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Effects of grinding and shaking on Cd, Pb and Zn distribution in anthropogenically impacted soils

Christophe Waterlot [a,b,]*, Géraldine Bidar [a,b], Christelle Pruvot [a,b], Francis Douay [a,b]

[a] Université Lille Nord de France, Lille 59046, France
[b] Groupe ISA, Equipe Sols et Environnement, Laboratoire Génie Civil et géoEnvironnement (LGCgE) Lille Nord de France (EA 4515), 48 Boulevard Vauban, 59046 Lille Cedex, France

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ABSTRACT

The effects of grinding size and shaking process on the results of Cd (cadmium), Pb (lead) and Zn (zinc) distribution measurements three agricultural and three kitchen garden soils highly contaminated by past atmospheric fallout of two lead and zinc smelters in northern France were studied. The physico-chemical parameters and pseudo-total concentration of metals within these soils were determined. The fractionation of metals was performed in triplicate, using the procedure recommended by the Standards, Measurements and Testing program (SM&T), on each air-dried soil sample, ground to pass through 2-mm, 0.315-mm and 0.250-mm sieves and using a reciprocating or rotary shaker. The samples were analysed by flame or electrothermal absorption atomic spectrometry using a self-reversal background system. For both shaking processes, the grinding size had no effect on the fractionation of metals in contaminated agricultural soils. In contrast, using a reciprocating shaker, the fractionation of metals in the kitchen garden samples sieved at <2 mm was so different that in the samples prepared to pass through the 0.315-mm and 0.250-mm sieves. Therefore changes (use of a 50 mL graduated polypropylene centrifuge tube, evaporation of the solution to a fixed volume in step 3 and the use of an automatic shaking heating bath) were made to the initial procedure and a rotary shaker was used to improve the suspension of the soil samples during extraction. For all grinding sizes, the fractionation of the three metals contained in the contaminated kitchen garden soil samples was successfully achieved. Nevertheless, some discrepancies from samples sieved at <2 mm were obtained. On the other hand, it is worth noting that the effect of the type of shaker on the distribution of metal depended on the soil and the grinding size. From an analytical point of view, precision and trueness were improved after optimisation of the procedure for all sequential extraction procedure steps. The best results were obtained for samples sieved at <0.250 mm. Similar trends were obtained using the CRM BCR®-701 certified material. All the results showed that optimisation of the sequential extraction procedure could be adopted for the Cd-, Pb- and Zn-partitioning in contaminated kitchen garden soils with high-level anthropogenic sources.

1. Introduction

The concentration level of metals in the environment has substantially increased over the past few years mainly because of industrial and human activities. It is now widely accepted that the determination of total metal contents is not sufficient to assess the impact of metals on soils [1–4] and on risks to human [2,5–9]. Since the toxicity of metals in soils is known to be related to the chemical form, techniques have been developed to manage the behaviour of metals within soils. Among these techniques, Fourier transform infrared spectrometry [10,11], nuclear magnetic resonance spectroscopy [12], X-ray diffractometry [13], scanning electron microscopy [14] and others have been used to determine the chemical speciation of metals [15–21]. However, in most environmental studies of contaminated soils, the behaviour of metals is studied through the assessment of their mobility and is often evaluated using chemical-extracting solutions. The first extraction procedures were published in the 1970s and 1980s [22] but the development of a unified sequential extraction procedure to determine the fractionation of metals in fluvial sediments started in the late 1970s [23]. Various sequential extraction procedures have been developed over the past 20 years and a variety of environmental samples such as sewage sludge, composts, oils, fly ashes, aerosols, sediments and soils have been studied [24]. The objectives of all of these studies were to evaluate the mobility and/or bioavailability of metals, and authors often suggested identifying binding sites of metals to assess metal accumulations and transport mechanisms using

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* Corresponding author. Tel.: +33 03 28 38 48 58; fax: +33 03 28 38 48 47.
E-mail address: christophe.waterlot@isa-lille.fr (C. Waterlot).

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relationships between the metal mobility in the operationally defined fractions and the chemical reagents used in the sequential extraction procedure [24–29].

The accuracy data provided by the sequential extraction procedures depends on the number of stages, commonly between three and nine [30–38], and the choice of the chemical reagents used in each of the extraction steps [24,39–40]. Ideally, each procedure uses a series of progressively harsher chemical extracting solutions to attack a geochemical compartment or a specific soil component with nominal dissolution of the non-target phases. However, it has been clearly demonstrated that the metal fractionation depended on the order in which the reagents were added, the type of reagents and the nature of the individual metal [35,41–43] in the sequential extraction procedure especially to access the oxy-hydroxides and organic matter bounds when heating is recommended [44]. This is why some authors have proposed sodium pyrophosphate for extraction of metals associated with humic acid prior to assessing the reducible fraction [30,35,45,46]. However, a number of errors have been detected using this reactant when soils contained higher organic matter and debris [46].

The most recent sequential extraction procedure was developed by the Community Bureau of Reference (BCR) [47]. Because a number of authors have highlighted pitfalls in the use of the BCR three-steps procedure [48–51], modifications were required to improve it and a modified protocol was proposed by the Standards, Measurements and Testing program (SM&T) of the European Community, focusing on the reproducibility of the extraction and the selectivity of the extracting solutions [42,52,53]. For instance, in the Tessier procedure [23], a hydroxylamine hydrochloride and acetic acid mixture was used. In the original BCR procedure, it was 0.1 M hydroxylamine hydrochloride at $\text{pH}=2$ [32,49] whereas 0.5 M hydroxylamine hydrochloride at room temperature and $\text{pH}=1.5$ was recommended by the SM&T program [54–57]. The optimised sequential extraction scheme was adopted for the certification of the extractable-metal concentrations in reference sediments and soils [51,54–56] and was therefore considered as an appropriate extraction procedure to evaluate the fractionation of metals in soils. For each extracting solution, nominal target phases were proposed as follows: (i) exchangeable, water soluble and acid-soluble, (ii) reducible and (iii) oxidisable [56,58–60].

Nowadays, the procedure is widely used throughout the world by scientists to predict metal partitioning in samples [36,54,61], sediments from estuaries, sewage sludge, non-polluted soils [62] as well as polluted soils characterised by a multi-element contamination [31,63,64]. Many studies have investigated the difference between metal contents in samples for different stirring times [42,62,65–67], the method used for the separation of the liquid and solid phases and centrifugation conditions [56,68]. It was observed that the reproducibility of extraction yields was strongly affected by the shaking speed and time [56] and, in a lesser extent, by the type of shaker used. Indeed some authors found differences between metal extracted using both end-over-end and reciprocating shakers [69,70] whereas others found no difference [71]. Arunachalam et al. [72] reported no difference in Cd and Pb extracted following the BCR procedure between the two modes of shaking whereas some effects were observed for Zn concentrations [72]. On the other hand, some authors mentioned the non-specificity of the reagent and also the possibility of redistribution of metals during the extraction procedure, depending on the physico-chemical parameters of soils and the procedure used to prepare them. With contaminated soil samples, the preparation is a major concern before conducting the extraction procedure [24]. Yet various procedures have been reported in the literature. After air-drying, the soil aggregates can be broken down using an agate mortar, a domestic cutter or a jaw-crusher [55,69]. Then the soil samples are generally ground and sieved to pass through a 1-mm nylon sieve [4,73], a 2-mm nylon sieve, a 2-mm stainless steel sieve [50], <2 mm [33,36,74,75] or a >2 mm [76].

One of the main sources of variability in sequential extraction results is the heterogeneity of the sample when anthropogenic contaminations are adding up over the industrial ones [50]. Some authors advocate to work with 3 g of samples to improve representativeness of the extracted metals [50,65] but the revised BCR sequential procedure applies this mass at 1 g [54–57]. There is no a real solution to the problem of heterogeneity but it is known that grinding increases the homogeneity of samples [68]. However, grinding also affects the characteristics of the particles and the specific surface of the material, reflecting changes in the extractability of metals [68,76,77]. Although the small particular size used in sediments is <63-μm, the most size used for the preparation of soil samples is <2-mm [78]. However, different grinding sizes (2 mm, 1 mm, 315 μm, 250 μm, 180 μm, 150 μm and 75 μm) can be found in the literature, reflecting that there is no agreed-upon size fraction to be analysed in environmental geochemistry.

Based on these considerations, we chose three agricultural and three kitchen garden soils. These garden soils have received various waste materials, slag, gravels, pieces of bricks, local contaminated composts. Compared to the agricultural soils, high heterogeneity of the garden soils, that could induce variability within the samples, was shown [79]. The sequential extraction scheme recommended by the SM&T program was applied on the six soils. The main objectives of the study were (1) to evaluate the effect of grinding size on the fractionation of cadmium (Cd), lead (Pb) and zinc (Zn), and (2) to compare the fractionation of these metals with regard to the type of shaker used. Other changes were made in order to improve the precision and the trueness of the sequential extraction procedure. Using the BCR®-701 material as a certified reference material, the bias value at each step of the procedure was calculated for each metal taking into account the literature data. Finally, these results are discussed.

2. Experiment

2.1. Sampling sites

Six sites were selected between 2001 and 2004 in the surroundings of two Pb–Zn smelters located in the north of France (Metaleurop Nord at Noyelles-Godault and Nyrstar at Auby). Sampling was undertaken in the surface layer of the soils (0–25 cm). Depending on the site surface, five to 13 elementary samples were taken, homogenised and pooled to constitute a representative sample. The six sample sites were divided into two groups, depending on their uses. The first group was composed of three agricultural soils located 3 km from the former Metaleurop Nord smelter. The second was made up of three kitchen garden soils chosen in the vicinity of the two smelters less than 2 km away. The criterion for selecting the kitchen garden soils was not their geographical location but the Pb contamination level.

