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Validation of a new method for monitoring trace elements in Mediterranean cereal soils

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Validation of a new method for monitoring trace elements in Mediterranean cereal soils

In the Mediterranean region, agricultural soils are seriously polluted with toxic trace elements (TEs) which could enter the food chain via the soil-plant trophic chain. For food safety reasons, the monitoring of TE concentrations in these agricultural soils is thus imperative. The most powerful monitoring method for TE measurements is based on perchloric acid (HClO₄) and hydrofluoric acid (HF) digestion, commonly used as reference total digestion (RTD) method, with consequent use of inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). Unfortunately, HF and HClO₄ manipulations are highly dangerous and ICP-OES and ICP-MS apparatus are very expensive, thus they are unaffordable, notably in developing countries. In this paper, an alternative, microwave sulphuric digestion (MSD) method, combined with atomic absorption spectroscopy (AAS) is proposed. First, the suggested method was validated on a soil certified reference material, for the determination of 7 TEs (As, Cd, Co, Cr, Cu, Ni and Pb). Second, the MSD method was applied on agricultural soil samples situated in the Bekaa valley, East Lebanon and results were compared to those of RTD method. The MSD method, coupled to AAS, offers a promising and feasible alternative to HF, as well as, *aqua regia* based methods.

Keywords: method validation; trace elements; soil; microwave digestion; atomic absorption spectroscopy; Bekaa; East Mediterranean.

1. Introduction

Trace elements (TEs) are the most studied soil contaminants in the world, and come both from lithogenic sources [1] and anthropogenic activities [2]. They are persistent, non-degradable and even though some are essential for living organisms, they can adversely affect microorganisms, animals, plants and humans at high concentrations. As a matter of fact, TEs can accumulate in plants to harmful concentrations, becoming a threat for human health through the food chain [3]. Accurate and routine determination of their concentration in soil is thus necessary to assess their risk to directly and indirectly enter

the food chain.

Cereal production soils in the Mediterranean basin are regularly amended with organic matter and phosphates bearing TEs and repeatedly irrigated with untreated wastewater [4,5]. A regular monitoring of TE concentrations in these agricultural soils is of great interest. Arsenic, Cd, Co, Cr, Cu, Ni and Pb are the most monitored elements based on previous studies of TE determination in cereal production soils of the Bekaa valley, East Lebanon [6]. Nevertheless, less than 6% of Lebanese arable lands have been checked so far for TE contamination [7].

Although some countries introduced the nondestructive thick target particle induced X-ray emission technique for the determination of trace elements in as received soil samples, digestion methods are still widely practiced [8]. However, the heterogeneous and complex soil matrix, associating organic compounds and mineral constituents with different physicochemical characteristics, does not allow a single solid phase digestion method for the determination of TEs. To be acceptable, precision and accuracy for digestion methods should be lower than 20% [9] for any set of targeted soil to be analysed on a routine basis.

The digestion of samples containing TEs can be done using a dry method: in this case, the sample is calcined in an oven at temperatures ranging between 450°C and 500°C. Dry ashing offers the advantage of requiring few reagents like acids to solubilize ashes. However, dry ashing can lead to a loss of analytes like As and Hg by volatilization.

To reduce the sample preparation time and losses by volatilization, wet digestion processes are an attractive alternative to dry ashing methods for several samples such as biological tissues, food and soils [3,10]. In this case, the sample is mineralized in a mixture of acid at their boiling point. Different acids are usually used to perform the wet

mineralization: hydrochloric acid (HCl), nitric acid (HNO₃), sulfuric acid (H₂SO₄), perchloric acid (HClO₄), hydrofluoric acid (HF). Several combinations were used for the mineralization of soil samples [11–14] but there is no consensus on the choice of the mineralization reagent.

It is admitted that HF combined with HClO₄ allows for a total dissolution of all elements present in the sample but induces Si volatilization. However, HF is dangerous to handle. High precautions and safe handling should be implemented in the laboratory to prevent accidents. Despite this, serious accidents can always occur.

To avoid handling HF, sample attacks using a mixture of other strong acids are usually adopted in routine analysis. HNO₃ has high oxidant properties, most often used in wet mineralization, especially when the analysis step is done by atomic absorption technics [15]. Its relatively low boiling point (120°C) limits its efficiency. Therefore, HNO₃ is commonly used in a mixture with H₂SO₄ or HClO₄ (boiling point at 340°C and 200°C respectively). HClO₄ should be handled carefully because when its concentration exceeds 85% above 150°C, it becomes unstable and presents a serious explosion hazard. The combination of HNO₃ with HCl is well-known as “*aqua regia* mixture”. It has a high dissolution power and is usually used to evaluate the trace element contamination level [16]. *Aqua regia* reagent gives a pseudo-total concentration compared with the HF total method, especially for the following elements (Cr, Ni, Zn and Pb) [17].

Ammonium fluoride (NH₄F) and ammonium bifluoride (NH₄HF₂) are used as a substitute for HF. These compounds are safer than HF for the operator and have high boiling points (260 °C and 239.5 °C) allowing sample digestion at high temperature in open vessels [18].

To optimize the mineralization time, microwave heating is often performed. Hydrogen peroxide (H_2O_2) is largely used in mixture with strong acids for the oxidation and destruction of organic matter [11–13]. Its oxidation power is greatly enhanced in the presence of H_2SO_4 due to the formation of H_2SO_5 [15]. Recently, Magladi et al. [19] used a mixture of NH_4HF_2 and HNO_3 in a microwave oven to dissolve completely a wide range of silicate rock samples for trace element determination. Certain analytes were not fully recovered using this method which limits its field of application.

