# A comparison of two digestion methods for the analysis of heavy metals by flame atomic absorption spectroscopy

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## Introduction

Heavy metals in soil samples can be determined by highly sensitive spectroscopic techniques, such as atomic absorption spectroscopy [e.g. Flame Atomic Absorption Spectroscopy (FAAS) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS)] and Inductively Coupled Plasma (ICP) spectrometry [e.g. ICP-Atomic Emission Spectrophotometry (ICP-AES) and ICP-Mass Spectrometry (ICP-MS)]. These techniques require the transformation of a solid sample into solution from which metal concentrations are determined. Heavy metals in soil samples are usually converted to a soluble form through acid digestion methods. The most important methods used for soil digestion consist of open vessel digestion by digester block and closed vessel digestion by microwave oven. Given that it is well known that different digestion methods may lead to different results, a validation process is needed to demonstrate data quality and select the most useful method according to the characteristics of the study area and, particularly, soil properties. Moreover, results obtained applying these digestion methods are generally used to assess the degree of pollution in soils. Therefore, the choice of the digestion method has to be suitable in order to provide information on the total content of heavy metals in soils and consequently about the pollution levels.

The aim of this study is to compare two of the most applied digestion methods for heavy metals determination in calcareous soils, evaluating the most effective and useful digestion method to be applied to agricultural soils devoted to growing vegetable crops in the Valencian Mediterranean region, a representative area of the European Mediterranean region. Open vessel digestion using a digester block with $HNO_3$, $HClO_4$ and HCl and microwave-assisted digestion using $HNO_3$ and HCl were carried out on soil samples. The first step was to validate both methods using a certified reference material (CRM) with similar properties and characteristics to soils from the study area. The second step was to apply the digestion methods on agricultural soils of the Lower Segura basin, as a pilot area of the Valencian Mediterranean region. The heavy metals determined were some of those considered being of great environmental concern in agricultural soils, such as Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. The determination of heavy metals in solutions was performed by FAAS for all the elements.

## Digestion methods and determination with FAAS

Heavy metal concentrations were initially analysed by the method proposed by the Ministry of Agriculture, Fisheries and Food (MAFF) of the United Kingdom.[1] A pre-digestion step with 10 mL of $HNO_3$ overnight was included to improve digestion of calcareous soils.[2] Soils were digested at 180°C for 120 min and 200°C for 30 min in a digester block. Then, 10 mL of $HNO_3$ and 3 mL of $HClO_4$ were added and samples were digested at 200°C until white fumes were given off and, finally, 4 mL of HCl were added to dissolve the residue. Separation of the extracts from the solid residue was carried out by centrifugation at 3500 rpm for 5 min and then diluted to 50 mL with Milli-Q® (Millipore), purified water.

Heavy metal concentrations were also analysed using a microwave oven according to the standard digestion method of the United States Environmental Protection Agency[3] (US-EPA), which consists of digesting soil samples in a microwave oven with 9 mL of $HNO_3$ and 3 mL of HCl for 5 min at 700 W (to reach 180°C) and 10 min at 500 W (to maintain 180°C). After cooling, separation of the extracts from the solid residue was carried out by centrifugation at 3500 rpm for 5 min and then dilution to 25 mL with Milli-Q water.

Heavy metals in solutions were determined by FAAS. The spectrometer, model Varian SpectrAA-220FS, was equipped with hollow cathode lamps (HCL) specific for each element and a deuterium lamp for continuous background correction. The instrument was also equipped with an autosampler controlled by SpectrAA system software (SpectrAA 220, version 2.0 FS). Instrumental operating parameters for each element (e.g. wavelength, slit width etc.) are shown in Table 1. Calibration with stock standard solutions

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of 1000 mg L$^{-1}$ of Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn was used to prepare a series of composite standards the concentrations of which in 0.5% nitric acid ranged between 0.15–3 mg L$^{-1}$ for cobalt, 0.07–3 mg L$^{-1}$ for chromium, 0.04–4 mg L$^{-1}$ for copper, 0.6–50 mg L$^{-1}$ for iron, 0.02–2 mg L$^{-1}$ for manganese, 0.10–3 mg L$^{-1}$ for nickel, 0.20–5 mg L$^{-1}$ for lead and 0.02–0.8 mg L$^{-1}$ for zinc. To analyse concentrations over the calibration range, solutions were diluted using the appropriate acid diluent. All calibration curves for heavy metals had a correlation coefficient of $R^2 \geq 0.999$. Instrument detection limit (IDL) and quantitation limit (QL) are also given in Table 1.