2.2. Physico-chemical parameters of soils

Each soil sample was air-dried at room temperature and then mixed, homogenised to constitute a representative soil sample of the site and crushed to pass through a 10-mm stainless steel sieve. Each representative sample was prepared according to the NF ISO 11464 standard. Granulometric analysis was carried out by the dispersion of mineral particles after destruction of the organic matter using hydrogen peroxide and separation of the particles into different classes by sedimentation (particles $< 50\ \mu m$ and sieving particles $> 50\ \mu m$) [80]. Soil pH was measured in water as described previously [81]. Organic carbon and total nitrogen were determined by dry combustion according to the NF ISO 10694 and NF ISO 13878 standards after burning 50 mg of samples at 1000 °C in the presence of O_2 [82]. Total carbonates were determined by measuring the volume of CO_2 released after a reaction with HCl, using a Bernard calcimeter, as described in the NF ISO 10693 standard. Assimilated phosphorous was extracted with ammonium oxalate solution (0.1 M) extraction following the revised procedure of Joret–Hébert [83] and was measured by spectrocolorimetry (NF X 31-161). The cation exchange capacity (CEC) was determined after percolation of 1.0 M ammonium acetate solution at pH 7 according to the NF X 31-130 standard [84]. All these analyses were performed by the INRA Soil Analysis Laboratory (Arras, France), accredited by COFRAC (French Accreditation Committee) according to the ISO 17025 standard.

2.3. Microwave-assisted digestion

Digestion of air-dried soil samples (0.3 g) ground and sieved to pass through a 0.250-mm sieve with an ultra-centrifugal mill (Retsch type ZM 200; Hann, Germany) was conducted in a microwave pressure digestion unit (Bergh of Speed waveTM MWS-2; Eningen, Germany) with a built-in *in-situ* IR temperature measurement device equipped with a Teflon-coated oven cavity, removal 10-position sample carousels and an exhaust tube. Soil samples were digested with a mixture of concentrated nitric acid (70% m/m, 1.5 mL, J.T. Baker for metal trace analysis; Deventer, Netherlands) and hydrochloric acid (37% m/m, 4.5 mL, J.T. Baker). After addition of these acids to the Teflon TFM containers (DAP-60K), the vessels were shaken until the carbon dioxide had escaped. The vessels were then closed and were capped under pressure using the capping station (PTFE-thread-cap) and aluminium disc seals. The digestion was conducted at controlled temperature and power was pulsed by the microwave oven to maintain temperature at a constant value. Quality control was based on the use of a certified sample (CRM BCR-141R, certified reference material composed of calcareous loam soil with a certified value of the *aqua regia*-extractable contents of metals). The microwave program was: stage 1: 7 min at 160 °C and 40 W; stage 2: 30 min at 210 °C and 80 W; stage 3: 210 °C at 30 min and 100 W.

2.4. Sequential extraction procedure

The quality of the analytical data for the sequential extraction procedure was assessed by carrying out analyses of the sediment CRM BCR®-701 (Certified reference material composed of sediment from Lake Orta, Piemonte, Italy). Certified values for extractable metals are available for the first three steps of the procedure whereas indicative values are available for the *aqua regia*-extractable metal values.

Each sample of soil was dried at room temperature, ground and sieved to pass through a 2-mm mesh to obtain 600 g of soil. Then a subsample of 300 g was sieved to pass through a 315-μm mesh and a gentle and very rapid size reduction (< 0.250-mm) of 150 g of this subsample was achieved using an ultra-centrifugal mill (Retsch type ZM 200) fitted with rotors and ring sieves made from titanium together with cassette and cover with titanium-niobium coating. The fractionation of Cd, Pb and Zn in the soil samples was carried out using the modified three-step extraction procedure recommended by the SM&T program and the *aqua regia* extractable concentrations were given [55].

Mixtures of soil samples with extracting solutions were reacted in a linear reciprocating shaker with an amplitude of 60 mm and speed range of 120 ± 10 rpm or rotary shaker at 10 ± 1 rpm. Although a simple decantation is recommended in the BCR protocol to recover the supernatant after the four extraction steps, the soil solution were extracted by decantation and filtered over an acetate Millipore membrane (Millipore, 0.45-μm porosity, Minisart). No significant effect (Kruskal–Wallis test, $p > 0.05$) of the filtration on the Cd, Pb and Zn measured in the four extracting solutions was observed using the CRM BCR®-701 as reference material (see supplementary data).

The bottle was manually shaken before sampling to maintain the homogeneous distribution of particles and to avoid segregation of particles after bottling [77].

The procedure was carried out in triplicate as described below:

Step 1, fraction 1 (F1): 40 mL of 0.11 M acetic acid (from glacial acetic, $d = 1.048$, Acrōs Organics, Noisy-le-Grand, France) was added to 1 g of the dry soil sample in a 250 mL polypropylene wide-mouthed bottle. The mixture was shaken at room temperature for 16 h on a linear mechanical reciprocating shaker/rotary shaker. The extract was separated from the solid by centrifugation (Rotanta 460 Hettich; Tuttingen, Germany) at 3000 *g* (4394 rpm) for 20 min. After filtration, the supernatant was put into a polyethylene container and stored at 4 °C until analysis. The residue was washed with 20 mL of distilled water by shaking for 15 min and was centrifuged at 3000 *g* for 20 min. The washings were discarded, taking care not to discard the solid residue.

Step 2, fraction 2 (F2): 40 mL of a freshly prepared solution of 0.5 M hydroxylamine hydrochloride (Acrōs Organics) adjusted to pH 1.5 with nitric acid (J.T. Baker) was added to the residue from step 1. The mixture was first resuspended by manual shaking and extraction was pursued with a mechanical reciprocating shaker/rotary shaker for 16 h. The residue was extracted and separated by centrifugation at 3000 *g* for 20 min. After filtration, the supernatant was put into a polyethylene container. Nitric acid (123 μL) was added to the supernatant and the mixture was stored at 4 °C for analysis. The residue was washed with 20 mL of distilled water by shaking for 15 min and then centrifuged, and the washings were discarded as in step 1.

Step 3, fraction 3 (F3): 10 mL of 8.8 M hydrogen peroxide (J.T. Baker) was added in small aliquots to the residue from step 2 into a 250-mL polypropylene wide-mouthed bottle. The bottle was loosely covered with its cap so that gas could escape. The residue was digested for 1 h at room temperature with occasional manual shaking. The digestion was followed by heating the bottle at 85 °C in a water bath for 1 h. The bottle cap was then removed and the mixture was evaporated to obtain a volume less than 3 mL. Another second 10 mL of hydrogen peroxide was added, the cap of the bottle was closed and the mixture was heated at 85 °C for the first 30 min with occasional manual shaking and without any shaking for the second 30 min. The bottle cap was then removed to reduce the volume of liquid to about 1 mL. After cooling the moist residue, 50 mL of 1.0 M ammonium acetate (Acrōs Organics), adjusted to pH 2 with nitric acid, was added. The mixture was shaken at room temperature for 16 h on a mechanical reciprocating shaker/rotary shaker. The extract was separated from the residue by centrifugation and after filtration, the supernatant was put on the polyethylene container and stored at 4 °C until analysis. The residue was washed with 20 mL of distilled water by shaking for 15 min, centrifuged and the washings were discarded as in step 1.

Step 4, pseudo-total residual fraction (F4): The dried residual soil was manually crushed and 0.3 g was digested with a mixture of concentrated nitric acid (1.5 mL) and hydrochloric acid (4.5 mL) according to the protocol described to determine the pseudo-total concentration in soils.

The residual moisture contents were measured by weighing 1 g of individual soil samples after drying at 105 °C in an oven until it reached a constant mass according to the NF ISO 11465 standard. From this, a correction to dry mass was obtained and was applied to the reported analytical values.

The accuracy of the method comprised two components: precision and trueness [85]. Precision for each step was based on analyses of three replicates from a single bottle of the CRM and was defined as (standard deviation/mean) × 100. Trueness was calculated following the relation:

[(measured concentration − certified or indicative concentration)/certified or indicative concentration] × 100.

2.5. Analytical technique

A Shimadzu AA-6800 (Tokyo, Japan) atomic absorption spectrometer with an ASC-6100 autosampler (Shimadzu, Tokyo, Japan) was used to determine the pseudo-total concentrations of Cd, Pb and Zn and their concentrations in F1, F2, F3 and F4 extracting solutions. The spectrometer was fitted with an air–acetylene flame and boosted discharge hollow-cathode lamps in order to avoid molecular, structured and spectral interferences [86–88]. The instrumental parameters for achieving maximum sensitivity and precision in flame absorption atomic spectrometry (FAAS) are described in Table 1.

For the lowest Cd and Pb concentrations, a digital control technology graphite furnace atomiser (GFA-EX7, Tokyo, Japan) that had been fitted with a high-density graphite tube was used. The furnace program steps and the analytical characteristics were described in previous studies [86,87]. All analytical determinations of metals were performed in triplicates.

2.6. Statistical analysis

The comparison between Cd, Pb and Zn concentrations measured in extracting solutions from the soils sieved to pass through 2-mm, 0.315-mm and 0.250-mm sieves were performed using Kruskal–Wallis ANOVA as non parametric tests, in order to evaluate the effects of grinding size on the fractionation of Cd, Pb and Zn. All statistical tests were performed using Statistica 6.0 (Statsoft, Tulsa, OK, USA) and the level of significance was set at $\alpha = 0.05$.

Table 1
Instrumental parameters used for the determination of Cd, Pb and Zn by FAAS.

Parameter	Cd	Pb	Zn
Wavelength (nm)	228.8	217.0	213.856
Slit (nm)	1.0	1.0	0.5
Burner height (mm)	7	7	7
Air/acetylene flow rate (L min^{-1})	1.8	2.0	2.0
Lamp intensity (mA)	8–100	8–300	10–300

3. Results and discussion

3.1. Analytical characteristics of FAAS

Reagent blanks were negligible and no detectable contamination was found when aliquots of the sequential extraction reagents were processed and analysed with the samples. The detection limits (three times the standard deviation of the blank, $n = 10$) of metals in the different extracting solutions are given in Table 2. The relatively low detection limits for Cd, Pb and Zn by FAAS were due to the boosted discharge hollow-cathode lamps used in this study instead of normal lamps combined with a deuterium lamp for the background correction. One of the advantages of the self-reversal background corrector is that it improves the stability of the baseline during the flame atomisation of metals.