Most often, TEs concentration in the mineralization solutions is determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) following ISO 22036 [20] and ISO 17294-2 [21] standards. These devices are very expensive and have high maintenance costs but combines low detection limits and a rapid multi-elemental determination in comparison with atomic absorption spectroscopy (AAS). Therefore, it is important to have available a method for TE analysis that is safe, green, inexpensive and accessible to a large number of laboratories. In agreement with green analytical chemistry, an eco-friendly infrared digestion method was recently developed for multi-element determination in soil sample by microwave induced plasma atomic emission spectrometry (MIP-OES) [22]. This method minimizes the reagents amounts, makes digestion safer and reduces waste.

The choice of a sample preparation method is crucial in the analysis of soil and sediment samples. Proper choice reduces matrix effects in complex samples and subsequently limits spectral and non-spectral interferences. The latter involves sample-introduction and plasma related-interferences. This last point becomes very important when using plasma-based atomic emission like ICP-OES and IC-PMS. Nitric acid is usually used to avoid spectral interferences caused by other acids [23].

In this study, two digestion methods were compared using a soil certified reference material: 1) heating 3 acid digestion (HAD) with a mixture of HNO₃, HClO₄ and H₂SO₄, and 2) microwave sulfuric digestion (MSD) using a mixture of H₂SO₄ and H₂O₂. The latter is proposed as a green, simple and fast method for soil sample digestion. The use of H₂SO₄ in sample digestion in combination with H₂O₂ is more advantageous than the use of NH₄F and NH₄F₂. The latter are safer than HF but remain more dangerous than H₂SO₄.

The measurement of As, Cd, Co, Cr, Cu, Ni and Pb concentrations was then conducted using a flame atomic absorption spectrometer (AAS). AAS is less efficient than the widely used plasma based methods (ICP-OES and ICP-MS) because it is less sensitive and only allows the determination of single elements. However, AAS is cheaper than plasma based methods and is available to a large number of laboratories. A validation of the selected digestion method was done using a certified reference material (CRM). Afterward, the validated method (MSD + AAS) was applied on agricultural soil samples collected from the Bekaa region, located in Lebanon, East Mediterranean. The results were compared to those obtained using the reference total digestion method (RTD) (HF-based digestion + ICP/ICPMS).

2. Material and methods

2.1. Chemicals

The standard solutions for the elements As, Cd, Co, Cr, Cu, Ni and Pb of concentrations $1000 \pm 1 \text{ mg L}^{-1}$ were obtained from Sigma-Aldrich. Nitric acid HNO₃ 65%, extra pure, sulphuric acid H₂SO₄ 95% and, perchloric acid HClO₄ 70% and hydrogen peroxide H₂O₂ 30%, obtained from Sigma-Aldrich, were used for digestion. A 1% HNO₃ nitric acid was prepared from concentrated acid (65% HNO₃) and was used in the standard solution

preparation. Ultrapure water ($>18 \text{ M}\Omega \text{ cm}^{-1}$) was obtained from a Milli-Q apparatus (Boeco pure, Germany). The Certified Reference Material (CRM) (SQC001-LRAA8753) for trace elements (As, Cd, Co, Cr, Cu, Ni and Pb) in soil was purchased from Sigma Aldrich.

2.2. Sites and sampling

Lebanon is located in the eastern Mediterranean basin between 35° and $36^\circ 40'$ E and between 33° and $34^\circ 40'$ N. The Bekaa Valley, an agricultural region where more than 90% of Lebanese wheat production is produced [24], is mainly a plain area located between Mount Lebanon in the west and the Anti-Lebanon mountains to the East. Eight sites where soil samples were taken are selected along a transect crossing the central Bekaa valley from southwest to northeast (**Figure 1**). The soils of the sampling sites are Eutric and Vertic Cambisols (B1, B2, B5, B6 and B7), and Eutric Fluvisols (B3, B4ab and B8) according to the World Reference Base [25]. They are deep, predominantly non-calcareous clay, with neutral to weakly basic pH values (7.4 - 7.8) and low to medium organic matter (1.4% - 2.7%).

Most of these sites were irrigated by private tube wells taking water from the deep groundwater reservoir, and by surface waters when available nearby (Berdawni and Litani rivers). Farmers generally practice an annual wheat/potato succession.

Topsoil layer (0 - 20 cm) was sampled using a stainless-steel shovel at the corners of the plot, in the centre and diagonally. The nine subsamples of the same plot were mixed, homogenized and carried in clean polyethylene bags. The samples were then brought to the laboratory where a quartering technique [26] was applied to the overall homogenized sample to reduce the sample size and form a composite sample. Only about 1 kg of soil sample underwent pretreatment.

2.3. Samples pretreatment and digestion

The composite soil samples were cleaned up from roots and gravels, dried according to the AFNOR NF X31-102 standard [27] in an oven (Model 700 Memmert) at 40°C, until constant weight and sieved at 2 mm. Then, soil samples were stored in plastic bottles protected from the direct sunlight and at a temperature below 4°C (in the refrigerator) until analysis.

Before mineralization, the soil samples were ground fine with a mortar and sieved (diameter < 250 µm) following the standard NF ISO 11464 [28]. Two methods of digestion were used in order to mineralize a representative part of the composite soil samples:

i) For the heating 3 acid digestion (HAD), a three-acid mixture (9 mL HNO₃, 2 mL HClO₄, 2 mL H₂SO₄) was added to 0.5 g of soil sample or CRM, followed by heating up to 350°C for 2h 30min in a heating block. The mixture was then stored at room temperature for one night. ii) For the microwave sulphuric digestion (MSD), a microwave type "Milestone - Ethos Easy" was used. Fifteen vessels can be processed simultaneously. This system controls the temperature in all vessels using a direct contactless temperature sensor. An acid mixture of 9 mL of H₂SO₄ and 3 mL of H₂O₂ was added to 0.5 g of soil sample or CRM in a Teflon receptacle tightly closed. A temperature of 200 °C was reached in 10 min and maintained at this level 10 min. Then, the temperature decreased gradually where the cooling step was maintained for 20 min. This method was suggested by Milestone and was subjected to a validation.

