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JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS

Validation of a horizontal method for trace elements in soil, sludge and biowaste

Carmen Cristache, Sara Comero, Giovanni Locoro, Isabelle Fissiaux, Agustín Alonso Ruiz, Gerard Tanet and Bernd Manfred Gawlik

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Joint Research Centre
Institute for Environment and Sustainability

Contact information
Bernd Manfred Gawlik
Address: Joint Research Centre, Via Enrico Fermi 2749, TP 290, 21027 Ispra (VA), Italy
E-mail: bernd.gawlik@ec.europa.eu
Tel.: +39 0332 78 9487
Fax: +39 0332 78 9831

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Abstract

Validation of an analytical method is a necessary step in controlling the quality of quantitative analysis. Method validation is an established process, which is the provision of documentary evidence that a system fulfils its pre-defined specification or the process of providing that an analytical method is acceptable for its intended purpose.

To implement a validated method for the analysis of 22000 soil samples stemming from 2009 LUCAS Soil Survey as well as from sewage sludge and treated biowaste samples from to FATE-Programme, a validation study was conducted with the following objectives:

(i) to validate these methods for the determination of heavy metals by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Could Vapour-Atomic Adsorption Spectrometry (CV-AAS) techniques, respectively, according to the ISO 17025 requirement and (ii) to implement these methods for the determination of heavy metals in soil, sludge and compost samples on a routine basis.

The two methods were validated using Certified Reference Materials (CRMs): BCR 141R 'Calcareous Loam Soil', BCR 142 'Light Sandy Soil', "San Joaquin Soil" SRM 2709 and LCG 6181 'sewage sludge'.

The calibration curves, detection and quantification limits, trueness as well as repeatability were determined. The budget uncertainty was also estimated (including a full uncertainty budget and Ishikawa-diagram).

The observed expanded uncertainty were establish for Ag (4.0%, Al (6.1%), As(7.6%), Ba (5.3%), Cd (4.5%), Co(7.4%), Cr (5.8%), Cu (3.5%), Fe (5.4%), Mg (6.5%), Mn (4.1%), Mo (2.5%), Ni (5.4%), Pb (7.0%), Sb (6.8%), Se (3.1%), Ti (8.3%), V (4.3%), Zn (5.9%), P (14.2%), K (20.0%).

List of Abbreviations and Symbols

Throughout this report the following abbreviations and symbols are used:

AMA	advanced mercury analyzer
BCR	Bureau Communautaire de Reference
CRMs	certified reference materials
CV	cold-vapour
DG	Directorate General
EC	European Commission
EU	European Union
ESDAC	European Soil Data Centre
ESTAT	European Statistical Office
ENV	Directorate General for Environment
ICP	inductively coupled plasma
IES	Institute for Environment and Sustainability
IUPAC	International Union for Pure and Applied Chemistry
JRC	Directorate General Joint Research Centre
k	coverage factor
LIMS	Laboratory Information Management System
LUCAS	Land Use/Cover Area frame Statistical Survey
LoD	limit of detection
LoQ	limit of quantification
OES	optical emission spectrometry
R	repeatability
RSD	relative standard deviation
SD	standard deviation
SOP	standard operation procedure
SRM	standard reference material
$u_{combined}$	combined uncertainty
U	expanded uncertainty
$u_{(r)}$	combined uncertainty for repeatability
$u_{(IP)}$	combined uncertainty for repeatability
$u_{(t)}$	combined uncertainty for repeatability

Note that chemical elements are identified and expressed according to IUPAC rules.

Table of Contents

Abstract ..... *III*
List of Abbreviations and Symbols ..... *IV*
Table of Contents ..... *V*
List of Figures ..... *VI*
List of Tables ..... *VII*
1. Introduction *8*
2. Experimental methods *9*
2.1 Reagents 9
2.2 Sample preparation prior to digestion 9
2.3 Microwave-assisted digestion 9
2.4 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) 10
2.5 Could Vapour-Atomic Adsorption Spectrometry (CV-AAS) 11
3. Method validation studies *11*
3.1 Method validation for ICP-OES 11
3.1.1 Linearity of calibration 12
3.1.2 Working range 14
3.1.3 Recovery 15
3.1.4 Repeatability and intermediate precision 17
3.1.5 Uncertainty budget 18
3.2 Method validation for Hg determination by CV-AAS 23
3.2.1 Linearity of calibration 23
3.2.2 Working range 26
3.2.3 Trueness 26
3.2.4 Repeatability and intermediate precision 27
3.3 Estimation of the measurement uncertainty 28
3.3.1 Combined uncertainty 28
3.3.2 Expanded uncertainty 31
4. Conclusions *31*
5. Annex A - Temporary storage of 2009 LUCAS soil samples *32*
6. Annex B - Calibration curve for low calibration method validation *33*
7. Annex C - Calibration curve for high calibration method validation *40*

List of Figures

Figure 1 - Micro-wave assisted digestion device 10
Figure 2 - Optima 2100 DV ICP-OES operated at the JRC 10
Figure 3 - Linear low calibration curves for Ag 12
Figure 4 - Residual plots for Ba – low calibration 13
Figure 5 - Linear high calibration curves obtained for Ag 13
Figure 6 - Residual plots for Ba – high calibration 14
Figure 7 - Comparison between certificate and measured concentration in SRM 2709 16
Figure 8 – Cause-effect diagram used for uncertainty assessment 18
Figure 9 - Linear low calibration curves for Hg 24
Figure 10 - Residual plots for low calibration 24
Figure 11 - Linear high calibration curves for Hg 25
Figure 12 - Residual plots for high calibration 26
Figure 13 - Comparison between certified and measured concentration for mercury in low and high calibration range 27
Figure 14 - Cause-effect (or Ishikawa) diagram used for uncertainty assessment 28

List of Tables

Table 1 - Operating conditions for the Optima 2100 DV ICP-OES 10

Table 2 - CV-AAS operational conditions 11

Table 3 – LoD and LoQ for the selected elements in soil and compost samples, by ICP-OES expressed in mg/kg 15

Table 4 - Average recoveries for the selected elements obtained in soil and compost samples 15

Table 5 – Repeatability, intermediate precision and between day variation for low content samples of sewage sludge as well as soil/compost 17

Table 6 - Repeatability, intermediate precision and between day variation for high content samples of sewage sludge as well as soil/compost 17

Table 7 - Relative standard deviation 20

Table 8 - Uncertainty of volumetric flask for final digested volume 20

Table 9 - Recovery contribution to uncertainty budget for soil and compost (rel %) 20

Table 10 – Estimated uncertainty for precision for sludge (%) 21

Table 11 - Expanded uncertainty calculated for sludge (%) 22

Table 12 - Regression coefficients of linear low calibration curves 23

Table 18 - Regression coefficients of linear calibration curves 25

Table 19 - CRM's results for Hg 26

Table 20 - Results obtained for high recovery 27

Table 21 - Results for repeatability and intermediate precision (low calibration) 27

Table 22 - Repeatability and intermediate precision of CV-AAS method (high calibration) 27

Table 23 - The combined uncertainty of Hg sock solution 29

Table 24 - Uncertainty due to balance for analysed mass of samples 29

Table 25 - Trueness 30

Table 26 - Results on precision uncertainty 30

Table 27 - Uncertainty as RSD (%) 31

1. Introduction

The Thematic Strategy for Soil Protection shows that soil degradation is a serious problem in Europe. This results in loss of soil fertility, carbon and biodiversity, lower water-retention capacity, disruption of gas and nutrient cycles and reduced degradation of contaminants. Soil degradation has a direct impact on water and air quality, biodiversity and climate change. It can also impair the health of European citizens and threaten food and feed safety. In this discussion, the issue of physic-chemical soil properties and the content of heavy metals and other trace elements plays an important role, when assessing soil quality.

In 2006, the JRC published the results of sub-contracted study [1] addressing the issue of establishing background values in European soils, which presented the results for the elements Cd, Cr, Cu, Hg, Ni, Pb, and Zn after harmonization to *aqua regia* basis by using conversion algorithms. Parallel to this study, a large-scale standardization project was mandated by the Commission to CEN (Mandate M 320), with the task to produce across-matrix applicable measurement standards for soil, sludge and bio-waste analyses.

The work carried out in this report presents the implementation and validation of a method based on the horizontal standards prEN 16170 [2] and prEN 16174 [3] for the determination of trace elements in soil, sewage sludge and treated biowaste following micro-wave assisted digestion and ICP-OES measurements and CV-AAS for Hg, respectively. The methods were designed to characterize the soil samples collected in the 2009 LUCAS Soil Survey [4]. The use of a horizontal standard, which is applied to different environmental matrices (soil, sewage sludge and treated biowaste), allowed the application of the method not only for the LUCAS Soil Survey, but also to other monitoring projects covering sewage sludge and biowaste.

In the context of the LUCAS Soil Project, the main challenge consisted in the elevated number of samples to be handled by the laboratory. The following validation contributed to perform the chemical analysis of selected trace elements in the LUCAS Soil Set. The data set is of particular relevance to establish a baseline for diffuse pollution across the EU. The studied elements include As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mg, Mn, P, Pb and V, Zn. The result of the analyses will be integrated to the database of the European Soil Data Centre (ESDAC).

The LUCAS campaign resulted in the collection of 22.000 soil samples over 25 Member States (EU-27 except Romania and Bulgaria) during April-October 2009. Soil samples were taken on points stratified on about 10% of the around 230.000 field sample points, so as to cover consistently the whole area surveyed. The JRC and DG ESTAT selected soil-sampling points jointly. Currently, the samples are located at the JRC IES temporary storage, building 29C (see Annex A). About 500 g of soil are available per each sample.

The main purpose of the method validation described in the following was to implement the laboratory procedure for trace element analysis by ICP-OES and mercury determination by CV-AAS as well as to establish the respective performance characteristics. The methods will than be used for different environmental matrix types such as soil, sludge and biowaste. With the validation, accuracy of the employed method is quantified, but also an expanded uncertainty is provided. The developed methods were validated according to the requirements laid down in ISO 17025. Traceability to the International System of Units (S.I.) was established by using appropriate certified reference materials.

To meet the challenge of processing 22.000 samples within 24 months, preference was given to a microwave-assisted digestion method instead of using a classical open-vessel approach. To evaluate the equivalence between microwave-assisted digestion and open-vessel digestion both techniques were evaluated in a comparison on a subset of soils (500 samples). The findings of this comparison are published in second technical report.

The JRC laboratory performing this study is certified according to ISO 9001 and operates all its procedures in full alignment with the general requirements of the organization's quality policy. All procedures are carefully documented and results of measurements are managed using a LIMS (Laboratory Information Management System), which fulfils the requirements of ISO 9001/ISO 17025.

2. Experimental methods

2.1 *Reagents*

Deionized water with a resistivity of 18.2 MΩ cm, produce by a Milli-Q Plus pure water generating system from MILLIPORE Gradient A10 was employed for all preparation of standard and samples solution.

Trace analytical-grade nitric acid (HNO_3) 65% and hydrochloric acid (HCl) 37%, from SUPRAPURE were used for sample dissolution.

Single elements stock standard solutions were diluted in order to obtain standards for calibration both in the low and in the high range of concentration.

2.2 *Sample preparation prior to digestion*

During method validation, certified reference materials (CRMs) were used. Since they were furnished in homogeneous and dried powders at a size lower to 90 µm, no pre-treatment was necessary, both before their microwave digestion used for ICP-OES analysis, and CV-AAS analysis for mercury determination.

However, in real samples an additional sample preparation is necessary to obtain well homogeneous and dried powders. All soils were air-dried, whereas sludge and compost samples were homogenized according to the following procedure. A GAMMA 1-16 LSC (Christ) freeze-drier was used to remove the water content. Obtained dried samples are grounded in an agate ball mixer up to a particle size of 630 µm in order to provide homogenized powder for further analysis.

While trace element analysis by ICP-OES technique required microwave-assisted acid digestion, mercury analysis, carried out by CV-AAS technique did not require any further sample pre-treatment.

2.3 *Microwave-assisted digestion*

A Multiwave 3000 microwave (Anton Paar) device was employed for samples digestion. prEN Standard 16174 [3] was followed. About 0.1g of each soil sample was then weighted. The electronic balance used was a Mettler AT261 (Mettler Instruments Corp., Hightsown, NJ) with a precision of 1 µg. The test portion was introduced into a high-pressure closed Teflon decomposition vessel. The mixture of "aqua regia", i.e. 1.5 mL of HNO_3 and 4.5 mL HCl was carefully added to each sample and the vessels were gently shaken, sealed and placedd in a microwave-oven under previously optimized operating conditions (see below). Blank solutions were prepared by applying the same procedure and reagent solutions without sample.

The microwave autoclave can digest up to 48 samples in the reaction chamber simultaneously under identical experimental conditions. The maximum pressure of the reaction chamber with sample vessels inside was set to 1225 bar. Then, the vessels were heated in the microwave autoclave for 35 min reaching a temperature of max 140 °C and a pressure of approximately 20bar. Before opening the reaction chamber, the digests were allowed to cool for about 180 min to well below the boiling point of the acid mixture at atmospheric pressure.

Each extract was filtered in a 50 mL glass flask using a vacuum pump system and a Millipore Millex® HN Nylon syringe Driven Filters with 0.45µm pore size. The vessel and the vessel cup were subsequently rinsed three times with Milli-Q water and the rinse water was filtered in the same flask. At the end, the flask was completed to volume. The experimental apparatus is shwon in Figure 1. The samples were stored at 4 ºC until analyses.

Final measured concentrations of determinate elements were expressed with respect to the dry material weight.

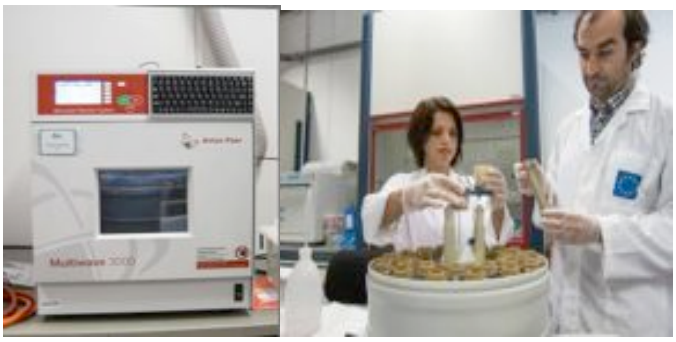


Figure 1 - Micro-wave assisted digestion device

2.4 *Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)*

An Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method (based on prEN 16170) for the evaluation of trace element content in soils, sludge and biowaste was optimized and then validated according to the ISO 17025 requirement.

The method describes the multi-elemental determination of elements in aqueous solutions and digests by simultaneous optical ICP-OES with axial or radial viewing of the plasma. The instrument measures characteristic emission spectra. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element specific emission spectra are produced by radio frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer and photosensitive devices monitor the intensities of the emission lines. Additional interferences and matrix effects must be recognized and appropriate correction made. The Optima 2100 DV ICP-OES device by Perkin Elmer was employed for this study. The operating conditions used are listed in Table 1.

Table 1 - Operating conditions for the Optima 2100 DV ICP-OES

Instrument part	***Parameter***	**Value**
Plasma condition	Plasma flow (Argon)	15 L/min
	Auxiliary flow (Argon)	0.2 L/min
	Nebulizer flow (Argon)	0.8 L/min
	Power	1300 W
	View distance	15
	Plasma view	axial
Peristaltic pump	Sample flow rate	1.5 L/min
Autosampler	Wash between samples	30s

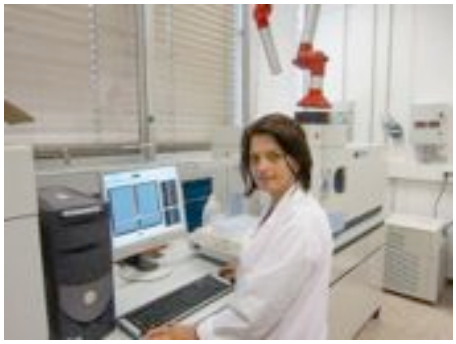


Figure 2 - Optima 2100 DV ICP-OES operated at the JRC

2.5 *Could Vapour-Atomic Adsorption Spectrometry (CV-AAS)*

The determination of Hg was carried out by Cold Vapour-Atomic Absorption Spectrometry (CV-AAS) technique using an Advanced Mercury Analyser instrument (AMA 254, Altec). CV-AAS is a commonly used technique for determining mercury concentration in matrices such as soil, sludge and compost. Samples are usually analysed fresh, or, if long-term storage is required, samples should be kept in the dark at low temperatures or lyophilized.

In this study samples were measured either after air-drying (soils) or after a lyophilisation (freeze-drying) process (sludges and biowastes). For the samples rich in organic matter, lyophilisation is considered to be the most useful drying method, because it reduces risks of losing the more volatile elements. The operational conditions of CV-AAS are given in Table 2.

Table 2 - CV-AAS operational conditions

Parameter	Value
Drying time	60s
Decomposition time	200s
Cuvette clear time	45s
Delay	0s
Cell to use for analysis	Low / High cell
Metric to use for calculation	Peak area

For Hg analyses the electronic balance used to weight the investigated materials was a Crystal 200SMI (Gibertini) with a precision of 1 µg. All measurements were done using an Advanced Mercury Analyser instrument, type AMA 254, Altec, which is equipped with a specific Hg lamp, a fixed 254nm filter, a photomultiplier tube for radiation detection and the specific software for processing the output signal.

