction procedure, together with the corresponding chemical information, are described in *Table 3*. Following the BCR protocol, the polymer vessels and tools were rinsed in 4M HNO₃ prior to use. Samples weighing 1.0000 (\pm 0.0050) g were placed in 50 mL Falcon tubes (Shimadzu ATX224).

Table 3
Reagents applied in the BCR (3+1) sequential extraction procedure to fractionate the mobilizable element content of sediments and soils

<i>Reagents applied in BCR sequential extraction steps</i>	<i>Chemical information</i>
Step 1: 1.0000 g sample + 40 mL 0.11 mol dm ⁻³ HOAc	Water-soluble, exchangeable and carbonate-bound element content
Step 2: solid residue from Step 1 + 40 mL 0.1 mol dm ⁻³ NH ₂ OH·HCl (pH = 2)	Element content bound to reducible Fe/Mn oxides and hydroxides
Step 3: solid residue from Step 2 + 20 mL 8.8 mol dm ⁻³ H ₂ O ₂ (open system digestion), then + 50 mL 1 mol dm ⁻³ NH ₄ OAc (pH = 2)	Element content bound to organic matter and sulphides
Step (+1): microwave-assisted digestion of the residue from Step 3 with HNO ₃ /H ₂ O ₂	Residual mobilizable element content (e.g. bound to silicate matrices)

Step 1: The samples were shaken (240 rpm) with 40 mL 0.11 mol dm⁻³ acetic acid (Merck, Darmstadt) for 16 h, then centrifuged at 4000 rpm for 20 min. The supernatant was filtered through filter paper No. 589 (Hahnemühle, Dassel). The solid residue was shaken (240 rpm) with 20 mL distilled water for 15 min to remove residual extractant, and then centrifuged for 10 min at 4000 rpm, after which the supernatant was discarded.

Step 2: The solid residue from Step 1 was shaken (240 rpm) with 40 mL 0.1 mol dm⁻³ hydroxylamine hydrochloride (Merck, Darmstadt) (pH=2) for 16 h, then centrifuged at 4000 rpm for 20 min. The supernatant was filtered through filter paper No. 589 (Hahnemühle, Dassel). The solid residue was shaken (240 rpm) with

20 mL distilled water for 15 min to remove residual extractant, and then centrifuged for 10 min at 4000 rpm, after which the supernatant was discarded.

Step 3: The solid residue from Step 2 was digested with 20 mL 8.8 mol dm⁻³ hydrogen peroxide (Thomasker, Budapest) in an open system at 85°C for 8 h. After the addition of 50 mL 1 mol dm⁻³ ammonium acetate (Merck, Darmstadt) (pH =2) the mixture was shaken (240 rpm) for 16 h, then centrifuged at 4000 rpm for 20 min. The supernatant was filtered through filter paper No. 589 (Hahnemühle, Dassel). The solid residue was shaken (240 rpm) with 20 mL distilled water for 15 min to remove residual extractant, and then centrifuged for 10 min at 4000 rpm, after which the supernatant was discarded.

Step (+1): The solid residue from Step 3 was digested with a mixture of 5 mL 65(m/m)% HNO₃ (Honeywell, Seelze) and 2 mL 30(m/m)% H₂O₂ (Thomasker, Budapest). A CEM MARS 5 closed device with temperature and pressure monitoring was used for microwave digestion with the operational parameters proposed by the manufacturer.

Pseudototal: The total soluble (mobilizable) element content was also determined with the procedure described above (Step (+1)), but in this case 0.5000 g original solid sample was weighed into the digestion vessel.

Elemental analysis

Instrument: A Horiba Jobin Yvon ACTIVA-M inductively coupled plasma optical emission spectrometer was used. The optimized operational parameters were: *incident power 1200 W; plasma argon gas flow 16.04 L/min; sheath argon gas flow 0.288 L/min; nebulizer pressure 2.89 bar; nebulizer sample uptake 0.83 mL/min; auxiliary gas flow 0.589 L/min; argon moistening; cyclon type spray chamber and Meinhard nebulizer; internal standard: Yttrium (1 mg/L).*

Calibration: Six elements (Cd, Cr, Cu, Ni, Pb and Zn) certified as BCR CRM 701 reference samples (USEPA, 1994; WAI & WANG, 1997, ZEMBERYOVÁ et al., 2006; ZHAN et al., 2014) and Ca measured. Calibration was performed with the MERCK CertiPUR 1.11355.0100 ICP multi-element standard solutions diluted with extractant-solvents.

Wavelengths of analytical lines: *Ca:* 315.887 nm and *Ca:* 393.366 nm, *Ni:* 222.296 nm, *Cd:* 214.441 nm, *Pb:* 220.353 nm, *Cr:* 205.571 nm, *Zn:* 231.857 nm and *Cu:* 324.754 nm.