All digested samples were filtered and adjusted to 25 mL using HNO₃ 1% before analysis.

2.4. Apparatus for TE analysis

The analyses of TEs were conducted by atomic absorption spectrometry (flame iCE 3000

series - Thermo type, Thermo Fischer Scientific, Germany). An acetylene/air flame was used. Liquid samples were introduced in the spectrometer with an auto sampler using blank and standards for the analysis of the following elements: As, Cd, Co, Cr, Cu, Ni and Pb.

Calibration standard solutions were prepared by successive dilution, all plastic labware used for sampling and sample treatment was new or cleaned by soaking 24 h first in 10% HNO₃ then in ultra-pure water.

2.5. Official or reference methods for trace element analysis

For the reference total digestion (RTD) method, soil sample mineralization was done by a mixture of HF (5 mL) and HClO₄ (1.5 mL) acids added to 0.25g previously ground soil below 250 µm following the standard ISO 14869-1 [29] (details are given in Supplementary Materials SM1). HF decomposes silicates to form volatile SiF₄. The use of HClO₄ is necessary to avoid the precipitation of calcium in the form of fluoride (CaF₂). To avoid the risks of a brutal oxidation (acid ejection) of the organic matter (OM) by HClO₄, the OM was previously destroyed by calcining at 450°C. The hydrofluoric and perchloric acids were eliminated by evaporation at the end of the reaction. The residue was dissolved in diluted nitric acid 2%. Trace elements Co, Cr, Cu and Ni were then analysed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Vista Pro, Varian, France) following the standard ISO 22036 [20], while As, Cd and Pb were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo series 2, France) following the standard ISO 17294-2 [21].

2.6. Statistical analysis

All calculations related to the working range, linearity domain, limit of detection and

limit of quantification were realised using R-4.0.0 [30] and RStudio 1.2.5042 [31] with packages: “car” [32], “lmtest” [33] and “dplyr” [34].

Analysis of variance was performed using XLSTAT 2018 to calculate repeatability and intermediate precision.

Information about various statistical analysis are detailed in following paragraphs, especially in section 3.2.1 for working range, linearity domain, limit of detection and limit of quantification and 3.2.2. for repeatability and intermediate precision.

3. Results and Discussion

3.1. Choice of the best digestion method using the soil Certified Reference Material CMR (SQC001)

To choose the best digestion method, the heating 3 acid digestion (HAD) and the microwave sulfuric digestion (MSD) methods were both applied on the soil CRM (SQC001).

HNO₃ is the most used acid in wet digestion. To overcome its limited efficiency due to its low boiling point, it was used in combination with H₂SO₄ and HClO₄ for the HAD digestion. Moreover, HClO₄ ameliorate the dissolution of sediment samples and insure a complete recovery of trace elements [35]. For the MSD digestion, H₂SO₄ was used because it has the highest boiling point. A mixture of H₂SO₄ and H₂O₂ was used because H₂SO₄ enhances the oxidation power of H₂O₂.

After digestion, the TEs As, Cd, Co, Cr, Cu, Ni and Pb were determined by AAS. The relative recovery, expressed in percentage was calculated using the following equation (Equation 1):

$$R(\%) = \frac{\bar{x}}{x_{ref}} \times 100 \quad (1)$$

where \bar{x} is the mean of CRM after digestion of the sample in triplicate, and x_{ref} is the assigned property value of CRM.

For the HAD method, the relative recovery for the elements analysed by AAS varied between 51.5% and 90.0% (**Table 1**). These values were very low and showed that the HAD method using the mixture HNO₃, HClO₄, H₂SO₄ was not efficient enough to sufficiently solubilize the analysed trace elements.

Table 1: Comparison of the heating 3 acid digestion method (HAD) and the microwave sulphuric digestion method (MSD) using their relative recovery percentage of the soil Certified Reference Material CMR (SQC001)

| Element | Soil CRM | HAD method | | MSD method | |
|---------|--|--|---------------|--|---------------|
| | Assigned value \pm SD mg kg ⁻¹ | Mean Value \pm SD mg kg ⁻¹ | Recovery % | Mean Value \pm SD mg kg ⁻¹ | Recovery % |
| As | 161.0 \pm 3.6 | 115.3 \pm 1.0 | 71.6 | 150.9 \pm 1.2 | 93.7 |
| Cd | 190.0 \pm 3.9 | 154.6 \pm 1.1 | 81.4 | 165.9 \pm 2.4 | 87.3 |
| Co | 177.0 \pm 3.6 | 91.2 \pm 1.0 | 51.5 | 199.3 \pm 4.7 | 112.6 |
| Cr | 87.9 \pm 2.3 | 67.8 \pm 0.7 | 77.1 | 98.7 \pm 1.0 | 112.3 |
| Cu | 258.0 \pm 5.3 | 232.3 \pm 0.3 | 90.0 | 230.1 \pm 2.3 | 89.2 |
| Ni | 127.0 \pm 3.2 | 111.2 \pm 1.1 | 63.5 | 125.0 \pm 1.1 | 98.4 |
| Pb | 138.0 \pm 4.0 | 80.6 \pm 1.2 | 80.6 | 155.4 \pm 1.0 | 112.6 |

SD = Standard deviation

For the MSD method, the relative recovery was in general higher and varied between 87.3% and 112.6%. These values were close to 100% and are within the accepted range for TE analysis (80 - 120%) [36]. Results show that sulphuric acid along with H₂O₂ represents an efficient mixture to solubilize TEs from different soil types, especially when microwave heating is used.