The mercury vapour, in the form of atomic gas, passes through a cell positioned in the light path of an atomic absorption spectrometer. Its absorbance at a wavelength of 253.7 nm is measured. The absorbance signal is a function of mercury concentration, and the concentration is calculated using a calibration curve.

3. Method validation studies

Process and extent of a validation study depend on the method to be validated. The applied protocol used for validation tested the following instrument performance for every investigated element: linearity of the calibration curve, working range, limits of detection and limits of quantification, and recovery. Moreover, an expanded uncertainty was estimated and associated with measurement results.

This approach is based on the EURACHEM document "*Fitness for purpose for analytical method*" with respect to the degrees of freedom of the individual parameters [5], but decreases the total number of measurements to be performed by using ANOVA for evaluation. Where applicable, the conditions of the IUPAC "*Harmonized Guidelines for Single-Laboratory Validation of Methods of Measurement*" [6] were applied.

3.1 *Method validation for ICP-OES*

The validation of the ICP-OES method was carried out using the following CRMs: BCR 141R "*Calcareous Loam Soil*", BCR 142 "*Light Sandy Soil*", "*San Joaquin Soil*" SRM 2709 and LCG 6181 thus establishing also traceability of the measurements to the International System of Units (SI). Single element standards (Perkin Elmer) with a concentration of 1000 mg/mL in aqueous solution (100 mL) were used for instrument calibration. For ICP analysis an aliquot (about 15 mL) of the digested samples was transferred to the ICP sample holder vials, made in Polypropylene. The following elements were determined and validated using the operating condition described in this section: Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, K, Mn, Mo, Ni, P, Pb, Sb, Se, Ti, V and Zn.

3.1.1 Linearity of calibration

Two ranges of concentration were chosen in order to obtain more accurate calibration curves, especially in the low range of concentrations.

3.1.1.1 Low calibration

The low calibration range was from 0.02 to 0.5 mg/L. In order to verify the linearity of the calibration curve, a blank and five standard concentrations were analysed in three replicates for five different days. For each daily calibration, low calibration curves present correlation coefficients higher than 0.998. All linear curves obtained, each one for every tested element, can be found in the Annex B of this report.

An example of obtained linear curves for each daily low calibration is shown in Figure 3 for silver.

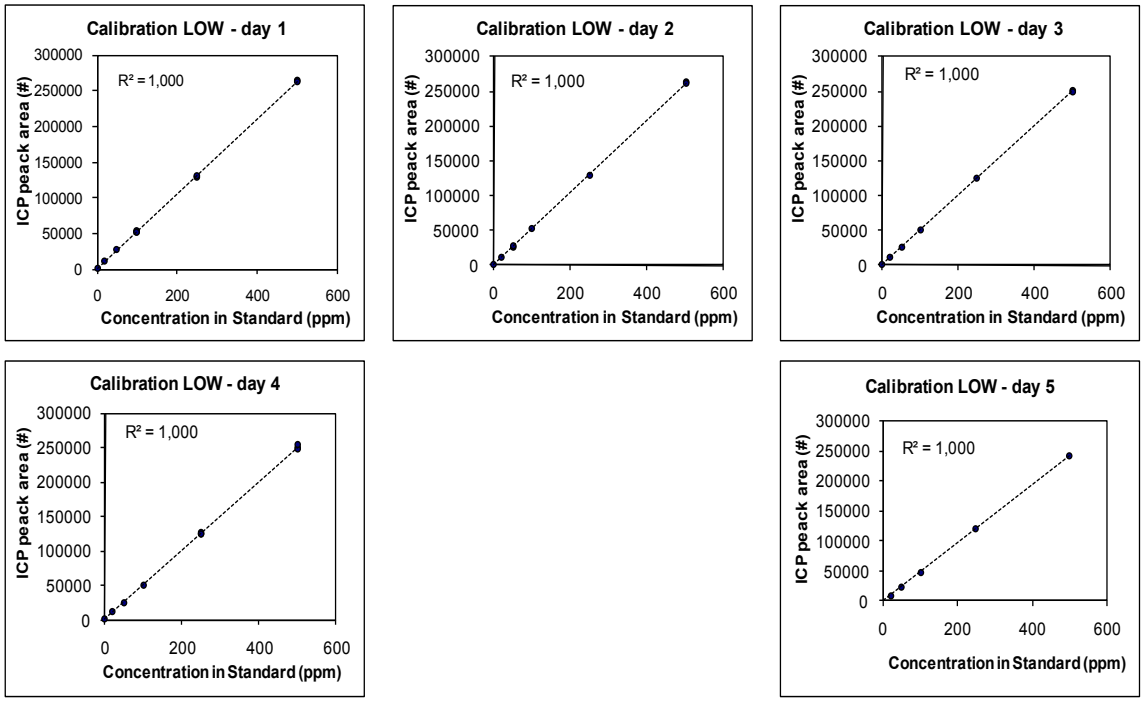


Figure 3 - Linear low calibration curves for Ag

The F-test shows that the calibration model is suitable for all the daily calibration curves, at the 99% confidence level. In order to test whether the data meet the assumptions of linear regression, even the assumption of homogeneity of variance (homoscedasticity) must be considered: the error variance should be constant. Bartlett's test and Fligner-Killeen's test were applied for detecting homoscedasticity. The first one is more sensitive to non-normality of data, while the Fligner-Killeen's test is more robust in the case of departure from normality [7].

Both, the tests of Bartlett and Fligner-Killeen, test the null hypothesis H_0 that the variance of the residuals is homogenous. Therefore, if the p-value is very small, we would have to reject the hypothesis and accept the alternative hypothesis that the variance is not homogenous.

For low calibration, Bartlett's test reject the hypothesis of homogeneity of variance for the elements As, Ba, Cd, Cr, Cu, Mn, Fe, Ti and Zn calibration curves while Fligner-Killeen's is significance for all calibration curves at the 95% confidence level.

There are also graphical methods for detecting homoscedasticity. A commonly used way to visualize homogeneity of variance is to plot the residuals versus predicted values by the linear model. If the model is well-fitted, there should be no pattern to the residuals plotted against the fitted values. If the variance of the residuals is non-constant, then the residual

variance is said to be "*heteroscedastic*". An example of residual plot is reported in Figure 4 for Ba.

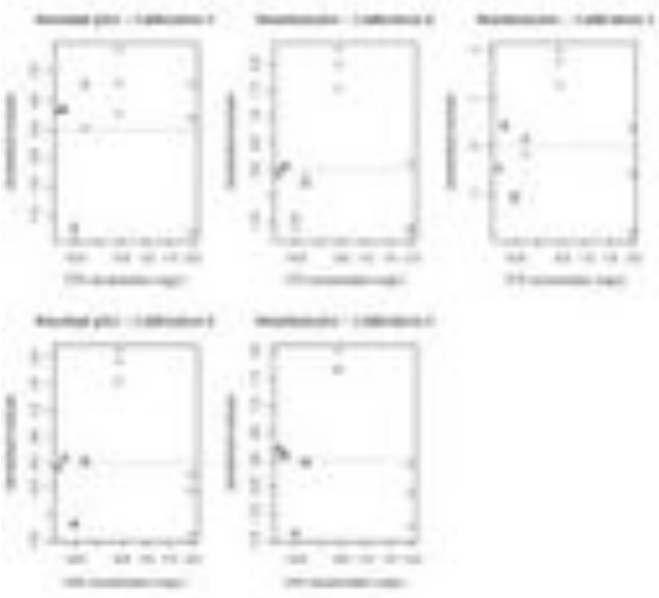


Figure 4 - Residual plots for Ba – low calibration

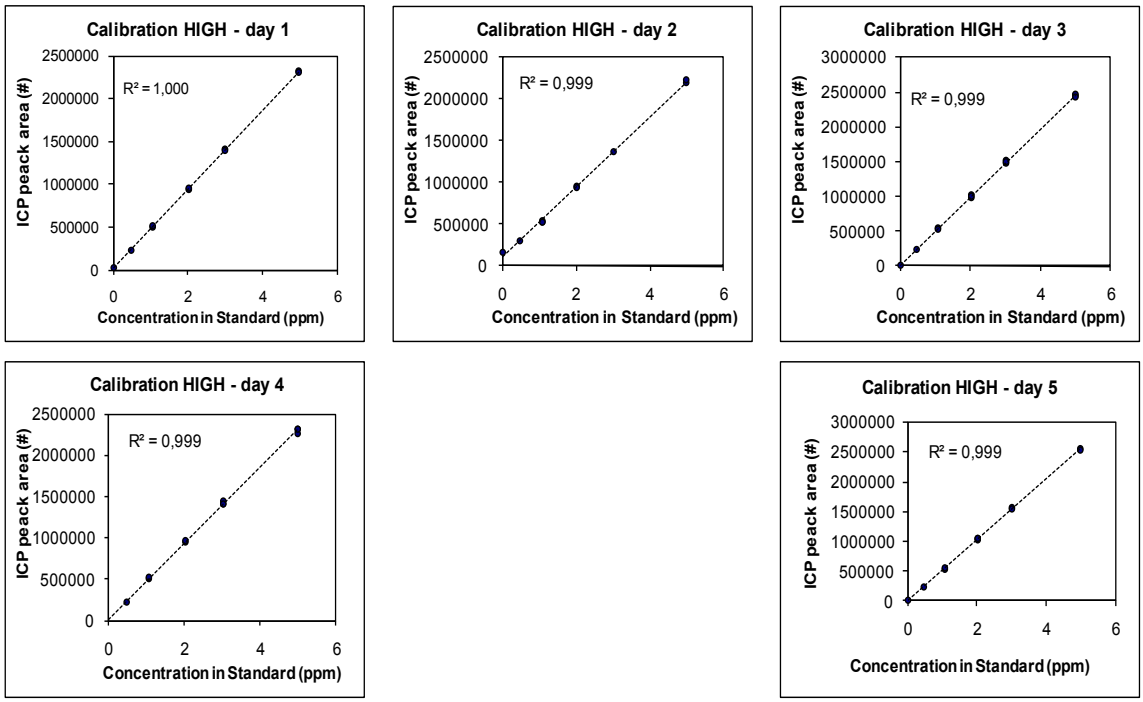


Figure 5 - Linear high calibration curves obtained for Ag

3.1.1.2 High calibration

The high calibration range was from 0.5 to 5 mg/L. All linear curves obtained, each one for every tested element, can be found in the Annex C of this report. An example of obtained linear curves for each daily high calibration is shown in Figure 5 for the element silver. The

F-test shows that the calibration model is suitable for all the daily calibration curves, at the 99% confidence level.

For high calibration, Bartlett's test reject the hypothesis of homogeneity of variance for all the elements calibration curves except for Pb, Se and P, while Fligner-Killeen's is significance for all calibration curves at the 95% confidence level. Residual plots for high calibration obtained for Ba are presented in Figure 6.

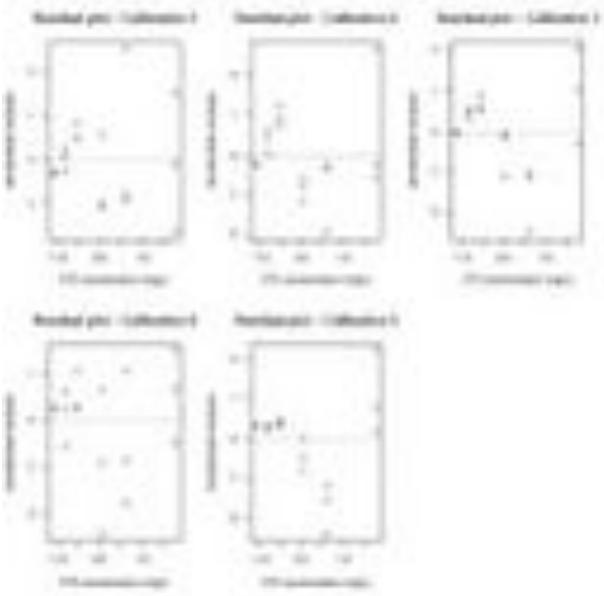


Figure 6 - Residual plots for Ba – high calibration

3.1.2 Working range

The working range is defined by the calibration curve (upper value) and the limit of quantification (LoQ). For higher concentration than those defined by calibration curve the measured solution has to be diluted and re-analysed.

3.1.2.1 LoD and LoQ

In order to estimate LoD (Limit of Detection) and LoQ (Limit of Quantification), soil samples containing the selected elements at very low concentration, were analysed. When an element was non available in the soil sample, a blank was used. The following formulas were used to compute LoD and LoQ are:

$$\mathrm{LOD} = \Phi_{n,\varepsilon} \cdot \frac{s_L}{b}$$

$$\mathrm{LOQ} = k \cdot \Phi_{n,\varepsilon} \cdot \frac{s_L}{b}$$

where s_L is the standard deviation of the ten replicates and b is the slope of the used calibration curve. $\Phi_{n,\alpha}$ is a multiplier factor that take into account the probability that certain response could be due to the standard deviation of the blank rather than the one of the analyte. The factor *k* corresponds to the reciprocal value of the desired accuracy.

For 10 measurements and at a 95% confidence level ($\alpha = 0.05$) the $\Phi_{n\,\alpha}$ factor is equal to 1.9. LOQ was computed using a k factor of 2, which give a 50% of accuracy. From this computation, the resulting values for LoD and LoQ are reported in

Table 3 for soil and compost samples and for sludge samples, respectively. All concentrations are expressed in mg/kg. For the elements Ba, Mn, Se and Ti, a blank was used for the computation.

Table 3 – LoD and LoQ for the selected elements in soil and compost samples, by ICP-OES expressed in mg/kg

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Ag	0,07	0,14	0,06	0,12
Al	1,53	3,06	1,53	3,06
As	2,84	5,67	2,63	5,25
Ba	0,02	0,04	0,02	0,04
Cd	0,07	0,15	0,09	0,18
Co	0,15	0,30	0,18	0,35
Cr	0,32	0,64	0,16	0,32
Cu	0,26	0,52	0,19	0,38
Fe	6,66	13,32	6,66	13,32
Mg	3,58	7,15	3,58	7,15
Mn	0,02	0,03	0,02	0,03
Mo	0,28	0,56	0,36	0,72
Ni	0,27	0,53	0,14	0,27
Pb	1,16	2,33	1,26	2,52
Sb	0,81	1,61	1,66	3,32
Se	1,78	3,56	1,78	3,56
Ti	0,03	0,05	0,03	0,05
V	0,66	1,33	0,81	1,62
Zn	2,12	4,23	2,12	4,23
P	3,03	6,06	3,03	6,06
K	4,83	9,66	4,83	9,66

3.1.3 Recovery

Recovery was computed using the following certified reference materials: BCR 141R "*Calcareous Loam Soil*", BCR 142 "*Light Sandy Soil*", "*San Joaquin Soil*" SRM 2709 and LCG 6181 and spiking solution when an element was not available in a given CRM. Prior to ICP analysis, an aliquot of the CRM was pre-treated using assisted microwave digestion.

For method validation, low and high concentration of the selected elements were analysed in CRM in triplicate for five different days, i.e. under conditions of intermediate precision. In Table 4 the average recovery obtained during the 5 days for low and high calibration are reported.

Table 4 - Average recoveries for the selected elements obtained in soil and compost samples

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Ag	84%	82%	101%	92%
Al	-	59%	-	103%

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
As	83%	94%	83%	90%
Ba	88%	45%	89%	95%
Cd	85%	90%	95%	88%
Co	96%	94%	98%	89%
Cr	66%	98%	96%	98%
Cu	89%	99%	93%	99%
Fe	-	85%	-	98%
Mg	-	88%	-	96%
Mn	87%	94%	87%	92%
Mo	91%	86%	87%	92%
Ni	81%	98%	97%	96%
Pb	66%	95%	94%	97%
Sb	83%	91%	101%	91%
Se	83%	92%	83%	92%
Ti	90%	92%	90%	92%
V	93%	97%	99%	93%
Zn	-	95%	-	89%
P	-	116%	-	122%
K	-	27%	-	102%

In Figure 7, a bar plot representing the comparison between the certified values in SRM 2709 material and the measured concentrations are shown. Error bars for measured values correspond to the standard deviation of replicates.

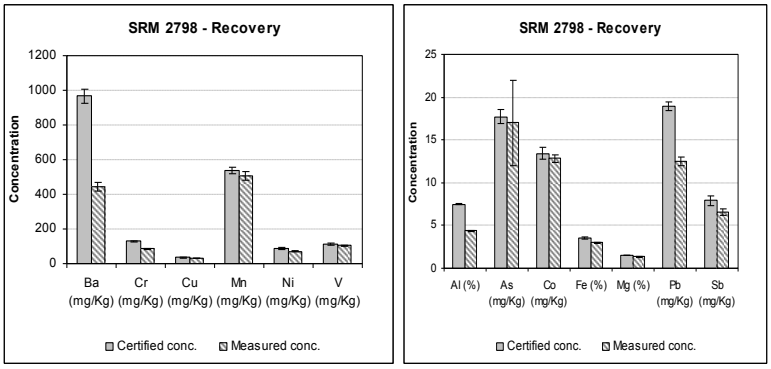


Figure 7 - Comparison between certificate and measured concentration in SRM 2709

It could be observed that, in general, the comparison between certified and ICP values is satisfactory. This applies to the elements As, Co, Cu, Fe, Mg, Mn, Ni, Sb and V. Findings for Al, Ba, and Pb, indicate an incomplete digestion with regard to the certified values. the lower recovery respect to the other elements in the high and low calibration, respectively, is the cause for difference in concentration values.