Results

Kinetic study

The dissolved Ca²⁺ amounts gained during 5-min intervals and the cumulative calcium content extracted during these 5-min intervals from the Nagyhörcsök soil sample with a subcritical H₂O/CO₂ mixture using the original preparative SFE column (2011) and the reduced-size column (2015) are shown in *Figures 2 and 3* respectively. It can be seen that the cumulative Ca²⁺ amounts extracted changed and

that more calcium was extracted with the modified method. In earlier experiments it was observed that a change in temperature may significantly influence the speed of the reactions, and rapid pressure changes may also occur, causing the samples to stick in the column, so the kinetic studies were performed at a well-controlled constant temperature ($T = 80^{\circ}\text{C}$).

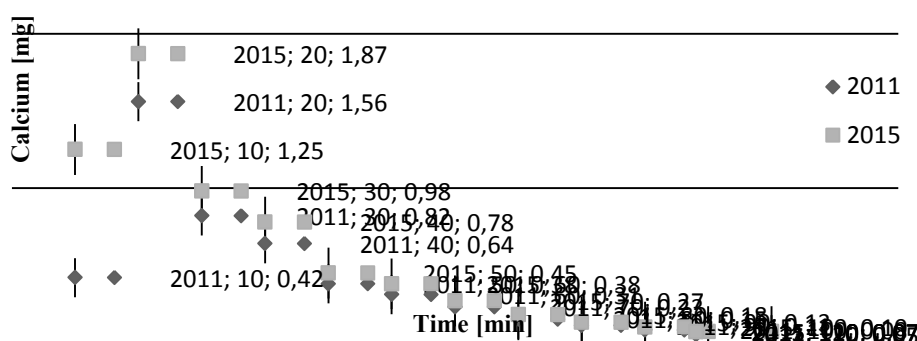


Figure 2

Calcium amounts extracted during 5-min intervals by the $\text{H}_2\text{O}/\text{CO}_2$ extractant from 0.5 g Nagyhörcsök soil samples as a function of time using the original (2011) and reduced-size (2015) columns

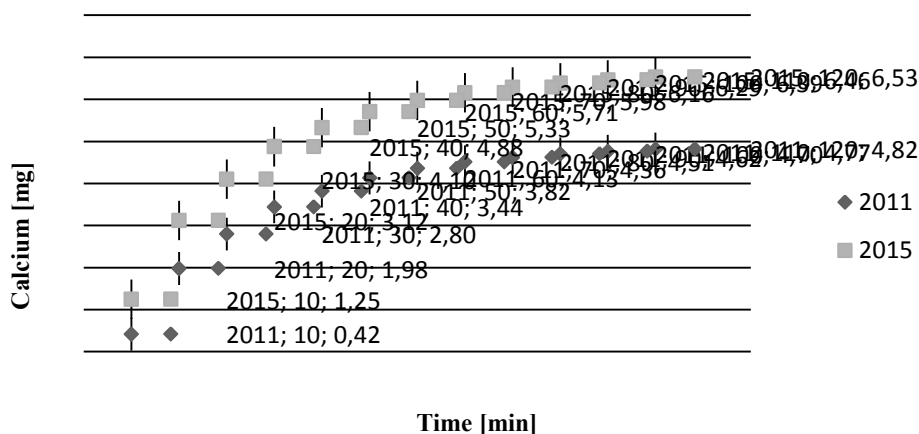


Figure 3

Cumulative calcium amounts extracted in 5-min intervals by the $\text{H}_2\text{O}/\text{CO}_2$ extractant from 0.5 g Nagyhörcsök soil samples as a function of time using the original (2011) and reduced-size (2015) columns

On the basis of *Figure 2* the extraction time necessary in the 3rd SFE step for samples with different carbonate contents can be determined when the dissolved Ca-carbonate content in the 5-min intervals approaches zero (*Table 4*)

Table 4
Extraction times necessary for samples with different lime contents in the 3rd SFE step

Kecskemét soil 3.8% CaCO ₃	120 min
Nagyhörcsök soil 4.8% CaCO ₃	120 min
Mosonmagyaróvár soil 22.1% CaCO ₃	180 min

Comparison of fractionation results obtained using the BCR and improved SFE procedures

The fractionation results achievable with the BCR sequential extraction procedure (*Table 5*) and with the improved SFE procedure (*Table 6*) were compared using the BCR CRM-701 sample and a sediment sample taken from pond No. VII in the Gödöllő-Isaszeg lake system.