As the microwave sulphuric digestion of CRM gave acceptable recovery percentages, a validation procedure using this method was performed to check its applicability in our laboratory for trace element analysis in some Mediterranean soils.

3.2. Validation of the microwave sulphuric digestion method followed by AAS using the soil Certified Reference Material CMR (SQC001)

To validate the new digestion using a mixture of H₂SO₄ and H₂O₂, before applying it for trace element analysis in the soils, the following parameters were considered and determined: working range including linearity, limit of detection of the analytical system (LOD), limit of quantification (LOQ), precision including repeatability and intermediate precision, specificity and trueness.

3.2.1. Working range, linearity domain, limit of detection (LOD) and limit of quantification (LOQ)

The linearity of a method is often confused with the linearity of the instrument calibration function. In fact, the linearity of a method characterizes its accuracy, whereas instrumental linearity is concerned only with its own response. However, a response function does not have to be linear for correct quantification. Many methods make effective use of calibration models that are much more complex than just linearity [37,38].

Calibration curves for each measured element were built using 4 or 5 points, depending on the element, each point was triplicated. For some elements, quadratic regression $y = \beta_0 + \beta_1x + \beta_2x^2 + \varepsilon$ gave better results and for some others, the best fit model was linear regression: $y = \beta_0 + \beta_1x + \varepsilon$. It can happen when wide dynamic ranges and/or not isotopically pure internal standards are considered (especially true in mass spectrometry) [39]. The independent variable x is assumed unaffected by error. β_0 , β_1 , and β_2 are the

parameters of the model and ε represents a normally distributed random error, with mean zero and constant variance σ^2 [homoscedastic condition and $\varepsilon \sim \mathcal{N}(0, \sigma^2)$]. The β parameters are unknown and ordinary least-squares regression provides their estimates “b” by using a set of experimental data points (x_i, y_i) [40].

The adequacy of the model has been tested in several ways: (i) by the use of an analysis of variance test (ANOVA) called the lack of fit test (with replicate data) [41]; (ii) by inspection of the behaviour of the residuals and (iii) by the evaluation of the determination coefficient R^2 (square of correlation coefficient r). Models and main results of ANOVA tests are summarized in **Table 2** and **Table 3**. More results are given in **Supplementary Materials SM2 and SM3**.

Estimated coefficients b_1 and b_2 were significantly different from zero and b_0 coefficient, depending on element, was not significantly different from zero. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following equations [42]:

$$LOD = \frac{3}{b_1} \sqrt{\frac{s_{Bl}^2}{n_{Bl}} + s_{b_0}^2} \quad (2)$$

Where b_1 is the sensitivity and, s_{Bl} is the standard deviation of the blank measurements, n_{Bl} is the number of blank measurements and s_{b_0} is the standard deviation of the intersection with the vertical axis. In case of the quadratic model, when the instrument detector is working close to zero concentrations, the contribution of the 2nd degree term in the calibration curve is negligible.

$$LOQ \cong 3 \times LOD \quad (3)$$

Table 2. Calibration curves of the measured elements

| Element | Model | Residual behaviour (normality, homoscedasticity, independence) |
|---------|-----------|--|
| As | quadratic | Passed |
| Cd | quadratic | Passed excepted normality* |
| Co | quadratic | Passed excepted normality* |
| Cr | linear | Passed excepted autocorrelation * |
| Cu | linear | Passed |
| Ni | linear | Passed excepted normality & autocorrelation, presence of 1 outlier |
| Pb | quadratic | Passed |

* Due to the presence of one or two extreme values (checked with Bonferroni Outlier Test) [32]. The calculation of a reliable calibration model needed in general many values to be at least between 8 and 10 to verify the normality of the data by the Shapiro–Wilk test (Shapiro & Wilk, 1965) for instance, and to ascertain their scedasticity; and the number of concentration levels must range between 7 and 10 [43]. In this study, the number of levels is between 4 and 5 with 3 replicates. So, the calibration models were evaluated with and without extreme values and coefficients were not drastically affected. It was decided to keep them.

Table 3. Working ranges, regression equations, limit of detection (LOD) and limit of quantification (LOQ) of the measured elements

| Element | Working range mg kg ⁻¹ | Regression equation y = ax ² + bx + c or y = ax + b | Standard errors on a, b and c | Regression quality R ² | LOD mg kg ⁻¹ | LOQ mg kg ⁻¹ |
|---------|--------------------------------------|--|----------------------------------|--------------------------------------|----------------------------|----------------------------|
| As | 1.25 – 12 | y = -0.0032x ² + 0.1599x - 0.0616 | 0.0089; 0.0035; 0.0003 | 0.9997 | 0.166 | 0.498 |
| Cd | 5 – 15 | y = -0.0017x ² + 0.0566x - 0.1077 | 0.0119; 0.0027; 0.0002 | 0.9983 | 0.630 | 1.890 |
| Co | 3 – 12 | y = -0.0075x ² + 0.1673x - 0.2153 | 0.0034; 0.0011; 0.0001 | 0.9999 | 0.062 | 0.186 |
| Cr | 1 – 50 | y = 0.0637x + 0.0204 | 0.0301; 0.0012 | 0.9956 | 1.736 | 5.041 |
| Cu | 5 – 15 | y = 0.1381x - 0.2347 | 0.0030; 0.0003 | 1.000 | 1.764 | 1.915 |
| Ni | 1 – 15 | y = 0.1036x + 0.2119 | 0.0610; 0.0065 | 0.9620 | 3.811 | 7.931 |
| Pb | 5 – 25 | y = -0.0158x ² + 2.1287x - 3.2825 | 0.2628; 0.0440; 0.0014 | 0.9999 | 0.370 | 1.110 |