3.1.4 Repeatability and intermediate precision

For repeatability and intermediate precision two samples at different levels of trace elements concentration (low and high) were measured for 5 different days in three replicates. Results obtained from two different sludge and soil samples with low concentration in trueness calculation were used (Table 5).

Repeatability, intermediate precision (or within laboratory reproducibility) and day-to-day variation were evaluated using one-way ANOVA (Analysis of Variance). The results are presented below. Results for high concentration of trace elements for the determination of repeatability, intermediate precision and day-to-day variation are presented in Table 6

Table 5 – Repeatability, intermediate precision and between day variation for low content samples of sewage sludge as well as soil/compost

	Repeatability		***Between day***		***Intermediate precision***	
Element	**Sludge**	**Soil/Compost**	**Sludge**	**Soil/Compost**	**Sludge**	**Soil/Compost**
Ag	6%	4%	2%	2%	6%	4%
Al	-	-	-	-	-	-
As	9%	3%	2%	5%	9%	6%
Ba	3%	3%	4%	3%	5%	4%
Cd	2%	3%	4%	2%	5%	3%
Co	10%	2%	5%	3%	11%	4%
Cr	8%	2%	4%	2%	9%	3%
Cu	3%	3%	1%	3%	3%	4%
Fe	-	-	-	-	-	-
Mg	-	-	-	-	-	-
Mn	3%	3%	2%	2%	4%	4%
Mo	3%	2%	2%	0%	4%	2%
Ni	8%	4%	2%	2%	8%	5%
Pb	9%	4%	4%	3%	10%	5%
Sb	8%	6%	3%	4%	8%	8%
Se	4%	3%	2%	1%	5%	3%
Ti	9%	9%	2%	2%	9%	10%
V	7%	7%	1%	3%	7%	7%
Zn	-	-	-	-	-	-
P	-	-	-	-	-	-
K	-	-	-	-	-	-

Table 6 - Repeatability, intermediate precision and between day variation for high content samples of sewage sludge as well as soil/compost

	Repeatability		***Between day***		***Intermediate precision***	
Element	**Sludge**	**Soil/Compost**	**Sludge**	**Soil/Compost**	**Sludge**	**Soil/Compost**
Ag	7%	6%	4%	3%	8%	7%
Al	10%	4%	3%	2%	10%	5%
As	1%	1%	3%	1%	3%	2%
Ba	9%	4%	1%	5%	9%	7%
Cd	2%	2%	4%	6%	5%	6%
Co	1%	1%	4%	2%	4%	2%
Cr	1%	1%	1%	1%	1%	1%
Cu	8%	1%	3%	2%	8%	2%
Fe	7%	5%	4%	2%	8%	6%
Mg	4%	4%	6%	3%	7%	5%
Mn	3%	3%	5%	5%	6%	6%
Mo	1%	3%	4%	5%	4%	6%
Ni	1%	1%	2%	1%	2%	1%
Pb	1%	1%	2%	2%	2%	2%
Sb	3%	3%	8%	8%	8%	8%
Se	5%	5%	7%	7%	8%	8%
Ti	1%	1%	8%	8%	8%	8%
V	1%	1%	3%	1%	3%	1%
Zn	5%	1%	3%	5%	6%	5%
P	1%	1%	7%	14%	7%	14%
K	2%	2%	6%	21%	6%	22%

3.1.5 Uncertainty budget

The aim of the uncertainty assessment was to provide the expanded uncertainty associated with the measurement of elements content in soil, sludge and compost samples by ICP-OES technique. In this way, it is first easier to keep the method under statistical control and, secondly, the number of replicate digested (i.e. independent sub-samples) can be reduced to a single digestion. In order to analyses each source of error an cause-effect diagram was designed (Figure 8). The combined uncertainty was computed using the error propagation law and the expanded uncertainty was obtained from the preceding one by multiplication of a coverage factor, k, which takes into account the confidence limit [8].

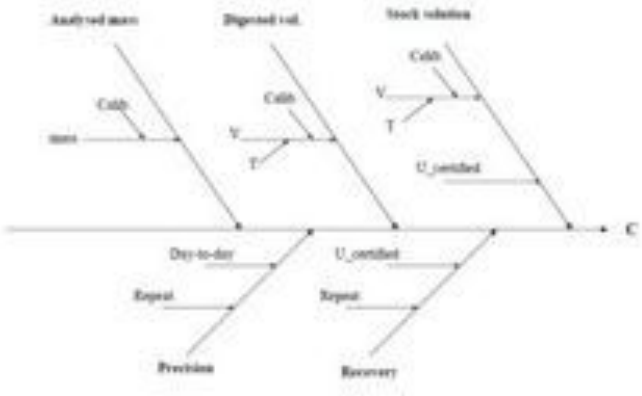


Figure 8 – Cause-effect diagram used for uncertainty assessment

3.1.5.1 Combined uncertainty

The concentration of the elements content in each sample, obtained after the mineralization procedure and ICP-OES analysis, was derived from the following equation:

$$C = C_{ICP} d$$

C_{ICP} is the value, mg/L, derived from ICP-OES analysis and *d* is the dilution factor after the mineralization process, expressed by:

$$d = V / \mathrm{m}_{sample}$$

with m_{sample} being the mass of the sample aliquot used in the mineralization process, and V the final diluting volume. Basing on the cause-effect diagram, the main factors that contribute to the overall uncertainty were found to be the method recovery, precision, concentration of diluted standards stock solutions and the final volume of sample digest. Starting from the contribution of the single uncertainties and using the error propagation low the combined uncertainty, expressed in terms of relative uncertainties u_i, can be calculated using the following equation:

$$u_{rel}(C) = \sqrt{u_{rel}^2(stok) + u_{rel}^2(mass) + u_{rel}^2(V_{fin}) + u_{rel}^2(rec) + u_{rel}^2(precision)}$$

In the next sections all this contributions are analysed individually. The uncertainty due to pipetting operations was taken into account in precision.

3.1.5.2 Uncertainty source: elements' standard stock solutions

The uncertainty associated with the elements standard stock solution used for calibration is a combination of the uncertainty associated with the standards uncertainty, given in the certificate of the solution, and uncertainty derived from the volumetric flask used for dilution. The certificate standard uncertainty (given by the manufacturer) is equal to:

1000 ± 2 mg/L for Ag, Al, Ba, Cd, Co, Cr, Cu, Mg, Mn, Pb, Ti, V, Zn and K

1001 ± 2 mg/L for Fe, P and Mo

1002 ± 2 mg/L for Ni

998 ± 2 mg/L for Se

As this value is not correlated with a confidence level or distribution information, a rectangular distribution is assumed and the uncertainty is divided by √3

$$u_{cert} = 2/\sqrt{3} = 1.15\, mg/L$$

The uncertainty of the 100 mL volumetric flask used for diluting the stock solution was computed combining the uncertainties arising from temperature and calibration effects. The tolerance, given by the manufacturer, of a 100 mL volumetric flask is set to 0.1 mL at a temperature of 20 ºC. As no confidence level is reported, a triangular distribution was assumed and the uncertainty associate with calibration effect is:

$$u_{calib} = 0.1/\sqrt{6} = 0.04\, mL$$

In order to account for the temperature variability in the laboratory within ±3 ºC in respect to the calibrating temperature (20°C), a rectangular distribution was assumed and the uncertainty associate to this effect is computed with the following formula:

$$u_{temp} = \frac{T.V.Q}{\sqrt{3}} = 0.04\, mL$$

Where T is the temperature variability (±3), V is the volume of the volumetric flask used and Q is the coefficient of volume expansion of the water (Q = 2.1 x 10^{-4} ºC^{-1}). The combined uncertainty of the 100 mL volumetric flask is then:

$$u_{volum} = \sqrt{u_{calib}^2 + u_{temp}^2} = 0.05\, mL$$

The combined uncertainty of the stock solution was calculated combining the three uncertainties above:

$$\frac{u_{stock}}{C_{stock}} = \sqrt{\left(\frac{u_{std}}{C_{stock}}\right)^2 + \left(\frac{u_{volum}}{V}\right)^2} = 0.13\%$$

3.1.5.3 Uncertainty source: analysed mass

The uncertainty associate to the analysed mass of sample used for digestion process is only due to the linearity uncertainty of the balance from calibration certificate. From the balance linearity (± 0.03 mg), a rectangular distribution is assumed to obtain a standard uncertainty; this contribution is considered twice, once for the tare and once for the gross weight. This gives for the standard uncertainty of sample mass, u_m, the following value:

$$u_m = \sqrt{2\left(\frac{0.03}{\sqrt{3}}\right)^2} = 0.02\,mg$$

The aliquot of sample used for digestion, was approximately identical. In order to calculate the relative standard deviation, the mean mass weight (0.1 g) was used as point of reference (Table 7).

Table 7 - Relative standard deviation

Description	Value	SD	Uncertainty as RSD (%)
Mass (u_m)	100 mg	0.02 mg	0.02

3.1.5.4 Uncertainty source: final digested volume

This uncertainty is due only to the 50 mL volumetric flask used to collect the sample after the microwave digestion process. As in the previous cases the uncertainty associated to the volumetric flask is a combination of the calibration and temperature effects.

$$u_{V\,final} = \sqrt{u_{calib}^2 + u_{temp}^2} = 0.04\,mL$$

Uncertainty as relative standard deviation is reported in Table 8.

Table 8 - Uncertainty of volumetric flask for final digested volume

Description	Value	SD	Uncertainty as RSD (%)
Volumetric flask (u_{Vfinal})	50 mL	0.04 mL	0.09

3.1.5.5 Uncertainty source: recovery

The uncertainty due to recovery is derived from the standard deviation of the mean of the trueness assessment study (u_{tr}). Usually, also the uncertainty associated with the nominal value of CRMs is taken into account. However, in this case both CRMs and spiking solution were used for recovery study and being the uncertainty associated with spike lower than the nominal uncertainty of CRMs elements could show very different uncertainty values. Moreover, large uncertainties in nominally CRMs values could have a high impact on the overall uncertainty, making comparability very poor [9]. In order to have more comparable data it was chosen not to use this term in the uncertainty formula. Recovery uncertainty for sludge and compost are presented in Table 9.

Table 9 - Recovery contribution to uncertainty budget for soil and compost (rel %)

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Ag	1	2	1	1.5
Al	-	2	-	0.6
As	2	1	2.9	0.6
Ba	2	2	1.9	2.6

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Cd	2	2	1.1	2.6
Co	1	2	1.6	0.9
Cr	1	0	1.2	0.2
Cu	1	2	1.5	0.9
Fe	-	1	-	1.1
Mg	-	3	-	1.6
Mn	2	2	1.6	2.3
Mo	2	1	0.4	0.7
Ni	2	1	1.5	0.2
Pb	2	1	1.7	0.9
Sb	2	4	2.5	3.6
Se	1	3	0.6	3.2
Ti	3	4	3.3	3.9
V	2	1	1.1	0.6
Zn	-	1	-	2.3
P	-	3	-	6.4
K	-	3	-	9.6

3.1.5.6 Uncertainty source: precision

Uncertainty associated to precision was derived from two contributions: repeatability and intermediate precision calculated in the validation study. Uncertainty due to repeatability was estimated as:

$$s_{rep} \,/\sqrt{n_{rep}}$$

where s_{rep} is the relative standard deviation due to repeatability experiment and n_{rep} the number of replicates. The uncertainty due to intermediate precision was estimated as $s_{day}\,/\sqrt{d}$ with s_{day} being the relative day-to-day variation and d the number of days. The precision uncertainty was derived combining these two uncertainties:

$$u_{prec} = \sqrt{u_{rep}^2 + u_{day}^2}$$

As precision varies with concentration level, three levels of uncertainty have been computed, associated to the concentration level from which they are derived from (low, medium, high) (Table 10).

Table 10 – Estimated uncertainty for precision for sludge (%)

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Ag	1.7%	2.5%	1.7%	2.5%
Al	-	3.0%	-	3.0%
As	2.5%	1.4%	2.5%	1.4%
Ba	1.9%	2.4%	1.9%	2.4%
Cd	2.0%	1.9%	2.0%	1.9%
Co	3.3%	1.7%	3.3%	1.7%
Cr	2.6%	0.4%	2.6%	0.4%
Cu	0.9%	2.4%	0.9%	2.4%
Fe	-	2.5%	-	2.5%
Mg	-	2.8%	-	2.8%
Mn	1.2%	2.4%	1.2%	2.4%
Mo	1.2%	1.6%	1.2%	1.6%
Ni	2.2%	0.9%	2.2%	0.9%
Pb	3.1%	0.8%	3.1%	0.8%
Sb	2.3%	3.6%	2.3%	3.6%
Se	1.4%	3.3%	1.4%	3.3%
Ti	2.5%	3.5%	2.5%	3.5%
V	1.9%	1.4%	1.9%	1.4%
Zn	-	1.9%	-	1.9%
P	-	3.0%	-	3.0%
K	-	2.7%	-	2.7%

3.1.5.7 Expanded uncertainty

In order to take into account a confidence level, the combined uncertainty is to be multiplied by a coverage factor, *k*, to produce the expanded uncertainty. The choice of this factor was done taking into account a 95% confidence level, which give a coverage factor of 2. The expanded uncertainty, equal for both level of precision, is given by:

$$u_{expanded} = k.u_{combined}$$

Values computed for sludge and compost are presented in Table 11.

Table 11 - Expanded uncertainty calculated for sludge (%)

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Ag	4.0%	5.9%	4.5%	6.2%
Al	-	6.1%	-	7.2%
As	7.6%	3.1%	6.3%	4.0%
Ba	5.3%	7.1%	6.1%	6.7%
Cd	4.5%	6.4%	5.6%	5.5%

Elements	Soil/Compost (LOW)	Soil/Compost (HIGH)	Sludge (LOW)	Sludge (HIGH)
Co	7.4%	3.9%	7.1%	5.0%
Cr	5.8%	1.1%	6.0%	1.3%
Cu	3.5%	5.1%	3.0%	5.8%
Fe	-	5.4%	-	5.2%
Mg	-	6.5%	-	7.9%
Mn	4.1%	6.8%	3.9%	6.9%
Mo	2.5%	3.5%	3.9%	3.5%
Ni	5.4%	1.9%	6.0%	2.6%
Pb	7.0%	2.4%	6.9%	2.3%
Sb	6.8%	10.1%	5.5%	10.1%
Se	3.1%	9.3%	3.3%	9.3%
Ti	8.3%	10.5%	8.3%	10.5%
V	4.3%	3.1%	5.3%	4.0%
Zn	-	5.9%	-	4.0%
P	-	14.2%	-	8.6%
K	-	20.0%	-	7.7%

3.2 ***Method validation for Hg determination by CV-AAS***

Determination of Hg content in soil, sludge and compost samples was validated according to approach than in case of ICP OES, which has been described in the previous chapter.

3.2.1 Linearity of calibration

A Carlo Erba mercury standard solution with a nominal mercury concentration of 1 mg/mL was used for instrument calibration. Also in this study, low and high calibration scenarios were investigated.

3.2.1.1 Low calibration

The low calibration range was from 0.05 to 0.5 mg/L. In order to verify the linearity of the calibration curve, a blank and five standard concentrations were analysed in three replicated for five different days. For each daily calibration low and high calibration curves present correlation coefficients higher that 0.995 and 0.996, respectively. Linear calibration curves for each daily low calibration are shown in Figure 9 and the correlation coefficients are reported in Table 12.

Table 12 - Regression coefficients of linear low calibration curves

	Day 1	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Regression coefficient (low)	>0.998	> 0.995	> 0.996	> 0.996	> 0.997

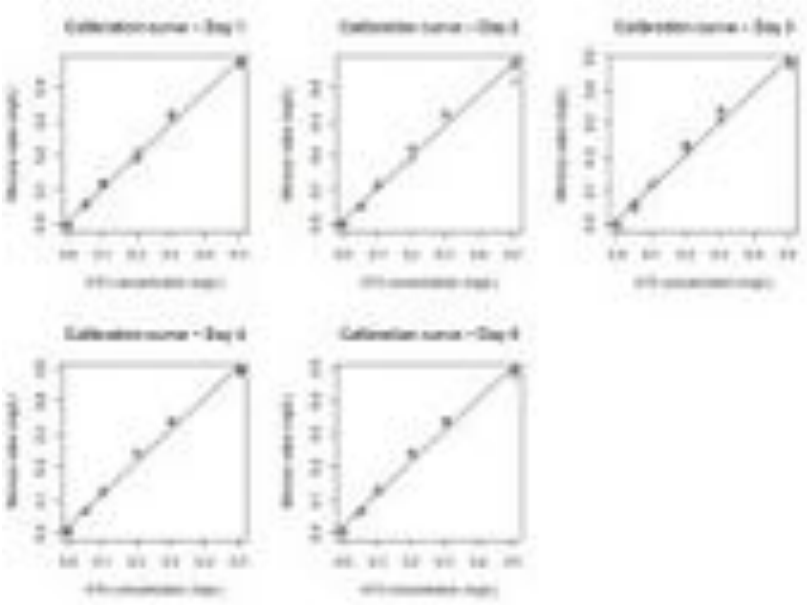


Figure 9 - Linear low calibration curves for Hg

Shape of calibration curves and regression coefficient values for each daily calibration prove the linearity and stability of the measurement in the 5-days range; calibration curves were also obtained up to 9 days confirming the stability of the system. In Figure 10 plots of standardized residuals for low calibrations are reported to prove the linearity.