3.2. Physico-chemical parameters of soils

Physico-chemical parameters of the sampled soils are shown in Table 3. The fraction of silt was predominant and all soils were alkaline in spite of a low $CaCO_3$ content in the ME-21 and ME-B2 agricultural soils. The highest organic carbon and available phosphorous contents were measured in the 04-173 kitchen garden soil. These high concentration values are explained by the gardeners' intensive use of phosphate and organic amendments. As a consequence, the values of the C/N ratio in kitchen gardens were higher than those in agricultural soils. In comparison with agricultural soils, Douay et al. [79] recently highlighted that the high contamination level of kitchen gardens in the studied area was the main reason explaining the slowed down degradation of the organic matter.

3.3. Pseudo-total metal concentrations of soils

Soil samples were subjected to microwave-assisted digestion with *aqua regia* according to the protocol described above. The certified reference material (CRM BCR-141R) was used to optimise the digestion procedure. The results are summarised in Table 4. Pseudo-total Cd, Pb and Zn concentrations were generally in good

Table 3
Physico-chemical parameters of agricultural and kitchen garden soils.

Soils	Clay g kg^{-1}	Silt g kg^{-1}	Sand g kg^{-1}	OC g kg^{-1}	$CaCO_3$ g kg^{-1}	P_2O_5 g kg^{-1}	C/N	pH
Agricultural								
ME-01	239	595	166	35.9	84	1.210	16.5	8.10
ME-21	188	686	126	17.1	7	0.352	11.7	7.80
ME-B2	211	592	197	14.6	10	0.348	11.1	8.30
Kitchen garden								
04-173	147	449	404	102.0	23.2	2.68	31.8	7.73
04-176	197	644	159	34.4	86.1	0.567	21.0	8.16
04-179	190	540	270	27.2	74.6	0.682	16.5	7.86

Table 2
Detection limits of FAAS for analytes in sequential extraction reagents.

Fraction	Reagent	Nominal target phase	Detection limit (mg kg^{-1})		
			Cd	Pb	Zn
F1	CH_3CO_2H (0.11 mol L^{-1})	Exchangeable, water and acid soluble species	3.40	29.20	1.90
F2	$NH_2OH.HCl$ (0.5 M) adjusted to pH = 1.5 with HNO_3 (2 M)	Reducible species, bound to Fe and Mn oxyhydroxides	5.80	41.50	1.60
F3	H_2O_2 (8.8 mol L^{-1}) followed by $CH_3CO_2^- \ NH_4^+$ (1.0 mol L^{-1}) at pH 2	Oxidizable species, bound to sulfides and organic matter	4.30	16.20	0.70
F4	HCl (4.5 mL) + HNO_3 (1.5 mL)	Pseudo-total	4.90	51.70	1.90

agreement with the certified *aqua regia* soluble concentrations of these metals. The mean recoveries ranged from 94.2 to 100.7% and the precision values were 3% for Cd, 11.5% for Pb and 5% for Zn.

Table 4
Concentrations of Cd, Pb and Zn (mg kg^{-1}) in the certified reference material ($n=10$) and contaminated soil samples.

Sample	Cd	Pb	Zn
BCR CRM 141 R[a]	14.0 ± 0.4	51.3 ± 2.0	270 ± 8
BCR CRM 141 R[b]	13.2 ± 0.4	49.5 ± 5.7	272 ± 14
ME-01	13.2	768	913
ME-21	6.6	294	320
ME-B2	10.9	495	683
04-173	13.6	827	971
04-176	4.8	254	515
04-179	10.1	413	1313

[a] Certified values are means ± uncertainty (half-width of the 95% confidence interval).
[b] Values are means ± standard deviations, $n=10$.

Table 5
Quality control and validation of the sequential extraction procedure using the CRM BCR®-701 material ($n=3$).

Fraction	Metal	Obtained value[a] (mg kg^{-1})	Certified value[b] (mg kg^{-1})
F1	Cd	7.43 ± 0.12 (1.6)	7.34 ± 0.35
	Pb	3.07 ± 0.13 (4.2)	3.18 ± 0.21
	Zn	205 ± 8 (3.9)	205 ± 6
F2	Cd	3.72 ± 0.07 (1.9)	3.77 ± 0.28
	Pb	134 ± 2 (1.5)	126 ± 3
	Zn	122 ± 6 (4.9)	114 ± 5
F3	Cd	0.27 ± 0.02 (7.4)	0.27 ± 0.06
	Pb	9.3 ± 1.1 (11.8)	9.3 ± 2.0
	Zn	43.0 ± 5.0 (11.6)	45.7 ± 3.4
F4[c]	Cd	0.12 ± 0.03 (25)	0.13 ± 0.08
	Pb	11.6 ± 0.7 (6.0)	11.0 ± 5.2
	Zn	98 ± 5 (5.1)	95 ± 13

[a] Values are means ± standard deviations, with precision (%) in parentheses.
[b] Values are means ± uncertainty (half-width of the 95% confidence interval).
[c] Indicative values (means ± standard deviations).

No significant difference (Kruskal–Wallis test, $p > 0.05$) was obtained between the mean contamination levels of Cd ($p=0.83$), Pb ($p=0.83$) and Zn ($p=0.27$) in the three agricultural soils and those in the three kitchen gardens. However, it is noteworthy the high Zn contamination level of the 04-179 sample soil (Table 4) which was sampled near the Nyrstar smelter, one of the largest zinc production plants in Europe.

3.4. *Effect of grinding size*

Prior to investigating the effect of grinding size on the fractionation of Cd, Pb and Zn, the sequential extraction procedure was applied using the BCR®-701 certified reference material. Regarding the data set in Table 5, the results showed good agreement between the obtained and certified values for the three metals and for each step of the SM&T procedure. Very low standard deviations were obtained, reflecting good reproducibility of the sequential extraction procedure in determining the fractionation of Cd, Pb and Zn in this reference material. The overall precision for each step was very good (Table 5), except for Cd in step 4 (25%) due to the low Cd concentrations. Fraction-specific trueness for Cd, Pb and Zn was good, with values less than 7.8% of certified and indicative values. The application of a paired *t*-test ($p < 0.05$) showed that the measured and certified (or indicative) values were not significantly different.

To assess the effect of the grinding size on the metal's extractable content and on the reproducibility of the BCR procedure, 1 g of each soil sieved to pass through 2-mm, 0.315-mm and 0.250-mm sieves was extracted in triplicate. The sums of metal concentrations in the four fractions of the sequential extraction scheme and recovery rates are presented in Table 6. The sum of the extracted metals from the four steps compared well with the pseudo-total concentrations from the original CRM and contaminated soil samples. However, it is worth noting that significant differences were obtained between Cd concentrations measured in each sample sieved at < 2-mm and those prepared with a < 0.250-mm grinding size whereas for Pb and Zn, no significant differences were systematically obtained. As shown in Table 6, recovery rates were in the range of 76 to 107% for contaminated

Table 6
Sum of the extractable metals in the four fractions of the SM&T procedure (mean ± standard deviation, $n=3$) and recovery rates.

Soils	Cd (mg kg^{-1})[a]	Pb (mg kg^{-1})[a]	Zn (mg kg^{-1})[a]	Cd[b]	Pb[b]	Zn[b]
Agricultural						
ME-01 2 mm	10.5 ± 0.1^{c}	653 ± 15^{c}	838 ± 25^{c}	79.3 ± 0.8^{c}	84.9 ± 1.9^{c}	91.7 ± 2.7^{c}
ME-01 0.315 mm	10.5 ± 0.1^{c}	704 ± 5^{d}	910 ± 16^{d}	79.5 ± 0.5^{c}	91.6 ± 0.6^{d}	99.7 ± 1.7^{d}
ME-01 0.250 mm	11.5 ± 0.2^{d}	730 ± 12^{d}	978 ± 34^{e}	87.5 ± 1.6^{d}	95.0 ± 1.0^{e}	107.0 ± 3.7^{e}
ME-21 2 mm	5.0 ± 0.1^{c}	267 ± 5^{c}	316 ± 8^{c}	75.7 ± 1.9^{c}	90.7 ± 1.7^{c}	98.8 ± 2.6^{c}
ME-21 0.315 mm	5.9 ± 0.1^{d}	265 ± 10^{c}	311 ± 6^{c}	90.1 ± 2.2^{d}	90.1 ± 3.4^{c}	97.2 ± 1.8^{c}
ME-21 0.250 mm	6.0 ± 0.1^{d}	269 ± 3^{c}	330 ± 11^{c}	91.3 ± 1.2^{d}	91.3 ± 1.0^{c}	103.1 ± 3.4^{c}
ME-B2 2 mm	8.3 ± 0.2^{c}	438 ± 5^{c}	570 ± 7^{c}	75.8 ± 1.7^{c}	88.4 ± 1.0^{c}	83.4 ± 1.0^{c}
ME-B2 0.315 mm	9.7 ± 0.1^{d}	$408 \pm 6^{d,e}$	538 ± 5^{d}	88.8 ± 0.7^{d}	$82.5 \pm 1.3^{d,e}$	78.8 ± 0.7^{d}
ME-B2 0.250 mm	9.4 ± 0.2^{d}	$432 \pm 21^{c,e}$	648 ± 11^{e}	86.1 ± 2.0^{d}	$87.3 \pm 4.2^{c,e}$	94.8 ± 1.6^{e}
Kitchen garden						
04-173 2 mm	12.3 ± 0.2^{c}	757 ± 21^{c}	963 ± 12^{c}	90.2 ± 1.8^{c}	91.5 ± 2.5^{c}	99.1 ± 1.2^{c}
04-173 0.315 mm	11.6 ± 0.2^{d}	771 ± 15^{c}	982 ± 9^{c}	85.0 ± 1.2^{d}	93.2 ± 1.8^{c}	101.1 ± 0.9^{c}
04-173 0.250 mm	11.8 ± 0.1^{d}	769 ± 13^{c}	979 ± 15^{c}	87.0 ± 0.9^{d}	93.0 ± 1.5^{c}	100.8 ± 1.5^{c}
04-176 2 mm	4.0 ± 0.1^{c}	276 ± 1^{c}	909 ± 12^{c}	83.9 ± 0.5^{c}	108.7 ± 0.6^{c}	176.5 ± 2.4^{c}
04-176 0.315 mm	3.8 ± 0.1^{d}	248 ± 8^{d}	522 ± 2^{d}	78.6 ± 0.2^{d}	97.6 ± 3.1^{d}	101.4 ± 0.4^{d}
04-176 0.250 mm	4.0 ± 0.1^{c}	251 ± 4^{d}	525 ± 6^{d}	82.3 ± 0.6^{c}	99.0 ± 1.6^{d}	102.0 ± 1.2^{d}
04-179 2 mm	10.3 ± 0.9^{c}	509 ± 22^{c}	1501 ± 35^{c}	101.6 ± 8.8^{c}	123.3 ± 5.2^{c}	114.3 ± 2.6^{c}
04-179 0.315 mm	7.9 ± 0.2^{d}	365 ± 11^{d}	1182 ± 13^{d}	78.1 ± 1.9^{d}	88.4 ± 2.7^{d}	90.0 ± 1.0^{d}
04-179 0.250 mm	8.5 ± 0.1^{e}	407 ± 3^{e}	1419 ± 9^{e}	83.8 ± 0.7^{e}	98.6 ± 0.6^{e}	108.0 ± 0.7^{e}