High regression quality was obtained for all elements. Low detection limits, below 1 mg kg⁻¹ were obtained for all elements except Cr, Cu and Ni for which LOD was 1.736, 1.764 and 3.811 mg kg⁻¹ respectively (**Table 3**). If we consider the threshold of Cr and Ni for multifunctional land use, where farmers can grow everything including the leaf succulent plants, are 50 and 40 mg kg⁻¹ respectively [44], this means an error less than 3% and 4.4% for these two TEs, respectively. For agricultural crops and fruit trees, these values are reduced by 4 and 2.5 times, which means high reliability of results to orient land use policy and protect public health from TE transfer risks to food chain.

3.2.2. Precision including repeatability and intermediate precision

Precision is a measure of how close results are to one another, usually expressed by the standard deviation [45]. Repeatability is the test results obtained using the same method,

on the same sample in the same laboratory, with the same equipment, by the same operator, in short intervals of time. Other terms are used to define repeatability, such as within-run, within-batch or intra-assay precision. Repeatability gives the smallest variation in results. Reproducibility is the test results obtained by different operators, different laboratories over a long period. It gives the largest variation in results. Between these two extremes, intermediate precision estimates result's variation in the same laboratory under routine conditions by different analysts and during an extended timescale. Other terms can be used to define intermediate precision such as between-run, between-batches, or inter-assay variation.

To determine repeatability and intermediate precision, two operators measured the metal concentrations in the soil CRM during each of the nine days. One-way ANOVA was applied on the obtained results. Repeatability was calculated as the within-group precision and expressed by the repeatability standard deviation S_r . It was calculated using the square root of the within-group mean square MS_w :

$$S_r = \sqrt{MS_w} \quad (4)$$

Intermediate precision was calculated as the within-group and between-group precision and expressed by the intermediate precision standard deviation S_i . It was calculated by combining the within- and between-group variance.

$$S_i = \sqrt{MS_w + \frac{MS_b - MS_w}{n}} \quad (5)$$

MS_b is the between-group mean square and MS_w the within-group mean square. Both values were calculated from the one-way ANOVA table. Relative standard deviation RSD varied from 0.3% to 1.5% for the first operator and from 0.1% to 1.2% for the second operator. The higher RSD was obtained for Co whatever the operator was.

Repeatability standard deviation varied from 0.43 to 2.46 mg kg⁻¹. These values were relatively low and reflected good repeatability conditions (**Table 4**).

Table 4. Repeatability and intermediate precision results obtained after TE analysis of CRM soil using the microwave sulphuric digestion method (n = 9 for both operators).

| Parameters | units | TE quantification | | | | | | |
|-----------------------|---------------------|-------------------|-------|-------|------|-------|-------|-------|
| | | As | Cd | Co | Cr | Cu | Ni | Pb |
| Operator 1 | | | | | | | | |
| mean | mg kg ⁻¹ | 151.5 | 165.5 | 186.3 | 98.5 | 233.8 | 123.7 | 126.4 |
| SD | mg kg ⁻¹ | 0.5 | 2.4 | 2.7 | 0.6 | 2.1 | 0.6 | 1.0 |
| RSD | % | 0.3 | 1.4 | 1.5 | 0.6 | 0.9 | 0.5 | 0.8 |
| Operator 2 | | | | | | | | |
| mean | mg kg ⁻¹ | 151.0 | 167.3 | 182.4 | 98.1 | 235.0 | 124.0 | 126.9 |
| SD | mg kg ⁻¹ | 0.9 | 0.7 | 2.2 | 0.4 | 0.2 | 0.1 | 0.5 |
| RSD | % | 0.6 | 0.4 | 1.2 | 0.4 | 0.1 | 0.1 | 0.4 |
| S_r | mg.kg ⁻¹ | 0.70 | 1.76 | 2.46 | 0.49 | 1.52 | 0.43 | 0.76 |
| S_i | mg kg ⁻¹ | 0.76 | 2.10 | 3.62 | 0.54 | 1.65 | 0.46 | 0.81 |
| RS_i | % | 0.5 | 1.3 | 2.0 | 0.5 | 0.7 | 0.4 | 0.6 |

SD = Standard Deviation; RSD = Relative Standard Deviation

S_r = repeatability standard deviation; S_i = intermediate precision standard deviation

RS_i = intermediate precision relative standard deviation

Intermediate precision varied between 0.46 and 3.62 mg kg⁻¹, which represented 0.4 to 2.0% as the relative standard deviation. These relatively low values reflect an acceptable variation of the results obtained by the two operators who applied the same method under routine conditions. The acceptable value for repeatability depends on concentration and was set at 8% for samples at the mg kg⁻¹ level [46].

3.2.3. Specificity

The lack of specificity of a method can be due to the widening of the absorption signal, but most of the time, it is due to the presence of interfering elements, which could increase or hinder the signal of a specific element. Interfering compounds are various constituents of the matrix and/or the reagents used to prepare the sample. It is what is called matrix effects. The International Conference on Harmonisation [47] defined specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, this might include impurities, degradants, matrix, etc.”