Lack-of-fit test is used to confirm the linear trend of the regression curve expressed by the correlation coefficient. The lack-of-fit test helps us to determine if the modelling error is significant different than the pure error and this is done comparing the variance of the lack-of-fit against the pure error variance.

Bartlett test rejects the hypothesis of homogeneity of variance for all calibration curves while Fligner-Killeen's is significance for all calibration curves at the 95% confidence level.

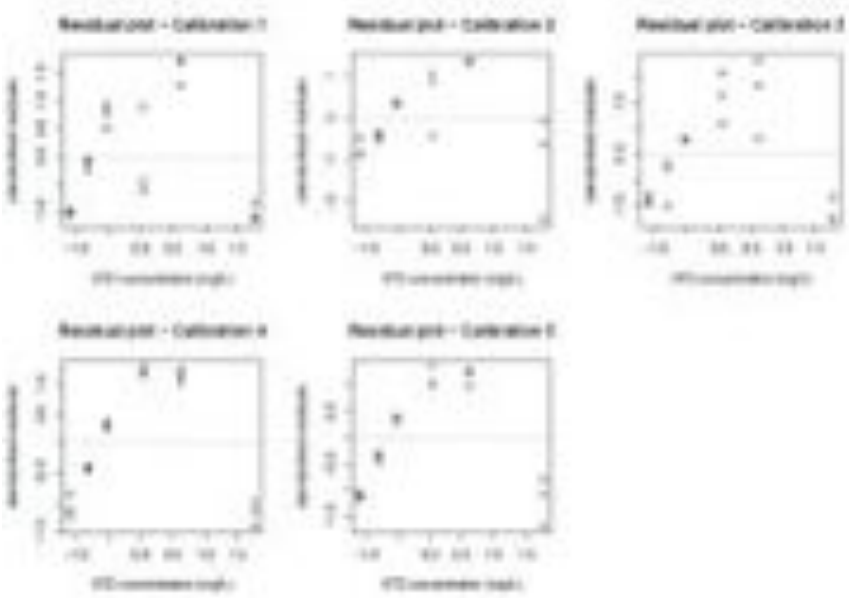


Figure 10 - Residual plots for low calibration

3.2.1.2 High calibration

The high calibration range was from 0.5 to 5 mg/l. For the high calibration we made the same procedure like low calibration described above. In Figure 11 and Table 13 regression coefficients and linear calibration curves in the high range were reported, respectively.

Table 13 - Regression coefficients of linear calibration curves

	Day 1	***Day 2***	***Day 3***	***Day 4***	***Day 5***
Regression coefficient (high)	>0.996	>0.999	>0.997	>0.999	>0.999

A simple method to check linearity is the use of residual plots, where residuals are the difference between the computed y-value and the predicted one using calibration function. Residual plot are used to graphically show deviation from the curve chosen and data homoscedasticity [6]. In Figure 12, plots of standardized residuals are reported to prove the linearity for high calibration curves. In the case of high calibration studies linear lack of fit was good (95% level of confidence) for the last two calibration curves (days 4 and 5) while lack of fit was good (95% level of confidence) for all the calibration curves in the case of quadratic calibration. Bartlett test reject the hypothesis of homogeneity of variance for all calibration curves, while Fligner-Killeen's is significance for all calibration curves at the 95% confidence level.

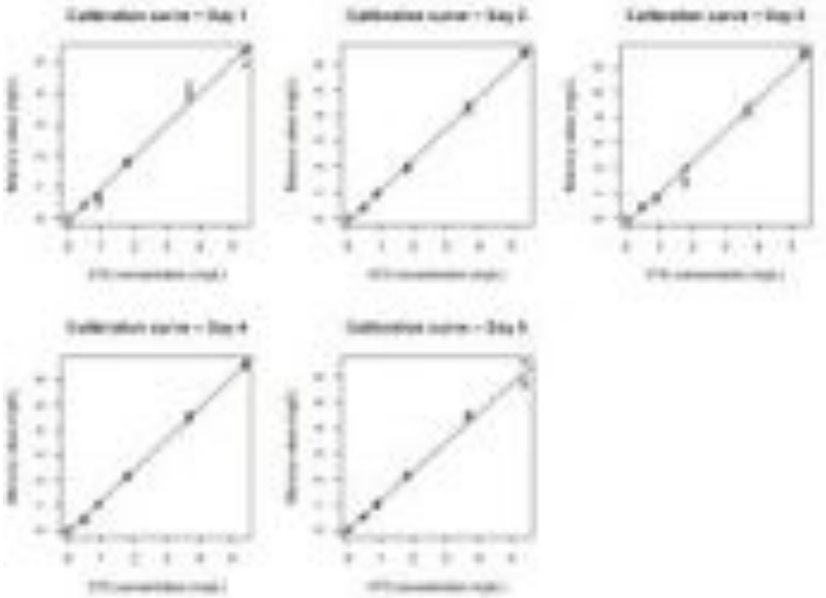


Figure 11 - Linear high calibration curves for Hg

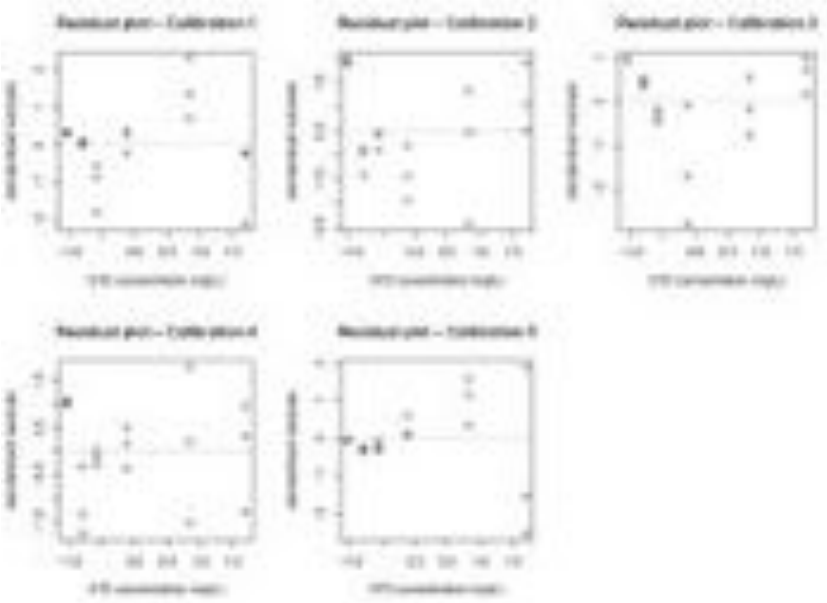


Figure 12 - Residual plots for high calibration

3.2.2 Working range

3.2.2.1 LoD and LoQ

In order to estimate LoD (Limit of Detection) and LoQ (Limit of Quantification), due to the non-availability of a soil, sludge and compost sample containing Hg at very low concentration, a blank was analysed. Ten replicates were made in order to compute the standard deviation. To compute LoD and LoQ equations below were used. For 10 measurements and at a 95% confidence level ($\alpha = 0.05$) the $\Phi_{n,\alpha}$ factor is equal to 1.9. LoQ is computed using a k factor of 2, which give a 50% of accuracy. Results for LoD and LoQ show the following values:

$$LOD = 4\ \mu g/L$$

$$LOQ = 8\ \mu g/L$$

3.2.3 Trueness

For mercury, a sludge CRM with concentration values in the low range calibration was not available for recovery computation. Therefore, it was decided to use two CRMs for soils instead. Low recoveries were computed using the following certified reference materials (CRMs): BCR 141R *Calcareous loam soil* (0.25mg/kg Hg) and BCR 142R *Light sandy soil (*0.067mg/kg Hg). For method validation, CRMs were analysed in triplicate for five different days. Results are presented in the Table 14.

For the high recovery the CRMs: SRM 2709 *San Joaquin Soil* (4.9 mg/kg Hg) and LCG 6181 (1.4 mg/kg Hg) were used. The results are presented in Table 15.

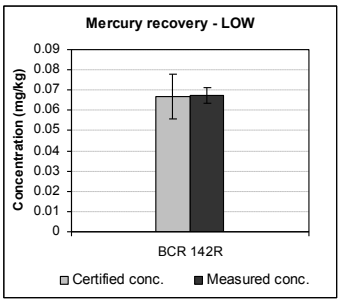
Figure 13 shows a graphical representation of recoveries obtained for mercury. Error bars for measured concentrations were determined by the standard deviation of replicates.

Table 14 - CRM's results for Hg

	Day 1	*Day2*	*Day3*	*Day4*	*Day5*	*Average*
BCR 141R	113%	103%	103%	104%	108%	106%
BCR 142R	107%	96%	95%	99%	106%	101%

Table 15 - Results obtained for high recovery

	Day1	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Average*
LCG 6181	110%	122%	117%	120%	115%	117%
SRM 2789	118%	120%	106%	109%	111%	113%



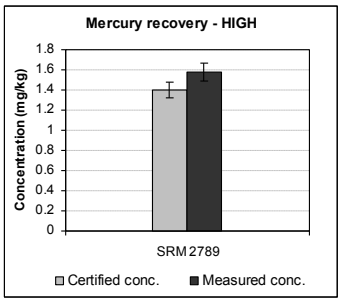


Figure 13 - Comparison between certified and measured concentration for mercury in low and high calibration range

3.2.4 Repeatability and intermediate precision

For the assessment of repeatability and intermediate precision two samples at different levels of mercury concentration (low and high) were measured for 5 different days in three replicates. Results obtained from two different soil samples with low concentration in trueness calculation were used. Repeatability, intermediate precision (or within laboratory reproducibility) and day-to-day variation were evaluated using one-way ANOVA (Analysis of Variance). The results are presented in Table 16.

Table 16 - Results for repeatability and intermediate precision (low calibration)

	Soil 1	*Soil 2*
Repeatability	7%	4%
Intermediate precision	8%	5%
Day-to-day	4%	3%

Soil and sludge samples with high Hg concentration were used, too, for determination repeatability, intermediate precision and day-to-day variation. The results obtained are presented in Table 17.

Table 17 - Repeatability and intermediate precision of CV-AAS method (high calibration)

	Soil	*Sludge*
Repeatability	8%	10%
Intermediate precision	8%	11%
Day-to-day	4%	10%

3.3 *Estimation of the measurement uncertainty*

The estimation of the measurement uncertainty was performed using the method expressed in the EURACHEM/CITAC Guide [8]. As in the case of ICP-OES validation, the aim of this uncertainty assessment was to provide the expanded uncertainty associated with the measurement of mercury content in soil, sludge and compost samples by CV-AAS technique. In order to analyse each source of error the cause-effect diagram was designed (Figure 14) The combined uncertainty was computed using the propagation error law and the expanded uncertainty was obtained from the preceding one by multiplication of a coverage factor, *k*, which takes into account the confidence limit.

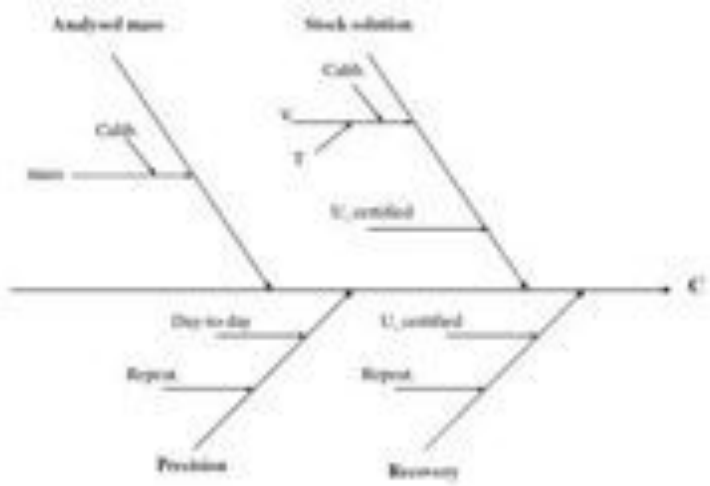


Figure 14 - Cause-effect (or Ishikawa) diagram used for uncertainty assessment

3.3.1 Combined uncertainty

3.3.1.1 Uncertainty source: mercury standard stock solution

The certified standard uncertainty of the mercury stock solution is equal to 1 ± 0.001 mg/mL and was provided by the manufacturer. As this value is not correlated with a confidence level or distribution information, a rectangular distribution is assumed and the uncertainty is divided by $\sqrt{3}$.

$$u_{cert} = 2/\sqrt{3} = 0.58\, mg/L$$

The uncertainty of the 100 mL volumetric flask used for diluting the stock solution was computed combining the uncertainties arising from temperature and calibration effects.

The tolerance of the 100 ml volumetric flask (given by the manufacturer) is set to 0.1 ml at a temperature of 20 ºC. As no confidence level is reported, a triangular distribution was assumed and the uncertainty associate with calibration effect is:

$$u_{calib} = 0.1/\sqrt{6} = 0.04\, mL$$

In order to account for the temperature variability in the laboratory within ±3 °C respects to the calibrating temperature (20°C), a rectangular distribution was assumed and the uncertainty associate to this effect is computed with the following formula:

$$u_{temp} = \frac{T.V.Q}{\sqrt{3}} = 0.04\,mL$$

where T is the temperature variability (±3), V is the volume of the volumetric flask used and Q is the coefficient of volume expansion of the water (Q = 2.1 x 10^{-4} $^{o}C^{-1}$). The combined uncertainty of the 100 ml volumetric flask is:

$$u_{volum} = \sqrt{u_{calib}^2 + u_{temp}^2} = 0.05\,mL$$

The combined uncertainty of the mercury stock solution was calculated combining the three uncertainties (Table 18).

$$\frac{u_{stock}}{C_{stock}} = \sqrt{\left(\frac{u_{std}}{C_{stock}}\right)^2 + \left(\frac{u_{volum}}{V}\right)^2} = 0.08\%$$

Table 18 - The combined uncertainty of Hg sock solution

Description	**Value**	**SD**	**Uncertainty as RSD (%)**
Hg stock solution (u_{cert})	1000 mg/L	1 mg/L	0.06
Volumetric flask (u_{volum})	100 mL	0.05 mL	0.05
Combined uncertainty (u_{stock})			0.08

3.3.1.2 Uncertainty source: analyzed mass

The contribution from the weight of samples is due to the linearity uncertainty of the balance from Calibration Certificate. From balance linearity (± 0.03 mg), a rectangular distribution is assumed to obtain a standard uncertainty; this contribution is considered twice, once for the tare and once for the gross weight. This gives for the standard uncertainty of samples mass, U_m, the following value:

$$U_m = \sqrt{2\left(\frac{0.03}{\sqrt{3}}\right)^2} = 0.16\,mg$$

Uncertainty due to balance for analysed mass is presented in Table 19.

Table 19 - Uncertainty due to balance for analysed mass of samples

Description	**Value**	**SD**	**Uncertainty as RSD (%)**
Mass (u_m)	90 mg	0.16 mg	0.18

3.3.1.3 Uncertainty source: Recovery

The overall bias of the analytical method is determined by the recovery study of the method validation procedure, using the standard addition method. The uncertainty due to recovery is derived from the standard deviation of the mean of the trueness assessment study u_{tr}.

$$u_{rec} = \sqrt{\frac{s_{tr}^2}{n_{tr}} + u_{std}^2}$$

where s_{tr} is the relative standard deviation derived from the average recovery of each day, and n_{tr} is the number of days. Like in the case of ICP-OES, the term associated with the CRMs nominal value uncertainty was not taken into account.

Table 20 - Trueness

Trueness (u_{tr})	*Value*	*SD*	*Uncertainty as RSD (%)*
BCR 141R			1.77
BCR 142 R			2.54
SRM 2709			2.52
LCG 6181			1.79

3.3.1.4 Uncertainty source: Precision

Uncertainty associated to precision was derived from two contributions: repeatability and intermediate precision calculated in the validation study. Uncertainty due to repeatability was estimated as $s_{rep}/\sqrt{n_{rep}}$ where s_{rep} is relative standard deviation due to repeatability experiment and n_{rep} the number of replicates. The uncertainty due to intermediate precision was estimated as $s_{day}/\sqrt{d}$ with s_{day} being the relative day-to-day variation and d the number of days. The precision uncertainty was derived combining these two uncertainties as follows:

$$u_{prec} = \sqrt{u_{rep}^2 + u_{day}^2}$$

Obtained uncertainties are summarized in Table 21.