[a] Sum of the metal concentrations measured in F1, F2, F3 and F4 fractions.
[b] Recovery rate $=[(F1+F2+F3+F4)/\text{pseudo-total}] \times 100$ [55].
For a given soil sample and metal, concentration values with the same letter are not significantly different at $\alpha=0.05$.

agricultural soils. The Kruskal–Wallis test showed significant differences between the recovery rates for samples prepared to pass through a 2-mm sieve and those calculated for the soils sieved at <0.250-mm excepted for Pb and Zn in the ME-21 agricultural soil. In the similar way, significant differences were observed for kitchen garden soils excepted for Pb and Zn in the 04-173 garden soil. Surprisingly, the clay contents of these two soils were the lowest (Table 3). Therefore, for the other four soils, particle segregations of soil samples sieved at <2-mm inside the bottle during the protocol steps cannot be excluded even if samples were rehomogenised prior to weighing [68,80]. For the overall kitchen garden samples, the Cd and Pb recovery rates ranged from 78 to 109%, except for Pb from the 04-179 sieved at 2 mm (123.3%, Table 6). This result could be explained by the heterogeneity within the kitchen garden sub-samples prepared to pass through a 2-mm sieve, which was related to the presence of small particles containing Pb (*e.g.*, slags) that would not have been mineralised during the microwave-assisted digestion procedure. On the other hand, gardening practices can make kitchen gardens inhomogenous. The standard deviation was the highest for Pb in the 04-173 soils. This result seems to be related to the greatest organic carbon value (102 g kg^{-1}, Table 3) in this soil. Although Bacon et al. [65] have clearly proved the reproducibility of the sequential extraction procedure, significant differences were obtained between the soils studied with high organic matter contents. The small mass used to perform the procedure can also explain some of the results. According to Davidson et al. [51], precision improves in the determination of Pb when a larger sample is processed.

Mean recovery values with respect to pseudo-total Zn concentrations in kitchen garden soils ranged from 90 to 114%, which is considered acceptable given the intrinsic heterogeneity of urban soils [89]. An exception occurred for Zn in the 04-176 soil prepared with a 2-mm sieve (176%, Table 6). Synergy of several factors may provide an initial explanation. Unlike the two gardens that are mainly under the influence of the former lead smelter Metaleurop, 04-176 is also under the influence of the Auby zinc plant (Nyrstar) because it is located midway between the two plants. Douay et al. [79] highlighted the effect of the anthropogenic actions on the variability of Zn concentrations in urban soils and on the high heterogeneity and variability of their physico-chemical parameters. Both of these factors may explain the highly pronounced heterogeneity of the Zn contents in this garden and therefore could contribute to the heterogeneity of sub-samples.

The statistical tests (Kruskal–Wallis test, $p<0.05$) showed that significant differences obtained between the sums of extracted metals from the four steps depended on the metal and the grinding fineness of the sample (Table 6). On the other hand, it appeared that the effect of grinding size depended on the soil uses (agricultural *vs.* kitchen garden). It seems to be difficult to conclude for Cd but the effect of grinding size on the determination of Pb and Zn is greater for the kitchen garden soils than the agricultural soils, reflecting anthropogenic enrichment (intake of contaminated materials by humans) of the kitchen gardens.

The relative fractionations of metals at each extraction stage of the SM&T procedure for the six contaminated soil samples are given as a distribution graphs for Cd, Pb and Zn in Fig. 1. The y-axis indicates the percentage of extraction with respect to the sum of the three fractions (F1+F2+F3) and the residual phase (F4) of the protocol [50,90]. For the agricultural and kitchen garden samples, the concentrations of metals in mobile phases, based on the sum of the first two fractions (F1+F2) and considered as easily available [38,50,51], were in the following order $\mathrm{Cd}>\mathrm{Pb}>\mathrm{Zn}$. However, the percentage of the Pb-exchangeable fraction (fraction F1) is the lowest. The high organic carbon contents in the soils studied suggest that Pb was preferentially bound to the strong binding sites of soil organic matter than to the weaker sites, forming labile complexes [91]. Despite the reported affinity of Pb for soil organic matter [92], the organically bound fraction was generally very small in the surface horizon (0–25 cm) of the soils studied probably because of the high dissolution of organo-Pb complexes at the alkaline pH of soils ($7.7<\mathrm{pH}<8.3$) [93]. The high availability of Pb is also attributed to the iron and manganese oxides and hydroxides in the soils studied (77–86%) as observed by Ettler et al. [75] and Guevara Riba et al. [94]. Therefore, the mobility of Pb can be highly affected under reducing and strong acidic conditions. As shown in Fig. 1, the fractionation of Pb in each contaminated agricultural soil is similar for all grinding sizes. In contrast, the effect of the reducing and acidic conditions on the Pb fractionation in the contaminated kitchen garden soils depended on grinding size. The major changes in the Pb fractionation were observed in the 04-179 sample. At the first grinding size (<2 mm), Pb was mainly bounded to the organic matter whereas it was associated with the Fe and Mn oxides and hydroxides for samples sieved at <0.315 mm and <0.250 mm. The presence of many organic debris in the kitchen garden samples sieved at 2-mm could explain why Pb appeared the least available (fractions F3 and F4). The organic components of the soil have a high affinity for metals by forming chelates [95], whose stability decreases as follows: $\mathrm{Pb}^{2+}\gg\mathrm{Cd}^{2+}>\mathrm{Zn}^{2+}$. Therefore, the decrease in extractability of Pb in the 2-mm sieved contaminated kitchen garden samples could be explained by changes in the organic matter–mineral phase interactions [42,77,95]. On the other hand, it was demonstrated that a significant amount of Pb on Mn oxides can be extracted using H_2O_2 at pH 2 [96]. The Pb fractionation in 04-179 soil sieved at <0.315 mm and <0.250 mm must be interpreted with caution. We cannot exclude the possibility of finding Pb in the reducible fraction, which may be attributed to binding on organic matter, as already observed in tilled soils and sediment samples by Yong et al. [97] and other authors using concentrated hydroxylamine hydrochloride (0.5 M) at pH 1.5 in the SM&T procedure instead of 0.1 M in the original BCR sequential extraction protocol [68]. In this regard, it is now well accepted that hydroxylamine hydrochloride can release substantial amounts of metals bound to organic matter and some sulfide minerals [71,98].

The fractionation of Cd in the three contaminated agricultural soil samples did not depend on the grinding size. Higher desorption yields of Cd with acetic acid – -0.11 mol L^{-1} – than Pb and Zn was obtained, corroborating that Cd was the most mobile metal in the agricultural soils around the former Metaleurop Nord plant. In the kitchen garden samples, the fractionation of Cd was highly modified depending on the sample preparation. For the 04-173 and 04-176 soil samples sieved at <2-mm, the percentage of acetic acid-extractable Cd was systematically the lowest (ranging from 7 to 10.2%). A substantial modification of the Cd fractionation in the 04-179 soil occurred in relation with the grinding size. At <2 mm, the distribution graph showed that Cd was preferentially bound to the organic matter, while for the samples sieved at <0.350 mm and <0.250 mm, Cd was mainly bound to the Fe and Mn oxides and hydroxides. Therefore, the acidic conditions used in the first and second steps of the SM&T procedure can be a factor causing remobilisations of Cd in the 04-179 soil sample sieved at <2-mm. Similar results were obtained by Sahuquillo et al. [4] after changing the acetic acid concentrations.

Differences in the distribution of Zn were obtained according to the preparation of contaminated soil samples. For agricultural samples, the percentage of Zn extracted from the two first fractions was 10% higher at <0.250 mm than that obtained when samples were sieved at <2 mm and <0.315 mm. The same trends were observed for the three contaminated kitchen gardens. It is worth noting that fractionation of Zn in the 04-176 and