It was evaluated by analysing the CRM soil alone and after addition of known quantities of each element. Two levels of concentration for each element were added to the CRM soil: 50 and 100 mg kg⁻¹. Samples were labelled CRM, CRM1 and CRM2. Samples were digested in triplicate and each sample was measured 3 times. The specificity was calculated using the recovery ratio expressed in percentage:

$$R(\%) = \frac{\text{experimental quantity after standard addition} - \text{experimental quantity before standard addition}}{\text{known added quantity}} \times 100 \quad (6)$$

The mean recovery of samples CRM, CRM1 and CRM2 and their respective standard deviations, for all the being studied elements are gathered in **Table 5**.

Table 5. Specificity results expressed as mean recovery percentage and standard deviation. Three samples were analysed: CRM soil alone and CRM after the addition of two concentration levels for each element (n = 9).

| Trace elements | Mean recovery percentage and standard deviation | | |
|----------------|---|---|--|
| | % | | |
| | Soil CRM | Soil CRM 1 + 50 mg kg ⁻¹ of each TE | Soil CRM 2 + 100 mg kg ⁻¹ of each TE |
| As | 93.7 ± 0.9 | 96.1 ± 9.6 | 104.0 ± 4.9 |
| Cd | 87.3 ± 4.7 | 94.7 ± 9.2 | 92.0 ± 6.3 |
| Co | 112.6 ± 0.1 | 116.9 ± 0.3 | 117.8 ± 0.8 |
| Cr | 112.3 ± 3.5 | 111.5 ± 7.2 | 109.9 ± 5.7 |
| Cu | 89.2 ± 3.9 | 90.6 ± 4.0 | 99.1 ± 4.9 |
| Ni | 98.4 ± 0.9 | 98.1 ± 2.1 | 97.7 ± 1.7 |
| Pb | 112.6 ± 0.1 | 116.9 ± 0.2 | 117.8 ± 0.8 |

The specificity results show that the recovery percentages of all elements are within [80 - 120%] interval. Specificity values for TEs in soil have not been found in the literature. However, EU commission regulation No 2001/22/EC [48] mentions that recovery between 80 - 120% is acceptable for TEs such as lead, cadmium and mercury

in foodstuffs. These results allow the application of the developed method in routine analysis without causing interference for As, Cd, Co, Cr, Cu, Ni and Pb.

3.2.4. Comparison between the validated method (MSD + AAS) and the RTD method (HF-based digestion + ICP/ICPMS) of TE analysis. Accuracy or trueness.

Accuracy is defined as “the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found” [47]. Accuracy is sometimes defined as trueness. Accuracy can be determined by analysing a CRM and comparing the experimental value to the assigned value. It is actually what we did in paragraph 3.1 where we obtained recovery percentages between 87 and 112%. Another way to assess accuracy is to work on real samples, to analyse them by the method being validated and to compare results with that obtained from a reference method. In this study, TEs in soil samples from the Bekaa governorate were determined by an MSD method using a mixture of H₂SO₄ and H₂O₂ followed by AAS measurements. Microwave digestion and element analysis were done in triplicate. Results from this method were compared to an external ISO reference which is the RTD method where the same samples were digested by HF and HClO₄ and analysed by ICP-AES or ICP-MS. The RTD method allows for total digestion of soil samples.

Results were compared in terms of recovery percentage calculated using equation 1. They are presented in **Table 6**.

1 Table 6. Comparison between the MSD method (H₂SO₄ + H₂O₂ digestion) being validated and the RTD method (HF + HClO₄ digestion) for TEs in soil samples from the Bekaa governorate sites.