Table 21 - Results on precision uncertainty

Description	RSD (%)	RSD (%)	RSD (%)	RSD (%)
	BCR 141R	BCR 142R	SRM 2709	LCG6181
Repeatability (u_{rep})	0.99	1.82	1.94	2.65
Intermediate precision (u_{day})	1.47	1.77	1.6	1.96
Combined uncertainty (u_{prec})	1.77	2.54	2.52	3.3

Starting from the contribution of the single uncertainties, the combined uncertainty, expressed in terms of relative uncertainties u_i, can be calculated as:

$$u_{rel}(C) = \sqrt{u_{rel}^2(stok) + u_{rel}^2(mass) + u_{rel}^2(V_{fin}) + u_{rel}^2(rec) + u_{rel}^2(precision)}$$

In Table 22 all the contributions are reported.

Table 22 - Uncertainty as RSD (%)

Description	BCR 141R	BCR 142R	SRM2709	LCG 6181
Stock solution (u_{stock})	0.1	0.1	0.1	0.1
mass (u_m)	0.2	0.2	0.2	0.2
Recovery (u_{rec})	1.8	2.5	1.8	4.5
Precision (u_{prec})	1.8	2.5	2.5	2.5
Combined	2.5	3.6	3.8	3.6

3.3.2 Expanded uncertainty

In order to take into account a confidence level, the combined uncertainty is to be multiplied by a coverage factor, *k*, to produce the expanded uncertainty. The choice of this factor was done taking into account a 95% confidence level, which give a coverage factor of 2.

The expanded uncertainty is given by:

$$u_{expanded} = k \cdot u_{combined}$$

To compute the expanded uncertainty we chose the higher combined uncertainty in both low and high calibration. In percentage terms, we obtain an expanded uncertainty of 7% in low calibration and 8% in high calibration

4. Conclusions

The performed method validation proofed that the horizontal standard methods were implemented successfully for the three matrices studied, i.e. soil, treated biowaste and sewage sludge. Analyses of certified reference materials showed generally good recoveries for the investigated elements with some minor difficulties in a few exceptional cases, e.g. Pb at very low concentration in compost.

Observed uncertainties showed further that the methods are fit for purpose, i.e. the characterisation of background values.

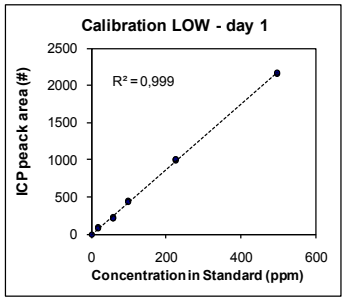
5. Annex A - Temporary storage of 2009 LUCAS soil samples

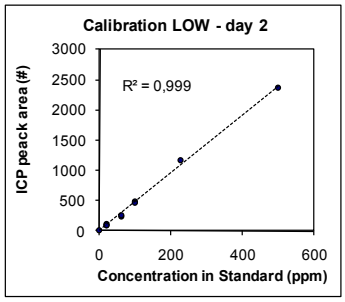
The pictures show the current storage facilities of the LUCAS 2009 Soil Set, located at the European Commission's Joint Research Centre, Ispra, Italy.

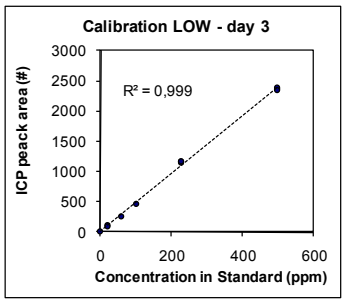
6. Annex B - Calibration curve for low calibration method validation

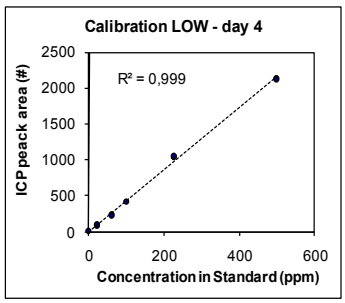
Aluminium (Al) No calibration curve in low range

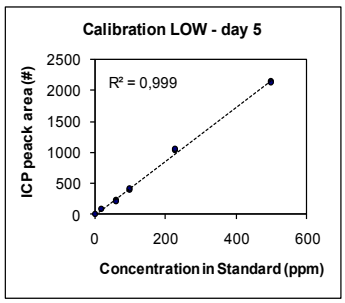
Arsenic (As)



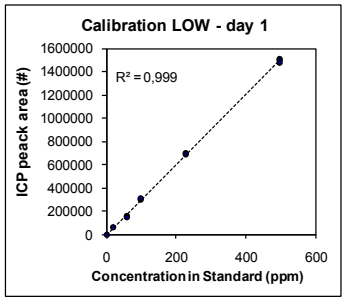


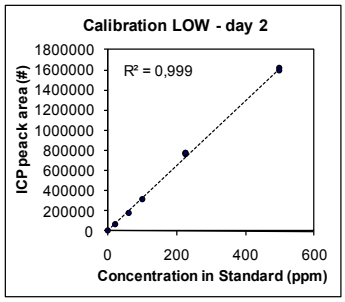


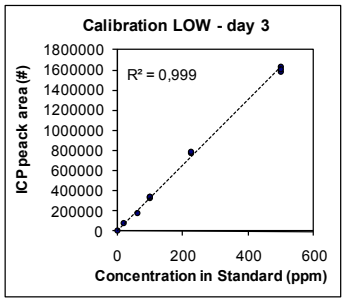


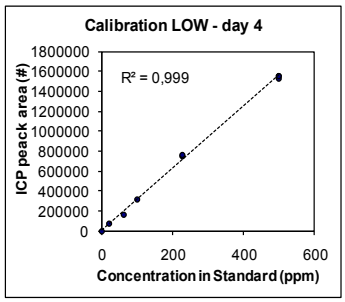


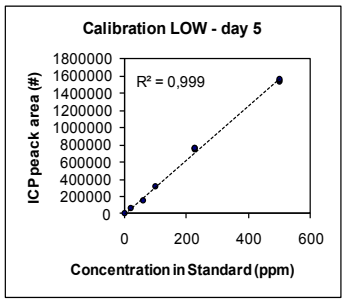
Barium (Ba)



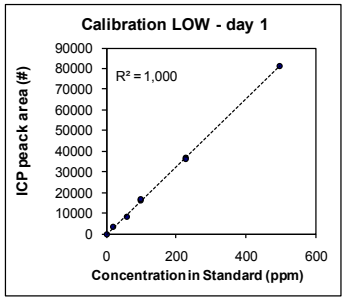


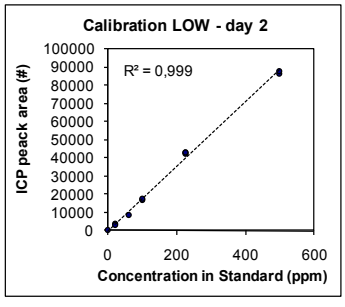


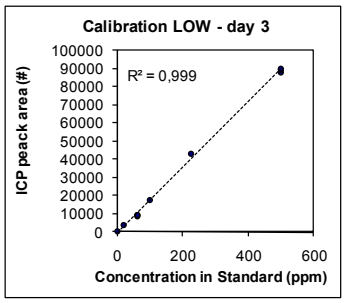


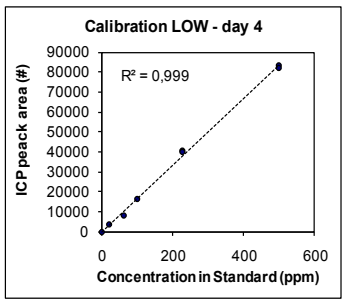


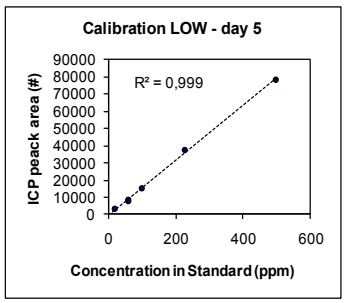
Cadmium (Cd)



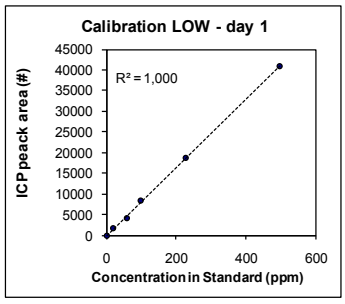


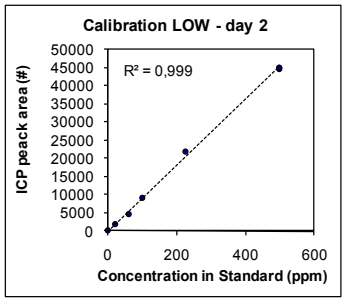


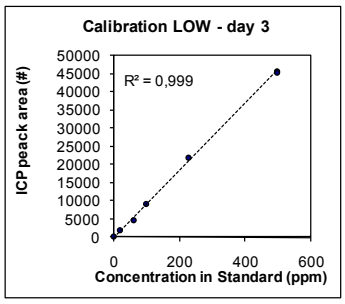


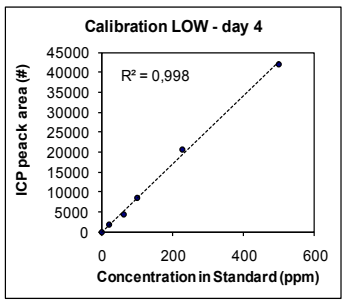


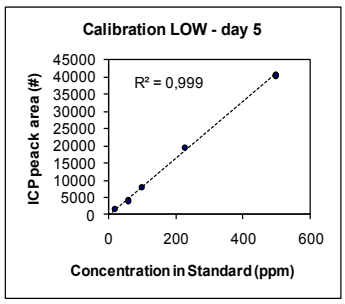
Cobalt (Co)



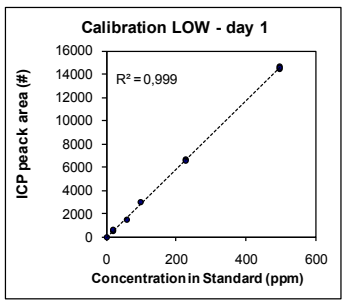


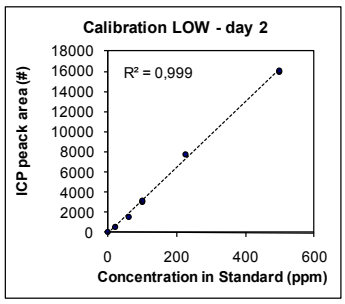


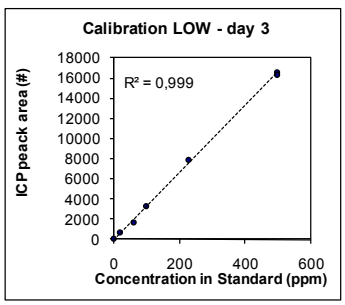


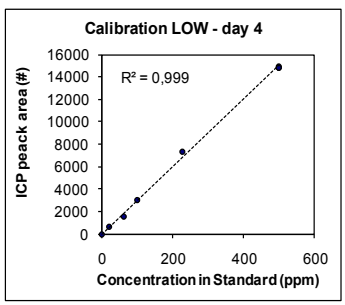


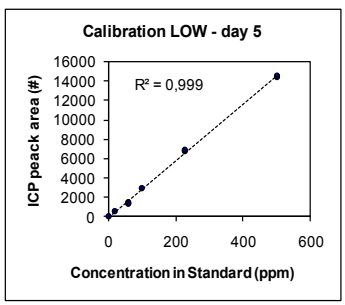
Chrome (Cr)



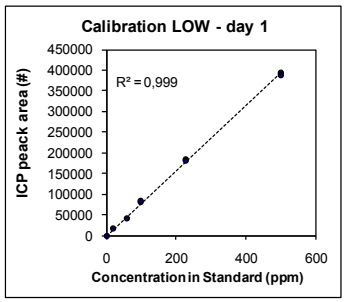


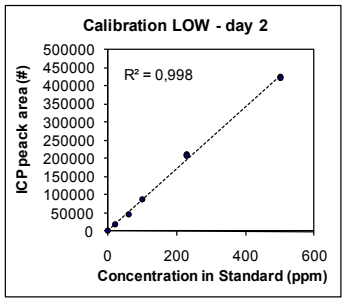


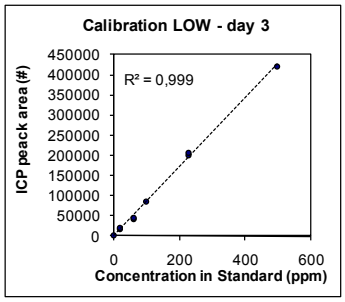


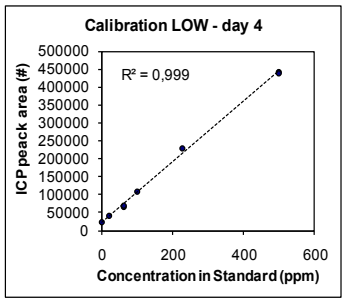


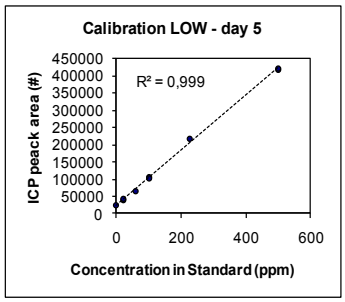
Copper (Cu)







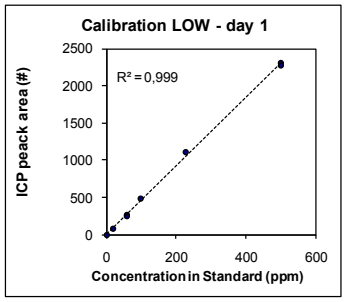


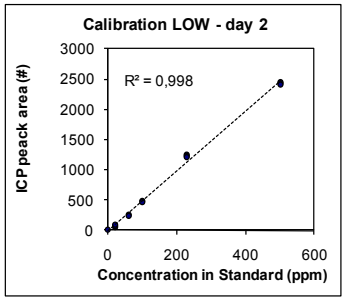


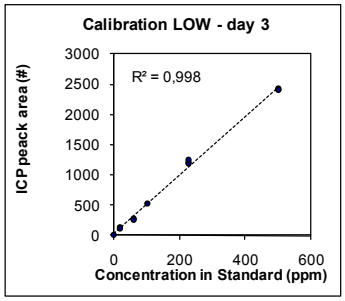
Iron (Fe)

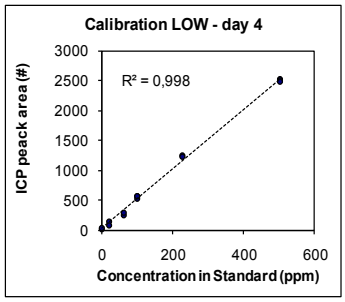
No calibration curve in low range

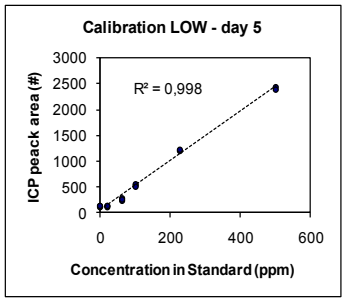
Magnesium (Mg)







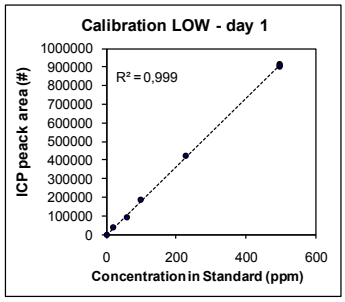


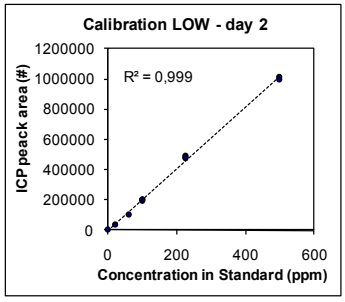


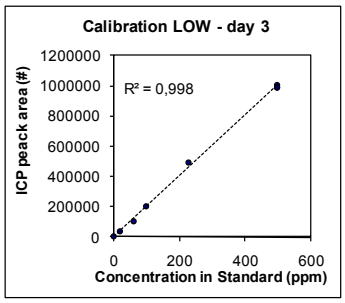
Potassium (K)

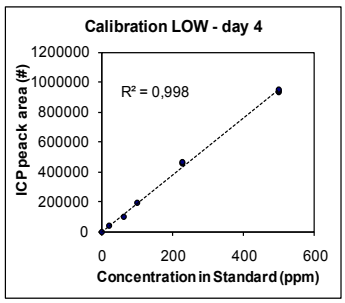
No calibration curve in low range

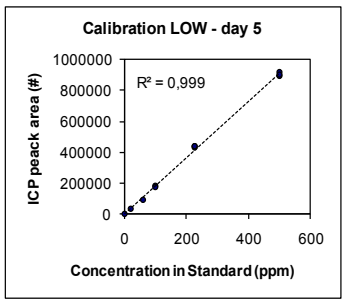
Manganese (Mn)