In *Table 5* figures marked with an asterisk* indicate that the measured and certified values for the BCR CRM-701 sample were significantly different at the p=5.0 % level. These differences were not significant at the p=0.1% level. It could be seen that the measured and certified concentrations for BCR CRM-701 exhibited acceptable agreement, i.e. the BCR sequential extraction was performed reliably and the fractionation results obtained for the Gödöllő lake sediment sample were also reliable.

A comparison of the fractionation results for the BCR CRM-701 and Gödöllő lake sediment samples, showed that both samples contained significant amounts of PTEs in all fractions and that the distribution of individual elements between the fractions gave a clear reflection of the origin of the contamination (HELTAI et al., 1998). It can be seen that the most easily mobilized 1st fraction contained PTE amounts of the same order of magnitude in both samples with the exception of copper.

It is clear from *Tables 5* and *6* that the 1st BCR step dissolved similar, though not exactly the same amounts of PTEs as the three SFE steps together, i.e. the SFE procedure characterized the easily mobilizable PTE fractions in a more detailed way.

Table 5

Results of fractionation of the BCR CRM-701 sample and the Gödöllő lake sediment sample using the BCR sequential extraction technique (** reference: European Commission, BCR-701 Lake Sediment (trace elements) - BCR-701 Certified)

BCR procedure steps	Element	BCR CRM-701 (mg/kg)	Certified value (mg/kg) **	Recovery as a % of certified value (%) **	Gödöllő (2003) (mg/kg)
BCR 1st	Cd	6.96 ± 0.09	7.3 ± 0.4	95.34	7.25 ± 0.18
	Cr	2.68 ± 0.02*	2.26 ± 0.16	118.58*	4.13 ± 0.13
	Cu	46.8 ± 0.1*	49.3 ± 1.7	94.93*	0.2 ± 0
	Ni	15.8 ± 1.6	15.4 ± 0.9	102.60	21.1 ± 0.4
	Pb	3.55 ± 0.22	3.18 ± 0.21	111.64	6.32 ± 0.25
	Zn	192 ± 2	205 ± 6	93.66	344 ± 13
BCR 2nd	Cd	3.66 ± 0.55	3.77 ± 0.28	97.08	21 ± 0.9
	Cr	46.6 ± 6	45.7 ± 2.0	101.97	294 ± 9
	Cu	112 ± 2	124 ± 3	90.32	14 ± 1
	Ni	29 ± 4.7	26.6 ± 1.3	109.02	83.9 ± 2.1
	Pb	116 ± 3	126 ± 3	92.06	257 ± 11
	Zn	117 ± 15	114 ± 5	102.63	737 ± 22
BCR 3rd	Cd	0.09 ± 0.05	0.27 ± 0.06	33.33	0.51 ± 0.04
	Cr	118 ± 9	143 ± 7	96.55	551 ± 61
	Cu	53.1 ± 1.2	55 ± 4	96.55	136 ± 2
	Ni	14.9 ± 4.2	15.3 ± 0.9	94.77	37.9 ± 0.4
	Pb	9.2 ± 0.8	9.3 ± 2.0	98.93	97.8 ± 0.6
	Zn	42.4 ± 2.5	46 ± 4	92.17	101 ± 1
BCR 4th (+1)	Cd	1.68 ± 0.03			0.97 ± 0.15
	Cr	131 ± 1			101 ± 2
	Cu	37.8 ± 1.3			52.2 ± 0.2
	Ni	59.6 ± 1.8			30.5 ± 0.1
	Pb	15.2 ± 0.58			14.4 ± 0.56
	Zn	139 ± 2			70.7 ± 2.2
Σ BCR(1+2+3+4)	Cd	12.39			29.73
	Cr	298.28			950.13
	Cu	249.7			202.4
	Ni	119.3			173.4
	Pb	143.95			375.52
	Zn	490.4			1252.7
Pseudo-total element content	Cd	9.52 ± 0.53			25.7 ± 0.9
	Cr	235 ± 12			932 ± 32
	Cu	210 ± 17			154 ± 54
	Ni	72 ± 5			127 ± 4
	Pb	112 ± 10			291 ± 10
	Zn	347 ± 18			1139 ± 39

Table 6
Results of fractionation of the BCR CRM-701 sample and the Gödöllő lake sediment sample using the SFE sequential extraction technique