| Sites | Trace elements | | | | | | | | | | | | | | | | | |
|---------------------|----------------|-----------|---------------------|------------|------------|---------------------|--------------|------------|---------------------|------------|------------|---------------------|------------|------------|---------------------|------------|------------|------|
| | As | | | Co | | | Cr | | | Cu | | | Ni | | | Pb | | |
| | RTD±SD | MSD±SD | Rec. | RTD±SD | MSD±SD | Rec. | RTD±SD | MSD±SD | Rec. | RTD±SD | MSD±SD | Rec. | RTD±SD | MSD±SD | Rec. | RTD±SD | MSD±SD | Rec. |
| mg kg ⁻¹ | | | mg kg ⁻¹ | | | mg kg ⁻¹ | | | mg kg ⁻¹ | | | mg kg ⁻¹ | | | mg kg ⁻¹ | | | |
| B1 | 6.8 ± 0.4 | 3.3 ± 0.0 | 48 | 18.3 ± 2.0 | 10.0 ± 0.0 | 55 | 94.1 ± 12.8 | 59.6 ± 0.1 | 63 | 22.0 ± 1.7 | 18.1 ± 0.1 | 82 | 56.1 ± 3.0 | 39.4 ± 0.2 | 70 | 13.8 ± 0.8 | 11.5 ± 0.0 | 83 |
| B2 | 4.5 ± 0.2 | 3.1 ± 0.0 | 69 | 13.3 ± 1.7 | 9.7 ± 0.0 | 73 | 83.8 ± 11.6 | 60.7 ± 0.1 | 72 | 19.8 ± 1.6 | 16.3 ± 0.1 | 83 | 47.3 ± 2.7 | 25.6 ± 0.2 | 54 | 16.8 ± 1.0 | 12.3 ± 0.0 | 73 |
| B3 | 4.4 ± 0.2 | 4.2 ± 0.0 | 95 | 11.4 ± 1.5 | 9.7 ± 0.1 | 85 | 75.1 ± 11.3 | 50.2 ± 0.1 | 67 | 17.2 ± 1.5 | 13.5 ± 0.1 | 78 | 43.6 ± 2.5 | 27.5 ± 0.2 | 63 | 24.5 ± 1.5 | 19.9 ± 0.0 | 81 |
| B4a | 7.3 ± 0.4 | 4.2 ± 0.0 | 57 | 11.4 ± 1.6 | 10.7 ± 0.0 | 94 | 55.8 ± 8.5 | 43.2 ± 0.1 | 77 | 16.6 ± 1.5 | 15.2 ± 0.0 | 91 | 39.4 ± 2.4 | 40.0 ± 0.2 | 102 | 9.1 ± 0.6 | 6.0 ± 0.0 | 66 |
| B4b | 7.5 ± 0.4 | 4.1 ± 0.0 | 55 | 11.7 ± 1.6 | 10.4 ± 0.1 | 89 | 57.2 ± 8.6 | 43.9 ± 0.0 | 77 | 14.5 ± 1.4 | 14.4 ± 0.2 | 100 | 39.7 ± 2.4 | 39.4 ± 0.6 | 99 | 8.8 ± 0.6 | 6.4 ± 0.0 | 73 |
| B5 | 11.3 ± 0.6 | 9.9 ± 0.0 | 87 | 25.2 ± 2.3 | 17.3 ± 0.1 | 69 | 129.0 ± 16.7 | 68.2 ± 0.1 | 53 | 27.9 ± 1.8 | 15.6 ± 0.1 | 56 | 78.9 ± 3.9 | 46.8 ± 0.1 | 59 | 17.5 ± 1.1 | 15.5 ± 0.1 | 89 |
| B6 | 6.4 ± 0.4 | 4.2 ± 0.0 | 66 | 14.9 ± 1.9 | 13.4 ± 0.1 | 90 | 71.7 ± 10.8 | 50.4 ± 0.0 | 70 | 23.0 ± 1.8 | 16.5 ± 0.0 | 72 | 52.0 ± 2.7 | 50.2 ± 0.1 | 96 | 15.0 ± 0.9 | 5.7 ± 0.0 | 38 |
| B7 | 7.7 ± 0.4 | 4.3 ± 0.0 | 55 | 19.1 ± 2.5 | 17.0 ± 0.0 | 89 | 94.3 ± 10.7 | 90.4 ± 0.0 | 96 | 23.9 ± 1.9 | 16.8 ± 0.0 | 70 | 58.5 ± 3.0 | 50.4 ± 0.0 | 86 | 18.6 ± 1.1 | 5.8 ± 0.0 | 31 |
| B8 | 9.7 ± 0.5 | 8.2 ± 0.0 | 85 | 23.0 ± 2.2 | 16.7 ± 0.1 | 72 | 114.0 ± 15.0 | 71.2 ± 0.1 | 62 | 25.9 ± 1.8 | 15.6 ± 0.0 | 60 | 64.2 ± 3.3 | 39.2 ± 0.1 | 61 | 19.5 ± 1.2 | 17.3 ± 0.1 | 89 |

2 RTD = Reference Total Digestion method mean value; MSD = Microwave Sulphuric Digestion method mean value; Rec. = mean Recovery; SD = Standard deviation

3

4 All recovery values were below 100%, which was predictable because the MSD with a
5 mixture of H₂SO₄ and H₂O₂ does not induce a total mineralization of silicates compared with
6 the RTD method using a mixture of HF and HClO₄. High recovery percentages were obtained
7 for Co, Cr and Cu. Two-thirds of the calculated recoveries were higher than 70%, which is an
8 acceptable value for trace analysis as defined by (AOAC 2002) [46]. The other third was
9 between 50 and 70% except for 3 values (As for B1 and Pb for B6 and B7). As, Co, Cr and Pb
10 concentrations measured using the validated MSD method were correlated with values obtained
11 using the RTD method. The relation was greatly improved for Pb ($R^2 = 0.9624^*$ with a slope =
12 1.0486) when B6 and B7 were removed. For Cr, the relationship was greatly improved when
13 B7 was not considered ($R^2 = 0.9074^*$) and indicated a reduction of the recovery percentage
14 with increased total concentrations. The most contaminated soil was B5, with the highest
15 concentrations for As, Co, Cr, Cu and Ni. This plot is occasionally irrigated by pumping water
16 from an irrigation canal from the Berdawni River, the catchment of which drains the town of
17 Zahleh. Nevertheless, all soils remained in the lower range of concentrations found in soils
18 worldwide for all elements [14]. Cobalt, Cr, Cu and Ni concentrations were correlated
19 indicating a probable common geogenic origin. Arsenic was only correlated to Co and Ni,
20 which may be related to a more scattered occurrence due to anthropogenic inputs [49]. The lead
21 concentration was not correlated to that of any of the other elements, probably because two
22 soils (B6 and B7) had a very low recovery rate. These results confirm the reported low content
23 in the soils of the Central Bekaa area and depicted from the spatial distribution of total Pb in
24 the soil surface [50]. The pattern of Pb concentration shows, above a weak geogenic background
25 (4 - 14 mg kg⁻¹), an anthropic contamination along heavy trafficked roads by atmospheric
26 deposition [51] and surface accumulation gradient of Pb from foot-slope, located downslope of
27 Zahleh town, towards the Litani River (14 - 34 mg kg⁻¹). This lead is carried by water runoff
28 from Zahleh agglomeration (see **Figure S1, Supplementary Materials SM4**).

29 Arsenic values obtained by the RTD method varied between 4.35 mg kg⁻¹ for B3 and
30 11.3 mg kg⁻¹ for B5. They were slightly above the commonly reported mean background As
31 concentrations in surface soils (worldwide data) [14], but still within the range for common,
32 possible background values and multifunctional land use [44].