## Heavy metal concentrations in CRM

Validation of digestion methods is necessary to assure that reliable results and conclusions are obtained.[4] The validation process was carried out using a certified reference material (CRM 141R, Calcareous Loam Soil) supplied by the Community Bureau of Reference of the European Communities. CRM 141R was analysed using the digestion methods described above in order to check data quality. Calculation of the recovery of each metal was based on the mean certified value [measured concentration (mg kg$^{-1}$) / mean certified value for CRM 141R (mg kg$^{-1}$) × 100]. Six replicate measurements were made for the CRM

141R by open vessel digestion and eight replicate measurements by microwave-assisted digestion for several days. The precision of replicate analyses of CRM 141R was defined as relative standard deviation (RSD), which is calculated from the standard deviation (SD) divided by the mean.

Heavy metal content in the blanks was close to or below the detection limit, indicating a null contamination effect in both methods. Recoveries for open vessel digestion varied from 88% to 96%. However, a high recovery was found for Co (124%). High recovery for Co, which has also been reported by other authors, is due to the great complexity of the dissolved matrix, which can affect the recovery of some minor elements. RSD values were lower than or equal to 5%, with the only exceptions being Co and Cr (27% and 19%, respectively), which showed higher RSDs due to variations in hot-plate temperatures and refluxing times, which affected the concentrations of minor elements. On the other hand, recoveries obtained by microwave-assisted digestion were satisfactory for all elements, ranging from 83% to 103%, although a low value was found for Zn (76%). This low value is probably due to the reported binding of Zn to organic matter and to mineral residue, which are moderately resistant to dissolution with $HNO_3$ and HCl. Recoveries from microwave digestion were slightly lower than

recoveries from the digester block. This could be due to the use of $HClO_4$ in open vessel digestion, which has a high oxidising power and improves recoveries. However, the use of $HClO_4$ in pressurised systems is recognised as a potential explosion risk and, therefore, this reagent was not used in closed vessels. RSD values for microwave digestion were lower than or equal to 7% with the exception of Co and Cr (12% and 10%, respectively). In this case, RSD values for both elements by microwave-assisted digestion were lower and more acceptable than values obtained by open vessel digestion. Microwave-assisted digestion is, therefore, a more precise method than open vessel digestion as determined from the validation process carried out, although higher recoveries were, in most cases, achieved by open vessel digestion.

## Heavy metal concentrations in soils

After the validation process, the digestion methods were applied to calcareous soil samples selected from agricultural soils in the Lower Segura basin, a pilot area of the Valencian Mediterranean region, in order to compare results obtained by open vessel digestion with those obtained by microwave-assisted digestion. A total of 29 calcareous soil samples were analysed using both methods to determine their heavy metal contents. Characteristics of the study area and sampling procedure are described in full in a previous paper.[5] Summary statistics are given in Table 2 and are recorded for each element. Further, the paired-samples t-test was used to compare heavy metal contents obtained by both methods. A low significance value ($p < 0.05$) indicates that there is a significant difference between both populations and, therefore, a significant difference between results achieved by both digestion methods for a given element. As it was expected, the results obtained are different for some heavy metals. Application of the paired-samples t-test demonstrated that there was a significant difference for Co, Cr, Cu, Mn, Ni and Pb when results were analysed sample by sample. On the other hand, linear regression showed a good correlation between open vessel digestion and microwave-

**Table 1.** Instrumental parameters used by Varian SpectrAA-220FS spectrometer.

| Metal | Wavelength (nm) | Slit width (nm) | Background correction | Air:acetylene (L min$^{-1}$) | Detection limit (mg L$^{-1}$) | Quantitation limit (mg L$^{-1}$) |
|-------|-----------------|-----------------|-----------------------|------------------------------|-------------------------------|----------------------------------|
| Co | 240.7 | 0.2 | Yes | 13.5:2.0 | 0.05 | 0.15 |
| Cr | 357.9 | 0.2 | No | 13.5:3.0 | 0.02 | 0.07 |
| Cu | 324.8 | 0.5 | No | 13.5:2.0 | 0.01 | 0.04 |
| Fe | 372.0 | 0.2 | No | 13.5:2.0 | 0.2 | 0.6 |
| Mn | 279.5 | 0.2 | No | 13.5:2.0 | 0.01 | 0.02 |
| Ni | 232.0 | 0.2 | Yes | 13.5:2.0 | 0.03 | 0.10 |
| Pb | 217.0 | 1.0 | Yes | 13.5:2.0 | 0.06 | 0.20 |
| Zn | 213.9 | 1.0 | Yes | 13.5:2.0 | 0.006 | 0.02 |

**Table 2.** Summary statistics for heavy metal content in the Lower Segura basin soil samples by digester block (D) and microwave oven (M) digestions.