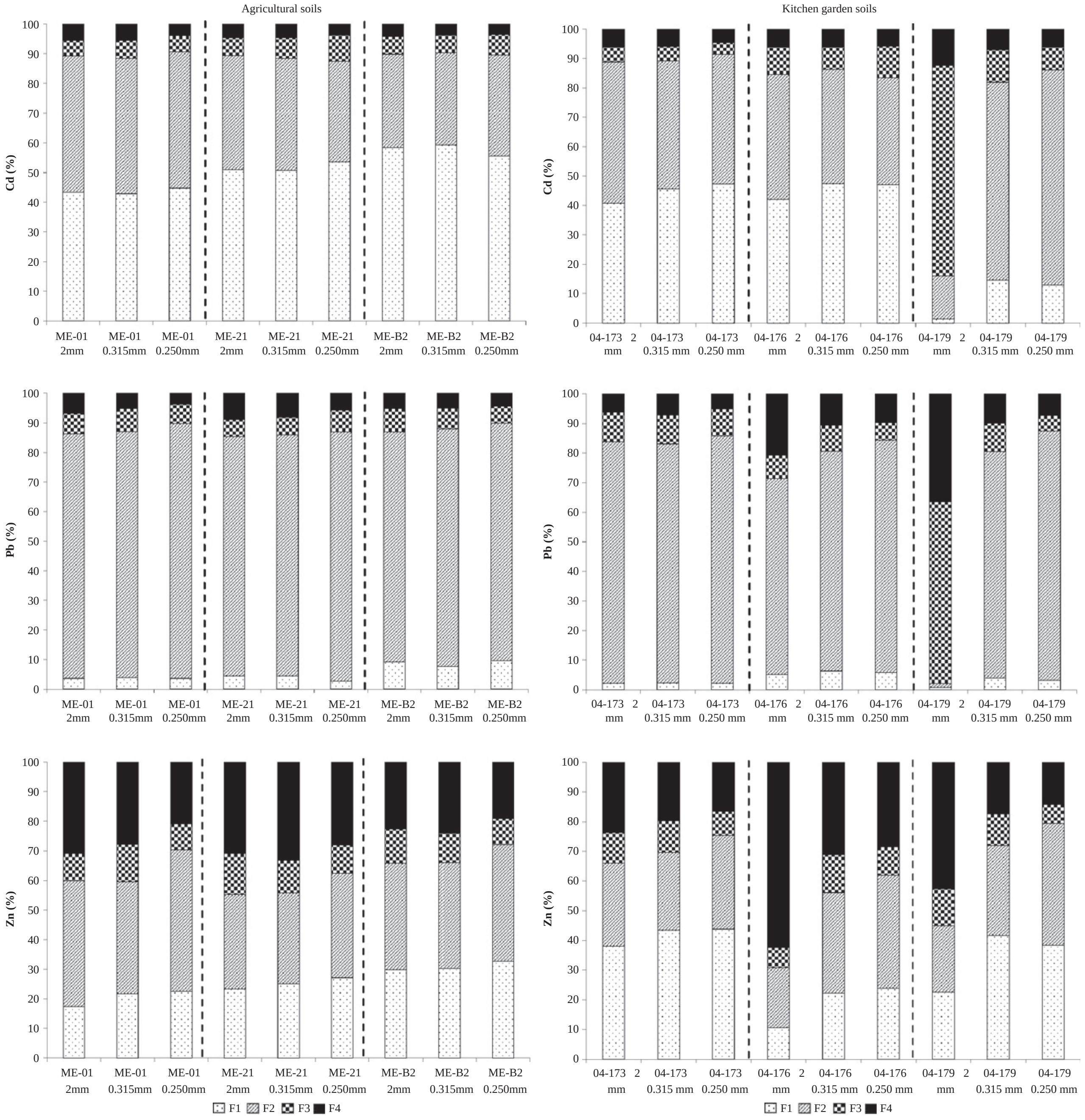

Fig. 1. Effect of grinding size on Cd, Pb and Zn fractionation using a reciprocating shaker.

04-179 soils sieved at <2 mm was very different than that obtained with the other two preparation methods. Zinc was essentially in both residual F4 fraction (ranging from 42 to 64%) from the two soils at <2 mm indicating a low mobility of Zn whereas it was mainly associated with the carbonates and Fe and Mn oxides/hydroxides for soils with smaller grind sizes. Therefore, a redistribution of Zn species (carbonate-bound, Fe–Mn oxide-bound) from the first two fractions to the residual fraction was suspected. On the other hand, more time is probably required for complete solubilisation of carbonates within samples sieved at <2 mm containing high-carbonate contents like 04-176 and 04-179 soils (86.1 and 76.4 g kg^{-1}, respectively).

With regards to the results obtained, the grinding size of contaminated soils, from <2 mm to <0.250 mm, caused an increase in extractability that can be explained by a decrease in the selectivity of the interaction between soil phases and metals due to the formation of new sites created by grinding the soil samples [45,99]. Depending on the metal and the extracting solution, relevant changes in metal extractability were observed in contaminated kitchen garden samples. Major modifications occurred overall when acetic acid was used as the extracting solution, reflecting that the changes introduced by the grinding size affect the sequential extraction procedure step in which low interactions between the extracting solution and soil particle can be identified.

Standard deviations were generally lower for contaminated soils sieved at <0.250-mm but no systematic effect of the grinding size on the precision was highlighted for Cd, Pb and Zn, except for Pb in the 04-173 kitchen garden soil. Surprisingly, the lower precision was obtained for samples sieved at

<0.250 mm and for Pb measurements in the F3 fraction (15.8%). This observation could be mainly attributed to the high amounts of organic carbon in this sample (102 g kg^{-1}). Indeed, the variable and possible incomplete destruction of organic matter in the third step as well as the difficulty oxidising sulphide may be a considerable source of uncertainty [49]. On the other hand, the need for a reference volume for the third step, in which the sample is dried to a "small volume" can contribute to the widely spread results.

3.5. *Improving the procedure*

The BCR protocol, in the revision modified according to the findings of the above cited SM&T program, recommends using a mechanical end-over-end shaker, at a speed of 30 ± 10 rpm to improve the reproducibility of extraction yields in sediments [68]. If some authors found no difference in metal extracted with both end-over-end and reciprocating shakers [71], others found differences for specific metals [72] whereas in other studies, differences were systematically obtained [69,70]. In contrast, there is no doubt about the effects of the type of shaker used but also the shaking time and speed on the reproducibility and the precision, whether the single or sequential extraction procedure was used [49,66,72]. Therefore, to complete this study and to avoid the uncertainty associated with the shaking conditions, a rotary shaker fitted with 24 bottle positions and adapted to 10 rpm was used in order to keep soil samples in suspension during extraction. Particular attention was focused on the pH adjustment of the 0.5 M hydroxylamine hydrochloride (pH = 1.5), known to cause variability between results obtained for the same sample [55]. Furthermore, a 50-mL graduated polypropylene centrifuge tube was chosen for greater precision in the determination of the "small volume" in step 3, fixed at 2 mL and then 1 mL. Finally, agitation during this step was regular and was performed with a reciprocal shaking heating bath (Memmert; Schwabach, Germany). As shown in Table 7, the sums of metal concentrations in the four fractions were in good agreement with the pseudo-total concentrations (Table 4). For a given soil and metal, no significant difference in metal concentration was found between the three-grain size fractions (excepted for Cd and Zn in the 04-173 soil; Table 7), indicating that the grinding size had only a slight effect on the extractable metal concentrations when the rotary shaker was used. Most of the data showed very low standard deviations, within the 0.8–9.5%, 0.45–2.60%, and 0.48–2.0% range for soils ground to pass through 2-mm, 0.350-mm and 0.250-mm sieves, respectively, indicating improved precision. Recovery rates ranged from 80 to 100.3%, 94 to 106.5% and 93.7 to 112.8% for Cd, Pb and Zn, respectively. These rates were generally better than those obtained with the reciprocating shaker. Compared to the previous agitation method, a continuous suspension of the mixture during the extraction procedure using the rotary shaker may explain these excellent results. The pattern observed is, however, somewhat at odds with a number of studies on sediment that report shaker type (reciprocating and end-over-end shakers) had no effect in the sequential extraction procedure [64,69,71,100–102]. Indeed, in some particular cases, no significant difference between the two types of shaking was obtained. For instance, Cd in ME-21 and 04-176, Pb in 04-176, Zn in ME-01, ME-B2 and 04-176 using the rotary shaker and these soils milled at <0.250-mm for these metals using the reciprocating shaker (Compare Tables 6 and 7).

The relative fractionations of metals at each extraction stage of the SM&T procedure for all soil samples are given as distribution graphs for Cd, Pb and Zn in Fig. 2. For each contaminated soil sample, this distribution was very similar for all three metals. The statistical tests showed that with the rotary shaker, the distribution of metals did not depend on the grinding size for contaminated agricultural soils and kitchen garden soils sieved at <0.350-mm and <0.250-mm. It should be noted that significant differences were obtained only for Cd, Pb and Zn in the 04-179 soil prepared at <2 mm and the two other grinding sizes. For the three metals, the two first steps were generally affected by changes in distribution and low metal concentrations were obtained in the first step of the extraction procedure when the 04-179 soil was sieved at <2 mm, reflecting lower solubilisation of carbonate compounds.

Table 7
Sum of the extractable metals in the four fractions after slight modifications to the SM&T procedure (mean ± standard deviation, $n = 3$) and recovery rates.

Soils	Sequential extraction[a]			Recovery[b] (%)		
	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cd	Pb	Zn
Agricultural						
ME-01 2 mm	12.1 ± 0.1^{c}	768 ± 12^{c}	997 ± 22^{c}	91.8	99.9	109.2
ME-01 0.315 mm	12.2 ± 0.1^{c}	778 ± 4^{c}	988 ± 19^{c}	92.1	101.3	108.2
ME-01 0.250 mm	12.1 ± 0.2^{c}	777 ± 9^{c}	986 ± 20^{c}	91.9	101.2	108.0
ME-21 2 mm	6.2 ± 0.1^{c}	313 ± 5^{c}	352 ± 6^{c}	94.1	106.3	110.1
ME-21 0.315 mm	6.0 ± 0.1^{c}	313 ± 7^{c}	361 ± 7^{c}	91.3	106.5	112.8
ME-21 0.250 mm	6.1 ± 0.1^{c}	310 ± 4^{c}	354 ± 5^{c}	92.9	105.5	110.7
ME-B2 2 mm	13.4 ± 0.2^{c}	506 ± 5^{c}	655 ± 9^{c}	98.2	102.3	96.0
ME-B2 0.315 mm	13.3 ± 0.1^{c}	502 ± 6^{c}	639 ± 7^{d}	97.8	101.4	93.5
ME-B2 0.250 mm	13.3 ± 0.2^{c}	508 ± 7^{c}	651 ± 7^{c}	97.4	102.6	95.3
Kitchen garden						
04-173 2 mm	13.6 ± 0.2^{c}	778 ± 18^{c}	1294 ± 12^{c}	100.3	94.0	98.5
04-173 0.315 mm	12.9 ± 0.1^{d}	783 ± 7^{c}	1227 ± 10^{d}	94.5	94.7	93.5
04-173 0.250 mm	12.6 ± 0.1^{d}	784 ± 4^{c}	1231 ± 12^{d}	93.0	94.8	93.7
04-176 2 mm	4.1 ± 0.1^{c}	250 ± 9^{c}	516 ± 12^{c}	85.4	98.3	100.1
04-176 0.315 mm	3.8 ± 0.1^{c}	250 ± 5^{c}	518 ± 8^{c}	80.0	98.6	100.7
04-176 0.250 mm	3.9 ± 0.1^{c}	252 ± 3^{c}	517 ± 10^{c}	80.7	99.4	100.4
04-179 2 mm	9.4 ± 0.9^{c}	408 ± 21^{c}	1233 ± 22^{c}	93.3	98.7	93.9
04-179 0.315 mm	8.8 ± 0.2^{c}	411 ± 8^{c}	1296 ± 12^{d}	86.8	99.5	98.7
04-179 0.250 mm	8.8 ± 0.1^{c}	408 ± 3^{c}	1302 ± 9^{d}	87.3	98.8	99.2

For a given soil sample and metal, concentration values with the same letter are not significantly different at $\alpha = 0.05$.