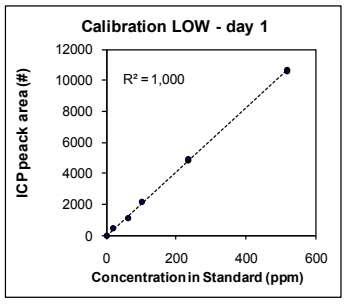


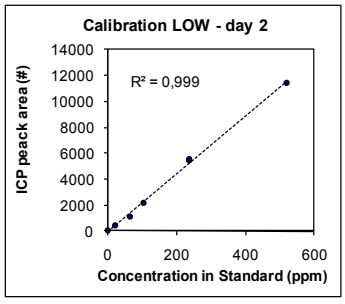


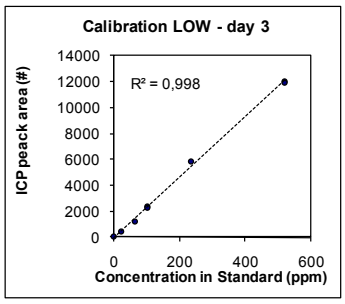


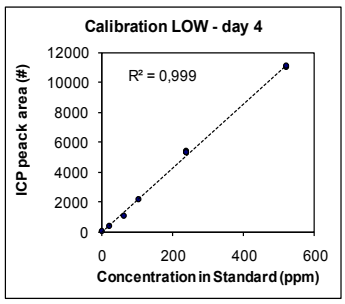


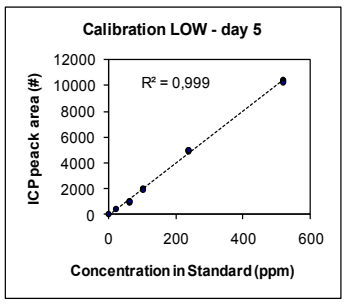
Molybdenum (Mo)



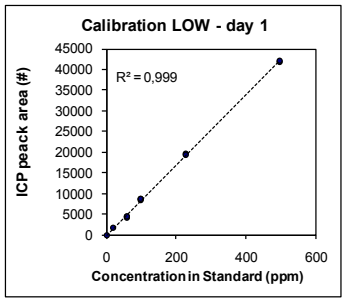


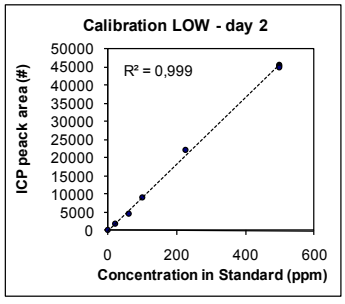


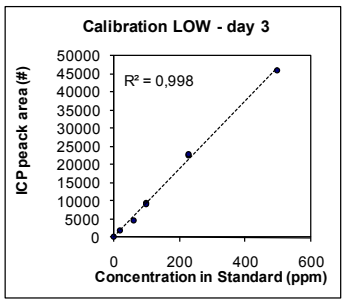


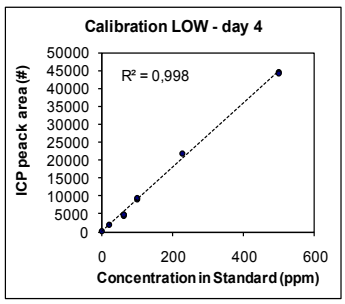


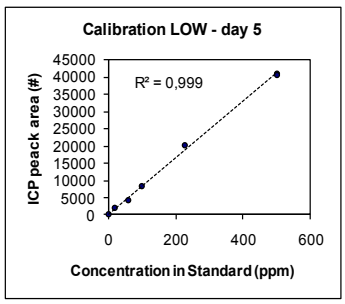
Nickel (Ni)







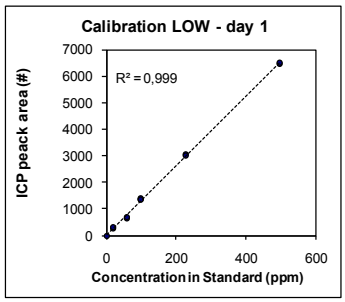


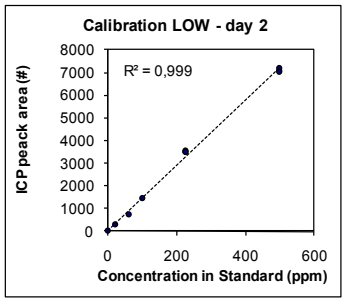


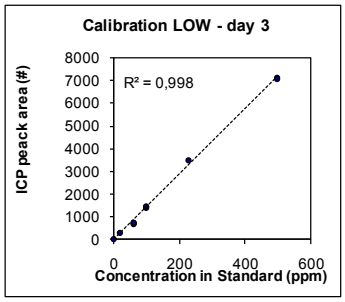
Phosphorus (P)

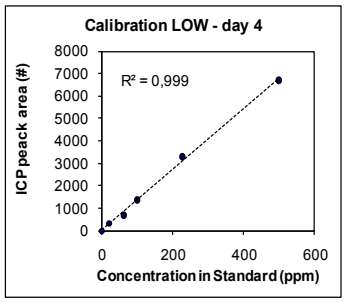
No calibration curve in low range

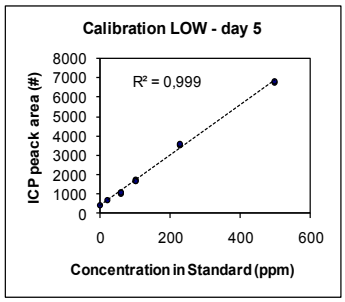
Lead (Pb)



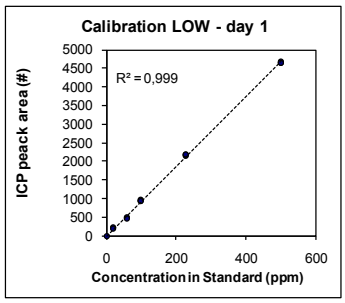


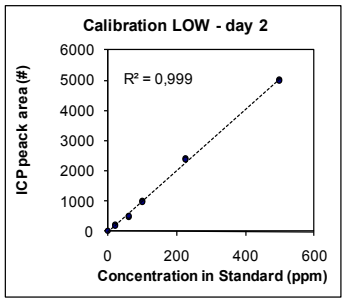


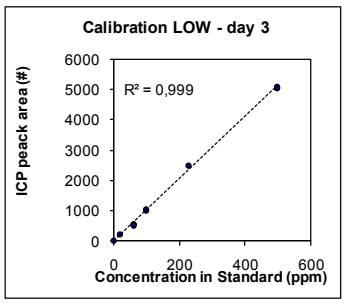


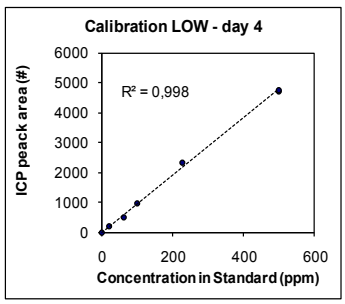


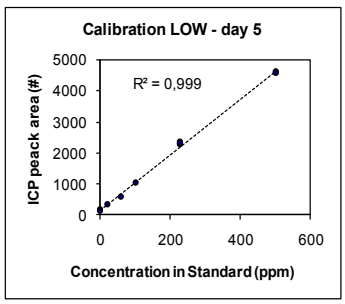
Antimony (Sb)



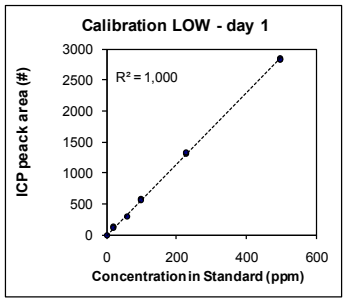


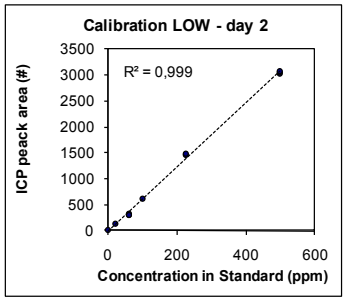


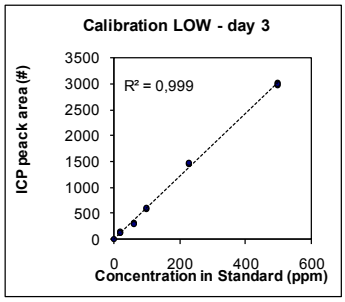


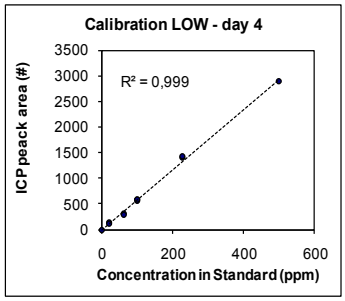


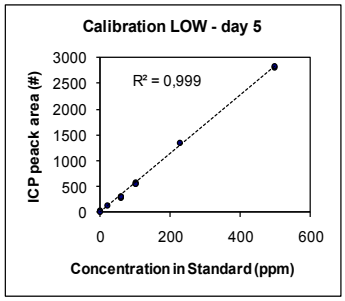
Selenium (Se)



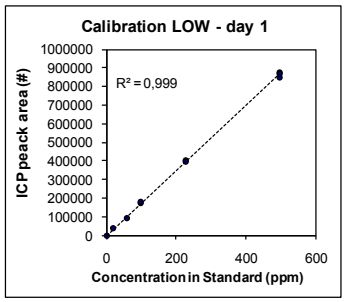


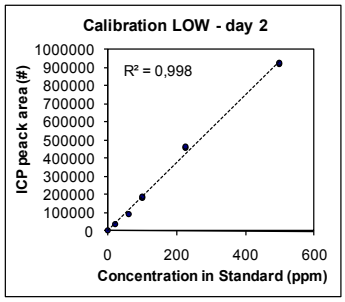


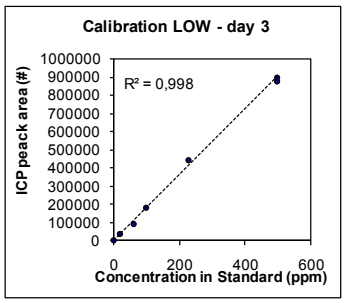


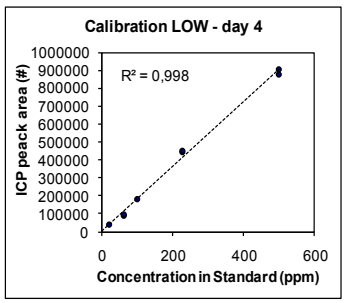


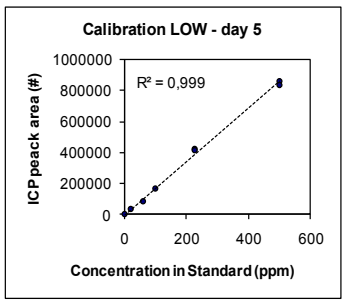
Titanium (Ti)



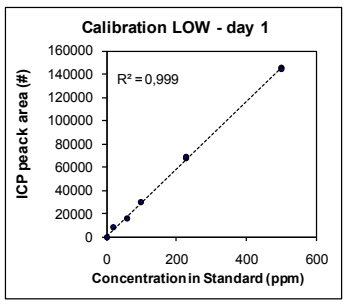


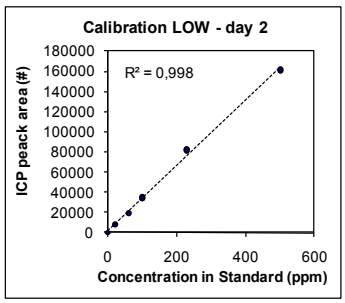


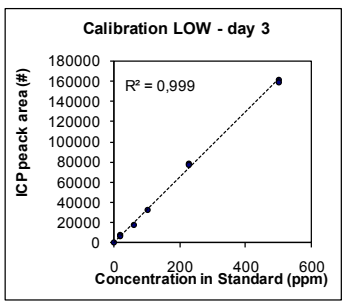


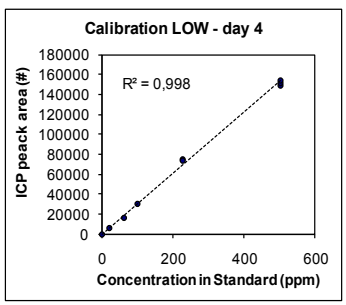


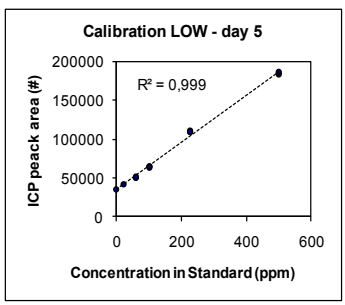
Vanadium (V)



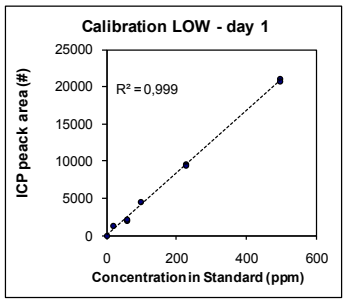


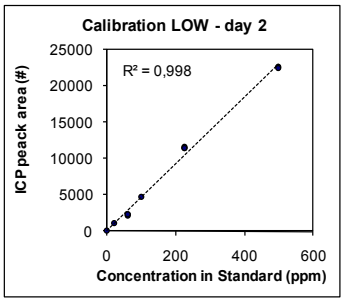


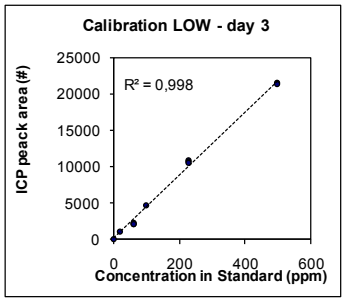


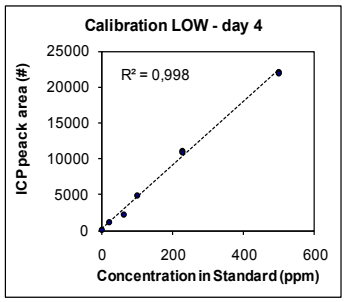


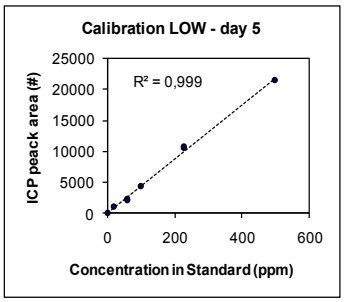
Zinc (Zn)


Calibration LOW - day 1
ICP peack area (#)
R² = 0,999
25000
20000
15000
10000
5000
0
0
200
400
600
Concentration in Standard (ppm)


Calibration LOW - day 2
ICP peack area (#)
R² = 0,998
25000
20000
15000
10000
5000
0
0
200
400
600
Concentration in Standard (ppm)

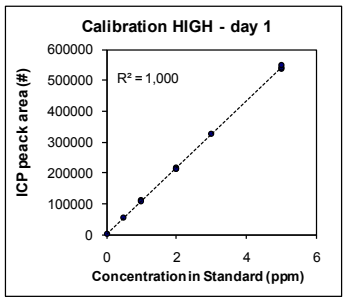

Calibration LOW - day 3
ICP peack area (#)
R² = 0,998
25000
20000
15000
10000
5000
0
0
200
400
600
Concentration in Standard (ppm)

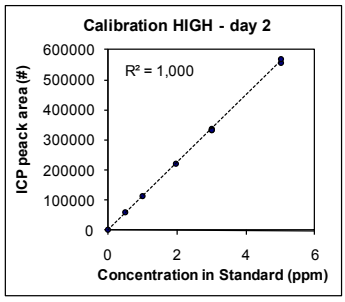

Calibration LOW - day 4
ICP peack area (#)
R² = 0,998
25000
20000
15000
10000
5000
0
0
200
400
600
Concentration in Standard (ppm)

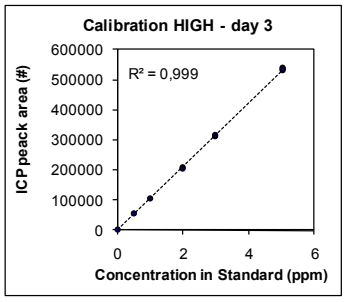

Calibration LOW - day 5
ICP peack area (#)
R² = 0,999
25000
20000
15000
10000
5000
0
0
200
400
600
Concentration in Standard (ppm)

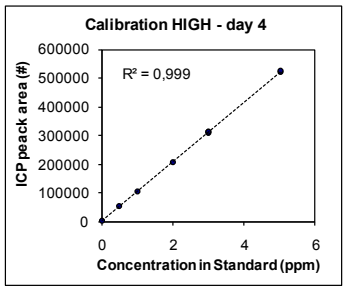
7. Annex C - Calibration curve for high calibration method validation

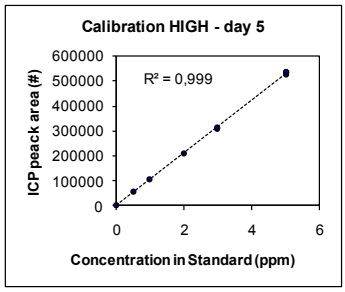
Aluminium (Al)