33 Cadmium values were not shown in this study because they were below quantification
34 limit (1.89 mg kg⁻¹) when using the MSD validated method. Cd could not be determined by this
35 method. However, all values determined by the RTD method varied between 0.23 mg kg⁻¹ for
36 B4b and 0.48 mg kg⁻¹ for B8 and were defined within the global average soil Cd concentration
37 (0.06 - 1.1 mg kg⁻¹) [14]. Our values are in agreement with those found by Kanbar et al.
38 (0.28 - 2.8 mg kg⁻¹) in different Lebanese soils [52] and those found by Darwish in Bekaa soils
39 (0.28 - 0.48 mg kg⁻¹) [7].

40 Cobalt values obtained by the RTD method varied between 11.4 mg kg⁻¹ for B3 and
41 25.2 mg kg⁻¹ for B5. These values are close to the range of world values in surface soils
42 (4.5 - 12 mg kg⁻¹) [14]. They are comparable to those obtained for arid and semiarid regions
43 (16.5 - 26.8 mg kg⁻¹) [53]. They are also in agreement with those found by Darwish in Bekaa
44 soils (28.1- 28.5 mg kg⁻¹) [7].

45 Chromium values obtained by the RTD method varied between 55.8 mg kg⁻¹ for B4a
46 and 129.0 mg kg⁻¹ for B5. These values are higher than the world median content of Cr in soils:
47 54 mg kg⁻¹. They are within the sandy and light loamy soils range, which contains Cr between
48 2 and 350 mg kg⁻¹ [14]. They are close to those found by Darwish in Bekaa soil samples (43.3
49 and 71.3 mg kg⁻¹) [7] and do not exceed the limits for safe agricultural land use.

50 Copper values obtained by the RTD method varied between 14.5 mg kg⁻¹ for B4b and
51 27.9 mg kg⁻¹ for B5. These results are within the range for Cu concentration in soils (8 mg kg⁻¹
52 ¹ in acid sandy soils to 80 mg kg⁻¹ in heavy loamy soils) [14]. They are in general lower than

53 those obtained in other Lebanese soils (25.3 - 54.2 mg kg⁻¹) [52], among others Taalabaya
54 (Zahleh district) soils (35.7 mg kg⁻¹) [7], but conform to the values obtained in Saadnaeal
55 (Zahleh district) soils (17.0 mg kg⁻¹).

56 Nickel values obtained by the RTD method varied between 39.4 mg kg⁻¹ for B4a and
57 78.9 mg kg⁻¹ for B5. These values are within the range of Ni concentration in soils worldwide
58 (0.2 - 450 mg kg⁻¹) [14]. They are in agreement with values obtained in Taalabaya soils
59 (60.7 mg kg⁻¹) but higher than values obtained in Saadnaeal soils sampled at the same depth
60 (33.7 mg kg⁻¹) [7].

61 Lead values obtained by the RTD method varied between 8.84 mg kg⁻¹ for B4b and
62 24.48 mg kg⁻¹ for B3. They are lower than the overall mean value of Pb in different soils
63 worldwide (25 mg kg⁻¹) [14]. They are in agreement with those obtained in other Lebanese soils
64 (11.3 - 22.9 mg kg⁻¹) [52] and those obtained in Taalabaya soils (16.7 mg kg⁻¹) and Saadnaeal
65 soils (10.0 mg kg⁻¹) [7].

66 In summary, the results obtained in this study are representative of soils with limited
67 trace elements contamination. The concentrations measured are within the same range than the
68 values obtained by Darwish for surface soils and soil profiles of the Bekaa plain. He points to
69 both a natural TE source with variation explained by the alluvial-colluvial origin of the parent
70 material but also to a limited anthropogenic pollution due to a combination of inputs from
71 cultivation and atmospheric deposition which is modulated by soil organic content.

72 The correlations between TEs values were higher following the RTD method than the
73 validated one, suggesting that the RTD method can better trace the common origin of the
74 elements, which was mostly of geogenic origin, as also indicated by the observed level of
75 concentrations, which were close to the background level detected in North Lebanon [8].

76 *Aqua regia*, which is a reference method for pseudo-total metal concentrations in soils,
77 gives extraction efficiency between 53 and 100%, depending on the element, as compared to
78 total concentrations (calculated for the certified material METRANAL-33 in Shahbazi and
79 Beheshti) [54]. Although it is widely used, resorption occurs (up to 470% for Cu) leading to
80 underestimation of element concentrations [55] and other mild extraction procedures may
81 provide accurate results when trace element reactive fraction, not bound to silicates, is to be
82 recovered [55]. Our results fall within the same range of efficiency as *aqua regia* and the
83 validated method including the microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ may offer a more
84 feasible and workable alternative.

85 **4. Conclusion**

86 The microwave digestion with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2$ combined with flame-AAS measurement was
87 validated with CRM and after comparison with HF-based digestion. It allows for routine but
88 accurate characterization of calcareous and Ca-saturated soils with limited contamination in As,
89 Cd, Cu, Co, Cr and Pb. The tested soils, used for wheat production, are irrigated with water of
90 poor quality and need to be monitored for their trace element concentrations regularly to prevent
91 transfer to the food chain.

92 Recent microwave digestion methods were implemented according to green chemistry,
93 they have proved their efficiency for the determination of trace elements in soil samples
94 [11,12,19,22]. In these studies, several reagents were used to dissolve soil or sediment samples
95 such as: HNO_3 , HClO_4 , HF, HCl, NH_4HF_2 and H_2O_2 before trace element analysis using ICP-
96 OES, ICP-MS or MIP-OES. These recent methods uses problematic reagents difficult to handle
97 safely and/or very expensive analytical devices.

98 The validated method offers an alternative to HF-based methods, as well as *aqua regia*.
99 It is green, safe and cheap method for trace element analysis in soil samples. Other soil types

- 100 will have to be tested in order to further extend its use to a larger range of soils to cover the soil
- 101 diversity in the Mediterranean area.

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