| Metal | Mean (mg kg$^{-1}$) | | SD (mg kg$^{-1}$) | | Min (mg kg$^{-1}$) | | Max (mg kg$^{-1}$) | | RSD (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | D | M | D | M | D | M | D | M | D | M |
| Co | 8.2 | 7.9 | 1.7 | 1.2 | 5.1 | 5.8 | 11.6 | 10.1 | 21 | 16 |
| Cr | 34.2 | 28.3 | 10.1 | 5.4 | 19.0 | 21.1 | 56.0 | 42.5 | 29 | 19 |
| Cu | 18.8 | 21.6 | 3.5 | 3.1 | 12.5 | 16.1 | 28.0 | 30.6 | 19 | 15 |
| Fe | 15,185 | 15,274 | 2486 | 2181 | 10,279 | 10,979 | 20,215 | 19,807 | 16 | 14 |
| Mn | 275 | 320 | 41 | 41 | 184 | 213 | 366 | 406 | 15 | 13 |
| Ni | 24.8 | 23.7 | 4.9 | 3.7 | 14.9 | 16.4 | 35.3 | 32.0 | 20 | 16 |
| Pb | 23.1 | 19.6 | 6.6 | 5.2 | 7.9 | 8.9 | 39.2 | 34.5 | 28 | 27 |
| Zn | 56.5 | 57.8 | 11.3 | 10.6 | 29.9 | 33.4 | 80.6 | 80.7 | 20 | 18 |

SD, standard deviation; Min, minimum value; Max, maximum value; RSD, relative standard deviation; D, open vessel digestion by digester block; M, closed vessel digestion by microwave

assisted digestion for Cu, Fe, Mn, Ni, Pb, and Zn ($R^2 > 0.75$) (Figure 1). This finding suggests that open vessel digestion may be equivalent to microwave-assisted digestion for determining these elements in the study area. However, Co and Cr presented a low determination coefficient ($R^2 < 0.50$) indicating a slight difference between the methods. Thus, open vessel digestion and microwave-assisted digestion are not interchangeable for evaluating these elements. On the other hand, RSD values recorded for microwave-assisted digestion were lower than values found for open vessel digestion, reflecting a lower variability in the whole population by microwave digestion. This is due to a greater variability with the digester block, since digestion is carried out in open vessels and splashing can be produced between vessels. In fact, the digester block produces loss of volatile elements and presents a high potential for contamination. Further, this conventional digestion system is the more time-consuming analytical process and, therefore, micro-

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wave oven digestion is preferred in some cases. It follows that the microwave-assisted digestion provides more reliable results and is also a quicker and safer extraction process with which to estimate heavy metal content for agricultural soils in the Lower Segura basin.

## Summary

Application of open vessel digestion and microwave-assisted digestion to Mediterranean agricultural soils showed a significant difference between results achieved by both digestion methods for Co, Cr, Cu, Mn, Ni and Pb. However, regression analysis indicated a good correlation between digester block and microwave oven digestion methods for Cu, Fe, Mn, Ni, Pb, and Zn. Therefore, the choice of the digestion method is more relevant for Co and Cr. Given the impracticality of applying different methods for each element, a compromise analysis is necessary to choose an optimal analytical method. It follows therefore, that microwave-assisted digestion seems to be a more reproducible method since it leads to lower *RSD* values. This digestion method provides more reliable results for all the elements studied due to the use of closed vessels and it assures a quick and safe extraction process to estimate most of the heavy metals for agricultural soils in the Valencian Mediterranean region. Moreover, closed vessel digestion is the preferred method to be used in the study area when Co and Cr are included, because of the better effectiveness of this method to minor elements. Further studies in the European Mediterranean region are needed to investigate if these results will be confirmed for other calcareous soils. Nevertheless, a prior comparison between different digestion methods is always required in order to ensure the correct choice is made.

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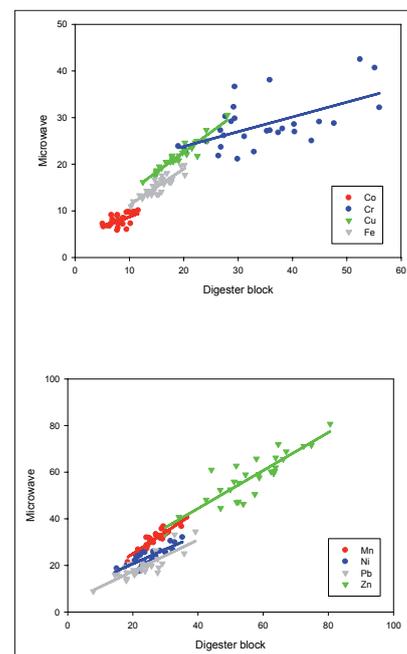
**Figure 1.** Comparison of heavy metal concentrations (mg kg⁻¹) obtained in the Lower Segura basin ($n=29$) by digester block and microwave oven. Fe concentrations have been divided by 1000 and Mn concentrations have been divided by 10.