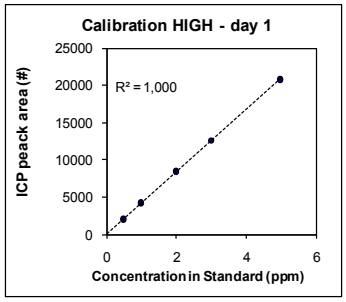


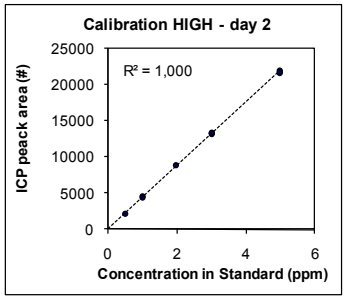


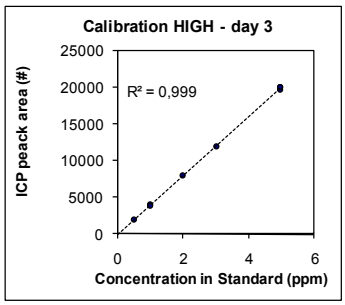


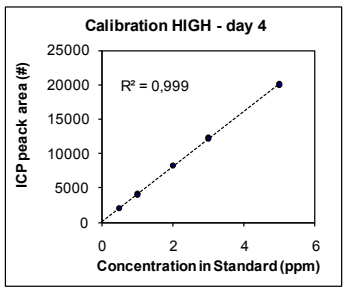


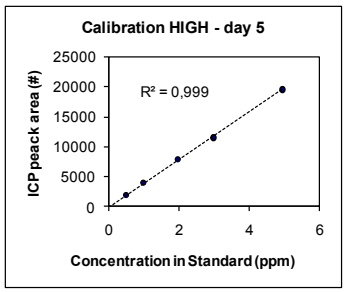
Arsenic (As)



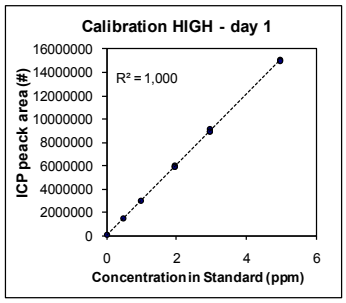


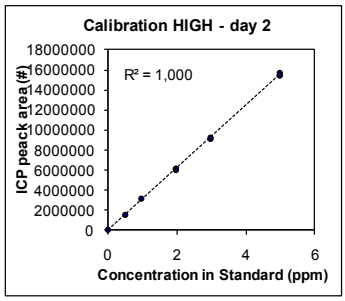


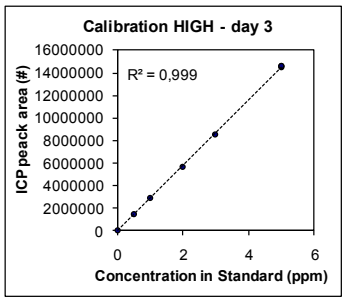


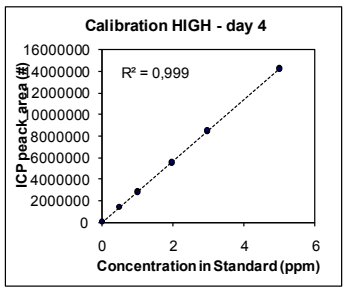


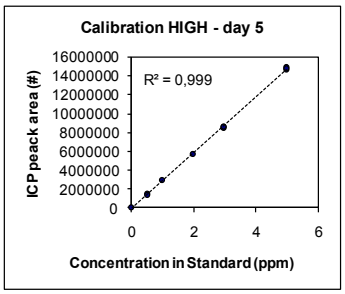
Barium (Ba)



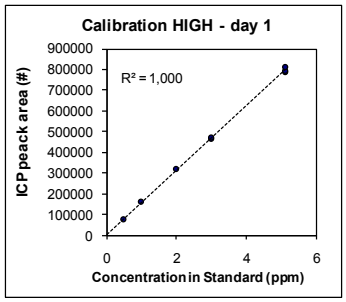


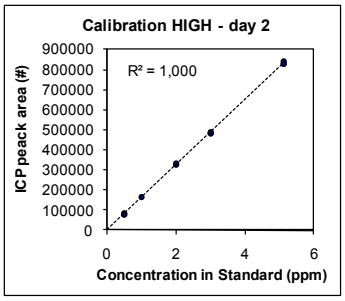


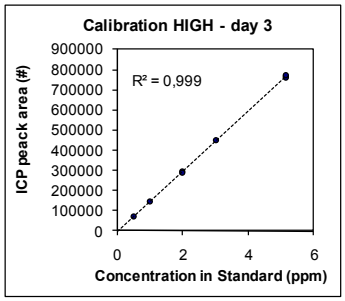


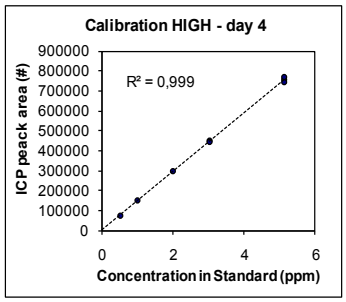


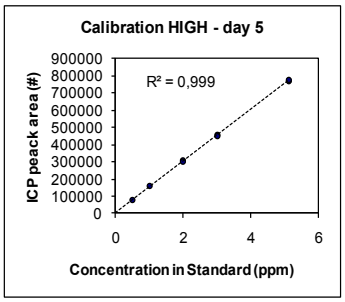
Cadmium (Cd)



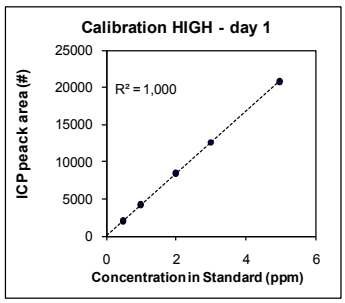


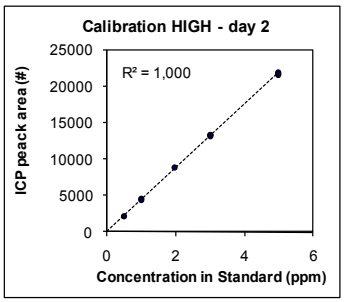


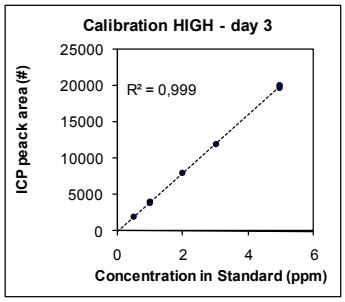


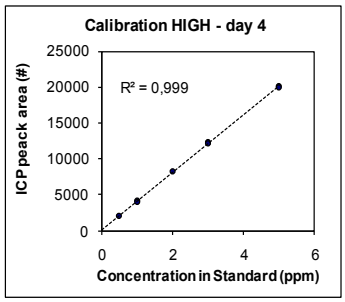


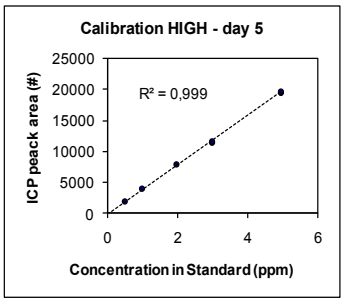
Cobalt (Co)



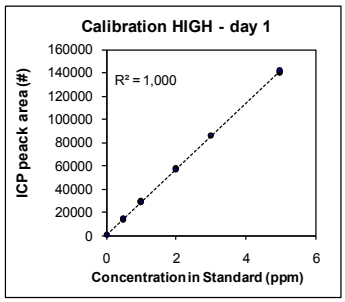


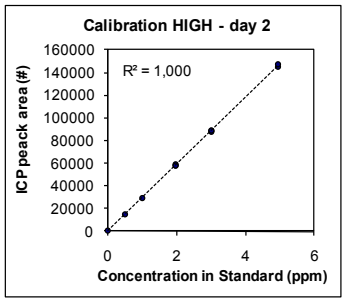


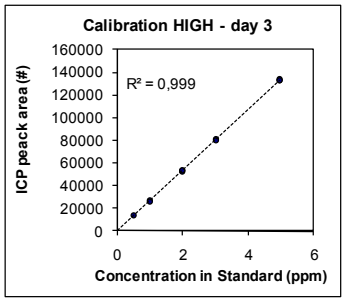


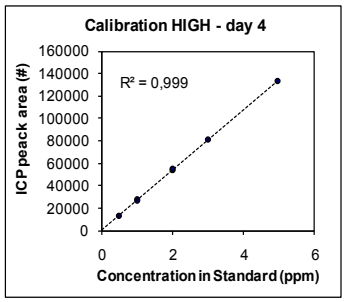


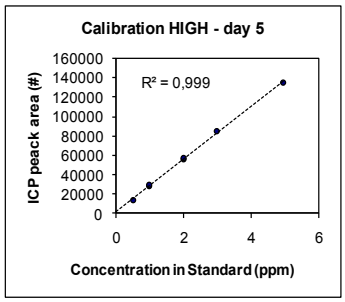
Chrome (Cr)



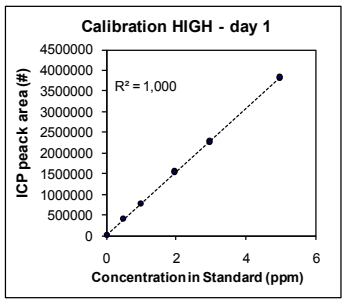


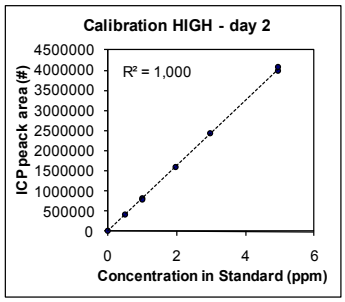


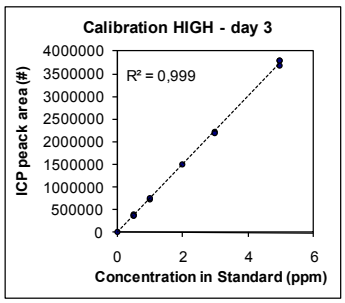


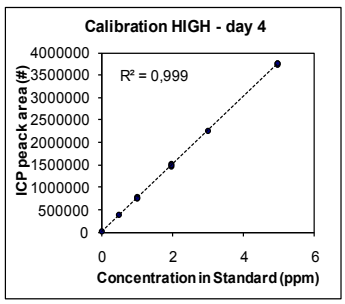


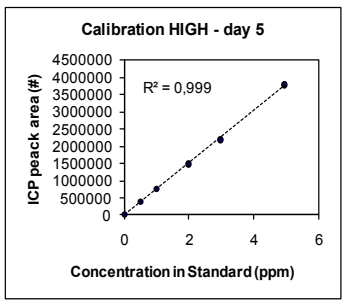
Copper (Cu)



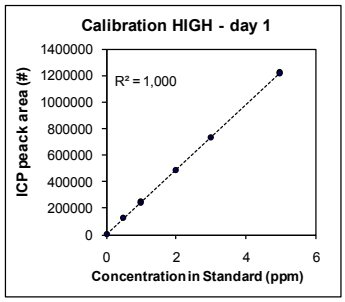


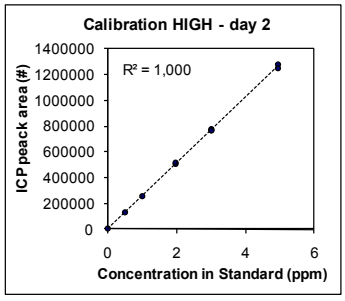


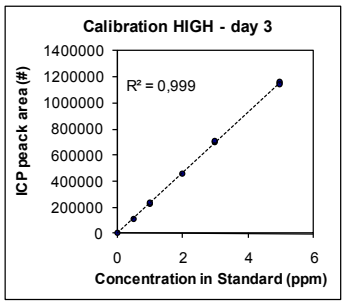


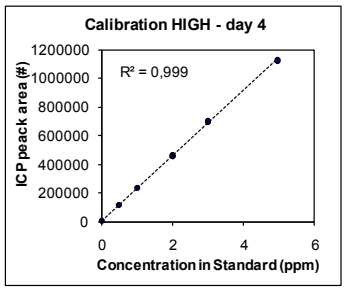


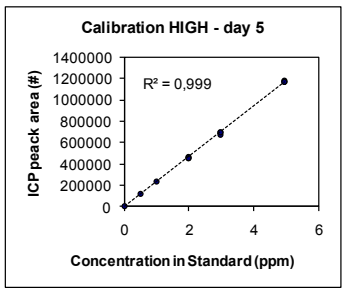
Iron (Fe)



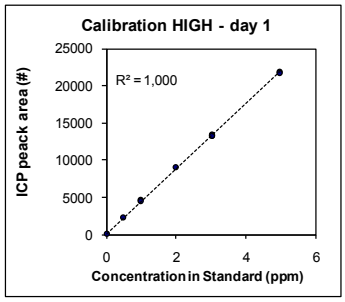


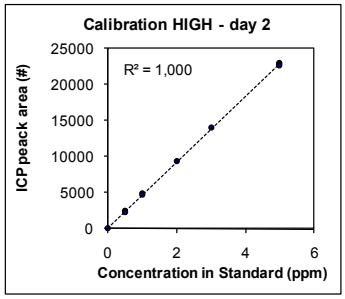


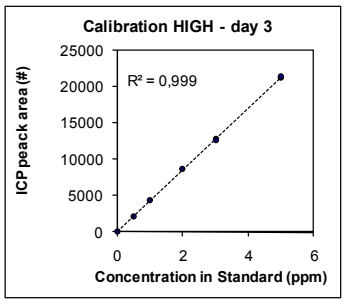


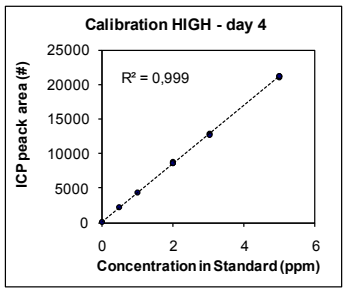


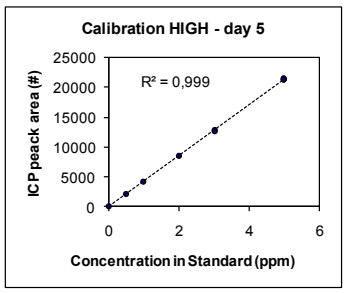
Magnesium (Mg)



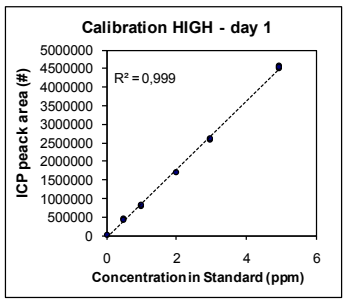


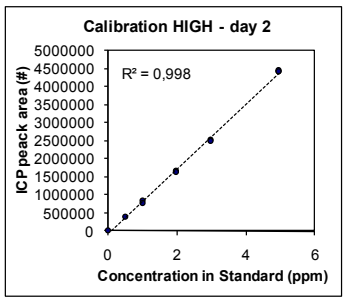


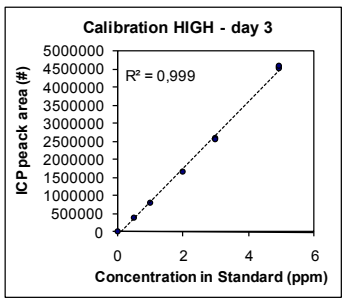


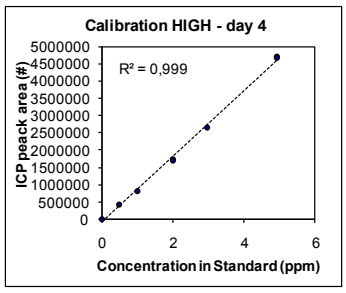


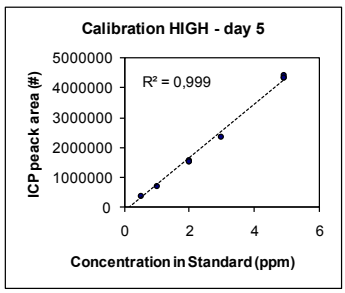
Potassium (K)



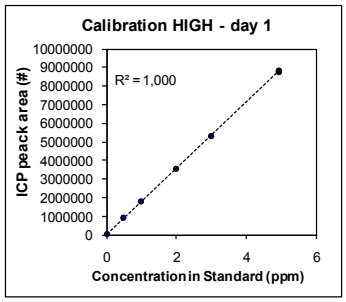


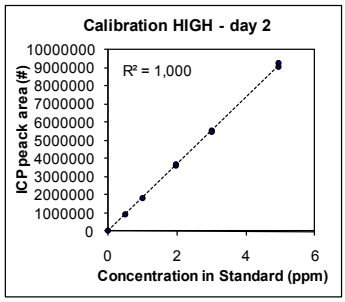


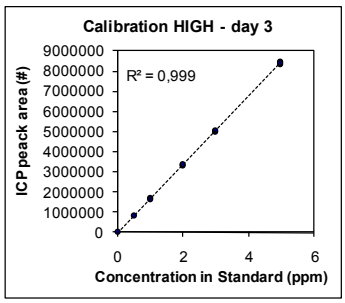


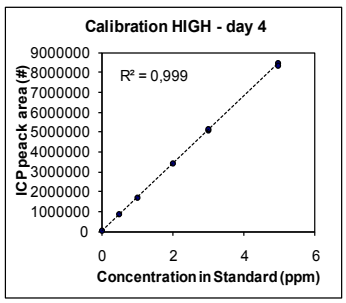


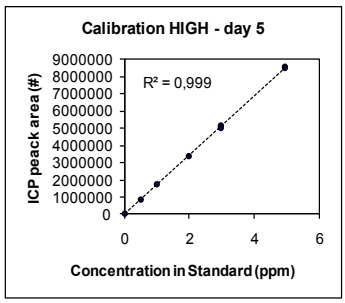
Manganese (Mn)