[a] Sum of the metal concentrations measured in F1, F2, F3 and F4 fractions.

[b] Recovery rate = $[(F1+F2+F3+F4)/\text{pseudo-total}] \times 100$ [55].

Using the rotary shaker, standard deviations were generally lower for contaminated soils sieved at <0.250 mm but no systematic effect of grinding size on precision was highlighted for Cd, Pb and Zn. In contrast, compared to the data set obtained with the reciprocating shaker, low precision values were obtained for Pb for all steps of the sequential extraction procedure, indicating a beneficial effect of the rotary shaking mode which promoted particle suspension and contributed to a more complete destruction of organic matter in the third step than with the reciprocating shaker. For instance, the precision of the third step for the soil samples ground at <2-mm, <0.315-mm and <0.250-mm were below 10%, 8.5% and 8%, respectively. Although no statistical difference was demonstrated between the distributions of Zn in soil samples sieved at <0.315-mm and 0.250-mm using the rotary shaker, a slight increase in the precision values was obtained in comparison with the reciprocating shaker in all steps and for all soils. This is probably due to the low shaking speed of the rotor disc (10 rpm). However, the similar distribution of the three metals for the soil samples sieved at <0.315-mm and <0.250-mm and the improvement in precision (except for Zn) obtained with this type of shaker may be explained by the continuous suspension of particles and the use of the 50-mL polypropylene centrifuge tube to ensure the removal of the solution up to 2 mL and 1 mL during the third step of the sequential extraction procedure.

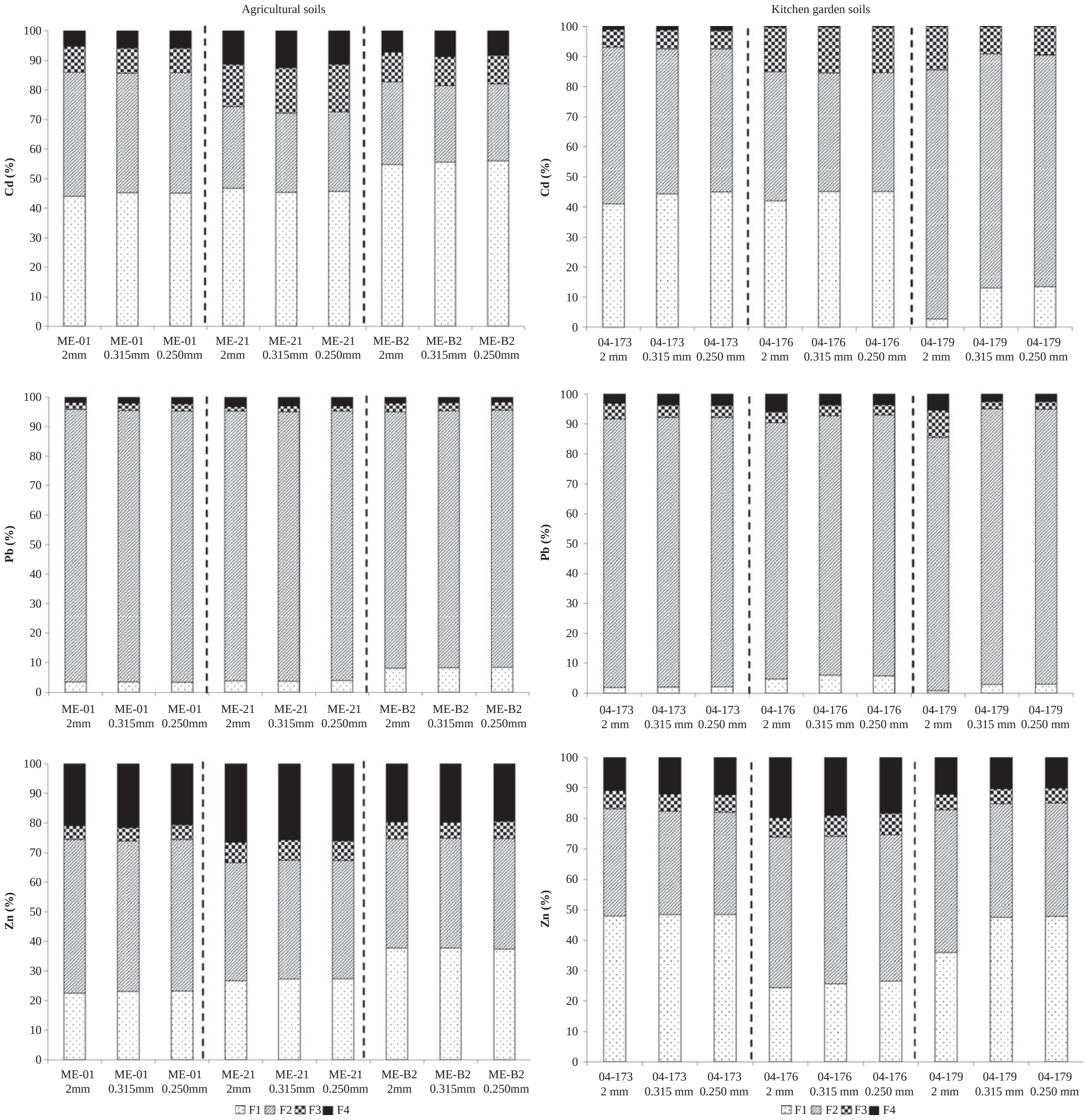

Fig. 2. Effect of grinding size on Cd, Pb and Zn fractionations using a rotary shaker.

3.6. *Application of the procedure for metal fractionation in the CRM BCR®-701*

As stated by Sutherland [103] in a review of the use of the CRM BCR®-701 certified material, the most common deviations between the data compiled from the literature and the certified values can be attributed to the sample mass, filtration, centrifugation speed, type of shaker, speed and duration of shaking, temperature of the experimentation procedure and correction to dry mass. Regarding the results obtained with the contaminated soil samples, it is undeniable that the bottle size and the automatic shaking heating bath were important in optimising the volume reduction and homogenising the solution between samples and replicates. All modifications reported above were applied for the determination on the Cd, Pb and Zn fractionation in the CRM BCR®-701 certified material. The results are recorded in Table 8. In comparison with the data in Table 5, the mean concentrations of Cd, Pb and Zn in each step were similar but low relative standard deviation values were obtained using the rotary shaker and appropriate analytical materials, indicating an improvement in precision. This is particularly true for Cd in fraction F4 (4.8% *vs.* 25%) and for Zn in F3 (2.7% *vs.* 11.6%). For Cd, the bias was positive for the first three steps, ranging from 0.51 to 1.64%, and negative for the fourth step (−2.31%). Very good agreement was observed between the sum of the Cd concentrations measured in the four steps and the indicative concentrations. As can be seen, precision ranged from 1.19 to 8.41% for Pb. Major improvement in precision was obtained for the third step, from 11.8% (Table 5) to 8.41% (Table 8), indicating that change related to the reduction of the extracting solution in the third step was essential. As recently reported by Sutherland [103], the high degree of imprecision (21.5%) of the certified Pb concentrations in the third step was probably associated with measurement during the certification process, due to the low

Table 8
Validation of the sequential extraction procedure after slight modifications using the CRM BCR®-701 material ($n=3$) – Comparison with the corresponding certified and indicative values.

Metals	Cd			Pb			Zn		
	Obtained value[a] (mg kg^{-1})	Precision (%)	Trueness (%)	Obtained value[a] (mg kg^{-1})	Precision (%)	Trueness (%)	Obtained value[a] (mg kg^{-1})	Precision (%)	Trueness (%)
F1	7.43 ± 0.11	1.44	1.31	3.07 ± 0.13	4.28	−3.31	194 ± 7	3.72	−5.48
F2	3.79 ± 0.08	2.08	0.51	135 ± 2	1.19	6.98	123 ± 3	2.42	8.42
F3	0.27 ± 0.01	5.16	1.64	9.3 ± 0.8	8.41	0.57	47 ± 1.2	2.67	1.94
F4	0.13 ± 0.01	4.76	−2.31	11.6 ± 0.9	7.56	5.87	97 ± 3	2.73	2.14
∑three steps+residual	11.52 ± 0.60	0.51	1.1	158.9 ± 1.4	0.91	6.63	461 ± 6	1.8	0.43
	11.50 ± 0.50[b]			149 ± 6.3[b]			459 ± 15[b]		

[a] Values are means ± standard deviations.