SFE procedure steps	Element	BCR CRM-701	Gödöllő (2013)
		(mg/kg)	(mg/kg)
SFE 1st	Cd	0.01 ± 0.0014	n. d.
	Cr	0.10 ± 0.0783	n. d.
	Cu	0.04 ± 0.0005	n. d.
	Ni	0.07 ± 0.0378	0.04 ± 0.0235
	Pb	0.03 ± 0.0115	0.01 ± 0.0056
	Zn	0.05 ± 0.0014	0.04 ± 0.0072
SFE 2nd	Cd	0.15 ± 0.0043	0.10 ± 0.0029
	Cr	0.54 ± 0.0043	0.84 ± 0.0137
	Cu	5.13 ± 0.0086	0.94 ± 0.0079
	Ni	1.84 ± 0.0115	1.99 ± 0.0734
	Pb	n. d.	n. d.
	Zn	2.60 ± 0.0129	3.56 ± 0.0122
SFE 3rd	Cd	3.51 ± 0.0277	6.48 ± 0.0086
	Cr	0.66 ± 0.0019	4.41 ± 0.0115
	Cu	28.22 ± 0.0630	0.12 ± 0.0202
	Ni	6.81 ± 0.2118	37.69 ± 0.3907
	Pb	1.76 ± 0.1317	n. d.
	Zn	82.67 ± 0.2748	464.55 ± 3.0058
Σ SFE(1+2+3)	Cd	3.67	6.58
	Cr	1.30	5.25
	Cu	33.39	1.06
	Ni	8.72	39.72
	Pb	1.79	0.01
	Zn	85.32	468.15
Pseudototal element content	Cd	9.52 ± 0.53	25.7 ± 0.9
	Cr	235 ± 12	932 ± 32
	Cu	210 ± 17	154 ± 54
	Ni	72 ± 5	127 ± 4
	Pb	111 ± 10	291 ± 10
	Zn	347 ± 18	1139 ± 39

In *Tables 7* and *8* fractions providing similar chemical information are compared for the two samples. The ratio of potentially toxic element contents extracted by the corresponding steps in the BCR and SFE extraction procedures were compared for both samples.

It could be established that:

- The ratio of the BCR 1 to SFE(H₂O) fraction was significantly higher than 1 in all cases, i.e. the water-soluble PTE content made up only a very low proportion of the BCR 1 fraction.
- The ratio of the BCR 1 to SFE(H₂O/CO₂) fraction was in the 0.5-2.5 range for the various elements in both samples, indicating that the carbonate-bound fraction made up the majority of the easily mobilizable element fraction.
- The ratio of the BCR 3 to SFE(CO₂) fractions showed that only a very small amount of PTEs could be found in sulfides or bound to organic matter substances.

Table 7

Ratios of the element contents extracted in the 1st and 3rd steps of the BCR procedure to the corresponding steps in the SFE sequential extraction for the BCR CRM-701 sample

Ratio of element contents in the two different fractions	Cd	Cr	Cu	Ni	Pb	Zn
BCR 1/SFE(H ₂ O)	51.9	4.6	10.4	9.1	n.d	85.1
BCR 1/SFE(H ₂ O/CO ₂)	2.3	3.7	1.9	2.4	1.9	2.7
BCR 1/SFE ((H ₂ O)+(H ₂ O/CO ₂))	2.2	2.1	1.6	1.9	1.9	2.6
BCR 3/SFE(CO ₂)	38.9	1889.9	2208.3	268.5	490.2	1066.6

Table 8

Ratios of the element contents extracted in the 1st and 3rd steps of the BCR procedure to the relevant steps in the SFE sequential extraction for the Gödöllő-Isaszeg lake sediment sample

Ratio of element contents in the two different fractions	Cd	Cr	Cu	Ni	Pb	Zn
BCR 1/SFE(H ₂ O)	69.5	4.9	0.2	10.6	n.d	96.5
BCR 1/SFE(H ₂ O/CO ₂)	1.1	0.9	0.5	0.6	n.d	0.7
(BCR 1)/SFE ((H ₂ O)+(H ₂ O/CO ₂))	1.1	0.8	0.2	0.5	n.d	0.7
(BCR 3)/SFE(CO ₂)	n.d	n.d	n.d	939.3	14409.5	2390.6

Conclusions

On the basis of the results it can be concluded that

- By using the improved SFE technique supplementary information can be obtained rapidly about the composition of the most mobile PTE fraction obtained by acetic acid extraction in the 1st step of the BCR procedure. The water-soluble and carbonate-bound fractions in sediment and soil samples were reliably distinguished by the SFE procedure.
- The use of a reduced-size SFE column allowed the solid dilution rate to be decreased, in an increase in the rate of carbonate dissolution via hydrocarbonate formation in the 3rd step in the SFE procedure and the

reliability of the analytical determination was improved simultaneously. We used to the smaller amount of quartz for solid phase dilution.

- The improved SFE sequential procedure takes only 7-8 hours, including the preliminary temperature stabilization of the system, whereas the BCR procedure needs at least 5 days, including pretreatments.

This work was financed by the Hungarian Scientific Research Fund (OTKA 108558) and the Research Centre of Excellence (11476-3/2016/FEKUT).

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