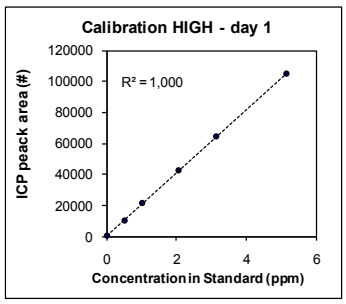


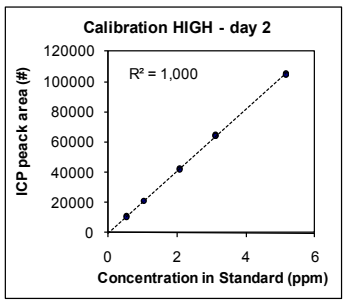


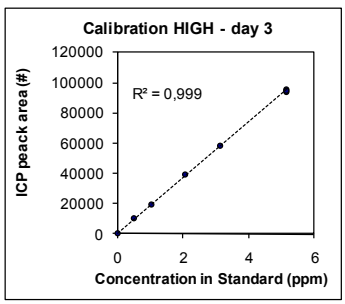


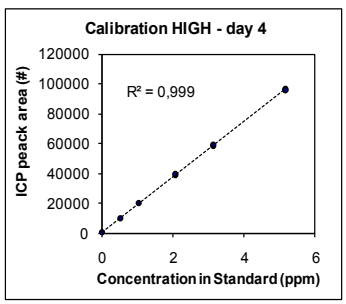


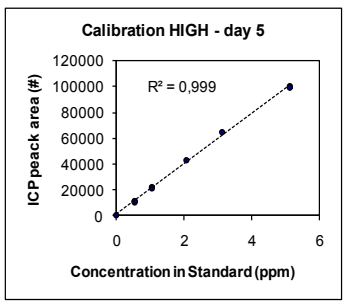
Molybdenum (Mo)



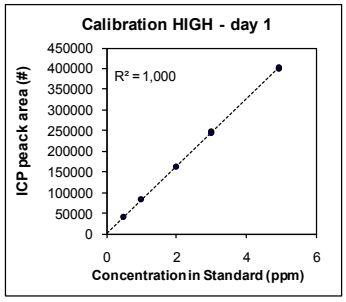


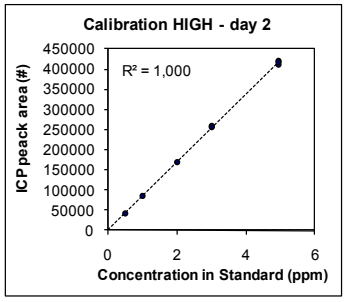


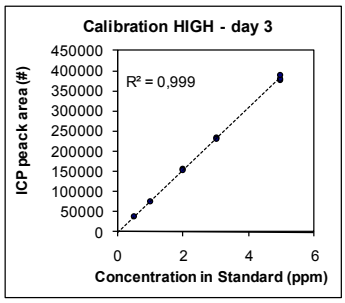


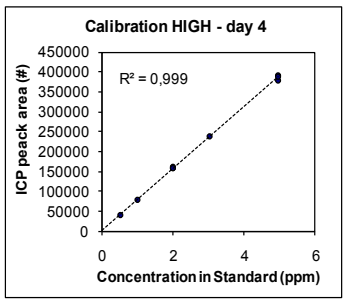


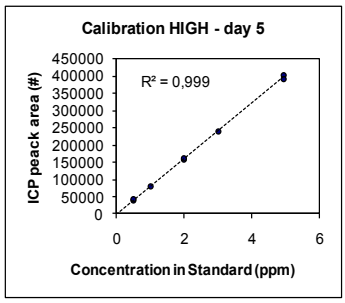
Nickel (Ni)



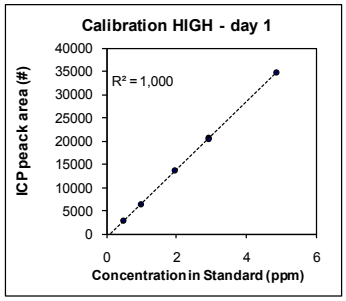


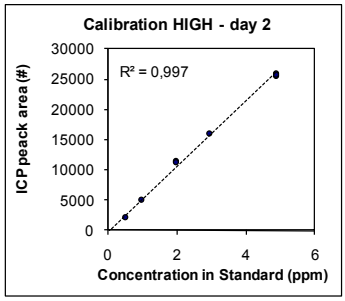


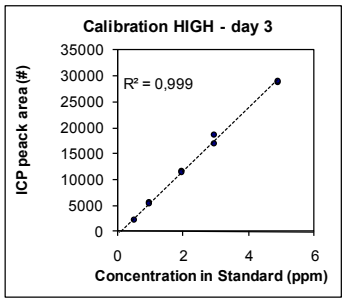


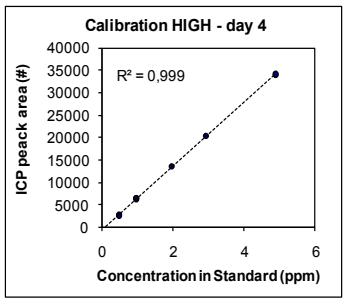


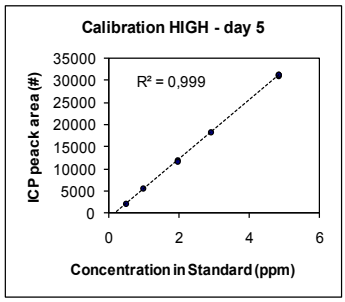
Phosphorus (P)



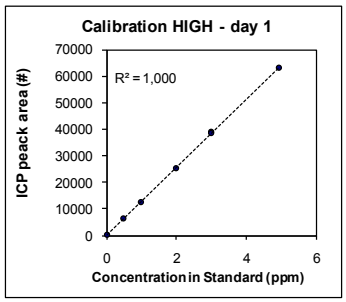


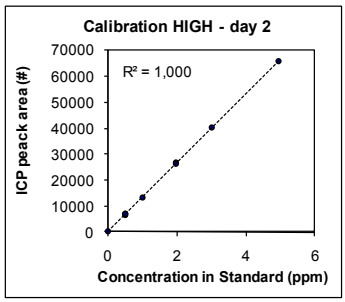


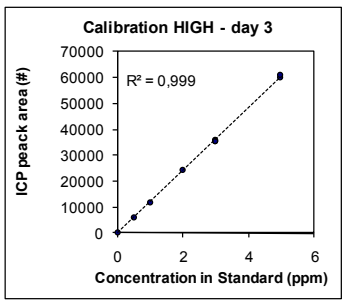


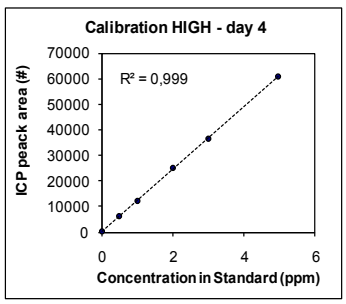


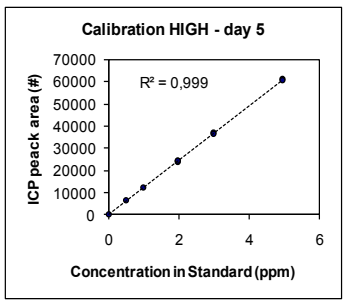
Lead (Pb)



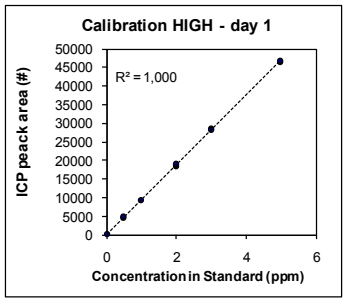


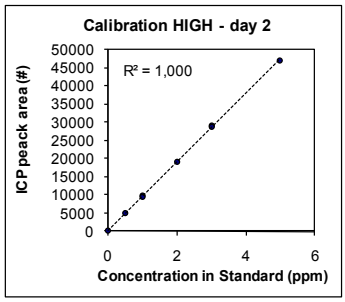


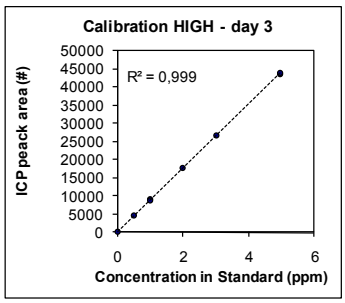


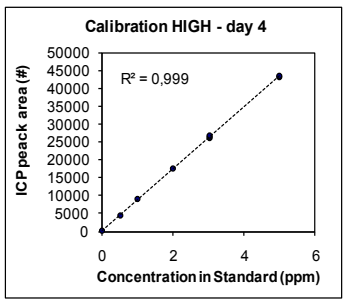


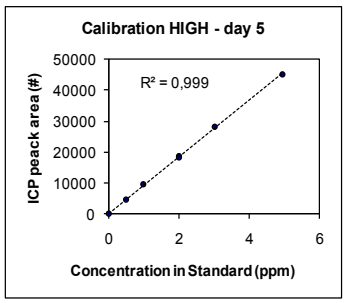
Antimony (Sb)



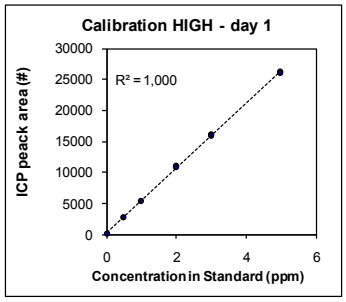


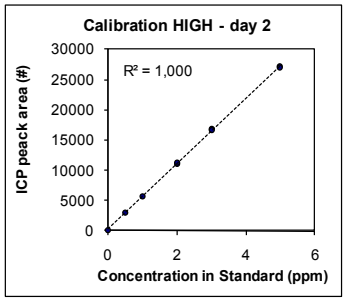


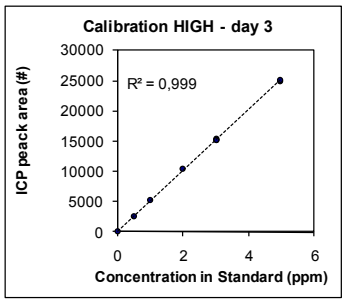


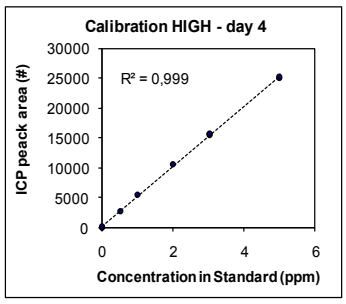


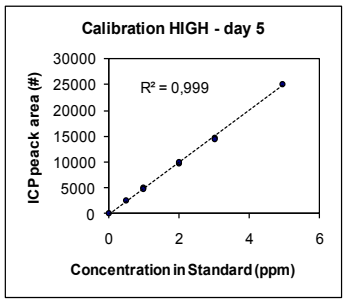
Selenium (Se)



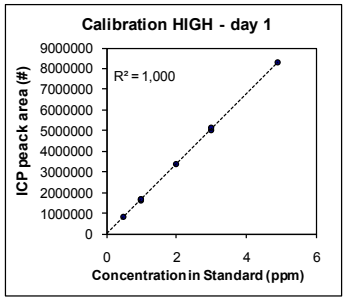


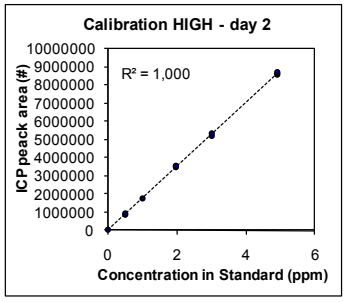


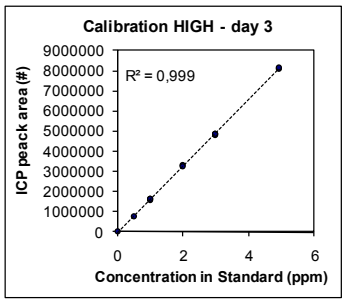


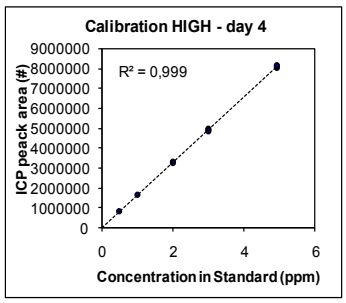


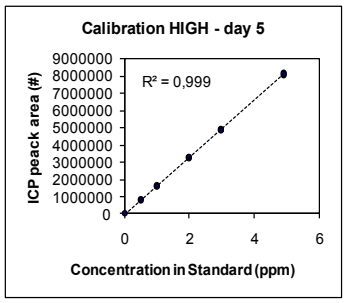
Titanium (Ti)



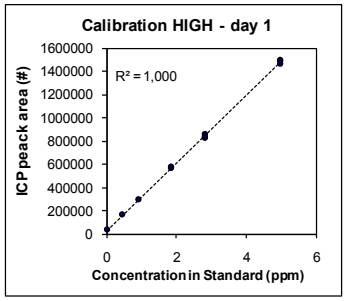


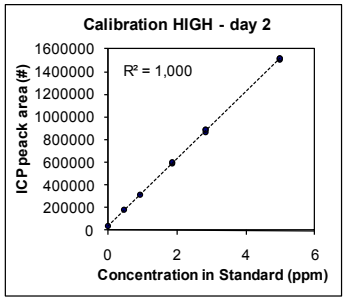


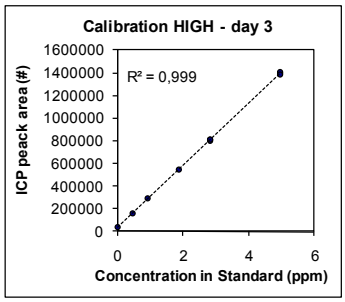


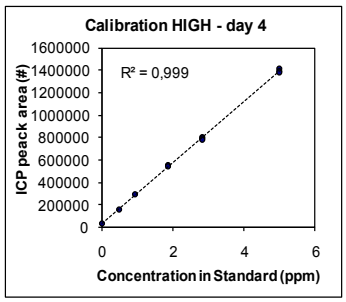


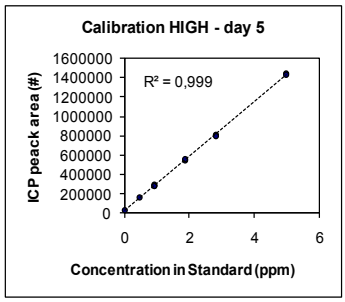
Vanadium (V)



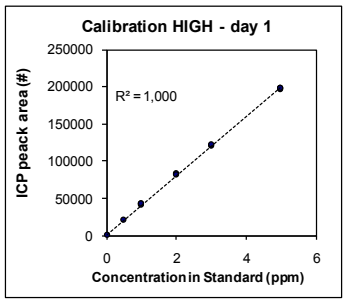


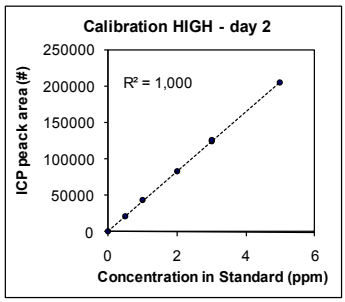


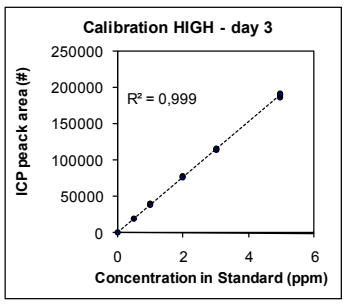


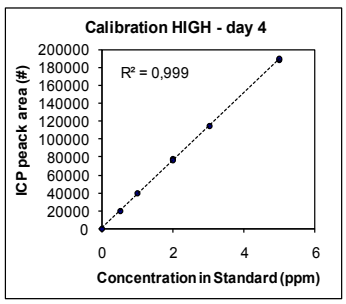


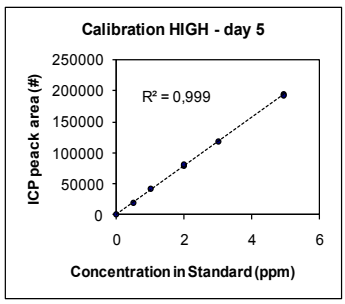
Zinc (Zn)











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Author(s): Carmen Cristache, Sara Comero, Giovanni Locoro, Isabelle Fissiaux, Agustín Alonso Ruiz, Gerard Tanet and Bernd Manfred Gawlik

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Abstract

To implement a validated method for the analysis of 22000 soil samples stemming from 2009 LUCAS Soil Survey as well as from sewage sludge and treated biowaste samples from to FATE-Programme, a validation study was conducted with the following objectives: (i) to validate these methods for the determination of heavy metals by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Could Vapour-Atomic Adsorption Spectrometry (CV-AAS) techniques, respectively, according to the ISO 17025 requirement and (ii) to implement these methods for the determination of heavy metals in soil, sludge and compost samples on a routine basis. The calibration curves, detection and quantification limits, trueness as well as repeatability were determined. The budget uncertainty was also estimated (including a full uncertainty budget and Ishikawa-diagram).

LB-NA-25850-EN-N

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Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security, including nuclear; all supported through a cross-cutting and multi-disciplinary approach.

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