[b] Values are means ± uncertainty (half-width of the 95% confidence interval).

concentration and to the fact that the third step accumulated the errors of the previous two. Average bias values (from −3.31 to 6.98%) were acceptable in comparison with the data reported by Sutherland [101]. However, the mean bias in the second step was positive (7%), indicating that the mean Pb concentration measured in the second step was higher than the certified concentration (135 ± 2 mg kg^{-1} instead of 126 ± 3 mg kg^{-1}). This result is somewhat at odds with a number of studies even if problems determining Pb in the second step were mentioned by Sutherland [108] with regard to the mean bias (−5.6%) and spread (80%) calculated by the author using data compiled from the literature, but it is worth noting that a similar Pb concentration in the second step was found when a reciprocating shaker was used (134 ± 2 mg kg^{-1}, Table 5). The high Pb concentration measured in the second step of the sequential extraction procedure can explain the difference between the sum of extractable Pb (158.9 ± 1.4 mg kg^{-1}) and the indicative concentration (149.0 ± 6.3 mg kg^{-1}), and the resulting positive bias value (6.63%). In contrast, the sum of extractable Pb was very close to the *aqua regia* extractable Pb using microwave-assisted digestion (162.4 ± 4.8 mg kg^{-1}, $n=3$) which implies a very low bias value (−2.1%). For Zn, precision was less than 4% in all instances and bias ranged from −5.48 to 8.42%. The mean concentration of Zn measured in the first step of the procedure was affected by the shaking process. It was 205 ± 8 mg kg^{-1} using the reciprocating shaker and was similar to the certified concentration (205 ± 6 mg kg^{-1}) while it was 194 ± 7 mg kg^{-1} after slightly modifying the protocol and using the rotary shaker, resulting in a negative bias value (−5.48%). This result is in accordance with the mean concentrations of Zn (193 mg kg^{-1}) and mean the bias value (−5.6%) calculated by Sutherland [103] from values compiled from the literature. The sum of the extracted metals in the four fractions compared well with the indicative values.

Taking into account all these results, the quality of analytical extraction data was ensured and all slight modifications (a polypropylene centrifuge tube with a nominal 50-mL volume to increase the precision in the reduction of the extracting solution at a volume of 2 mL and 1 mL during the third step of the sequential extraction procedure using an automatic shaking heating bath) and the use of a rotary shaker appeared suitable for the analysis of Cd, Pb and Zn in contaminated soils.

4. Conclusion

The fractionation of Cd, Pb and Zn in highly contaminated soils was studied focusing on the sample grinding size and the shaker used during the sequential extraction procedure. The first observation from the data obtained using the reciprocating shaker was the wide variation in the fractionation of Cd, Pb and Zn for kitchen garden soils depending on the grinding size. In comparison with the fractionation of metals within the kitchen garden samples sieved at < 0.350 mm and < 0.250 mm, the metal partitioning within the samples sieved at < 2 mm was sufficiently different to make the comparison of results in terms of mobility and environmental availability impossible. In contrast, no grinding size effect was found for metal fractionation within the contaminated agricultural soils. After optimisation of the sequential extraction procedure to the specific characteristics of kitchen garden samples, the fractionation of Cd, Pb and Zn within these soil samples was achieved for all grinding sizes selected even if discrepancies from samples sieved at < 2 mm were identified. From an analytical point of view, precision and trueness were improved for all sequential extraction procedure steps and the best results were obtained for samples ground to pass through a 0.250-mm sieve. Regarding the results obtained using the CRM BCR®-701 certified material, use of the rotary shaker and the minor changes in the sequential extraction procedure (the use of a 50 mL graduated polypropylene centrifuge tube, evaporation of the solution to a fixed volume in step 3 and the use of an automatic shaking heating bath) can be adopted for Cd, Pb and Zn partitioning in contaminated kitchen garden soils affected by a high degree of anthropogenisation.

Appendix: Standards

NF ISO 11464. Soil quality—Pretreatment of samples for physico-chemical analyses. AFNOR, 1994, 9 p.

NF X 31-107. Soil quality – Particle size determination by sedimentation – Pipette method. AFNOR, 1983, 15 p.

NF ISO 10694. Soil quality—Determination of organic and total carbon after dry combustion (elementary analysis). AFNOR, 1995, 7 p.

NF ISO 13878. Soil quality—Determination of total nitrogen content by dry combustion content. AFNOR, 1998, 5 p.

NF ISO 10693. Soil quality – Determination of carbonate content – Volumetric method. AFNOR, 1995, 7 p.

NF X 31-161. Soil quality – Determination of phosphorus soluble in a 0.1 mol l^{-1} ammonium oxalate solution – Joret–Hébert method. AFNOR, 1993, 9 p.

NF X 31-130. Soil quality – Chemical methods – Determination of cationic exchange capacity (CEC) and extractible cations. AFNOR, 1993, 14 p.

NF ISO 17025. General requirements for the competence of testing and calibration laboratories. AFNOR, 2005, 28p.

NF ISO 11465. Soil quality – Determination of dry matter and water content on a mass basis – Gravimetric method. AFNOR, 1994, 4 p.

NF X 31–147. Soil quality – Soils, sediments – Total solubilizing by acid attack. AFNOR, 1996, 12 p.

Appendix. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.06.068.

References

[1] D. Baize, T. Sterckeman, Sci. Total Environ. 264 (2001) 127–139.
[2] H.F. Clark, D.J. Brabander, R.M. Erdil, J. Environ. Qual. 35 (2006) 2066–2074.
[3] X.P. Li, C.C. Huang, Environ. Geol. 52 (2007) 1631–1637.
[4] A. Sahuquillo, A. Rigol, G. Rauret, J. Environ. Monit. 4 (2002) 1003–1009.
[5] P. Glorennec, M. Ledrans, B. Fabres, Rev. Epidemiol. Sante Publique 54 (2006) 117–125.
[6] A.G. Oomen, J.P.A. Lijzen, (2004). Relevancy of Human Exposure via House Dust to the Contaminants Lead and Asbestos, RIVM Report 711701037, RIVM-Bilthoven, The Netherlands, 2004.
[7] C. Pruvot, F. Douay, H. Fourrier, C. Waterlot, J. Soils Sediments 6 (2006) 215–220.
[8] C.W. Versluijs, P.F. Otte, Accumulation of Metals in Plants, a Contribution to the Evaluation of the Intervention Values and the Location-specific Risk Assessment of Contaminated Soils, RIVM Report 711701024, RIVM-Bilthoven, The Netherlands, 2001.
[9] C.W. Versluijs, R. Koops, P. Kreule, M.F.W. Waitz, The Accumulation of Soil Contaminants in Crops, Location-specific Calculation Based on the CSOIL Module, RIVM Report 711701008, RIVM-Bilthoven, The Netherlands, 2006.
[10] N. Dupuy, F. Douay, Spectrochim. Acta, Part A 57A (2001) 1037–1047.
[11] S. Orsetti, M.D.L.M. Quiroga, E.M. Andrade, Chemosphere 65 (2006) 2313–2321.
[12] W.H. Otto, W.R. Carper, C.K. Larive, Environ. Sci. Technol. 35 (2001) 1463–1468.
[13] P.E. Jensen, L.M. Ottosen, A.J. Pedersen, Water Air Soil Pollut. 170 (2006) 359–382.
[14] R. Moral, R.J. Gilkes, M.M. Jordan, Water Air Soil Pollut. 162 (2005) 127–142.
[15] K. Flogeac, E. Guillon, M. Aplincourt, E. Marceau, L. Stievano, P. Beaunier, Y.M. Frapart, Agron. Sustainable Dev. 25 (2005) 345–353.
[16] N. Jørgensen, J. Laursen, A. Viksna, N. Pind, P.E. Holm, Environ. Int. 31 (2005) 43–52.
[17] F. Liu, W.-F. Tan, G.-Q. Liu, X.-Y. Li, J.-Z. He, in: 17th WCSS, Thailand, Adsorption of Heavy Metals on Mn Oxide Surfaces of Fe–Mn Modules from Several Soils of China, 28 (2002) 1–8.
[18] A. Manceau, M.A. Marcus, N. Tamura, O. Proux, N. Geoffroy, B. Lanson, Geochim. Cosmochim. Acta 68 (2004) 2467–2483.
[19] J.F. Ranville, D.J. Chittleborough, R. Beckett, Soil Sci. Soc. Am. J. 69 (2005) 1173–1184.
[20] W. Tan, F. Liu, X. Feng, Q. Huang, X. Li, J. Colloid Interf. Sci 284 (2005) 600–605.
[21] T. Yoshida, T. Yamaguchi, Y. Iida, S. Nakayama, J. Nucl. Sci. Technol. 40 (2003) 672–678.
[22] A. Hass, P. Fine, Environ. Sci. Technol. 40 (2010) 365–399.
[23] A. Tessier, P.G.C. Campbell, M. Bisson, Anal. Chem. 51 (1979) 844–851.
[24] A.V. Filgueiras, I. Lavilla, C. Bendicho, J. Environ. Monit. 4 (2002) 823–857.
[25] A. Barona, F. Romero, Environ. Technol. 17 (1996) 63–70.
[26] J.J. Sloan, R.H. Dowdy, M.S. Dolan, D.R. Linden, J. Environ. Qual. 26 (1997) 966–974.
[27] N. Basta, R. Gradwohl, J. Soil Contam. 9 (2000) 149–164.
[28] C.N. Mulligan, R.N. Yong, B.F. Gibbs, J. Hazard. Mater. 85 (2001) 145–163.
[29] S. Tokalioglu, S. Kartal, G. Birol, Turk J. Chem. 27 (2003) 333–346.
[30] G.S.R. Krishnamurti, P.M. Huang, K.C.J. Van Rees, L.M. Kozak, H.P.W. Rostad, Analyst 120 (1995) 659–665.
[31] D. McGrath, Sci. Total Environ. 178 (1996) 37–44.
[32] P. Quevauviller, G. Rauret, J.-F. Lpez-sanchez, R. Rubio, A. Ure, H. Muntau, Sci. Total Environ. 205 (1997) 223–234.
[33] I. Maiz, I. Arambarri, R. Garcia, E. Millan, Environ. Pollut. 110 (2000) 3–9.
[34] T. Xie, W.D. Marshall, J. Environ. Monit. 3 (2001) 411–416.
[35] M. Turek, T. Korolewicz, J. Ciba, J. Cebula, Water Air Soil Pollut. 135 (2002) 311–323.
[36] M. Pueyo, J. Sastre, E. Hernandez, M. Vidal, J.F. Lpez-Sanchez, G. Rauret, J. Environ. Qual. 32 (2003) 2054–2066.
[37] S. Tandy, K. Bossart, R. Mueller, J. Ritschel, L. Hauser, R. Schulin, B. Nowack, Environ. Sci. Technol. 38 (2004) 937–944.
[38] C.M. Davidson, G.J. Urquhart, F. Ajmone-Marsan, M. Biasioli, A. da Costa Duarte, E. Dıaz-Barrientos, H. Grcman, I. Hossack, A.S. Hursthouse, L. Madrid, S. Rodrigues, M. Zupan, Anal. Chim. Acta 565 (2006) 63–72.
[39] Z. Mester, C. Cremisini, E. Ghiara, R. R. Morabito, Anal. Chim. Acta 359 (1998) 133–142.
[40] J. Usero, M. Gamero, J. Morillo, I. Gracia, Environ. Int. 24 (1998) 487–496.
[41] J.M. Jouanneau, C. Latouche, F. Pautrizel, Environ. Technol. Lett. 4 (1983) 509–514.
[42] B. Kim, M.B. McBride, Environ. Pollut. 144 (2006) 475–482.
[43] C. Parat, J. Leveque, S. Dousset, R. Chaussod, F. Andreux, Anal Bioanal Chem. 376 (2003) 243–247.
[44] S. Cornu, B. Clozel, Etude et Gestion des sols 7 (2000) 179–189.
[45] G.E.M. Hall, J.E. Vaive, A.I. MacLaurin, J. Geochem. Explor. 56 (1996) 23–36.
[46] N.L. Benitez, J.P. Dubois, Int. J. Environ. Anal. Chem 74 (1999) 289–303.
[47] P. Quevauviller, A.M. Ure, H. Muntau, B. Griepink, Int. J. Environ. Anal. Chem. 51 (1993) 129–134.
[48] E. Tipping, N.B. Hetherington, J. Hilton, D.W. Thompson, E. Bowles, J. Hamilton-Taylor, Anal. Chem. 57 (1985) 1944–1946.
[49] P. Quevauviller, G. Rauret, H. Muntau, A.M. Ure, R. Rubio, J.F. Lpez-Sanchez, H.D. Fiedler, B. Griepink, Fresenius J. Anal. Chem. 349 (1994) 808–814.
[50] C.M. Davidson, A.L. Duncan, D. Littlejohn, A.M. Ure, Anal. Chim. Acta 363 (1998) 45–55.
[51] C.M. Davidson, P.C.S. Ferreira, A.M. Ure, J. Fresen., Anal. Chem. 363 (1999) 446–451.
[52] N. Belzile, P. Lecomte, A. Tessier, Environ. Sci. Technol. 23 (1989) 1015–1020.
[53] Y.N. Vodyanitskii, Eurasian Soil Sci. 39 (2006) 1074–1083.
[54] G. Rauret, J.F. Lpez-Sanchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, P. Quevauviller, J. Environ. Monit. 1 (1999) 57–61.
[55] G. Rauret, J.F. Lpez-Sandez, A. Sahuquillo, E. Barahona, M. Lachica, A.M. Ure, C.M. Davidson, A. Gomez, D. Luick, J. Bacon, M. Yli-Halla, H. Muntau, P. Quevauviller, J. Environ. Monit. 2 (2000) 228–233.
[56] G. Rauret, G.,,J.F. Lpez Sanchez, Int. J. Environ. Anal. Chem. 79 (2001) 81–95.
[57] M. Pueyo, G. Rauret, D. Luck, M. YliHalla, H. Muntau, P. Quevauviller, J.F. Lpez Sanchez, J. Environ. Monit. 3 (2001) 243–250.
[58] M.D. Ho, G.J. Evans, Anal. Commun. 11 (1997) 363–364.
[59] R.A. Sutherland, F.M.G. Tack, Anal. Chim. Acta 454 (2002) 249–257.
[60] J. Kubova, V. Stresko, M. Bujdos, P. Matus, J. Medved, Anal. Bioanal. Chem. 379 (2004) 108–114.
[61] A.V. Filgueiras, I. Lavilla, C. Bendicho, Anal. Bioanal. Chem. 374 (2002) 103–108.
[62] R. Santamaria Fernandez, M.R. Cave, S.J. Hill, J. Environ. Monit. 5 (2003) 929–934.
[63] T. Asami, M. Kubota, K. Orikasa, Water Air Soil Pollut. 83 (1995) 187–194.
[64] E. Fernandez, R. Jimenez, A.M. Lallena, J. Aguilar, Environ. Pollut. 131 (2004) 355–364.
[65] J.R. Bacon, I.J. Hewitt, P. Cooper, Sci. Total Environ. 337 (2005) 191–205.
[66] M.K. Jamali, T.G. Kazi, H.I. Afridi, M.B. Arain, N. Jalbani, A.R. Memon, J. Environ. Sci. Health A 42 (2007) 649–659.
[67] S. Tokalioglu, S. Kartal, Bull. Environ. Contam. Toxicol. 75 (2005) 180–188.
[68] A. Sahuquillo, H. Bosch, G. Rauret, H. Muntau, J. Fresen., Anal. Chem. 360 (1998) 304–307.
[69] P. Quevauviller, Trends Anal. Chem. 17 (1998) 289–298.
[70] H.D.L. Fiedler, Método Común de Extracciones Secuenciales de Metales en Sedimentos: Estudio Sobre la Preparación de un Material de Referencia Certificado. Ph.D. Thesis, University of Barcelona, 1995.
[71] J.L. Gomez Ariza, I. Giraldez, D. Sanchez Rodas, E. Morales, Int. J. Environ. Anal. Chem. 75 (1999) 3–18.
[72] J. Arunachalam, H. Emons, B. Krasnodebska, C. Mohl, Sci. Total Environ. 181 (1996) 147–159.
[73] A. Fernández Alborés, B. Pérez Cid, E. Fernández Gómez, E. Falqué López, Analyst 125 (2000) 1353–1357.
[74] N. Sabienë, D.M. Brazauskienë, D. Rimmer, Ekologija 1 (2004) 36–41.
[75] V. Ettler, A. Vanek, M. Mihaljevic, P. Bezdicka, Chemosphere 58 (2005) 1449–1459.
[76] S.R. Claff, E.D. Burton, L.A. Sullivan, R.T. Bush, Geoderma 159 (2010) 156–164.
[77] T.E. Clevenger, Water Air Soil Pollut. 50 (1990) 241–254.
[78] C.M. Rao, A. Sahuquillo, J.F.Lopez Sanchez, Water Air Soil Pollut. 189 (2008) 291–333.
[79] F. Douay, C. Pruvot, H. Roussel, H. Ciesielski, H. Fourrier, N. Proix, C. Waterlot, Water Air Soil Pollut. 188 (2008) 247–260.
[80] G.W. Gee, J.W. Bauder, Particle-size analysis, in: A. Klute (Ed.), Methods of Soil Analysis, Part 1, Second eds.,American Society of Agronomy and Soil Science Society of Agronomy, Madison, 1986, pp. 383–411.
[81] C. Waterlot, C. Pruvot, H. Ciesielski, F. Douay, Ecol. Eng. 37 (2011) 1081–1093.
[82] D.W. Nelson, L.E. Sommers, Total carbon, organic carbon and organic matter, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis, Soil Science Society of America, Madison, WI, 1982, pp. 574–577.
[83] G. Joret, J. Hébert, Ann. Agron. 2 (1955) 233–299.
[84] P. Lefevre, Ann. Agron. 12 (1961) 169–206.
[85] I. Taverniers, M. De Loose, E. Van Bockstaele, Trends Anal. Chem. 23 (2004) 535–552.
[86] C. Waterlot, F. Douay, Talanta 80 (2009) 716–722.
[87] M. Savio, S. Cerutti, L.D. Martinez, P. Smichowski, R.A. Gil, Talanta 82 (2010) 523–527.

[88] C. Waterlot, A. Pelfrêne, F. Douay, F.J. Anal., Methods Chem. 2012 (2012) 512709.

[89] Y. Lu, Z.T. Gong, G.L. Zhang, W. Burghardt, Geoderma 115 (2003) 101–111.

[90] D. Bakircioglu, Y.B. Kurtulus, H. Ibar, Environ. Monit. Assess. 175 (2011) 303–314.

[91] A.L.R. Sekaly, R. Mandal, N.M. Hassan, J. Murimboh, C.L. Chakrabarti, M.H. Back, D.C. Grégoire, W.H. Schroeder, Anal. Chim. Acta 402 (1999) 211–221.

[92] C. Kabala, L. Szerzseń, Water Air Soil Pollut. 138 (1992) 307–317.

[93] S. Sauvé, M.B. McBride, W.H. Hendershot, Soil Sci. Soc. Am. J. 62 (1998) 618–621.

[94] A. Guevara Riba, A. Sahuquillo, R. Rubio, G. Rauret, Sci. Total Environ. 321 (2004) 241–255.

[95] C. Kabala, B.R. Singh, J. Environ. Qual. 30 (2001) 485–492.

[96] N. Meguellati, D. Robbe, P. Marchandise, M. Astruc, A new chemical extraction procedure in the fractionation of heavy metals in sediments-interpretation, Proceedings of the International Conference on Heavy Metals in the Environment, Edimburg CEP, Consulting, 1983, 1090–1093.

[97] R.N. Yong, S.P. Bentley, C. Harris, W.Z.W. Yaacob, Selective sequential extraction analysis (SSE) on estuarine alluvium soils, in: R.N. Yong, H.R. Thomas (Eds.), Geoenvironmental Engineering Ground Contamination: Pollutant Management and Remediation, Thomas Telford, London, 1999, pp. 118–126.

[98] J.L. Gomez Ariza, I. Giraldez, D. Sanchez Rodas, E. Morales, Sci. Total Environ. 246 (2000) 271–283.

[99] N.G. Kim, J.E. Fergusson, Sci. Total Environ. 105 (1991) 191–209.

[100] B. Pérez-Cid, I. Lavilla, C. Bendicho, Anal. Chim. Acta 360 (1998) 35–41.

[101] R. Santamaría-Fernández, A. Moreda-Piñeiro, S.J. Hill, J. Environ. Monit. 4 (2002) 330–336.

[102] M.K. Jamali, T.G. Kazi, M.B. Arain, H.I. Afridi, J.A. Baig, A.Q. Shah, Acta Agric. Hung. 57 (2009) 215–230.

[103] R.A. Sutherland, Anal. Chim. Acta 680 (2010) 10–20.